Phylum XXVI. Actinobacteria phyl. nov.

Michael Goodfellow

Ac.ti.no.bac.te′ri.a. Gr. n. *actis -inos* a ray, beam, N.L. n. *bacter* a rod; suff. *-ia* ending denoting phylum; N.L. pl. neut. n. *Actinobacteria* actinomycete bacteria with diverse morphologies.

This taxon is one of the major phyla in the domain *Bacteria*, as inferred from its branching pattern in the 16S rRNA gene tree (Garrity and Holt, 2001; Ludwig and Klenk, 2005) and taxon-specific 16S rRNA signatures (Zhi et al., 2009). The separation of the phylum from other bacterial taxa is supported by conserved indels in some proteins (e.g., cytochrome-*c* oxidase subunit 1, CTP synthase and glutamyl-tRNA synthase), by the presence of a large insert in 23S rRNA (Gao and Gupta, 2005; Gao et al., 2006) and by distinctive gene arrangements (Kunisawa, 2007). The nearest phylogenetic neighbor to the phylum is not clear (Ventura et al., 2007) though the *Firmicutes* are usually considered in this context. In this volume of the *Manual*, the phylum *Actinobacteria* encompasses five classes, 19 orders, 50 families, and 221 genera. However, many new taxa continue to be discovered so this listing is inevitably incomplete. The constituent classes are *Acidimicrobiia* class. nov., *Actinobacteria* (Stackebrandt et al., 1997), *Coriobacteriia* class. nov., *Rubrobacteria* class. nov., and *Thermoleophilia* class. nov.

Actinobacteria are Gram-stain-positive or Gram-stain-variable aerobes, facultative anaerobes or anaerobes, which have a rigid cell wall that contains muramic acid. Some contain wall teichoic acids. Most are chemo-organotrophs which grow at neutral pH, but some are acidophiles or alkalophiles. Others are halophiles and some are thermophiles. The phylum includes phenotypically diverse organisms which show diverse morphological properties that range from cocci to highly differentiated mycelia. Most are saprophytic, but some are pathogenic for plants and animals. The DNA G+C content ranges from just under 50 mol% (e.g., *Hoyosella* and *Tropheryma*) to over 70 mol% (e.g., *Frankia* and *Streptomyces*). Members of the taxon are widely distributed in aquatic and terrestrial habitats (Goodfellow and Williams, 1983; Jensen and Lauro, 2008; Stach and Bull, 2005; Williams et al., 1984).

Type order: **Actinomycetales** Buchanan 1917, 162^{AL} emend. Zhi, Li and Stackebrandt 2009, 594.

Taxonomic comments

The class *Actinobacteria* and constituent taxonomic ranks above the genus level were proposed by Stackebrandt et al. (1997) solely on the basis of 16S rRNA gene sequence-based groups and taxon-specific 16S rRNA gene sequences. This classification represented a step-change in the classification of actinomycetes above the genus level as it showed that previous classifications based on form and function were wanting as they did not reflect natural relationships. Comprehensive accounts of earlier classifications of actinobacteria are available (Cross and Goodfellow, 1973; Goodfellow and Williams, 1983; Stackebrandt and Schumann, 2006). In addition, the evolutionary history of the phylum has been traced by Ventura et al. (2007). At the genus level, good correlation has been found between the phylogenetic position of organisms and the discontinuous distribution of key chemical markers (Embley and Stackebrandt, 1994; Goodfellow, 1989; Goodfellow and Maldonado, 2006; Kroppenstedt and Goodfellow, 2006). The class *Actinobacteria* and constituent higher taxa have been updated by Zhi et al. (2009). In this volume, the actinobacteria have been assigned the rank of a phylum as the phylogenetic depth represented by the lineage corresponds to that of existing phyla (Garrity and Holt, 2001; Ludwig and Klenk, 2005).

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Class I. **Actinobacteria** Stackebrandt, Rainey and Ward-Rainey 1997, 483

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Ac.ti.no.bac.te′ri.a. Gr. n. *actis -inos* a ray, beam; N.L. n. *bacter* a rod; suff. *-ia* ending denoting a class; N.L. pl. neut. n. *Actinobacteria* actinomycete bacteria with diverse morphologies.

Upon publication of the class *Actinobacteria*, a type order was not designated. Hence, this name is not validly published under Rule 27 of the *Bacteriological Code* (1990 Revision) (Euzéby and Tindall, 2001; Lapage et al., 1992). Nevertheless, because of its wide use, the name is adopted for this volume of the *Manual*. If so designated by the Judicial Commission, the type order is likely to be *Actinomycetales* Buchanan 1917, 162[AL] emend. Zhi, Li and Stackebrandt 2009, 594.

The class *Actinobacteria sensu stricto* encompasses 15 orders, 43 families, and 203 genera. Members of the taxon show a remarkable range of morphologies, these include organisms that form cocci (e.g. *Dermacoccus* and *Micrococcus*), short rods (e.g. *Mycobacterium* and *Tropheryma*), irregular rods (e.g. *Mycetocola*), rods and cocci (e.g. *Arthrobacter* and *Brevibacterium*), and mycelia that fragment into coccoid and rod-like elements (e.g. *Nocardia* and *Oerskovia*). Others show more extensive morphological differentiation ranging from those which produce extensively branched substrate hyphae that bear spores (e.g. *Micromonospora*) or spore vesicles (e.g. *Actinoplanes* and *Dactylosporangium*) to those that form a stable branched mycelium that carries aerial hyphae which differentiate into short or long chains of spores (e.g. *Actinomadura* and *Streptomyces*) or into spore vesicles (e.g. *Planobispora* and *Streptosporanigium*). In general, spores are nonmotile though those released from spore vesicles tend to be motile.

Amongst prokaryotes, members of the class *Actinobacteria*, notably *Streptomyces* strains, are the richest source of natural products, especially clinically useful antibiotics, antimetabolites and antitumor agents (Bérdy, 2005; Newman and Cragg, 2007; Olano et al., 2009). Filamentous actinobacteria account for about 45% of all microbial bioactive secondary metabolites with about 80% of these 7600 compounds being produced by streptomycetes (Bérdy, 2005). Despite this amazing metabolic diversity, it has been estimated that only about 10% of the total number of natural products that can be synthesized by these organisms have been discovered (Watve et al., 2001). *Amycolatopsis*, *Actinoplanes*, *Micromonospora* and strains are also a source of clinically significant antibiotics, whereas members of less well-known genera, such as *Salinispora* and *Verrucosispora*, are showing promise in this respect (Goodfellow and Fiedler, 2010; Jensen, 2010). It seems likely that filamentous actinobacteria belonging to such taxa will remain a source of new chemical entities as the whole-genomes of representatives of such genera contain 20 or more natural-product biosynthetic gene clusters for the production of known or predicted secondary metabolites, as exemplified by *Amycolatopsis mediterranei* strain U32 (Zhao et al., 2010). There is also evidence that actinobacteria isolated from the extremobiosphere will be a rich source of novel natural products (Bull, 2010).

Actinobacteria are widely distributed in aquatic and terrestrial habitats, including extreme habitats, such as deep-sea sediments (Pathom-aree et al., 2006) and hyper-arid desert soils (Okoro et al., 2009). They are particularly common in soil where they have roles in the turnover of organic matter and recalcitrant molecules (Goodfellow and Simpson, 1983; Goodfellow and Williams, 1983). Others are pathogens of animals (e.g. *Actinomadura*, *Mycobacterium*, *Nocardia*, *Propionibacterium*, and *Tropheryma* spp.) and plants (e.g. *Clavibacter*, *Curtobacterium*, *Rhodococcus*, and *Streptomyces* spp.), plant commensals (e.g. *Leifsonia* spp.), nitrogen-forming symbionts (e.g. *Frankia*) and inhabitants of the gastrointestinal tract (*Bifidobacterium* and *Scardovia* spp.). A range of procedures are available for the selective isolation of specific components of actinobacterial communities present in natural habitats (Goodfellow, 2010).

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Order I. **Actinomycetales** Buchanan 1917, 162[AL] emend. Zhi, Li and Stackebrandt 2009, 594

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Ac.ti.no.my.ce.ta′les. N.L. masc. n. *Actinomyces* type genus of family, suff. *-ales* ending to denote an order; N.L. pl. fem. n. *Actinomycetales* the *Actinomyces* order.

This taxon is one of 15 orders classified in the class *Actinobacteria*, as recognized in the present volume of the *Manual*. As currently defined the order contains the family *Actinomycetaceae*. Besides the genus *Actinomyces*, the family includes the genera *Actinobaculum*, *Arcanobacterium*, *Mobiluncus*, and *Varibaculum*. The current composition of the order is determined by 16S rRNA gene sequence analysis and by the presence of family-specific patterns of 16S rRNA nucleotide sequences, as cited in the description of the family.

Type genus: **Actinomyces** Harz 1877, 133[AL] emend. Georg, Pine and Gerencser 1969, 292[VP].

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Family I. **Actinomycetaceae** Buchanan 1918, 403 (emend. Stackebrandt, Rainey and Ward-Rainey 1997, 484), emend. Zhi, Li and Stackebrandt 2009, 594[VP]

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Ac.ti.no.my.ce.ta.ce′a.e. N.L. masc. n. *Actinomyces* the type genus of the family; suff. *-aceae* ending to denote the family; N.L. fem. pl. n. *Actinomycetaceae* the *Actinomyces* family.

Most members of the family appear as **Gram-stain-positive, straight or slightly curved, predominantly diphtheroidal, non-motile rods some of which tend to form branched filaments of up to 1 µm in diameter**. **Fragmentation of the filaments readily occurs** producing rod-shaped or coccoid forms. **Several members** of the family are **chiefly or exclusively coccobacillary or coccoid**. However, members of one genus, namely the **genus** *Mobiluncus*, exclusively exhibit **curved, non-branching rods with tapered ends which stain Gram-variable to Gram-negative** although they possess a multilayered Gram-positive cell wall without an outer membrane. Depending on the species, cells are <0.5 µm wide and have a mean length of 1.7 µm or 2.9 µm. They occur singly or in pairs producing a **gull wing appearance** and **are motile by means of multiple flagella**. **None of the *Actinomycetaceae* is acid-fast or forms endospores or conidia**.

Colonies may be filamentous giving a more or less mycelial appearance, but aerial mycelium is usually not formed. Most species produce non-filamentous predominantly white or gray colonies, while some species may develop pigmented colonies (deep red, reddish, brown, pink, pinkish, or yellowish). A few species are β- or α-hemolytic. Most members of the family grow as **facultative anaerobes**, but **some are anaerobic** and **some are able to grow in air.** Carbon dioxide usually stimulates growth. **Catalase may be produced.** Nitrate reduction varies between genera, species, and strains within one species (*Varibaculum cambriense*). **All members of the family are chemo-organotrophs** with comparatively exacting nutritional requirements.

The cell walls of members of the family do not contain diaminopimelic acid. The **amino acid composition of the peptidoglycan** as far as it has been determined **varies between the genera as well as between species of some of the genera** (*Actinobaculum*, *Actinomyces*). The **cellular fatty acid profiles** of the *Actinomycetaceae* comprise **straight-chain saturated** as well as **mono- and diunsaturated fatty acids**, but genera and species differ in the presence or absence of iso- and anteiso-methyl-branched and cyclopropane ring fatty acids. **10-Methyl-branched fatty acids (tuberculostearic acid) and mycolic acids are absent**. Menaquinones are the only respiratory quinones detected so far with menaquinone-9 (MK-9) and/or menaquinone-10 (MK-10) in different structural types occurring at least in three of the five genera of the family. The genera *Actinobaculum*, *Actinomyces*, and *Arcanobacterium* show a phospholipid pattern consisting of diphosphatidylglycerol, phosphatidylglycerol, phosphatidylinositol, and phosphatidylinositol mannosides with or without phosphatidylcholine corresponding to **phospholipid types PI, PII, and PIII**, respectively.

The end products from glucose fermentation include acetic, lactic, and succinic acids with some variation in the amounts of these acids produced by members of different genera. The pattern of 16S rRNA signatures consists of nucleotides at positions 127:234 (R–U), 598:640 (Y–G), 828 (R), 829:857 (G–Y), 952:1229 (C–G), and 986:1219 (A–U) and is identical with that of the suborder *Actinomycineae*. All members of the family occur worldwide as commensals and/or pathogens of man and other animals.

DNA G+C content (mol%): 48–71 (HPLC, T_m).

Type genus: **Actinomyces** Harz 1877, 133[AL], emend. Georg, Pine and Gerencser 1969, 292[VP].

Further descriptive information

The family *Actinomycetaceae* was created by Buchanan in (1918) and originally served as the home of a diverse collection of organisms such as members of the genera *Actinobacillus*, *Actinomyces*, *Leptotrichia*, and *Nocardia*. After several revisions, membership of the family was restricted to bacterial species that appeared to be linked taxonomically by their ability to produce Gram-stain-positive, branching, and subsequently fragmenting filaments without aerial hyphae and spores, by comparatively exacting nutritional requirements, by facultatively anaerobic to anaerobic growth, and by a fermentative type of carbohydrate metabolism (Slack, 1974; Slack and Gerencser, 1975).

Defined on the basis of these common characteristics, the family *Actinomycetaceae* included the genera *Actinomyces*, *Arachnia*, *Bacterionema*, *Bifidobacterium*, and *Rothia* (Slack, 1974). However, the validity of this family concept was increasingly questioned after modern taxonomic techniques such as numerical phenetic, chemotaxonomic, and molecular genetic methods had been applied to the respective organisms (Schaal et al., 2006).

As currently defined, the family *Actinomycetaceae* Buchanan 1918 with the type genus *Actinomyces* Harz 1877, emend. Georg, Pine and Gerencser 1969, is a member of the suborder *Actinomycineae* Stackebrandt, Rainey and Ward-Rainey 1997, emend. Zhi, Li and Stackebrandt 2009, in the order *Actinomycetales* Buchanan 1917 (Approved Lists, 1980), emend. Stackebrandt, Rainey and Ward-Rainey 1997, emend. Zhi, Li and Stackebrandt 2009. Besides the genus *Actinomyces*, the family includes the genera *Actinobaculum* Lawson, Falsen, Åkervall, Vandamme and Collins 1997, *Arcanobacterium* Collins, Jones and Schofield 1983 (effective publication Collins et al., 1982b), emend. Lehnen, Busse, Frölich, Krasinska, Kämpfer and Speck 2006, *Mobiluncus* Spiegel and Roberts 1984b, emend. Hoyles, Collins, Falsen, Nikolaitchouk and McCartney 2004, and *Varibaculum* Hall, Collins, Lawson, Hutson, Falsen, Inganäs and Duerden 2003e.

Actinomyces bovis belonged to one of the first actinomycete species subjected to 16S rRNA gene sequence analysis (Stackebrandt and Woese, 1981). In the meantime, almost complete 16S rRNA gene sequences have been determined for essentially all of the type strains of the species of the family *Actinomycetaceae*. Phylogenetic trees have been included in various publications covering descriptions of novel species and phylogenetic analyses. As the size of databases, selection of members of the family, outgroup organisms, and treeing algorithms differ in these surveys, the respective phylogenetic trees also differ from each other both in the intrafamily structure and in the position of the family within the suborder *Actinomycineae* (Schaal et al., 2006). In this and the following chapter, the interpretation of relatedness at the intrafamily level, as in a previous analysis (Schaal et al., 2006), is again based on the maximum-likelihood (ML) algorithm (Figure 19), included

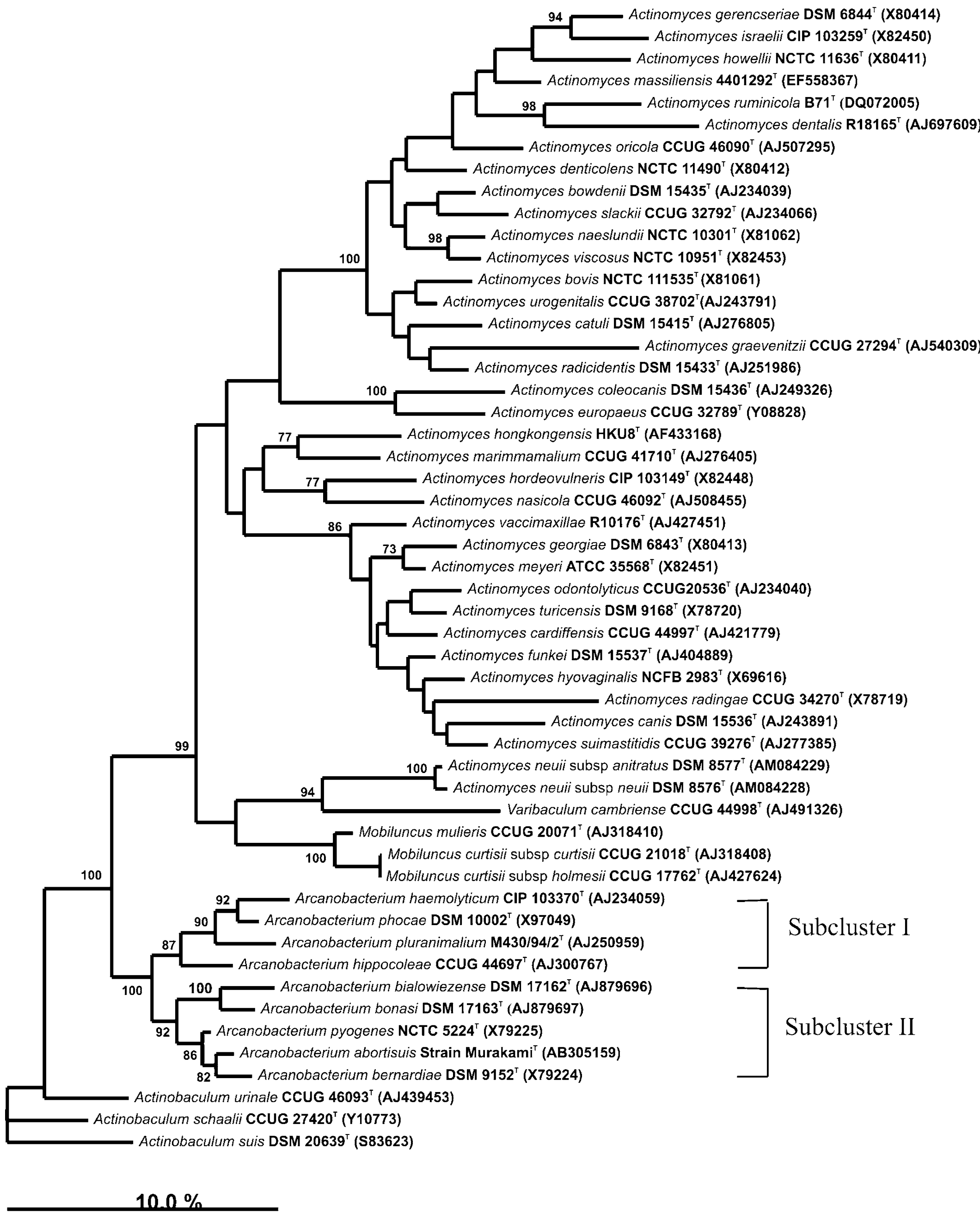

FIGURE 19. Maximum-likelihood tree (Felsenstein, 1993) based on nearly complete 16S rRNA gene sequences showing relationships between representatives of validly described species in the family *Actinomycetaceae*. The numbers at the nodes refer to the level of bootstrap support (%) based upon 500 resamplings. ^{T}, type strain.

in the PHYLIP V version of Felsenstein (1993). Differences in analyses based upon other algorithms such as the neighbor-joining method (Felsenstein, 1993) or the distance matrix algorithm (De Soete, 1983) are rather slight and were discussed earlier (Schaal et al., 2006), but the current ML dendrogram, although including additional species, is in good agreement with that published in 2006.

Based upon both, the phylogenetic position of the type strains of species available in 1997 and on signature nucleotides of the 16S rRNA gene sequences, Stackebrandt et al. (1997) described the suborder *Actinomycineae* for the family *Actinomycetaceae* that branched between the suborders *Micrococcineae* and *Corynebacterineae* (Schaal et al., 2006). The description of these suborders has recently been emended (Zhi et al., 2009). In the description of novel or reclassified *Arcanobacterium* species and a phylogenetic analysis of the genus *Actinomyces* (Pascual Ramos et al., 1997b), the *Actinomycetaceae* branched next to the *Micrococcineae* (only two sequences included) and the *Propionibacterineae*.

Similarity values of 16S rRNA gene sequence analyses for members of the family *Actinomycetaceae* range from 88 to 99%, indicating the presence of several phylogenetically different groups (Figure 19), which are separated by intra-cluster similarity values ranging from 88–90% to 89–92%. As already assumed on the basis of previous numerical phenetic (Schaal and Schofield, 1981a, 1981b, 1984; Schofield and Schaal, 1981) and phylogenetic (Pascual Ramos et al., 1997b) analyses, the genus *Actinomyces* does not represent a monophyletic taxon. Owing to the lack of detailed chemotaxonomic and phenotypic information, the family still serves as a phylogenetic dumping ground. Chemotaxonomic properties, found to correlate well with the phylogenetic structure in other actinobacterial genera, are either not available or not exclusive in phylogenetic clusters. Although tempting, a purely phylogeny-based dissection is not favored as novel sequences may blur the present topology of the family and its genera as is underlined by a comparison between the phylogenetic dendrogram published in 2006 (Schaal et al., 2006) and that presented in Figure 19.

Three clusters in the phylogenetic ML analysis (Figure 19) correspond to the genera *Actinobaculum*, *Arcanobacterium*, and *Mobiluncus*, and *Varibaculum* apparently also deserves genus rank (Collins et al., 1993; Funke et al., 1997a; Hoyles et al., 2001c, 2002b; Nikolaitchouk et al., 2000; Schaal et al., 2006). *Actinobaculum* and *Arcanobacterium* appeared to be phylogenetic neighbors in a previous phylogenetic analysis (Schaal et al., 2006), but may be less related according to the data presented in Figure 19. Furthermore, current 16S rRNA gene sequence data, signature nucleotides and chemotaxonomic characteristics appear to indicate that the genus *Arcanobacterium* is also not monophyletic (see genus *Arcanobacterium*, below).

Mobiluncus and *Varibaculum* species were found to branch adjacent to *Actinomyces neuii* in both the previous and the current analysis. The genera *Actinobaculum*, *Arcanobacterium*, and *Mobiluncus* are all defined by individual signature nucleotides (Table 1). From a phylogenetic point of view, members of the genera *Actinobaculum*, *Arcanobacterium*, *Mobiluncus*, and *Varibaculum* were thought to have originated as descendants of an *Actinomyces* ancestor (Schaal et al., 2006), but even this assumption may change with more sequences included as the present phylogenetic analysis appears to indicate.

Members of the genus *Actinomyces* form several phylogenetic clusters, the number of which depends on the cut-off branching points. As the branching is rarely supported by high bootstrap values, the decision on what level these clusters should be considered taxonomically coherent entities should be based upon results of additional taxonomic investigations such as chemotaxonomic, physiological, and morphological studies. Even within individual clusters, the majority of species are remotely related showing more than 3% dissimilarity between their 16S rRNA gene sequences. Following the suggestion of Stackebrandt and Goebel (1994) that at this low level of relatedness DNA–DNA hybridization would result in values significantly below the species delineation threshold value of around 70% (Wayne et al., 1987), most authors refrained from confirming the species status of the more recently described species by applying this technique to the type strains of the novel species.

Based upon similar cut-off points, *Actinomyces* species (excluding *Actinomyces neuii*) form two major clusters (Figure 19). Cluster 1 contains 15 or 17 (16 or 18 when the novel species *Actinomyces timonensis* is included) species, depending on whether *Actinomyces graevenitzii* and *Actinomyces radicidentis* are included or whether they represent individual lineages. As far as 16S rRNA gene signature nucleotides (Table 1) are concerned, *Actinomyces radicidentis* should be considered a member of cluster 1, whereas *Actinomyces graevenitzii* exhibits deviations from the set of signature nucleotides of core cluster 1 and should thus be treated as a separate phylogenetic unit.

In a previous phylogenetic analysis (Schaal et al., 2006), *Actinomyces coleocanis* and *Actinomyces europaeus* formed a subcluster loosely affiliated with cluster 2. In the current dendrogram (Figure 19), however, these two species appear as a distantly related subgroup of cluster 1 or they form a subgroup similar to that of the two subspecies of *Actinomyces neuii* when the recently described novel species *Actinomyces hominis* is included in the phylogenetic tree (Funke et al., 2010).

All of the remaining *Actinomyces* species except for *Actinomyces neuii* form cluster 2. While the majority of species of cluster 2 (core cluster 2) (Figure 19) is moderately related, two distantly related subclusters of cluster 2 emerge, containing *Actinomyces hongkongensis* and *Actinomyces marimammalium* on the one hand and *Actinomyces hordeovulneris* and *Actinomyces nasicola* on the other hand. In the previous dendrogram (Schaal et al., 2006), *Actinomyces radingae* appeared to form an additional subcluster of cluster 2, while in Figure 19, this species appears to be a member of core cluster 2. This is in good agreement with the signature nucleotides of *Actinomyces radingae* which differ only slightly from those of the members of core cluster 2 (Table 1). The other subclusters mentioned above represent individual lineages that are defined by the signature nucleotides shown in Table 1.

While signature nucleotides or 16S rRNA gene sequences help to differentiate the genera of the family *Actinomycetaceae* from each other, reliable additional differential characteristics are hardly available, especially because detailed morphological, chemotaxonomic, and physiological characterizations remain to be performed for many of the recently described taxa. Nevertheless, Table 2 shows some characteristics that may aid in the differentiation of the genera of the family *Actinomycetaceae*.

Key to the genera of the family *Actinomycetaceae*

I. Cells are Gram-stain-positive, filamentous or rod-shaped and possibly branching, or coccobacillary to coccoid and nonmotile.

1. **DNA G+C content (mol%): 55–71**

Because the genus as currently recognized is obviously polyphyletic, a pattern of 16S rRNA signature nucleotides common to all members of the genus would be of little taxonomic relevance. The signature nucleotides characterizing the phylogenetic clusters and subclusters as shown in Figure 19 are listed in Table 1. Optimal growth occurs at 36±1°C in air with added CO_2 or anaerobically. Only *Actinomyces ruminicola* grows best at 46°C. Members of the genus belong to peptidoglycan types A5α (L-Lys[L-Orn]-L-Lys–D-Glu, two species having L-Lys–L-Lys–D-Glu), A5β (L-Orn–L-Lys–D-Glu), or A4 (L-Lys–D-Asp), respectively (Schleifer and Kandler, 1972; Schleifer and Seidl, 1985). Characteristic cell-wall sugars include galactose, glucose, mannose, rhamnose, and 6-deoxytalose

with characteristic species differences, but not arabinose. Respiratory quinones include different combinations of fully unsaturated menaquinones with eight, nine, and ten isoprene units or the same compounds with varying degrees of saturation and a methyl-substituted menaquinone with ten isoprene units (*methyl*-MK-10) as major components depending on the species involved. The phospholipid patterns belong to phospholipid type I (with no diagnostic phospholipid) and phospholipid type III (with phosphatidylcholine as diagnostic phospholipid) *sensu* Lechevalier et al. (1977). Nearly all species examined so far contain diphosphatidylglycerol, phosphatidylglycerol, phosphatidylinositol, and phosphatidylinositol mannosides as major phospholipids. Straight-chain saturated and monounsaturated fatty acids are generally present as long-chain cellular fatty acids, and some species contain iso-methyl-branched, anteiso-methyl-branched and/or cyclopropane ring fatty acids in addition. Catalase and nitrate reductase may or may not be produced (Table 2).

→ Genus *Actinomyces*

2. **DNA G+C content (mol%): 48–64**

The pattern of 16S rRNA signatures consists of nucleotides at positions 125:236 (U–A), 153:168 (G–U), 316:337 (U–G), 378:385 (A–U), 407:435 (G–C), 419:424 (U–G), 603:635 (U–A), 615:625 (G–Y), 668:738 (U–A), 669:737 (A–U), 722:733 (A.A), and 1308:1329 (U–A) (Table 1). Members of the genus are facultatively anaerobic and usually require an increased CO_2-tension (~5%) for optimal growth at 36±1°C. The cell-wall chemotype is chemotype V (Lechevalier and Lechevalier, 1970) with L-lysine as wall diamino acid and rhamnose as diagnostic whole-cell sugar. The *Arcanobacterium* species examined so far belong to peptidoglycan type A5, but can be assigned to two different subtypes, namely the L-Lys– L-Ala– L-Lys– D-Glu subtype and the L-Lys– L-Lys– D-Glu subtype, corresponding by and large with the 16S rRNA gene sequence similarity-based subdivision of the genus. The muramic acid residue of the glycan moiety of the cell-wall peptidoglycan is *N*-acetylated. The phospholipids identified in members of the genus include diphosphatidylglycerol and phosphatidylglycerol and possibly various additional polar lipids. The principal respiratory quinones are menaquinones with eight, nine, ten, or eleven isoprene units, tetrahydrogenated menaquinones with nine [MK-9(H_4)] or ten [MK-10(H_4)] isoprene units, respectively, being the major components. The cellular fatty acid profile consists predominantly of straight-chain saturated and monounsaturated fatty acids, but iso- and anteiso-branched fatty acids may occur in small amounts. Catalase production is consistently negative in six species (*Arcanobacterium abortisuis*, *Arcanobacterium bernardiae*, *Arcanobacterium bialowiezense*, *Arcanobacterium bonsai*, *Arcanobacterium hippocoleae*, and *Arcanobacterium pyogenes*) and positive in one species (*Arcanobacterium pluranimalium*). One additional species (*Arcanobacterium phocae*) is catalase-variable and some strains of *Arcanobacterium haemolyticum* may show a weakly positive catalase reaction. The ability to reduce nitrate to nitrite varies between species.

→ Genus *Arcanobacterium*

3. **DNA G+C content (mol%): 55–57**

The pattern of 16S rRNA signatures consists of nucleotides at positions 153:168 (G–U), 316:337 (U–G), 407:435 (G–U), 419:424 (U–G), 603:635 (U–A), 615:625 (G–U), 668:738 (U–A), and 722:733 (A.A) (Table 1). Members of the genus are anaerobic to facultatively anaerobic growing best at 36±1°C. The cell-wall murein types differ between species consisting of either L-Lys– L-Ala– L-Lys– D-Glu or of L-Lys– L-Lys– D-Glu. The cell-wall sugar pattern includes galactose, mannose, and rhamnose (*Actinobaculum suis*). The phospholipid type is PI *sensu* Lechevalier et al. (1977) comprising diphosphatidylglycerol, phosphatidylglycerol, phosphatidylinositol, and phosphatidylinositol mannosides. The respiratory quinones consist of a tetrahydrogenated menaquinone with ten isoprene units [MK-10(H_4)] as the only isoprenoid quinone. The cellular fatty acid profile comprises straight-chain saturated and monounsaturated fatty acids. However, the chemotaxonomic pattern of *Actinobaculum schaalii* appears to differ considerably from that of *Actinobaculum suis*. Catalase- and nitrate reductase-negative.

→ Genus *Actinobaculum*

4. **DNA G+C content (mol%): 52**

Members of the genus are preferentially anaerobic and catalase-negative. The majority of strains reduce nitrate to nitrite. Major end products of glucose fermentation are lactic and succinic acids. Chemotaxonomic properties have not yet been determined. Whole-cell protein profiling using SDS-PAGE demonstrated a considerable phenotypic coherence of members of the single species of the genus and their clear distinctness from *Mobiluncus* species.

→ Genus *Varibaculum*

II. Cells are Gram-stain-variable to Gram-stain-negative, strongly curved rods with tapered ends which occur singly or in pairs producing a gull wing appearance and which are motile by means of multiple, either subpolar or more centrally located flagella.

1. **DNA G+C content (mol%): 49–55**

The pattern of 16S rRNA signatures consists of nucleotides at positions 154:167 (G–U), 378:385 (A–U), 407:435 (G–C), 408:434 (A–U), 441 (U), 450:483 (A–U), 613:627 (A–U), 668:738 (U–A), 722:733 (A.A), 840:846 (G–U), and 1308:1329 (U–A) (Table 1). Despite the variable to negative Gram reaction, members of the genus possess a multilayered Gram-stain-positive cell wall lacking an outer membrane. The whole-cell sugar pattern includes galactose, glucose, and rhamnose. The cellular fatty acid profile consists of straight-chain saturated and mono- and diunsaturated fatty acids with hexadecanoic (16:0), octadecadienoic (18:2), and octadecenoic (18:1) acids being the major components. At least for primary isolation at the optimal growth temperature of 36±1°C, anaerobic growth conditions are required. Addition of serum to the culture medium stimulates growth. Nitrate reduction varies between species or within one species, respectively. Catalase is not produced.

→ Genus *Mobiluncus*

TABLE 1. Signature nucleotides of 16S rRNA gene sequences, defining individual phylogenetic subclusters within the genus *Actinomyces*, as well as the genera *Arcanobacterium*, *Actinobaculum*, and *Mobiluncus*[a]

Position[b]	*A. gerencseriae*, *A. israelii*, *A. howellii*, *A. massiliensis*, *A. ruminicola*, *A. dentalis*, *A. oricola*, *A. denticolens*, *A. bowdenii*, *A. slackii*, *A. naeslundii*, *A. viscosus*, *A. bovis*, *A. urogenitalis*, *A. catuli*, *A. radicidentis*[c]	*A. graevenitzii*	*A. vaccimaxillae*, *A. georgiae*, *A. meyeri*, *A. odontolyticus*, *A. turicensis*, *A. cardiffensis*, *A funkei*, *A. hyovaginalis*, *A. canis*, *A. suimastitidis*	*A. radingae*
125:236	**U–G**	**U–G**	**U–G**	**U–G**
146:176	**U–G**	**U–G**	**G–Y**	**G–U**
153:168	**C–G**	**C–G**	**C–G**	**C–G**
154:167	Y–R	U–A	**G–U**	**G–U**
316:337	C–G	C–G	**U–G**	**U–G**
378:385	G–C	G–C	R–Y	G–C
407:435	A–U	A–U	A–U	A–U
408:434	G–C	G–C	G–C	G–C
419:424	C–G	C–G	C–G	C–G
441	G	G	G	G
450:483	G–C	**C–G**	C–G	C–G
501:544	C–G	C–G	C–G	C–G
502:543	G–C	G–C	G–C	G–C
586:755	C–G	C–G	C–G	C–G
590:649	U–G	U–G	U–G	U–G
591:648	**G–Y**	**G–Y**	**G,u–V**	U–A
601:637	G–U	G–U	G–U	G–U
602:636	Y–G	Y–G	Y–G	U–G
603:635	C–G	C–G	C–G	C–G
614:626	**C–G**	**C–G**	**C–G**	**C–G**
615:625	U–G	C–G	U–G	U–G
613:627	Y–G	U–G	U–G	U–G
835:851	G–C	**A–U**	G–C	G–C
668:738	A–U	**U–A**	A–U	A–U
669:737	G–C	**A–U**	G–C	G–C
722:733	G.G	G.G	G.G	G.G
838:848	G–Y	**C–G**	G–U	N–U
839:847	V–V	U–G	C–G	C–G
840:846	Y–G	C–G	C–G	C–G
997:1044	U–A	U–A	U–G	U–G
1118:1155	U–A	U–A	C–G	C–G
1122:1151	G–C	G–C	A–U	A–U
1123:1150	U–G	U–G	U–G	U–G
1244:1293	C–R	U–G	U–R	A–U
1243:1294	C–G	C–G	C–G	C–G
1245:1292	G–C	G–U	G–Y	G–U
1246:1291	G–C	G–C	G–C	G–C
1308:1329	C–G	**U–A**	Y–G	C–G
1310:1327	G–C	G–C	R–Y	G–U
1311:1326	**U–A**	**U–A**	R–Y	G–C
1308:1329	C–G	**U–G**	Y–R	C–G

[a] Modified from Schaal et al. (2006). Abbreviations: V, variable nucleotide composition; Y, pyrimidine; R, purine.

[b] *Escherichia coli* position (Brosius et al., 1978).

[c] The position of *Actinomyces radicidentis* in core cluster 1 remains to be confirmed.

[d] *Actinomyces nasicola* forms a subcluster together with *Actinomyces hordeovulneris*, but the signature nucleotides of the former species have not been determined.

[e] *Actinomyces hongkongensis* forms a subcluster together with *Actinomyces marimammalium*, but the signature nucleotides of the former species have not been determined. A signature is defined as the presence of a unique nucleotide in members (>90%) of a single cluster or more clusters but not in all clusters of the family.

A. hordeovulneris, A. nasicola[d]	*A. europaeus, A. coleocanis*	*A. marimammalium, A. hongkongensis*[e]	*Actinobaculum*	*Arcanobacterium*	*Mobiluncus*	*A. neuii*
U–G	**U–G**	**U–G**	**U–G**	**U–A**	**U–G**	**U–G**
G–C	**U–A**	**G–U**	**G–Y**	**G–Y**	**G–U**	**G**
C–G	**C–G**	**G–U**	**G–U**	**G–U**	**C–G**	**G–U**
G–U	Y–G	C–G	C–G	C–G	**G–U**	C–G
C–G	C–G	C–G	**U–G**	**U–G**	C–G	C–G
G–C	G–C	G–C	G–C	**A–U**	**A–U**	**A–U**
A–U	A–U	A–U	**G–U**	**G–C**	**G–C**	**G–C**
G–C	G–C	G–C	G–Y	G–U	**A–U**	G–U
C–G	C–G	C–G	**U–G**	**U–G**	C–G	C–G
A	G	A	G	A	**U**	G
G–C	R–Y	G–C	C–G	Y–R	**A–U**	**A–U**
C–G	**U–A**	C–G	C–G	C–G	C–G	C–G
G–C	**A–U**	G–C	G–C	G–C	G–C	G–C
C–G	**U–A**	C–G	C–G	C–G	U–R	C–G
U–G	U–G	**G–U**	U–G	U–G	U–G	U–G
U–A	U–A	U–A	U–A	U–A	C–G	U–A
G–U	G–U	G–U	G–U	G–U	G–U	**A–U**
U–G	U–G	U–G	C–G	C–G	C–G	**G–U**
C–G	C–G	C–G	**U–A**	**U–A**	C–G	C–G
A–U	R–U	A–U	G–C	G–C	G–C	C.C
C–G	V–V	U–G	**G–U**	**G–Y**	C–G	G–C
C–G	Y–G	**A–U**	C–G	C–G	**A–U**	U–G
G–C	G–C	G–C	G–C	G–C	G–C	G–C
A–U	A–U	A–U	**U–A**	**U–A**	**U–A**	**U–A**
G–C	G–C	G–C	G–C	**A–U**	G–C	**A–U**
G.G	G.G	G.G	**A.A**	**A.A**	**A.A**	G.G
A–U	G–U	G–U	V–V	C–G	A–U	G.G
A–U	C–G	C–G	V–V	C–G	U–G	C–G
U–G	Y–G	U–G	C–G	U–R	**G–U**	U.U
U–A	U–G	U–G	U–A	U–A	U–A	U–G
U–A	U–A	U–A	U–A	U–A	C–G	U–A
A–U	G–C	G–C	G–C	G–C	A–U	A–U
U–G	U.U	U–G	U–A	U–A	U–G	U–G
U–G	Y–G	C–G	C–G	Y–R	C–G	**G–C**
C–G	**R–U**	C–G	C–G	C–G	C–G	**U–A**
A–U	G–Y	G–U	G–Y	G–Y	A–U	**U–G**
G–C	G–C	G–C	G–C	G–C	G–C	**C–G**
U–A	C–G	C–G	C–G	**U–A**	**U–A**	C–G
G–C	**U–A**	A–U	G–U	G–C	G–C	**C–G**
G–C	A–U	**U–A**	G–C	G–C	G–C	A–U
U–A	C–G	C–G	C–G	U–A	U–A	C–G

TABLE 2. Characteristics differentiating the genera of the family *Actinomycetaceae* from each other[a,b]

Characteristic	*Actinomyces*	*Arcanobacterium*	*Actinobaculum*	*Varibaculum*	*Mobiluncus*
Aerobic growth (with added CO_2)	D	+	D	–	–(+)
Filaments and/or branched rods	D	nr	D	–	–
Cells coccoid or coccobacillary	D	D	–	–	–
Curved rods with tapered ends	–	–	–	–	+
Gram reaction	+	+	+	+	v/–
Microcolonies filamentous	D	–	–	–	–
Catalase	D	–/+	–	–	–
Nitrate reduction	D	D	–	d	D
Cell-wall components:					
A_2pm[c]	–	–	–	–	–
Ornithine	D	–	nr	nr	nr
Lysine	+	+	+	nr	nr
Aspartic acid	–(+)	–	nr	nr	nr
Glycine	–	–	nr	nr	nr
Glucose	D	+/–	nr	nr	+
Galactose	D	–	+	nr	+
Rhamnose	D	+	+	nr	+
6-Deoxytalose	D	–	nr	nr	nr
Arabinose	–	–	nr	nr	nr
Mannose	D	–/+	+	nr	nr
Mycolic acids	–	–	–	–	–
Acyl type	Acetyl (glycolyl)	Acetyl	Acetyl	nr	nr
End products from glucose fermentation:					
Acetic acid	+	+	+	–	+
Propionic acid	–	–	–	–	–
Volatile fatty acids with more than three C-atoms	–	–	–	–	–
Pyruvic acid	–	–	–	–	–
Lactic acid	+	+	D	+	(+)
Succinic acid	+	+	(+)	+	+
Phospholipids[c]	P type I or P type III: DPG, (PG), (PC), PI, PIM	DPG, PG and several other unknown phospholipids	P type I: DPG, PG, PI, PIM	nr	nr
Menaquinones[e]	MK-8, MK-9, MK-10, methyl-MK-10, MK-9(H_4), MK-10(H_4)	MK-9(H_4), MK-10(H_4), [MK-8(H_4), MK-11(H_4)]	MK-10(H_4)	nr	nr
Cellular fatty acids[f]	S, U, (C), (I), (A)	S, U, (I), (A)	S,U	nr	nr
Motility	–	–	–	–	+
DNA G+C content (mol%)	54–70	48–65	55–57	51.7	49–55

[a]Symbols: +, positive/present; –, negative/absent; +/–, predominantly positive/present; –/+, predominantly negative/absent; (+), weakly or irregularly positive/present; D, differences between species; d, differences between strains within one species; v, variable; nr, not reported.

[b]Data compiled from Holdeman et al. (1977), Schofield and Schaal (1981), Schaal (1986b), and Schaal et al. (2006).

[c]A_2pm, Diaminopimelic acid.

[d]DPG, Diphosphatidylglycerol; PG, phosphatidylglycerol; PC, phosphatidylcholine; PI, phosphatidylinositol; PIM, phosphatidylinositol mannosides; P type I/P type III, phospholipid types I or III *sensu* Lechevalier et al. (1977).

[e]Menaquinones with 8, 9, 10 or 11 isoprene units and varying degrees of saturation; methyl-MK-10, methyl-substituted menaquinone-10.

[f]S, Straight-chain, saturated; U, unsaturated; A, anteiso-methyl-branched; I, iso-methyl-branched; C, cyclopropane ring fatty acids.

Genus I. **Actinomyces** Harz 1877, 133[AL] emend. Georg, Pine and Gerencser 1969, 292[VP]

Klaus P. Schaal and Atteyet A. Yassin

Ac.ti.no.my′ces. Gr. fem. n. *aktis*, *aktinos* ray; Gr. masc. n. *mukês* fungus; N.L. masc. n. *Actinomyces* ray fungus referring to the radial arrangement of filaments in *Actinomyces bovis* sulfur granules.

Straight or slightly curved rods, 0.2–1.0 µm in diameter, which vary considerably in shape and size, and **slender filaments**, 1 µm or less in width and 10–50 µm or more in length, **with true branching. Short rods** (0.5–5.0 µm in length) **with or without clubbed ends** are frequently seen and **may occur singly, in pairs with diphtheroidal arrangements** (Y, V, T forms and palisades), **in short chains or in small clusters.** Longer (5.0–10.0 µm in length) branched rods are also common. Several members

of the genus appear chiefly or exclusively as **coccobacillary or even coccoid elements**. **Filaments** which may predominate in certain species **are either straight or wavy**, show varying degrees of **branching** and may have **swollen, clubbed, or clavate ends. Gram-stain-positive**, but irregular staining giving rise to a beaded or barred appearance frequently occurs, and two species may be Gram-stain-variable. **Non-acid-fast, nonmotile, and non-endospore-forming.** Conidia are not produced.

Facultatively anaerobic; several species are preferentially anaerobic, some grow well aerobically. **Carbon dioxide is usually required for maximum growth.**

A few species produce characteristic **filamentous microcolonies (spider- or cobweb-like colonies)** after 24–48 h of incubation at 36±1°C which are composed of branched, septate or non-septate filaments with or without signs of central fragmentation. The microcolonies of the remaining species are predominantly or exclusively non-filamentous and consist chiefly of diphtheroidal and/or branched rods, or of coccobacillary or coccoid elements. Several of the recently described new *Actinomyces* species form pinpoint surface colonies after 48 h.

Mature colonies (3–14 d of incubation at 36±1°C depending on the species examined) measure between 0.5 and 5.0 mm in diameter and are **either rough and dry to crumbly in texture or smooth and soft to mucoid, or** they show various degrees of transition between these forms. Most colonies are **white to gray-white** or **creamy white**, but *Actinomyces odontolyticus* develops a **deep red or brown to red pigmentation** when grown on blood agar. The colony pigmentation of a few *Actinomyces* spp. has been described as follows: cream to pinkish (*Actinomyces cardiffensis*), pink (when grown on horse blood agar; *Actinomyces denticolens*), dark brown (*Actinomyces graevenitzii*), brown (*Actinomyces radicidentis*), and reddish (*Actinomyces urogenitalis*). Aerial filaments are usually absent although an occasional rough isolate of *Actinomyces israelii* or *Actinomyces gerencseriae* may produce short aerial threads under certain cultural conditions. **Optimum temperature predominantly 35–37°C** (*Actinomyces meyeri* grows equally well at 30°C and the optimum growth temperature of *Actinomyces ruminicola* **is 46°C**).

Chemo-organotrophic, having a **fermentative type of carbohydrate metabolism.** Carbohydrates are fermented with the production of acid but no gas. **End products from glucose fermentation** include **formic, acetic, lactic, and succinic acids;** the amount of succinic acid formed depends on the concentration of CO_2/HCO_3^- available in the medium or the gaseous environment, respectively. Catalase-negative or -positive; nitrate reduction positive or negative; **indole not produced. Organic nitrogen is required for growth.** Some species may show greening or complete lysis on agar media containing rabbit, sheep, horse, or human red blood cells.

Characteristic **amino acids of the cell-wall peptidoglycan** of those species whose peptidoglycan has been examined in detail are L-lysine and either or neither L-ornithine nor D-β-aspartic acid (peptidoglycan types A4, A5α, and A5β, respectively) ; **diaminopimelic acid (A_2pm) or glycine do not occur.** Cell-wall sugars may include 6-deoxytalose, fucose, galactose, glucose, mannose, and rhamnose, but not arabinose and xylose.

DNA G+C content (mol%): 55–71 (T_m, HPLC).

Type species: **Actinomyces bovis** Harz 1877, 133[AL].

Further descriptive information

Phylogeny. The genus *Actinomyces* is the type genus of the family *Actinomycetaceae* Buchanan (1918) (emend. Stackebrandt et al. 1997, emend. Zhi et al. 2009), within the suborder *Actinomycineae* Stackebrandt et al. 1997, emend. Zhi et al. 2009, of the order *Actinomycetales* Buchanan 1917 (Approved Lists; Skerman et al., 1980) emend. Stackebrandt et al. 1997, emend. Zhi et al. 2009. In addition to *Actinomyces*, the family currently comprises the genera *Arcanobacterium* Collins et al. 1983 (effective publication Collins et al., 1982b), *Actinobaculum* Lawson et al. 1997, *Varibaculum* Hall et al. 2003e, and *Mobiluncus* Spiegel and Roberts 1984b emend. Hoyles et al. 2004. The genera *Actinobaculum* and *Arcanobacterium* both contain species formerly classified in the genus *Actinomyces* (Schaal et al., 2006).

The genus *Actinomyces* apparently does not represent a monophyletic taxon, but forms several phylogenetic clusters (Figure 19) as had already been noted in previous phylogenetic analyses (Lawson et al., 1997; Pascual Ramos et al., 1997b; Schaal et al., 2006) and had even been presumed from the results of numerical taxonomic studies (Schaal and Gatzer, 1985; Schaal and Schofield, 1984; Schofield and Schaal, 1981). As shown in Figure 19, *Actinomyces* species may be separated into two major (core clusters 1 and 2) and four to five additional minor phylogenetic subgroups on the basis of 16S rRNA gene sequence similarity. The taxonomic relevance of this classification scheme will be discussed later.

Cell morphology. The cellular morphology of *Actinomyces* species has traditionally been described as being both diphtheroidal and filamentous (Slack, 1974; Slack and Gerencser, 1975). However, members of different species, as well as individual strains of a single species, may vary considerably with regard to the proportion in which rods and filaments occur. Furthermore, some *Actinomyces* species appear to produce only coccoid or coccobacillary cells. It should also be noted, however, that the cellular appearance may be considerably influenced by factors such as composition of the growth medium, cultural conditions, or age of cultures.

Gram-stained smears or wet mounts prepared from young broth (2–3 d) or agar (3–5 d) cultures, rough mature colonies (7–14 d), or clinical specimens often show groups or clusters of Gram-stain-positive, intertwining filaments, and branching rods which resemble small microcolonies (Figure 20). The staining may be uniform (Figure 20) or irregular (Figure 21), sometimes producing a beaded or barred appearance (Schaal, 1986b; Schaal et al., 2006; Slack and Gerencser, 1975). A granular cytoplasm that might account for the irregular staining properties can also be demonstrated in unstained cells observed in darkfield (Slack and Gerencser, 1975).

The filaments are often wavy, sometimes straight, slender, and of varying length (Figure 20 and Figure 21). Some may have swollen, bulbous ends, and *Actinomyces hordeovulneris* may occasionally form sphaeroplast-like bodies at the tips of the filaments (Buchanan and Scott, 1984). *Actinomyces gerencseriae*, *Actinomyces hordeovulneris*, *Actinomyces israelii*, and fresh isolates of *Actinomyces viscosus sensu stricto* may occur in these branching filamentous structures (Figure 21) and the formation of beaded filaments was also described to be characteristic of *Actinomyces dentalis* (Hall et al., 2005). Filament formation is less common in

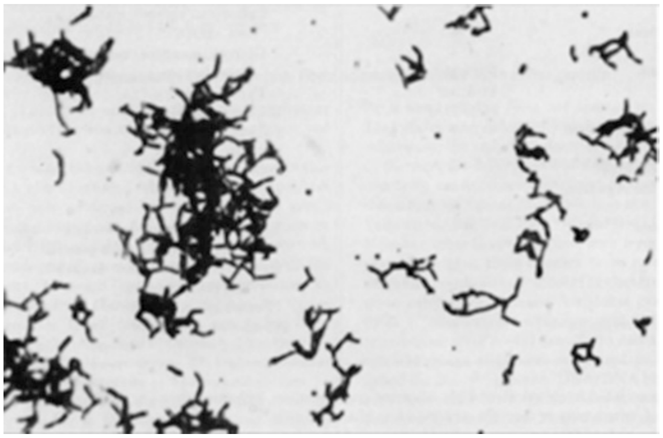

FIGURE 20. *Actinomyces israelii.* Gram-stained smear from a 24-h culture in Tarozzi broth, showing branching filaments, branched rods and diphtheroidal forms; micrograph (1200×).

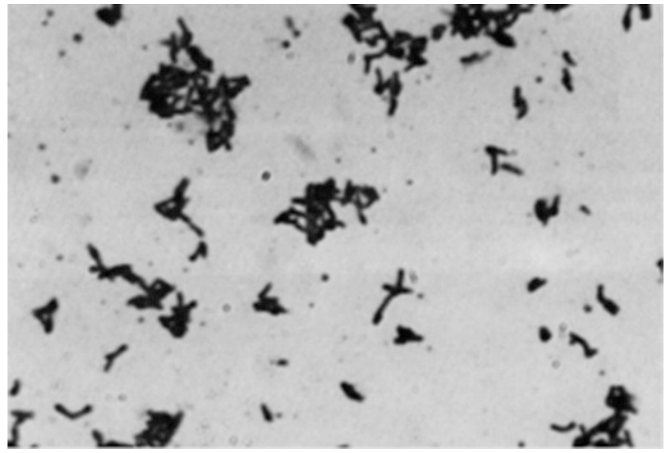

FIGURE 22. *Actinomyces oris* (*Actinomyces viscosus*, serovar II). Gram-stained smear from a 24-h culture in Tarozzi broth, showing irregular rods with some branching and diphtheroidal arrangements; micrograph (1200×).

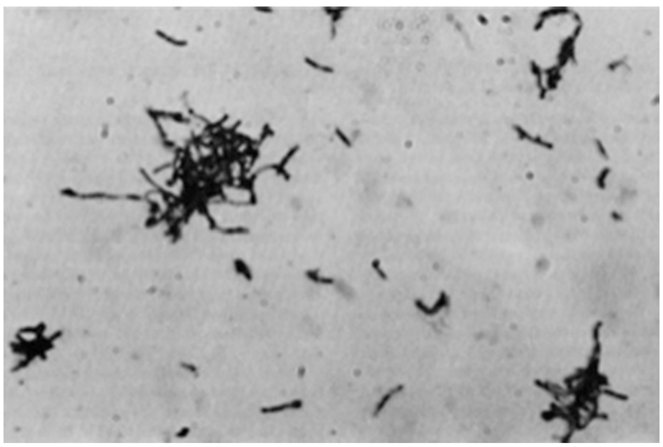

FIGURE 21. *Actinomyces bovis*, serovar II. Gram-stained smear from a 24-h culture in Tarozzi broth, showing wavy, irregularly stained filaments as well as branched and diphtheroidal rods; micrograph (1200×).

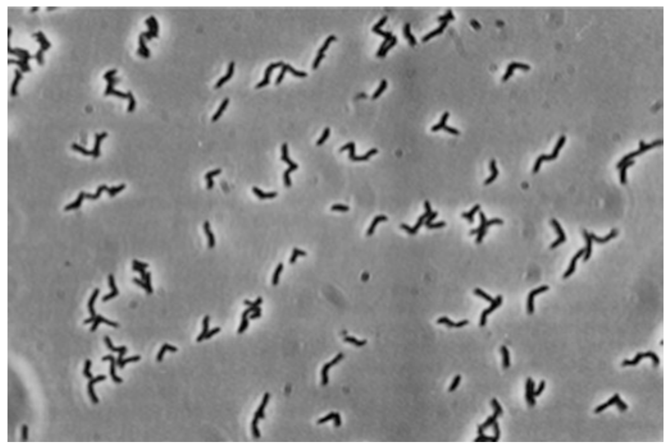

FIGURE 23. *Actinomyces naeslundii.* Wet mount in lactophenol cotton blue mounting fluid prepared from a mature colony, showing curved and branched rods in diphtheroidal arrangements (BHIA, 14 d, 36°C); phase-contrast micrograph (1200×).

Actinomyces johnsonii, *Actinomyces naeslundii*, *Actinomyces oris*, laboratory strains of *Actinomyces denticolens*, *Actinomyces oricola*, and *Actinomyces viscosus* (Hall et al., 2003c), rare in *Actinomyces bovis*, *Actinomyces howellii*, and *Actinomyces odontolyticus*, and very rare in *Actinomyces meyeri* although very young cultures or an occasional rough strain of the latter four species (Figure 21) may be definitely filamentous. All of the other *Actinomyces* species have not been reported as being predominantly filamentous or have not been characterized morphologically in sufficient detail.

The cellular morphology of many *Actinomyces* cultures is predominantly or completely diphtheroidal, and even pus specimens derived from *Actinomyces* infections may only contain short or medium-sized rods with or without branching unless one of the coccoid species is present. *Actinomyces viscosus* serovar II strains (Figure 22), which were assigned to *Actinomyces naeslundii* genospecies 2 and were recently classified as *Actinomyces oris*, smooth *Actinomyces naeslundii sensu stricto* (Figure 23) and *Actinomyces viscosus sensu stricto* strains, *Actinomyces bowdenii* (Pascual et al., 1999), *Actinomyces canis* (Hoyles et al., 2000), *Actinomyces cardiffensis* (Hall et al., 2002), *Actinomyces catuli* (Hoyles et al., 2001b), *Actinomyces coleocanis* (Hoyles et al., 2001c), *Actinomyces funkei* (Lawson et al., 2001b), *Actinomyces georgiae* (Johnson et al., 1990), *Actinomyces graevenitzii* (Pascual Ramos et al., 1997a), *Actinomyces howellii*, *Actinomyces johnsonii*, *Actinomyces marimammalium* (Hoyles et al., 2001c), *Actinomyces suimastitidis* (Hoyles et al., 2001a), *Actinomyces turicensis* (Vandamme et al., 1998; Wüst et al., 1995a), *Actinomyces urogenitalis* (Nikolaitchouk et al., 2000), and some *Actinomyces gerencseriae* isolates produce comparatively long to medium-sized, straight to slightly curved rods which often show branching and/or characteristic arrangements in Y, V, and T forms. However, Gram-stained smears of *Actinomyces cardiffensis* may also contain beaded branching filaments. *Actinomyces hongkongensis* (Woo et al., 2003), *Actinomyces massiliensis* (Renvoise et al., 2009), and *Actinomyces ruminicola* (An et al., 2006) are characterized by straight, short to medium-sized rods. Shorter irregular rods resembling propionibacteria are frequently seen in *Actinomyces bovis* (Figure 24), *Actinomyces*

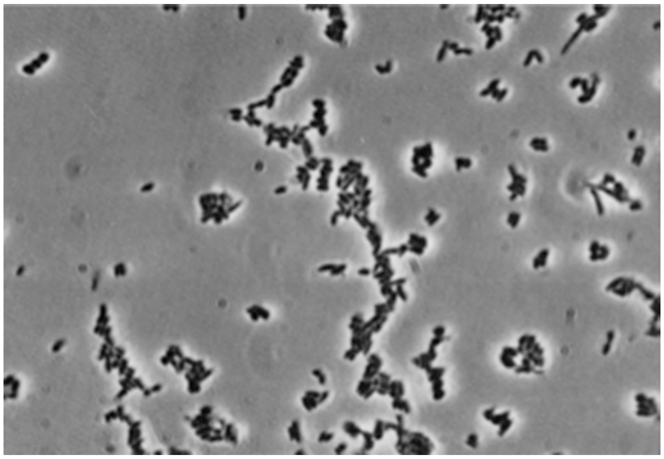

FIGURE 24. *Actinomyces bovis*, serovar I. Wet mount in lactophenol cotton blue mounting fluid prepared from a mature colony, showing short and a few longer rods (BHIA, 14 d, 36°C); phase-contrast micrograph (1200×).

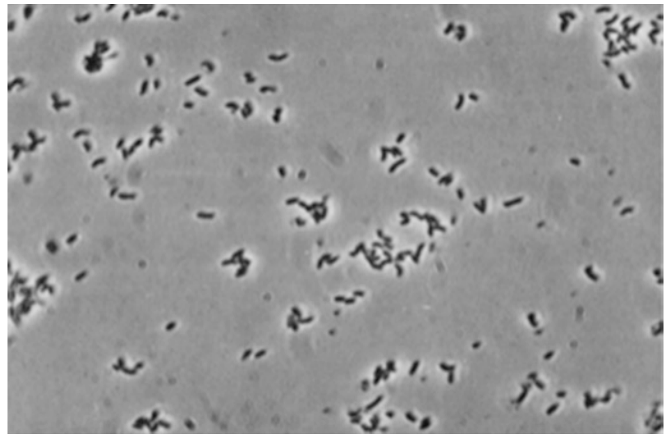

FIGURE 26. *Actinomyces odontolyticus*, serovar I. Wet mount in lactophenol cotton blue mounting fluid prepared from a mature colony, showing short diphtheroidal rods (BHIA, 14 d, 36°C); phase-contrast micrograph (1200×).

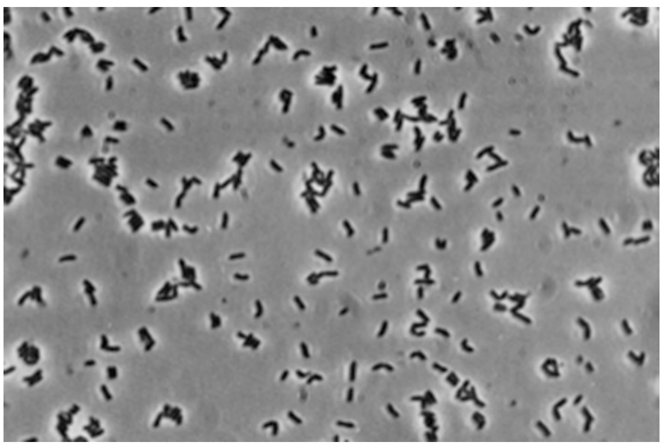

FIGURE 25. *Actinomyces denticolens*. Wet mount in lactophenol cotton blue mounting fluid prepared from a mature colony, showing predominantly diphtheroidal rods (BHIA, 10 d, 36°C); phase-contrast micrograph (1200×).

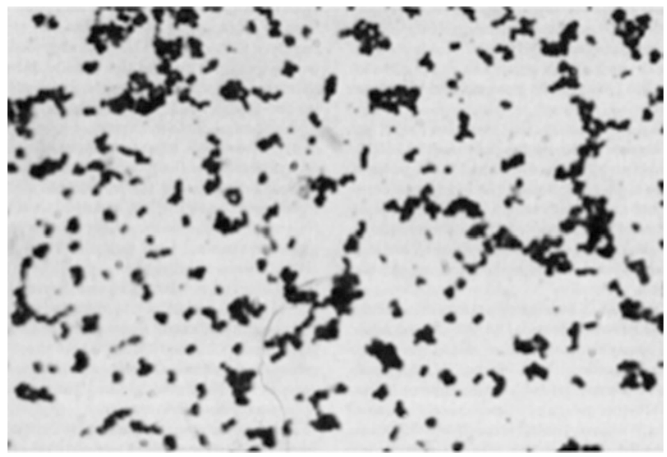

FIGURE 27. *Actinomyces odontolyticus*, serovar II. Gram-stained smear from a 48-h culture in Tarozzi broth, showing coccobacillary and coccoid elements; micrograph (1200×).

denticolens (Dent and Williams, 1984b) (Figure 25), *Actinomyces meyeri* (Cato et al., 1984), and *Actinomyces odontolyticus* (Figure 26) cultures, and may be arranged in palisades as well as in other diphtheroidal arrangements. *Actinomyces hyovaginalis* (Collins et al., 1993), *Actinomyces nasicola* (Hall et al., 2003d), and *Actinomyces neuii* (Funke et al., 1994) usually appear as short, diphtheroidal rods, but some branching and/or coccoid or coccobacillary forms may occur. Coccobacillary or coccoid elements are uncommon in the "classical" *Actinomyces* species although they may predominate in certain *Actinomyces bovis* and *Actinomyces odontolyticus* cultures (Figure 27), and they are also found in *Actinomyces vaccimaxillae* (Hall et al., 2003a) together with short diphtheroidal rods. *Actinomyces europaeus* (Funke et al., 1997a), *Actinomyces radicidentis* (Collins et al., 2000), *Actinomyces radingae* (Vandamme et al., 1998), and *Actinomyces slackii* (Dent and Williams, 1986) characteristically form only coccoid or coccobacillary elements.

Cell-wall composition. The species currently included in the genus *Actinomyces* display considerable chemotaxonomic heterogeneity in particular with regard to their amino acid composition of the cell-wall peptidoglycan, DNA base ratio values, cell-wall sugars, as well as their menaquinone, polar lipid, and cellular fatty acid composition.

In most of the members of the genus *Actinomyces*, the muramic acid residue of the glycan moiety of the cell-wall peptidoglycan is *N*-acetylated; up to the present, only *Actinomyces hordeovulneris* was found to possess the *N*-glycolated form of this cell-wall constituent (Schaal, 1999), at least as far as the acyl types of *Actinomyces* species have already been determined. The mode of cross-linkage and the amino acid composition of the tetrapeptide bridge of the peptidoglycan layer of members of the genus *Actinomyces* is complex. Using the methods of Schleifer and Kandler (1972), modified by the application of cellulose sheets (Merck, Darmstadt, Germany) for ascending thin-layer chromatography (Weiss, unpublished), the cross-linking between the

amino group of the diamino acid in position 3 of the peptide side chain and the carboxyl group of D-alanine in position 4 of the adjacent tetrapeptide varies between *Actinomyces* species.

The peptidoglycan of *Actinomyces bovis*, the type species of the genus, was characterized by Schleifer and Kandler (1972) as containing a typical L-alanine, D-glutamic acid, L-lysine, D-alanine sequence in the peptide moiety and a D-aspartic acid cross-link between L-lysine in position 3 of one tetrapeptide unit and D-alanine of the corresponding subunit (L-Lys–D-Asp). This peptidoglycan type was abbreviated as peptidoglycan type A4. However, the findings of Schleifer and Seidl (1985) appear to indicate that the *Actinomyces bovis* peptidoglycan is of the L-Lys–L-Lys–D-Asp type with L-lysine in position 3 of the tetrapeptide and L-lysine and D-aspartic acid in the interpeptide bridge. As both variations of the peptidoglycan contain D-aspartic acid, those filamentous strains of *Actinomyces bovis* that were reported to lack aspartic acid (Pine and Boone, 1967) would thus belong to a different murein type, a finding which still awaits further elucidation.

All of the remaining *Actinomyces* species studied so far belong to peptidoglycan type A5 (Schleifer and Seidl, 1985), which is characterized by the presence of a diamino acid in the interpeptide bridge. This type is common, though not exclusive, among *Actinomycetaceae* strains and appears to be restricted to this group of bacteria. Type A5 occurs in several variations designated A5α, A5β, etc. *Actinomyces europaeus* and *Actinomyces neuii* were found to belong to subtype A5α (L-Lys–L-Lys–D-Glu) (Funke et al., 1997a). *Actinomyces radingae* also shows this subtype with L-lysine in position 3 of the tetrapeptide subunit being partially replaced by L-ornithine (L-Lys[L-Orn]-L-Lys–D-Glu) (Wüst et al., 1995a). *Actinomyces turicensis* is characterized by peptidoglycan subtype A5β with L-ornithine as the diamino acid in position 3 of the tetrapeptide (L-Orn–L-Lys–D-Glu) which is identical to that of *Actinomyces israelii* (Schleifer and Seidl, 1985; Wüst et al., 1995a).

The detailed structures of the peptidoglycan of *Actinomyces* species as far as they have been determined do not support the taxonomic homogeneity of the genus and are therefore in at least partial agreement with the findings derived from 16S rRNA gene sequencing (Schaal et al., 2006). For example, *Actinomyces georgiae*, *Actinomyces gerencseriae*, *Actinomyces hyovaginalis*, *Actinomyces israelii*, and *Actinomyces turicensis* all exhibit the type A5β (L-Orn–L-Lys–D-Glu), *Actinomyces radingae* has type A5α with L-Lys(L-Orn)-L-Lys–D-Glu, and *Actinomyces europaeus* and *Actinomyces neuii* have the type A5α with L-Lys–Lys–D-Glu.

Apart from *Actinomyces bovis*, *Actinomyces europaeus*, and *Actinomyces neuii*, all of the other species of the genus *Actinomyces* studied so far contain L-ornithine in their cell-wall peptidoglycan and have therefore definitely or presumably the peptidoglycan type A5β. With the exception of *Actinomyces radingae*, which was assigned to A5α, these are *Actinomyces denticolens*, *Actinomyces gerencseriae*, *Actinomyces hordeovulneris*, *Actinomyces howellii*, *Actinomyces hyovaginalis*, *Actinomyces israelii*, *Actinomyces naeslundii*, *Actinomyces odontolyticus*, and *Actinomyces slackii*, as well as presumably *Actinomyces johnsonii*, *Actinomyces oris*, and *Actinomyces viscosus* (Buchanan et al., 1984; Collins et al., 1993; Dent and Williams, 1984b, 1984c, 1986; Schleifer and Kandler, 1972).

The cell-wall sugars of *Actinomyces* species may include 6-deoxytalose, fucose, galactose, glucose, mannose, and rhamnose which may be present singly or in various combinations (Buchanan et al., 1984; Dent and Williams, 1984b, 1986; Pine, 1963; Schaal, 1999; Slack, 1974; Slack and Gerencser, 1975). *Actinomyces gerencseriae* and *Actinomyces israelii* contain only one detectable sugar, galactose, in their walls (Cummins and Harris, 1958; Schaal et al., 2006). Galactose is also the only or principal sugar component of the walls of both subspecies of *Actinomyces neuii* and of *Actinomyces georgiae* and *Actinomyces radingae* (Schaal, 1999). In contrast, rhamnose was the sole cell-wall sugar detected in *Actinomyces denticolens* (Dent and Williams, 1984b).

All of the other *Actinomyces* species investigated so far exhibit more complex sugar patterns: In *Actinomyces bovis* cell walls, 6-deoxytalose and rhamnose were identified together with smaller amounts of glucose and mannose (Cummins and Harris, 1958, 1959; Schaal, 1999; Slack, 1974; Slack and Gerencser, 1975). Whether fucose is usually present remains to be clarified (Schaal et al., 2006). According to the previous definition of the species, *Actinomyces naeslundii* walls were reported to contain 6-deoxytalose, glucose, mannose, rhamnose, and occasionally fucose (Boone and Pine, 1968; Cummins and Harris, 1958; Hammond et al., 1973; Slack, 1974; Slack and Gerencser, 1975). However, a re-evaluation of these data (Dent and Williams, 1984b) showed that certain of these *Actinomyces naeslundii* strains lack 6-deoxytalose, and mannose was not detected at all. It remains to be seen whether these differences correlate with the subdivision of the species into *Actinomyces naeslundii sensu stricto*, *Actinomyces oris*, and *Actinomyces johnsonii*. The sugar pattern of *Actinomyces viscosus* as previously defined is similar to that of *Actinomyces naeslundii sensu lato* in that it encompasses 6-deoxytalose, glucose, and rhamnose as characteristic components (Dent and Williams, 1984b; Schaal, 1999; Slack, 1974; Slack and Gerencser, 1975). However, some strains may have varying amounts of galactose and small amounts of mannose in addition to the above components (Dent and Williams, 1984b; Schaal, 1999; Slack, 1974) while isolates from dogs may lack 6-deoxytalose (Buchanan et al., 1984). Results on the sugar pattern of *Actinomyces odontolyticus* have remained contradictory: Hammond et al. (1973) and Slack and Gerencser (1975) claimed that galactose, glucose, and mannose, but no 6-deoxytalose or rhamnose were present in walls of this species. On the other hand, Slack (1974), in his contribution to the 8th edition of *Bergey's Manual of Determinative Bacteriology*, listed 6-deoxytalose, fucose, galactose, glucose, mannose, and rhamnose as sugar components characteristic of *Actinomyces odontolyticus*. The findings of Dent and Williams (1984b) who analyzed two strains of *Actinomyces odontolyticus* (NCTC 9931, NCTC 9935) and of Schaal et al. (1999) are similar to those reported by Slack in (1974) and support the view that at least certain strains of this species may have a sugar pattern that includes 6-deoxytalose, glucose, mannose, and rhamnose as principal components. *Actinomyces howellii* cell-wall carbohydrates were reported to comprise glucose and rhamnose, but not 6-deoxytalose (Dent and Williams, 1984c). However, Schaal et al. (1999) also identified galactose and mannose in addition to the above two sugars in this organism. The walls of *Actinomyces hordeovulneris* contain galactose and glucose, while 6-deoxytalose and rhamnose are absent (Buchanan et al., 1984; Schaal, 1999). The sugar pattern of *Actinomyces slackii* is composed of glucose and rhamnose, and may also include galactose (Dent and Williams, 1986; Schaal, 1999). *Actinomyces*

hyovaginalis, *Actinomyces meyeri*, and *Actinomyces turicensis* all contain fucose in their walls (Schaal, 1999). In addition, *Actinomyces turicensis* walls were reported to contain 6-deoxytalose and rhamnose (Schaal, 1999). Besides fucose, the sugar pattern of *Actinomyces hyovaginalis* comprises galactose, glucose, and rhamnose (Schaal, 1999). The sugar content of *Actinomyces meyeri* is most complex as it includes galactose, glucose, mannose, and rhamnose in addition to fucose (Schaal, 1999).

Other chemotaxonomic characteristics. Early reports (Amdur et al., 1978; Kroppenstedt and Kutzner, 1976, 1978; Schaal, 1986b) appeared to indicate that the cellular fatty acid profiles of members of the genus *Actinomyces* were rather simple, encompassing predominantly even numbered, *n*-saturated and *n*-unsaturated, but no branched-chain, odd-numbered, or hydroxyl substituted fatty acids. Thus, whole-organism extracts of *Actinomyces bovis*, *Actinomyces israelii*, *Actinomyces naeslundii*, and *Actinomyces viscosus* as previously defined, as well as *Actinomyces odontolyticus* were reported to contain tetradecanoic ($C_{14:0}$, myristic acid), hexadecanoic ($C_{16:0}$, palmitic acid), octadecanoic ($C_{18:0}$, stearic acid) and octadecenoic ($C_{18:1}$ ω9*c*, oleic acid) acids, possibly together with small amounts of dodecanoic ($C_{12:0}$, *Actinomyces naeslundii sensu lato*) and C_{20} acids (Amdur et al., 1978; Kroppenstedt and Kutzner, 1978). *Actinomyces bovis* may contain larger amounts of octadecanoic acid (Amdur et al., 1978) while these acids constitute only a small part of the fatty acid profiles of the remaining species mentioned before.

In the description of the two genospecies of *Actinomyces naeslundii* and of *Actinomyces* serovar WVA 963 (Johnson et al., 1990), a detailed analysis of their fatty acid profiles was included. According to this analysis, *Actinomyces naeslundii* genospecies 1 which represents *Actinomyces naeslundii sensu stricto* (Henssge et al., 2009), contains $C_{16:0}$, $C_{18:1}$ ω9*c*, and $C_{18:0}$ as major fatty acids together with small amounts of $C_{10:0}$, $C_{12:0}$, $C_{14:0}$, $C_{16:1}$ ω7 *c*, and $C_{16:1}$ ω9*c*. The profile of *Actinomyces naeslundii* genospecies 2, which has recently been described formally as *Actinomyces oris*, is very similar to that of *Actinomyces naeslundii sensu stricto*. The same is true for the profile of *Actinomyces* serovar WVA 963 which has recently been described as *Actinomyces johnsonii* (Henssge et al., 2009). The fatty acid composition of *Actinomyces viscosus* serovar I, which now represents *Actinomyces viscosus sensu stricto*, differs from that of the other species from the *Actinomyces naeslundii/Actinomyces viscosus* complex both qualitatively and quantitatively: According to its new definition, *Actinomyces viscosus* contains small amounts of $C_{18:2}$ ω9,12*c* fatty acids which have been identified neither in *Actinomyces naeslundii sensu stricto* nor in *Actinomyces oris* or *Actinomyces johnsonii*. Furthermore, the relative amount of $C_{16:0}$ present is comparatively low while the content of $C_{10:0}$ is comparatively high.

Other studies on the fatty acid composition of *Actinomyces* species showed that methyl branched-chain fatty acids of the iso and anteiso type, as well as fatty acids which contain a cyclopropane ring, are also not uncommon (Schaal, 1999). The occurrence of cyclopropane fatty acids in a strain of *Actinomyces israelii* (DSM 43305) had already been claimed by Kroppenstedt and Kutzner (1978), but could not be confirmed by Amdur et al. (1978). In the comparative taxonomic study of Schaal et al. (1999), *Actinomyces denticolens*, *Actinomyces gerencseriae*, *Actinomyces hordeovulneris*, *Actinomyces slackii*, and *Actinomyces viscosus* serovar II (*Actinomyces oris*) were found to contain branched-chain fatty acids of the iso and anteiso type. In the same study, saturated and unsaturated fatty acids were detected in all of the *Actinomyces* species examined (*Actinomyces denticolens*, *Actinomyces gerencseriae*, *Actinomyces howellii*, *Actinomyces meyeri*, *Actinomyces slackii*, and *Actinomyces viscosus* serovar II), apart from *Actinomyces hordeovulneris* which only contained saturated fatty acids in addition to the branched-chain ones.

The fatty acid profiles of the two subspecies of *Actinomyces neuii*, as well as those of *Actinomyces europaeus*, *Actinomyces oricola*, and *Actinomyces ruminicola* comprise palmitic ($C_{16:0}$), stearic ($C_{18:0}$), and oleic ($C_{18:1}$ ω9*c*) acids as predominant components (An et al., 2006; Funke et al., 1997a; Funke et al., 1994; Hall et al., 2003c). In addition to these acids, whole-cell extracts of *Actinomyces massiliensis* were found to contain a mixture of $C_{18:2}$ ω6,9*c* and C_{18} anteiso, as well as $C_{12:0}$, $C_{14:0}$, $C_{16:1}$ ω9*c*, and $C_{16:1}$ ω7*c* (Renvoise et al., 2009). The spectrum of cellular fatty acids identified in *Actinomyces radingae* and *Actinomyces turicensis* consisted of $C_{10:0}$, $C_{14:0}$, $C_{16:1}$ ω9*c*, $C_{16:0}$, and $C_{18:1}$ ω9*c*, and another $C_{18:1}$ fatty acid in addition (Wüst et al., 1995a). *Actinomyces graevenitzii* cells were reported to contain $C_{12:0}$, $C_{14:0}$, $C_{16:1}$ ω9, $C_{16:0}$, $C_{18:1}$ ω9, and $C_{18:0}$ fatty acids (Pascual Ramos et al., 1997a). In *Actinomyces nasicola*, $C_{15:0}$ anteiso, $C_{16:0}$, $C_{18:0}$, and $C_{18:1}$ ω9*c* were identified as the major fatty acids (Hall et al., 2003d).

Yribarren and Vilkas (1974) and Pandhi and Hammond (1978) were the first to determine polar lipids in four *Actinomyces viscosus sensu lato* strains: The total lipid content of this species ranged from 3.5 to 5.5% of dry weight. Pandhi and Hammond (1978) identified the polar lipids as cardiolipin, galactosyldiglyceride, phosphatidylcholine, phosphatidylglycerol, and phosphatidylinositol dimannosides. Phosphatidylethanolamine and phosphatidylinositol were not detected. This is in contrast to the findings of Yribarren and Vilkas (1974) who reported that their *Actinomyces viscosus sensu lato* strains contained phosphatidylethanolamine.

In a comparative chemotaxonomic study of *Actinomyces* species (Schaal, 1999), two different phospholipid types were identified, namely phospholipid type I (PI) (with no diagnostic phospholipid) and phospholipids type III (PIII) (with phosphatidylcholine as diagnostic lipid) *sensu* Lechevalier et al. (1977). *Actinomyces bovis* (DPG, PC, PI?, PIM?), *Actinomyces gerencseriae*, *Actinomyces israelii*, *Actinomyces meyeri* (DPG, PC, PI), *Actinomyces naeslundii sensu lato*, *Actinomyces neuii*, and *Actinomyces viscosus sensu lato* were assigned to phospholipid type III (DPG, PG, PC, PI, and PIM*). Phospholipid type I (DPG, PG, PI, and PIM) was found to be characteristic of *Actinomyces denticolens*, *Actinomyces hordeovulneris*, and *Actinomyces howellii*.

The principal respiratory quinones found in *Actinomyces* species are menaquinones with eight, nine, ten, and eleven isoprene units. *Actinomyces bovis*, *Actinomyces canis*, *Actinomyces graevenitzii*, *Actinomyces hordeovulneris*, *Actinomyces nasicola*, and *Actinomyces ruminicola* thus far appear to be the only members of the genus which contain fully saturated menaquinones, whereas the remaining species possess menaquinones with varying degrees of saturation (Schaal et al., 2006). In *Actinomyces bovis* and *Actinomyces ruminicola*, menaquinones with nine and ten isoprene units (MK-9, MK-10) were identified (An et al., 2006; Schaal, 1999); *Actinomyces bovis* was found to contain *methyl-*

*DPG, diphosphatidylglycerol; PG, phosphatidylglycerol; PC, phosphatidylcholine; PI, phosphatidylinositol; PIM, phosphatidylinositol mannosides.

MK-10 in addition (Schaal, 1999). *Actinomyces hordeovulneris* contains MK-8 and MK-9 (Schaal, 1999) and the major menaquinone of *Actinomyces nasicola* is MK-9 (Hall et al., 2003d). The major menaquinone of *Actinomyces bowdenii* (Pascual et al., 1999), *Actinomyces gerencseriae*, *Actinomyces howellii*, *Actinomyces israelii*, *Actinomyces naeslundii sensu lato*, *Actinomyces slackii* (Schaal, 1999), and *Actinomyces viscosus* serovar II (*Actinomyces oris*), is MK-10(H_4) although at least *Actinomyces naeslundii sensu lato*, *Actinomyces slackii*, and *Actinomyces viscosus* serovar II (*Actinomyces oris*) may also contain substantial amounts of MK-9(H_4). The two subspecies of *Actinomyces neuii* are characterized by MK-8(H_4) and MK-9(H_4) (Schaal, 1999). In preparations of *Actinomyces meyeri*, MK-9(H_4), MK-10(H_4), MK-11(H_4), and *methyl*-MK-12(H_4) were identified (Schaal, 1999). Only for *Actinomyces coleocanis*, the complete absence of respiratory menaquinones was reported (Hoyles et al., 2002a), a result which awaits confirmation.

The chemotaxonomic data as outlined above underpin the heterogeneity of the genus *Actinomyces* as demonstrated by 16S rRNA gene sequencing although the chemotaxonomic patterns obtained so far are not in full agreement with the different subclusters that can be discerned in phylogenetic dendrograms.

Fine structure. The cell morphology of *Actinomyces* species as observed by electron microscopy of ultrathin sections reflects the picture obtained by light microscopy: cells of most species present as bacillary structures of varying length with or without clubbed ends. In the "classical" *Actinomyces* species, branching can readily be demonstrated (Duda and Slack, 1972; Lai and Listgarten, 1980; Overman and Pine, 1963; Slack and Gerencser, 1975). It should be noted, however, that detailed electron microscopy studies of the recently described newer species have not been performed.

Septum formation can easily be seen in most of the ultrathin sections analyzed so far (Duda and Slack, 1972; Slack and Gerencser, 1975). The septa are not always at a right angle to the longitudinal axis of the cell and the completed cross-walls are often curved (Duda and Slack, 1972). Cells divided by cross-wall formation do not necessarily separate, so that a straight row of rods may result (Duda and Slack, 1972).

Cross-walls dividing rod-shaped elements into two approximately equal parts were taken as an indication for the occurrence of binary fission (Slack and Gerencser, 1975). However, bacteria which typically multiply by binary fission synthesize new cell-wall material either along the whole length of the cell body or in the center part of the rod leading to conservation of the apices (Locci and Schaal, 1980). In contrast, the *Actinomyces* species investigated so far exclusively showed apical growth at one or both ends of the rod-shaped propagule. As evidenced by scanning electron microscopy (Locci, 1978) and immunofluorescence labeling (Locci and Schaal, 1980), new cell-wall material is only formed at the tips of the cell while the interpolar part of the envelope remains unchanged. The unipolar or bipolar extension of the rods resulting from the apical synthesis of new wall material leads to elongated cells or filaments which divide by septum formation and may separate into rod-shaped elements by fragmentation (Locci, 1976; Locci and Schaal, 1980).

The mode of cell growth and division outlined above may be described more appropriately by the term "budding" than by "binary fission". Budding is obviously also responsible for the formation of side branches (Locci and Schaal, 1980). In this respect, electron microscopy of ultrathin sections provided essentially the same information (Slack and Gerencser, 1975). Whether the recently described coccoid or coccobacillary *Actinomyces* species follow the same way of synthesizing new cell-wall material remains to be demonstrated.

The Gram-stain-positive cell walls of *Actinomyces* species have a bilayered structure (Duda and Slack, 1972; Lai and Listgarten, 1980). They consist of a moderately electron-dense outer layer and a thinner, more electron-dense, inner layer. The thickness of the inner layer is 3.6–9.0 nm depending on the species examined. In *Actinomyces bovis*, this inner layer of the cell wall was not detectable (Lai and Listgarten, 1980). Measurements of the thickness of the outer layer indicated pronounced interspecies variation ranging from 14 nm in *Actinomyces bovis* to 36 nm in *Actinomyces israelii* (Lai and Listgarten, 1980). Analogous differences were observed for the overall width of the walls of various *Actinomyces* species although the absolute figures given by different investigators vary considerably. In the following compilation, wall thickness data are listed in this order: Overman and Pine (1963), Duda and Slack (1972), and Lai and Listgarten (1980). According to these authors, the wall thickness was found to be as follows: *Actinomyces bovis*, 10:31:14–22 nm; *Actinomyces israelii*, 29:65:35–50 nm; *Actinomyces naeslundii sensu lato*, 20:45:25–35 nm; *Actinomyces odontolyticus*, not determined (nd):31:22–27 nm; *Actinomyces viscosus sensu lato*, (nd):35:23–28 nm.

In most of the *Actinomyces* strains investigated so far, the external contour of the outer cell-wall layer was described as fuzzy or shaggy (Duda and Slack, 1972; Lai and Listgarten, 1980). *Actinomyces bovis*, *Actinomyces naeslundii sensu lato*, and certain strains of *Actinomyces israelii* and *Actinomyces viscosus sensu lato* may produce long filamentous projections radiating from the cell-wall surface (Lai and Listgarten, 1980; Slack and Gerencser, 1975). Using negatively stained preparations, electron microscopy revealed the presence of hair-like fimbriae protruding through a thick surface coat on some strains of *Actinomyces israelii* (Figdor and Davies, 1997). These surface fibrils or fimbriae obviously play an important role in the attachment of these microbes to epithelial cells, tooth surfaces, or other bacteria (Ellen et al., 1978; Figdor and Davies, 1997; Girard and Jacius, 1974; McIntire et al., 1978). The detection of the "surface fuzz" is markedly influenced by the culture conditions used, with broth-grown cells usually exhibiting longer appendages than blood agar-grown organisms (Lai and Listgarten, 1980). Nevertheless, *Actinomyces odontolyticus* and some strains of *Actinomyces israelii* and *Actinomyces viscosus sensu lato* were reported to produce neither long nor short surface fibrils (Lai and Listgarten, 1980).

Actinomyces odontolyticus cells show a marked increase in electron-density at the cell-wall surface. This increase can also be demonstrated, although to a lesser extent, in *Actinomyces israelii*, *Actinomyces naeslundii sensu lato*, and *Actinomyces viscosus sensu lato*, but is apparently absent in *Actinomyces bovis* (Lai and Listgarten, 1980).

The cytoplasmic membrane of *Actinomyces* species has the usual trilaminar structure (Lai and Listgarten, 1980), but is not always readily discernible (Slack and Gerencser, 1975). A nucleoid without a membrane could be visualized in most species (Duda and Slack, 1972; Lai and Listgarten, 1980). The occurrence of mesosomes has been described for all species examined so far (Duda and Slack, 1972; Lai and Listgarten, 1980; Overman

and Pine, 1963). However, they vary widely in number, size, and type. Simple tubular structures were observed most frequently, but coiled forms were also seen (Slack and Gerencser, 1975). Most of these membranous figures appeared to be derived from the cytoplasmic membrane with which they were often found to be in continuity (Lai and Listgarten, 1980; Overman and Pine, 1963). The cytoplasm of actively growing cells may be densely packed with ribosomes, and electron-dense cytoplasmic inclusions were observed in most species (Duda and Slack, 1972; Lai and Listgarten, 1980).

Cultural characteristics. The morphology of *Actinomyces* microcolonies, although previously thought to be indicative of the genus, may vary considerably, depending on both species affiliation of the strain under study and the media and incubation conditions employed. Nevertheless, standardization of the latter factors usually results in morphological features of the colonies which are fairly reproducible and comparatively diagnostic of some of the various species (Schaal and Pulverer, 1981; Schaal et al., 2006; Slack and Gerencser, 1975). However, most of the recently published *Actinomyces* species are rather fast-growing so that their colonies are macroscopically visible after 24 or 48 h when the traditional species of the genus usually only appear as microcolonies (e.g. An et al., 2006; Hall et al., 2003a, 2003c, 2003d, 2005; Lawson et al., 2001b; Renvoise et al., 2009; Woo et al., 2003).

Microcolonies are observed most appropriately on transparent agar media [brain heart infusion agar (BHIA) or CC-medium*] incubated anaerobically or in air with added CO_2 for 18–48 h and viewed *in situ* under the microscope in transmitted light (Erikson, 1940; Lentze, 1938b) or using the slide culture technique (Schaal, 1986b; Schaal et al., 2006).

Using these culture conditions and observation techniques, several characteristic types of *Actinomyces* microcolonies can be discerned. *Actinomyces gerencseriae*, *Actinomyces hordeovulneris*, and *Actinomyces israelii*, produce microcolonies which are regularly filamentous (Buchanan et al., 1984; Schaal, 1986b; Schaal et al., 2006; Slack and Gerencser, 1975), but may vary in the number and length of the filaments formed. A definitely filamentous type which is commonly referred to as "spider" colony may be considered most characteristic of *Actinomyces israelii* and many strains of *Actinomyces gerencseriae* and *Actinomyces hordeovulneris* (Figure 28). This microcolony consists of branching threads which often appear to originate from a single, central point with radial symmetry. The filaments are slender and moderately long with branches arising at an acute angle (Slack and Gerencser, 1975). In certain strains, the microcolonies may be smaller consisting of only one or two branched filaments (Figure 29), or the colonies may be larger with many radial threads which cross and fragment to bacillary elements at the center (Figure 30). Occasionally, small colonies with few, short projecting filaments and a fragmented center are seen (Schaal and Pulverer, 1981; Schaal et al., 2006; Slack and Gerencser, 1975) (Figure 31).

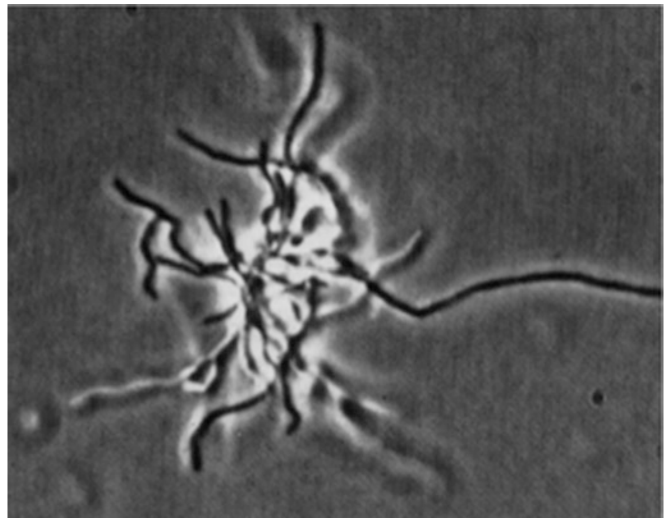

FIGURE 28. *Actinomyces gerencseriae.* Spider-like, highly filamentous microcolony on BHIA (slide culture, 26 h at 36°C); phase-contrast micrograph *in situ* in lactophenol cotton blue mounting fluid (1500×).

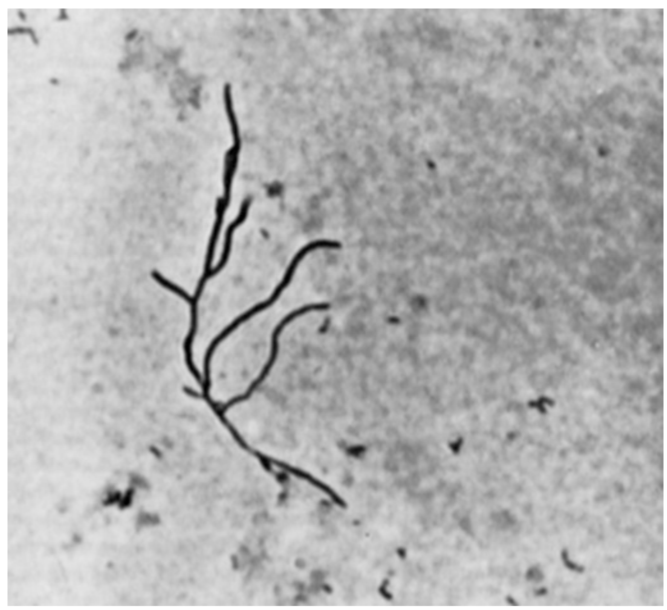

FIGURE 29. *Actinomyces gerencseriae.* Microcolony on BHIA, showing a long filament with many branches (slide culture, 24 h at 36°C); phase-contrast micrograph *in situ* in lactophenol cotton blue mounting fluid (1200×).

*CC-medium (Heinrich and Korth, 1967) : I. Solution of minerals and trace elements, containing per liter of distilled water (can be stored in refrigerator for months): $MgSO_4 \cdot 7H_2O$, 20 g; $CaCl_2 \cdot 2H_2O$, 2 g; $FeSO_4 \cdot 7H_2O$, 400 mg; $MnSO_4 \cdot 2H_2O$, 15 mg; $NaMoO_4 \cdot 2H_2O$, 15 mg; $ZnSO_4$, 4 mg; $CuSO_4 \cdot 5H_2O$, 0.4 mg; $CoCl_2 \cdot 4H_2O$, 0.4 mg; boric acid, 20 mg; KI, 10 mg. The solution is acidified with 10 ml 10% HCl. *II. Vitamin solution*, containing per 100 ml of distilled water (should be prepared freshly): thiamine-HCl, 20 mg; pyridoxine-HCl, 20 mg; biotin, 1 mg; folic acid, 5 mg; vitamin B_{12} (1 mg/100 ml), 1 ml; *p*-aminobenzoic acid, 20 mg; *m*-inositol, 20 mg; nicotinamide, 10 mg; nicotinic acid, 10 mg; Ca-pantothenate, 20 mg. III. Solution of amino acids and vitamins, containing per 100 ml of distilled water (should always be prepared freshly): casein hydrolysate, 12 g; L-cysteine-HCl, 500 mg; L-asparagine, 30 mg; DL-tryptophan, 20 mg; solution II, 12 ml. Solution III is sterilized by Seitz filtration. Preparation of the final medium: KH_2PO_4, 4 g, is dissolved in 250 ml of distilled water and adjusted to pH 7.6 with NaOH. Then, 10 ml of solution I, 500 mg of potato starch dissolved in 70 ml of boiling distilled water, about 20 g of agar (depending on quality), and distilled water are added to give a final volume of 900 ml. The mixture is autoclaved at 121°C for 15 min. After cooling to 50°C, solution III is added aseptically and the final pH adjusted to 7.3. The medium is poured in 15-ml amounts into glass Petri dishes.

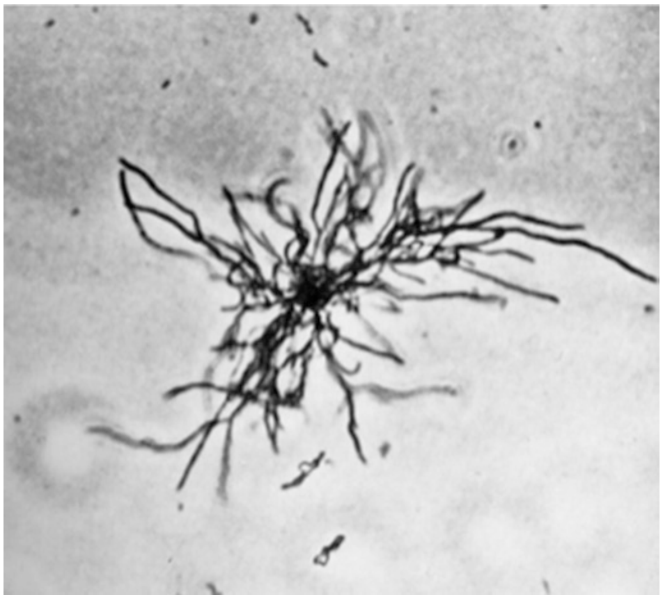

FIGURE 30. *Actinomyces israelii.* Spider- or cobweb-like microcolony on BHIA with beginning fragmentation in the center (slide culture, 48 h at 36°C); phase-contrast micrograph *in situ* in lactophenol cotton blue mounting fluid (1200×).

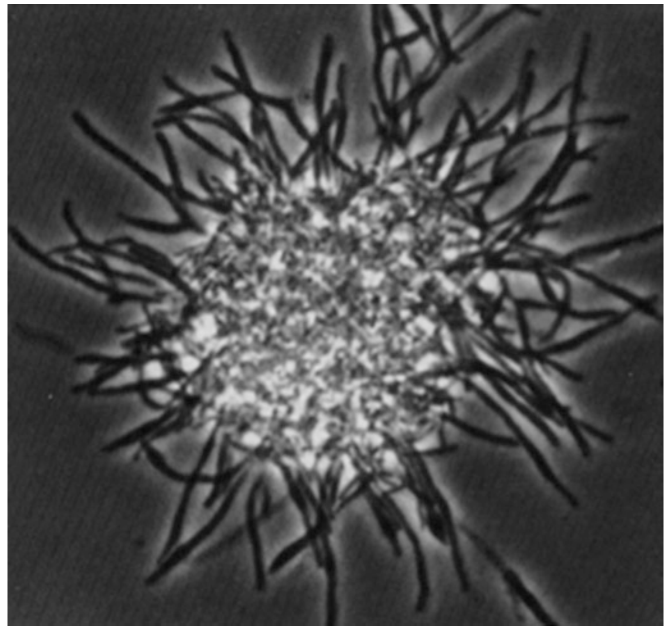

FIGURE 32. *Actinomyces naeslundii.* Filamentous microcolony on BHIA with numerous rigidly projecting filaments (slide culture, 24 h at 36°C); phase-contrast micrograph *in situ* in lactophenol cotton blue mounting fluid (1600×).

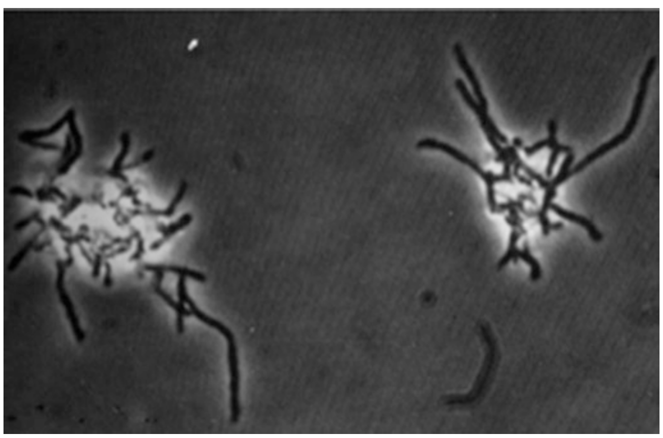

FIGURE 31. *Actinomyces gerencseriae.* Spider-like microcolony on BHIA with comparatively short filaments (slide culture, 24 h at 36°C); phase-contrast micrograph *in situ* in lactophenol cotton blue mounting fluid (1600×).

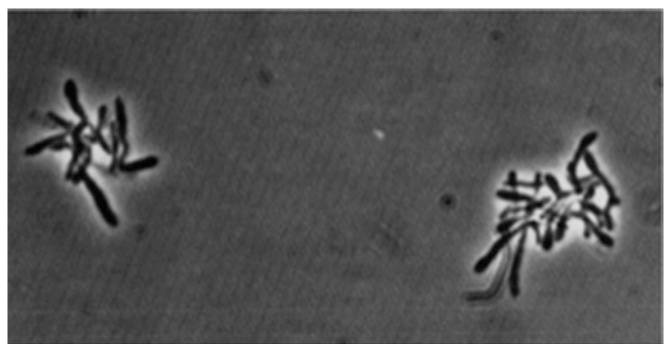

FIGURE 33. *Actinomyces oris (Actinomyces viscosus,* serovar II). Microcolony on BHIA consisting of short filaments with clubbed ends, grown under strictly anaerobic conditions (slide culture, 24 h at 36°C); phase-contrast micrograph *in situ* in lactophenol cotton blue mounting fluid (1600×).

Actinomyces naeslundii and, more characteristically, fresh hamster isolates of *Actinomyces viscosus* usually produce filamentous microcolonies. In very early growth stages, these may resemble the spider colonies of *Actinomyces israelii* (Coleman et al., 1969; Howell, 1963; Howell et al., 1959, 1965; Locci and Schaal, 1980). However, after 18–24 h of incubation, *Actinomyces naeslundii* microcolonies are usually larger than those of *Actinomyces israelii* and have a dense center composed of diphtheroidal cells and/or partly fragmented filaments which are surrounded by long, branched, and irregularly curved threads projecting in all directions (Schaal and Pulverer, 1981; Slack and Gerencser, 1975) (Figure 32). Occasionally, the filamentous fringe may be very short.

In principle, the microcolonies of human and hamster isolates of *Actinomyces viscosus* (*Actinomyces oris* and *Actinomyces viscosus sensu stricto*) resemble those of *Actinomyces naeslundii* presenting as dense cores with a filamentous periphery. However, the projecting threads are often shorter or even very short (Slack and Gerencser, 1975), and the colonies may be small when the organisms (*Actinomyces viscosus* serovar II = *Actinomyces oris*; *Actinomyces viscosus sensu stricto*) are cultured under anaerobic conditions (Figure 33). Spider-like very young microcolonies have also been reported (Howell, 1963). After serial laboratory subcultivation, both *Actinomyces viscosus sensu stricto* and *Actinomyces viscosus* serovar II (*Actinomyces oris*) tend to produce "smooth" microcolonies which are circular and have a smooth or granu-

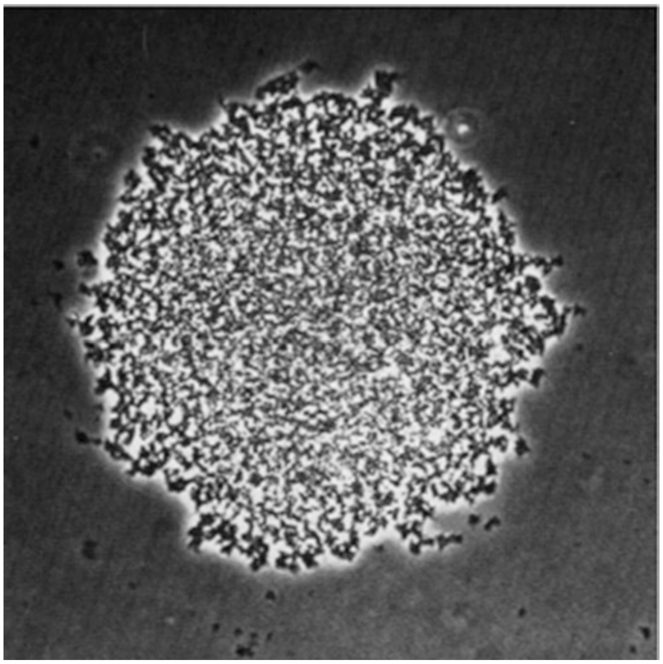

FIGURE 34. *Actinomyces viscosus.* Microcolony on BHIA chiefly consisting of short rods, grown under aerobic conditions with added CO_2 (slide culture, 48 h at 36°C); phase-contrast micrograph *in situ* in lactophenol cotton blue mounting fluid (200×).

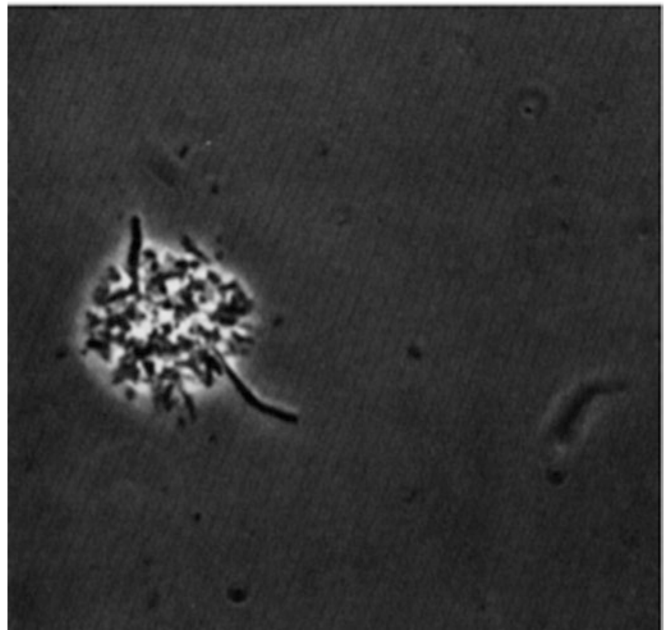

FIGURE 35. *Actinomyces bovis*, serovar I. Microcolony on BHIA, consisting of a few filaments and short rods (slide culture, 24 h at 36°C); phase-contrast micrograph *in situ* in lactophenol cotton blue mounting fluid (1200×).

lar surface and an entire or irregular edge (Slack and Gerencser, 1975) (Figure 34). In such predominantly smooth cultures, a few filamentous colonies may be present. The heterogeneous picture resulting from this morphological dissociation is easily mistaken for contamination of the culture.

Actinomyces bovis, *Actinomyces denticolens*, *Actinomyces hyovaginalis*, *Actinomyces marimammalium*, *Actinomyces meyeri*, *Actinomyces nasicola*, *Actinomyces neuii*, *Actinomyces odontolyticus*, *Actinomyces radicidentis*, *Actinomyces radingae*, *Actinomyces slackii*, *Actinomyces suimastitidis*, *Actinomyces turicensis*, and *Actinomyces vaccimaxillae* all form predominantly or exclusively non-filamentous microcolonies. These have a smooth or finely granular surface, an entire or irregular edge, are slightly raised to convex, white to colorless and soft, and may show a few short projecting filaments (Figure 35, Figure 36, and Figure 37). In addition, they may have an optically dark central spot, and typical *Actinomyces bovis* microcolonies often have irregular, jagged edges, but do not show radiating filaments (Slack and Gerencser, 1975). Similar colonies, occasionally with a dark center, are produced by *Actinomyces denticolens* (Figure 38). Nevertheless, truly filamentous strains have been reported for both *Actinomyces bovis* (Figure 39) and *Actinomyces odontolyticus* (Georg et al., 1964; Pine et al., 1960; Slack and Gerencser, 1975) and may also occur in *Actinomyces denticolens* (Figure 40). Completely smooth, circular microcolonies with entire edges are characteristic of *Actinomyces hyovaginalis*, *Actinomyces marimammalium*, *Actinomyces meyeri*, *Actinomyces nasicola*, *Actinomyces neuii*, *Actinomyces radicidentis*, *Actinomyces radingae*, *Actinomyces slackii*, *Actinomyces suimastitidis*, *Actinomyces turicensis*, and *Actinomyces vaccimaxillae* although the majority of the recently described new *Actinomyces* species have not been characterized in detail as far as their microcolony morphology is concerned.

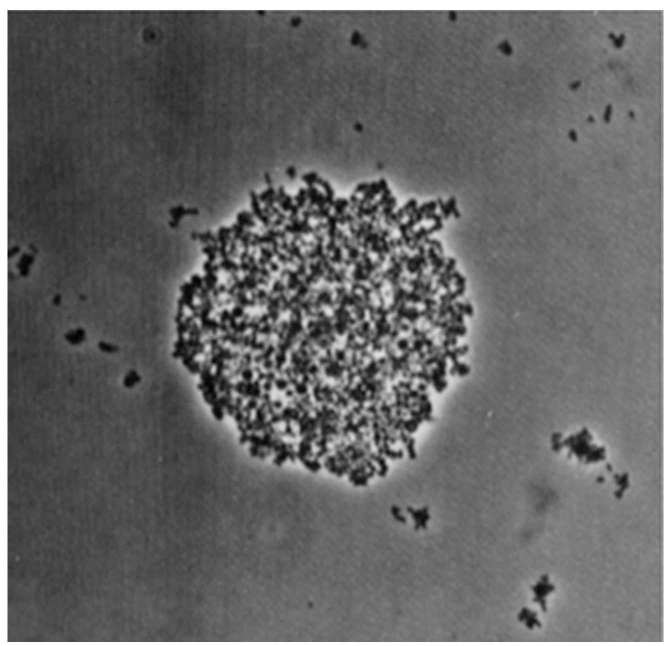

FIGURE 36. *Actinomyces odontolyticus*, serovar I. Microcolony on BHIA entirely consisting of short rods (slide culture, 48 h at 36°C); phase-contrast micrograph *in situ* in lactophenol cotton blue mounting fluid (480×).

Detailed information on the microcolony appearance of *Actinomyces howellii*, as well as that of *Actinomyces bowdenii*, *Actinomyces canis*, *Actinomyces cardiffensis*, *Actinomyces catuli*, *Actinomyces coleocanis*, *Actinomyces europaeus*, *Actinomyces funkei*, *Actinomyces georgiae*, *Actinomyces graevenitzii*, *Actinomyces hongkongensis*, *Actinomyces*

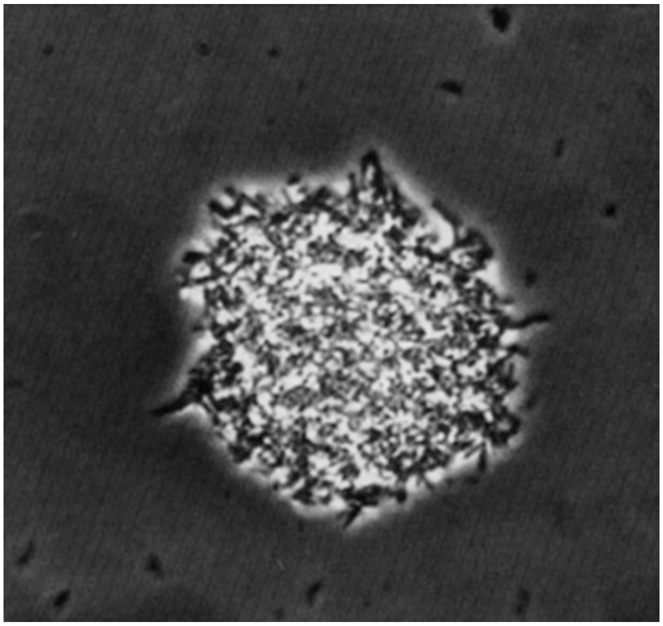

FIGURE 37. *Actinomyces denticolens.* Microcolony on BHIA, consisting of shorter and longer rods (slide culture, 24 h at 36°C); phase-contrast micrograph *in situ* in lactophenol cotton blue mounting fluid (1200×).

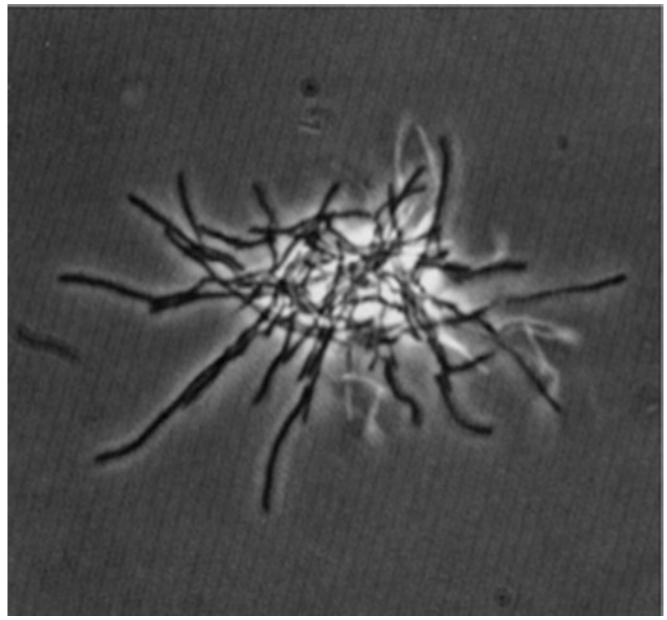

FIGURE 39. *Actinomyces bovis*, serovar II. Spider-like microcolony on BHIA, showing long, scarcely branched filaments and little signs of central fragmentation (slide culture, 24 h at 36°C); phase-contrast micrograph *in situ* in lactophenol cotton blue mounting fluid (1200×).

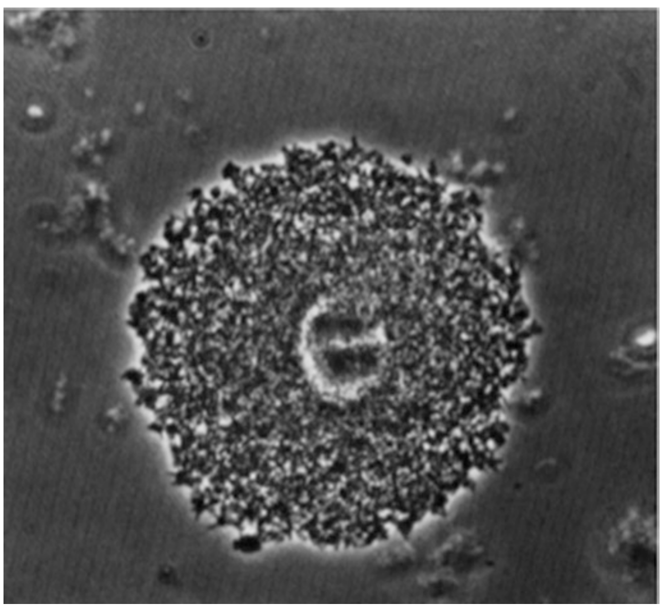

FIGURE 38. *Actinomyces denticolens.* Microcolony on BHIA, consisting of irregular rods (slide culture, 48 h at 36°C); phase-contrast micrograph *in situ* in lactophenol cotton blue mounting fluid (480×).

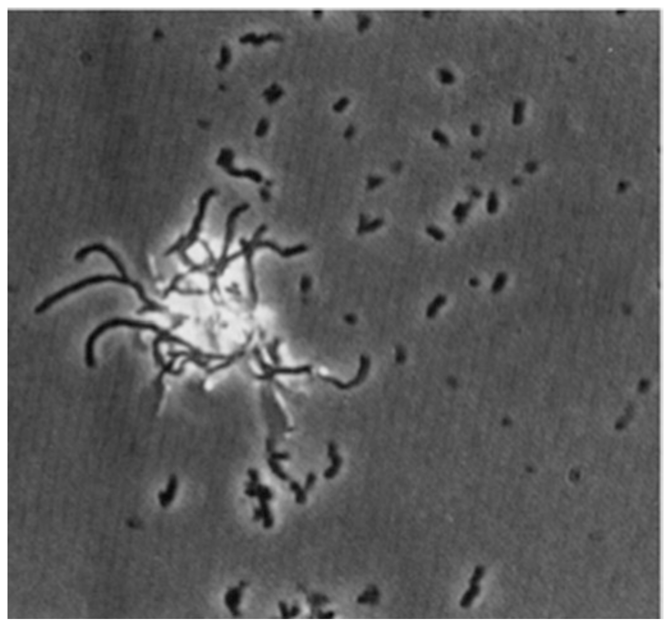

FIGURE 40. *Actinomyces denticolens.* Filamentous microcolony on BHIA (slide culture, 24 h at 36°C); phase-contrast micrograph *in situ* in lactophenol cotton blue mounting fluid (1200×).

massiliensis, *Actinomyces oricola*, and *Actinomyces urogenitalis* is not available (Schaal et al., 2006).

The morphological properties of microcolonies of the "classical" *Actinomyces* species, as demonstrated by light microscopy, have been confirmed and elucidated in more detail by scanning electron microscopy (Locci, 1976, 1978; Slack and Gerencser, 1975). A sharp contrast was noted between the compact non-filamentous colonies of *Actinomyces bovis* and *Actinomyces odontolyticus* and the filamentous ones of *Actinomyces gerencseriae*, *Actinomyces israelii*, *Actinomyces naeslundii sensu lato*, and *Actinomyces viscosus sensu lato.*

Colonies of the "classical" *Actinomyces* species mature within 5–14 d of incubation on BHIA at 36±1°C. The colony

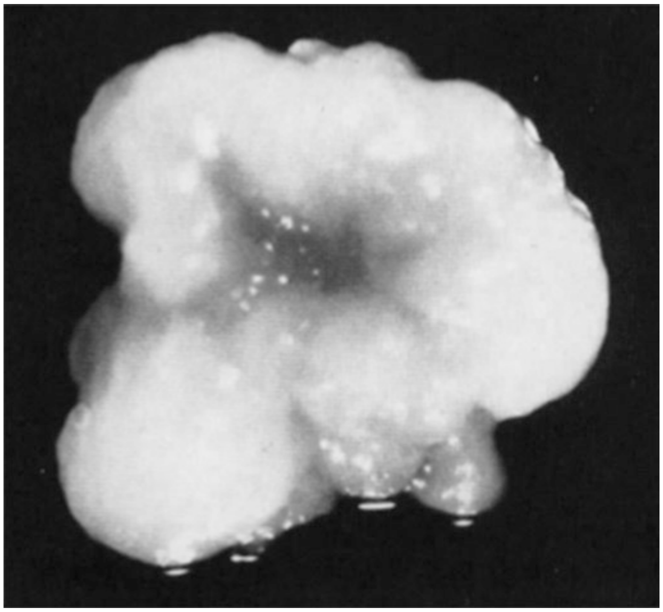

FIGURE 41. *Actinomyces israelii.* Mature "molar-tooth" colony on CC-medium (14 d at 36°C); micrograph (30×).

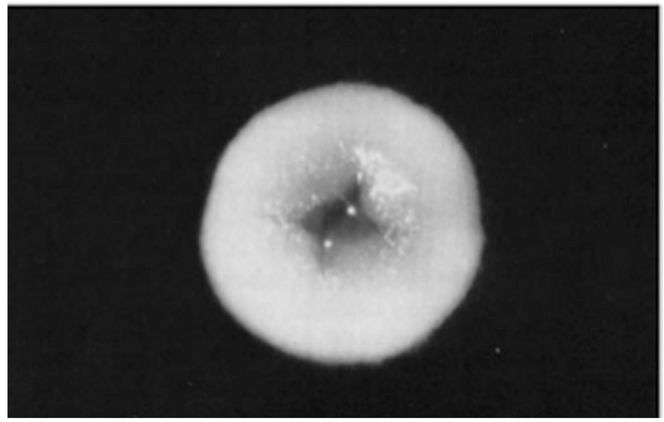

FIGURE 42. *Actinomyces israelii.* Mature colony with central depression on CC-medium (14 d at 36°C); micrograph (30×).

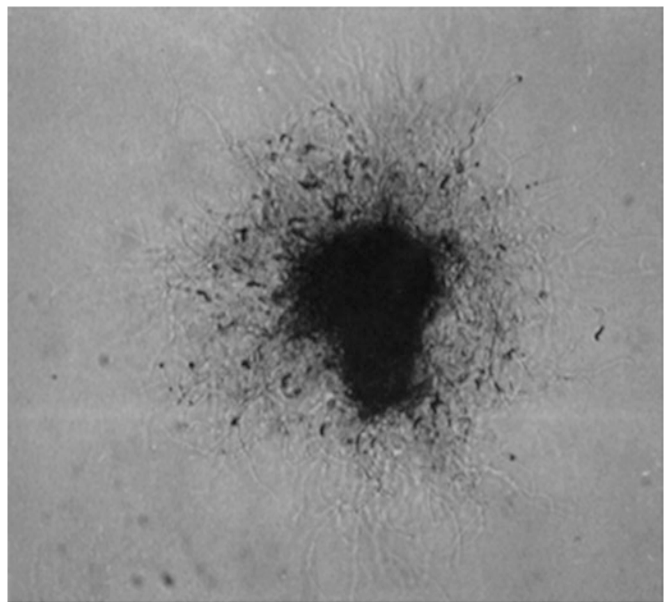

FIGURE 43. *Actinomyces gerencseriae.* Small mature colony on CC-medium with a highly filamentous fringe and short aerial hyphae (dark parts of the filaments [14 d at 36°C]); micrograph (200×).

morphology may slightly differ when growth on BHIA is compared with that on CC medium (Heinrich and Korth, 1967) or on blood agar, but the general growth characteristics may be observed on either medium. Strains which produce highly filamentous microcolonies usually form rough mature colonies. The morphology of the recently published new *Actinomyces* species has only been described in some detail after 24–48 h of incubation, predominantly on media containing blood. Details on the colony morphology of *Actinomyces canis* (Hoyles et al., 2000), *Actinomyces coleocanis* (Hoyles et al., 2002a), *Actinomyces massiliensis* (Renvoise et al., 2009), *Actinomyces radicidentis* (Collins et al., 2000), *Actinomyces suimastitidis* (Hoyles et al., 2001a), and *Actinomyces urogenitalis* (Nikolaitchouk et al., 2000), were not given in the original descriptions of these species, except that the colonies of *Actinomyces radicidentis* may be brownish and those of *Actinomyces urogenitalis* reddish (Schaal et al., 2006).

Mature colonies of *Actinomyces israelii* and *Actinomyces gerencseriae* are 0.5–2.0 mm in diameter, opaque, and white to gray-white in color. Rough colonies, with or without central depressions, are considered most characteristic of *Actinomyces israelii* and have been described as molar-tooth, bread-crumb, or raspberry-like colonies (Schaal and Pulverer, 1981; Slack and Gerencser, 1975) (Figure 41 and Figure 42). These colonies are circular to irregular in shape with undulate, lobate, or erose edges and, often, with a highly filamentous fringe (Figure 43). In addition, they are convex, pulvinate, or umbonate with a convoluted or granular surface and a dry to crumbly texture. Although *Actinomyces gerencseriae* may show the same colonial picture, this closely related species tends to produce more often smooth mature colonies with entire, circular or irregular edges, a white to gray-white to creamy-white, opaque appearance, and soft texture (Schaal and Pulverer, 1981; Slack and Gerencser, 1975) (Figure 44), which is comparatively rarely seen in *Actinomyces israelii.* The surface of this colony type is usually shiny, but may also be granular and matte. Rough *Actinomyces gerencseriae* and *Actinomyces israelii* colonies may be very hard and they often adhere to the agar medium or come off *in toto* when an attempt is made to pick them. Soft and certain bread-crumb colonies, on the other hand, are friable and easy to disintegrate or remove from the agar surface.

Although the formation of aerial hyphae is by no means characteristic of the genus, a few *Actinomyces gerencseriae* and *Actinomyces israelii* isolates from various sources may produce numerous, short aerial filaments (Figure 43) especially when grown on comparatively poor agar media (Erikson, 1940; Locci, 1978; Schaal, 1986b; Slack and Gerencser, 1975).

Mature colonies of *Actinomyces hordeovulneris* are similar to those of *Actinomyces israelii.* On bovine blood agar, this species produces white, agar-adherent, molar toothed colonies with a tendency to shift to a white, conical, domed, buttery, less adherent type upon

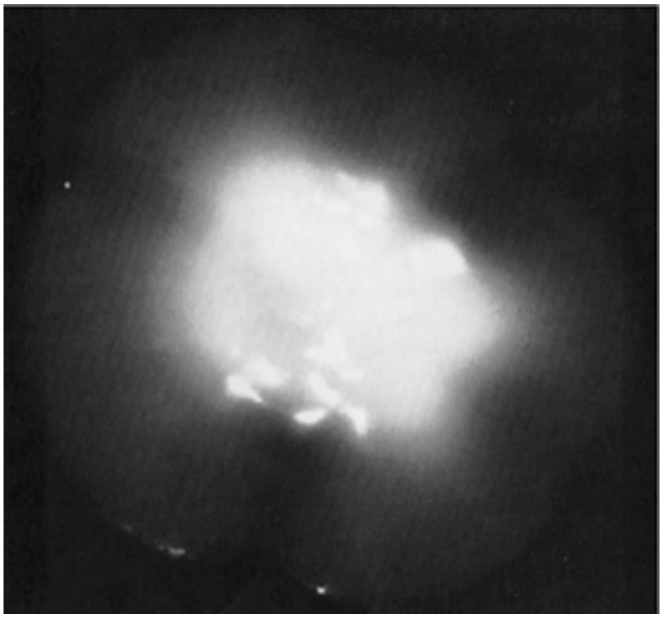

FIGURE 44. *Actinomyces gerencseriae*. Mature colony on CC-medium with a smooth surface and an undulate edge (14 d at 36°C); micrograph (30×).

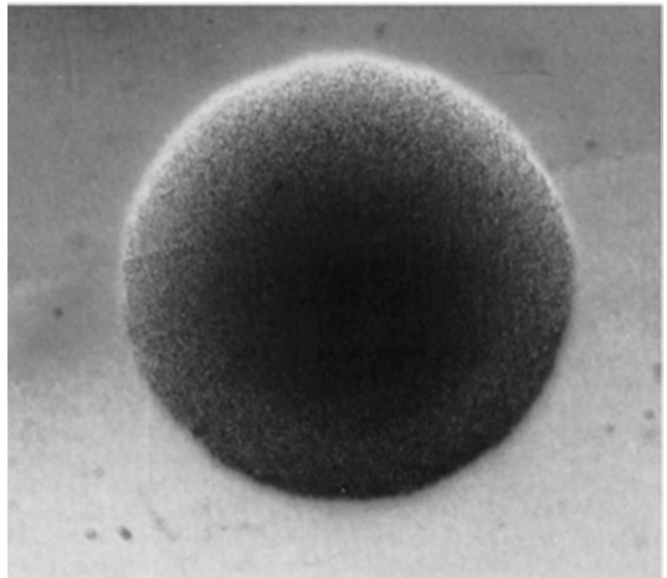

FIGURE 46. *Actinomyces naeslundii*. Small mature colony on CC-medium with a granular surface and a circular, entire edge (10 d at 36°C); micrograph (120×).

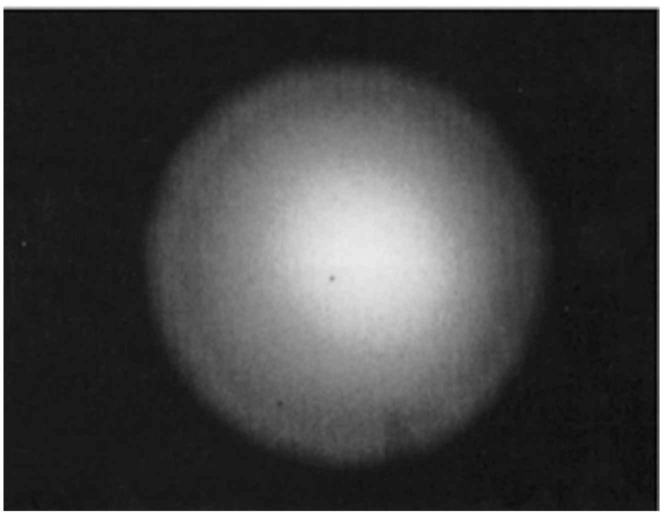

FIGURE 45. *Actinomyces oris* (*Actinomyces naeslundii*, serovar II). Mature colony on CC-medium with a smooth surface and a circular, entire edge (14 d at 36°C); micrograph (30×).

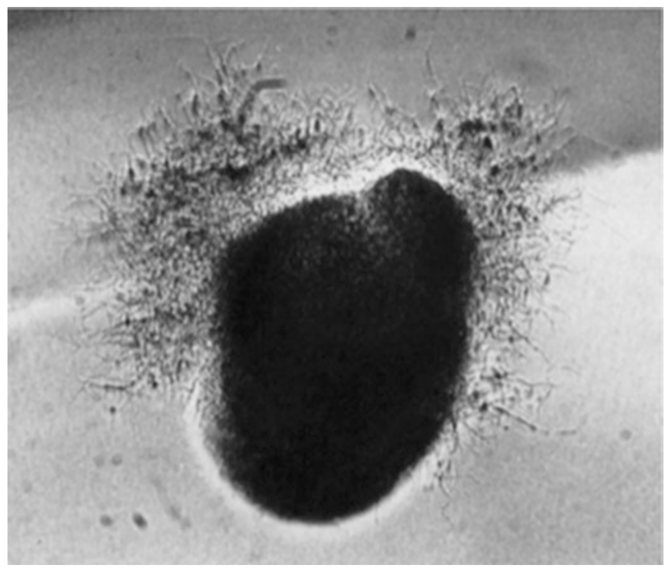

FIGURE 47. *Actinomyces naeslundii*. Small mature colony on CC-medium with a granular surface and an irregular edge from which long branching filaments project over the surrounding agar surface in two main directions (10 d at 36°C); micrograph (120×).

laboratory passage. Diameters are 0.5–1.0 mm after 48 h at 37°C and 2 mm after 3 d (Buchanan et al., 1984).

Macrocolonies of *Actinomyces naeslundii sensu lato* are predominantly soft, circular, low convex to umbonate, and entire measuring 1.0–5.0 mm in diameter (Figure 45 and Figure 46). Their color is white to gray- or creamy-white and the surface structure is usually granular. Frequently, some peripheral projecting filaments or even mycelial structures can be observed under the microscope (Figure 47) and often show an asymmetrical distribution. Furthermore, various degrees of roughness may be found so that isolates may appear in the bread-crumb or molar-tooth colony type (Figure 48).

Mature colonies of *Actinomyces viscosus sensu lato* grown under reduced oxygen tension for 7 d are approximately 0.5 mm in diameter while cultures incubated aerobically with added CO_2 produce larger colonies (4–5 mm in diameter). Their color is creamy-white, the texture is usually soft and may be viscous, in both animal (Howell, 1963) and human isolates. The overall picture is very similar to that described for *Actinomyces naeslundii sensu lato* (Figure 49 and Figure 50) although hamster isolates

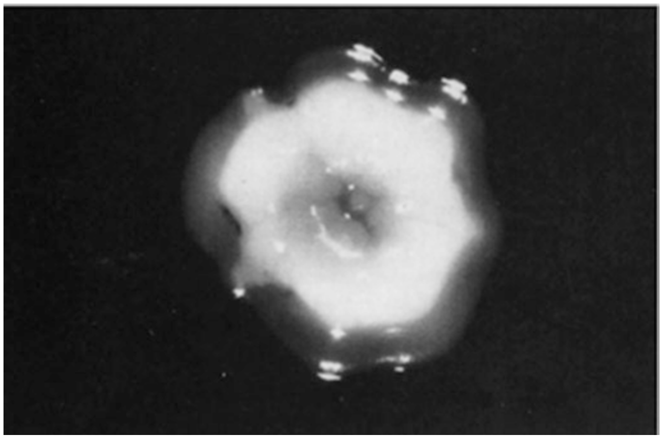

FIGURE 48. *Actinomyces naeslundii*. Molar tooth colony on CC-medium with central depression and an undulate edge (14 d at 36°C); micrograph (30×).

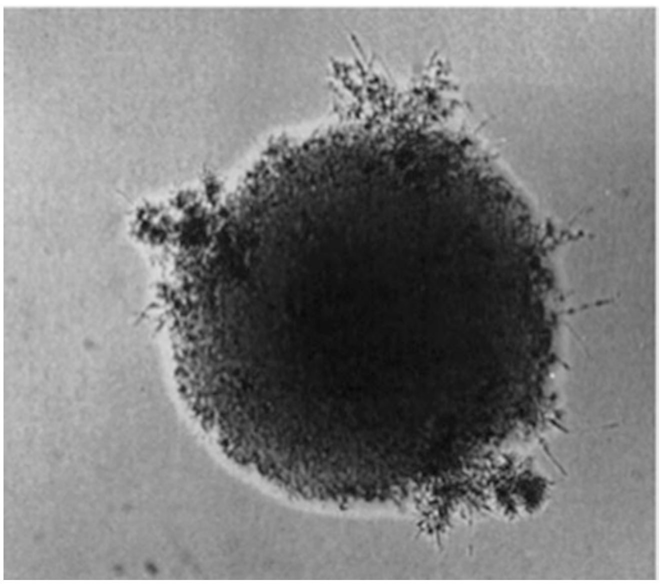

FIGURE 49. *Actinomyces oris* (*Actinomyces viscosus*, serovar II). Small mature colony on CC-medium with a granular structure and a few projecting short and branching filaments in the periphery (14 d at 36°C); micrograph (120×).

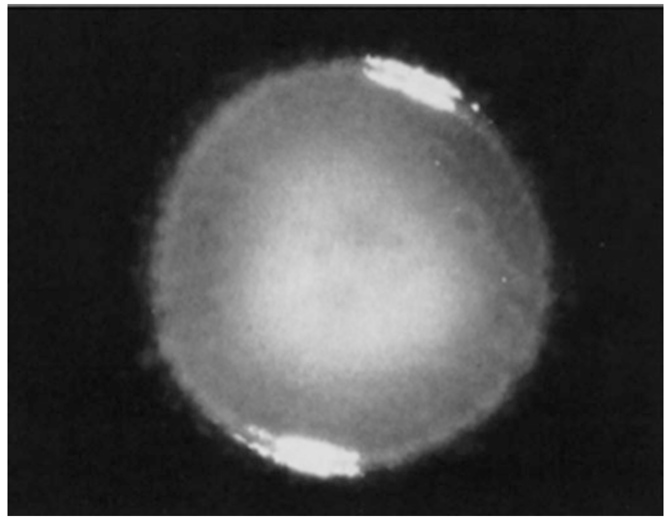

FIGURE 50. *Actinomyces oris* (*Actinomyces viscosus*, serovar II). Mature colony on CC-medium with a smooth, shiny surface and an irregular, "fuzzy" edge (14 d at 36°C); micrograph (30×).

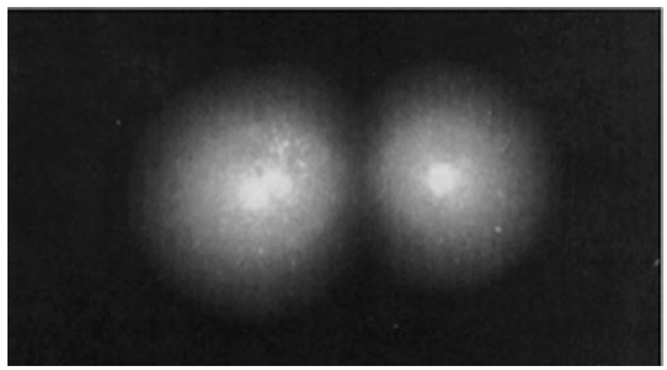

FIGURE 51. *Actinomyces bovis*, serovar I. Mature colony on CC-medium with a smooth to finely granular surface and an entire edge (14 d at 36°C); micrograph (30×).

(*Actinomyces viscosus sensu stricto*) with short, scattered aerial filaments have been reported (Slack and Gerencser, 1975). Completely smooth colonies may resemble those of cutaneous propionibacteria.

The typical macrocolony of *Actinomyces bovis* measures 0.5–1.0 mm in diameter and is circular, convex, entire, white, and predominantly soft, with a smooth or finely granular surface and a dark center (Figure 51). Rough mature colonies (Figure 52) may resemble the molar-tooth or bread-crumb colony type of *Actinomyces israelii* (Slack and Gerencser, 1975).

Actinomyces odontolyticus forms mature colonies which are 1.0–2.0 mm in size, circular to irregular with an entire or irregular edge, low convex to umbonate with a smooth to finely granular surface and a soft texture (Figure 53). In transmitted light, they may show a dark center spot and/or dark irregular granules which decrease in size from the center to the periphery (Figure 54). Rough strains may have a colony morphology similar to that of *Actinomyces israelii*. On BHIA, macrocolonies of *Actinomyces odontolyticus* are opaque and white. However, older colonies grown on blood agar develop a deep red color (Batty, 1958). This pigment may appear during anaerobic incubation in as little as 48 h, but it usually requires 5–10 d to develop or it may only be formed after the cultures have been left standing in air at room temperature following primary anaerobic incubation (Slack and Gerencser, 1975). Occasionally, the pigment formation cannot be demonstrated.

Mature colonies of *Actinomyces denticolens* reach at least 2.0 mm in diameter and are circular, entire, convex, or umbonate and smooth (Figure 55). On horse blood agar, they develop a slightly pink pigmentation when grown anaerobically, but they are white if grown in air (Dent and Williams, 1984b).

On horse blood agar plates after 3–5 d of anaerobic incubation at 37°C, colonies of *Actinomyces howellii* are white, smooth,

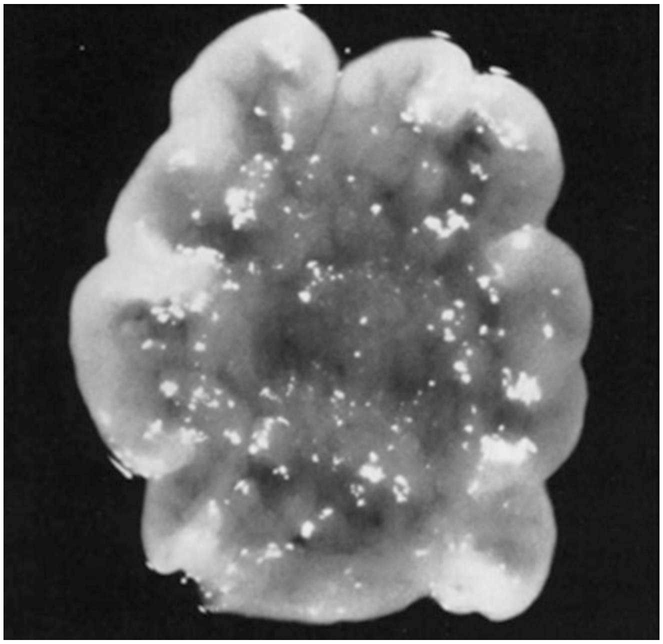

FIGURE 52. *Actinomyces bovis*, serovar II. Bread-crumb colony on CC-medium with a highly uneven surface and an irregular, lobate edge (14 d at 36°C); micrograph (30×).

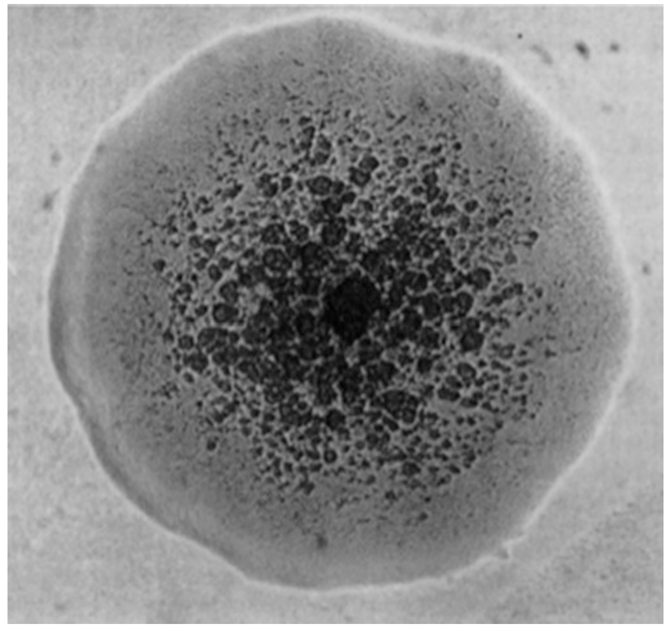

FIGURE 54. *Actinomyces odontolyticus*, serovar I. Mature colony on CC-medium with a dark center spot and granular inclusions decreasing in size from the center to the periphery (14 d at 36°C); micrograph in transmitted light (120×).

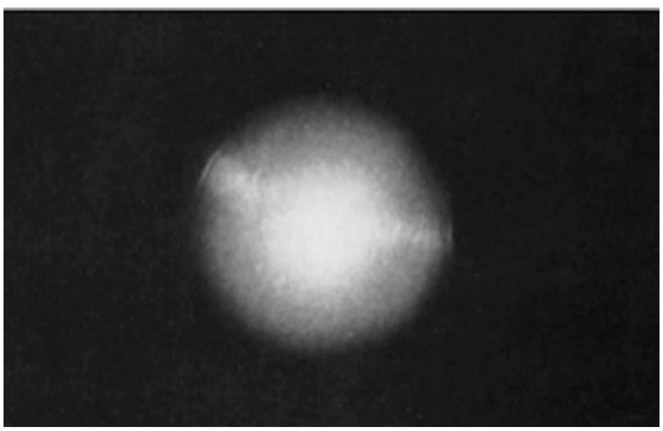

FIGURE 53. *Actinomyces odontolyticus*, serovar II. Mature smooth colony on CC-medium with an entire edge (14 d at 36°C); micrograph (40×).

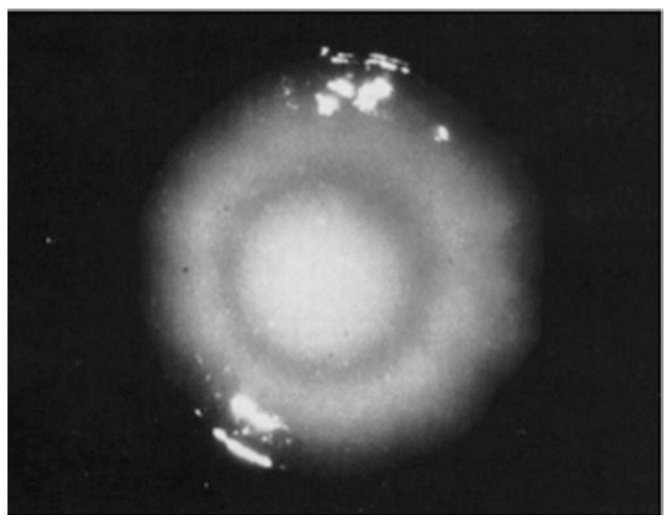

FIGURE 55. *Actinomyces denticolens*. Mature umbonate colony on CC-medium with a shiny surface and an irregular edge (14 d at 36°C); micrograph (30×).

shiny, translucent, and convex with entire margins. Their diameter is up to 2 mm (Dent and Williams, 1984c).

Mature surface colonies of *Actinomyces meyeri* on supplemented BHI blood agar are pinpoint to 1 mm in diameter, circular, flat to convex, translucent to opaque and white with shiny, smooth surfaces and entire margins. They may be α- or non-hemolytic (Cato et al., 1984). In transmitted light, their appearance is similar to those of *Actinomyces odontolyticus*. On horse blood agar after 3–5 d of incubation at 37°C, colonies of *Actinomyces slackii* are white, smooth, shiny, translucent, and convex, having entire margins and measuring up to 2 mm in diameter (Dent and Williams, 1986). Colonies of *Actinomyces georgiae*, incubated anaerobically, are 0.5–2 mm in diameter, usually circular, entire, low convex, opaque (smaller colonies are transparent to translucent), white (27% were described as tan or beige), shiny, and smooth (Johnson et al., 1990). Colonies of *Actinomyces radingae* on sheep blood agar are small (0.3–0.5 mm in diameter), gray, circular, low convex, with a glistening surface, opaque, of a butyrous consistency and with an entire edge after 48-h aerobic incubation with 5% added CO_2. Growth on sheep blood agar is the same under aerobic conditions with added CO_2 and under anaerobic conditions. A hemolytic zone appears slowly and starts mostly with α-hemolysis (Vandamme et al., 1998; Wüst et al., 1995a). Colonies of *Actinomyces turicensis*, grown on sheep blood agar aerobically with 5% CO_2 for 48 h,

are also small, gray, circular, convex, with a glistening surface, opaque, with a butyrous consistency and an entire edge. They produce a small zone of β-hemolysis, especially when grown under CO_2 enrichment or anaerobically, and growth under these conditions is slightly better compared to aerobiosis (Vandamme et al., 1998).

Within the species *Actinomyces hyovaginalis*, two biovars ("vaginal" biovar and "general" biovar) have been discerned which chiefly differ in colony morphology and a few physiological reactions (Storms et al., 2002). When grown on horse blood agar in an anaerobic or CO_2-enriched atmosphere, colonies of the "vaginal" biovar of *Actinomyces hyovaginalis* are flat with outrunning edges and non-hemolytic (Collins et al., 1993). The "general" biovar is characterized by smaller colonies that are more convex than those of the "vaginal" type and which lack the characteristic outrunning edges of the latter type resembling colonies of *Arcanobacterium pyogenes* apart from being non-hemolytic or at best weakly hemolytic (Storms et al., 2002).

Actinomyces neuii subsp. *neuii* forms circular, smooth, convex, opaque colonies, more white than creamy in color, and with entire edges. Their size is 0.5–1.5 mm in diameter after 48 h of incubation in 5% CO_2 on sheep blood agar. Most strains produce α-hemolysis on sheep blood agar and all strains do so on human blood agar. The colonies of *Actinomyces neuii* subsp. *anitratus* are similar to those of the subspecies *neuii*, except that the latter are non-hemolytic (Funke et al., 1994).

Colonies of *Actinomyces europaeus* were described as circular and smooth with a translucent grayish appearance. After 48 h of incubation in 5% CO_2-enriched atmosphere at 37°C, they measure not more than 0.5 mm in diameter (Funke et al., 1997a). Grown on blood agar in the presence of 5% CO_2 for 24 h, *Actinomyces graevenitzii* produces non-hemolytic, opaque colonies which measure approximately 0.2 mm in diameter. They characteristically adhere to agar surfaces (Pascual Ramos et al., 1997a). *Actinomyces bowdenii* forms non-hemolytic, grayish-white, rounded colonies after 24–48 h of aerobic incubation on blood agar at 37°C. Their diameter is approximately 2 mm. Growth is not enhanced by increased concentrations of CO_2 between 1.5 and 10% (Pascual et al., 1999). After 48 h of incubation on blood agar, colonies of *Actinomyces marimammalium* are gray, entire, circular, convex, and pinpoint to 0.5 mm in diameter. Hemolysis is not observed (Hoyles et al., 2001c). *Actinomyces catuli* produces tiny, irregular, convex, white colonies adherent to the medium at colony base (Hoyles et al., 2001b). When grown on Columbia blood agar for 24 h, colonies of *Actinomyces funkei* are small (<1 mm in diameter), non-hemolytic, and gray in color (Lawson et al., 2001b). On Fastidious Anaerobe Agar with 5% horse blood after 48 h of incubation, colonies of *Actinomyces cardiffensis* are pinpoint, convex, smooth-surfaced, entire-edged, and opaque cream to pinkish. As most other *Actinomyces* species, they are non-hemolytic (Hall et al., 2002). *Actinomyces hongkongensis* forms non-hemolytic, pinpoint colonies after 24 h of incubation at 37°C on sheep blood agar in an anaerobic atmosphere (Woo et al., 2003). Colonies of *Actinomyces vaccimaxillae* are <1 mm in diameter, convex, smooth, entire-edged, opaque white, and non-hemolytic when grown anaerobically on Fastidious Anaerobe Agar with 5% horse blood for 48 h (Hall et al., 2003a). After 48 h of anaerobic incubation on Fastidious Anaerobe Agar, colonies of *Actinomyces nasicola* are pinpoint, white or gray, opaque, shiny, entire, and convex (Hall et al., 2003d). Under analogous growth conditions on Fastidious Anaerobe Agar with 5% horse blood, *Actinomyces oricola* forms pinpoint, breadcrumb-like, white, and non-hemolytic colonies (Hall et al., 2003c). *Actinomyces dentalis* produces tiny, white, breadcrumb-like, agar-pitting colonies on Fastidious Anaerobe Agar with 5% horse blood after 48 h of anaerobic incubation (Hall et al., 2005). Colonies of *Actinomyces ruminicola* when grown on PYRG agar (peptone-yeast extract-glucose agar with 15% rumen fluid) for 48 h at 46°C are circular, slightly convex, white, and measure approximately 0.5–0.8 mm in diameter (An et al., 2006). *Actinomyces massiliensis* was reported to produce circular, white, shiny, pinpoint colonies after 48 h of anaerobic incubation at 37°C on sheep blood agar (Renvoise et al., 2009). In liquid media (e.g., thioglycolate broth or Tarozzi broth*), *Actinomyces* species either grow as discrete compact masses of variable size leaving the medium clear, or they produce a diffuse growth with varying amounts of a granular, flaky, or pellicular sediment (Schaal, 1986b; Slack, 1974). The cotton pad- or snowball-like growth, which represents filamentous colonies and may be suspended in the broth or adhere to the glass or the surface of the liver piece (Figure 56), is commonly seen

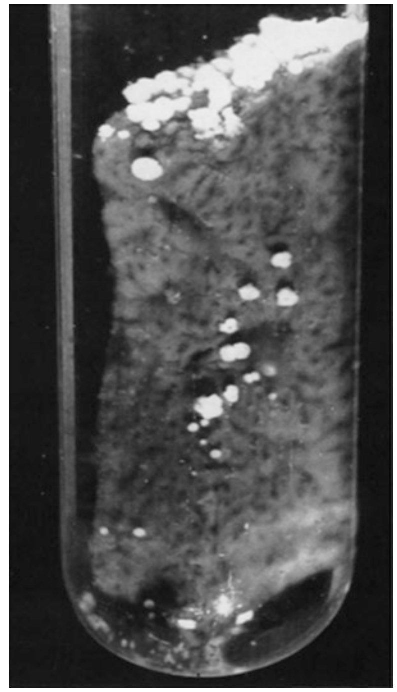

FIGURE 56. *Actinomyces israelii.* Mature snowball- or cotton pad-like colonies adhering to the liver piece in Tarozzi broth (14 d at 36°C) (2.5×).

*Tarozzi broth, modified according to Schaal et al. (2006): I: Basal nutrient broth: meat extract, 10 g; peptone (e.g., peptone P – Oxoid), 12 g; NaCl, 3 g; K_2HPO_4, 2 g; distilled water to equal a total of 1 liter. Dissolve the ingredients under heat at 80°C and boil subsequently for 20 min. Adjust the pH to 7.5 with NaOH solution. II. Preparation of liver pieces: Cut fresh guinea pig or beef liver into pieces of approximately 2 × 1 × 1 cm and wash them thoroughly in several changes of fresh saline. III. Preparation of the medium: Place liver pieces into test tubes (1 piece per tube). Add 8 ml of broth I supplemented with 0.1% sodium thioglycolate (w/v) to each tube. Stopper the tubes with cotton plugs and sterilize by autoclaving at 121°C for 15 min. Inoculate immediately after cooling, seal the tubes by adding about 1 ml of sterilized melted petrolatum (petroleum jelly, Vaseline) or paraffin wax (less suitable), and incubate at 36±1°C.

in *Actinomyces gerencseriae* and *Actinomyces israelii* strains, but also in rough filamentous isolates of other species. Broth cultures of *Actinomyces bovis*, *Actinomyces denticolens*, *Actinomyces meyeri*, *Actinomyces odontolyticus*, and *Actinomyces viscosus sensu lato* are often evenly turbid and, upon further incubation, may show a granular, flocculent, pellicular, stringy, or smooth sediment. Many strains of *Actinomyces viscosus sensu stricto* and a few *Actinomyces bovis* and *Actinomyces gerencseriae* isolates produce a viscous growth which, in the case of *Actinomyces viscosus sensu stricto*, may give rise to a mucoid sediment (Howell, 1963). Cultures of *Actinomyces meyeri* in peptone-yeast extract-glucose broth are only slightly turbid with a smooth or stringy sediment (Cato et al., 1984). Growth of *Actinomyces hordeovulneris* in trypticase soy broth supplemented with ≥5% fetal calf serum is flocculent, but weak without added serum (Buchanan et al., 1984). Cultures of *Actinomyces georgiae* in pre-reduced anaerobically sterilized peptone-yeast extract-glucose-Tween 80 broth were described as being predominantly turbid (85%) with smooth (38%), flaky (26%), crumbly or granular (24%), or stringy to ropy (12%) sediment that occasionally adheres to the bottom of the tube. Broth cultures without turbidity often have a crumbly to coarse granular sediment (Johnson et al., 1990). Details on the appearance of broth cultures of the remaining *Actinomyces* species have not been published.

Life cycle. Filamentous *Actinomyces* strains show a characteristic life cycle when cultivated on or in artificial media. The initial developmental step includes elongation of the original, usually rod-shaped propagule by apical growth at one or both ends and possibly lateral budding (Locci and Schaal, 1980; Morris, 1951) (Figure 57). When growth proceeds, a network of interwoven, branching filaments may form whose central parts sooner or later exhibit signs of septation and fragmentation (Figure 28, Figure 30, Figure 31, Figure 32, and Figure 40). Degree of filament formation, as well as intensity and rate of fragmentation and cell separation vary depending on species and strain peculiarities so that it may sometimes be difficult to detect the developmental cycle. Variation in filament formation and fragmentation results in mature colonies which either remain predominantly or partly filamentous (Figure 42, Figure 43, and Figure 47) and have a rough appearance and a dry to crumbly or even very hard texture or become smooth and soft (Figure 45, Figure 46, Figure 51, Figure 53, Figure 54, and Figure 55) being composed of bacillary or coccobacillary forms only. In addition, various transitions between these extremes may be found.

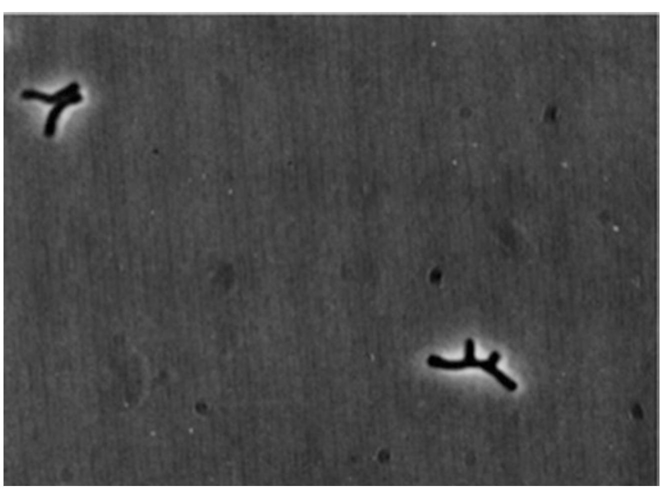

FIGURE 57. *Actinomyces odontolyticus*, serovar I. Filament with signs of budding in a very young (16 h) slide culture; phase-contrast micrograph *in situ* in lactophenol cotton blue mounting fluid (1600×).

Nutrition and growth conditions. The minimal nutritional requirements of *Actinomyces* species are not well understood. However, good growth has only been obtained on or in complex media containing either rich biological ingredients (e.g., BHI, meat extract, yeast extract, and serum) or a defined and complex mixture of a large variety of organic and inorganic compounds. Studies using synthetic media were reported (Christie and Porteous, 1962a, 1962b, 1962c; Howell and Pine, 1956; Keir and Porteous, 1962), but were mostly restricted to a few strains. Modifications and/or simplifications of the synthetic medium of Howell and Pine (1956) have been developed (Georg et al., 1964; Heinrich and Korth, 1967; Pine and Watson, 1959) and were tested with a greater number of strains. Attempts to further simplify these media have so far always resulted in insufficient growth rates or rapid loss of cultures during subcultivation.

It can, therefore, be concluded that the anabolic capacity of *Actinomyces* species is rather limited (Slack and Gerencser, 1975). These organisms apparently require organic nitrogen (e.g., peptides, amino acids), a fermentable carbohydrate and possibly also vitamins and other growth factors (e.g., Tween 80) for optimum growth. *Actinomyces meyeri* was found to have an absolute requirement for vitamin K_1, and its replication is greatly stimulated by 0.02% Tween 80 and by a fermentable carbohydrate (Cato et al., 1984). Serum added to the medium also enhances growth of this organism. The same is true for *Actinomyces hordeovulneris* whose growth is considerably stimulated when the medium is supplemented with 10–20% (v/v) fetal calf serum (Buchanan et al., 1984). Addition of 0.02% Tween 80 to broth media also enhanced growth and carbohydrate fermentation of the majority (62%) of the *Actinomyces georgiae* strains tested (Johnson et al., 1990). On the other hand, purines, pyrimidines, and potential other growth factors were found to be either stimulatory or inhibitory to the replication of certain *Actinomyces* strains (Christie and Porteous, 1962a; Keir and Porteous, 1962). For example, one strain of *Actinomyces israelii* apparently required adenine and thymine to be added to the simplest medium while another strain of the same species was inhibited by one or more of the bases guanine, xanthine, and uracil (Christie and Porteous, 1962a).

Though the nutritional requirments of all *Actinomyces* species described during the last two decades have not been characterized, essentially all of them grow only on complex media such as trypticase soy, Columbia, BHI, or Fastidious Anaerobe Agar usually supplemented with sheep, horse, or human blood (Buchanan et al., 1984; Hall et al., 2003a, 2003c, 2003d; Lawson et al., 2001b; Pascual et al., 1999). *Actinomyces ruminicola* was primarily isolated by inoculating rumen content into the xylan medium of Krause et al. (2001) and subsequently subcultured in or on peptone-yeast extract-glucose media containing 15% rumen fluid (PYRG with or without agar). Whether rumen fluid is an obligate additive for good growth of this organism remains to be seen.

Fermentable carbohydrates are the preferred sources of carbon and energy and are therefore usually necessary for optimum growth. When utilized, they are fermented with the production of short-chain fatty acids but no gas (Howell et al., 1959; Lentze, 1938a; Schaal et al., 2006; Slack, 1974; Slack and Gerencser, 1975). Mono-, di-, tri-, and polysaccharides, as well as sugar alcohols and glucosides, may serve as fermentable substrates, however, with considerable species differences so that carbohydrate fermentation patterns form the traditional basis for phenotypic differentiation of these actinomycetes and related bacteria (Table 4, Table 5, Table 7, and Table 9). Only *Actinomyces johnsonii*, *Actinomyces naeslundii sensu stricto*, and *Actinomyces oris*, which were formally described recently (Henssge et al., 2009), as well as *Actinomyces viscosus sensu stricto*, cannot be differentiated reliably using phenotypic characteristics (Johnson et al., 1990) although it remains to be clarified whether more carefully adapted techniques could overcome this problem.

Glucose and fructose are utilized by nearly all of the organisms currently included in the genus *Actinomyces* (Schaal et al., 2006; Schofield and Schaal, 1979a, 1979b, 1980, 1981; Slack, 1974; Slack and Gerencser, 1975). However, *Actinomyces hongkongensis* was reported to be unable to produce acid from glucose (Woo et al., 2003) and even appeared to be completely asaccharolytic (Table 4 and Table 7). *Actinomyces nasicola* was found to produce acid from glucose, cellobiose, and fructose but not from a large variety of other carbohydrates (Hall et al., 2003d) (Table 4 and Table 7). Detectable acid production from carbohydrates does not only depend on the presence of the respective enzymes in the species in question, but also on the test procedure used and especially on the relation between inoculum size and substrate volume.

Members of the genus *Actinomyces* vary considerably with respect to their oxygen requirements. *Actinomyces bowdenii* (Pascual et al., 1999), *Actinomyces canis* (Hoyles et al., 2000), *Actinomyces cardiffensis* (Hall et al., 2002), *Actinomyces catuli* (Hoyles et al., 2001b), *Actinomyces coleocanis* (Hoyles et al., 2002a), *Actinomyces denticolens* (Dent and Williams, 1984b), *Actinomyces europaeus* (Funke et al., 1997a), *Actinomyces funkei* (Lawson et al., 2001b), *Actinomyces georgiae* (Johnson et al., 1990), *Actinomyces graevenitzii* (Pascual Ramos et al., 1997a), *Actinomyces hordeovulneris* (Buchanan et al., 1984), *Actinomyces hyovaginalis* (Collins et al., 1993), *Actinomyces marimammalium* (Hoyles et al., 2001c), *Actinomyces meyeri* (Cato et al., 1984), *Actinomyces naeslundii sensu stricto* (Howell et al., 1959), *Actinomyces nasicola* (Hall et al., 2003d), *Actinomyces neuii* (Funke et al., 1994), *Actinomyces odontolyticus* (Schaal, 1986b), *Actinomyces oricola* (Hall et al., 2003c), *Actinomyces. oris* and *Actinomyces johnsonii* (Johnson et al., 1990), *Actinomyces radicidentis* (Collins et al., 2000), *Actinomyces radingae* and *Actinomyces turicensis* (Wüst et al., 1995a), *Actinomyces slackii* (Dent and Williams, 1986), *Actinomyces suimastitidis* (Hoyles et al., 2001a), *Actinomyces urogenitalis* (Nikolaitchouk et al., 2000), *Actinomyces vaccimaxillae* (Hall et al., 2003a), and *Actinomyces viscosus sensu stricto* (Howell, 1963; Howell et al., 1965) are all able to grow in air on the surface of suitable agar media, although growth of some species (e.g., *Actinomyces viscosus sensu stricto*) may be very weak or they may even fail to grow when no CO_2 is available in the medium or the incubation atmosphere. In liquid media, aerobic growth may be better, provided that a large inoculum is used. However, aerobic growth yields are usually greatly increased when the atmosphere contains carbon dioxide (approx. 5%) or when HCO_3^- is added to the medium. *Actinomyces massiliensis* and most strains of *Actinomyces viscosus sensu stricto* appear to obligately require 5% CO_2 for growth and do not grow at all in air without added CO_2 (Howell, 1963; Renvoise et al., 2009). The aerotolerant *Actinomyces* species with an obligate or facultative requirement of CO_2 may be described most appropriately as capnophilic.

While some of the species mentioned above grow equally well in air, usually with added CO_2, and under anaerobic conditions (e.g., *Actinomyces denticolens*, *Actinomyces hyovaginalis*, *Actinomyces massiliensis*, *Actinomyces radicidentis*, *Actinomyces radingae*, *Actinomyces slackii*, and *Actinomyces turicensis*), others definitely prefer anaerobic growth conditions and produce weaker or even very weak growth when incubated in air with added CO_2 (e.g., *Actinomyces bovis*, *Actinomyces cardiffensis*, *Actinomyces georgiae*, *Actinomyces gerencseriae*, *Actinomyces israelii*, *Actinomyces meyeri*, *Actinomyces nasicola*, *Actinomyces oricola*, and *Actinomyces vaccimaxillae*) (Cato et al., 1984; Hall et al., 2002, 2003a, 2003c, 2003d; Johnson et al., 1990; Schaal, 1986b). As reported by Pascual et al. (1999), growth of *Actinomyces bowdenii* is not enhanced by increased concentrations of CO_2 between 1.5 and 10%. Only certain strains of *Actinomyces bovis*, *Actinomyces gerencseriae*, *Actinomyces israelii*, *Actinomyces meyeri* and the recently described new *Actinomyces* species *Actinomyces dentalis* (Hall et al., 2005), *Actinomyces hongkongensis* (Woo et al., 2003), and *Actinomyces ruminicola* (An et al., 2006) may be considered or were described as strict anaerobes.

It has been known for decades that species such as *Actinomyces bovis*, *Actinomyces gerencseriae*, (*Actinomyces israelii* serovar II), *Actinomyces israelii*, and *Actinomyces meyeri* exhibit a marked strain variation as far as aerotolerance is concerned (Schaal, 1986b). Furthermore, consistently good anaerobic growth is usually only obtained with added CO_2 or HCO_3^- while under anaerobic conditions without CO_2 most strains will not grow at all or only produce poor growth (Howell and Pine, 1956; Schofield and Schaal, 1981). On the other hand, nearly all of the *Actinomyces* species, including the aerotolerant ones, will multiply well at low oxygen tension with added CO_2 so that the genus *Actinomyces* as a whole may be considered a taxon of facultative anaerobes with obligate carbon dioxide requirement and variation in oxygen tolerance (Erikson, 1940; Howell, 1963; Schaal, 1986b; Slack and Gerencser, 1975). For these reasons, sophisticated anaerobic techniques such as the roll tube method or the anaerobic glove box are usually not required for the cultivation of these actinomycetes. It remains to be seen if this also holds true for the supposedly strictly anaerobic species *Actinomyces dentalis*, *Actinomyces hongkongensis*, and *Actinomyces ruminicola*.

The optimum growth temperature of most of the *Actinomyces* species validly published so far is 30–37°C (Schaal et al., 2006; Slack, 1974; Slack and Gerencser, 1975). Detailed studies on the temperature requirements of these organisms have rarely been performed. Most strains of *Actinomyces gerencseriae*, *Actinomyces israelii*, *Actinomyces naeslundii sensu lato*, and *Actinomyces viscosus sensu lato* were reported to produce also visible growth at lower temperatures ranging from 28 to 32°C (Howell, 1963), Schaal, unpublished results; Thompson and Lovestedt, 1951). Growth of hamster strains of *Actinomyces viscosus* (*Actinomyces viscosus sensu stricto*) even occurred at 23°C when the organism was incubated in air with added CO_2 or in an atmosphere of CO_2 and N_2 on BHIA (Howell, 1963). *Actinomyces naeslundii sensu*

lato and *Actinomyces viscosus sensu lato* may also grow at 45°C (Holmberg and Nord, 1975). *Actinomyces meyeri* strains were reported to grow equally well at 30 and 37°C and nearly as well at 25°C. No growth was obtained at 45°C (Cato et al., 1984). *Actinomyces massiliensis* produced good growth at 37°C and weak growth at 30°C, but no growth was observed at 25°C or 44°C, respectively (Renvoise et al., 2009). *Actinomyces ruminicola* is the only *Actinomyces* species described so far which differs greatly in growth temperature (20–55°C, with optimum growth at 46°C; An et al., 2006).

Actinomyces species including most of the new members of the genus tested so far usually ferment glucose to formic, acetic, lactic, and succinic acids when grown anaerobically with added CO_2 (Buchanan and Pine, 1965; Holdeman et al., 1977; Howell and Pine, 1956; Schofield and Schaal, 1981). The amount of succinic acid formed depends on the concentration of CO_2/HCO_3^- available in the medium, but lactic and succinic acids are thought to be the most characteristic fermentation end products of *Actinomyces* species. Anaerobically without added CO_2, the fermentation of "*Actinomyces israelii*" ATCC 10049 which is now considered to belong to the species *Actinomyces gerencseriae* was described as being homolactic (Buchanan and Pine, 1965). In the presence of CO_2, however, the fermentation was heterolactic with formate, acetate, lactate, and succinate as end products. Carbon dioxide when present is utilized (Howell and Pine, 1956).

Metabolism and metabolic pathways. In a detailed study on the carbohydrate metabolism of *Actinomyces viscosus sensu lato* in continuous culture under glucose or nitrogen limitation (Hamilton and Ellwood, 1983), it was shown that the molar growth yields were lower under nitrogen limitation than under glucose limitation. This indicates that the presence of utilizable nitrogen sources may be equally important for the growth of this organism in the oral cavity as the presence of suitable carbohydrates.

Actinomyces meyeri only occasionally forms small amounts of lactic acid and may also produce small quantities of pyruvic acid (Cato et al., 1984). The latter acid was also detected together with lactate in culture supernatants of *Actinomyces georgiae* when the tubes were inoculated aerobically and then restoppered, but not when the cultures were inoculated under CO_2 (Johnson et al., 1990). Formic acid was reported to be the main end product of glucose fermentation by *Actinomyces ruminicola* in addition to smaller amounts of acetic and lactic acids (An et al., 2006).

Energy is produced by substrate phosphorylation, but the amount of ATP produced depends on the cultural conditions. The results of Buchanan and Pine (1967) indicate that 4 mol of ATP are formed per mol of glucose when "*Actinomyces israelii*" (= *Actinomyces gerencseriae*) is incubated aerobically with added CO_2. Anaerobically without CO_2, only 2 mol of ATP are produced.

Several *Actinomyces* enzymes involved in the initial dissimilation of exogenous carbohydrates or the energy yielding glycolytic pathway have been identified and some were characterized in more detail. α-Galactosidase activities were observed in *Actinomyces denticolens*, *Actinomyces israelii* and in about one half of the tested isolates of *Actinomyces johnsonii*, *Actinomyces naeslundii sensu stricto*, and *Actinomyces oris* (Henssge et al., 2009). β-Galactosidase was found in *Actinomyces denticolens*, *Actinomyces israelii*, *Actinomyces massiliensis*, *Actinomyces viscosus sensu lato*, and in the majority of the *Actinomyces johnsonii*, *Actinomyces naeslundii sensu stricto*, and *Actinomyces oris* isolates examined (Henssge et al., 2009; Renvoise et al., 2009; Schaal et al., 2006). *Actinomyces denticolens*, *Actinomyces israelii*, *Actinomyces johnsonii*, and *Actinomyces naeslundii sensu stricto* were found to dispose of α- and β-glucosidases and the former enzyme was also present in *Actinomyces massiliensis*, *Actinomyces meyeri*, and *Actinomyces viscosus sensu lato*, and the latter in *Actinomyces odontolyticus* and the majority of the *Actinomyces oris* isolates. *Actinomyces israelii*, presumably *Actinomyces gerencseriae* and a few *Actinomyces naeslundii sensu lato* and *Actinomyces viscosus sensu lato* isolates showed β-xylosidase activities. *Actinomyces odontolyticus* strains were the only "classical" fermentative actinomycetes which contained α-fucosidase. α-Mannosidase was not detected in any of these actinomycetes (Dent and Williams, 1984b; Kilian, 1978; Paddick et al., 2005; Peros and Gibbons, 1981; Renvoise et al., 2009; Schofield and Schaal, 1981).

The β-galactosidase of *Actinomyces viscosus sensu lato* was reported to have a molecular mass of 4.2×10^5, a K_m for lactose of about 6 mM, and a pH optimum between 6.0 and 6.5 (Kiel et al., 1977). Invertase activity has been demonstrated in *Actinomyces naeslundii sensu lato* (Miller, 1974) and *Actinomyces viscosus sensu lato* (Kiel et al., 1977; Palenik and Miller, 1975). The characteristics of the *Actinomyces viscosus* invertase were a molecular mass of 8.6×10^4, a K_m for sucrose of about 71 mM, and a pH optimum between 5.8 and 6.3. The enzyme was noncompetitively inhibited by fructose 6-phosphate and fructose 1,6-diphosphate (Kiel and Tanzer, 1977).

Fructose-1,6-diphosphate aldolase and phosphate acetyltransferase activities were demonstrated in cell-free extracts of "*Actinomyces israelii*" (= *Actinomyces gerencseriae*) (Buchanan and Pine, 1967). A nicotinamide adenine dinucleotide-dependent lactate dehydrogenase of *Actinomyces viscosus sensu lato* purified by Brown et al. (1975) had a molecular mass of 1×10^5 and a pH optimum of 5.5–6.2 and was under negative control by adenosine 5′-triphosphate and inorganic phosphate. Malate and glutamate dehydrogenases were detected in extracts of *Actinomyces gerencseriae*, *Actinomyces israelii*, *Actinomyces naeslundii sensu lato*, and *Actinomyces viscosus sensu lato*, but differed in their electrophoretic mobilities (Fillery et al., 1978). 6-Phosphogluconate dehydrogenase was only demonstrated in *Actinomyces naeslundii sensu lato* and *Actinomyces viscosus sensu lato*, but not in *Actinomyces gerencseriae* and *Actinomyces israelii* (Fillery et al., 1978).

Certain *Actinomyces* species synthesize extracellular and cell-associated polymers such as levan, dextran, or glycogen which enable them to attach directly to tooth surfaces. Such polymers produced by *Actinomyces viscosus sensu stricto* and *sensu lato* have been studied in detail (Hamilton and Ellwood, 1983; Howell and Jordan, 1967; Imai and Kuramitsu, 1983; Komiyama et al., 1988; Warner and Miller, 1978). The levan produced by these organisms formed a capsule tenaciously adhering to the cells under certain conditions (Warner and Miller, 1978). The enzyme levansucrase which is responsible for the production of the high-molecular-weight polymers was found to occur both in the growth medium and associated to the cell wall (Pabst, 1977; Pabst et al., 1979). On the other hand, *Actinomyces viscosus sensu lato* have levan-hydrolyzing activity (Miller and Somers, 1978) due to an extracellular and cell-associated enzyme that degrades the levan of *Actinomyces viscosus* as well as inulin and other levans. Similar synthetic

and degrading capacities for such polymers were also observed in *Actinomyces naeslundii sensu lato* (Komiyama et al., 1988; Miller and Somers, 1978).

Most *Actinomyces* species do not exhibit any detectable proteolytic activity (Schofield and Schaal, 1981; Slack and Gerencser, 1975). *Actinomyces bovis*, *Actinomyces gerencseriae*, *Actinomyces israelii*, *Actinomyces meyeri*, *Actinomyces naeslundii sensu lato*, *Actinomyces odontolyticus*, *Actinomyces viscosus sensu lato*, and most of the recently described species do not liquefy gelatin or Loeffler's serum nor do they hydrolyze casein, peptonize milk, or digest meat (Holdeman et al., 1977; Schaal et al., 2006; Schofield and Schaal, 1981; Slack, 1974; Slack and Gerencser, 1975). However, 40–60% of the *Actinomyces georgiae* strains tested (Johnson et al., 1990) were reported to liquefy gelatin, and *Actinomyces radicidentis* and one strain of *Actinomyces radingae* were described as being weakly gelatin-positive (Collins et al., 2000; Vandamme et al., 1998). Litmus milk may be acidified, reduced and/or clotted by several *Actinomyces* species apparently due to their saccharolytic activities (Slack and Gerencser, 1975).

The ability to deaminate or decarboxylate amino acids is also uncommon in the genus *Actinomyces* (Schaal, 1986b). Nevertheless, most strains of *Actinomyces israelii* and a few *Actinomyces naeslundii sensu lato* and *Actinomyces viscosus sensu lato* strains are capable of producing ammonia from arginine. Furthermore, some *Actinomyces odontolyticus* strains may decarboxylate lysine (Schofield and Schaal, 1980, 1981). *Actinomyces bovis*, *Actinomyces israelii*, *Actinomyces naeslundii sensu lato*, *Actinomyces odontolyticus*, and *Actinomyces viscosus sensu lato* may form hydrogen sulfide when grown on triple-sugar iron agar (TSIA), although they do not blacken the indicator in the medium, but only lead acetate paper strips suspended over the medium. Similarly, certain strains of *Actinomyces bovis*, *Actinomyces naeslundii sensu lato*, and *Actinomyces odontolyticus* were reported to show H_2S production over BHIA (Slack and Gerencser, 1975). Indole is not produced by any member of the genus *Actinomyces* (Henssge et al., 2009; Schaal et al., 2006).

With the exception of some strains of *Actinomyces johnsonii*, *Actinomyces meyeri*, *Actinomyces naeslundii*, *Actinomyces oris*, and *Actinomyces radicidentis*, all other *Actinomyces* species tested so far are urease-negative (Collins et al., 2000; Henssge et al., 2009; Schaal, 1986b; Schaal et al., 2006; Schofield and Schaal, 1981).

Adenine, guanine, hypoxanthine, tyrosine, and xanthine are not hydrolyzed (Schofield and Schaal, 1981). Hippurate hydrolysis is rare among members of the genus *Actinomyces* and was thus far only reported for *Actinomyces funkei* (Lawson et al., 2001b) and the "vaginal" biovar of *Actinomyces hyovaginalis* (Collins et al., 1993).

Esculin hydrolysis was previously thought to be very common among *Actinomyces* species (Schaal, 1986b; Schofield and Schaal, 1981; Slack and Gerencser, 1975) although strain variation had been noted. The latter particularly applies to the species *Actinomyces bovis*, *Actinomyces europaeus*, *Actinomyces georgiae*, *Actinomyces odontolyticus*, and *Actinomyces ruminicola* which encompass both esculin-positive and -negative strains. *Actinomyces meyeri*, *Actinomyces radingae*, and *Actinomyces turicensis* strains are, apart from a few exceptions, esculin-negative. Completely negative are, however, members of the new *Actinomyces* species *Actinomyces canis*, *Actinomyces cardiffensis*, *Actinomyces coleocanis*, *Actinomyces funkei*, *Actinomyces graevenitzii*, *Actinomyces hongkongensis*, *Actinomyces marimammalium*, *Actinomyces nasicola*, *Actinomyces neuii*, and *Actinomyces slackii* (Schaal et al., 2006).

Starch hydrolysis is a consistent feature of *Actinomyces bovis* and possibly *Actinomyces viscosus sensu stricto* (Howell and Jordan, 1963) although exceptions occur. It may also be found in some strains of a few other *Actinomyces* species such as *Actinomyces georgiae* or *Actinomyces johnsonii* (Johnson et al., 1990; Schaal et al., 2006; Schofield and Schaal, 1981; Slack and Gerencser, 1975). Hydrolysis of Tweens is weak or absent; lipase as observed on egg yolk-containing agar media or using the API system has not been detected (Schaal et al., 2006; Schofield and Schaal, 1981).

Lecithinase activity has only been found in a few *Actinomyces israelii sensu lato* isolates (Schofield and Schaal, 1981). The Voges–Proskauer reaction is usually negative (Holmberg and Nord, 1975; Howell and Jordan, 1963; Slack and Gerencser, 1975); weakly positive test results were reported for *Actinomyces suimastitidis* (Hoyles et al., 2001a) and *Actinomyces radicidentis* (Collins et al., 2000). Acetoin production of *Actinomyces urogenitalis* was found to be variable (Nikolaitchouk et al., 2000). The methyl red test is usually positive (Holmberg and Nord, 1975; Slack and Gerencser, 1975). No chondroitin sulfatase activity has been detected and hyaluronidase has only been found in a few *Actinomyces odontolyticus* strains. DNase is absent in *Actinomyces gerencseriae*, *Actinomyces israelii*, *Actinomyces meyeri*, *Actinomyces naeslundii sensu lato*, and *Actinomyces viscosus sensu lato*, but present in *Actinomyces bovis* and some strains of *Actinomyces odontolyticus* (Schofield and Schaal, 1981).

Using API test kits (API-BioMérieux), a large variety of further enzymic activities can be observed: All of the *Actinomyces* species tested previously were found to be negative for α-arabinosidase, β-mannosidase, β-glucuronidase (except for *Actinomyces catuli*), lipase C14, chymotrypsin, and trypsin (Kilian, 1978; Schaal et al., 2006; Schofield and Schaal, 1981). However, Renvoise et al. (2009) reported that single strains (the type strains) of *Actinomyces gerencseriae*, *Actinomyces israelii*, and *Actinomyces catuli* were α-arabinosidase-positive. Henssge et al. (2009) claimed that 88% of *Actinomyces naeslundii sensu stricto*, 93% of *Actinomyces oris*, and 100% of *Actinomyces johnsonii* strains tested were α-arabinosidase-positive when using a method other than the API Rapid ID32A kit. Essentially all of the other substrates included in the API enzyme test system give results that can be used for identification purposes because they exhibit characteristic species differences (Schaal et al., 2006) (Table 4, Table 5, Table 7, and Table 9). Leucine arylamidase activity is present in all members of the genus except *Actinomyces radingae* which was reported to be consistently negative in this trait (Vandamme et al., 1998). Phosphoamidase activity appears to be a typical characteristic of *Actinomyces denticolens*, but may also be found in certain strains of *Actinomyces gerencseriae*, *Actinomyces israelii*, and *Actinomyces odontolyticus*. Phospholipase A activity has not been detected in the "classical" *Actinomyces* species tested (Bulkacz et al., 1979).

The absence of catalase was once thought to be a characteristic of all *Actinomyces* species except *Actinomyces viscosus* (Schofield and Schaal, 1981; Slack, 1974; Slack and Gerencser, 1975).

However, with the description of many new members of the genus, the number of catalase-positive species has increased considerably. In addition to *Actinomyces viscosus sensu stricto*, *Actinomyces bowdenii*, *Actinomyces canis*, *Actinomyces hordeovulneris*,

Actinomyces neuii, *Actinomyces radicidentis*, and *Actinomyces slackii* have been shown to possess this enzyme. Catalase activity can also be observed in about half of the *Actinomyces oris* isolates and in very few strains of *Actinomyces naeslundii sensu stricto*. As far as *Actinomyces johnsonii* is concerned, conflicting results have been reported in the literature (Henssge et al., 2009; Johnson et al., 1990). Catalase activity is usually weak to moderate in *Actinomyces hordeovulneris* (Buchanan et al., 1984) and is present in *Actinomyces catuli* isolates (Hoyles et al., 2001b).

Cytochrome oxidase activity has been reported for some strains of *Actinomyces naeslundii sensu lato* and *Actinomyces viscosus sensu lato* (Schofield and Schaal, 1981). However, the results of the benzidine test used for the detection of cytochrome oxidase do not necessarily correspond with the detection of cytochromes in cell extracts of actinomycetes by low temperature spectrophotometry (Taptykova and Kalakoutskii, 1973). *Actinomyces bovis*, *Actinomyces israelii*, *Actinomyces viscosus sensu lato*, and *Arcanobacterium pyogenes* apparently all contain cytochrome *b* (Reddy et al., 1977; Taptykova and Kalakoutskii, 1973). It was assumed that in *Arcanobacterium pyogenes*, cytochrome *b* mediates the reduction of fumarate to succinate, with reduced nicotinamide adenine dinucleotide as the electron donor (Reddy et al., 1982). *Actinomyces viscosus sensu lato* may contain cytochromes *a* and *c* in addition to cytochrome *b* (Taptykova and Kalakoutskii, 1973).

Nearly all of the members of the species or subspecies of *Actinomyces bowdenii*, *Actinomyces catuli*, *Actinomyces denticolens*, the "vaginal" biovar of *Actinomyces hyovaginalis*, *Actinomyces israelii*, *Actinomyces johnsonii*, *Actinomyces massiliensis*, *Actinomyces naeslundii sensu stricto*, *Actinomyces neuii* subsp. *neuii*, *Actinomyces odontolyticus*, and *Actinomyces ruminicola* are able to reduce nitrate to nitrite (An et al., 2006; Henssge et al., 2009; Johnson et al., 1990; Renvoise et al., 2009; Schaal et al., 2006). Nitrate reduction is variable in *Actinomyces cardiffensis*, *Actinomyces funkei*, *Actinomyces oris*, *Actinomyces radicidentis*, and *Actinomyces viscosus sensu stricto*. It is rarely positive in *Actinomyces georgiae* and *Actinomyces gerencseriae*. *Actinomyces neuii* subsp. *anitratus* is *per definitionem* nitrate reductase-negative (Funke et al., 1994) and the remaining *Actinomyces* species known so far are also unable to reduce nitrate (Hall et al., 2005; Johnson et al., 1990; Schaal et al., 2006; Schofield and Schaal, 1981; Slack, 1974; Slack and Gerencser, 1975). The ability to reduce nitrite appears to be uncommon in the genus, but may be seen in many *Actinomyces naeslundii sensu lato* strains (Schofield and Schaal, 1980; Schofield and Schaal, 1981) although this feature may often be difficult to detect (Dent and Williams, 1984b).

Some *Actinomyces* species exhibit β-hemolytic activities which may vary depending on the erythrocytes used (Slack, 1974). *Actinomyces bovis* usually shows β-hemolysis on media containing human erythrocytes and may also lyse sheep and horse red blood cells. *Actinomyces odontolyticus* strains vary in their β-hemolytic activity irrespective of the erythrocytes used. However, *Actinomyces odontolyticus* isolates, some *Actinomyces bovis* and *Actinomyces meyeri* strains, about one half of *Actinomyces oris* isolates, the majority (78%) of *Actinomyces naeslundii sensu stricto* cultures, and a few *Actinomyces viscosus sensu stricto* isolates may produce an area of greening around the colonies (α-hemolysis) when grown on human, sheep, or rabbit blood agar (Johnson et al., 1990; Schofield and Schaal, 1981; Slack, 1974). A few *Actinomyces hordeovulneris* isolates were reported to exhibit a weak hemolytic activity on bovine blood agar while most strains of this species were non-hemolytic (Buchanan et al., 1984).

Most of the remaining *Actinomyces* species are non-hemolytic or their hemolytic properties have not been reported. Weak hemolysis was observed in strains of *Actinomyces radingae*, which appeared slowly and started mostly as α-hemolysis, and in *Actinomyces turicensis*, especially when incubated with added CO_2 or anaerobically (Vandamme et al., 1998). Most strains of *Actinomyces neuii* subsp. *neuii* exhibit α-hemolysis on sheep blood agar as do all strains on human blood agar. *Actinomyces neuii* subsp. *anitratus* is non-hemolytic (Funke et al., 1994).

Adherence mechanisms. Certain *Actinomyces* species, in particular *Actinomyces naeslundii sensu lato* and *Actinomyces viscosus sensu lato*, have been shown to possess mechanisms which enable them to adhere to hard and smooth surfaces and epithelial cells, to coaggregate with each other or with other bacteria, or to agglutinate red blood cells. Further studies revealed that obviously more than one mechanism is involved in these adherence and coaggregation functions. The simplest of these attachment mechanisms is the production of extracellular or cell-associated polymers (glycocalyx), especially dextran, levan, glycogen, and *N*-acetylglucosamine-rich slime polysaccharides, which make the cells sticky so that they become able to attach to enamel, artificial denture materials and even glass. *Actinomyces viscosus sensu stricto* (Howell and Jordan, 1963, 1967) and *Actinomyces viscosus* serovar II strains (*Actinomyces oris*) (Bourgeau and McBride, 1976; Hamilton and Ellwood, 1983; Imai and Kuramitsu, 1983; Miller et al., 1978; Ooshima and Kuramitsu, 1985; Pabst, 1977), as well as *Actinomyces naeslundii sensu lato* (Komiyama et al., 1988; Slack and Gerencser, 1975), have been shown to produce these polymers.

Enhancement of aggregation by low pH values indicated that alterations of the cell surface potential might be involved in the aggregation process (Miller et al., 1978). Most importantly, however, the so-called surface fibrils or fimbriae that are produced by several *Actinomyces* species were found to mediate both interbacterial aggregation and adhesion processes of fermentative actinomycetes. The fimbriae of *Actinomyces viscosus* T14V consist of high molecular mass proteins with some carbohydrate and up to 14.3% nitrogen (Cisar and Vatter, 1979; Masuda et al., 1983). That such fimbrial proteins might be responsible for attachment and coaggregation, was first suggested by the finding that treatment with proteolytic enzymes or heat could impair the coaggregation between *Actinomyces naeslundii sensu lato* or *Actinomyces viscosus sensu lato* on the one hand and *Streptococcus sanguinis* and *Streptococcus mitis* on the other hand (Ellen and Balcerzak-Raczkowski, 1977; McIntire et al., 1978).

It soon became apparent that a lectin-like mechanism was involved in some of these interbacterial coaggregations. McIntire et al. (1978) found that the coaggregation between *Actinomyces viscosus* T14V and *Streptococcus sanguinis* 34 required a protein or glycoprotein on *Actinomyces viscosus* and a carbohydrate on *Streptococcus sanguinis*. This coaggregation as well as the binding of *Actinomyces viscosus* to glycoprotein-coated latex beads and its hemagglutinating properties were specifically inhibited by lactose, β-methyl-D-galactoside, and D-galactose, but not or only weakly inhibited by α-methyl-D-galactoside, melibiose, maltose, cellobiose, sucrose, and a number of monosaccharides (Costello et al., 1979; Heeb et al., 1985). Costello et al. (1979)

and Ellen et al. (1980) reported that *Actinomyces naeslundii sensu lato* and *Actinomyces viscosus sensu lato* were able to agglutinate human AB and horse erythrocytes. This agglutinating property was enhanced by pretreatment of the red blood cells with neuraminidase and specifically inhibited by the compounds mentioned before. Furthermore, it was suggested that the actinomycetes could prime erythrocytes and human buccal epithelial cells for hemagglutination or attachment by removing sialic acid to expose more penultimate β-galactosides on the surface of the cells. Thus, neuraminidase removal of terminal sialic acid and lectin-like binding to exposed β-galactoside-associated sites on the erythrocytes appeared to be responsible for the hemagglutination and adherence capabilities of *Actinomyces naeslundii sensu lato* and *Actinomyces viscosus sensu lato* (Saunders and Miller, 1983). Lectin-dependent attachment of *Actinomyces naeslundii sensu lato* to human epithelial cells could clearly be related to the lactose-sensitive fimbriae (type 2 fimbriae) of strain WVU 45 (Brennan et al., 1984). Further characterizing the epithelial cell receptor for the bacterial lectin, Brennan et al. (1984) showed that plant lectins from *Bauhinia purpurea* and *Arachis hypogaea* had specificities similar to that of the *Actinomyces naeslundii* fimbrial lectin.

Numerous studies have demonstrated that the coadhesion mechanisms between oral microbes are complex. *Actinomyces viscosus* T14V was shown to possess a highly specific aggregation factor for *Streptococcus sanguinis* H1 (AFH1) which mediated coadhesion that was not inhibited by lactose (Mizuno et al., 1983). The most active fraction of AFH1 reacted as a single antigen with anti-*Actinomyces viscosus* T14V serum and was unrelated to the fimbrial antigens of this organism. It was composed of approximately 53% cell-wall components, 40% polysaccharide, and 7% protein, but did not contain teichoic acid. Thus, it was concluded that a carbohydrate-protein interaction might be dominant in the aggregation of *Streptococcus sanguinis* H1 by AFH1 and in the coaggregation of *Streptococcus sanguinis* H1 with *Actinomyces viscosus* T14V.

In the coaggregation between *Capnocytophaga gingivalis* DR2001 and *Actinomyces israelii* PK16, an adhesin on *Capnocytophaga gingivalis* was identified that mediates the interaction (Tempro et al., 1989). This adhesin was a 140-kDa polypeptide located in the outer membrane of *Capnocytophaga gingivalis* whose molecules were arranged non-uniformly on the bacterial surface, occurred singly, in pairs, and in small clusters, and reacted with carbohydrate receptors on *Actinomyces israelii*. In contrast, fimbriae of *Porphyromonas gingivalis* were found to mediate the coadhesion of this organism with *Actinomyces viscosus sensu lato* (Goulbourne and Ellen, 1991). Similarly, fimbriae-associated proteins of *Prevotella (Bacteroides) loescheii* were found to induce coaggregation with *Streptococcus sanguinis* 34 and *Actinomyces israelii* PK14 (Weiss et al., 1987). However, while coadhesion with *Streptococcus sanguinis* was inhibited by lactose, that with *Actinomyces israelii* was not.

Denaturing polyacrylamide gel electrophoresis and immunoblot analysis revealed the presence of both a 75- and a 43-kDa protein associated with parental fimbriae, the former protein being responsible for the recognition of *Streptococcus sanguinis* and the latter for that of *Actinomyces israelii*. Studying the coaggregation of *Prevotella intermedia* with oral *Actinomyces* species, Nesbitt et al. (1993) found that a protein or glycoprotein located on *Prevotella intermedia* strain 27 interacted with carbohydrate-containing molecules on the surface of *Actinomyces viscosus* T14V, *Actinomyces naeslundii* ATCC 12104, and additional *Actinomyces naeslundii sensu lato* strains. Comparing these coaggregations with those between *Prevotella intermedia* 113 and *Actinomyces odontolyticus* WVU 1546 or *Actinomyces israelii* WVU 838, it was suggested that different surface molecules of *Prevotella intermedia* 113 mediated each of the latter coaggregations.

More detailed studies have shown that *Actinomyces viscosus sensu lato* possesses two types of fimbriae: type 1 fimbriae are involved in the attachment of this organism to saliva-coated hydroxyapatite whereas type 2 fimbriae mediate the lectin-dependent coaggregation with *Streptococcus sanguinis* that can be inhibited by lactose (Cisar et al., 1984a, 1984b, 1988; Clark et al., 1986; Mergenhagen et al., 1987). Using specific polyclonal and monoclonal antibodies, Ellen et al. (1989) demonstrated that anti-type 2 antibodies bound to antigens on long fibrils whereas anti-type 1 antibodies were mostly localized close to the cell body or on short appendages.

Typical human isolates of *Actinomyces naeslundii*, which possibly represent members of *Actinomyces naeslundii sensu stricto*, were reported to elaborate a single fimbriae type, namely type 2 fimbriae. Thus, the two genospecies of *Actinomyces naeslundii*, defined by Johnson et al. (1990) (*Actinomyces naeslundii sensu stricto* and *Actinomyces oris*), apparently differ with respect to the fimbriae expressed: Type 1 fimbriae are mainly present on *Actinomyces oris* while type 2 fimbriae are expressed by both *Actinomyces naeslundii sensu stricto* and *Actinomyces oris* (Cisar et al., 1984b; Strömberg et al., 1996).

The specificity of the lectins present on the fimbriae of certain *Actinomyces naeslundii* strains was found to be similar to but not identical with that of certain *Actinomyces viscosus* lectins (Cisar et al., 1984a, 1984b; McIntire et al., 1983). These lectins mediate binding to β-1,3-linked galactose or galactosamine structures in cell surface glycolipids and glycoproteins (Brennan et al., 1984; McIntire et al., 1983; Strömberg and Boren, 1992; Strömberg and Karlsson, 1990), salivary glycoproteins (Strömberg et al., 1992), and streptococcal capsular polysaccharides (Abeygunawardana et al., 1991). However, *Actinomyces naeslundii sensu stricto* ATCC 12104 and *Actinomyces oris* LY7 showed different binding patterns to a panel of saccharides containing β-linked galactose or galactosamine structures (Strömberg et al., 1996; Strömberg and Boren, 1992).

A 57-kDa protein from *Actinomyces viscosus* WVA 963 (*Actinomyces johnsonii*) was implicated as the type 2 fimbrial subunit while a 95-kDa protein appeared to be a putative *Actinomyces* adhesin mediating the lactose-inhibitable coaggregation with streptococci (Klier et al., 1997). Further studies with a coaggregation-defective mutant of this *Actinomyces johnsonii* strain confirmed the role of the 95-kDa protein which, in its secreted form, was bound to streptococcal partner cells and to lactose-agarose affinity beads (Klier et al., 1998).

In contrast, type 1 fimbriae mediate attachment to saliva-treated surfaces (Clark et al., 1984, 1986). Gibbons and coworkers (Gibbons, 1989; Gibbons and Hay, 1988; Gibbons et al., 1988) have implicated acidic proline-rich proteins (APRPs) and statherin derived from human parotid or submandibular saliva as promoting the attachment of *Actinomyces viscosus* (*Actinomyces oris*) to apatitic surfaces. *In vitro* studies with PRP- or PRG (proline-rich glycoproteins)-coated polystyrene or hydroxyapatite surfaces confirmed and extended these observations

(Clark et al., 1989). Subsequent findings using mutant strains of *Actinomyces viscosus sensu lato* indicated that a particular adhesive molecule probably associated with type 1 fimbriae allows for the interaction of *Actinomyces viscosus sensu lato* with constituents of the salivary pellicle (Nesbitt et al., 1992).

Analyzing the binding specificities of *Actinomyces naeslundii* genospecies 1 and 2 (*Actinomyces naeslundii sensu stricto* and *Actinomyces oris*, respectively) in detail, Hallberg et al. (1998a) found that *Actinomyces oris* commonly bound to *N*-acetyl-β-D-galactosamine and acidic proline-rich proteins. The *N*-acetyl-β-D-galactosamine binding specificity was signified by *N*-acetyl-β-D-galactosamine-inhibitable coaggregation with four specific streptococcal strains. Isolates of *Actinomyces naeslundii sensu stricto* also bound commonly to *N*-acetyl-β-D-galactosamine but less commonly to acidic proline-rich proteins. All of these isolates possessed another *N*-acetyl-β-D-galactosamine specificity in that they differed in their binding specificities when the same set of streptococcal strains was used.

In studies of coaggregation between *Streptococcus gordonii* and *Actinomyces naeslundii sensu lato*, six coaggregation groups of *Actinomyces naeslundii* were defined. These differed by heat and protease sensitivity and by the ability of spontaneous mutants to coaggregate with members of various groups of partner strains (Kolenbrander, 1989). The *Streptococcus gordonii* surface proteins SspA and SspB were found to be primarily responsible for defining these coaggregation groups (Egland et al., 2001) whose interaction modes are either inhibitable or unaffected in the presence of lactose (Kolenbrander, 1988; Kolenbrander and Williams, 1981). More recently, it was demonstrated (Hallberg et al., 1998b) that *Actinomyces naeslundii sensu lato* exhibits variant fimbrial subunit genes *fimP* (type 1) and *fimA* (type 2) which correspond to diverse APRP and Gal-*N*Acβ specificities, respectively, while *Actinomyces odontolyticus* was found to have genetically related but distinct adhesion binding specificity.

16S rRNA gene sequence and other molecular data. It has long been recognized that the genus *Actinomyces* exhibits considerable phenotypic diversity (Schaal, 1986b, 1992a; Schofield and Schaal, 1981). The dramatic increase in the number of *Actinomyces* species and the corresponding availability of nearly complete 16S rRNA gene sequence data of these species have allowed the construction of phylogenetic trees, which became more and more complex. Trees differed greatly from one another depending on the size of databases, the selection of strains and outgroups, and the treeing algorithms (Schaal et al., 2006). Nevertheless, all of these surveys confirmed that the genus *Actinomyces* is not only heterogeneous phenotypically, but shows pronounced phylogenetic diversity as well (Lawson et al., 1997; Pascual Ramos et al., 1997b; Schaal et al., 2006). Recent 16S rRNA gene sequence similarity analyses demonstrated unanimously that *Actinomyces* species form several phylogenetic clusters whose number depends on the cut-off branching points (Figure 19). However, the decision on what level these clusters should be considered taxonomically coherent entities apparently requires further chemotaxonomic, physiological, and morphological investigations (Schaal et al., 1999, 2006).

According to these phylogenetic analyses, *Actinomyces* species can be subdivided into two major (core clusters 1 and 2) and at least four minor phylogenetic clusters (Schaal et al., 2006) (Figure 19). Core cluster 1 is supported by high statistical significance (100% bootstrap value) and contains 15 or 17 species depending on whether *Actinomyces graevenitzii* and *Actinomyces radicidentis* are included or are considered individual lineages. The latter view was based on phylogenetic dendrograms published earlier (see Schaal et al., 2006) and, as far as *Actinomyces graevenitzii* is concerned, was supported by deviations in its set of signature nucleotides (see Table 1) while the 16S rRNA gene signature nucleotides of *Actinomyces radicidentis* suggested its inclusion in core cluster 1. The topical dendrogram shown in Figure 19 is of no additional help concerning the phylogeny of these species.

Actinomyces bovis, the type species of the genus *Actinomyces*, is a member of core cluster 1 as are the “classical” species *Actinomyces gerencseriae*, *Actinomyces israelii*, *Actinomyces naeslundii sensu lato*, and *Actinomyces viscosus sensu lato*. As strains of *Actinomyces naeslundii sensu stricto* and of *Actinomyces oris* exhibit >99% 16S rRNA gene sequence similarity between one another, and *Actinomyces johnsonii* strains exhibit >98.5% similarity with the two former species, these organisms cannot be reliably identified by 16S rRNA gene sequence comparison. The new species *Actinomyces johnsonii* and *Actinomyces oris* were, therefore, not included in the phylogenetic dendrogram which still contains *Actinomyces naeslundii sensu lato* and *Actinomyces viscosus sensu lato*. However, *Actinomyces oris* and *Actinomyces johnsonii* can apparently be identified and differentiated from *Actinomyces naeslundii sensu stricto* and *Actinomyces viscosus sensu stricto* by comparison of partial sequences of the housekeeping genes *atpA* and *metG* (Henssge et al., 2009). In addition, core cluster 1 comprises the recently described species *Actinomyces dentalis*, *Actinomyces denticolens*, *Actinomyces massiliensis*, *Actinomyces oricola*, and *Actinomyces ruminicola*, together with *Actinomyces bowdenii*, *Actinomyces catuli*, *Actinomyces howellii*, *Actinomyces slackii*, and *Actinomyces urogenitalis*, the latter species having been described between 1984 and 2001. The pattern of 16S rRNA signatures of core cluster 1 consists of nucleotides at positions 146:176 (U–G), 591:648 (G–Y), 614:626 (C–G), and 1311:1326 (U–A).

Members of core cluster 1, sharing 94–99% sequence similarity, may be considered authentic *Actinomyces* species as the cluster contains the type species *Actinomyces bovis* (Schaal et al., 2006). This species is closely related to *Actinomyces urogenitalis* as had already been pointed out in the original description of the latter species (Nikolaitchouk et al., 2000). Additional pairs of phylogenetic neighbors are *Actinomyces israelii* and *Actinomyces gerencseriae*, *Actinomyces slackii* and *Actinomyces bowdenii*, and *Actinomyces naeslundii sensu stricto* and *Actinomyces viscosus sensu stricto*. All other species form individual lines of descent. Results of DNA–DNA reassociation studies (Dent and Williams, 1984a; Johnson et al., 1990) indicated that *Actinomyces denticolens*, *Actinomyces gerencseriae*, *Actinomyces israelii*, *Actinomyces naeslundii*, and *Actinomyces viscosus* form genomically well separated species. *Actinomyces gerencseriae* ATCC 23860^{T}, formerly considered a representative of *Actinomyces israelii* serovar II, was identified as a taxon distinct from *Actinomyces israelii* serovar I strains by oligonucleotide probing (Stackebrandt and Charfreitag, 1990) and low DNA reassociation values with DNA of *Actinomyces israelii sensu stricto* (Johnson et al., 1990).

Actinomyces graevenitzii, sharing less than 94% 16S rRNA gene sequence similarity with other members of the genus *Actinomyces* (Pascual Ramos et al., 1997a), appears to occupy a separate phylogenetic position as has been verified in subsequent

descriptions of new *Actinomyces* species (Hoyles et al., 2001a; 2001b; 2001c; 2002b; Nikolaitchouk et al., 2000; Pascual et al., 1999). The 16S rRNA gene sequence of the type strain of *Actinomyces graevenitzii* (CCUG 27294^T) contained several undetermined nucleotides, and erroneous stretches and deletions. The corrected sequence (Yassin unpublished) neither changed the similarity values nor the branching point but supported the distinct position of the organism. The pattern of 16S rRNA signatures of *Actinomyces graevenitzii* consists of nucleotides at positions 146:176 (U–G), 450:483 (C–G), 591:648 (G–Y), 614:626 (C–G), 835:851 (A–U), 668:738 (U–A), 669:737 (A–U), 1308:1329 (U–A), 1311:1326 (U–A), and 1308:1329 (U–G). However, the dendrogram depicted in the description of *Actinomyces radicidentis* (Collins et al., 2000) indicated that the latter species is only loosely attached to core cluster 1 so that both *Actinomyces graevenitzii* and *Actinomyces radicidentis* may represent distinct phylogenetic entities although this is not as obvious in the dendrogram shown in Figure 19.

Except for *Actinomyces odontolyticus*, *Actinomyces meyeri*, and *Actinomyces georgiae* which were described in 1958, 1984, and 1990, respectively, the second major cluster, core cluster 2 (86% bootstrap value) only contains species that have been described within the past 15 years, namely *Actinomyces canis*, *Actinomyces cardiffensis*, *Actinomyces funkei*, *Actinomyces hyovaginalis*, *Actinomyces radingae*, *Actinomyces suimastitidis*, *Actinomyces turicensis*, and *Actinomyces vaccimaxillae*. This means that core cluster 2 encompasses 11 species. The two biovars of *Actinomyces hyovaginalis* display a high 16S rRNA gene sequence similarity (98.8%) so that they cannot be clearly separated by this trait (Storms et al., 2002). They also show very similar DNA fingerprints of the amplified spacer regions in between the tRNA-genes and high DNA–DNA hybridization values (Storms et al., 2002). The pattern of 16S rRNA signatures of core cluster 2 consists of nucleotides at positions 154:167 (G–U), 316:337 (U–G), 591:648 (G,u–V), and 614:626 (C–G). In general, it should be noted that the subdivision of the genus *Actinomyces* into these two major clusters does not correspond with the human or animal sources of these species, respectively.

Actinomyces georgiae, *Actinomyces meyeri*, and *Actinomyces odontolyticus* are phylogenetic neighbors that have been subjected to DNA–DNA reassociation studies (Johnson et al., 1990). Low hybridization values confirmed their separate species status. *Actinomyces radingae*, which appeared as a remote relative of cluster 2 in a previous phylogenetic analysis (Schaal et al., 2006), seems to be a true member of core cluster 2 in Figure 19 so that its position in this dendrogram is more similar to that shown by Pascual Ramos et al. (1997b). Its membership in core cluster 2 is also supported by the set of signature nucleotides (Table 1) which is similar to that of other members of this cluster.

Actinomyces coleocanis and *Actinomyces europaeus* share a remote relationship of 94.2% sequence similarity. The branching point of this lineage is not settled as seen by different affiliations of either one or both species in recently published dendrograms (Collins et al., 2000; Hoyles et al., 2000, 2001b, 2002b; Nikolaitchouk et al., 2000; Pascual et al., 1999; Schaal et al., 2006). The presence of unique 16S rRNA gene nucleotide signatures (Table 1) highlights the separate phylogenetic position of these two species which are only distantly related to core clusters 1 and 2. The pattern of their 16S rRNA signatures consists of nucleotides at positions 146:176 (U–A), 501:544 (U–A), 502:543 (A–U), 586:755 (U–A), 1243:1294 (R–U), and 1310:1327 (U–A).

Actinomyces marimammalium and *Actinomyces hongkongensis* branch deeply within the tree of species of the *Actinomycetaceae* and are only loosely affiliated with core cluster 2 in the dendrogram shown in Figure 19. The bootstrap value for the two type strains is low. The signature nucleotides (Table 1) of *Actinomyces marimammalium* support its isolated phylogenetic position. This pattern of 16S rRNA signatures consists of nucleotides at positions 153:168 (G–U), 590:649 (G–U), 613:627 (A–U), and 1311:1326 (U–A).

An additional pair of outgroup species loosely affiliated with core cluster *2* is represented by *Actinomyces hordeovulneris* and *Actinomyces nasicola*. The phylogenetic position of *Actinomyces hordeovulneris* was not determined in the original description of this species (Buchanan et al., 1984), but was determined in the phylogenetic analysis of the genus *Actinomyces* published by Pascual Ramos et al. (1997b). An isolated phylogenetic position of *Actinomyces hordeovulneris* has been demonstrated since then in many subsequent studies (Schaal et al., 2006). Several deviations in the set of 16S rRNA gene sequence signatures from those of members of core cluster 2 support the phylogenetic relevance of these findings. The pattern of 16S rRNA signatures of this species consists of nucleotides at positions 154:167 (G–U), 839:847 (A–U), and 1308:1329 (U–A). The species *Actinomyces nasicola* has recently been added to this lineage.

Actinomyces neuii was described (Funke et al., 1994) before the phylogenetic diversity of the genus *Actinomyces* had been recognized. However, the separate position of this species, apart from other members of the genus, became apparent in several subsequent studies, but lack of distinct and differentiating phenotypic properties has hindered the formal description of a new genus for the former CDC group 1 (subspecies *neuii*) and CDC group 1-like coryneform bacteria (subspecies *anitratus*). The two groups were considered subspecies because of phenotypic differences observed for the phylogenetically highly related type strains (99.7% sequence similarity). Nonquantitative DNA hybridization (Funke et al., 1993), however, showed a low degree of overall genomic relatedness between members of the two subspecies. The pattern of 16S rRNA signatures of *Actinomyces neuii* consists of nucleotides at positions 153:168 (G–U), 378:385 (A–U), 407:435 (G–C), 450:483 (A–U), 601:637 (A–U), 602:636 (G–U), 668:738 (U–A), 669:737 (A–U), 1243:1294 (U–A), 1245:1292 (U–G), 1246:1291 (C–G), and 1310:1327 (C–G). The branching of *Varibaculum cambriense* adjacent to the *Actinomyces neuii* lineage is obviously without statistical significance.

Besides the 16S rRNA gene and certain housekeeping genes of the *Actinomyces naeslundii/Actinomyces viscosus* complex whose sequences were chiefly used for taxonomic purposes, several other genes have been sequenced and functionally characterized; these genes are or may be involved in the pathogenicity of the respective actinomycetes. For example, Yeung et al. (1998) determined the sequence of the *Actinomyces viscosus (Actinomyces oris?*) T14V type 2 fimbrial structural subunit gene, *fimA*, and the 3′ flanking DNA region. The *fimA* gene encoded a 535-amino-acid precursor subunit protein (FimA). A second

gene, *orf365*, that encoded a 365-amino-acid protein which contained a putative transmembrane segment was identified immediately 3′ to *fimA*. Deletion mutants of these genes did not participate in the type 2 fimbriae-mediated coaggregation with *Streptococcus oralis* 34. In addition, the nucleotide sequence of the chromosomal DNA flanking the *Actinomyces viscosus* T14V type 1 fimbrial structural subunit gene (*fimP*) was determined (Yeung and Ragsdale, 1997) in which six open reading frames were identified. These were further characterized in respect to their additional role in the synthesis and function of the fimbriae of *Actinomyces viscosus* T14V.

As the multisubunit enzyme urease may also be considered a virulence factor, the urease gene regulation and nitrogen metabolism of *Actinomyces naeslundii* WVU45 (=ATCC 12104 = *Actinomyces naeslundii sensu stricto*) was studied (Morou-Bermudez and Burne, 2000). In this study, it was demonstrated that urease activity and urease-specific mRNA levels in this strain can increase up to 50-fold during growth under nitrogen limitation. Using primer extension analysis, a putative, proximal, nitrogen-regulated promoter of the *Actinomyces naeslundii* urease gene cluster was identified whose functionality and nitrogen responsiveness were confirmed using reporter gene fusions and 5′ deletion analysis. From the data obtained, it was concluded that regulation of urease expression by nitrogen availability in *Actinomyces naeslundii* may require a positive transcriptional activator.

Mutants, plasmids, phages, and bacteriocins. Spontaneous mutants of certain *Actinomyces* species such as *Actinomyces viscosus sensu lato* or *Actinomyces naeslundii sensu lato* appear to occur comparatively frequently. The first of these mutant strains, T14AV, was characterized in some detail. It appeared spontaneously in a culture of *Actinomyces viscosus* T14V, a human oral strain which, according to the recent taxonomic revisions, belongs to the new species *Actinomyces oris* and it was used to study the periodontopathic potential of this species (Hammond et al., 1976). T14AV differed from the parent strain T14V as it proved to be non-virulent under certain experimental conditions. Upon further investigation, the morphological, chemical, and antigenic differences between the strains which could account for the impaired virulence of T14AV were shown to be quantitative rather than qualitative. For example, the virulent parent strain produced more fimbriae and more virulence-associated antigen than the mutant strain (Brecher et al., 1978; Cisar et al., 1978, 1980), a microcapsule was apparent on the mutant strain (Powell et al., 1978), and the mutant strain produced increased amounts of a viscous, extracellular polysaccharide (Brecher et al., 1978; Hammond et al., 1976). Due to these changes, the mutant strain T14AV displayed various adherence defects (Brecher et al., 1978; McIntire et al., 1978; Wheeler et al., 1979) and a decreased ability to colonize germ-free rats and to initiate periodontal pathology (Brecher et al., 1978; Hammond et al., 1976). Thus, the lower virulence of strain T14AV appeared to be related to several different factors and is probably not associated with a distinct structural change of the mutant cell surface.

Meanwhile, various other mutants were used to elucidate in detail adherence and pathogenicity mechanisms, particularly in relation to the development of caries and periodontal disease. For example, Cisar et al. (1983) described a spontaneous coaggregation-defective (COG$^-$) mutant, *Actinomyces viscosus* (*Actinomyces oris*) T14V(PK455), which associated the lectin activity with type 2 fimbriae, whereas strain T14AV provided little insight into the mechanisms of lactose-sensitive adherence. In addition, the precise nature of the cell surface modification displayed by strain T14V(PK455) provided clear evidence for the distinct and independent functions of type 1 and type 2 fimbriae of *Actinomyces viscosus* (*Actinomyces oris*) T14V.

Using rabbit antibodies against either or both fimbrial antigens, spontaneous mutants of *Actinomyces viscosus* (*Actinomyces oris*) T14V were isolated which lacked type 1, type 2, or both types of fimbriae (Cisar et al., 1988). These results allowed further insight into the function of both types of fimbriae, especially the ability of salivary acidic proline-rich proteins and statherin to serve as receptors for type 1 fimbriae. Mutagenesis with ethyl methanesulfonate of *Actinomyces viscosus* T14V-J1 and enrichment for cells non-reactive with proline-rich proteins (PRPs) by successive adsorption with PRP-treated latex beads resulted in mutants that exhibited reduced binding to hydroxyapatite coated with whole saliva or salivary protein preparations, but were still capable of reaction with antiserum to type 1 and type 2 fimbriae suggesting that an adhesive molecule was probably associated with type 1 fimbriae and allowed for the interaction of *Actinomyces viscosus* (*Actinomyces oris*) with constituents in the salivary pellicle (Nesbitt et al., 1992). Another coaggregation-defective mutant (*Actinomyces* serovar WVA 963, strain PK1259, which is now considered a member of the new species *Actinomyces johnsonii*) lacking type 2 fimbriae was shown to synthesize the putative adhesin, but appeared unable to present it properly on its surface (Klier et al., 1998).

The plasmid content of *Actinomyces* species appears to be very limited. Vandenbergh et al. (1982) were not able to detect plasmids in 17 strains of *Actinomyces viscosus sensu lato*, four strains of *Actinomyces odontolyticus*, and one strain of *Actinomyces israelii*. However, integration plasmids were used to generate site-specific mutations in the *Actinomyces viscosus* (*Actinomyces oris*) T14V chromosome (Yeung, 1995).

Although several groups of oral bacteria abundantly produce bacteriocins or bacteriocin-like activities, there is little evidence that *Actinomyces* species synthesize such substances. Holmberg and Hallander (1972) were unable to detect bacteriocin-like activities in strains of *Actinomyces viscosus sensu lato* and *Actinomyces naeslundii sensu lato*. Tompkins and Tagg (1986) observed inhibitory effects in 62 *Actinomyces* strains when incubated anaerobically, but little inhibitory activity was found under "microaerophilic" conditions and anaerobically in a medium buffered by 0.5% (w/v) calcium carbonate. It was therefore concluded that the inhibitory effect was due to the production of large amounts of lactic and succinic acids and the concomitant fall in the pH rather than to the production of bacteriocin-like substances. Turner and Jordan (1981) also reported that interstrain antagonism was low and, when present, induced only small and poorly defined inhibition zones. Only one report (Franker et al., 1977) suggests that a strain of *Actinomyces odontolyticus* isolated from dental plaque produced a non-dialyzable, trypsin-sensitive substance that was bactericidal for certain strains of bifidobacteria at 42°C but not at 37°C. However, treatment of the producer cells with curing agents did not induce a high frequency of non-bacteriocinogenic cells and the presumed bacteriocin was adsorbed by susceptible as well as resistant bacteria.

Information on bacteriophages of *Actinomyces* species is also rather scarce: Delisle et al. (1978) isolated a lytic phage from a sample of raw domestic sewage. This phage Av-1 produced clear plaques on a human isolate primarily identified as *Actinomyces viscosus* and was found to belong to Bradley's morphological group C (Bradley, 1967). It possessed a small polyhedral head measuring 40 nm in diameter and a short tail of 26 nm in length. At first, its host range appeared to be very narrow as it only infected one human isolate of *Actinomyces viscosus* (*Actinomyces oris*?) (strain MG-1), but it did not show lysis or growth inhibition when plated with 10 other *Actinomyces viscosus sensu lato* and 10 *Actinomyces naeslundii sensu lato* strains. Later, Delisle and Donkersloot (1995) showed this phage to be lytic for several human strains of *Actinomyces naeslundii sensu lato*. Recently, the lysis genes of Av-1 were isolated and characterized; the complete lysis region of Av-1 is comprised of two holin-like genes which were designated *holA* and *holB*, and an endolysin gene, designated *lysA* (Delisle et al., 2006).

When screening 336 human dental plaque samples for the presence of bacteriophage for *Actinomyces viscosus sensu lato*, 10 were found to contain virulent *Actinomyces* phages (Tylenda et al., 1985a). Again, a high host cell specificity of these phages was noted. One phage isolate only infected *Actinomyces viscosus* (*Actinomyces oris*) T14V, eight phage isolates infected only *Actinomyces viscosus* MG-1, and one infected both strains. One of the plaque samples yielding phage was examined for the presence of a natural host strain for that particular phage. Consequently, two actinomycetes strains identified as *Actinomyces viscosus* serovar II (*Actinomyces oris*) and *Actinomyces naeslundii* serovar I (*Actinomyces naeslundii sensu stricto*) were isolated.

Yeung and Kozelsky (1997) isolated bacteriophages from 22 of 124 samples of fresh human dental plaque. All *Actinomyces naeslundii sensu stricto*, *Actinomyces oris*, and *Actinomyces viscosus sensu stricto* strains tested were susceptible to infection with one or more of these phages. In contrast, none of the *Actinomyces odontolyticus*, *Actinomyces israelii*, or *Actinomyces bovis* isolates included in the study was sensitive. The genomes of these phages were found to consist of double-stranded DNA molecules 16–60 kbp in size. A temperate phage, designated Φ 225, produced a lysogenized strain of *Actinomyces viscosus* MG-1.

The use of such *Actinomyces* phages as tools for studying coaggregation between actinomycetes and streptococci revealed that the phages AV-1, AV-2, AV-3, and 1281 bound to coaggregation group A of *Actinomyces viscosus* and to group E of *Actinomyces naeslundii* (Tylenda et al., 1985b) but not to groups B, C, D, or F. Studies of another lytic *Actinomyces viscosus (Actinomyces oris)* T14V phage isolated from raw sewage and designated BF307 indicated that this phage recognizes a receptor on *Actinomyces viscosus* strains MG-1 and T14V that is related to one of the structures that mediates coaggregation with oral streptococci (Delisle et al., 1988). Hence such phages were thought to be useful for probing surface components of human oral actinomycetes.

Antigenic structure. Agglutination (Bellack and Jordan, 1972; Erikson, 1940; Holm, 1930; King and Meyer, 1957; Lentze, 1938a; Putnins and Bowden, 1993; Slack et al., 1951, 1955; Snyder et al., 1967), complement fixation (Kwapinski and Snyder, 1961; Snyder et al., 1967), cell-wall agglutination (Bowden and Fillery, 1978; Cummins, 1962, 1970; Fillery et al., 1978; Putnins and Bowden, 1993), immunofluorescence (Bellack and Jordan, 1972; Brock and Georg, 1969b; Buchanan et al., 1984; Cato et al., 1984; Holmberg and Forsum, 1973; Johnson et al., 1990; Lai and Listgarten, 1979; Lambert et al., 1967; Marucha et al., 1978; Schaal and Gatzer, 1985; Schaal and Pulverer, 1973; Slack and Gerencser, 1966, 1970, 1975; Slack et al., 1961, 1966), immunoassay (Gillis and Thompson, 1978), immunodiffusion (Bellack and Jordan, 1972; Bowden et al., 1976; Georg et al., 1968; King and Meyer, 1963; Schaal and Gatzer, 1985; Snyder et al., 1967), immunoelectrophoresis (Bowden et al., 1976; Fillery et al., 1978; Holmberg et al., 1975), immunoblotting (Putnins and Bowden, 1993), passive hemagglutination (Wicken et al., 1978), and immunoelectron microscopy (Garant et al., 1979) procedures have all been used to elucidate the antigenic structure of *Actinomyces* species, to define the serological relationships between species and serovars, and to locate antigenic determinants in or on *Actinomyces* cells. Among these techniques, cell-wall agglutination, immunodiffusion, immunoblotting, and fluorescent antibody tests have proved especially useful for application to the fermentative actinomycetes. Immunofluorescence has been employed most extensively, and the majority of data reported below are based upon direct or indirect modifications of this technique.

The species *Actinomyces bovis*, *Actinomyces israelii*, *Actinomyces meyeri*, *Actinomyces naeslundii*, *Actinomyces odontolyticus*, and *Actinomyces viscosus*, were all considered to be serologically heterogeneous in previous studies and were therefore subdivided into two or more serovars (Brock and Georg, 1969a; Cato et al., 1984; Slack and Gerencser, 1975). Varying degrees of cross-reactivity between the serovars within one species and between different species were noted, but there has always been general agreement that species and serovar-specific antigens do exist (Bowden et al., 1976; Schaal and Gatzer, 1985; Slack and Gerencser, 1975). Furthermore, a certain variability within some of the serovars and significant serological differences between the subclusters of the *Actinomyces israelii* phenon delineated by numerical taxonomy (Schaal and Gatzer, 1985; Schofield and Schaal, 1981) appear to indicate that additional serovars might exist. This might be of particular importance since the two original serovars of *Actinomyces israelii* have been assigned to two different species, namely *Actinomyces israelii* corresponding to *Actinomyces israelii* serovar I strains and *Actinomyces gerencseriae* which represents the former *Actinomyces israelii* serovar II strains (Johnson et al., 1990).

Serological grouping of members of the genus *Actinomyces* was first introduced by Slack and co-workers (Slack and Gerencser, 1966, 1970; Slack et al., 1961) and these serological groups could be related later on to the various *Actinomyces* species known at that time. Depending on the quality of antisera and the serological techniques used, species and/or serovar-specific antisera can be obtained either by simple dilution or by absorption procedures (Johnson et al., 1990; Marucha et al., 1978; Putnins and Bowden, 1993; Schaal and Gatzer, 1985; Schaal and Pulverer, 1973; Slack and Gerencser, 1975).

Actinomyces bovis shows no or only low grade cross-reactions with other species or between the two serovars known among strains of this species for a long time (Johnson et al., 1990; Schaal and Gatzer, 1985; Slack and Gerencser, 1975). From the comparatively small number of strains studied so far, it can be concluded that *Actinomyces bovis* serovar I strains usually produce smooth, non-filamentous microcolonies (Figure 35), whereas

serovar II microcolonies are often definitely filamentous (Slack and Gerencser, 1975) (Figure 39).

Serological heterogeneity of the taxon *Actinomyces israelii* as originally described (Lachner-Sandoval, 1898) had already been assumed by Lentze (1938a). The existence of two serovars was first reported by Lambert et al. (1967) and confirmed by Blank and Georg (1968), Brock and Georg (1969a, 1969b), Slack et al. (1969), Cummins (1970), and Schaal and Pulverer (1973). Cross-reactions occur between these two serovars which are now considered separate species. However, Brock and Georg (1969b) found only one-way cross-reactions in which serovar I (*Actinomyces israelii sensu stricto*) reacted with serovar II cells (*Actinomyces gerencseriae*), while others (Holmberg and Hallander, 1973; Schaal and Pulverer, 1973; Slack et al., 1969) obtained reciprocal cross-reactions.

Actinomyces israelii serovar I strains (*Actinomyces israelii sensu stricto*) were reported to exhibit antigenic variation (Bowden et al., 1976; Slack and Gerencser, 1975). When analyzing the serological reactivity of the four subclusters delineated within the *Actinomyces israelii* phenon in a numerical taxonomic study (Schofield and Schaal, 1981), Schaal and Gatzer (1985) confirmed these findings as the two serovar I strains, ATCC 10048 and ATCC 12102, were not only found in two different subclusters (1a and 1b), but also showed distinct serological reactivity. On the other hand, subcluster 1b contained both serovar I and II strains including the type strains of both *Actinomyces israelii sensu stricto* (ATCC 12102) and *Actinomyces gerencseriae* (ATCC 23860) thereby indicating that in the numerical phenetic analysis of Schofield and Schaal (1981) a clear-cut separation of *Actinomyces israelii* serovars I and II or *Actinomyces israelii sensu stricto* and *Actinomyces gerencseriae*, respectively, had not been possible. However, members of the subclusters 1c and 1d, which only contained human clinical isolates without any type or reference strain and which were phenotypically closely related to the two serovars of *Actinomyces israelii sensu lato* (*Actinomyces israelii sensu stricto* and *Actinomyces gerencseriae*), exhibited little or no cross-reactivity with each other and with members of the two defined serovars. It therefore remains to be seen whether the isolates of subclusters 1c and 1d represent additional serovars of *Actinomyces israelii* or *Actinomyces gerencseriae* or whether they are new *Actinomyces* species. Nonetheless, at least *Actinomyces israelii* as currently defined appears to be serologically heterogeneous although two (or more) distinct serovars have not been formally described. Furthermore, both *Actinomyces israelii sensu stricto* and *Actinomyces gerencseriae* may show noticeable cross-reactivity with *Actinomyces hordeovulneris*, *Actinomyces naeslundii*, *Actinomyces odontolyticus*, *Actinomyces oris* and even with *Arcanobacterium pyogenes* and *Propionibacterium propionicum* at low dilutions of the antisera (Buchanan et al., 1984; Schaal and Gatzer, 1985), although these cross-reactions are usually eliminated by further dilution or absorption (Schaal and Gatzer, 1985; Slack and Gerencser, 1975).

Serological differences between *Actinomyces naeslundii sensu lato* and *Actinomyces viscosus sensu lato* strains were noted more than 30 years ago. A second serovar of *Actinomyces naeslundii sensu lato* was first reported by Bragg et al. (1972) on the basis of four strains which cross-reacted with both *Actinomyces naeslundii* serovar I (*Actinomyces naeslundii sensu stricto*) and *Actinomyces viscosus*, serovar II (*Actinomyces oris*). Further studies indicated, however, that three of these strains should be considered untyped (Bragg et al., 1975) or serovar I variants (Gerencser, 1979) and only one strain (WVU 1523 = CDC W1544 = ATCC 49339) should be accepted as serovar II. Jordan et al. (1974) described strain N 16 (WVU 820 = ATCC 49340) as serovar III of *Actinomyces naeslundii sensu lato*. This strain and a few similar isolates appeared to be more closely related to *Actinomyces naeslundii*, serovar II, than to serovar I and showed strong cross-reactions with *Actinomyces viscosus*, serovar II. These serological findings underpin the inclusion of serovars II and III of *Actinomyces naeslundii sensu lato* and of serovar II of *Actinomyces viscosus* in the new species *Actinomyces oris*. The organisms tentatively designated serovar IV of *Actinomyces naeslundii* (Gerencser and Slack, 1976) were found to be unrelated to *Actinomyces viscosus*, serovar II, and were therefore provisionally designated *Actinomyces* sp., serovar WVA 963 (Gerencser, 1979). This serovar was recently described as a separate *Actinomyces* species and named *Actinomyces johnsonii* (Henssge et al., 2009).

These findings were largely obtained using a direct immunofluorescence technique and largely confirmed using whole-cell and cell-wall agglutination as well as by immunoblotting (Putnins and Bowden, 1993). Whole-cell agglutination placed *Actinomyces viscosus*, serovar II, *Actinomyces naeslundii*, serovars II and III, as well as *Actinomyces*, serovar NV, which could not be typed by immunofluorescence, into a single group corresponding to the new species *Actinomyces oris*. *Actinomyces viscosus sensu stricto* cross-reacted weakly with this group.

The two serovars primarily recognized in the species *Actinomyces viscosus sensu lato* (Gerencser and Slack, 1969; Slack and Gerencser, 1970) differ in their ecology: serovar I strains represent the original hamster isolates of *Actinomyces viscosus*, while all of the serovar II strains originated from human sources. The latter were included in the new species *Actinomyces oris* whereas the hamster isolates (serovar I) retained the designation *Actinomyces viscosus (sensu stricto)* (Henssge et al., 2009; Johnson et al., 1990; Putnins and Bowden, 1993). Cross-reactions were reported between *Actinomyces hordeovulneris* and an antiserum to serovar I of *Actinomyces viscosus* (ATCC 15987 = *Actinomyces viscosus sensu stricto*). *Actinomyces viscosus*, serovar II, antisera (*Actinomyces oris*) showed no reaction with *Actinomyces hordeovulneris* (Buchanan et al., 1984).

The two serovars of *Actinomyces odontolyticus* described so far (Slack and Gerencser, 1970) exhibit only low grade cross-reactivity between each other (Schaal and Gatzer, 1985). However, very pronounced cross-reactions can be observed between *Actinomyces odontolyticus*, serovar II, and *Arcanobacterium pyogenes* (Schaal and Gatzer, 1985; Slack and Gerencser, 1975). Unfortunately, this cross-reactivity, which resulted in indirect immunofluorescence staining of the heterologous antigens at titers that were only one dilution below the homologous reaction (Schaal and Gatzer, 1985), has not been studied in more detail. Cross-reactions with other *Actinomyces* species are weak or do not occur at all.

The serological relationships between *Actinomyces naeslundii* genospecies 1 (*Actinomyces naeslundii sensu stricto*) and *Actinomyces naeslundii* genospecies 2 (*Actinomyces oris*) on the one hand and *Actinomyces denticolens*, *Actinomyces howellii*, and *Actinomyces slackii* on the other hand have been studied by Putnins and Bowden (1993) using cell-wall and whole-cell agglutination as well as immunoblotting. Whole-cell agglutination placed *Actinomyces viscosus* serovar II, *Actinomyces naeslundii* serovars II and

III and several other related strains in a single group thereby confirming the inclusion of these organisms in the new species *Actinomyces oris*. *Actinomyces viscosus* serovar I (*Actinomyces viscosus sensu stricto*) cross-reacted weakly with this group. *Actinomyces naeslundii* serovar I (*Actinomyces naeslundii sensu stricto*), *Actinomyces denticolens*, and *Actinomyces howellii* were distinct and appeared to be serologically homogeneous. The agglutination results for *Actinomyces slackii* were equivocal. Immunoblot analyses of cell-wall extracts developed with non-absorbed antisera showed cross-reactivity among all of the organisms tested including *Actinomyces israelii*.

As far as *Actinomyces denticolens* is concerned, preliminary immunofluorescence data obtained by Schaal and Gatzer (unpublished observation) confirm that this species represents a separate antigenic entity. Cross-reactions with antisera to several other *Actinomyces* species did not occur or were only very low-grade. In good agreement with cell-wall agglutination results (Putnins and Bowden, 1993), striking cross-reactions were not observed even with *Actinomyces naeslundii sensu lato* with which *Actinomyces denticolens* shares many physiological characters.

In principle, the various serological techniques give comparable results when applied to *Actinomyces* strains. However, the degree of cross-reactivity may vary depending on the method, the antigens, and the antisera used. Cross-reactions with members of other genera have occasionally been encountered (Schaal and Gatzer, 1985; Slack and Gerencser, 1975), but nearly always low-titered. They may be especially pronounced with cytoplasmic antigens (Kwapinski and Seeliger, 1964), but can also be demonstrated with other antigen sources including whole cells used for agglutination or immunofluorescence procedures. Apart from the striking cross-reactivity between *Actinomyces odontolyticus*, serovar II, and *Arcanobacterium pyogenes* that has been mentioned above, cross-reactions between *Actinomyces israelii* and *Actinomyces gerencseriae* on the one side and *Propionibacterium propionicum* on the other side can be observed by immunofluorescence and by the Ouchterlony technique (Schaal and Gatzer, 1985). Occasionally, *Actinomyces israelii sensu lato* may also cross-react with *Propionibacterium acnes* (Slack and Gerencser, 1975) or *Rothia dentocariosa* (Schaal and Gatzer, 1985). Similar cross-reactions may occur between *Actinomyces naeslundii sensu lato* and *Actinomyces viscosus sensu lato* on the one hand and *Propionibacterium propionicum* and/or *Rothia dentocariosa* on the other hand (Schaal and Gatzer, 1985; Slack and Gerencser, 1975). *Actinomyces bovis*, serovar I, antisera show low-grade cross-staining with *Actinomyces meyeri* cells in indirect fluorescent antibody tests (Schaal and Gatzer, 1985).

Knowledge of the number, chemical composition, and cellular location of *Actinomyces* antigens has remained fragmentary although some antigens of oral *Actinomyces* strains which are or might be associated with their virulence have been characterized in more detail (Hammond et al., 1976). Antigenic components were found in the cytoplasm (Holmberg et al., 1975; Kwapinski and Snyder, 1961), in or on the cell wall (Bowden and Fillery, 1978; Bowden et al., 1976; Cummins, 1962; Fillery et al., 1978; Firtel and Fillery, 1988; Hammond et al., 1976; Powell et al., 1978; Putnins and Bowden, 1993; Wheeler and Clark, 1980; Wicken et al., 1978) and in culture supernatants (Georg et al., 1968; King and Meyer, 1963; Lambert et al., 1967).

Firtel and Fillery (1988) investigated the antigenic structure of *Actinomyces viscosus sensu lato* and *Actinomyces naeslundii sensu lato* using a set of 18 monoclonal antibodies against whole cells of these species. These antibodies identified 11 different antigenic determinants which were arranged in a complicated mosaic. The profiles derived from the application of these 18 monoclonal antibodies corresponded at least to some extent to the distinctions made within the *Actinomyces naeslundii/Actinomyces viscosus* complex by DNA hybridization experiments (Johnson et al., 1990), which finally led to the description of *Actinomyces naeslundii sensu stricto*, *Actinomyces viscosus sensu stricto*, *Actinomyces oris*, and *Actinomyces johnsonii* (Henssge et al., 2009).

One group of *Actinomyces* antigens consists of cell-wall associated carbohydrates or polysaccharides which may be released into the culture fluid (Bowden and Hardie, 1973; Bowden et al., 1976; Cummins, 1962; King and Meyer, 1963; Pirtle et al., 1965). These antigens are usually heat stable and resistant to treatment with proteases. They may carry species and serovar specificity (Bowden and Hardie, 1973; Bowden et al., 1976; Cummins, 1962; Putnins and Bowden, 1993). Wicken et al. (1978) characterized an amphipathic antigen of *Actinomyces viscosus* Ny 1 in more detail. This antigen was found to be a fatty acid-substituted heteropolysaccharide which accounted for 1% of the cell mass. It contained 16.8% (by weight) *O*-esterified fatty acids ($C_{16:0}$, $C_{18:0}$, $C_{18:1}$) and a polysaccharide composed of mannose, glucose, and galactose in a molar ratio of 1:2:3. Glycerophosphate, *N*-acetylgalactosamine, alanine, and lysine were further constituents, but 6-deoxytalose and rhamnose were not identified. Evidence for the presence of lipoteichoic acids was not found.

In contrast, Hammond et al. (1976) characterized a virulence-associated, high molecular mass antigen of *Actinomyces viscosus* T14-V and *Actinomyces naeslundii sensu stricto* that was composed of a polysaccharide moiety containing 6-deoxytalose as the major sugar and as determinant of serologic specificity, and a small peptide resembling to some extent part of the peptidoglycan.

Landfried (1972) isolated an antigen from the culture supernate of *Actinomyces israelii* ATCC 12102 which was non-migratory in immunoelectrophoresis and was detected in several strains of both *Actinomyces israelii* and *Actinomyces gerencseriae*. Chemical analysis indicated that this antigen might be a glycan or a mixture of two or three glycans. The peptidoglycan itself may also possess antigenic properties (Hammond et al., 1976; Reed, 1972).

A second group of *Actinomyces* antigens is composed of polypeptides or polypeptide-containing compounds (Bowden and Hardie, 1973; Bowden et al., 1976; Putnins and Bowden, 1993). These were found to be pronase-sensitive, but trypsin resistant, charged molecules which were species-specific although they contained cross-reacting components. Employing two-dimensional (crossed) immunoelectrophoresis, Ayakawa et al. (1983) identified a major heat-stable surface antigen in *Actinomyces israelii* serovar II (*Actinomyces gerencseriae*) which was protease-sensitive, non-dialyzable, and found in relatively large amounts. *Actinomyces israelii sensu stricto* lacked this antigen or contained small amounts of a cross-reacting antigen which was not identical with the *Actinomyces gerencseriae* component. Despite its heat-stable property, the antigen was assumed to be, at least in part, a protein. Trypsin digestion split the antigen into two components with considerably faster electrophoretic mobilities than the original antigen.

The cytoplasmic antigens prepared by Kwapinski and Snyder (1961) exhibited pronounced cross-reactivity. In contrast, Holmberg et al. (1975) reported that the crossed immunoelectrophoresis or crossed immunoelectrofocusing of their cytoplasmic preparations gave specific and reproducible results. It can be assumed that at least some of the cytoplasmic antigens are proteins or polypeptides.

The proteinaceous nature has been proven for the virulence-associated antigen 1 of *Actinomyces viscosus* T14V (Cisar and Vatter, 1979; Cisar et al., 1978; Wheeler and Clark, 1980) which is identical with the fimbriae (type 1 fimbriae) produced by this organism. These fimbriae were reported (Wheeler and Clark, 1980) to consist of 95.2% protein and less than 2% carbohydrate and contained large quantities of aspartic acid, threonine, glutamic acid, and alanine. A minimum molecular mass of 24,960 was calculated for this fimbrial protein.

The antigens on type 2 fimbriae differ clearly from those of type 1 fimbriae so that the different attachment properties mediated by each of the two fimbrial types can be inhibited separately by specific neutralizing antibodies (Cisar et al., 1988; Revis et al., 1982).

Susceptibility to antibiotics and various chemical compounds. *Actinomyces* species display a moderate to high susceptibility to many of the antibacterial drugs currently in use, thereby showing comparatively little species and strain variation (Abrahams and Miller, 1946; Blake, 1964; Buchanan et al., 1984; Cato et al., 1984; Chow and Bednorz, 1978; Fritsche, 1964a; Hanf, 1956; Hanf et al., 1953; Howell, 1953; Lentze, 1957, 1967; Lerner, 1967, 1974; Niederau et al., 1982; Schaal and Pape, 1980; Schaal et al., 1979; Smith et al., 2005; Spieckermann, 1970; Suter and Vaughan, 1955; Sutter and Finegold, 1976). A tendency towards an increasing antibiotic resistance of these actinomycetes has as yet never been documented with sufficient certainty. However, susceptibility results obtained in different laboratories often differ considerably even though certain reference strains were common to many studies. This indicates that methodological problems obviously play an important role in the assessment of drug susceptibility of fermentative actinomycetes (Schaal and Pape, 1980; Schaal et al., 2006).

In susceptibility testing of fermentative actinomycetes, the only procedure found to give reproducible and clinically useful results is the agar dilution test using DST agar (Oxoid) as test medium and microscopic assessment of results after only 24 (at maximum 48) hours of incubation (Schaal and Pape, 1980). The different growth rate and degrees of filament formation usually do not allow sufficient standardization of the agar dilution or broth dilution tests, respectively. Whether the E-test methodology is applicable to all of the *Actinomyces* species known so far remains unproven. The results obtained by Smith et al. (2005) using this technique appear to indicate that filamentous and slowly growing species might be more susceptible than the same species tested by the agar dilution method. Which of these differing results is clinically more relevant awaits clarification.

The minimal inhibitory concentrations (MICs; <0.016–3.12 mg/l) reported for *Actinomyces bovis*, *Actinomyces europaeus*, *Actinomyces funkei*, *Actinomyces graevenitzii*, *Actinomyces israelii sensu lato* as well as for *Actinomyces israelii sensu stricto*, and *Actinomyces gerencseriae*, *Actinomyces naeslundii sensu lato*, *Actinomyces viscosus sensu lato*, and *Actinomyces turicensis* reveal that all of these species are usually highly susceptible to β-lactam antibiotics (penicillins, cephalosporins, and carbapenems) (Niederau et al., 1982; Schaal and Pape, 1980; Schaal et al., 1979; Smith et al., 2005). This also applies to the combinations of β-lactams and β-lactamase inhibitors such as amoxicillin plus clavulanic acid, ampicillin plus sulbactam, and piperacillin plus tazobactam. However, Smith et al. (2005) using the E-test found that two *Actinomyces europaeus*, five *Actinomyces funkei*, and nine *Actinomyces turicensis* strains displayed reduced susceptibility (≥1 mg/l) to piperacillin/tazobactam.

Tetracyclines, chloramphenicols, macrolides, lincomycins, rifamycins, fusidic acid, and glycopeptide antibiotics (vancomycin and teicoplanin) also exhibit a moderate to high inhibitory activity against all of the "classical" *Actinomyces* species (Niederau et al., 1982; Schaal and Pape, 1980; Schaal et al., 1979). As far as the newer members of the genus are concerned, 2 of 10 *Actinomyces europaeus* and 2 of 11 *Actinomyces turicensis* isolates showed resistance to erythromycin (Smith et al., 2005).

All isolates of *Actinomyces europaeus*, *Actinomyces funkei*, *Actinomyces gerencseriae*, *Actinomyces graevenitzii*, *Actinomyces israelii*, and *Actinomyces turicensis* tested by Smith et al. (2005) were reported to be resistant to ciprofloxacin. In contrast, all but three of 87 isolates of the above six species were found to be susceptible to linezolid. The three strains having MICs of >1.0 mg/l comprised one *Actinomyces europaeus* and two *Actinomyces turicensis* isolates. *Actinomyces radingae* and *Actinomyces turicensis* were both found to be resistant to mupirocin (Vandamme et al., 1998).

MICs for *Actinomyces hordeovulneris* were reported as follows: penicillin G, ≤0.25 mg/l; chloramphenicol, 1.0 mg/l; cotrimoxazole, 1.0 mg/l; tetracycline, 0.5–4.0 mg/l. All strains of *Actinomyces meyeri* tested so far were susceptible to chloramphenicol, clindamycin, erythromycin, penicillin G, and tetracyclines (Cato et al., 1984).

Complete resistance has only been observed to aminoglycosides, nitroimidazole compounds, polypeptide antibiotics (polymyxin B, colistin), antifungal drugs, and certain antituberculotics (Niederau et al., 1982; Suter and Vaughan, 1955). Susceptibility data for the remaining newer *Actinomyces* species are not available.

The resistance patterns of members of the genus *Actinomyces* to other chemical and biological inhibitory or toxic compounds are as follows: sodium chloride at a concentration of 2% (w/v) was found to be inhibitory only to *Actinomyces odontolyticus*, while many strains of *Actinomyces bovis*, *Actinomyces israelii sensu lato*, *Actinomyces meyeri*, *Actinomyces naeslundii sensu lato*, and *Actinomyces viscosus sensu lato* were able to grow at this concentration. Certain *Actinomyces meyeri*, *Actinomyces naeslundii sensu lato*, and *Actinomyces viscosus sensu lato* strains also tolerated 4% (w/v) NaCl, but 6% inhibited growth of all of the *Actinomyces* isolates tested. Bile at concentrations of 5 and 10% (w/v) inhibited many *Actinomyces bovis*, *Actinomyces israelii sensu lato*, *Actinomyces meyeri*, and all of the *Actinomyces odontolyticus* strains, whereas *Actinomyces naeslundii sensu lato* and *Actinomyces viscosus sensu lato* isolates were resistant. Even at 20% bile, about one half of the *Actinomyces naeslundii sensu lato* and *Actinomyces viscosus sensu lato* strains were able to grow. The results obtained with sodium taurocholate (0.2%, w/v) were at variance to these findings. Sodium selenite (0.01%, w/v) caused complete growth inhibition of the majority of the *Actinomyces israelii sensu lato* and *Actinomyces odontolyticus* strains tested while about one third of the *Actinomyces bovis*, *Actinomyces naeslundii sensu lato*,

and *Actinomyces viscosus sensu lato* isolates grew in the presence of this inhibitor. Potassium tellurite (0.01%, w/v) and sodium azide (0.005%, w/v) were tolerated by most of the *Actinomyces* strains except *Actinomyces radingae* and *Actinomyces turicensis* isolates, whereas crystal violet (0.005%, w/v) inhibited all of the *Actinomyces israelii sensu lato* and *Actinomyces odontolyticus* strains and many of the *Actinomyces bovis*, *Actinomyces naeslundii sensu lato*, and *Actinomyces viscosus sensu lato* isolates tested (Cato et al., 1984; Schofield and Schaal, 1981; Vandamme et al., 1998).

Trace element requirements. Gallagher and Cutress (1977) studied the effect of trace elements on growth and fermentation of *Actinomyces naeslundii sensu lato* and *Actinomyces viscosus sensu lato*, and found that sodium selenite (50 ppm) inhibited growth and acid production of both species groups tested. This is in accord with the results of Schofield and Schaal (1981). Similar inhibitory effects were obtained with sodium fluoride (1000 ppm), manganous chloride (500 ppm), zinc sulfate (400 ppm), strontium chloride (10,000 ppm, only *Actinomyces viscosus sensu lato*), silver nitrate (30 ppm), and antimony potassium tartrate (100 ppm). Ammonium vanadate (12.5 ppm), nickelous chloride (100 ppm), copper sulfate (200 ppm), cadmium iodide (2 ppm), and barium chloride (400 ppm) showed no, or only slight, inhibition of growth and/or acid production.

Pathogenicity. Since the description of the first member of the genus *Actinomyces*, namely *Actinomyces bovis* (Bollinger, 1877), it has been known that fermentative actinomycetes can cause various forms of potentially severe inflammatory diseases in man, as well as in feral, domestic, and laboratory animals. However, virulence, types of pathological lesions induced, and host specificity vary considerably between members of different *Actinomyces* species (Schaal, 1979, 1981; Schaal and Beaman, 1984; Schaal and Pulverer, 1981; Schaal et al., 2006; Slack, 1974; Slack and Gerencser, 1975). Essentially all of these pathogenic actinomycetes apparently belong to the normal indigenous microflora of their respective hosts, so that they must be considered facultative rather than obligate pathogens that only invade tissue under certain facilitating conditions and are usually not transmissible (Lentze, 1938a, 1938b, 1969; Pulverer and Schaal, 1978; Pulverer and Schaal, 1984b, 1984a; Schaal, 1979, 1986b; Schaal, 1996; Schaal, 1998; Schaal and Beaman, 1984; Schaal and Pulverer, 1984; Schaal and Lee, 1992; Schaal et al., 2006; Slack and Gerencser, 1975). These facilitating conditions do not necessarily include dysfunctions of the host's immune system!

Human diseases and impairments. Typical diseases and impairments caused by *Actinomyces* species in humans are: actinomycoses, lacrimal canaliculitis and other ocular infections, periodontal disease, caries, and intrauterine infections. Among these pathological conditions, actinomycoses are undoubtedly the most characteristic disease entities caused by members of the genus *Actinomyces* in humans.

As currently recognized, actinomycoses are endogenous, subacute to chronic, granulomatous inflammatory processes that give rise to suppuration, abscess formation, and development of draining sinus tracts (Pulverer and Schaal, 1978, 1984a, 1984b; Schaal, 1979, 1996, 1998; Schaal and Beaman, 1984; Schaal et al., 2006; Slack and Gerencser, 1975). The etiology of actinomycotic infections is complex in two respects: i) Clinically typical human actinomycoses are produced not only by several *Actinomyces* species but also by *Propionibacterium propionicum*, and, rarely, by *Bifidobacterium dentium*. ii) Apart from the causative actinomycetes, additional microbes, so-called concomitant bacteria, are associated with actinomycoses (Holm, 1950, 1951; Lentze, 1948, 1953, 1969; Pulverer and Schaal, 1978; Pulverer et al., 2003; Schaal, 1979, 1981, 1985a, 1986a, 1986b, 1996, 1998; Schaal and Beaman, 1984; Schaal and Pulverer, 1981, 1984; Schaal and Lee, 1992; Schaal et al., 1984, 2006). Although *Aggregatibacter actinomycetemcomitans* may be considered the most characteristic companion of the actinomycetes, other companions include various Gram-stain-negative and Gram-stain-positive bacterial species. The composition of this mixed flora varies from case to case, but is always present to boost the relatively low invasive potential of the causative actinomycetes.

In 10–25% of cases, depending on the course of the disease (Pulverer and Schaal, 1978, 1984a, 1984b; Pulverer et al., 2003; Schaal, 1979, 1981; Schaal, 1996, 1998; Schaal and Beaman, 1984; Schaal et al., 2006), the purulent discharge from actinomycotic lesions contains macroscopically visible (≤1 mm in diameter), yellowish to brownish or reddish particles (usually referred to as "Drusen" or "sulfur granules") that are conglomerates of filamentous actinomycete microcolonies formed *in vivo*, various other bacteria, and tissue reaction material, especially polymorphonuclear granulocytes, surrounding the microbial center (Schaal et al., 2006). Corresponding filamentous colony-like structures are commonly found in tissue sections, but are often smaller than those from pus and usually show a club-shaped layer of hyaline material on the tips of peripheral hyphae (Schaal and Beaman, 1984; Schaal et al., 2006; Slack and Gerencser, 1975). The chemical composition of this hyaline material, though not elucidated in detail, is a polysaccharide-protein complex containing high concentrations of various salts (Crawford, 1971; Frazier and Fowler, 1967; Pine and Overman, 1966; Widra, 1963). It should also be noted that morphology and chemical composition of human and animal sulfur granules are apparently not completely identical (Schaal et al., 2006).

Human actinomycoses most frequently affect the face and neck, the cervicofacial area, but they may also be encountered in thoracic and abdominal or pelvic sites (Lentze, 1938a, 1948, 1953, 1969, 1970, 1971; Pulverer and Schaal, 1978; Pulverer et al., 2003; Schaal, 1979, 1981; Schaal and Beaman, 1984; Schaal and Pulverer, 1981; Schaal et al., 2006; Slack and Gerencser, 1975; Wolff and Israel, 1891). Cervicofacial actinomycoses predominantly develop in the soft tissue around the mandible, but they may also be found adjacent to the maxilla or may extend to the orbita, the sinuses, the ear, or the neck (Olson et al., 1989; Pape et al., 1984). Even primary actinomycoses of the thyroid gland have been observed (Dan et al., 1984).

In contrast to animal actinomycoses, human actinomycoses rarely affect the skeleton (Schaal et al., 2006). However, actinomycotic osteitis or osteomyelitis of the mandible, the maxillae, and even the humerus, the temporal bone, the skull, a phalanx of the big toe (Toumi et al., 2005), and the vertebrae have been reported sporadically (Gupta et al., 1986; Honda et al., 2008; Kannangara et al., 1981; King et al., 1998; Kumar et al., 2008; Mehta et al., 2007; Mtaallah et al., 2005; Slack and Gerencser, 1975; Vannier et al., 1986; Winston, 1951; Yenson et al., 1983). Furthermore, recent observations suggest that osteoradionecrosis and biphosphonate-associated osteonecrosis of the jaw greatly promote the development of actinomycotic

osseous infections (Danic et al., 2008; Hansen et al., 2006a; Hansen et al., 2007; Hansen et al., 2006; Magremanne et al., 2006).

Thoracic actinomycoses usually have a history of preceding cervicofacial lesions or of aspiration, but may also develop hematogenously. Their incidence appears to be much higher in the U.S.A. than in Europe, but difficulties in diagnosing the disease may contribute to this difference (Kedmi et al., 2007; Massart et al., 1986; Masters et al., 1985; Philipsen et al., 1988; Schaal, 1996, 1998; Suzuki and Delisle, 1984). From the lungs, the infection may extend to the pleural space (Schaal, 1996), the pericardium (Kedmi et al., 2007), and the chest wall (Reyes, 2007), or may even track down to the groin and appear as a psoas abscess (Schaal, 1996).

The nature and clinical relevance of a disease termed “endobronchial actinomycosis” remains to be definitely clarified as nearly all of the reports on this condition that can be found in the literature were solely based upon histopathological findings without confirmation by bacteriological techniques (Chouabe et al., 2002; Jin et al., 2000; Kim et al., 2002; Lee et al., 1999; Ocal et al., 2004; Rad and Milani, 2007; Toth et al., 2007). Furthermore, it should be noted that all of these cases of so-called endobronchial actinomycoses did not display the pronounced invasiveness of typical actinomycoses and many of them were associated with foreign bodies (inhaled chicken bones, fish bones, teeth or tooth fragments, grape seeds, beans, or broncholithiasis).

Abdominal actinomycoses often follow appendicitis, abdominal trauma, injuries caused by ingested bone splinters or fish bones, bowel surgery, or laparoscopy, and may be located retro- or intraperitoneally (Adachi et al., 1985; Baierlein et al., 2007; Ghannouchi Jaafoura et al., 2008; Karagulle et al., 2008; Levine and Doyle, 1988; Wohlgemuth and Gaddy, 1986; Yamada et al., 2006). They may involve a variety of organs such as the colon (Heer et al., 1986; Saha et al., 2007), the rectum (Stein and Schaal, 1987), the perianal tissue (Gayraud et al., 2000; Shimada et al., 1986), the gallbladder (Lee et al., 2009; Lee et al., 2007; van Steensel and Kwan, 1988), the liver (Braun et al., 2009; Forgan-Smith et al., 1989; Logan et al., 1989; Mongiardo et al., 1986; Yamashita et al., 2007), the kidneys (Homberg et al., 2008; Yenarkarn et al., 2007), the urinary bladder with sulfur granules in the urine (Wajszczuk et al., 1984), and the spleen (Jabr and Skeik, 2007).

The incidence of pelvic infections appears to be increasing in recent years and this is obviously related to the widespread use of intrauterine contraceptive devices (IUCDs), vaginal pessaries, or tampons (Barnham et al., 1978; Bhagavan and Gupta, 1978; Drew, 1981; Gupta et al., 1978; Hager and Majmudar, 1979; Hart et al., 1977; Kohoutek and Nozicka, 1978; Pine et al., 1985; Szabo et al., 1981; Witwer et al., 1977). But pelvic actinomycoses can apparently also be induced by other foreign bodies such as retained intrauterine fetal bones (White and Felix, 2007). Pelvic infections may remain restricted to tissue in the small pelvis (uterus, Fallopian tubes, ovaries), but also may spread continuously or hematogenously to the liver and other intra-abdominal organs or to the abdominal wall (Brihmer et al., 1987; Eibach et al., 1992; Harmouch et al., 2008; Kaszuba et al., 2008; Lininger and Frable, 1984; Mtaallah et al., 2005; Mubiayi et al., 2007; O’Connor et al., 1989; Peitsidis et al., 2008; Persson and Christina, 1986; Shurbaji et al., 1987; Taga, 2007; White and Felix, 2007). Even involvement of the placenta has been observed (Zakut et al., 1987).

Actinomycoses of the central nervous system (CNS) are rare and may either develop hematogenously from distant sites (lung, abdomen, pelvis) or by direct extension from contiguous foci such as ears, orbital cavity, sinuses, or cervicofacial lesions (Fetter et al., 1967; Hutton and Behrens, 1979; Koshi et al., 1981; Nithyanandam et al., 2001; Slack and Gerencser, 1975; Smego, 1987; Stevenson and Gossman, 1968). The types of CNS lesions include brain or cerebellar abscess, meningitis or meningoencephalitis, subdural empyema, and epidural abscess (Benito León et al., 1998; Peacock et al., 1984; Prager et al., 1984; Schwarz et al., 1993; Smego, 1987; Soto-Hernándes et al., 1999; Tsai et al., 2001; Tvede et al., 1985; Winking et al., 1996).

Primary actinomycoses of the skin and the extremities are also very rare and usually have a history of trauma resulting from human bites or fist fights (“punch actinomycosis”) thereby representing the only exogenously acquired forms of the disease (Slack and Gerencser, 1975; Southwick and Lister, 1979). These infections are usually located on the hands or arms, but may also be found on the back (Patil et al., 2008). Hematogenous dissemination to the soft tissue, the bones, or the joints of the extremities have been observed (Blinkhorn et al., 1988; Legum et al., 1978; Mesgarzadeh et al., 1986; Reiner et al., 1987) and even a total hip arthroplasty was found to be infected with fermentative actinomycetes (Strazzeri and Anzel, 1986). The detailed pathogenesis of lesions of the vulva that were suspected of being malignant, but appeared to have been caused by *Actinomyces israelii* in co-infection with *Propionibacterium acnes* and *Peptostreptococcus* sp. remains to be clarified (McElroy et al., 2006).

The predominant causative agents of human actinomycoses are *Actinomyces israelii* and *Actinomyces gerencseriae* (Lentze, 1969; Pulverer and Schaal, 1978; Pulverer and Schaal, 1984b; Pulverer et al., 2003; Schaal, 1981; Schaal and Lee, 1992; Schaal and Pulverer, 1973; Schaal et al., 2006; Slack and Gerencser, 1975; Wolff and Israel, 1891). In addition, *Propionibacterium propionicum* is also a typical causative agent of the disease (Brock et al., 1973; Conrad et al., 1978; Gerencser and Slack, 1967; Pulverer and Schaal, 1978, 1984a, 1984b; Pulverer et al., 2003; Schaal, 1979, 1981, 1985a, 1986a, 1988; Schaal and Lee, 1992; Schaal et al., 2006; Slack and Gerencser, 1975). Other *Actinomyces* species may occasionally be isolated from characteristic human actinomycotic lesions. *Actinomyces naeslundii sensu lato* has been recovered comparatively frequently from cervicofacial infections (Pulverer and Schaal, 1978; Pulverer et al., 2003; Schaal, 1979, 1981; Schaal, 1984; Schaal and Lee, 1992) and sporadically from pulmonary (Karetzky and Garvey, 1974) and abdominal (Scharfen, 1975) lesions. Similarly, *Actinomyces viscosus sensu lato* has been recovered from cervicofacial (Larsen et al., 1978; Pulverer et al., 2003; Schaal, 1979, 1981; Schaal and Lee, 1992) and thoracic (Eng et al., 1981; Lewis and Gorbach, 1972; Mosimann et al., 1979; Spiegel and Telford, 1984; Thadepalli and Rao, 1979) actinomycoses. It should be kept in mind, however, that these actinomycetes may be found in mixed culture together with *Actinomyces israelii* or *Actinomyces gerencseriae* so that their etiological role in a given case of human actinomycosis may be questioned (Schaal et al., 2006). *Actinomyces odontolyticus* appears to be rarely involved in the etiology of invasive infections although this species has been isolated from a few cases of cervicofacial lesions (Mitchell et al., 1977; Pulverer

et al., 2003; Schaal and Pulverer, 1984; Schaal and Lee, 1992) and from pleural fluid and lung abscesses, respectively (Baron et al., 1979; Guillou et al., 1977). *Actinomyces meyeri* has been isolated frequently from brain abscesses and pleural fluid and less often from cervicofacial suppurations and bite wounds (Cato et al., 1984; Pulverer et al., 2003; Schaal and Lee, 1992).

Actinomycete eye infections may present as conjunctivitis, lacrimal canaliculitis, dacryocystitis, keratitis, or even intraocular infection, lacrimal canaliculitis being the most characteristic disease entity among these conditions. Branching, filamentous organisms in lacrimal concretions were already described by Ferdinand Cohn in (1875). Detailed bacteriological examinations revealed that these filamentous microbes can be identified as *Actinomyces gerencseriae*, *Actinomyces israelii*, *Actinomyces naeslundii sensu lato*, *Actinomyces odontolyticus*, *Actinomyces viscosus sensu lato*, *Propionibacterium propionicum*, *Bifidobacterium dentium*, *Rothia dentocariosa*, and even as *Nocardia farcinica* (Blanksma and Slijper, 1977; Buchanan and Pine, 1962; Ellis et al., 1961; Jones and Robinson, 1977; Pine and Hardin, 1959; Pine et al., 1960; Schaal, 1986a, 1988; Schaal and Pulverer, 1984; Schaal and Lee, 1992; Schütt-Gerowitt et al., 1999; Slack and Gerencser, 1975; Takemura et al., 2002). Unlike human actinomycoses, eye infections are usually not invasive, and *Propionibacterium propionicum* is the predominant causative agent of lacrimal canaliculitis followed by *Actinomyces israelii* and *Actinomyces gerencseriae* (Schaal, 1986a; Schaal and Lee, 1992; Schütt-Gerowitt et al., 1999). The etiology of the other inflammatory processes of the eye mentioned above differs to some extent from that of canaliculitis in that *Actinomyces naeslundii sensu lato*, *Actinomyces viscosus sensu lato*, and *Actinomyces odontolyticus* appear to be the prevailing causative agents (Schaal, 1988; Schaal and Lee, 1992; Schütt-Gerowitt et al., 1999). Even two different *Actinomyces* species, such as *Actinomyces israelii* and *Actinomyces naeslundii sensu lato*, may be isolated from a single ocular specimen (Schaal and Pulverer, 1984).

Apart from actinomycosis and lacrimal canaliculitis which represent clinically well defined disease entities, various other inflammatory processes may be induced by *Actinomyces* species. This primarily applies to nearly all of the recently described novel species of human origin as well as to some of the classical ones. Although *Actinomyces naeslundii sensu lato* and *Actinomyces viscosus sensu lato* have been recovered rather frequently from actinomycosis-like lesions (Pulverer et al., 2003; Schaal and Lee, 1992), a study of such infections (comparing the ways of obtaining specimens for bacteriological culture [intraoral incision/puncture vs extraoral incision/puncture]) showed that the isolation of these two species was often related to contamination of the specimen with oral secretions (Schaal and Pulverer, 1984). Furthermore, *Actinomyces naeslundii* or *Actinomyces viscosus* may occasionally be isolated together with *Actinomyces gerencseriae*, *Actinomyces israelii*, or *Propionibacterium propionicum* so that the etiological role of the former species remains obscure in these instances, especially in cases of clinically characteristic actinomycoses (Pulverer et al., 2003). However, *Actinomyces naeslundii sensu lato* has been convincingly reported as a causative agent of gallbladder infections (Freland et al., 1987), pelvic infections (Bonnez et al., 1985), a disseminating infection in a mentally retarded child (Dobson and Edwards, 1987), of a recurring empyema of the knee joint (Schaal and Pulverer, 1984), and of an infection of a total knee arthroplasty (Ruhe et al., 2001) and a hip prosthesis (Wüst et al., 2000).

Apart from its involvement in cervicofacial and pulmonary infections, *Actinomyces viscosus sensu lato* has been isolated from an abscess following a dog bite (del Carmen Pinilla and Ciniglio, 1983), and even from blood samples of patients with endocarditis (Mardis and Many, 2001).

The number of reports on human *Actinomyces odontolyticus* infections appears to have increased recently. Schaal and Lee (1992) found that 1.8% of all actinomycete isolates encountered belonged to this species. Clinically, *Actinomyces odontolyticus* infections should not be termed actinomycoses (Klaaborg et al., 1985; Peloux et al., 1983). Nonetheless, various types of inflammatory processes have been attributed to *Actinomyces odontolyticus*: an ulcer of the oral mucosa (Alamillos-Granados et al., 2000), peritonsillar abscesses (Civen et al., 1993), enterocutaneous fistulation (Klaaborg et al., 1985), multiple liver abscesses (Ruutu et al., 1982), thoracic infections including lung infections with and without extension to the pleural space, to the mediastinum, or the pericardium (Baron et al., 1979; Bassiri et al., 1996; Dontfraid and Ramphal, 1994; Hooi et al., 1992; Ibanez-Nolla et al., 1993; Litwin et al., 1999; Perez-Castrillon et al., 1997; Verrot et al., 1993), brain abscess (Simpson et al., 1996), and even septicemia (Raoult et al., 1982).

Actinomyces meyeri has been isolated frequently from brain abscesses and pleural fluid (Cato et al., 1984) and less often from cervicofacial abscesses (Pordy, 1988), lung and breast abscesses (Allen, 1987; Ferrier et al., 1986; Rippon and Kathuria, 1984; Rose et al., 1982), pneumonitis (Allworth et al., 1986), and abscesses with and without osteomyelitis of hip, symphysis pubis, leg, and foot (Cato et al., 1984; Ferrier et al., 1986; Long et al., 2007; Machet et al., 1993; Pang and Abdalla, 1987), as well as from spleen, liver, or perianal infections, and infected bite wounds (Bauer et al., 2006; Cato et al., 1984; Garcia-Corbeira and Esteban-Moreno, 1994; Garduño et al., 2000; Gayraud et al., 2000; Harsch et al., 2001). A striking tendency of the organism to disseminate has led to involvement of various other organs including heart valves, spleen, or brain (Apotheloz and Regamey, 1996; Chaumentin et al., 1997; Colmegna et al., 2003; Ferrier et al., 1986; Garduño et al., 2000; Huang et al., 1998; Kuijper et al., 1992; Lentino et al., 1985; Marty and Wüst, 1989; van Mook et al., 1997). An infection of the umbilical cord has also been described (Wright et al., 1994).

Actinomyces radingae and *Actinomyces turicensis* have been primarily isolated from various polymicrobial infections (Wüst et al., 1995a), but occasionally also as pure cultures (Clarridge and Zhang, 2002). More recent studies by Vandamme et al. (1998), Sabbe et al. (1999), and Clarridge and Zhang (2002) revealed that human *Actinomyces radingae* infections are relatively rare and mostly present as skin-related processes and soft tissue conditions including perineal abscesses, an infected pilonidal cyst, pleural empyema, and axillar hydradenitis (Junquera-Bañares and Sanz-de La Fuente, 2007; Lepe et al., 1998). In contrast, *Actinomyces turicensis* infections appear to be quite common and usually involve the urogenital tract and the skin (Clarridge and Zhang, 2002; Sabbe et al., 1999; Zautner et al., 2009). In addition, appendicitis, cholecystitis, hepatic abscess, ear, nose and throat infections, breast abscesses, vaginal infections, vulvar abscesses, as well as bacteremia due to *Actinomyces turicensis* or double infections together with *Actinomyces radingae*, may be observed (Attar et al., 2007; Riegert-Johnson et al., 2002; Sabbe et al., 1999; Vandamme et al., 1998).

The two subspecies of *Actinomyces neuii* have been mainly isolated from abscesses or infected atheromas in association with mixed anaerobic flora and from human blood cultures (Funke et al., 1994; Funke and von Graevenitz, 1995), but not from typical actinomycotic lesions. The organism has also been recovered from infected mammary prostheses (Brunner et al., 2000), chronic osteomyelitis (Van Bosterhaut et al., 2002), postoperative endophthalmitis (Garelick et al., 2002; Perez-Santonja et al., 2007; Raman et al., 2004), neonatal sepsis following chorioamnionitis (Mann et al., 2002), infective endocarditis (Cohen et al., 2007), and from a ventriculoperitoneal shunt infection (Watkins et al., 2008).

Actinomyces europaeus has been cultured from abscesses at various locations (breast, suprapubic, perianal, and labial abscesses), but also from femur tissue, decubital ulcers, and an atheroma cyst (Clarridge and Zhang, 2002; Funke et al., 1997a; Sabbe et al., 1999). It has also been recovered from patients suffering from urinary tract infections (Sabbe et al., 1999) and, together with *Actinomyces turicensis*, from subcutaneous fistulae in a patient with femoral hypoplasia (Zautner et al., 2009).

The four isolates of *Actinomyces graevenitzii* reported upon in its original description had been isolated from bronchial secretions (three strains) and osteitis material (one strain) (Pascual Ramos et al., 1997a). In addition, a case of disseminated coinfection with *Actinomyces graevenitzii* and *Mycobacterium tuberculosis* as causal agents has been recently published (Tietz et al., 2005). *Actinomyces radicidentis* was derived from infected root canals of human teeth (Collins et al., 2000; Kalfas et al., 2001) although the role played by this species in endodontic infections has not been fully clarified (Siqueira and Rocas, 2003). Nevertheless, *Actinomyces radicidentis* was shown to form large aggregates of cells held together within extracellular matrix where it may more easily evade host defense mechanisms (Nair et al., 2008). *Actinomyces urogenitalis* has been primarily isolated from samples from the human urogenital tract such as urine, and urethral and vaginal secretions (Nikolaitchouk et al., 2000); recently, this species was found to be etiologically involved in a penis ulcer (Schaal, unpublished observation) and in an intrauterine contraceptive device-associated pelvic actinomycosis (Elsayed et al., 2006). The three strains of *Actinomyces funkei* reported so far were derived from a case of human tricuspid valve endocarditis of a female intravenous drug user, a sternum wound, and an abdominal incision (Lawson et al., 2001b; Westling et al., 2002). The eight isolates of *Actinomyces cardiffensis* described to date originated from intrauterine contraceptive devices without reported signs of inflammation (three strains), from multiple abscesses in a patient four weeks after mastoidectomy together with a complex concomitant flora, from pleural fluid of a patient suffering from shortness of breath and wheezing, from pus of an actinomycotic jaw abscess, from a pericolic abscess, and from a right antral washout of a patient with sinusitis (Hall et al., 2002). The single strains described as *Actinomyces nasicola* (Hall et al., 2003d), *Actinomyces oricola* (Hall et al., 2002), *Actinomyces dentalis* (Hall et al., 2005), *Actinomyces hongkongensis* (Woo et al., 2003), and *Actinomyces massiliensis* (Renvoise et al., 2009) originated from pus from the nasal antrum, two dental abscesses, a patient with suspected pelvic actinomycosis, and a blood culture from a patient with pleuropneumonia, respectively.

Actinomyces georgiae had long been thought to be nonpathogenic to humans although it belongs, like the other *Actinomyces* species mentioned above, to the human indigenous oral microflora (Johnson et al., 1990; Schaal et al., 2006). However, a recent report appears to indicate that *Actinomyces georgiae* might be a rare agent of endocarditis (Jitmuang, 2008).

In the complex etiology of caries and periodontal disease, *Actinomyces* species apparently constitute only one link in a long chain of cause and effect. Nevertheless, evidence has been growing that at least *Actinomyces naeslundii sensu stricto*, *Actinomyces oris*, and *Actinomyces odontolyticus*, because of their adherence and coaggregation mechanisms, play an important role in the formation of dental plaque which is thought to be a prerequisite for the development of these conditions (Batty, 1958; Jordan and Hammond, 1972; Jordan and Sumney, 1973; Slack and Gerencser, 1975; Socransky, 1970; Winford and Haberman, 1966). Additional factors that might contribute to the pathogenesis of caries and periodontitis are acid production from carbohydrates (Wikström et al., 1983), mitogenicity for splenocytes including the B-cell subpopulation (Baker, 1985; Engel et al., 1984; Halfpap et al., 1985), induction of T-cell proliferation (Burckhardt, 1978), activation of the alternate complement pathway (Baker and Billy, 1983), the production of chemotactic effects and the marking of fibroblasts for immune-mediated damage (Engel et al., 1976, 1978; Wicken et al., 1978). Furthermore, the type 2 fimbrial lectin of *Actinomyces oris* was demonstrated to stimulate superoxide and lactoferrin release from polymorphonuclear leukocytes and obviously also mediates phagocytosis and subsequent killing of bacteria (Sandberg et al., 1986; Taichman et al., 1978). *Actinomyces meyeri* has been cultured from subgingival crevices in patients with severe periodontitis (Cato et al., 1984) although its etiological role in this condition remains to be definitely proven.

Apart from producing typical pelvic actinomycoses, *Actinomyces* species, in particular *Actinomyces israelii* and *Actinomyces gerencseriae*, are possibly also able to produce cervicitis and endometritis in women using IUCDs (Bhagavan and Gupta, 1978; Eibach et al., 1989; Luff et al., 1978).

Animal diseases. The best known natural *Actinomyces* infection occurring in animals is bovine actinomycosis (lumpy jaw) (Bollinger, 1877) which (as in human infections) is usually located adjacent to the mandible or maxilla, but in contrast to human infections frequently involves the bone (Slack and Gerencser, 1975). Primary lung infections in cattle have been reported (Biever et al., 1969). The principal causative agent of bovine actinomycosis is *Actinomyces bovis* which may also occur in other animals, but has never been identified with certainty in material from human lesions (Bollinger, 1877; Schaal and Beaman, 1984; Slack and Gerencser, 1975). Whether *Actinomyces vaccimaxillae* (Hall et al., 2003a) is a second agent of typical bovine actinomycoses, remains to be proven. Bovine infections caused by *Actinomyces israelii sensu lato* (Cummins and Harris, 1959; King and Meyer, 1957; Pine et al., 1960) have also been reported, but remain to be confirmed using modern identification methods. The same is true for the isolation of *Actinomyces israelii* from different organs of dead, disabled, diseased, and dying sheep (Goda et al., 1986) and for the role of *Actinomyces viscosus sensu lato* as an etiologic agent of abortion in cattle (Okewole et al., 1989).

Actinomycosis in swine appears to involve udders, lungs, or other internal organs more frequently than neck and bones (Franke, 1973; Grässer, 1957; Magnusson, 1928; Thompson,

1933). "*Actinomyces suis*" (Franke, 1973; Grässer, 1957), *Actinomyces israelii* (Magnusson, 1928), and *Actinomyces viscosus sensu lato* (Georg et al., 1972) were previously incriminated as etiologic agents of pig infections. "*Actinomyces suis*" Franke (1973) has remained a *species incertae sedis* (Schaal, 1986b). The *Actinomyces*-like isolates described by Murakami et al. (1998, 1999) appeared to differ from other actinomycetes occurring in swine, but were not described in detail. Nevertheless, one of these pig species that had been isolated from purulent discharge and resembled "*Actinomyces suis*" phenotypically, was described as *Actinomyces hyovaginalis* (Collins et al., 1993). The strains which were used for the original description of *Actinomyces hyovaginalis* were exclusively derived from purulent vaginal secretions and aborted fetuses of pigs. These were recently assigned to the "vaginal" biovar of *Actinomyces hyovaginalis*. Similar strains that could not be differentiated on the basis of 16S rRNA gene sequence similarity and which had originated from necropsy samples from a variety of body sites of pigs differed from the original isolates in colony morphology and a few physiological characteristics, and were therefore designated members of the "general" biovar of the species. A new species from pig mastitis was named *Actinomyces suimastitidis* (Hoyles et al., 2001a). To make the situation even more complicated, an organism formerly known as *Eubacterium suis* (Wegienek and Reddy, 1982) was first transferred to the genus *Actinomyces* as *Actinomyces suis* (Ludwig et al., 1992), but later to the new genus *Actinobaculum* as *Actinobaculum suis* (Lawson et al., 1997). The latter organism has been isolated from cystitis and pyelonephritis as well as metritis of pregnant sows (Soltys, 1961; Soltys and Spratling, 1957), so that it remains to be clarified which of these species are the most important swine pathogens.

Canine and feline actinomycoses have been reported comparatively frequently (Davenport et al., 1974, 1975; Georg et al., 1972; Hardie and Barsanti, 1982; Kawamura et al., 2005; McGaughey et al., 1951; Moens and Verstraeten, 1980) and include infections of the soft tissue of the jaw, as well as thoracic and abdominal cases. Tail, scrotum, epidural space, meninges and brain, vertebrae and other bones, as well as the eye or heart valves, may also be affected (Barnes and Grahn, 2007; Bestetti et al., 1977; Couto et al., 2000; Johnson et al., 1984; Junius et al., 2004; Murakami et al., 1997). Most of the actinomycotic processes encountered in dogs and cats have been caused by *Actinomyces viscosus sensu lato* (Bestetti et al., 1977; Davenport et al., 1975; Davenport et al., 1974; Donohue and Brightman, 1995; Georg et al., 1972; Hardie and Barsanti, 1982; Johnson et al., 1984; Moens and Verstraeten, 1980; Murakami et al., 1997). *Actinomyces odontolyticus* was identified as the causative agent in a dog with a subvertebral mass and quadriplegia (Edwards et al., 1988) and a case of feline pyothorax was also assumed to have been caused by this species (Thompson et al., 1992).

Several additional *Actinomyces* species may be considered specific pathogens of dogs and cats: *Actinomyces hordeovulneris* (Buchanan et al., 1984) was first isolated from pleuritis, peritonitis, visceral abscesses, septic arthritis, and recurrent localized infections of dogs in California and was found to be frequently associated with injuries caused by awns of the grass *Hordeum*. These awns, which easily penetrate skin or mucous membranes, are propelled forward through the affected tissue with any adjacent muscle contraction, leaving trails of inflammation and necrosis (Brennan and Ihrke, 1983). Infections in dogs due to *Actinomyces hordeovulneris* have also been reported from Hungary (Pelle et al., 2000) and occasionally observed in Germany (Schaal, unpublished observation). The ability of this organism to produce L-phase variants spontaneously with coincident calcium deposition (Buchanan and Scott, 1984) was believed to be related to sulfur granule formation *in vivo*. As *Actinomyces hordeovulneris* can be recovered from the gingival margin of cats (Love et al., 1990), it appears sensible to assume that the organism is introduced into tissue from the oral cavity or intestinal tract when mucous membranes are penetrated by the awns or when the animal licks or bites a wound. In addition or alternatively, the pathogen may have spread hematogenously to the necrotic focus resulting from an awn injury.

Actinomyces bowdenii (Pascual et al., 1999) is another new species that has been isolated from canine and feline clinical specimens, in particular from an abscess under the mandible of a dog, from feline pleural fluid, from a canine neck abscess, and from a pyogranuloma in a dog. All of these isolates were found to be associated with aerobically or anaerobically growing concomitant organisms such as *Pasteurella multocida*, *Prevotella* species, or *Fusobacterium* species. *Actinomyces canis* (Hoyles et al., 2000) has been recovered in mixed culture with other bacteria from the vagina and pus specimens of dogs. The two strains identified so far as *Actinomyces catuli* (Hoyles et al., 2001b) were of similar origin as was the single isolate of *Actinomyces coleocanis* (Hoyles et al., 2002a) from vaginal secretions of a cocker spaniel dog together with "*Corynebacterium genitalium*". Even in a case of mitral valve endocarditis in a labrador retriever, an *Actinomyces* species, namely *Actinomyces turicensis*, was identified as causal agent (Junius et al., 2004).

Actinomycosis-like lesions have been reported from sheep, goats, horses, deer, moose, antelope, mountain sheep, gazelles, and even from an arctic fox (Raju et al., 1986; Slack and Gerencser, 1975; Snyder et al., 1987). However, detailed information on the *Actinomyces* species etiologically involved is sparse. In one case of pyogenic granulomas found in the abdomen of a mandrill, *Actinomyces israelii* was identified as the etiologic agent using immunofluorescence (Altman and Small, 1973). *Actinomyces marimammalium* (Hoyles et al., 2001c) was isolated from two dead seals and a dead porpoise, but its pathogenic potential has not been determined yet.

Actinomyces viscosus sensu stricto ("hamster strains of *Actinomyces viscosus*") was recovered from dental plaque of hamsters with naturally occurring periodontal disease (Howell, 1953; Jordan and Keyes, 1964, 1965; Syed et al., 1981) indicating that this organism apparently contributes to the development of this disease in hamsters.

Various domestic and laboratory animals including horses, cattle, sheep, goats, pigs, dogs, rabbits, guinea pigs, rats, hamsters, and mice have been used to produce experimental infections with *Actinomyces* species (Slack and Gerencser, 1975). After intraperitoneal, intravenous, or subcutaneous injection, *Actinomyces bovis*, *Actinomyces israelii sensu lato*, *Actinomyces naeslundii sensu lato*, *Actinomyces odontolyticus*, and *Actinomyces viscosus sensu lato* were all found to cause abscess formation which resembled, at least to some extent, naturally occurring actinomycotic lesions (Schaal, 1986b). However, progressive inflammatory processes rarely developed from these abscesses and the infected animals usually survived. Only certain rough strains of *Actinomyces israelii* were found to produce slowly

progressive chronic infections extending into other body areas which finally led to the animals' death (Behbehani and Jordan, 1982). Furthermore, detailed histopathological examinations showed that the actinomycotic lesions produced by *Actinomyces israelii* in mice differed considerably from those following challenge with *Actinomyces naeslundii sensu lato* or *Actinomyces viscosus sensu lato* (Behbehani et al., 1983a). These and other findings indicate that the virulence of *Actinomyces* species apparently varies between both species and individual strains, and the susceptibility of the test animals may also vary considerably (Beaman et al., 1979; Coleman and Georg, 1969; Georg et al., 1972; Georg and Coleman, 1970; Pine et al., 1960; Wolff and Israel, 1891). In addition, it has been shown that the simultaneous challenge with *Actinomyces israelii* and *Eikenella corrodens* decreased the minimal infecting dose of *Actinomyces israelii* markedly stressing the role of concomitant bacteria in the naturally occurring disease (Jordan et al., 1984). Similar results were obtained when *Actinomyces israelii* cells were packed in alginate gel particles as this led to the formation of structures comparable with sulfur granules (Sumita et al., 1998). Using an ischemic mouse thigh model for demonstration of pathogenicity, Chatterjee and Chakraborti (1989) found that *Actinomyces naeslundii sensu lato*, under these experimental conditions, exhibited an especially high degree of virulence, causing death in all test animals within 24 hours. Mice and hamsters appear to be the most suitable laboratory animals for pathogenicity studies of fermentative actinomycetes (Slack and Gerencser, 1975), although an animal model satisfactorily resembling naturally occurring actinomycoses has not been developed.

The only animal *Actinomyces* species that appear to be nonpathogenic are *Actinomyces denticolens*, *Actinomyces howellii*, *Actinomyces ruminicola*, and *Actinomyces slackii* although detailed pathogenicity studies with these organisms have not been performed (An et al., 2006; Dent and Williams, 1984b, 1984c, 1986).

Numerous animal experiments were undertaken on the ability of *Actinomyces* species to colonize teeth (Crawford and Clark, 1986; de Jong et al., 1983) and on their role in the development of caries and periodontitis. After oral inoculation, *Actinomyces naeslundii* (human strains) and *Actinomyces viscosus* (human and hamster strains) were shown to produce periodontal disease with alveolar bone loss and/or root surface caries in hamsters (Hernandez, 1985; Jordan and Keyes, 1964; Kametaka et al., 1989), as well as in conventional and gnotobiotic rats (Brecher and van Houte, 1979; Brecher et al., 1978; Burckhardt et al., 1981; Crawford et al., 1978; Firestone et al., 1987; Jordan et al., 1965; Llory et al., 1971; Shakespeare et al., 1985; Socransky et al., 1970). Similar observations were made with *Actinomyces israelii* orally implanted into gnotobiotic rats: this organism produced extensive plaque formation accompanied by root surface caries and bacterial invasion of the pulp (Behbehani et al., 1983b).

The factors of pathogenicity and/or virulence which enable *Actinomyces* species to invade tissue and to cause necrosis and abscess formation remain to be elucidated in detail. In contrast, *Actinomyces naeslundii sensu lato*, *Actinomyces viscosus sensu lato*, and possibly *Actinomyces israelii sensu lato*, and *Actinomyces odontolyticus* were shown to possess a large variety of properties which apparently contribute to the formation of dental plaque and to the development of periodontal disease, gingivitis, and caries. Adherence and coaggregation mechanisms involved in plaque formation have already been described. The same is true for certain surface components which may represent virulence antigens. In addition, *Actinomyces naeslundii sensu lato* and *Actinomyces viscosus sensu lato* were found to form a food chain with *Veillonella* (Distler and Kröncke, 1981; Distler et al., 1980), to produce chemotactic effects, a polyclonal B-cell activator and a stimulus for lysosome release in polymorphonuclear phagocytes, to induce release of mediators of inflammation and immunoglobulin production, and to mark fibroblasts for immune-mediated damage (Burckhardt, 1978; Clagett et al., 1980; Engel et al., 1976; Engel et al., 1978; Mangan and Lopatin, 1981; Taichman et al., 1978; Wicken et al., 1978).

Ecology. All of the recognized *Actinomyces* species appear to occur primarily as normal inhabitants of the mucosal surfaces of man and other homoiothermic animals although this has not been proven definitely for all of them. Thus, the typical pathogenic species may be considered facultative pathogens which invade the body of their hosts endogenously and are not transmissible (exception: "punch actinomycosis" following human bites).

The oral cavity of man and animals is obviously the principal natural habitat of members of the genus *Actinomyces*. There, they colonize teeth and oral mucosal surfaces, are able to resist natural and artificial cleaning mechanisms and utilize organic compounds present in the oral cavity as nutritional sources. Furthermore, they show complex interactions with other oral microbes thereby being involved in the formation of dental plaque. Thus, dental plaque or calculus and saliva are the materials from which actinomycetes can consistently be recovered by either immunofluorescence or culture (Bergey, 1907; Emmons, 1936, 1938; Ennever et al., 1951; Howell et al., 1959; Morris, 1954; Naeslund, 1925; Rosebury et al., 1944; Slack, 1942; Slack and Gerencser, 1975; Sullivan and Goldsworthy, 1940). *Actinomyces israelii sensu lato*, *Actinomyces naeslundii sensu lato*, *Actinomyces odontolyticus*, and *Actinomyces viscosus sensu lato* have all been identified in such samples since the early days of bacteriology (Batty, 1958; Collins et al., 1973; Ellen et al., 1978; Gerencser and Slack, 1969; Hill et al., 1977; Howell et al., 1959, 1962; Lentze, 1948; Naeslund, 1925; Russell and Melville, 1978; Slack, 1942; Slack et al., 1971; Snyder et al., 1967; Socransky, 1970; Sutter, 1984; Thompson and Lovestedt, 1951). Human periodontal sulci appear to be the principal habitat of *Actinomyces meyeri* (Cato et al., 1984).

Gram-stain-positive, facultative and anaerobic bacteria were reported to constitute 15–29% of the cultivable flora in saliva and on the tongue, 40% of that in dental plaque and 35% of that in the gingival crevice (Socransky and Manganiello, 1971). Actinomycetes amounted to about 40% of the cultivable organisms in plaque (Brown et al., 1986; Ellen et al., 1985; Loesche et al., 1972; Nyvad and Fejerskov, 1989). The total count of fermentative actinomycetes in human dental calculus was estimated at 1.9×10^7 cells per gram wet weight (Collins et al., 1973). For individual species, the counts were: *Actinomyces israelii sensu lato*, 6.32×10^6; *Actinomyces naeslundii sensu lato*, 5.37×10^6; *Actinomyces odontolyticus*, 0.1×10^6; *Actinomyces viscosus sensu lato*, 4.39×10^6; when no signs of inflammation were present. In patients with periodontal disease, the respective figures were: *Actinomyces israelii sensu lato*, 14.01×10^6; *Actinomyces naeslundii sensu lato*, 2.38×10^6; *Actinomyces*

odontolyticus, 1.26×10^6; *Actinomyces viscosus sensu lato*, 4.39×10^6 (Collins et al., 1973).

The numbers of actinomycetes in the oral environment may be influenced by inhibitory factors released by other bacteria (Rogers et al., 1978), by fluorides or other measures for preventing caries (Beighton and McDougall, 1977; Johansen et al., 1997; Phan et al., 2000), or by the eating habits of the host (Minah et al., 1985; Pfister et al., 1984). Furthermore, actinomycete bacteriophages with high host cell specificity may play an active role in the oral microbial ecology (Tylenda et al., 1985a). In any case, the qualitative and quantitative composition of the oral microflora differs from the saliva (in the planktonic state) to the mucosal surface (in the biofilm state), from the saliva to the tooth surface (in the biofilm state), and even from tooth to tooth and at different sites on one single tooth.

Recent studies on the oral colonization with *Actinomyces* species in healthy infants between the 2nd and 24th month of their life-time have provided insight into the composition of the actinomycete oral flora in relation to the early age period (Sarkonen et al., 2000). Within the first two years, the relative frequency of the total actinomycete flora increased from 31% to 97%. *Actinomyces odontolyticus* appeared to be the most prominent colonizer at all of the sampling occasions (after 2, 6, 12, 18, and 24 months of age). *Actinomyces naeslundii sensu lato* was also commonly encountered but not before the age of one year, as this species has been regarded one of the pioneer colonizers of tooth surfaces (Kolenbrander et al., 1999). *Actinomyces graevenitzii* was also rather regularly identified in the mouths of children (Sarkonen et al., 2000) as were *Actinomyces georgiae*, *Actinomyces gerencseriae*, *Actinomyces israelii*, and *Actinomyces viscosus sensu lato* in the oral cavity of children and in gingival crevices of periodontally healthy adults (Cato et al., 1984; Johnson et al., 1990).

Actinomyces israelii sensu lato, *Actinomyces naeslundii sensu lato* and possibly other *Actinomyces* species have also been demonstrated in human tonsils (Blank and Georg, 1968; Emmons, 1938; Garcia Ramos et al., 1984; Grüner, 1969; Hotchi and Schwarz, 1972). Occasionally, *Actinomyces viscosus sensu lato* and other *Actinomyces* species may also be recovered from the uninfected conjunctiva and/or cornea (Jones and Robinson, 1977; Schaal and Beaman, 1984).

Information on the occurrence of *Actinomyces* species as normal inhabitants of the human intestinal tract is very scarce although cases of abdominal actinomycoses that developed endogenously after bowel surgery, appendix perforation, or injuries of the intestine suggest that these organisms might form a small but significant component of the intestinal flora. The observations of Minsker and Moskovskaya (1979) who recovered *Actinomyces israelii* from 60.3% of diseased appendices examined support this view. The same is true for the findings of Fritsche ((1964b) and unpublished observations) who was able to isolate *Actinomyces israelii* from stool specimens of healthy individuals when using strong selective measures. Noack-Loebel and coworkers (1983), when comparing the composition of the fecal flora in two groups of children of primary school age, reported that the numbers of fermentative actinomycetes were significantly influenced by individual dietary habits: Children with a lacto-ovo-vegetarian diet presented with increased numbers of fermentative actinomycetes along with increased *Bifidobacterium* and *Enterobacteriaceae* counts as compared to school children with normal, *ad libitum* diets. Furthermore, studies on the composition of the microflora in duodenal and jejunal fluids suggest that *Actinomyces* species frequently form an important component of the cultivable flora of the upper intestinal tract (Bernhardt and Knoke, 1984; Justesen et al., 1984a; Justesen et al., 1984b). However, as the actinomycetes occurred together with other oral bacteria, it remains to be determined whether they represent true indigenous microbiota of the upper small intestine or simply "washed-down" oral organisms.

Previously, the vagina, the cervical canal, or the *cavum uteri* were not considered natural habitats of fermentative actinomycetes (Hanf and Hanf, 1955). Also in more recent studies, it was often not possible to demonstrate these organisms in healthy women who did not use IUCDs (Jones et al., 1983). However, other reports indicate that at least *Actinomyces israelii* and *Propionibacterium propionicum* may occur in the female genital tract independently of the use of IUCDs. When IUCDs were not used, the percentage of positive specimens ranged from 3% for *Actinomyces israelii sensu lato* (Persson et al., 1983) and 19% for *Actinomyces* species in general (Grice and Hafiz, 1983) to more than 70% for *Actinomyces israelii sensu lato* alone (Persson and Holmberg, 1984). In women wearing IUCDs or vaginal pessaries, it is usually easier to demonstrate these organisms either in cervicovaginal secretions or on the IUCD and, in particular, on its thread reaching from the *cavum uteri* to the vagina (Pine et al., 1985).

Nevertheless, the isolation or microscopic demonstration rates of actinomycetes in materials from the female genital tract varied widely between 0% (Schiffer et al., 1978) to more than 80% (Duguid et al., 1982; Eibach et al., 1989, 1992; Grice and Hafiz, 1983; Gupta et al., 1978; Gupta et al., 1976; Hager et al., 1979; Jarvis, 1985; Persson et al., 1983; Pine et al., 1981; Schaal and Pulverer, 1984; Traynor et al., 1981; Valicenti et al., 1982). These differing results indicate that sensitivity and specificity of the detection methods used are apparently not comparable and that, in addition, there might also exist regional, ethnic, social, or even religious differences. Furthermore, it remains to be clarified whether (as with oral cavity actinomycetes) genital actinomycetes also occur in association with amoebae (Arroyo and Quinn, 1989).

Apart from *Actinomyces israelii* and *Propionibacterium propionicum*, several additional *Actinomyces* species have been identified in specimens from the female genital tract. These included *Actinomyces cardiffensis*, *Actinomyces gerencseriae*, *Actinomyces meyeri*, *Actinomyces naeslundii sensu lato*, *Actinomyces odontolyticus*, and *Actinomyces viscosus sensu lato* (Eibach et al., 1989, 1992; Hall et al., 2002; Mitchell and Crow, 1984; Schaal and Pulverer, 1984; Schaal and Lee, 1992). Occasionally, two different *Actinomyces* species such as *Actinomyces israelii* plus *Actinomyces oris* or *Actinomyces israelii* plus *Actinomyces odontolyticus* may be isolated from a single genital specimen (Schaal, unpublished observation).

The occurrence of *Actinomyces* species in healthy animals is less well documented. Nevertheless, *Actinomyces viscosus* has been isolated from subgingival plaque of hamsters and from cervical plaque of rats (Bellack and Jordan, 1972; Howell, 1963; Howell and Jordan, 1963; Jordan and Keyes, 1964, 1965). Beighton (1985) in a study on the microbial colonization of the mouths of neonatal monkeys (*Macaca fascicularis*) found that *Actinomyces naeslundii sensu lato* and, to a lesser extent, *Actinomyces viscosus sensu lato* was established in the dental plaque of

these animals during the period of breast-feeding, suggesting that these organisms belong to the basic plaque flora which is altered by diet as the animals mature. Similarly, *Actinomyces denticolens*, *Actinomyces howellii*, and *Actinomyces slackii* were recovered from the dental plaque of cattle (Dent and Williams, 1984b, 1984c, 1986) as were *Actinomyces denticolens*, *Actinomyces hordeovulneris*, and *Actinomyces viscosus* from the gingival margin of healthy cats (Love et al., 1990). The natural habitat of the "vaginal" biovar of *Actinomyces hyovaginalis* was assumed to be the porcine genital tract (Collins et al., 1993); members of the "general" biovar of the species were recovered from many different body sites including the genital tract, but their natural habitat remains to be determined (Storms et al., 2002). It also remains to be definitely demonstrated to which biovar the isolates of *Actinomyces hyovaginalis* belong: these strains were recovered from a large number of tonsils of piglets before and after weaning (Baele et al., 2001). *Actinomyces ruminicola* originated from cattle rumen (An et al., 2006). Interestingly, however, Dent and Williams (1984c), in their study on the microflora of the bovine dental plaque, were not able to identify any *Actinomyces*-like isolate from healthy cattle as *Actinomyces bovis* so that the normal habitat of this species still awaits clarification. The same is true for essentially all of the other recently described animal *Actinomyces* species that were as yet solely derived from various clinical conditions.

Enrichment and isolation procedures

Although *Actinomyces* species are rather fastidious as far as the composition of the growth medium is concerned, several complex general purpose media have been found to be useful for isolating these organisms from clinical specimens, the oral cavity of man and animals, or from other natural habitats. These media include fluid thioglycolate broth (THIO) possibly supplemented with 0.1–0.2% (w/v) sterile rabbit serum, Todd–Hewitt broth supplemented with rabbit serum, peptone-yeast extract-glucose broth or agar (PYGB, PYGA), brain heart infusion broth or agar (BHIB, BHIA), cooked-meat medium, trypticase soy broth (TSB) or agar (TSA), heart infusion agar or TSA with 5% (w/v) defibrinated rabbit, sheep or horse blood, Columbia agar with 5% (w/v) defibrinated sheep or horse blood, brucella agar with or without addition of defibrinated blood, Schaedler broth or agar, or Fastidious Anaerobe Agar (Neogen, Lansing, MI, USA) (Schaal, 1986b; Schaal and Pulverer, 1981; Schaal et al., 2006; Slack and Gerencser, 1975).

For culturing *Actinomyces meyeri*, addition of vitamin K_1 to the medium (PYGB or PYGA) is obligatory and growth of this species is greatly stimulated by 0.02% (w/v) Tween 80 and a fermentable carbohydrate (Cato et al., 1984). Similarly, *Actinomyces hordeovulneris* requires addition of 10–20% (v/v) fetal bovine serum to the medium for adequate growth (Buchanan et al., 1984). *Actinomyces ruminicola* is best grown in PYGB or PYGA supplemented with 15% rumen fluid. Furthermore, this organism requires elevated growth temperatures between 39 and 46°C (An et al., 2006). All of the other *Actinomyces* species grow well at 36±1°C (Schaal, 1986b; Schaal et al., 2006; Slack and Gerencser, 1975).

For diagnosing actinomycete infections, which usually contain numerous concomitant other bacteria, as for detecting actinomycetes in dental plaque or similar materials, broth enrichment media are less suitable because the slowly growing actinomycetes are mostly overgrown by the concomitant or indigenous flora. Consequently, they are easily overlooked or their growth is even completely suppressed. The general purpose agar media mentioned above, especially when supplemented with defibrinated blood, may pose problems in that characteristic filamentous colonies (of the typical human pathogens) may not develop or may not be discernible because abundant growth of additional microbes often masks actinomycete growth (Schaal, 1986b; Schaal et al., 2006). Furthermore, non-transparency of the medium due to blood cells hampers the recognition of filamentous growth. The detection of the classical pathogenic species can be improved by employing complex semisynthetic media such as those of Pine and Watson (1959) or Heinrich and Korth (1967) (CC-medium: see footnote †). The latter media promote the development of characteristic filamentous micro- and macrocolonies and reduce growth of the concomitant flora. In addition, CC-medium plates facilitate the recognition of spider-like colonies when viewed under a microscope at low magnifications (80–100×) (Figures 28–32). If a long-distance objective is used, cultures can be examined through the transparent medium without opening sealed plates such as Fortner plates (see below) (Lentze, 1938a).

Although Tarozzi broth (see footnote ‡) is in principle an enrichment medium, characteristic snow ball or cotton pad-like colonies of pathogenic actinomycetes can often be seen on the surface of the liver piece (Figure 56) especially when they are present together with *Aggregatibacter actinomycetemcomitans*. These colonies can easily be picked for subculture using a Pasteur pipette. The above procedures are particularly useful inasmuch as studies on the selective isolation of *Actinomyces* species from pus specimens have rarely been performed. Columbia blood agar (with defibrinated horse blood) supplemented with mupirocin (128 mg/l) and metronidazole (2.5 mg/l) (MMBA) was reported to greatly increase the percentage of *Actinomyces* isolates (up to 71% of specimens) from dentofacial infections as well as from IUCDs (Lewis et al., 1995). However, further studies are needed to decide whether these additional actinomycete isolates are of etiological relevance because they included relatively large proportions of *Actinomyces meyeri*, *Actinomyces naeslundii*, *Actinomyces odontolyticus*, and *Actinomyces viscosus* which are not known as frequent causative agents of cervicofacial actinomycoses.

Selective principles have, however, been used successfully for isolating and enumerating actinomycetes from dental plaque or saliva. The four different selective media devised for this purpose differ to some extent in their selectivity against other oral microbes and in their inhibitory activity to individual *Actinomyces* species. Ellen and Balcerzak-Raczkowski (1975) described a partially selective medium for *Actinomyces naeslundii sensu lato* (human strains) and *Actinomyces viscosus sensu lato* (CNAC-20 medium*). After inoculation, the medium is incubated at 36±1°C in 90% air and 10% CO_2 in order to encourage growth of *Actinomyces johnsonii*, *Actinomyces naeslundii*, and *Actinomyces oris* while inhibiting the more anaerobic actinomycetes and other Gram-stain-

*CNAC-20 Medium (Ellen and Balcerzak-Raczkowski, 1977): I. Basal culture medium: Columbia CNA agar base (Difco). II. Preparation of the selective medium: Add $3CdSO_4\ 8H_2O$ to the basal medium to give a final concentration of 20 mg/ml. Autoclave. Incubate plates containing the medium at 36±1°C in 90% air and 10% CO_2.

positive oral anaerobes. The medium of Beighton and Colman* (1976) was developed for the selective isolation of oral actinomycetes in general, while that of Kornman and Loesche (1978) (GMC Medium†) was designed for the preferential detection of *Actinomyces naeslundii* and *Actinomyces viscosus (Actinomyces oris* and *Actinomyces johnsonii*). The latter medium was reported to allow 98% recovery of *Actinomyces viscosus sensu lato* (*Actinomyces oris* and *Actinomyces johnsonii*) and 73% recovery of *Actinomyces naeslundii sensu lato* while suppressing 76% of the total count of other oral organisms. *Propionibacterium propionicum* also grew quite well on this medium whereas *Actinomyces israelii sensu lato* and *Actinomyces odontolyticus* were inhibited (Kornman and Loesche, 1978). Zylber and Jordan (1982) described a medium for the detection and enumeration of *Actinomyces naeslundii sensu lato* and *Actinomyces viscosus sensu lato* (CFAT medium‡) which contains a complex mixture of antimicrobial substances. This medium gave considerably higher counts for *Actinomyces naeslundii* and *Actinomyces viscosus* than the FC medium and the CNAC-20 medium, respectively. The major interfering organisms on this medium were *Aerococcus* and *Rothia* species, whereas FC and CNAC-20 media were less selective and supported growth of significant numbers of other oral microbes (1982).

A different means of selective isolation of fermentative actinomycetes, especially *Actinomyces israelii*, from heavily contaminated materials such as stool specimens has been described by Fritsche** (1964b). This method utilizes the inactivating effect of toluene on Gram-stain-negative bacteria and, although the colony morphology of actinomycetes may be altered upon primary isolation, it was found to be very useful for separating *Actinomyces israelii* from contaminating *Enterobacteriaceae*.

Occasionally, commercial disks for antibiotic susceptibility testing can aid in obtaining pure *Actinomyces* subcultures. Depending on the contaminating organisms, disks containing metronidazole, colistin, or nalidixic acid in common concentrations may be employed. From their inhibition zones, actinomycete colonies can often be picked easily and transferred to another plate.

To avoid loss of viability of actinomycetes by oxygen contact, clinical samples, as well as samples for ecological studies, should be inoculated onto suitable culture media as soon as possible. Alternatively, reduced transport media should be used. For clinical specimens, the Stuart transport medium or its commercial modifications (e.g. Port-A-Cul, Becton Dickinson) has proved satisfactory (Loesche et al., 1972; Syed and Loesche, 1972). For ecological studies, the reduced transport fluid (RTF††) of Syed and Loesche (1972) may be more appropriate. This medium can also be used for sonic or mechanical dispersal of more solid materials.

Anaerobic growth conditions may be obtained by using a Torbal anaerobic jar with a gas mixture of N_2-H_2-CO_2 (80:10:10%), a GasPak jar (Becton Dickinson) with a H_2-CO_2-generating envelope and an active catalyst or an anaerobic chamber or a glove box with appropriate gas mixtures. Use of pre-reduced, anaerobically sterilized media or the roll-tube technique are usually not necessary. The capnophilic members of the genus may be grown in a candle jar or in a GasPak jar with a CO_2-generating unit. For routine use in the diagnostic laboratory, Fortner's method‡‡ (Fortner, 1928, 1929) has proved especially useful and reliable, because cultures can be checked for growth without disturbing the gaseous conditions and the semi-anaerobic atmosphere of this technique allows growth of essentially all of the pathogenic *Actinomyces* species including the more aerophilic ones (Schaal and Pulverer, 1981).

All *Actinomyces* cultures apart from those of *Actinomyces ruminicola* are incubated at 35–37°C and should be checked at 2, 4, 7, and 14 d for growth of characteristic micro- or macrocolonies, respectively (Schaal, 1986b; Schaal et al., 2006). Optimum growth temperature of *Actinomyces ruminicola* is 46°C (An et al., 2006).

Maintenance procedures

Actinomyces species may cause problems in continuous subculture, especially when the growth medium contains significant

*Selective medium for oral actinomycetes (Beighton and Colman, 1976): I. Basal culture medium (BYS medium): Brain heart infusion broth, 3.7 g; yeast extract powder, 0.5 g; polyvinylpyrrolidone, 1.0 g; cysteine-HCl, 0.1 g; agar, 1.5 g. Add these ingredients to 100 ml of distilled water and autoclave for 15 min at 121°C. Cool to 45°C and supplement with 5 ml of sterile horse serum. II. Selective enrichment medium (FC medium), containing per 100 ml BYS medium: NaF solution (25 mg/ml), 1.0 ml; colistin sulfate solution (1 mg/ml), 0.5 ml. The inhibitor solutions are sterilized separately by autoclaving at 121°C for 15 min before they are added to the BYS medium.

†Selective GMC medium for (human) *Actinomyces viscosus* and *Actinomyces naeslun*dii strains (Kornman and Loesche, 1978): I. Basal medium: The enriched gelatin agar of Syed (1976) is recommended, but other complex media may yield similar results. II. Selective medium, containing per liter basal medium: cadmium sulfate ($3CdSO_4$ $8H_2O$), 20 mg; metronidazole, 10 mg. Autoclave cadmium sulfate together with the basal medium. Cool to 45–50°C and add a filter sterilized solution of metronidazole aseptically.

‡Selective medium for detection and enumeration of *Actinomyces viscosus* and *Actinomyces naeslundii* (CFAT medium) (Zylber and Jordan, 1982): Composition per liter: trypticase soy broth (Becton Dickinson), 30.0 g; glucose, 5.0 g; agar, 15.0 g; cadmium sulfate ($3CdSO_4$ $8H_2O$), 13.0 g; sodium fluoride (NaF), 80.0 mg; neutral acriflavin, 1.2 mg; potassium tellurite, 2.5 mg; basic fuchsin, 0.25 mg; defibrinated sheep blood, 50 ml. Adjust the final pH to 7.3.

**Selective isolation of Actinomyces israelii (Fritsche, 1964b): The specimen is suspended and dispersed in a suitable transport fluid (e.g., reduced transport fluid of Syed and Loesche (1972) or in thioglycolate broth). Add 1 ml of the suspension thus obtained to 1 ml of toluene in a screw-cap tube. Agitate the tube on a mechanical shaker (high speed) for 20–25 min. Remove the watery suspension at the bottom of the tube carefully with a capillary pipette and add it to 10 ml of the same transport fluid or broth. Remove remaining droplets of toluene on the surface of the fluid with a Bunsen flame. Centrifuge so that the *Actinomyces* cells can settle and streak the sediment onto non-selective agar media.

††Reduced transport fluid (RTF) (Syed and Loesche, 1972): I. Stock mineral salts solution No. 1, containing: K_2HPO_4, 0.6% (w/v). II. Stock mineral salts solution No. 2, containing: NaCl, 1.2% (w/v), $(NH_4)_2SO_4$, 1.2% (w/v), KH_2PO_4, 0.6% (w/v), $MgSO_4$, 0.25% (w/v). III. Final transport medium, containing per liter: Stock solution No. 1, 75 ml; stock solution No. 2, 75 ml; 0.1 M ethylenediaminetetraacetate solution, 10 ml; 8% Na_2CO_3 solution, 5 ml; 1% dithiothreitol solution (freshly prepared), 20 ml; 0.1% resazurin solution (optional), 1 ml; distilled water, 814 ml. Sterilize by membrane filtration (pore size, 0.22 µm). Dispense into 16 × 125 mm screw-cap tubes (dilution tubes) and 18 × 150 mm test tubes (sample-collection tubes). The pH should be 8±0.2 without adjustment, and it decreases to pH 7 in 48 h in the anaerobic glove box atmosphere (85% N_2, 10% H_2, 5% CO_2).

‡‡Fortner's method for obtaining (semi-)anaerobic growth conditions (Fortner, 1928): Use only agar media, preferably transparent ones, in glass Petri dishes! Inoculate one-half to two-thirds of the agar surface with the material to be examined or with the strain to be subcultured. Inoculate the remaining agar surface heavily with a *Serratia marcescens* culture using a spatula. Place the dish upside down upon a glass sheet of appropriate size. Fix and seal the dish with plasticine to make the system airtight. Incubate at 36±1°C for up to 14 days and examine for microbial growth and especially filamentous colonies every 2 days using either a hand lens or a microscope equipped with a long-distance 10× objective in transmitted light without opening the plate. (Leakage in the plasticine seal which may occur when the plasticine is too brittle or the base glass sheet is damp, can easily be detected because pigment-producing strains of *Serratia marcescens* form nonpigmented growth under shortage of oxygen, but become red when the system is not completely airtight.)

amounts of fermentable carbohydrates which rapidly lead to toxic low pH values (Schaal et al., 2006). In addition, cultures of these organisms are liable to contamination with propionibacteria which are often difficult to recognize.

Cultures in routine use may be maintained by weekly transfer in thioglycolate broth containing 0.2% (v/v) rabbit serum or by monthly transfer on Fortner plates containing CC-medium or in Tarozzi broth. For longer preservation (up to 1 year) and long-term storage (up to 10 years or longer) freezing or lyophilization is recommended (Schaal, 1986a, 1986b; Schaal et al., 2006; Slack and Gerencser, 1975).

For freezing, 3–5 ml brain heart infusion broth in screw-capped tubes are inoculated with 0.1 ml of a fresh broth culture or a dense suspension of the organism in BHIB prepared from a 3–7 d-old agar culture. The inoculated broth is incubated at 36±1°C (*Actinomyces ruminicola*: 46°C) for 3–5 d under appropriate gaseous conditions (in air, usually with added CO_2, or in an anaerobic jar or chamber) with the caps loosened. As soon as good growth is obtained, the caps are tightened and the tubes are placed in a freezer at −70°C.

For freeze-drying, the sediment of broth cultures or – preferably – biomass scraped from agar plates or dialysis membranes are suspended in sterilized skim milk or lyophilization medium* and lyophilized using standard techniques (Schaal, 1986a, 1986b; Schaal et al., 2006; Slack and Gerencser, 1975). Lyophilized *Actinomyces* cultures have been reported to remain viable for 10 years when stored at room temperature (Slack and Gerencser, 1975). Most lyophilisates can even be revived after 20–30 years of storage at room temperature (Schaal et al., 2006). Frozen broth cultures will survive for at least 1 year.

Procedures for testing of special characters

Pus from actinomycotic abscesses or the purulent discharge from sinus tracts occasionally contain large amounts of sulfur granules which give these exudates the macroscopic appearance of semolina soup (Lentze, 1969; Schaal, 1984; Schaal et al., 2006b). More often, however, sulfur granules are not immediately apparent so that the laboratory personnel have specifically to search for hard, yellowish to brownish to reddish particles of up to 1 mm in diameter in the purulent discharge. As pus specimens may contain other structures that resemble sulfur granules in terms of size, color, and texture (e.g., granules of actinomycetomas), the nature of suspected sulfur granules has to be confirmed by subsequent microscopic examination. For this purpose, the granule is placed in the middle of a microscopic glass slide, one drop of 1% methylene blue solution is added, and the slide is covered with a cover glass. After having gently pressed down the cover slip, the preparation is viewed under the microscope in transmitted light at about 100× magnification. Under these conditions, the sulfur granules appear as cauliflower-like structures which, because of the reducing capacity, retain an unstained center whereas surrounding leukocytes and peripheral hyphae take up the blue color of the dye. After slightly harder pressing onto the coverslip, sulfur granules usually disintegrate into spherical or partially spherical elements that represent actinomycete colonies formed *in vivo* (Schaal, 1992a; Schaal et al., 2006).

For detailed microscopic examination at high magnifications, the particles are crushed between two slides and the smears thus obtained are Gram-stained and viewed at 800–1000× magnification. Stained smears of sulfur granules typically show nests of Gram-stain-positive, branched, unevenly curved and irregularly stained filaments and rods. In addition, various other Gram-stain-positive and Gram-stain-negative bacteria together with leukocytes can be seen indicating the presence of the concomitant flora.

The morphology of *Actinomyces* microcolonies is demonstrated most easily on BHIA or CC-medium plates that have been incubated for 18–24 h. The colonies may be observed directly on the medium (Erikson, 1940; Lentze, 1938a) within the plate, or small sections of agar may be removed and placed on a glass slide to facilitate the use of the microscope. Magnifications of 100–400× are usually satisfactory (Slack and Gerencser, 1975). If higher magnification is required for the detailed study of young microcolonies (oil immersion), a slide culture technique† together with lactophenol cotton-blue staining of the microbes may be more appropriate. Mature colonies are observed on BHIA or blood agar plates after 5–14 d of incubation using a hand lens or a dissecting microscope.

Tests for oxygen requirements are performed according to Slack and Gerencser‡ (1975). The analysis of the acid end products resulting from the fermentative carbohydrate metabolism of *Actinomyces* species is usually performed using gas-liquid chromatography (GLC) according to the procedures described in the VPI Anaerobe Laboratory Manual (Holdeman et al.,

*Lyophilization medium for long-term storage of Actinomyces species (Schaal, 1986a; Schaal et al., 2006): Brain heart infusion broth, 1 part; horse or fetal bovine serum, 1 part; sucrose to give a final concentration of 7% (w/v) in the whole mixture.

†Slide culture technique for the microscopic observation of filamentous actinomycete microcolonies (Schaal, 1986b; Schaal et al., 2006): I. Preparation of slide cultures: Cover glass slides with a thin layer of BHIA by briefly immersing one surface of the slide in melted BHIA at about 50°C. For solidification of the agar, transfer the slide immediately to a sterile Petri dish containing a sheet of filter paper moistened with sterile water. Perform all of the necessary manipulations under strict aseptic conditions (a clean bench). II. Culture: Inoculate the agar-coated slides with the strain to be examined using a very thin, freshly drawn glass filament. Incubate the slide cultures inside their moist dishes in an anaerobic or CO_2-enriched atmosphere. III. Microscopy: After 5-10-20 h incubation, the agar films are air-dried 10–15 min. Then, 1–3 drops of lactophenol-cotton blue mounting fluid (formula see below) are placed in the center of the agar layer and covered with a cover glass. View under the microscope using phase-contrast equipment and objectives between 50× and 100× magnification. IV. Lactophenol-cotton blue mounting fluid (Hendrickson, 1985): Phenol crystals, 20 g; lactic acid, 20 ml; glycerol, 40 ml; distilled water, 20 ml. Dissolve the ingredients by heating the flask in a hot water bath. Add 0.05 g of cotton blue (Poirier blue).

‡Tests for oxygen requirements (Slack and Gerencser, 1975): I. Agar slant method: Suspend cells obtained from about 3-day-old cultures in 0.85% saline and adjust the suspension to a density matching the MacFarland 3 standard. Inoculate eight BHIA slants in cotton-plugged tubes with the suspension using a capillary pipette. Incubate the slants, in duplicate, under the following conditions: 1. For aerobic growth, place 2 slants with the original cotton plugs directly in the incubator. 2. For aerobic conditions with added CO_2, clip off the cotton plugs of two tubes and push the remaining parts into the tube to just above the slant. Place small pieces of absorbent cotton on top of the plugs, add 5 drops of 10% Na_2CO_3 and 5 drops of 1 M KH_2PO_4 to each tube and close it immediately with a rubber stopper. 3. For anaerobic conditions with added CO_2, tubes are prepared as under 2. Then, add 5 drops of 10% Na_2CO_3 and 5 drops of pyrogallol solution (100 g pyrogallic acid in 150 ml of distilled water) to the absorbent cotton. 4. For anaerobic conditions without CO_2, add 5 drops of 10% KOH and 5 drops of pyrogallol solution to the properly prepared tubes (see 2). Record results after 3 and 7 days of incubation. If the growth in two corresponding tubes does not seem equal, the test has to be repeated. II. Agar deep method: Inoculate melted and cooled agar medium, while still liquid, with a suspension of the test organism using a capillary pipette. Push the tip of the pipette to the bottom of the tube and then withdraw slowly while expelling a drop of inoculum. Gently mix the agar by rotating the tube and allow the agar to solidify in an upright position. Read the results after 3 and 7 days of incubation by measuring the distance in millimeters between the surface and the zone of maximum growth.

1977), but more modern techniques such as high performance liquid chromatography (HPLC) may also be applied.

Comparatively simple modifications of several chemotaxonomic procedures have been developed that are easily applicable for identification purposes under diagnostic routine conditions (Alshamaony et al., 1977; Becker et al., 1964; Schaal, 1985b). This is especially true for the demonstration of diaminopimelic acid (A_2pm) or certain sugars in whole-cell hydrolysates, for the analysis of long-chain fatty acids and mycolic acids in whole-cell methanolysates, and for the determination of cytoplasmic polypeptide patterns. The former procedures have been greatly improved and facilitated by using commercially available cellulose-coated plastic sheets (Merck 5577) or silica gel-coated aluminum sheets (Merck 5554) for thin-layer chromatography (Kutzner, 1981; Schaal, 1985b, 1992b).

A prerequisite for the successful use of these techniques is the production of sufficient amounts of biomass that should not contain larger quantities of contaminating material from the culture media. Although this requirement is readily fulfilled by using carefully washed cell mass from fluid cultures, broth cultures of fermentative actinomycetes are particularly prone to microbial contamination. The latter is more easily controlled when the organisms are grown on solid media. However, the substrate mycelium of certain *Actinomyces* species may be difficult to remove from agar media so that special precautions have to be taken to secure cell mass without contaminating material from the medium. A suitable method for preparing biomass* of actinomycetes from agar cultures has been described by Schaal (1985b).

The demonstration of A_2pm in whole-cell hydrolysates can be achieved comparatively easily following the procedure of Schaal (1985b). The same is true for the determination of sugar patterns in whole-cell hydrolysates and the demonstration of mycolic acids. Analyses for the presence of A_2pm and/or mycolic acids are useful for differentiating *Propionibacterium propionicum*, *Corynebacterium matruchotii*, and other corynebacteria from members of the family *Actinomycetaceae*, while sugar patterns are helpful in differentiating *Actinomyces* species from each other. The analysis of long-chain fatty acids, isoprenoid quinones, and whole-cell protein patterns can be performed using the methods described in the literature (Collins and Jones, 1981; Dent and Williams, 1985; Jackman, 1985; Kroppenstedt, 1985; Mauff et al., 1981).

Whole-cell protein patterns have been widely used to demonstrate differences or relatedness between different collections of otherwise similar strains (Collins et al., 2000; Dent and Williams, 1985; Hoyles et al., 2000; Pascual Ramos et al., 1997a), thus possibly avoiding more laborious DNA–DNA hybridization experiments. Cell-free, more or less purified soluble protein fractions may be obtained by disintegrating bacterial cell suspension in a Mickle shaker (Mickle Engineering Ltd., Gomshall, UK) using grade 12 glass beads for 1.5 h or in a "French pressure cell" at 2200 p.s.i. and subsequent centrifugation in two steps at 20,000 to 150,000 g for 15 min and 1 h (Dent and Williams, 1985; Mauff et al., 1981). The cell-free extracts are then electrophoresed in 10% polyacrylamide-sodium dodecyl sulfate (SDS) gels as described by Swindlehurst et al. (1977). A comprehensive description of the methods useful for producing electrophoretic whole-organism protein fingerprints, including densitometric analysis and numerical analysis of the normalized protein profiles, has been published by Pot et al. (1994). Alternatively, sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) may be applied directly to packed *Actinomyces* cells incubated with an equal volume of a 6.4 M urea solution (McCormick et al., 1985). The patterns thus obtained consist of many more bands than those derived from cell-free extracts, but they appear to be reproducible and well suited for classification, identification, or typing purposes.

The catalase test when applied to fermentative actinomycetes is best performed by flooding aerobic growth on slants with 3% H_2O_2 and looking for active production of gas bubbles. Anaerobic cultures must be exposed to air for 30 min before testing. The presence of cytochrome oxidase may be demonstrated by the method of Deibel and Evans (1960).

The results of the other biochemical and physiological tests listed in Table 4, Table 5, Table 7, and Table 9 may be considerably influenced by the media and methods used (Scharfen, 1973; Schofield and Schaal, 1979a; Slack and Gerencser, 1975). The data compiled in the diagnostic and descriptive tables were chiefly derived from the techniques described by Slack and Gerencser (1975) and Schofield and Schaal (1979a, 1979b, 1980, 1981), as far as the species described before 1984 are concerned. The physiological characters of all of the other *Actinomyces* species described since 1984 were derived from the original descriptions of these species and, thus, based upon the methods reported in the respective publications.

The traditional procedure for testing acid production from carbon compounds (fermentation tests), as described by Slack and Gerencser (1975), is based on thioglycolate broth without glucose or indicator supplemented with glucose-free yeast extract (0.2%, w/v) and bromocresol purple (1% aqueous solution) added in 2-ml amounts to 1 liter of the medium. To this medium, low molecular-weight carbohydrates (mono- and disaccharides) are added to give final concentrations of 1% and other compounds are used at a concentration of 0.5%.

These and similar fluid media, when used in amounts of 1 ml or more, do not always yield satisfactory and reproducible results especially when highly filamentous strains are tested as was shown in several numerical taxonomic studies (Holmberg and Hallander, 1973; Holmberg and Nord, 1975; Melville, 1965). These problems are obviously due to the inadequacy of the inoculum size and growth rate of the organisms compared to the volume of the substrate-containing medium. In contrast, the results obtained from miniaturized test procedures (Buchanan et al., 1984; Schofield and Schaal, 1980; Schofield and Schaal, 1979a, 1979b, 1980, 1981) indicate that these modifications of the traditional physiological tests are much more suitable for testing fermentative actinomycetes. However, it has also been

*Preparation of biomass from fermentative actinomycetes for chemotaxonomic tests (Schaal, 1985b): I. Media: BHIB is usually satisfactory, but other media may also be used. II. Membrane culture technique: Place autoclaved sheets of dialysis tubing on top of the agar surface of BHIA plates and inoculate them with a heavy suspension of the test strain. Incubate the plates under suitable conditions (aerobically, under increased CO_2 tension, or anaerobically), until good growth is obtained. III. Preparation of cell mass: Remove the sheets of dialysis tubing with the bacterial growth on top with sterile forceps and transfer them to Erlenmeyer flasks containing either distilled water or saline and a few glass beads (~5 mm in diameter). Inactivate the organism either by adding formaldehyde or by heating the flask in a water bath at 75°C for 45 min. Wash growth off the sheets by shaking the flasks on a rotary shaker at 200-250 rpm and collect the cell mass by centrifugation. Wash the sediment in two changes of distilled water. Dry the cells by lyophilization (amino acid and sugar analyses may also be carried out on cells dried overnight in absolute ethanol).

TABLE 3. Characteristics differentiating the genus *Actinomyces* from other genera of the family *Actinomycetaceae* and morphologically related, fermentative organisms[a,b]

Characteristics	*Actinomyces*	*Arcanobacterium*	*Actinobaculum*	*Varibaculum*	*Mobiluncus*	*Propionibacterium*	*Corynebacterium*	*Bifidobacterium*	*Rothia*	*Lactobacillus*	*Eubacterium*	*Listeria*	*Gardnerella*	*Erysipelothrix*
Aerobic growth (with added CO_2)	D	+	D	–	–(+)	D	+	–	+	D	–	+	d	+
Curved rods with tapered ends	–	–	–	–	+	–	–	–	–	–	–	–	–	–
Gram reaction	+	+	+	+	v/–	+	+	+	+	+	+	+	+	+
Microcolonies filamentous	D	–	–	–	–	D	D	–	D	–	–	–	–	–
Catalase	D	–/+	–	–	–	D	+	–/D	+	–	–	+	–	–
Nitrate reduction	D	D	–	d	D	D	D	–	+	–	D	D	–	–
Cell-wall components:														
DL-A_2pm[c]	–	–	–	–	–	D	+	–	–	–/+	+/–	+	–	–
LL-A_2pm[c]	–	–	–	–	–	+/–	–	–	–	–	–	–	–	–
Ornithine	D	–	nr	nr	nr	–	nr	D	–	+/–	nr	–	nr	nr
Lysine	+	+	+	nr	nr	D	nr	D	+	+/–	nr	–	+	+
Aspartic acid	–(+)	–	nr	nr	nr	+?	nr	D	nr	+/–	nr	nr	+	nr
Glycine	–	–	nr	nr	nr	D	+	–	–	nr	nr	nr	+	nr
Glucose	D	+/–	nr	nr	+	+	+/–	nr	+	nr	nr	nr	nr	+
Galactose	D	–	+	nr	+	D	+	–	+	nr	nr	nr	nr	+
Rhamnose	D	+	+	nr	+	D	–	nr	–	nr	nr	nr	nr	nr
6-Deoxytalose	D	–	nr	nr	nr	–	–	nr	–	nr	nr	nr	nr	nr
Arabinose	–	–	nr	nr	nr	–	+	nr	–	nr	nr	–	nr	+
Mannose	D	–/+	+	nr	nr	+	(+)	–	nr	nr	nr	nr	nr	nr
Mycolic acids	–	–	–	–	–	–	+/–	–	–	–	–	–	–	–
Acyl type	Acetyl (glycolyl)	Acetyl	Acetyl	nr	nr	Acetyl	Acetyl	nr	nr	nr	nr	Acetyl	Acetyl	nr
End products from glucose fermentation:														
Acetic acid	+	+	+	–	+	+	+	+	(+)	(+)	+	+/–	+	+
Propionic acid	–	–	–	–	–	+	D	–	–	–	D	–	–	–
iso-Butyric acid	–	–	–	–	–	–	–	–	–	–	D	–	–	–
n-Butyric acid	–	–	–	–	–	–	–	–	–	–	D	–	–	–
iso-Valeric acid	–	–	–	–	–	D	–	–	–	–	D	–	–	–
n-Valeric acid	–	–	–	–	–	–	–	–	–	–	D	–	–	–
iso-Caproic acid	–	–	–	–	–	–	–	–	–	–	D	–	–	–
n-Caproic acid	–	–	–	–	–	–	–	–	–	–	D	–	–	–
Pyruvic acid	–	–	–	–	–	–	–	–	(+)	nr	nr	–	–	nr
Lactic acid	+	+	D	+	(+)	+	+	(+)	+	+	D	+	(+)	+
Succinic acid	+	+	(+)	+	+	D	D	+	(+)	(+)	D	+/–	(+)	+

Phospholipids[d]	DPG, PC, PI, PIM, (PG)	DPG, PG and several unknown phospholipids	DPG, PG, PC, PI, PIM	nr	nr	nr	PI, PIM	PG	nr	nr	nr	PG, DPG	nr	nr
Menaquinones[e]	MK-8, MK-9, MK-10, *methyl*-MK-10, MK-9(H_4), MK-10(H_4)	MK-9(H_4), MK-10(H_4), [MK-8(H_4), MK-11(H_4)]	MK-10(H_4)	nr	nr	MK-9(H_4)	MK-9(H_2), MK-8(H_2)	nr	MK-7	– (MK-8, MK-9)	nr	MK-7 (MK-6, MK-5)	–	nr
Cellular fatty acids[f]	S,U, (C),(I), (A)	S,U, (I),(A)	S,U	nr	S,U	S,U, (I),(A)	S,U,(T)	S,U	S,I,A	S,U, (C)	nr	S,A, I	S,U	S,U, (I),(A)
Motility	–	–	–	–	+	–	–	–	–	–/+	D	+	–	–
DNA G+C content (mol%)	55–70	48–65	55–57	51.7	49–55	59–66	51–63	55–67	47–53	32–53	30–55	36–38 (42)	42–44	36–40

[a] Symbols: +, positive/present; –, negative/absent; +/–, predominantly positive/present; –/+, predominantly negative/absent; (), weakly or irregularly positive/present; D, differences between different taxa; d, differences between strains within one species; v, variable; nr, not reported.

[b] Data compiled from Holdeman et al. (1977), Holländer and Pohl (1980), Schofield and Schaal (1981), Collins et al. (1982a, 1982b), Collins and Cummins, (1986a, 1986b), Cummins and Johnson (1986), Gerencser and Bowden (1986), Greenwood and Pickett (1986), Jones (1986), Jones and Collins (1986), Kandler and Weiss (1986), Moore and Holdeman (1986), Scardovi (1986), Schaal (1986a, 1986b), Seeliger and Jones (1986), Schaal et al. (2006).

[c] DL-A_2pm, *meso*-diaminopimelic acid; LL- A_2pm, LL-diaminopimelic acid.

[d] DPG, Diphosphatidylglycerol; PC, phosphatidylcholine; PI, phosphatidylinositol; PIM, phosphatidylinositol mannosides; PG, phosphatidylglycerol.

[e] Menaquinones with 7, 8, 9, 10, or 11 isoprene units and varying degrees of saturation.

[f] S, Straight-chain, saturated; U, unsaturated; A, anteiso-methyl-branched; I, iso-methyl-branched; C, cyclopropane ring; T, 10-methyl-branched (tuberculostearic acid).

shown that among the various miniaturized commercial systems, only the Minitek differentiation system (Becton Dickinson) is equally well applicable to all members of the genus *Actinomyces* (Schofield and Schaal, 1979b). As the Minitek system is no longer available on the market, a home-made modification* of this system can be used. A slightly modified procedure allows the reliable assessment of nitrate and nitrite reduction as well as of urease and arginine dihydrolase activities† (Schofield and Schaal, 1980, 1981). The agar plate method of Phillips (1976) has only sporadically been used for testing fermentation reactions of fermentative actinomycetes (Hall et al., 2005) so that its suitability remains to be proven.

Tests for enzymic activities detectable by means of chromogenic or fluorogenic substrates were shown to possess differential power when applied to classification and identification of fermentative actinomycetes (Brander and Jousimies-Somer, 1992; Casin et al., 1984; Kilian, 1978; Maiden et al., 1996; Sarkonen et al., 2001; Schofield and Schaal, 1981), but they were also found to be difficult to standardize because many of the enzymes which might be detected in this way are inducible so that the test results highly depend on the composition of the growth medium used for preparation of the inoculum.

Various commercial test systems which are based upon such enzyme tests completely or in part, have been used for the characterization and identification of *Actinomyces* species, in particular with the recently described species. These include the API ZYM, RapID ANA II, API 20 anaerobes, API 20 Strep, API Rapid ID 32 Strep, API Coryne, API (rapid) Coryne (bioMérieux), and Rosco Diagnostic Tablets (Rosco, Taastrup, Denmark) systems. A test based on 4-methylumbelliferyl (4-UM) derivatives of various substrates has also been applied successfully to the phenotypic identification of actinomycetes (Sarkonen et al., 2001; Whiley et al., 1990). Automated systems such as the Vitek System (bioMérieux) have rarely been used (Woo et al., 2003) and their performance for identifying *Actinomyces* species was generally found to be unsatisfactory (Schaal, unpublished observation).

Miniaturized multitest systems such as the API 20 anaerobes (Casin et al., 1984), API 20 Strep (Morrison and Tillotson, 1988), API Rapid ID 32 Strep (Nikolaitchouk et al., 2000), API Coryne (Hoyles et al., 2001a, 2001b, 2001c) and API (rapid) Coryne (Funke et al., 1997b) systems contain carbohydrate fermentation reactions in addition to enzyme tests or they consist exclusively of fermentation reactions such as the API 50 CH system (bioMérieux). When applied to the characterization and identification of fermentative actinomycetes, the reliability and reproducibility of the results highly depend on the growth characteristics of the individual strain under investigation especially as far as acid production from carbohydrates is concerned. Only species and strains which predominantly form soft, nonfilamentous growth, so that homogeneous cell suspensions can easily be prepared, may give satisfactory results (Funke et al., 1997b). Furthermore, it has been shown (Hall et al., 2003d) that the patterns of physiological characters produced by different test systems may differ considerably for a given actinomycete strain. In a recent comparative study of four commercial systems (RapID ANA II, Rapid ID 32 A, RapID CN Plus, BD Crystal) for identification of *Actinomyces* species and some closely related bacteria (Santala et al., 2004), a strikingly poor applicability of these systems to the fermentative actinomycetes was found not only with new, but also with "classical" species of this genus. These observations confirm the results of Moll et al. (1996) who obtained similar results when comparing the BBL Crystal ANR ID and the API Rapid ID 32 A systems.

For the above reasons, biotyping has gained no practical importance for differentiating *Actinomyces* species below the species level. A bacteriophage typing system has also not been developed. Thus, only serological, SDS-PAGE, and molecular typing methods appear to be suitable for the application to the fermentative actinomycetes.

For serological identification and typing, both direct and indirect modifications of the fluorescent antibody technique have been applied successfully to the fermentative actinomycetes (Schaal and Pulverer, 1973; Slack et al., 1961, 1971) and can be used on culture material as well as on clinical specimens including pus, dental plaque, and calculus or mucosal secretions (Pine et al., 1985). A simple direct fluorescent antibody (FA) procedure was devised by Slack and Gerencser (1975). However, the use of this procedure is limited because fluorescein isothiocyanate (FITC)-conjugated specific antisera to the *Actinomyces* species and serovars are not commercially available, although such antisera for a few species and serovars used to be obtainable from the Biological Reagents Section of the Centers for Disease Control and Prevention (CDC), Atlanta, GA, USA.

*Modified miniaturized system for testing acid production from carbohydrates by fermentative actinomycetes and related organisms (Schofield and Schaal, 1979b, 1980, 1981): I. Basal medium: Thioglycolate broth without glucose or indicator, supplemented with 0.2% (w/v) yeast extract. II. Preparation of carbohydrate-containing filter paper disks: Soak sterile blank filter paper disks (Oxoid) in 3% (w/v) solutions of the various test carbohydrates (filter-sterilized) and dry by lyophilization. III. Test procedure: Prepare the inoculum from 4–7 day-old cultures on BHIA or CC-medium by removing colony material carefully without adhering agar particles and suspend it in basal medium I in a test tube. For strains firmly adhering to the agar, use the dialysis tubing method described above. Homogenize the suspension using a Vortex mixer or a similar mixing device after addition of a few sterile glass beads to the tube. Add 2–3 drops of the densely turbid suspension thus obtained to each well of a microtiter tray with flat bottoms containing one of the sugar-containing disks. Cover the tray with a plastic tape or a plastic cover (when using a tape, perforate it over each well with a sterile injection needle to allow gaseous exchange), and incubate for 2–3 days under adequate gaseous conditions. After incubation, add 1 drop of a 0.2% (w/v) solution of bromothymol blue to each well. Acid production is indicated by a yellow color of the indicator; when the carbohydrate has not been fermented, the color of the indicator is green. A blue-green or blue color is usually due to contamination by organisms not belonging to the genus *Actinomyces*.

†Assessment of nitrate and nitrite reduction and of urease and arginine dihydrolase activities (Schofield and Schaal, 1980, 1981): *I. Basal medium:* Bacto peptone (Difco), 5.0 g; yeast extract (Difco), 3.0 g; glucose, 0.5 g; KH_2PO_4, 2.0 g; agar, 0.5 g; distilled water to reach a total of 1 liter. Adjust the pH to 6.8 and sterilize the medium by autoclaving at 121°C for 15 min. II. Test procedure: Add the test substances to basal medium I from filter-sterilized solutions to obtain the following final concentrations: sodium nitrate, 0.1%; sodium nitrite, 0.005%; urea, 0.5%; arginine, 0.5%. Pipette the various solutions of the test substances into the wells of microtiter trays in volumes of 0.15 ml. Inoculate with suspensions of the test organisms in basal medium I without agar, seal the wells with plastic tape which is perforated as described above, or use a plastic cover. After incubation for 3–4 days, the formation (= nitrate reduction) or disappearance (= nitrite reduction) of nitrite is assessed by adding one drop of the usual nitrite reagent (solution 1: 8.0 g of sulfanilic acid + 1000 ml of 5N acetic acid; solution 2: 5.0 g of α-naphthylamine + 1000 ml of 5N acetic acid. Both solutions are mixed in equal amounts immediately before use). The development of a brick-red color immediately after addition of the reagent indicates the presence of nitrite. If no color develops in the nitrate reduction test, a negative nitrate reduction test is differentiated from the reduction of both nitrate and nitrite by adding a small amount of finely granular zinc which would reduce nitrate to nitrite if the former is still present in the well. Urease activity and deamination of arginine are assessed by means of the ammonia released into the medium when the test is positive. Presence of ammonia is detected by adding 1–2 drops of Nessler's reagent to the wells whereupon a heavy, dark-yellow to ochre precipitate forms immediately. Slowly developing precipitates must be considered as negative results.

To avoid the elaborate and time-consuming labeling procedure for many antisera, Schaal and Pulverer (1973) described an indirect immunofluorescence test* that provides similarly specific results and is even more sensitive than the direct FA modification. For this method, commercially available, FITC-conjugated goat antisera to rabbit globulins are used. The production of the specific rabbit antisera required for serological tests can be performed according to Schaal and Pulverer (1973) or Schaal and Gatzer† (1985).

Whole-cell or cell-wall agglutination as well as immunoblotting procedures (Putnins and Bowden, 1993) may help to solve taxonomic problems, but are not easily applicable to routine identification or differentiation.

Differentiation of the genus *Actinomyces* from other genera

Actinomyces species resemble a variety of other Gram-stain-positive, filamentous, diphtheroidal, or coccobacillary genera morphologically and/or physiologically. This is especially true for the remaining members of the family *Actinomycetaceae* with the exception of *Mobiluncus*, but also for members of the genera *Propionibacterium*, *Bifidobacterium*, and *Erysipelothrix*. Particularly pronounced is the similarity between *Actinomyces israelii* and *Actinomyces gerencseriae* on the one hand and *Propionibacterium propionicum* on the other hand because these species share many diagnostic characters. However, as shown in Table 3, these *Actinomyces* species can be differentiated reliably from *Propionibacterium propionicum* by the presence of LL-A_2pm in the cell walls of and the production of propionic acid as a major end product of glucose fermentation by the latter organism.

Mobiluncus is the only genus that can easily be differentiated from the other members of the family *Actinomycetaceae* by its unique cellular morphology, motility, and variable to negative Gram reaction (Table 3). The other members of the family are much more difficult to differentiate: Only certain *Actinomyces* species produce filamentous cells and/or colonies, a morphological trait that is characteristic, but not exclusive to the genus *Actinomyces* and may, therefore, aid in their differentiation from members of the genera *Actinobaculum*, *Arcanobacterium*, and *Varibaculum* which only form straight to curved, eventually diphtheroidal cells or coccoid structures, possibly with some signs of branching. Additional differential characters may be obtained from chemotaxonomic and acid end product analyses (Table 3).

Other genera which may be confused with *Actinomyces* species can be separated using the characteristics summarized in Table 3. If uncertainties concerning the taxonomic affiliation of an unknown isolate still exist, 16S rRNA gene sequence analyses or other molecular techniques usually help to clarify the situation.

Taxonomic comments

The genus *Actinomyces* was created in 1877, when Harz described the causative agent of bovine actinomycosis which he named *Actinomyces bovis*. Breed and Conn (1919) recommended that *Actinomyces* be considered a *genus conservandum* with *Actinomyces bovis* as the type species. This recommendation was accepted by the Winslow Committee in 1920 (Winslow et al., 1920), thereby confirming that *Actinomyce* Meyen 1827, a name proposed for a fungus (*Tremella meteorica*), was not valid.

Originally, the description of the genus was solely based upon the morphology of the filamentous elements produced by *Actinomyces bovis* in the granules of bovine infections. The discovery of similar, but not identical granules in human infections and the successful cultivation of both *Actinomyces bovis* and *Actinomyces israelii* which included *Actinomyces gerencseriae* at that time added little to this description. Many years later, cultural, physiological, and chemical properties slowly began to influence the definition of the taxon. Pine and Georg (1969) used cellular and colonial morphology, cell-wall composition, fermentation end products, and certain physiological characteristics to define the family *Actinomycetaceae* and the genus *Actinomyces* in light of more modern approaches to taxonomy. Georg et al. (1969) modified the genus description of *Actinomyces* to include catalase-positive organisms to permit the inclusion of *Actinomyces viscosus*. At the same time, *Actinomyces propionicus* was removed from the genus and reclassified as *Arachnia propionica* (now: *Propionibacterium propionicum*) as it produced propionic acid as a major fermentation end product and contained LL-diaminopimelic acid in its cell walls (Pine and Georg, 1969) (Table 3).

Because of persisting taxonomic and nomenclatural uncertainties as well as lack of suitable differential characters, it was essentially impossible for decades to separate *Actinomyces bovis* reliably from *Actinomyces israelii* so that both names were used interchangeably until the reports of Erikson (1940), Thompson (1950), and Pine et al. (1960) finally proved that the causative agents of human and bovine actinomycosis were two taxonomically distinct species.

At the same time, improved taxonomic techniques allowed the delineation of a first group of additional *Actinomyces* species: Thompson and Lovestedt (1951) proposed the name *Actinomyces naeslundii* for a filamentous bacterium that had first been isolated from the human oral cavity by Carl Naeslund in (1925). *Actinomyces viscosus*, which had primarily been described under the genus designation *Odontomyces* (Howell et al., 1965), was

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*Indirect immunofluorescence procedure for identifying and typing *Actinomyces* species (Schaal and Gatzer, 1985; Schaal and Pulverer, 1973): I. Preparation of smears: a) Clinical material: Make two smears on a clean glass slide containing two marked circles. Fix air-dried smears by flooding with methanol for 1 min. Pour off the alcohol and allow the slide to air-dry. b) Cultures: Make smears of a suspension of the organism obtained by centrifugation of a broth culture or from an agar culture on glass slides, air dry, and gently fix by heating in a Bunsen flame. II. Staining procedure: 1. Place 1 drop of specific rabbit antiserum (possibly absorbed before use) on each smear and incubate the slide in a moist chamber for 30 min at ambient temperature. 2. Pour off excess antiserum and wash the slide in two changes of pH 7.2 buffer (FTA, hemagglutination buffer, BD) for 5 min each. 3. Briefly air-dry the slide. 4. Place 1 drop of FITC-conjugated goat antiserum to rabbit immunoglobulins on the same smear and incubate again in a moist chamber for about 30 min at ambient temperature. 5. Pour off excess conjugate and wash the slide in two changes of pH 7.2 buffer. 6. Counterstain the smear in 0.5% Evans blue for 5 min. 7. Remove excess Evans blue by dipping the slide briefly into distilled water and wash it in two changes of pH 9.0 buffer for 1 min each. 8. Allow the smear to air dry. 9. Place 1 drop of buffered glycerol mounting fluid (9 parts c.p. glycerol, 1 part pH 9.0 buffer) on each smear and cover with a coverslip. 10. For examination with a microscope equipped for FA work, use a 50× water-immersion or an analogous oil-immersion objective.

†Immunization scheme for producing antisera to whole-cell antigens of fermentative actinomycetes (Schaal and Gatzer, 1985; Schaal and Pulverer, 1973): I. Preparation of antigens: Biomass of the reference strains is produced using the dialysis tubing membrane technique (see footnote) on BHIA. The cell suspension is inactivated by heating at 60°C for 20 min in a waterbath, homogenized in a Potter tissue homogenizer, and adjusted to a concentration of 20 mg wet weight per ml. II. Immunization scheme: Ordinary laboratory rabbits can be used, but cross-bred animals may give better immune responses. The immunization schedule with the above suspension is as follows: 1st week: day 1, 1.5 ml i. m.; day 3, 0.5 ml i. v.; day 4, 1.0 ml i. v.; day 5, 1.5 ml i. v.; day 7, 1.5 ml i. m. 2nd week: day 10, 1.5 ml i. v.; day 13, 1.5 ml i. v. 4th week: day 22, test for antibody production. If the antibody titers are below 1:1000, make 2–4 booster i. v. injections at half-weekly intervals. Antiserum is obtained by cardiac puncture in ether anesthesia and lyophilized in 1-ml aliquots until use.

included in the genus *Actinomyces* after its definition changed to accommodate both catalase-positive and catalase-negative species (Georg et al., 1969).

Actinomyces naeslundii and *Actinomyces viscosus* long appeared to be closely related in terms of traditional physiological characters and antigenic structure (Fillery et al., 1978; Holmberg and Hallander, 1973; Schaal and Gatzer, 1985; Schofield and Schaal, 1981). It was even suggested (Gerencser, 1979) that both taxa were varieties of a single species. However, detailed numerical phenetic analyses (Schaal and Schofield, 1981a, 1981b; Schofield and Schaal, 1981) revealed that strains labeled *Actinomyces naeslundii* and *Actinomyces viscosus* formed a set of comparatively stable subclusters, two of which appeared to represent typical *Actinomyces naeslundii* and *Actinomyces viscosus* isolates, respectively. Similarly, in a recent study using a new physiological differentiation system (Taxa Profile, Merlin, Germany), four distinct numerical phenetic clusters could be delineated within the *Actinomyces naeslundii*/*Actinomyces viscosus* complex (N.A. Dahlen, MD thesis, Bonn 2004) showing the heterogeneity of this group of actinomycetes. This heterogeneity had also been suggested by results of DNA–DNA hybridization studies (Coykendall and Munzenmaier, 1979; Johnson et al., 1990) and was definitely confirmed by 16S rRNA gene sequencing (Henssge et al., 2009). The latter study led to the description of *Actinomyces naeslundii sensu stricto* for the former *Actinomyces naeslundii* serovar I or *Actinomyces naeslundii* genospecies 1 according to Johnson et al. (1990), *Actinomyces oris* for the former *Actinomyces naeslundii* serovars II, III, and NV and the former *Actinomyces viscosus* serovar II, which correspond to *Actinomyces naeslundii* genospecies 2 of Johnson et al. (1990), and of *Actinomyces johnsonii* for the former *Actinomyces naeslundii* serovar WVA 963. This revision of the species concept of the *Actinomyces naeslundii*/*Actinomyces viscosus* complex leaves the name *Actinomyces viscosus sensu stricto* for the former serovar I of *Actinomyces viscosus*, which represents the hamster isolates of *Actinomyces viscosus*. *Actinomyces odontolyticus* had primarily been isolated from advanced human carious lesions and was described as a separate *Actinomyces* species by Batty (1958).

After further improvement of the chemotaxonomic and physiological methods and especially after the introduction of DNA–DNA pairing and 16S rRNA gene sequencing technologies into the field of actinomycete taxonomy, numerous additional taxa were added to the list of *Actinomyces* species. Dent and Williams (1984b, 1984c, 1984a, 1986) described three groups of Gram-stain-positive bacteria isolated from the dental plaque of dairy cattle which resembled to some extent *Actinomyces naeslundii* and *Actinomyces viscosus* phenotypically. However, they could be differentiated from these species on the basis of cell wall and DNA base composition, DNA–DNA homology data, polypeptide molecular mass distribution, and a few physiological reactions. These isolates were designated *Actinomyces denticolens*, *Actinomyces howellii*, and *Actinomyces slackii*, respectively, and are all members of the phylogenetic core cluster 1. In contrast, actinomycetes that were isolated from canine infections and named *Actinomyces hordeovulneris* (Buchanan et al., 1984) appear to represent a separate phylogenetic line of descent, possibly together with the species *Actinomyces nasicola*, which was created for a strain recovered from purulent discharge in the nose of a 81-year-old male patient (Hall et al., 2003d).

Actinomyces meyeri, first described by Kurt Meyer (1911), was included in the genus "*Actinobacterium*" by Prévot (1938). However, the genus designation "*Actinobacterium*" lost its standing in nomenclature after the type species of this genus, "*Actinobacterium*" *israelii*, had been transferred to the genus *Actinomyces* by Breed and Conn (1919), so that "*Actinobacterium*" *meyeri* remained "a species in search of a genus" for many years. In 1984, Cato et al. reclassified the organism as *Actinomyces meyeri* and this reclassification is underpinned by 16S rRNA gene sequence data which place the species in the phylogenetic core cluster 2.

The dramatically increasing application of 16S rRNA gene sequencing to the Gram-stain-positive, rod-shaped bacteria in recent years has led to a host of new and reclassified *Actinomyces* species. As far as the classical human species *Actinomyces israelii* is concerned, Stackebrandt and Charfreitag (1990) as well as Johnson et al. (1990) found that strains formerly known as *Actinomyces israelii* serovar II showed a surprisingly low level of DNA relatedness to *Actinomyces israelii* serovar I which prompted the latter authors to propose the new species *Actinomyces gerencseriae* for the serovar II strains. In the same study, Johnson et al. (1990) created the species *Actinomyces georgiae* whose members were recovered from gingival crevices of humans with and without periodontitis. However, while *Actinomyces gerencseriae* belongs to the phylogenetic core cluster 1, *Actinomyces georgiae* is found in core cluster 2.

Another group of organisms which had primarily been termed CDC group 1 coryneform bacteria and CDC group 1-like coryneform bacteria were assigned to the genus *Actinomyces* as *Actinomyces neuii* subsp. *neuii* and *Actinomyces neuii* subsp. *anitratus*, respectively (Funke et al., 1994) although current phylogenetic data indicate that these bacteria represent a separate line of descent only loosely affiliated with *Actinomyces* core cluster 2. *Actinomyces radingae* and *Actinomyces turicensis*, which were both chiefly isolated from human mixed infections, are members of core cluster 2.

Actinomyces-like bacteria that had predominantly been recovered from human respiratory tract secretions were described as *Actinomyces graevenitzii* (Pascual Ramos et al., 1997a). However, several trees derived from 16S rRNA gene sequences show unequivocally that *Actinomyces graevenitzii* occupies an isolated phylogenetic position within the *Actinomycetaceae*. Similarly, *Actinomyces europaeus* (which was proposed as a new *Actinomyces* species by Funke et al. (1997a) is only remotely related to the classical *Actinomyces* species in terms of 16S rRNA gene sequence similarity, but exhibits some relationship to *Actinomyces coleocanis*, a species found in the vagina of dogs (Hoyles et al., 2002a).

In contrast, *Actinomyces urogenitalis* which had been isolated from the human urogenital tract (Nikolaitchouk et al., 2000) is a true member of phylogenetic core cluster 1 while *Actinomyces funkei* (which was proposed by Lawson et al. (2001b) for three human strains of different sources including blood cultures) is found in core cluster 2. The most recent novel species of the genus *Actinomyces* from human sources apparently belong to different phylogenetic lines of descent. *Actinomyces cardiffensis* which was described by Hall et al. (2002) had been isolated from various clinical specimens including IUCDs and is also a member of core cluster 2. In contrast, strains recovered from infected human dental root canals and designated *Actinomyces radicidentis* (Collins et al., 2000) are associated with core cluster 1 (Schaal et al., 2006) or possibly more closely with *Actinomyces graevenitzii* (Figure 19).

Another new *Actinomyces* species of human origin, *Actinomyces oricola* Hall et al. 2003c, was isolated from a dental abscess and shows some relatedness to the subgroup in core cluster 1 which contains *Actinomyces gerencseriae* and *Actinomyces israelii*. An additional new species from a human clinical specimen and designated *Actinomyces hongkongensis* (Woo et al., 2003) shows some 16S

rRNA gene sequence relatedness to the new animal species *Actinomyces marimammalium* (Hoyles et al., 2001c), which was isolated from seals and a porpoise. These two species form a subcluster in the phylogenetic tree related to core cluster 2 and may represent a separate line of descent. The definition of *Actinomyces houstonensis* which was proposed by Clarridge and Zhang in (2002) for three human isolates from serious subcutaneous abscesses, was primarily based upon 16S rRNA gene sequence data, while its phenotypic characterization remained fragmentary. In addition, the species has not yet been validated so that it will be listed under "*Species incertae sedis*". Details on a species named *Actinomyces lingnae* mentioned in the paper of Clarridge and Zhang (2002) could be found neither in the international literature nor in the catalogs of relevant international culture collections.

Additional novel *Actinomyces* species have been cultured from various animal sources: The designation *Actinomyces hyovaginalis* was proposed for a group of *Actinomyces*-like isolates that were derived from purulent discharge and aborted fetuses of pigs (Collins et al., 1993). These organisms are members of phylogenetic core cluster 2. Fermentative Gram-stain-positive bacteria isolated from pigs and designated "*Actinomyces suis*" have caused taxonomic and nomenclatural problems over many years. This species epithet was first used by Gasperini (1892), but had not been validly published and was therefore declared a *nomen dubium* (Slack, 1974). Grässer (1957) cultured bacteria from mastitis of swine and named them also "*Actinomyces suis*". In this case, the name was validly published, but the description was inadequate and cultures were not available. More recently, Franke (1973) once more described actinomycetes isolated from the udders of swine as "*Actinomyces suis*". The description of Franke's isolates appears to differ from that of Grässer's strains, but is strikingly similar to that of *Actinomyces hyovaginalis* (Collins et al., 1993) at least as far as physiological characteristics are concerned. Another single bacterial isolate from pig mastitis was found to represent a further new *Actinomyces* species belonging to core cluster 2 and was named *Actinomyces suimastitidis.*

Several additional new animal *Actinomyces* species originated from canine and/or feline clinical lesions. *Actinomyces bowdenii* Pascual et al., 1999 was recovered from various pathological processes in dogs and cats, predominantly in mixed culture with other microbes, and is a member of core cluster 1. *Actinomyces canis* Hoyles et al., 2000 was cultured from either vaginal secretions or pus of dogs and belongs to core cluster 2. A third canine species, *Actinomyces catuli* Hoyles et al., 2001c, was isolated from a polymicrobial infection of the lungs and the pleural space and is a border-line member of core cluster 1, possibly with some phylogenetic relationship to *Actinomyces graevenitzii* and *Actinomyces radicidentis.* The phylogenetic affiliation of *Actinomyces coleocanis* (Hoyles et al., 2002b) from the vagina of a cocker spaniel dog has already been mentioned.

The most recent animal *Actinomyces* species, *Actinomyces vaccimaxillae* (Hall et al., 2003d) from a jaw lesion of an adult cow and *Actinomyces ruminicola* (An et al., 2006) from cattle rumen, are members of core cluster 2 and core cluster 1, respectively. The species "*Actinomyces humiferus*" Gledhill and Casida 1969 differs considerably from typical members of the genus *Actinomyces* in that it grows at 30°C, has a high G+C content of its DNA (73 mol%), is sensitive to lysozyme, and occurs in high numbers in organically rich soils (Gledhill and Casida, 1969). Also, it was found to be unrelated phylogenetically to true *Actinomyces* species and was thus reclassified as *Cellulomonas humilata* (Collins and Pascual, 2000).

Similarly, "*Actinomyces eriksonii*" Georg, Robertstad, Brinkman, and Hicklin 1965, when primarily characterized, appeared to resemble members of the genus *Actinomyces* in terms of cellular and colonial morphology, oxygen requirements, cell-wall composition, and pathogenicity. However, additional and more detailed analyses showed that its fermentation end products as well as its cell-wall structure were those of bifidobacteria (Moore, 1970; Slack and Gerencser, 1975). Mitsuoka et al. (1974) claimed that "*Actinomyces eriksonii*" was identical with *Bifidobacterium adolescentis* Reuter (1963). As *Bifidobacterium adolescentis* and *Bifidobacterium dentium* are closely related and as it is even impossible to differentiate biovars *b* and *d* of *Bifidobacterium adolescentis* phenotypically from *Bifidobacterium dentium* (Scardovi, 1986), it is not very surprising that DNA of the type strain of "*Actinomyces eriksonii*" (ATCC 15423) is completely homologous with that of a *Bifidobacterium dentium* strain (Scardovi, 1986).

Further reclassifications involved "*Actinomyces bernardiae*" Funke et al. 1995 and "*Actinomyces pyogenes*" Reddy et al. 1982, which were both transferred to the genus *Arcanobacterium* as *Arcanobacterium bernardiae* and *Arcanobacterium pyogenes*, respectively (Pascual Ramos et al., 1997b). "*Actinomyces suis*" Ludwig et al. 1992 which had primarily been described as *Eubacterium suis* Wegienek and Reddy 1982 and differed from the swine isolates mentioned as "*Actinomyces suis*" above, was reclassified as *Actinobaculum suis* Lawson et al. 1997.

The heterogeneity of the genus *Actinomyces* as currently recognized is suggested by 16S rRNA gene sequence data that separate the genus into two major phylogenetic clusters (core clusters 1 and 2) and at least five subclusters possibly representing additional separate lines of descent. A subdivision of the genus is also suggested by numerical phenetic results (Schofield and Schaal, 1981) and chemotaxonomic findings (Schaal et al., 2006). In this respect, the prediction Schaal (1986b) made in the previous edition of *Bergey's Manual* concerning the future taxonomic development of the genus is now known to be true without reservation. However, as many *Actinomyces* species have as yet been characterized incompletely, especially as far as chemotaxonomy is concerned, it still appears to be premature to formally subdivide the genus *Actinomyces* into three or even more new genera. Nevertheless, as the type species of the genus, *Actinomyces bovis*, is found in core cluster 1, species such as *Actinomyces gerencseriae*, *Actinomyces israelii*, *Actinomyces johnsonii*, *Actinomyces naeslundii*, *Actinomyces oris*, and the remaining species of core cluster 1 may retain the genus designation *Actinomyces* in the future.

Further reading

Schaal, K.P., A. Crecelius, G. Schumacher and A.A. Yassin. 1999. Towards a new taxonomic structure of the genus *Actinomyces* and related bacteria. Nov. Acta Leopold. *80*: 83–91.

Slack, J.M and M.A. Gerencser. 1975. *Actinomyces.* Filamentous Bacteria. Biology and Pathogenicity. Burgess Publishing Company, Minneapolis.

Identification and descriptive characteristics of *Actinomyces* species

The phenotypic characteristics useful for identification of the recognized *Actinomyces* species are given in Table 4 and Table 5. Table 6 and Table 8 summarize the morphological properties of human and animal *Actinomyces* species. Additional descriptive details of both groups of *Actinomyces* species are listed in Table 7 and Table 9.

TABLE 4. Differential characteristics of the human species of the genus *Actinomyces*[a,b]

Characteristic	*A. cardiffensis*	*A. europaeus*	*A. funkei*	*A. georgiae*	*A. gerencseriae*	*A. graevenitzii*	*A. hongkongensis*[c]	*A. israelii*	*A. meyeri*	*A. massiliensis*	*A. nasicola*	*A. neuii* subsp. *neuii*	*A. neuii* subsp. *anitratus*	*A. odontolyticus*	*A. oricola*	*A. radicidentis*	*A. radingae*	*A. turicensis*	*A. urogenitalis*	*A. viscosus sensu stricto*[d]	*A. naeslundii sensu stricto*	*A. oris*	*A. johnsonii*	*A. dentalis*
Cells coccoid or coccobacillary	–	d	–	d	–	–	–	–	d	–	d	d	d	(d)	–	+	+	–	–	–	–	–	–	–
Microcolonies filamentous	–	–	–	–	+	nr	–	+	–	–	–	–	–	(d)	nr	–	–	–	nr	+/–	d	d	d	nr
Aerobic growth (without CO_2)	–/+	+	+	–/+	–	+	–	–	–/+	–	–/+	+	+	+	–/+	+	+	+	+	–/(+)	+	+	+	–
Acid production from:																								
L-Arabinose	–	–	–	–/+	–	nr	–	+/–	d	–	–	d	–	d	–	–	d	d	d	–	–	–	–	–
Cellobiose	–	nr	nr	–	+/–	–	nr	+	–	d	+	d	d	–	+	nr	d	d	nr	–	d	d	d	+
D-Glucose	+	+	+	+	+	+	–	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
myo-Inositol	nr	nr	nr	–/+	+/–	+	nr	+/–	–	–	nr	+	+	–	nr	nr	d	d	nr	+/–	+	+	+	nr
Lactose	–	–	d	+/–	+	+	nr	+	d	+	–	+	+	d	–	+	d	–	+	+/(+)	d	d	d	(+)/–
D-Mannitol	–	–	–	–/+	+/–	–	nr	+	–	–	–	+	+	–	–	+	–	–	d	–	–	–	–	–
Melezitose	–	d	–	–/+	+/–	–	nr	–/+	–	–[+]	–	+	+	–	–	+	d	d	+	–	–/+	d	–	–
D-Raffinose	d	–	–	–/+	+/–	nr	–	+	–	–	–	+	+	–	d	+	d	d	+	+/–	+	+	+	+
L-Rhamnose	nr	–	nr	+	–/+	–	nr	–	–/+	–	nr	–	–	d	nr	nr	–	–	nr	–	–/+	–	–	nr
D-Ribose	d	d	d	+	+/–	+	nr	+/–	+	–[+]	–	+	+	d	–	+	+	+	d	–	d	d	–/+	–
D-Sorbitol	–	–	–	–/+	–/+	–	nr	–/+	–	–	–	–	–	–	–	–	–	–	–	–	–/+	d	d	–
Sucrose	d	d	+	+	+	+	nr	+	+	+	–	+	+	d	d	+	+	+	+	+	+	+	+	+
Trehalose	–	d	–	+	+	–	–	+/–	–	+	–	+	+	d	d	+	d	+	+	d	d	d	d	+
D-Xylose	–	–	+	+/–	+/–	–	–	+	+	–[+]	–	+	+	d	–	nr	+	+	+	–	–/+	–/+	–	–
Hydrolysis of:																								
Esculin	–	d	–	+/–	+	–	–	+	–/+	nr	–	–	–	d	+	+	+	–	+	+	+	+	+	+
Gelatin	–	nr	–	d	–	nr	nr	–	–	nr	–	–	–	–	–	+	–/(+)	–	–	–	–	–	–	–
Hippurate	–	nr	+	nr	–	–	nr	–	–	nr	–	nr	nr	–	–	–	nr	nr	–	nr	nr	nr	nr	–
Enzyme activities:																								
N-Acetyl-β-glucosaminidase	–	–	d	nr	–	+	–	–	–	–	+	nr	nr	–	–	–	+	–	+	nr	–	–	–	–
Alkaline phosphatase	–	–	+	nr	–	nr	+	–	–	–	–	–	d	–	d	–	–	–	d	nr	–	–	d	–
α-Fucosidase	–	nr	–	nr	–	–	–	–	–	–	–	nr	nr	d	–	–	nr	nr	–	nr	–	–	–/+	–
α-Galactosidase	–	+	–	nr	nr	–	–	nr	–	–	–	+	+	–	+	+	+	–	+	nr	+	+	+	+
β-Galactosidase	–	+	nr	nr	+	+	–	+	–	+	+	+	+	–	d	+	+	–	+	nr	+	+	+	d
α-Glucosidase	+	+	+	nr	+	–	–	+	+	+	+	+	+	–	+	+	+	+	+	nr	+/–	d	+	+
β-Glucosidase	–	–	–	nr	+	–	–	+	–	–	d	–	–	d	+	+	+	–	+	nr	+/–	d	–/+	+
α-Mannosidase	nr	–	–	nr	–	–	–	–	–	–	–	+	+	–	nr	–	–	–	d	nr	nr	nr	nr	nr
Pyrazinamidase	–	–	d	nr	nr	nr	nr	nr	nr	nr	nr	+	+	nr	+	+	d	–	–	nr	nr	nr	nr	–
Valine arylamidase	nr	+	–	nr	–	–	nr	–	+	+	+	–	–	–	nr	–	nr	–	+	–	–	–	nr	nr
Urease	–	–	–	–	–	–	–	–	d	–	–	–	–	–	–	d	–	–	–	nr	–	–	d	–
Catalase production	–	–	–	–	–	–	–	–	–	–	–	+	+	–	–	+	–	–	–	+	–	d	–	–
Nitrate reduction	d	–	d	–/+	–/+	–	–	+/–	–	+	–	+	–	+	–	d	–	–	+	d	+	d	+/–	–
β-Hemolysis	–	(d)	–	–	–	–	–	–	–	–	nr	–	–	d	–	nr	(+)	(+)	nr	–	–	–	–	nr

[a]Symbols: +, positive/present; –, negative/absent; +/–, predominantly positive/present; –/+, predominantly negative/absent; (+), weakly or irregularly positive/present; d, strain differences; (d), some strains weakly positive; –[+], acid production by *Actinomyces massiliensis* usually detected after 24 h, with some strains showing a positive result after 48 h; nr, not reported.

[b]Data compiled from Schaal (1986b), Johnson et al. (1990), Vandamme et al. (1998), Hall et al. (2005), Schaal et al. (2006), Henssge et al. (2009), and Renvoise et al. (2009).

[c]The negative acid production tests from carbohydrates may be due to the use of an inadequate commercial test system in the original description of the species.

[d]Animal species included for comparison.

TABLE 5. Differential characteristics of the animal species of the genus *Actinomyces*[a,b]

Characteristic	*A. bovis*	*A. bowdenii*	*A. canis*	*A. catuli*	*A. coleocanis*	*A. denticolens*	*A. hordeovulneris*	*A. howellii*	*A. hyovaginalis*	*A. marimammalium*	*A. slackii*	*A. suimastitidis*	*A. vaccimaxillae*	*A. viscosus sensu stricto*	*A. ruminicola*
Cells coccoid or coccobacillary	d	−	−	−	−	−	−	−	+	−	+	−	+	−	−
Microcolonies filamentous	d	nr	nr	nr	nr	d	+	nr	−	−	−	−	−	+/−	nr
Aerobic growth (without CO_2)	−	+	+	+	+	d	−	nr	+	nr	+	nr	(+)	−/(+)	−
Acid production from:															
L-Arabinose	−	−	+	d	−	−	−	+/−	+	−	−	(+)	+	−	nr
Cellobiose	−	nr	nr	nr	nr	−/(+)	+	−	d	nr	−	nr	nr	−	+
D-Glucose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
myo-Inositol	d	nr	nr	nr	nr	+/−	−	−	d	nr	−/+	nr	nr	+/−	d
Lactose	d	+	+	+	+	+	+	+/−	d	+	d	−	d	+/(+)	+
D-Mannitol	−	−	−	−	−	d	−	−	nr	−	−	−	d	−	d
Melezitose	−	+	−	−	−	−/(+)	nr	nr	−	−	−	−	−	−	+
D-Raffinose	−	+	d	+	−	+	(+)	+	−	−	+	+	−	+/−	nr
L-Rhamnose	−	nr	nr	nr	nr	−(+)	−	−	−	nr	−	nr	nr	−	+
D-Ribose	d	+	+	+	−	+/−	−	−	nr	−	−/+	(+)	+	−	+
D-Sorbitol	−	−	−	−	−	−	nr	nr	−	−	−	−	−	−	+
Sucrose	+	+	d	+	−	+	nr	+	+	−	d	+	d	+	+
Trehalose	−	+	−	+	−	−	+	+/−	−	−	d	−	+	d	+
D-Xylose	−	−	+	+	−	−	+	+/−	+	−	−	+	+	−	+
Hydrolysis of:															
Esculin	d	+	−	+	−	+	+	nr	+	−	−	+	+	+	d
Gelatin	−	−	−	−	−	nr	nr	nr	−	−	nr	−	−	−	nr
Hippurate	−	−	−	−	−	nr	nr	nr	+	−	nr	−	−	nr	−
Enzyme activities:															
N-Acetyl-β-glucosaminidase	+	−	+	d	nr	−	nr	nr	nr	nr	nr	d	−	nr	nr
Alkaline phosphatase	−	d	−	−	−	−	nr	nr	+	d	nr	d	d	nr	nr
α-Fucosidase	−	−	+	−	nr	−	nr	nr	nr	nr	nr	nr	+	nr	nr
α-Galactosidase	−	+	+	d	−	+/−	nr	nr	+	−	nr	+	−	nr	nr
β-Galactosidase	−	+	+	+	+	+	nr	nr	+	+	nr	+	−	nr	nr
α-Glucosidase	−	d	+	+	+	+	nr	nr	nr	−	nr	+	d	nr	nr
β-Glucosidase	−	+	−	+	−	+/−	nr	nr	nr	−	nr	+	d	nr	nr
α-Mannosidase	−	−	−	−	nr	−	nr	nr	nr	nr	nr	nr	−	nr	nr
Pyrazinamidase	nr	+/−	+	+	+	nr	nr	nr	−	−	nr	(+)	+	nr	nr
Valine arylamidase	−	−	−	−	nr	−	nr	nr	nr	nr	nr	nr	−	nr	nr
Urease	−	−	−	−	−	nr	−	nr	−	−	nr	−	−	nr	−
Catalase production	−	+	+	d	−	−	(+)	+	−	−	+	−	−	+	−
Nitrate reduction	−	+	−	+	−	+	−	nr	+	−	nr	−	−	d	+
β-Hemolysis	d	−	nr	nr	nr	−	−	−	−	−	−	nr	−	−	nr

[a] Symbols: +, positive/present; −, negative/absent; (+), weakly positive; +/−, predominantly positive/present; −/+, predominantly negative/absent; d, strain differences; nr, not reported.

[b] Data compiled from Schaal (1986b), An et al. (2006), Schaal et al. (2006).

TABLE 6. Morphological characteristics of *Actinomyces* species from human sources[a,b]

Characteristic	*A. cardiffensis*	*A. europaeus*	*A. funkei*	*A. georgiae*	*A. gerencseriae*	*A. graevenitzii*	*A. hongkongensis*	*A. israelii*	*A. meyeri*	*A. massiliensis*	*A. nasicola*	*A. neuii* subsp. *neuii*	*A. neuii* subsp. *anitratus*	*A. odontolyticus*	*A. oricola*	*A. radicidentis*	*A. radingae*	*A. turicensis*	*A. urogenitalis*	*A. viscosus sensu stricto*[c]	*A. naeslundii sensu stricto*	*A. oris*	*A. johnsonii*	*A. dentalis*
Cellular morphology:																								
Coccoid elements	–	–	–	–	–	–	–	–	–	–	d	–	–	–	–	+	–	–	–	–	–	–	–	–
Coccobacillary elements	–	d	–	d	–	–	–	–	d	–	d	d	d	(d)	–	–	+	–	–	–	–	–	–	–
Irregular rods	+	nr	nr	d	+	d	–	+	d	–	d	d	d	+	(+)	–	d	(+)	–	+	+	+	+	+
Rods with swollen ends	nr	nr	nr	d	d	d	nr	d	d	nr	nr	nr	nr	d	nr	nr	–	d	nr	d	d	d	d	nr
Branched rods	d	–	d	(d)	+	d	nr	+	–	nr	nr	–	–	(d)	d	nr	d	d	–	d	+	d	d	nr
Round-ended ends	nr	nr	nr	nr	+	nr	nr	+	d	nr	nr	nr	nr	d	nr	nr	nr	nr	nr	+	+	nr	nr	nr
Square-ended ends	nr	nr	nr	nr	–	nr	nr	–	d	nr	nr	nr	nr	–	nr	nr	nr	nr	nr	–	–	nr	nr	nr
Large spherical bodies	nr	nr	nr	nr	–	nr	nr	–	d	nr	nr	nr	nr	–	nr	nr	nr	nr	nr	–	–	nr	nr	nr
Short filaments	d	–	–	–	+	–	–	+	(d)	–	–	–	–	d	d	–	–	–	–	d	d	d	d	+
Long filaments	–	–	–	–	d	–	–	d	–	–	–	–	–	(d)	–	–	–	–	–	d	d	d	d	+
Gram-reaction positive	+	+	+	+	+	+	+	+	d	+	+	+	+	+	+	+	+	d	+	+	+	+	+	+
Gram-reaction variable	–	–	–	–	–	–	–	–	d	–	–	–	–	–	–	–	–	d	–	–	–	–	–	–
Motility	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Colony morphology:																								
Microcolonies filamentous	–	–	–	–	+	nr	–	+	–	–	–	–	–	(d)	nr	–	–	–	nr	d	d	d	d	nr
Macrocolony size																								
≤2 mm	+	+	+	+	+	+	+	+	+	+	+	d	d	+	+	+	+	+	nr	d	d	d	d	+
>2 mm	–	–	–	–	–	–	–	–	–	–	–	d	d	–	–	–	–	–	nr	d	d	d	d	–
Flat	–	nr	nr	–	–	–	nr	–	d	nr	–	–	–	d	–	nr	–	–	nr	(d)	(d)	(d)	nr	–
Raised	+	nr	nr	+	+	+	nr	+	d	nr	+	+	+	d	+	nr	+	+	nr	+	+	+	+	+
Umbonate	–	nr	+	–	d	nr	nr	d	–	nr	nr	–	–	d	nr	nr	nr	nr	nr	d	d	nr	nr	nr
Central depression	–	nr	nr	–	d	nr	nr	d	–	nr	nr	–	–	–	nr	nr	nr	nr	nr	–	–	nr	nr	nr
Entire margin	+	+	nr	+	–	nr	nr	–	+	nr	+	+	+	d	–	+	+	+	nr	d	d	d	nr	–
Undulate margin	–	–	nr	–	d	nr	nr	d	–	nr	–	–	–	d	–	nr	–	–	nr	d	d	d	nr	+
Filamentous margin	–	–	–	–	d	nr	nr	d	–	nr	–	–	–	–	–	nr	–	–	nr	d	d	d	nr	nr
Transparent	–	+	nr	d	(d)	–	nr	–	d	nr	–	–	–	d	–	nr	–	–	nr	–	–	–	nr	–
Opaque	+	–	nr	d	+	+	nr	+	d	nr	+	+	+	d	+	nr	+	+	nr	+	+	+	+	+
White/gray	–	+	+	d	+	d	nr	d	+	nr	+	d	d	d	+	+	+	+	nr	d	d	d	d	+
Creamy white/beige	d	–	–	d	d	–	nr	d	–	nr	–	d	d	–	–	nr	–	–	nr	d	d	d	d	–
Brown/tan	–	–	–	d	–	d	–	–	–	nr	–	–	–	d	–	d	–	–	nr	–	–	–	–	–
Orange	–	–	–	–	–	–	nr	–	–	nr	–	–	–	–	–	–	–	–	nr	–	–	–	–	–
Pink	d	–	–	–	–	–	nr	–	–	nr	–	–	–	–	–	–	–	–	nr	–	–	–	–	–
Red/reddish	–	–	–	–	–	–	nr	–	–	nr	–	–	–	d	–	–	–	–	d	–	–	–	–	–
Rough	–	–	nr	–	+	nr	nr	+	–	nr	–	–	–	d	+	–	–	–	nr	d	d	d	nr	+
Smooth	+	+	nr	+	d	nr	nr	(d)	+	nr	+	+	+	d	–	+	+	+	nr	d	d	d	nr	–
Dry to crumbly	–	–	nr	–	d	nr	nr	d	–	nr	–	–	–	–	+	–	–	–	nr	d	d	d	nr	+
Soft	nr	Nr	nr	nr	d	nr	nr	–	+	nr	nr	nr	nr	+	nr	nr	+	+	nr	d	d	d	nr	nr
Mucoid	–	–	nr	nr	–	nr	nr	–	–	nr	nr	nr	nr	–	nr	–	–	–	nr	d	(d)	d	nr	–

[a]Symbols: +, present; –, absent; (+), weakly or irregularly present; d, strain differences; (d), occasional strain difference; nr, not reported.

[b]Data compiled from: Schaal (1986b); Vandamme et al. (1998); Hall et al. (2005); Schaal et al. (2006); Renvoise et al. (2009).

[c]Animal species included for comparison.

TABLE 7. Physiological characteristics of *Actinomyces* species from human sources[a,b]

Characteristic	*A. cardiffensis*	*A. europaeus*	*A. funkei*	*A. georgiae*	*A. gerencseriae*	*A. graevenitzii*	*A. hongkongensis*[c]	*A. israelii*	*A. meyeri*	*A. massiliensis*	*A. nasicola*	*A. neuii* subsp. *neuii*	*A. neuii* subsp. *anitratus*	*A. odontolyticus*	*A. oricola*	*A. radicidentis*	*A. radingae*	*A. turicensis*	*A. urogenitalis*	*A. viscosus sensu stricto*[d]	*A. naeslundii sensu stricto*	*A. oris*	*A. johnsonii*	*A. dentalis*
Acid production from:																								
N-Acetyl-β-glucosamine	–	nr	nr	nr	nr	+	nr	nr	nr	–	nr	d	d	nr	nr	nr	nr	nr	nr	–	nr	nr	nr	nr
Adonitol	nr	d	nr	nr	nr	nr	nr	nr	–	–	nr	d	+	d	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr
Amygdalin	–	–	nr	–	+/–	–	nr	d	d	–	–	nr	nr	–	(+)	nr	–	–	nr	–	–	–	–	nr
D-Arabinose	nr	nr	nr	nr	nr	–	nr	nr	nr	–	nr	–	–	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	–
L-Arabinose	–	–	–	–/+	–	nr	–	+/–	d	–	–	d	–	d	–	–	d	d	d	–	–	–	–	–
D-Arabitol	–	nr	–	nr	nr	–	nr	nr	nr	nr	–	+	+	nr	–	–	nr	nr	+	nr	nr	nr	nr	–
L-Arabitol	nr	nr	nr	nr	nr	–	nr	nr	nr	nr	nr	d	d	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr
Arbutin	nr	–	nr	nr	nr	nr	nr	nr	nr	–	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr
Cyclodextrin	–	nr	–	nr	nr	nr	nr	nr	nr	nr	–	nr	nr	nr	–	–	nr	nr	–	nr	nr	nr	nr	–
Cellobiose	–	nr	nr	–	+/–	–	nr	+	–	d	+	d	d	–	+	nr	d	d	nr	–	d	d	d	+
Dextrin	nr	nr	nr	nr	+	nr	nr	+	+	nr	nr	nr	nr	d	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr
Dulcitol	nr	–	nr	nr	–	–	nr	–	–	–	nr	d	d	–	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr
iso-Erythritol	nr	nr	nr	nr	nr	–	nr	nr	–	–	nr	+	+	–	nr	nr	d	d	nr	nr	nr	nr	nr	nr
D-Fructose	nr	+	nr	+/–	+	+	nr	+	+	+	+	+	+	+	+	nr	d	d	nr	+/–	+	+	+	+
D-Fucose	nr	nr	nr	nr	nr	–	nr	nr	nr	–	nr	d	d	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr
L-Fucose	nr	nr	nr	nr	nr	–	nr	nr	nr	–	nr	d	d	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr
D-Galactose	nr	+	nr	nr	nr	+	nr	nr	d	+	nr	+	+	d	nr	nr	d	d	nr	d	nr	nr	nr	nr
β-Gentiobiose	nr	–	nr	nr	nr	–	nr	nr	nr	–	nr	d	d	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr
D-Glucose	+	+	+	+	+	+	–	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Glycerol	nr	nr	nr	d	nr	+	nr	nr	d	–	nr	+	+	d	nr	nr	nr	nr	nr	–	nr	nr	nr	nr
Glycogen	–	d	–	+	–/+	–	nr	–/+	d	–	–	d	d	d	–	–	–	–	–	–	d	d	d	–
myo-Inositol	nr	nr	nr	–/+	+/–	+	nr	+/–	–	–	nr	+	+	–	nr	nr	d	d	nr	+/–	+	+	+	nr
Inulin	nr	–	nr	nr	nr	–	nr	nr	–	–	nr	d	–	–	nr	nr	nr	nr	nr	–/+	nr	nr	nr	nr
2-Ketogluconate	nr	–	nr	nr	nr	nr	nr	nr	nr	nr	nr	–	–	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr
5-Ketogluconate	nr	nr	nr	nr	nr	+	nr	nr	nr	–	nr	+	+	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr
Lactose	–	–	d	+/–	+	+	nr	+	d	+	–	+	+	d	–	+	d	–	+	+/(+)	d	d	d	(+)/–
D-Lyxose	nr	nr	nr	nr	nr	nr	nr	nr	nr	–[+]	nr	+	+	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr
Maltose	d	+	d	+	+	+	nr	+	d	–	–	+	+	d	d	+	+	+/d	+	+	+	+	+	+
D-Mannitol	–	–	–	–/+	+/–	–	nr	+	–	–	–	+	+	–	–	+	–	–	d	–	–	–	–	–
D-Mannose	d	d	nr	–/+	+	nr	–	+	–	–/(+)	–	+	+	–	nr	nr	+	d	nr	+/–	+/–	+	d	+
Melezitose	–	d	–	–/+	+/–	–	nr	–/+	–	–[+]	–	+	+	–	–	+	d	d	+	–	–/+	d	–	–
Melibiose	–	d	–	–	+/–	–	nr	+/–	–	–	–	+	+	–	–	+	d	d	+	+	+/–	d	+	+
α-Methyl-D-glucoside	nr	d	nr	nr	nr	–	nr	nr	–	nr	nr	d	+	–	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr
α-Methyl-D-mannoside	nr	d	nr	nr	nr	–	nr	nr	–	nr	nr	d	–	–	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr
Methyl-β-D-glucopyranoside	–	nr	–	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	–	+	nr	nr	d	nr	nr	nr	nr	–
Pullulan	–	nr	–	nr	nr	nr	nr	nr	nr	nr	–	nr	nr	nr	–	–	nr	nr	–	nr	nr	nr	nr	–
D-Raffinose	d	–	–	–/+	+/–	nr	–	+	–	–	–	+	+	–	d	+	d	d	+	+/–	+	+	+	+
L-Rhamnose	nr	–	nr	+	–/+	–	nr	–	–/+	–	nr	–	–	d	nr	nr	–	–	nr	–	–/+	–	–	nr
D-Ribose	d	d	d	+	+/–	+	nr	+/–	+	–[+]	–	+	+	d	–	+	+	+	d	–	d	d	–/+	–
Salicin	–	–	nr	–/+	+/–	–	nr	+/–	–	–	–	–	d	d	+	nr	+	–	nr	–/(+)	d	d	d	nr
D-Sorbitol	–	–	–	–/+	–/+	–	nr	–/+	–	–	–	–	–	–	–	–	–	–	–	–	–/+	d	d	–
L-Sorbose	nr	nr	nr	nr	nr	–	nr	nr	–	–	nr	–	–	–	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr
Starch	nr	nr	nr	+	d	nr	nr	+/–	+/–	–	nr	nr	nr	d	nr	nr	nr	+	nr	d	d	d	d	nr
Sucrose	d	d	+	+	+	+	nr	+	+	+	–	+	+	d	d	+	+	+	+	+	+	+	+	+
D-Tagatose	–	nr	–	nr	nr	+	nr	nr	nr	–	–	d	d	nr	–	–	nr	nr	–	nr	nr	nr	nr	–

(continued)

TABLE 7. (continued)

Characteristic	*A. cardiffensis*	*A. europaeus*	*A. funkei*	*A. georgiae*	*A. gerencseriae*	*A. graevenitzii*	*A. hongkongensis*[c]	*A. israelii*	*A. meyeri*	*A. massiliensis*	*A. nasicola*	*A. neuii* subsp. *neuii*	*A. neuii* subsp. *anitratus*	*A. odontolyticus*	*A. oricola*	*A. radicidentis*	*A. radingae*	*A. turicensis*	*A. urogenitalis*	*A. viscosus sensu stricto*[d]	*A. naeslundii sensu stricto*	*A. oris*	*A. johnsonii*	*A. dentalis*
Trehalose	–	d	–	+	+	–	–	+/–	–	+	–	+	+	d	d	+	d	+	+	d	d	d	d	+
D-Turanose	nr	d	nr	nr	nr	nr	nr	nr	nr	–[+]	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr
Xylitol	nr	nr	nr	nr	nr	–	nr	nr	nr	–	nr	d	–	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr
D-Xylose	–	–	+	+/–	+/–	–	–	+	+	–[+]	–	+	+	d	–	nr	+	+	+	–	–/+	–/+	–	–
L-Xylose	nr	nr	nr	nr	nr	–	nr	nr	nr	–	nr	–	–	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr
Hydrolysis of:																								
Casein	nr	nr	nr	nr	–	nr	nr	–	–	nr	nr	nr	nr	–	nr	nr	nr	nr	nr	–	nr	nr	nr	nr
Esculin	–	d	–	+/–	+	–	–	+	–/+	nr	–	–	–	d	+	+	+	–	+	+	+	+	+	+
Gelatin	–	nr	–	d	–	nr	nr	–	–	nr	–	–	–	–	–	+	–/ (+)	–	–	–	–	–	–	–
Hippurate	–	nr	+	nr	–	–	nr	–	–	nr	–	nr	nr	–	–	–	nr	nr	–	nr	nr	nr	nr	–
Starch	–	nr	nr	–/+	–	nr	nr	–	–	nr	–	nr	nr	d	–	nr	nr	nr	nr	+	–	–	–	–
Enzyme activities:																								
N-Acetyl-β-glucosaminidase	–	–	d	nr	–	+	–	–	–	–	+	nr	nr	–	–	–	+	–	+	nr	–	–	–	–
Acid phosphatase	nr	nr	+	nr	d	+	nr	d	–	–	–	–	–	–	nr	+	nr	nr	–	nr	nr	nr	nr	nr
Alanine arylamidase	+	nr	nr	nr	nr	nr	+	nr	nr	-	+	nr	nr	nr	+	nr	nr	nr	nr	nr	–	–	–	+
Alanine phenylalanine proline arylamidase	+	nr	+	nr	nr	nr	nr	nr	nr	nr	+	nr	nr	nr	+	+	nr	nr	+	nr	nr	nr	nr	–
Alkaline phosphatase	–	–	+	nr	–	nr	+	–	–	–	–	–	d	–	d	–	–	–	d	nr	–	–	d	–
α-Arabinosidase	–	nr	nr	nr	nr	nr	–	nr	nr	–	–	nr	nr	nr	–	nr	d	–	nr	nr	–	–	–	–
Arginine arylamidase	+	nr	nr	nr	nr	nr	+	nr	nr	–	+	nr	nr	nr	+	nr	+	+	nr	nr	–	–	–	+
Arginine dihydrolase	–	nr	–	nr	+	nr	+	+	–	–	d	nr	nr	–	–	–	nr	nr	–	nr	–	–	–	–
Benzoyl-arginine arylamidase	nr	nr	nr	nr	nr	nr	–	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr
Chymotrypsin	nr	–	–	nr	–	–	nr	–	–	–	–	–	–	–	nr	–	–	–	–	nr	nr	nr	nr	nr
Cysteine arylamidase	nr	nr	nr	nr	–	nr	nr	–	+	–	–	nr	nr	–	nr	–	–	–	d	nr	nr	nr	nr	nr
DNase	nr	nr	nr	nr	–	nr	nr	–	–	nr	nr	nr	nr	d	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr
Esterase C4	nr	+	–	nr	d	nr	nr	d	–	–	–	nr	nr	–	nr	nr	+	+/–	–	nr	nr	nr	nr	nr
Ester lipase C8	nr	+	–	nr	–	nr	nr	–	–	–	–	nr	nr	–	nr	–	+	+/–	–	nr	nr	nr	nr	nr
α-Fucosidase	–	nr	–	nr	–	–	–	–	–	–	–	nr	nr	d	–	–	nr	nr	–	nr	–	–	–/+	–
β-Fucosidase	nr	nr	nr	nr	nr	nr	–	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	+/–	+/–	+	nr
α-Galactosidase	–	+	–	nr	nr	–	–	nr	–	–	–	+	+	–	+	+	+	–	+	nr	+	+	+	+
β-Galactosidase	–	+	nr	nr	+	+	–	+	–	+	+	+	+	–	d	+	+	–	+	nr	+	+	+	d
α-Glucosidase	+	+	+	nr	+	–	–	+	+	+	+	+	+	–	+	+	+	+	+	nr	+/–	d	+	+
β-Glucosidase	–	–	–	nr	+	–	–	+	–	–	d	–	–	d	+	+	+	–	+	nr	+/–	d	–/+	+
β-Glucuronidase	–	–	–	nr	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	nr	–	–	–	–
Glutamic acid decarboxylase	nr	nr	nr	nr	nr	nr	–	nr	nr	–	–	nr	nr	nr	–	nr	nr	nr	nr	nr	–	–	–	–
Glutamyl glutamic acid arylamidase	nr	nr	nr	nr	nr	nr	–	nr	nr	–	d	nr	nr	nr	–	nr	nr	nr	nr	nr	–	–	–	–
Glycine arylamidase	+	nr	nr	nr	nr	nr	+	nr	nr	–	+	nr	nr	nr	+	nr	+	+	nr	nr	–	–	–	–
Glycyl tryptophan arylamidase	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	–	nr	nr	nr	–	nr	nr	nr	nr	nr	nr	nr	nr	–
Histidine arylamidase	+	nr	nr	nr	nr	nr	+	nr	nr	nr	+	nr	nr	nr	–	nr	nr	nr	nr	nr	–	–	nr	(+)
β-Lactosidase	nr	nr	nr	nr	nr	nr	–	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr
Lecithinase	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	–	nr	nr	nr	–	nr	nr	nr	nr	nr	nr	nr	nr	nr

(continued)

TABLE 7. (continued)

Characteristic	*A. cardiffensis*	*A. europaeus*	*A. funkei*	*A. georgiae*	*A. gerencseriae*	*A. graevenitzii*	*A. hongkongensis*[c]	*A. israelii*	*A. meyeri*	*A. massiliensis*	*A. nasicola*	*A. neuii* subsp. *neuii*	*A. neuii* subsp. *anitratus*	*A. odontolyticus*	*A. oricola*	*A. radicidentis*	*A. radingae*	*A. turicensis*	*A. urogenitalis*	*A. viscosus sensu stricto*[d]	*A. naeslundii sensu stricto*	*A. oris*	*A. johnsonii*	*A. dentalis*
Lipase C14	–	nr	–	nr	–	–	nr	–	–	–	–	nr	nr	–	–	nr	–	–	–	nr	nr	nr	nr	nr
Leucine arylamidase	+	+	+	nr	nr	+	+	nr	+	+	+	+	+	d	+	+	+	+/–	+	nr	+	+	+	+
Leucyl glycine arylamidase	+	nr	nr	nr	nr	nr	+	nr	nr	–	nr	nr	nr	nr	+	nr	+	+	nr	nr	–	–	–	–
Lysine arylamidase	nr	nr	nr	nr	nr	nr	+	nr	nr	nr	nr	nr	nr	nr	nr	nr	–	nr	nr	nr	nr	nr	nr	nr
α-Mannosidase	nr	–	–	nr	–	–	–	–	–	–	–	+	+	–	nr	–	–	–	d	nr	nr	nr	nr	nr
β-Mannosidase	–	nr	–	nr	nr	nr	nr	nr	nr	nr	–	nr	nr	nr	–	–	nr	nr	–	nr	nr	nr	nr	–
Proline arylamidase	+	nr	nr	nr	nr	nr	+	nr	nr	+	+	nr	nr	nr	+	nr	+	+	nr	nr	+	+	+	+
Phenylalanine arylamidase	+	nr	nr	nr	nr	nr	+	nr	nr	+	+	nr	nr	nr	+	nr	+	+	nr	nr	+	+	+	+
Phosphoamidase	nr	nr	nr	nr	d	nr	nr	d	–	nr	–	nr	nr	d	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr
Pyrazinamidase	–	–	d	nr	nr	nr	nr	nr	nr	nr	nr	+	+	nr	+	+	d	–	–	nr	nr	nr	nr	–
Pyroglutamic acid arylamidase	nr	nr	nr	nr	nr	nr	–	nr	nr	–	d	nr	nr	nr	–	nr	nr	nr	nr	nr	–	–	–	–
Pyrrolidonyl arylamidase	–	–	–	nr	nr	–	nr	nr	nr	nr	–	nr	nr	nr	nr	nr	–	–	+	nr	nr	nr	nr	nr
Serine arylamidase	+	nr	nr	nr	nr	nr	+	nr	nr	–	+	nr	nr	nr	–	nr	+	+	nr	nr	–	–	–	–
Trypsin	nr	–	–	nr	–	–	nr	–	–	–	–	–	–	–	nr	–	–	–	–	nr	nr	nr	nr	nr
Tyrosine arylamidase	+	nr	nr	nr	nr	nr	+	nr	nr	(+)	+	nr	nr	nr	+	nr	nr	nr	nr	nr	+	+	+	–
Urease	–	–	–	–	–	–	–	–	d	–	–	–	–	–	–	d	–	–	–	nr	–	–	d	–
Valine arylamidase	nr	+	–	nr	–	–	nr	–	+	+	+	–	–	–	nr	–	nr	–	+	nr	nr	nr	nr	nr
β-Xylosidase	nr	nr	nr	nr	+	nr	–	+	nr	nr	nr	nr	nr	–	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr
Catalase production	–	–	–	–	–	–	–	–	–	–	–	+	+	–	–	+	–	–	–	+	–	d	–	–
Nitrate reduction	d	–	d	–/+	–/+	–	–	+/–	–	+	–	+	–	+	–	d	–	–	+	d	+	d	+/–	–
Nitrite reduction	nr	nr	nr	nr	nr	nr	nr	nr	–	nr	nr	nr	nr	–	nr	nr	nr	nr	nr	–	nr	nr	nr	nr
Acetoin production	–	nr	–	nr	–	nr	nr	–	–	–	–	nr	nr	–	–	(+)	nr	nr	d	–	nr	nr	nr	–
Indole production	–	nr	nr	nr	–	nr	–	–	–	–	–	nr	nr	–	–	nr	–	nr	nr	–	–	–	–	–

[a]Symbols: +, positive/present; –, negative/absent; d, positive or negative, possibly depending on the system used; +/–, usually positive; –/+, usually negative; (), weak reaction; –[+], acid production by *Actinomyces massiliensis* usually detected after 24 h, with some strains showing positive reactions after 48 h.

[b]Data compiled from: Schofield and Schaal (1981); Cato et al. (1984); Schaal (1986b); Johnson et al. (1990); Funke et al. (1994, 1997a); Wüst et al. (1995a); Pascual Ramos et al. (1997a); Collins et al. (2000); Nikolaitchouk et al. (2000); Lawson et al. (2001b); Sarkonen et al. (2001); Hall et al. (2002, 2003c, 2003d, 2005); Woo et al. (2003); Henssge et al. (2009); Renvoise et al. (2009).

[c]The negative acid production tests from carbohydrates may be due to the use of an inadequate commercial test system in the original description of the species.

[d]Animal species included for comparison.

TABLE 8. Morphological characteristics of *Actinomyces* species from animal sources[a,b]

Characteristic	*A. bovis*	*A. bowdenii*	*A. canis*	*A. catuli*	*A. coleocanis*	*A. denticolens*	*A. hordeovulneris*	*A. howellii*	*A. hyovaginalis*, "general" biovar	*A. hyovaginalis*, "vaginal" biovar	*A. marimammalium*	*A. slackii*	*A. suimastitidis*	*A. vaccimaxillae*	*A. viscosus sensu stricto*	*A. ruminicola*
Cellular morphology:																
Coccoid elements	–	–	–	–	–	–	–	–	d	d	–	–	–	–	–	–
Coccobacillary elements	d	–	–	–	–	–	–	–	d	d	–	+	–	d	–	–
Irregular rods	+	–	–	–	–	(+)	+	+	(+)	(+)		–	–	+	+	–
Rods with swollen ends	d					d	+								d	
Branched rods	d	d	d	–	d	d	+		–	–	d	–	–		d	–
Round-ended ends	+					+									+	
Square-ended ends	–					–									–	
Large spherical bodies	–					–	d	–							–	
Short filaments	d	–		–	–	d	d	d	–	–	–	–	–	–	d	–
Long filaments	d	–		–	–	–	d	–	–	–	–	–	–	–	d	–
Gram-reaction positive	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Gram-reaction variable	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Motility	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Colony morphology:																
Microcolonies filamentous	d					d	+		–	–	–	–	–	–	d	
Macrocolony size:																
≤2 mm	+	d		+		+	d	+	+	+		+		+	d	+
>2 mm	–	d		–		–	d	–				–		–	d	–
Flat	–			–		–	–	–	+	–	–	–		–	(d)	–
Raised	+			+		+	+	+	–	+	+	+		+	+	+
Umbonate	d					–	d	–							d	
Central depression	d					–	–	–							–	
Entire margin	d	+		–		+	–	+	–	+	+	+		+	d	+
Undulate margin	d	–		+		–	d	–	+	–	–	–		–	d	–
Filamentous margin	–	–				–	d	–	–	–	–	–		–	d	–
Transparent	–			–		–	–	d				+		–	–	
Opaque	+			+		+	+	d				–		+	+	
White/gray-white	d	+		+		d	+	+	+	+	+	+		+	d	+
Creamy white/beige	d	–		–		–	–	–	–	–	–	–		–	d	–
Brown/tan	–	–		–		–	–	–	–	–	–	–		–	–	–
Orange	–	–		–		–	–	–	–	–	–	–		–	–	–
Pink	–	–		–		d	–	–	–	–	–	–		–	–	–
Red/reddish	–	–		–		–	–	–	–	–	–	–		–	–	–
Rough	d	–		d		–	+	–	–	–	–	–		–	d	
Smooth	d	+		d		+	–	+	+	+	+	+		+	d	
Dry to crumbly	d			+		–	d	–	–	–					d	
Soft	d					+	d	+							d	
Mucoid	–	–		–		–	–	–	–		–	–		–	d	–

[a]Symbols: +, present; –, absent; (+), weakly or irregularly present; d, strain differences; (d), occasional strain difference.

[b]Data compiled from: Schaal (1986b), An et al. (2006), Schaal et al. (2006).

TABLE 9. Physiological properties of *Actinomyces* species from animal sources[a,b]

Property/characteristic	*A. bovis*	*A. bowdenii*	*A. canis*	*A. catuli*	*A. coleocanis*	*A. denticolens*	*A. hordeovulneris*	*A. howellii*	*A. hyovaginalis*, "general" biovar	*A. hyovaginalis*, "vaginal" biovar	*A. marimammalium*	*A. slackii*	*A. suimastitidis*	*A. vaccimaxillae*	*A. viscosus*	*A. ruminicola*
Acid production from:																
N-Acetyl-β-glucosamine	nr	nr	nr	nr	–	nr	nr	nr	+	+	+	nr	nr	nr	–	nr
Adonitol	–	nr	nr	nr	nr	nr	nr	nr	+	+	nr	nr	nr	nr	nr	–
Amygdalin	–	nr	nr	nr	nr	–/(+)	nr	–	nr	nr	nr	–	nr	nr	–	nr
D-Arabinose	nr	nr	nr	nr	nr	nr	nr	nr	+	+	nr	nr	nr	nr	nr	nr
L-Arabinose	–	–	+	d	–	–	–	+/–	+	+	–	–	(+)	+	–	nr
D-Arabitol	nr	–	–	–	–	nr	nr	nr	–	–	–	nr	–	–	nr	+
L-Arabitol	nr	nr	nr	nr	nr	nr	nr	nr	nr	+	nr	nr	nr	nr	nr	nr
Cyclodextrin	nr	–	–	–	–	nr	nr	nr	nr	nr	–	nr	–	–	nr	+
Cellobiose	–	nr	nr	nr	nr	–/(+)	+	–	+/–	–	nr	–	nr	nr	–	+
Dextrin	d	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr
Dulcitol	–	nr	nr	nr	nr	nr	nr	nr	nr	–	nr	nr	nr	nr	nr	–
iso-Erythritol	–	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	+
D-Fructose	+	nr	nr	nr	nr	nr	nr	nr	+	+	nr	nr	nr	nr	+/–	(+)
D-Galactose	+	nr	nr	nr	nr	nr	nr	nr	+	+	nr	nr	nr	nr	d	+
β-Gentiobiose	nr	nr	nr	nr	nr	nr	nr	nr	nr	–	nr	nr	nr	nr	nr	nr
D-Glucose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Glycerol	–	nr	nr	nr	nr	–/(+)	–	–	nr	nr	nr	–	nr	nr	–	–
Glycogen	d	nr	+	–	+	nr	nr	nr	nr	nr	d	nr	nr	d	–	d
myo-Inositol	d	nr	nr	nr	nr	+/–	–	–	–	–	nr	–/+	nr	nr	+/–	d
Inulin	–	nr	nr	nr	nr	+/–	nr	–	nr	–	nr	–/+	nr	nr	–/+	–
Lactose	d	+	+	+	+	+	+	+/–	nr	d	+	d	–	d	+/(+)	+
D-Lyxose	nr	nr	nr	nr	nr	nr	nr	nr	nr	+	nr	nr	nr	nr	nr	nr
Maltose	+	+	+	+	+	+	+	+	+	+	+	nr	d	–	+	+
D-Mannitol	–	–	–	–	–	d	–	–	–	–	–	–	–	d	–	d
D-Mannose	d	nr	nr	nr	nr	–/(+)	(+)	+/–	+	+	nr	d	nr	-	+/–	d
Melezitose	–	+	–	–	–	–/(+)	nr	nr	–	–	–	–	–	–	–	+
Melibiose	d	+	–	d	–	nr	(+)	+/–	nr	–	–	nr	+	–	+	+
α-Methyl-D-glucoside	–	nr	nr	nr	nr	nr	nr	nr	nr	–	nr	nr	nr	nr	nr	nr
α-Methyl-D-mannoside	–	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr
Methyl-β-D-glucopyranoside	nr	+	–	d	–	nr	nr	nr	nr	nr	–	nr	–	–	nr	nr
Pullulan	nr	–	+	–	+	nr	nr	nr	nr	nr	–	nr	+	–	nr	nr
D-Raffinose	–	+	d	+	–	+	(+)	+	–	–	–	+	+	–	+/–	nr
L-Rhamnose	–	nr	nr	nr	nr	–/(+)	–	–	nr	–	nr	–	nr	nr	–	+
D-Ribose	d	+	+	+	–	+/–	–	–	+	+	–	–/+	(+)	+	–	+
Salicin	–	nr	nr	nr	nr	+	nr	–	+	+	nr	+/(+)	nr	nr	–/(+)	d
D-Sorbitol	–	–	–	–	–	–	nr	nr	nr	–	–	–	–	–	–	+
L-Sorbose	–	nr	nr	nr	nr	nr	nr	nr	–	–	nr	nr	nr	nr	nr	nr
Starch	+/–	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	d	nr
Sucrose	+	+	d	+	–	+	nr	+	+	+	–	d	+	d	+	+
D-Tagatose	nr	d	–	–	–	nr	nr	nr	nr	–	–	nr	–	–	nr	nr
Trehalose	–	+	–	+	–	–	+	+/–	–	–	–	d	–	+	d	+
D-Turanose	nr	nr	nr	nr	nr	nr	nr	nr	nr	+	nr	nr	nr	nr	nr	nr
Xylitol	nr	nr	nr	nr	nr	nr	nr	nr	–	–	nr	nr	nr	nr	nr	d
D-Xylose	–	–	+	+	–	–	+	+/–	+	+	–	–	+	+	–	+
L-Xylose	nr	nr	nr	nr	nr	nr	nr	nr	–	–	nr	nr	nr	nr	nr	nr
Hydrolysis of:																
Casein	–	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	–	nr
Esculin	d	+	–	+	–	+	+	nr	+	+	–	–	+	+	+	d

(continued)

TABLE 9. (continued)

Property/characteristic	*A. bovis*	*A. bowdenii*	*A. canis*	*A. catuli*	*A. coleocanis*	*A. denticolens*	*A. hordeovulneris*	*A. howellii*	*A. hyovaginalis*, "general" biovar	*A. hyovaginalis*, "vaginal" biovar	*A. marimammalium*	*A. slackii*	*A. suimastitidis*	*A. vaccimaxillae*	*A. viscosus*	*A. ruminicola*
Gelatin	–	–	–	–	–	nr	nr	nr	–	–	–	nr	–	–	–	nr
Hippurate	–	–	–	–	–	nr	nr	nr	–	+	–	nr	–	–	nr	–
Starch	+/–	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	–	+	nr
Enzyme activities:																
N-Acetyl-β-glucosaminidase	+	–	+	d	nr	–	nr	nr	+	+	nr	nr	d	–	nr	nr
Acid phosphatase	–	+	–	+	nr	+/–	nr	nr	nr	nr	nr	nr	nr	(+)	nr	nr
Alanine arylamidase	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	+	nr	nr
Alanine phenylalanine proline arylamidase	nr	+	+	–	+	nr	nr	nr	nr	nr	+	nr	+	+	nr	nr
Alkaline phosphatase	–	d	–	–	–	–	nr	nr	+	+	d	nr	d	d	nr	nr
α-Arabinosidase	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	–	nr	nr
Arginine arylamidase	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	+	nr	nr
Arginine dihydrolase	–	–	–	d	–	nr	nr	nr	nr	–	–	nr	–	–	nr	nr
Chymotrypsin	–	–	–	–	nr	–	nr	nr	nr	nr	nr	nr	nr	–	nr	nr
Cystine arylamidase	–	–	+	–	nr	–	nr	nr	nr	nr	nr	nr	nr	–	nr	nr
DNase	+	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr
Esterase C4	–	–	–	d	nr	–	nr	nr	nr	nr	nr	nr	nr	–	nr	nr
Ester lipase C8	–	–	+	+	nr	–	nr	nr	nr	nr	nr	nr	nr	–	nr	nr
α-Fucosidase	–	–	+	–	nr	–	nr	nr	nr	nr	nr	nr	nr	+	nr	nr
α-Galactosidase	–	+	+	d	–	+/–	nr	nr	+	+	–	nr	+	–	nr	nr
β-Galactosidase	–	+	+	+	+	+	nr	nr	+	+	+	nr	+	–	nr	nr
α-Glucosidase	–	d	+	+	+	+	nr	nr	+	+	–	nr	+	d	nr	nr
β-Glucosidase	–	+	–	+	–	+/–	nr	nr	nr	nr	–	nr	+	d	nr	nr
β-Glucuronidase	–	–	–	+	–	–	nr	nr	–/(+)	–	–	nr	–	–	nr	nr
Glycine arylamidase	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	+	nr	nr
Histidine arylamidase	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	+	nr	nr
Lipase C14/Tween 80	–	–	–	–	nr	–	nr	nr	+	+	nr	nr	nr	–	nr	nr
Leucine arylamidase	+	+	+	+	nr	+	nr	nr	+	+	+	nr	nr	+	nr	nr
Leucyl glycine arylamidase	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	+	nr	nr
α-Mannosidase	–	–	–	–	nr	–	nr	nr	nr	nr	nr	nr	nr	–	nr	nr
β-Mannosidase	nr	–	–	–	–	nr	nr	nr	nr	nr	–	nr	–	–	nr	nr
Proline arylamidase	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	+	nr	nr
Phenylalanine arylamidase	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	+	nr	nr
Pyrazinamidase	nr	+/–	+	+	+	nr	nr	nr	nr	–	–	nr	(+)	+	nr	nr
Pyrrolidonyl arylamidase	nr	nr	–	+	–	nr	nr	nr	–	–	–	nr	nr	–	nr	nr
Serine arylamidase	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	+	nr	nr
Trypsin	–	–	–	–	nr	–	nr	nr	nr	nr	nr	nr	nr	–	nr	nr
Tyrosine arylamidase	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	+	nr	nr
Urease	–	–	–	–	–	nr	–	nr	nr	–	–	nr	–	–	nr	–
Valine arylamidase	–	–	–	–	nr	–	nr	nr	nr	nr	nr	nr	nr	–	nr	nr
β-Xylosidase	–	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr
Catalase production	–	+	+	d	–	–	(+)	+	–	–	–	+	–	–	+	–
Nitrate reduction	–	+	–	+	–	+	–	nr	–/+	+	–	nr	–	–	d	+
Nitrite reduction	–	nr	nr	nr	nr	–	nr	nr	nr	nr	nr	nr	nr	nr	–	nr
Acetoin production	–	–	–	–	–	nr	–	nr	nr	nr	–	nr	(+)	–	–	–
Indole production	–	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	–	–	nr

[a]Symbols: +, positive/present; –, negative/absent; d, strain differences; +/–, predominantly positive; –/+, predominantly; (), weakly or irregularly positive; nr, not reported.

[b]Data compiled from: Howell and Jordan (1963), Schofield and Schaal (1981), Buchanan et al. (1984), Dent and Williams (1984b, 1984c, 1986), Schaal (1986b), Johnson et al. (1990), Collins et al. (1993), Pascual et al. (1999), Hoyles et al. (2000, 2001a, 2001b, 2001c, 2002a, 2002b); Hall et al. (2003a), and An et al. (2006).

List of species of the genus *Actinomyces*

1. **Actinomyces bovis** Harz 1877, 133[AL] (*Discomyces bovis* Harz 1877) Rivolta 1878; *Sarcomyces bovis* (Harz 1877) Rivolta 1879; *Oospora bovis* (Harz 1877) Sauvageau and Radais 1892; *Actinocladothrix bovis* (Harz 1877) Gasperini 1892; *Nocardia bovis* (Harz 1877) Blanchard 1896; *Streptothrix bovis* (Harz 1877) Chester 1901; *Cladothrix bovis* (Harz 1877) Macé 1901; *Sphaerotilus bovis* (Harz 1877) Engler 1907; *Proactinomyces bovis* (Harz 1877) Henrici 1939

bo′vis. L. masc. and fem. n. *bos* ox, cow; L. gen. n. *bovis* of the ox/cow.

Cells of *Actinomyces bovis* are usually short and diphtheroidal (Figure 24), but short branching rods (Figure 21) may occur while long multibranched filaments are rarely seen. The two serovars recognized within the species are most easily and reliably identified by direct or indirect fluorescent antibody techniques. In addition, the microcolonies of serovar I strains are usually smooth with an entire to irregular edge, slightly raised to convex, white to colorless and soft and show only occasionally short radiating filaments at the edge (Figure 35), while serovar II microcolonies tend to be filamentous (Figure 39) and may even resemble *Actinomyces israelii* microcolonies (Figure 30). Mature *Actinomyces bovis* colonies are 0.5–1.0 mm in diameter, circular, with a smooth or finely granular surface, convex, entire-edged, and usually soft (Figure 49). Strains producing filamentous microcolonies also form rough macrocolonies resembling the molar-tooth or bread-crumb colony type of *Actinomyces israelii* (Figure 51). Catalase-negative. Further morphological details, ultrastructure, cell-wall composition, nutrition and growth conditions, metabolism, and metabolic pathways as well as drug susceptibility are as given for the genus and as summarized in Table 8. Physiological and other descriptive and differential characteristics are listed in Table 5 and Table 9. The natural habitat is not definitely known especially because in studies on the plaque flora of dairy cattle, *Actinomyces bovis* could not be identified (Dent and Williams, 1984b, 1984c, 1986). However, given the apparently endogenous origin of bovine infections, the natural habitat is assumed to be the oral cavity and/or the intestinal tract of cattle and possibly other animals. *Actinomyces bovis* is the causative agent of actinomycosis in cattle (bovine actinomycosis, lumpy jaw). The etiology of similar infections in other animals, including a llama, has not been clarified with certainty although it has been claimed that at least some of these diseases might also be due to *Actinomyces bovis*. In contrast, this species has never been proved to cause actinomycosis in man, nor has it been isolated from human mucosal surfaces or other human sources. Experimental infections have been produced in hamsters, mice and a few other animal species after intraperitoneal, intravenous or subcutaneous injections of viable cell suspensions.

Source: a typical case of lumpy jaw in a cow.

DNA G+C content (mol%): 57–63 (T_m).

Type strain: ATCC 13683, CIP 103258, NCTC 11535, CCUG 31996, DSM 43014 (serovar I).

Reference strain for serovar II: WVU 292.

Sequence accession no. (16S rRNA gene): X81061.

2. **Actinomyces bowdenii** Pascual, Foster, Falsen, Bergström, Greko and Collins 1999, 1876[VP]

bow.de′ni.i. N.L. gen. masc. n. *bowdenii* of Bowden, to honor George H. Bowden, a contemporary British microbiologist, who made many valuable contributions to actinomycete microbiology.

Cells are straight or slightly curved rods, 2–4 µm in length, some of which exhibit branching. Colonies on blood agar are non-hemolytic, grayish-white, circular, and approximately 2 mm in diameter after 24–48 h of aerobic incubation. Growth is not enhanced by increased concentrations of CO_2. Catalase-positive. Additional morphological details, chemotaxonomic markers, nutrition, and growth conditions and metabolic activities are as given for the genus and as summarized in Table 8. Physiological and other descriptive and differential characteristics are listed in Table 5 and Table 9. The natural habitat is not definitely known.

Source: clinical materials from dogs and a cat. The type strain was from an abscess under the mandible of a dog.

DNA G+C content (mol%): not reported.

Type strain: M1327/96/1. CCUG 37421, CIP 106647, DSM 15435.

Sequence accession no. (16S rRNA gene): AJ234039.

3. **Actinomyces canis** Hoyles, Falsen, Foster, Pascual, Greko and Collins 2000, 1549[VP]

ca′nis. L. masc. and fem. n. *canis* dog, bitch; L. gen. n. *canis* of the dog/bitch.

Cells are straight to slightly curved rods, some of which exhibit branching. Catalase-positive. Additional morphological details, nutrition and growth conditions, and metabolic activities are as given for the genus and as summarized in Table 8. Physiological and other descriptive and differential characteristics are listed in Table 5 and Table 9. *Actinomyces canis* can be readily distinguished from other *Actinomyces* species by physiological characteristics and SDS-PAGE analysis of whole-cell proteins. The natural habitat is not known.

Source: canine vagina, a fatal pyogranulomatous pleurisy of a foxhound bitch, and a subcutaneous abscess in a female hunting dog, exclusively in mixed culture with other aerobic and anaerobic bacteria. The type strain was isolated from the vagina of a dog with a history of infertility.

DNA G+C content (mol%): not reported.

Type strain: M2289/98/2, CCUG 41706, CIP 106351, DSM 15536.

Sequence accession no. (16S rRNA gene): AJ243891.

4. **Actinomyces cardiffensis** Hall, Collins, Hutson, Falsen and Duerden 2003f, 1[VP] (Effective publication: Hall, Collins, Hutson, Falsen and Duerden 2002, 3429.)

car.dif.fen′sis. N.L. masc. adj. *cardiffensis* of or pertaining to Cardiff, a city in Wales.

Cells are pleomorphic, slender, straight to curved rods; beaded branching filaments occur. On Fastidious Anaerobe Agar with 5% horse blood after 48 h of incubation, colonies are pinpoint, convex, smooth-surfaced, entire-edged, opaque, cream to pinkish, and non-hemolytic.

Catalase-negative. Additional morphological details, nutrition and growth conditions as well as metabolism and metabolic pathways are as given for the genus and as summarized in Table 6. Physiological and other descriptive and differential characteristics are listed in Table 4 and Table 7. The natural habitat is not known.

Source: human clinical sources, including IUCDs, pleural fluid, brain, jaw, pericolic and ear abscesses, and nasal antrum. The type strain was isolated from an intrauterine contraceptive device, which had been *in situ* for 7 years in a 35-year-old woman.

DNA G+C content (mol%): not reported.

Type strain: R10394, CCUG 44997, CIP 107323, DSM 15803.

Sequence accession no. (16S rRNA gene): AJ421779.

5. **Actinomyces catuli** Hoyles, Falsen, Pascual, Sjödén, Foster, Henderson and Collins 2001b, 681[VP]

ca'tu.li. L. masc. n. *catulus* young dog; L. masc. gen. n. *catuli* of a small dog.

Cells are straight to slightly curved rods. Colonies are tiny, irregular, convex, white, and adherent to medium at colony base. Catalase-negative or weakly catalase-positive. Additional morphological details, nutrition and growth conditions, and metabolism and metabolic pathways are as given for the genus and as summarized in Table 8. Physiological and other descriptive and differential characteristics are listed in Table 5 and Table 9. In SDS-PAGE analysis, the *Actinomyces catuli* isolates formed a distinct group which was separate from all *Actinomyces* species described until 2001. The natural habitat is not known.

Source: polymicrobial infection of a dog with pneumonia and exudative pleurisy and from a dog from which no information on the clinical background was available. The type strain was isolated from mediastinal tissue and blood clot of a dog with pneumonia and exudative pleurisy together with *Bacteroides fragilis*, *Fusobacterium nucleatum*, *Peptostreptococcus anaerobius*, and *Ruminococcus (Peptostreptococcus) productus.*

DNA G+C content (mol%): not reported.

Type strain: M1192/98/1, CCUG 41709, CIP 106507, DSM 15415.

Sequence accession no. (16S rRNA gene): AJ276805.

6. **Actinomyces coleocanis** Hoyles, Falsen, Foster and Collins 2002a, 1203[VP]

co.le.o.ca'nis. Gr. masc. n. *koleos* sheath, vagina; L. masc. and fem. n. *canis* dog, bitch; N.L. gen. n. *coleocanis* of the vagina of a bitch.

Cells are straight to slightly curved rods, some of which exhibit branching. Catalase-negative. Additional morphological details, nutrition, and growth conditions, as well as metabolism and metabolic pathways, are as given for the genus and as summarized in Table 8. Physiological and other descriptive and differential characteristics are listed in Table 5 and Table 9. Upon comparative analysis of SDS-PAGE whole-cell protein profiles, the single strain of *Actinomyces coleocanis* formed a distinct line and did not show a particularly close affinity with any recognized *Actinomyces* species. The natural habitat is not known.

Source: mixed culture with "*Corynebacterium genitalium*" from the vagina of a cocker spaniel dog. The type strain was isolated from the vagina of a dog.

DNA G+C content (mol%): not reported.

Type strain: M343/98/2, CCUG 41708, CIP 106873, DSM 15436.

Sequence accession no. (16S rRNA gene): AJ249326.

7. **Actinomyces dentalis** Hall, Collins, Lawson, Falsen and Duerden 2005, 430[VP]

den.ta'lis. L. masc. n. *dens, dentis* tooth; L. masc. suff. *-alis* suffix denoting pertaining to; N.L. masc. adj. *dentalis* pertaining to teeth.

Cells are rod-shaped or filamentous and beaded. After 48 h of anaerobic incubation on Fastidious Anaerobe Agar with 5% horse blood, colonies are tiny, white and breadcrumb-like and pit the agar. Catalase-negative. Additional morphological details, nutrition and growth conditions, as well as metabolism and metabolic pathways, are as given for the genus and as summarized in Table 6. Physiological and other descriptive and differential characteristics are listed in Table 4 and Table 7. The natural habitat is not known.

Source: pus of a dental abscess from a 72-year-old female patient with facial swelling. The type strain was isolated from a human dental abscess.

DNA G+C content (mol%): 62 (HPLC).

Type strain: R18165, CCUG 48064, CIP 108337, DSM 19115.

Sequence accession no. (16S rRNA gene): AJ697609.

8. **Actinomyces denticolens** Dent and Williams 1984d, 503[VP] (Effective publication: Dent and Williams 1984b, 188.)

den.ti.co'lens. L. masc. n. *dens, dentis* tooth; L. v. *colere* to inhabit, to dwell; L. part. adj. *colens* dwelling; N.L. part. adj. *denticolens* tooth-dwelling.

Cells are rod-shaped and straight or slightly curved (mean, 0.7 µm × 5 µm; Figure 25), but may also be very short. Microcolonies are usually flat and non-filamentous (Figure 38), but may show a few peripheral hyphae radiating from the granular center (Figure 37); very young microcolonies may be completely filamentous (Figure 40). Mature colonies on horse blood agar are slightly pink when grown anaerobically, but white if grown aerobically. They are circular, entire, convex, and smooth (Figure 55). Catalase-negative. Additional morphological details, cell-wall composition, nutrition and growth conditions, metabolism and metabolic pathways, and drug susceptibility are as given for the genus and as summarized in Table 8. Physiological and other descriptive and differential characteristics are listed in Table 5 and Table 9. The patterns of polypeptides as analyzed by SDS-PAGE are unique, although with some intraspecies variation, and allow the species to be distinguished from both *Actinomyces naeslundii* and *Actinomyces howellii* with which it shares several physiological and biochemical characters. Immunoblotting showed that *Actinomyces denticolens* was between 39% and 72% similar to *Actinomyces naeslundii* and *Actinomyces viscosus*, while whole-cell agglutination clearly differentiated *Actinomyces denticolens* from *Actinomyces naeslundii sensu stricto.* The normal habitat of *Actinomyces denticolens* is the oral cavity, especially dental plaque, of cattle. Pathogenicity has not been reported.

Source (type strain): supragingival plaque of dairy cattle.

DNA G+C content (mol%): 66–68 (T_m).

Type strain: Sh 8/4303, CIP 103126, ATCC 43322, CCUG 32758, DSM 20671, NCTC 11490.

Sequence accession no. (16S rRNA gene): X80412.

9. **Actinomyces europaeus** Funke, Alvarez, Pascual, Falsen, Åkervall, Sabbe, Schouls, Weiss and Collins 1997a, 690[VP]

eu.ro.pa′e.us. L. masc. adj. *europaeus* European, referring to the fact that six different European laboratories contributed to the description of the species.

Cells are short (0.5–1.5 µm in length) rods that sometimes are arranged in clusters. Colonies are circular and smooth with a translucent grayish appearance. After 48 h of incubation in a 5% CO_2-enriched atmosphere, their diameter is not greater than 0.5 mm. Catalase-negative. Additional morphological details, cell-wall composition, nutrition and growth conditions, metabolism and metabolic pathways as well as drug susceptibility are as given for the genus and as summarized in Table 6. Physiological and other descriptive and differential characteristics are listed in Table 4 and Table 7.

The natural habitat of *Actinomyces europaeus* is not definitely known.

Source: human abscesses. The type strain was isolated from femur tissue of a 63-year-old man.

DNA G+C content (mol%): 61–63 (HPLC).

Type strain: ATCC 700353, CCUG 32789A, CIP 105308, LMG 18454.

Sequence accession no. (16S rRNA gene): Y08828.

10. **Actinomyces funkei** Lawson, Nikolaitchouk, Falsen, Westling and Collins 2001b, 855[VP]

fun′ke.i. N.L. gen. masc. n. *funkei* of Funke, to honor Guido Funke, a contemporary German microbiologist, for his contributions to the clinical microbiology of *Actinobacteria*.

Cells are slender, straight to slightly curved rods, some of which exhibit branching. When grown on Columbia horse blood agar for 24 h in air plus 5% CO_2, colonies are small (<1 mm in diameter), gray, and non-hemolytic. Older cultures display fried-egg-type colony morphology. Catalase-negative. Additional morphological details as well as nutrition and growth conditions are as given for the genus and as summarized in Table 6. Physiological and other descriptive and differential characteristics are listed in Table 4 and Table 7. In SDS-PAGE analysis, the *Actinomyces funkei* isolates were highly related to each other and were distinct from recognized *Actinomyces* species. The natural habitat is not definitely known.

Source: human clinical specimens (blood cultures, sternum and abdominal incision). The type strain was isolated from three blood cultures from a 40-year-old female intravenous drug user who had a history of *Staphylococcus aureus* endocarditis of the tricuspid valve.

DNA G+C content (mol%): not reported.

Type strain: CCUG 42773, CIP 106713, DSM 15537.

Sequence accession no. (16S rRNA gene): AJ404889.

11. **Actinomyces georgiae** Johnson, Moore, Kaneko and Moore 1990, 276[VP]

ge.or.gi′a.e. N.L. gen. fem. n. *georgiae* of Georg, to honor Lucile K. Georg, a pioneer in *Actinomyces* taxonomy.

Cells grown in peptone-yeast extract-glucose broth for 24 h are 0.9–1.0 µm wide and 3.3–6.1 µm long. Without addition of a fermentable carbohydrate, cells are shorter and may even be coccoid. The rods occur in pairs and short chains and may have swellings; branching is seldom seen. Best growth is obtained when the cultures are incubated anaerobically. Colonies are circular, entire, pulvinate, translucent to opaque, white (27% are tan or beige), shiny, and smooth. Their diameter ranges from 0.5 to 2.0 mm. Addition of 0.02% Tween 80 to broth media enhances growth and fermentation of 62% of the strains. Catalase-negative. Additional morphological details, nutrition and growth conditions, metabolism and metabolic pathways are as given for the genus or as summarized in Table 6. Physiological and other descriptive or differential characteristics are listed in Table 4 and Table 7. *Actinomyces georgiae* cells do not react with FITC-labeled antisera to *Actinomyces gerencseriae*, *Actinomyces israelii*, *Actinomyces johnsonii*, *Actinomyces meyeri*, *Actinomyces naeslundii*, *Actinomyces odontolyticus* serovars I and II, and *Actinomyces oris*. *Actinomyces georgiae* has been isolated from human gingival crevices. It accounts for about 1.4% of the human healthy periodontal flora. The species has usually been considered nonpathogenic. However, a recent report suggests that it might be a rare causative agent of endocarditis in man.

Source (type strain): gingival crevice of a child.

DNA G+C content (mol%): 65–69 (T_m).

Type strain: ATCC 49285, CCUG 32935, CIP 104749, DSM 6843, VPI D145A-7.

Sequence accession no. (16S rRNA gene): X80413.

12. **Actinomyces gerencseriae** Johnson, Moore, Kaneko and Moore 1990, 278[VP]

ge.renc.se.ri′ae. N.L. gen. fem. n. *gerencseriae* of Gerencser, to honor the American microbiologist Mary Ann Gerencser, an authority on *Actinomyces* species.

Cellular and colonial morphology are similar to those of *Actinomyces israelii*: microcolonies may be highly filamentous and spider-like (Figure 28 and Figure 29), but may also be less filamentous with a few projecting short filaments and a fragmented center (Figure 31). Mature colonies may be highly filamentous even showing short aerial hyphae (Figure 43), but smooth and soft colonies are quite common (Figure 44). Catalase-negative. Additional morphological details, cell-wall composition, nutrition and growth conditions, metabolism and metabolic pathways as well as drug susceptibility are as given for the genus and as summarized in Table 6. Physiological and other descriptive and differential characteristics are listed in Table 4 and Table 7. The type strain and 12% of the other isolates tested were obligately anaerobic. The pH in peptone-yeast extract-glucose-Tween 80 medium incubated 3–5 d is 4.3–5.3. Addition of Tween 80 to broth medium increases the growth of 25% of the cultures and does not affect the growth of 75%. The species is distinct from *Actinomyces israelii sensu stricto* as determined by polyacrylamide gel electrophoresis banding patterns, serological reactions, and the inability to ferment L-arabinose.

The normal habitat of *Actinomyces gerencseriae* is the oral cavity of man including tonsillar crypts and dental plaque. The species was found to account for 1.2% of the human healthy periodontal flora and occurred in 14% of the samples examined. The respective figures in patients with

gingivitis and periodontitis were 1.5% and 17%. It apparently also occurs in lower numbers in the human intestinal and female genital tracts. *Actinomyces gerencseriae* is one of the typical causative agents of human cervicofacial and other actinomycoses. It has also been isolated from retroperitoneal abscesses, hand abscesses, and various other clinical conditions. Experimental animal infections are similar to or identical with those produced by *Actinomyces israelii*.

Source (type strain): human parotid abscess.

DNA G+C content (mol%): 70–71 (T_m).

Type strain: ATCC 23860, CCUG 32936, CCUG 34703, CDC W 838, CIP 105418, DSM 6844, JCM 12963, VPI 12594.

Sequence accession no. (16S rRNA gene): X80414.

13. **Actinomyces graevenitzii** Pascual Ramos, Falsen, Alvarez, Åkervall, Sjöden and Collins 1997a, 887[VP]

gra.e.ve.nit′zi.i. N.L. gen. masc. n. *graevenitzii* of Graevenitz, to honor Alexander von Graevenitz, a contemporary clinical microbiologist, for his many contributions to clinical microbiology.

Cells are straight or slightly curved rods that exhibit some branching and have swollen ends. Colonies on blood agar, incubated in the presence of 5% CO_2, are non-pigmented, opaque, and approximately 0.2 mm in diameter after 24 h of incubation; they adhere to agar surfaces. Catalase-negative. Additional morphological details, chemotaxonomic markers, nutrition and growth conditions, metabolism and metabolic pathways as well as drug susceptibility are as given for the genus and as summarized in Table 6. Physiological and other descriptive and differential characteristics are listed in Table 4 and Table 7. The natural habitat of *Actinomyces graevenitzii* appears to be the oral cavity of man, especially of children.

Source: human clinical specimens, in particular respiratory tract secretions and an osteitis of the jaw. The type strain was isolated from a bronchus brush of a 57-year-old woman.

DNA G+C content (mol%): not reported.

Type strain: CCUG 27294, CIP 105737, DSM 15540.

Sequence accession no. (16S rRNA gene): AJ540309.

14. **Actinomyces hominis** Funke, Englert, Frodl, Bernard, and Stenger 2010, 1679[VP]

ho′mi.nis. L. masc. n. *homo, hominis* man; L. gen. n. *hominis* of man, indicating that the type strain was isolated from a human.

Cells are Gram-stain-positive, diphtheroidal rods that do not stain partially acid-fast; filamentous forms are not observed. Mycolic acids are absent. The main straight-chain saturated cellular fatty acids are palmitic (32%) and stearic acids (15%); small amounts of decanoic, dodecanoic, and tetradecanoic acids are also detected. Oleic acid (39%) is the predominant unsaturated fatty acid. The type strain grows equally well under aerobic and anaerobic conditions. After 72 h of incubation on sheep blood agar at 35°C in a CO_2-enriched atmosphere, colonies are white-grayish, convex, non-lipophilic (i.e., do not take up Sudan Black or Oil Red-O), and 1 mm in diameter. Catalase-positive. When using the API Coryne, API ZYM, and API 50 CHB systems, acid is produced from D-adonitol, L-arabinose, D- and L-arabitol, erythritol, D-fructose, D-galactose, potassium gluconate, potassium 5-ketogluconate, methyl α-D-glucopyranoside, D-glucose, glycerol, glycogen, inositol, maltose, D-mannose, melezitose, raffinose, D-ribose, sucrose, starch, and turanose, but not from amygdalin, D-arabinose, arbutin, cellobiose, dulcitol, D- and L-fucose, gentiobiose, potassium 2-ketogluconate, *N*-acetylglucosamine, inulin, lactose, D-mannitol, methyl α-D-mannopyranoside, melibiose, L-rhamnose, salicin, D-sorbitol, L-sorbose, D- and L-xylose, or methyl β-D-xylopyranoside. Nitrate is reduced to nitrite. Pyrazinamidase, acid and alkaline phosphatase, leucine arylamidase, naphthol-AS-BI-phosphohydrolase, α- and β-galactosidase, α-glucosidase, *N*-acetyl-β-glucosaminidase, and α-mannosidase are produced, but not urease, gelatinase, pyrrolidonyl arylamidase, β-glucuronidase, esterase (C4), lipase (C14), valine arylamidase, cystine arylamidase, trypsin, α-chymotrypsin, β-glucosidase, or α-fucosidase. CAMP reaction with *Staphylococcus aureus* ATCC 25923 is positive. Using the microdilution method for coryneform bacteria, the type strain exhibited susceptibility to cefotaxime, doxycycline, erythromycin, linezolid, meropenem, penicillin G, and vancomycin. In the neighbor-joining phylogenetic tree based on 16S rRNA gene sequences, *Actinomyces hominis* clustered near *Actinomyces europaeus* and *Actinomyces coleocanis*. The natural habitat is not known.

Source (type strain): a wound swab taken from an 89-year-old human female patient.

DNA G+C content (mol%): not reported.

Type strain: 1094, 7894GR, CCUG 57540, DSM 22168.

Sequence accession no. (16S rRNA gene): FJ617539.

15. **Actinomyces hongkongensis** Woo, Fung, Lau, Teng, Wong, Wong, Hon, Tang and Yuen 2004, 307[VP] (Effective publication: Woo, Fung, Lau, Teng, Wong, Wong, Hon, Tang and Yuen 2003, 521.)

hong.kong.en′sis. N.L. masc. adj. *hongkongensis* of or pertaining to Hong Kong, where the type strain was isolated.

Cells are straight rods. On sheep blood agar after 24 h of anaerobic incubation, colonies are pinpoint and non-hemolytic. Catalase-negative. Additional morphological details as well as nutrition and growth conditions are as given for the genus and as summarized in Table 6. Physiological and other descriptive and differential characteristics are listed in Table 4 and Table 7. The natural habitat is not known.

Source (type strain): pus from the Fallopian tubes of a 36-year-old Chinese woman with IUCD-related pelvic actinomycosis.

DNA G+C content (mol%): not reported.

Type strain: HKU 8, CCUG 48484, DSM 15629, CIP 107949, LMG 21939.

Sequence accession no. (16S rRNA gene): AF433168.

16. **Actinomyces hordeovulneris** Buchanan, Scott, Gerencser, Beaman, Jang and Biberstein 1984, 442[VP]

hor.de.o.vul′ne.ris. L. neut. n. *hordeum* barley; N.L. n. *Hordeum* genus of grass; L. neut. n. *vulnus, vulneris* wound, injury; N.L. gen. n. *hordeovulneris* of (isolated from) injuries produced by awns of *Hordeum*.

Cells are pleomorphic rods and filaments that are 0.5–1.0 μm in diameter and usually exhibit a noticeable degree of

branching on blood agar. In broth supplemented with 15% fetal bovine serum, the filaments are long and extensively branched and have swollen ends, where sphaeroplast-like bodies occasionally develop. Fragmentation into diphtheroidal cells is incomplete in broth cultures.

Microcolonies on BHIA supplemented with 10–20% fetal bovine serum after 24 h of incubation are regularly filamentous and similar to those of *Actinomyces israelii*. Mature colonies on bovine blood agar are white, agar-adherent, and molar-toothed, with a tendency to shift to a white, conical, domed, buttery, less adherent type upon laboratory passage. Diameters are 0.5–1.0 mm after 48 h and 2 mm after 3 d. Growth is weak in trypticase soy broth both aerobically and anaerobically unless the medium is supplemented with serum. Catalase activity was found to be weak to moderate in all of the strains tested. Additional morphological details, cell-wall composition, nutrition and growth conditions, metabolism and metabolic pathways as well as drug susceptibility are as given for the genus or as summarized in Table 8. Physiological and other descriptive and differential characteristics are listed in Table 5 and Table 9.

Preliminary serological data obtained with FITC-conjugated antisera to various *Actinomyces* and *Rothia* species showed some degree of cross-reactivity between conjugates of the type strains of *Actinomyces israelii* (ATCC 12102) and *Actinomyces viscosus* (ATCC 15987) and *Actinomyces hordeovulneris* cells. Significant cross-reactions were not observed with antisera to *Actinomyces oris*, *Actinomyces gerencseriae*, *Actinomyces bovis* serovars I and II, *Actinomyces naeslundii*, *Actinomyces odontolyticus* serovars I and II, "*Actinomyces suis*", *Arcanobacterium pyogenes*, or to the serovars of *Rothia dentocariosa*. The normal habitat of *Actinomyces hordeovulneris* remains to be definitely clarified. However, the organism has been isolated from the gingival margin of healthy cats so that it might be assumed that it belongs to the indigenous microflora of the oral cavity of cats and possibly dogs. *Actinomyces hordeovulneris* is well established as a pathogen of dogs in which it may produce pleuritis, pericarditis, peritonitis, visceral abscesses, septic arthritis, and recurrent localized suppurations in which sulfur granule-like particles may be found. These infections often occurred secondary to injuries caused by tissue-migrating awns of several members of the grass genus *Hordeum* (commonly called foxtails) which are common in the Western United States, especially California. Some of the infected animals died despite ampicillin treatment although this drug was effective *in vitro*. The unfavorable responses to ampicillin were attributed to a marked tendency of *Actinomyces hordeovulneris* to produce L-phase variants spontaneously with coincident uptake of calcium and may additionally be due to the formation of sulfur granules.

Source (type strain): An infection secondary to tissue-wandering foxtail awns.

DNA G+C content (mol%): 67–68 (Bd).

Type strain: ATCC 35275, CCUG 32937, CIP 103149, DSM 20732, UCD 81-332-9.

Sequence accession no. (16S rRNA gene): X82448.

17. **Actinomyces howellii** Dent and Williams 1984c, 319[VP]

how.el′li.i. N.L. gen. masc. n. *howellii* of Howell, to honor the American microbiologist Arden Howell Jr, who studied oral actinomycetes from animals, particularly *Odontomyces* (*Actinomyces*) *viscosus*.

Cells are rod-shaped (0.8 µm wide × 1.5–5.0 µm long) and may show characteristic Y, V, and T forms. Short filaments may be present. Mature colonies when grown anaerobically on horse blood agar for 3–5 d are white, smooth, shiny, translucent, convex with entire margins, and up to 2 mm in diameter. Catalase-negative. Additional morphological details, cell-wall composition, nutrition and growth conditions as well as metabolism and metabolic pathways are as given for the genus or as summarized in Table 8. Physiological and other descriptive and differential characteristics are listed in Table 5 and Table 9. *Actinomyces howellii* strains exhibit characteristic although not completely identical polypeptide patterns when analyzed by SDS-PAGE which allow clear separation of *Actinomyces howellii* from both *Actinomyces naeslundii* and *Actinomyces denticolens*. Whole-cell agglutination results showed that *Actinomyces naeslundii* and *Actinomyces howellii* were serologically distinct. Immunoblotting revealed some antigenic relationship between *Actinomyces howellii* and *Actinomyces denticolens* on the one hand and *Actinomyces naeslundii* and *Actinomyces viscosus* on the other hand.

Source: oral cavity of cattle, especially dental plaque. Pathogenicity has not been reported. The type strain was isolated from supragingival plaque of dairy cattle.

DNA G+C content (mol%): 66–67 (T_m).

Type strain: Sh 7/4276, ATCC 43323, CCUG 32757, CIP 103127, NCTC 11636.

Sequence accession no. (16S rRNA gene): X80411.

18. **Actinomyces hyovaginalis** Collins, Stubbs, Hommez and Devriese 1993, 472[VP]

hy.o.va.gi.na′lis. Gr. masc. and fem. n. *hys*, *hyos* pig, sow; L. fem. n. *vagina* sheath, vagina; L. masc. suff. *-alis* suffix denoting pertaining to; N.L. masc. adj. *vaginalis* of (pertaining to) the vagina; N.L. masc. adj. *hyovaginalis* pertaining to the vagina of a sow.

The species *Actinomyces hyovaginalis* has been differentiated into two biovars, which were designated the "vaginal" and the "general" biovars, respectively. The "vaginal" biovar resembles "*Actinomyces suis*" Franke (1973) phenotypically for which no type strain exists. Cells of both biovars are predominantly diphtheroidal and the rods are arranged in clusters or V or Y forms; coccoid elements may occur. Colonies of the "vaginal" biovar are flat with outrunning edges, particularly when grown anaerobically or under CO_2 on horse blood agar. Colonies of the "general" biovar are smaller and more convex than those commonly seen in vaginal isolates and the typical outrunning edges of the latter are absent. As demonstrated by 16S rRNA gene sequence similarity and DNA–DNA hybridization values, the two biovars belong to one species although they also differ from each other by a few physiological reactions. Catalase-negative. Additional morphological details, cell-wall composition, and nutrition and growth conditions are as given for the genus or as summarized in Table 8. Physiological and other descriptive or differential characteristics including those differentiating the two biovars are listed in Table 5 and Table 9. The natural habitat of the "vaginal" biovar of *Actinomyces hyovaginalis*

appears to be the porcine genital tract; the natural habitat of the "general" biovar is not known definitely.

Source: purulent vaginal discharge and aborted fetuses of sows ("vaginal" biovar) and various other porcine purulent lesions ("general" biovar). The type strain was isolated from the vagina of a sow.

DNA G+C content (mol%): 63–68 (T_m).

Type strain: BM 1192/5, ATCC 51367, CCUG 35604, CCUG 35715, CIP 103923, DSM 10695, NCIMB 702983 (formerly NCFB 2983).

Sequence accession no. (16S rRNA gene): X69616.

19. **Actinomyces israelii** (Kruse 1896) Lachner-Sandoval 1898, 64[AL], emend. Johnson, Moore, Kaneko and Moore 1990, 279[VP] (*Streptothrix israeli* Kruse 1896, 56; *Discomyces israeli* (Kruse 1896) Gedoelst 1902; *Actinobacterium israeli* (Kruse 1896) Sampietro 1908; Cohnistreptothrix *israeli* (Kruse 1896) Pinoy 1913; *Nocardia israeli* (Kruse 1896) Castellani and Chalmers 1913; *Oospora israeli* (Kruse 1896) Sartory 1920; *Brevistreptothrix israeli* (Kruse 1896) Lignières 1924; *Corynebacterium israeli* (Kruse 1896) Haupt and Zeki 1933; *Proactinomyces israeli* (Kruse 1896) Negroni 1934)

is.ra.e′li.i. N.L. gen. masc. n. *israelii* of Israel, to honor James Israel, a German surgeon, who was one of the original describers of the organism.

Cells frequently present as shorter and longer, branching filaments (Figure 20), but diphtheroidal forms may occur simultaneously or may even predominate. Microcolonies are filamentous, and the most common and characteristic form is the "spider colony" (Figure 30). Mature colonies measure 0.5–2.0 mm in diameter and are predominantly rough with or without central depression. They have been described as molar-tooth-, bread-crumb-, and raspberry-like (Figure 41 and Figure 42), are white to grayish to creamy in color and usually hard and adhering to the medium. In liquid media, they may present as snow ball- or cotton pad-like structures (Figure 56). Catalase-negative. Additional morphological details, ultrastructure, cell-wall composition, nutrition and growth conditions, metabolism, metabolic pathways, and drug sensitivity are as given for the genus and as summarized in Table 6. Physiological and other descriptive and differential characteristics are listed in Table 4 and Table 7. Although the species *Actinomyces israelii* as currently defined was restricted to the former *Actinomyces israelii* serovar I strains, it appears to be still antigenically heterogeneous (Schaal and Gatzer, 1985). Furthermore, two additional serological types were found among strains fitting the general description of *Actinomyces israelii sensu lato.* As these serological variants differed from typical members of the taxon in a number of physiological characteristics, it remains to be seen whether they constitute new serovars of *Actinomyces israelii sensu stricto* or *Actinomyces gerencseriae* or deserve species rank.

The normal habitat of *Actinomyces israelii* is the oral cavity of man including tonsillar crypts and dental plaque. In humans with healthy gingivae, it could be identified in 6.5% of the samples examined and accounted for 0.4% of the total flora analyzed. In patients with gingivitis and periodontitis, the respective figures were 9.4% and 0.7%. Furthermore, *Actinomyces israelii* may be recovered from the mucosal surfaces of the human intestinal and female genital tracts. *Actinomyces israelii* is one of the principal causative agents of human cervicofacial, thoracic, abdominal, and pelvic actinomycoses. In addition, it may cause eye infections such as lacrimal canaliculitis, conjunctivitis or dacryocystitis and is apparently also etiologically involved in the development of cervicitis and endometritis in women using intrauterine contraceptive devices or vaginal pessaries. Occasionally, *Actinomyces israelii* (or *Actinomyces gerencseriae*) has been reported from animal infections such as actinomycoses in cattle or swine and pyogenic granulomas in a mandrill. However, it is not definite whether these isolates were correctly identified. Sulfur granules are characteristically, but not obligatorily produced in human infections. After intraperitoneal, intravenous, or subcutaneous injection, *Actinomyces israelii* produces abscess formation in a variety of experimental animals including hamsters, mice, and rabbits. The pathological lesions thus induced resemble naturally occurring actinomycoses to a certain extent, but are usually self-limited and not progressive unless at least one typical concomitant organism is added to the challenge injection.

Source (type strain): a human brain abscess.

DNA G+C content (mol%): 63–70 (T_m).

Type strain: ATCC 12102, CCUG 18307, CIP 103259, DSM 43320, JCM 12964, NCTC 12972.

Additional reference strain: ATCC 10048, which differs serologically from the type strain.

Sequence accession no. (16S rRNA gene): X82450.

20. **Actinomyces johnsonii** Henssge, Do, Radford, Gilbert, Clark and Beighton 2009, 515[VP]

john.so′ni.i. N.L. gen. masc. n. *johnsonii* of Johnson, to honor the American molecular biologist John L. Johnson, who undertook extensive studies on the genetic relationships between oral actinomycetes.

The species contains strains previously identified as *Actinomyces naeslundii* serotype WVA 963 or *Actinomyces naeslundii* genospecies WVA 963. Cellular and colonial morphology are similar to those described for *Actinomyces naeslundii* and *Actinomyces oris.* Catalase-negative. Additional morphological details, chemotaxonomic properties, nutrition and growth conditions, and metabolism are as given for the genus and as summarized in Table 6. Physiological and other descriptive or differential characteristics are listed in Table 4 and Table 7. However, morphological and physiological properties usually do not suffice for reliable identification. The latter can be achieved by sequence comparison of partial gene sequences of *atpA* or *metG.* The normal habitat of *Actinomyces johnsonii* is the oral cavity of man. It was found to account for 3.4% of the healthy periodontal flora and was detected in 34.5% of the samples examined. The respective figures for patients with gingivitis and periodontitis were 2.4% and 26.2%. Whether and to what extent *Actinomyces johnsonii* may be present in other human body areas remains to be determined. Although *Actinomyces johnsonii* (*Actinomyces naeslundii* serovar WVA 963) represents a major component of the periodontal flora (the oral biofilm), its contribution

to the development of gingivitis and periodontitis remains uncertain given its relative incidence in human periodontal floras of healthy and diseased individuals (see above). No reliable information is available on the potential role of the organism in invasive human infections. Also, detailed experimental animal studies have apparently not been performed yet.

Source (type strain): gingival crevice of a healthy child.

DNA G+C content (mol%): 67 (T_m).

Type strain: ATCC 49338, CCUG 34287.

Sequence accession no. (16S rRNA gene): EU667411.

21. **Actinomyces marimammalium** Hoyles, Pascual, Falsen, Foster, Grainger and Collins 2001c, 154[VP]

ma.ri.mam.ma′li.um. L. neut. n. *mare*, *maris* sea, ocean; L. fem. n. *mamma* breast, mammary gland; N.L. neut. pl. n. *mammalia* mammals; N.L. gen. pl. n. *marimammalium* of marine mammals.

Cells are straight to slightly curved rods, some of which exhibit branching. Colonies are gray, entire, circular, convex, non-hemolytic and pin-point to 0.5 mm in diameter. Catalase-negative. Additional morphological details, chemotaxonomic markers, nutrition and growth conditions, metabolism, and metabolic pathways are as given for the genus and as summarized in Table 8. Physiological and other descriptive and differential characteristics are listed in Table 5 and Table 9. In SDS-PAGE analysis, the *Actinomyces marimammalium* isolates were recovered in a single group which was well separated from all other reference strains examined. The natural habitat is not known. The strains were isolated from multiple organs of a dead male hooded seal, the small intestine of a dead gray seal which had been shot, and from the lung of a harbor porpoise.

Source (type strain): lung, liver, kidney, and mesenteric lymph node of a dead male hooded seal.

DNA G+C content (mol%): 66 (method not reported).

Type strain: M1749/98/1, CCUG 41710, CIP 106509, DSM 15383.

Sequence accession no. (16S rRNA gene): AJ276405.

22. **Actinomyces massiliensis** Renvoise, Raoult and Roux 2009, 541[VP]

mas.si.li.en′sis. L. masc. adj. a *massiliensis* of or belonging to *Massilia*, the old Greek and Roman name for Marseille, where the type strain had been isolated.

Cells are anaerobic, straight rods (0.5–1.7 µm in length and 0.35–0.74 µm in diameter). After 48 h of incubation on sheep blood agar, colonies are pinpoint, circular, white, shiny, and non-hemolytic. Catalase-negative. Additional morphological details, chemotaxonomic markers as well as nutrition and growth conditions are as given for the genus and as summarized in Table 6. Physiological and other descriptive and differential characteristics are listed in Table 4 and Table 7.

The natural habitat is not known.

Source (type strain): blood culture of a 38-year-old man with pleuropneumonia.

DNA G+C content (mol%): not reported.

Type strain: 4401292, CSUR P18, CCUG 53522.

Sequence accession no. (16S rRNA gene): EF558367.

23. **Actinomyces meyeri** (Prévot 1938) Cato, Moore, Nygard and Holdeman 1984, 487[VP] (*Actinobacterium meyeri* Prévot 1938, 303)

me.ye′ri. N.L. gen. masc. n. *meyeri* of Meyer, to honor Kurt F. Meyer, a German bacteriologist, who described the organism as a "new anaerobic *Streptothrix* species" in 1911.

Cells grown in peptone-yeast extract-glucose broth supplemented with vitamin K_1, hemin, and Tween 80 are short rods (0.5–0.9 µm by 0.9–3.0 µm) and occur in pairs, short chains, or clumps. Terminal swellings are present occasionally, and branching may be difficult to demonstrate. Microcolonies are smooth and non-filamentous resembling those of *Actinomyces odontolyticus*. Mature surface colonies on supplemented BHIA incubated anaerobically are pinpoint to 1 mm in diameter, circular, flat to convex, translucent to opaque and white, with shiny, smooth surfaces, and entire margins. They may be α- or non-hemolytic. Growth in air with 5% CO_2 added is very slight or absent. *Actinomyces meyeri* has an absolute requirement for vitamin K_1, and growth is greatly stimulated by 0.02% Tween 80 and by a fermentable carbohydrate. Addition of serum may also stimulate growth. Catalase-negative. Additional morphological details, nutrition and growth conditions, metabolism and metabolic pathways, and drug susceptibility are as given for the genus and as summarized in Table 6. Physiological and other descriptive and differential characteristics are listed in Table 4 and Table 7. The patterns of soluble cellular proteins as determined by polyacrylamide gel electrophoresis indicate that the strains tested so far represent a homogeneous taxon. Most of the isolates studied gave a positive fluorescent antibody reaction with monovalent FITC-conjugate prepared against strain ATCC 33972. Cross-reactions have not been observed with conjugates to *Actinomyces gerencseriae*, *Actinomyces israelii*, *Actinomyces johnsonii*, *Actinomyces naeslundii*, *Actinomyces odontolyticus*, serovars I and II, and *Actinomyces oris*. However, as several isolates of *Actinomyces meyeri* did not react with the antiserum to strain ATCC 33972, there may exist additional serovars of the species. The principal natural habitat of *Actinomyces meyeri* is the human periodontal sulcus. In addition, the organism has been isolated frequently from brain abscesses and pleural fluid and less often from abscesses of the cervicofacial area, hips, hands, feet and spleen and from bite wounds.

Source (type strain): Purulent human pleurisy.

DNA G+C content (mol%): 64–67 (spectrometry/chromatography).

Type strain: Prévot 2477B, ATCC 35568, CCUG 21024, CIP 103148, DSM 20733, LMG 16161, VPI 8617.

Sequence accession no. (16S rRNA gene): X82451.

24. **Actinomyces naeslundii** Thompson and Lovestedt 1951, 175[AL], emend. Henssge, Do, Radford, Gilbert, Clark and Beighton 2009, 516[VP]

na.es.lun′di.i. N.L. gen. masc. n. *naeslundii* of Naeslund, to honor Carl Naeslund from Sweden, who first described this organism in some detail, but did not give it a species epithet.

The species contains strains previously identified as *Actinomyces naeslundii* serovar I, also known as *Actinomyces*

naeslundii genospecies 1. Cells of *Actinomyces naeslundii* are usually diphtheroidal showing some branching (Figure 23) and/or arrangement in Y, V, or T forms. Microcolonies may be highly filamentous (Figure 32), but may also show the result of early fragmentation leading to less to non-filamentous colony forms. Mature colonies are either smooth or finely granular (Figure 46) and circular, or they may show branching filaments in the periphery of the colony (Figure 47) or even present as a molar-tooth colony (Figure 48). Their diameter may be greater than 2 mm. Catalase-negative. Additional morphological details, ultrastructure, cell-wall composition, nutrition and growth conditions, metabolism and metabolic pathways as well as drug susceptibility are as given for the genus and as summarized in Table 6. Physiological and other descriptive and differential characteristics are listed in Table 4 and Table 7. *Actinomyces naeslundii* can reliably be differentiated from the closely related species of the former *Actinomyces naeslundii/Actinomyces viscosus* complex on the basis of sequence comparison of partial gene sequences of *atpA* and *metG*. A clear-cut distinction from *Actinomyces oris* and *Actinomyces johnsonii* is as yet not possible by phenotypic testing. After emendation of the species, it appears to be serologically homogeneous. The normal habitat of *Actinomyces naeslundii* is the oral cavity of man including tonsillar crypts and dental plaque. It was found to account for 7.6% of the human healthy periodontal flora and occurred in 54.6% of the samples examined. The respective figures for patients with gingivitis and periodontitis were 2.7% and 30.7%. The species may also be detected in cervicovaginal secretions from women using and not using IUCDs. Human infections due to *Actinomyces naeslundii* most frequently present as cervicofacial, thoracic, or abdominal actinomycotic lesions clinically indistinguishable from those produced by *Actinomyces israelii* or *Actinomyces gerencseriae* although some of the reports blaming *Actinomyces naeslundii* as causative agent might have been biased by mixed infections with *Actinomyces israelii* or *Actinomyces gerencseriae* or by contamination of the specimen from mucosal secretions. However, eye infections (e.g., lacrimal canaliculitis) and infections of the female genital tract as well as gallbladder infections, pelvic infections, mediastinal actinomycosis, a disseminated infection in a mentally retarded child, a recurring empyema of the knee joint, and infections of a total knee arthroplasty and a hip prosthesis have also been reported. Furthermore, the species obviously plays an important role in the complex etiology and pathogenesis of caries and periodontitis. After parenteral inoculation, *Actinomyces naeslundii* was found to cause abscess formation in experimental animals. After oral inoculation, the organism is apparently able to initiate periodontitis with alveolar bone loss and/or fissure lesions in hamsters and conventional as well as gnotobiotic rats.

Source (type strain): human sinus secretions.

DNA G+C content (mol%): 63 (T_m).

Type strain: ATCC 12104, CCUG 2238, CCUG 18310, CCUG 32832, CCUG 35333, CDC W826, CIP 103128, DSM 43013, JCM 8349, NCTC 10301.

Sequence accession no. (16S rRNA gene): X81062.

25. **Actinomyces nasicola** Hall, Collins, Lawson, Falsen and Duerden 2003d, 1447[VP]

na.si′co.la. L. masc. n. *nasus, nasi* nose; L. masc. suff. *-cola* from L. masc. n. *incola* inhabitant, dweller; N.L. masc. n. *nasicola* inhabitant of the nose, referring to the body site from which the type strain had been isolated.

Cells are short, diphtheroid-shaped rods; some branching and coccoid forms occur. After 48 h of anaerobic incubation on Fastidious Anaerobe Agar, colonies are pinpoint, white or gray, opaque, shiny, entire, and convex. Catalase-negative. Additional morphological details, chemotaxonomic markers, nutrition and growth conditions as well as metabolism and metabolic pathways are as given for the genus and as summarized in Table 6. Physiological and other descriptive and differential characteristics are listed in Table 4 and Table 7. The organism produced a unique 16S rDNA restriction pattern with *Hae*III and *Hpa*II that was distinct from the profiles of over 400 *Actinomyces* strains. The natural habitat is not known. The single strain encountered so far was isolated from a nasal antrum washout.

Source (type strain): nasal antrum aspirate.

DNA G+C content (mol%): 66.5 (HPLC).

Type strain: R2014, CCUG 46092, CIP 107668, DSM 19116.

Sequence accession no. (16S rRNA gene): AJ508455.

26. **Actinomyces neuii subsp. neuii** and **Actinomyces neuii subsp. anitratus** Funke, Stubbs, von Graevenitz and Collins 1994, 170[VP]

neu′i.i. N.L. gen. masc. n. *neuii* of Neu, to honor Harold Neu, an authority in antimicrobial chemotherapy and infectious diseases.

DNA G+C content (mol%): 55–58 (T_m).

26a. **Actinomyces neuii subsp. neuii**

Cells are predominantly diphtheroidal and rods are arranged in clusters or V or Y forms; coccobacillary elements may occur. Colonies are circular, smooth, convex, opaque, more white than creamy in color, and with entire edges, and 0.5–1.5 mm in diameter after 48 h of incubation. α-Hemolysis is observed on sheep blood agar for most strains. Catalase-positive. Nitrate is reduced to nitrite.

Source (type strain): human blood.

Type strain: 97/90, ATCC 51847, CCUG 32252, CIP 104015, DSM 8576.

Sequence accession no. (*16S rRNA gene):* AM084228 (ATCC 51847).

26b. **Actinomyces neuii subsp. anitratus**

a.ni.tra′tus. Gr. pref. *a-* not; N.L. n. *nitras*, *nitratis* nitrate; L. masc. suff. *-atus* suffix used in adjectives meaning provided with; N.L. masc. adj. *anitratus* intended to mean not reducing nitrate.

The morphological characteristics are similar to those of the subspecies *neuii*, except that *Actinomyces neuii* subsp. *anitratus* is non-hemolytic. Catalase-positive. Nitrate is **not** reduced to nitrite. Additional morphological details, chemotaxonomic characteristics and nutrition and growth conditions for both subspecies are as given for the genus or

as summarized in Table 6. Physiological and other descriptive or differential characteristics including those suitable for differentiating the two subspecies from one another are listed in Table 4 and Table 7. Strains of both subspecies of *Actinomyces neuii* have mainly been isolated from human abscesses in association with mixed anaerobic flora as well as from blood cultures. The natural habitat of the subspecies of *Actinomyces neuii* is not definitely known.

Source (type strain): human mammary hematoma.

Type strain: 50/90, ATCC 51849, CCUG 32253, CIP 104016, DSM 8577, LMG 14788.

Sequence accession no. (*16S rRNA gene*)*:* AM084229 (ATCC 51849).

27. **Actinomyces odontolyticus** Batty 1958, 455[AL]

o.don.to.ly′ti.cus. Gr. masc. n. *odous*, *-ontos* tooth; N.L. masc. adj. *lyticus* (from Gr. masc. adj. *lutikos*) able to loosen, able to dissolve; N.L. masc. adj. *odontolyticus* tooth-dissolving.

Cells of *Actinomyces odontolyticus* are usually diphtheroidal (Figure 26), but may also be coccobacillary (Figure 27). In very early growth stages, cells may show signs of budding (Figure 57). Microcolonies are non-filamentous (Figure 36) and mature colonies are 1–2 mm in size, smooth, with an entire to irregular edge and granular structures inside the colony (Figure 53 and Figure 54). When grown on BHIA, the colonies are opaque to translucent and white to gray-white. After 5–10 d of incubation on blood agar, however, the colonies usually become deep red. This pigmentation may be seen on plates incubated anaerobically or it may become apparent after the cultures have been left standing in air at room temperature following primary anaerobic incubation. Catalase-negative. Additional morphological details, ultrastructure, cell-wall composition, nutrition and growth conditions, metabolism and metabolic pathways, and drug susceptibility are as given for the genus and as summarized in Table 6. Physiological and other descriptive or differential characteristics are listed in Table 4 and Table 7. Two serovars of *Actinomyces odontolyticus* have been described which show only low-grade cross-reactivity between each other. However, considerable cross-reactions appear to occur between *Actinomyces odontolyticus* serovar II strains and *Arcanobacterium pyogenes*. The natural habitat of *Actinomyces odontolyticus* is the oral cavity of man, in particular dental plaque and calculus. Progressive actinomycotic infections of this organism in man have been reported, but are apparently rare. More frequently, *Actinomyces odontolyticus* is etiologically involved in the development of eye infections such as lacrimal canaliculitis and possibly also of periodontitis and caries. Primarily, it had been isolated from deep carious lesions in man. Naturally occurring animal infections by *Actinomyces odontolyticus* have only been reported twice, from one dog and one cat. Experimental animal infections were produced with varying success.

Source (type strain): a deep carious lesion in man.

DNA G+C content (mol%): 61–62 (T_m).

Type strain: ATCC 17929, CCUG 20536, CIP 101124, DSM 43760, JCM 14871, LMG 18080, NCTC 9935.

Sequence accession no. (16S rRNA gene): AJ234040.

28. **Actinomyces oricola** Hall, Collins, Hutson, Inganäs, Falsen and Duerden 2003c, 1517[VP]

o.ri′co.la. L. neut. n. *os*, *oris* mouth; L. masc. suff. *-cola* from L. masc. n. *incola* inhabitant; N.L. masc. n. *oricola* inhabitant of the mouth.

Cells are rod-shaped and some of them display branching; filaments may be observed. After 48 h of anaerobic incubation on Fastidious Anaerobe Agar with 5% horse blood, colonies are pinpoint, breadcrumb-like, and nonhemolytic. Catalase-negative. Additional morphological details, chemotaxonomic markers, nutrition and growth conditions as well as metabolism and metabolic pathways are as given for the genus and as summarized in Table 6. Physiological and other descriptive and differential characteristics are listed in Table 4 and Table 7. The natural habitat is not known.

Source (type strain): a human dental abscess.

DNA G+C content (mol%): not reported.

Type strain: R5292, CCUG 46090, CIP 107639, DSM 17234.

Sequence accession no. (16S rRNA gene): AJ507295.

29. **Actinomyces oris** Henssge, Do, Radford, Gilbert, Clark and Beighton 2009, 514[VP]

o′ris. L. neut. n. *os* mouth; L. gen. n. *oris* of the mouth.

The species comprises strains previously identified as *Actinomyces naeslundii* serovars II, III, and NV as well as *Actinomyces viscosus* serovar II and has also been known as *Actinomyces naeslundii* genospecies 2. Cellular and colonial morphology resemble those described for *Actinomyces naeslundii* (Figure 49) although less filamentous forms predominate (Figure 33 and Figure 50). Quite often, smooth, entire, and soft colonies are seen (Figure 45) which resemble those of propionibacteria. Catalase may or may not be produced. Additional morphological details, chemotaxonomic properties, nutrition and growth conditions, and metabolism are as given for the genus and as summarized in Table 6. Physiological and other descriptive or differential characteristics are listed in Table 4 and Table 7. However, morphological and physiological properties usually do not suffice for reliable identification. The latter can be achieved by sequence comparison of partial gene sequences of *atpA* or *metG*. As the species *Actinomyces oris* contains strains formerly classified as *Actinomyces naeslundii* serovars II, III, and NV and *Actinomyces viscosus* serovar II, it must be antigenically heterogeneous, but the definite antigenic structure of the new species remains to be described. The normal habitat of *Actinomyces oris* is the oral cavity of man. It accounts for 8.3% of the healthy periodontal flora and was detected in 79.8% of the samples examined. The respective figures for patients with gingivitis and periodontitis were 8.2% and 77.4%. *Actinomyces oris* has obviously also been recovered from cervicovaginal secretions of women with or without IUCDs and from the uninfected conjunctiva and/or cornea of man. Occasionally, *Actinomyces oris* (*Actinomyces viscosus* serovar II) has been isolated from cervicofacial and abdominal cases of human actinomycoses and at least in some of these cases, *Actinomyces oris* appeared to be the primary pathogen. Furthermore, the species may be involved

in the etiology of lacrimal canaliculitis and other eye infections and possibly also in the etiology of cervicitis and endometritis of women using IUCDs. Among the members of the genus *Actinomyces*, *Actinomyces oris* represents a species whose contribution to the development of caries and periodontitis has been established convincingly. Experimental animal studies using *Actinomyces viscosus sensu lato* strains need to be re-evaluated in light of modern taxonomic revisions.

Source (type strain): human sputum.

DNA G+C content (mol%): 66 (T_m).

Type strain: ATCC 27044, CCUG 34288.

Sequence accession no. (16S rRNA gene): EU667403.

30. **Actinomyces radicidentis** Collins, Hoyles, Kalfas, Sundquist, Monsen, Nikolaitchouk and Falsen 2001, 1[VP] (Effective publication: Collins, Hoyles, Kalfas, Sundquist, Monsen, Nikolaitchouk and Falsen 2000, 3402.)

ra.di.ci.den′tis. L. fem. n. *radix*, *radicis* root; L. masc. n. *dens*, *dentis* tooth; N.L. gen. n. *radicidentis* of the root of the tooth.

Cells are coccoid. Catalase-positive. Additional morphological details, nutrition and growth conditions and metabolic activities are as given for the genus and as summarized in Table 6. Physiological and other descriptive and differential characteristics are listed in Table 4 and Table 7. In SDS-PAGE analysis the two isolates of *Actinomyces radicidentis* dealt with in the original description clustered together and were clearly separated from other *Actinomycetaceae*. The natural habitat is not known. The two strains were isolated from infected root canals of human teeth.

Source (type strain): infected root canal of the upper right canine of an 80-year-old woman who had suffered from persistent symptoms after conventional root canal treatment.

DNA G+C content (mol%): not reported.

Type strain: CCUG 36733, CIP 106352, DSM 15433.

Sequence accession no. (16S rRNA gene): AJ251986.

31. **Actinomyces radingae** Wüst, Stubbs, Weiss, Funke and Collins 1995b, 619[VP] (Effective publication: Wüst, Stubbs, Weiss, Funke and Collins 1995a, 80.), emend. Vandamme, Falsen, Vancanneyt, Van Esbroek, Van de Merwe, Bergmans, Schouls and Sabbe 1998, 509[VP]

ra.din′ga.e. M.L. fem. n. *Radinga* the Latin name for Reading, UK; M.L. fem. gen. n. *radingae* of (pertaining to) Reading.

Cells are coccoid rods (0.65 µm in diameter and 1 µm long). Pseudobranching is rare.

After 48 h of incubation on sheep blood agar in a 5% CO_2 atmosphere, colonies are small, gray, convex, circular, and opaque with a glistening surface, an entire edge, and a butyrous consistency. A hemolytic zone appears slowly and mostly starts with α-hemolysis. Catalase-negative. Additional morphological details, cell-wall composition and other chemotaxonomic properties, nutrition and growth conditions, metabolism and metabolic pathways as well as drug susceptibility are as given for the genus and as summarized in Table 6. Physiological and other descriptive and differential characteristics are listed in Table 4 and Table 7. *Actinomyces radingae* strains have chiefly been isolated from human mixed infections including otitis, empyema, pilonidal cyst, perianal abscesses, and decubitus ulcers. The isolates have usually been found to be associated with anaerobes, "*milleri*" streptococci, enterococci, and Gram-stain-negative rod-shaped bacteria. The natural habitat is not definitely known.

Source (type strain): a human perianal abscess.

DNA G+C content (mol%): 60 (T_m).

Type strain: APL1, ATCC 51856, CCUG 32394, (CCUG 34270), CIP 105358, DSM 9169, LMG 15960.

Sequence accession no. (16S rRNA gene): X78719.

32. **Actinomyces ruminicola** An, Cai and Dong 2006, 2045[VP]

ru.mi.ni′co.la. L. n. *rumen*, *ruminis* first stomach of ruminants, rumen; L. masc. suffix *-cola* from L. masc. n. *incola* inhabitant; N.L. masc. n. *ruminicola* inhabitant of rumen from which the type strain had been isolated.

Cells are straight strictly anaerobic rods (0.5–1.0 µm in diameter and 2.5–4.0 µm in length). Colonies on peptone-yeast extract-glucose agar with 15% rumen fluid (PYGR agar) are circular, slightly convex, and white and reach approximately 0.5–0.8 mm in diameter after 48 h of anaerobic incubation at 46°C. The organism grows between 20 and 55°C with optimum growth at 46°C. Catalase-negative. Additional morphological details, ultrastructure, chemotaxonomic markers, nutrition and growth conditions as well as metabolism and metabolic pathways are as given for the genus and as summarized in Table 8. Physiological and other descriptive and differential characteristics are listed in Table 5 and Table 9. The natural habitat appears to be cattle rumen. Pathogenicity has not been reported.

Source (type strain): cattle rumen.

DNA G+C content (mol%): not reported.

Type strain: B71, JCM 13352, CGMCC 1.5030.

Sequence accession no. (16S rRNA gene): DQ072005.

33. **Actinomyces slackii** Dent and Williams 1986, 394[VP]

slac′ki.i. N.L. gen. masc. n. *slackii* of Slack, to honor John M. Slack, an expert on *Actinomyces* and actinomycosis.

Cells are short rods (0.56 µm by 0.8 µm). Colonies on horse blood agar after 3–5 d of incubation are white, smooth, shiny, translucent, and convex, have entire margins and measure up to 2 mm in diameter. Catalase-positive. Additional morphological details, cell-wall composition, nutrition and growth conditions, and metabolism and metabolic pathways are as given for the genus and as summarized in Table 8. Physiological and other descriptive or differential characteristics are listed in Table 5 and Table 9. The patterns of polypeptides produced by SDS-PAGE are homogeneous within the species, but quite distinct from those of *Actinomyces howellii*, *Actinomyces viscosus*, and *Actinomyces naeslundii*. *Actinomyces slackii* has been isolated from the oral cavities of a number of individual dairy cattle, in particular from dental plaque. Pathogenicity has not been reported.

Source: oral cavity of dairy cattle, in particular dental plaque.

DNA G+C content (mol%): 65–67 (T_m).

Type strain: Sh 13/4563, ATCC 49928, CCUG 32792, CIP 103130, NCTC 11923.

Sequence accession no. (16S rRNA gene): AJ234066.

34. **Actinomyces suimastitidis** Hoyles, Falsen, Holmström, Persson, Sjödén and Collins 2001a, 1326[VP]

su.i.mas.ti'ti.dis. L. fem. n. *sus*, *suis* pig; N.L. n. *mastitis*, *-idis* inflammation of the milk gland; N.L. gen. n. *suimastitidis* of porcine mastitis.

Cells are straight to slightly curved rods. Catalase-negative. Additional morphological details as well as nutrition and growth conditions are as given for the genus and as summarized in Table 8. Physiological and other descriptive and differential characteristics are listed in Table 5 and Table 9. In SDS-PAGE analysis, the *Actinomyces suimastitidis* isolate was found to be different from all *Actinomyces* species described until 2001. The natural habitat is not known. The single strain of the species encountered so far was isolated from pig mastitis, but its pathogenic potential has not been proven.

Source (type strain): mammary gland of a sow with chronic granulomatous mastitis.

DNA G+C content (mol%): not reported.

Type strain: CCUG 39276, CIP 106779, DSM 15538.

Sequence accession no. (16S rRNA gene): AJ277385.

35. **Actinomyces timonensis** Renvoise, Raoult and Roux 2010, 1520[VP]

ti.mo.nen'sis. N.L. masc. adj. *timonensis* of or pertaining to the Hôpital de la Timone, the hospital in Marseille, France, where the type strain was isolated.

Cells are Gram-stain-positive, straight rods. As estimated by electron microscopy, they measure 1.0–3.2 µm in length and 0.3–0.5 µm in diameter. Catalase- and oxidase-negative. Optimal growth occurs under anaerobic conditions at 37°C; growth was also obtained at 25°C and 30°C, but not at 44°C, and occurred in an atmosphere of 5% CO_2 as well as under "microaerophilic" and anaerobic conditions, but was weak in air. After 48 h of incubation on sheep blood agar, colonies are pinpoint, circular, white, dry, embedded in the agar medium, and α-hemolytic. When using API 50 CH strips, after 24 h of incubation, acid is produced from D-galactose, D-glucose, D-fructose, inositol, D-mannitol, *N*-acetylglucosamine, esculin, ferric citrate, salicin, maltose, lactose, melibiose, sucrose, trehalose, melezitose, and raffinose. After 48 h of incubation, acid is also produced from glycerol, D-ribose, D-mannose, and glycogen and weakly from D-arabitol. Acid is not produced from erythritol, D- and L-arabinose, D- and L-xylose, methyl-β-D-xylopyranoside, L-sorbose, L-rhamnose, dulcitol, D-sorbitol, methyl-α-D-mannopyranoside, methyl-α-D-glucopyranoside, amygdalin, cellobiose, inulin, starch, xylitol, gentiobiose, turanose, D-lyxose, D-tagatose, D- and L-fucose, L-arabitol, potassium gluconate, potassium 2-ketogluconate, or potassium 5-ketogluconate. After 24 h of incubation, acid production from arbutin is variable. After 48 h of incubation, acid production is variable from D-adonitol and arbutin. Using the API ZYM system, leucine arylamidase activity is detected, but valine arylamidase, β-galactosidase, α-glucosidase, alkaline phosphatase, esterase (C4), esterase lipase (C8), lipase (C14), cystine arylamidase, trypsin, α-chymotrypsin, acid phosphatase, naphthol-AS-BI-phosphohydrolase, α-galactosidase, β-glucuronidase, β-glucosidase, *N*-acetyl-β-glucosaminidase, α-mannosidase, and α-fucosidase activities are not. Using the API Rapid ID32A system, β-glucosidase, proline arylamidase, phenylalanine arylamidase, leucine arylamidase, and tyrosine arylamidase are detected. Variable reactions are obtained for α-glucosidase, mannose fermentation, and raffinose fermentation. Urease, arginine dihydrolase, α-galactosidase, β-galactosidase-6-phosphate, β-galactosidase, α-arabinosidase, β-glucuronidase, *N*-acetyl-β-glucosaminidase, glutamic acid decarboxylose, α-fucosidase, nitrate reduction, indole production, alkaline phosphatase, arginine arylamidase, leucyl glycine arylamidase, pyroglutamic acid arylamidase, alanine arylamidase, glycine arylamidase, histidine arylamidase, glutamyl glutamic acid arylamidase, and serine arylamidase reactions were negative. The type strain was found to be susceptible to penicillin G, amoxicillin plus clavulanic acid, cefotetan, vancomycin, and imipenem, but resistant to metronidazole. In the phylogenetic tree produced from 16S rRNA gene sequences by the neighbor-joining method *Actinomyces timonensis* clustered close to *Actinomyces denticolens* so that it appears to be a member of the *Actinomyces* core cluster 1. MALDI-TOF MS analysis confirmed that *Actinomyces timonensis* is a novel *Actinomyces* species. The natural habitat is not known.

Source (type strain): a clinical osteo-articular sample from a 13-year-old girl.

DNA G+C content (mol%): not reported.

Type strain: 7400942, CSUR P35, CCUG 55928.

Sequence accession no. (16S rRNA gene): EU484334.

36. **Actinomyces turicensis** Wüst, Stubbs, Weiss, Funke and Collins 1995b, 619[VP] (Effective publication: Wüst, Stubbs, Weiss, Funke and Collins 1995a, 80.), emend. Vandamme, Falsen, Vancanneyt, Van Esbroek, Van de Merwe, Bergmans, Schouls and Sabbe 1998, 509)

tu.ri.cen'sis. L. masc. adj. *turicensis* of or pertaining to *Turicum* (Zürich) referring to the place where the type strain had been isolated.

Cells are straight and slightly curved, Gram-stain-variable rods (0.5–0.8 µm in diameter and 1.3–4.0 µm in length) with some (pseudo)branching and a beaded appearance. A few club-shaped cells may be present. After 48 h of incubation on sheep blood agar in air with 5% CO_2, colonies are small, gray, convex, circular, and opaque with a glistening surface, an entire edge and a butyrous consistency. A small hemolytic zone is more visible when strains are grown under CO_2 enrichment or anaerobically. Catalase-negative. Additional morphological details, cell-wall composition and other chemotaxonomic properties, nutrition and growth conditions, metabolism and metabolic pathways as well as drug susceptibility are as given for the genus and as summarized in Table 6. Physiological and other descriptive and differential characteristics are listed in Table 4 and Table 7. *Actinomyces turicensis* strains have chiefly been isolated from human mixed infections including otitis, empyema, pilonidal cyst, perianal abscesses, and

decubitus ulcers. The isolates were usually found to be associated with anaerobes, "*milleri*" streptococci, enterococci, and Gram-stain-negative rod-shaped bacteria. The natural habitat is not definitely known.

Source (type strain): human perianal abscess.

DNA G+C content (mol%): 57.5 (T_m).

Type strain: APL10, ATCC 51857, CCUG 32401, CCUG 34269, CIP 105357, DSM 9168, LMG 15961.

Sequence accession no. (16S rRNA gene): X78720.

37. **Actinomyces urogenitalis** Nikolaitchouk, Hoyles, Falsen, Grainger and Collins 2000, 1653[VP]

u.ro.ge.ni.ta′lis. Gr. n. *ouron* urine; L. adj. *genitalis* belonging to the genital system or progenitive tract; N.L. masc. adj. *urogenitalis* belonging to the urogenital tract.

Cells are straight to slightly curved rods. Catalase-negative. Additional morphological details, nutrition and growth conditions, and metabolic activities are as given for the genus and as summarized in Table 6. Physiological and other descriptive and differential characteristics are listed in Table 4 and Table 7. *Actinomyces urogenitalis* strains formed a homogeneous group in the SDS-PAGE analysis that was distinct from all other *Actinomyces* species described until 2000. The natural habitat is not known.

Source: urine, vaginal secretions, and the human urethra. The type strain was isolated from vaginal secretions of a 33-year-old woman with abnormal vaginal discharge.

DNA G+C content (mol%): 61 (T_m).

Type strain: CCUG 38702, CIP 106421, DSM 15434.

Sequence accession no. (16S rRNA gene): AJ243791.

38. **Actinomyces vaccimaxillae** Hall, Collins, Hutson, Inganäs, Falsen and Duerden 2003a, 604[VP]

vac.ci.ma.xil′la.e. L. fem. n. *vacca* cow; L. fem. n. *maxilla* the jaw; N.L. gen. n. *vaccimaxillae* of a cow's jaw.

Cells are coccobacillary to short diphtheroidal rods. After 48 h of anaerobic incubation on Fastidious Anaerobe Agar with 5% horse blood, colonies are <1 mm in diameter, convex, smooth, entire-edged, opaque white, and non-hemolytic. Catalase-negative. Additional morphological details, nutrition and growth conditions as well as metabolism and metabolic pathways are as given for the genus and as summarized in Table 8. Physiological and other descriptive and differential characteristics are listed in Table 5 and Table 9. The natural habitat is not known.

Source (type strain): a purulent lesion in the jaw of a cow.

DNA G+C content (mol%): not reported.

Type strain: R10176, CCUG 46091, CIP 107423, DSM 15804.

Sequence accession no. (16S rRNA gene): AJ427451.

39. **Actinomyces viscosus** (Howell, Jordan, Georg and Pine 1965) Georg, Pine and Gerencser 1969, 292[AL] (*Odontomyces viscosus* Howell, Jordan, Georg and Pine 1965, 65)

vis.co′sus. L. masc. adj. *viscosus* sticky.

After the taxonomic revision of the *Actinomyces naeslundii/ Actinomyces viscosus* complex, the species only contains the so-called hamster strains of the former *Actinomyces viscosus* taxon, which have also been known as *Actinomyces viscosus* serovar I. Cells from broth cultures present as dense clumps of long Gram-stain-positive filaments which vary from finely beaded or stippled thread-like forms to solidly stained, slightly clavate structures. Branching is frequently seen. Growth on BHIA in air without added CO_2 is usually negligible or very poor. However, when plates are incubated in air plus 5% CO_2, growth is rapid and profuse. Microcolonies under such growth conditions at about 12 h of incubation are composed of branched, non-septate filaments (1 µm in diameter), which rapidly undergo septation and angular branching to produce diphtheroidal cells. Mature colonies develop within 2–3 d and vary from 0.5 to 5.0 mm in diameter. They are usually round, strongly convex to heaped, smooth, granular, often with an eccentric pit, cream to white, and dry to viscous in consistency (Figure 34). Occasionally, especially after serial transfer, colonies may become round, entire, low convex, smooth, transparent with an optically dark central "core" visible in transmitted light producing an appearance similar to that of propionibacteria. Catalase-positive. Additional morphological details, ultrastructure, cell-wall composition, nutrition and growth conditions, and metabolism and metabolic pathways are as given for the genus and as summarized in Table 8. Physiological and other descriptive or differential characteristics are listed in Table 5 and Table 9. As morphological and physiological properties may not suffice to reliably identify *Actinomyces viscosus* and differentiate it from *Actinomyces naeslundii*, *Actinomyces oris*, and *Actinomyces johnsonii*, serology can help in identification because cross-reactions with the above group of actinomycetes were found to be weak. *Actinomyces viscosus* has been isolated from subgingival plaque of hamsters and cervical plaque of rats. Thus, the oral cavity of these (and other?) animals appears to be the natural habitat of this species. Bacteria named *Actinomyces viscosus* have also been reported as the causative agents of animal diseases. These included actinomycosis-like lesions in swine, cats, and dogs. However, it remains to be proven whether all of these infections were really due to *Actinomyces viscosus* as currently defined. In contrast, periodontal disease spontaneously occurring in hamsters can be accepted as a disease definitely caused by *Actinomyces viscosus*.

Experimental infections have been produced in mice and hamsters. After oral inoculation, the organism produced periodontitis and/or caries in hamsters as well as in conventional and gnotobiotic rats.

Source (type strain): periodontitis in a hamster.

DNA G+C content (mol%): 59–63 (T_m).

Type strain: ATCC 15987, CCUG 14476, CIP 103147, DSM 43327, JCM 8353, NCTC 10951.

Sequence accession no. (16S rRNA gene): X82453.

Species *incertae sedis*

a. **"Actinomyces houstonensis"** Clarridge and Zhang 2002, 3448

hous.to.nen′sis. N.L. masc. adj. *houstonensis* of or pertaining to Houston, where the organism was described.

Cells are Gram-stain-positive, pleomorphic rods, which have a tendency to form half circles. Colonies on sheep blood agar after 48 h of incubation at 36°C are α-hemolytic, gray, and 0.2 mm in diameter. Growth is equal in air with added CO_2 and under anaerobic conditions. Catalase-negative. Esculin, urea, and gelatin are not hydrolyzed. Nitrate is reduced to nitrite. α-Glucosidase, leucyl-glycine arylamidase, glycine arylamidase, proline arylamidase, phenylalanine arylamidase, arginine arylamidase, and serine arylamidase are produced, but not alkaline phosphatase, pyrrolidonyl arylamidase, and indole. Acid is produced from glucose and sucrose, but not from xylose. All three strains of the species encountered so far originated from serious human subcutaneous abscesses requiring drainage. The natural habitat is not known.

DNA G+C content (mol%): not reported.

Type strain: Houston VAMC strain 3971 was isolated from an abscess from the back of a patient. The strain has not been deposited in one of the international culture collections. The species designation has also not appeared on a Validation List in the IJSEM.

b. **"Actinomyces suis"** Franke 1973, 123 (Not *Actinomyces suis* Gasperini 1892, 183, *Actinomyces suis* Grässer 1957, 148, or *Actinobaculum* (*Eubacterium*, *Actinomyces*) *suis* Lawson, Falsen, Åkervall, Vandamme and Collins 1997, 901, but phenotypically related to *Actinomyces hyovaginalis* Collins, Stubbs, Hommez and Devriese 1993, 472)

su′is. L. masc. and fem. n. *sus, suis* pig, hog; L. gen. n. *suis* of the hog.

Cells are predominantly diphtheroidal and may be arranged in clusters or in V, Y, or T forms. Coccoid elements as well as branched filaments may also occur. Microcolonies may be filamentous, but entire-edged and granular or smooth forms may also be seen. Mature colonies are up to 2 mm in diameter, opaque and raised, and may have entire, undulate, or filamentous margins. Catalase-negative. Growth at 36±1°C is good in air with added CO_2 and anaerobically with and without CO_2. Aerobic growth without CO_2 is weak. Nitrate is reduced to nitrite, gelatin is not liquefied, and indole is not produced. Acid is produced from fructose, galactose, glucose, *myo*-inositol, inulin, lactose, maltose, mannose, raffinose, salicin, starch, sucrose, and trehalose, but not from L-arabinose, rhamnose, sorbitol, and D-xylose. Acid production is variable from adonitol, glycerol, mannitol, and ribose. Using an agar gel precipitation procedure, all of the "*Actinomyces suis*" isolates tested appeared to be serologically homogeneous, but differed clearly from other *Actinomyces* species. Only low-titered cross-reactions were observed with *Actinomyces israelii sensu lato*, *Actinomyces naeslundii sensu lato*, and *Propionibacterium propionicum*. The natural habitat of "*Actinomyces suis*" is not known. The organisms have been isolated from actinomycosis of the mammary gland of swine. The taxonomic and nomenclatural problems associated with "*Actinomyces suis*" have been discussed in the taxonomy section of this chapter.

DNA G+C content (mol%): not reported.

Type strain: none designated or deposited.

Genus II. **Actinobaculum** Lawson, Falsen, Åkervall, Vandamme and Collins 1997, 902[VP]

Paul A. Lawson

Ac.ti.no.ba′cu.lum. Gr. n. *actis*, *actinos* ray; L. neut. n. *baculum* rod, stick; N.L. neut. n. *Actinobaculum* ray stick.

On blood agar, **cells are straight to slightly curved rods, some of which exhibit branching. Cells are Gram-stain-positive, not acid-fast, and nonmotile and do not form spores. Anaerobic or facultatively anaerobic. Catalase-negative.** Acid may or may not be produced from glucose. **The major end product of glucose and/or maltose metabolism is acetate or lactate.** Esculin and gelatin are not hydrolyzed. Nitrate is not reduced to nitrite. Acetoin is not produced. **The cell-wall murein type is type A5a** (L-Lys–L-Ala–Lys–D-Glu or L-Lys–Lys–D-Glu). The major long-chain cellular fatty acids are straight-chain saturated and monounsaturated components.

DNA G+C content (mol%): 55–57 (T_m).

Type species: **Actinobaculum suis** (Wegienek and Reddy 1982) Lawson, Falsen, Åkervall, Vandamme and Collins 1997, 902[VP].

Further descriptive information

Grows on 5% (v/v) sheep or horse blood. Cells are straight to slightly curved. Strains can be isolated on a variety of rich agar-containing media (such as heart infusion agar) supplemented with blood. Strains grow at 37°C in a 5% CO_2 atmosphere or under anaerobic conditions although *Actinobaculum suis* appears to be the only species that prefers anaerobic growth conditions. None of the species produce acid from lactose, mannitol, melibiose, melezitose, raffinose, or sorbitol.

The cell-wall murein of *Actinobaculum* is type A5a. *Actinobaculum suis* contains L-Lys–L-Ala–Lys–D-Glu and *Actinobaculum schaali* L-Lys–Lys–D-Glu (see Schleifer and Kandler, 1972, for nomenclature; Lawson et al., 1997). The murein type for *Actinobaculum urinale* has not been determined.

Actinobaculum species have been recovered from a variety of human and animal sources. *Actinobaculum massiliense*, *Actinobaculum schaalii*, and *Actinobaculum urinale* have been isolated from a number of human clinical sources that include blood (Clarridge and Zhang, 2002; Fendukly and Osterman, 2005; Lawson et al., 1997) and urine (Greub and Raoult, 2002; Hall et al., 2003b). *Actinobaculum suis* has been isolated from several animal sources (Walker and MacLachlan, 1989; Wegienek and Reddy, 1982; Woldemeskel et al., 2002; Yamini and Slocombe, 1988).

Isolation procedures

Strains can be isolated on a variety of rich agar-containing media (such as heart infusion agar) supplemented with 5% (v/v) animal blood (sheep or horse). Strains grow at 37°C in a 5% CO_2 atmosphere or under anaerobic conditions. There is no information on enrichment or selective media for this genus.

Maintenance procedures

Strains can be maintained on agar media (such as heart infusion or Columbia agar) supplemented with blood (5% v/v). Strains grow in brain heart infusion broth supplemented with 5% (v/v) serum. For long-term preservation, strains can either be stored at –70°C on cryogenic beads or lyophilized.

Taxonomic comments

The genus *Actinobaculum* was proposed by Lawson et al. (1997) to accommodate bacterial strains that had previously been assigned to various other genera and that were named "*Actinobaculum suis*", together with *Actinomyces*-like isolates from human clinical specimens. The genus forms a distinct subline, related to but distinct from the genera *Actinomyces*, *Arcanobacterium*, and *Mobiluncus* (Figure 58). Currently, three species, *Actinobaculum*

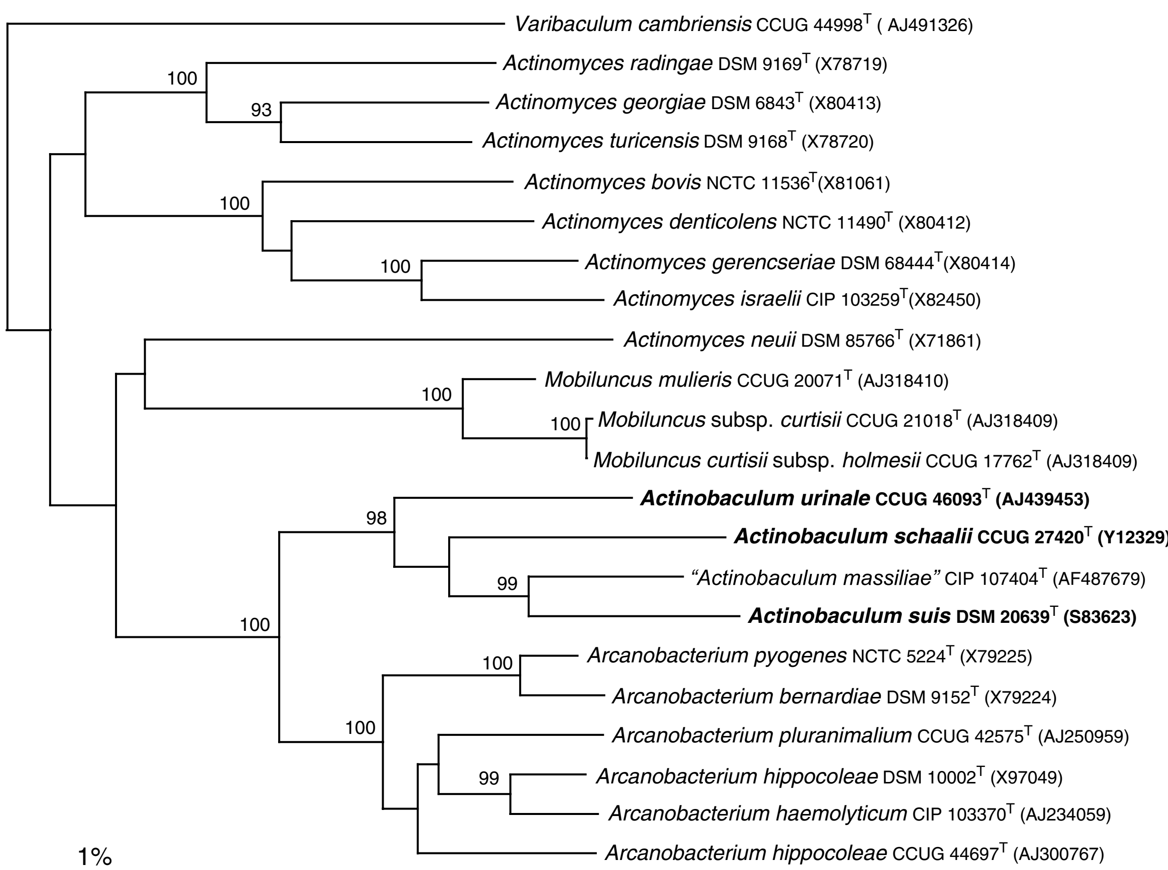

FIGURE 58. Unrooted neighbor-joining tree depicting the estimated phylogenetic relationships of members of the genus *Actinobaculum* and their close relatives. The numbers on the branches refer to bootstrap values, determined from 1000 replications. Only values above 90% are shown. Bar = 1% sequence divergence.

suis, *Actinobaculum schaalii*, and *Actinobaculum urinale* are recognized. *Actinobaculum suis*, the type species of the genus, is an established pathogen of pigs and has had a somewhat confused taxonomic and nomenclatural history, having been assigned to a variety of genera including *Actinomyces*, *Corynebacterium*, and *Eubacterium* (Wegienek and Reddy, 1982). This species designation was first introduced by Gasperini (1892), but in the eighth edition of the *Bergey's Manual of Determinative Bacteriology* (Slack, 1974) it was cited as a *nomen dubium* because this species designation had not been validly published. Grässer (1957) isolated bacteria named *Actinobaculum suis* from mastitic swine, but in this case while the name was validly published, the description was inadequate and cultures were not available. Hence, the species *Actinobaculum suis* was not included in the Approved Lists of Bacterial Names (Skerman et al., 1980). More recently, Franke (1973) described actinomycetes isolated from the udder of swine which he called *Actinobaculum suis*. The morphological and biochemical characteristics of these isolates appeared to be somewhat different from those of Grasser's strains, but they seemed to fit well within the genus *Actinomyces* (Slack and Gerencser, 1975). At present, it is impossible to decide whether *Actinobaculum suis* Grasser and *Actinobaculum suis* Franke are identical.

In a taxonomic study of "*Corynebacterium suis*", Wegienek and Reddy (1982) found that "*Corynebacterium suis*" strain Soltys 50052 is anaerobic, has rhamnose and lysine as major cell components, and produces acetate, formate, and ethanol as major end products of carbohydrate metabolism. In contrast, representatives of the genus *Corynebacterium* are aerobic or facultatively anaerobic organisms characterized by cell walls containing arabinose and galactose as the major sugar components and *meso*-diaminopimelic acid as the major diamino acid. Furthermore, corynebacteria produce major amounts of acetate, propionate, and formate and variable amounts of other acids as products of carbohydrate metabolism (Reddy and Kao, 1978). Thus, Wegienek and Reddy (1982) concluded that this strain did not belong to the genus *Corynebacterium* and they proposed, despite differences in cell-wall composition and DNA base ratios, that the organism should be included in the genus *Eubacterium*. Their proposal was based on a rather limited number of characteristics such as anaerobiosis, morphology, as well as absence of lactate, propionate, and succinate among the end products of carbohydrate metabolism. Using comparative sequence analysis of the 16S rRNA gene, Ludwig et al. (1992) showed that *Eubacterium suis* was a relatively close relative (93.8% sequence similarity) of *Actinomyces pyogenes*. These two organisms have the same cell-wall composition (lysine is the diamino acid; rhamnose and traces of mannose are the cell-wall sugars), contain type *c* cytochromes, and have a similar DNA G+C content, 55 and 56–58 mol%, respectively (Schaal, 1986b; Schaal and Pulverer, 1981; Soltys and Spratling, 1957; Wegienek and Reddy, 1982). Since the type strain of "*Actinomyces suis*" Franke is extant, Ludwig et al. (1992) proposed that "*Eubacterium suis*" should be transferred to the genus *Actinomyces* as "*Actinomyces suis*".

Following a study of *Actinomyces*-like organisms from clinical materials, Lawson et al. (1997) proposed the genus *Actinobaculum* to accommodate these organisms. 16S rRNA gene sequencing showed that they were distinct from previously described *Actinomyces* and *Arcanobacterium* species. However, *Actinomyces suis* was demonstrated to be a close phylogenetic relative (94.2% sequence similarity) of the newly isolated clinical strains and presented similar phenotypic and biochemical characteristics. Therefore it was reclassified as *Actinobaculum suis* and as the type species of the genus. A third species of *Actinobaculum* was proposed by Hall et al. (2003b) to accommodate an organism isolated from human urine, and this taxon exhibited phylogenetic and biochemical characteristics consistent with its assignment to the genus *Actinobaculum*. In 2002, Greub and Raoult described an organism isolated from human urine, and based on phylogenetic and biochemical studies, they identified it as a member of the genus *Actinobaculum* and proposed the name "*Actinobaculum massiliae*". Subsequently this organism was validly published as *Actinobaculum massiliense* (Greub and Raoult, 2006).

Differentiation of the genus *Actinobaculum* from other genera

Actinobaculum can be distinguished readily from its closest phylogenetic relatives using a combination of morphological, biochemical, and chemotaxonomic criteria. Differential characteristics of the species of *Actinobaculum* are presented in Table 10.

Differentiation of species of the genus *Actinobaculum*

Differential characteristics of the species of *Actinobaculum* are presented in Table 11.

TABLE 10. Characteristics that differentiate *Actinobaculum* from *Actinomyces*, *Arcanobacterium*, and *Mobiluncus* species[a,b]

Characteristic	*Actinobaculum massiliense*	*Actinobaculum urinale*	*Actinobaculum schaalii*	*Actinobaculum suis*	*Actinomyces georgiae*	*Actinomyces gerencseriae*	*Actinomyces israelii*	*Actinomyces meyeri*	*Actinomyces naeslundii*	*Actinomyces neuii* subsp. *neuii*	*Actinomyces neuii* subsp. *anitratus*	*Actinomyces odontolyticus*	*Actinomyces radingae*	*Actinomyces turicensis*	*Actinomyces viscosus*	*Arcanobacterium bernardiae*	*Arcanobacterium haemolyticum*	*Arcanobacterium hippocoeae*	*Arcanobacterium pyogenes*	*Arcanobacterium phocae*	*Mobiluncus curtisii*	*Mobiluncus muleris*
Nitrate reduction	–	–	–	–	d	d	d	–	d	+	–	+	–	–	+	–	–	–	–	–	–	–
Urease activity	–	+	–	+	–	–	–	d	+	–	–	–	–	–	d	–	–	–	–	–	–	–
Esculin hydrolysis	–	–	–	–	+	d	+	–	+	–	–	d	+	–	d	–	–	+(w)	–	–	–	–
Acid produced from mannitol	–	–	–	–	d	d	d	–	–	+	+	–	d	d	–	–	–	–	d	d	–	–
Acid produced from xylose	+	–	+	–	+	+	+	+	d	+	+	d	+	+	d	–	–	–	+	–	–	–
β-Galactosidase	–	–	–	+	nd	nd	+	–	d	+	+	–	+	–	d	–	+	+	d(w)	+(w)	d	–
α-Glucosidase	+	–	+	nd	nd	nd	+	+	d	+	+	–	+	+	d	+	nd	+	–	+(s)	d	+
N-Acetyl-β-glucosaminidase	–	–	–	nd	nd	nd	–	–	–	d	d	–	+	–	–	–	+	d	+	–	–	–
Murein type	nd	nd	A5a (L-Lys–Lys–D-Glu)	A5a (L-Lys–L-Ala–Lys–D-Glu)	A5b (L-Orn–Lys–D-Glu)	A5b (L-Orn–Lys–D-Glu)	A5b (L-Orn–Lys–D-Glu)	nd	nd	A5a (L-Lys–Lys–D-Glu)	A5a (L-Lys–Lys–D-Glu)	nd	A5a (L-Lys [L-Orn]–Lys–D-Glu)	A5b (L-Orn–L-Lys–D-Glu)	nd	A5a (L-Lys–L-Ala–Lys–D-Glu)	A5a (L-Lys–Lys–D-Glu)	nd	A5a (L-Lys–L-Ala–Lys–D-Glu)	nd	nd	nd

[a] Symbols and abbreviations: –, negative; +, positive; (w), weak; (s), slow; d, differences between strains; nd, not determined.

[b] Data from Greub and Raoult (2002), Funke et al. (1997a, 1995, 1997c), Pascual Ramos et al. (1997b), Schaal (1986b), Lawson et al. (1997), Hall et al. (2003b), Spiegel and Roberts (1984b), and Hoyles et al. (2004).

TABLE 11. Tests that are useful for distinguishing *Actinobaculum* species[a]

Test	*A. massiliense* CCUG 47753[T]	*A. schaalii* (*n* = 5)	*A. suis* CCUG 19206[T]	*A. urinale* CCUG 46093[T]
API Rapid ID 32Strep system				
Acid from:				
L-Arabinose	–	d	–	–
Maltose	+(w)	+	–	–
Sucrose	–	d	–	–
Production of:				
Alanine-phenylalanine-proline arylamidase	+	+	+	–
Alkaline phosphatase	–	–	+	–
β-Glucuronidase	–	–	+	+
Pyroglutamic acid arylamidase	–	+	+	–
API Rapid ID 32A system				
Production of:				
Alanine arylamidase	+	+	+	–
Alkaline phosphatase	–	–	+	–
Glycine arylamidase	+	+	+	–
α-Glucosidase	–	+	+	–
β-Glucuronidase	–	–	+	+
Leucine-glycine arylamidase	+	+	+	–
Pyroglutamic acid arylamidase	+	+	+	–
Tyrosine arylamidase	+	+	+	–
Urease	–	–	+	+
Murine type	nd	A5a (L-Lys–Lys–D-Glu)	A5a (L-Lys–L-Ala–Lys–D-Glu)	nd

[a]Symbols and abbreviations: +, positive; –, negative; d, differences between strains; (w), weak; nd, not determined.

[b]Data from Lawson et al. (1997) and CCUG (http://www.ccug.se).

List of species of the genus *Actinobaculum*

1. **Actinobaculum suis** Wegienek and Reddy 1982; "*Corynebacterium suis*" Soltys and Spratling 1957; Lawson, Falsen, Åkervall, Vandamme and Collins 1997, 902[VP]

su′is. L. gen. n. *suis* of a hog, of a pig.

Slender, nonmotile, pleomorphic rods (1–3 by 0.5 μm) are arranged singly, in pairs (cells often at an angle to each other or in palisades), or in small clusters. Gram-stain-positive, but rather easily decolorized, especially in old cultures. Non-acid-fast and nonsporulating; does not survive heating at 80°C for 10 min. Capsules are not observed by capsule staining; however, a fringelike outer coat external to the cell wall is seen in thin-section electron micrographs. Colonies on anaerobic blood agar plates are 0.5–3.0 mm in diameter after 48 h, white, circular, and granular and have entire to slightly irregular margins. Colonies often have slightly raised centers, which gives them a fried-egg appearance. After 1 week, colonies are 3–5 mm in diameter and flatter. Growth is barely discernible after incubation for 7 d under 6% CO_2 or air. Peptone-yeast extract-starch broth supports excellent growth. The optimal pH is 7–8; no growth occurs at pH 5.0 or less. Growth is optimal at 37°C and in the temperature range, 30–43°C; no growth occurs at 22–23°C. Anaerobic. Metabolism is strictly fermentative. Glycogen, maltose, and starch are fermented. Acetate, ethanol, and formate are the main products from maltose fermentation. Adonitol, amygdalin, arabinose, cellobiose, dulcitol, erythritol, esculin, fructose, galactose, glucose, glycerol, inositol, inulin, lactose, mannitol, mannose, melezitose, melibiose, raffinose, rhamnose, salicin, sorbitol, sucrose, trehalose, lactate, pyruvate, and threonine are not fermented. Strongly urease-positive. Does not produce catalase, indole, acetylmethylcarbinol, hydrogen sulfide, lipase, or lecithinase; ammonia is not produced from peptone. Esculin and gelatin are not hydrolyzed. Meat and milk are not digested. Nitrates are not reduced. Major amounts of type *b* cytochrome and minor amounts of type *c* cytochrome are synthesized. The cell-wall sugars are mannose and rhamnose. The cell-wall murein type is type A5a (L-Lys–L-Ala–Lys– D-Glu). Originally isolated from cases of cystitis and pyelonephritis and cases of metritis in pregnant sows. Not isolated from healthy sows but frequently recovered from urine and semen of apparently healthy boars. Sows can be infected artificially by intrarenal injection of live organisms plus 5% saponin. No demonstrable exotoxin is produced.

DNA G+C content (mol%): 55–57 (T_m).

Type strain: Soltys 50052, ATCC 33144, CCUG 19206, CIP 105361, DSM 20639, LMG 18291.

Sequence accession no. (16S rRNA gene): S83623.

2. **Actinobaculum massiliense** Greub and Raoult 2006, 2025[VP] (Effective publication: Greub and Raoult 2002, 3941.)

mas.si.li.en′se L. neut. adj. *massiliense* of or pertaining to *Massilia*, the Latin name of Marselle, where the organism was isolated.

The organism forms straight to slightly curved rods, some of which exhibit branching. It has nonmotile, non-acid-fast, Gram-stain-positive (easily decolorized) cells. Colonies on

sheep blood agar are 1 mm in diameter after 72 h of incubation at 37°C in a 5% CO_2 atmosphere, and there is no hemolysis. It is facultatively aerobic. Esculin and gelatin are not hydrolyzed. Nitrate is not reduced to nitrite. Acid is produced from glucose, glycogen, maltose, ribose, and xylose. The cellular fatty acids are of the straight chain saturated and monounsaturated types, with $C_{16:0}$, $C_{16:1}$, $C_{18:0}$, and $C_{18:1}$ ω9c predominating.

Source (type strain): the urine of an 81-year-old woman with cystitis. Habitat is not known.

DNA G+C content (mol%): not determined.

Type strain: CCUG 47753, CIP 107404, DSM 19118.

Sequence accession no. (16S rRNA gene): AF487679.

3. **Actinobaculum schaalii** Lawson, Falsen, Åkervall, Vandamme and Collins 1997, 902[VP]

schaa′li.i. N.L. gen. masc. n. *schaalii* of Schaal, to honor Klaus P. Schaal, contemporary German microbiologist, for his contributions to actinomycete microbiology.

Cells are straight to slightly curved rods, some of which exhibit branching, Gram-stain-positive, non-acid-fast, nonmotile, and non-spore-forming. Nonhemolytic. A weak CAMP reaction occurs. Facultatively anaerobic and catalase-negative. Acetate and succinate are the major end products of glucose fermentation. Acid is produced from glucose, maltose, ribose, and D-xylose. Some strains produce acid from L-arabinose, mannose, starch, sucrose, and trehalose. Acid is not produced from D-arabitol, *N*-acetyl-β-glucosamine, cyclodextrin, glycogen, lactose, mannitol, melezitose, melibiose, methyl-β-D-glucopyranoside, pullulan, raffinose, sorbitol, and D-tagatose. Hippurate is hydrolyzed. Esculin, gelatin, and urea are not hydrolyzed. α-Glucosidase, alanine-phenylalanine-proline arylamidase, and pyroglutamic acid arylamidase are produced. Alkaline phosphatase, arginine dihydrolase, α-galactosidase, β-galactosidase, β-glucuronidase, glycyl-tryptophan arylamidase, and β-mannosidase activities are not detected. Pyrazinamidase and pyrrolidonylarylamidase activities are shown by some strains. Nitrate is not reduced to nitrite. Acetoin is not produced. The cell-wall murein type is A5a (L-Lys–Lys–D-Glu). The major cellular fatty acids are hexadecanoic, octadecanoic, and *cis*-delta-9-octadecenoic acids. Strains have been isolated from human blood and urine. The habitat is unknown but is probably the genital or urinary tract.

Source: human blood (chronic pyelonephritis, 64-year-old male).

DNA G+C content (mol%): 55–57 (HPLC).

Type strain: CCUG 27420, CIP 105739, CCM 4738, DSM 15541, LMG 18293.

Sequence accession no. (16S rRNA gene): Y12329.

4. **Actinobaculum urinale** Hall, Collins, Hutson, Falsen, Inganäs and Duerden 2003b, 682[VP]

u.ri.na′le. L. neut. adj. *urinale* of or belonging to urine.

Cells are straight to slightly curved Gram-stain-positive rods. Branching is not observed. Cells are non-acid-fast and non-spore-forming. After 48 h of anaerobic incubation on fastidious anaerobe agar supplemented with 5% horse blood, colonies are <1 mm in diameter, convex, smooth, entire-edged, gray or white, and weakly β-hemolytic. Facultatively anaerobic and catalase-negative. Lactic acid is the major end product of glucose metabolism together with minor amounts of acetic acid. Using the commercially available API systems, acid is produced from glucose and ribose; acid production from maltose and sucrose is variable and dependent on the test system used. Acid is not formed from D-arabitol, L-arabinose, cyclodextrin, glycogen, lactose, mannitol, mannose, melibiose, melezitose, methyl-β-D-glucopyranoside, pullulan, raffinose, sorbitol, tagatose, trehalose, or D-xylose. Hippurate is hydrolyzed, but gelatin and esculin are not. Arginine arylamidase, β-glucuronidase, and proline arylamidase are produced and urease may or may not be detected. Alanine phenylalanine proline arylamidase, arginine dihydrolase, acid phosphatase, alkaline phosphatase, alanine arylamidase, arginine arylamidase, α-arabinosidase, chymotrypsin, trypsin, cysteine arylamidase, esterase C4, ester lipase C8, α-fucosidase, α-galactosidase, β-galactosidase, β-galactosidase-6-phosphate, α-glucosidase, β-glucosidase, glycyl tryptophan arylamidase, glutamic acid decarboxylase, glutamyl glutamic acid arylamidase, glycine arylamidase, histidine arylamidase, leucine arylamidase, leucyl glycine arylamidase, lipase C14, α-mannosidase, β-mannosidase, *N*-acetyl-β-glucosaminidase, phosphoamidase, phenylalanine arylamidase, pyroglutamic acid arylamidase, pyrazinamidase, serine arylamidase, tyrosine arylamidase, and valine arylamidase are not detected. Acetoin is not produced. Indole-negative. Nitrate is not reduced to nitrite. The cellular fatty acids are of the straight chain saturated and monounsaturated types, with $C_{14:0}$, $C_{16:0}$, $C_{16:1}$, $C_{18:0}$, and $C_{18:1}$ ω9*c* predominating.

Source (type strain): human urine. Habitat is not known.

DNA G+C content (mol%): not determined.

Type strain: R9242, CCUG 46093, CIP 107424, DSM 15805.

Sequence accession no. (16S rRNA gene): AJ439453.

Genus III. **Arcanobacterium** Collins, Jones and Schofield 1983, 438[VP] (Effective publication: Collins, Jones and Schofield 1982b, 1280.), emend. Lehnen, Busse, Frölich, Krasinska, Kämpfer and Speck 2006, 864[VP]

Atteyet F. Yassin and Klaus P. Schaal

Ar.ca.no.bac.te′ri.um. L. adj. *arcanus* secret, hidden, secretive; L. neut. n. *bacterium* a small rod; N. L. neut. n. *Arcanobacterium* secretive bacterium.

Slender, irregular, bacillary forms predominate during the first **18** h of growth; many cells are **arranged at an angle to give V-formations**. As growth proceeds cells become granular and segmented so that they resemble small and irregular cocci. Both rods and coccoid cells are Gram-stain-positive, non-acid fast and non-motile; endospores are not formed. **Facultatively anaerobic**; growth is considerably enhanced in an atmosphere of increased CO_2-tension. Growth is sparse on ordinary media

but enhanced by blood or serum. The optimum temperature for growth is **37°C**. Organisms will not withstand heating at **60°C** for **15 min**. **Chemoorganotrophic**, having a fermentative type of carbohydrate metabolism. Fermentation end products include acetic and lactic acids; the amount of succinic acid produced may vary from species to species and may even be difficult to detect. Catalase-negative. Nitrate reduction is usually negative. Characteristic **amino acids of the cell-wall peptidoglycan** of those species whose peptidoglycan has been examined in detail are based on L-lysine. Other chemotaxonomic properties include **rhamnose** as diagnostic whole-cell sugar, lack of mycolic acids and *N*-acetylated muramic acid residue of the **peptidoglycan**.

DNA G+C content (mol%): 50–63.8 (T_m, HPLC).

Type species: **Arcanobacterium haemolyticum** Collins, Jones and Schofield 1983, 438[VP] (Effective publication: Collins, Jones and Schofield 1982b, 1280).

TABLE 12. 16S rRNA signature nucleotides that define clusters 1 and 2 of *Arcanobacterium* species and their occurrence in the 16S rRNA sequence of *Arcanobacterium hippocoleae*

Position[a]	Cluster 1	Cluster 2	*A. hippocoleae*
157:164	G–U	U–G	U–G
440	C	U	C
443:491	C–G	U–G	U–G
446:488	U–G	A–U	A–U
492	U	C	C
497	U	G	G
598	C	U	U
625	C	U	C
631	C	U	C
659	C	U	C
776	G	U	G
1278	G	U	G

[a] *Escherichia coli* position (Brosius et al., 1978).

Further descriptive information

Phylogeny. Current phylogenetic data indicate that members of the genus *Arcanobacterium* constitute a separate phylogenetic lineage within the *Actinomycetaceae* (Figure 19). It is evident from the tree that the genera *Arcanobacterium* and *Actinobaculum* have a close phylogenetic affinity. From a phylogenetic point of view, it appears that members of the genera *Arcanobacterium* and *Actinobaculum* have originated as descendants of a common ancestor. The phylogenetic coherence of the genus *Arcanobacterium* is supported by a set of distinct signature nucleotides (Table 1).

The intrageneric relationships based on comparisons of the almost complete 16S rRNA gene sequences of the nine *Arcanobacterium* species are shown in Figure 19 of the *Actinomyces* chapter. The type strains of the nine *Arcanobacterium* species form two 16S rRNA gene sequence subclusters in which the species of each subcluster show a high degree of interspecies relatedness. Bootstrap values of 100% indicate that these subclusters are of statistical significance. Subcluster 1 contains the type species of the genus, *Arcanobacterium haemolyticum*, as well as *Arcanobacterium phocae* and *Arcanobacterium pluranimalium*. The 16S rRNA gene sequence similarities within members of this subcluster range from 95.4 to 97.4%. Subcluster 2 contains the species *Arcanobacterium abortisuis*, *Arcanobacterium bernardiae*, *Arcanobacterium bialowiezense*, *Arcanobacterium bonasi* and *Arcanobacterium pyogenes*. The 16S rRNA gene sequence similarities within the type strains of these species range from 95.3 to 98.6%. Members of subclusters 1 and 2 can be separated by 4.4–7.5% sequence divergence. In addition, members of the two subclusters can be clearly distinguished by a set of subcluster-specific nucleotides, which can be considered molecular signatures. The positions of these nucleotides in the 16S rRNA sequence are indicated in Table 12.

The branching point of the *Arcanobacterium hippocoleae* sequence was close to the bifurcation point of the two *Arcanobacterium* lineages. However, this species branched with members of subcluster 1 in the neighbor-joining (Saitou and Nei, 1987) and maximum likelihood (Felsenstein, 1981) trees. The finding that *Arcanobacterium hippocoleae* shows 1% more 16S rRNA gene sequence similarity to members of subcluster 1 than to members of subcluster 2 may be indicative of a specific relatedness to the former cluster as indicated in Figure 19 of the *Actinomyces* chapter. This relationship is also expressed by the presence of six signature nucleotides that are shared between *Arcanobacterium hippocoleae* and members of subcluster 1, while five signatures are common to members of subcluster 2. Bootstrap values of the branching points (98% significance for subcluster 1 members) indicate that members of subcluster 1 are the closest phylogenetic neighbors of *Arcanobacterium hippocoleae*.

Cell morphology. The cellular morphology of the genus *Arcanobacterium* as described by Collins et al. (1982b) for bacteria formerly named "*Corynebacterium haemolyticum*" is as follows: "On blood agar plates, slender, irregular, bacillary forms predominate during the first 18 h; many cells are arranged at an angle to give V-formations. As growth proceeds, organisms become granular and segmented so that they resemble small and irregular cocci. Both rods and coccoid cells are Gram-stain-positive, non-acid fast and non-motile; endospores are not formed".

The morphology of *Arcanobacterium pyogenes* by and large conforms to this description (Reddy et al., 1982; Schaal, 1969). The same is true for *Arcanobacterium abortisuis*, *Arcanobacterium phocae* and *Arcanobacterium bernardiae*, whereas *Arcanobacterium hippocoleae* and *Arcanobacterium pluranimalium* appear to be less coccoid. The cells of *Arcanobacterium bialowiezense* and *Arcanobacterium bonasi* are short pleomorphic rods.

Surface colonies of *Arcanobacterium haemolyticum* on blood agar were described to be small after 24 h of incubation at 36±1°C, becoming larger upon extended incubation (Collins and Cummins, 1986a). They are circular, discoid, slightly raised, opaque and nonpigmented with a butyrous consistency, and they show β-hemolysis. Colonies of *Arcanobacterium pyogenes* are very similar, while *Arcanobacterium bernardiae* varies concerning β-hemolysis. *Arcanobacterium phocae* also produces a potent hemolysin, whereas *Arcanobacterium hippocoleae* is weakly β-hemolytic, and *Arcanobacterium pluranimalium* was reported to be α-hemolytic.

After 48 h of growth under aerobic conditions on sheep blood agar, colonies of *Arcanobacterium bialowiezense* and *Arcanobacterium bonasi* are translucent, convex, approximately 0.5 mm in diameter and surrounded by a narrow zone of β-hemolysis. Colonies of *Arcanobacterium abortisuis* have a translucent smooth surface with a diameter of about 2 mm on TMVL agar (Azuma et al., 2009).

Cell-wall composition. The species currently included in the genus *Arcanobacterium* display considerable chemotaxonomic heterogeneity in particular with regard to their menaquinone, polar lipid and cellular fatty acid composition (Table 13).

TABLE 13. Chemotaxonomic characteristics of members the genus *Arcanobacterium*[a]

	A. haemolyticum	*A. phocae*	*A. pluranimalium*	*A. hippocoleae*	*A. abortisuis*	*A. bernardiae*	*A. bialowiezense*	*A. bonasi*	*A. pyogenes*
Subcluster	1	1	1		2	2	2	2	2
Peptidoglycan[b]	L-Lys–L-Lys–D-Glu	L-Lys–L-Lys–D-Glu	L-Lys–L-Lys–D-Glu	nd	nd	L-Lys–L-Ala–L-Lys–D-Glu	nd	nd	L-Lys–L-Ala–L-Lys–D-Glu
Cell-wall sugars[c]	Rhamnose + glucose[d]	Rhamnose + glucose	Rhamnose + glucose	Rhamnose + glucose	Rhamnose + glucose	Rhamnose + glucose	Rhamnose + glucose	Rhamnose + glucose	Rhamnose + glucose[d]
Acyl[c] type	Acetyl	Acetyl	Acetyl	Acetyl	Acetyl	Acetyl	Acetyl	Acetyl	Acetyl
Menaquinones[c]	MK-8(H_4) + MK-9(H_4)	MK-8(H_4) + MK-9(H_4)	MK-8(H_4) + MK-9(H_4)	Not determined	MK-9(H_4) + MK-10(H_4)	MK-9(H_4) + MK-10(H_4)	MK-9(H_4) + MK-10(H_4)	MK-9(H_4) + MK-10(H_4)	MK-9(H_4) + MK-10(H_4)
Phospholipids[c]	DPG, PG, PI	DPG, PG, PI	Not determined	Not determined	DPG, PG, PI, PGL	DPG, PG, PI, PGL	DPG, PG, PI, PGL	DPG, PG, PI, PGL	DPG, PG, PI, PGL
Fatty acids[c]	S, U, I, A	S, U, I, A	S, U, I, A	S, U, I, A	S, U, I, A	S, U, I, A	S, U, I, A	S, U, I, A	S, U, I, A

[a]DPG, diphosphatidylglycerol; PG, phosphatidylglycerol; PGL, unknown phosphoglycolipid; PI, phosphatidylinositol. S, straight-chain, saturated; U, monounsaturated; A, *anteiso*-methyl-branched; I, *iso*-methyl-branched. L-Lys, L-lysine; L-Ala, L-alanine; D-Glu, D-glutamate; nd, not determined.

[b]Data for *Arcanobacterium haemolyticum* from Lawson et al. (1997); data for *Arcanobacterium phocae* from Schaal et al. (2006); data for *Arcanobacterium pluranimalium* from Lawson et al. (2001a); data for *Arcanobacterium bernardiae* from Funke et al. (1995); data for *Arcanobacterium pyogenes* from Pascual Ramos et al. (1997b).

[c]A.F. Yassin (unpublished).

[d]Data from Cummins and Harris (1956).

Menaquinone composition. The principal respiratory quinones among the *Arcanobacterium* species are menaquinones with eight, nine, ten and eleven isoprene units. Two different menaquinone patterns could be distinguished within the *Arcanobacterium* species (Table 13). *Arcanobacterium bernardiae* possesses a tetrahydrogenated menaquinone with ten isoprene units [MK-10(H_4)] as the major component and small amounts of tetrahydrogenated menaquinones with nine [MK-9(H_4)] and eleven [MK-11(H_4)] isoprene units. A similar pattern was also found in *Arcanobacterium abortisuis*, *Arcanobacterium bialowiezense*, *Arcanobacterium bonasi* and *Arcanobacterium pyogenes*. In contrast, tetrahydrogenated menaquinones with nine isoprene units [MK-9(H_4)] appear to be the major components present in *Arcanobacterium haemolyticum*, *Arcanobacterium phocae*, and *Arcanobacterium pluranimalium* with trace amounts of MK-8(H_4). The menaquinone structure of *Arcanobacterium hippocoleae* has not been reported.

Polar lipids. The reports on the distribution of polar lipids among the *Arcanobacterium* species are controversial. Schaal et al. (2006) reported a type PIII phospholipid pattern (Lechevalier et al., 1977) with phosphatidylcholine as key diagnostic phospholipid in *Arcanobacterium bernardiae*, *Arcanobacterium haemolyticum*, *Arcanobacterium phocae*, and *Arcanobacterium pyogenes*. Lehnen et al. (2006) reported the presence of diphosphatidylglycerol, phosphatidylglycerol, three unknown phosphoglycolipids (PGL), an unknown aminolipid (AL2), two unknown phospholipids and an unknown aminophospholipid as predominant compounds in *Arcanobacterium bonasi* and *Arcanobacterium bialowiezense*. The two species could be distinguished by the presence of hydrophilic lipids in *Arcanobacterium bialowiezense*; an unknown aminoglycolipid and an unknown aminophosphoglycolipid. Similar, but not identical, profiles were detected for *Arcanobacterium pyogenes* DSM 20630[T] and *Arcanobacterium haemolyticum* DSM 20595[T] (Lehnen et al., 2006). On the other hand, Azuma et al. (2009) reported that the phospholipid pattern of *Arcanobacterium abortisuis* belongs to phospholipid type PII (Lechevalier et al., 1977). This pattern contained basically phosphatidylethanolamine as characteristic phospholipid, in addition to two unknown phosphatidylinositol mannosides.

Recent investigations (A. F. Yassin, unpublished) of the phospholipid pattern from *Arcanobacterium abortisuis*, *Arcanobacterium bernardiae*, *Arcanobacterium haemolyticum*, *Arcanobacterium phocae*, and *Arcanobacterium pyogenes* indicated that the phospholipids composition of these species were basically similar to the phospholipid pattern detected in *Arcanobacterium bialowiezense* and *Arcanobacterium bonasi* as reported by Lehnen et al. (2006). These five species contained diphosphatidylglycerol (DPG), phosphatidylglycerol (PG) and phosphatidylinositol (PI), in addition to a number of unknown phospholipids. In contrast to *Arcanobacterium haemolyticum* and *Arcanobacterium phocae*, a phosphoglycolipid with unknown composition was detected in *Arcanobacterium abortisuis*, *Arcanobacterium bernardiae*, and *Arcanobacterium pyogenes*. Similar phosphoglycolipid was detected in *Arcanobacterium bialowiezense* and *Arcanobacterium bonasi* (Lehnen et al., 2006). No nitrogen-containing phospholipids, i.e. phosphatidyl ethanolamine or phosphatidyl choline, were detected in the phospholipid extracts of these species. Thus, the phospholipid patterns of *Arcanobacterium abortisuis, Arcanobacterium bernardiae, Arcanobacterium haemolyticum*, *Arcanobacterium phocae*, and *Arcanobacterium pyogenes* belonged to phospholipid type PI according to Lechevalier et al. (1977). Apart from the unkown aminolipids, aminophospholipids and aminophosphoglycolipids detected in *Arcanobacterium bialowiezense* and *Arcanobacterium bonasi* (as determined by Lehnen et al., 2006), the phospholipid pattern of the latter two species should belong to phospholipid type PI.

Fatty acid composition. Earlier reports (Collins et al., 1982a; Lechevalier, 1977) and recent investigations (A.F. Yassin, unpublished) on cellular fatty acid of members of the genus *Arcanobacterium* showed that the fatty acid profiles composed of predominantly straight-chain saturated and monounsaturated acids. The major monounsaturated fatty acid was of the oleic acid series ($C_{18:1}$ ω9*c*). Small amounts of *cis*-vaccenic acid ($C_{18:1}$ ω7*c*) and *trans*-vaccenic acid ($C_{18:1}$ ω7*t*) were variably distributed in members of the genus *Arcanobacterium*. Furthermore, substantial amounts of octadecadienoate ($C_{18:2}$ ω6,9*c*) was regularly distributed in all the type strains of the validly published *Arcanobacterium* species. Moreover, trace amounts of methyl-branched-chain fatty acids (anteiso-, iso-) were detected in all *Arcanobacterium*. The reports on the distribution of cyclopropanoic acids among the *Arcanobacterium* species are controversial. Collins et al. (1982b) reported the presence of *cis*-9,10-methylenehexadecanoic acid (17Δ7*c*) and lactobacillic acid (*cis*-11,12 methylene-octadecanoic acid; 19Δ7*c*) in one strain of *Arcanobacterium haemolyticum* (strain NCTC 9697). However, Lehnen et al. (2006) and Azuma et al. (2009) did not report the presence of 17Δ7*c* or 19Δ7*c* in the cellular fatty acid profiles of the type strains of *Arcanobacterium abortisuis*, *Arcanobacterium bialowiezense*, *Arcanobacterium bonasi*, *Arcanobacterium haemolyticum* and *Arcanobacterium pyogenes*. The presence of cyclopropanoic acids in members of the genus *Arcanobacterium* as well as the presence of the tetraenoic acid ($C_{20:4}$ ω6,9,12,15*c*) reported in *Arcanobacterium bialowiezense*, *Arcanobacterium bonasi* and *Arcanobacterium haemolyticum* by Lehnen et al. (2006) are not verified by gas-chromatography-mass-spectrometric analysis (GC-MS) of the fatty acid methyl esters of the type strains of validly published *Arcanobacterium* species (A.F. Yassin, unpublished).

Peptidoglycan composition. The mode of cross-linkage and the amino acid composition of the tetrapeptide bridge of the peptidoglycan layer of several *Arcanobacterium* species have been published previously (Collins et al., 1982a; Lawson et al., 2001a; Pascual Ramos et al., 1997b; Schaal et al., 2006). This corresponds to peptidoglycan type A5 according to the nomenclature of Schleifer and Seidl (1985). Thus, *Arcanobacterium haemolyticum*, *Arcanobacterium phocae* and *Arcanobacterium pluranimalium* exhibit subtype A5α (L-Lys–L-Lys–D-Glu), while *Arcanobacterium bernardiae* and *Arcanobacterium pyogenes* possess subtype A5α (L-Lys–L-Ala–L-Lys–D-Glu). The cell-wall peptidoglycan of *Arcanobacterium abortisuis* contains glutamic acid, alanine and lysine in the molar ratio of 1:2:1 (Azuma et al., 2009). Although almost one half of the validly published species of the genus *Arcanobacterium* have not yet been analyzed with respect to peptidoglycan composition, the distribution of peptidoglycan types matches by and large the phylogenetic clustering of members of the genus *Arcanobacterium*. The muramic acid residue of the glycan moiety of the cell-wall peptidoglycan, as far as examined for members of the genus *Arcanobacterium*, is *N*-acetylated. Analysis of whole-cell hydrolysates from *Arcanobacterium*

species reveals the regular presence of rhamnose and glucose as diagnostic cell-wall sugar.

Metabolism and metabolic pathways. Arcanobacteria are facultatively anaerobic having a fermentative type of carbohydrate metabolism. Fermentation end products are acetic and lactic acids; the amount of succinic acid produced may vary from species to species and may even be difficult to detect.

The catalase production is negative in *Arcanobacterium abortisuis*, *Arcanobacterium bernardiae*, *Arcanobacterium bialowiezense*, *Arcanobacterium bonasi*, *Arcanobacterium hippocoleae* and *Arcanobacterium pyogenes*; it is positive in *Arcanobacterium pluranimalium* and variable in *Arcanobacterium phocae*. Catalase activity in *Arcanobacterium haemolyticum* is usually negative, but some strains may show weak catalase production.

Acid production from various carbohydrates, enzyme activities of *Arcanobacterium* species, and several other physiologic properties are summarized in Table 14. Nearly all members of the genus ferment glucose and maltose, and none produce urease or hydrolyze esculin. All of the other physiological characters vary among the *Arcanobacterium* species and may therefore be used for differentiating or identifying them (Table 14).

Pathogenicity. Arcanobacteria are known as opportunistic pathogens of human and a number of important livestock species, and usually infect from an endogenous, commensal source (Baumann et al., 2001; Dieleman et al., 1989; Jonsson et al., 1991; Jost and Billington, 2005; Lechtenberg et al., 1988; Therriault et al., 2008; Volante et al., 2008). Infections can be transmitted from animal to animal, from animals to humans, or even between humans. However, members of different species vary considerably with regard to virulence, types of pathological lesions induced and host specificity.

Human infections. Man is the main reservoir of *Arcanobacterium haemolyticum*. The clinical symptoms of *Arcanobacterium haemolyticum* infections are similar, this organism being predominantly isolated from pharyngeal infections, skin lesions and septicemia (Carlson et al., 1994b; Collins and Cummins, 1986a; Dethy et al., 1986; Gaston and Zurowski, 1996; Jurankova and Votava, 2001; Moreno Montesinos et al., 1989; White and Foshee, 2000) with and without metastatic abscess formation (Dieleman et al., 1989). Primary deep-seated abscesses (Barnham and Bradwell, 1992), cellulitis including a fulminant tubo-ovarian soft tissue infection (in coculture with fusobacteria; Batisse-Milton et al., 1995) and an orbital cellulitis (Ford et al., 1995), surgical wound infections, an infected foot wound, or endocarditis due to this organism have also been reported (Alos et al., 1995; Dobinsky et al., 1999; Esteban et al., 1994; Ritter et al., 1993; Skov et al., 1998). *Arcanobacterium haemolyticum* was identified in blood culture of a woman with developed symptoms of pharyngitis with necrotising pneumonia (van der Eerden et al., 2006) and from multiple peritonsillar abscesses in a young male with a four-week history of sore throat (Bomke et al., 2009). A case of severe sepsis caused by *Arcanobacterium haemolyticum* in male with a medical history significant for mild asthma has been reported by Therriault et al. (2008). However, on the whole this organism has been rarely documented in systemic infections (Dobinsky et al., 1999; Minarik et al., 1997; Parija et al., 2005) and even less in brain abscesses (Chhang et al., 1991; Vargas et al., 2006).

Human infections with *Arcanobacterium pyogenes* are uncommon and the microorganism has hitherto primarily been considered a zoonotic pathogen (Smith, 1966; Sørensen, 1974). Human infections are usually presented as acute pharyngitis, urethritis or as cutaneous or subcutaneous suppurative processes (Barksdale et al., 1957; Collins and Jones, 1982; Gahrn-Hansen and Frederiksen, 1992; Reddy et al., 1982), but bacteremia has also been reported (Barnham, 1988). Additional *Arcanobacterium pyogenes* infections include septic arthritis in a diabetic farmer (Lynch et al., 1998) and endemic leg ulcers in Thailand (Kotrajaras and Tagami, 1987).

Arcanobacterium bernardiae has been isolated from various clinical sources including blood, abscesses, and eye infections (Funke et al., 1995). More recently, urinary tract infections, septic arthritis and an unusual case of prosthetic joint infection due to this organism were also described (Adderson et al., 1998; Lepargneur et al., 1998; Loïez et al., 2009). The remaining *Arcanobacterium* species appear to occur only in animals.

Animal infections. *Arcanobacterium pyogenes* is a ubiquitous inhabitant of the mucous membranes of cattle, swine, and many other animal species (Jost and Billington, 2005). As an opportunistic pathogen, *Arcanobacterium pyogenes* can cause a variety of suppurative diseases in animals compromised by previous microbial infection or trauma. Economically significant disease includes mastitis in cows, often associated with *Peptoniphilus* (*Peptostreptococcus*) *indolicus*, and peritonitis and pleuritis in swine (Glage, 1903; Reddy et al., 1982; Roberts, 1968). *Arcanobacterium pyogenes* was also found to be etiologically involved in pneumonia in calves (Vogel et al., 2001), hematogenous osteomyelitis in cattle (Firth et al., 1987), purulent osteomyelitis in fattening pigs (Bürgi et al., 2001), a pyothorax in a one-month-old female kitten (Gulbahar and Gurturk, 2002), chronic otitis externa in a cat and urinary tract infection in a dog (Billington et al., 2002), hypertrophic osteopathy in a dog, the retention of fetal membranes in ewes (Tzora et al., 2002), facial and mandibular abscesses in blue duiker, often associated with *Fusobacterium necrophorum* (Roeder et al., 1989), intracranial abscesses in white-tailed deer (Baumann et al., 2001) and a steer (Strain et al., 1987), and in lymphadenitis in camels (Moustafa, 1994). The development of renal medullary amyloidosis in Dorcas gazelles also appeared to be related to chronic or recurring *Arcanobacterium pyogenes* infections (Rideout et al., 1989).

Experimental mastitis induced by intramammary challenge with *Arcanobacterium pyogenes* was found to be more severe in dry than in lactating glands and was very difficult to eliminate in the dry period even by antibiotic treatment, whereas lactating glands recovered more easily either spontaneously or after administration of antibiotics. Combined infections of *Arcanobacterium pyogenes* with *Peptoniphilus indolicus* took an especially severe clinical course and showed a higher frequency of systemic involvement (Hillerton and Bramley, 1989).

Arcanobacterium haemolyticum has been known as an occasional cause of infections in farm animals (Collins and Jones, 1982). Recently, the organism was identified as one of the pathogens responsible for mandibular and maxillary abscesses in pet rabbits (Tyrrell et al., 2002).

Of the remaining *Arcanobacterium* species, *Arcanobacterium bernardiae* has as yet only been isolated from human sources. *Arcanobacterium phocae* has been recovered in mixed culture from various tissues and fluids of common seals (*Phoca vitu-*

TABLE 14. Differential characteristics of *Arcanobacterium* species[a,b,c]

Characteristic	*A. abortisuis*	*A. bernardiae*	*A. bialowiezense*	*A. bonasi*	*A. haemolyticum*	*A. hippocoleae*	*A. phocae*	*A. pluranimalium*	*A. pyogenes*
Aerobic growth (without CO_2)	+	–	+	+	–	–	–	–	–
Acid production from:									
Cellobiose	+	–	nd	nd	–	nd	–	nd	–
D-Glucose	+	+	+	+	+	+	+	+	+
Glycerol	+	+	–	–	+	–	+	–	–
Glycogen	+	+	–	–	–	–	+	–	+
D-Lactose	w	–	–	–	+	+	+	–	+
D-Maltose	+	+	–	–	+	w	+	d	+
D-Mannitol	+	–	–	–	–	–	d	–	d
Mannose	+	–	–	–	+	nd	d	–	+
D-Ribose	+	+	–	–	+	–	+	+	+
Sucrose	–	–	–	–	d	–	+	–	d
Trehalose	–	–	–	–	–	–	d	–	+
D-Xylose	–	–	–	–	–	–	–	–	+
Hydrolysis of:									
Gelatin	–	–	–	–	–	–	–	+	+
Hippurate	+	–	+	+	–	+	–	+	+
Enzyme activities:									
Catalase	–	–	–	–	d	–	d	+	d
Acid phosphatase	–	–	+	+	+	–	+	+	–
Alkaline phosphatase	–	–	–	–	–	+	+	–	–
α-Galactosidase	+	–	–	–	–	–	+	–	–
β-Galactosidase	+	–	–	–	+	+	+	–	–
α-Glucosidase	+	+	+	–	–	+	+	–	+
β-Glucuronidase	+	–	+	+	–	+	–	+	+
N-Acetyl-β-glucosaminidase	–	+	–	–	+	+	–	–	–
Leucine arylamidase	+	+	+	+	+	+	+	+	+
Esterase Lipase C8	–	–	+	+	+	–	+	–	–
Pyrazinamidase	–	+	+	+	+	–	+	–	–
Pyrrolidonyl arylamidase	–	–	+	+	–	–	–	+	–
Acetoin production	–	–	–	–	–	–	–	–	–

[a]Symbols: +, positive/present; –, negative/absent; w, weakly positive; nd, not determined; d, differences between strains within one species.

[b]Data compiled from Collins et al. (1982b); Funke et al. (1995); Pascual Ramos et al. (1997b); Lawson et al. (2001a); Hoyles et al. (2002b); Lehnen et al. (2006); Azuma et al. (2009); A. F. Yassin (unpublished).

[c]All strains are negative for acid production from L-arabinose, raffinose, L-rhamnose, salicin, D-sorbitol and sucrose as well as arginine dihydrolase, β-glucosidase, nitrate reductase and urease activity.

lina) and gray seals (*Halichoerus grypus*), but its pathological significance was unclear (Pascual Ramos et al., 1997b). Johnson et al. (2003) reported on the isolation of *Arcanobacterium phocae* in animals from the Pacific Ocean. The overall prevalence of *Arcanobacterium phocae* among cultured stranded marine mammals was 8%. *Arcanobacterium hippocoleae* was cultured from a female Arab horse with vaginal discharge (Hoyles et al., 2002b) and isolated from a case of necrosuppurative placentitis and stillbirth in an American Quarterhorse mare (Bemis et al., 2008). *Arcanobacterium pluranimalium* has been isolated from the spleen of a dead harbor porpoise and a lung abscess of a dead sallow deer (Lawson et al., 2001a), whereas *Arcanobacterium abortisuis* has been isolated from a sow's placenta after an abortion (Azuma et al., 2009). *Arcanobacterium bialowiezense* and *Arcanobacterium bonasi* were isolated from the prepuce of European bison (*Bison bonasus*) bulls suffering from balanoposthitis (Lehnen et al., 2006).

Antigenic structure. *Arcanobacterium haemolyticum* infection induces an antibody response in the host. This was demonstrated in sera of patients with acute infection or from convalescents (Nyman et al., 1997). These antibodies react primarily with four distinct cell-wall-associated proteins with estimated molecular masses of 80, 60, 50, and 30 kDa.

Arcanobacterium haemolyticum has been reported to produce phospholipase D (PLD), an extracellular toxin with antigenic properties (Cuevas and Songer, 1993; McNamara et al., 1995; Skalka et al., 1998; Votava et al., 2001). Neutralizing antibodies against PLD of *Arcanobacterium haemolyticum* have been detected in patients with tonsillitis (Votava et al., 2001). Skalka et al. (1998) used PLD neutralization to examine sera of humans with a spontaneous infection by *Arcanobacterium haemolyticum*. They found that the titres of sera neutralizing the homologous PLD were always significantly higher than those neutralizing the heterologous PLD. The antigenic properties of PLD from *Arcanobacterium haemolyticum* (PL D-A) and from *Corynebacterium pseudotuberculosis* (PL D-C) were similar but not identical (Skalka et al., 1998).

Arcanobacterium pyogenes secret pyolysin (PLO), a cholesterol-dependent cytolysin, an important host-protective antigen (Billington et al., 1997). However, this molecule is toxic and its usefulness as a vaccine without prior inactivation is limited. Three genetically toxoided, nonhemolytic PLO molecules, HIS-PLO.F_{497}, HIS-PLO.P_{499}, and HIS-PLO.A_{522}, were found to be nontoxic, and vaccinated mice were protected from infection, indicating the potential of these toxoids as vaccines (Jost et al., 2003).

Antigens of the other *Arcanobacterium* species have not been studied.

Virulence factors. *Arcanobacterium pyogenes* expresses several known virulence factors that may contribute to its pathogenicity. These include pyolysin, which is a hemolysin with cytolytic activity for immune cells; two neuraminidases (NanH and NanP) required for adhesion to epithelial cells (Billington et al., 1997; Jost et al., 1999, 2001, 2002); a collagen-binding protein (CbpA) required for adhesion to collagen rich tissues (Pietrocola et al., 2007); and fimbriae (similar to type 2 fimbriae of *Actinomyces naeslundii*) that might be involved in host adhesion (Jost and Billington, 2005).

Arcanobacterium haemolyticum produces phospholipase D as one of its soluble toxins (Cuevas and Songer, 1993). This toxin is a protein of approximately 31.5 kDa and is related to, but not identical with, the phospholipases D of *Corynebacterium pseudotuberculosis* and *Corynebacterium ulcerans*. Virulence factors of the other *Arcanobacterium* species have not been studied.

Susceptibility to antibiotics. *Arcanobacterium haemolyticum* is susceptible to most classes of antimicrobial drugs, including carbapenems, cephalosporins, clindamycin, macrolides, penicillins, tetracyclines, and vancomycin (Almuzara et al., 2002; Arikan et al., 1997; Carlson et al., 1994a, 1999). General resistance has been reported against trimethoprim/sulfamethoxazole (Therriault et al., 2008). While treatment options are numerous, Therriault et al. (2008) recommend the use of intravenous penicillin or a cephalosporin as first-line pharmacologic management of deep-seated infections caused by this organism, whereas Van der Eerden et al. (2006) found that a macrolide antibiotic was the treatment of choice for a case of pharyngitis caused by *Arcanobacterium haemolyticum*.

Although *Arcanobacterium haemolyticum* is susceptible (thus far, universally) to penicillin by *in vitro* MIC testing, treatment failures have been documented (Bomke et al., 2009; Nyman et al., 1990; Osterlund, 1995; Volante et al., 2008). Clinical failures that have been reported could be associated with penicillin tolerance (Carlson et al., 1999). Isolates resistant to ciprofloxacin (Vargas et al., 2006), vancomycin (French et al., 1992), macrolides and quinolones (Carlson et al., 1999) have also been reported.

Arcanobacterium pyogenes is susceptible to third generation cephalosporins (Sheldon et al., 2004). Penicillin was reported to be highly effective against *Arcanobacterium pyogenes* (Ali, 2000; Guerin-Faublee et al., 1993). On the other hand, antimicrobial susceptibility of *Arcanobacterium pyogenes* strains isolated from cattle showed that some isolates were resistant to tetracyclines, macrolides, and sulfonamides (Trinh et al., 2002; Werckenthin et al., 2007). All 18 isolates of *Arcanobacterium phocae* recovered from marine mammals were 100% susceptible to amikacin, ampicillin, amoxicillin/clavulanate, cefazolin, ceftiofur, ceftizoxime, chloramphenicol, enrofloxacin, erythromycin, gentamicin, oxacillin, penicillin, rifampin, tetracycline, ticarcillin/clavulanate, trimethoprim-sulphamethoxazole (Johnson et al., 2003). This suggests that beta-lactam antibiotics should be useful in treating *Arcanobacterium phocae* infections in marine mammals. Detailed studies on the antibiotic susceptibility of the remaining *Arcanobacterium* species have not been reported.

Habitat and ecology. Information on the natural habitat of arcanobacteria is scarce, although it has been assumed that these organisms occur as commensals of the mucous membranes of domestic animals such as cattle, sheep, swine, and goats (Billington et al., 2002; Carter and Chengappa, 1991; Reddy et al., 1982). In a study on the Gram-positive tonsillar and nasal flora of piglets, Baele et al. (2001) found that *Arcanobacterium pyogenes* was one of the common colonizers of the tonsils of pigs before and after weaning but not of the nasal conchae. In addition, this organism has been isolated from semen of beef breed bulls (Sprecher et al., 1999) without a significant relationship between the cultural results and possible sperm abnormalities. This is in accordance with the findings of Olson et al. (2002). *Arcanobacterium pyogenes*, as certain *Actinomyces* species, is able to produce biofilms experimentally.

Another interesting finding is that *Arcanobacterium haemolyticum* could be identified as one of the major secondary colonizers of leprosy skin ulcers (Sturm et al., 1996) and may contribute to the inflammatory reaction of these lesions. The vast majority of *Arcanobacterium* isolates originated, however, from various pathological conditions that were thought to be etiologically related to these microbes and that has been discussed in the section on Pathogenicity.

Isolation, enrichment and maintenance procedures

For primary isolation and maintenance, complex media such as Columbia blood agar supplemented with 5% horse, sheep or human blood, BHI broth (or agar) or trypticase soy broth (or agar) have been recommended and are usually satisfactory. Good growth is obtained for *Arcanobacterium abortisuis*, *Arcanobacterium bernardiae*, *Arcanobacterium bialowiezense*, and *Arcanobacterium bonasi* in BHI broth incubated at 37°C under aerobic conditions. Best growth of *Arcanobacterium bialowiezense* occurred on BHI agar supplemented with 1% Tween 80 at 37°C. *Arcanobacterium haemolyticum* and *Arcanobacterium hippocoleae* are more difficult to grow in liquid media. However, addition of serum may enhance growth of *Arcanobacterium haemolyticum* considerably. Thus, a suitable liquid medium for *Arcanobacterium haemolyticum* consists of brain heart infusion broth supplemented with 5% horse serum (Collins and Jones, 1982). All of the other *Arcanobacterium* species apparently grow well on Columbia blood agar supplemented with 5% horse or sheep blood and incubated under increased CO_2 tension (~5%) at 36±1°C.

Recognition of *Arcanobacterium haemolyticum* in cultures from clinical specimens is often hampered by its delayed β-hemolysis and the presence of additional pathogens (e.g., streptococci) or microbes from the indigenous flora of the affected mucous membranes. Thus, media for selective isolation or improved recognition of this organism were developed: Coman et al. (1996) used a medium containing 5% sheep blood agar and 3.5% NaCl for selective isolation of *Arcanobacterium haemolyticum* from pharyngeal swabs of children. Jurankova and Votava (2001) applied sheep blood agar with a streak of *Staphylococcus aureus* to recognize *Arcanobacterium haemolyticum* on the basis of the reverse CAMP (Christie, Atkins and Munch-Petersen) phenomenon. The medium of Votava et al. (2000) containing Columbia blood agar base and 5% washed sheep erythrocytes sensitized with equi factor (EF) of *Rhodococcus equi* is based upon the same principle.

Cultures in routine use may be maintained by weekly transfer on Columbia blood agar supplemented with 5% sheep blood and incubated under increased CO_2 tension (~5%) at 36±1°C. For longer preservation (1–10 years or longer) freezing or lyophilization is recommended.

Differentiation of the genus *Arcanobacterium* from other genera

Arcanobacterium species resemble a variety of other Gram-stain-positive, diphtheroidal or coccobacillary genera morphologically and/or physiologically. However, comparative chemotaxonomic studies of cell-wall components allowed well differentiation between members of the arcanobacteria from *Actinomyces bovis* (as representative of the genus *Actinomyces sensu stricto), Actinobaculum*, and *Mobiluncus* (Table 15). Thus, the salient chemotaxonomic features of the type strains of *Arcanobacterium haemolyticum*, the type species of the genus, include cell-wall chemotype V (Lechevalier and Lechevalier, 1970) with the peptidoglycan subtype A5α (L-Lys–L-Lys–D-Glu), rhamnose as diagnostic whole-cell sugar; tetrahydrogenated menaquinones with eight [MK-8(H_4)] and nine [MK-9(H_4)] isoprene units, the predominant menaquinone being MK-9(H_4); the long-chain fatty acids are straight-chain saturated, monounsaturated as well as branched chain fatty acids of the iso- and anteiso-types; a muramic acid residue of the peptidoglycan that is N-acetylated, and DNA G+C contents between 50 and 58 mol%.

TABLE 15. Chemotaxonomic characteristics differentiating members the genus *Arcanobacterium* from other genera of the family *Actinomycetaceae*[a]

	Arcanobacterium haemolyticum	*Arcanobacterium pyogenes*	*Actinomyces bovis*	*Actinobaculum schaalii*	*Mobiluncus curtisii* subsp. *holmesii*
Peptidoglycan[b]	L-Lys–L-Lys–D-Glu	L-Lys–L-Ala–L-Lys–D-Glu	L-Lys–D-Asp	L-Lys–L-Lys–D-Glu	nd
Cell-wall sugars[c]	Rhamnose + glucose	Rhamnose + glucose	Glucose + mannose + rhamnose + 6-deoxytalose	Glucose + rhamnose + 6-deoxytalose	Galactose + rhamnose
Menaquinones[c]	MK-8(H_4) + MK-9(H_4)*	MK-9(H_4) + MK-10(H_4)*	MK-8 + MK-9* + MK-10	MK-7*	MK-8 + MK-9*
Phospholipids[c]	DPG, PG, PI	DPG, PG, PI, PGL	DPG, PG, PC, PI, PIM	DPG, PG, PGL	DPG, PG, PGL
Fatty acids[c]	S, U, I, A	S, U, I, A	S, U	S, U	S, U, I, A
DNA G+C content (mol%)	50–52	56–58	57–63	55–57	49–52

[a]Symbols/abbreviations: *Major component; DPG, diphosphatidylglycerol; PG, phosphatidylglycerol; PC, phosphatidylcholine; PI, phosphatidylinositol; PIM, phosphatidylinositol mannosides; PGL, unkown phosphoglycolipid. S, straight-chain, saturated; U, monounsaturated; A, anteiso-methyl-branched; I, iso-methyl-branched. L-Ala, L-alanine; D-Asp, D-aspartic acid; D-Glu, D-glutamic acid ; L-Lys, L-lysine; nd, not determined.

[b]Data for *Arcanobacterium haemolyticum* from Schaal et al. (2006); data for *Arcanobacterium pyogenes* from Pascual Ramos et al. (1997b); data for *Actinomyces bovis* from Schleifer and Kandler (1972); data for *Actinobaculum schaalii* from Lawson et al. (1997).

[c]A.F. Yassin (unpublished).

Taxonomic comments

The genus *Arcanobacterium* was described by Collins et al. (1982b) to accommodate bacterial strains originally isolated from infected American soldiers and previously named "*Corynebacterium haemolyticum*" (MacLean et al., 1946). However, the species exhibits little similarity to typical corynebacteria and its placement in the genus *Corynebacterium* was questioned by several workers (Barksdale, 1970; Barksdale et al., 1957; Collins et al., 1982a; Cummins and Harris, 1956; Jones, 1975; Minnikin et al., 1978; Schofield and Schaal, 1981). Furthermore, the relationship of "*Corynebacterium haemolyticum*" to the species "*Corynebacterium pyogenes*" (Glage) remained unclear. In a study of cell-wall compositions in some Gram-stain-positive bacteria, Cummins and Harris (1956) noted that the cell-wall compositions of "*Corynebacterium pyogenes*" and "*Corynebacterium haemolyticum*" were obviously similar to one another but differed both in sugar and amino acid composition from the other corynebacteria (i.e., neither species contains arabinose or galactose, lysine is the diamino acid of the peptidoglycan, and A_2pm is absent). On the other hand, Cummins and Harris (1956) found that rhamnose was present in both organisms, and this, together with the fact that alanine, glutamic acid and lysine were the major amino acid components, led these authors to suggest that the two organisms were related to the streptococci. This view was upheld by Barksdale et al. (1957) who suggested not only that "*Corynebacterium haemolyticum*" and "*Corynebacterium pyogenes*" should be reclassified in the genus *Streptococcus*, but also that "*Corynebacterium haemolyticum*" was a mutant form of "*Corynebacterium pyogenes.*"

In the 8th edition of *Bergey's Manual of Determinative Bacteriology*, both taxa were listed in an addendum to the genus *Corynebacterium* (Cummins et al., 1974), and "*Corynebacterium haemolyticum*" does not appear in the Approved Lists of Bacterial Names (Skerman et al., 1980). Later, numerical phenetic (Schofield and Schaal, 1981) and chemical (Collins et al., 1982a) studies showed that "*Corynebacterium haemolyticum*" and "*Corynebacterium pyogenes*" are two distinct taxa. The discovery of tetrahydrogenated menaquinones in "*Corynebacterium pyogenes*" and "*Corynebacterium haemolyticum*" (Collins et al., 1982a) was not in accord with the inclusion of these taxa in the genus *Streptococcus*. The majority of streptococci completely lack respiratory quinones, although some unsaturated naphthoquinones have been detected in a few group D and group N streptococci (Collins and Jones, 1979b; Collins and Jones, 1979a). In addition, fatty acid data (Collins et al., 1982a) did not support the view of Barksdale et al. (1957) that "*Corynebacterium haemolyticum*" should be reclassified in the genus *Streptococcus*. "*Corynebacterium haemolyticum*" possesses major amounts of monounsaturated fatty acids of the oleic acid series (synthesized via an aerobic pathway; Collins et al., 1982a). In contrast, members of the genus *Streptococcus* possess monounsaturated fatty acids of the *cis*-vaccenic acid series (synthesized via an anaerobic pathway; Kroppenstedt and Kutzner, 1978). Furthermore, the menaquinone patterns of "*Corynebacterium pyogenes*" and "*Corynebacterium haemolyticum*" were also incompatible with the retention of these species in the genus *Corynebacterium*. True corynebacteria generally possess dihydrogenated menaquinones with eight and nine isoprene units (Collins et al., 1977; Yamada et al., 1976). Tetrahydrogenated menaquinones with ten and nine isoprene units have, however, been reported in the genera *Actinomyces* (Collins et al., 1977) and *Propionibacterium* (Schwartz, 1973; Sone, 1974), respectively. Therefore, Reddy et al. (1982) as well as Collins and Jones (1982) proposed that "*Corynebacterium pyogenes*" should be reclassified in the genus *Actinomyces*, as "*Actinomyces pyogenes*". The presence of a peptidoglycan based upon L-lysine in "*Corynebacterium pyogenes*" supported this view since this amino acid is present in the peptidoglycan of *Arcanobacterium bovis* (Schleifer and Kandler, 1972). "*Corynebacterium pyogenes*" is physiologically similar to *Arcanobacterium bovis*, although it differs from *Arcanobacterium bovis* in being actively proteolytic (Cummins et al., 1974; Slack and Gerencser, 1975). However, the DNA base ratio of "*Corynebacterium pyogenes*" (58 mol% G+C) was not incompatible with its inclusion in the genus of *Arcanobacterium bovis*, which has 63 mol% G+C (Johnson and Cummins, 1972).

The taxonomic position of "*Corynebacterium haemolyticum*" remained equivocal. The results of lipid analyses did not support the view of Barksdale et al. (1957) that "*Corynebacterium haemolyticum*" is a mutant of "*Corynebacterium pyogenes.*" Phenotypically, "*Corynebacterium haemolyticum*" is very similar to *Arcanobacterium bovis* and also contains lysine in the cell-wall peptidoglycan. The menaquinone composition of "*Corynebacterium haemolyticum*" is distinct from that of *Arcanobacterium bovis* (Collins et al., 1977), and resembles that of the propionibacteria (Schwartz, 1973; Sone, 1974), but the results of cell wall and fatty acid analyses do not support this latter relationship. On the basis of cell-wall and lipid composition, the taxon "*Corynebacterium haemolyticum*" appeared quite distinct from all other coryneform and actinomycete taxa, thus warranting a new genus (Collins et al., 1982a). Therefore, on the basis of phenetic, peptidoglycan, fatty acid, menaquinone and DNA data (Collins et al., 1982a; Schleifer and Kandler, 1972; Schofield and Schaal, 1981), this taxon was reclassified by Collins et al. (1982b) in the new genus *Arcanobacterium* as *Arcanobacterium haemolyticum*.

The genus *Arcanobacterium* currently comprises nine validly published species, namely *Aracanobacterium abortisuis*, *Arcanobacterium bernardiae*, *Arcanobacterium bialowiezense*, *Arcanobacterium bonasi*, *Arcanobacterium haemolyticum*, *Arcanobacterium hippocoleae*, *Arcanobacterium phocae*, *Arcanobacterium pluranimalium*, and *Arcanobacterium pyogenes*. Arriving at this current taxonomy has involved the inclusion of species transferred from other genera. For example, *Arcanobacterium pyogenes* was created by Pascual et al. (1997c) for the invalid "*Actinomyces pyogenes*" which was in turn created from the invalid "*Corynebacterium pyogenes*" by Reddy et al. (1982) and *Arcanobacterium bernardiae* was created from the invalid "*Actinomyces bernardiae*" (Pascual Ramos et al., 1997b). The chemotaxonomic features, as described previously (Collins et al., 1982b), include a cell-wall peptidoglycan based on lysine as the dibasic amino acid, rhamnose as the diagnostic cell-wall sugars, tetrahydrogenated menaquinone with nine isoprene units MK-9(H_4) as the major respiratory quinone, the long-chain fatty acids are straight-chain saturated and monounsaturated (oleic acid series) acids, lack of mycolic acids, and the DNA base composition was 50–52 mol% G+C.

In 1982, Collins and Jones found that tetrahydrogenated menaquinones with ten isoprene units MK-10(H_4) is the principal isoprenoid quinone of "*Actinomyces pyogenes*". This result is supported by recent analysis of menaquinone of this species (Lehnen et al., 2006). Furthermore, Lehnen et al. (2006) found that MK-10(H_4) is the principal isoprenoid quinone in *Arcanobacterium bialowiezense* and *Arcanobacterium bonasi*. This

incompatible menaquinone pattern with that of *Corynebacterium haemolyticum* [MK-9(H_4)], the type species of the genus, in addition to phylogeny points out toward a possible subdivision within the genus *Arcanobacterium* (Lehnen et al., 2006).

Similarity values of 16S rRNA gene sequence analyses for members of the genus range from 92.5–98.6%, indicating the presence of several phylogenetically defined clusters (Figure 19 in the *Actinomyces* chapter), which are separated by intra-cluster similarity values ranging from 95.4–97.4% to 95.3–98.6%. The idea that the genus *Arcanobacterium* may not represent a monophyletic taxon has been expressed before by Lehnen et al. (2006). Chemotaxonomic properties were demonstrated to correlate well with the phylogenetic structure in other actinobacterial genera. In general, the quinone system is a rather conserved characteristic. The finding of MK-10(H_4) in one lineage within the genus *Arcanobacterium*, represented by *Arcanobacterium abortisuis*, *Arcanobacterium bernardiae*, *Arcanobacterium bialowiezense*, *Arcanobacterium bonasi*, and *Arcanobacterium pyogenes*, is rather exciting because the representatives of the second lineage, *Arcanobacterium haemolyticum*, *Arcanobacterium phocae*, and *Arcanobacterium pluranimalium*, are characterized by having the MK-9(H_4) quinone system. Hence, both quinone systems and phylogeny indicate that a subdivision of the genus *Arcanobacterium* might be appropriate, which would result in the restriction of the genus *Arcanobacterium* to the species *Arcanobacterium haemolyticum*, *Arcanobacterium phocae*, and *Arcanobacterium pluranimalium*. This would require the reclassification of *Arcanobacterium abortisuis*, *Arcanobacterium bernardiae*, *Arcanobacterium bialowiezense*, *Arcanobacterium bonasi*, and *Arcanobacterium pyogenes* in a novel genus. In a reclassified genus *Arcanobacterium*, the presence of a MK-9(H_4) quinone system might be a genus-specific trait, while MK-10(H_4) might characterize the neighboring genus. The suitability of the quinone system as the basis for such differentiation is supported by cluster-specific nucleotides, which can be considered a molecular signature (Table 12).

From a phylogenetic point of view, *Arcanobacterium hippocoleae* appears to be a phylogenetic neighbor of subcluster 1, while this species branches adjacent to *Arcanobacterium pluranimalium* (Figure 19 in the *Actinomyces* chapter), shows 95.1–95.8% sequence similarities (in contrast to 94.0–95.0% sequence similarities to members of subcluster 2) and shares six signature nucleotides that are common to members of subcluster 1. Although the branching of *Arcanobacterium hippocoleae* with members of subcluster 1 is supported by 87% bootstrap, the level at which this clustering be considered a taxonomically coherent entity should be decided on the basis of results of chemotaxonomic investigations.

Further reading

Horner, K.L. and G.J. Raugi. 2007. *Arcanobacterium haemolyticum.* http://www.emedicine.com/derm/topic758.htm

Identification and descriptive characteristics of *Arcanobacterium* species

The phenotypic characteristics useful for identification of the recognized *Arcanobacterium* species are given in Table 14.

List of species of the genus *Arcanobacterium*

1. **Arcanobacterium haemolyticum** (MacLean, Liebow and Rosenberg 1946) Collins, Jones, and Schofield 1983, 438[VP] (*Corynebacterium haemolyticum* MacLean, Liebow and Rosenberg 1946, 69)

hae.mo.ly′ti.cum. Gr. neut. n. *haema* blood; N.L. adj. *lyticus -a -um* (from Gr. adj. *lutikos -ê -on*) able to loosen, able to dissolve; N.L. neut. adj. *haemolyticum*, blood-dissolving, hemolytic.

Surface colonies on blood agar are small (0.75 mm in diameter) after 24 h, becoming large (1.5–2.5 mm in diameter) on extended incubation. Colonies are circular discoid and slightly raised, and β-hemolytic. Growth is sparse on ordinary media but is enhanced by blood or serum. Slender, irregular rods predominate during the first 18 h on blood agar; many cells exhibit V-forms. Upon extended incubation, organisms become granular and segmented, and resemble small irregular cocci. On Loeffler's medium, they maintain the slender, irregular, bacillary form, but become pleomorphic at 48 h, with numerous club and comma forms. Facultatively anaerobic. Growth is considerably enhanced in an atmosphere of CO_2. The optimum temperature for growth is 37°C. The organism will not withstand heating at 60 °C for 15 min. Acid is produced from glucose, lactose and some other sugars. Catalase is negative. Extracellular DNase is produced. Gelatin, esculin, and casein are not hydrolysed. β-Galactosidase and *N*-acetyl-β-glucosaminidase are produced, but β-glucuronidase and α-fucosidase are not. The organism is resistant to oxytetracycline (30 pg per disc). The interpeptide bridge within the peptidoglycan is L-Lys–L-Lys–D-Glu (Type A5α). The principal menaquinone is MK-9(H_4).

The type strain was isolated from infections amongst American soldiers.

DNA G+C content (mol%): 50–52 (T_m).

Type strain: ATCC 9345, CIP 103370, DSM 20595, IFO (now NBRC) 15585, LMG 16163, NCTC 8452.

Sequence accession no. (16S rRNA gene): AJ234059.

2. **Arcanobacterium abortisuis** Azuma, Murakami, Ogawa, Okada, Miyazaki and Makino 2009, 1471[VP]

a.bor.ti.su′is. L. n. *abortus, abortus* an abortion; L. n. *sus, suis* a pig, a sow; N.L. gen. n. *abortisuis* from an abortion of a sow.

Cells are short and diphtheroid-shaped and are arranged in either V-forms or palisades. Colonies have a translucent smooth surface and are strictly anaerobic. Good growth also occur under aerobic conditions. Catalase- and indole-negative. Reduces nitrate. Soluble starch is hydrolyzed, but gelatin is not. The CAMP-test is negative. Produces acid from adonitol, cellobiose, fructose, galactose, glucose, glycerol, inulin, lactose, maltose, melezitose, raffinose, sucrose, soluble starch, and trehalose. Does not ferment amygdalin, arabinose, arbutin, dulcitol, erythritol, inositol, mannitol, mannose, melibiose, rhamnose, ribose, salicin, sorbitol, sorbose or xylose.

Produces α-galactosidase, β-galactosidase, α-glucosidase, and leucine arylamidase but not urease, β-glucosidase or pyroglutamic acid arylamidase. Fermentation end products are lactic, succinic, and acetic acids. Whole-cell sugars include rhamnose and ribose. Cell-wall peptidoglycan contains glutamic acid, alanine and lysine in the molar ratio of 1:2:1. Major fatty acids are $C_{14:0}$, $C_{16:1}$ ω7, $C_{16:0}$ and $C_{18:1}$ω9. The major menaquinone is MK-10(H_4). Polar lipids are phosphatidylethanolamine and two unknown phosphatidylinositol mannosides. The interpeptide bridge within the peptidoglycan has not been reported.

The type strain was isolated from a sow's placenta after an abortion.

DNA G+C content (mol%): 63.8 (HPLC).

Type strain: Murakami, ATCC BAA-1522, DSM 19515, JCM 14813.

Sequence accession no. (16S rRNA gene): AB305159.

3. **Arcanobacterium bernardiae** (Funke, Pascual Ramos, Fernández-Garayzábal, Weiss and Collins 1995), comb. nov. Pascual Ramos, Foster and Collins 1997b, 51[VP] (*Actinomyces bernardiae* Funke, Pascual Ramos, Fernández-Garayzábal, Weiss and Collins 1995, 59)

ber.nar.di′a.e. N.L. gen. fem. n. *bernardiae* of Bernard, named after the contemporary Canadian microbiologist Kathryn A. Bernard for her contributions to the study of asporogenous, Gram-positive rods.

Cells are gram-positive rods, with coccobacilli predominating. Cells are sometimes arranged in clusters, but primary branching is not observed. Cells are non-motile and do not form spores. Colonies are circular, smooth, and slightly convex with a glassy appearance. Colony diameters range from 0.2 to 0.5 mm after 48 h of incubation in the presence of 5% CO_2 on sheep blood agar. Facultatively anaerobic. Catalase is not produced. Acid is produced from adonitol, D-arabitol, D-fructose, D-glucose, glycerol, maltose, ribitol, starch, xylitol, and 5-keto-gluconate. Most strains ferment L-arabitol, erythritol, glycogen, and ribose. Acid is not produced from amygdalin, arbutin, xylose, mannitol, α-methyl-D-mannoside, sucrose, and 2-ketogluconate. Leucine arylamidase and α-glucosidase positive. Alkaline phosphatase, cystine arylamidase, chymotrypsin, trypsin, acid phosphatase, α-galactosidase, β-glucuronidase, β-glucosidase, α-mannosidase, and α-fucosidase negative. Esculin and urea are not hydrolyzed. Nitrate is not reduced. Palmitic and stearic acids are the main straight-chain cellular fatty acids, while oleic acid is the predominant unsaturated fatty acid. The major menaquinone is MK-10(H_4). The interpeptide bridge within the peptidoglycan is L-Lys–L-Ala–L-Lys–D-Glu (type A5α).

The type strain was isolated from human blood.

DNA G+C content (mol%): 63–66 (T_m).

Type strain: LCDC 89–0504, CCUG 33419, CIP 104252, DSM 9152, LMG 18721.

Sequence accession no. (16S rRNA gene): X79224.

4. **Arcanobacterium bialowiezense** Lehnen, Busse, Frölich, Krasinska, Kämpfer and Speck 2006, 864[VP]

bi.a.lo.wi.e.zen′se. N.L. neut. adj. *bialowiezense* of or pertaining to Bialowieza, Poland, where the type strain was isolated.

Cells are short pleomorphic rods. After 48 h of growth under aerobic conditions on sheep blood agar, colonies are translucent, convex, approximately, 0.5 mm in diameter and surrounded by a narrow zone of β-hemolysis. Cells are non-motile (motility agar), facultatively anaerobic and are catalase- and oxidase-negative. Best growth occurs at 37°C; no growth at 42°C. No growth is observed on Gassner or MacConkey agar. Nitrate reduction is negative. Hydrolysis of gelatin and esculin negative. Hippurate hydrolysis is positive. The CAMP-test is negative. Acid is produced from D-fructose, D-glucose, and glycerol. Acid is not produced from adonitol, D-arabitol, L-arabitol, erythritol, glycogen, inulin, D-lactose, maltose, D-mannitol, raffinose, ribose, D-sorbitol, starch, sucrose, trehalose, and D-xylose. Acid phosphatase, esterase lipase (C8), naphthol-AS-BI-phosphohydrolase, β-glucuronidase, α-glucosidase and pyrazinamidase positive. Alkaline phosphatase, cystine arylamidase, chymotrypsin, α-galactosidase, *N*-acetyl-β-glucosaminidase, β-glucosidase, α-fucosidase, leucine arylamidase, α-mannosidase, pyrrolidonyl arylamidase, trypsin and urease negative. The quinone system contains MK-10(H_4) as the predominant compound. The interpeptide bridge within the peptidoglycan has not been reported.

Source (type strain): prepuce of a European bison.

DNA G+C content (mol%): not reported.

Type strain: 1(W3/01), DSM 17162, NCTC 13354.

Sequence accession no. (16S rRNA gene): AJ879696.

5. **Arcanobacterium bonasi** Lehnen, Busse, Frölich, Krasinska, Kämpfer and Speck 2006, 864[VP]

bo. na′ si. L. n. *bonasus*, *-i* a species of bull in Pœonia, with the hair of a horse, and with horns unfit for fighting; L. gen. n. *bonasi* of *bonasus*, of the European bison (*Bison bonasus*) from which the type strain was isolated.

Cells are short pleomorphic rods. After 48 h of growth under aerobic conditions on sheep blood agar, colonies are translucent, convex, approx. 0·5 mm in diameter and surrounded by a narrow zone of β-hemolysis. Cells are non-motile (motility agar), facultatively anaerobic and catalase- and oxidase-negative. Best growth occurs at 37°C; no growth at 42°C. No growth is observed on Gassner or MacConkey agar. Nitrate reduction is negative. Hydrolysis of gelatin and esculin negative. Hippurate hydrolysis is positive. The CAMP-test is negative. Acid is produced from D-arabitol, L-arabitol, D-fructose and trypsin. Acid is weakly produced from adonitol, erythritol, and glycerol. Acid is not produced from erythritol, D-glucose, glycogen, inulin, D-lactose, maltose, D-mannitol, raffinose, ribose, D-sorbitol, starch, sucrose, trehalose, and D-xylose. Acid phosphatase, esterase lipase (C8), lipase (C14), naphthol-ASBI-phosphohydrolase, β-glucuronidase, pyrazinamidases, and pyrrolidonyl arylamidase positive. Alkaline phosphatase, cystine arylamidase, chymotrypsin, α-galactosidase, α-glucosidase, β-glucosidase, *N*-acetyl-β-glucosaminidase, α-fucosidase, leucine arylamidase, α-mannosidase, and urease negative. The quinone system contains MK-10(H_4) as the predominant compound. The interpeptide bridge within the peptidoglycan has not been reported.

Source (type strain): prepuce of a European bison.

DNA G+C content (mol%): not reported.

Type strain: 2(W106/04), DSM 17163, NCTC 13355,

Sequence accession no. (16S rRNA gene): AJ879697.

6. **Arcanobacterium hippocoleae** Hoyles, Falsen, Foster, Rogerson and Collins 2002b, 619[VP]

hip.po.co.le'a.e. Gr. masc. and fem. n. *hippos* horse; Gr. masc. n. *koleos* sheath, vagina; N.L. masc. gen. n. *hippocoleae* of the horse vagina.

Cells are non-branching, irregular-shaped rods which stain Gram-positive, are non-acid-fast and non-motile. Colonies on blood agar are convex, circular, entire, shiny, opaque and grey. Weakly hemolytic. Facultatively anaerobic and catalase-negative. Acid is produced from D-glucose and lactose, but not from D-arabitol, L-arabinose, cyclodextrin, glycogen, pullulan, mannitol, melibiose, melezitose, methyl β-D-glucopyranoside, D-ribose, D-raffinose, sucrose, D-sorbitol, tagatose, trehalose or D-xylose. Acid may or may not be produced from maltose depending on the test system used. α-Glucosidase, β-galactosidase, β-glucuronidase, leucine arylamidase, and phosphoamidase are detected, but not acid phosphatase, alanine phenylalanine proline arylamidase, arginine dihydrolase, chymotrypsin, esterase C-4, ester lipase C8, α-fucosidase, α-galactosidase, β-glucosidase, lipase C14, α-mannosidase, β-mannosidase, pyrolidonyl arylamidase, pyroglutamic acid arylamidase, pyrazinamidase, trypsin, valine arylamidase, urease or glycyl tryptophan arylamidase. *N*-Acetyl-β-glucosaminidase and alkaline phosphatase may or may not be detected depending on the test system used. Esculin (weak reaction) and hippurate are hydrolysed, but not gelatin. Acetoin is not produced. Nitrate is not reduced to nitrite.

Habitat is not known.

Source (type strain): vaginal discharge from a horse.

DNA G+C content (mol%): not reported.

Type strain: M401624/00/2, CCUG 44697, CIP 106850, DSM 15539.

Sequence accession no. (16S rRNA gene): AJ300767.

7. **Arcanobacterium phocae** Pascual Ramos, Foster and Collins 1997b, 52[VP]

pho'ca.e. L. gen. n. *phocae* of a seal, isolated from common seals (*Phoca vitulina*) and grey seals (*Halichoerus grypus*).

Cells are coccobacilli and short rods that occur singly, in pairs, or in clusters; primary branching is not observed. Short diphtheroid forms may occur. Gram-stain-positive, non-motile, non-spore-forming, non-acid-fast. Colonies on blood agar (incubated for 24 h) are white, tiny, circular, low convex and surrounded by a zone of β-hemolysis which may be two to three times the diameter of the colony. Facultatively anaerobic. Growth is not enhanced by increased concentrations of CO_2, (5–10%). Catalase reaction is variable. Metabolism is strictly fermentative. Acid but not gas is produced from glucose. Acid is produced from D-fructose, galactose, D-glucose, N-acetylglucosamine, glycerol, glycogen, lactose, maltose, D-ribose, starch, sucrose, D-turanose, and 5-keto-gluconate. Most strains ferment inositol, mannose, melezitose, and trehalose, and a few strains ferment gluconate, mannitol, D-tagatose, and D-xylose. Acid is not produced from adonitol, amygdalin, D-arabinose, L-arabinose, D-arabitol, L-arabitol, cellobiose, dulcitol, erythritol, D-fucose, L-fucose, gentiobiose, 2-keto-gluconate, α-methyl-D-glucoside, D-lyxose, rhamnose, salicin, D-sorbitol, sorbose, α-methyl-mannoside, arbutin, melibiose, inulin, D-raffinose, L-xylose, β-methyl-D-xyloside, and xylitol. Nitrate reduction is negative. Esculin, gelatin, and urea are not hydrolyzed. Acid phosphatase, alkaline phosphatase, α-galactosidase, β-galactosidase, α-glucosidase, pyrazinamidase, esterase (C4), esterase-lipase (C8), cystine arylamidase, and leucine arylamidase are produced. Production of trypsin is variable. Chymotrypsin, β-glucosidase, *N*-acetyl-β-glucosaminidase, β-glucuronidase, α-fucosidase, α-mannosidase lipase (C14), naphthol-AS-BI-phosphohydrolase, pyrrolidonyl arylamidase, and valine arylamidase are not produced. The interpeptide bridge within the peptidoglycan is L-Lys–Lys-D-Glu (Type A5α). The principal menaquinone is MK-9(H_4).

Arcanobacterium phocae has been isolated from various tissues and fluids of common seals (*Phoca vitulina*) and grey seals (*Halichoerus grypus*), for which its pathological significance is unclear, but it has been recovered in mixed cultures from pneumonic and septicemic seals.

Source (type strain): the lung of common seals (*Phoca vitulina*).

DNA G+C content (mol%): not reported.

Type strain: M1590/94/3, CIP 105740, DSM 10002, LMG 18722. The type strain possesses the characteristics of the species except that it is Catalase-positive and produces trypsin. M1590/94/3 ferments inositol, mannitol, mannose, melezitose, trehalose, and D-xylose but does not ferment D-tagatose and gluconate.

Sequence accession no. (16S rRNA gene): EU1945657.

Additional remarks: the type strain possesses the characteristics of the species except that it is catalase-positive and produces trypsin. M1590/94/3 ferments inositol, mannitol, mannose, melezitose, trehalose, and D-xylose but does not ferment D-tagatose and gluconate.

8. **Arcanobacterium pluranimalium** Lawson, Falsen, Foster, Eriksson, Weiss and Collins 2001a, 58[VP]

plur.a.ni.ma'li.um. L. comp. adj. *plus, pluris* more, a greater quantity; L. gen. pl. n. *animalium* from animals; N.L. gen. pl. n. *pluranimalium* from many animals.

Cells are straight to slightly curved, non-branching, slender rods which stain Gram-positive, are non acid fast and non-motile. Facultatively anaerobic and catalase-positive. Acid is produced from D-glucose and D-ribose, but not from D-arabitol, L-arabinose, cyclodextrin, glycogen, pullulan, lactose, mannitol, melibiose, melezitose, raffinose, D-sorbitol, sucrose, tagatose, trehalose or D-xylose. Maltose is variable. Alanine phenylalanine proline arylamidase, pyroglutamic acid arylamidase and β-glucuronidase are detected, but not alkaline phosphatase, arginine dihydrolase, α-galactosidase, β-galactosidase, β-galacturonidase, α-glucosidase, β-glucosidase, *N*-acetyl-β-glucosaminidase, β-mannosidase or urease. Pyrazinamidase and glycyl tryptophan arylamidase may or may not be detected. Esculin (weak reaction), gelatin and hippurate are hydrolyzed. Acetoin is not produced. Nitrate is not reduced to nitrite. The interpeptide bridge within the peptidoglycan is L-Lys–Lys-D-Glu (type A5α). The principal menaquinone is MK-9(H_4).

Habitat is not known.

Source (type strain): a dead harbor porpoise and a dead sallow deer.

DNA G+C content (mol%): 57 (method not reported).

Type strain: M430/94/2, CCUG 42575, CIP 106442, DSM 13483.

Sequence accession no. (16S rRNA gene): AJ250959.

9. **Arcanobacterium pyogenes** (Glage 1903), Pascual Ramos, Foster and Collins 1997b, 51[VP] [*Bacillus pyogenes* Glage 1903, 1973; *Corynebacterium pyogenes* (Glage) Eberson 1918, 23; *Actinomyces pyogenes* Reddy, Cornell and Fraga 1982, 427]

py.o′ge.nes. Gr. neut. n. *pyon* pus; N.L. suff. *-genes* (from Gr. v. *gennaô* to produce) producing; N. L. adj. *pyogenes* pus-producing).

Gram-stain-positive, nonmotile, non-spore-forming coccobacilli and short rods that occur singly, in pairs (V, T, and palisade formations), or in clusters. Short diphtheroid forms with clubs are also seen. Streptococcal forms in small clumps and short crooked chains are occasionally observed. Cells vary in shape and size (0.2–0.9 by 0.3–2.5 μm) in different media. Cells from 24-h-old broth cultures are Gram-stain-positive, but cells from older cultures may be Gram-stain-variable. The cell-wall ultrastructure is typical of Gram-stain-positive bacteria. The cell walls are 29 to 30 nm thick and have a characteristic double-track appearance. Pinpoint, β-hemolytic colonies occur on sheep blood agar after 24 h of incubation. The zones of hemolysis are typically two to three times the diameter of the colony. After 48–72 h, the colonies (diameter 0.5–1.5 mm) are convex, circular, opaque, white, and soft with entire edges. Colonies develop faster and are bigger (diameter 1.5–3.0 mm) on SFM agar plates. Good growth occurs under aerobic and strictly anaerobic conditions. Metabolism is strictly fermentative. Acid but not gas is produced from cellobiose, dextrin, fructose, galactose, glucose, glycogen, lactose, maltose, mannose, melezitose, starch, trehalose, and xylose. The fermentation of adonitol, arabinose, erythritol, glycerol, sucrose, mannitol, and sorbitol varies with the strain. No acid is produced from amygdalin, esculin, melibiose, raffinose, rhamnose, or salicin. Alanine, arginine, aspartate, glycine, and threonine are not fermented. Most strains are catalase-negative, although one strain has been reported to be catalase-positive. Acid clotting and digestion of clots in litmus milk and liquefaction of gelatin are characteristic of all strains. Nitrates are not reduced, and indole is not produced. The optimum growth temperature is 37°C; the temperature range is 20–40°C.

Lactic acid is the primary metabolic product in brain heart infusion or tryptose broth with no added HCO_3^-; acetate is a minor product. Glucose is fermented in the presence of CO_2/HCO_3^- and this reaction yields succinate, acetate, formate, and lactate as major end products. For each mol of CO_2/HCO_3^- fixed, 1 mol of succinate, 1 mol of acetate, and 1 mol of formate are produced. In identical media without added bicarbonate or hemin, lactate is the major product, and smaller amounts of acetate, succinate, and formate are produced. CO_2/HCO_3^- is required for growth. Hemin is highly stimulatory or required for growth. Peptides are required for growth even in the presence of a complete complement of 20 amino acids and $(NH_4)_2SO_4$. Inositol can replace the peptide requirement for growth. Riboflavin and nicotinic acid are required for optimal growth. Adenine and uracil are required for optimal growth of some strains. Characteristic cell-wall sugar components are rhamnose and glucose. No mycolic acids are present. Culture filtrates are fatal to mice and rabbits after intravenous injection. The soluble hemolysin produced is active against human, guinea pig, sheep, horse, and rabbit erythrocytes. Both toxic and hemolytic activities of crude cell extracts are neutralized by antitoxin. The interpeptide bridge within the peptidoglycan is L-Lys–L-Ala–L-Lys–D-Glu (type A5α). The principal menaquinone is MK-10(H_4).

This organism is frequently isolated from a wide variety of pyogenic disease conditions in many species of domestic animals and in humans. Presumably, *Arcanobacterium pyogenes*, occurs as a commensal organism on the mucous surfaces of warm-blooded animals.

DNA G+C content (mol%): 56–58 (T_m).

Type strain: C-100, ATCC 1941, CCUG 13230, CIP 103129, DSM 20630, LMG 16162, NCTC 5224.

Sequence accession no. (16S rRNA gene): X79225.

Genus IV. **Mobiluncus** Spiegel and Roberts 1984a, 180[VP] emend. Hoyles, Collins, Falsen, Nikolaitchouk and McCartney 2004

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Mo.bi.lun′cus. L. adj. *mobilis* capable of movement, active; L. masc. n. *uncus* hook; N.L. masc. n. *Mobiluncus* a motile curved rod.

Curved rods with tapered ends, 1–5 × 0.4–0.6 μm. Depending on the growth medium, cells occur singly or in pairs with a gull-wing appearance. **Gram-stain-negative or Gram-stain-variable**. Cells are **motile** by means of **multiple subpolar flagella** (exact numbers are yet to be determined, but are thought to be between one and eight), and possess a multi-layered Gram-stain-positive cell-wall type. **Endospores are not formed**. **Anaerobic**, growing best in an enriched CO_2 atmosphere. Some strains become aerotolerant after several passages, being able to grow in a partially reduced atmosphere containing 5% O_2. Optimum growth at 35–37°C, with no or poor growth at 20, 43, or 45°C. Growth is stimulated by horse or rabbit serum and by whole blood. Growth is not stimulated by formate/fumarate. Slow growth on solid media. After 3 d incubation at 37°C, colonies on peptone/yeast extract/glucose/sheep blood or Columbia agar supplemented with human blood are pin-point to 1 mm in diameter. After 5 d, the colonies are larger, 1–2.5 mm in diameter, and gray-white or slightly yellowish. Cells are killed by heating at 55°C for 15 minutes. **Oxidase- and catalase-negative**. Weakly (terminal pH 5.5–6.5) or strongly (terminal pH <5.5) saccharolytic. Fermentation products include acetic and succinic acids, with or without lactic acid, when grown in peptone/yeast extract/glucose medium. Propionic acid is not produced. Do not produce acid from L-arabinose, D-arabitol, lactose, mannitol, methyl-β-D-galactopyranoside, or sorbitol. Negative for alkaline phosphatase, α-arabinosidase,

chymotrypsin, β-galactosidase-6-phosphate, β-glucosidase, trypsin, and urease (RapID 32Strep), cysteine arylamidase, lipase (C14), α-mannosidase, β-mannosidase, *N*-acetyl-β-glucosaminidase, glutamyl glutamic acid arylamidase, pyroglutamic acid, and valine arylamidase activity. Variable activity for phenylalanine arylamidase (weak- to strong-positive reaction). **Acid phosphatase- and pyrollydonyl arylamidase-negative**. **Acetoin is not produced**. Gelatin is not hydrolyzed. **Nitrate may be reduced**. Sensitive to ampicillin, cefazolin, cefoxitin, cephalothin, clindamycin, gentamicin, imipenem, moxalactam, penicillin G, rifampin, streptomycin, tobramycin, vancomycin, and virginiamycin. Resistant to colistin, cycloserine, nalidixic acid, neomycin, and mezlocillin. Associated with the human vaginal flora and bacterial vaginosis (BV).

DNA G+C content (mol%): 49–52 (T_m; Spiegel and Roberts, 1984a); 50–54 (T_m; Hammann et al., 1984).

Type species: **Mobiluncus curtisii** Spiegel and Roberts 1984a, 181[VP].

Further descriptive information

Phylogeny. Partial reverse transcriptase sequencing of the 16S rRNA from *Mobiluncus curtisii* and *Mobiluncus mulieris* and subsequent sequence analysis led Lassnig et al. (1989) to conclude that the genus *Mobiluncus* belonged to the order *Actinomycetales*, not to the family *Bacteroidaceae* (as assigned by Spiegel and Roberts, 1984a), and that the species were moderately related to the genus *Actinomyces*. More recent analyses have confirmed that the genus *Mobiluncus* shares common ancestry with the two subspecies of [*Actinomyces*] *neuii* (Pascual Ramos et al., 1997b) and with *Varibaculum cambriense* (Hoyles et al., 2004), but the relationships are not statistically significant (Hall et al., 2003e; Hoyles et al., 2004; Pascual Ramos et al., 1997b). Sequence divergence values among these taxa of between 10 and 11% indicate that they represent three distinct bacterial lineages. Schaal et al. (2006) have suggested that the family *Actinomycetaceae* should be considered to comprise six phylogenetic clusters, with *Mobiluncus* spp. belonging to cluster 4. Assignment to specific clusters is based upon cut-off branching points and signature nucleotides of 16S rRNA gene sequences (Schaal et al., 2006; Stackebrandt et al., 1997).

Cell-wall composition. Although they observed that the Gram reactions of *Mobiluncus* spp. vary with age of culture and species, Spiegel and Roberts (1984a) noted that electron micrographs of both species revealed multi-layered Gram-stain-positive cell walls without an outer membrane. They postulated that the thinness of the peptidoglycan layer may explain the tendency of the curved, rod-shaped organisms to stain Gram-negative. Carlone et al. (1986) demonstrated the presence of peptidoglycan by analyzing *o*-methyloxime acetate derivatives of whole-cell carbohydrates of four strains of *Mobiluncus*. No 2-keto-3-deoxy-octulosonic acid (KDO) or degradation products of KDO were present in either species, nor was L-glycero-D-mannoheptose (heptose), consistent with a Gram-stain-positive cell-wall type. Comparable amounts of galactose, glucosamine, muramic acid, and rhamnose were observed in both species. Three times as much ribose was present in *Mobiluncus mulieris* strains as in *Mobiluncus curtisii* strains, whereas *Mobiluncus curtisii* strains had twice as much glucose as *Mobiluncus mulieris*. Mannosamine was present in *Mobiluncus curtisii* subsp. *curtisii* (4%) and *Mobiluncus curtisii* subsp. *holmesii* (2%), but was absent from *Mobiluncus mulieris*. Galactosamine was present in *Mobiluncus mulieris* (1%) and *Mobiluncus curtisii* (0.5%). A relatively large amount of an unidentified compound was also found in the two *Mobiluncus mulieris* strains studied. Diaminopimelic acid was not detected in cell-wall hydrolysates of either species (Hammann et al., 1984). Lipopolysaccharide was absent from the cell wall (Carlone et al., 1986).

Fatty acids. Hydroxylated fatty acids are absent from the cell walls of *Mobiluncus* spp. (Carlone et al., 1986; Spiegel and Roberts, 1984a). The major cellular fatty acids include $C_{16:0}$ (hexadecanoic), $C_{18:2}$ (octadecadienoic), and $C_{18:1}$ (octadecenoic), representing greater than 50% of the total fatty acids; $C_{14:0}$ (myristic), $C_{16:1}$ (hexadecenoic), $C_{17:0}$ (heptadecanoic), and $C_{18:0}$ (octadecanoic) are also present (Carlone et al., 1986; Christiansen et al., 1984; Skarin et al., 1982, 1984b). The presence of fatty acid aldehydes needs further investigation, as there is conflicting evidence regarding their presence (Skarin et al., 1982) or absence (Carlone et al., 1986).

Flagella. Flagella have been observed in electron micrographs of both *Mobiluncus* species, although the exact number is yet to be determined. Curtis (1913) noted that *Mobiluncus* spp. were motile by means of up to six flagella, and that exposure to air led to a decrease in the motility of the organisms. Moore (1954) observed motility in two strains isolated from women attending fertility clinics; the strains had up to six flagella, but were overgrown by contaminants before they were investigated further. He also collected ten strains that were motile by means of a single flagellum. Moore (1954) stated that the most distinctive morphological property of the organisms studied was their corkscrew motion. In all strains investigated, he found that the organisms were monotrichate, with a flagellum attached at or very near one pole, or in the center of the concave side of the organism, though most cultures showed occasional forms with two flagella in one or other of these positions. Moore (1954) also noted that the organisms became nonmotile after exposure to air and rapidly adhered to cellular debris, so that epithelial cells in particular were often furred with large numbers of immobilized vibrios ("clue cells"). Spiegel and Roberts (1984a) observed clusters of two to six flagella; sometimes a subterminal attachment site was observed. *Mobiluncus curtisii* has one to six flagella per cell with a common point of origin, while *Mobiluncus mulieris* has one to eight flagella with multiple origins (Hammann et al., 1984; Hernández et al., 1994; Sprott et al., 1983, 1984; Taylor and Owen, 1984). Pili (fimbriae) have not been observed (Hammann et al., 1984).

Colonial and cultural characteristics. On blood agar after 5 d incubation, colonies of *Mobiluncus mulieris* are small (<1 mm in diameter), convex, glossy, and colorless (Hernández et al., 1994). On Columbia agar base containing human blood (5%) and vitamin K_1 (1 mg/l), colonies are minute and transparent gray after anaerobic incubation for 48 h (Sprott et al., 1983): *Mobiluncus mulieris* strains produce α-hemolysis after prolonged culture on this medium, and develop a dark-brown pigment after 10–14 d incubation. Clinical isolates grown on Rlk and SA media containing laked rabbit blood (Smith and Moore, 1988) form colonies that are very small (0.25 mm, *Mobiluncus mulieris*; 0.32–0.35 mm, *Mobiluncus curtisii*), convex, glossy, and translucent after 48 h incubation; reference strain colonies

have a similar appearance when grown on these agars, but have much larger diameters (0.70–0.75 mm, *Mobiluncus mulieris*; 0.98–1.0 mm, *Mobiluncus curtisii*). When laked sheep blood is used instead of rabbit blood, 7 d incubation are required to achieve the diameters indicated for rabbit blood. On brain heart infusion, Brucella, and Wilkins–Chalgren agars supplemented with 5% horse blood, strains produce colonies that are small (1–2 mm diameter), slightly convex, entire, and transparent (Vetere et al., 1987): the colony morphology of *Mobiluncus curtisii* and *Mobiluncus mulieris* is indistinguishable, but β-hemolysis develops around *Mobiluncus mulieris* colonies more often than *Mobiluncus curtisii* colonies

Nutrition and growth conditions. *Mobiluncus* spp. require anaerobic conditions for growth (Hammann et al., 1984; Spiegel and Roberts, 1984a). After primary isolation and multiple passages, growth has been reported in 10% CO_2 or 5% O_2 (Hammann et al., 1984). The organisms are fastidious. Details of media (solid and broth) used to successfully isolate and culture *Mobiluncus* spp. can be found in the Enrichment and Isolation Procedures section of this chapter. Optimum growth is achieved at 35–37°C (Hammann et al., 1984; Spiegel and Roberts, 1984a).

Antibodies. One of the earliest studies into the use of polyclonal antibodies (pAbs) to distinguish strains of *Mobiluncus* spp. from one another was carried out by Moi et al. (1984b). They used hyperimmune rabbit pAbs to demonstrate the heat-labile and heat-stable antigens of *Mobiluncus curtisii* and *Mobiluncus mulieris*, and to study the antigenic relationship of these micro-organisms and other bacteria. Co-agglutination assays were used to demonstrate surface-specific antigens of the two species, and immunofluorescence assays were used to study the immunomorphology of the organisms. The main conclusions of their study were that the species could be separated into two distinct biochemical and serological groups, and that the antigenic markers of *Mobiluncus* spp. could be used in taxonomic, ecological, and epidemiological studies. Although they found differences in the heat-labile and heat-stable antigens of *Mobiluncus curtisii* and *Mobiluncus mulieris*, they did not investigate the potential of these antigens for the detection/identification of *Mobiluncus* spp. in routine diagnostic tests or in vaginal samples from patients with BV.

Since the study of Moi et al. (1984b), monoclonal antibodies (mAbs) have been used by a number of different groups to detect the presence of *Mobiluncus* spp. in vaginal samples and blood samples, and to detect strain differences among the two species and subspecies of the genus (Fohn et al., 1988; Hallén et al., 1987; Ison et al., 1989).

In a later study by Påhlson et al. (1986a), 51 strains of *Mobiluncus* from clinical isolates were studied using phenotypic traits and a set of mAbs to defined *Mobiluncus* antigens. Analysis of the phenotypic and mAb reactivity data showed that the strains could be split into two distinct species, *Mobiluncus curtisii* and *Mobiluncus mulieris*. However, Påhlson et al. (1986a) also noted strain variation. To give a better definition of the cell-wall proteins that differed between strains of *Mobiluncus*, a number of hybridomas secreting mAbs were constructed. The mAbs produced were shown to be specific to the genus *Mobiluncus*, as they did not react with *Bacillus cereus*, *Bacteroides fragilis*, *Escherichia coli*, *Haemophilus influenzae*, *Klebsiella* sp., *Neisseria* sp., *Peptostreptococcus magnus*, *Propionibacterium acnes*, *Proteus mirabilis*, and a number of the lactic acid cocci. The reactivity of the mAbs with members of the genus *Mobiluncus* was examined by immunoblot analysis of the soluble preparations from the type strains of the *Mobiluncus* species used to prepare the mAbs. The proteins were separated by SDS-PAGE and the distinct differences between the *Mobiluncus curtisii*, *Mobiluncus mulieris*, and atypical strains were clearly observed, providing a fingerprint for each individual strain (Påhlson et al., 1986a). Upon blotting onto nitrocellulose membranes and enzyme assay, the authors detected specific mAbs for each species. The antigens had molecular masses of 78 kDa for *Mobiluncus curtisii* and 84–92 kDa for *Mobiluncus mulieris*. The proteinaceous nature of the antigens was confirmed by the detection of a drop in binding in the enzyme assay (decrease in optical density readings) when they were treated with pronase. After showing the specificity of their mAbs for members of the genus *Mobiluncus*, Påhlson et al. (1986a) used their mAbs to detect *Mobiluncus* spp. in clinical samples. A comparison of their results for the detection of *Mobiluncus* by mAbs or by examination of a wet smear showed high accord between the two methods for detection of *Mobiluncus* spp.

Hallén et al. (1987) used mAbs in a study (immunofluorescence microscopy) to detect *Mobiluncus* spp. in vaginal samples from women with BV. They provided the first evidence that detection of *Mobiluncus* spp. by mAbs was far more sensitive than the previously relied upon traditional methods (i.e., Gram stains of vaginal smears, direct examination of vaginal suspensions for the presence of curved motile rods), and also demonstrated that *Mobiluncus* spp. could be detected in women who did not show signs of BV. Of the 90 strains of *Mobiluncus* isolated from vaginal specimens, 44% were *Mobiluncus curtisii* and 34% were *Mobiluncus mulieris* (Hallén et al., 1987). In 21% of the cases, *Mobiluncus curtisii* and *Mobiluncus mulieris* were found together. Previously, Påhlson and Forsum (1985) had detected *Mobiluncus* spp. by the use of mAbs in the examination of vaginal smears by indirect immunofluorescence. They used five different mAbs to cover all known *Mobiluncus* phenotypes. In a study of 59 women attending a clinic for sexually transmitted diseases (STDs), they found that indirect immunofluorescence gave a 30% higher yield of *Mobiluncus* spp. than did conventional cultivation.

Fohn et al. (1988) used SDS-PAGE and immunoblotting to demonstrate production of four murine mAbs specific for an 82-kDa molecule of *Mobiluncus curtisii* subsp. *curtisii* and five antibodies that detected a major band of *Mobiluncus mulieris* at 93 kDa. To confirm the application of their panel of mAbs, the authors tested them against 25 *Mobiluncus* isolates from women with BV. They detected the presence of *Mobiluncus* spp. in 24 of the clinical isolates, and also used the mAbs to detect the presence of *Mobiluncus* spp. directly in the vaginal fluid of a patient with BV. The use of an immunofluorescence assay with the mAbs in the examination of the vaginal fluid sample revealed that the patient was harboring *Mobiluncus curtisii* subsp. *holmesii*; subsequent isolation confirmed this identification.

Fohn et al. (1988) also used murine mAbs against the ATCC type strains of *Mobiluncus curtisii* subsp. *curtisii*, *Mobiluncus curtisii* subsp. *holmesii* and *Mobiluncus mulieris* in an ELISA/indirect immunofluorescence assay. Four of the mAbs tested were subspecies-specific and reacted with *Mobiluncus curtisii* subsp. *curtisii*, but not with *Mobiluncus curtisii* subsp. *holmesii*; four others

were specific for *Mobiluncus mulieris*. The remaining antibodies tested showed some cross-reactivity with both subspecies of *Mobiluncus curtisii*, with one defining a common epitope of *Mobiluncus curtisii* subsp. *holmesii* and *Mobiluncus mulieris* (mAb F3F1). It was also shown that the mAbs tested were specific for *Mobiluncus,* as they did not react with a test panel of bacteria commonly isolated from the vaginas of healthy women or women with BV (e.g., *Bacteroides* spp., *Gardnerella vaginalis*, *Lactobacillus* spp., *Neisseria gonorrhoeae*, and *Peptostreptococcus saccharolyticus*).

An extensive study was carried out by Ison et al. (1989) of 37 *Mobiluncus curtisii* and 25 *Mobiluncus mulieris* strains (clinical samples and the NCTC type cultures for the three recognized species). The intention was to develop a rapid detection system for *Mobiluncus* spp. Initially, the strains were identified as *Mobiluncus* spp. if they were anaerobic, motile, Gram-stain-variable curved rods that were resistant to 5 μg of metronidazole. The two species were distinguished by their ability to reduce nitrate, to hydrolyze hippurate, and to ferment various carbohydrates, and were divided according to criteria set out by Spiegel and Roberts (1984a). They were also tested for enzyme activity using the API ZYM system. Following separation of the strains into their respective species, seven mAbs were produced after a single fusion of spleen cells from mice inoculated with each type strain. Four were chosen for further study on the basis of their reactivity with various strains of *Mobiluncus*. In immunofluorescence studies, three of the four mAbs showed reactivity against the type strains of *Mobiluncus curtisii*, but not against the type strain of *Mobiluncus mulieris* (mAb 179.1 showed equal activity against both subspecies of *Mobiluncus curtisii*, but at different titers; mAb 180.1 showed highest activity against *Mobiluncus curtisii* subsp. *curtisii*, whereas mAb 179.3 showed highest activity against *Mobiluncus curtisii* subsp. *holmesii*). The mAb 181.1 showed activity against *Mobiluncus mulieris*, but not against *Mobiluncus curtisii*. Overall, 100% sensitivity for *Mobiluncus curtisii* (both subspecies) was only achieved when all three reactive mAbs were used together. No relationship was found between the reactivity of the three mAbs and the results of the biochemical tests performed. With regard to the mAb raised against the type strain of *Mobiluncus mulieris* and reactive with only a small proportion of the clinical isolates tested, the authors concluded that antigenic heterogeneity in this species may limit the value of a single mAb. They also suggested that the non-reactive strains they had identified may have been atypical of the generally recognized *Mobiluncus mulieris* phenotype.

In a study designed specifically to investigate the use of mAbs against *Mobiluncus* spp. as a serological marker of BV (in pregnant women whose sexual history and vaginal microbiological data were available), Schwebke et al. (1996) screened sera for antibodies to *Mobiluncus* spp. by an indirect immunofluorescence assay. The frequency of women with IgG antibodies against *Mobiluncus curtisii* was 75%, which was higher than the authors had anticipated. This was attributed to nonspecificity of the detected antibody response or higher prevalence of BV than first anticipated. However, the demonstration that antibodies against *Mobiluncus curtisii* were completely removed by adsorption with *Mobiluncus curtisii*, but not by adsorption with *Mobiluncus mulieris*, strongly suggests that the antibodies were specific. Schwebke et al. (1996), Fohn et al. (1988), and Ison et al. (1989) agreed that the prevalence of BV was poorly defined, and that, if the true incidence of *Mobiluncus* spp. in BV and in normal women was to be determined, good detection systems would be necessary to overcome the problems associated with more classical (cultivation) techniques.

Gatti et al. (1997) obtained the antigenic profiles for 30 strains of *Mobiluncus* (22 *Mobiluncus curtisii* and 8 *Mobiluncus holmesii*) isolated from vaginal samples using mouse immune ascitic fluids (MIAFs) from mice immunized with the ATCC type strains for the genus. Initially, the 30 strains were split into *Mobiluncus curtisii* and *Mobiluncus holmesii* on the basis of biochemical tests (*Mobiluncus curtisii* positive for the presence of α-galactosidase and β-galactosidase and for hydrolysis of hippurate; *Mobiluncus holmesii* negative for all three tests). Division of the *Mobiluncus curtisii* strains into its two subspecies was based on nitrate reduction. SDS-PAGE profiles were obtained for each of the 30 strains and these were transferred to nitrocellulose membranes for blotting with the different MIAFs. The major antigenic proteins of *Mobiluncus curtisii* and *Mobiluncus mulieris*, as determined by their homologous MIAF, showed that *Mobiluncus curtisii* subsp. *holmesii* had five major antigenic proteins (63-, 54-, 52-, 46-, and 30-kDa bands), whereas *Mobiluncus curtisii* subsp. *curtisii* had only four of these antigenic proteins (no 54-kDa band). Of the 22 *Mobiluncus curtisii* samples tested, eight had the *Mobiluncus curtisii* subsp. *holmesii* pattern, whereas 14 had the *Mobiluncus curtisii* subsp. *curtisii* pattern. The authors found no correlation between the antigenic patterns of the vaginal isolates of *Mobiluncus curtisii* and the biochemical identification of the subspecies, because the antigenic patterns of the two subspecies were found to be interchangeable. *Mobiluncus mulieris* was shown to contain three major protein bands (64, 48, and 45 kDa), and a series of antigenic proteins (64–100 kDa). None of the clinical isolates of *Mobiluncus mulieris* gave identical profiles to that of the type strain. Gatti et al. (1997) also found cross-reactivity between the species and subspecies by immunoblot analysis with heterologous pAbs. Within the 22 isolates of *Mobiluncus curtisii* studied, only two different antigenic profiles were found based on the recognition, or lack, of a 54-kDa protein shown when *Mobiluncus curtisii* blots were probed with anti-*Mobiluncus curtisii* subsp. *holmesii* MIAF, whereas all of the *Mobiluncus mulieris* strains contained some major antigenic proteins of the type strain and showed great variability only in the 64–100-kDa antigenic proteins. However, *Mobiluncus curtisii* and *Mobiluncus mulieris* could be distinguished from each other by their antigenic protein profiles, particularly by the presence or absence of a 30- or 34-kDa protein.

In summary, antigenic characterization of *Mobiluncus* spp. with polyvalent rabbit antisera and mAbs has shown *Mobiluncus curtisii* and *Mobiluncus mulieris* to be fairly distinct, whereas the two subspecies of *Mobiluncus curtisii* show significant antigenic homogeneity. Also, the use of mAbs and pAbs to detect and identify *Mobiluncus* spp. is limited by the range of available antibodies. Ison et al. (1989) stated that, if mAbs are to be used successfully in studies to elucidate the role of *Mobiluncus* spp. in BV (and one would assume to detect and identify *Mobiluncus* spp. routinely), several antibodies will be required to detect the species and subspecies of the genus. The advantages of using mAb techniques over classical techniques are that they are simpler, less cumbersome, and less time-consuming. mAb techniques also allow for the selection of antigens specific to *Mobiluncus* in highly complex antigenic mixtures (Påhlson et al., 1986a).

Antibiotic sensitivity. Spiegel (1987) performed a standardized study of the sensitivity of 12 *Mobiluncus curtisii* strains and ten *Mobiluncus mulieris* strains to 23 antimicrobials and 15 other compounds known to inhibit bacterial growth: tests were done either by microbroth dilution (antimicrobial agents) or agar dilution (other agents). MIC ranges (μg/ml) are given in parentheses for *Mobiluncus curtisii* and *Mobiluncus mulieris*, respectively, where values are known for both species. All strains were sensitive to ampicillin (0.125–0.25 and ≤0.015–0.062), cefazolin (≤0.2–1.56 and ≤0.2–0.78), cefoxitin (1–4 and 0.25–0.5), cephalothin (0.78–1.56 and ≤0.2–0.4), chloramphenicol (4–8 and 2–4), clindamycin (0.062–0.125 and ≤0.015–4), imipenem (0.031–0.125 and 0.031–0.062), moxalactam (1–4 and ≤0.062–0.5), penicillin G (0.015–0.125 and 0.008–0.015), rifampin (≤0.004 and ≤0.004), tobramycin (0.25–1 and 0.25–1), vancomycin (0.5 and 0.5–1), and virginiamycin (≤0.2 and ≤0.2–0.39). All strains were resistant to colistin (32–64 and 64–256), cycloserine (≥200 and 100–>200), nalidixic acid (≥200 and 100–200), and neomycin (2–8 and 4–8). In addition, Hammann et al. (1984) demonstrated that both species were resistant to mezlocillin, slightly sensitive to gentamicin, and resistant to, or showed microcolony formation with, erythromycin and oxytetracycline. Sprott et al. (1983) also showed that *Mobiluncus curtisii* and *Mobiluncus mulieris* were sensitive to gentamicin (0.5–1.0 and 1.0–2 mg/l, respectively) and streptomycin (8–16 and 4–8 mg/l, respectively). The occurrence of aminoglycoside sensitivity in anaerobic organisms is rare, and its significance is unknown (Spiegel, 1987; Sprott et al., 1983). *Mobiluncus* spp. have also been shown to be sensitive to rifaximin (≤1 μg/ml; Hoover et al., 1993).

Mobiluncus curtisii was resistant to tinidazole (>256 μg/ml), while only one of ten strains of *Mobiluncus mulieris* was resistant to this antimicrobial (4–>256) (Spiegel, 1987). *Mobiluncus curtisii* strains were resistant to metronidazole (35–512) and hydroxymetronidazole (128–512); there was variability in the resistance of *Mobiluncus mulieris* strains to these two antimicrobials (2–>512 and 4–>512, respectively) (Spiegel, 1987). Sprott et al. (1983) also noted a difference in the sensitivity of *Mobiluncus curtisii* and *Mobiluncus mulieris* to metronidazole (0.5–4 mg/l and 16–1000 mg/l, respectively) but did not find increased activity of hydroxymetronidazole against their strains. The relative resistance of both species to metronidazole and tinidazole may aid in the development of selective media for the isolation of *Mobiluncus* spp. from clinical samples (Spiegel, 1987). However, some strains are inhibited by metronidazole (<8 μg/ml) (Hammann et al., 1984).

Spiegel (1987) also examined the effects of 15 compounds (alizarin red, azure II, basic fuchsin, brilliant green, deoxycholate, Evans blue, Gentian violet, Janus green, malachite green, methyl orange, Nile blue A, oxgall, resazurin, safranine, and sodium fluoride) known to inhibit growth of *Mobiluncus* strains. No quality control values were available for these compounds; therefore, no interpretations of sensitivity or resistance could be made. However, the information generated on these compounds may be of use in choosing selective agents for *in vitro* use (Spiegel, 1987). Of the 15 compounds tested, alizarin red, Evans blue, and sodium fluoride were found to inhibit *Mobiluncus mulieris* growth more than *Mobiluncus curtisii* growth. The use of sodium fluoride in selective media is limited, however, as it is a poison and cannot be placed in glass (Spiegel, 1987).

Only one study has been done regarding the presence of resistance determinants in *Mobiluncus* spp. Leng et al. (1997) examined *Mobiluncus curtisii* and *Mobiluncus mulieris* isolates for the presence of *tetQ* and *tetO*, encoding proteins that protect bacterial ribosomes from tetracycline both *in vivo* and *in vitro*. Five of the 12 *Mobiluncus curtisii* strains examined carried the *tetO* gene, two carried the *tetQ* gene, one carried both genes, and the remaining strains carried neither gene. Of the eight *Mobiluncus mulieris* strains examined, two carried *tetO*, two carried *tetQ*, one carried both genes, and three strains carried neither gene. Tetracycline resistance was observed in some of the strains that carried neither *tet* gene, suggesting that other novel tetracycline-resistance determinants are present in the genus *Mobiluncus*. One *Mobiluncus curtisii* strain is known to carry the *tetQ* gene on its chromosome (Leng et al., 1997).

Ecology

Association with BV. *Mobiluncus* spp. form part of the indigenous microbiota of the human vagina. Cultivation of strains from vaginal smears from healthy women often proves problematic, due to their fastidious nature and their overgrowth by less-fastidious, faster-growing organisms, such as *Lactobacillus* spp. (Hammann et al., 1984; Smith and Moore, 1988). However, the isolation of *Mobiluncus* spp. from vaginal samples from women afflicted by bacterial vaginosis (BV) has been well documented (Fontaine et al., 1982; Hjelm et al., 1982; Holst et al., 1982, 1984a; Skarin et al., 1984a; Sprott et al., 1982, 1983). It is thought that the increase in pH associated with the onset of BV allows less prominent members of the vaginal flora, such as *Mobiluncus* species, to become prominent. However, isolation of these organisms from BV samples is still difficult and their role, if any, in BV has not been determined: they are considered an indicator organism for BV, rather than the causative agent (Fox and Phillips, 1984; Hallén et al., 1987; Hjelm et al., 1984; Schwebke et al., 1996). Notably, *Mobiluncus* spp. are not present in all patients with BV (Hallén et al., 1987). Where *Mobiluncus* spp. are associated with BV, it is not unusual to detect both *Mobiluncus* species simultaneously (Hallén et al., 1987, 1988).

In addition to the detection of *Mobiluncus* spp. in BV-affected women, Hallén et al. (1987) also demonstrated the presence of *Mobiluncus* spp. in infections associated with candidal vaginitis (14 of 148 patients), chlamydial infection (14 of 65), gonorrhea (7 of 14), trichomoniasis (3 of 9), non-specific urethritis (3 of 21), and non-specific cervicitis (3 of 19).

Prevalence in healthy women. There are conflicting reports with regard to the prevalence of *Mobiluncus* spp. in vaginal secretions from healthy women. Older studies (Hallén et al., 1987; Skarin and Mardh, 1982; Sprott et al., 1983) show detection levels of up to 6%, whereas more recent conventional bacteriological studies have shown that the prevalence of *Mobiluncus* spp. in healthy women is 0.7–4% (Hillier et al., 1991). Using a PCR-based method, Schwebke and Lawing (2001) demonstrated that 38% of women showing no signs of infection harbored *Mobiluncus* spp. They also showed that *Mobiluncus mulieris* predominates in the vaginas of healthy women (*Mobiluncus curtisii* was rarely detected). Hallén et al. (1987, 1988) reported the isolation of *Mobiluncus* spp. from the rectums of women (21–54%) with *Mobiluncus*-associated BV, and have suggested that, like *Candida* spp., *Mobiluncus* spp. contaminate the vagina from the rectum, which would explain finding these organisms in healthy women

as well as those with other *Mobiluncus*-related infections. Holst (1990) isolated *Mobiluncus* spp. from rectal samples of healthy men, women, and children, and suggested that they could be part of the normal intestinal microbiota, probably occurring in low numbers. Because of the complex nature of this microbiota, isolation of *Mobiluncus* spp. from intestinal samples would therefore, be difficult. Consequently, molecular methods are more appropriate for detection of these organisms in vaginal and intestinal samples.

Role in pre-term delivery. In the past decade there has been an increased recognition that BV can contribute to pre-term delivery (Calderas et al., 1999; Hillier et al., 1995; Royce et al., 1999). In pregnant women showing pre-term labor signs and BV, Calderas et al. (1999) showed that ~7% had *Mobiluncus* spp. in their vaginal microbiota. However, no correlation between presence of *Mobiluncus* and pre-term birth has been shown. Micro-organisms associated with BV, and other genital infections, can ascend into the intra-amniotic cavity from the vagina and cervix and produce biochemical changes responsible for pre-term labor. Production of phospholipase C by *Bacteroides thetaiotaomicron*, *Gardnerella vaginalis*, *Prevotella bivia*, *Prevotella melaninogenica*, and group B streptococci has been demonstrated (McGregor et al., 1991). This enzyme has been shown to induce lysis and cellular rupture, producing loss of integrity in tissues and an increase in arachidonic acid production. These events are precursors to prostaglandin synthesis, which induces and maintains uterine contractions. However, the production of phospholipase C by *Mobiluncus* spp. has yet to be demonstrated. McGregor et al. (1994), however, did demonstrate that *Mobiluncus* spp. produce sialidase, an enzyme associated with periodontal infections. While *Mobiluncus* spp. may play a role in initiating pre-term delivery, it is more likely that the factors affecting pre-term birth are polymicrobial, and clearly more work is needed to elucidate the mechanisms by which the production of phospholipase C, sialidase, and other factors, are upregulated.

Presence in men. The presence of *Mobiluncus* spp. in male hosts has been documented by a number of authors; the general consensus is that these organisms are detectable only in a small number of men. Holst et al. (1984b) examined 309 men attending a clinic for sexually transmitted diseases and found that ten of them carried *Mobiluncus curtisii*. Four of the men found to be carrying this organism had urethritis. Vetere et al. (1987) reported the isolation of a strain of *Mobiluncus* from the seminal fluid of an infertile man. Hillier et al. (1990) obtained semen samples from 37 men attending a Special Infertility Clinic and found that 74 of the 188 isolates they obtained were anaerobic bacteria. *Mobiluncus* spp. were recovered from fewer than 10% of the specimens, with coagulase-negative streptococci, α-hemolytic streptococci, *Bacteroidaceae*, and *Gardnerella vaginalis* predominating. However, the clinical relevance, if any, of the presence of *Mobiluncus* spp. in seminal fluid was not elucidated. In a search for the possible reservoir and spread of four BV-associated micro-organisms (*Mobiluncus curtisii*, *Mobiluncus mulieris*, *Mycoplasma hominis*, and *Gardnerella vaginalis*), Holst (1990) examined the occurrence of these species in rectal, oral, and pharyngeal specimens from the male consorts of women with and without BV, homosexual men, and children. A total of 47 of 148 women with BV harbored both species, 45 only *Mobiluncus curtisii*, 20 only *Mobiluncus mulieris*, and 20 harbored neither species in their rectums. Only one healthy woman and one male partner of a healthy woman harbored *Mobiluncus curtisii*. Among the children studied, one boy and one girl harbored *Mobiluncus curtisii* in the rectum. Three girls harbored *Mobiluncus mulieris* in the rectum. *Mobiluncus* spp. were never recovered from the genital specimens of male consorts of healthy women. However, *Mobiluncus curtisii* and *Mobiluncus mulieris* were isolated from genital specimens of male partners of women with BV (*Mobiluncus curtisii* was isolated more frequently than *Mobiluncus mulieris*). None of the individuals examined in the study of Holst (1990) harbored *Mobiluncus* spp. in their throats or mouths.

Extragenital infections. There has also been increased interest in the incidence of extragenital infections caused by species of this genus. The isolation of members of the genus *Mobiluncus* from non-specific vaginal infections has been well documented. There have been a number of reports in recent years of members of the genus being isolated from extragenital infections. Extragenital infections associated with *Mobiluncus* were reported by Glupczynski et al. (1984) in four women. The strains were isolated over an 18-month period at Brugmann University Hospital, Belgium. Initially, the isolated unidentified organisms were thought to be related to *Wolinella succinogenes*. In the first reported case, a 29-year-old woman presented with a breast abscess. Her medical history indicated that she had had a benign cystadenoma removed from the opposite breast 6 months earlier, but symptoms for the breast abscess did not appear for another 5 months. Any link between the cyst removal and *Mobiluncus*-associated infection therefore seems unlikely. Direct puncture of the abscess using a needle yielded a yellowish, foul-smelling pus, suggesting the presence of anaerobic bacteria. Culture of the specimen produced "*unidentified curved anaerobic bacilli*" in association with *Bacteroides intermedius*, *Bacteroides urealyticus*, and *Peptococcus* spp.

The second case reported by Glupczynski et al. (1984) concerned a 60-year-old woman suffering from recurrent abdominal pain and bloody stools lasting 2 months. She also had a brownish discharge through the navel. The patient had a significant medical history, which included appendectomy, hysterectomy, and cholecystectomy within the previous 4 years. Laboratory findings for abdominal and rectal examinations were shown to be normal. However, bacteriological analysis of the umbilical discharge showed a mixed anaerobic flora comprising *Bacteroides intermedius*, *Wolinella succinogenes*, and anaerobic, comma-shaped rods.

The third case concerned a 41-year-old woman admitted to hospital with an exudative wound following a radical mastectomy. One month earlier the patient had undergone a total mastectomy of the left breast. On examination, a foul-smelling discharge was obtained from the lower part of the wound. Chloramphenicol powder was administered onto the wound, and total recovery was observed within 1 week. Laboratory examination of swabs taken from the wound showed the presence of *Bacteroides assacharolyticus*, *Peptostreptococcus anaerobius*, *Eubacterium* spp., and another unidentified anaerobic curved bacilli. Again, this organism was retrospectively identified as *Mobiluncus* spp.

Sturm and Sikkenk (1984) also reported the isolation of short anaerobic rods in pure culture from the discharge of a breast abscess in a 33-year-old woman. Although motile curved rods

were not isolated from the vagina of the patient, the infection was thought to be due to transfer of vaginal *Mobiluncus* to the breast area during sexual intercourse, or auto-infection via the hands. Sturm (1989) came to a similar conclusion to explain the presence of *Mobiluncus* spp., and other vagina-associated bacteria, in samples taken from breast abscesses. Weinbren et al. (1986) described the isolation of a *Mobiluncus* spp. from the non-puerperal breast abscess of a woman who had undergone a bilateral submammary excision 3 years previously. The organism was isolated in mixed culture with a *Bacteroides* spp., but the significance of the *Mobiluncus* strain in this infection is not known. Edmiston et al. (1989) also described the isolation of *Mobiluncus curtisii* (in combination with *Bacteroides bivius*, *Bacteroides capillosus*, *Bacteroides ureolyticus*, several *Peptostreptococcus* spp. and *Staphylococcus epidermidis*) from a breast abscess in a woman 5 months post-partum. The authors postulated that the trauma caused by childbirth 5 months previously may have precipitated the hematogenous seeding of the breast tissue.

The final case reported by Glupczynski et al. (1984) pertained to a 39-year-old alcoholic woman with a 1-week history of abdominal pain, fever, and jaundice. In the previous 6 months she had suffered an episode of hepatic encephalopathy. Blood cultures were taken, leading to the isolation of pure cultures of *Mobiluncus* spp. from two separate anaerobic blood bottles 9 d after they had been inoculated. In all four cases described by Glupczynski et al. (1984), none of the patients had symptoms or signs of genital tract infections. The clinical significance and source of infection were unclear for the first three cases, as the *Mobiluncus* spp. were isolated in mixed culture. In the final case, the authors suggested the colon as the likely portal of entry, as "*patients with liver cirrhosis show a high frequency of spontaneous bacteremia originating from the digestive tract*". However, this link is still tentative, as the isolation of *Mobiluncus* from the patient's stool specimen was not attempted.

Curtis (1913) isolated *Mobiluncus* spp. (in mixed culture) from a woman who suffered an infection following an instrumental abortion, and from a woman who had an infection that complicated labor at full-term. *Mobiluncus* spp. have also been isolated from an abdominal abscess of a woman with pelvic inflammatory disease (PID; cited in Fohn et al., 1988). A correlation has been found between presence of *Mobiluncus* spp. and "clue cells" in vaginal discharge and the incidence of PID after first-trimester abortions (Larsson et al., 1989); however, the role, if any, of *Mobiluncus* spp. in this disease is not known. Larsson et al. (1986) also described the identification of *Mobiluncus*-specific antibodies in serum samples from a woman with a post-operative infection (the woman had undergone a hysterectomy). Severe sepsis caused by *Mobiluncus curtisii* in a previously healthy 54-year-old woman has been documented (Hill et al., 1998). *Mobiluncus mulieris* has been isolated from an abdominal abscess in a 62-year-old woman (Mayer et al., 1994).

Hosts other than humans. The prevalence of *Mobiluncus* spp. in hosts other than man has not been investigated thoroughly. In a study of vaginal samples from 37 rhesus macaques, Doyle et al. (1991) observed the presence of curved Gram-stain-negative rods in Gram stains of vaginal smears. The macaques (age, 4–22 years old) had no history of vaginitis or septic abortion. Samples were taken in the middle of the menstrual cycle prior to bleeding. Using tests such as catalase production, oxidase production, motility, and GLC analysis of acid end products, the rods were characterized as *Mobiluncus curtisii* subsp. *curtisii* (found in 46% of all the animals tested). Half of the animals that carried *Mobiluncus curtisii* subsp. *curtisii* also harbored *Gardnerella*-like organisms. This is the first and only case describing the isolation of *Mobiluncus* spp. in hosts other than man. Notably, the *Mobiluncus curtisii* subsp. *curtisii* strains were isolated from healthy animals. To date, no environmental sources of members of the genus *Mobiluncus* have been reported.

Pathogenicity. While the presence of *Mobiluncus* spp. in vaginal fluids is associated with BV, the etiological role of these organisms in this condition is unknown and difficult to assess. *Mobiluncus* spp. have never been recovered in pure culture from vaginal discharges, and they have been identified in healthy and BV-affected women (Schaal et al., 2005). In addition, there has been no demonstration of a difference in the severity of BV symptoms when *Mobiluncus* spp. are present in or absent from the vaginal flora (Jones et al., 1985). Taking these factors into account, one cannot exclude the pathogenic potential of these organisms, as these observations are true for other anaerobic species that predominantly or exclusively occur in synergistic mixed infections (Schaal et al., 2005). It is interesting to note, however, that *Mobiluncus curtisii* appears to be more prevalent than *Mobiluncus mulieris* in BV patients (Moi et al., 1991; Schwebke and Lawing, 2001).

Potential virulence factors. Several studies have shown that *Mobiluncus* spp. can attach to vaginal epithelial cells. Fredricsson et al. (1984) showed that, compared to *Gardnerella vaginalis*, *Mobiluncus* spp. had a greater degree of adherence to vaginal epithelial cells. Moi et al. (1984a) examined the influence of pH on the adhesion of *Mobiluncus* spp. to vaginal epithelial cells obtained from healthy women. They found that adherence of *Mobiluncus* spp. increased as the pH increased, with *Mobiluncus mulieris* adhering significantly better than *Mobiluncus curtisii* at pH 7.5 when compared with pH 4.0. They also demonstrated that "clue cells" seen in wet mounts from vaginal discharge of women with BV were very similar to *Mobiluncus*-covered vaginal epithelial cells in *in vitro* tests. Catalanotti et al. (1994) also demonstrated the adhesion of *Mobiluncus curtisii* to vaginal epithelial cells at pH 5.4, the normal vaginal pH on the 22nd day of the menstrual cycle, but showed that it adhered less well at 4.8 (the pH on the 24th day of the menstrual cycle) and 4.4 (the pH during peak ovulatory phases). De Boer and Plantema (1988) showed that *Mobiluncus* spp. attached both directly to epithelial cell surfaces and at various distances from them. They concluded that, after initial attachment, *Mobiluncus* spp. can grow at the epithelial surface in sessile microcolonies. Also conspicuous was the ability of the curved bacilli to attach to the epithelial cells via their cell tips. However, *in situ*, no specialized bacterial cell surface structures were seen that might explain this polar attachment. Using ruthenium red staining, they also demonstrated the presence of precipitated glycocalyx material on both the flagellae and surfaces of the bacilli. This led the authors to postulate that the bacilli were enclosed in a very hydrated matrix of exopolysaccharides. In fact, the production of a glycocalyx was demonstrated *in vitro* for *Mobiluncus curtisii* subsp. *curtisii* and *Mobiluncus mulieris* (De Boer and Plantema, 1988). Using luminol-enhanced chemiluminescence, *Mobiluncus curtisii* was shown to induce a significantly less pronounced oxidative metabolism of polymorphonuclear leukocytes than

Mobiluncus mulieris, implying that the former species is able to escape phagocytosis more easily (Schaal et al., 2006). This observation led Schaal et al. (2006) to postulate that the curved rods seen inside phagocytic vesicles of vaginal polymorphonuclear leukocytes by De Boer and Plantema (1988) were *Mobiluncus mulieris* rather than *Mobiluncus curtisii*.

Eleven strains of *Mobiluncus curtisii* and four strains of *Mobiluncus mulieris* were shown to produce a cytotoxin after growth in three different liquid media (Taylor-Robinson et al., 1993). The cytotoxin, present in supernatant fluids after centrifugation, was active against Vero cells and four other cell lines tested, producing a marked cytopathic effect within 72 h. Of the supernatant fluids from eight *Mobiluncus curtisii* strains and two *Mobiluncus mulieris* strains tested, all caused loss of ciliary vigor of bovine oviduct organ cultures. This process was usually complete after 60 h and caused raggedness of the epithelial cell border and loss of cilia. The toxin was extracellular, relatively heat-stable, inactivated under extremes of pH, and not found in colonies isolated from solid medium nor in centrifuged deposits from organisms grown in broth culture. However, the demonstration of the ability of *Mobiluncus* spp. to produce toxin suggests that these organisms could have a role in BV. The findings of Menolascina et al. (1999) corroborated the results of Taylor-Robinson et al. (1993), since filtered supernatants of 11 of 12 *Mobiluncus* strains tested produced a cytopathic effect on HeLa cells at dilutions of up to 1:16.

Ison et al. (1989) presented preliminary data showing that one of their mAbs (180.1) inhibited attachment of *Mobiluncus curtisii* cells to exfoliated vaginal epithelial cells *in vitro*. They postulated that this was a situation analogous to that observed in *Neisseria gonorrhoeae* with regard to its PII family of proteins: these proteins enhance the ability of gonococci to adhere to vaginal epithelial cells. Therefore, Ison et al. (1989) may have detected an outer-membrane protein in *Mobiluncus curtisii* that had a role in colonization; however, this hypothesis requires support through more-detailed studies.

Clearly, more work is required to elucidate the adhesin(s) present in the cell wall of *Mobiluncus* spp. and to characterize the toxin(s).

Enrichment and isolation procedures

Members of the genus *Mobiluncus* are fastidious anaerobic organisms and, as such, require enriched media to grow. Their isolation from vaginal samples has proven difficult over the years as they are slow growing and are rapidly overgrown by co-habiting organisms, such as *Bacteroides* spp., *Gardnerella vaginalis*, *Lactobacillus* spp., and *Streptococcus* spp. (Hammann et al., 1984; Hernández et al., 1994; Smith and Moore, 1988). *Mobiluncus* spp. are easily grown on non-selective, nutrient-rich medium once they have been isolated from the other abundant anaerobic micro-organisms associated with BV. The process of isolating *Mobiluncus* spp. in pure culture can be lengthy, taking weeks of subculturing before pure cultures are obtained. For this reason, a number of different selective media have been designed over the years, with a view to improving the yield of *Mobiluncus* strains from vaginal samples. Most of these media rely on the addition of animal blood or serum to provide the enriched environment that the micro-organisms require. Recovery of *Mobiluncus* spp. from vaginal samples can also be enhanced by the use of antibiotics/inhibitors in media, and the use of selective techniques. Table 16 details some of the selective agars that have been used for the isolation of *Mobiluncus* spp. from human vaginal samples.

TABLE 16. Examples of agars used to isolate and culture *Mobiluncus* strains

Agar used[a]	Reference(s)
Blood agar	Curtis (1913); Moore (1954)
Colistin/nalidixic acid agar + 2.5% horse serum, nalidixic acid (5 µg/ml)	Durieux and Dublanchet (1980); Holst et al. (1982)
Enriched tryptone soya agar	Fontaine et al. (1982)
Brain heart infusion agar + 5% sheep blood, hemin (5 µg/ml), 0.5% yeast extract, menadione (0.5 µg/ml)	Hjelm et al. (1982)
Columbia blood agar + hemin (0.5 µg/ml), vitamin K_1 (0.5 µg/ml)	Holst et al. (1982)
Columbia blood agar	Phillips and Taylor (1982)
Oxoid DST agar + 5% defibrinated horse blood, 1% hemin, 0.2% vitamin K, 0.5% yeast extract	Sprott et al. (1982)
Columbia agar + 5% human blood, 0.2% vitamin K	Sprott et al. (1983)
Columbia agar + tinidazole (1 mg) + colistin (10 mg) + nalidixic acid (15 mg)	Holst et al. (1984a)
Brain heart infusion agar + 5% horse blood	Vetere et al. (1987)
Brucella agar + 5% horse blood	Vetere et al. (1987)
Wilkins–Chalgren agar + 5% horse blood	Vetere et al. (1987)
SA agar. Columbia blood agar + laked rabbit or sheep blood (16 ml) + rabbit serum (20 ml)	Smith and Moore (1988)
Rlk agar. Columbia CNA agar + yeast extract (6 g) + peptone (20 g)	Smith and Moore (1988)
Mobi agar. Columbia broth + Bacto agar + cysteine hydrochloride (0.4 g) + soluble starch (10 g) + resazurin solution (4 ml of 11 mg/44 ml solution) + colistin methane sulfonate (10 mg) + nalidixic acid (15 mg)	Spiegel (1992)
Horse blood agar	Gatti (2000)
Schaedler blood agar 107 (with nalidixic acid, 1 µg/ml)	Calderas et al. (1999)
Schaedler blood agar 108 (with nalidixic acid, 1 µg/ml, and vancomycin, 2.5 µg/ml)	Calderas et al. (1999)

[a]All formulations are for the preparation of 1 liter of medium. All % values are v/v percents, except for the % of yeast extract, which is a w/v percent.

In the past, the blood from a wide variety of mammals has been used in the preparation of these selective media: e.g., human (Curtis, 1913; Hammann et al., 1984; Hjelm et al., 1984; Moore, 1954; Sprott et al., 1983), goat (Curtis, 1913), sheep (Curtis, 1913; Hammann et al., 1984; Moore, 1954; Smith and Moore, 1988), rabbit (Smith and Moore, 1988), guinea pig (Moore, 1954) and horse (Fox and Phillips, 1984; Moore, 1954; Sprott et al., 1982). Although a wide variety of blood types has been used, it is generally recognized that the source of the blood used in the medium does not greatly affect the growth of

strains of *Mobiluncus* spp. (although hemolytic activity is always observed on rabbit blood; Thomason et al., 1984). A wide range of blood concentrations (4–20%) have been used for the cultivation of *Mobiluncus* spp. from vaginal samples, although blood concentration above 4% has no effect on the growth or hemolytic activity of strains of *Mobiluncus* spp. (Holst et al., 1982). In addition, no appreciable difference in colony size is noted when laked blood is used as a substitute to whole blood (Thomason et al., 1984). The addition of serum to solid medium appears to stimulate the growth of *Mobiluncus* strains. Thomason et al. (1984) noted that, although serum improves the growth of *Mobiluncus* spp., only the addition of fetal calf serum to the medium increases the size of colonies. They suggested the use of a colistin/nalidixic acid (CNA)-based agar containing 5% rabbit blood and 7% fetal calf serum to maximize the growth of *Mobiluncus* strains.

It is questionable whether both colistin and nalidixic acid should be added to the selective agars described, as no synergistic effect has been demonstrated between them (Holst et al., 1984a). Also the usefulness of CNA-based selective agars to enrich for *Mobiluncus* strains is somewhat controversial, as Hammann et al. (1984) have suggested that the CNA agar of Durieux and Dublanchet (1980) regularly allowed good growth of *Bacteroides* spp., *Gardnerella vaginalis*, lactobacilli, and anaerobic cocci. Other inhibitory agents added to solid media have included trimethoprim plus polymyxin B, tinidazole plus colistin or nalidixic acid, tinidazole (to which some strains of *Mobiluncus mulieris* are susceptible at concentrations greater than 4 μg/ml), and Nile blue A (Spiegel, 1992).

Growth of *Mobiluncus* spp. in broth culture is relatively poor. However, Vetere et al. (1987) showed that strains grew best in broth cultures when the pH of the medium was 6–8 (sparse growth was observed at pH <6). Numerous broths have been tested for their suitability as growth media, including peptone/yeast, peptone/yeast/glucose, thioglycollate and Mueller–Hinton (Holst et al., 1982), Todd–Hewitt broth and Robertson's cooked meat medium (with and without hemin, sodium fumarate, sodium formate, and vitamin K; Sprott et al., 1983), and tryptone soya and brain heart infusion (Fontaine et al., 1982). Curtis (1913) was unable to culture *Mobiluncus* spp. in broth culture, but this was likely due to the limitations on anaerobic culturing at the beginning of the twentieth century. Sprott et al. (1983) reported that they were unable to grow *Mobiluncus* spp. consistently well in liquid culture, and that the addition of vitamin K to a medium had no effect on the level of growth. There are conflicting reports regarding the growth benefits of serum addition to broths (Holst et al., 1982; Taylor-Robinson and Taylor-Robinson, 2002; Vetere et al., 1987). Several authors have reported that fumarate/formate solution has no effect on the growth of *Mobiluncus* spp., in either solid or liquid medium (Hjelm et al., 1982; Spiegel and Roberts, 1984a; Sprott et al., 1983; Vetere et al., 1987). However, Holst et al. (1982) observed that the addition of 0.3% fumarate/formate slightly improved growth in chopped meat, peptone/yeast, and peptone/yeast/glucose broths. Holst et al. (1982) also observed that the addition of 3% horse serum to thioglycollate broth led to the development of heavy, turbid, granular growth. Spiegel and Roberts (1984a) found that growth in peptone/yeast broth was improved by the addition of maltose and glucose (carbohydrates found in the human vagina). Moore (1954) obtained best growth of the species in Brewer's thioglycollate broth enriched with 10% serum. Taylor-Robinson and Taylor-Robinson (2002) evaluated the suitability of nine different broths for growth of *Mobiluncus* strains. They concluded that a few commercial liquid media were excellent for growth, and even suggested that Columbia blood broth or peptone starch glucose broth would suffice if supplemented with horse serum (10%). A bacterial strain was selected that was known to multiply to high numbers (as some strains, whatever the medium, consistently reached maximal titers greater than others), and an initial inoculum of 10^5–10^6 organisms per milliliter was used.

Two different enrichment techniques have been suggested for improving the yield of *Mobiluncus* spp. from vaginal samples. The first method involves pre-treatment of the vaginal sample with alkali (Påhlson and Forsum, 1985; Påhlson et al., 1986b) and is based on the knowledge that *Mobiluncus* spp. are more resistant to alkaline solutions than most of the other bacterial species present in vaginal discharge. Påhlson and colleagues suggested that the high content of extractable lipids (approx. 0.7% wet weight in packed cells, compared with 0.3 and 0.4% for *Escherichia coli* and *Bacteroides fragilis*, respectively) may account for the success of this technique. Samples of vaginal discharge were diluted in buffer (pH 12.0) for 10 min, leading to a tenfold drop in the recovery of organisms other than members of the genus *Mobiluncus*. Treatments at pH 12.3 and 12.6 for 5–10 min were also shown to be effective, but the recovery of *Mobiluncus* spp. after 10 min was drastically reduced or not possible due to death of the bacteria, respectively. Within the genus *Mobiluncus*, species and strains differ in their tolerance to alkali, with *Mobiluncus curtisii* strains having prolonged survival times in high-pH buffers. These authors also pre-cultured samples in an enrichment broth, such as brain heart infusion broth supplemented with 2% inactivated serum and filter-sterilized maltose solution (1% final concentration) and pH 12.0. After 2 d growth, the samples were subcultured onto plates, and pure cultures of *Mobiluncus* spp. were available within 1–2 weeks.

The second method involves cold enrichment at 4 or 5°C (Smith and Moore, 1988). These authors used Rlk agar, SA agar, Brucella blood agar, and Mobi agar (Table 16). Storage of pure cultures of *Mobiluncus curtisii* and *Mobiluncus mulieris* for 1, 5, and 25 h in anaerobic storage medium under cold enrichment conditions prior to inoculation onto each of the four different agars did not enhance the recovery of *Mobiluncus* spp.; in fact, recovery of these organisms decreased (10–40 c.f.u. to 1–9 c.f.u.) on Brucella blood agar when compared with cultures kept at room temperature (for 24 h) prior to inoculation. In mixed cultures containing *Bacteroides* spp., *Gardnerella vaginalis*, *Peptostreptococcus* spp., *Staphylococcus aureus*, *Mobiluncus mulieris*, *Mobiluncus curtisii* subsp. *curtisii*, and *Mobiluncus curtisii* subsp. *holmesii*, recovery of *Mobiluncus* spp. increased after cold enrichment on Rlk and SA media (1–9 c.f.u. after 1 h at room temperature to 10–40 c.f.u. after 1, 5, and 24 h at cold enrichment). Growth of *Mobiluncus* spp. at room and cold temperatures was poor on Brucella blood agar (0 to 1–9 c.f.u.) and prone to overgrowth by other organisms on Mobi agar. The most abundant growth of *Mobiluncus* spp. was obtained on SA medium. However, Smith and Moore (1988) recommend the use of both media in clinical isolation of *Mobiluncus* spp., since differences in chemical composition (i.e., yeast extract, additional peptone and laked horse blood versus serum and laked

blood) between media allows the growth of different organisms. Hence, overgrowth does not usually occur in both media. Use of this method allows *Mobiluncus* spp. to grow at rates similar to those of other anaerobic members of the vaginal flora, and allows their isolation even when they are present in very low numbers. This method is effective in the isolation of *Mobiluncus* spp. from mixed populations according to Smith and Moore (1988) because cold enrichment slows the metabolism of competing flora present during BV to a rate that allows *Mobiluncus* spp. to compete successfully with them; cold temperature, in combination with the selective media, provides *Mobiluncus* spp. with the advantage they need to survive among the less-fastidious, rapidly multiplying mixed flora.

Maintenance of *Mobiluncus* strains is also relatively easy. A number of suitable methods have been suggested for storing strains of *Mobiluncus*. These include traditional skimmed-milk/freeze-drying treatments before storage at 10°C or at −70°C. Hammann et al. (1984) suggested maintenance in Rosenow broth (48 h, 37°C) under sterile paraffin wax or under CO_2 and black stoppers and then freezing at −20 or −70°C. Samples were stored by Spiegel and Roberts (1984a) at −20 or −70°C in double-strength skimmed milk and used within five passages of recovery from frozen stocks. Samples stored at −70°C in 50% fetal calf serum plus 50% trypticase soy broth (Fohn et al., 1988) were still viable two years after freezing.

Differentiation of the genus *Mobiluncus* from other genera

Characteristics useful for differentiating *Mobiluncus* spp. from related genera can be found in Table 17.

Taxonomic comments

Members of the genus *Mobiluncus* have been known by different names: "Vibrion de Curtis", "*Vibrio mulieris*" (Prévot, 1940), and "vibrions succinoproducteurs" (Durieux and Dublanchet, 1980). However, after a number of studies on strains of curved rods from the vagina (Blackwell et al., 1983; Fontaine et al., 1982; Hammann et al., 1984; Hjelm et al., 1982; Holst et al., 1982; Phillips and Taylor, 1982; Skarin and Mardh, 1982; Spiegel and Roberts, 1984a; Sprott et al., 1982, 1983; Taylor and Owen, 1984) these organisms were shown to differ from organisms of the genera recognized at that time.

On the basis of these data, studies were carried out to characterize the relationship of these organisms. Two studies were undertaken concurrently, leading to descriptions of the genera *Falcivibrio* (Hammann et al., 1984) and *Mobiluncus* (Spiegel and Roberts, 1984a). Although the name *Falcivibrio* was published first, it was not validated in the *International Journal of Systematic Bacteriology* first; therefore, the name *Mobiluncus* takes precedence. However, the genus name *Falcivibrio* has not been removed from the approved list of bacterial names. Spiegel and Roberts (1984a) tentatively placed the genus *Mobiluncus* in the family *Bacteroidaceae*; however, a later 16S rRNA gene-based study by Lassnig et al. (1989) demonstrated that the genus seemingly belongs to the order *Actinomycetaceae*, family *Actinomycetaceae*.

Currently, two species of the genus are recognized: *Mobiluncus curtisii* (*Falcivibrio vaginalis*) and *Mobiluncus mulieris* (*Falcivibrio grandis*). *Mobiluncus mulieris* is negative for hippurate hydrolysis, arginine dihydrolase activity, and β-galactosidase activity, while *Mobiluncus curtisii* is positive for these traits. SDS-PAGE, DNA–DNA hybridization, serological, and 16S rRNA gene sequence studies have supported the delineation of the two species (Baron et al., 1984; Hoyles et al., 2004; Moi and Danielson, 1984; Spiegel and Roberts, 1984a; Taylor and Owen, 1984; Tiveljung et al., 1996). *Mobiluncus curtisii* was divided into two subspecies, *Mobiluncus curtisii* subsp. *curtisii* (nitrate-negative and able to migrate through soft agar) and *Mobiluncus curtisii* subsp. *holmesii* (nitrate-positive and unable to migrate through soft agar) (Spiegel and Roberts, 1984a), but evidence for the need of subspecies is conflicting. DNA–DNA hybridization studies on "short curved rod" (*Mobiluncus curtisii*) strains showed that strains comprising *Mobiluncus curtisii* may represent several species (Christiansen et al., 1984); however, further biochemical and serological studies were required for delineation of this species. The case for subspecies was supported by the absence of surface antigens of *Mobiluncus curtisii* subsp. *curtisii* from *Mobiluncus curtisii* subsp. *holmesii* (Fohn et al., 1988). However, Påhlson et al. (1986a) were able to distinguish between *Mobiluncus curtisii* and *Mobiluncus mulieris* (and atypical) strains but not able to split the *Mobiluncus curtisii* strains into the two subspecies using mAbs. Hoyles et al. (2004) found that the type strains of the two subspecies were unable to reduce nitrate or pit agar. Other examples of the phenotypic complexity among *Mobiluncus* spp., particularly within *Mobiluncus curtisii* strains, are available within the literature (Carlone et al., 1986; Garlind et al., 1989; Skarin, 1986; Vetere et al., 1987). The identification of atypical strains has also clouded the issue of speciation within the genus *Mobiluncus* (Christiansen et al., 1984; Påhlson et al., 1986a; Vetere et al., 1987).

Hoyles et al. (2004) also found that the system used to biochemically characterize strains produced varying results. While useful for characterizing *Mobiluncus* spp., biochemical tests produce results that are highly method-dependent. This may be especially true for the detection of weak or very weak acid production from carbohydrates, which may also be influenced by size and viability of inoculum, or the techniques used for obtaining anaerobic growth conditions (Schaal et al., 2005). Clearly, fermentation pattern is not a reliable criterion for differentiation of *Mobiluncus* spp. (Vetere et al., 1987). Spiegel (1992) is of the opinion that differences between the subspecies are due mainly to normal strain variation and differences in media and methods. In contrast, Hoyles et al. (2004) think that *Mobiluncus curtisii* strains should be considered a single but complex entity that cannot be separated into distinct subspecies on the basis of physiological markers alone. The taxonomy of the genus *Mobiluncus* is still unclear because not all strains fulfil the criteria proposed for the two species (Vetere et al., 1987). However, molecular tools provide the opportunity to determine the true taxonomy of this interesting group of bacteria.

Further reading

Mårdh, P.-A. and Taylor-Robinson, D. (editors). 1984. Bacterial Vaginosis. Almqvist and Wilkinson International, Stockholm.

Differentiation of the species of the genus *Mobiluncus*

The traits used to differentiate *Mobiluncus curtisii* and *Mobiluncus mulieris* are shown in Table 18. Strains can be allocated to species using 16S rRNA gene sequencing. *Mobiluncus curtisii* and *Mobiluncus mulieris* can be separated on the basis of differences

TABLE 17. Characteristics useful for distinguishing *Mobiluncus* from related genera[a,b]

Genus	*Actinomyces*	*Arcanobacterium*	*Actinobaculum*	*Varibaculum*	*Mobiluncus*
End products of glucose fermentation	Acetic, lactic, and succinic (major/minor) acids[c]	Acetic (major/minor), lactic, and succinic (minor) acids	Acetic and succinic acids	Lactic, acetic (minor), and succinic acids	Acetic, lactic (minor/absent), and succinic acids
DNA G+C content (mol%)	57.5–71	48.4–66	55–57	51.7	49–54
Aerobic growth	v	+	v	–	–
Motile	–	–	–	–	+
Filamentous microcolonies	+	–	–	–	–
Nitrate reduction	v	v	–	v	v
Catalase	v	v	–	–	–
Habitat	Mucosal surfaces of humans and animals[d]	Presumed to be commensals of mucous surfaces of warm-blooded animals	Unknown, but probably genital or urinary tract[e]	Not known, but isolated from human clinical sources[f]	Human vagina and rectum; vagina of rhesus macaque

[a]Symbols and abbreviations: v, variability among species and strains of the genus; –, negative; +, positive.

[b]All data are from Bowden (1998), Buchanan et al. (1984), Cato et al. (1984), Collins and Cummins (1986a), Collins et al. (1982b, 1993), Dent and Williams (1984b, 1984c, 1986), Funke et al. (1994, 1995, 1997a), Hall et al. (2003e), Hammann et al. (1984), Johnson et al. (1990), Lawson et al. (1997, 2001b), Lehnen et al. (2006), Pascual et al. (1999, 1997a), Pascual Ramos (1999), Pascual Ramos et al. (1997b), Reddy et al. (1982), Schaal (1986b), Schaal et al. (2006), Spiegel and Roberts (1984a), Vandamme et al. (1998), and Wüst et al. (1995a).

[c]Formic and pyruvic acids are sometimes produced by strains of *Actinomyces georgiae*, *Actinomyces gerencseriae*, and *Actinomyces meyeri*. Lactic and pyruvic acids only detected in *Actinomyces georgiae* cultures when incubated aerobically (Johnson et al., 1990).

[d]*Actinomyces radingae* and *Actinomyces turicensis* strains have been isolated from human feces (L. Hoyles, unpublished data).

[e]An *Actinobaculum schaalii*-like bacterium has been isolated from human feces (L. Hoyles, unpublished data).

[f]*Varibaculum cambriense*-like strains have been isolated from human feces (L. Hoyles, unpublished data).

TABLE 18. Tests useful for differentiating *Mobiluncus curtisii* and *Mobiluncus mulieris*[a]

Biochemical test	*M. curtisii* ($n = 12$)	*M. mulieris* ($n = 10$)
Arginine dihydrolase	+	−
β-Galactosidase	$+^-$	−
β-Galacturonidase	−/+	−
α-Galactosidase	$+^-$	−
α-Fucosidase	−	−/+
Pyrazinamidase	−/+	−
Tyrosine arylamidase	−/w	+
Alanine arylamidase	−/w	+
α-Glucosidase	−/+	+
Serine arylamidase	−	−/w
Leucine arylamidase	−/+	+
Hippurate hydrolysis	$+^-$	−
Maltose fermentation	$-^+$	+
Sucrose fermentation	−	$+^-$
Ribose fermentation	−	$+^-$
Trehalose fermentation	−	+
Nitrate reduction	$-^+$	−
Leucyl glycine arylamidase	+	−/w

Symbols and abbreviations: +, All or ≥95% strains positive; −, all or ≥95% strains negative; −/+, variable reaction; $+^-$, majority of strains positive, but some negative; −/w, strains negative or weak; $-^w$, majority of strains negative, but some weak; $-^+$, majority of strains negative, but some positive.

[a]Results presented here are representative for four different API strips (Hoyles et al., 2004).

in the V3 (GATGGTTCCAGAGATGGGCCAGCCTT→GA CA-T GCCAGAGATGG TGTGGCCTT) and V4 (AGCG-GTTCGGCCGGGG→AGC ACGTT ATG GTGGGG) regions (Hoyles et al., 2004; Tiveljung et al., 1995, 1996) of their 16S rRNA gene sequences. There appears to be sequence heterogeneity among strains of *Mobiluncus curtisii*, while none is observed among *Mobiluncus mulieris* strains (Hoyles et al., 2004). SDS-PAGE has been used by a number of different groups to split *Mobiluncus* strains into the two species (Baron et al., 1984; Drouet et al., 1991; Hoyles et al., 2004; Taylor and Owen, 1984; Vetere et al., 1987). Intraspecies levels of association suggest that members of the two species represent several distinct groups, as correlations within strains designated *Mobiluncus curtisii* were as low as ~55%, and those within strains of *Mobiluncus mulieris* were as low as ~65% (Hoyles et al., 2004). High correlation values (~84%) for SDS-PAGE profiles and the corresponding biochemical profiles of the same *Mobiluncus curtisii* strains did not correlate. This was also observed for *Mobiluncus mulieris* strains (even though some pairs showed correlation values of >90%). SDS-PAGE analysis is a useful tool for differentiating between the two species of *Mobiluncus* and in intraspecies typing; however, it is of little use in subspecies delineation. Although analysis of whole-cell proteins allows intraspecies clustering of strains (Drouet et al., 1991), these clusters are meaningless because the biochemical properties of the strains are complex.

Preliminary work using pulsed-field gel electrophoresis has demonstrated that *Mobiluncus curtisii* and *Mobiluncus mulieris* can be separated using the enzyme *Spe*I (Hoyles et al., 2004). Although further work is required to optimize conditions for isolation of high-quality DNA from *Mobiluncus mulieris* strains, use of *Spe*I demonstrated strain heterogeneity among *Mobiluncus curtisii* isolates, supporting the conclusion of heterogeneity within this species drawn by researchers using SDS-PAGE.

Ampicillin, cefotoxin, cephalothin, and moxalactam are more active against *Mobiluncus mulieris* strains than *Mobiluncus curtisii*, while colistin is more active against *Mobiluncus curtisii* than *Mobiluncus mulieris* (Spiegel, 1987). *Mobiluncus mulieris* is more susceptible than *Mobiluncus curtisii* to alizarin red, Evans blue, and sodium fluoride (Spiegel, 1987). As discussed in the Antibodies section of this chapter, *Mobiluncus curtisii* and *Mobiluncus mulieris* can be distinguished by using species-specific mAbs.

List of species of the genus *Mobiluncus*

1. **Mobiluncus curtisii** Spiegel and Roberts 1984a, 181[VP] emend. Hoyles, Collins, Falsen, Nikolaitchouk and McCartney 2004 (Later heterotypic synonym: *Falcivibrio vaginalis* Hammann, Kronibus, Viebahn and Brandis 1984.)

   cur.ti′si.i. N.L. gen. masc. n. *curtisii* of Curtis, named after A.H. Curtis, who isolated the first strain.

   The species description is based on those of Spiegel and Roberts (1984a), Hammann et al. (1984), and Hoyles et al. (2004). Members of the species have the characteristics of the genus. Cells are anaerobic, Gram-stain-variable or Gram-stain-negative, slightly curved, non-spore-forming, rod-shaped (0.4–0.6 µm wide and 2–3 µm long, with ends slightly rounded and often pointed), and occur singly or in pairs. Motile by means of multiple subterminal flagella. Colonies on chocolate agar are cream to light-brown in color, convex, smooth, and entire after 5 d anaerobic incubation at 37°C, reaching 2–4 mm in diameter. Cultures are more turbid in peptone/yeast extract broth supplemented with rabbit serum and glycogen or maltose than in peptone/yeast extract broth supplemented with glucose and rabbit serum or rabbit serum alone. Growth is stimulated by arginine, and by fumarate, which is reduced to succinate. Traces of formate are formed from malate. Ammonia, citrulline, and ornithine are produced from arginine. Positive for arginine dihydrolase and histidine arylamidase (except for CCUG 21018[T]); negative for α-fucosidase and serine arylamidase activity. Variable activity for β-galacturonidase (RapID 32Strep system), α-galactosidase (positive API ZYM, variable API CORYNE), α-glucosidase (positive API 32AN and API ZYM, variable API CORYNE), β-galactosidase (API ZYM), pyrazinamidase (API CORYNE), tyrosine arylamidase (API 32AN), alanine arylamidase (API 32AN), phosphoaminidase (API ZYM), and leucine arylamidase (API ZYM). Negative for serine arylamidase activity. Does not produce acid from cyclodextrin, glucogen, melezitose, melibiose (except strain CCUG 22338), pullulan, ribose, sucrose, tagatose, or trehalose. Using the API CORYNE system, production of acid from glucose, lactose, maltose, and D-xylose is variable;

negative for acid production from maltose using the RapID 32Strep system; variable acid production from mannose and raffinose using the API 32AN system. Fermentation products from glycogen include succinate, trace amounts of fumarate, and moderate amounts of acetate. Products in peptone/yeast extract/glucose broth are acetic, lactic, and succinic acids; the same products are formed in peptone/yeast extract broth but in smaller amounts. Minor amounts of gas formed under a paraffin wax seal over a broth culture. Most strains hydrolyze hippurate (strains CCUG 44166B and CCUG 14425 do not). Esculin is generally not hydrolyzed (CCUG 22339 and CCUG 44116B positive). Starch and hippurate are hydrolyzed in the absence of serum. Reduction of nitrate to nitrite is variable. May "pit" agar (1.5%) after 5 d anaerobic incubation at 37°C. May migrate through soft (0.25%) agar. A weak CAMP reaction is produced on Brucella agar supplemented with 5% sheep blood when *Staphylococcus aureus* ATCC 25923 is used. Associated with BV, but role in pathogenicity is not known.

Source: human vagina.

DNA G+C content (mol%): 51–52 (T_m; Spiegel and Roberts, 1984a); 53–54 (T_m; Hammann et al., 1984).

Type strain: BV345-16, ATCC 35241, CCUG 21018, NCTC 11656.

Sequence accession no. (16S rRNA gene): AJ427623.

1a. **Mobiluncus curtisii subsp. curtisii** Spiegel and Roberts 1984a, 181[VP]

Strains have the characteristics of the genus and those given for *Mobiluncus curtisii.*

DNA G+C content (mol%): 51–52 (T_m; Spiegel and Roberts, 1984a); 53–54 (T_m; Hammann et al., 1984).

Type strain: BV345-16, ATCC 35241, CCUG 21018, NCTC 11656.

Sequence accession no. (16S rRNA gene): AJ427623.

1b. **Mobiluncus curtisii subsp. holmesii** Spiegel and Roberts 1984a, 181[VP]

holme′si.i. N.L. gen. masc. n. *holmesii* of Holmes, named after K.K. Holmes, a researcher of sexually transmitted diseases.

Strains have the characteristics of the genus and those given for *Mobiluncus curtisii.*

DNA G+C content (mol%): 51–52 (T_m; Spiegel and Roberts, 1984a); 53–54 (T_m; Hammann et al., 1984).

Type strain: BV376-6, ATCC 35242, CCUG 17762, LMG 7786, NCTC 11657.

Sequence accession no. (16S rRNA gene): AJ427624.

2. **Mobiluncus mulieris** Spiegel and Roberts 1984a, 181[VP] (Later heterotypic synonym: *Falcivibrio grandis* Hammann, Kronibus, Viebahn and Brandis 1984.)

mu.li′e.ris. L. gen. n. *mulieris* of a woman.

Species description is based on those of Spiegel and Roberts (1984a), Hammann et al. (1984), and Hoyles et al. (2004). Members of the species have the characteristics of the genus. Cells are anaerobic, Gram-stain-variable or Gram-stain-negative, curved, non-spore-forming, rod-shaped, and occur singly, sometimes in pairs (then S- or U-shaped). Motile by means of multiple subterminal flagella. Cells are 0.4–0.6 μm wide and 2.5–5 μm long, and are usually thicker in the middle, with tapering ends. Colonies on chocolate agar are light-brown to brown in color, convex, smooth, and entire after 5 d anaerobic incubation at 37°C, reaching 2–4 mm in diameter. Growth is stimulated by fumarate, which is reduced to succinate and partially oxidized to malate. Cultures are more turbid in peptone/yeast extract broth supplemented with rabbit serum and glycogen or maltose than in peptone/yeast extract broth supplemented with rabbit serum alone. Growth is not stimulated by arginine. Citrulline, ornithine, and ammonia are not produced from arginine. Positive for alanine arylamidase, ester lipase (C8) (CCUG 17992 and 30101 weak-positive; API ZYM), α-glucosidase, and tyrosine arylamidase activity. Negative for arginine dihydrolase, β-galacturonidase, α-galactosidase, β-galactosidase, β-glucosidase, pyrazinamidase, and phosphoamidase (CCUG 20071^T weak-positive; API ZYM) activity. Negative to weak activity for esterase (C4) (API ZYM) and leucyl glycine arylamidase, glycine arylamidase, histidine arylamidase, and serine arylamidase (API 32AN). Variable activity for α-fucosidase. Produces acid from glucose (except for CCUG 30101, which is negative for this trait; API CORYNE), maltose, and trehalose. Does not produce acid from lactose. Variable acid production from ribose and sucrose using the RapID 32Strep and API CORYNE systems; variable acid production from melezitose, pullulan, and tagatose (RapID 32Strep), glucogen and D-xylose (API CORYNE), and mannose (API 32AN). Weak to negative acid production from cyclodextrin and raffinose. Fermentation products from glycogen include acetate, succinate, and trace amounts of fumarate; some strains produce trace amounts of oxaloacetate, pyruvate, and/or lactate. Products in peptone/yeast extract/glucose broth are acetic, lactic, and succinic acids; the same products are formed in peptone/yeast extract broth but in smaller amounts. Traces of gas formed under a paraffin wax seal over a broth culture. Esculin and hippurate are not hydrolyzed. Starch is hydrolyzed in the absence of serum. May "pit" agar after 5 d anaerobic incubation at 37°C. May migrate through soft (0.25%) agar. Nitrate is not reduced to nitrite. A strong CAMP reaction is produced on Brucella agar supplemented with 5% sheep blood when *Staphylococcus aureus* ATCC 25923 is used. Associated with BV, but role in pathogenicity is not known.

Source: human vagina.

DNA G+C content (mol%): 49–50 (T_m; Spiegel and Roberts, 1984a); 50–53 (T_m; Hammann et al., 1984).

Type strain: SV17J, ATCC 35243, CCUG 20071, LMG 7787, NCTC 11658.

Sequence accession no. (16S rRNA gene): AJ427625.

Genus V. **Varibaculum** Hall, Collins, Lawson, Hutson, Falsen, Inganas and Duerden 2003e, 644[VP]

Val Hall

Va.ri.ba′cu.lum. L. adj. *varus* bent, crooked; L. neut. n. *baculum* a small rod; N.L. neut. n. *Varibaculum* small bent rod.

Short, straight or curved diphtheroidal cells. Gram-stain-positive. Non-acid-fast. Non-spore-forming. Nonmotile. Facultatively anaerobic, may grow poorly in air and air plus 5% CO_2. Colonies are tiny, nondescript, and non-hemolytic. **Catalase-negative**. **End products of glucose metabolism are lactic and succinic acids with small amounts of acetic acid**. Acid is produced from glucose and some other sugars. **Hippurate is hydrolyzed**, but esculin, gelatin, and starch are not. Acetoin is not produced. Leucine arylamidase and α-glucosidase are weakly positive. Arginine dihydrolase, indole, pyrazinaminidase, and urease-negative. **Most isolates reduce nitrate to nitrite**. Major fatty acids are $C_{16:0}$, $C_{18:0}$, $C_{18:1}$ ω9*c*, and $C_{18:2}$ ω6,9*c*.

DNA G+C content (mol%): 51.7 (HPLC).

Type species: **Varibaculum cambriense** Hall, Collins, Lawson, Hutson, Falsen, Inganas and Duerden 2003e, 644[VP].

Further descriptive information

Phylogenetic analysis has demonstrated that the genus *Varibaculum* belongs to the high G+C Gram-stain-positive class *Actinobacteria* and is most closely related to, but clearly distinct from, the genera *Actinomyces*, *Actinobaculum*, *Arcanobacterium*, and *Mobiluncus*.

Cells are short, straight or curved Gram-stain-positive rods and form diphtheroid-like to almost completely ring-shaped arrangements. All forms may be seen in a single culture. Nonhemolytic, translucent white to gray, pinpoint, convex, entire edged, and glistening colonies are formed after anaerobic incubation for 48 h at 37°C on Fastidious Anaerobe Agar supplemented with 5% horse blood. Cultures grown in brain heart infusion broth incubated anaerobically at 37°C produce a granular sediment and an almost clear supernate. Optimal growth occurs under anaerobic conditions. Strains grow well or poorly in air plus 5% CO_2 and poorly or not at all in air. Good growth occurs on Fastidious Anaerobe Agar supplemented with 5% horse blood at neutral pH and at 35–37°C. Growth on other media and at other temperatures has not been examined.

Principal end products of glucose metabolism are lactic and succinic acids with small amounts of acetic acid. Acid is produced from D-glucose, D-ribose, and sucrose in conventional biochemical tests. Acid production from fructose, mannitol, and xylose is variable, but acid is not produced from amygdalin, L-arabinose, cellobiose, lactose, mannose, D-raffinose, salicin, or trehalose. Most strains reduce nitrate to nitrite; indole production is negative; neither lecithinase nor lipase are produced. Hippurate is hydrolyzed but not esculin, gelatin, or starch.

The almost complete 16S rRNA gene sequence of the type strain (CCUG 44998[T]) shares 99.1–100% similarity with several other *Varibaculum cambriense* strains and shows 10–12% divergence from *Actinomyces neuii* and *Mobiluncus* species, their nearest phylogenetic neighbors; other *Actinomyces* species and *Arcanobacterium* species are more distantly related. These relationships are supported by similar findings by Hoyles et al. (2004) and by whole-cell protein profiles obtained in SDS-PAGE analyses (Hall et al., 2003e).

Varibaculum cambriense is susceptible to benzylpenicillin, chloramphenicol, and imipenem and is resistant to metronidazole. Susceptibility to clindamycin, erythromycin, and tetracycline is variable. MICs demonstrate a bi-modal distribution as they are either sensitive or highly resistant. Clindamycin resistance is associated with erythromycin resistance.

Varibaculum cambriense has been isolated from various human soft-tissue infections in mixed cultures of anaerobic and aerobic bacteria. The roles of individual species in polymicrobial infections are difficult to ascertain and may involve complex interactions between component organisms, hence the pathogenicity of *Varibaculum cambriense* is not clear. In the United Kingdom and Scandinavia, this organism has been isolated from sebaceous cysts, pilonidal sinus, vagina, intrauterine contraceptive devices and from abscesses of the axilla, brain, breast, chest wall, ear, ischio-rectum, mastoid, mouth, and neck.

Isolation procedures

Varibaculum cambriense may be cultured on Fastidious Anaerobe Agar supplemented with 5–7% horse or sheep blood, and on Columbia Blood Agar or equivalent agars. For isolation from polymicrobial infections, culture on Actinomyces selective agar supplemented with metronidazole 10 mg/l and nalidixic acid 30 mg/l may be advantageous. Cultures should be incubated for a minimum of 48 h under anaerobic conditions at 35–37°C. Broth enrichment cultures are rarely useful due to overgrowth from other organisms.

Maintenance procedures

Viability has been maintained for >10 years in strains stored at –70°C in commercially available vials of beads or in brain heart infusion broth containing 10% glycerol. Reference strains held in culture collections are stored as lyophilized cultures.

Procedures for testing special characters

Commercially available identification systems. API Coryne, API Rapid 32A, API Rapid ID 32Strep, and API ZYM systems (BioMérieux, Marcy l'Étoile, France) may aid identification. However, *Varibaculum cambriense* is not listed in the manufacturer's databases hence results should be interpreted with reference to the findings of Hall et al. (2003e) or as listed below.

Amplified 16S rDNA restriction analysis (ARDRA). *Varibaculum cambriense* is reliably differentiated from other facultatively anaerobic non-sporeforming, Gram-stain-positive rods in ARDRA using universal 16S rDNA primers, namely AGAGTTTGATCCTGGCTCAG (pA) and AAGGAGGTGATCCAGCCGA (pH′) and endonucleases *Hae*III and *Hpa*II, as described by Hall et al. (1999).

Differentiation of the genus *Varibaculum* from other genera

Phylogenetically, *Varibaculum cambriense* is most closely related to *Mobiluncus* spp. and *Actinomyces neuii* and less closely to other *Actinomyces* spp., but it can be clearly differentiated from these

taxa by comparison of 16S rRNA gene sequences sharing only 91–92% similarity with its nearest relatives.

In phenotypic tests, *Varibaculum cambriense* is differentiated from *Mobiluncus* spp. by its lack of motility and in the API Rapid ID 32Strep system by its ability to hydrolyze hippurate (*Mobiluncus mulieris* is negative) and its negative reactions for α-galactosidase, β-galactosidase, and arginine dihydrolase, all of which are positive in *Mobiluncus curtisii*. An inability to produce catalase differentiates *Varibaculum* from *Actinomyces neuii* and other catalase-positive *Actinomyces* spp. and it is further distinguished from *Actinomyces neuii* by being negative for α-galactosidase, β-galactosidase, and pyrazinamidase. A combination of reactions in the API Rapid ID 32Strep system are useful to differentiate *Varibaculum cambriense* from catalase-negative, hippurate-hydrolyzing *Actinomyces* spp. isolated from human sources (see Table 19).

In ARDRA, as described above, *Varibaculum cambriense* isolates yielded one or other of the profiles designated 035/023 and 034/014 and were described as "unidentified actinomycete group 3" by Hall et al. (2001b). These profiles are distinct from those of *Actinomyces* spp., *Actinobaculum* spp., *Arcanobacterium* spp., *Mobiluncus* spp., *Gardnerella vaginalis*, and clinically relevant species of *Bifidobacterium*, *Lactobacillus*, and *Propionibacterium* (Hall et al., 2001a, 2001b).

TABLE 19. Differentiation of *Varibaculum cambriense* from catalase-negative, hippurate-hydrolyzing *Actinomyces* species isolated from human sources[a]

	V. cambriense	*A. funkei*	*A. hongkongensis*	*A. turicensis*
Acid from:[b]				
Lactose	–	+	–	–
Ribose	d	d	–	d
Sucrose	d	+	–	+
Trehalose	d	–	–	–
Production of:				
β-Galactosidase	d	d	–	–
Alkaline phosphatase	–	+	–	–
Alanine phenylalanine proline arylamidase	d	+	+	+
Nitrate reduction	+	d	–	–

[a]Symbols: +, >85% positive; d, different strains give different reactions (16–84% positive); –, 0–15% positive.

[b]Tests other than nitrate reduction were performed in the API Rapid ID 32Strep system.

Taxonomic comments

The genus *Varibaculum* was described by Hall et al. in 2003 to define a number of *Actinomyces*-like isolates from human clinical sources and it comprises a single taxon, originally defined as *Varibaculum cambriensis* (sic). The species epithet was corrected to *Varibaculum cambriense* upon its validation (*In* Validation of publication of new names and new combinations previously effectively published outside the IJSEM. List no. 91, 2003). *Varibaculum cambriense* remains the sole species. However, a small number of isolates have been found to be genotypically and phenotypically indistinguishable from *Varibaculum cambriense* except for their ability to rapidly hydrolyze urea. To date, these isolates have not been formally described as a distinct taxon.

List of species of the genus *Varibaculum*

1. **Varibaculum cambriense** Hall, Collins, Lawson, Hutson, Falsen, Inganas and Duerden 2003e, 644[VP]

cam.bri.en′se. N.L. neut. adj. *cambriense* pertaining to Cambria, the Latin name of Wales.

The characteristics are as described for the genus with the following additional information. With the API ZYM system, α-glucosidase and leucine arylamidase are positive; acid phosphatase, phosphoamidase, esterase C4, and ester lipase C8 are weakly positive or negative, and all other tests are negative. With the API Coryne system, acid is produced from D-glucose, maltose and sucrose; α-glucosidase is positive; acid production from D-ribose and D-xylose is variable; acid is not formed from glycogen, lactose, or mannitol. Alkaline phosphatase, β-galactosidase, β-glucuronidase, *N*-acetyl-β-glucosaminidase, pyrolidonyl arylamidase, pyrazinamidase, and urease reactions are negative. With the API 32A system, proline arylamidase is positive; α-glucosidase and arginine-, phenylalanine-, leucine-, tyrosine-, and serine arylamidases are positive or weakly positive. β-galactosidase, nitrate reduction, and alanine-, glycine-, and histidine arylamidases are variable. Urease, arginine dihydrolase, α-galactosidase, β-6-phosphate-galactosidase, β-glucosidase, α-arabinosidase, β-glucuronidase, *N*-acetyl-β-glucosaminidase, mannose, raffinose, indole, alkaline phosphatase, leucyl glycyl arylamidase, pyroglutamic acid arylamidase, glutamic acid decarboxylase, α-fucosidase, and glutamyl glutamic acid arylamidase are negative.

In a study of 23 clinical isolates, including the type strain, MICs were determined by the Etest. All isolates were susceptible to benzylpenicillin (range 0.032–1 mg/l), chloramphenicol (0.19–3), and imipenem (0.047–0.5). Eight isolates were susceptible to clindamycin, erythromycin, and tetracycline, eight were resistant to tetracycline but susceptible to clindamycin and erythromycin, four were resistant to clindamycin and erythromycin but susceptible to tetracycline. Three isolates, including the type strain, were resistant to clindamycin, erythromycin, and tetracycline. MICs (in mg/l) for the 23 isolates were: clindamycin MIC_{50} = 0.125, MIC_{90} = >256, range 0.016–>256; erythromycin MIC_{50} = 0.047, MIC_{90} = >256, range 0.016–>256; tetracycline MIC_{50} = 3.0, MIC_{90} = >256, range 0.5–>256.

DNA G+C content (mol%): 51.7 (HPLC).

Type strain: R12359, CCUG 44998, CIP 107344.

Sequence accession no. (16S rRNA gene): AJ428402, AJ491326.

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Order II. **Actinopolysporales** ord. nov.

Michael Goodfellow and Martha E. Trujillo

Ac.ti′no.po.ly.spo.ra.les. N.L. fem. n. *Actinopolyspora* the type genus of the order; suff. *-ales* ending to denote an order; N.L. fem. pl. n. *Actinopolysporales* the *Actinopolyspora* order.

The order was formed by elevation of the suborder *Micromonosporineae* Zhi, Li and Stackebrandt 2009, 594[VP]. It contains the family *Actinopolysporaceae*. It is defined on the basis of its phylogeny in 16S rRNA gene trees and the **16S rRNA signature consisting of nucleotides at positions 127:234 (A–U), 242:284 (C–G), 657:749 (G–C), 672:734 (C–G), 828(A), 829:857 (G–C), 833–853 (U–G), 840:846 (C–G), 986:1219 (U–A), 1100 (U), 1183 (C), 1117:1183 (G–C) and 1309:1328 (G–U).**

Type genus: **Actinopolyspora** Gochnauer, Leppard, Komaratat, Kates, Novitsky and Kushner 1975, 1510[AL].

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Gochnauer, M.B., G.G. Leppard, P. Komaratat, M. Kates, T. Novitsky and D.J. Kushner. 1975. Isolation and characterization of *Actinopolyspora halophila*, gen. et sp. nov., an extremely halophilic actinomycete. Can. J. Microbiol. *21*: 1500–1511.

Zhi, X.-Y., W.-J. Li and E. Stackebrandt. 2009. An update of the structure and 16S rRNA gene sequence-based definition of higher ranks of the class *Actinobacteria*, with the proposal of two new suborders and four new families and emended descriptions of the existing higher taxa. Int. J. Syst. Evol. Microbiol. *59*: 589–608.

Family I. **Actinopolysporaceae** Zhi, Li and Stackebrandt 2009, 595[VP]

Michael Goodfellow and Martha E. Trujillo

Ac.ti′no.po.ly.spo.ra′ce.ae. N.L. fem. n. *Actinopolyspora* the type genus of the family; -*aceae*, ending to denote a family; N.L. fem. pl. n. *Actinopolysporaceae* the *Actinopolyspora* family.

The description is the same as for the genus *Actinopolyspora*. The 16S rRNA nucleotide signature is that of the order.

DNA G+C content (mol%): 64–68 (T_m, HPLC).

Type genus: **Actinopolyspora** Gochnauer, Leppard, Komaratat, Kates, Novitsky and Kushner 1975, 1510[AL].

Genus I. **Actinopolyspora** Gochnauer, Leppard, Komaratat, Kates, Novitsky and Kushner 1975, 1510[AL]

Martha E. Trujillo and Michael Goodfellow

Ac.ti.no.po.ly.spo′ra. Gr. n. *aktis* -*inos* ray; Gr. adj. *polus* many; Gr. fem. n. *spora* seed and in biology a spore; N.L. fem. n. *Actinopolyspora* the many-spored ray (fungus).

Aerobic, Gram-stain-positive, acid-fast, nonmotile actinomycetes that **form an extensively branched substrate mycelium about 1 μm in diameter**. Fragmentation of the substrate mycelium is occasionally observed near the colony center, but substrate hyphae are mostly non-fragmented. **Sporophores containing 10 or more smooth-walled coccobacillary and coccoid spores are produced basipetally on aerial hyphae.** Spores are not observed on the substrate mycelium. **Chemoorganotrophic and halophilic; 10–20% (w/v) NaCl is required for optimal growth. Temperature growth range is 30–50°C.** Whole-organism hydrolyzates contain *meso*-diaminopimelic acid (*meso*-A_2pm), arabinose, and galactose. Muramic acid moieties are *N*-acetylated. **Cells contain phosphatidylcholine as the diagnostic phospholipid and are rich in branched-chain fatty acids, notably $C_{17:0}$ anteiso and $C_{15:0}$ iso, but lack mycolic acids. The predominant menaquinones are MK-9(H_4) and MK-9(H_6).**

DNA G+C content (mol%): 64–68 (HPLC, T_m).

Type species: **Actinopolyspora halophila** Gochnauer, Leppard, Komaratat, Kates, Novitsky and Kushner 1975, 1510[AL].

Further descriptive information

Phylogeny. The genus *Actinopolyspora* currently contains three species with validly published names: *Actinopolyspora halophila*, *Actinopolyspora iraqiensis*, and *Actinopolyspora mortivallis*. The three species are moderately related based on 16S rRNA gene sequence analysis (Figure 59). *Actinopolyspora halophila* and *Actinopolyspora mortivallis*, the most closely related species, share a sequence similarity of 96.7%. *Actinopolyspora iraqiensis* shares 16S rRNA gene sequence similarities of 89.3% and 89.7% with *Actinopolyspora halophila* and *Actinopolyspora mortivallis*, respectively. It shows a much closer phylogenetic relationship with *Saccharomonospora halophila* and *Saccharomonospora paurometabolica* (98.9–99.0%) and probably belongs to this genus. *Actinopolyspora halophila* and *Actinopolyspora mortivallis* share a DNA–DNA relatedness value of 34% (Yoshida et al., 1991). A thermophilic species, "*Actinopolyspora thermovinacea*" was described by Lu and Yan (1983), but the name has not been validly published.

Cell morphology. Strains currently classified in the genus *Actinopolyspora* produce well developed, irregularly branched,

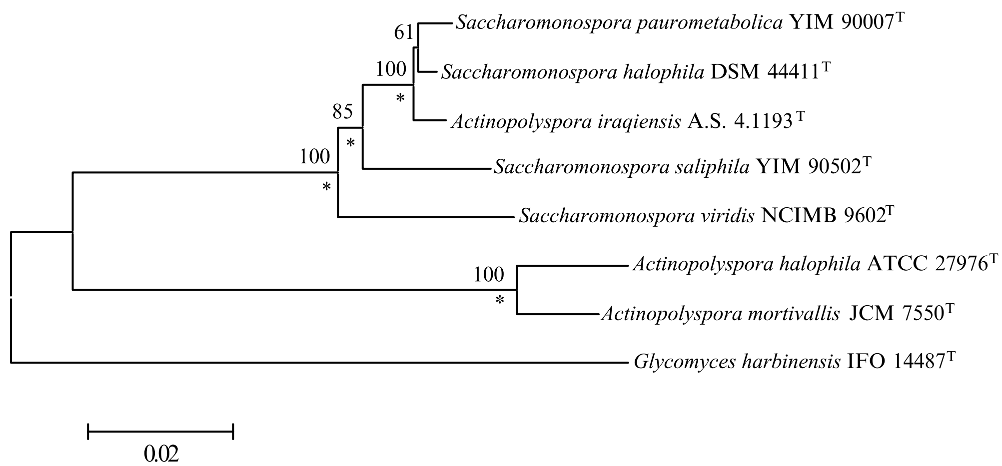

FIGURE 59. Neighbor-joining tree based on almost complete 16S rRNA gene sequences showing relationships between *Actinopolyspora* species and related taxa. Asterisks indicate branches of the tree that were also recovered using the maximum-parsimony tree-making algorithm. Numbers at the nodes indicate the levels of bootstrap support based on a neighbor-joining analysis of 1000 resampled datasets. *Glycomyces harbinensis* IFO 14487[T] was used as the outgroup. Bar = 2 substitutions per 100 nucleotides.

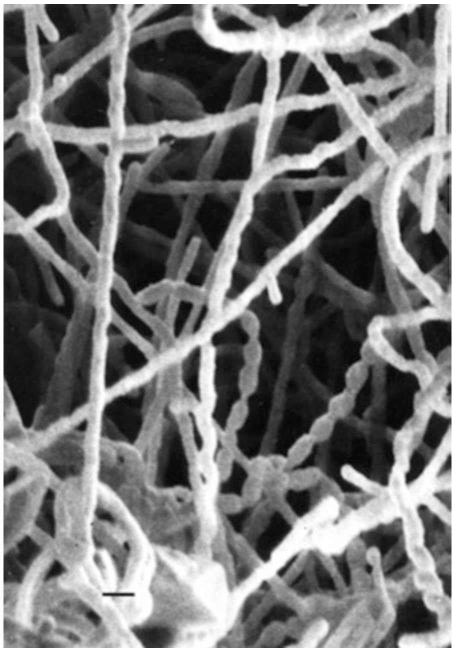

FIGURE 60. Scanning electron micrograph of *Actinopolyspora halophila* ATCC 27976ᵀ. Filamentous growth on 20% (w/v) NaCl. Bar, 1 μm. (Reproduced from *Bergey's Manual of Systematic Bacteriology*, 1st edn, vol. 4.)

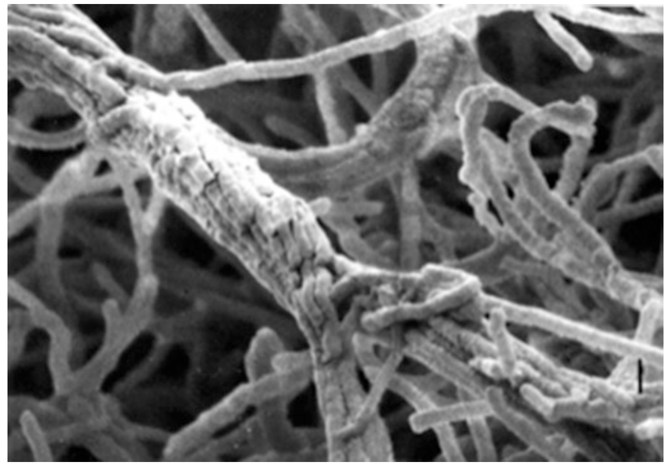

FIGURE 61. Aggregate of filaments of *Actinopolyspora halophila* ATCC 27976ᵀ grown on 10% (w/v) NaCl. SEM. Bar, 1 μm. (Reproduced from *Bergey's Manual of Systematic Bacteriology*, 1st edn, vol. 4.)

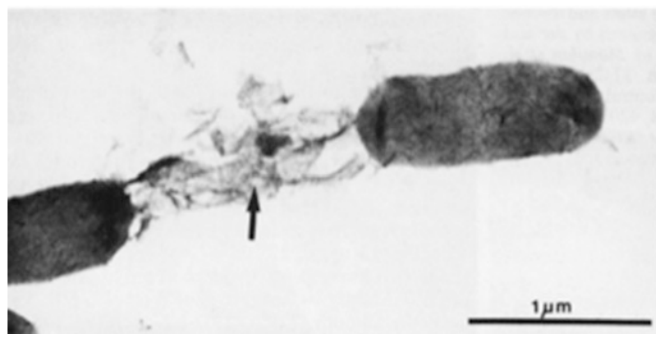

FIGURE 62. Negatively stained aerial hypha of *Actinopolyspora halophila* ATCC 27976ᵀ with sheath, partially detached (arrow). TEM. (Reproduced with permission from Kothe et al., 1989. Syst. Appl. Microbiol. *12*: 61–69.)

substrate hyphae (0.4–1.0 μm in diameter) which rarely fragment, although *Actinopolyspora halophila* may show fragmentation near the centers of colonies. Spores are produced on aerial hyphae, but have not been observed on the substrate mycelium. Sporophores containing 20 or more smooth-walled coccobacillary and coccoid spores (0.7–1 × 1–2 μm) are produced basipetally on aerial hyphae in *Actinopolyspora halophila*. Straight to flexuous chains of 10 or more spores are formed by *Actinopolyspora mortivallis*; the spores are oval to cylindrical (0.5–0.7 × 0.8–1.3 μm) and have smooth surfaces. *Actinopolyspora iraqiensis* forms short chains of spores (1–15) that are spherical (1 μm in diameter) and have smooth surfaces.

Fine structure. The fine structure of *Actinopolyspora halophila* has been examined using transmission and scanning electron microscopy (Gochnauer et al., 1975; Kothe et al., 1989). The mycelium at the air-surface interface consists of long cylindrical filaments that are about 1 μm in diameter and which terminate in rounded tips (Figure 60); branching occurs at either acute or right angles to the main filament. Not all branches have the same diameter as the main filament; irregular branching patterns include some opposite branching. Filaments frequently have several bands or pronounced curvatures, and some are helical. Cross walls have not been detected in vegetative filaments. Ropelike aggregates of vegetative filaments are frequently formed on agar media containing 10% (w/v) NaCl (Figure 61), but rarely on media containing 30% (w/v) NaCl.

Aerial hyphae are enclosed by an outer sheath (Figure 62) which corresponds to the sheath of *Streptomyces* (Williams et al., 1972). Long chains of smooth spores are formed on the aerial mycelium (Figure 63). Small cells may be observed between mature spores in ultrathin sections (Figure 64 and Figure 65). Some (possibly immature) sporophores are branched. Spores vary in size and shape within a single sporophore. They may be spherical to short rods with rounded ends. The spore morphology of cultures grown on agar media containing 12.5 or 15.0% (w/v) NaCl is similar to that of strains grown in the presence of 20% (w/v) NaCl. Spores are not formed on media containing 25% (w/v) NaCl, but are produced, albeit infrequently, on media containing 10% (w/v) NaCl.

Colony morphology. Actinopolysporae grow well on a variety of culture media. *Actinopolyspora halophila* forms wrinkled colonies covered with white aerial hyphae and a buff colored substrate mycelium on the complex medium of Sehgal and Gibbons (1960) supplemented with 15% (w/v) NaCl. The color of the substrate mycelium is influenced by the salt concentration and becomes darker with reduced salt; at a concentration of 10% (w/v) salt, the substrate mycelium is black (Gochnauer et al., 1975). A diffusible brown pigment is formed on low salt medium. *Actinopolyspora iraqiensis* forms small, thin, elevated to convex colonies (2–4 mm in diameter) that are covered by white aerial hyphae when grown on agar media containing 10–15 (w/v) salt; the substrate mycelium is yellowish to brownish (Ruan et al., 1994). *Actinopolyspora mortivallis* forms yellowish white colonies and a pale diffusible pigment on oatmeal agar (Yoshida et al., 1991).

Actinopolysporae grow best under aerobic conditions. If stab-inoculated into soft agar media, *Actinopolyspora halophila* grows

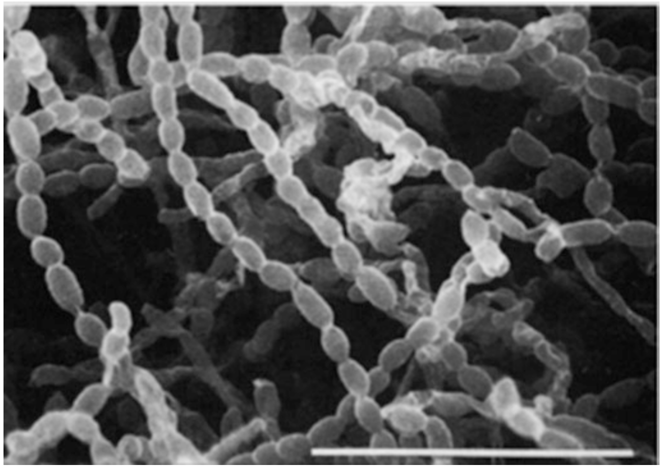

FIGURE 63. Aerial hyphae of *Actinopolyspora halophila* ATCC 27976[T] with chains of smooth spores. SEM. (Reproduced with permission from Kothe et al., 1989. Syst. Appl. Microbiol. *12*: 61–69.)

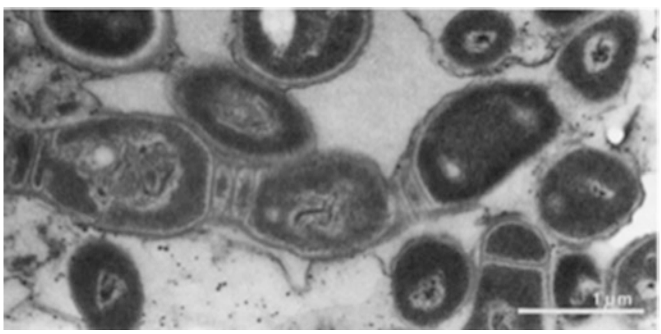

FIGURE 64. Ultrathin section of aerial hyphae of *Actinopolyspora halophila* ATCC 27976[T]. (Reproduced with permission from Kothe et al., 1989. Syst. Appl. Microbiol. *12*: 61–69.)

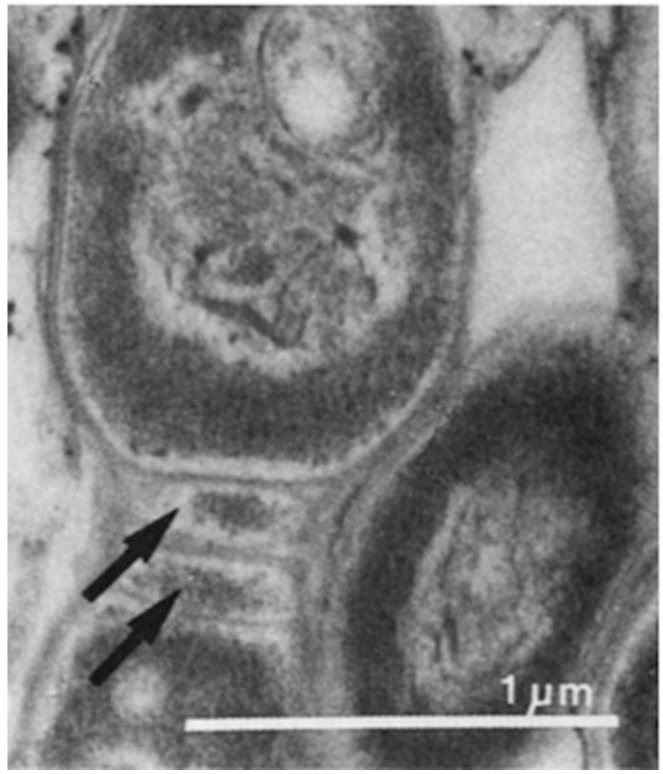

FIGURE 65. Part of a spore chain of *Actinopolyspora halophila* ATCC 27976[T] with characteristic small cells (arrow) between spores. TEM. (Reproduced with permission from Kothe et al., 1989. Syst. Appl. Microbiol. *12*: 61–69.)

only at the surface. Growth of the organism in liquid media is proportional to the degree of aeration; in stationary cultures, growth occurs on the surface as a wrinkled hydrophobic pellicle.

Chemotaxonomy. *Actinopolyspora* strains have cell walls that contain *meso*-A_2pm, arabinose, and galactose, i.e., they have a wall chemotype IV *sensu* Lechevalier and Lechevalier (1970). In addition, *Actinopolyspora iraqiensis* contains traces of LL-A_2pm and ribose (Ruan et al., 1994). Almost all the available glycan disaccharide units are peptide substituted in the walls of *Actinopolyspora halophila* strains; peptide cross-linkage is facilitated by direct peptide linkage between N^E-A_2pm and COOH-terminal alanine. The peptidoglycans of *Actinopolyspora halophila* WT (wild-type) and ER (mutant) strains are 50 and 67% peptide cross-linked, respectively. The WT strain contains 15.7% non-*N*-substituted muramic acid and 35% non-*N*-substituted glucosamine; corresponding figures for the ER strain are 11 and 48.8%, respectively (Johnson et al., 1986b). In addition, the walls of both the wild-type and the mutant strains contain glutamic acid, alanine, and A_2pm in the molar ration 1:2:1. There is also evidence of a direct cross-linkage between A_2pm and the terminal D-alanine in the intact peptidoglycan (type A1γ; Schleifer and Kandler, 1972) and for a wall-associated arabino-galactan polymer. The type strain of *Actinopolyspora halophila* contains a number of ester-linked phospholipids, as well as glycolipids and neutral lipids, but lacks ether-linked phospholipids (Ross et al., 1981).

Actinopolysporae contain major amounts of phosphatidylcholine (taxonomically significant polar lipid), diphosphatidylglycerol, phosphatidylglycerol, and *lyso*-phosphatidylglycerol (Embley et al., 1988b; Gochnauer et al., 1975; Kothe et al., 1989; Ruan et al., 1994) and, hence, they have a phospholipid type III *sensu* Lechevalier et al. (1981, 1977). They are rich in branched-chain fatty acids (Gochnauer et al., 1975; Kothe et al., 1989; Yoshida et al., 1991), but lack mycolic acids (Kates et al., 1987; Minnikin and Goodfellow, 1980; Ruan et al., 1994; Yoshida et al., 1991). The predominant menaquinone is MK-9(H_4), although *Actinopolyspora halophila* and *Actinopolyspora mortivallis* also contain MK-10(H_4) (Embley et al., 1988b; Yoshida et al., 1991), and *Actinopolyspora iraqiensis* also contains MK-9(H_6) (Ruan et al., 1994). The electrophoretic mobilities of ribosomal AT-L30 proteins of the type strains of *Actinopolyspora halophila* and *Actinopolyspora mortivallis*, determined by two dimensional PAGE, have been shown to be similar (Ochi and Yoshida, 1991).

Nutrition and growth conditions. *Actinopolyspora* strains require salt for growth. On solid media, growth occurs in the presence of 5–30% (w/v) NaCl, with optimal growth in media containing 10 and 20% (w/v) NaCl. Growth does not occur in media containing 5% (w/v) NaCl. *Actinopolyspora halophila* strain WT (wild-type) is extremely halophilic: it needs at least 12% (w/v) NaCl to grow in liquid medium and 10% (w/v) NaCl to grow on agar medium. It grows well in the presence of 15–20% (w/v) NaCl and can be cultivated in media containing 30% (w/v) NaCl. In contrast, mutant *Actinopolyspora halophila*

strain ER can grow in 6% (w/v) complex medium (CM*) broth (Gochnauer and Kushner, 1969; Sehgal and Gibbons, 1960). These strains can be washed in distilled water without loss of viability. Salt cannot be effectively replaced by KCl. On CM agar, the WT strain grows and sporulates at pH 6.0–8.6. Strain ER grows from pH 5.0 to 8.6 (poorly at higher pH) though sporulation is slow and occurs only from pH 6.0 to 7.0.

Optimal growth of the type and mutant strains of *Actinopolyspora halophila* occurs at 37°C in shake flasks containing CM broth (Sehgal and Gibbons, 1960) supplemented with 20% (w/v) NaCl (Johnson et al., 1986a). Good growth is usually achieved within 14 d, but there may be a long lag phase followed by rapid growth. Lysis may occur in stationary phase cultures. *Actinopolyspora mortivallis* shows abundant growth on nutrient, oatmeal, potato-glucose, sucrose-nitrate, and yeast extract-malt extract agars, and moderate growth on peptone-yeast extract-iron agar, but grows poorly on glucose-asparagine agar (Yoshida et al., 1991). *Actinopolyspora iraqiensis* grows well on modified complex medium (SGA†). Actinopolysporae grow between 30 and 50°C (Ruan et al., 1994).

Metabolism and genetics. Actinopolysporae have a chemoorganotrophic metabolism and are able to use diverse carbon sources, including fructose, galactose, glucose, glycerol, maltose, mannitol, raffinose, rhamnose, sucrose, xylose, isopropyl alcohol, sodium citrate, and sodium succinate. *Actinopolyspora mortivallis*, unlike *Actinopolyspora halophila*, degrades xylan and peptonizes milk, but is unable to reduce nitrate, it also has a distinctive temperature range and carbon utilization profile (Yoshida et al., 1991). An *Actinopolyspora* strain isolated from a salt marsh off the southeast coast of India was found to be resistant to mercury (Senthilkumar et al., 2005).

Actinopolyspora halophila synthesizes a range of extracellular enzymes when grown in CM broth containing 15% (w/v) NaCl; these include α-amylase, carboxymethylcellulase, proteases, and a xylanase (Johnson and Lanthier, 1986; Johnson et al., 1986a). The production of the carboxymethylcellulase and xylanase is enhanced by the presence of xylan. Cells grown in CM supplemented with lactose synthesize calcium-dependent (0.1 mM) cellular and extracellular β-galactosidase and 6-phospho-β-galactosidase. Both of these enzymes and a cellular glutamate dehydrogenase are NADP-dependent; it has also been shown that succinate dehydrogenase uses either NAD or NADP. *Actinopolyspora halophila* also produces a cellular and an active exocellular β-lactamase (Johnson and Lanthier, 1986). These enzymes degrade compounds containing 6-aminopenicillanic acid (including methicillin) and 6-aminocephalosporanic acid. It has also been shown that these enzymes are stimulated in their activities and protected from thermal denaturation by NaCl. "*Actinopolyspora thermovinacea*" is thermophilic (Lu and Yan, 1983).

Halophilic bacteria living in high salt concentrations accumulate small organic compounds in order to balance the osmotic pressure. These molecules have been named compatible solutes because of their compatibility with cellular metabolism at high concentrations (Brown, 1976). Glycine betaine (betaine), the best-known compatible solute, is synthesized from simple carbon sources by halophilic and halotolerant phototrophic bacteria and by extremely halophilic methanogenic archaea, an ability that is rare among heterotrophic bacteria. In an extensive study using ^{13}C-NMR, Severin et al. (1992) found that *Actinopolyspora halophila* and a related isolate were the only heterotrophic strains in which *de novo* synthesis of betaine could be detected. They also found that trehalose, like betaine, was present in the compatibility solute pool of *Actinopolyspora halophila*. It was subsequently shown that *Actinopolyspora halophila* synthesized betaine by threefold methylation of glycine with *S*-adenosylmethionine acting as the methyl group donor (Nyyssölä et al., 2000). *Actinopolyspora halophila* oxidizes choline to betaine and appears to be the only bacterium that is able to do this and use the glycine methylation pathway for *de novo* synthesis of betaine (Nyyssola and Leisola, 2001). These workers also found that trehalose was synthesized as a compatible solute and accounted for up to 9.7% of the cellular dry weight. The betaine concentration was shown to rise with increasing NaCl concentration, whereas that of trehalose was highest at the lowest concentration (15%, w/v).

Actinopolyspora strain YIM 90600, a putatively novel member of the genus *Actinopolyspora*, was isolated from a dried salt lake in Xinjiang Province, northwest China, and shown to produce erythronolides H and I, new erythromycin congeners (Huang et al., 2009). The authors noted that the presence of the C-14 hydroxyl moieties and the C-6/C-18-epoxide in erythronolide H and the spiroketal moiety of erythronolide I shed new insights into the structural diversity of erythromycin analog libraries that might be accessible by combinatorial biosynthesis. The organism was also shown to produce a high titer of erythromycin C.

The genetics of *Actinopolyspora* have received little attention. Erythromycin resistance in *Actinopolyspora halophila* strain ER is genetically stable, but neither this strain nor the wild-type strain was found to contain plasmids (Johnson et al., 1986a). Holes which resemble viral plaques appear most frequently in colonies grown at lower NaCl concentrations suggesting the presence of bacteriophages, but no phage has been isolated (Gochnauer et al., 1975).

Antibiotic sensitivity. *Actinopolyspora halophila* is highly resistant to β-lactam antibiotics, but is sensitive to amoxycillin. The antibiotic sensitivity profiles of individual *Actinopolyspora* species are given in the species descriptions.

Ecology. *Actinopolyspora* strains have been isolated from hypersaline environments, including salt marsh soil and marine sediments (Kokare et al., 2004; Senthilkumar et al., 2005; Vijayakumar et al., 2007). *Actinopolyspora halophila* was recovered as a contaminant in a culture medium containing 25% NaCl (Gochnauer et al., 1975), *Actinopolyspora mortivallis* was from a salty soil sample collected in Death Valley, California (Yoshida et al., 1991), and *Actinopolyspora iraqiensis* was from an extremely saline soil sample in Iraq (Al-Tai and Ruan, 1994; Ruan et al., 1994). *Actinopolyspora* strains identified by 16S rRNA gene sequencing have been isolated from salt-lakes in China (Huang et al., 2009; Zhi et al., 2007). "*Actinopolyspora thermovinacea*" was isolated from soil samples (Lu and Yan, 1983).

*Complex medium (all ingredients except Fe^{2+} given in g/l): Difco Casamino acids (vitamin free), 7.5; Difco yeast extract, 10.0; $MgSO_4 \cdot 7H_2O$, 10.0; sodium citrate, 3.0; KCl, 1.0; NaCl, 200; Fe^{2+}, prepare 4.98% $FeSO_4 \cdot 7H_2O$ (10,000 ppm Fe^{2+}; 0.001 M in HCl; add 1 ml/l medium). Adjust to pH 6.8 and sterilize at 15 lbs pressure for 10 min. For tryptone medium, substitute 1.5 g Difco tryptone for Casamino acids-yeast extract. Also, 27.5 g Difco trypticase-soy broth can be substituted for casamino acids and yeast extract. All support good growth.

†Modified complex medium (per liter): Difco Casamino acids, 7.5 g; Difco yeast extract, 10.0 g; $MgSO_4 \cdot 7H_2O$, 20 g; sodium citrate, 3.0 g; KCl, 2.0 g; NaCl, 200 g; plus 1 ml of a trace salts solution (0.1 g $FeSO_4 \cdot 7H_2O$, $MnCl_2 \cdot 4H_2O$, and $ZnSO_4 \cdot 4H_2O$ in 1 liter distilled water).

Isolation procedures

Actinopolyspora halophila was initially recognized as a contaminant in a flask of nonsterile CM broth containing 25% (w/v) solar salt. The broth was left to stand at room temperature for 50 d when *Actinopolyspora halophila* was observed as a surface mycelial mat (Gochnauer et al., 1975). The organism may well have been a contaminant of the solar salt which suggests that it might occur in salt ponds (salterns) where sea salt is harvested. It may also occur in muds and soils adjacent to salterns and salt lakes. The procedures used to isolate *Actinopolyspora iraqiensis* and *Actinopolyspora mortivallis* were not given in the original publications (Ruan et al., 1994; Yoshida et al., 1991). "*Actinopolyspora thermovinacea*" was isolated from soil samples (Lu and Yan, 1983).

The following procedure was suggested by Gochnauer et al. (1989) for the selective isolation of actinopolysporae from saline habitats. Cells are washed thoroughly with distilled water to eliminate osmotically sensitive halobacteria and other microorganisms, collected on membrane filters and incubated on an agar medium containing 20% (w/v) NaCl and the basic minerals in CM medium plus low concentrations of yeast extract (1–2%, w/v) and Casamino acids (0.1%, w/v); cycloheximide is added to inhibit the growth of salt-tolerant and halophilic eukaryotic cells. The medium is adjusted to pH 6.5–7.0 and the inoculated plates are incubated at 35–40°C in plastic bags to provide a humid atmosphere. For enrichment, stationary liquid cultures in the same medium should be incubated for up to 6 weeks. Typical white mycelial growth on the surface and on the walls at the air-liquid interface should be examined, diluted, and plated on solid medium of the same formulation (2% agar) until pure cultures are obtained.

Maintenance procedures

Well grown cultures may be stored for up to a year in screw-capped tubes at 4°C in CM agar containing 15–20% (w/v) NaCl. Such cultures can also be maintained at −70°C and, on thawing, should be grown on fresh CM agar for three successive 72–96 h periods prior to use. For longer term preservation, cultures may be lyophilized. Cells are grown to the beginning of the stationary phase, centrifuged, and suspended in the following medium: Dextran T-500 (Pharmacia), 5 g; sucrose, 9.5 g; monosodium glutamate, 1.0 g; NaCl, 20 g; and distilled water to a final volume of 100 ml. After the ingredients are dissolved, the medium is dispensed into tubes and sterilized at 121°C for 15 min. Cells are then resuspended in the same medium, and 1–2 ml suspension per ampoule is frozen in acetone-dry ice. Because of the high NaCl concentration, it is especially important to coat the inside of ampoules with a very thin layer of frozen material before applying a vacuum in order to ensure complete lyophilization.

Differentiation of the genus *Actinopolyspora* from other genera

The genus *Actinopolyspora* is phylogenetically different from other genera with a type IV cell wall containing *meso*-A_2pm, arabinose, and galactose. It can be differentiated as it forms a deep branch in the 16S rRNA gene tree and hence can be readily distinguished from other genera classified in the order *Actinomycetales* (Zhi et al., 2009), including those which encompass strains with a wall chemotype IV. Zhi and his colleagues also noted that members of the genus shared unique 16S rRNA gene signatures. In addition, *Actinopolyspora*-specific oligonucleotide primers are available for the identification of members of the genus (Zhi et al., 2007). Actinopolysporae can also be distinguished from other actinomycetes which produce long chains of spores on the aerial mycelium by using chemical markers (Embley et al., 1988b; Yoshida et al., 1991), on the basis of the electrophoretic mobilities of their ribosomal AT-L30 proteins (Ochi, 1995b) and by partial amino acid sequencing of the AT-L30 protein (Ochi, 1995a). The genus has traditionally been separated from other sporoactinomycetes by its requirement for high salt concentrations for growth (Gochnauer et al., 1975; Yoshida et al., 1991), although the reliability of this feature can be questioned as a mutant of the type strain of *Actinopolyspora halophila* only requires 6% (w/v) NaCl for growth (Johnson et al., 1986a).

Taxonomic comments

The genus *Actinopolyspora* was proposed by Gochnauer et al. (1975) to accommodate an extremely halophilic strain that had been isolated as a contaminant from CM broth containing 25% (w/v) NaCl. The chemotaxonomic and morphological properties of the organism led to its assignment to the family *Nocardiaceae* which, at the time, contained an assortment of actinomycetes that included the genera *Micropolyspora*, *Mycobacterium*, *Nocardia*, *Pseudonocardia*, *Saccharopolyspora*, and *Thermomonospora* (Lacey and Goodfellow, 1975; Lechevalier et al., 1971, 1973). The genus was classified with the "nocardioform actinomycetes" in the last edition of *Bergey's Manual of Systematic Bacteriology* (Lechevalier, 1989). It subsequently became a founder member of the family *Pseudonocardiaceae* Embley et al. (1988a), together with the genera *Amycolatopsis*, *Faenia*, *Pseudonocardia*, *Saccharomonospora*, and *Saccharopolyspora*. These taxa, which have wall chemotype IV and contain MK-9(H_4) as the predominant isoprenolog, were considered to form a coherent phylogenetic group based on reverse transcriptase sequencing of 16S rRNA. The genus *Kibdelosporangium* was later added to the family (Bowen et al., 1989).

Stackebrandt et al. (1997) retained *Actinopolyspora* in the family *Pseudonocardiaceae* in their proposal for a new hierarchic classification of actinobacteria based on 16S rRNA gene sequences even though the genus had been separated from other wall chemotype IV actinomycetes that lacked mycolic acids by PAGE of ribosomal AT-L30 preparations and partial amino acid sequencing of ribosomal protein AT-L30 (Ochi, 1995b; Ochi and Yoshida, 1991). In addition to the genus *Actinopolyspora*, the revised family *Pseudonocardiaceae* (Warwick et al., 1994) emend. Stackebrandt et al. (1997) included the genera *Actinosynnema*, *Amycolatopsis*, *Kibdelosporangium*, *Kutzneria*, *Saccharomonospora*, *Saccharopolyspora*, *Saccharothrix*, *Streptoalloteichus*, and *Thermocrispum*. The introduction of the family *Actinosynnemataceae* by Labeda and Kroppenstedt (2000) to encompass the genera *Actinosynnema*, *Lenztia*, and *Saccharothrix* left the family *Pseudonocardiaceae* as a more homogeneous taxon. However, it is now apparent from 16S rRNA gene sequence studies that the genus *Actinopolyspora* is not related to members of the family *Pseudonocardiaceae* and merits recognition as the sole member of the family *Actinopolysporaceae* in the order *Actinopolysporales*, formerly suborder *Actinopolysporineae* (Zhi et al., 2009).

The genus *Actinopolyspora* currently contains three species with validly published names, i.e., *Actinopolyspora halophila* (Gochnauer et al., 1975), the type species, *Actinopolyspora iraqiensis* (Ruan et al., 1994), and *Actinopolyspora mortivallis* (Yoshida et al., 1991). However, it seems likely that *Actinopolyspora iraqiensis* is misclassified as it falls into the *Saccharomonospora* 16S rRNA gene clade (Figure 59).

TABLE 20. Differential characteristics of the type strains of *Actinopolyspora* species[a]

Characteristic	*Actinopolyspora halophila*	*Actinopolyspora mortivallis*	"*Actinopolyspora iraqiensis*"
Spore-chain morphology	Long (around 20), elongated	Short (≤10), oval to cylindrical	Short (≤15), spherical
Color of colony in 10% (w/v) NaCl	Black	Yellowish white	Pale brown
Soluble pigments (in 10% NaCl)	Black	Pale brown	None
Growth in (w/v) NaCl:			
5%	–	+	+
25%	+	+	–
NaCl concentration for optimum growth (%, w/v)	15–20	10–15	10–15
Sensitivity to[b]*:*			
Amoxycillin	S	R	S
Clindamycin	S	S	R
Vancomycin	S	S	R
Hydrolysis of carboxymethylcellulose	+	nd	–
Acid production from:			
L-Arabinose	–	–	–
Fructose	–	nd	–
Inositol	–	–	–
Mannitol	–	–	+
Rhamnose	–	–	–
Xylose	–	–	–
Growth at 42°C	+	+	–

[a] Modified from Ruan et al. (1994). +, Positive; –, negative; nd, not determined.

[b] S, Sensitive; R, resistant.

Differentiation of the species of the genus *Actinopolyspora*

Actinopolyspora species can be distinguished from one another by using a combination of morphological, nutritional, and physiological characteristics (Table 20). The type strains are also readily distinguished by 16S rRNA gene sequencing.

List of species of the genus *Actinopolyspora*

1. **Actinopolyspora halophila** Gochnauer, Leppard, Komaratat, Kates, Novitsky and Kushner 1975, 1510[AL]

ha.lo′phi.la. Gr. n. *hals halos* salt; Gr. adj. *philos* loving; N.L. fem. adj. *halophila* salt-loving.

An extensively branched substrate mycelium is formed (1 µm in diameter) which does not penetrate agar surfaces. Fragmentation is observed occasionally in the center of colonies. Straight sporophores composed of 20 or more smooth-walled spores are produced basipetally on aerial hyphae. Spores vary in size and shape within a single sporophore, but are not formed on the substrate mycelium. Colonies have a wrinkled appearance and produce white aerial mycelium in CM medium; the substrate mycelium is buff at high salt concentrations, darker at lower concentrations, and black in the presence of 10% (w/v) NaCl. Growth does not occur on solid or in liquid CM medium when NaCl is replaced with 30% (w/v) KCl. The temperature growth range is 10–43°C and the optimal growth temperature is 37°C.

The most distinctive characteristic of the organism is its absolute requirement for at least 10–12% (w/v) NaCl for growth; optimal growth occurs with 20% (w/v) NaCl. When grown in liquid shake culture in CM supplemented with 1% (w/v) starch and 20% (w/v) NaCl, a pineapple-like odor is produced; the odor is musty when the organism is grown in the presence of nutrients such as casein and yeast extract, 1% (w/v) tryptone, or 1% (w/v) trypticase soy.

Does not hydrolyze urea. Degrades casein, gelatin, and Tweens 20, 40, 60 and 80, but not starch or xanthine. Arabinose, fructose, inositol, mannitol, rhamnose, and xylose are used as sole carbon sources.

The organism is sensitive to lysozyme and to (IU) amikacin (30), amoxycillin (30), bacitracin (10), cefamandole (30), cefoxitin (30), chloramphenicol (30), clindamycin (2), erythromycin (15), gentamicin (10), kanamycin (30), naladixic acid (30), novobiocin (30), and vancomycin (30).

Additional phenotypic properties are cited in Table 20.

Whole-organism hydrolyzates are rich in arabinose and galactose. The cellular fatty acid profile contains major proportions of $C_{15:0}$ iso (20%), $C_{16:0}$ iso (14%), $C_{17:0}$ iso (11%), and $C_{17:0}$ anteiso (32%), and smaller proportions (<10%) of $C_{16:0}$ (2%), $C_{16:1}$ (2%), $C_{17:0}$ (2%), $C_{18:0}$ iso (3%), and $C_{15:0}$ anteiso (8%). Does not contain mycolic acids. The polar lipid pattern contains phosphatidylcholine, phosphatidylglycerol, *lyso*-phosphatidylglycerol, and two glycolipids. The predominant menaquinones are MK-9(H_4) (65%) and MK-10(H_4) (22%); also contains minor proportions of MK-8(H_4), MK-9(H_2), and MK-9(H_6).

Source: isolated from contaminated CM medium supplemented with 25% (w/v) NaCl.

DNA G+C content (mol%): 64–68 (HPLC, T_m).

Type strain: ATCC 27976, DSM 43834, IFO (now NBRC) 14106, JCM 3278, VKM Ac-871.

Sequence accession no. (16S rRNA gene): X54287.

Additional comments: Actinopolyspora halophila strain ER was detected when the type strain was screened against 15 µg/ml erythromycin (Johnson et al., 1986a). The colonial and cell morphology of mutant strain ER was indistinguishable from

that of the parent strain, it had the same optimal growth temperature and grew over the same range of NaCl concentrations though its minimal NaCl concentration for growth is 6% (w/v) whereas that for the type strain is 12% (w/v). *Actinopolyspora halophila* ER is resistant to (IU) cefamandole (30), clindamycin (2), and erythromycin (15). Optimal growth is at pH 6.0–7.0, but unlike strain WT it grows at pH 5.0.

2. **Actinopolyspora mortivallis** Yoshida, Matsubara, Kudo and Horikoshi 1991, 19[VP]

mor.ti.val′lis. L. gen. n. *mortis* of death; L. gen. n. *vallis* of the valley; N.L. gen. n. *mortivallis* of Death Valley, the source of the soil sample from which the type strain was isolated.

A well developed, nonfragmenting, irregularly branched mycelium (0.4–0.6 µm in diameter) is formed. The aerial mycelium is branched monopodially and forms spore chains with more than 10 spores per chain. The spores are oval to cylindrical (0.5–0.7 × 0.8–1.3 µm) with smooth surfaces. Colonies on oatmeal (ISP medium 3) and potato-glucose agars are yellowish white, but are pinkish white on yeast extract-malt extract agar (ISP medium 2). A diffusible pale brown pigment is produced on oatmeal, nutrient, and peptone-yeast extract-iron agars.

The NaCl tolerance range is 5–30% (w/v), with optimal growth occurring in the presence of 10–15% (w/v) NaCl. The growth temperature range is 10–50°C, with optimal growth occurring at 45°C.

Milk is peptonized. Does not hydrolyze urea or reduce nitrate. Degrades casein, gelatin, Tweens 20, 40, 60 and 80, and xanthine. Fructose, glucose, raffinose, sucrose, and xylose are used as sole carbon sources, but not arabinose, inositol, mannitol, or rhamnose.

Susceptible to (µg/ml) ampicillin (10), carbenicillin (100), cephalothin (10), chloramphenicol (100), erythromycin (<10), methicillin (100), novobiocin (100), penicillin G (10), and vancomycin (100).

Additional phenotypic properties are cited in Table 20.

Whole-organism hydrolyzates are rich in arabinose and galactose. The cellular fatty acid profile contains major proportions of $C_{15:0}$ iso (16%), $C_{16:0}$ iso (18%), $C_{17:0}$ iso (28%), and $C_{17:0}$ anteiso (29%), and minor proportions of $C_{17:0}$ (2%) and $C_{15:0}$ anteiso (3%). Does not contain mycolic acids. The polar lipid pattern includes phosphatidylcholine. The predominant menaquinones are MK-9(H_4) (32%) and MK-10(H_4) (47%); minor amounts of MK-8(H_4), MK-9(H_2), and MK-10(H_2) are also present.

Source: the type strain was isolated from a soil sample collected in Death Valley, California.

DNA G+C content (mol%): 68.0 (HPLC).

Type strain: HS-1, ATCC 49777, DSM 44261, IFO (now NBRC) 15162, JCM 7550, VKM Ac-1974.

Sequence accession no. (16S rRNA gene): DQ883812.

Species *incertae sedis*

1. **Actinopolyspora iraqiensis** Ruan, Al-Tai, Zhou and Qu 1994, 760[VP]

ira.qi.en′sis. N.L. fem. adj. *iraqiensis* of or belonging to Iraq, the source of the soil sample from which the organism was isolated.

Well developed/branched substrate mycelium (0.5–0.7 µm in diameter) which rarely fragments in either solid or liquid media. Aerial hyphae differentiate into short chains of 1–15 spherical spores (1 µm in diameter) with smooth surfaces.

Colonies are small (2–4 mm in diameter), thin, elevated, or convex. The aerial spore mass is white and appears abundant on modified complex solid medium containing 10–15% (w/v) NaCl. The reverse side of colonies is yellow to brownish; diffusible pigments are not formed. Optimal growth occurs when culture media are supplemented with 10–15% (w/v) NaCl. Does not grow in the presence of 25% (w/v) salt or in the absence of salt. The optimal growth temperature is between 30 and 35°C; grows poorly at 16 and 40°C, does not grow at 42°C.

Neither esculin nor urea are hydrolyzed. Degrades starch, tyrosine, Tween 80, and xanthine, but not cellulose. L-Arabinose, fructose, galactose, glucose, inositol, maltose, mannitol, raffinose, and sucrose are used as sole carbon sources, but not mannose, L-rhamnose, or xylose.

Sensitive to lysozyme and to amoxycillin, bacitracin, carbenicillin, cephalexin, chloramphenicol, erythromycin, and novobiocin, but resistant to clindamycin, oxacillin, and vancomycin. Shows antimicrobial activity against *Saccharomyces pombe*.

Additional phenotypic properties are cited in Table 20.

The cell wall contains *meso*-A_2pm and trace amounts of L-A_2pm. Whole-organism hydrolyzates contain arabinose, galactose, and ribose. Does not contain mycolic acids. The polar lipid pattern includes diphosphatidylglycerol, *lyso*-diphosphatidylglycerol, phosphatidylcholine, and phosphatidylglycerol. The predominant menaquinone is MK-9(H_4).

Source: isolated from saline soil collected in Iraq.

DNA G+C content (mol%): not determined.

Type strain: IQ-H1, CCIM A.S. 4.1193, DSM 44640, JCM 9891, NBRC 103187.

Sequence accession no. (16S rRNA gene): EF372523.

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Order III. **Bifidobacteriales** Stackebrandt, Rainey and Ward-Rainey 1997, 487[VP]

Bruno Biavati

Bi.fi.do.bac.te.ri.a′les. N.L. neut. n. *Bifidobacterium* type genus of the order; suff. *-ales* ending denoting an order; N.L. fem. pl. n. *Bifidobacteriales* the *Bifidobacterium* order.

Type genus: **Bifidobacterium** Orla-Jensen 1924, 472[AL].

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Family I. **Bifidobacteriaceae** Stackebrandt, Rainey and Ward-Rainey 1997, 487[VP]

Bruno Biavati

Bi.fi.do.bac.te.ri.a.ce′a.e. N.L. neut. n. *Bifidobacterium* type genus of the family; suff. *-aceae* ending to denote a family; N.L. fem. pl. n. *Bifidobacteriaceae* the *Bifidobacterium* family.

The pattern of 16S rRNA gene signatures consists of nucleotides at positions 122:239 (G–U), 128:233 (C–G), 450:483 (C–G), 602:636 (G–C), 681:709 (C–G), 688:699 (A–U), 823:877 (A–U), 1118:1155 (C–G), and 1311:1326 (A–U).

The delineation of this bacterial family is based on phylogenetic criteria supported by biochemical characteristics such as phosphoketolase activity. The family contains the genera *Bifidobacterium* (32 species), *Alloscardovia* (one species), *Aeriscardovia* (one species), *Metascardovia* (one species), *Parascardovia* (one species), *Scardovia* (one species), which are close phylogenetic neighbors, and the genus *Gardnerella* (one species). The phylogenetic relationships between the genera classified in the family *Bifidobacteriaceae* have been the subject of several studies (Leblond-Bourget et al., 1996; Maidak et al., 1994; Okamoto et al., 2008). A phylogenetic tree based on 16S rRNA gene sequences showing the relationships within and between the genera classified in the family *Bifidobacteriaceae* is shown in Figure 66.

Cells of members of this family consist of pleomorphic rods that occur singly or in many-celled chains or clumps. Cells do not have capsules, are nonsporeforming, nonmotile, and nonfilamentous, and are Gram-stain-positive, except for *Gardnerella vaginalis*, which is Gram-stain-variable. They are anaerobic (among the *Bifidobacterium* species, some can tolerate O_2, but only in the presence of CO_2; *Bifidobacterium psychraerophilum*, *Bifidobacterium scardovii*, and *Bifidobacterium tsurumiense* can grow under aerobic conditions) or facultatively anaerobic (*Gardnerella*). However, *Aeriscardovia* can grow under aerobic conditions. Members of the family are negative for gelatin hydrolysis, indole production, and oxidase activity, but possess fructose 6-phosphoketolase (F6PPK; EC 4.1.2.22), which cleaves fructose 6-phosphate into acetylphosphate and erythrose 4-phosphate. The DNA G+C content varies from 42 to 67 mol%. They are chemo-organotrophs, having a fermentative type of metabolism, and produce acid, but not gas, from a variety of carbohydrates. Optimum growth temperature is 30–39°C.

Members of the family have been isolated from animal and human sources. They are non-pathogenic except for *Bifidobacterium dentium*, *Scardovia inopinata*, and *Parascardovia denticolens*, which have been isolated from dental caries and are probably involved in caries pathology. *Gardnerella vaginalis* often assumes pathogenic characteristics, such as in bacterial vaginosis and in urogenital tract infections of both sexes.

DNA G+C content (mol%): 42–67 (T_m).

Type genus: **Bifidobacterium** Orla-Jensen 1924, 472[AL].

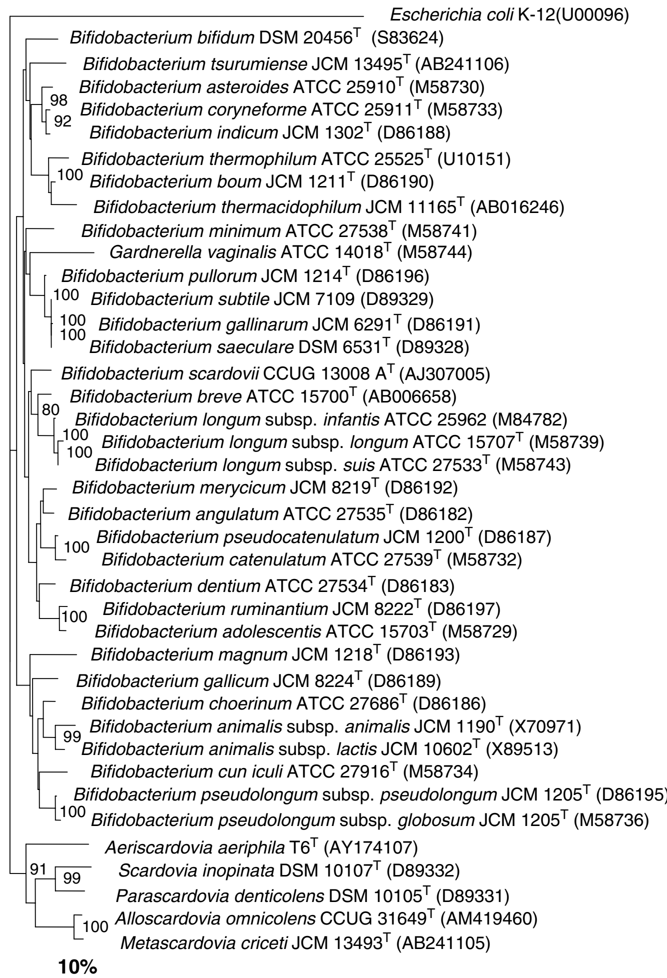

FIGURE 66. Phylogenetic tree constructed from partial 16S rRNA gene sequences of members of the family *Bifidobacteriaceae*. The tree was rooted with *Escherichia coli*, and constructed by using the neighbor-joining method with bootstrap values calculated from 1000 trees (represented as percentages and given at each branch). For each species, the GenBank accession number for the respective 16S rRNA gene sequence is indicated. The scale bar shows the number of nucleotide substitutions per site. This figure was kindly provided by Professor Maasaki Okamoto (University of Tsurumi, Japan).

Genus I. **Bifidobacterium** Orla-Jensen 1924, 472[AL]

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Bi.fi.do.bac.te′ri.um. L. adj. *bifidus* cleft, divided; L. neut. n. *bacterium* a small rod; N.L. neut. n. *Bifidobacterium* a cleft rodlet.

Rods of various shapes: short, regular, thin cells with pointed ends, coccoidal regular cells, or long cells with slight bends or protuberances or with a large variety of branching; pointed, with slightly bifurcated **club-shaped or spatulated extremities**; may occur singly or in chains of many elements; may occur in star-like aggregates or in "V" or "palisade" arrangements.

Colonies are smooth, convex with entire edges, cream to white, glistening, and of soft consistency. **Gram-stain-positive, non-acid-fast, nonsporeforming, and nonmotile. Cells often stain irregularly with methylene blue**. **Anaerobic**; some species can tolerate O_2, but only in the presence of CO_2 and the more recently described species, such as *Bifidobacterium psychraerophilum*, *Bifidobacterium scardovii*, and *Bifidobacterium tsurumiense*, can grow under aerobic conditions (Okamoto et al., 2008). Optimum growth temperature is 37–41°C, except for *Bifidobacterium mongoliense*, which exhibits an optimal growth temperature of 30°C; minimum growth temperature is 25–28°C, with the exception of *Bifidobacterium mongoliense* and *Bifidobacterium psychraerophilum*, which can grow at 15°C and 8°C, respectively; maximum growth temperature is 43–45°C, with the exception of *Bifidobacterium thermacidophilum*, which exhibits a maximal growth temperature of 49.5°C. Growth at 45°C seems to discriminate between animal and human strains, since most of the animal but not the human strains are able to grow at this temperature (Gavini et al., 1991). Optimum pH for initial growth is 6.5–7.0; does not grow at pH 4.5–5.0 (except for *Bifidobacterium thermacidophilum*, which can grow at pH 4.5) or pH 8.0–8.5.

Saccharoclastic. Acetic and lactic acid are formed primarily in a 3:2 molar ratio. CO_2 is not produced (except during the degradation of gluconate). Small amounts of ethanol, formic acid, and succinic acid are produced. **Butyric and propionic acids are not produced. Glucose is degraded exclusively and characteristically by the fructose 6-phosphate shunt in which fructose-6-phosphoketolase** (F6PPK; EC 4.1.2.22) **cleaves fructose 6-phosphate into acetylphosphate and erythrose 4-phosphate.** End products are formed through the sequential action of transaldolase (EC 2.2.1.2), transketolase (EC 2.2.1.1), xylulose-5-phosphate phosphoketolase (EC 4.1.2.9) and enzymes of the Embden–Meyerhof pathway (EMP) acting on glyceraldehyde 3-phosphate. Additional acetic and formic acids may be formed through cleavage of pyruvate. Glucose-6-phosphate dehydrogenase (G6PDH; EC 1.1.1.49, $NADP^+$- or NAD^+-dependent) activity is not generally measurable.

Catalase-negative except for *Bifidobacterium asteroides* and *Bifidobacterium indicum*, which are catalase-positive when grown in the presence of air with or without added hemin. Ammonium is generally utilized as a source of nitrogen.

Source: present in the intestine of man, various animals, and honey bees. Also found in sewage, fermented milk products, and human clinical samples.

DNA G+C content (mol%): 47–67 (Bd or T_m).

Type species: **Bifidobacterium bifidum** (Tissier 1900) Orla-Jensen 1924, 472[AL] (*Bacillus bifidus* Tissier 1900, 86).

Further descriptive information

Phylogeny. Tissier discovered bifidobacteria in infant feces and called them "*Bacillus bifidus*" (Tissier, 1900, 1899). In 1924, Orla-Jensen recognized the existence of the genus *Bifidobacterium* as a separate taxon, but given their similarities to the genus *Lactobacillus*, bifidobacteria were included in the genus *Lactobacillus* as listed in the 7th edition of *Bergey's Manual of Determinative Bacteriology* (Breed et al., 1957).

In the 8th edition of *Bergey's Manual of Determinative Bacteriology* (Buchanan and Gibbons, 1974), bifidobacteria were classified in the genus *Bifidobacterium*, the name initially adopted by Orla-Jensen. The genus comprised eight species and was included in the family *Actinomycetaceae* of the order *Actinomycetales*, although with regard to the murein structure of the cell wall, bifidobacteria are more similar to the family *Lactobacillaceae* than to the family *Actinomycetaceae* (Kandler and Lauer, 1974).

In the 1st edition of *Bergey's Manual of Systematic Bacteriology* (Sneath et al., 1986), as well as the 9th edition of *Bergey's Manual of Determinative Bacteriology* (Holt et al., 1993), members of the genus *Bifidobacterium* were grouped and defined as "irregular, nonsporing, Gram-positive rods".

The introduction of rRNA gene sequence analyses in studies of bacterial phylogeny confirmed the assignment of bifidobacteria to an "*Actinomyces*" group (Fox et al., 1980). rRNA gene sequence analyses were used mainly to determine relationships between genera, families, and other ranks. Molecular phylogeny, based on high correlations between several molecules, e.g., 16S rRNA genes (Woese, 1987), 23S rRNA genes (Collins and Wallbanks, 1992), and genes encoding elongation factors involved in translation and the subunit of ATPase (Ludwig et al., 1993), led to the description of domains for the three highest taxa: *Archaea*, *Bacteria*, and *Eukarya* (Woese et al., 1990).

Stackebrandt et al. (1997) proposed a novel hierarchical structure for the phylogenetic group of "*Actinomycetes*, bacteria and relatives" based on 16S rRNA gene sequence data. This proposal did not change the descriptions of species and genera based upon morphological, chemotaxonomic, and physiological properties, but provided descriptions of taxa above the genus level. The genus *Bifidobacterium* was classified as a single taxon in the family *Bifidobacteriaceae* of the order *Bifidobacteriales*, subclass *Actinobacteridae*, in the class *Actinobacteria*, phylum *Firmicutes* of the domain *Bacteria* (Stackebrandt et al., 1997). Garrity and Holt (2001), based on an extensive principal component analysis of 16S rRNA gene sequence data, proposed elevating the class *Actinobacteria* (Stackebrandt et al., 1997) to the rank of phylum, recognizing that the phylogenetic depth represented in this lineage is equivalent to that of existing phyla and that the group shows clear separation from the *Firmicutes*. Within the phylum *Actinobacteria*, the single class *Actinobacteria* was preserved as in the complete hierarchical structure proposed by Stackebrandt et al. (1997). The order *Bifidobacteriales* is classified in subclass *Actinobacteridae*, class *Actinobacteria*, phylum *Actinobacteria* in the domain *Bacteria*. The *Bifidobacteriales* comprise a separate group lying adjacent to, but away from the major lineages within the order *Actinomycetales*, a classification consistent with the published phylogenetic model of Stackebrandt et al. (1997). A 16S rRNA gene sequence-based phylogenetic tree of the family *Bifidobacteriaceae* is shown in Figure 66.

Cell morphology. Cell morphology and its variations, which are affected by different culture conditions, have been investigated widely (Poupard et al., 1973). However, recent discoveries of novel *Bifidobacterium* species from a variety of sources have permitted a clearer picture of the morphology of members of the genus. A comparison of the cell morphology of large numbers of strains grown anaerobically (Merck system) in stabs of trypticase-phytone-yeast extract (TPY) medium showed that some species had distinct cell shapes or arrangements, which might help in their identification. These traits are exemplified in Figure 67.

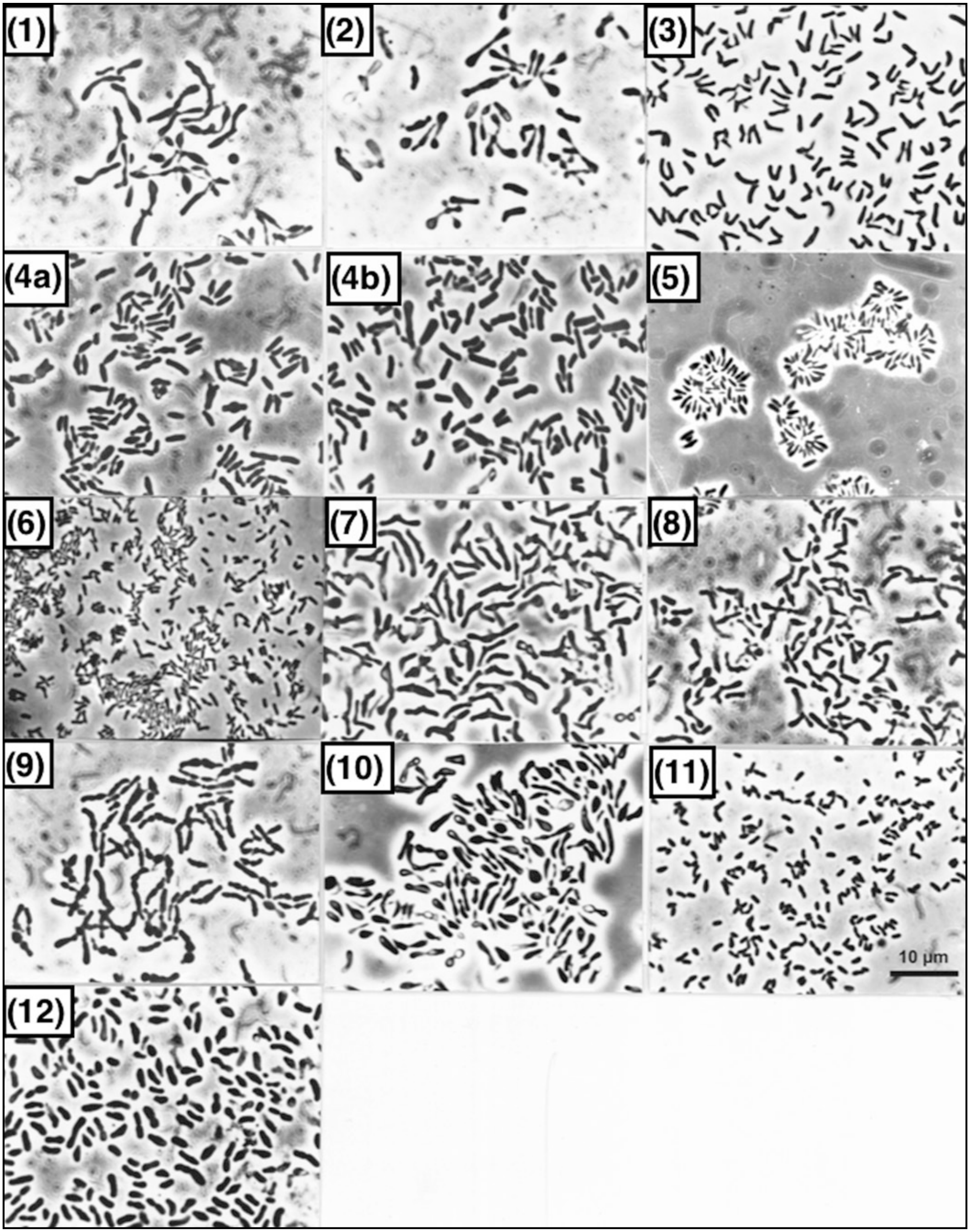

FIGURE 67 (A). (continued)

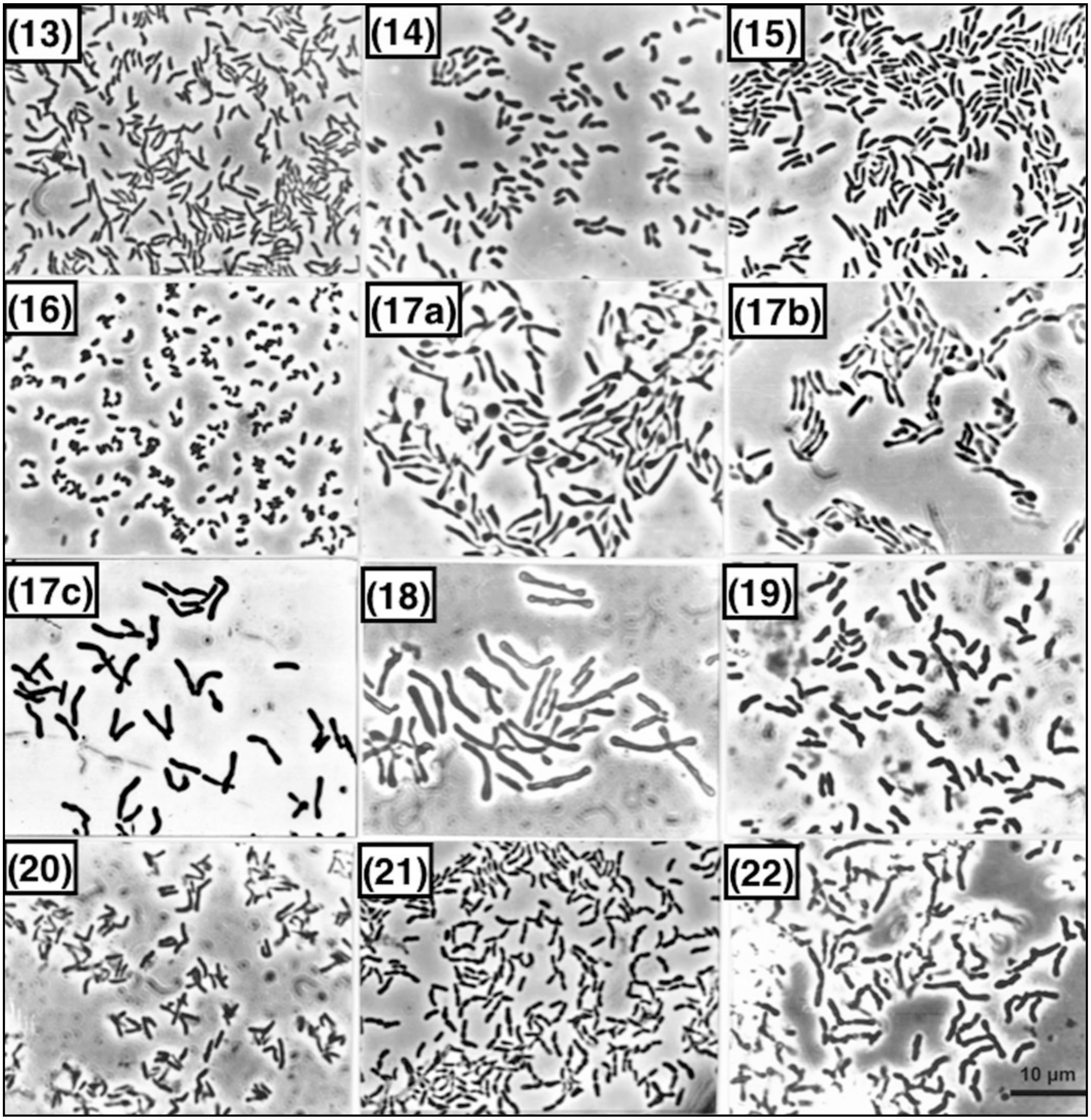

FIGURE 67 (B). (continued)

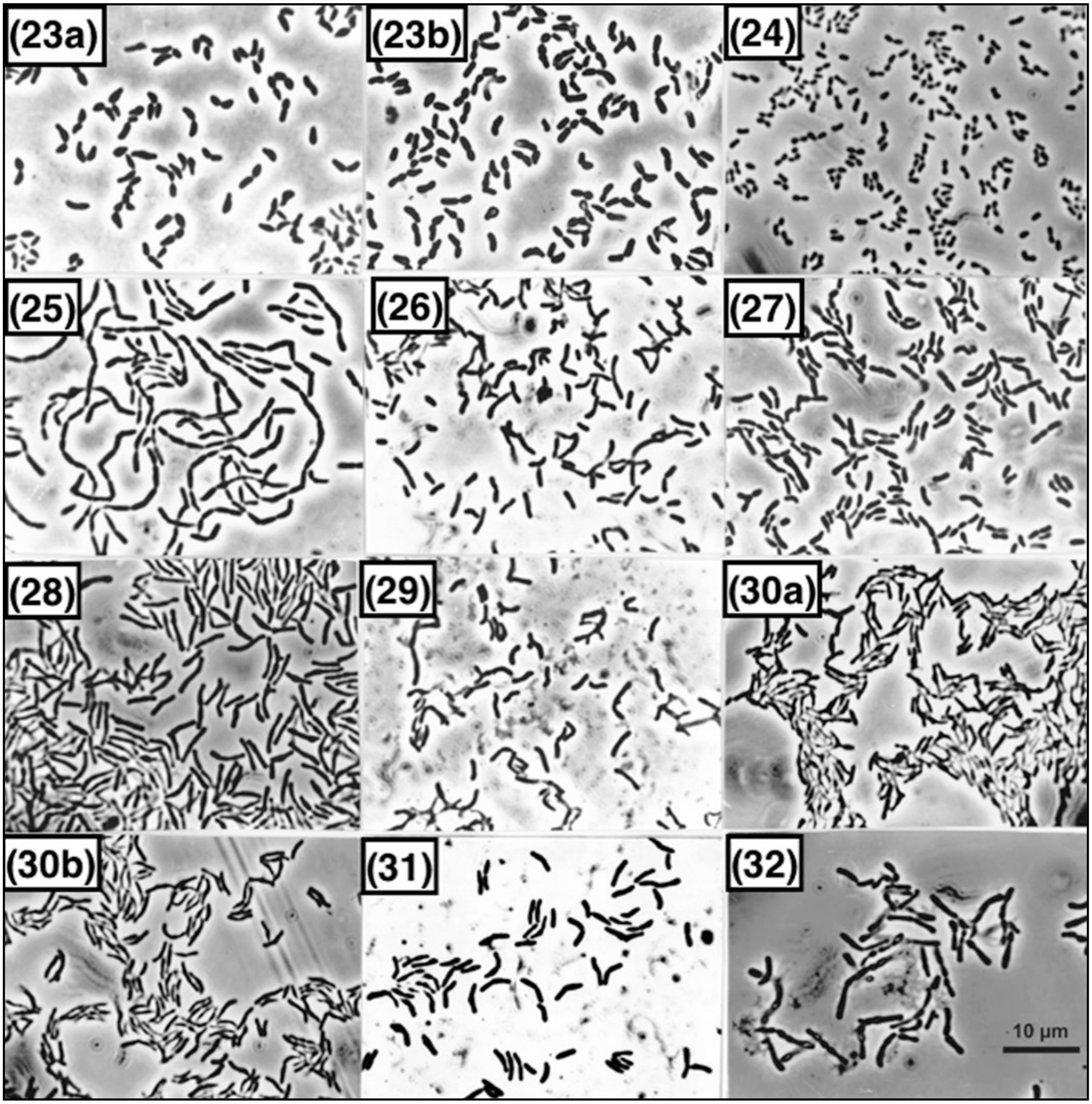

FIGURE 67 (C). (A) Panels 1–12; (B) panels 13–22; (C) panels 23–32 (panel numbers correspond to the species number in the List of species). Cellular morphology in the genus *Bifidobacterium*. Cells of the type strains were grown in TPY medium stabs. Phase-contrast photomicrographs (magnification 1500×). Part A: 1, *B. bifidum*; 2, *B. adolescentis*; 3, *B. angulatum*; 4a, *B. animalis* subsp. *animalis*; 4b, *B. animalis* subsp. *lactis*; 5, *B. asteroides*; 6, *B. bombi*; 7, *B. boum*; 8, *B. breve*; 9, *B. catenulatum*; 10, *B. choerinum*; 11, *B. coryneforme*; 12; *B. cuniculi*. Part B: 13, *B. dentium*; 14, *B. gallicum*; 15, *B. gallinarum*; 16, *B. indicum*; 17a, *B. longum* subsp. *longum*; 17b, *B. longum* subsp. *infantis*; 17c, *B. longum* subsp. *suis*; 18, *B. magnum*; 19, *B. merycicum*; 20, *B. minimum*; 21, *B. mongoliense*; 22, *B. pseudocatenulatum*. Part C: 23a, *B. pseudolongum* subsp. *pseudolongum*; 23b, *B. pseudolongum* subsp. *globosum*; 24, *B. psychraerophilum*; 25, *B. pullorum*; 26, *B. ruminantium*; 27, *B. saeculare*; 28, *B. scardovii*; 29, *B. subtile*; 30a, *B. thermacidophilum* subsp. *thermacidophilum*; 30b, *B. thermacidophilum* subsp. *porcinum*; 31, *B. thermophilum*; 32, *B. tsurumiense*.

Of particular interest are the well known amphora-like cells of *Bifidobacterium bifidum* (Sundman et al., 1959) (Figure 67, panel 1), the V or palisade arrangement of cells in *Bifidobacterium angulatum* (Figure 67, panel 3), the linear groups of globular elements in *Bifidobacterium catenulatum* (Figure 67, panel 9), the long chains of regular cells in *Bifidobacterium pullorum* (Figure 67, panel 25), the middle-enlarged cells of *Bifidobacterium animalis* (Figure 67, panels 4a and 4b), the cellular dimensions of *Bifidobacterium magnum* (Figure 67, panel 18), the small cells of *Bifidobacterium minimum* (Figure 67, panel 20) and *Bifidobacterium psychraerophilum* (Figure 67, panel 24), and the unusual star-like arrangements of cells of *Bifidobacterium asteroides* (Figure 67, panel 5).

The cellular shape most frequently encountered in *Bifidobacterium* species that do not have a distinct morphology as observed on TPY stabs (see above) is depicted in Figure 67, panel 26. Details of cellular morphologies are given under single species descriptions. *Bifidobacterium asteroides* (star-like clusters) and *Bifidobacterium indicum* (small rods or coccobacilli), the species with the most nonbifid-like morphology in the classic sense, show features that are common to the morphology of the other bifidobacteria only when grown in nutritionally deficient media (Scardovi and Trovatelli, 1969); this seems to be a general trend in this group of bacteria (Glick et al., 1960; Sundman and Björksten, 1958).

Cell-wall composition. Several workers have studied the cell-wall murein structure of bifidobacteria (Kandler and Lauer, 1974; Lauer and Kandler, 1983) (Table 21). Closely related species can be distinguished clearly on this basis, for example, *Bifidobacterium boum* from *Bifidobacterium thermophilum*, and *Bifidobacterium minimum* from *Bifidobacterium subtile*. These authors also reported that bifidobacterial cell walls contained significant amounts of polysaccharides, normally galactose and glucose often associated with rhamnose. The structure and composition of cell-wall polysaccharides have been investigated (Habu et al., 1987; Nagaoka et al., 1988, 1995). The presence of lipoteichoic acid, which remains insoluble after lysozyme treatment, in the cell wall has been reported in *Bifidobacterium bifidum* (Veerkamp et al., 1983); its chemical composition and structure have been studied (Fischer et al., 1987; Op den Camp et al., 1984). Immunochemical studies have indicated that lipoteichoic acid is a common antigen within the genus *Bifidobacterium* (Op Den Camp et al., 1985b). Furthermore, proteins and lipoteichoic acid are responsible, either independently or in dynamic complexes, for the hydrophobic character of the bifidobacterial surface (Op den Camp et al., 1985a).

Mukai et al. (1997) suggested that cell surface proteinaceous components are involved in the adhesion of *Bifidobacterium adolescentis*. Studies on cellular structures (lipoteichoic acids, proteins) and the interaction of cells with substrates (*in vivo* and *in vitro* adhesion) are needed to understand the mechanisms that regulate bacterial adhesion and, therefore, colonization. Protein ligands present on the cell surfaces and/or in the culture medium have been identified by Bernet et al. (1993) in some strains of bifidobacteria of human origin (*Bifidobacterium bifidum*, *Bifidobacterium breve*, *Bifidobacterium longum* subsp. *infantis*, and *Bifidobacterium longum* subsp. *longum*). These investigations have shown that different strains belonging to the same species adhere differently to intestinal cells of Caco-2 and HT29MTX cell lines. More recently, Pérez et al. (1998) studied the adhesive capacity of some strains of human origin showing that, in many cases, adhesion was linked to the self-agglutination capacity of such strains: organisms with a high self-agglutination capacity tested positive by hemagglutination and showed a high surface hydrophobicity.

An important contribution to the study of cell-wall-associated proteins in *Bifidobacterium pseudolongum* subsp. *globosum* was made by Mattarelli et al. (1993). Using plasmid-free clones, obtained by "curing", the involvement of certain plasmids in determining the surface protein patterns was ascertained. Sensitivity to lytic and bactericidal activities of lysozyme and cationic polypeptides was found to be related to the presence of a plasmid-dependent surface protein of high molecular mass (Mattarelli et al., 1997). The effect of temperature on the biosynthesis of cell-wall proteins and cell hydrophobicity has been studied in *Bifidobacterium pseudolongum* subsp. *globosum*. The production of cell-wall proteins is temperature-dependent: expression at low temperature is considerably attenuated, whereas it increases at medium and high growth temperatures. At medium growth temperatures, cell hydrophobicity was strictly correlated with cell-wall protein expression, whereas at low and high growth temperatures, the presence of cell-wall protein only partially influenced the hydrophobic features (Mattarelli et al., 1999).

The relationship between interaction sites in the gut, hydrophobicity, mucosal immunomodulating capacities, and cell-wall protein profiles in bifidobacteria was investigated by Vinderola et al. (2004). The differences in the immunopotentiating capacity of the various strains might be related to differences in their cell-wall protein profiles. The relationship between the presence of cell-wall proteins and autoaggregating ability in *Bifidobacterium adolescentis* and *Bifidobacterium bifidum* was investigated by Canzi et al. (2005). These authors also characterized the cell surface properties of *Bifidobacterium* strains isolated from human fecal material.

A deeper understanding of the ecological variability of the surface structure in members of the genus *Bifidobacterium* and its function in the multifaceted relationships of host and bacterium are thought to be important prerequisites for a better understanding of the colonization mechanism of bifidobacteria.

The phospholipid composition of some *Bifidobacterium* and *Lactobacillus* species were studied by Exterkate et al. (1971). Differences in polyglycerol phospholipid and aminoacyl phosphatidylglycerol composition helped to differentiate between the two genera. The effects of growth conditions on the lipid and ionic composition of *Bifidobacterium bifidum* were examined by Veerkamp (1977a, 1977b). The changes in bacterial lipid surface composition during growth in bile-containing medium were studied by Gómez Zavaglia et al. (2002), who found that lipid composition was not directly related to adhesive properties.

Fine structure. The ultrastructure of bifidobacteria has received little attention. Overman and Pine (1963) presented ultrastructure micrographs of *Bifidobacterium bifidum* (strain Reuter, S 28a variant, ATCC 15696). Zani and Severi (1982) made a more extensive investigation of the ultrastructure of this strain. Scanning and transmission electron microscopy studies revealed a possible sequence of morphological events during transformation from one pleomorphic form to another in

Bifidobacterium bifidum and *Bifidobacterium longum* subsp. *longum* (Bauer et al., 1975). An ultrastructural study of *Bifidobacterium pseudolongum* subsp. *pseudolongum* and *Bifidobacterium thermophilum* revealed the production of extensive amounts of extracellular material only in the former. The cell walls of these organisms differed in thickness: approximately 0.2 μm and approximately 0.05 μm for *Bifidobacterium pseudolongum* subsp. *pseudolongum* and *Bifidobacterium thermophilum*, respectively (Kudo et al., 1989).

Nutrition and growth conditions. Little was known about bifidobacterial nutrition up to 1969. The majority of studies focused on *Bifidobacterium bifidum* (strain Gyorgy and Rose 212A, ATCC 11863, *ex Lactobacillus bifidus* var. *pennsylvanicus*). Some of these studies demonstrated that biotin and calcium pantothenate, in association with cysteine, were essential components for growth promotion and that calcium pantothenate could be replaced by pantethine (Hassinen et al., 1951). This strain was found to require pantethine, a "bifidus factor" oligosaccharide-type compound present in human milk and a "supplementary factor" present in human and cow's milk and in pancreatic extract (György and Rose, 1955). The growth stimulating effect of pancreatin had previously been shown by Tomarelli et al. (1949).

In 1969, Scardovi and Trovatelli showed that only *p*-aminobenzoic and folic acids, together with their bases, were indispensable for the growth of *Bifidobacterium asteroides*, a species isolated from honey bees, when it was cultivated on synthetic medium (Gyllenberg and Carlberg, 1958). Later, a survey on the nutritional requirements of nine *Bifidobacterium* species of human and animal origin, including *Bifidobacterium dentium*, demonstrated greater heterogeneity among the species, with calcium pantothenate and riboflavin being the most frequently required nutrients for growth (Trovatelli and Biavati, 1978). The results of Matteuzzi et al. (1978) from a survey of 18 *Bifidobacterium* species were in agreement with those of Hassinen et al. (1951), namely that a large number of bifidobacteria grew on ammonium salts as a sole nitrogen source in a relatively simple synthetic medium, although *Bifidobacterium choerinum*, *Bifidobacterium cuniculi*, *Bifidobacterium magnum*, *Bifidobacterium pseudolongum* subsp. *globosum*, and *Bifidobacterium longum* subsp. *suis* did not grow in the absence of organic nitrogen (Mattarelli and Biavati, 1999b). The pantothenate-type compounds biotin and riboflavin were also reported to be required for growth of human species of the genus, such as *Bifidobacterium adolescentis*, *Bifidobacterium bifidum*, *Bifidobacterium breve*, *Bifidobacterium longum* subsp. *infantis*, and *Bifidobacterium longum* subsp. *longum* (Deguchi et al., 1985). In addition, nicotinic acid and thiamin have been found to be essential for some *Bifidobacterium* strains.

The activity of enzymes such as glutamate dehydrogenase and glutamine synthetase allows the assimilation of ammonia. Glutamine synthetase has been isolated and characterized from *Bifidobacterium bifidum*, *Bifidobacterium breve*, and *Bifidobacterium pseudolongum* subsp. *pseudolongum* (Hatanaka et al., 1987).

In organic nitrogen-free media, considerable amounts of various amino acids are excreted by strains of *Bifidobacterium adolescentis*, *Bifidobacterium animalis*, *Bifidobacterium dentium*, *Bifidobacterium longum* subsp. *infantis*, and *Bifidobacterium thermophilum*. In general, the amino acids produced in the largest amounts are alanine, aspartic acid, and valine. *Bifidobacterium bifidum* can produce up to 150 mg/l threonine (Matteuzzi et al., 1978).

Analog-resistant mutants obtained from *Bifidobacterium thermophilum* (*ex Bifidobacterium ruminale*) show increased production of isoleucine and valine (Crociani et al., 1977; Matteuzzi et al., 1976). Homoserine dehydrogenase and threonine deaminase activities, as well as some aspects of their regulation, have been studied in many *Bifidobacterium* species (Selli et al., 1986).

Studies on the nutrition of plasmid-negative and plasmid-positive clones of *Bifidobacterium pseudolongum* subsp. *globosum* revealed auxotrophy for L-methionine in both clones (Mattarelli and Biavati, 1999b). Auxotrophy for L-leucine was shown only for plasmid-positive clones, thereby showing the involvement of plasmids in this requirement. The lack of β-isopropyl malate dehydrogenase enzyme activity is considered to be responsible for the L-leucine auxotrophy in the plasmid-positive clones. Minimal amino acid requirements were also investigated. Only six or seven amino acids were required for growth, regardless of the ones chosen, including L-leucine and/or L-methionine for plasmid-positive and plasmid-negative clones, respectively (Mattarelli and Biavati, 1999a).

A survey of growth factor and vitamin requirements was made in 10 species of bifidobacteria and the different species were shown to have a great deal of heterogeneity. Comparative studies on the synthesis of water-soluble vitamins (cyanocobalamin, folic acid, nicotinic acid, pyridoxine, riboflavin, and thiamin) among species isolated from human feces have shown that many strains can synthesize all of the vitamins tested, with the exception of pantothenic acid and riboflavin (Tamura, 1983; Trovatelli and Biavati, 1978). The concentration of the vitamins accumulated varies widely among different species or strains (Deguchi et al., 1985). The ability of bifidobacteria to synthesize biotin has also been demonstrated (Noda et al., 1994).

Some factors, called "bifidus factors", are able to stimulate growth of bifidobacteria. The first "bifidus factor" was proposed by György et al. (1954a, 1954b); it has been found in human milk and also, in minor amounts, in the milk of several other mammals. This factor was later found to be composed of glycoproteins and oligosaccharides containing *N*-acetyl-D-glucosamine (Bezkorovainy and Topouzian, 1981). Another bifidogenous factor has been identified in the low protein content of human milk. A reduction in the protein content of milk formulae should lower the buffering capacity and thus allow the formation of an acidic milieu in the gut that can promote the growth of bifidobacteria (Heine et al., 1992). However, in an experimental study, the use of protein-reduced formula milk (1.2 g/100 ml) did not increase the number of bifidobacteria.

Lactoferrin, an iron-binding protein, promotes the growth of bifidobacteria. The study by Petschow et al. (1999) suggested that bovine lactoferrin from mature milk increases the growth of *Bifidobacterium breve* and *Bifidobacterium infantis* in a concentration-dependent way *in vitro*, whereas human lactoferrin promotes the growth of *Bifidobacterium bifidum*, but not that of *Bifidobacterium breve* and *Bifidobacterium infantis*. Oligo- and polysaccharides are also classified as "bifidogenic factors"; this may be explained by the evolution of these bacteria in the gastrointestinal tract where competition for nutrients is intense.

Pure culture growth studies have shown that, in addition to a wide spectrum of suitable energy sources, bifidobacteria show

TABLE 21. Type strains, chemotaxonomic properties, and sources of species of the genus *Bifidobacterium*

Species	Murein type[a]	DNA G+C content[b]	Electrophoretic patterns of enzymes:[c] 6PGD	Transaldolase	Source
B. bifidum	L-Orn–D-Ser–D-Asp	61	**7**, (8)	7	Feces of human adults, infants, and suckling calves; human vagina
B. adolescentis	L-Lys(L-Orn)–D-Asp	59	5	8	Feces of human adults; bovine rumen; sewage
B. angulatum	L-Lys–D-Asp	59	5	5	Sewage; feces of human adult
B. animalis					
subsp. *animalis*	L-Lys(L-Orn)–L-Ser–(L-Ala)–L-Ala_2	61.3	8, **9**	5	Feces of guinea pigs and rats
subsp. *lactis*	L-Lys(L-Orn)–L-Ser–(L-Ala)–L-Ala_2	61	nt	nt	Feces of calves, chickens, and rabbits; sewage; fermented milk (yogurt)
B. asteroides	L-Lys–Gly	59	(9), (9a), (9b), (10), **10a**, 10b, (11), (12), (13)	(6), (7), (7a), **8**, (8a), (8b), (9), (9a)	Intestine of *Apis mellifera* subsp. *caucasica*, *ligustica*, and *mellifera*
B. bombi		47.3	nt	nt	Digestive tract of different bumblebees
B. boum	L-Lys–D-Ser–D-Glu	60	**8**, 9, 9a	6	Bovine rumen; feces of piglets
B. breve	L-Lys–Gly	58 (Bd)	(5), 6, 6a, 7	6	Feces of infants and suckling calves; human vagina; sewage
B. catenulatum	L-Lys(L-Orn)–L-Ala_2–L-Ser	54.7	**6**, 8	5	Feces of infants and human adults; human vagina; sewage
B. choerinum	L-Lys(L-Orn)–L-Ser–(L-Ala)–L-Ala_2	66.3	4	3	Feces of piglets; sewage
B. coryneforme	L-Lys–D-Asp	nt	6	6	Intestine of *Apis mellifera* subsp. *mellifera*
B. cuniculi	L-Lys(L-Orn)–L-Ser–(L-Ala)–L-Ala_2	64.1	4	1	Feces of rabbits
B. dentium	L-Lys(L-Orn)–D-Asp	61.2	(2)	4	Human dental caries and oral cavity; feces of human adults; human vagina; human abscess and appendix
B. gallicum	L-Lys–L-Ala–L-Ser	61	nt	Present	Human feces
B. gallinarum	L-Lys–D-Asp	65.7	Absent	Present	Chicken cecum
B. indicum	L-Lys–D-Asp	60	6, 6a, (7), 8, (**9**), (9a), (9b)	(6), 7, 8, **9**	Intestine of *Apis cerana* and *A. dorsata*
B. longum					
subsp. *longum*	L-Orn–L-Ser–L-Ala–L-Thr–L-Ala	61	5, (**6**)	(5), 6, **8**	Feces of human adults, infants, and suckling calves; human vagina; sewage
subsp. *infantis*	L-Orn–L-Ser–L-Ala–L-Thr–L-Ala	60.5	(3), **4**, (5)	5, (6), (**8**)	Feces of infants and suckling calves; human vagina
subsp. *suis*	L-Orn–L-Ser–L-Ala–L-Thr–L-Ala	62	5, 8	6	Feces of piglets
B. magnum	L-Lys(L-Orn)–L-Ala_2–L-Ser	60	7	5	Feces of rabbits
B. merycicum	L-Lys(L-Orn)–D-Asp	59	Present	Present	Bovine rumen
B. minimum	L-Lys–L-Ser	61.5	6	10	Sewage; pig cecum
B. mongoliense	L-Lys–D-Asp	61.1	nt	nt	Fermented milk (airag)
B. pseudocatenulatum	L-Lys(L-Orn)–L-Ala_2–L-Ser	57.5	**1**, 3	**4**, (5)	Feces of infants and suckling calves; sewage

B. pseudolongum					
subsp. *pseudolongum*	L-Orn(L-Lys)–L-Ala_{2-3}	64.8	(**3a**), (4), (5), (**6**), (7)	2	Feces of bulls, calves, chickens, dogs, guinea pigs, pigs, and rats
subsp. *globosum*	L-Orn(L-Lys)–L-Ala_{2-3}	64.1	7	2	Bovine rumen; feces of lambs, piglets, rabbits, rats, and suckling calves; sewage
B. psychraerophilum		59.2	nt	nt	Pig cecum (content and epithelium)
B. pullorum	L-Lys–D-Asp	67.4	nd	2	Feces of chickens
B. ruminantium	L-Lys(L-Orn)–L-Ser–(L-Ala)–L-Ala_2	57	Present	Present	Bovine rumen
B. saeculare	L-Lys(L-Orn)–D-Asp	63	Absent	Present	Feces of rabbit
B. scardovii	L-Lys–L-Ser–L-Ala	60 (HPLC)	nt	nt	Human blood
B. subtile	L-Lys–D-Asp	61.5	2	3	Sewage
B. thermacidophilum					
subsp. *thermacidophilum*		56.8	nt	nt	Wastewater
subsp. *porcinum*		61	nt	nt	Piglet feces
B. thermophilum	L-Orn(L-Lys)–D-Glu	60	7, 8, **9**, (9a)	(7), **8**	Feces of chickens, pigs, and suckling calves; bovine rumen; sewage
B. tsurumiense	Glu–Lys–Asp–$(Ala)_2$	53	nt	nt	Hamster dental plaque

[a] Data from Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH (DSMZ). Amino acids given in parentheses partly replace the other one cited.

[b] Mean values (mol%) are given; data from Scardovi (1986). For data for recently described species, see species descriptions.

[c] Numbers 1–13 and 1–10 were given to 6-phospho-D-gluconate dehydrogenase isoenzymes:NAD(P)oxidoreductase (6PGD) and transaldolase, respectively, based on the order of decreasing mobility ("a" and "b" indicate additional isozymes). Numbers in bold are the isozymes of the type strains. Numbers in parentheses are isozymes found in less than 10% of the strains studied. 6PGD is undetectable by spot staining in most strains of *Bifidobacterium dentium* and in all strains of *Bifidobacterium pullorum*. "Present" and "Absent" indicate the presence and absence, as determined by other methods. Data from Scardovi et al. (1979a). nt, Not tested; nd, not detectable.

preferences for different molecules. Substrate utilization shows growth rates with considerable interspecies and interstrain differences (Gibson and Wang, 1994b; Hopkins et al., 1998). Furthermore, it has also been found that oligomeric forms of some monosaccharides are utilized more efficiently than the monomer itself (Anderson et al., 2001). Among the numerous oligosaccharides, soybean oligosaccharides, which consist mainly of the trisaccharide raffinose and the tetrasaccharide stachyose (Benno et al., 1987; Hayakawa et al., 1990; Yazawa et al., 1978), stimulate growth of bifidobacteria. More recently, other oligosaccharides have been identified and termed "bifidogenic factors", such as fructooligosaccharides (FOS) (Gibson and Wang, 1994a; Roberfroid et al., 1998), synthetic oligosaccharides, and transgalactosylated oligosaccharides (TOS) (Bouhnik et al., 1997; Ito et al., 1993). Glucooligosaccharides also stimulate the growth of bifidobacteria, although less intensely than FOS and TOS (Djouzi and Andrieux, 1997; Jaskari et al., 1998). The polysaccharide inulin also increases fecal bifidobacteria, having a similar prebiotic effect to oligofructose (Gibson and Roberfroid, 1995). In conclusion, "bifidogenic factors" can be classified into two large groups: oligosaccharides and protein hydrolyzates. In recent years, the need for bifidus factors for growth has been questioned because it is possible to obtain bifidobacterial multiplication in the absence of these factors and, hence, they cannot be defined as growth factors.

The metalloelement requirements of *Bifidobacterium bifidum* and *Bifidobacterium thermophilum* have been studied extensively by Bezkorovainy and colleagues (Bezkorovainy and Topouzian, 1983; Bezkorovainy et al., 1986, 1987; Topouzian et al., 1984). It is to be hoped that these investigations, which are important for bifidobacterial ecology, will be extended to other species of the genus. Bifidobacteria accumulate iron when it is presented in the ferrous oxidation state. This form of iron would be expected under anaerobic conditions, such as those prevalent in the human colon or cattle rumen. The iron accumulation by bifidobacteria under these conditions may serve a host defense function ("nutritional immunity") preventing the growth of pathogens for want of iron (Bezkorovainy and Solberg, 1989). Bifidobacteria possess a membrane-bound ferrireductase, which ensures the conversion of iron from the ferric to the ferrous state at the cell surface. In *Bifidobacterium*, iron uptake does not depend on siderophore-type carriers, but on a proton-motive-force-associated electrogenic pump or pumps (Kot and Bezkorovainy, 1993).

Anaerobiosis. Bifidobacteria are anaerobic micro-organisms. They usually do not develop in plates under aerobic conditions. However, oxygen sensitivity differs depending on the strain or species, the reasons for which are equivocal (de Vries and Stouthamer, 1969). Most species do not develop in slants incubated under an atmosphere of CO_2-enriched air (90% air/10% CO_2), but *Bifidobacterium pseudolongum* subsp. *globosum*, *Bifidobacterium thermophilum*, and *Bifidobacterium longum* subsp. *suis* do so without the cells becoming catalase-positive even if hemin is added to the medium. *Bifidobacterium asteroides* grows under these conditions and becomes catalase-positive. *Bifidobacterium indicum* behaves similarly, but is catalase-positive only if hemin is added to the medium (Matteuzzi et al., 1971; Scardovi and Trovatelli, 1969; Scardovi et al., 1969). Most of the recently described species, *Bifidobacterium mongoliense*, *Bifidobacterium psychraerophilum*, and *Bifidobacterium tsurumiense*, can grow on agar under aerobic conditions (Okamoto et al., 2008; Simpson et al., 2004; Watanabe et al., 2009).

Metabolism and metabolic pathways. The fermentation of hexose occurs in the genus *Bifidobacterium* through the sequence of reactions (bifid shunt) shown in Figure 68 (de Vries and Stouthamer, 1967; Scardovi and Trovatelli, 1965; Veerkamp, 1969a, 1969b). However, the theoretical ratio of acetate/lactate of 1.5:1.0 is scarcely ever found in growing cultures of bifidobacteria. Phosphoroclastic cleavage of some pyruvate to formic acid and acetyl phosphate and reduction of acetyl phosphate to ethanol often alters the fermentation balance in favor of the production of acetate and some formic acid and ethanol. Given X as the amount of formic acid produced, the general reaction postulated is: glucose → $(1.5 + 0.5X)$acetate + $(1.0 - X)$lactate + 0.5Xethanol + Xformate (de Vries and Stouthamer, 1968; Lauer and Kandler, 1976). G6PDH and aldolase are either considered to be absent or not detectable, thereby ruling out the monophosphate pathway and the glycolytic system (de Vries and Stouthamer, 1967), although low but detectable levels of these two enzymes have been found in some species (Scardovi and Sgorbati, 1974).

The enzymes of the Leloir pathway of galactose metabolism, i.e., galactokinase (EC 2.7.1.6), hexose-1-phosphate uridylyltransferase (EC 2.7.7.12), and UDP-galactose 4-epimerase (EC 5.1.3.2) are constitutive in glucose-grown cells of *Bifidobacterium*, whereas in other micro-organisms these enzymes are induced by either galactose or fucose (Lee et al., 1980).

The existence of UDP-galactose pyrophosphorylase (Lee et al., 1978) suggests that, at least in *Bifidobacterium* species from human sources, an alternative pathway based on galactose is operative (Lee et al., 1979).

The enzymic carboxylation of phosphoenolpyruvate to oxaloacetate in some bifidobacteria from human feces and from honey bees has been compared with the corresponding activity in strains of *Actinomyces bovis* and *Actinomyces israelii*. In bifidobacteria, this activity is independent of the phosphate acceptor and is irreversible, whereas in *Actinomyces* it is inosine- or guanosine-diphosphate-dependent (Chiappini, 1966).

Many enzymes for feeding mono- and disaccharides into the fructose 6-phosphate shunt have been identified but, in addition, arabinosidases, α- and β-galactosidases, glucosidases, hexosaminidases, inulinase, isomaltase, mannosidases, neopullanase, and xylanases are also present. In addition to those enzymes responsible for degrading complex carbohydrates, eight high-affinity oligosaccharide transporters were found and this may explain preferences for oligomerized sugars.

FOS are among the sugars that qualify as prebiotics. They comprise a diverse family of naturally occurring oligosaccharides used commercially in food products and nutritional supplements. They represent nondigestible oligosaccharides and are composed of short- to medium-size chains of fructose moieties connected by β(2–1) linkages (degree of polymerization, 4–60), which are in turn attached to a terminal glucose unit [also by a β(2–1) bond]. Because of these β(2–1) linkages, FOS are resistant to mammalian enzymes and, thus, are able to reach the colon, where they are reported to serve as a source of highly digestible substrates for bifidobacteria (Cummings et al., 1989; Gilliland, 1990; McKellar and Modler, 1989; Modler et al., 1990).

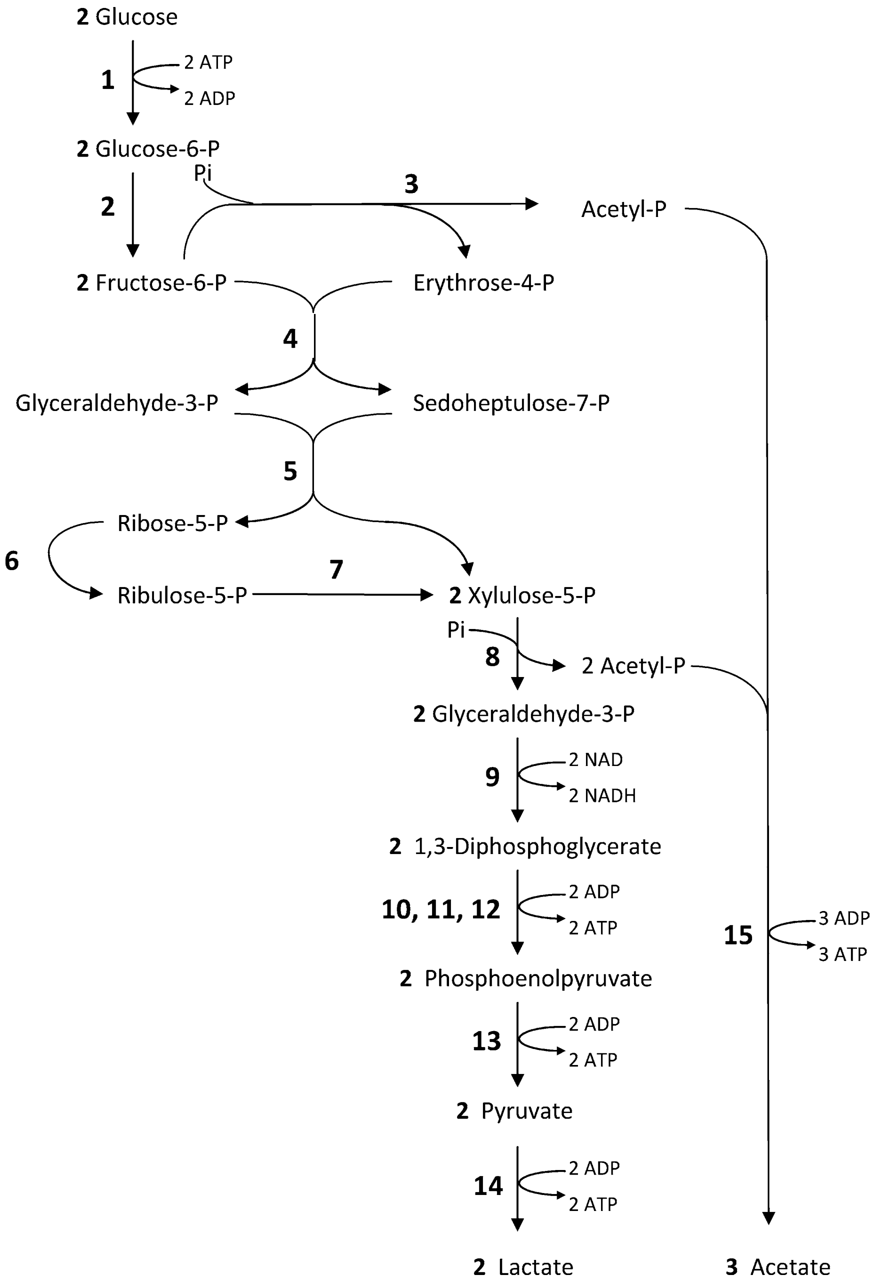

FIGURE 68. *Bifidobacterium* shunt. 1, Hexokinase (EC 2.7.1.2); 2, glucose-6-phosphate isomerase (EC 5.3.1.9); 3, fructose-6-phosphate phosphoketolase (EC 4.1.2.22); 4, transaldolase (EC 2.2.1.2); 5, transketolase (EC 2.2.1.1); 6, ribose-5-phosphate isomerase (EC 5.3.1.6); 7, ribulose-5-phosphate epimerase (EC 4.1.2.9); 8, xylulose-5-phosphate phosphoketolase (EC 4.1.2.9); 9, glyceraldehyde-3-phosphate dehydrogenase (EC 1.2.1.12); 10, phosphoglycerate kinase (EC 2.7.2.3); 11, phosphoglyceromutase (EC 5.4.2.1); 12, enolase (EC 4.2.1.11); 13, pyruvate kinase (EC 2.78.1.40); 14, lactate dehydrogenase (EC 1.1.1.27); 15, acetate kinase (EC 2.7.2.1).

Invertase (EC 3.2.1.26; β-fructofuranosidase) catalyzes hydrolysis of the β(2-1) glycosidic linkage of sucrose releasing an equimolar mixture of glucose and fructose. Recent research on invertase activities found in bifidobacteria has demonstrated that invertase breaks down FOS as well as sucrose (Ehrmann et al., 2003; Imamura et al., 1994; Janer et al., 2004; McKellar and Modler, 1989; Muramatsu et al., 1992; Warchol et al., 2002). An exoinulinase that exhibits β-fructofuranosidase activity has been purified from *Bifidobacterium infantis* ATCC 15697[T]. This enzyme was a monomeric protein (M_r of 70,000) that catalyzes the degradation of both sucrose and inulin (Warchol et al., 2002). A β-fructofuranosidase identified from *Bifidobacterium infantis* was shown to be composed of three identical subunits (M_r of 75,000) and to be capable of catalyzing the hydrolysis of inulin, 1-kestose, nystose, raffinose, and sucrose (Imamura et al., 1994).

The ability of β-fructofuranosidase from *Bifidobacterium animalis* subsp. *lactis* DSM 10140^T to cleave a variety of FOS has been characterized (Janer et al., 2004). Its gene was cloned and the deduced amino acid sequence of 532 residues (59.4 kDa) appeared to be identical to the β-fructofuranosidase from the same strain that was described by Ehrmann et al. (2003). However, there were some discrepancies between the results reported in the two papers, namely differences in the similarity to CscA from *Escherichia coli* (41% vs 16%), activity of the enzyme towards sucrose (6% vs 100%), and a high activity (100%) with the oligofructose Raftilose.

An operon involved in FOS breakdown has been identified in the genome of *Bifidobacterium breve* UCC 2003. This 2.6 kb transcriptional unit was comprised of three genes that encoded a putative permease, a conserved hypothetical protein, and a β-fructofuranosidase (Ryan et al., 2005).

In *Bifidobacterium animalis* subsp. *lactis*, an intracellular endopeptidase (PepO) was identified and characterized. The gene encoding an endopeptidase O was cloned. The product is a 74 kDa monomer member of the M13 peptidase family of zinc metallopeptidases and displays 67.4% sequence similarity with the predicted PepO protein from *Bifidobacterium longum* subsp. *longum* (Janer et al., 2005).

Urease activity. Four hundred and fourteen *Bifidobacterium* strains representing 21 species of the genus were examined for urease activity. The strongest ureolytic strains belonged mainly to *Bifidobacterium longum* subsp. *suis*; more than 80% of the strains of this species were ureolytic. Ureolytic strains were found in all species except for *Bifidobacterium cuniculi*. The enzyme is apparently not inducible; neither organic nitrogen nor urea influenced urease production. Less than 10% of *Bifidobacterium breve* and *Bifidobacterium longum* subsp. *longum* strains are ureolytic, i.e., "human" bifidobacteria species, and *Bifidobacterium bifidum* is only weakly ureolytic (Crociani and Matteuzzi, 1982).

Nitrate reduction. Bifidobacteria are generally not considered to reduce nitrate, although cells grown in the presence of lysed red cells may do so. Cytochrome *b* and cytochrome *d* are synthesized under these growth conditions (Van der Wiel-Korstanje and Vries, 1973).

Genetics. The acquisition of the completed genome sequence of a microbe is an essential step in deciphering the function of each gene within a genome and provides invaluable information on the functional characteristics of the organism and on relationships between the microbes and the host. Currently, one genome of *Bifidobacterium adolescentis* (ATCC 15703^T, GenBank accession no. AP009256), one of *Bifidobacterium animalis* subsp. *lactis* (AD011, GenBank no. CP001213), two genomes of *Bifidobacterium longum* subsp. *longum* (NCC2705, GenBank no. AE014295; DJO10A, GenBank no. CP000605), and one of *Bifidobacterium longum* subsp. *infantis* (ATCC 15697^T, GenBank no. CP001095) have been sequenced completely (Kim et al., 2009; Leahy et al., 2005; Schell et al., 2002; Sela et al., 2008). These genomes show high sequence similarity throughout the chromosome with some regions exclusive to each strain (Ventura et al., 2009). Sequencing of the genomes of the following strains are in progress (GENOME PROJECT; http://www.ncbi.nlm.nih.gov/sites/entrez): *Bifidobacterium adolescentis* strain L2-32; *Bifidobacterium angulatum* DSM 20098^T; *Bifidobacterium animalis* subsp. *lactis* strains BB12, B1-04, DSM 10140^T, HN019, and V9; *Bifidobacterium bifidum* strains BGN4, DSM 20456^T, and NCIMB 41171; *Bifidobacterium breve* DSM 20213^T and UCC 2003; *Bifidobacterium catenulatum* DSM 16992; *Bifidobacterium dentium* ATCC 27678, Bd1, and JCVIHMP023; *Bifidobacterium gallicum* DSM 20093^T; *Bifidobacterium longum* subsp. *longum* strain BORI; *Bifidobacterium longum* subsp. *infantis* ATCC 55813 and CGUG 52486; and *Bifidobacterium pseudocatenulatum* DSM 20438^T.

The 2.26 Mbp genome sequence of an infant-derived strain of *Bifidobacterium longum* subsp. *longum* has been determined and 1730 possible coding sequences organized in a circular chromosome with a G+C content of 60 mol% have been identified. Bioinformatic analysis revealed several physiological traits that could partially explain the successful adaptation of this organism in the colon. An unexpectedly large number of the predicted proteins appeared to be specialized for catabolism of a variety of oligosaccharides, some possibly released by rare or novel glycosyl hydrolases acting on "nondigestible" plant polymers or host-derived glycoproteins and glycoconjugates. This ability to scavenge from a large variety of nutrients probably contributes to the competitiveness and persistence of bifidobacteria in the colon (Schell et al., 2002).

The genome of *Bifidobacterium longum* subsp. *infantis* strain ATCC 15697^T has also been sequenced. It is the largest bifidobacterial genome reported to date with its single circular chromosome consisting of 2,832,748 bp (Sela et al., 2008). This genome reflects a competitive nutrient utilization strategy targeting milk-borne molecules that lack a nutritive value to the neonate. Several chromosomal loci reflect potential adaptation to the infant host including a 43 kbp cluster encoding catabolic genes, extracellular solute-binding proteins, and permeases predicted to be active on milk oligosaccharides. An examination of *in vivo* metabolism has detected the hallmarks of milk oligosaccharide utilization via the central fermentative pathway using metabolomic and proteomic approaches. Finally, conservation of gene clusters in multiple isolates corroborates the genomic mechanism underlying milk utilization for this infant-associated phylotype (Sela et al., 2008). The analyses of oligosaccharides present in human milk and of the metabolic capabilities of infants and of this strain to utilize these compounds provide the basis for the hypothesis of a three-way evolutionary mechanism: human milk–infant–bifidobacteria. Human milk is the source of oligosaccharides, which pass undigested through the infant intestine and are utilized by bifidobacteria, which are beneficial for the infant host.

Analysis of the *Bifidobacterium breve* genome has revealed that the system for exporting proteins in this organism is similar to that of other Gram-stain-positive bacteria. These proteins contain either a signal peptide or a number of transmembrane regions which can be important in the interaction of *Bifidobacterium breve* with the environment of its host (MacConaill et al., 2003). The dnaK operon organization of *Bifidobacterium breve* is almost identical to other presumed dnaK regions of other sequenced *Bifidobacterium* species and to that of other *Actinobacteridae* (Ventura et al., 2005). Other genes important in the shock response of *Bifidobacterium* have been characterized, such as *dnaJ*, *hrcA*, *groEL*, *clpB*, and *clpP* (Ventura et al., 2004).

Different kinds of analytical genetic methods have been used to discriminate between bifidobacteria at the genus, species, and strain levels.

16S rRNA gene sequence analyses are replacing 16S rRNA cataloguing and reverse transcriptase sequencing (Garrity and Holt, 2001; Ludwig and Klenk, 2001). The 16S rRNA gene sequence is considered to be a convenient phylogenetic characteristic and has been used extensively in taxonomic studies of the genus *Bifidobacterium*. Results from these genetic techniques are in fair agreement with 16S rRNA ribotyping data (Sakata et al., 2006).

Pulsed-field gel electrophoresis (PFGE), following genomic digestion with rare-cutting restriction enzymes, has been reported to discriminate effectively between strains of several human and animal *Bifidobacterium* species (O'Riordan and Fitzgerald, 1997; Roy et al., 1996; Simpson et al., 2003). A number of PFGE macro-restriction patterns from porcine cecum strains differed by only a single DNA fragment. It is possible that the extra band seen in these patterns represents an insertion of a fragment with two restriction sites separated by a distance equal to the size of the insertion. However, it was considered more likely that the fragment represented the presence of a putative plasmid.

Fluorescent *in situ* hybridization (FISH) allows direct enumeration of fecal microbiota. It is dependent on the availability of the 16S rRNA gene sequence in databases and effective probe design and validation (Harmsen et al., 2000; Langendijk et al., 1995). FISH has also been adapted for use with flow cytometry; this procedure allows for higher throughput analysis (Rochet et al., 2004; Zoetendal et al., 2002).

PCR of targeted genes, such as 16S rRNA (Matsuki et al., 1999, 1998) or RecA (Kullen et al., 1997), has been used to detect members of the genus and particular species of bifidobacteria.

Quantitative PCR, i.e. real-time PCR, is a sensitive and discriminating method that has been used to quantify bifidobacterial nucleic acids. The real-time PCR TaqMan (Applied Biosystems) assay has been used for bifidobacterial and intestinal microbiota assessment (Gueimonde et al., 2004; Hopkins et al., 2005; Matsuki et al., 2004; Requena et al., 2002).

Another method used to differentiate between *Bifidobacterium* species is amplified rDNA restriction analysis (ARDRA) (Heyndrickx et al., 1996; Krizová et al., 2006; Roy and Sirois, 2000; Ventura et al., 2001). In addition, a PCR technique targeting the transaldolase gene followed by denaturing gradient gel electrophoresis (DGGE) has been designed by Requena et al. (2002). The multiplex PCR technique can simultaneously accomplish the rapid identification and correct differentiation of several bifidobacterial species (Mullié et al., 2003).

PCR-restriction fragment length polymorphism (RFLP) based on 16S rRNA gene sequences has been used to distinguish between human- and animal-borne bifidobacterial strains (Delcenserie et al., 2004). This technique has also been used to differentiate between *Bifidobacterium* species and subspecies (Ventura et al., 2003).

DGGE and variations of this method, such as temperature gradient gel electrophoresis (TGGE), nested-PCR-DGGE, or combinations of DGGE and TGGE, have been used to monitor microbiota community shifts rapidly and to compare microbial communities between different people, different intestinal locations, and diet (Favier et al., 2002; Mangin et al., 2006; Satokari et al., 2001; Temmerman et al., 2003).

Plasmids. When 1461 isolates, representing 24 species of the genus, were examined for the presence of plasmids about 20% of them were found to be positive (Sgorbati and London, 1982). However, only members of four species contained these elements, namely *Bifidobacterium longum* subsp. *longum*, the predominant bifidobacterial species in the human intestine, *Bifidobacterium pseudolongum* subsp. *globosum*, the most common bifidobacterium in animals, and *Bifidobacterium asteroides* and *Bifidobacterium indicum*, which are found exclusively in the intestines of honey bees. *Bifidobacterium longum* subsp. *longum* strains have multiple-plasmid patterns (1.25–9.5 MDa), and *Bifidobacterium pseudolongum* subsp. *globosum* strains each contain one plasmid of three molecular mass classes of (13.5, 24.5, and 46 MDa). Multiple patterns were seen in *Bifidobacterium asteroides* (1.2–22 MDa), whereas 60% of the plasmid-bearing *Bifidobacterium indicum* isolates contained one 22 MDa plasmid. It is noteworthy that *Bifidobacterium longum* subsp. *infantis*, the species most closely related to *Bifidobacterium longum* subsp. *longum*, does not contain plasmids, although strains of both species have been isolated from the same specimens. Phenotypic properties have yet to be correlated with the presence of plasmids (Sgorbati and London, 1982). The 13 plasmid patterns of *Bifidobacterium longum* subsp. *longum* contain only a few homologous structures, whereas the 14 patterns found in *Bifidobacterium asteroides* are structurally more heterogeneous (Sgorbati et al., 1986b). Strains of *Bifidobacterium longum* subsp. *longum* liberate phage particles after UV or mitomycin C treatment, but there is no correlation with their plasmid complement (Sgorbati et al., 1986a, 1983). Plasmids have also been isolated from *Bifidobacterium breve* strains (Bourget et al., 1993; Iwata and Morishita, 1989).

All the plasmids investigated so far are cryptic. Only in *Bifidobacterium pseudolongum* subsp. *globosum* was a relationship found between plasmid and auxotrophy for L-leucine (Mattarelli and Biavati, 1999a). Some plasmids have been characterized at the sequence level (Corneau et al., 2004; Mattarelli et al., 1994; Matteuzzi et al., 1990; Park et al., 1997; Tanaka et al., 2005). Two plasmids were reported to replicate via a rolling circle replication mechanism (O'Riordan and Fitzgerald, 1999; Park et al., 1999) and one has been shown to replicate via theta replication (Lee and O'Sullivan, 2006). A number of cloning vectors have been constructed with plasmids from *Bifidobacterium* and *E. coli* and transformed into both species by electroporation (Lee and O'Sullivan, 2006; Matsumura et al., 1997; Missich et al., 1994; Rossi et al., 1996, 1998). In all cases, transformation efficiency in *Bifidobacterium* was very low and attempts have been made to optimize it (Argnani et al., 1996).

Antibiotic or drug sensitivity. Resistance (with respect to achievable serum levels) to aminoglycosides (framycetin, gentamicin, kanamycin, neomycin, paramycin sulfate, and streptomycin), polypeptides (polymixin B), quinolones (nalidixic acid and ofloxacin), and other antibiotics (fusidic acid and metronidazole), and susceptibility to β-lactams (amoxycillin, ampicillin, cefalothin, cefotaxine, cefoxitin, co-amoxiclav, imipenem, oxacillin, penicillin G, piperacillin, and ticarcillin), coumarin

(novobiocin), ketolide (telithromicin), lincosamide (lindamycin), macrolides (erythromycin, oleandomycin, and spiramycin), streptogramin (pristinamycin), oxazolidinone (linezolid), polypeptide (bacitracin), glycopeptide (teicoplanin), and other antibiotics (chloramphenicol, lincomycin, and rifampin) are a general feature among *Bifidobacterium* species (Charteris et al., 1998; Lim et al., 1993; Matteuzzi et al., 1983). The newly commercialized molecules gatifloxacin, linezolid, and telithromycin were found to be active with MIC_{50} of 1 µg/ml (Moubareck et al., 2005). It can be concluded that the genus *Bifidobacterium* exhibits uniform sensitivity towards the most common antibiotics. However, sensitivity towards antibiotics such as cefhalotin, cefotetan, gentamicin, kanamycin, metronidazole, nalidixic acid, neomycin, ofloxacin, polymyxin B, rifampin, and streptomycin varies greatly within species, ranging from 10–500 µg/ml or more of antibiotic.

Intrinsic resistance to aminoglycosides is probably due to the lack of cytochrome-mediated drug transport among anaerobes (Bryan et al., 1979). Resistance to metronidazole varies among *Bifidobacterium* species. Metronidazole activity is due to the preferential reduction of the parent compound by the bacterial ferridoxin system leading to the generation of an intermediate product responsible for breaks in double-stranded DNA (Bezkorovainy and Miller-Catchpole, 1989). Some strains of bifidobacteria lack the ferridoxin system responsible for the reduction of the parent compound and present a high degree of resistance to metronidazole, a drug active against virtually all obligate anaerobes (Moubareck et al., 2005).

Discordant results have been reported regarding susceptibility to the glycopeptide vancomycin. Some workers (Lim et al., 1993; Matteuzzi et al., 1983; Moubareck et al., 2005) demonstrated vancomycin susceptibility as a general characteristic of bifidobacteria, whereas Charteris et al. (1998) reported resistance to vancomycin. This discrepancy may be due to the use of different assay methodologies, since vancomycin is reported to diffuse poorly in agar media (Thomson et al., 1995). Sensitivity to tetracyclines (tetracycline and minocycline) was shown to be inter- and intra-specifically variable (Matteuzzi et al., 1983). The tetracycline-resistance gene tet(W) has been detected in *Bifidobacterium animalis* subsp. *lactis* DSM 10140^T (Kastner et al., 2006). In *Bifidobacterium longum* subsp. *longum*, the presence of a cholate transporter has been shown and seems to be functionally related to the efflux system encoded by the *ctr* gene, which may contribute to antibiotic and bile resistance (Price et al., 2006). *Bifidobacterium breve* is generally more resistant to antibiotics than other *Bifidobacterium* species (Moubareck et al., 2005). Margolles et al. (2005) were the first to provide evidence for the involvement of a membrane protein from *Bifidobacterium breve* that conferred moderate resistance to macrolides when expressed in the heterologous host *Lactococcus lactis*.

The susceptibility of bifidobacteria to antibiotics is highly relevant to an understanding of alterations in the normal gut flora when antibiotics are used, as well as in selecting bifidobacterial strains as probiotics. Bifidobacteria are natural inhabitants of gastrointestinal microbiota and exert several health-promoting effects. When the intestinal microbiota are exposed to antibiotics, the presence of antibiotic resistance in bifidobacteria might be positive in reducing alterations of the normal gut flora. The relative bifidobacterial resistance to metronidazole might be beneficial in cases of co-administration of bifidobacteria with this antibiotic in preventing antibiotic-associated diarrhea. It should be noted that the therapeutic antibiotic dosage which reaches the colon may be lower than the initial dose because antibiotics are adsorbed mainly in the ileum. Bifidobacteria therefore survive better *in vivo* than *in vitro*.

In other instances, the presence of microbial resistance to antibiotics might be negative. Thus, antibiotic resistance may be transferred to other micro-organisms leading to a rapid increase in antibiotic resistance in human-pathogenic bacteria, particularly with respect to nosocomial infections (Scott et al., 2000). It is important, therefore, that bifidobacterial probiotic strains are free of transferable antibiotic-resistance determinants. However, bifidobacteria, with their low natural and acquired resistance to antibiotics, appear to be safe for use in healthy populations (Moubareck et al., 2005).

Bacteriocins. These antimicrobial peptides have a relatively narrow specificity against closely related Gram-stain-positive organisms (Riley and Wertz, 2002). They have the potential to inhibit the growth of pathogens and to prevent microbial food spoilage. The bactericidal action of bifidobacteria is ascribed to the production of some kind of antimicrobial agent in addition to their pH-reducing effect (Fujiwara et al., 1997; Gibson and Wang, 1994a; Meghrous et al., 1990). Only a limited number of studies have focused on the characterization of antimicrobial peptides synthesized by *Bifidobacterium* species. So far, only some infant *Bifidobacterium* isolates have been shown to produce antimicrobial peptides against *Listeria monocytogenes*. A unique bacteriocin, bifidocin B, from *Bifidobacterium bifidum* NCFB 1454, which is active against Gram-stain-positive bacteria, has been purified (Touré et al., 2003; Yildirim and Johnson, 1998; Yildirim et al., 1999). Bacteriocin-like compounds have been identified in human fecal *Bifidobacterium* strains that show antibacterial activity towards species of the genera *Aeromonas*, *Arcobacter*, *Campylobacter*, *Helicobacter*, and *Vibrio* (Collado et al., 2005).

Pathogenicity. Bifidobacteria have been isolated occasionally from clinical material (Biavati et al., 1982) and, although implicated in some opportunistic infections (Bezkorovainy and Miller-Catchpole, 1989), they are generally regarded as nonpathogens. *Bifidobacterium breve* and *Bifidobacterium longum* have been isolated occasionally from human clinical material (Biavati et al., 1982). Predisposing factors such as ongoing infections (Prévot et al., 1967) or contact with contaminated material (Ha et al., 1999) may lead to bifidobacteria being associated with infections. Some bifidobacterial strains have been reported to participate in soft-tissue infections initiated by mixed bacterial populations. Most strains of *Bifidobacterium dentium* have been isolated from human dental caries and plaque; this organism is the only *Bifidobacterium* species found at this site. *Scardovia inopinata* and *Parascardovia denticolens*, members of the family *Bifidobacteriaceae*, have also been isolated from this location (Crociani et al., 1996; Jian and Dong, 2002; Scardovi and Crociani, 1974). Strains of *Bifidobacterium appendicitis*, a synonym of *Bifidobacterium dentium* (Scardovi and Crociani, 1974), have been found in human clinical material (Prévot et al., 1967). *Actinomyces eriksonii* (Georg et al., 1965), later named *Bifidobacterium eriksonii* by Holdeman and Moore (1970) and recognized genetically as *Bifidobacterium dentium* (Scardovi et al., 1979a), has been isolated

primarily from human abscesses. Thus, *Bifidobacterium dentium* appears to have the most likely pathogenic potential amongst members of the genus *Bifidobacterium* (Beighton, 2005).

Ecology. Bifidobacteria are typically found in the alimentary tracts of human infants and adults. Many factors control the number and the composition of microbial populations in different regions of the gastrointestinal tract. Ways of restoring or maintaining a proper microbial balance in the gastrointestinal tract have been investigated extensively (see Bifidobacteria as health-promoting agents below). Bifidobacteria coexist with a large variety of bacteria, most of which are obligate anaerobes. Components of this microbiota are different in the different areas of the gastrointestinal tract (Moore et al., 1969). The obligate anaerobes in this complex microbiota are in part unknown, although significant advances have been made using appropriate identification techniques, such as the use of molecular probes. Furthermore, it seems likely that particular ecotypes (or biovars) probably have more ecological significance in these habitats than the species to which they belong (Benno and Mitsuoka, 1986; Mitsuoka, 1984; Mitsuoka and Kaneuchi, 1977). Indeed, all factors that pertain to and influence natural genetic variation of single bacterial populations should be studied (Milkman, 1975).

In studies on the ecology of bifidobacteria performed in the Microbiology Section of the Department of Agroenvironmental Technology, Bologna University, Italy, a large number of strains (at present more than 7000) has been isolated from many different sources (Table 21). They are now maintained in the BUSCOB collection (Bologna University Scardovi Collection of Bifidobacteria). Studies on the distribution of bifidobacteria in feces of both infants (Biavati et al., 1984) and adults (Biavati et al., 1986), the human vagina (Crociani et al., 1973), or "pathological conditions" such as dental caries (Crociani et al., 1996) and stomach hypochlorhydria (unpublished data) demonstrate that the adaptation of the species of human origin to the five habitats is different. *Bifidobacterium bifidum* and *Bifidobacterium longum* subsp. *longum*, for example, are the most represented species in the intestinal tract of infants and adults, whereas *Bifidobacterium dentium* together with the *Bifidobacteriaceae* taxa *Scardovia inopinata* and *Parascardovia denticolens* are typical of dental caries and hypochlorhydric stomach. *Bifidobacterium adolescentis*, *Bifidobacterium bifidum*, *Bifidobacterium longum* subsp. *longum*, and *Bifidobacterium breve* are typically found in the vagina.

Furthermore, bifidobacteria have been isolated from many animals and from honey bees. In general, *Bifidobacterium* species are specific either to humans or animals. The same *Bifidobacterium* species found in the intestinal microbiota of suckling calves and breast-fed infants are exceptions. Some species, 15 out of 32, are host-specific and they are typical of a given animal habitat.

Eleven species of *Bifidobacterium* have been found in sewage: six are from humans and five are from animals. *Bifidobacterium minimum* and *Bifidobacterium subtile* were originally isolated from sewage (Biavati et al., 1982). These two species have not been isolated from different habitats for two decades, thus raising the exciting question of the possible development of bifidobacteria in extraenteral ecological niches. Unexpectedly, *Bifidobacterium minimum* was isolated from a rabbit cecum (Simpson et al., 2003) and, in 2009, *Bifidobacterium subtile* was isolated from human caries lesions (Mantzourani et al., 2009). The isolation of *Bifidobacterium minimum* and *Bifidobacterium subtile* confirm that sewage is not a habitat for bifidobacteria.

Bifidobacterium animalis subsp. *lactis* was isolated from yogurt, but this cannot be considered an extra-body habitat as these bifidobacteria are added to fermented milk because of their probiotic properties. Their isolation from human feces (Gavini et al., 2001; Masco et al., 2004; Mättö et al., 2004), therefore, is caused by the consumption of probiotic food containing this species. *Bifidobacterium mongoliense* was isolated recently from fermented mare's milk (Watanabe et al., 2009). *Bifidobacterium thermacidophilum* subsp. *thermacidophilum* and *Bifidobacterium thermacidophilum* subsp. *porcinum* (Dong et al., 2000; Zhu et al., 2003) were described recently. They were isolated from an anaerobic digester used to treat wastewater from a bean-curd farm and from the feces of a piglet, respectively. These species differed from other *Bifidobacterium* species by their ability to grow at 49.5°C and in media with an initial pH of 4.0. *Bifidobacterium scardovii*, isolated from human blood, was described recently by Hoyles et al. (2002). Other recently described species are *Bifidobacterium tsurumiense*, isolated from hamster dental plaque (Okamoto et al., 2008), and *Bifidobacterium bombi*, isolated from the digestive tract of bumblebees (Killer et al., 2009).

Bifidobacteria as health-promoting agents: probiotic, prebiotic, and symbiotic substances. The term probiotic (Parker, 1974) is a relatively new word meaning "for life" and it is used currently to indicate bacteria associated with beneficial effects for humans and animals. A more recent, but probably not the last definition for probiotic is "live micro-organisms, which when consumed in adequate amounts, confer a health effect on the host" (Guarner and Schaafsma, 1998).

Members of the genera *Bifidobacterium* and *Lactobacillus* are considered health-promoting constituents of the gut microbiota and are mainly, but not exclusively, used as probiotic micro-organisms (Leahy et al., 2005). A growing number of probiotic foods are available to the consumer. In the last 20 years, there has been an increased commercial and scientific interest in members of the genus *Bifidobacterium*. Health benefits associated with bifidobacteria include the treatment and prevention of gastrointestinal disorders and the maintenance of digestive health (Snelling, 2005), alleviation of lactose intolerance (Vesa et al., 2000), resistance to microbial infections (Servin, 2004), protection against pathogens (Gill, 2003), enhancement of the immune system (Roller et al., 2004; Tlaskalová-Hogenová et al., 2004), management of allergic disorders (Bienenstock et al., 2002), cancer prevention (Rafter, 2002), and reduction of serum cholesterol (St-Onge et al., 2000; Xiao et al., 2003). Recently, the interesting properties of folate production by bifidobacteria have been investigated (Pompei et al., 2007).

Another approach to increasing the beneficial bifidobacterial population of the gastrointestinal microbiota is to add prebiotic substances to the diet. The word prebiotic is defined as "non-digestible food ingredients that beneficially affect the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon that can improve the host's health" (Gibson and Roberfroid, 1995). In order to be effective, prebiotic components have to be partially undigested and unabsorbed in the small intestine to provide fermentable substrates for the colon microbiota. They should also stimulate the growth of health-promoting bacteria (mainly bifidobacteria and lactobacilli) and not that of pathogenic bacteria. FOS, lactulose, and GOS are all popular prebiotics (Manning and Gibson, 2004). The concept of prebiotics fundamentally shares

the same objective as probiotics albeit via a different mechanism. The simultaneous administration of prebiotics and probiotics goes under the name "synbiotics" (Tuohy et al., 2005).

An area which promises to be very interesting in the immediate future is that of probiogenomics, which should cast light on how the *Bifidobacterium* genome has evolved from its ancestral prototype to the that present in the species we know today. Bioinformatic analysis of the *Bifidobacterium* genome has revealed a high number of components that can be studied in order to elucidate both the physiology of the microbiota and microbe–host interactions (Ventura et al., 2009). These new approaches to studying probiotics will help in the selection of strains on the basis of physiologically relevant characteristics for the host.

Isolation procedures

A large variety of media have been devised for the isolation and enumeration of bifidobacteria in natural habitats. Ingredients include human milk, liver or meat extracts, sheep or horse blood, tomato juice, and a variety of peptones (Biavati and Mattarelli, 2001). In addition, antibiotics or other ingredients have been used to improve selectivity. Iodoacetic acid, kanamycin, nalidixic acid, polymixin and 2,3,5-triphenyltetrazolium chloride (Muñoa and Pares, 1988), bromocresol green, nalidixic acid and neomycin (Resnick and Levin, 1981), lithium chloride, neomycin, paramomycin and sodium propionate (Mitsuoka et al., 1965), propionic acid (Beerens, 1990), lithium chloride, raffinose and sodium propionate (Hartemink et al., 1996), and lithium chloride and sodium propionate (Lapierre et al., 1992) have all been used as selective agents.

Known bifidobacteria are resistant to certain antibiotics (see above), but the intraspecific variations are large. At present, preference should be given to substrates that permit satisfactory growth of the largest number of known bifidobacteria added with mupirocin. The ingredients of choice are trypticase and phytone and the formula which, in our hands, has proved to be satisfactory for isolating bifidobacteria from all known habitats is TPY medium (trypticase, 10 g; phytone, 5 g; glucose, 5 g; yeast extract, 2.5 g; Tween 80, 1 ml; cysteine hydrochloride, 0.5 g; K_2HPO_4, 2 g; $MgCl_2 \cdot 6H_2O$, 0.5 g; agar, 15 g; and distilled water to 1000 ml) plus 1 ml/l of a freshly prepared ethanol stock solution of 100 mg/ml of mupirocin which is added to the already sterilized medium. Dilutions can be made with physiological solutions. Petri dishes with vents are incubated at 37–39°C in anaerobic jars with a gas generator system (Merck system). Colonies are transferred to stabs of the same medium with 0.5% agar. After growth has reoccurred, stabs are kept at 3–4°C in anaerobic jars. Transfers should be made every 2 weeks.

Maintenance procedures

Cultures may be maintained for short periods on TPY slants or as TPY liquid cultures under anaerobic conditions and should be subcultured every 2–4 weeks. This procedure is not satisfactory for long-term storage as some properties are liable to change on repeated subculture. However, strains may be maintained for 1–2 years at low temperatures (−135°C) after centrifugation and addition of a cryoprotective agent containing 10% skim milk and 0.3% yeast extract in distilled water (0.3 g wet pellet of cells/ml cryoprotective agent). Freeze-drying is the best maintenance procedure for long-term storage (40 years).

Enzymes used for species and group differentiation

Starch gel electrophoresis revealed three types of F6PPK in bifidobacteria (Table 21) (Scardovi et al., 1971a). The most anodal type was detected in species found in the intestine of honey bees; the type found in species from humans (human type) migrated less, but the least anodal type characterized species found in animals (animal type). F6PPK from *Bifidobacterium pseudolongum* subsp. *globosum* (animal type) and *Bifidobacterium dentium* (human type) have been purified (Sgorbati et al., 1976). The animal type has properties similar to those found in *Acetobacter xylinum* (Schramm et al., 1958) and is also active towards xylulose 5-phosphate. The human type has different properties (activators, pH range of activity, heat inactivation) and only cleaves fructose 6-phosphate (Sgorbati et al., 1976). The molecular mass of F6PPK can be used to differentiate between animal and human strains (Grill et al., 1995; Sgorbati et al., 1976).

The ecological groups of *Bifidobacterium* species have also been distinguished on an immunological basis (Sgorbati and London, 1982). Isozyme patterns can also be used to identify species. Isozymes of transaldolase and 6-phosphogluconate dehydrogenase (6PGD) were studied by starch gel electrophoresis in 1206 strains belonging to 24 *Bifidobacterium* species (Scardovi et al., 1979a). Fourteen isozymes of transaldolase and 19 of 6PGD were identified and numbered and patterns or zymograms were thus obtained for each species (Table 21); 60% of the strains were identified on this basis. An additional 20% of the strains were assigned to species based on the electrophoretic behavior of their 3-phosphoglyceraldehyde dehydrogenase (Scardovi et al., 1979a).

Antisera against eight purified transaldolases further established natural relationships among *Bifidobacterium* species (Sgorbati, 1979; Sgorbati and London, 1982; Sgorbati and Scardovi, 1979). Transaldolases selected on the basis of their electrophoretic behavior (see above) were examined from *Bifidobacterium angulatum*, *Bifidobacterium asteroides*, *Bifidobacterium cuniculi*, *Bifidobacterium globosum* subsp. *globosum*, *Bifidobacterium infantis*, *Bifidobacterium minimum*, *Bifidobacterium longum* subsp. *suis*, and *Bifidobacterium thermophilum*. Indices of dissimilarity and immunological distances were determined using microcomplement fixation data. The various purified transaldolases cannot be distinguished on the basis of the usual parameters, such as molecular mass, substrate affinity, pH range, and type of acceptors (V. Scardovi, unpublished data).

The segregation of *Bifidobacterium* species into four distinct clusters that correlated with the groups made on the basis of their ecological distribution suggests that a "subdivision of the genus into four subgenera would more accurately reflect the group's natural history" (Sgorbati and London, 1982).

The starch-gel horizontal electrophoresis system of Smithies (1955) is recommended for the detection of enzymes.

Transaldolases. Tris (16.3 g) and citric acid monohydrate (9.0 g/l) (pH 7.0) is used as the bridge buffer. It is diluted 1:15 for use as a gel buffer. Hydrolyzed starch, 90 g/l, is added, the mixture is boiled for 5 min (keep agitated) and gas removed under reduced pressure. The liquid is poured into a plastic three-frame mold (12.0 × 37.0 × 0.9 cm accommodates 12 samples at a time). Samples of bacterial extracts [cells suspended in 0.05 M phosphate buffer (pH 7.0), sonicated, and centrifuged],

5–10 µl in 0.5 × 0.5 cm Whatman 3-mm paper cuts, are generally run for 15–20 h with a current of 15–20 mA.

The middle slab is used preferably for staining by the flooding technique. The developing solution contains (per 100 ml of distilled water): fructose 6-phosphate (sodium salt, 98% purity; Sigma), 400 mg; sodium arsenate, 370 mg; glycine, 240 mg; NAD, 13 mg; D-erythrose 4-phosphate (60–75% purity; Sigma), 16 mg; phenazine methosulfate, 2 mg; Nitro Blue Tetrazolium (NBT; Sigma), 20 mg; and about 130–150 IU glyceraldehyde-3-phosphate dehydrogenase.

6-Phosphogluconate dehydrogenases (6PGD). Use trisodium citrate·$2H_2O$ (120 g/l) (pH 7.0 with citric acid) as the bridge buffer, and histidine (0.75 g) plus NaC1 (1.5 g/l) (pH 7.0) as gel buffer. Prepare the gel as for detecting transaldolases. The developing solution is made as follows: 0.5 M Tris/HCl buffer, pH 7.0, 10 ml; 6-phosphogluconate (trisodium salt; Sigma) 250 mg; NADP, 20 mg; NBT, 20 mg; phenazine methosulfate, 2 mg; and distilled water, 90 ml.

The electrophoretic patterns of cellular proteins on PAGE (Moore et al., 1980) were used to confirm the taxonomic identification of 1094 bifidobacteria strains representing all known species of the genus. Excellent correlation was found between electrophoretic and DNA–DNA hybridization data. On this basis, one species was revived (*Bifidobacterium coryneforme*) and two others were proposed from previously recognized DNA homology groups (*Bifidobacterium minimum* and *Bifidobacterium subtile*) (Biavati et al., 1982).

In addition, a sort of "genus band" is clearly visible in all bifidobacteria gel electrophoretic patterns. This band migrates to the same position for all strains with the exception of *Bifidobacterium boum* where it is somewhat less anodic. The presence of this "genus band" is a good indication that an unknown organism belongs to the genus *Bifidobacterium* (Biavati et al., 1982). In *Bifidobacterium animalis*, it is possible to discriminate between the subspecies *animalis* and *lactis* based on PAGE patterns. Strains of *Bifidobacterium animalis* subsp. *animalis* isolated from rats appear to differ from strains of *Bifidobacterium animalis* subsp. *lactis* isolated from chickens, rabbits, and sewage (Biavati et al., 1982). The intestinal origin of *Bifidobacterium animalis* subsp. *lactis* strains isolated from sewage and fermented milk products has been determined by comparing electrophoretograms of cellular soluble proteins (Mattarelli et al., 1992). This procedure was also found to be useful for the rapid identification of unknown bifidobacterial isolates. Computer-assisted numerical comparisons of protein patterns are feasible and a database can be created for identification purposes provided that highly standardized conditions are used for cultivation and electrophoresis; this allows large numbers of strains to be compared and grouped into closely related clusters (Pot et al., 1994).

Procedures for testing special identification characteristics

Identification of a bacterial strain as a member of the genus *Bifidobacterium* is unreliable unless special procedures are used. Morphological properties can be misleading due to unusual and unique traits shown by some species such as *Bifidobacterium angulatum*, *Bifidobacterium asteroides*, and *Bifidobacterium pullorum*. Culture conditions and physiological characteristics are largely shared by other bacteria such as strains of *Actinomyces*, *Corynebacterium*, and *Lactobacillus*. Identification of fermentation products by GC (Holdeman et al., 1977) may be difficult, especially for inexperienced workers, as side reactions forming substantial and variable amounts of formic and succinic acids and ethanol may occur.

The most direct and reliable property for assigning an organism to the genus *Bifidobacterium* is the demonstration of F6PPK activity in cellular extracts. F6PPK is the characteristic key enzyme of "the bifid shunt". This enzyme activity, tested with fructose 6-phosphate as substrate, is apparently absent in anaerobic Gram-stain-positive bacteria with a "pseudobifid" morphology, i.e., *Actinomyces*, *Arthrobacter*, *Corynebacterium*, and *Propionibacterium* (Scardovi and Trovatelli, 1965). Proof of the validity of this test has been furnished by (a) the isolation of anaerobic bacteria from the bovine rumen and from sewage with the morphology and gross physiology of bifidobacteria, but which do not possess F6PPK (V. Scardovi, unpublished data) and (b) recognition of bifidobacteria on this basis, i.e., of bacteria with nonbifid morphology such as *Bifidobacterium pullorum* (Trovatelli et al., 1974) and of bifidobacteria from honey bees (Scardovi and Trovatelli, 1969).

F6PPK test. Reagents are as follows: 1, 0.05 M phosphate buffer, pH 6.5, plus 500 mg/l cysteine; 2, a solution containing 6 mg/ml NaF and 10 mg/ml iodoacetate (potassium or sodium salt); 3, hydroxylamine·HC1, 13.9 g/100 ml water, freshly neutralized with NaOH to pH 6.5; 4, trichloroacetic acid (TCA), 15% (w/v) in water; 5, 4 M HC1; 6, 5% (w/v) $FeC1_3 \cdot 6H_2O$ in 0.1 M HCl; and 7, fructose 6-phosphate (sodium salt; 70% purity), 80 mg/ml in water.

The formation of acetyl phosphate from fructose 6-phosphate is shown by the reddish violet color (absorption maximum at 505 nm) formed by the ferric chelate of its hydroxamate (Lipmann and Tuttle, 1945).

Classical procedure. Cells harvested from 20 ml TPY broth are washed twice with reagent 1 and resuspended in 1.0 ml of the same reagent. Cells are disrupted by sonication in the cold, and 0.25 ml each of reagents 2 and 7 are added to the sonicate. After 30 min incubation at 37°C, the reaction is stopped with 1.5 ml of reagent 3. After 10 min at room temperature, 1.0 ml each of reagents 4 and 5 are added. The mixture may be left at room temperature before the final addition of 1.0 ml of the color-developing reagent 6. Invert tube for mixing. Any reddish violet color that develops immediately is taken as a positive result. A tube without fructose 6-phosphate can serve as a blank to aid the visual comparison. The color becomes more evident after standing, as this allows particles to settle. Warning: avoid heating during sonication because of heat sensitivity of the enzyme.

Modified procedure. A modified procedure of the phosphoketolase test involves an alternative chemical method for cell disruption (Orban and Patterson, 2000). Cells harvested and resuspended in 1 ml reagent 1, as above, are incubated with 0.4 ml cetrimonium bromide (hexadecyltrimethylammonium bromide or CTAB) (450 µg/ml stock solution) for 5 min at room temperature; the same steps as above are then followed. This method is less time-consuming and is an effective alternative to sonication and French press disruption.

Differentiation of the species of the genus *Bifidobacterium*

Differential characteristics for distinguishing between *Bifidobacterium* species are indicated in Table 21. Other diagnostic characteristics are cited in Table 22.

TABLE 22. Fermentative characteristics distinguishing species of the genus *Bifidobacterium*[a]

Substrate	*B. bifidum*	*B. adolescentis*	*B. angulatum*	*B. animalis* subsp. *animalis*	*B. animalis* subsp. *lactis*	*B. asteroides*[e]	*B. bombi*	*B. boum*	*B. breve*	*B. catenulatum*	*B. choerinum*	*B. coryneforme*	*B. cuniculi*	*B. dentium*	*B. gallinarum*	*B. gallicum*	*B. indicum*[e]	*B. longum* subsp. *longum*	*B. longum* subsp. *infantis*	*B. longum* subsp. *suis*	*B. magnum*	*B. merycicum*	*B. minimum*	*B. mongoliense*	*B. pseudocatenulatum*	*B. pseudolongum* subsp. *pseudolongum*	*B. pseudolongum* subsp. *globosum*	*B. pullorum*	*B. ruminantium*	*B. saeculare*	*B. scardovii*	*B. subtile*	*B. thermacidophilum* subsp. *thermacidophilum*	*B. thermophilum*	*B. tsurumiense*
α-L-Fucose	–	–[d]	–	–	nd	–	nd	–	+	–	–	–	–	–	–	–	–	–	+[b]	–	–	–	–	–	v	–	–	–	–	–	nd	–	nd	–	nd
Amylopectin	v	+[b]	+[b]	v	nd	–[d]	nd	+	v	–[d]	+	–[d]	+	+	–	+	–	–	–	v	–[d]	v	+	nd	+[b]	+	+	–	–[d]	–	nd	+[b]	nd	+[b]	nd
Amylose	v	v	–	–	nd	–[d]	nd	+[b]	v	v	+	–[d]	+[b]	+	–	+	–[d]	–	–	v	–[d]	v	+	nd	+[b]	+	v	–	–[d]	–	nd	+[b]	nd	+[b]	nd
Arabic gum	–	v	–	–	nd	–	nd	–	–	–	–	–	–	–	–	–	–	v	–	–	–	–	–	nd	–	–	–	–	–	–	nd	–	nd	–	nd
Arabinogalactan	–	–	–	–	nd	–[d]	nd	–	–	–	–	–[d]	–	–	–	–	v	+[b]	–	v	–	–	–	–	–	–	–[d]	–	–	–	nd	–	nd	–[d]	nd
L-Arabinose	–	+	+	+	+	–	–	–	–	+	–	–	+	+	+	+	–	+	–	+	+	+	–	+	+	+	v	+	–	+	+	–	+[g]	–	+
Cellobiose	–	+	–	v	–	+	–	–	–	+	–	+	–	+	v	–	+	–	v	–	–	v	–	+[b]	v	v	–	–	–	–	nd	–	nd	v	+
D-Fructose	+[b]	+	+	+	–	+	nd	+	+	+	–	+	–	+	+	+	+	+	+	v[i]	+	+[g]	+	–	+	+	+[k]	+	+	+	nd	+	+	+	+
D-Galactosamine	–	–	v	+[b]	nd	v	nd	+	v	v	+[b]	+	v	–[d]	–	–	+	v	–	–[d]	v	+[b]	–	nd	–	–	v	+	+	+	nd	+	nd	+[b]	nd
D-Galactose	+	+	+	+	–	v	–	+[g]	+	+	+	nd	+	+	+	+	v	+	+	+	+	+	–	+	+	+	+	+[h]	+	+[h]	nd	+	+	+	+
Ghatti gum	–	–	–	–	nd	–	nd	–	–	–	–	–	–	–[d]	–	–	–	v	–	–	–	–	–	nd	v	–	–	–	–	–	nd	–	nd	–	nd
Gluconate	–	+	v	–	–	+	nd	–	–	v	–	+	–	+	nd	–	+	–	–	–	–	–	–	+[b]	v	–	–	–	–	–	nd	+	–[g]	–	+
D-Glucosamine	v	–	v	v	nd	–[d]	nd	+	v	v	–[d]	+	+[b]	v	–	+	+	v	v	–[d]	+	–	–	–	–[d]	v	v	+	+[b]	+	nd	+	nd	v	nd
D-Glucuronate	–	–	–	–	nd	–	nd	–	nd	–	–	–	–	–	–	–	–	–	+	–	–	–	–	nd	–	–	–	–	–	–	nd	–	nd	–	nd
Guar gum	–	–	–	–[d]	nd	–	nd	–[d]	–	–	v	–	–	+	–	–	–	–	–	–	–	–	–	nd	–	–	–	–	–	–	nd	–	nd	–[d]	nd
Inulin	–	v	+	–	–	–	–	+	v	v	–	nd	–	–	+	–	–	–	v	–	–	–	–	–	–	–	–	+	–	+	nd	v	–	v	–
D-Lactose	+	+	+	+	+	–	–	v	+	+	+	–	–	+	+	–	–	+	+	+	+	+	–	+	+	v	+	–	+	+[h]	+	–	–[d]	v	+
Locust bean gum	–	–	–	v	nd	–	nd	–	–	–	v	–	–	+	–	–	–	–[d]	–	–	–	–	–	nd	–	–	–	–	–	–	nd	–	nd	–[d]	nd
Maltose	–[d]	+	+	+	+	v	–	+	+	+	+	+	+	+	+	+	v	+	+	+	+	+	+	+	+	+	+	+	+	+[b]	+	+	nd	+	+
D-Mannitol	–	v	–	–	–	–	–	–	v	v	–	–	–	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	+	–	–	–	–	–	+
D-Mannose	–	v	–	v	–	–[d]	+	–	+	–	–	–	–	+	v	–	v	v	v	v[i]	–	–	–	–	+	+[h]	–	+	–	+[b]	+	–	–	–[j]	+
Melezitose	–	+	–	v	–	–	nd	–	–	–	–	–	–	+	v	–	–	+	v	–	–	–	–	–	–	v	–	–	–	+	v	+	v	v	–
Melibiose	v	+	+	+	+	+	+	+	+	+	+	+	+	+	+	–	+	+	+	+	+	+	–	+	+	+	+	+	+	+	+	+	+	+	+
Pectin	–[d]	–	–	v	nd	–[d]	nd	–	v	v	–	–[d]	–	v	–	–	v	–[d]	–	–	–	–	–	nd	–	–	–	–	–	–	nd	v	nd	v	nd
Porcine gastric mucin	+	–	–	–	nd	–	nd	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	nd	–	–	–	–	–	–	nd	–	nd	–	nd
Raffinose	–	+	+	+	+	+	nd	+	+	+	+	+	–	+	+	–	+	+	+	+	+	+	–	+	+	+	+	+	+	+	+	+	+	+	+
D-Ribose	–	+	+	+	+	+	+	–	+	+	–	+	–	+	+	+	+	+	+	–	+	+	–	+[b]	+	+	+	+	+	+	+	+	+[g]	–	+
Salicin	–	+	+	+	–	+	+	–	+	+	–	+	–	+	+	+[h]	+	–	–	–	–	+[g]	–	+[b]	+	–	–[l]	+	+[g]	v	nd	v	–[d]	v	+
D-Sorbitol	–	v	v	–	–	–	–	–	v	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	v	–	–	–	–	–[d]	–	+	+	–	–
Starch	–	+	+	+	–	–	nd	+	–	–	+	–	+	+	–	+	–	–	–	–	–	+	+	+	+	+	+	–	+	–	nd	+	v	+	+
Sucrose	v[c]	+	+	+	+	+	nd	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Tragacanth gum	–	–	–	–	nd	–	nd	–	–	–	–	–	–	–	–	–	v	v	–	–	–	–	–	nd	–	–[d]	–	–	–	–	nd	–	nd	–	nd
Trehalose	–	v	–	v	–	–	nd	–	v	v	–	–	–	+	+	–	–	–	–	–	–	–	–	–	v	–	–	+	–	+	+	v	–	v	+
Xylan	–[d]	–	–	–[d]	nd	–[d]	nd	–	–	v	–	–	–	v	–	–	–	–	–	–	–	–	–	nd	v	–	–[d]	+	–	+	nd	–	nd	v	nd
D-Xylose	–	+	+	+	+	+[f]	nd	–	–	+	–	+	+	+	+	+	–	v	v	+	+	+	–	–	+	+	v	+	–	+	nd	–	–[d]	–	+

[a] Symbols: +, 90% or more strains positive; –, 90% or more strains negative; v, 11–89% of strains positive; nd, not determined. All strains tested ferment glucose, but not alginate, bovine submaxillary mucin, chondroitin sulfate, dextran, α-D-fucose, D-galacturonate, glycerol, gum karaya, heparin, hyaluronate, lactate, laminarin, ovomucoid, polygalacturonate, or L-rhamnose. *Bifidobacterium psychraerophilum* and *Bifidobacterium thermacidophilum* subsp. *porcinum* have not been tested. All data relating to the degradation of complex carbohydrates are from Crociani et al. (1994). Data for *Bifidobacterium animalis* subsp. *lactis* from Meile et al. (1997); data for *Bifidobacterium gallinarum* from Watabe et al. (1983); data for *Bifidobacterium gallicum* from Lauer (1990); data for *Bifidobacterium thermacidophilum* subsp. *thermacidophilum* from Dong et al. (2000).

[b] A few strains do not ferment this sugar.

[c] A few strains do not ferment this sugar, but for strains that do, fermentation is slow.

[d] Some strains ferment this sugar.

[e] Sugars listed as "v" mainly give erratic results.

[f] A few strains do not ferment pentoses.

[g] Some strains are weak fermenters.

[h] Generally delayed or slight fermentation.

[i] Reported as "sometimes not fermented" (Matteuzzi et al., 1971).

[j] Some strains from sewage ferment this sugar.

[k] Some strains, especially those from rabbit and rat feces, do not ferment this sugar.

[l] Some strains can ferment this sugar weakly.

List of species of the genus *Bifidobacterium*

1. **Bifidobacterium bifidum** (Tissier 1900) Orla-Jensen 1924, 472[AL] (*Bacillus bifidus* Tissier 1900, 86)

bi′fi.dum. L. neut. adj. *bifidum* cleft, divided.

Cells are highly variable in appearance, but some traits observed with cells grown in TPY agar stabs are distinct (Figure 67, panel 1). Groupings of "amphora-like" cells are characteristic (Sundman et al., 1959).

Two biovars are recognized; biovar a predominates in the feces of human adults and biovar b in that of neonates (variants a and b; Reuter, 1963); these variants differ in their fermentation of maltose, melibiose, and sucrose.

Source: the type strain was isolated from the feces of a human infant.

DNA G+C content (mol%): 61 (T_m).

Type strain: Ti (Tissier), ATCC 29521, DSM 20456, JCM 1255, LMG 11041.

Sequence accession no. (16S rRNA gene): M38018.

2. **Bifidobacterium adolescentis** Reuter 1963, 502[AL]

a.do.les.cen′tis. L. n. *adolescens* adolescent; L. gen. n. *adolescentis* of an adolescent.

The cellular morphology is common to that of many other *Bifidobacterium* species (Figure 67, panel 2).

Reuter (1963) named the pentose-fermenting bifidobacteria he first found to predominate in the feces of human adults as *Bifidobacterium adolescentis* and *Bifidobacterium longum* subsp. *longum*. Four biovars were identified, a, b, e, and d, that varied in their ability to ferment mannitol and sorbitol and were serologically distinct (Reuter, 1963).

Among those species which are regularly found in man, *Bifidobacterium adolescentis* occurs most frequently in sewage (Scardovi et al., 1979a). Reuter's biovars b and d, namely those which do not ferment sorbitol, cannot be distinguished phenotypically from *Bifidobacterium dentium* (Table 22) (Scardovi et al., 1979a). Alternatively, the PAGE procedure can be used for differentiation (Biavati et al., 1982).

Source: the type strain was isolated from the feces of a human adult.

DNA G+C content (mol%): 59 (T_m).

Type strain: E194a, ATCC 15703, DSM 20083, JCM 1275, LMG 10502, NCIMB 702204.

Sequence accession no. (16S rRNA gene): M58729.

Additional comments: the genome of *Bifidobacterium adolescentis* ATCC 15703[T] (GenBank accession no. AP009256) has been completely sequenced; its single circular chromosome consists of 2,089,645 bp (GENOME PROJECT, http://www.ncbi.nlm.nih.gov/sites/entrez).

3. **Bifidobacterium angulatum** Scardovi and Crociani 1974, 19[AL]

an.gu.la′tum. L. neut. adj. *angulatum* with angles, angular.

Cells grown in TPY agar stabs generally and characteristically form V (angular) or palisade arrangements similar to corynebacteria; cells are rarely enlarged at the extremities. Branching is absent. This morphological type is unique among bifidobacteria (Figure 67, panel 3).

Anaerobic, but more sensitive to O_2 than most bifidobacteria (measured by the depth of growth in stabs). CO_2 does not affect this sensitivity, but it strongly enhances anaerobic growth.

Some *Bifidobacterium angulatum* strains (20%) ferment sorbitol and thus can be readily distinguished from other *Bifidobacterium* species that ferment this sugar (Table 22). Strains that do not ferment sorbitol can be confused, in the case of doubtful morphology, with *Bifidobacterium pseudolongum* subsp. *globosum*, *Bifidobacterium pseudolongum* subsp. *pseudolongum*, and with sorbitol-negative strains of *Bifidobacterium pseudocatenulatum* from calf feces [nearly half of the *Bifidobacterium pseudocatenulatum* strains from this source are inactive toward sorbitol (Scardovi et al., 1979b)].

Bifidobacterium angulatum possesses the least anodal transaldolase (number 5) with respect to named *Bifidobacterium* taxa.

Source: first isolated from adult human feces and later found in sewage; the type strain was isolated from the feces of a human adult.

DNA G+C content (mol%): 59 (T_m).

Type strain: B677, ATCC 27535, DSM 20098, JCM 7096, LMG 11039, NCIMB 702236.

Sequence accession no. (16S rRNA gene): M84775.

4. **Bifidobacterium animalis** (Mitsuoka 1969) Scardovi and Trovatelli 1974, 26[AL] (*Bifidobacterium longum* subsp. *animalis* biotype a Mitsuoka 1969, 60)

a.ni.ma′lis. L. neut. gen. n. *animalis* of a living being, an animal.

Cells grown on TPY characteristically show that the central parts are slightly enlarged (Figure 67, panels 4a and 4b). Branching can occur to form cross-like aggregates of four cells that are distally inflated.

Bifidobacteria isolated from feces of calves, sheep, rats, and guinea pigs are very similar phenotypically to *Bifidobacterium longum*, but inactive toward melezitose, and were referred to as a subspecies of *Bifidobacterium longum* (*Bifidobacterium longum* subsp. *animalis* Mitsuoka (1969). Two distinct biovars, a and b, were identified: biovar a was mannose-negative and biovar b was mannose-positive (Mitsuoka, 1969). Mitsuoka's strains R101-8[T] biovar a (ATCC 25527[T]) and C10-45 biovar b were not related to *Bifidobacterium longum* based on DNA homology studies, but one of them (C10-45 biovar b) was related to *Bifidobacterium pseudolongum* (Scardovi et al., 1971b). Strain R101-8[T] was subsequently allotted to a DNA homology group of bifidobacteria isolated from chickens, rats, rabbits, and sewage and proposed as the distinct species, *Bifidobacterium animalis* comb. nov. (Scardovi and Trovatelli, 1974).

CO_2 has no effect upon O_2 sensitivity or anaerobic growth. Cells survive exposure to pH 3–5 for 3 h (Matsumoto et al., 2004). Glucose is fermented using the characteristic enzyme F6PPK in the so-called bifid shunt. Dextrin, D-glucose, maltose, maltotriose, raffinose, and sucrose are fermented, but not starch. Growth occurs in the presence of 4.2% NaCl at 37°C and in 3.0% NaCl at 45°C (Yaeshima et al., 1991).

Isozymes of transaldolase (isozyme 5) and 6PGD (isozymes 8 or 9) possessed by this species show a pattern shared with only a strains of *Bifidobacterium catenulatum* (Table 21).

Fructose-bisphosphate aldolase and G6PDH activities are observed (Scardovi and Trovatelli, 1974).

Biavati et al. (1992a) observed the phenomenon called "phase variation" (Trüper and Krämer, 1981) in members of the two subspecies of *Bifidobacterium animalis*. The transition of colony phenotype accompanied by a dramatic change in cell morphology and dimensions was described. Cells forming transparent (T) colonies, which were minute and mostly spherical, were seen to develop into cells that formed opaque (O) colonies, which had species-specific shapes and dimensions (Figure 69.)

Source: found in calves, chickens, rabbits, feces of rats, sewage, and fermented milk.

DNA G+C content (mol%): 60.1 ± 0.3 (T_m; Scardovi and Trovatelli, 1974).

Type strain: Mitsuoka R101-8, ATCC 25527, DSM 20104, NCIMB 702242, JCM 1190, LMG 10508.

Sequence accession no. (16S rRNA gene): D86185.

Additional comments: Bifidobacterium animalis can be readily differentiated from other *Bifidobacterium* species found in animal habitats that ferment arabinose and xylose (namely, *Bifidobacterium cuniculi*, *Bifidobacterium magnum*, *Bifidobacterium pseudolongum* subsp. *globosum*, *Bifidobacterium pseudolongum* subsp. *pseudolongum*, *Bifidobacterium pullorum*, and *Bifidobacterium longum* subsp. *suis*) by lactose and salicin fermentation. If a strain ferments both sugars, it can be retained as *Bifidobacterium animalis* [of the species cited above, *Bifidobacterium pullorum* ferments salicin, but not lactose (Table 22)]. "Human" species that ferment pentoses but not starch, such as *Bifidobacterium adolescentis* and *Bifidobacterium dentium*, can be distinguished from *Bifidobacterium animalis* by the absence of gluconate fermentation in *Bifidobacterium animalis* (Table 22).

Bifidobacterium animalis strains were subdivided into the subspecies *animalis* and *lactis* by Masco et al. (2004). This taxonomic rearrangement has been supported by genotypic and phenotypic data. *Bifidobacterium animalis* subsp. *animalis* contains strains isolated from rat feces, whereas *Bifidobacterium animalis* subsp. *lactis* includes strains isolated from chicken and rabbit feces, and from fermented milk and sewage. The two subspecies can also be distinguished by BOX-PCR, fluorescent amplified fragment length polymorphism (FAFLP) DNA fingerprinting, by *atpD* and *groEL* gene sequence typing, and by PAGE electrophoresis (Masco et al., 2004; Mattarelli et al., 1992), and also by their ability to grow in a milk-based medium (Masco et al., 2004). The PAGE protein pattern is distinct from that of all other *Bifidobacterium* species (Biavati et al., 1982). PAGE analysis provides a reliable way of separating *Bifidobacterium animalis* subsp. *lactis* strains isolated from different sources (Mattarelli et al., 1992).

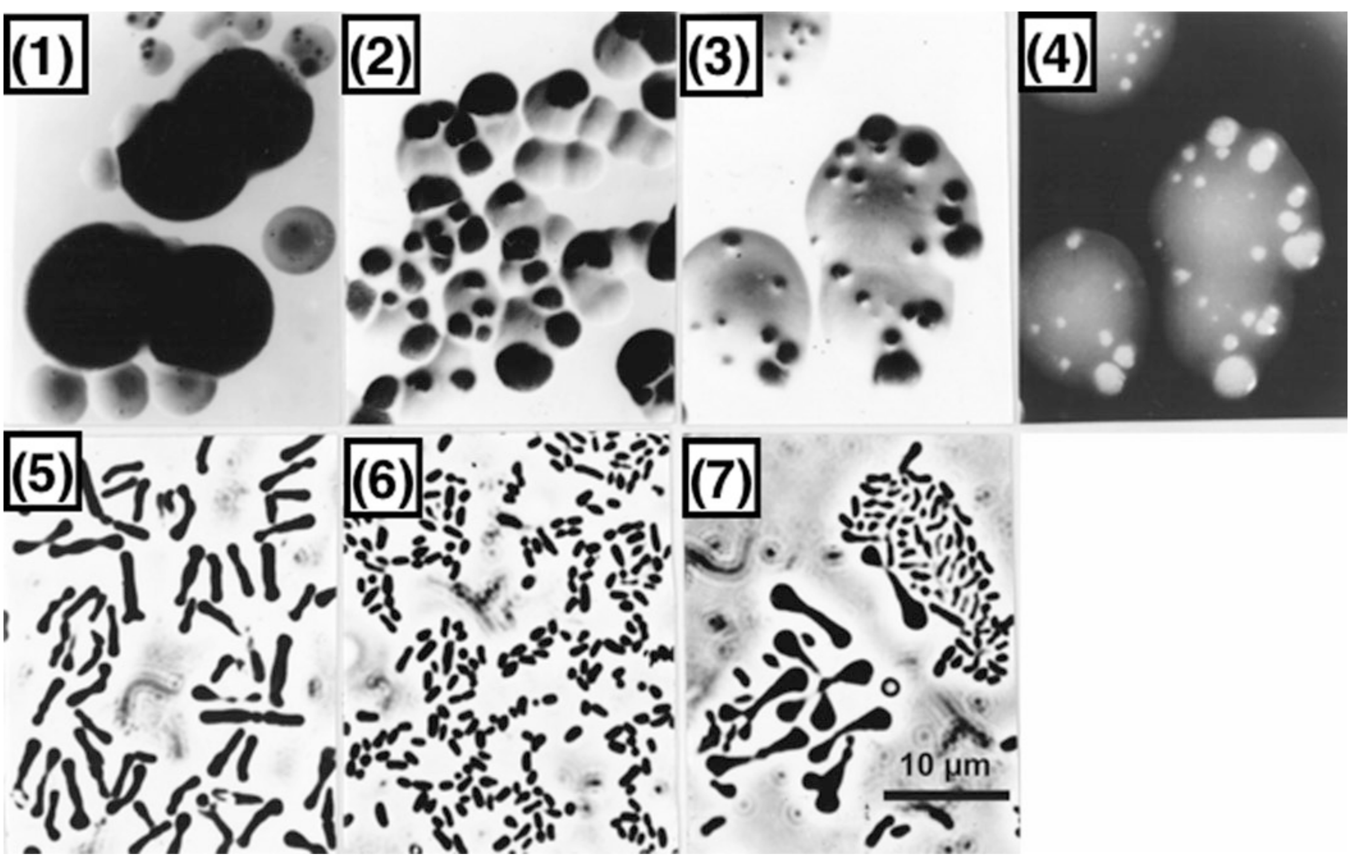

FIGURE 69. *Bifidobacterium animalis* subsp. *lactis* strain P 23 (=ATCC 27536): (1) and (2), transparent (T) and opaque (O) colonies, respectively; (3) and (4), T colonies with O papillae viewed under different illumination; (5) and (6), cells from O and T colonies, respectively; (7), mixed type from TPY stab. Phase-contrast photomicrographs (magnification 1500×).

4a. **Bifidobacterium animalis subsp. animalis** (Scardovi and Trovatelli 1974) Masco, Ventura, Zink, Huys and Swings 2004, 1142[VP] (*Bifidobacterium animalis* Scardovi and Trovatelli 1974, 26; *Bifidobacterium longum* subsp. *animalis* biotype a Mitsuoka 1969, 60)

a.ni.ma′lis. L. neut. gen. n. *animalis* of a living being, an animal.

Strains display characteristics that are typical of *Bifidobacterium animalis* as described above. No growth occurs in slants incubated in air or in air enriched with carbon dioxide. The optimum growth temperature is 39–41°C. Growth does not occur in milk or milk-based media.

Source: strains, including the type strain have been isolated from rat feces.

DNA G+C content (mol%): 61.3 ± 0.0 (T_m).

Type strain: Mitsuoka R101-8, ATCC 25527, DSM 20104, NCIMB 702242, JCM 1190, LMG 10508.

Sequence accession no. (16S rRNA gene): X70971.

4b. **Bifidobacterium animalis subsp. lactis** (Meile, Ludwig, Rueger, Gut, Kaufmann, Dasen, Wenger and Teuber 1997) Masco, Ventura, Zink, Huys and Swings 2004, 1142[VP] (*Bifidobacterium lactis* Meile, Ludwig, Rueger, Gut, Kaufmann, Dasen, Wenger and Teuber 1997, 63)

lac′tis. L. neut. gen. n. *lactis* of milk.

Strains display characteristics that are typical of *Bifidobacterium animalis*. Growth is not seen on agar plates exposed to air, but is evident in cultures containing 10% oxygen in the headspace atmosphere above liquid media. The molar ratio of acetate to lactate from glucose metabolism is about 10:1 under anaerobic conditions, e.g. lactate production is replaced by formate production. The optimum growth temperature is 39–42°C. Growth occurs in milk or milk-based media.

Source: strains have been isolated from chicken and rabbit feces, fermented milk samples, sewage, and recently from human feces (infants and adults) (Gavini et al., 2001; Masco et al., 2004; Mättö et al., 2004). *Bifidobacterium animalis* subsp. *lactis* is a probiotic supplement in the human diet (Biavati et al., 1995; Bouhnik et al., 1992) and, hence, it is reasonable to consider finding it in human feces due to the ingestion of milk products. However, the natural habitat of the taxon is the intestine of chickens and rabbits. The type strain was isolated from fermented milk.

DNA G+C content (mol%): 61.0 ± 0.5 (T_m).

Type strain: UR1, DSM 10140, JCM 10602, LMG 18314.

Sequence accession no. (16S rRNA gene): X89513.

Additional comments: the genome of *Bifidobacterium animalis* subsp. *lactis* AD011 (GenBank accession no. CP001213) has been completely sequenced (Kim et al., 2009); it is smaller than other completely sequenced bifidobacterial genomes reported to date with its single circular chromosome consisting of 1,933,695 bp.

5. **Bifidobacterium asteroides** Scardovi and Trovatelli 1969, 83[AL]

as.te.roi′des. N.L. neut. adj. *asteroides* (from Gr. adj. *asteroeides -es*) star-like.

Cells grown in TPY agar stabs are 2–2.5 µm long, generally occur in pairs with pointed ends, and are slightly curved. They usually show a radial disposition around a common mass of hold-fast material (Figure 67, panel 5). Nutritional or CO_2 deficiencies or growth with certain sugars may induce clavate or spatulate cells with occasional swellings, and irregular or cross-like branching in the central part of the cell body. Colonies are circular, smooth, and convex, with entire edges. The consistency is such that colonies removed by needle can hardly be dispersed in water. Growth in static fluid culture generally causes bacteria to adhere to the glass walls and leaves the liquid clear. CO_2 is required for growth in all media, including stabs. Aerobic growth occurs on slopes only if air is enriched with 10% CO_2. H_2O_2 is decomposed vigorously by cells grown in 90% air + 10% CO_2. In stabs, incubated in CO_2/air, only the lower portions of the growth are catalase-negative. Temperature optimum is 35–36°C; growth does not occur at 21°C or at 42°C after incubation for 7 d. Biotin, nicotinic acid, pantothenate, pyridoxine, riboflavin, and thiamin are required for continued growth in a Bacto vitamin-free Casamino acids-containing substrate.

Fructose-bisphosphate aldolase is not detected in cell-free extracts or in iodoacetate-poisoned cell suspensions. Cell-free extracts possess $NADP^+$-dependent hexose monophosphate dehydrogenases. When 85 strains from very different geographical origins were examined electrophoretically for their transaldolase and 6PGD isozyme content at least eight transaldolases and nine 6PDG were detected; each strain exhibited only one band for each allozyme. *Bifidobacterium asteroides* possesses the least mobile variant of 6PGD (isozyme 13 moves 53, whereas isozyme 1, the most anodal variant, found in *Bifidobacterium pseudocatenulatum*, moves 100) (Scardovi et al., 1979a).

A large variety of extrachromosomal elements of diverse molecular masses ranging from 1.2 to 22 MDa were found in 74 out of 224 members of this species (Sgorbati et al., 1982). The functions encoded by these plasmids are still not known.

Source: normally found in the intestine of western honey bees and occasionally in the hind-gut of *Apis cerana*, an asiatic honey bee. The type strain was isolated from the intestine of *Apis mellifera* subsp. *ligustica*.

DNA G+C content (mol%): 59 (T_m).

Type strain: C 51, ATCC 25910, DSM 20089, JCM 8230, LMG 10735.

Sequence accession no. (16S rRNA gene): M58730.

Additional comments: DNA–DNA homolgy with *Bifidobacterium indicum*, another bifid found in honey bees, is about 30%. Unexpectedly, 50% DNA–DNA homology was shown with *Bifidobacterium choerinum*, a bifid from piglets; otherwise, the DNA is unrelated to that of any other *Bifidobacterium* species.

6. **Bifidobacterium bombi** Killer, Kopečný, Mrázek, Rada, Benada, Koppová, Havlík and Straka 2009, 2023[VP]

bom′bi. L. n. *bombus* a boom, a deep hollow noise, buzzing, also the zoological genus name of the bumblebee; N.L. gen. n. *bombi* of *Bombus*, of a bumblebee

Short and irregularly shaped rods (0.4–0.5 μm wide and 0.9–1.1 μm long) with occasional bifurcations (Figure 67, panel 6). Along the cells are irregular constrictions. Colonies on TPY agar under anaerobic conditions are white, circular, and low convex with sharp undulate edges. Their size can reach 1.0–1.7 mm after 3 d incubation. In culture, the appearance of the cells resembles dividing bacilliform cells frequently forming filaments with irregular contractions and bifurcations. Strictly anaerobic. Optimal temperature for growth is 30°C; maximal temperature is 37°C. Good growth occurs from 10 to 37°C. Very limited growth is observed at 47°C. Minimal initial pH for growth is 4.5.

All strains utilize arbutin, esculin, α-galactoside, gentiobiose, D-glucose, D-mannose, melibiose, D-ribose, salicin, L-arabinose, cellobiose, dulcitol, erythritol, D-galactose, glycerol, glycogen, inositol, inulin, D-lactose, maltose, D-mannitol, L-rhamnose, D-sorbitol, sorbose, and turanose; D- and L-xylose are not utilized.

Isolates produce arginine arylamidase, histidine arylamidase, leucine arylamidase, phenyl arylamidase, proline arylamidase, serine arylamidase, tyrosine arylamidase, β-galactosidase, and α- and β-glucosidases. Catalase-, oxidase-, and urease-negative.

Fatty acids in the cultures contain a low proportion of branched-chain components. In 145 isolates, the dominant fatty acids are oleic ($C_{18:1}$; 7.49%) and arachidic ($C_{20:0}$; 7.18%) acids. *Bifidobacterium bombi* contains a high level of linoleic acid ($C_{18:2}$; 7.34%). Heneicosanoic acid is not present. Four other fatty acids are present: palmitic ($C_{16:0}$; 7.14%), stearic ($C_{18:0}$; 5.91%), behenic ($C_{22:0}$; 5.87%), and tricosanoic ($C_{23:0}$; 5.38%) acids. The content of odd chain fatty acids is high (41.2%, w/v). Increased unsaturated fatty acid content corresponds with lower growth temperature.

Source: isolated from the digestive tract of different bumblebees (*Bombus lucorum*, *Bombus pascuorum*, and *Bombus lapidarius*) in Central Bohemia. The type strain was isolated from the digestive tract content of *Bifidobacterium lucorum* from Central Bohemia, Czech Republic.

DNA G+C content (mol%): 47.3 (T_m), the lowest value found so far in the bifidobacteria.

Type strain: BluCI/TP, ATCC BAA-1567, DSM 19703.

Sequence accession no. (16S rRNA gene): EU127549.

Additional comments: the type strain, BluCI/TP^T, and related isolates were located in the actinobacterial cluster and were closely related to members of the genera *Bifidobacterium*, *Aeriscardovia*, *Parascardovia*, and *Scardovia*. The closest relative of BluCI/TP^T was *Bifidobacterium asteroides* (93% 16S rRNA gene sequence similarity). Because of the low G+C content, the phylogenetic location of this species was verified by phylogenetic analysis using the *hsp60* gene; highest *hsp60* gene sequence similarity of BluCI/TP^T was to *Bifidobacterium asteroides* (82%).

7. **Bifidobacterium boum** Scardovi, Trovatelli, Biavati and Zani 1979b, 308[AL]

bo′um. L. n. *bos* a cow; L. pl. gen. n. *boum* of cattle.

Cells grown on TPY agar are, in general, more irregular than those of *Bifidobacterium thermophilum* and vary greatly between strains (Figure 67, panel 7). Most branched forms are seen in cells grown in air + CO_2. Grows in 90% air + 10% CO_2 without becoming catalase or catalase-like (hemin)-positive. Requirements for growth factors are unknown.

Fructose-bisphosphate aldolase and G6PDH are present in cell-free extracts, as with *Bifidobacterium thermophilum*. The transaldolase isozyme of *Bifidobacterium boum* (number 6) is clearly electrophoretically distinct from that of the majority (93%) of *Bifidobacterium thermophilum* strains (number 8).

The interpeptide bridge of the cell-wall peptidoglycan is Lys–D-Ser–D-Glu and differs from that of the closely related species *Bifidobacterium thermophilum*, which is Orn(Lys)–D-Glu.

The PAGE protein pattern of *Bifidobacterium boum* is distinct and easily distinguishable from that of *Bifidobacterium thermophilum*. The so called "genus band" is slightly less anodal in *Bifidobacterium boum* than in any other *Bifidobacterium* species (Biavati et al., 1982).

Source: the type strain was isolated from bovine rumen.

DNA G+C content (mol%): 60.0 ± 0.2 (T_m).

Type strain: RU917, ATCC 27917, DSM 20432, JCM 1211, LMG 10736.

Sequence accession no. (16S rRNA gene): D86190.

Additional comments: the practical distinction of *Bifidobacterium boum* from *Bifidobacterium choerinum* and *Bifidobacterium thermophilum*, which are often found in the same ecological niches, can be achieved with transaldolase analysis or protein PAGE (see Additional comments under *Bifidobacterium choerinum*).

A few strains isolated from the bovine rumen as biovars of *Bifidobacterium thermophilum* (*Bifidobacterium ruminale*) (Scardovi et al., 1969) were later found to show nearly 70% DNA–DNA relatedness to *Bifidobacterium thermophilum* (Scardovi et al., 1971b). Subsequently, other strains isolated from the bovine rumen were assigned to DNA homology group IV, as they showed 55–75% relatedness to *Bifidobacterium thermophilum* (Trovatelli and Matteuzzi, 1976). Out of a large number of animal strains analyzed by DNA–DNA hybridization, 36 strains from the rumen and five from piglet feces were assigned to the novel species *Bifidobacterium boum* (Scardovi et al., 1979b).

Although *Bifidobacterium boum* does not ferment cellobiose, melezitose, D-mannose, or trehalose, and thus has a more stable sugar fermentation pattern than *Bifidobacterium thermophilum*, the fermentation patterns of the two species are often the same. DNA relatedness between *Bifidobacterium boum* and *Bifidobacterium thermophilum* has been studied extensively (Scardovi et al., 1979b). DNA relatedness values of 36–74% have been reported between *Bifidobacterium boum* and *Bifidobacterium thermophilum* (Table 23).

8. **Bifidobacterium breve** Reuter 1963, 502[AL]

bre′ve. L. neut. adj. *breve* short.

The cell morphology is responsible for the designation of the specific epithet: the species encompasses organisms with the thinnest and shortest cells among bifids found in the human intestine (Figure 67, panel 8).

One hundred and six strains of *Bifidobacterium breve*, including a few that ferment arabinose and xylose, have been identified by DNA–DNA hybridization studies and their transaldolase and 6PGD isozymes were determined. Of these, 57% had unique zymograms and 25% displayed the same zymogram as *Bifidobacterium coryneforme*, a bifid found only in the intestine of the honey bee (Scardovi et al., 1979a).

The genome of *Bifidobacterium breve* UCC 2003 is the largest *Bifidobacterium* genome sequenced to date; it has roughly 47 kilobases (Leahy et al., 2005).

Source: the type strain was isolated from the feces of a human infant.

DNA G+C content (mol%): 58 (Bd).

Type strain: S1, ATCC 15700, DSM 20213, JCM 1192, NCIMB 702257.

Sequence accession no. (16S rRNA gene): M58731.

Additional comments: among bifids isolated from newborn infants that do not ferment pentoses, the rank of species was attributed to those fermenting D-mannitol and D-sorbitol. Two biovars, a and b, with differences towards melezitose fermentation were recognized. Serologically related strains isolated from breast-fed older infants and that did not ferment mannitol or sorbitol were referred to as a separate species, namely "*Bifidobacterium parvulorum*" (Reuter, 1963).

Strain S50 (=ATCC 15698), the type strain of "*Bifidobacterium parvulorum*" biovar a, "*Bifidobacterium parvulorum*" biovar b strain S17c (=ATCC 15699), and *Bifidobacterium breve* biovar b strain S46 (ATCC 15701) had 88, 94, and 86% DNA relatedness, respectively, to strain $S1^T$ (=ATCC 15700^T), the type strain of *Bifidobacterium breve* biovar a; other DNA relatedness tests proved the genetic identity of the two Reuters' species (Scardovi et al., 1971a). On the basis of DNA relatedness data, *Bifidobacterium breve* is more closely related to *Bifidobacterium longum* subsp. *infantis* and *Bifidobacterium longum* subsp. *longum* than to any other species of the genus (40–60% relatedness).

9. **Bifidobacterium catenulatum** Scardovi and Crociani 1974, 18[AL]

ca.te.nu.la′tum. L. n. *catenula* a small chain; L. neut. suff. *-atum* suffix used in adjectives meaning provided with; N.L. neut. adj. *catenulatum* having small chains.

Cells grown in TPY agar stabs are generally and characteristically arranged in chains of three, four, or more globular elements. The distal ends of the chains are usually tapered. Distinct branchings, club-like swellings, or spatula-like extremities are rare (Figure 67, panel 9). Anaerobic. CO_2 does not have any effect on O_2 sensitivity or anaerobic growth.

Most strains of *Bifidobacterium catenulatum* ferment D-sorbitol, but not D-mannitol, similar to *Bifidobacterium adolescentis* biovar c and *Bifidobacterium pseudocatenulatum* (see below), but they can be distinguished from the former because they ferment melezitose, and from the latter as they do not ferment starch (Table 22). Riboflavin and pantothenate are required for growth.

Source: found in feces of human adults and in sewage. The type strain was isolated from the feces of a human adult.

DNA G+C content (mol%): 54.7 ± 0.2 (T_m).

Type strain: B669, ATCC 27539, DSM 20103, JCM 1194, LMG 11043, NCIMB 702246.

Sequence accession no. (16S rRNA gene): M58732.

Additional comments: most strains show DNA relatedness to *Bifidobacterium adolescentis* (reference DNA from strain E298b, ATCC 15705, biovar c of Reuter) in the range 30–57% (Scardovi and Crociani, 1974). *Bifidobacterium catenulatum* should be considered, as should *Bifidobacterium pseudocatenulatum* (see below), to be more closely related to *Bifidobacterium adolescentis* than to any other species of the genus *Bifidobacterium* on this basis.

10. **Bifidobacterium choerinum** Scardovi, Trovatelli, Biavati and Zani 1979b, 307[AL]

choe.ri′num. N.L. neut. adj. *choerinum* (from Gr. adj. *khoireos*) pertaining to a pig.

Cells grown in TPY agar stabs are often short or coccoid and are similar to those of *Bifidobacterium pseudolongum* subsp. *globosum* (Figure 67, panel 10). In liquid medium, cells may be elongated to 10–12 µm, bent, with rounded or spatulated ends. Anaerobic. The effect of CO_2 is not detectable.

Strains of this species that ferment raffinose, but not L-arabinose, D-ribose, or D-sorbitol, cannot be distinguished from *Bifidobacterium boum* or *Bifidobacterium thermophilum* on this basis (Table 22).

Transaldolase and 6PGD isozymes are 3 and 4, respectively, a pattern unique among bifidobacteria. The species has a distinct PAGE protein pattern (Biavati et al., 1982).

Source: found in feces of piglets or occasionally in sewage. The type strain was isolated from the feces of a pig.

DNA G+C content (mol%): 66.3 ± 0.15 (T_m).

Type strain: Su806, ATCC 27686, DSM 20434, JCM 1212, LMG 10510.

Sequence accession no. (16S rRNA gene): D86186.

Additional comments: Bifidobacterium choerinum cannot be distinguished by its sugar fermentation pattern from either *Bifidobacterium thermophilum* biovar c (Mitsuoka, 1969) or from strains of *Bifidobacterium boum* that ferment D-lactose but not melezitose (Table 22). These three species are found frequently in the feces of piglets. However, transaldolase electrophoresis can be used to distinguish between them. The most anodal isozyme is that of *Bifidobacterium choerinum* (migration 100) and the least anodal is that of *Bifidobacterium thermophilum* (isozyme 8; migration 84); an intermediate form is shown by *Bifidobacterium boum* (isozyme 6; migration 90). Alternatively, PAGE patterns of soluble proteins can be used to distinguish between these taxa. The DNA of this species shows 26–57% and 37–62% relatedness to DNA of *Bifidobacterium pseudolongum* subsp. *globosum* and *Bifidobacterium pseudolongum* subsp. *pseudolongum*, respectively. An unexpected DNA relatedness value of 50% is observed between *Bifidobacterium choerinum* and *Bifidobacterium asteroides*.

11. **Bifidobacterium coryneforme** (*ex* Scardovi and Trovatelli 1969) Biavati, Scardovi and Moore 1982, 368[VP] (*Bifidobacterium coryneforme* Scardovi and Trovatelli 1969, 85)

co.ry.ne.for′me. Gr. n. *coryne* a club; L. neut. suff. *-forme* of the shape of; N.L. neut. adj. *coryniforme* club-shaped.

Grows poorly in TPY medium, but profusely in MRS medium. Cells grown in MRS agar stabs are short (1–1.5 µm long), often lanceolate, single, or in pairs, sometimes with short branches or simple knobs (Figure 67, panel 11). Radial groupings of cells are very rare and are formed under extreme growth conditions (Scardovi and Trovatelli, 1969). Anaerobic. Does not develop in slants inoculated under 90% air + 10% CO_2. CO_2 does not influence anaerobic growth on either solid or in liquid medium.

TABLE 23. DNA–DNA hybridization between *Bifidobacterium* species[a,b]

Competitor DNA from	Hybridization (%) to reference DNA from: *B. bifidum*	*B. longum* subsp. *longum*	*B. longum* subsp. *infantis*	*B. longum* subsp. *suis*	*B. breve*	*B. adolescentis*	*B. angulatum*	*B. catenulatum*	*B. pseudocatenulatum*	*B. dentium*	*B. gallicum*	*B. pseudolongum* subsp. *globosum*	*B. pseudolongum* subsp. *pseudolongum*
B. bifidum	100	2	20–28	0	10–20	0	7	25	15		15–16	0	
B. longum subsp. *longum*	40	75–101	50–76	73–75	12	0	8	20	22		18	0	
B. longum subsp. *infantis*	42	50–79	74–101	70	17–50			20	15		18	0	
B. longum subsp. *suis*	28	61–80	67	100	22		20		25		16		23
B. breve	25	40	40	0	100		10	24	10		11	0	
B. adolescentis	14		22		5–10	70–102	20–44	20–57	30	24–49	24		
B. angulatum	24	6–13	20	20	12	20–30	76–100	20–35	20	8–20	18	20	
B. catenulatum	20	0–26	15	25	10	22–57	2–37	78–101	50–80	3–48	15–18	5	
B. pseudocatenulatum								46–88	78–115		21		
B. dentium	20	0–13	20	20	12	15–57	5–26	16–45	18	69–110	19	12	
B. gallicum											100		
B. pseudolongum subsp. *globosum*	29		25	10	25		20		5			100	75
B. pseudolongum subsp. *pseudolongum*	35		17	9–27	7				5		22–25	65–70	100
B. magnum	10	4	25	9	9	6	5	26	20	21	23	6	
B. cuniculi	7		17		22				10		17		
B. choerinum	32		8		15				25		18		8–16
B. animalis subsp. *animalis*	10	8	10	27	20	25					20		
B. animalis subsp. *lactis*				20			10	10	10	4		30	34
B. thermophilum	22		29	5–12	10–22		18		16		18–20	5	0–23
B. boum	38		7		23				5		16		
B. thermacidophilum subsp. *thermacidophilum*				3									
B. merycicum													
B. ruminantium				23									
B. pullorum	27		39		33				12		22		
B. gallinarum													2–7
B. saeculare				10									
B. subtile	5	20	5	11	14	15	11	8	10	0	13		8
B. minimum	5	10	6	7	10	4	20	25	25	0	16		5
B. coryneforme				0	6								5
B. asteroides					0						8–9		0
B. indicum					11						12		0

[a] Except where indicated otherwise, data from Biavati et al. (1991), Biavati and Mattarelli (1991), Dong et al. (2000), Lauer (1990), Sakata et al. (2002), Scardovi et al. (1970, 1971b, 1979b), Scardovi and Trovatelli (1969), Scardovi and Zani (1974), Scardovi and Crociani (1974), Trovatelli et al. (1974), and Watabe et al. (1983).

[b] *Bifidobacterium coryneforme* was not used as reference: the DNA from this species was used as competitor DNA. *Bifidobacterium mongoliense* showed 13% and 7% DNA–DNA homology values with *Bifidobacterium minimum* and *Bifidobacterium psychraerophilum*, respectively (Watanabe et al., 2009). *Bifidobacterium bombi*, *Bifidobacterium scardovii*, *Bifidobacterium psychraerophilum*, *Bifidobacterium thermacidophilum* subsp. *porcinum*, and *Bifidobacterium tsurumiense* have not been tested.

[c] DNA from *Bifidobacterium thermophilum* RU 326 (=ATCC 25866) used as reference.

[d] Data from Dong et al. (2000) and von Ah et al. (2007).

B. cuniculi	*B. choerinum*	*B. animalis* subsp. *animalis*	*B. animalis* subsp. *lactis*	*B. thermophilum*[c]	*B. boum*	*B. thermacidophilum* subsp. *thermacidophilum*	*B. magnum*	*B. merycicum*	*B. ruminantium*	*B. pullorum*	*B. gallinarum*	*B. saeculare*	*B. subtile*	*B. minimum*	*B. asteroides*	*B. indicum*
10	27	10	10	5	11	35	5	36	30	20	3–9	18	4	4	0	0
17	16	6–9	5	0	23	28	0–29	19–32	25–30	9	6–10	19	23	7	0	
16–23	20–22		15	5	11	26	15	21	25–40	13	1–9	19–24	5	4	0	
21	21		5	10	7	3	10	11	23	10	3–8	30	8	10		
12	21		20	5	18	9	10	23	0–29	18	1–8	24	17	10	0	
25	20		30		25	18	14	16–27	40–72	21	1–6	25	13	5		
17	16		11	18	10	39	5	36	23	0	1–4	14	8	23		
10	32		10	20	8	23	17	28	40	12	1–8	20	12	30		
10	32				5	31		20	30		1	20				
17	15		6	20	0	42	8	34	25	0	3–6	9	0	5		
						26		12	10			8				
12	3		40	14	14	37	32	9–18	12–16	16		15	10	35	13	12
3	4				10	31		22	28		4–7	15				
10	10		23	20	12	0	75–106	0	27	2	2–4	17	7	20		
94–102	9–15		18–25	12–20	5	41		8	20	2	3–6	5				
8–20			4–20	15–35	20	25		13	23	4	1–5	25				
31	18		72–99			29				0	2–9	5				
26	20		85–100	25	21	35	15	23	18				10	5	5	0
15–25	15–20		23	79–117	27–80	59	11	36	25–31	10	3–6	8	15	4	0	0
8	23		11	36–74	69–96	34		0–29	19–26	0	3–6	2				
				60–82[d]		87–100		25	32		34	28	19	23	30	
8	13	23			36	25	0–29	88–100	16–22		32					
						32		16–22	16–22		32	34	8	12		
25	37		35		13	25		35	27	94–107	59–66	60–64				
					2–7	34		31	32		64–105	61				
						28		32	34		61	96–100	30	24	20	
14	34		5	18	0	19	0	15	8	8	3–9	30	70–100	10–31	5	4
23	36		5	10	0	23	19	20	12	10	1–8	24	16–20	100–103	3	0
20	36		0			30		10	8	0		0	0	4	23	60
11	50		5	0	0	32		8	6	6		20	2	0	100	30
20	44			10				12	8	17	1–4	25	0	0	23	100

Aldolase is not detected in cell-free extracts. Hexose monophosphate dehydrogenases are present. Transaldolase and 6PGD isozyme patterns (6-6) are shared by some strains of *Bifidobacterium breve*. However, zymograms of 3-phosphoglyceraldehyde dehydrogenase (Scardovi et al., 1979a) and PAGE protein patterns are distinct (Biavati et al., 1982).

Source: strains assigned to this species have been isolated occasionally from the intestine of the honey bees *Apis mellifera* subsp. *mellifera* and *Apis mellifera* subsp. *caucasica*, from Germany (Bayern), Norway (Billingstad), England (Buckfast, Buckfastleigh), and Bulgaria. The type strain was isolated from the intestine of *Apis mellifera* subsp. *caucasica* from Norway.

DNA G+C content (mol%): not determined.

Type strain: C215, ATCC 25911, DSM 20216, JCM 5819, LMG 18911.

Sequence accession no. (16S rRNA gene): M58733.

Additional comments: Bifidobacterium coryneforme Scardovi and Trovatelli (1969) was not included in the Approved Lists of Bacterial Names (Skerman et al., 1980). Its revival was proposed on the basis of its distinct electrophoretic cellular protein pattern and previously confirmed differential characteristics (Biavati et al., 1982). It is not related to other *Bifidobacterium* species, except *Bifidobacterium indicum*, on the basis of DNA relatedness. An interspecific DNA relatedness value of 60% has been reported (Scardovi et al., 1970).

12. **Bifidobacterium cuniculi** Scardovi, Trovatelli, Biavati and Zani 1979b, 307[AL]

cu.ni′cu.li. L. n. *cuniculus* rabbit; L. gen. n. *cuniculi* of the rabbit.

Cells grown in TPY agar stabs present a morphology that is very similar to that of *Bifidobacterium pseudolongum* subsp. *globosum* and *Bifidobacterium pseudolongum* subsp. *pseudolongum*. However, the short knobs or protuberances often in the center of the cells are rarely seen in other *Bifidobacterium* species (Figure 67, panel 12). Highly anaerobic. CO_2 has no effect on O_2 sensitivity or anaerobic growth.

D-Lactose and raffinose are characteristically not fermented. D-Fructose is regularly fermented, but only in prereduced media.

Transaldolase isozyme 1 and 6PGD isozyme 4 were found in seven strains studied by Scardovi et al. (1979a). This 1-4 pattern is unique among bifidobacteria.

Source: found in feces of adult rabbits. The type strain was isolated from the feces of a rabbit.

DNA G+C content (mol%): 64.1 ± 0.35 (T_m).

Type strain: RA93, ATCC 27916, DSM 20435, JCM 1213, LMG 10738.

Sequence accession no. (16S rRNA gene): M58734.

Additional comments: Bifidobacterium cuniculi can be distinguished easily from the morphologically similar taxa *Bifidobacterium pseudolongum* subsp. *globosum*, *Bifidobacterium pseudolongum* subsp. *pseudolongum*, and *Bifidobacterium animalis* subsp. *lactis*, which are also frequently found in rabbit feces, by its inability to ferment D-lactose, raffinose, and D-ribose. The same fermentation pattern can be used to distinguish *Bifidobacterium cuniculi* from the morphologically diverse *Bifidobacterium magnum*, another species isolated from the same source.

The DNA of the species is 50–67% related to that of *Bifidobacterium pseudolongum* subsp. *globosum* [reference strain RU 230 (=ATCC 25864)], but far less related to *Bifidobacterium pseudolongum* subsp. *pseudolongum* and other species of the genus *Bifidobacterium*.

13. **Bifidobacterium dentium** Scardovi and Crociani 1974, 18[AL]

den′ti.um. L. masc. n. *dens* tooth; L. pl. gen. n. *dentium* of teeth.

Cells grown in TPY agar show a general morphology resembling that of *Bifidobacterium infantis* (Figure 67, panel 13); hence, they are without any characteristic appearance. Anaerobic. CO_2 does not affect sensitivity to O_2 or anaerobic growth. Requires pantothenate and riboflavin for growth, similar to *Bifidobacterium angulatum* and *Bifidobacterium catenulatum*.

Source: found in human dental caries and plaque (Modesto et al., 2006). The type strain was isolated from human dental caries.

DNA G+C content (mol%): 61.2 ± 0.4 (T_m).

Type strain: B764, ATCC 27534, DSM 20436, JCM 1195, LMG 11045, NCIMB 702243.

Sequence accession no. (16S rRNA gene): D86183.

Additional comments: Bifidobacterium dentium cannot be distinguished phenotypically from *Bifidobacterium adolescentis* biovars b and d (strains not fermenting sorbitol; see Table 22). The distinction between these two species should be based either on transaldolase isozymes or on electrophoresis of total cellular proteins patterns (see *Bifidobacterium adolescentis*).

A number of bifid strains isolated from human dental caries, feces of human adults, and from the human vagina were assigned to *Bifidobacterium adolescentis* using phenotypic properties, but were subsequently recognized as forming a distinct “dentium” DNA homology group together with strains isolated from the oral cavity (Beerens et al., 1957) and strain 3859, which was labeled as *Bifidobacterium appendicitis*. *Bifidobacterium dentium* (Table 23) is most closely related to *Bifidobacterium adolescentis* on the basis of DNA relatedness, but is less related or unrelated to other *Bifidobacterium* species. DNA of “*Actinomyces eriksonii*” ATCC 15423 and ATCC 15424 are completely homologous to that of *Bifidobacterium dentium* reference strain B764^T (Scardovi et al., 1979a).

A study on the presence and distribution in human plaque and dental caries of *Bifidobacterium dentium*, *Scardovia inopinata*, and *Parascardovia denticolens*, which all belong to the family *Bifidobacteriaceae*, showed that *Scardovia inopinata* was the species most frequently isolated in dental caries, whereas *Bifidobacterium dentium* was more numerous in dental plaque. The prevalence of *Parascardovia denticolens* was similar in the two habitats (Modesto et al., 2006).

14. **Bifidobacterium gallicum** Lauer 1990, 100[VP]

gal′li.cum. L. neut. adj. *gallicum* of or belonging to the Gauls, although ethnic relationships to the French remains speculative.

Short rods with rounded ends, generally 0.7–0.9 µm by 1.5–3 µm, mostly arranged in pairs or short chains when grown in liquid culture (Figure 67, panel 14). Irregular elongations and swellings occur in cells obtained from colony growth. A slimy, capsule-like material is excreted in complex media containing meat extract, bacteriological peptone, and fermentable carbohydrates. Colonies are whitish, opaque, round, entire, and have a soft consistency. Optimal growth is at 37–39°C. Growth does not occur below 30°C or at 45°C and above. Grows at pH 5.0–8.0; optimal growth occurs between pH 6.0 and 7.0.

L-Arabinose, dextrin, D-fructose, D-galactose, D-glucose, glycogen, maltose, D-ribose, salicin (slowly), starch, sucrose, and D-xylose are fermented, but not D-arabinose, D-gluconate, glycerol, inulin, D-mannitol, D-mannose, melezitose, *myo*-inositol, raffinose, L-rhamnose, D-sorbitol, trehalose, or L-xylose.

The following enzyme activities are present in cell-free extracts: fructose-6-phosphate, L-(+)-lactate dehydrogenase (NAD-dependent and allosterically regulated by fructose 1,6-diphosphate), transaldolase, and xylulose-5-phosphate phosphoketolases. There is no fructose-1,6-diphosphate aldolase activity. Relative electrophoretic mobilities are: lactate dehydrogenase (R_{LDH}), 1.10 relative to rabbit-iso-I lactate dehydrogenase of muscle; and transaldolase (R_{TA}), 1.65 relative to type X transaldolase of yeast.

The peptidoglycan type is L-Lys–L-Ala–L-Ser (A3α); some lysine moieties are replaced to a variable degree by ornithine. The cell-wall polysaccharide comprises a galactan polymer of unknown structure. Minor amounts of rhamnose may be found in addition to galactose after growth on less complex media.

Source: habitat is not known. The type, and only strain, was isolated from human feces.

DNA G+C content (mol%): 61 (T_m).

Type strain: P6, ATCC 49850, DSM 20093, JCM 8224, LMG 11596.

Sequence accession no. (16S rRNA gene): D86189.

15. **Bifidobacterium gallinarum** Watabe, Benno and Mitsuoka 1983, 130[VP]

gal.li.na′rum. L. n. *gallina* hen; L. gen. pl. n. *gallinarum* of hens.

Slightly curved, short rods (0.5–1.0 µm wide by 1.0–2.5 µm long) with tapered ends, usually arranged singly or in short chains (Figure 67, panel 15). Swellings or club-shaped cells are frequently observed. In older liquid cultures, some cells have short branches. Surface colonies on BL agar (Mitsuoka et al., 1965) after 2 d of anaerobic incubation are punctiform to 1 mm in diameter, circular, entire, flat to convex, gray to grayish white, transparent to translucent, and glistening. Strictly anaerobic. Gelatin is not liquefied. Gas is not formed from fructose or glucose. Fermentation products from fructose or glucose are lactic acid and acetic acid in a molar ratio of 1:4.0 in EG broth (Mitsuoka et al., 1965); butyric and propionic acids are not formed. Final pH of glucose or fructose broth is 4.5–5.1.

Acid is produced from amygdalin, dextrin, esculin, D-fructose, inulin, maltose, melibiose, raffinose, D-ribose, salicin, sucrose, and D-xylose. L-Arabinose, cellobiose, D-galactose, D-glucose, D-lactose, D-mannose, methyl α-glucoside, and trehalose are usually fermented, but not glycogen, inositol, D-mannitol, L-rhamnose, D-sorbitol, or starch. Melezitose is not usually fermented. Nitrate is not reduced and neither indole nor hydrogen sulfide are produced. Ammonia is not produced from arginine. Litmus milk is usually acidified.

Source: the type strain was isolated from chicken cecum.

DNA G+C content (mol%): 65.7 ± 1.5 (T_m).

Type strain: Ch206-5, ATCC 33777, DSM 20670, JCM 6291, LMG 11586.

Sequence accession no. (16S rRNA gene): D86191.

Additional comments: DNA relatedness values with *Bifidobacterium pullorum* and *Bifidobacterium saeculare* are higher than those with other *Bifidobacterium* species (Biavati et al., 1991).

16. **Bifidobacterium indicum** Scardovi and Trovatelli 1969, 84[AL]

in′di.cum. L. neut. adj. *indicum* of India, Indian, named after the specific epithet of *Apis indica* F.

Cells grown in TPY agar stabs are generally short, occur in pairs, often in an angular disposition, and are more or less globular, sometimes suggesting a minute morphovar of *Bifidobacterium pseudolongum* subsp. *globosum* (Figure 67, panel 16). Some strains have slender and longer cells, but star-like clusters of cells never occur. Cells grown on gluconate are extremely small and regular in shape (Sgorbati et al., 1970). Colonies do not have the consistency of those of *Bifidobacterium asteroides*. Cells grown in liquid media do not adhere to the walls and sediment very slowly. Oxygen tolerance is similar to that of *Bifidobacterium asteroides*. CO_2 is required for aerobic growth, whereas the effect of CO_2 is equivocal for anaerobic growth. H_2O_2 is decomposed only by cells grown in 90% air + 10% CO_2 in the presence of hemin.

Cell-free extracts are fructose-bisphosphate aldolase-negative, but possess hexose monophosphate dehydrogenases. G6PDH uses both $NADP^+$ and NAD^+, whereas only $NADP^+$ is effective in 6-phosphogluconate dehydrogenation (Scardovi and Trovatelli, 1969). A total of 122 strains from different sources (Japan, Malaysia, Philippines) was examined for their transaldolase and 6PGD zymograms. Starch gel electrophoresis showed the presence of isozymes 4 and 7, respectively. Most strains isolated from *Apis dorsata* possess a transaldolase isozyme (number 7) that differs from that found in strains isolated from the intestine of *Apis cerana* subsp. *indica* (number 9) (Scardovi et al., 1979a).

Extrachromosomal elements were found in 73 out of 106 strains, 57% of which had a 22 MDa plasmid, whereas 33% showed a two-banded pattern at 2.0 and 3.5 MDa (Sgorbati et al., 1982). The cellular functions encoded by these plasmids are not known.

Source: the type strain was isolated from the intestine of *Apis cerana* subsp. *indica* from Malaysia.

DNA G+C content (mol%): 60 (T_m).

Type strain: C 410, ATCC 25912, DSM 20214, JCM 1302, LMG 11587.

Sequence accession no. (16S rRNA gene): D86188.

17. **Bifidobacterium longum** Reuter 1963, 502[AL]

lon.gum. L. neut. adj. *longum* long.

D-Galactose, D-lactose, maltose, melibiose, raffinose, and sucrose are fermented, but not gluconate, D-mannitol, salicin, D-sorbitol, starch, or trehalose. Variable responses are shown for L-arabinose, cellobiose, D-fructose, inulin, D-mannose, melezitose, and D-xylose. Cell-wall amino acids consist of L-Orn–L-Ser–L-Ala–L-Thr–L-Ala.

Source: a similar ecological distribution is shared by *Bifidobacterium longum* subsp. *longum* and *Bifidobacterium longum* subsp. *infantis*, which are particularly found in the gastrointestinal tracts of humans and infants, respectively; *Bifidobacterium longum* subsp. *suis* is typically found in the pig gastrointestinal tract.

Type strain: see subspecies below.

Additional comments: Bifidobacterium longum, *Bifidobacterium infantis*, and *Bifidobacterium suis* strains have DNA relatedness values around or above 70% (Bahaka et al., 1993; Lauer and Kandler, 1983; Scardovi et al., 1979a). Phylogenetic analyses of the genus *Bifidobacterium* based on 16S rRNA gene sequences (Miyake et al., 1998) and a heat-shock protein (Jian et al., 2001) also showed *Bifidobacterium infantis*, *Bifidobacterium longum*, and *Bifidobacterium suis* to be closely related. On the basis of these data, Sakata et al. (2002) placed the three taxa into the single species *Bifidobacterium longum* and established the three biotypes “longum”, “infantis”, and “suis” for strains belonging to the former species. However, a variety of other genotypic techniques, including ribotyping, ARDRA, BOX-PCR, PCR-DGGE, randomly amplified polymorphic DNA-PCR and comparison of *ldh*, *recA*, and *tuf* gene sequences (Masco et al., 2003; Matsuki et al., 1998; Requena et al., 2002; Roy et al., 1996; Sakata et al., 2006; Ventura et al., 2003), and plasmid profiling (Sgorbati et al., 1982), clearly distinguished between *Bifidobacterium longum*, *Bifidobacterium infantis*, and *Bifidobacterium suis*, as do carbohydrate fermentation patterns (Crociani et al., 1994; Sakata et al., 2002; Scardovi, 1986), starch hydrolysis (Scardovi et al., 1979a), and PAGE analysis (Biavati et al., 1982; Roy et al., 1994).

The genotypic and phenotypic variations described above revealed considerable differences between members of the three former species and supported the description of “*Bifidobacterium longum*”, “*Bifidobacterium infantis*”, and “*Bifidobacterium suis*” as subspecies of *Bifidobacterium longum*. Consequently, Mattarelli et al. (2008) described three *Bifidobacterium longum* subspecies: *Bifidobacterium longum* subsp. *longum* subsp. nov., *Bifidobacterium longum* subsp. *infantis* comb. nov. and *Bifidobacterium longum* subsp. *suis* comb. nov. The “International Committee on Systematic Bacteriology Subcommittee on *Bifidobacterium*, *Lactobacillus* and related organisms” supported this proposal (Klein, 2006).

17a. **Bifidobacterium longum subsp. longum** (Reuter 1963) Mattarelli, Bonaparte, Pot and Biavati 2008, 770[VP] (*Bifidobacterium longum* Reuter 1963, 502)

lon.gum. L. neut. adj. *longum* long.

Most strains show very elongated and relatively thin cellular elements with slightly irregular contours and rare branching on TPY agar (Figure 67, panel 17a). In TPY broth, most strains develop a uniform turbidity; clearing is slow and the sediment is viscous.

Two biovars can be distinguished. Biovar a [E194 (variant a)^{T} (=ATCC 15707^{T})] is more frequent in human adults and slowly ferments mannose, whereas biovar b [S3 (variant b) (=ATCC 15708)] is more frequent in neonates and is mannose-negative (Reuter, 1963).

Bifidobacterium longum is apparently the only species among those usually found in human feces that possesses a large variety of plasmids (Sgorbati et al., 1982).

Source: the type strain was isolated from the feces of an adult human.

DNA G+C content (mol%): 61 (T_m).

Type strain: E194b (variant a), ATCC 15707, DSM 20219, JCM 1217, NCIMB 702259, LMG 13197.

Sequence accession no. (16S rRNA gene): M58739.

Additional comments: Bifidobacterium longum subsp. *longum* NCC2705 (GenBank accession no. for genome sequence AE014295) was the first *Bifidobacterium* strain to be completely sequenced. Sequencing the DNA of this strain revealed much about its metabolic diversity and generated insights into other members of the genus (Schell et al., 2002). Bioinformatic analysis revealed several physiological traits that partially explain the successful adaptation of this organism to the colon. An unexpectedly large number of the predicted proteins appear to be specialized for the catabolism of a variety of oligosaccharides, some possibly released by rare or novel glycosyl hydrolases acting on “nondigestible” plant polymers or host-derived glycoproteins and glycoconjugates. The genome of *Bifidobacterium longum* DJO10A has also been completely sequenced (GenBank accession no. CP000605) (Lee et al., 2008).

In 1969, Mitsuoka described “*Bifidobacterium longum* subsp. *animalis*” with biovars a and b, which can be distinguished from *Bifidobacterium longum* subsp. *longum* on the basis of melezitose and D-mannose fermentation, DNA relatedness, and by their ecology; they have been subsequently recognized as belonging to *Bifidobacterium animalis* [biovar a, R101-8^{T} (=ATCC 25527^{T}), from rat feces (Scardovi and Trovatelli, 1974)] and *Bifidobacterium pseudolongum* subsp. *pseudolongum* [biovar b, C10-45 (=DSM 20097), from calf feces (Scardovi et al., 1971b)], respectively.

17b. **Bifidobacterium longum subsp. infantis** (Reuter 1963) Mattarelli, Bonaparte, Pot and Biavati 2008, 770[VP] (*Bifidobacterium infantis* Reuter 1963, 502)

in.fan′tis. L. gen. n. *infantis* of an infant.

The cellular morphology does not present any specific traits as it is similar to that of many other *Bifidobacterium* species (Figure 67, panel 17b). Pentoses are not fermented.

Source: isolated as predominant forms from breast-fed infant feces. The type strain was isolated from the feces of a human infant.

DNA G+C content (mol%): 60.5 (T_m).

Type strain: S12, ATCC 15697, DSM 20088, JCM 1222, NCIMB 702205.

Sequence accession no. (16S rRNA gene): X70974.

Additional comments: the genome of *Bifidobacterium longum* subsp. *infantis* ATCC 15697[T] (GenBank accession no. CP001095) has been completely sequenced (Sela et al., 2008); it is the biggest bifidobacterial genome sequenced so far. Reuter (1963) proposed the name *Bifidobacterium infantis* for biochemical and seroliquid distinct strains isolated from infant feces. Bifid strains from the same source but with the ability to ferment D-xylose were separated and classified into two additional species, namely *Bifidobacterium liberorum* and *Bifidobacterium lactentis*, based on differences in other fermented sugars (Reuter, 1963). DNA relatedness data subsequently showed that these species were identical to *Bifidobacterium infantis* (Scardovi et al., 1971b).

Bifidobacterium parabifidum (Weiss and Rettger) Kandler and Lauer (1974) is considered here to be a synonym of *Bifidobacterium infantis* because strain ATCC 17930 [Timberlain strain isolated by Norris et al. (1950) and studied by Pine and Howell (1956), under the label 308]: (a) shows 82% DNA relatedness to ATCC 27920, one of the reference strains of *Bifidobacterium infantis*; (b) displays 76% DNA relatedness to ATCC 15707[T], the type strain of *Bifidobacterium longum*; and (c) possesses the protein pattern of *Bifidobacterium infantis* (Biavati et al., 1982).

17c. **Bifidobacterium longum subsp. suis** (Matteuzzi, Crociani, Zani and Trovatelli 1971) Mattarelli, Bonaparte, Pot and Biavati 2008, 771[VP] (*Bifidobacterium suis* Matteuzzi, Crociani, Zani and Trovatelli 1971, 393)

su′is. L. gen. n. *suis* of a hog.

Cells grown in TPY agar stabs show a similar morphology to those of many other *Bifidobacterium* species (Figure 67, panel 17c). Anaerobic. CO_2 does not affect O_2 sensitivity or anaerobic growth. Riboflavin is the only required growth factor.

Cell-free extracts possess fructose-bisphosphate aldolase and hexose monophosphate dehydrogenases. Most strains show a constitutive urease activity, which is not influenced by urea or organic nitrogen sources. Half of the strains tested share the transaldolase-6PGD isozyme electrophoretic pattern 6-5 with some *Bifidobacterium longum* subsp. *longum* and *Bifidobacterium longum* subsp. *infantis* strains; others display a pattern (6-8) that is common in *Bifidobacterium longum* subsp. *longum* (Scardovi et al., 1979a). The PAGE protein pattern is quite distinct from that of other *Bifidobacterium* species (Biavati et al., 1982).

Source: only found in the feces of piglets. The type strain was isolated from feces of a piglet.

DNA G+C content (mol%): 62 (T_m).

Type strain: SU 859, ATCC 27533, DSM 20211, JCM 1269.

Sequence accession no. (16S rRNA gene): M58743.

Additional comments: Bifidobacterium longum subsp. *suis* can be readily distinguished from other bifid species commonly found in pig feces, namely *Bifidobacterium boum*, *Bifidobacterium choerinum*, *Bifidobacterium pseudolongum* subsp. *globosum*, *Bifidobacterium pseudolongum* subsp. *pseudolongum*, and *Bifidobacterium thermophilum* on the basis of carbohydrate fermentation. The latter three species do not ferment arabinose or xylose, whereas *Bifidobacterium longum* subsp. *suis* does; the former two species ferment starch, but *Bifidobacterium longum* subsp. *suis* does not (Table 22; Zani et al., 1974).

18. **Bifidobacterium magnum** Scardovi and Zani 1974, 31[AL]

mag′num. L. neut. adj. *magnum* large, great.

Cells grown in TPY agar are usually characteristically long and thick, with irregular contours, measuring 2 × 10–20 µm and occurring frequently in aggregates (Figure 67, panel 18). Anaerobic. CO_2 does not affect O_2 sensitivity or anaerobic growth. The only acidophilic *Bifidobacterium* species; its optimal pH for growth is 5.3–5.5; growth is retarded at pH 5.0 and 5.9 and it does not grow at pH 4.2 or 7.0 (after 2 d). Sparse growth in TPY medium without Tween 80, as this component is highly stimulatory (Scardovi and Zani, 1974).

Fructose-bisphosphate aldolase is present in cell-free extracts (4–5 mU/mg proteins). $NADP^+$-dependent G6PDH is measurable. Aldolase is spot-stainable on electrophoresis (Scardovi and Sgorbati, 1974). The isozyme pattern 5-7 (transaldolase and 6PGD types, respectively) is unique among bifidobacteria (Scardovi et al., 1979a). The PAGE protein pattern is clearly distinct from that of any other *Bifidobacterium* species (Biavati et al., 1982).

Source: strains, including the type strain, found in the feces of rabbits.

DNA G+C content (mol%): 60.0 ± 0.6 (T_m).

Type strain: RA3, ATCC 27540, DSM 20222, JCM 1218, LMG 11591.

Sequence accession no. (16S rRNA gene): M58740.

Additional comments: the recognition of *Bifidobacterium magnum* should not be based solely on the unusually large dimensions of its cells. Fermentation of lactose and starch are useful in differentiating *Bifidobacterium magnum* from the “animal” species *Bifidobacterium animalis*, *Bifidobacterium pseudolongum* subsp. *globosum*, *Bifidobacterium pseudolongum* subsp. *pseudolongum*, and *Bifidobacterium pullorum*, which also ferment pentoses. *Bifidobacterium magnum* ferments lactose, but not starch, whereas the other species ferment either both or neither of these sugars (*Bifidobacterium pullorum*). *Bifidobacterium magnum* can be distinguished from the human species *Bifidobacterium longum* subsp. *infantis* and *Bifidobacterium longum* subsp. *longum* only by virtue of having different 6PGD isozymes. The transaldolase isozyme of *Bifidobacterium magnum* is shared by *Bifidobacterium infantis* (number 5). Alternatively, PAGE analysis can be used to separate these taxa.

19. **Bifidobacterium merycicum** Biavati and Mattarelli 1991, 167[VP]

me.ry′ci.cum. Gr. n. *meryx* (implied by verb *merykazo*) rumen; N.L. neut. adj. *merycicum* pertaining to the rumen.

Irregularly shaped rods that are 2.0–5.0 µm long and are sometimes arranged in angled pairs (Figure 67, panel 19). Surface colonies on TPY agar are soft, smooth, convex, circular with entire margins, cream to white, and glistening. Carbon dioxide has no effect on oxygen sensitivity, but it enhances anaerobic growth. The optimal temperature for growth is 38–42°C; growth does not occur below 25°C or above 45°C. The optimal initial pH for growth is 6.5–6.9; growth is delayed at pH 6.3 and 7.2, and does not occur at pH 4.5 or 8.

L-Arabinose, dextrin, D-fructose (weak), D-galactose, D-glucose, D-lactose, maltose, melibiose, raffinose, D-ribose, starch, sucrose, and D-xylose are all fermented, but glycerin, gluconate, lactate, D-mannitol, D-mannose, melezitose, L-rhamnose, D-sorbitol, and trehalose are not. Salicin and inulin are either not fermented or are occasionally fermented slowly. Cellobiose fermentation is variable.

Source: strains, including type strain, isolated from bovine rumen.

DNA G+C content (mol%): 59 (T_m).

Type strain: Ru915B, ATCC 49391, DSM 6492, JCM 8219, LMG 11341.

Sequence accession no. (16S rRNA gene): D86192.

20. **Bifidobacterium minimum** Biavati, Scardovi and Moore 1982, 368[VP]

min′i.mum. L. neut. adj. sup. *minimum* the least.

Cells grown in TPY agar stabs are characteristically very small (0.3 × 1.3–1.5 µm) with tapered ends and sometimes irregularly branched (Figure 67, panel 20). This morphology resembles that of *Bifidobacterium asteroides*, but star-like aggregates, characteristic for that species, are absent. Anaerobic. CO_2 has no affect on O_2 sensitivity or anaerobic growth. Sugars fermented include D-fructose, D-glucose, maltose, starch, and sucrose.

Cells possess the least anodal form of transaldolase among bifidobacteria (pattern 10, i.e. migration 66, whereas the most anodal isozyme, pattern 1, in *Bifidobacterium cuniculi*, migrates 100) (Scardovi et al., 1979a). Does not contain aldolase or G6PDH. The interpeptide bridge of the cell-wall peptidoglycan is Lys–Ser, which is unique amongst bifidobacteria. The PAGE protein pattern is distinct.

Source: the type strain was isolated from wastewater (sewage). Some isolates identified as *Bifidobacterium minimum* have been found in the cecum of pigs (Simpson et al., 2003); this was the first time that this species had been found in a natural habitat. These isolates had a type F PFGE profile.

DNA G+C content (mol%): 61.5 (T_m).

Type strain: F392, ATCC 27538, DSM 20102, JCM 5821, LMG 11592.

Sequence accession no. (16S rRNA gene): M59741.

Additional comments: this taxon was described previously and referred to as the "*minimum*" DNA homology group. It consisted of two strains isolated from sewage (Scardovi and Trovatelli, 1974).

21. **Bifidobacterium mongoliense** Watanabe, Makino, Sasamoto, Kudo, Fujimoto and Demberel 2009, 1539[VP]

mon.go.li.en′se. N.L. neut. adj. *mongoliense* pertaining to Mongolia, from where the type strain was isolated.

Cells grown in modified GAM broth are rods of various shapes, 0.4–0.6 × 0.8–2 µm, with rounded or tapered ends, sometimes curved, swollen, and branched (Figure 67, panel 21). Pinpoint colonies (0.2–0.4 mm in diameter) are formed under aerobic condition after 2 d incubation at 30°C. After anaerobic growth at 30°C for 5–6 d, colonies on modified GAM agar are 2–3 mm in diameter, convex, white, opaque, smooth, and circular with entire edges. Facultatively anaerobic. Catalase- and oxidase-negative. The temperature range for growth is 15–35°C; growth does not occur at 10°C or 40°C. The optimal temperature for growth is 25–30°C. Grows between pH 4.5 and 8.0, with optimum growth at pH 6.5–7.0.

Acid is produced from L-arabinose, D-galactose, D-glucose, glycogen, maltose, D-lactose, melibiose, methyl α-D-glucopyranoside, raffinose, starch, sucrose, and turanose, but not from *N*-acetylglucosamine, D-adonitol, D-arabinose, D- or L-arabitol, dulcitol, erythritol, D-fructose, D- or L-fucose, glycerol, inositol, inulin, 2- or 5-keto-gluconate, D-lyxose, D-mannitol, D-mannose, melezitose, methyl α-D-mannopyranoside, methyl β-D-xylopyranoside, L-rhamnose, D-sorbitol, L-sorbose, D-tagatose, -trehalose, xylitol, or D- or L-xylose. Acid production from amygdalin, arbutin, cellobiose, β-gentiobiose, gluconate, D-ribose, and salicin is strain-dependent. Esculin is hydrolyzed.

Peptidoglycan structure is L-Lys–D-Asp type in the presence of Lys, Glu, Ala, and Asp.

Source: strains, including the type strain, were isolated from airag, a traditional fermented mare's milk, which was collected in Umnugobi and Uburhangai Provinces in Mongolia in 2004.

DNA G+C content (mol%): 61.1 (T_m).

Type strain: YIT 10443, JCM 15461, DSM 21395.

Sequence accession no. (16S rRNA gene): AB433856.

Additional comments: phylogenetic analysis of the 16S rRNA gene sequence places *Bifidobacterium mongoliense* in the *Bifidobacterium minimum* subgroup of bifidobacteria.

22. **Bifidobacterium pseudocatenulatum** Scardovi, Trovatelli, Biavati and Zani 1979b, 309[AL]

pseu.do.ca.te.nu.la′tum. Gr. adj. *pseudês* false; N.L. neut. adj. *catenulatum* specific epithet; N.L. neut. adj. *pseudocatenulatum* the false (*Bifidobacterium*) *catenulatum*.

Cell morphology is one of the most variable amongst the bifidobacteria and highly diverse traits reflect the origin of the strains (Figure 67, panel 22). Anaerobic. CO_2 does not affect O_2 sensitivity or anaerobic growth. Nicotinic acid, pantothenate, and riboflavin are required for growth. The structure of the interpeptide bridge of the cell-wall peptidoglycan is shared by *Bifidobacterium catenulatum* and *Bifidobacterium angulatum* (Table 21).

Source: found abundantly in sewage, in the feces of breast- and bottle-fed infants, and in those of suckling calves. The type strain was isolated from feces of a human infant.

DNA G+C content (mol%): 57.5 (T_m).

Type strain: B1279, ATCC 27919, DSM 20438, JCM 1200, LMG 10505.

Sequence accession no. (16S rRNA gene): M84785.

Additional comments: there is no doubt that *Bifidobacterium catenulatum* and *Bifidobacterium pseudocatenulatum* are closely related species as indicated by DNA hybridization data. However, the G+C content of their DNA differs by 3 mol% (Table 21). None of the strains shown genetically to be *Bifidobacterium catenulatum* ferment mannose or starch, whereas strains recognized by DNA hybridization as *Bifidobacterium pseudocatenulatum* ferment these carbohydrates. Unlike *Bifidobacterium pseudocatenulatum, Bifidobacterium catenulatum*

does not require nicotinic acid for growth. Furthermore, *Bifidobacterium catenulatum* has never been isolated from the feces of suckling calves.

When 41 strains of *Bifidobacterium catenulatum* and 120 strains of *Bifidobacterium pseudocatenulatum* were studied for their transaldolase and 6PGD isozyme content, the isozymes of the two species were found to migrate very differently (Table 21). The few *Bifidobacterium pseudocatenulatum* strains (7 out of 120) that possessed a transaldolase that was electrophoretically identical to that of *Bifidobacterium catenulatum* (number 5) displayed a much more anodal 6PGD isozyme (Table 21). Although some data suggest the existence of "intermediate" strains (Scardovi et al., 1979b), like those found between *Bifidobacterium longum* subsp. *longum* and *Bifidobacterium longum* subsp. *infantis* (see *Bifidobacterium longum*), and although PAGE analysis give uncertain results (Biavati et al., 1982), it is advisable at present to maintain the rank of species for these two taxa.

Sorbitol is fermented by all strains found in the feces of infants and by 50% of those present in calf feces. The sorbitol-fermenting strains can be distinguished from *Bifidobacterium adolescentis* strains as they are melezitose-negative and from *Bifidobacterium catenulatum* as they invariably ferment starch. The sorbitol-negative strains (so far found only in calf feces) can be distinguished from *Bifidobacterium pseudolongum* subsp. *globosum* and *Bifidobacterium pseudolongum* subsp. *pseudolongum* strains, which are also found in calf feces, and from *Bifidobacterium angulatum*, not only on the basis of a different morphology, but also by means of their transaldolase zymogram (Table 21).

The DNA of this species is 46–88% related to that of *Bifidobacterium catenulatum*, but is virtually unrelated to that of any other *Bifidobacterium* species (Table 21; Scardovi et al., 1979b).

23. **Bifidobacterium pseudolongum** Mitsuoka 1969, 60[AL]

pseu.do.long′um. Gr. adj. *pseudês* false; L. neut. adj. *longum* specific epithet; N.L. neut. adj. *pseudolongum* false (*Bifidobacterium*) *longum*.

Cells grown anaerobically in TPY agar are generally short, coccoid, or almost spherical to curved or tapered, arranged singly, doubly or, rarely, in short chains (Figure 67, panels 23a and 23b). This morphology does not change with strain or source. Only cells grown in air + CO_2 have bifurcations or short cross-branchings, often with enlarged ends (Scardovi et al., 1969). The interpeptide bridge of the cell-wall peptidoglycan is L-Orn(L-Lys)–L-Ala$_{2–3}$.

Because of the genetic and phenotypic similarity between *Bifidobacterium pseudolongum* (Mitsuoka, 1969) and *Bifidobacterium globosum* (Biavati et al., 1982), these two species have been rearranged taxonomically as subspecies: *Bifidobacterium pseudolongum* subsp. *pseudolongum* and *Bifidobacterium pseudolongum* subsp. *globosum* (Yaeshima et al., 1992b). The two subspecies share a similar ecological distribution, but *Bifidobacterium pseudolongum* subsp. *globosum* is found mainly in the rumen and intestine of ruminants, whereas no strain isolated from the rumen has been assigned to *Bifidobacterium pseudolongum* subsp. *pseudolongum*.

23a. **Bifidobacterium pseudolongum subsp. pseudolongum** (Mitsuoka 1969) Yaeshima, Fujisawa and Mitsuoka 1992a, 656[VP] (Effective publication: Yaeshima, Fujisawa and Mitsuoka 1992b, 385; *Bifidobacterium pseudolongum* Mitsuoka 1969, 60.)

pseu.do.long′um. Gr. adj. *pseudês* false; L. neut. adj. *longum* specific epithet; N.L. neut. adj. *pseudolongum* false (*Bifidobacterium*) *longum*.

Four biovars (a, b, c, and d) are recognized on the basis of differences in the fermentation of cellobiose, D-lactose, D-mannose, and melezitose. The effect of O_2 and CO_2 on growth has not been studied in detail. Requirements for growth factors are unknown. Growth in the presence of 4.5% sodium malate at 30°C is positive or weakly positive (Yaeshima et al., 1992b).

Dextrin, D-galactose, D-glucose, D-lactose, maltose, melibiose, methyl α-glucoside, raffinose, D-ribose, and sucrose are fermented, but not amygdalin, esculin, sodium gluconate, inositol, inulin, D-mannitol, D-mannose, melezitose, L-rhamnose, D-sorbitol, or trehalose. Cellobiose fermentation is variable.

Cells were not examined for the presence of fructose-bisphosphate aldolase or G6PDH. Using electrophoresis, the type strain PNC-2-9G^T (=ATCC 25526^T, biovar a), strain 29-Sr-T representing biovar e, and strain Mo-2-10 representing biovar d, all display transaldolase isozyme 2 and 6PGD isozyme 7, i.e. a pattern identical to that possessed only by two *Bifidobacterium pseudolongum* subsp. *globosum* strains of the 103 studied (Table 21; Scardovi et al., 1979a). PAGE protein patterns of some strains, including strain ATCC 25526^T, are clearly distinct from those of *Bifidobacterium pseudolongum* subsp. *globosum* (Biavati et al., 1982). Plasmids were not detected in the three strains studied (Sgorbati et al., 1982).

Source: isolated from the feces of bulls, calves, chickens, dogs, guinea pigs, hamsters, pigs, and rats. The type strain was isolated from pig feces.

DNA G+C content (mol%): 64.8 ± 0.7 (T_m).

Type strain: PNC-2-9G, ATCC 25526, DSM 20099, JCM 1205, LMG 11571, NCIMB 702244.

Sequence accession no. (16S rRNA gene): D86195.

Additional comments: DNA relatedness studies show that *Bifidobacterium pseudolongum* subsp. *pseudolongum* is 45–70% related to *Bifidobacterium pseudolongum* subsp. *globosum* and 10–15% related to *Bifidobacterium cuniculi* (Scardovi et al., 1971b; Yaeshima et al., 1992b). The Mitsuoka strain C10-45 ("*Bifidobacterium longum* subsp. *animalis*" biotype b; Mitsuoka, 1969) is completely homologous to strain Mo-2-10, a representative of biovar d of *Bifidobacterium pseudolongum* subsp. *pseudolongum* (Scardovi et al., 1971b).

Mitsuoka (1969) recognized that bifid strains isolated from a variety of animals belonged to this species. The strains fermented arabinose, glycogen, starch, and xylose and slowly fermented fructose, thereby differing from strains of *Bifidobacterium thermophilum* isolated from the same animal sources (Mitsuoka, 1969).

23b. **Bifidobacterium pseudolongum subsp. globosum** (Scardovi, Trovatelli, Crociani and Sgorbati 1969) Yaeshima, Fujisawa and Mitsuoka 1992a, 656[VP] (Effective publication: Yaeshima, Fujisawa and Mitsuoka 1992b, 385; *Bifidobacterium globosum*

Scardovi, Trovatelli, Crociani and Sgorbati 1969, 290; *Bifidobacterium globosum* Biavati, Scardovi and Moore 1982, 368.)

glo.bo′sum. L. neut. adj. *globosum* round as a ball, spherical, globular.

Anaerobic aerotolerant organism. CO_2 does not affect anaerobic growth, but permits growth under high O_2 tensions. Grows in 90% air + 10% CO_2. Aerobically grown cells do not show catalase or pseudocatalase activity (Scardovi et al., 1969).

Initially, the strains allotted to this species were isolated from the bovine rumen and did not ferment cellobiose, mannose, or xylose, and only rarely fermented arabinose (Scardovi et al., 1969). Pentose-fermenting strains subsequently isolated from piglets were recognized as *Bifidobacterium pseudolongum* subsp. *globosum* based on DNA relatedness data (Zani et al., 1974). Many other strains genetically assigned to *Bifidobacterium pseudolongum* subsp. *globosum* have been isolated from feces of various animals, including strains which ferment cellobiose, mannitol, or mannose, as well as some that slowly ferment or are inactive towards fructose (Scardovi et al., 1979a). Dextrin, galactose, glucose, glycogen, lactose, maltose, melibiose, raffinose, ribose, sucrose, and starch are all fermented, but not amygdalin, esculin, sodium gluconate, inositol, inulin, mannitol, mannose, melezitose, rhamnose, salicin, sorbitol, or trehalose. Fermentation of arabinose, fructose, and xylose give variable results (Scardovi et al., 1969). Methyl α-glucoside is either weakly or strongly fermented.

Folic acid, pantothenate, riboflavin, and thiamin are required for anaerobic growth. Ammonia satisfies nitrogen requirements. Growth in the presence of 4.5% sodium malate at 30°C is weakly positive or negative (Yaeshima et al., 1992b).

Unlike most species of the genus *Bifidobacterium*, the enzyme fructose-bisphosphate aldolase and hexose monophosphate dehydrogenases can be detected in cell-free extracts. Aldolase activity in intact cells was proven by the expected increase of acetate production from glucose degradation in the presence of iodoacetic acid (Scardovi and Trovatelli, 1969; Schramm et al., 1958). Glucose-6-phosphate dehydrogenase, undetectable in some strains, has been found to be either NAD^+ or $NADP^+$-dependent (Scardovi and Sgorbati, 1974).

Zymograms of 103 strains of *Bifidobacterium pseudolongum* subsp. *globosum* were shown to have transaldolase isozyme 2 and more than 80% of them contained 6PGD isozyme 6, four other 6PGD isozymes were found (Scardovi et al., 1979a; Table 21). However, Yaeshima et al. (1992b) found that the 6PGD mobilities of *Bifidobacterium pseudolongum* subsp. *globosum* strains RU 224^T, RU 230, and RU 256 were identical to those of *Bifidobacterium pseudolongum* subsp. *pseudolongum* PNC-2-9G^T.

PAGE protein patterns of selected strains isolated from various sources were identical and quite distinct from those of any other *Bifidobacterium* species (Biavati et al., 1982).

A study on the presence of bifidobacterial outer proteins (BIFOPs) in the cell-wall of 150 strains of *Bifidobacterium pseudolongum* subsp. *globosum* isolated from different animals was conducted by Mattarelli et al. (1993); 60% of the strains examined were apparently devoid of BIFOPs. BIFOP expression changes caused by growth temperature were observed in 70 strains of *Bifidobacterium pseudolongum* subsp. *globosum* isolated from different animals. In general, BIFOP expression at low temperature was considerably attenuated, whereas it increased at medium and high growth temperature (Mattarelli et al., 1999).

Large molecular mass plasmids (13.5, 24.5, and 46 MDa) were found in 22% of the *Bifidobacterium pseudolongum* subsp. *globosum* strains studied by Sgorbati et al. (1982).

Source: originally isolated from the bovine rumen (Scardovi et al., 1969) and later found in the feces of calves, chickens, lambs, piglets, rabbits, and rats, in a single specimen of feces from a human infant, and in sewage (Scardovi et al., 1979a). The type strain was isolated from a bovine rumen.

DNA G+C content (mol%): 64.1 ± 0.7 (T_m).

Type strain: RU 224, ATCC 25865, DSM 20092, JCM 5820, LMG 11569, NCIMB 702245.

Sequence accession nos (16S rRNA gene): M58736, D86194.

Additional comments: Rogosa (1974) considered this organism to be a synonym of *Bifidobacterium pseudolongum* Mitsuoka and, hence, the taxon was not included in the Approved Lists of Bacterial Names (Skerman et al., 1980). Its revival as *Bifidobacterium globosum* was proposed by Biavati et al. (1982); Yaeshima et al. (1992b) reclassified *Bifidobacterium globosum as Bifidobacterium pseudolongum* subsp. g*lobosum*.

The DNA–DNA similarities found between the two subspecies reported by Scardovi et al. (1971b), Lauer and Kandler (1983), and Yaeshima et al. (1992b) are 69–73%, 76%, and 45–69%, respectively. Two intermediate groups, which showed 54–72% DNA relatedness with the two type strains, were reported by Yaeshima et al. (1992b). There is disagreement on the DNA similarity values to *Bifidobacterium cuniculi*: these have been reported to be 50–67% by Scardovi et al. (1979b) and 12% by Yaeshima et al. (1992b).

Members of the two subspecies and the two intermediate groups were divided into distinct subclusters basing on ribotyping analysis (Sakata et al., 2006). In addition, Masco et al. (2003) showed that the two subspecies could be divided into different intermediate groups using DNA fingerprinting, whereas HSP60 DNA sequence similarity between the two subspecies was 96%, a value corresponding to the interspecies level (Jian et al., 2001). Yaeshima et al. (1992b) found that the G+C content of the two subspecies was higher than 63 mol%, whereas Scardovi et al. (1971b) reported different values, such as 60.3 mol% for *Bifidobacterium pseudolongum* subsp. *pseudolongum* and 63.8 mol% for *Bifidobacterium pseudolongum* subsp. *globosum*.

Differences in plasmid content and in PAGE total cellular protein patterns have been demonstrated between the two subspecies (Biavati et al., 1982; Sgorbati et al., 1982), whereas the mobility of 6PGD of *Bifidobacterium pseudolongum* subsp. *pseudolongum* PNC-2-96^T against *Bifidobacterium pseudolongum* subsp. *globosum* Ru 224^T, Ru 230, and Ru 256 was found to be identical by Yaeshima et al. (1992b), but different by Scardovi et al. (1979a). However, the variability of 6PGD mobility observed among other strains of the two subspecies examined by Yaeshima et al. (1992b) was reported previously by Scardovi et al. (1979a). Considering all of these contrasting pieces of evidence, further studies are needed to clarify the taxonomic position of the two subspecies.

24. **Bifidobacterium psychraerophilum** Simpson, Ross, Fitzgerald and Stanton 2004, 404[VP]

psych.rae.ro′phi.lum. Gr. n. *psychros* cold; Gr. n. *aer aeros* air; N.L. adj. *philus -a -um* (from Gr. adj. *philos -ê -on*) friend, loving; N.L. neut. adj. *psychraerophilum* cold- and air-loving.

Short and irregularly shaped rods, approximately 0.7–1.0 μm wide and 0.8–1.5 μm long with occasional bifurcations, are arranged singly or in pairs (Figure 67, panel 24). Colonies on MRS agar under anaerobic conditions are white, circular and convex with smooth edges and reach a diameter of up to 3 mm after incubation for 3 d at 37°C. Colonies of ~1 mm are formed after 3 d incubation under aerobic conditions. A high tolerance is shown to oxygen. The organism grows on agar media under aerobic conditions, a trait that may be related to its cecal habitat. The optimal temperature for growth is 37°C and the maximum growth temperature is 42°C; growth does not occur at 46°C. Growth is evident at 4°C though reduced. The lowest pH recorded for growth is 4.0.

The highest similarity values for the partial HSP60 gene sequences, 83–58%, are shared with *Bifidobacterium asteroides*, *Bifidobacterium indicum*, and *Bifidobacterium minimum* (Simpson et al., 2004).

Source: the type strain was isolated from a pig cecum (contents and epithelium) in Fermoy, Ireland. The type strain has a PFGE type F profile (Simpson et al., 2004).

DNA G+C content (mol%): 59.2 (T_m).

Type strain: T16, LMG 21775, NCIMB 13940.

Sequence accession no. (16S rRNA gene): not available.

Additional comments: previously called "*Bifidobacterium pyschroaerophilum*" (Simpson et al., 2003).

25. **Bifidobacterium pullorum** Trovatelli, Crociani, Pedinotti and Scardovi 1974, 197[AL]

pul.lo′rum. L. n. *pullus* a chicken; L. pl. gen. n. *pullorum* of chickens.

Cells grown in TPY agar stabs are slightly curved, 2–8 μm long with tapered ends, and are mostly arranged in irregular chains which are often very long (Figure 67, panel 25). Cells are frequently poorly refractile and appear to be empty or vacuolized. Branching is rare. Anaerobic. CO_2 does not affect O_2 sensitivity or anaerobic growth. Lactic and acetic acids are produced in a ratio of 1:3.5 ± 0.2 in TPY medium but, unlike all other *Bifidobacterium* species, the isomeric type of lactic acid formed is DL. Requires *p*-aminobenzoic acid, folic acid, nicotinic acid, pyridoxine, thiamin, and Tween 80 for satisfactory growth.

Fructose-bisphosphate aldolase is present in cell-free extracts in considerable amounts (20–30 mU/mg protein). As with most *Bifidobacterium dentium* strains, neither 6PGD ($NADP^+$-or NAD^+-dependent) nor G6PDH can be detected.

Source: the type strain was isolated from chicken feces.

DNA G+C content (mol%): 67.4 ± 0.4 (T_m), the highest value found so far in the bifidobacteria.

Type strain: P145, ATCC 27685, DSM 20433, JCM 1214, LMG 21816.

Sequence accession no. (16S rRNA gene): D86196.

Additional comments: morphology is helpful in the recognition of this species. The taxon can be easily distinguished from the "animal" species of the genus, which also ferment arabinose, ribose, and xylose, because neither lactose nor starch is fermented (see Additional comments under *Bifidobacterium magnum*). It can be distinguished from other *Bifidobacterium* species by its fermentation characteristics (Table 22).

DNA relationships with *Bifidobacterium gallinarum* and *Bifidobacterium saeculare* are higher than with any other species of the genus *Bifidobacterium* (Biavati et al., 1991).

26. **Bifidobacterium ruminantium** Biavati and Mattarelli 1991, 165[VP]

ru.mi.nan′ti.um. L. part. adj. *ruminans, -antis* ruminating; N.L. gen. pl. n. *ruminantium* of ruminants.

Irregular rods (3.0–6.0 μm in length), rarely have terminal bifurcations, but often occur in pairs which form rare angles (Figure 67, panel 26). Surface colonies on TPY agar are soft, smooth, convex, circular with entire margins, cream to white, and glistening. Carbon dioxide has no effect on oxygen sensitivity, but it enhances anaerobic growth. The optimum temperature for growth is 38–42°C; growth does not occur below 25°C or above 45°C. The optimum initial pH for growth is 6.5–6.9; growth is delayed at pH 6.3 and 7.2, and does not occur at pH 4.5 or 8.

Dextrin, D-fructose, D-galactose, D-glucose, D-lactose, maltose, D-mannitol, melibiose, raffinose, D-ribose, sucrose, and starch are all fermented, but not L-arabinose, cellobiose, gluconate, glycerin, inulin, lactate, D-mannose, melezitose, L-rhamnose, D-sorbitol, trehalose, or D-xylose. Salicin fermentation, if it occurs, is slow.

Source: isolated from the bovine rumen, the source of the type strain.

DNA G+C content (mol%): 57 (T_m).

Type strain: Ru687, ATCC 49390, DSM 6489, JCM 8222, LMG 21811.

Sequence accession no. (16S rRNA gene): D86197.

Additional comments: Bifidobacterium ruminantium is more closely related to *Bifidobacterium adolescentis* than to any other *Bifidobacterium* species.

27. **Bifidobacterium saeculare** Biavati, Mattarelli and Crociani 1992b, 191[VP] (Effective publication: Biavati, Mattarelli and Crociani 1991, 391.)

sae.cu.la′re. L. neut. adj. *saeculare* of or belonging to a saeculum, centenary, commemorating the ninth centenary of the foundation of Bologna University.

Short, slightly curved rods, 2.0–4.0 μm long, occur in pairs. Longer, thicker, or spindle-shaped cells are common in some successive transfers. Branching cells are numerous at a low incubation temperature in liquid cultures (Figure 67, panel 27). Surface colonies on TPY agar are convex with entire edges, cream to white, glistening, and soft. Carbon dioxide does not affect oxygen sensitivity, but does enhance anaerobic growth. The optimal temperature for growth is 36–38°C; growth does not occur below 28°C or above 47°C. The initial optimal pH is 6.8–7.3; growth is delayed at pH 4.6 and 7.6, and does not occur at 5.5 or 8.9.

D-Glucosamine hydrochloride and xylan are fermented, but not alginate, amylopectin, amylose, arabinogalactan, bovine submaxillary mucin, chondroitin sulfate, α-D-fucose,

α-L-fucose, α-D-galacturonate, D-glucuronate, heparin, hyaluronate, laminarin, pectin, polygalacturonate, ovomucoid, or porcine gastric mucin. Penicillin-binding protein analysis has shown one detectable band with a mass of 52 kDa (Biavati et al., 1991).

Source: isolated from rabbit feces, the source of the type strain.

DNA G+C content (mol%): 63 (T_m).

Type strain: RA161, ATCC 49392, DSM 6531, JCM 8223, LMG 14934.

Sequence accession no. (16S rRNA gene): D89328.

Additional comments: the DNA relationships with *Bifidobacterium gallinarum* and *Bifidobacterium pullorum* are higher than with any other species of the genus *Bifidobacterium* (Biavati et al., 1991).

28. **Bifidobacterium scardovii** Hoyles, Inganäs, Falsen, Drancourt, Weiss, McCartney and Collins 2002, 998[VP]

scar.do′vi.i. N.L. gen. n. *scardovii* of Scardovi, named after Vittorio Scardovi, in recognition of his contribution to our knowledge of the bifidobacteria.

Rod-shaped cells, some of which are curved (Figure 67, panel 28). Colonies are 2–3 mm in diameter, convex, shiny, with a dense white center. Facultatively anaerobic and catalase-negative. Weak hemolysis is observed on Columbia blood agar.

Using API kits, acid is produced from L-arabinose, D-lactose, maltose, D-mannose, melibiose, raffinose, D-ribose, sucrose, and trehalose, but not from D-arabitol, cyclodextrin, glycogen, D-mannitol, methyl β-D-glucopyranoside, pullulan, D-sorbitol, or tagatose; acid may or may not be produced from melezitose.

Hippurate is not hydrolyzed. Positive for alanine phenylalanine proline arylamidase, arginine arylamidase, α-arabinosidase, α-fucosidase, α-glucosidase, β-glucosidase, α-galactosidase, β-galactosidase, glycine arylamidase, histidine arylamidase, leucine arylamidase, phenylalanine arylamidase, pyroglutamic acid arylamidase, serine arylamidase, and tyrosine arylamidase, but negative for alkaline phosphatase, arginine dihydrolase, β-galactosidase-6-phosphate, glutamic acid decarboxylase, glutamyl glutamic acid arylamidase, glycyl tryptophan arylamidase, proline arylamidase, and leucyl glycine arylamidase; a variable response is shown for alanine arylamidase, β-glucuronidase, *N*-acetyl-β-glucosaminidase, β-mannosidase, and urease.

Cell-wall murein is of the A3α type, L-Lys–L-Ser–L-Ala_2, a murein type that is unique amongst members of the genus *Bifidobacterium.*

Protein profiles clearly show that isolates of this species are not only separate from other closely related bifidobacteria, but represent a phenotypically homogeneous cluster displaying a very high intra-group similarity.

Source: isolated from human clinical sources. Habitat is not known. The type strain was isolated from human blood and another four strains were from human urine (three strains) and from the human hip (one strain).

DNA G+C content (mol%): 60±1 (HPLC).

Type strain: ATCC BAA-773, DSM 13734, CCUG 13008, JCM 12489, LMG 21589.

Sequence accession no. (16S rRNA gene): AJ307005.

29. **Bifidobacterium subtile** Biavati, Scardovi and Moore 1982, 369[VP]

sub′ti.le. L. neut. adj. *subtile* slender.

Cells grown in TPY agar stabs are slender, 0.5 × 2–3 µm, with rounded or tapered ends, and are sometimes curved. Branching is rare. Morphology (Figure 67, panel 29) is similar to that of *Bifidobacterium breve*, but cells of the latter are usually shorter and thicker, swollen and branched. Anaerobic. CO_2 does not affect O_2 sensitivity or anaerobic growth.

Optimal temperature for growth is 34–35.5°C, which is markedly lower than the range shown by other *Bifidobacterium* species (37–41°C). The sugar fermentation pattern is similar to that of *Bifidobacterium breve* but, unlike the latter, D-lactose is not fermented (Table 22). In addition, *Bifidobacterium subtile* ferments gluconate and starch, whereas *Bifidobacterium breve* does not.

Possesses high levels (10–15 mU/mg proteins in strain F395) of $NADP^+$/NAD^+-dependent G6PDH, but aldolase is not measurable. Transaldolase and 6PGD isozymes are among the most anodal amongst bifidobacteria (numbers 3 and 2, respectively); the 3-2 pattern occurs only in this species (Scardovi et al., 1979a). There are distinct PAGE protein patterns. Both human and animal types of F6PPK have been detected in cell-free extracts (Scardovi and Trovatelli, 1974).

Source: originally isolated in 1982 from specimens of wastewater and isolated very recently from caries lesions (Mantzourani et al., 2009); this was the first time that this species was found in a natural habitat. The type strain was isolated from wastewater.

DNA G+C content (mol%): 61.5 (T_m).

Type strain: F395, ATCC 27537, DSM 20096, LMG 11597.

Sequence accession no. (16S rRNA gene): D89378.

Additional comments: this taxon was previously described and referred to as the "*subtile*" DNA homology group and includes five strains isolated from sewage (Scardovi and Trovatelli, 1974).

30. **Bifidobacterium thermacidophilum** Dong, Xin, Jian, Liu and Ling 2000, 124[VP]

therm.ac.id.o′phil.um. Gr. n. *thermê* heat; N.L. n. *acidum* (from L. adj. *acidus* sour) acid; N.L. adj. *philus -a -um* (from Gr. adj. *philos -ê -on*) friend, loving; N.L. neut. adj. *thermacidophilum* heat/acid-loving.

Irregular rods, arranged singly or occasionally as "V" shapes (Figure 67, panels 30a and 30b); rods are 0.5 × 3–8 µm in size after 12–24 h in TPYG medium at 37°C. The fermentation products from glucose are acetic and lactic acids, in a molar ratio of 2.46–4.9:1. The optimal temperature for growth is 37–41°C and the temperature range for growth is 30–49.5°C. The optimal initial pH is 7.0–7.2; growth at pH 4.5 is quite good, but is delayed at pH 4.0.

D-Fructose, D-galactose, D-glucose, glycogen, maltose, melibiose, sucrose, raffinose, starch, and turanose are all fermented, but not mannitol, L-rhamnose, or sorbose. Fermentation of L-arabinose, gluconate, inulin, D-lactose, D-mannose, methyl α-D-glucoside, D-ribose, salicin, trehalose, and D-xylose is variable. Litmus milk is neither acidified nor coagulated by most strains.

Source: the type strain was isolated from an anaerobic digester treating wastewater from a bean-curd farm.

DNA G+C content (mol%): 56.9–61.0 (T_m).

Type strain: 36, AS 1.2282, DSM 15837, JCM 11165, LMG 21395.

Sequence accession no. (16S rRNA gene): AB016246.

Additional comments: deduced HSP60 protein sequences show 100% similarity among the thermophilic group that comprises *Bifidobacterium boum*, *Bifidobacterium thermacidophilum*, and *Bifidobacterium thermophilum*, although interspecies DNA homologies were 92.9–95.5%. Three signature amino acid residues at positions 156, 158, and 179 (Lys, Arg, and Ser) differentiate members of this group. The shared primary structures of HSP60 might relate to their thermophilic characteristics. High DNA reassociation values of 58.9% (Dong et al., 2000) and 82.2% (von Ah et al., 2007) are shown with *Bifidobacterium thermacidophilum* (see also *Bifidobacterium thermophilum*).

30a. **Bifidobacterium thermacidophilum subsp. thermacidophilum** (Dong, Xin, Jian, Liu and Ling 2000) Zhu, Li and Dong 2003, 1622[VP] (*Bifidobacterium thermacidophilum* Dong, Xin, Jian, Liu and Ling 2000, 124)

therm.ac.id.o′phil.um. Gr. n. *thermê* heat; N.L. n. *acidum* (from L. adj. *acidus* sour) acid; N.L. adj. *philus -a -um* (from Gr. adj. *philos -ê -on*) friend, loving; N.L. neut. adj. *thermacidophilum* heat/acid-loving.

All strains meet the species description. Colonies on TPY-glucose agar are circular, convex with entire edges, white, and reach 1.5–2 mm in diameter after 24 h cultivation. Anaerobic growth occurs in standing culture under 90% air plus 10% CO_2. Fermentation products from glucose are acetic and lactic acids at a molar ratio of 4.9:1. Liquid cultures of some strains can form a homogeneous sediment, which is readily dispersed by shaking. Maximum temperature for growth is 49.5°C. L-Arabinose, inulin, and D-mannose are fermented, but not trehalose. All strains grow well in media containing 2.0% (w/v) NaCl, but not 2.5% (w/v) NaCl.

Source: the type strain was isolated from the wastewater of a bean-curd farm in Beijing.

DNA G+C content (mol%): 56.85 ± 2.05 (T_m).

Type strain: 36, AS 1.2282, DSM 15837, JCM 11165, LMG 21395.

Sequence accession no. (16S rRNA gene): AB016246.

Additional comments: the DNA relationships with *Bifidobacterium thermophilum* are higher than with any other *Bifidobacterium* species.

30b. **Bifidobacterium thermacidophilum subsp. porcinum** Zhu, Li and Dong 2003, 1622[VP]

por.ci′num. L. neut. adj. *porcinum* of a hog.

All strains meet the species description. The fermentation products from glucose are acetic and lactic acids at a molar ratio of 2.46–2.72:1. Maximum temperature for growth is 46.5°C. Trehalose is fermented, but not L-arabinose, inulin, or D-mannose.

Source: isolated from piglet feces.

DNA G+C content (mol%): 61.02 ± 0.7 (T_m).

Type strain: P3-14, AS 1.3009, LMG 21689.

Sequence accession no. (16S rRNA gene): AY148470.

31. **Bifidobacterium thermophilum** Mitsuoka 1969, 59[AL]

ther.mo′phil.um. Gr. n. *thermê* heat; N.L. adj. *philus -a -um* (from Gr. adj. *philos -ê -on*) friend, loving; N.L. neut. adj. *thermophilum* heat-loving.

Cells grown in TPY agar stabs are long, slender, curved, and are arranged singly or in pairs, but never in clumps (Figure 67, panel 31). This morphology is shared by many other species of the genus *Bifidobacterium*.

Four biovars (a, b, c, and d) were distinguished by Mitsuoka (1969) based on differences in the fermentation of lactose and melezitose. Similar differences were found amongst strains isolated from bovine rumen, calf feces, and sewage (Scardovi et al., 1979b), and from piglet feces (Zani et al., 1974).

Bifidobacterium thermophilum, like *Bifidobacterium pseudolongum* subsp. *globosum*, can grow in 90% air plus 10% CO_2 without the cells becoming catalase- or pseudocatalase (hemin)-positive.

A few strains genetically assigned to *Bifidobacterium thermophilum* that ferment arabinose and xylose have been found in sewage and in piglets (Scardovi et al., 1979b).

Factors required for growth are pantothenate, pyridoxine, and riboflavin. Bases are not required and ammonia is the optimal nitrogen source.

Fructose-bisphosphate aldolase and hexose monophosphate dehydrogenases are always present in cell-free extracts (Scardovi et al., 1969); aldolase was spot-stained after electrophoresis (Scardovi and Sgorbati, 1974).

Source: isolated from bovine rumen, calf feces, sewage, and piglet feces. von Ah et al. (2007) reported the isolation of *Bifidobacterium thermophilum* from baby feces. The type strain was isolated from pig feces.

DNA G+C content (mol%): 60.0 (T_m).

Type strain: P2-91, ATCC 25525, DSM 20210, JCM 1207, LMG 11573, NCIMB 702253.

Sequence accession no. (16S rRNA gene): U10151.

Additional comments: Mitsuoka (1969) proposed the specific epithet *thermophilus* for strains of bifids which he isolated from the feces of chickens and swine, owing to their ability to grow at 46.5°C and their resistance to heating at 60°C for 30 min; Scardovi et al. (1969) named strains that fermented neither pentoses nor lactose, which he isolated from bovine rumens, as "*Bifidobacterium ruminale*". These strains did not ferment pentoses. Subsequent comparative data have shown that these species are identical (Biavati et al., 1982; Scardovi et al., 1971b, 1979a). See *Bifidobacterium choerinum* for details of how this organism and *Bifidobacterium boum* and *Bifidobacterium thermophilum* can be distinguished.

DNA relatedness values of 27–80% are shown to *Bifidobacterium boum. Bifidobacterium thermophilum* shows high DNA reassociation values of 58.9% (Dong et al., 2000) and 82.2% (von Ah et al., 2007) with *Bifidobacterium thermacidophilum*. The close DNA–DNA relationship between these two species reported by von Ah et al. (2007) questions the current classification of *Bifidobacterium thermacidophilum* as a discrete species. *Bifidobacterium thermophilum* and *Bifidobacterium thermacidophilum* share similar thermophilic characteristics.

The original spelling of the species was *Bifidobacterium thermophilium*, which was corrected by Hill et al. (1984) to *Bifidobacterium thermophilum*.

32. **Bifidobacterium tsurumiense** Okamoto, Benno, Leung and Maeda 2008, 146[VP]

tsu.ru.mi.en′se. N.L. neut. adj. *tsurumiense* pertaining to Tsurumi University, Yokohama, Japan.

Cells are short rods or coccoid-like when grown on *Brucella* HK blood agar under anaerobic conditions for 24 h (Figure 67, panel 32). Colonies on this medium are white, rough, and slightly convex. Growth occurs at 25 and 45°C; the optimal temperature for growth is 37°C. Facultatively anaerobic. F6PPK-positive; catalase- and oxidase-negative. End products of glucose fermentation are acetic and lactic acids at a molar ratio of 3:2.

Amygdalin, L-arabinose, arbutin, cellobiose, D-fructose, D-galactose, gentiobiose, gluconate, D-glucose, glycogen, 2-ketogluconate, 5-ketogluconate, D-lactose, maltose, D-mannitol, D-mannose, melibiose, methyl α-D-glucopyranoside, raffinose, D-ribose, salicin, starch, sucrose, trehalose, turanose, and D-xylose are fermented, but not *N*-acetylglucosamine, D-adonitol, D-arabinose, D-arabitol, L-arabitol, dulcitol, erythritol, D-fucose, L-fucose, glycerol, inositol, inulin, D-lyxose, melezitose, methyl α-D-mannopyranoside, methyl β-D-xylopyranoside, L-rhamnose, D-sorbitol, L-sorbose, D-tagatose, xylitol, or L-xylose. The cell-wall amino acids consist of Glu–Lys–Asp–$(Ala)_2$.

Source: isolated from dental plaque from hamsters fed with a high-carbohydrate diet.

DNA G+C content (mol%): 53.0 (T_m).

Type strain: OMB115, DSM 17777, JCM 13495

Sequence accession no. (16S rRNA gene): AB241106.

Genus II. **Aeriscardovia** Simpson, Ross, Fitzgerald and Stanton 2004, 405[VP]

Paola Mattarelli and Bruno Biavati

Aer.i.scar.do′vi.a. L. masc. n. *aer, aeris* air; N.L. fem. n. *Scardovia* a bacterial generic name to honor Vittorio Scardovi, an Italian microbiologist; N.L. fem. n. *Aeriscardovia* cells similar to those of the genus *Scardovia* that can grow in air.

Gram-stain-positive, catalase- and oxidase-negative, nonmotile, non-spore-forming actinomycete that forms short and irregularly shaped rods. Optimal growth occurs under anaerobic conditions, with aerobic growth yielding elongated cells. Isolated from a porcine cecum. 16S rRNA and HSP60 gene sequence comparisons indicate that *Aeriscardovia* belongs to the family *Bifidobacteriaceae*. Only the type species, *Aeriscardovia aeriphila*, has been described.

DNA G+C content (mol%): 54.7 (HPLC).

Type species: **Aeriscardovia aeriphila** Simpson, Ross, Fitzgerald and Stanton 2004, 405[VP].

Further descriptive information

Phylogeny. In a study of 160 bifidobacterial strains isolated from either the lumen or the epithelium of a porcine cecum, two groups were highlighted, one containing 83 isolates (group I) and the other containing 77 isolates (group II) (Simpson et al., 2003). Group 1 showed a PFGE F profile and group II showed PFGE profiles B, E, and Ea. Based primarily on 16S rRNA gene sequencing, groups I and II were considered to represent novel species, namely "*Bifidobacterium pyschroaerophilum*" and "*Bifidobacterium aerophilum*", respectively. DNA G+C content and partial heat-shock protein 60 (HSP60) gene sequence analysis confirmed the proposal for "*Bifidobacterium pyschroaerophilum*", corrected to *Bifidobacterium pyschraerophilum* (Simpson et al., 2004). The DNA G+C content of "*Bifidobacterium aerophilum*" was relatively low and consistent with its assignment to a subcluster comprising *Alloscardovia*, *Metascardovia*, *Parascardovia*, and *Scardovia* of the 16S rRNA gene sequence-based phylogenetic tree (Figure 66). However, partial HSP60 gene sequencing showed that "*Bifidobacterium aerophilum*" could be distinguished from members of the genera *Bifidobacterium*, *Parascardovia*, and *Scardovia*, sharing similarity values with these taxa of 73.8%, 72.0%, and 71.4%, respectively; the former value is comparable to those reported for *Parascardovia denticolens* and *Scardovia inopinata* (Jian et al., 2001). Consequently, "*Bifidobacterium aerophilum*" was reclassified as *Aeriscardovia aeriphila* gen. nov., sp. nov. (Simpson et al., 2004).

Anaerobiosis. *Aeriscardovia* strains show a high tolerance to oxygen and grow on agar media under aerobic conditions, a trait that may relate to their cecal habitat. Under aerobic growth conditions, the short-rod morphology of *Aeriscardovia aeriphila* is lengthened considerably, possibly due to incomplete cell division.

Maintenance procedures

Aeriscardovia aeriphila can be maintained at −135°C or as freeze-dried cultures by using standard procedures described for the genus *Bifidobacterium*.

List of species of the genus *Aeriscardovia*

1. **Aeriscardovia aeriphila** Simpson, Ross, Fitzgerald and Stanton 2004, 405[VP]

ae.ri′phi.la. L. masc. n. *aer, aeris* air; N.L. adj. *philus -a -um* (from Gr. adj. *philos -ê -on*) friend, loving; N.L. fem. adj. *aeriphila* air-loving.

Short, irregularly shaped rods, approximately 0.6–0.9 µm wide and 0.8–1.5 µm long, are arranged singly or in pairs, but not in chains (Figure 70). Colonies on modified de Man–Rogosa–Sharpe (mMRS) medium supplemented with 0.05% (w/v) cysteine/HCl under anaerobic conditions are gray–white, circular, and flat to convex with entire edges and reach a diameter of up to 3 mm after 3 d incubation at 37°C. Colonies are formed under aerobic conditions, but a diameter of only ~1 mm is attained after 5 d incubation. Under aerobic conditions, cell morphology includes elongated cells that are approximately 4–6 mm in length.

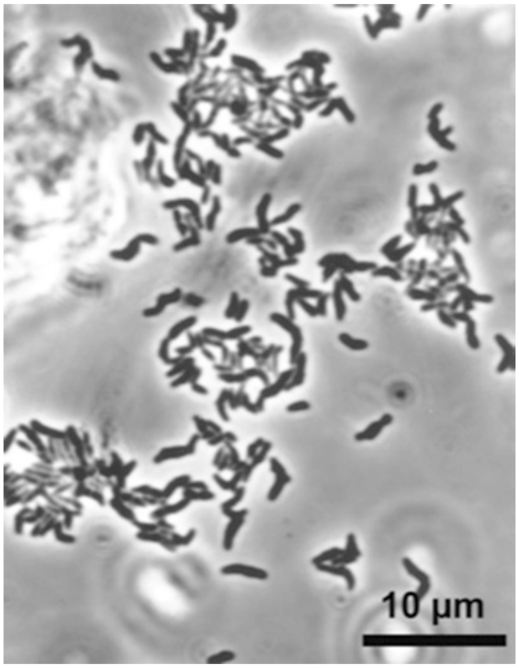

FIGURE 70. Cellular morphology of *Aeriscardovia aeriphila* T6^T grown in liquid TPY medium. Phase-contrast photomicrograph (magnification 1500×).

A high tolerance to oxygen is shown. This species grows under aerobic conditions on agar media, as does *Alloscardovia omnicolens*, *Bifidobacterium psychraerophilum*, *Bifidobacterium scardovii*, *Bifidobacterium tsurumiense*, *Gardnerella vaginalis*, and *Metascardovia criceti*. The optimal growth temperature is 37°C, with a maximum of 46°C and a minimum of 30°C; growth does not occur at 25 or 48°C. In mMRS medium, the lowest pH value recorded for growth is 4.2 and the minimum initial pH for growth is 4.5.

Acid is formed from arabinose, maltose, mannose, raffinose, salicin, sucrose, and xylose, but not from cellobiose, lactose, mannitol, melezitose, sorbitol, or trehalose. L-Arabinose, D-glucose, maltose, D-mannose, raffinose, and salicin are fermented, but not cellobiose, glycerol, D-lactose, D-mannitol, L-rhamnose, D-sorbitol, or trehalose. Fermentation of melezitose, sucrose, and D-xylose is variable.

Positive for α-arabinosidase, arginine arylamidase, glycine arylamidase, α- and β-galactosidases, α- and β-glucosidases, histidine arylamidase, leucine arylamidase, phenylalanine arylamidase, proline arylamidase, tyrosine arylamidase, and serine arylamidase; negative for *N*-acetyl-β-glucosaminidase, alanine arylamidase, alkaline phosphatase, β-galactosidase-6-phosphate, β-glucuronidase, glutamic acid decarboxylase, glutamyl glutamic acid arylamidase, leucyl glycine arylamidase, pyroglutamic acid arylamidase, and urease. Does not liquefy gelatin, produce indole, or reduce nitrate to nitrite.

A single plasmid (30 kbp) may be present in strain T8 and other isolates with the same PFGE type Ea based on macrorestriction pattern data.

Source: isolated from a pig cecum (contents and epithelium) in Fermoy, Ireland.

DNA G+C content (mol%): 54.7 (HPLC).

Type strain: T6, LMG 21773, NCIMB 13939.

Sequence accession no. (16S rRNA gene): AY174107.

Additional comments: from PFGE analysis, the 77 pig cecal isolates belonging to *Aeriscardovia* appeared to represent three strains, termed PFGE types B, E, and Ea. Previously known as "*Bifidobacterium aerophilum*".

Genus III. **Alloscardovia** Huys, Vancanneyt, D'Haene, Falsen, Wauters and Vandamme 2007, 1445^{VP}

Paola Mattarelli and Bruno Biavati

Al.lo.scar.do′vi.a. Gr. adj. *allos* different; N.L. fem. n. *Scardovia* a bacterial generic name; N.L. fem. n. *Alloscardovia* an organism related to, but different from, *Scardovia* and related genera.

Gram-stain-positive, catalase- and oxidase-negative, nonmotile, non-spore-forming actinomycete that forms short irregularly shaped rods. **Optimal growth occurs under anaerobic conditions on modified Columbia agar (MCA) or modified de Man–Rogosa–Sharpe medium (mMRS) after 24 h at 37°C, but slow aerobic growth (72 h), producing pinpoint-sized colonies, is also observed on MCA**. Isolated from various human clinical samples, but data are not currently available on potential pathogenic relevance or virulence factors. On the basis of 16S rRNA and HSP60 gene sequence analyses, *Alloscardovia* represents a new genus of the family *Bifidobacteriaceae*. Only one species has been described, *Alloscardovia omnicolens*.

DNA G+C content (mol%): 47.8 ± 0.5 (T_m).

Type species: **Alloscardovia omnicolens** Huys, Vancanneyt, D'Haene, Falsen, Wauters and Vandamme 2007, 1445^{VP}.

Further descriptive information

16S rRNA gene sequence data show that *Alloscardovia omnicolens* is most closely related to *Scardovia inopinata* (92.9–93.1%) and *Parascardovia denticolens* (93.0–93.2%). Its sequence similarities to *Aeriscardovia aeriphila*, *Gardnerella vaginalis*, and *Bifidobacterium* species range from 89.8 to 91.8%. Partial *hsp60* gene sequence data clearly separate *Alloscardovia* from its closest phylogenetic neighbors, namely *Parascardovia denticolens* (74.6–77.3% sequence similarity), *Scardovia inopinata* (75.0–77.7%), and *Gardnerella vaginalis* (80.3–81.5%). 16S rRNA and *hsp60* gene sequence analyses indicate that *Alloscardovia* forms a distinct branch within the family *Bifidobacteriaceae* and is sufficiently divergent from the other members of this family to be proposed as a new genus (Huys et al., 2007). A 16S rRNA phylogenetic tree of the family *Bifidobacteriaceae* is shown in Figure 66.

The DNA G+C contents of *Alloscardovia omnicolens* strains (47.3–48.3 mol%) are consistent with their phylogenetic positioning in the family *Bifidobacteriaceae* with *Gardnerella vaginalis* (41.8–43.0 mol%), *Metascardovia criceti* (53 mol%), *Parascardovia denticolens* (55 mol%), and *Scardovia inopinata* (45 mol%) as the closest relatives.

Maintenance procedures

Alloscardovia omnicolens can be maintained at −135°C or as freeze-dried cultures by using standard procedures described for the genus *Bifidobacterium*.

List of species of the genus *Alloscardovia*

1. **Alloscardovia omnicolens** Huys, Vancanneyt, D'Haene, Falsen, Wauters and Vandamme 2007, 1445^VP^

om.ni.co′lens. L. adj. *omnis* every; L. v. *colere* to dwell; L. part. adj. *colens* dwelling; N.L. part. adj. *omnicolens* dwelling everywhere in the human body.

Short irregularly shaped rods arranged singly or in pairs (Figure 71). Optimal growth occurs under anaerobic conditions on modified Columbia agar (MCA) and on modified MRS (mMRS) after 24 h at 37°C, but slow aerobic growth (72 h), resulting in pinpoint-sized colonies, is observed on MCA. Anaerobic growth on MCA occurs at 28°C (after 72 h), 37–40°C (after 24 h), and 45°C (after 96 h), but not at 50°C (after 96 h).

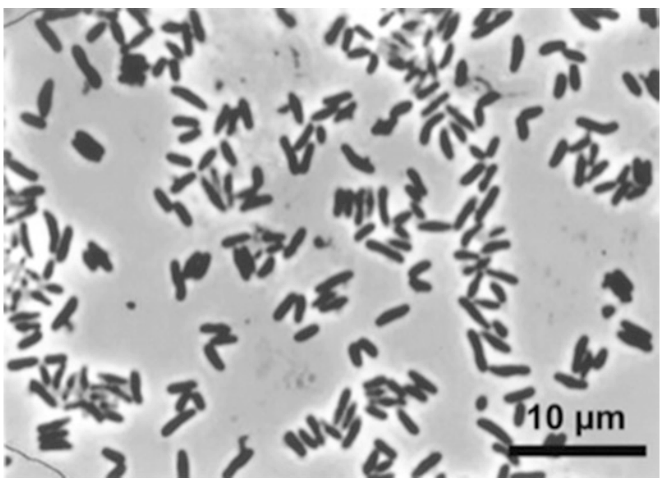

FIGURE 71. Cellular morphology of *Alloscardovia omnicolens* CCUG 31649^T^ grown in liquid TPY medium. Phase-contrast photomicrograph (magnification 1500×).

L-Arabinose, D-glucose, maltose, raffinose, salicin, sucrose, and D-xylose are fermented, but not glycerol (except for strain CCUG 31736), mannitol (except for strains CCUG 27412 and CCUG 31736), rhamnose (except for strain CCUG 31736), or sorbitol (except for strains CCUG 27412 and CCUG 31736).

Produces α-arabinosidase (except for strain CCUG 44766), arginine arylamidase, glycine arylamidase, histidine arylamidase, leucine arylamidase, phenylalanine arylamidase, proline arylamidase, serine arylamidase, tyrosine arylamidase, α-galactosidase, β-galactosidase, α-glucosidase, and β-glucosidase, but not alanine arylamidase, glutamyl glutamic acid arylamidase, leucyl glycine arylamidase, pyroglutamic acid arylamidase, glutamic acid decarboxylase, arginine dihydrolase, α-fucosidase, β-galactosidase 6-phosphate, *N*-acetyl-β-glucosaminidase, β-glucuronidase, alkaline phosphatase, or urease.

Esculin is hydrolyzed. Nitrate is not reduced to nitrite. Gelatin is not liquefied nor is indole produced. Little, if any, β-hemolysis is observed on blood-supplemented MCA.

Source: isolated from various human clinical samples, including blood and urine and from the urethra, oral cavity, tonsils, abscesses of lungs, and aortic valves. The type strain was isolated from the tonsils of a 25-year-old woman from Kristianstad, Sweden, in 1993.

DNA G+C content (mol%): 47.8 ± 0.5 (T_m).

Type strain: CCUG 31649, LMG 23792.

Sequence accession no. (16S rRNA gene): AM419460.

Additional comments: strains CCUG 7132, CCUG 50589, and CCUG 26938 have been deposited in the BCCM/LMG Bacteria Collection as LMG 23793–23795, respectively.

Genus IV. **Gardnerella** Greenwood and Pickett 1980, 176^VP^

VIRGINIE STORMS AND PETER VANDAMME

Gard.ner.el′la. N.L. fem. dim. n. *Gardnerella* named after H.L. Gardner.

Pleomorphic rods ~0.5 μm in diameter and 1.5–2.5 μm in length. Filaments do not occur. Neither capsules nor endospores are formed. **Gram-stain-negative to Gram-stain-variable. Nonmotile.** Facultatively anaerobic. Fastidious in growth requirements. **Catalase- and oxidase-negative.** Chemo-organotrophic, with a fermentative type of metabolism. **Acid, but no gas, is produced** from a variety of carbohydrates, including **maltose and starch.** Acetic acid is the major product of fermentation. **Hippurate is hydrolyzed. Human blood, but not sheep blood, is hemolyzed.** Found in the human genital and urinary tracts.

DNA G+C content (mol%): 42–44 (Bd).

Type species: **Gardnerella vaginalis** (Gardner and Dukes 1955) Greenwood and Pickett 1980, 176^VP^ (*Haemophilus vaginalis* Gardner and Dukes 1955, 963).

Further descriptive information

Cell morphology. Cells appear as small pleomorphic bacilli and coccobacilli which may stain Gram-stain-negative or Gram-stain-variable (Zinnemann and Turner, 1963). They do not give an acid-fast reaction. Sudanophilic inclusions and metachromatic granules occur in the cells.

Cell-wall composition. The cell walls contain the amino acids *N*-acetylglucosamine, alanine, aspartic acid, glutamic acid, glycine, histidine, lysine, methionine, proline, serine, threonine, and tryptophan, but diaminopimelic and teichoic acids have not been detected (Criswell et al., 1971). The presence of alanine, glycine, glutamic acid, and lysine was confirmed by O'Donnell et al. (1984). Cellular fatty acid profiles consist of straight-chain saturated and unsaturated non-hydroxylated fatty acids with hexadecanoic acid (16:0) and octadecenoic acid (18:1) as major components (Greenwood and Pickett, 1980; Moss and Dunkelberg, 1969; O'Donnell et al., 1984; Van Esbroeck et al., 1996). Other cellular fatty acid components include tetradecanoic acid (14:0), hexadecanoic acid (16:1 ω7*c*), octadecadenoic acid (18:2 ω6,9*c*), and octadecanoic acid (18:0).

Carbohydrate analysis has indicated 6-deoxytalose and no arabinose in the cell walls of the type strain (Vickerstaff and Cole, 1969). *Gardnerella vaginalis* strains contain very characteristic polar lipid patterns consisting of diphosphatidylglycerol, phosphatidylglycerol, phosphatidylinositol, five partially identified glycolipids, and an uncharacterized phospholipid (O'Donnell et al., 1984).

Fine structure. Electron microscopy of cell walls has presented conflicting results. Reyn et al. (1966) reported a microscopic morphology, particularly of the cell walls and septa, closely resembling that of a Gram-stain-positive organism. On the other hand, Criswell et al. (1971, 1972) reported that the fine structure of walls was more typical of a Gram-stain-negative organism. A re-examination of the cell-wall ultrastructure of the *Gardnerella vaginalis* type strain and of clinical isolates demonstrated that the cell walls of these bacteria have a Gram-stain-positive type of organization and that the unusual thinness of the cell walls accounts for the Gram-stain-negative staining tendency observed regularly (Sadhu et al., 1989).

Nutrition and growth conditions. Although most strains of *Gardnerella* are facultatively anaerobic, obligately anaerobic strains have been described.

Slight, if any, growth occurs on nutrient agar (Piot et al., 1980). Growth does not occur on most common selective media including tellurite (0.01%) agar, sodium chloride (3%) agar, bile (1%) agar, Rogosa agar, or Thayer–Martin agar. On Vaginalis agar* colonies are pinpoint after incubation for 24 h and are 0.4–0.5 mm in diameter after 48 h. The colonies are round, opaque, and smooth. They become larger than 0.5 mm after incubation beyond 48 h, but their viability decreases rapidly.

*Vaginalis agar consists of Columbia agar base (BBL) containing 1% proteose peptone no. 3 (Difco). After the medium has been autoclaved and cooled to 45–50°C, human blood (5%, v/v) preserved with citrate, phosphate, and glucose is added aseptically to the medium.

Colonial and cultural characteristics. Colonies are nonhemolytic on sheep blood agar, but the majority of strains exhibit diffuse β-hemolysis on human or rabbit blood. Little or no hemolysis is observed on horse blood.

The optimum growth temperature is 35–37°C. Growth may also occur at 25 and 42°C. The optimum pH range is 6.0–6.5. Slight growth occurs at pH 4.5, but not at pH 4.0 (Greenwood and Pickett, 1979). Obligately anaerobic strains have been described (Malone et al., 1975).

Gardnerella strains are fastidious in their nutritional requirements, but do not need nicotinamide adenine dinucleotide (V factor), hemin (X factor), or coenzyme-like substances (Dunkelberg and McVeigh, 1969; Edmunds, 1962). They have been reported to require biotin, folic acid, niacin, thiamine, riboflavin, and two or more purines/pyrimidines (Dunkelberg and McVeigh, 1969). Growth is improved with fermentable carbohydrates and certain peptones.

Metabolism and metabolic pathways. The major product of sugar fermentation is acetic acid (Moss and Dunkelberg, 1969), but some strains also produce one or more of lactic, formic, or succinic acids (Malone et al., 1975). Gas is not formed from sugar fermentation. Acid is produced from dextrin, glucose, maltose, ribose, and starch, but not from D-arabitol, arbutin, cellobiose, glycerol, methyl β-D-glucopyranoside, inositol, lactose, mannitol, melezitose, melibiose, raffinose, rhamnose, salicin, sucrose, tagatose, trehalose, or xylose. Production of acid from L-arabinose, fructose, galactose, inulin, and mannose is strain dependent.

Starch is hydrolyzed, but not esculin, gelatin, tributyrin, or urea. Voges–Proskauer test is negative; methyl red test is positive. Does not produce indole, H_2S, or acetoin, show phenylalanine deaminase activity, or degrade Tween 80. Does not produce nitrite from nitrate, or have arginine and ornithine decarboxylase or lysine dihydrolase activities. Gluconate is oxidized to 2-ketogluconate. Hydrolysis of *O*-nitrophenyl-β-D-galactopyranoside and casein, and lipase activity are strain dependent.

Positive for β-D-galactosidase, *N*-acetyl-α-D-glucosaminidase, α-D-glucosidase, α-maltosidase, F6PPK, and pyrazinamidase activities and also for the following arylamidases: L-alanine, alanyl-phenylalanyl-proline, β-alanine, L-arginine, *S*-benzyl-cysteine, α-L-glutamate, α-L-glutamyl-L-histidine, glycine, glycyl-L-arginine, glycyl-proline, L-histidine, L-histidyl-L-leucyl-L-histidine, L-histidyl-L-serine, L-hydroxyproline, L-leucyl-L-alanine, leucyl-glycine, L-lysine, L-lysyl-L-lysine, L-lysyl-L-serine-4-methoxy, methionine, L-ornithine, L-phenylalanine, L-phenylalanyl-L-arginine, L-phenylalanyl-L-prolyl-L-alanine, L-proline, L-prolyl-L-arginine, L-pyrrolidone, L-serine, L-seryl-tyrosine, L-threonine, and L-tyrosine.

Negative for α-L-arabinosidase, esterase (C_6, C_{16}, and C_{18}), α-L-fucosidase, β-D-fucosidase, β-L-fucosidase, α-D-galactosidase, phospho-β-D-galactosidase, β-D-galacturonohydrolase, *N*-acetyl-β-D-glucosaminidase, β-D-glucosidase, β-D-glucuronidase, γ-glutamyltransferase, β-D-lactosidase, β-maltosidase, β-D-mannosidase, α-D-xylosidase, and β-D-xylosidase activities and for the following arylamidases: *N*-acetyl-glycyl-L-lysine, α-aspartyl-L-arginine, *N*-benzoyl-L-alanine-4-methoxy, *N*-carboxybenzoxy-glycyl-L-arginine, *N*-carboxybenzoxy-glycyl-glycyl-arginine, α-L-glutamyl-α-L-glutamine, glycyl-tryptophan, and pyroglutamic acid.

Antibiotic or drug sensitivity. All strains are susceptible to ampicillin, carbenicillin, oxacillin, penicillin, and vancomycin, and all are uniformly resistant to colistin, nalidixic acid, neomycin, and sulfadiazine at the usual therapeutic levels. Strains differ in susceptibility to kanamycin, tetracycline, gentamicin, and tobramycin (Greenwood and Pickett, 1980; McCarthy et al., 1979). Metronidazole is effective *in vivo*, but gives variable results in *in vitro* susceptibility tests (Balsdon et al., 1980).

Pathogenicity. *Gardnerella vaginalis* has been considered to be the cause of bacterial nonspecific vaginitis or bacterial vaginosis. It has also occasionally been reported to cause bacteremia in postpartum women and in patients following septic abortion and transurethral resection of the prostate. *Gardnerella vaginalis* has been isolated from reproductive tracts of mares (Salmon et al., 1991). Nowadays, bacterial vaginosis is known to be associated with a diverse spectrum of mostly anaerobic bacteria, including species of the genera *Anaerococcus*, *Atopobium*, *Bacteroides*, *Finegoldia*, *Gemella*, *Mobiluncus*, and *Prevotella* (Bradshaw et al., 2006; Fredricks et al., 2005). In addition, recent studies (Fredricks et al., 2007) demonstrated that *Gardnerella vaginalis* is an unreliable indicator of bacterial vaginosis, as demonstrated by the high rate of detection in subjects without bacterial vaginosis.

Ecology. *Gardnerella vaginalis* has been isolated from the anorectal flora of healthy adults of both sexes as well as from that of children. It is part of the endogenous flora in women of reproductive age. The organism can be recovered from the urethras of male partners of women with bacterial vaginosis and, more rarely, from blood samples. It can also cause infections in newborns and appears to have worldwide distribution.

Isolation and detection procedures

Most commercial blood culture broths used for the isolation of fastidious bacteria contain sodium polyetholsulfonate as an anticoagulant which inhibits the growth of *Gardnerella*. Isolation of *Gardnerella vaginalis* from genital samples can be accomplished by adding gelatin to such media or by using selective media, namely human blood bilayer Tween agar or Vaginalis agar. One quadrant is inoculated with the swab and the plate is streaked for isolation of colonies. Inoculated plates are incubated for 48 h at 35°C in either a candle extinction jar lined with water-saturated absorbent paper or in a CO_2 incubator. *Gardnerella vaginalis* forms opaque, domed, entire colonies, ~0.5 mm in diameter, that are surrounded by a small zone of diffuse β-hemolysis. *Gardnerella vaginalis* colonies appear as pinpoint and nonhemolytic on 5% sheep blood agar. *Gardnerella* cells appear as small pleomorphic Gram-stain-negative or Gram-stain-variable coccobacilli and short rods upon Gram stain.

Another isolation medium is peptone-starch-glucose medium* (Dunkelberg et al., 1970). This medium is inoculated and incubated as outlined above for Vaginalis agar. On this medium, colonies of *Gardnerella vaginalis* are 0.5–2.0 mm in diameter, dull white, convex, domed, somewhat conical in shape, and entire.

Detection of *Gardnerella vaginalis* in vaginal specimens by means of a DNA-probe-based system, Affirm VPIII (Becton Dickinson), has been reported to be useful as a surrogate for wet mount cell examination (Briselden and Hillier, 1994). Several PCR tests that allow a more sensitive detection and identification of *Gardnerella vaginalis* have been described, but the specificity of these assays is not always optimal (Fredricks et al., 2007; Nath et al., 2000; Obata-Yasuoka et al., 2002; van Belkum et al., 1995; Zariffard et al., 2002).

Maintenance procedures

Working stock cultures should be transferred every 48 h to ensure viability. This requirement for frequent transfer appears to be independent of the type of maintenance medium or temperature of storage. Stock strains may be preserved indefinitely by lyophilization in either rabbit serum or 10% skim milk.

Differentiation of the genus *Gardnerella* from other genera

Several commercial systems for the identification of fastidious Gram-stain-negative bacteria can be used adequately to identify *Gardnerella vaginalis*. However, typical appearance on Gram stain, β-hemolytic behavior on human blood bilayer Tween agar or Vaginalis agar, the lack of hemolysis on 5% sheep blood agar, the absence of catalase and oxidase activities, and the presence of hippurate hydrolysis allow presumptive identification.

Gardnerella vaginalis-like organisms recovered from patients with bacterial vaginosis have been demonstrated to represent *Actinomyces turicensis* strains (Van Esbroeck et al., 1996; Vandamme et al., 1998). *Gardnerella vaginalis* can be differentiated from these organisms as it has acetic acid as the main end product of glucose fermentation and is unable to produce acid from xylose, whereas *Actinomyces turicensis* strains have succinic acid as the end product and produce acid from xylose.

Taxonomic comments

Before the study of bacterial phylogeny through sequence analysis of conserved chronometers like rRNA genes, the generic assignment of this organism was the subject of debate. It has been classified as a member of the genera *Corynebacterium* and *Haemophilus* and its assignment to many other Gram-stain-positive and Gram-stain-negative genera has been discussed (Greenwood and Pickett, 1980; Lapage, 1974; Piot et al., 1980). Nevertheless, its unique cell-wall structure and biochemical characteristics led Greenwood and Pickett (1980) and Piot et al. (1980) to conclude that this organism was best classified in a distinct genus, *Gardnerella*. Analysis of the 16S rRNA gene sequence of the *Gardnerella vaginalis* type strain (Van Esbroeck et al., 1996) confirmed its unique phylogenetic position among the *Actinobacteria* with members of the genus *Bifidobacterium* as its closest phylogenetic neighbors (Figure 72). A phylogenetic analysis based on *hsp60* gene sequences revealed the same taxonomic neighborhood (Huys et al., 2007).

*Peptone-starch-glucose agar contains (g/l): proteose peptone no. 3 (Difco), 20.0; soluble starch, 10.0; glucose, 2.0; Na_2HPO_4, 1.0; $NaH_2PO_4 \cdot H_2O$, 1.0; agar, 15.0. A more recent formulation of this medium omits the phosphate buffer.

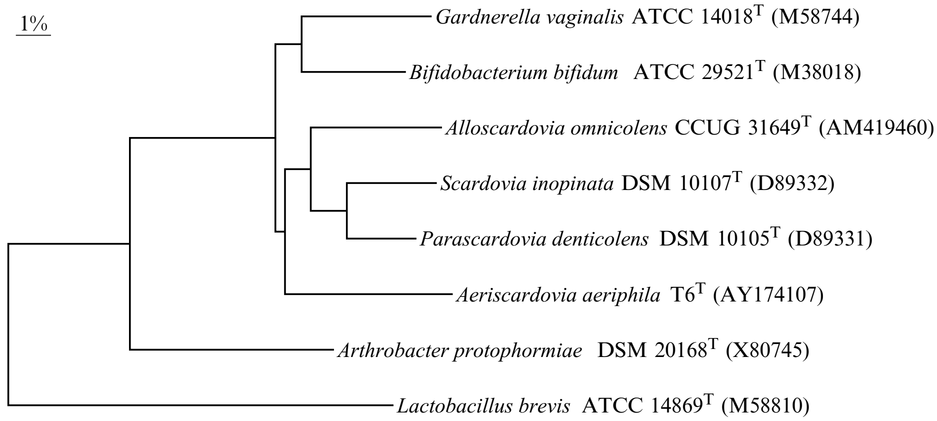

FIGURE 72. Neighbor-joining phylogenetic tree derived from analysis of nearly complete 16S rRNA gene sequences of the type strains of *Gardnerella vaginalis*, the type species of its closest phylogenetic neighbors, and of *Arthrobacter protophormiae* and *Lactobacillus brevis* as outgroups. Bar = 1% difference in 16S rRNA nucleotide sequences.

Further reading

Catlin, B.W. 1992. *Gardnerella vaginalis*: characteristics, clinical considerations, and controversies. Clin. Microbiol. Rev. *5*: 213–237.

Dunkelberg, W.E. 1977. *Corynebacterium vaginale*. Sex. Transm. Dis. *4*: 69–75.

Gardner, H.L. 1980. *Haemophilus vaginalis* vaginitis after twenty-five years. Am. J. Obstet. Gynecol. *137*: 385–391.

List of species of the genus *Gardnerella*

1. **Gardnerella vaginalis** (Gardner and Dukes 1955) Greenwood and Pickett 1980, 176[VP] (*Haemophilus vaginalis* Gardner and Dukes 1955, 963)

va.gi.na′lis. L. n. *vagina* sheath, vagina; L. fem. suff. *-alis* suffix denoting pertaining to; N.L. fem. adj. *vaginalis* pertaining to vagina, of vagina.

The morphological, cultural, physiological, and nutritional characteristics are as described for the genus.

Source: isolated from the human genital and urinary tracts.

DNA G+C content (mol%): 42–44 (Bd).

Type strain: ATCC 14018 (strain 594 of Gardner and Dukes, 1955).

Sequence accession no. (16S rRNA gene): M58744.

Genus V. **Metascardovia** Okamoto, Benno, Leung and Maeda 2007b, 2449[VP] (Effective publication: Okamoto, Benno, Leung and Maeda 2007a, 752.)

Paola Mattarelli and Bruno Biavati

Me.ta.scar.do′vi.a. Gr. adv. *meta* besides; N.L. fem. n. *Scardovia* a bacterial genus name; N.L. fem. n. *Metascardovia* a genus besides *Scardovia*.

Gram-stain-positive, facultatively anaerobic actinomycete that produces short and irregular shaped rods. F6PPK-positive; catalase- and oxidase-negative. Growth occurs at 25°C and 45°C (optimal at 37°C). Able to grow on cadmium fluoride acriflavin tellurite (CFAT) and *Lactobacillus* selection (LBS) agars (Okamoto et al., 2007a). Fermentation products from glucose are acetic and L-(+)-lactic acids in the molar ratio 3:2. 16S rRNA and HSP60 gene sequence data place *Metascardovia* in the family *Bifidobacteriaceae*. One species, *Metascardovia criceti*, has been described.

DNA G+C content (mol%): 53 (T_m).

Type species: **Metascardovia criceti** Okamoto, Benno, Leung and Maeda 2007b, 2449[VP] (Effective publication: Okamoto, Benno, Leung and Maeda 2007a, 752.).

Further descriptive information

Phylogeny. A phylogenetic tree based on 36 partial 16S rRNA gene sequences, including those from members of the genus *Bifidobacterium* and related genera, and rooted using *Escherichia coli* K-12, showed that *Metascardovia* was sharply separated from a cluster of *Bifidobacterium* species and was most closely related to

Aeriscardovia aeriphila, *Parascardovia denticolens*, and S*cardovia inopinata* (Okamoto et al., 2007a) (Figure 66). The 16S rRNA gene sequence similarity values of *Metascardovia criceti* OMB105^T with the type strains of *Parascardovia denticolens*, *Scardovia inopinata*, and *Aeriscardovia aeriphila* were 91.7%, 91.1%, and 90.0%, respectively. A phylogenetic tree based on 35 HSP60 gene sequences, including those from members of the genus *Bifidobacterium* and related genera, using 528 bp (corresponding to positions within the 304–831 nt region of *Escherichia coli* K-12 HSP60 gene) showed that *Metascardovia criceti* was well separated from a cluster of *Bifidobacterium* species, and was most closely related to *Gardnerella vaginalis* and *Scardovia inopinata*. It shared partial HSP60 gene similarities with *Parascardovia denticolens*, *Scardovia inopinata*, and *Gardnerella vaginalis* of 76.2%, 75.6%, and 74.8%, respectively.

Anaerobiosis. *Metascardovia* strains grow under aerobic conditions, as do other members of the family *Bifidobacteriaceae*, such as *Aeriscardovia aeriphila*, *Bifidobacterium psychraerophilum*, *Bifidobacterium scardovii*, and *Gardnerella vaginalis* (Hoyles et al., 2002; Simpson et al., 2004; Van Esbroeck et al., 1996). It would be of interest to understand the mechanism by which some *Bifidobacteriaceae* species are able to grow under aerobic conditions.

Maintenance procedures

Metascardovia criceti can be maintained at −135°C or as freeze-dried cultures by using standard procedures described for the genus *Bifidobacterium*.

List of species of the genus *Metascardovia*

1. **Metascardovia criceti** Okamoto, Benno, Leung and Maeda 2007b, 2449^{VP} (Effective publication: Okamoto, Benno, Leung and Maeda 2007a, 752.)

cri.ce′ti. N.L. gen. n. *criceti* of hamster.

Brownish-gray, rough, slightly convex colonies are formed on *Brucella* HK blood agar. When grown on media under anaerobic conditions for 24 h, irregular rods, with occasional bifurcated extremities and tapered ends, are formed (Figure 73). Longer incubation under similar conditions leads to changes in the Gram reaction and the formation of shorter rods.

Amygdalin, L-arabinose, arbutin, cellobiose, fructose, galactose, gentiobiose, 2-ketogluconate, glucose, maltose, mannose, melezitose, melibiose, raffinose, ribose, salicin, sucrose, trehalose, turanose, D-xylose, and lactose are fermented, but not adonitol, D-arabinose, D-arabitol, L-arabitol, dulcitol, erythritol, D-fucose, L-fucose, gluconate, 5-ketogluconate, methyl α-D-glucopyranoside, *N*-acetylglucosamine, glycerol, glycogen, inositol, inulin, D-lyxose, mannitol, methyl α-D-mannopyranoside, rhamnose sorbitol, sorbose, starch, D-tagatose, xylitol, methyl β-D-xylopyranoside, or L-xylose.

α-Arabinosidase, alanine arylamidase, arginine arylamidase, glycine arylamidase, histidine arylamidase, leucine arylamidase, phenylalanine arylamidase, proline arylamidase, serine arylamidase, tyrosine arylamidase, esterase, α-galactosidase, lipase, α-galactosidase, β-galactosidase, α-glucosidase, β-glucosidase, acid phosphatase, and α-phosphoamidase are formed, but not cysteine arylamidase, glutamyl glutamic acid arylamidase, leucyl glycine arylamidase, pyroglutamine arylamidase, valine arylamidase, arginine dihydrolase, catalase, glutamic acid decarboxylase, α-fucosidase, β-galactosidase-6-phosphate, *N*-acetyl-β-glucosaminidase, β-glucuronidase, lipase, α-mannosidase, alkaline phosphatase, trypsin, chymotrypsin, or urease.

Metascardovia criceti, like *Bifidobacterium dentium*, grows on selective media such as LBS (Becton Dickinson) and CFAT agars, unlike *Parascardovia denticolens*, *Scardovia inopinata*, and other members of the family *Bifidobacteriaceae* (Okamoto et al., 2007a). Does not hydrolyze esculin or gelatin, or produce indole. Nitrate is not produced.

The cell-wall type is Glu–Lys–Ser–(Ala)$_2$.

Source: isolated from dental plaque of hamsters fed with a high-carbohydrate diet.

DNA G+C content (mol%): 53 (T_m).

Type strain: OMB105, JCM 13493, DSM 17774.

Sequence accession no. (16S rRNA gene): AB241105.

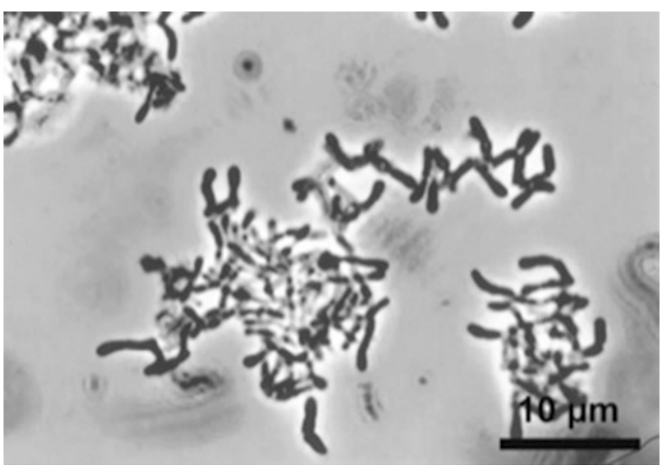

FIGURE 73. Cellular morphology of *Metascardovia criceti* OMB 105^T grown in liquid TPY medium. Phase-contrast photomicrograph (magnification 1500×).

Genus VI. **Parascardovia** Jian and Dong 2002, 811[VP]

Paola Mattarelli and Bruno Biavati

Pa.ra.scar.do′vi.a. Gr. prep. *para* beside, alongside of, near, like; N.L. fem. n. *Scardovia* a bacterial generic name; N.L. fem. n. *Parascardovia* resembling *Scardovia*.

Gram-stain-positive, non-acid-fast. Nonsporeforming. Nonmotile, small, slender rods of variable shape. **Anaerobic**. **Saccharoclastic**. **Glucose is degraded exclusively and characteristically by the fructose 6-phosphate** (F6PPK; EC 4.1.2.22) **shunt** (see treatment of the genus *Bifidobacterium*, above). Fermentation products from glucose are acetic acid and L-(+)-lactic acid in the molar ratio of 2:1. **Dextran is fermented by most strains.** Found in human dental caries and plaque. 16S rRNA and HSP60 gene sequence analyses have shown that the genus *Parascardovia* belongs to the family *Bifidobacteriaceae*. The type and only species is *Parascardovia denticolens*.

DNA G+C content (mol%): 55 ± 1 (T_m).

Type species: **Parascardovia denticolens** (Crociani, Biavati, Alessandrini, Chiarini and Scardovi 1996) Jian and Dong 2002, 811[VP] (*Bifidobacterium denticolens* Crociani, Biavati, Alessandrini, Chiarini and Scardovi 1996, 570).

Further descriptive information

Phylogeny. See treatment of *Scardovia*, below.

Nutrition. None of the *Parascardovia denticolens* strains studied could grow in a synthetic medium even when all of the amino acids and vitamins were added (Modesto et al., 2003). They are able to grow well when the synthetic medium contains, in addition to the amino acids and vitamins, pantethine (0.15 g/l), pancreatin (0.15 g/l), and lysozyme (0.4 g/l, inactivated by boiling for 10 min), but not when these compounds are added one at a time. For additional information, see treatment of *Scardovia inopinata*.

Ecology. *Bifidobacteriaceae* strains are inhabitants of the oral cavity, but can reach the stomach, although normally they do not survive there due to the acidic conditions. However, under some circumstances, such as stomach achlorydria induced by pharmacological treatments or disease, microbial populations may develop. *Bifidobacterium dentium*, *Parascardovia denticolens*, and *Scardovia inopinata* have been detected in the human achlorydric stomach (unpublished data).

Plasmids. *Parascardovia denticolens* strains do not contain plasmid DNA or produce any band in addition to chromosomal DNA bands (Crociani et al., 1996).

Enrichment and isolation procedures

The procedures for enrichment and isolation of *Parascardovia denticolens* are the same as those described for the genus *Scardovia*.

Maintenance procedures

Parascardovia denticolens can be maintained at −135°C or as freeze-dried cultures by using the standard procedures described for the genus *Bifidobacterium*.

List of species of the genus *Parascardovia*

1. **Parascardovia denticolens** (Crociani, Biavati, Alessandrini, Chiarini and Scardovi 1996) Jian and Dong 2002, 811[VP] (*Bifidobacterium denticolens* Crociani, Biavati, Alessandrini, Chiarini and Scardovi 1996, 570)

den.ti.co′lens. L. n. *dens*, *dentis* tooth; L. v. *colere* to dwell; L. part. adj. *colens* dwelling; N.L. part. adj. *denticolens* tooth-dwelling.

Small, slender rods (0.8–1.5 μm long), occasionally arranged in V-shapes. Morphology is variable depending on the culture conditions (Figure 74). In the presence of lactose, cells of arabinose-negative strains are very long (4–5 μm), sometimes branched, and have wide ends, whereas those of arabinose-positive strains are smaller and coccoid (0.4–0.6 μm long). At an initial pH of 7.7 or 8.0, cells are very small (0.4–0.6 μm long), but at an initial pH of 4.9 or 5.5 they are longer and branched or short (0.8–2.5 μm long) and frequently club-shaped. In liquid cultures incubated in an air/CO_2 mixture (1:10) at 37°C, much longer rods with swollen ends are formed. However, cells become very long (3–10 μm long) and branched when cultures are incubated

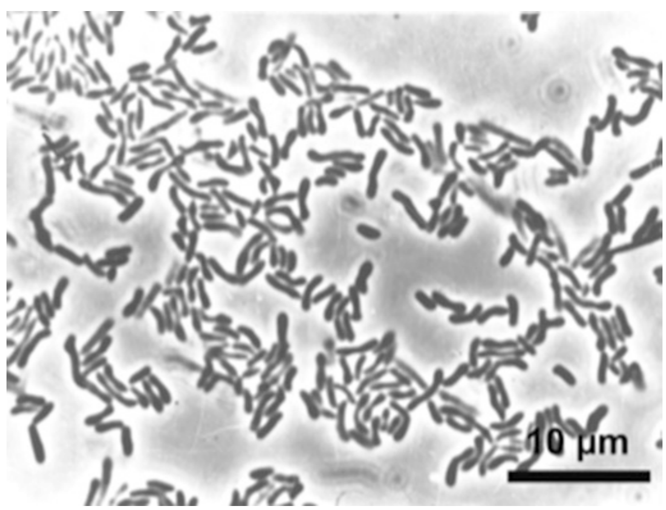

FIGURE 74. Cellular morphology of *Parascardovia denticolens* B3028[T] grown in liquid TPY medium. Phase-contrast photomicrograph (magnification 1500×).

at 30°C, but they are unbranched and flexible with protuberances if grown at 42°C. Liquid cultures of this species always have clear supernatants with compact sediment in clumps that cannot be dispersed with vigorous swirling. Colonies on TPY agar are soft, smooth, circular, convex with irregular margins, glistening, and cream to white. Anaerobic. CO_2 enhances anaerobic growth.

Fermentation products from glucose are L-(+)-lactic acid and acetic acid in the molar ratio of 1:2. The optimal temperature for growth is 36.5–38.5°C; the minimum temperature is 27.0–30.5°C and the maximum temperature is 42–44°C. Growth does not occur at 25 or 46°C, or at pH 4.4 or 8.5.

Amylose, amylopectin, cellobiose, dextrin, fructose, galactose, glucose, inulin, lactose, maltose, melibiose, raffinose, D-ribose, salicin, starch, and sucrose are fermented, but not alginate, arabinogalactan, bovine submaxillary mucin, chondroitin sulfate, D-fucose, L-fucose, D-galactosamine, α-D-galacturonate, D-glucitol, gluconate, D-glucuronate, glycerol, gum arabic, ghatti gum, guar gum, locust bean gum, karaya gum, tragacanth gum, heparin, hyaluronate, lactate, laminarin, D-mannitol, mannose, melezitose, ovomucoid, pectin, polygalacturonate, porcine gastric mucin, L-rhamnose, xylan, or D-xylose. L-Arabinose, D-glucosamine, and trehalose fermentation is variable. Dextran is fermented by 75% of strains.

Acetylmethylcarbinol is produced and asparagine is hydrolyzed. Negative for nitrate reduction, and indole, hydrogen sulfide, catalase, and pseudocatalase (hemin) production. Gelatin is not liquefied. Ammonia is not produced from arginine or urea. Litmus milk is always acidified and coagulated.

Source: found in human dental caries and plaque. In a study on the occurrence of the family *Bifidobacteriaceae*, the prevalence of *Parascardovia denticolens* in human dental caries and plaque was similar (Modesto et al., 2006).

DNA G+C content (mol%): 55 ± 1 (T_m).

Type strain: B3028, CCUG 35728, DSM 10105, LMG 18312, NCTC 12936.

Sequence accession no. (16S rRNA gene): D89331.

Genus VII. **Scardovia** Jian and Dong 2002, 811[VP]

PAOLA MATTARELLI AND BRUNO BIAVATI

Scar.do'vi.a. N.L. fem. n. *Scardovia* named after Vittorio Scardovi, an Italian microbiologist who has made many contributions to our knowledge of bifidobacteria.

Gram-stain-positive, non-acid-fast. Nonsporeforming. Nonmotile, anaerobic, rod-shaped cells that are small and coccoid and of variable shape. Saccharoclastic. Glucose is degraded exclusively and characteristically through the fructose 6-phosphate (F6PPK; EC 4.1.2.22) **shunt** (see treatment of the genus *Bifidobacterium*, above). The products from glucose fermentation are L-(+)-lactic acid and acetic acid in the molar ratio of 1:2.9. **Dextran is fermented.** Found in human dental caries and plaque.

DNA G+C content (mol%): 45 ± 1 (T_m).

Type species: **Scardovia inopinata** (Crociani, Biavati, Alessandrini, Chiarini and Scardovi 1996) Jian and Dong 2002, 811[VP] (*Bifidobacterium inopinatum* Crociani, Biavati, Alessandrini, Chiarini and Scardovi 1996, 569).

Further descriptive information

Phylogeny. The proposal of the genus *Scardovia* was based primarily on 16S rRNA and HSP60 gene sequence data and the genus is classified in the family *Bifidobacteriaceae*. Bifidobacteria were divided into two subclusters based on 16S rRNA gene sequence data (Miyake et al., 1998). Subcluster 2 encompassed *Bifidobacterium inopinatum* DSM 10107^T and *Bifidobacterium denticolens* DSM 10105^T (another species isolated from dental caries and described by Crociani et al., 1996), whereas subcluster 1 contained all of the other members of the genus *Bifidobacterium* together with *Gardnerella vaginalis*. The 16S rRNA gene sequence similarities to members of subcluster 1 ranged from 90.2 to 91.9% for *Bifidobacterium inopinatum* DSM 10107^T and from 90.7 to 92.8% for *Bifidobacterium denticolens* DSM 10105^T. *Gardnerella vaginalis* ATCC 14018^T, another member of the family *Bifidobacteriaceae* (Stackebrandt et al., 1997), shared higher 16S rRNA gene sequence similarities (91.6–95.3%) to other *Bifidobacterium* species than *Bifidobacterium inopinatum* DSM 10107^T and *Bifidobacterium denticolens* DSM 10105^T. A 16S rRNA phylogenetic tree of the family *Bifidobacteriaceae* is shown in Figure 66.

HSP60 has been used in bacterial phylogenetic analyses (Kwok et al., 1999; Viale et al., 1994). Jian et al. (2001) analyzed the sequence similarities of 538 bp fragments in the highly conserved HSP60 genes of 35 *Bifidobacterium* strains and of *Gardnerella vaginalis* ATCC 14018^T and found that *Bifidobacterium inopinatum* DSM 10107^T, *Bifidobacterium denticolens* DSM 10105^T, and the other *Bifidobacterium* species grouped into three distinct subclusters. *Bifidobacterium inopinatum* DSM 10107^T and *Bifidobacterium denticolens* DSM 10105^T showed exceptionally low levels of similarity to all other members of the genus *Bifidobacterium* (76–80 and 73–78.8%, respectively), whereas all other members exhibited sequence similarities of 81–98%. In the phylogenetic tree, *Gardnerella vaginalis* ATCC 14018^T and *Bifidobacterium inopinatum* DSM 10107^T were assigned to one subcluster, a result in line with DNA base composition data (42 mol% for *Gardnerella vaginalis* ATCC 14018^T and 45 mol% for *Bifidobacterium inopinatum* DSM 10107^T). However, the sequence similarity between *Bifidobacterium denticolens* and *Bifidobacterium inopinatum* was just 79%. *Bifidobacterium inopinatum* and *Bifidobacterium denticolens* were eventually reclassified as *Scardovia inopinata* and *Parascardovia denticolens*, respectively, based on DNA G+C content, 16S rRNA and HSP60 gene sequence analysis, and some phenotypic data (Jian and Dong, 2002).

Cell morphology and phase variations. Crociani et al. (1996) isolated 32 strains that were subsequently classified as *Bifidobacterium inopinatum* from dental caries. These isolates showed unusual phenotypic characteristics, notably a distinct cellular morphology, a characteristic that has significance for this group, and the presence of two types of colonies associated with different cellular morphologies. This latter characteristic has been observed and studied as a phenomenon of phase variation in only one member of the *Bifidobacteriaceae* family, namely *Bifidobacterium animalis* (Biavati et al., 1992a).

Dextran fermentation. Another phenotypic characteristic, also shown by *Parascardovia denticolens*, which distinguishes *Scardovia inopinata* from bifidobacteria is the ability to ferment dextran (Crociani et al., 1994). This feature was observed by Clarke (1959) in a rumen strain that closely resembled bifidobacteria and was therefore named *Lactobacillus bifidus*. Later, Kaster and Brown (1983) isolated three dextran-degrading, F6PPK-positive strains from human dental plaque and assigned them to the genus *Bifidobacterium*, but not to any species because they did not appear to be members of *Bifidobacterium dentium*.

Nutrition. The nutritional requirements of *Scardovia inopinata* and *Parascardovia denticolens* were compared to those of *Bifidobacterium dentium* by Modesto et al. (2003). The *Bifidobacterium dentium* strains showed optimal growth in a synthetic medium supplemented with vitamins, thereby confirming that this species requires pantothenate and riboflavin for growth. The *Scardovia inopinata* and *Parascardovia denticolens* strains also needed vitamins, as well as amino acids for growth. The results obtained with the *Scardovia inopinata* and *Parascardovia denticolens* strains isolated from human dental caries confirmed the observations reported on the nutritional requirements of *Bifidobacterium* species of human origin (Bezkorovainy and Miller-Catchpole, 1989; Tamura, 1983), namely that pantethine, a bifidus factor present in pancreatin and inactivated lysozyme, is indispensable for optimal growth of bifidobacteria of human origin. It can also be hypothesized that: (i) isolates of *Scardovia inopinata* and *Parascardovia denticolens* are unable to use pantothenic acid, the latter supplies a substitute growth factor that is converted within cells to coenzyme A, which is essential for all metabolic pathways; (ii) the growth-promoting effect of pancreatin is probably due either to a bifidus factor or to the presence of some enzymes (lacking in the strains examined) capable of hydrolyzing complex compounds that are otherwise not utilizable by the micro-organisms; and (iii) the inactivated lysozyme by hydrolyzing the polysaccharides of glycoproteins provides simpler nutrients which exert a growth-promoting effect on bifidobacteria. The requirement for lysozyme, a component of saliva, could be as a result of these bacteria living in dental caries

Ecology. A study on the occurrence of members of the family *Bifidobacteriaceae* in the human oral cavity revealed the presence of *Bifidobacterium dentium*, *Scardovia inopinata*, and *Parascardovia denticolens* in dental caries and plaque. *Scardovia inopinata* was more frequently isolated from caries samples than plaque (Modesto et al., 2006).

Pathogenicity. Like other oral micro-organisms, *Bifidobacteriaceae* strains might play a role in promoting the cariogenic process (acid production) and in the reduction of plaque formation (glucanase activity). Recently, in a study on the molecular analysis of bacterial species associated with childhood dental caries, the high incidence of members of the family *Bifidobacteriaceae* has been considered to play a large role in caries pathogenesis (Becker et al., 2002). However, the actual influence of members of this taxon on dental caries is unknown; hence, further studies are needed in order to characterize their role.

Plasmids. *Scardovia inopinata* strains do not contain plasmid DNA or produce any band in addition to chromosomal DNA bands (Crociani et al., 1996).

Enrichment and isolation procedures

Samples removed with sterile tooth-picks from the oral cavity (normally plaque or dental caries) are immediately suspended in 0.5 ml pre-reduced anaerobically sterilized dilution blanks (Holdeman et al., 1977) in 3 ml cryovials. They should then be maintained under anaerobiosis at 4°C. All microbiological analyses should be carried out 1–6 h later. Two slopes of each sample should then be plated on *Bifidobacterium*-selective TPY medium supplemented with 5% propionic acid (pH 5.0) (Beerens, 1990). The inoculated plates should be incubated anaerobically for 5 d at 37°C, then 12–15 randomly picked colonies should be subcultured in 0.5% agar TPY stabs. Identification and characterization assays should be carried out using isolates grown anaerobically in TPY liquid medium at 37°C for 24 h.

Maintenance procedures

Scardovia inopinata can be maintained at −135°C or as freeze-dried cultures by using the standard procedures described for the genus *Bifidobacterium*.

List of species of the genus *Scardovia*

1. **Scardovia inopinata** (Crociani, Biavati, Alessandrini, Chiarini and Scardovi 1996) Jian and Dong 2002, 811^{VP} (*Bifidobacterium inopinatum* Crociani, Biavati, Alessandrini, Chiarini and Scardovi 1996, 569)

   in.o.pin.a′ta. L. fem. adj. *inopinata* unexpected, referring to the very unusual morphology.

   An actinomycete which forms very small cocci (0.3–0.6 µm long; Figure 75). Cells become longer and more slender when strains are grown in liquid cultures containing fermentable sugars, such as lactose and raffinose. Much longer cells with swellings (1.3–3.0 µm in length) are produced in the presence of maltose and starch. This micromorphology also occurs on MRS medium and on tomato juice agar. The homogeneous sediment found in liquid cultures can be dispersed easily and never forms clumps.

   Circular, soft, smooth, convex, cream to white colonies with unbroken edges are formed on TPY agar. Two colony types

are produced. O colonies are made up of longer, slightly irregular rods and rarely contain club-shaped cells (Figure 76), whereas T colonies are formed when liquid cultures are incubated in an air/CO_2 mixture (1:10); in this instance, cells are much longer, more flexible, and exhibit wider ends and rare branches (2.0–8.0 µm long). These cells are very similar to those of typical bifidobacteria (Figure 76). Anaerobic. CO_2 enhances anaerobic growth. The fermentation products from glucose are L-(+)-lactic acid and acetic acid in a molar ratio of 1:2.9.

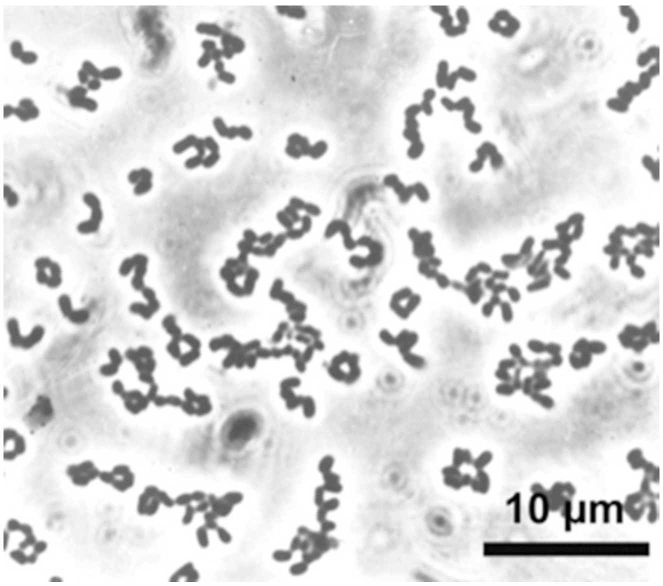

FIGURE 75. Cellular morphology of *Scardovia inopinata* B3109^T grown in liquid TPY medium. Phase-contrast photomicrograph (magnification ×1500).

The optimal temperature for growth is 38.5–42°C; minimum and maximum temperatures are 27–30.5 and 44°C, respectively. Growth does not occur at 26.5 or 46°C. The optimal initial pH is 6.9–7.4; growth is delayed at pH 4.9 and 8.0, and does not occur at pH 4.3 or 8.5.

Amylopectin, α-D-galacturonate, amylose, bovine submaxillary mucin, cellobiose, chondroitin sulfate, dextrin, fructose, glucose, maltose, D-ribose, starch, sucrose, and D-xylose are fermented, but not alginate, arabinogalactan, L-arabinose, D-fucose, L-fucose, D-galactosamine, D-glucitol, D-glucosamine, D-mannitol, glycerol, gluconate, gum arabic, ghatti gum, guar gum, locust bean gum, karaya gum, tragacanth gum, heparin, hyaluronate, lactate, laminarin, mannose, porcine gastric mucin, ovomucoid, pectin, polygalacturonate, L-rhamnose, trehalose, or xylan. Galactose is characteristically not fermented. Fermentation of D-glucuronate, inulin, lactose, melezitose, melibiose, raffinose, and salicin is variable. Thirty out of 31 isolates were found to ferment dextran. Acetylmethylcarbinol is produced and asparagine is hydrolyzed. Nitrate reduction, and indole, hydrogen sulfide, catalase, and pseudocatalase (hemin) production are negative. Gelatin is not liquefied. Ammonia is not produced from arginine or urea. Litmus milk is always acidified and coagulated.

Source: found in human dental caries and plaque. *Scardovia inopinata* was the species most frequently isolated in dental caries compared to dental plaque in a study on the occurrence of members of the family *Bifidobacteriaceae* in human dental caries and plaque (Modesto et al., 2006).

DNA G+C content (mol%): 45 ± 1 (T_m).

Type strain: B3109, CCUG 35729, DSM 10107, LMG 18313, NCTC 12937.

Sequence accession no. (16S rRNA gene): AB029087.

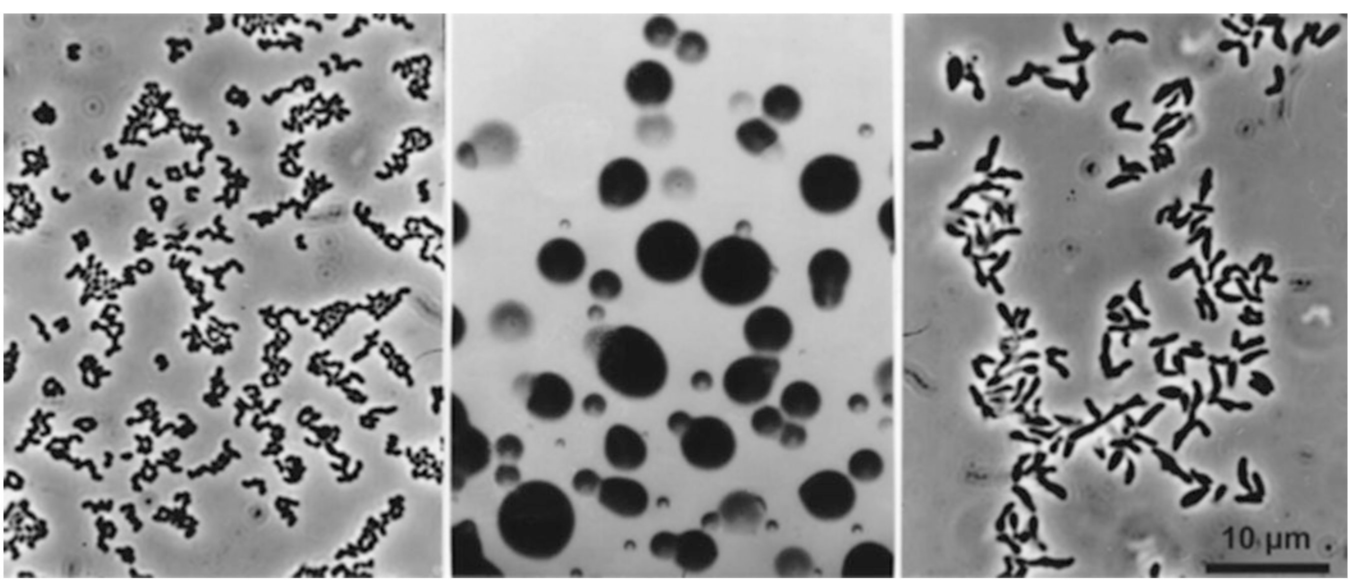

FIGURE 76. *Scardovia inopinata* B3109^T grown on TPY agar: (*left*) cells from transparent (T) colonies; (*center*) transparent and opaque (O) colonies; (*right*) cells from opaque (O) colonies. Phase-contrast photomicrograph (magnification 1500×).

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Order IV. **Catenulisporales** ord. nov.

Stefano Donadio, Linda Cavaletti and Paolo Monciardini

Ca.te.nu.li.spo′ra.les. N.L. fem. n. *Catenulispora*, type genus of the order; suff. -*ales*, ending to denote an order; N.L. fem. pl. n. *Catenulisporales*, the *Catenulispora* order.

The order *Catenulisporales* is formed by elevation of the suborder *Catenulisporineae*. The suborder was defined on the basis of phylogenetic analysis of 16S rRNA gene sequences to accommodate the families *Catenulisporaceae* and *Actinospicaceae*. Bootstrap analysis suggests a monophyletic origin for the two families (Figure 77), which also share a common pattern of 16S rRNA gene signatures consisting of nucleotides 127:234 (G–C), 138:225 (U–A), 139:224 (C–G), 140:223 (C–G), 141:222 (A–U), 157:164 (G–C), 449 (C), 589:650 (C–G), 602:636 (R–U), 603:635 (A–U), 694 (G), and 1251 (G).

Type genus: **Catenulispora** Busti, Cavaletti, Monciardini, Schumann, Rohde, Sosio and Donadio 2006, 1745[VP].

Key to differentiating the families of the order *Catenulisporales*

Family *Catenulisporaceae* vs family *Actinospicaceae*:

Chemotaxonomic parameters: LL-Dpm; C_{16} iso, C_{17} anteiso predominant fatty acids; absence of glucosamine-containing phospholipids vs 3-OH-Dpm; C_{15} iso, C_{16} iso, C_{15} ante predominant fatty acids; presence of glucosamine-containing phospholipids.

A subset of 16S rRNA gene signature nucleotides (129:232, U–A vs C–G; 591:648, C–G vs U–R; 952:1229, U–A vs C–G; no nucleotides vs 7–9 extra nucleotides between positions 1134 and 1140).

Physiological parameters: growth vs no growth at pH 6.5.

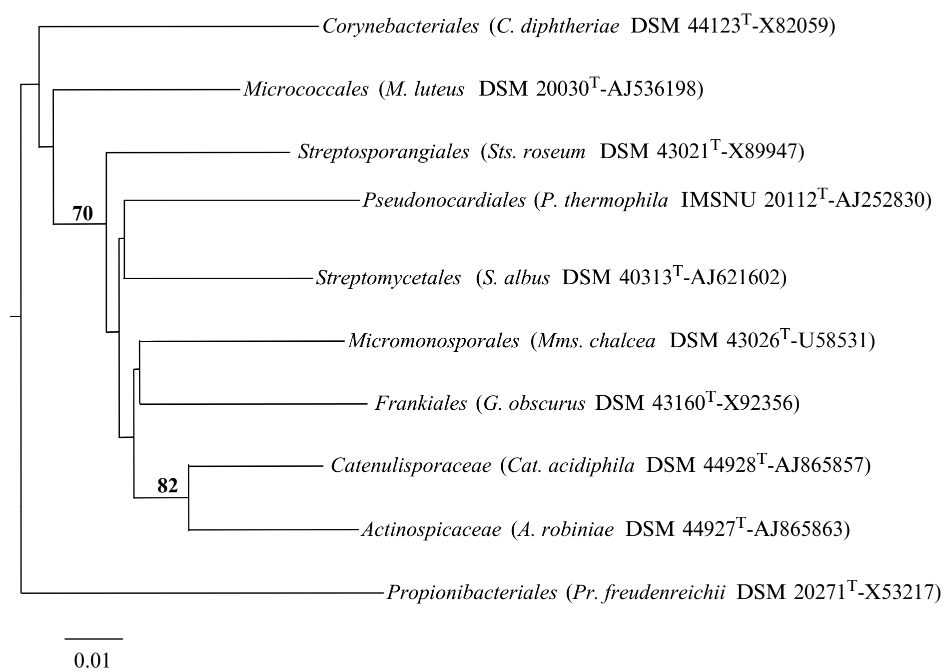

FIGURE 77. Neighbor-joining tree showing the placement of the order *Catenulisporales* families *Catenulisporaceae* and *Actinospicaceae* relative to other orders in the class *Actinobacteria*. For each order, the 16S rRNA gene sequence of the type strain from the type species is used in the alignment, with the exception of the *Frankiales*, for which it is replaced by the type strain of the family *Geodermatophilaceae*. Genus abbreviations are as follows: *A.*, *Actinospica*; *C.*, *Corynebacterium*; *Cat.*, *Catenulispora*; *G.*, *Geodermatophilus*; *M.*, *Micrococcus*; *Mms.*, *Micromonospora*; *P.*, *Pseudonocardia*; *Pr.*, *Propionibacterium*; *S.*, *Streptomyces*; *Sts.*, *Streptosporangium*. The tree is based on 1291 aligned positions within the 16S rRNA gene, omitting regions of ambiguous alignment, and was rooted using the 16S rRNA gene sequence of *Bifidobacterium bifidum* IFO 14252[T] (GenBank S83624). Numbers at nodes are bootstrap values based on 100 resamplings, only values higher than 60 are shown. Scale bar indicates 1 inferred nucleotide substitution per 100 nt.

Reference

Busti, E., L. Cavaletti, P. Monciardini, P. Schumann, M. Rohde, M. Sosio and S. Donadio. 2006. *Catenulispora acidiphila* gen. nov., sp. nov., a novel, mycelium-forming actinomycete, and proposal of *Catenulisporaceae* fam. nov. Int. J. Syst. Evol. Microbiol. *56*: 1741–1746.

Family I. **Catenulisporaceae** Busti, Cavaletti, Monciardini, Schumann, Rohde, Sosio and Donadio 2006a, 1745VP

Stefano Donadio, Linda Cavaletti and Paolo Monciardini

Ca.te.nu.li.spo.ra.ce′a.e. N.L. fem. n. *Catenulispora* type genus of the family; suff. *-aceae* ending to denote a family; N.L. fem. pl. n. *Catenulisporaceae* the *Catenulispora* family.

The family *Catenulisporaceae* was created to accommodate the genus *Catenulispora* on the basis of phylogenetic analysis of 16S rRNA. Members of the family share a common pattern of 16S rRNA gene signatures, represented by nucleotides 127:234 (G–C), 129:232 (U–A), 449 (C), 580:761 (U–A), 586:755 (U–A), 591:648 (C–G), 824:876 (A–U), 825:875 (A–U), 834:852 (G–U), 838:848 (U–G), 952:1229 (U–A), 999:1041 (U–U), and 1000:1040 (U–U). These signatures are defined on the basis of sequences from the four validly published species and of 7 additional sequences, from strains NEO 1, NEO 2, NEO 6 (Busti et al., 2006b), and strains ELLIN 5116, 5119, 5034, and 5062 (Joseph et al., 2003). The description of the family *Catenulisporaceae* matches that of the type genus.

DNA G+C content (mol%): 69–72.

Type genus: **Catenulispora** Busti, Cavaletti, Monciardini, Schumann, Rohde, Sosio and Donadio 2006a, 1745VP.

Genus I. **Catenulispora** Busti, Cavaletti, Monciardini, Schumann, Rohde, Sosio and Donadio 2006a, 1745VP

Stefano Donadio, Linda Cavaletti and Paolo Monciardini

Ca.te.nu.li.spo′ra. L. fem. n. *catenula* small chain; Gr. fem. n. *spora* seed and in biology a spore; N.L. fem. n. *Catenulispora* a thin chain of spores.

Stable, extensively-branched vegetative mycelium. Monopodially or dichotomously branched aerial hyphae produced on various media. Rod shaped and nonmotile spores, formed on the aerial mycelium and arranged in straight or flexuous chains, are 0.4–1.2 × 0.5–1.5 μm. Gram-stain-positive. Aerobic and non-acid-fast. **Some species acidophilic**. Optimal temperature is 25–30°C. Peptidoglycan contains **LL-diaminopimelic acid and glycine (A3γ type** *sensu* Schleifer and Kandler, 1972) plus glutamic acid and alanine. Arabinose is common to all, although additional sugars complete the whole-cell sugar pattern in the different species. Predominant menaquinones are MK-9 (H_8), (H_6), (H_4), in different amounts depending on the species. Predominant fatty acids are **saturated C_{16} iso and C_{17} anteiso**. Polar lipids containing glucosamine are not detected. Natural habitat is soil.

DNA G+C content (mol%): 69–72.

Type species: **Catenulispora acidiphila** Busti, Cavaletti, Monciardini, Schumann, Rohde, Sosio and Donadio 2006a, 1745VP.

Further descriptive information

All the described species share a common aerial structure characterized by chains of rod-shaped spores produced on aerial hyphae. The chains are straight or flexuous and spores are nonmotile (Figure 78). Colonies show the typical aspect of actinomycetes, with pigmentations ranging from yellow to red or brownish for the different species. In some cases the same strain shows different pigmentation on the different media used for growth.

Chemotaxonomically, the genus *Catenulispora* represents a homogeneus group in terms of peptidoglycan composition (all contain LL-diaminopimelic acid, glycine, glutamic acid, and alanine), cellular fatty acids (46–67% of $C_{16:0}$ iso and 12–28% $C_{17:0}$ anteiso in the different species), and isoprenoid quinones (all with nine isoprene units and 2, 3, or 4 saturated units), although the latter are present with different relative amounts: *Catenulispora acidiphila* has MK-9(H_6) and -(H_4) as the predominant compounds; *Catenulispora rubra* MK-9(H_6) and -(H_8); and *Catenulispora subtropica* and *Catenulispora yoronensis* MK-9(H_8) and -(H_6), respectively (Table 24). Less homogeneity is present in the polar lipids and especially in the whole-cell sugars pattern. In terms of polar lipids, all species lack glucosamine-containing phospholipids, but *Catenulispora acidiphila* contains phosphatidylglicerol (PG), diphosphatidylglicerol (DPG), phosphatidylinositol (PI), phosphatidylinositol mannosides, and two unknown phospolipids; *Catenulispora rubra* contains PG and PI; and *Catenulispora subtropica* and *Catenulispora yoronensis* contain only DPG. With respect to the whole-cell sugars, *Catenulispora acidiphila* contains arabinose in large amounts plus xylose, ribose, rhamnose, and glucose; *Catenulispora rubra* contains rhamnose, mannose, arabinose, and galactose; *Catenulispora subtropica* and *Catenulispora yoronensis* contain mannose, arabinose, and galactose.

Acidophilia is characteristic only for *Catenulispora acidiphila* and *Catenulispora rubra* as they grow between pH 4.0–4.5 and 6.5 with optima at 6.0 and 5.0, respectively, with the first showing growth even at pH 7.0. On the contrary, the other two species grow well in the pH range 5–7 (optima at 6–7) with *Catenulispora subtropica* able to grow also at pH 8.

Strains are mesophilic but perform best below 30°C, and *Catenulispora acidiphila* does not grow at 40°C.

The genus *Catenulispora* comprises at present four species, one isolated from Italian forest soils (*Catenulispora acidiphila*) and the other three from Japanese forest or paddy soils. Phylogenetically related strains have also been isolated from Australian pasture soils (Joseph et al., 2003). Moreover, 16S rRNA gene sequences belonging to this genus have been amplified from soil DNA isolated from samples of European, American, and African origin (Busti et al., 2006b). This genus can thus be defined as ubiquitous, and new species are expected to be described.

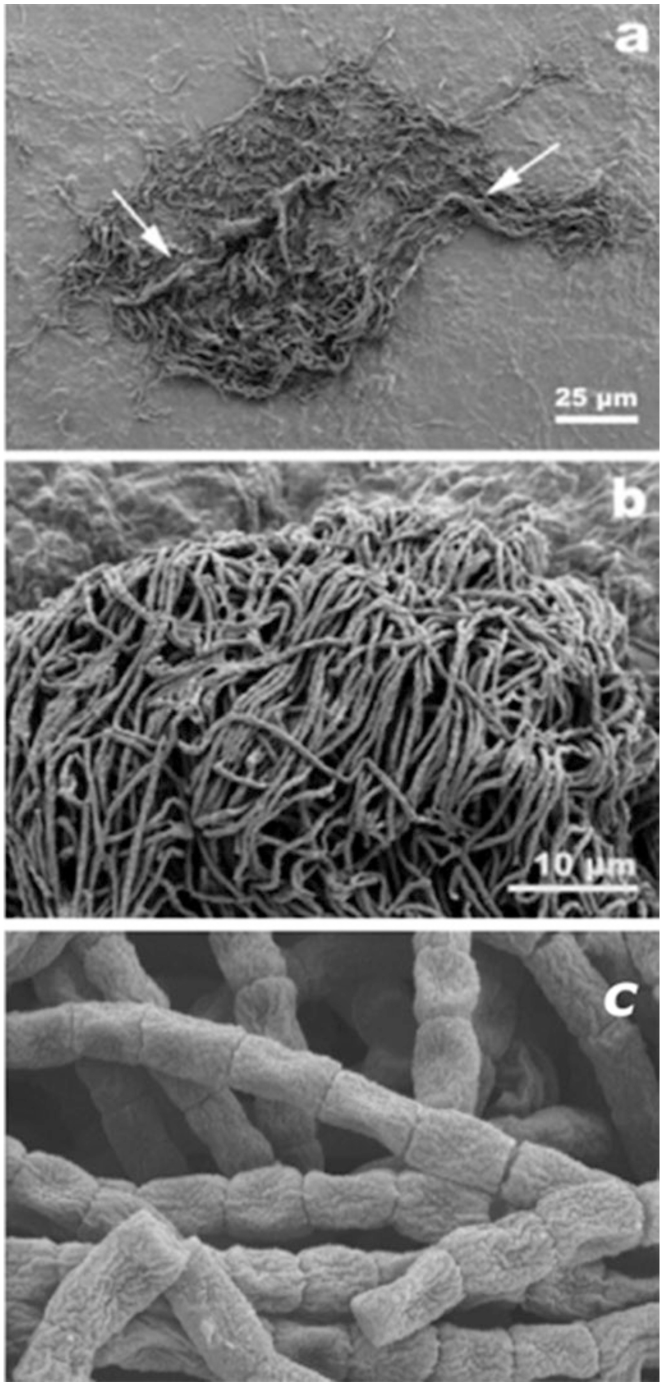

FIGURE 78. Field emission scanning electron microscopy (FESEM) of strain *Catenulispora acidiphila* grown on HSA5 agar for 3 weeks at 28°C. (a) Panoramic view of aerial hyphae. Arrows indicate bundles of hyphae running together. (b) Septation of hyphae gives origin to chains of more than 20 spores. (c) Cylindrical arthrospores (0.4–1.0 µm length × approximately 0.5 µm diameter) with a rugose surface are visible. Collapsing of the central part of spores walls results in a wavy appearance of spore chains as seen in (b).

Enrichment and isolation procedures

Acidic isolation media are useful to enrich for acidophilic strains of the genus *Catenulispora*, while other species can be isolated on neutral media. Moreover, in our experience acidophilic strains of *Catenulispora* can only be retrieved from acidic soils. This finding is consistent with the observation that 16S rRNA gene sequences related to these strains were amplified from one third of the soils with a pH of 5.5, or lower, while no signal was observed from 19 soils with pH >7 (Busti et al., 2006b). Nutrient-poor media, having only soil extract or humic acid as carbon and nitrogen sources, favor the isolation of these organisms.

Catenulispora acidiphila was isolated on GTV agar medium [500 ml/l soil extract (prepared by autoclaving 100 g fresh soil suspended in 500 ml H_2O and filtered through sterile gauze), 10 g/l gellan gum, 3 mM $CaCl_2$, pH 5] supplemented with 0.1% (v/v) CMM vitamin solution (25 µg/ml thiamin hydrochloride, 250 µg/ml calcium pantothenate, 250 µg/ml nicotinic acid, 500 µg/ml biotin, 1.25 mg/ml riboflavin, 6 µg/ml vitamin B_{12}, 25 µg/ml *p*-aminobenzoic acid, 500 µg/ml folic acid, and 500 µg/ml pyridoxal hydrochloride) and 50 µg/ml cycloheximide, after serial dilution of a soil suspension in 18.2 mM citric acid, 164 mM Na_2HPO_4, pH 7. Plates were incubated at 28°C for 8 weeks.

Catenulispora subtropica and *Catenulispora yoronensis* were isolated by pretreating soil suspensions with sodium dodecyl sulfate (SDS)-yeast extract solution (6% yeast extract and 0.05% SDS) at 40°C for 30 min (Hayakawa and Nonomura, 1989) before plating onto humic acid-vitamin agar (Hayakawa and Nonomura, 1987); 1 g/l humic acid, 0.5 g/l Na_2HPO_4, 1.71 g/l KCl, 0.05 g/l $MgSO_4 \cdot 7H_2O$, 0.01 g/l $FeSO_4 \cdot 7H_2O$, 0.02 g/l $CaCO_3$, 18 g/l agar, plus 0.5 mg each of thiamine-HCl, riboflavin, niacin, pyridoxin-HCl, inositol, Ca-pantothenate, *p*-aminobenzoic acid, and 0.25 mg biotin) supplemented with 20 µg/ml nalidixic acid and 200 µg/ml cycloheximide.

Catenulispora rubra was instead isolated on an acidic medium made through mixing 1:1 (v:v) two separately autoclaved media: 8 g/l glucose, 4 g/l Difco yeast extract, 36 g/l agar, pH adjusted to 5.0 with H_2SO_4; and 4 g/l $(NH_4)_2SO_4$, 1 g/l K_2HPO_4, 1 g/l $MgSO_4 \cdot 7H_2O$, 0.2 g/l KCl, final pH approximately 4.

Maintenance procedures

For preservation, organisms can be stored in nutrient broth containing 20% glycerol at −80°C, either as harvested cells from cultures in ATSB medium (17 g/l casitone, 3 g/l soytone, 2.5 g/l glucose, 10 mM 2-[*N*-Morpholino]ethanesulfonic acid, adjusted to pH 5.8 with HCl; Busti et al., 2006a) or as cultures collected directly from acidic ISP-3 medium (see footnote to Table 25). Under these conditions, strains remain viable for at least 12 months. For longer storage, lyophilization is recommended.

Taxonomic comments

Apart from the four described species with validly published names, 16S rRNA gene sequences are available for other isolated strains that appear to be members of the genus *Catenulispora*. Most of these sequences have high identity values and show a common phylogenetic origin, sustained by high bootstrap values (Figure 79). Two lineages of *Catenulispora* can be clearly differentiated in the phylogenetic tree shown in Figure 78, one comprising the two recently described species *Catenulispora subtropica* and *Catenulispora yoronensis*, and the second including the validly published species *Catenulispora acidiphila* and *Catenulispora rubra*, as well as the strains NEO 1, NEO 2, and NEO 6 (Busti et al., 2006b) and ELLIN 5116, 5119, 5034, and 5062 (Joseph et al., 2003). This phylogenetic

TABLE 24. Characteristics differentiating species of the genus *Catenulispora*[a]

Characteristic	*C. acidiphila*	*C. rubra*	*C. subtropica*	*C. yoronensis*
Pigment on ISP-2	Dark brown	Strong red	Grayish, reddish-orange	Yellowish, light brown
Optimal pH	6.0	5.0	6–7	6–7
Whole-cell sugars	Arabinose, xylose, ribose, rhamnose, glucose	Rhamnose, mannose, arabinose, galactose	Mannose, arabinose, galactose	Mannose, arabinose, galactose
Main menaquinones	MK-9(H)$_6$, -(H)$_4$	MK-9(H)$_6$, -(H)$_8$	MK-9(H)$_8$, -(H)$_6$	MK-9(H)$_8$, -(H)$_6$
Nitrate reduction	–	–	+	–
Gelatin hydrolysis	+	–	+	–
DNA G+C content (mol%)	71.9	69.1	70–71	69

[a]Symbols: +, >85% positive; –, 0–15% positive.

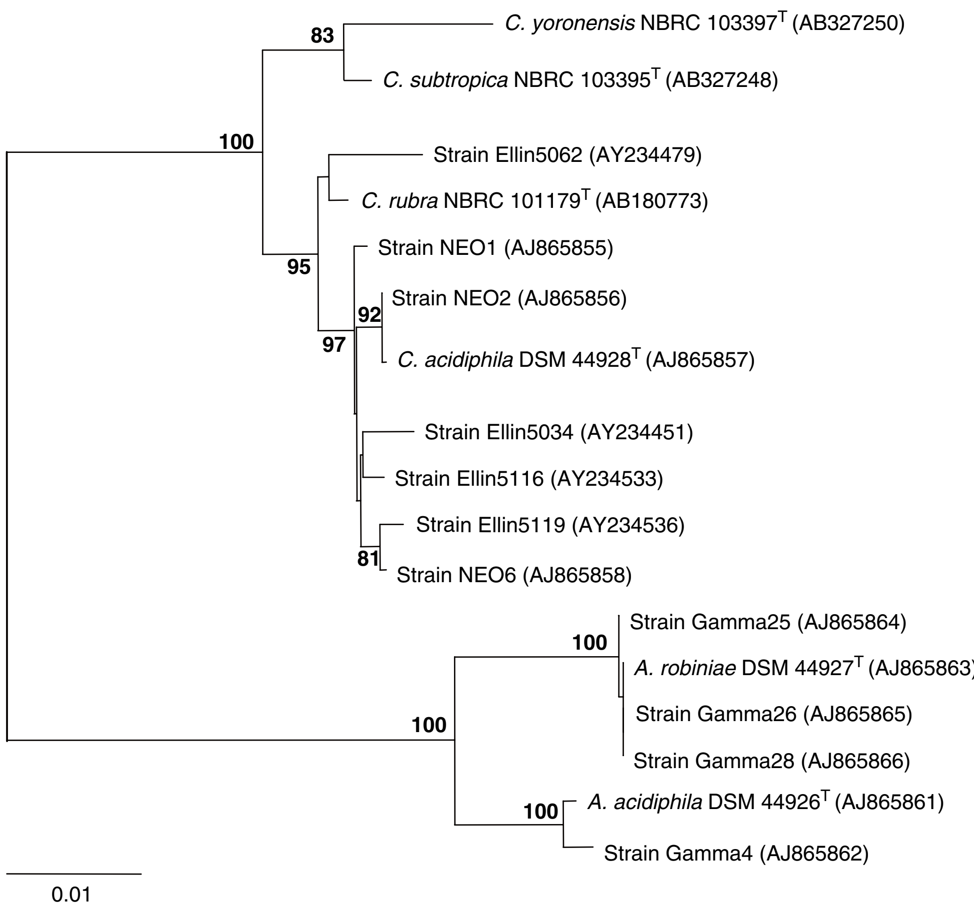

FIGURE 79. Neighbor-joining tree based on 1330 aligned positions within 16S rRNA gene sequences. *C.*, *Catenulispora*; *A.*, *Actinospica*. Sequences from not formally described strains (Busti et al., 2006b; Joseph et al., 2003) are also included. Accession numbers are in parentheses. The tree was rooted using the 16S rRNA gene sequence of *Geodermatophilus obscurus* DSM 43160^T (GenBank X92356). Numbers at nodes are bootstrap values based on 100 resamplings. Only values higher than 80 are shown. Scale bar indicates 1 inferred nucleotide substitution per 100 nt.

differentiation is congruent with physiological and chemotaxonomical differences: *Catenulispora subtropica* and *Catenulispora yoronensis* are not acidophilic while the other strains are; and *Catenulispora subtropica* and *Catenulispora yoronensis* have the same composition of whole-cell sugars and polar lipids pattern, but different from those of the described species of the other lineage. However, the >97% identity levels of the 16S rRNA genes between the two groups and other common chemotaxonomic characteristics are consistent with either a diverse *Catenulispora* genus or with *Catenulispora subtropica* and *Catenulispora yoronensis* belonging to a novel genus within the family *Catenulisporaceae*.

Differentiation of the species of the genus *Catenulispora*

Species of *Catenulispora* can be differentiated by the features summarized in Table 24. Cultural and physiological characteristics of the four species of *Catenulispora* are shown in Table 25 and Table 26 respectively.

TABLE 25. Cultural characteristics of *Catenulispora* species[a]

Medium[b]/character	*C. acidiphila*	*C. rubra*	*C. subtropica*	*C. yoronensis*
ISP-2:				
Growth	Abundant	Good	Moderate	Good
Color	Dark brown	Strong red	Grayish/reddish orange	Light to moderate yellowish brown, moderate yellow
Soluble pigment	Brown	Absent	Absent	Absent
ISP-3:				
Growth	Good	Weak	Moderate	Good
Color	Cream/light brown	Pale yellow	Grayish white	Moderate yellow, light to moderate yellowish brown
Soluble pigment	Brownish	Absent	Absent	Absent
ISP-4:				
Growth	Good	Good	Poor	Good
Color	Cream/light orange	Strong/dark purplish red	Pale yellow	Pale yellow
Soluble pigment	Brownish	Absent	Absent	Absent
ISP-5:				
Growth	Good	Good	Poor	Poor
Color	Brown	Strong/dark purplish red	Grayish white	Grayish white
Soluble pigment	Light brown	Absent	Absent	Absent
ISP-6:				
Growth	Absent	Absent	Moderate	Poor
Color	na	na	Grayish white	Grayish white
Soluble pigment	na	na	Absent	Absent
ISP-7:				
Growth	Abundant	Good	Moderate	Poor
Color	Brown	Strong/dark purplish red	Grayish white	Grayish white
Soluble pigment	Brown	Absent	Absent	Absent

[a] na, Not applicable.

[b] Media are according to Shirling and Gottlieb (1966), with pH adjusted to 5.5 –6 with HCl and to 5.0 with H_2SO_4 for *Catenulispora acidiphila* and *Catenulispora rubra*, respectively: ISP-2, 4 g/l yeast extract, 10 g/l malt extract, 4 g/l dextrose, 20 g/l agar; ISP-3, 20 g/l oat meal,18 g/l agar, 1 ml/l trace salts solution*; ISP-4, 10 g/l soluble starch, 1 g/l K_2HPO_4, 1 g/l $MgSO_4 \cdot 7H_2O$, 1 g/l NaCl, 2 g/l $(NH_4)_2SO_4$, 2 g/l $CaCO_3$, 20 g/l agar, 1 ml/l trace salts solution*; ISP-5, 1 g/l aspargine, 10 g/l glycerol, 1 g/l K_2HPO_4, 20 g/l agar, 1 ml/l trace salts solution*; ISP-6, 36 g/l Bacto-peptone iron agar (Difco), 1 g/l yeast extract; and ISP-7, 15 g/l glycerol, 0.5 g/l L-tyrosine, 1 g/l L-aspargine, 0.5 g/l K_2HPO_4, 0.5 g/l $MgSO_4 \cdot 7H_2O$, 0.5 g/l NaCl, 0.01 g/l $FeSO_4 \cdot 7H_2O$, 20 g/l agar, 1 ml/l trace salts solution*. *Trace salts solution is: 1 g/l $FeSO_4 \cdot 7H_2O$, 1 g/l $MnCl_2 \cdot 7H_2O$, 1 g/l $ZnSO_4 \cdot 7H_2O$.

TABLE 26. Physiological characteristics of *Catenulispora* species[a]

Characteristic	*C. acidiphila*	*C. rubra*	*C. subtropica*	*C. yoronensis*
Utilization of (API 50CH test):				
N-Acetyl-glucosamine	w	+	+	–
D-Adonitol	–	–	–	–
D-Arabinose	–	–	–	–
L-Arabinose	–	+	+	–
D-Arabitol	–	–	–	–
L-Arabitol	–	–	–	–
Arbutin	+	+	+	+
D-Cellobiose	+	+	+	+
Dulcitol	–	–	–	–
Erythritol	–	–	–	–
Esculin ferric citrate	+	+	+	+
D-Fructose	+	+	–	–
D-Fucose	–	–	–	–
L-Fucose	+	+	+	+
D-Galactose	+	–	+	–
Gentiobiose	+	+	+	+
D-Glucose	+	+	+	+
Glycerol	–	+	+	–
Glycogen	+	+	+	+
Inositol	–	–	–	–
Inulin	–	–	–	–
2-Keto-gluconate	–	–	–	–
5-Keto-gluconate	–	–	–	–
D-Lyxose	–	–	–	–
D-Maltose	+	+	+	+

(continued)

TABLE 26. (continued)

Characteristic	*C. acidiphila*	*C. rubra*	*C. subtropica*	*C. yoronensis*
D-Mannitol	−	+	−	−
D-Mannose	+	+	+	+
D-Melezitose	−	−	−	−
Methyl β-D-xylopyranoside	−	−	w	−
D-Ribose	−	−	−	−
Salicin	+	w	+	−
D-Sorbitol	−	−	−	−
L-Sorbose	−	−	−	−
D-Sucrose	−	+	−	w
Starch	+	+	+	+
D-Tagatose	−	−	−	−
D-Trehalose	+	+	+	+
Xylitol	−	−	−	−
D-Xylose	+	+	+	+
L-Xylose	−	−	−	−
Enzyme activities (API ZYM test):				
N-Acetyl-β-glucosaminidase	+	+	+	+
Acid phosphatase	+	+	+	+
Alkaline phosphatase	+	+	+	+
Cystine aminopeptidase	−	−	−	−
α-Fucosidase	−	−	+	+
α-Galactosidase	−	−	−	−
β-Galactosidase	+	w	+	−
β-Glucoronidase	−	+	−	−
α-Glucosidase	+	+	+	+
β-Glucosidase	+	+	+	+
Leucine aminopeptidase	+	+	+	+
Lipase	−	−	−	−
α-Mannosidase	+	+	+	+
Phosphohydrolase	+	+	+	+
Trypsin	−	+	+	−
Valine aminopeptidase	+	+	+	+
Reaction for (API coryne):				
Catalase activity	+	−	+	+
Esculin hydrolysis	+	+	+	+
Gelatin hydrolysis	+	−	+	−
Nitrate reduction	−	−	+	−
Pyrazinamidase	+	+	+	+
Pyrrolidonyl arylamidase	−	−	−	−
Urea hydrolysis	−	−	−	−

[a]Abbreviations: −, negative; +, positive; w, weakly positive.

List of species of the genus *Catenulispora*

1. **Catenulispora acidiphila** Busti, Cavaletti, Monciardini, Schumann, Rohde, Sosio and Donadio 2006a, 1745[VP]

a.ci.di′phi.la. N.L. n. *acidum* (from L. adj. *acidus* sour) an acid; N.L. adj. *philus -a -um* (from Gr. adj. *philos -ê -on*) friend, loving; N.L. fem. adj. *acidiphila* acid-loving.

Stable vegetative mycelium; straight to flexuous, relatively short aerial hyphae produce chains of more than 20 cylindrical arthrospores. Spores are long 0.4–1.0 µm with a mean diameter of about 0.5 µm. Spore surface is rugose. Brown-mahogany soluble pigment produced on tyrosine agar but not on tyrosine-supplemented Suter synthetic medium. Acidophilic, good growth at pH 4.3–6.8, optimum at 6. Mesophilic, best growth at 22–28°C, significant growth at 11 and 37°C, no growth at 4 or 40°C. H_2S produced. Nitrates not reduced. Starch and casein hydrolyzed. Gelatin liquefied. Catalase-positive. Up to 3% (w/v) NaCl is tolerated, as well as 100 µg/ml lysozyme. Glucose, arabinose, xylose, mannitol, fructose, and glycerol are utilized as sole carbon source in acidic ISP-9 whereas sucrose, inositol, rhamnose, and cellulose are not. Resistant to 10 µg/ml nalidixic acid, oxacillin, apramycin, daunomycin, GE2270, nisin, rifampin, and kanamycin, but sensitive to the same concentration of novobiocin, thiostrepton, A40926, and ramoplanin.

Produces metabolites with anti-Gram-stain-positive activity (Busti et al., 2006b).

DNA G+C content (mol%): 71.9 (HPLC).

Type strain: ID139908, DSM 44928, JCM 14897, NBRC 102108, NRRL B-24433.

Sequence accession no. (16S rRNA gene): AJ865857.

2. **Catenulispora rubra** Tamura, Ishida, Sakane and Suzuki 2007, 2273[VP]

ru′bra. L. fem. adj. *rubra* red.

Extensively branched, nonfragmenting vegetative mycelium. Monopodially or dichotomously branched aerial mycelia, sparsely developed; produce straight or flexuous chains of spores. Spores are rod shaped, 0.7–1.5 μm in diameter, nonmotile with a smooth surface. Colonies are red on most media. Acidophilic, growth at pH 4–6.5, optimum at pH 5. Mesophilic, grows at 20–30°C. Nitrates not reduced. Starch and esculin are hydrolyzed, but gelatin or urea are not. Catalase negative. D-Glucose, raffinose, L-arabinose, D-xylose, sucrose, and fructose are used as sole carbon source whereas inositol and rhamnose are not.

DNA G+C content (mol%): 69.1 (HPLC).

Type strain: Aac-30, DSM 44948, NBRC 101179.

Sequence accession no. (16S rRNA gene): AB180773.

3. **Catenulispora subtropica** Tamura, Ishida, Otoguro and Suzuki 2008, 1554[VP]

sub.tro.pi.ca. N.L. fem. adj. *subtropica* pertaining to subtropical zone, the origin of the soil sample from which the type strain was isolated.

Extensively and finely branched, nonfragmenting vegetative mycelium. Monopodially or dichotomously branched aerial mycelia develop sparsely. Spores are rod shaped and arranged in rectiflexible or straight chains comprising rod-shaped spores 0.5–0.8 μm in diameter and 0.8–1.2 μm long. Grayish reddish-orange colonies are formed on ISP-2 medium. The pH for growth range is 5–8, and the optimum is 6–7. The temperature for growth range is 10–37°C, and the optimum is 25–30°C. The strain hydrolyzes esculin and gelatin, but not urea. Glycerol, L-arabinose, D-xylose, methyl-β-D-xylopyranoside, D-galactose, D-glucose, D-mannose, *N*-acetyl-glucosamine, arbutin, esculine ferric citrate, salicin, D-celiobiose, D-maltose, starch, glycogen, gentiobiose, L-fucose, and gluconate are used as sole carbon sources. Positive reaction is shown for catalase, nitrate reduction, pyrazinamidase, leucine aminopeptidase, valine aminopeptidase, trypsin, acid phosphatase, phosphohydrolase, β-galactosidase, α-glucosidase, β-glucosidase, *N*-acetyl-β-glucosaminidase, α-mannosidase, and α-fucosidase; while negative reaction is shown for pyrrolidonyl arylamidase, esterase (c-4), esterase lipase (c-8), lipase (c-14), cystine aminopeptidase, chymotrypsin, α-galactosidase, and β-glucuronidase.

DNA G+C content (mol%): 70–71 (HPLC).

Type strain: TT 99-48, KCTC 19328, NBRC 103395.

Sequence accession no. (16S rRNA gene): AB327248.

4. **Catenulispora yoronensis** Tamura, Ishida, Otoguro and Suzuki 2008, 1555[VP]

yo.ro.en′sis. N.L. fem. adj. *yoronensis* pertaining to Yoro Valley, Chiba, Japan, the origin of the soil sample from which the type strain was isolated.

Extensively branched substrate hyphae. The vegetative hypha is finely branched and does not fragment. Monopodially or dichotomously branching aerial mycelia develop sparsely. Spores are arranged in rectiflexible or straight chains, rod-shaped, 0.5–0.8 μm in diameter and 0.8–1.2 μm long. They are nonmotile. Light to moderate yellowish brown and/or moderate yellow colonies are formed on ISP-2. Grows at pH 5–7, optimum is 6–7. Growth temperature ranges from 10–37°C, optimum at 25–30°C. Esculin is hydrolyzed, but gelatin and urea are not. D-Xylose, D-glucose, D-mannose, arbutin, esculine ferric citrate, D-celiobiose, D-maltose, D-melibiose, D-trehalose, starch, glycogen, L-fucose, and gluconate are used as sole carbon sources. Positive reaction is shown for catalase, pyrazinamidase, alkaline phosphatase, leucine aminopeptidase, valine aminopeptidase, acid phosphatase, α-glucosidase, β-glucosidase, *N*-acetyl-β-glucosaminidase, α-mannosidase, and α-fucosidase, while negative reaction is shown for nitrate reduction, pyrrolidonyl arylamidase, esterase (c-4), esterase lipase (c-8), lipase (c-14), cystine aminopeptidase, trypsin, chymotrypsin, β-galactosidase, and β-glucuronidase.

Contrary to the family signatures, nucleotides 586:755 of the 16S rRNA gene are C–G and not U–A.

DNA G+C content (mol%): 69 (HPLC).

Type strain: TT N02-20, KCTT 19327, NBRC 103397.

Sequence accession no. (16S rRNA gene): AB327250.

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Family II. **Actinospicaceae** Cavaletti, Monciardini, Schumann, Rohde, Bamonte, Busti, Sosio and Donadio 2006, 1751^VP

Stefano Donadio, Linda Cavaletti and Paolo Monciardini

Ac.ti.no.spi.ca.ce′a.e. N.L. fem. n. *Actinospica* type genus of the family; suff. *-aceae* ending to denote a family; N.L. fem. pl. n. *Actinospicaceae* the *Actinospica* family.

The family contains the single genus *Actinospica* and was defined on the grounds of its clear phylogenetic distance from all other actinomycete families. A distinctive pattern of 16S rRNA gene signatures is present, consisting of nucleotides 127:234 (G–C), 129:232 (C–G), 344 (G), 449 (C), 450:483 (C–G), 560 (U), 576 (G), 590:649 (C–G), 591:648 (U–R), 859 (G), 952:1229 (C–G), 1122:1151 (G–C), 1123:1150 (U–G), 1124:1149 (A–U), and of 7–9 extra nucleotides between positions 1134 and 1140.

DNA G+C content(mol%): 69–71 (HPLC).

Type genus: **Actinospica** Cavaletti, Monciardini, Schumann, Rohde, Bamonte, Busti, Sosio and Donadio 2006, 1751^VP.

Genus I. **Actinospica** Cavaletti, Monciardini, Schumann, Rohde, Bamonte, Busti, Sosio and Donadio 2006, 1751^VP

Stefano Donadio, Linda Cavaletti and Paolo Monciardini

Ac.ti.no.spi′ca. Gr. n. *aktis -inos* a ray; L. fem. n. *spica* tuft; N.L. fem. n. *Actinospica* an actinomycete with tufts of aerial hyphae.

Strains produce nonfragmenting vegetative mycelium and aerial mycelium appearing as tufts of straight to slightly flexuous hyphae, at maturity carrying chains of cylindrical spores with a slightly rugose surface. Chains contain up to 30 (or more) spores. Tufts originate from very short sporophorous hyphae branching in few sporogenous hyphae (Figure 80 and Figure 81). Spores are not motile.

Diagnostic diaminoacid of the peptidoglycan is 3-hydroxydiaminopimelic acid, in addition to glycine, glutamic acid, and alanine. Only small traces of *meso*-diaminopimelic acid are detected.

Predominant fatty acids are **saturated C_{15} iso, C_{16} iso and C_{15} anteiso**. The major menaquinones are MK-9(H_4), MK-9(H_6), and MK-9(H_8). Gram-stain-positive. Aerobic. They are **obligate acidophiles**, growth occurs at pH not exceeding 6.2, optimum at 5.0–5.5. Temperature range for growth is 17–33°C, optimum at 22–28°C. Strains are catalase positive, produce H_2S, are negative for nitrate reduction, gelatin liquefaction, and tyrosine reaction.

DNA G+C content (mol%): 69–71 (HPLC).

Type species: **Actinospica robiniae** Cavaletti, Monciardini, Schumann, Rohde, Bamonte, Busti, Sosio and Donadio 2006, 1752^VP.

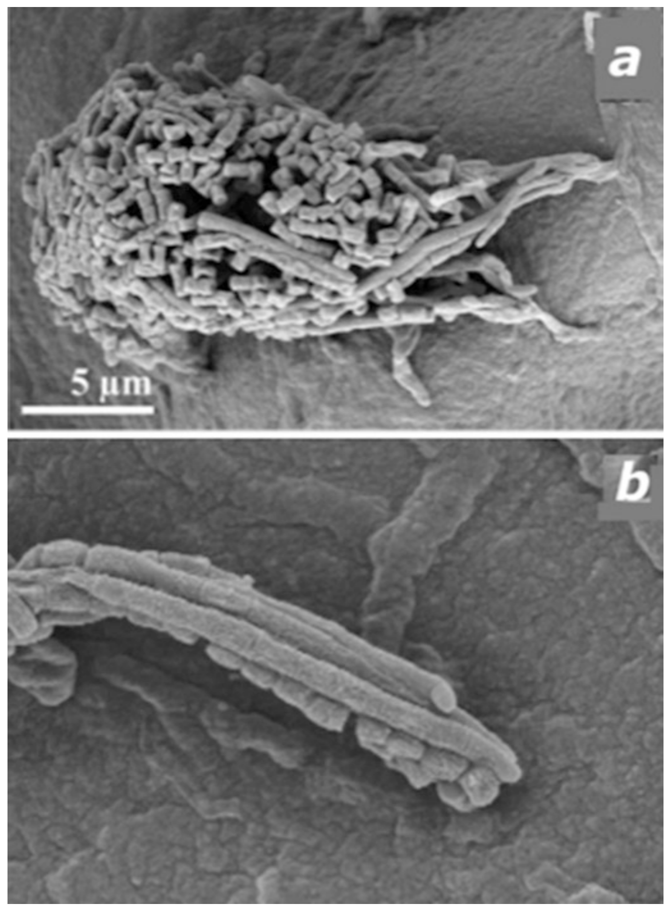

FIGURE 80. Field emission scanning electron microscopy of *Actinospica acidiphila* grown on HSA5 agar for 3 weeks at 28°C. (a) Conic tufts of sporogenous aerial hyphae originated by the branching of single and short sporophorous hyphae can be seen. (b) A young, unseptated hypha runs together with a partially septated one and with a mature one, divided into a spore chain. Panel (b) is from Cavaletti et al. (2006).

Further descriptive information

Strains grow on various agar media, but only at acidic pH, with *Actinospica acidiphila* slightly more acidophilic than *Actinospica robiniae* (Table 27). Cell-wall sugars comprise mannose and rhamnose plus additional sugars differentiating the species (see Table 27). Ratios of the major menaquinones MK-9(H_4), MK-9(H_6), and MK-9(H_8) vary according to the species (23:40:20 and 2:25:59 for *Actinospica acidiphila* and *Actinospica robiniae*, respectively). The polar lipids pattern is common to both species and consists of diphosphatidylglycerol, phosphatidylethanolamine, methyl-phosphatidylethanolamine, and phosphatidylinositol. Glucosamine-containing phospholipids are also present. Production of metabolites with antibiotic activity for strains of this genus (Busti et al., 2006b).

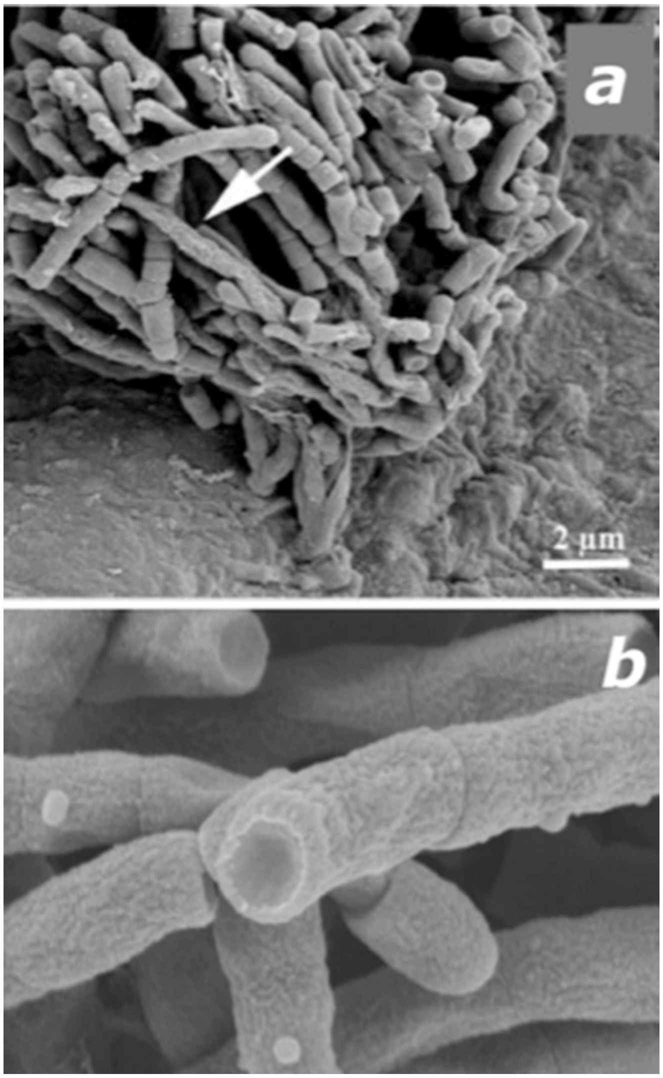

FIGURE 81. Field emission scanning electron microscopy of *Actinospica robiniae* grown on HSA5 agar for 3 weeks at 28°C. (a) Tufts of sporogenous aerial hyphae undergoing division into squat to cylindrical arthrospores are shown. Branching of sporophorous hyphae giving origin to tufts appears very close to the agar surface. Arrow indicates the rugosity of the surface of a hypha that is not fragmented. (b) Cylindrical spores with slightly rugose surface. Panel (b) is from Cavaletti et al. (2006).

Enrichment and isolation procedures

Described strains can be isolated after prolonged incubation on acidified HSA5 medium [0.5 g/l humic acid, 1 mg/l $FeSO_4 \cdot 7H_2O$, 1 mg/l $MnCl_2 \cdot 4H_2O$, 1 mg/l $ZnSO_4 \cdot 7H_2O$, 1 mg/l $NiSO_4 \cdot 6H_2O$, 10 mM 2-*N*-cyclohexyl-2-aminoethanesulfonic acid, 20 g/l agar; adjusted to pH5.5 with HCl, autoclaved, and then supplemented with 0.1% (v/v) CMM vitamin solution (see recipe in the *Catenulispora* chapter, above)] (Busti et al., 2006a) and transferred to acidified ISP-3 (see footnote to Table 25) medium for maintenance.

TABLE 27. Characteristics differentiating species of the genus *Actinospica*[a]

Characteristic	*A. robiniae*	*A. acidiphila*
Tolerance to 1% NaCl	–	+
Optimal pH	5.5	5
Whole-cell sugars	Mannose, rhamnose, galactose	Mannose, rhamnose, arabinose, xylose
Starch hydrolysis	–	+
DNA G+C content (mol%)	70.8	69.2

[a]Symbols: +, positive; –, negative.

Maintenance procedures

For preservation, organisms can be stored in nutrient broth containing 20% glycerol at –80°C, both as harvested cells from cultures in ATSB (17 g/l casitone, 3 g/l soytone, 2.5 g/l glucose, 10 mM 2-[*N*-Morpholino]ethanesulphonic acid, adjusted to pH 5.8 with HCl) medium (Busti et al., 2006a), or as cultures collected directly from acidic ISP-3 medium. Under these conditions, strains remain viable for at least 12 months. For longer storage, lyophilization is recommended.

Taxonomic comments

Although only two species are published with validated names, several 16S rRNA gene sequences with >97% identity to *Actinospica robiniae* and *Actinospica acidiphila* are available in public databases suggesting that the corresponding strains belong to the *Actinospica* genus. The pattern of signature nucleotides for the family is actually defined based on a set that, in addition to those of the two validly described species, contain other 28 sequences from cultured but not formally described strains. These strains were isolated from samples collected in several locations all over the world, indicating a ubiquitous distribution of *Actinospica*.

Differentiation of the species of the genus *Actinospica*

Species of *Actinospica* can be differentiated by the features summarized in Table 27.

Cultural characteristics of the two *Actinospica* species are shown in Table 28.

List of species of the genus *Actinospica*

1. **Actinospica robiniae** Cavaletti, Monciardini, Schumann, Rohde, Bamonte, Busti, Sosio and Donadio 2006, 1752[VP]

ro.bi.ni'a.e. N.L. fem. n. *Robinia* scientific name of a genus; N.L. fem. gen. n. *robiniae* of *Robinia*, isolated from a wood of *Robinia pseudoacacia*.

Stable vegetative mycelium. Aerial mycelium appears as enlarged tufts of sporogenous aerial hyphae that upon division form chains of arthrospores (squat to cylindrical, 0.6–0.7 to 1–1.2 × 0.6 µm) with slightly rugose surface. Grows only at acidic pH in the range 4.8–6.2, with optimal growth at pH 5.5. Grows in the range 17–33°C; optimal temperature for growth is in the range 22–28°C; no growth occurs at 14 or 37°C. Does not hydrolyze starch. NaCl at 1% (w/v) significantly inhibits growth. The type strain contains galactose as additional whole-cell sugar.

TABLE 28. Cultural characteristics of *Actinospica* species[a]

Medium[b]/characteristic	*A. robiniae*	*A. acidiphila*
ISP-2:		
Growth	Good	Good
Color	Light yellowish/brown	Beige
Aerial mass	Scant, thin, whitish	Scant, thin, whitish
Soluble pigment	Absent	Absent
ISP-3:		
Growth	Good	Good
Color	Beige to yellowish/brown	Cream to greenish
Aerial mass	Discrete, thin, whitish	Scant to abundant along margins, whitish
Soluble pigment	Absent	Absent
ISP-4:		
Growth	Discrete	Good
Color	Whitish	Whitish
Aerial mass	Absent	Absent
Soluble pigment	Absent	Absent
ISP-5:		
Growth	Scant	Discrete
Color	Whitish	light greenish
Aerial mass	Absent	Scant, whitish
Soluble pigment	Absent	greenish
ISP-6:		
Growth	Absent	Scant
Color	na	Whitish
Aerial mass	na	Absent
Soluble pigment	na	Absent
ISP-7:		
Growth	Discrete	Good
Color	Whitish	Greenish
Aerial mass	Absent	Discrete, white
Soluble pigment	Absent	Brownish

[a] na, Not applicable.

[b] Media according to Shirling and Gottlieb (1966), with pH adjusted to 4.8–5.5 with HCl (see footnote to Table 25, chapter *Catenulispora* for description).

DNA G+C content (mol%): 70.8 (HPLC).

Type strain: GE134769, DSM 44927, JCM 14908, NBRC 102112, NRRL B-24432.

Sequence accession no. (16S rRNA gene): AJ865863.

2. **Actinospica acidiphila** Cavaletti, Monciardini, Schumann, Rohde, Bamonte, Busti, Sosio and Donadio 2006, 1752[VP]

a.ci.di′phi.la. N.L. n. *acidum* (from L. adj. *acidus* sour) an acid; N.L. adj. *philus -a -um* (from Gr. adj. *philos -ê -on*) friend, loving; N.L. fem. adj. *acidiphila* acid-loving.

Stable vegetative mycelium. The aerial mycelium appears as tufts of straight to slightly flexuous hyphae which at maturity carry long chains of up to 30 cylindrical spores, occasionally more. Spores are 0.6–0.8 × 0.5 µm in size and have a rugose surface. Grows only at acidic pH in the range 4.2–6.0, with optimal growth at pH 5.0. Temperature optimum for growth is 28°C; grows in the range 17–33°C; no growth at 14°C or 37°C. The strain hydrolyzes starch. NaCl at 1% (w/v) does not affect growth, while at 2% (w/v) it is not tolerated. The type strain contains arabinose and xylose as additional whole-cell sugars.

DNA G+C content (mol%): 69.2 (HPLC).

Type strain: GE134766, DSM 44926, JCM 14896, NBRC 102107, NRRL B-24431.

Sequence accession no. (16S rRNA gene): AJ865861.

Acknowledgements

We are grateful to Tomohiko Tamura, National Institute of Technology and Evaluation, Japan, for sharing unpublished results, and Manfred Rohde, GBF-Gesellschaft für Biotechnologische Forschung, Germany, for the electron micrographs.

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Order V. **Corynebacteriales** ord. nov.

Michael Goodfellow and Amanda L. Jones

Co.ry.ne.bac.te.ri'a.les. N.L. neut. n. *Corynebacterium* type genus of order; suff. *-ales* ending to denote order; N.L. fem. pl. n. *Corynebacteriales* the *Corynebacterium* order.

Aerobic or facultatively anaerobic, Gram-stain-positive, catalase-positive actinomycetes which may form a branched substrate mycelium that fragments into coccoid- to rod-shaped elements or present as branched filaments, cocci, or as pleomorphic forms. Chemoorganotrophic. Some strains form aerial hyphae. **The wall peptidoglycan contains *meso*-diaminopimelic acid and is of the A1γ type. Arabinose and galactose are major wall sugars. Fatty acid profiles are rich in saturated and unsaturated components and usually contain tuberculostearic acid. Typically contain mycolic acids.** The pattern of 16S rRNA signatures consists of nucleotides at positions 127:134 (G–Y), 564 (C), 672:734 (U–G), 833:835 (U–G), 952:1229 (U–A), and 986:1219 (U–A). Forms a distinct monophyletic branch in the 16S rRNA actinobacterial gene tree.

The order encompasses the families *Corynebacteriaceae* Lehmann and Neumann 1907 emend. Zhi et al. 2009; *Dietziaceae* Rainey et al. 1997 emend. Zhi et al. 2009; *Gordoniaceae* Rainey et al. 1997; *Mycobacteriaceae* Chester 1897 emend. Zhi et al. 2009; *Nocardiaceae* Castellani and Chalmers 1919 emend. Zhi et al. 2009; *Segniliparaceae* Butler et al. 2005 emend. Zhi et al. 2009; *Tsukamurellaceae* Rainey et al. 1997 emend. Zhi et al. 2009; and the genera *Hoyosella* Jurado et al. 2009; *Tomitella* Katayama et al. 2010; and *Turicella* Funke et al. 1994.

Members of the order are found in diverse environments, notably in the soil ecosystem. Some strains are serious pathogens of humans and domesticated animals.

Type genus: **Corynebacterium** Lehmann and Neumann 1896, 350[AL] emend. Bernard, Wiebe, Burdz, Reimer, Ng, Singh, Schindle and Pacheco 2010, 877.

Further descriptive information

Phylogeny. Until recently, actinomycetes with *meso*-diaminopimelic acid (*meso*-A_2pm), arabinose, and galactose in the peptidoglycan (wall chemotype IV *sensu* Lechevalier and Lechevalier (1970a, 1970b) were assigned to two distinct aggregate groups (Goodfellow, 1992; Goodfellow and Lechevalier, 1989; Goodfellow and Minnikin, 1984). Wall chemotype IV actinomycetes that contain mycolic acids (high-molecular-weight 3-hydroxy fatty acids with a long branch in the 2-position) were assigned to the genera *Corynebacterium*, *Dietzia*, *Gordonia*, *Mycobacterium*, *Nocardia*, and *Tsukamurella* in the suborder *Corynebacterineae* Stackebrandt et al. 1997 and most of their mycolateless counterparts to the suborder *Pseudonocardineae* (Warwick et al., 1994) Stackebrandt et al. 1997. The group of mycolic acid-containing actinomycetes encompassed a few species which appear to have lost the capacity to synthesize mycolates, including *Turicella otitidis*, the type species of the monospecific genus *Turicella* (Funke et al., 1993, 1994).

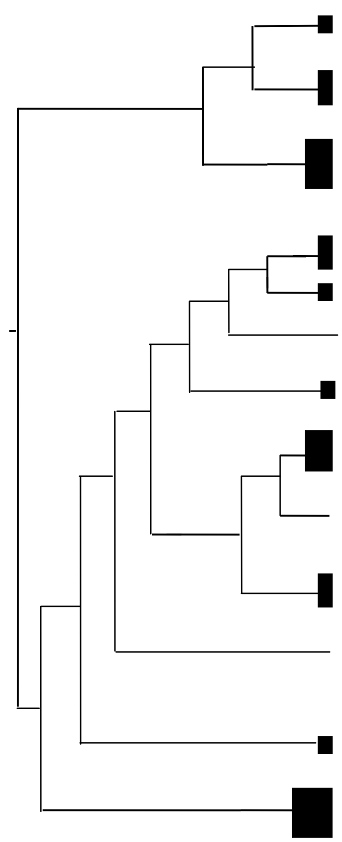
Mycolic acid-containing strains were found to have many phenotypic properties in common (Goodfellow and Cross, 1984; Goodfellow and Wayne, 1982) and formed a recognizable suprageneric group (Mordarski et al., 1980; Stackebrandt and Woese, 1981; Stackebrandt et al., 1980). In addition, representative strains were seen to be closely related using serological techniques (Magnusson, 1976; Pier, 1984), notably immunological procedures which showed the presence of common precipitinogens among corynebacteria, gordoniae, mycobacteria, nocardiae, and rhodococci (Lind et al., 1980; Lind and Ridell, 1976).

The suborders *Corynebacterineae* and *Pseudonocardineae* have been recast in this volume into the orders *Corynebacteriales* and *Pseudonocardiales*, respectively. The order *Corynebacteriales* contains the established mycolic acid-containing genera (Goodfellow and Maldonado, 2006; Stackebrandt et al., 1997) and the recently described taxa *Millisia* Soddell et al. 2006, *Segniliparius* Butler et al. 2005, *Skermania* Chun et al. 1997, *Smaragdicoccus* Adachi et al. 2007, *Tomitella* Katayama et al. 2010, and *Williamsia* Kämpfer et al. 1999. It is becoming increasingly apparent that the order provides a home for a range of mycolateless wall chemotype IV taxa including the genera *Amycolicococcus* Wang et al. 2010 and *Hoyosella* Jurado et al. 2009. Corynebacteria that lack mycolic acids have been assigned to several species including *Corynebacterium amycolatum* Collins et al. 1988a, *Corynebacterium atypicum* Hall et al. 2003, *Corynebacterium capsum* Collins et al. 2004, *Corynebacterium ciconiae* Fernández-Garayzábal et al. 2004, *Corynebacterium kroppenstedtii* Collins et al. 1998, which form distinct phyletic lines in the 16S rRNA *Corynebacterium* tree. Members of the species *Corynebacterium amycolatum* encompass strains previously were misidentified as *Corynebacterium minutissimum*, *Corynebacterium striatium*, and *Corynebacterium xerosis* (Funke et al., 1996; Wauters et al., 1996; Zinkernagel et al., 1996).

Initially, the genera *Corynebacterium*, *Mycobacterium*, and *Nocardia* were assigned to the families *Corynebacteraceae* Lehmann and Neumann 1907, *Mycobacteraceae* Chester 1897, and *Nocardiaceae* Castellani and Chalmers 1919, respectively, based on morphological and staining properties. The composition of these taxa varied over time as classifications were generated using different combinations of phenotypic properties, especially chemotaxonomic and morphological features (Bergey et al., 1939; Goodfellow and Magee, 1998; Lechevalier and Lechevalier, 1970a, 1970b; McClung, 1974). In contrast, mycolic acid-containing genera are now assigned to families based on 16S rRNA gene sequence similarity values and taxon specific 16S rRNA signature nucleotides (Stackebrandt et al., 1997; Zhi et al., 2009). In general, the taxonomic integrity of these families is underpinned by the discontinuous distribution of some chemical markers, as shown in Figure 82.

The current assignment of mycolic acid-containing genera to families is a marked improvement on earlier taxonomies of the group, but even so present classifications need to be seen as part of a progression towards better classifications in the future. It is already clear that the current classification needs to be reassessed with the recognition of novel taxa, as exemplified by the assignment of the mycolateless genus *Amycolicococcus* to the family *Mycobacteriaceae* (Wang et al., 2010). In addition, further comparative taxonomic studies are needed to establish family assignments for the genera *Hoyosella* (Jurado et al., 2009), *Tomitella* (Katayama et al., 2010), and *Turicella* (Funke et al., 1994).

Suprageneric relationships based on 16S rRNA sequence data



Family/genus	Fatty acid pattern[a]	Mycolic acids: Overall size (no. of carbons)[b]	Mycolic acids: Fatty acids released on pyrolysis[b]	Major menaquinones[c]	Phospholipid type[d]
Dietziaceae *Dietzia*	1b	34–38	nd	MK-8(H_2)	PI/PII
Tsukamurellaceae *Tsukamurella*	1b	62–78	22–26	MK-9	PII
Corynebacteriaceae *Corynebacterium*	1a	22–38	8–18	MK-8(H_2),-9(H_2)	PI
Gordoniaceae *Gordonia*	1b	46–77	16–18	MK-9(H_2)	PII
Williamsia	1b	50–56	nd	MK-9(H_2)	PII
Skermania	1b	58–64	16–20	MK-8(H_2,-ωcycl)	PII
Millisia	1b	44–52	nd	MK-8(H_2)	PII
Nocardiaceae *Nocardia*	1b	46–64	12–18	MK-8(H_4,-ωcycl)	PII
Smaragdicoccus	1b	43–49	nd	SQA-8(H_4-ωcycl) and SQB (H_4 dicycl)	PII
Rhodococcus	1b	30–54	12–16	MK-8(H_2)	PII
Tomitella	1a	42–52	nd	MK-9(H_2)	nd
Segniliparaceae *Segniliparus*[e]	nd	nd	nd	nd	nd
Mycobacteriaceae *Mycobacterium*	1b	60–90	22–26	MK-9(H_2)	PII

FIGURE 82. Chemotaxonomic profiles of genera and families of wall chemotype IV actinomycetes, including *Segniliparus*, classified in the order *Corynebacteriales*. Symbols: [a]Fatty acid classification according to Kroppenstedt (1985). Numbers refer to the type of fatty acid biosynthetic pathway and letters to the types of fatty acids (FA) synthesized. Type 1, pathway generating straight-chain fatty acids, including saturated and unsaturated (FA-type 1a), 10-methyl-branched (FA-type 1b), and cyclopropane fatty acids (FA-type 1c), the latter two being derived from the unsaturated compounds. Type 2, pathway yielding terminally-branched fatty acids (FA-type 2), i.e. iso- and anteiso-branched fatty acids. Type 3 encompasses organisms that have complex branched fatty acid types, i.e., both 10-methyl-branched (type 1) and iso- and/or anteiso-branched (type 2) fatty acids. [b]Mycolic acid size (number of carbons) and fatty acids released on pyrolysis were detected by the use of GC-MS and MS (Chun et al., 1997). [c]MK-9(H_8), notation for octahydrogenated menaquinone with nine isoprene units. [d]Characteristic phospholipids: PI, nitrogenous phospholipids absent (with phosphatidylglycerol variable); PII, only phosphatidylethanolamine (Lechevalier et al., 1977; Lechevalier et al., 1981). [e]The wall chemotype of *Segniliparus* has still to be established.

Chemotaxonomy. Chemical characterization of actinomycete cells provides invaluable data for the delineation of suprageneric groups such as the orders *Corynebacteriales* and *Pseudonocardiales*, and for the recognition of genera encompassed by them (Goodfellow and Maldonado, 2006; Katayama et al., 2010; Minnikin and Goodfellow, 1980; Minnikin et al., 1978; Wang et al., 2010). The most useful chemical markers for the classification of wall chemotype IV actinomycetes have been obtained from analyses of cellular fatty acids, menaquinones, mycolic acids, polar lipids, wall peptidoglycans, and DNA base composition (Collins, 1994; Embley and Wait, 1994; Goodfellow and Magee, 1998; Kroppenstedt, 1985; Minnikin and Goodfellow, 1976; Schleifer and Kandler, 1972; Schleifer and Seidl, 1985; Suzuki et al., 1994; Uchida et al., 1999). Some of these analyses have provided qualitative information, as exemplified by the detection of mycolic acid and polar lipid patterns, whereas others have yielded quantitative data, as in the case of cellular fatty acid and menaquinone analyses. The detection of variations in the structures, chain lengths, and degree of unsaturation of mycolic acids have proved to be especially useful in the delineation of mycolic acid-containing genera (Goodfellow et al., 1998, 1999; Goodfellow and Maldonado, 2006; Goodfellow and Magee, 1998; Katayama et al., 2010; Minnikin et al., 1980, 1984a, 1984b, 1984c). It is now known that mycolic acids are involved in the formation of an outer cellular membrane that has a bilayer structure (Hoffmann et al., 2008; Zuber et al., 2008).

The chemical profiles of genera assigned to the families which encompass mycolic acid-containing actinomycetes are shown in Figure 82. The genera classified in the families *Gordoniaceae*, *Mycobacteriaceae*, *Nocardiaceae*, and *Tsukamurellaceae* contain *N*-glycolated muramic acid (Uchida and Aida, 1977, 1979a, 1979b), a phospholipid pattern which includes diphosphatidylglycerol,

phosphatidylethanolamine (taxonomically significant nitrogenous phospholipid), phosphatidylinositol, and phosphatidylinositol mannosides (phospholipid type II *sensu* Lechevalier et al., (1977, 1981) and a fatty acid profile rich in straight-chain, unsaturated, and tuberculostearic acids (Kroppenstedt, 1985), but can be distinguished on the basis of menaquinone composition and the overall chain length of mycolic acids. The genera assigned to the families *Corynebacteriaceae* and *Dietziaceae* can be distinguished on this basis but are united in having a peptidoglycan containing *N*-acetylated muramic acid. In turn, members of the genus *Segniliparus* of the family *Segniliparaceae* are characterized by the presence of multiple chemical functional groups of high-molecular-weight, non-polar mycolic acids (Butler et al., 2005). *Tomitella* strains cannot be readily assigned to any of the mycolic acid-containing families but have a chemotaxonomic profile that distinguishes them from genera assigned to these families (Figure 82). Additional chemotaxonomic properties of the mycolic acid-containing genera are shown in Table 29.

The chemotaxonomic profiles of wall chemotype IV genera which lack mycolic acids are shown in Table 30. All of the genera contain unsaturated menaquinones, *N*-acetylated muramic acid moieties, and a combination of chemical markers which distinguish them from mycolic acid-containing taxa. It is interesting that the type strain of *Hoyosella altamirensis* has a DNA G+C content of 49.3 mol%, the lowest recorded value among all of the taxa classified in the order *Corynebacteriales* (Jurado et al., 2009). Little is known about the chemotaxonomic properties of mycolateless members of the genus *Corynebacterium*; these organisms are rich in saturated and unsaturated fatty acids though only *Corynebacterium kroppenstedtii* contains tuberculostearic acid (Collins et al., 1998; Fernández-Garayzábal et al., 2004; Hall et al., 2003). Ultrarapid pyrosequencing of *Corynebacterium kroppenstedtii* DSM 44385^T indicates that its inability to synthesize mycolic acids may be due to gene loss, including a condensase gene cluster and a mycolate reductase gene (Tauch et al., 2008). *Corynebacterium amycolatum* synthesizes unsaturated menaquinones with nine isoprene units and can thereby be distinguished from *Corynebacterium kroppenstedii* which has corresponding menaquinones, albeit with eight isoprene units like *Hoyosella altamirensis* (Jurado et al., 2009).

Lipoglycans found in the walls of some actinomycetes have been assigned to a number of structural archetypes (Sutcliffe, 1995). The most extensively studied lipoglycans are the mycobacterial lipoarabinomannans (Bricken et al., 2004; Chatterjee and Khoo, 1998; Nigou et al., 2003). However, lipoarabinomannans (LAM) and LAM-like/lipomannan components have been extracted and characterized from other mycolic acid-containing taxa including *Corynebacterium diphtheriae* (Moreira et al., 2008), *Dietzia maris* (Sutcliffe, 2000), *Gordonia rubripertincta* (Flaherty and Sutcliffe, 1999), *Rhodococcus equi* (Garton et al., 2002), *Rhodococcus ruber* (Gibson et al., 2003a), and *Tsukamurella paurometabola* (Gibson et al., 2004), and mycolateless *Amycolatopsis sulfurea* (Gibson et al., 2003b), *Lechevalieria aerocolonigenes* (Gibson et al., 2005), and *Turicella otitidis* (Gilleron et al., 2005). Consequently, it is clear that the distribution of the LAM family of lipoglycans is not limited to mycolic acid-containing genera.

The cell walls of some mycolic acid-containing actinomycetes contain one or more cell-wall channels (Lichtinger et al., 2000, 2001, 2004; Riess et al., 1998; Trias et al., 1992). These channels allow the uptake of hydrophilic solutes through the thick mycolic acid layers which form an effective permeability barrier on the outer surface of cells (Brennan and Nikaido, 1995; Jarlier and Nikaido, 1994) that is analogous to the outer membrane of Gram-stain-negative bacteria. There is evidence that the cell wall of *Corynebacterium amycolatum* contains sufficient lipids to form a permeability barrier on the outer wall surface, thereby accounting for the presence of channel-forming proteins in this organism (Dörner et al., 2009). There are grounds for believing that *Micromonospora purpurea* and *Streptomyces griseus* contain cell-wall channels with properties similar to those of mycolic acid-containing genera (Kim et al., 2002).

Detection of chemotaxonomic markers

Members of genera and families classified in the order *Corynebacteriales* can be distinguished from one another and from corresponding taxa in the phylum *Actinobacteria* by 16S rRNA similarity values and by taxon-specific 16S rRNA oligonucleotide sequences (Stackebrandt et al., 1997; Zhi et al., 2009). However, actinobacterial phylogenies need to be supported by additional evidence, as phylogenetic relationships are not always sufficiently robust to allow groups to be delineated with confidence (Labeda et al., 2011) while major differences can be found in actinobacterial trees based on different algorithms (e.g. Ludwig and Klenk, 2005; Zhi et al., 2009).

Chemical markers have provided an effective way of evaluating actinobacterial phylogenies as they are discontinuously distributed across taxa, as exemplified in Figure 82 and Table 29 and Table 30. Standard chemotaxonomic procedures are available for the detection of wall diamino acids (Hancock, 1994; Staneck and Roberts, 1974), fatty acids (Kroppenstedt, 1985; Suzuki and Komagata, 1983) including mycolic acids (Minnikin et al., 1975, 1980), menaquinones (Collins, 1994; Kroppenstedt, Collins, 1982, Collins, 1985; Minnikin et al., 1984a), muramic acid residues (Uchida and Aida, 1977, 1979a, 1979b; Uchida et al., 1999), and polar lipids (Minnikin et al., 1977, 1984a; Tindall, 1990).

Examination of whole-organism hydrolysates for the presence of *meso*-A_2pm, arabinose, and galactose, and the detection of mycolic acids in whole-cell methanolysates are the first steps in the chemotaxonomic procedure. The thin-layer-chromatographic (TLC) procedures of Staneck and Roberts (1974) provide an easy way of detecting the diagnostic amino acid and sugar markers, while mycolic acids can be detected using the acid methanolysis protocol described by Minnikin et al. (1975); modification of these procedures are described in detail by Goodfellow (1996).

The detection of *meso*-A_2pm, arabinose, galactose, and mycolic acids serves to distinguish *Corynebacteriales* strains from corresponding taxa such as the orders *Actinopolysporiales*, *Calenulosporiales*, *Micrococciniales*, *Micromonosporiniales*, *Pseudonocardiales*, *Streptomycetiales*, and *Streptosporangiales*. The mycolateless wall chemotype IV genera *Amycolicicoccus*, *Hoyosella*, and *Turicella* can be distinguished from corresponding actinomycetes classified in the order *Pseudonocardiales* as they contain unsaturated menaquinones (Table 30); a variety of chromatographic procedures can be used to establish menaquinone profiles (Collins, 1994; Minnikin et al., 1984a). The menaquinone composition of most corynebacteria which lack mycolic acids has still to be established.

TABLE 29. Characteristics of wall chemotype IV genera, including *Segniliparus*[a], classified in the *Corynebacteriales*[b]

Characteristic	*Corynebacterium*	*Dietzia*	*Gordonia*	*Millisia*	*Mycobacterium*	*Nocardia*	*Rhodococcus*	*Segniliparus*	*Skermania*	*Smaragdicoccus*	*Tomitella*	*Tsukamurella*	*Williamsia*
Cell morphology	Pleomorphic rods, often club-shaped; commonly in angular and palisade arrangement	Short rods and cocci	Rods and cocci or moderately branching hyphae	Rudimentary right angled branching	Rods, occasionally branched filaments which fragment into rods and coccoid elements	Mycelium which fragments into rods and cocci	Rods to extensive substrate mycelium which fragments into irregular rods and cocci	Rods	Mycelium resembling a pine tree	Coccoid cells	Irregular rods that exhibit snapping division; cells turn to short coccoid rods after prolonged culture	Rods occur singly, in pairs, or in masses; cocobacillary forms occur	Thin irregular rods or cocci occur singly or in small clusters
Aerial hyphae	Absent	Absent	Absent	Present	Usually absent	Present	Absent	Absent	Present but not visible to the naked eye	Absent	Absent	Absent	Present
Time to growth of visible colonies (d)	1–2	1–3	1–3	1–3	2–40	1–5	1–3	3–4	9–21	7	nd	1–3	1–4
Acid-fastness	Sometimes weakly acid-fast	Not acid-fast	Partially acid–alcohol-fast	Acid–alcohol-fast	Strongly acid-fast	Partially acid fast	Partially acid-fast at some stage of the growth cycle	Acid–alcohol-fast	Not acid fast	nd	nd	Partially acid–alcohol-fast	nd
Strictly aerobic	–	+	+	+	+	+	+	+	–	+	+	+	+
Fatty acid composition[c]	S, U[d]	S, U, T	S, U, T	S, U, T	S, U, T	S, U, T	S, U, T	S, U, T	S, U, T	S, U	S, U	S, U, T	S, U, T
Major menaquinone(s)[e]	MK-8(H_2)	MK-8(H_2)	MK-9(H_2)	MK-8(H_2)	MK-9(H_2)	MK-8(H_4,ω-cycl)	MK-8(H_2)	nd	MK-8(H_4,ω-cycl)	SQA-8(H_4 ω cycl) and SQB (H_4 dicycl)	MK-9(H_2)	MK-9	MK-9(H_2)
Muramic acid type	Acetylated	Acetylated	Glycolated	Glycolated	Glycolated	Glycolated	Glycolated	nd	Glycolated	Glycolated	Glycolated	Glycolated	Glycolated
Mycolic acid pattern[f]	Single spot	Single spot	Single spot	Single spot	Multiple spots	Single spot	Single spot	Multiple spots	Single spot	nd	nd	Two spots	Single spot
Mycolic acids:													
Overall size (number of carbons)	22–38	34–38	46–77	44–52	60–90	46–64	30–54	nd	58–64	43–49	42–52	62–78	50–56
No. double bonds	0–2	0–1	1–6	nd	1–4	0–3	0–4	nd	2–6	nd	nd	1–7	nd
Fatty acids released on pyrolysis	8–18	nd	16–18	nd	22–26	12–18	12–16	nd	16–20	nd	nd	22–26	nd
Phosphatidylethanalamine present in polar lipid patterns	–[g]	+/–	+	+	+	+	+	nd	+	+	+	+	+
DNA G+C content (mol%)	51–67	66–73	63–69	64.7	57–73	63–72	63–73	68–72	67.5	63.7	69.3–71.6	68–78	64–65

[a]The wall chemotype of *Segniliparus* has yet to be established.

[b]Symbols: +, positive; –, negative; and nd, not determined. Data taken from Butler et al., (2005); Soddell et al., (2006); and Adachi et al. (2007).

[c]Abbreviations: S, straight chain; U, unsaturated; T, tuberculostearic acid (10-methyloctadecanoic acid).

[d]*Corynebacterium bovis*, *Corynebacterium minutissimum*, *Corynebacterium urealyticum* and *Corynebacterium variabile* contain tuberculostearic acid (Kämpfer et al., 1999; Lechevalier et al., 1977).

[e]Examples of abbreviations: MK-9(H_2), menaquinone with two of the nine isoprene units hydrogenated; SQA and SQB, smaradiquinones A and B.

[f]Number of mycolic acid spots produced from whole organism methanolysates (Minnikin et al., 1975, 1980). In mycobacterial mycolic acids, double bonds may be converted to cyclopropane rings; methyl branches and other oxygen functions may be present (Dobson et al., 1985).

[g]Present in *Corynebacterium bovis* and *Corynebacterium urealyticum* (Kämpfer et al., 1999).

TABLE 30. Characteristics of the mycolic acid-less type strains of type species of wall chemotype IV genera classified in the order *Corynebacteriales*[a,b]

Characteristic	*Amycolicococcus*	*Hoyosella*	*Turicella*
Micromorphology	Cocci	Cocci found singly, in pairs, or in small groups	Single cells, V-shaped, or in palisades
Aerial hyphae	Absent	Absent	Absent
Major polar lipids[c]	DPG, PC, PE, PG, PI, GluNu	DPG, PE, PG, PI	nd
Muramic acid moieties	Acetylated	Acetylated	Acetylated
Predominant menaquinones	MK-7, 8	MK-8	MK-10, 11
Presence of tuberculostearic acid	+	+	+
DNA G+C content (mol%)	66.0	49.3	65–72

[a] Symbols: +, present; nd, not determined.

[b] Data taken from Funke et al. (1994), Jurado et al. (2009), and Wang et al. (2010).

[c] Abbreviations: DPG, diphosphatidylglycerol; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; PI, phosphatidylinositol and GluNu, unknown glucosamine containing phospholipid.

Mycolic acids show structural variations which range from relatively simple mixtures of saturated and unsaturated components in corynebacteria to the highly complex mixtures characteristic of mycobacteria (Goodfellow and Magee, 1998; Minnikin, 1982, 1993; Minnikin and Goodfellow, 1980). Mycobacterial mycolates have 60–90 carbon atoms with up to 4 double bonds; those of the remaining genera vary in chain length and the number of double bonds (Table 30). Qualitative TLC analyses of mycolic acids after Minnikin et al. (1975) show that the methanolysates of mycobacteria, with the exception of *Mycobacterium brumae*, *Mycobacterium fallax*, and *Mycobacterium triviate* (Dobson et al., 1985; Luquin et al., 1993), give a multispot pattern, tsukamurellae two spots, and most of the remaining taxa a single spot (Table 29); the different Rf values of the mycolic acid spots reflect differences in chain lengths and structures (Embley and Wait, 1994; Minnikin, 1988, 1993; Minnikin et al., 1980; Yassin et al., 1997). *Segniliparus* strains also produce a multispot pattern (Butler et al., 2005). Mycolic esters can be positively identified on thin-layer chromatograms by their characteristic immobility when plates are subsequently washed in methanol water (5:2, v/v) (Minnikin et al., 1975). Mycobacterial species can be assigned to one of several groups based on the presence or absence of different types of mycolic acids (Goodfellow and Magee, 1998; Minnikin, 1988, 1993; Minnikin et al., 1984a, 1984c, 1985); the latter can be detected more precisely by two dimensional TLC using appropriate protocols (Dobson et al., 1985; Embley and Wait, 1994; Minnikin, 1988; Minnikin et al., 1980).

Several precipitation methods have been recommended to distinguish between mycobacterial mycolic acids and those from related taxa. Mycobacterial mycolic acids can be precipitated from ether solution by the addition of an equal (Kanetsuna and Bartoli, 1972) or double volume (Hecht and Causey, 1976) of ethanol. In contrast, mycolic acids from corynebacteria, gordoniae, and rhodococci are not precipitated in these procedures though Hecht and Causey (1976) detected their presence in supernatants by TLC. A more sensitive and reliable precipitation procedure was introduced by Hamid et al. (1993) to separate mycobacteria from other mycolic acid-containing genera. This method is based on the solubility of mycolic acid methyl esters in acetonitrile/toluene (1:2, v/v) and the insolubility of those from mycobacteria in acetonitrile/toluene (3:2, v/v).

Once the presence of mycolic acids has been detected, their esters can be extracted and studied further to yield additional taxonomic data, notably on the overall size of mycolic acids, their degree of unsaturation, and the length of alkyl side chains (Table 29). Methods used for such purposes include pyrolysis gas chromatography (Collins et al., 1982b; Kroppenstedt, 1985), mass spectrometry (Collins et al., 1982a), gas chromatography-mass spectrometry of trimethylsilyl ether and tetrabutyldimethyl derivatives of mycolic acids (Athalye et al., 1984; Pommier and Michel, 1985; Tomiyasu et al., 1981; Yano et al., 1978), and high performance liquid chromatography (HPLC) of *para*-bromophenyl and *N*-methyl-*N*-nitro-*p*-toluenesulfonamide derivatives of mycolic acids (Butler et al., 2005; Butler et al., 1996; Lévy-Trébault et al., 1986). Reverse-phase HPLC of *para*-bromophenyl esters of mycolic-acids provides a particularly rapid way of distinguishing between mycolic acid-containing genera (Butler et al., 1986); appropriate columns, detectors, and solvent gradients have been described by Butler and his colleagues (Butler et al., 1986, 1987; Butler and Kilburn, 1988; De Briel et al., 1992). In addition, different classes of mycobacterial mycolic acids can be separated by HPLC (Butler et al., 2005; Kaneda et al., 1995; Ramos, 1994; Steck et al., 1978). This separation is based upon chain lengths, the degree of unsaturation, and other functional groups in mycolic acids (Svensson et al., 1982).

10-Methyloctadecanoic (tuberculostearic) acid, phosphatidylethanolamine, and the acyl types of muramyl residues in wall peptidoglycans are important markers in the classification and identification of genera which belong to the order *Corynebacteriales* (Table 29 and Table 30). In addition, phosphatidylethanolamine has not been detected in some *Dietzia* species (Li et al., 2008). The genus *Corynebacterium* stands apart from other mycolic acid-containing genera as nearly all of its constituent species lack both phosphatidylethanolamine and tuberculostearic acid, though *Millisia*, *Smaragdicoccus*, and *Tomitella* strains do not contain this latter component. The genera *Corynebacterium* and *Dietzia* can be distinguished from the other mycolic acid-containing genera as they contain *N*-acetyl, as opposed to *N*-glycolyl residues in the glycan moiety of the peptidoglycan. The genera *Amycolicococcus*, *Hoyosella*, and *Tomitella* contain tuberculostearic acid and *N*-acetylated muramic acid residues, though differences are seen in the polar lipid patterns of the

former two taxa. Standard procedures are available for the detection of tuberculostearic acid (Kroppenstedt, 1985), phosphatidylethanolamine (Minnikin et al., 1984a), and the acyl residues of wall peptidoglycans (Uchida et al., 1999). A small-scale integrated procedure can be used for the detection of wall and lipid markers (O'Donnell et al., 1985).

Most of the genera assigned to the order *Corynebacteriales* can be separated solely on the basis of their predominant menaquinones (Table 29 and Table 30). The presence of fully unsaturated menaquinones with nine isoprene units (MK-9) serves to distinguish tsukamurellae from all other mycolic acid-containing genera (Collins et al., 1988b; Nam et al., 2004) and from the unsaturated menaquinones characteristic of the genera *Amycolicoccus*, *Hoyosella*, and *Turicella* (Funke et al., 1994; Jurado et al., 2009; Wang et al., 2010). Nocardiae and skermaniae can be distinguished from all of the other taxa as they contain predominant proportions of hexahydrogenated menaquinones with eight isoprene units where the last two are cyclized (Blackall et al., 1989; Chun et al., 1997; Collins et al., 1987; Howarth et al., 1986). In turn, smaragdicocci contain two novel cyclic forms of menaquinones, smaragdiquinone A-8 (H_4, ω-cycl) and smaragdiquinone B-8 (H_4, ω-cycl) (Adachi et al., 2007). Corynebacteria, dietziae, millisiae, and rhodococci all contain major amounts of dihydrogenated menaquinones with eight isoprene units as the predominant components; gordoniae, mycobacteria, and williamsiae have major proportions of the corresponding component with nine isoprene units (Collins et al., 1977, 1985; Goodfellow and Maldonado, 2006). Detailed analytical procedures for establishing menaquinone profiles have been described (Collins, 1994; Kroppenstedt, 1982, 1985), including the integrated method introduced for the analysis of bacterial quinones and polar lipids (Minnikin et al., 1984a).

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Family I. **Corynebacteriaceae** Lehmann and Neumann 1907[AL] emend. Stackebrandt, Rainey and Ward-Rainey 1997, 485 emend. Zhi, Li and Stackebrandt 2009, 593

Hans-Jürgen Busse

Co.ry.ne.bac.te.ri.a.ce′a.e. N.L. neut. n. *Corynebacterium* type genus of the family; suff. *-aceae* ending to denote a family; N.L. fem. pl. n. *Corynebacteriaceae* the *Corynebacterium* family.

The pattern of 16S rRNA signatures consists of nucleotides at positions 250 (U), 316:337 (U–G), 418:425 (C–G), 586:755 (U–G), 599:639 (C–G), 662:743 (U–G), 987:1218 (G–C), and 1059:1198 (U–A). **The peptidoglycan type is A1γ with directly cross-linked *meso*-diaminopimelic acid. The cell wall contains arabinogalactan**. The muramic acid type is acetyl. **Major fatty acids are $C_{16:0}$, $C_{18:1}$ ω9*c***; tuberculostearic acid (10-methyl $C_{18:0}$) may be present. **The quinone system consists exclusively of menaquinones**; most strains contain major amounts of **menaquinones with either eight or nine isoprenoic units in the side chain, one of which is saturated [MK-8(H_2), MK-9(H_2) or a mixture of both]**, but completely unsaturated menaquinones are found in *Turicella otitidis*. Most *Corynebacterium* species contain **mycolic acids with 22–38 carbons, but some species lack** mycolic acids, as does *Turicella otitidis*.

The family contains the type genus *Corynebacterium* and the genus *Turicella*.

DNA G+C content (mol%): 46–74.

Type genus: **Corynebacterium** Lehmann and Neumann 1896, 350[AL] emend. Bernard, Wiebe, Burdz, Reimer, Ng, Singh, Schindle and Pacheco 2010, 877.

Further descriptive information

The family *Corynebacteriaceae* is composed of the genera *Corynebacterium* with more than 80 species and the monospecific genus *Turicella*. These taxa form a clade which is clearly separated from related families classified in the order *Corynebacteriales*. 16S rRNA gene sequence similarities between some *Corynebacterium* species are down to <92%, whereas *Turicella otitidis* shares similarity values higher than 92% with several *Corynebacterium* species. Phylogenetic calculations based on 16S rRNA gene sequences (Brennan et al., 2001; Collins et al., 1998; Ludwig et al., 2009b; Pascual Ramos et al., 1997; Ruimy et al., 1995) place *Turicella otitidis* within the radiation of *Corynebacterium* species and not as a separated line of descent within the family.

Most *Corynebacterium* species contain mycolic acids, though *Corynebacterium amycolatum*, *Corynebacterium atypicum*, *Corynebacterium caspium*, *Corynebacterium ciconiae*, *Corynebacterium kroppenstedtii*, and *Turicella otitidis* lack this component (Collins et al., 1988a, 1998, 2004; Fernández-Garayzábal et al., 2004; Funke et al., 1994; Hall et al., 2003). The length of the mycolic acids found in strains varies between 22 and 38 carbons, but usually only one or two mycolic acid species predominate (Collins et al., 1982b). *Corynebacterium bovis* contains major amounts of a mycolic acid composed of 28 carbons with a single double bond, though two members of the species exhibit a major mycolic acid with 24 carbons with a single double bond; in *Corynebacterium diphtheriae* the major mycolic acid contains either 32, 34, or 36 carbons each with two double bonds or 32 carbons with a single double bond and a second major compound consisting of 34 carbons with two double bonds. Mycolic acids with 34 carbons with one double bond have been found in *Corynebacterium callunae*, *Corynebacterium glutamicum*, *Corynebacterium minutissimum*, and *Corynebacterium mycetoides*; *Corynebacterium pseudodiphtheriticum* strains show a major, completely unsaturated mycolic acid with 32 carbons and 34 carbons with one double bond. Strains of *Corynebacterium pseudotuberculosis* contain major amounts of mycolic acids with either 32 or 36 carbons with one or two double bonds, 34 and 36 carbons each with one double bond, 32 and 36 carbons with the smaller mono-unsaturated and the larger di-unsaturated or two mycolic acid species with 32 carbons with one completely saturated and the other mono-unsaturated.

Like other members of the order *Corynebacteriales*, the fatty acid composition of representatives of the family *Corynebacteriaceae* is mainly composed of saturated and unsaturated, unbranched acids with $C_{16:0}$ and $C_{18:1}$ ω9*c* predominating, though some species also contain tuberculostearic acid (10-methyl-$C_{18:0}$), including *Corynebacterium ammoniagenes* (Collins et al., 1982a), *Corynebacterium bovis* (Collins et al., 1998), *Corynebacterium cystitidis*, *Corynebacterium minutissimum*, and *Corynebacterium pilosum* (Herrera-Alcaraz et al., 1990), *Corynebacterium kroppenstedtii* (Collins et al., 1998), *Corynebacterium terpenotabidum* (Takeuchi et al., 1999), *Corynebacterium urealyticum* (Pitcher et al., 1992), and *Corynebacterium variabilis* (Collins, 1987a; Collins et al., 1982b). The peptidoglycan is of A1γ type with the characteristic diamino acid diaminopimelic acid.

Quinone systems reported for *Corynebacterium* species are MK-8(H_2), MK-9(H_2), or a mixture of both (Bernard et al., 2010; Collins, 1987b; Collins et al., 1979, 2001b; Collins and Jones, 1983b; Fudou et al., 2002; Pitcher et al., 1992; Takeuchi et al., 1999; Zimmermann et al., 1998); significant amounts of MK-7(H_2) may also be present (Kämpfer et al., 2009). In contrast, *Turicella otitidis* contains completely unsaturated menaquinones (MK-10, MK-11). Some significant variability in the G+C content of genomic DNA of *Corynebacterium* species has been reported. The lowest value was determined for *Corynebacterium kutscheri* (46 mol%) and the highest for *Corynebacterium auris* (74 mol%).

Taxonomic comments

The family *Corynebacteriaceae* contains the type genus *Corynebacterium* and the monospecific genus *Turicella*. The status of *Turicella* as a genus is supported by phenotypic characteristics (completely unsaturated menaquinones MK-10 and MK-11, lack of mycolic acids). In contrast, 16S rRNA gene sequence data, G+C content, and heterogeneity in several chemotaxonomic characteristics that are considered to be useful for defining genera suggest that the status of the genus *Corynebacterium* and affiliated species needs to be revisited.

A strong case can be made for assigning *Corynebacterium* species to several genera based on 16S rRNA gene sequence similarities. The intra-generic 16S rRNA gene similarities of most prokaryotic genera are at or above 95%, whereas the lowest sequence similarities among *Corynebacterium* species are between 91–92%. The G+C content of genomic DNA within

genera is rarely more than 10 mol% (Goodfellow et al., 1997). In contrast, the DNA base composition of the genus *Corynebacterium* varies between 46 (*Corynebacterium kutscheri*; Pitcher, 1983) and 74 mol% (*Corynebacterium auris*; Funke et al., 1995c). Other chemical markers which might be useful in the assignment of *Corynebacterium* species to novel genera include menaquinone composition and the presence/absence of mycolic acids. However, the task of unraveling the structure of the genus *Corynebacterium* is a daunting one, as the descriptions of many of the constituent species lack information on key chemical markers.

Genus I. **Corynebacterium** Lehmann and Neumann 1896, 350[AL] emend. Bernard, Wiebe, Burdz, Reimer, Ng, Singh, Schindle and Pacheco 2010, 877

KATHRYN A. BERNARD AND GUIDO FUNKE

Co.ry.ne.bac.te′ri.um. Gr. n. *coryne* a club; L. neut. n. *bacterium* a rod, and in biology a bacterium (so called because the first ones observed were rod-shaped); N.L. neut. n. *Corynebacterium* a club bacterium.

Straight to slightly curved rods with tapered ends. Rods are usually short or of medium length. Club-shaped forms may be observed; sometimes ellipsoidal, ovoid or rarely, "whip handles" (see below, *Corynebacterium matruchotii*) or thinner rods with bulges (see below, *Corynebacterium sundsvallense*) observed. **Snapping division produces angular and palisade arrangements of cells. Gram-stain-positive; some cells stain unevenly**. Metachromatic (synonym being polyphosphate) granules may be observed for some species. Not-acid-fast (Ziehl–Neelsen stain), and no species has aerial mycelium. Nonsporeforming. **All species are nonmotile**. **All species are catalase positive**. All species are oxidase negative except for *Corynebacterium bovis*, *Corynebacterium aurimucosum*, *Corynebacterium doosanense*, and *Corynebacterium maris* (below). **Many species are facultatively anaerobic and some are aerobic**. Chemoorganotrophs. Some species are lipophilic. Many species produce acid from glucose and some other sugars in peptone media. Several species alkalinize citrate as sole carbon sources, but most do not.

Cell-wall peptidoglycan is based on *meso*-diaminopimelic acid (*meso*-DAP) (variation of A1γ of Schleifer and Kandler). Glycan type of cell walls contains acetyl residues. **Major cell-wall sugars are arabinose and galactose** (also referred to as arabinogalactan), but occasionally other sugars detected. **Short-chain mycolic acids** (also referred to as corynomycolates, corynemycolates, or α-alkyl-β-hydroxy-long-chain fatty acids) **22–36 carbons in length may be present**, but some species lack mycolates entirely (see below, *Corynebacterium amycolatum*, *Corynebacterium atypicum*, *Corynebacterium caspium*, *Corynebacterium ciconiae*, and *Corynebacterium kroppenstedtii*). Long-chained cellular fatty acids are of the straight-chain saturated and monounsaturated types, with significant amounts of hexadecanoic (palmitic, $C_{16:0}$), octadecanoic ($C_{18:0}$), and *cis*-9-octadecenoic ("oleic", $C_{18:1}$ ω9*c*) acids as major components. Small or moderate amounts of tuberculostearic acid (TBSA) (10-methyl $C_{18:0}$) and other cellular fatty acids may also be present. *Corynebacterium diphtheriae*, *Corynebacterium ulcerans*, *Corynebacterium pseudotuberculosis*, and *Corynebacterium vitaeruminis* are the only species where significant amounts of the 16:1 isomer, $C_{16:1}$ ω7*c*, are observed and corynemycolates may be observed to coelute with cellular fatty acids (Bernard et al., 1991). Branched-chain or hydroxylated fatty acids are absent or found only in trace amounts. Metabolic products of fermentation may include small volumes of acetic, succinic, and lactic acids, but production of propionic acid is species specific (Bernard et al., 2002). Dihydrogenated menaquinones with either **eight [MK-8(H_2)] and/or nine [MK-9(H_2)] isoprene units** are present. In addition, MK-7(H_2) has been detected for *Corynebacterium glaucum* and *Corynebacterium lubricantis*; small amounts of MK-10(H_2) have also been found for *Corynebacterium thomssenii* (below). Phospholipids include simple, phosphatidylinositol, phosphatidylinositol dimannoside(s), phosphatidylglycerol trehalose dimycolates, and other glycolipids (Collins and Cummins, 1986; Yague et al., 1997). Phosphatidyethanolamine is absent except for *Corynebacterium bovis* and *Corynebacterium urealyticum* (Kämpfer et al., 1999).

DNA G+C content (mol%): 46–74.

Type species: **Corynebacterium diphtheriae** (Kruse 1886) Lehmann and Neumann 1896, 350 ("*Bacillus diphtheria*" Kruse *in* Flügge1886, 225).

Further descriptive information

The genus *Corynebacterium* contains 84 validly published named species at the time of writing (May 2010). Phylogenetic relatedness among *Corynebacterium* species was first inferred by nearly full 16S rRNA gene sequence relationship studies in 1995 (Pascual et al., 1995; Ruimy et al., 1995) (Figure 83). Since the 1st edition of the *Manual*, members of this genus have been restricted to species most closely related by 16S rRNA gene sequencing to the type species, *Corynebacterium diphtheriae.* The genus *Corynebacterium* and the closely related genus, *Turicella*, are sole genera in the family *Corynebacteriaceae* (Ludwig et al., 2009a; Zhi et al., 2009). In addition to a close relationship by 16S rRNA or *rpoB* gene sequence analyses (described below), *Corynebacterium* species share phenotypic, chemotaxonomic, and other commonalities, and so taxa from the 1st edition of this *Manual* which do not fit this description have now been reassigned to other genera and families (outlined below). The degree of variance using 16S rRNA gene sequencing is 2% or greater for many species in this genus, and so most are discernable from each other without study of additional gene targets if comparison of nearly full (~1400–1500 bp) sequence of the 16S rRNA gene is used (Stackebrandt and Ebers, 2006). Shorter, but more rapidly analyzed lengths (400–500 bp) of 16S rRNA gene sequence have been used for characterization of *Corynebacterium* species for rapid identification (Tang et al., 2000). However, when full sequencing data for the 16S rRNA gene for all taxa are analyzed, some species are related with a degree of variance which is ≤2%. These include: *Corynebacterium afermentans*, *Corynebacterium coyleae*, *Corynebacterium mucifaciens* (<2%); *Corynebacterium aurimucosum*, *Corynebacterium minutissimum*, and *Corynebacterium singulare* (<2%); *Corynebacterium sundsvallense*

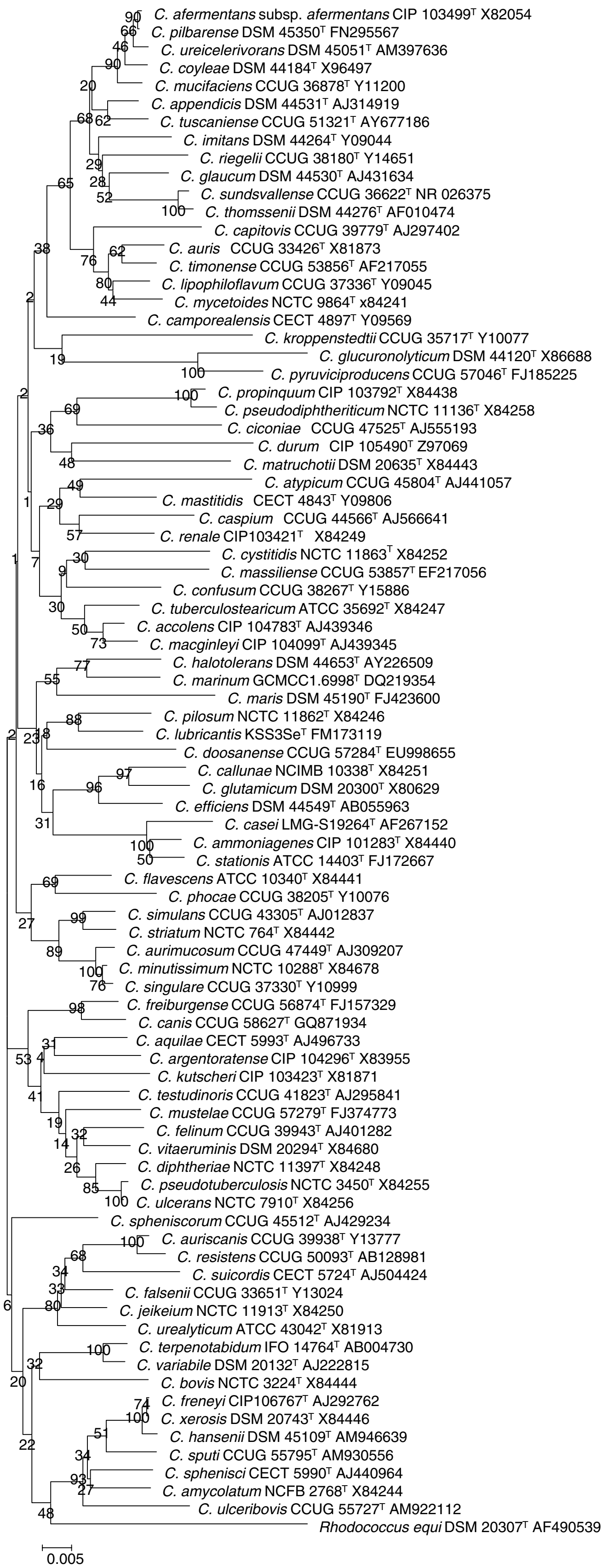

FIGURE 83. Phylogenetic tree of 16S rRNA gene sequences using type strains of *Corynebacterium* species, with *Rhodococcus equi* as outlier. Bar represents % substitutions. Alignment was done using CLUSTAL W

and *Corynebacterium thomssenii* (<1.5%); *Corynebacterium ulcerans* and *Corynebacterium pseudotuberculosis* (<1% to each other, both <2% to *Corynebacterium diphtheriae*); *Corynebacterium propinquum* and *Corynebacterium pseudodiphtheriticum* (<2%); *Corynebacterium xerosis*, *Corynebacterium freneyi*, and *Corynebacterium hansenii* (<2%); and *Corynebacterium macginleyi* and *Corynebacterium accolens* (<2%).

Corynebacterium species which cannot be discerned by 16S rRNA gene sequencing can be further characterized by sequencing of other gene targets. One widely used method is that of *rpoB* gene sequencing, as an adjunct to or in lieu of, 16S rRNA gene sequencing. When full or partial *rpoB* gene sequences were used, taxa with ≥95% identity are thought to be members of the same species and those with ≥ 98% identity were deemed to be members of the same subspecies (Khamis et al., 2004, 2005). *Corynebacterium xerosis*, *Corynebacterium freneyi*, and *Corynebacterium hansenii*, which could not be discerned using 16S rRNA, 16S–23S spacer region or *rpoB* gene sequencing, were determined only by DNA–DNA hybridization to be separate species (Renaud et al., 2007). Restriction length polymorphism using *cfo*I fragment of the 16S–23S region has been used to characterize *Corynebacterium freneyi* strains (Funke and Frodl, 2008b). Sequencing of a gene associated with cell division, *div*IVA, has been applied to discern *Corynebacterium amycolatum* from closely related species (Letek et al., 2006). A PCR-based assay to detect *dtxR*, a chromosomal iron-dependent repressor gene associated with *Corynebacterium diphtheriae* strains, has been used as a method to screen for these organisms (Pimenta et al., 2008). A number of other approaches have been used to try to separate closely related *Corynebacterium* species. Amplified rRNA-restriction analysis (ARDRA) using the enzymes *alu*I, *cfo*I, and *rsa*I was applied as a means to differentiate among otherwise closely related species (Vaneechoutte et al., 1995). Length polymorphisms of the16S–23S rRNA gene spacer region were used to reveal unexpected heterogeneity among strains of species otherwise thought to be monophyletic (Aubel et al., 1997). Ribotyping and RFLP (restriction fragment length polymorphisms) have been used to differentiate species using various enzymes (Björkroth et al., 1999). Entire genome studies of *Corynebacterium* species have been completed to date for *Corynebacterium glutamicum* (ATCC 13032^{T}) (Kalinowski et al., 2003), *Corynebacterium efficiens* (YS-314^{T}) (Nishio et al., 2003), *Corynebacterium diphtheriae* (NCTC 13129) (Cerdeno-Tarraga et al., 2003), *Corynebacterium jeikeium* (strain K411) (Tauch et al.,

FIGURE 83. (continued) software with MEGA 4 software (Kumar et al., 2008). Numbers represent neighbor joining (NJ) distances, with robustness estimated after 1000 bootstraps. Sequence from strains found in collections cited on the World Federation of Culture Collections website (www.wfcc.info) were used. ATCC, American Type Culture Collection, Manassas, VA, USA; CCUG, Culture Collection, University of Goteborg, Goteborg, Sweden; CECT, Coleccion Espanola de Cultivos Tipo, Valencia, Spain; CIP, Collection Institut Pasteur, Paris, France; DSM, Deutsche Sammlung von Mikroorganismen und Zellkulturen, Braunschweig, Germany; LMG, Laboratorium voor Microbiologie, Universiteit Gent (part of the) Belgian Coordinated Collections of Microorganisms, Gent, Belgium; NBRC, National Institute of Technology and Evaluation (NITE) Biological Resource Center, Chiba, Japan. NCIMB, National Collections of Industrial Food and Marine Bacteria (incorporating the NCFB), Aberdeen Scotland and NCTC, National Collection of Type Cultures, London UK, both are part of the United Kingdom National Culture Collection (UKNCC).

2005), *Corynebacterium urealyticum* (DSM 7109^T) (Tauch et al., 2008b), *Corynebacterium kroppenstedtii* (DSM 44385^T) (Tauch et al., 2008a), and a black-pigmented *Corynebacterium aurimucosum* strain (CN-1) (Trost et al., 2010). These range in size from *Corynebacterium urealyticum* (2,369,217 bp) to *Corynebacterium glutamicum* (3,309,401 bp), with a number of additional projects involving *Corynebacterium* species under way as of this writing. Although still in its infancy, comparative analysis of these entire genomes has provided insight into common or different protein expressions found among these taxa including a set of conserved, DNA-binding transcriptional regulators consisting of 28 proteins that is involved with the regulation of cell division, septation, SOS and stress response, carbohydrate metabolism, and macroelement and metal homeostasis (Brune et al., 2005).

Corynebacterium species epidemiologically linked in an outbreak have been characterized comprehensively using several molecular typing schemes. Concerted efforts to track a large diphtheria outbreak in former Soviet Union (FSU) states, spearheaded by the European laboratory working group on diphtheria (DIPNET), have been studied by various methods, particularly rRNA gene restriction pattern determination (ribotyping). Multilocus enzyme electrophoresis (MEE), yielding an electromorph (ET) type, pulsed field gel electrophoresis (PFGE), randomly amplified polymorphic DNA (RAPD), and amplified fragment length polymorphism (AFLP) analyses have been used to characterize outbreak strains (De Zoysa et al., 1995b; Popovic et al., 1996) and isolates which predated the outbreak from those geographic areas (Skogen et al., 2002). By these means, a clonal group of closely related strains, particularly ribotypes Sankt-Peterburg, Rossija and others, were found to be largely responsible for the Russian and FSU state countries outbreak. With this information, movement of clonal strains to other countries could be tracked (Mokrousov et al., 2005). Four molecular methods (ribotyping, AFLP, PFGE, and RAPD) for typing *Corynebacterium diphtheriae* strains have been compared. Ribotyping was found to provide the most useful and discriminatory information (De Zoysa et al., 2008) and used to propose international nomenclature rules (Grimont et al., 2004). A multi-method approach was used to characterize a large number of American and Canadian isolates; clonal groups distinct from European outbreak strains and in circulation for many years, in spite of universal vaccination programs in both countries, were found to persist (Marston et al., 2001). Further discrimination among strains recovered during the Russian/FSU state outbreak have been studied using a microarray approach, the use of which could arguably eliminate inherent laboratory inter-reproducibility issues or cumbersome methods particularly associated with PFGE, MEE, and ribotyping (Mokrousov et al., 2009). A multilocus, sequence typing (MLST) scheme has been described for *Corynebacterium diphtheriae* surveillance and data derived from sequencing seven housekeeping genes (*atpA*, *dnaE*, *dnaK*, *fusA*, *leuA*, *odhA*, *rpoB*) were curated, compared to a database, assigned a sequence type, and interpreted (Bolt et al., 2010; Dallman et al., 2008).

Molecular typing methods for *Corynebacterium* species other than *Corynebacterium diphtheriae* have been occasionally described. Thirty-two strains of *Corynebacterium urealyticum* from humans or animals, recovered over several years, were extensively characterized by ribotyping. There, most human isolates were generally found to be multidrug-resistant (MDR) and clustered into ribotypes called 8, 9, and 10, in contrast with animal strains which were assigned to ribotypes 5 and 6 and were more susceptible to antibiotics (Nieto et al., 2000). A unique strain of *Corynebacterium striatum* which produced a diffusible brown pigment and was associated with respiratory, wound, or blood infections in an intensive care unit (ICU) was found to be clonal, when studied by DNA restriction fragment patterns and Southern hybridization with an *att* site probe, whereas apigmented, contemporary strains to the outbreak had diverse genetic patterns (Leonard et al., 1994). RAPD method was used to study an outbreak among patients in a surgical ICU, where a genetically and biochemically identical clone with identical antibiogram, had spread from patient to patient and could be discerned from other strains circulating in the ward over a 1 year period (Brandenburg et al., 1996). Ribotyping and RAPD were used to prove that cutaneous sites and blood culture isolates of *Corynebacterium striatum* from the same patient were identical (Martin et al., 2003). PFGE was used to evaluate 36 MDR *Corynebacterium striatum* strains recovered primarily from ventilatory-related respiratory or central venous catheter infections from three hospitals over a 2-year period, where infection was associated with a single clone possessing *ermX*, *tet A/B*, *cmx A/B*, and *aphA1* resistance genes (Campanile et al., 2009). PFGE was used to type 48 *Corynebacterium striatum* strains recovered from patients in a community hospital over a 5-year period, primarily from respiratory specimens; these could be assigned into 14 patterns with 20 subtypes and provide definitive linkages for nosocomial acquisition (Otsuka et al., 2006). Strains of *Corynebacterium ulcerans* recovered from a patient and her dog were found to be identical but differed from reference strains when characterized by ribotyping (Lartigue et al., 2005). Ribotyping was also used to link infection by a toxigenic strain of *Corynebacterium ulcerans* with a patient and companion animals (two dogs, one cat) (Hogg et al., 2009). An outbreak of fluoroquinolone-resistant *Corynebacterium macginleyii* was studied by MLST of seven housekeeping genes (*adk*, *dnaA*, *fumC*, *gltA*, *gyrB*, *icd*, and *purA*) in parallel to RAPD analysis, with the two methods being found to be equally discriminating (Eguchi et al., 2008). *Corynebacterium pseudotuberculosis* outbreaks in sheep and goats have been studied using PFGE (Connor et al., 2007).

Metabolic pathways and metabolism. *Corynebacterium* species historically have been characterized as having metabolisms which are fermentative, oxidative, or neither fermentative or oxidative (von Graevenitz and Bernard, 2003). Three closely related species, *Corynebacterium glutamicum*, *Corynebacterium callunae*, and *Corynebacterium efficiens*, have had very extensive metabolic, chemotaxonomic, and genetic properties studied because of their usefulness in biotechnological production of amino acids (Bayan et al., 2003; Wendisch et al., 2006). Comprehensive metabolic pathways and putative functions have been conjectured for species where complete genome studies have been done (Brune et al., 2005; Cerdeno-Tarraga et al., 2003; Kalinowski et al., 2003; Mokrousov, 2009; Nishio et al., 2003; Nishio et al., 2004; Tauch et al., 2008a, 2008b; Wendisch et al., 2006).

Pathogenicity, ecology, miscellaneous. *Corynebacterium diphtheriae*, *Corynebacterium ulcerans*, and *Corynebacterium. pseudotuberculosis* are the only species which may produce diphtheria toxin (DT), a potent exotoxin which plays a significant role in pathogenicity. The resultant disease, diphtheria, has histori-

cally been associated with significant morbidity and mortality in humans and animals, prior to universal use of an efficacious vaccine in countries around the world with higher socio-economic means (Funke et al., 1997f). In diphtheria, DT contributes to the formation of a pseudomembrane in the nasopharynx of the patient; although the organism is rarely found outside the infected area, the toxin, once absorbed by the circulatory system, can cause systemic complications, such as myocarditis and neuritis. Therefore, early administration of diphtheria antitoxin (DAT), a commercial immunoglobulin which neutralizes circulating DT, is critical for patient care, but local stockpiles of DAT may not exist or be in limited supply in countries where diphtheria has low prevalence (Wagner et al., 2009). Diphtheria is still endemic in some subtropical and tropical countries as well as among individuals of certain ethnic groups (e.g. indigenous peoples in the Americas and Australia). At population level, endemic geographically-specific variants of *Corynebacterium diphtheriae* are influenced by human host factors, public health control efforts (i.e. universal vaccination), and socio-economic conditions. Importation of (toxigenic) strains, or lysogenization by a bacteriophage (also called corynephage) of local strains, may instigate an outbreak (Mokrousov, 2009).

It has been known since the 1950s that nontoxigenic strains of *Corynebacterium diphtheriae* could become toxigenic after infection by temperate bacteriophages and a one-to-one correlation exists between the presence of the *tox* gene mechanism on a bacteriophage and subsequent DT production. The biology, structure, and molecular epidemiology of DT and the *tox* gene have been comprehensively reviewed (Holmes, 2000; Yates et al., 2006). Fragment A of the *tox* gene contains ADP-ribosyltransferase activity; fragment B contains the receptor-binding and membrane-associating domains, with the structural *tox* gene being under the control of the chromosomal iron-dependant repressor, *dtxR*, and a promoter-operator region (Nakao et al., 1996).

Most nontoxigenic strains of *Corynebacterium diphtheriae* and most *tox* gene-negative bacteriophages do not contain detectable *tox*-related DNA sequences (Holmes, 2000). However, some strains contain detectable *tox* genes but do not express DT. It has been suggested that these strains have one or more of a variety dysfunctional genetic mechanisms which preclude DT production (von Graevenitz and Bernard, 2003); such isolates are considered as non-toxigenic with respect to public health response (Efstratiou et al., 1998; Efstratiou and George, 1999). The *dtxR* gene has been demonstrated to be heterogeneous, but all subtypes of this gene, if functional in strains lyosogenized by corynephage, could theoretically produce DT, and the possibility exists that non-toxigenic strains could revert to toxigenic status (De Zoysa et al., 2005).

Corynephage insert into the *Corynebacterium diphtheriae* chromosome at either one of two specific attachment sites, *attB1* and *attB2* (Oram et al., 2007; Seto et al., 2008). Protein transcribed by the DT repressor gene (*dtxR*), serves as a global repressor of metabolism in *Corynebacterium diphtheriae*. Cellular functions which are negatively regulated by iron (Fe^{2+}) levels include production of DT, synthesis of a siderophore (also called corynebactin), corynebactin-dependant iron uptake, and utilization of iron from heme (Qian et al., 2002). DT catalyzes the NAD-dependant ADP ribosylation of elongation factor 2 and inhibits protein synthesis and death in cells from humans or susceptible animals (Qian et al., 2002).

Diphtheria-like disease in humans as manifested by *Corynebacterium ulcerans*, although long understudied, is now being described as an emerging, possibly zoonotic disease (Bonmarin et al., 2009; Hogg et al., 2009). Different opinions exist on the efficacy of diphtheria toxoid vaccine to protect against the effects of infection by toxigenic *Corynebacterium ulcerans* (Schuhegger et al., 2008a, 2008b). Differences (up to ~5%) exist between nucleotide and amino acid sequences of *Corynebacterium diphtheriae* and *Corynebacterium ulcerans tox* genes (Sing et al., 2003); therefore, further study is required to clarify the efficacy of diphtheria toxoid to protect against disease caused by *Corynebacterium ulcerans*. Features of the attachment site in *Corynebacterium ulcerans* have been reviewed (Seto et al., 2008).

Complete genome analysis of a strain of *Corynebacterium jeikeium*, an opportunistic pathogen which is usually MDR and always lipophilic, revealed a 14,323-bp bacteriocin-producing plasmid which is designated pKW4. This analysis revealed that "lipophila" in *Corynebacterium jeikeium*, but probably in all other lipophilic species described in this section, is expression of a fatty acid auxotrophy due to the absence of fatty acid synthase (Tauch et al., 2005). This phenomenon was also observed from complete genome analysis of the lipophile *Corynebacterium kroppenstedtii* which, as a species which lacks mycolates, was also found to lack a mycolate reductase gene (Tauch et al., 2008a).

The role of plasmids, as bearers of virulence factors or in association with increased pathogenicity among *Corynebacterium* species, is poorly understood. The complete genome of an unusual black-pigmented strain of *Corynebacterium aurimucosum* (ATCC 700975, designated the type strain of *Corynebacterium nigricans*, CN-1^T) that was associated with the female genital tract of a woman who experienced a spontaneous abortion during month 6 of her pregnancy, was described. A 29,037 bp plasmid designated pET44827 was found to code for a non-ribosomal peptide synthetase, which appeared to play a key role in the synthesis of the black pigment; this pigment was conjectured to play a role in both protecting the bacterium from the high hydrogen peroxide concentration of the vagina and play a role in causing complications in pregnancy (Trost et al., 2010).

Phospholipase D (Pld) is a major virulence factor of *Corynebacterium pseudotuberculosis*, the organism causing caseous lymphadenitis in sheep and goats in addition to being able to harbor corynephage and express DT. Pld is detected in *Corynebacterium ulcerans* as well as *Arcanobacterium hemolyticum*; in clinical microbiology laboratories, the presence of Pld is demonstrated by the inhibition of the Christie–Atkins–Munch–Petersen (CAMP) reaction (also called the reverse CAMP test) (Barksdale et al., 1981). Pld regulation and expression has been found to be a complex process which includes assisting with dissemination of the bacterium from site of infection to the lymph nodes and thereafter reducing macrophage viability (McKean et al., 2007). Rapid detection of *Corynebacterium pseudotuberculosis* among diseased animals has been made possible by the use of a multiplex assay to detect the 16S rRNA, *rpoB*, and *pld* genes of this bacterium (Pacheco et al., 2007).

Pathogenicity, virulence factors and ecological niches for other species remain understudied and largely unknown.

Antibiotic susceptibility and mechanisms of resistance. Antibiotic susceptibility of *Corynebacterium* species is less well studied compared with many other Gram-stain-positive rods because, for many years, "diphtheroids" were dismissed as contaminants and did not merit either the resources or interest required for further study. In addition, there was no consensus on standardized protocols and breakpoint interpretation for many years, so a wide variety of methods were independently used for clinically relevant isolates. Application of the broth microdilution method and specific MIC breakpoints were formally recommended by the CLSI in 2006 (Clinical Laboratory Standards Institute, 2006). However, Etest (e.g. Martinez-Martinez et al., 1995a), variations of agar dilution (e.g. Lagrou et al., 1998; Soriano et al., 1995), and disk diffusion (e.g. Riegel et al., 1996a; Weiss, et al., 1996) have also been used. A lack of precise species assignments and a lack of published historical data have made it difficult to compare older data to contemporary data to see if significant changes in resistance for specific taxa have occurred over time.

In 1971, *Corynebacterium diphtheriae* strains were found to be susceptible to commonly used drug classes tested, particularly those used for acute care treatment (penicillin and erythromycin) (McLaughlin et al., 1971). In 1995, diphtheria strains were found to be susceptible to beta lactams, third generation cephalosporins, vancomycin, pefloxacin, imipenam but not pefixime, azreonam, cefpodoxime, and some earlier generation cephalosporins; occasional strains were resistant to lincomycin and erythromycin (Patey et al., 1995). Antibiotic susceptibility patterns for species other than *Corynebacterium diphtheriae* confirmed observations that some or most strains of specific species, including *Corynebacterium amycolatum*, *Corynebacterium striatum*, *Corynebacterium minutissimum*, *Corynebacterium afermentans*, *Corynebacterium argentoratense*, *Corynebacterium auris*, *Corynebacterium glucuronolyticum*, lipophilic species *Corynebacterium jeikeium*, *Corynebacterium tuberculostearicum* (or as provisionally identified as CDC group G2), and *Corynebacterium urealyticum*, were often resistant to two or more drug classes (Funke et al., 1996b; Lagrou et al., 1998; Riegel et al., 1996a; Soriano et al., 1995; Williams et al., 1993). The new glycylcycline, tigecycline, and other commonly used antibiotics were evaluated against various *Corynebacterium* species, where tigecycline, as well as linezolid, daptomycin, vancomycin, and quinupristin/dalfopristin demonstrated good *in vitro* activity against isolates, but *Corynebacterium coyleae* and *Corynebacterium aurimucosum* strains were found to be MDR (Fernandez-Roblas et al., 2009). A daptomycin-resistant strain of *Corynebacterium jeikeium* has been reported (Schoen et al., 2009). *Corynebacterium resistens* was described as being susceptible only to vancomycin and minocycline but resistant to other drugs tested (Otsuka et al., 2005a).

In 1980, the presence of a 9.5 MDa plasmid was described as being associated with erythromycin resistance for strains of *Corynebacterium diphtheriae* (Schiller et al., 1980). In 1990, PCR was used to detect *erm* genes, i.e. genes encoding erythromycin ribosome methylases (rRNA methylases) that are linked to erythromycin resistance, from a variety of species (Arthur et al., 1990). Subsequently, studies of resistance mechanisms for various taxa have been reviewed including, among others, *mef* genes (macrolide efflux pump) and *erm* family genes, of which *ermB*, *ermC*, and particularly *ermX* have been detected among *Corynebacterium* species (Roberts, 2008). The *ermX* gene, believed to be borne on plasmids or transposons, adds one or two methyl groups to a single adenine in the 23S rRNA moiety and confers high level resistance to macrolides, lincosamides, and streptogramin B (MLS phenotype), as evidenced by resistance to at least erythromycin and clindamycin but often to other drug classes (Roberts, 2008). The *ermX* gene from *Corynebacterium jeikeium* has been linked to transposon Tn*5432* for some, but not all, isolates studied (Rosato et al., 2001). To date, resistance as linked to the presence of *ermX* has been reported in *Corynebacterium amycolatum* and *Corynebacterium jeikeium* (Rosato et al., 2001; Tauch et al., 2005; Yague Guirao et al., 2005), *Corynebacterium coyleae* (Fernandez-Natal et al., 2008), *Corynebacterium striatum* (Campanile et al., 2009; Otsuka et al., 2006), *Corynebacterium diphtheriae* (Tauch et al., 2003), and *Corynebacterium urealyticum* (Tauch et al., 2008b). The *ermX* gene was not detected among complete genome studies of *Corynebacterium kroppenstedtii* and a black pigmented *Corynebacterium aurimucosum* strain (Tauch et al., 2008b; Trost et al., 2010). Resistance to quinolones, due to a mutation in the *gyrA* gene, was found among clinical isolates of *Corynebacterium amycolatum* and *Corynebacterium striatum* (Sierra et al., 2005). A single amino acid substitution at position 83 of the *gyrA* gene generated a norfloxacin-resistance phenotype, but double mutation with amino acid substitutions at positions 83 and 87 gave rise to high level resistance to other fluoroquinolones in a study of *Corynebacterium macginleyi* isolates involved in ocular infections (Eguchi et al., 2008). *Corynebacterium striatum* strains were found to possess *ermX*, *tet A/B* (related resistance, tetracycline, oxytetracycline, and oxacillin), *cmx A/B* (related resistance chloramphenicol) and *aphA1* (related resistance, aminoglycoside) genes (Campanile et al., 2009). With time, other antibiotic resistance mechanisms among *Corynebacterium* species will be elucidated.

Ecology and habitat. There are at time of writing, ~51 medically relevant species have been described that cause occasional infections in humans or are transmitted to humans by zoonotic means. Most are deemed to be rare opportunistic pathogens. Some species thought to be part of the common skin flora, such as *Corynebacterium amycolatum*, *Corynebacterium jeikeium*, and some of the other lipophilic species, have been found to be resistant to multiple drug classes and can cause significant and occasionally fatal disease, particularly in immunocompromised patients or nosocomially in hospitals or nursing homes (Funke and Bernard, 2007). However, many of the medically relevant species can also be recovered as commensals or contaminants from a variety of clinical specimens. Therefore, it is recommended that identification to species level should be attempted if the organism is isolated (i) from normally sterile body sites, e.g. blood (except if only one of multiple specimens became positive), (ii) from adequately collected clinical material if the *Corynebacterium* species is the predominant organism, and (iii) if recovered from urine specimens, e.g. *Corynebacterium urealyticum* (described further below), is the sole bacterium encountered with a bacterial count >10^4/ml or if it is the predominant organism recovered and the total bacterial count is >10^5/ml (Funke and Bernard, 2007).

Corynebacterium species can cause significant infection or appear to be commensals in animals or birds. Virulence factors and mechanisms of pathogenicity remain understudied. To date, 31 species associated with animals or birds have

been described including: *Corynebacterium amycolatum* (mastitis in cattle) (Hommez et al., 1999), *Corynebacterium aquilae* and *Corynebacterium falsenii* (from eagles) (Fernández-Garayzábal et al., 2003), *Corynebacterium auriscanis* (otitis and suppurations in dogs) (Collins et al., 1999b), *Corynebacterium bovis* (mastitis and abscesses in cattle) (Watts et al., 2000), *Corynebacterium camporealensis* (mastitis in sheep) (Fernández-Garayzábal et al., 1998), *Corynebacterium canis* (dog mouth) (Funke et al., 2010a), *Corynebacterium capitovis* (sheep skin scrapings) (Collins et al., 2001a), *Corynebacterium caspium* (from a seal) (Collins et al., 2004), *Corynebacterium ciconiae* (stork trachea) (Fernández-Garayzábal et al., 2004), *Corynebacterium cystitidis* (pyelonephritis in cattle) (Yanagawa and Honda, 1978), *Corynebacterium diphtheriae* (mastitis, dermatitis, and wound infection in cattle or horses) (Corboz et al., 1996; Greathead and Bisschop, 1963; Henricson et al., 2000), *Corynebacterium felinum* (Scottish wild cat) (Collins et al., 2001b), *Corynebacterium freiburgense* (dog mouth) (Funke et al., 2009), *Corynebacterium glucuronlyticum* (genital tract of pigs) (Devriese et al., 2000), *Corynebacterium kutscheri* (rats, mice, and hamsters) (Collins and Cummins, 1986), *Corynebacterium mastitidis* (mastitis in sheep) (Fernández-Garayzábal et al., 1997). *Corynebacterium minutissimum* (mastitis in cattle) (Hommez et al., 1999), *Corynebacterium mustelae* (sepsis in ferrets) (Funke et al., 2010b), *Corynebacterium phocae* (nasal cavity of seal) (Pascual et al., 1998), *Corynebacterium pilosum* (urogenital tract of cattle) (Yanagawa and Honda, 1978), *Corynebacterium pseudotuberculosis* (ovine caseous lymphadenitis and other sheep or goat diseases) (Baird and Fontaine, 2007; Dorella et al., 2006), *Corynebacterium renale* (pyelonephritis in cattle) (Collins and Cummins, 1986), *Corynebacterium sphenisci* (from wild penguins) (Goyache et al., 2003a), *Corynebacterium spheniscorum* (from cloacae of wild penguins) (Goyache et al., 2003a), *Corynebacterium suicordis* (pericarditis, pneumonia, and lymph enlargements of pigs) (Vela et al., 2003), *Corynebacterium testudinoris* (from a tortoise) (Collins et al., 2001b), *Corynebacterium ulcerans* (goats, pigs, cattle, horses, cats, dogs, otters, and other) (Bonmarin et al., 2009; Schuhegger et al., 2009), *Corynebacterium ulceribovis* (bovine ulcers) (Yassin, 2009), *Corynebacterium urealyticum* (UTIs of dogs and cats) (Bailiff et al., 2005; Cavana et al., 2008), and *Corynebacterium vitaeruminis* (derived from cow rumen) (Collins and Cummins, 1986).

Corynebacterium species which have been recovered or detected in animals have also been documented to be potentially the cause of infection in humans by zoonotic transmission. These infections have been found to have occurred by occupationally related handling of the animals, by animal bites, or by unknown means. This has been observed with *Corynebacterium auriscanis* (Bygott et al., 2008), *Corynebacterium bovis* (Achermann et al., 2009; Bernard et al., 2002), *Corynebacterium canis* (Funke et al., 2009), *Corynebacterium diphtheriae* (Bonmarin et al., 2009), *Corynebacterium freiburgense* (Funke et al., 2009), *Corynebacterium kutscheri* (Holmes and Korman, 2007), a *Corynebacterium mastitidis*-like organism (Eguchi et al., 2008), *Corynebacterium pseudotuberculosis* (Bonmarin et al., 2009; Join-Lambert et al., 2006; Peel et al., 1997), and particularly of interest as an emerging zoonotic agent, toxigenic strains of *Corynebacterium ulcerans* (Bonmarin et al., 2009; De Zoysa et al., 2005b; Hogg et al., 2009; Wagner et al., 2001). *Corynebacterium glucuronolyticum*, *Corynebacterium minutissimum*, and *Corynebacterium urealyticum* (described further below) are usually associated with human disease, rather than animal disease, and evidence of transmission between human and animals has not been described.

Other *Corynebacterium* species have been described as being recovered from foodstuffs, the environment, or water, and some of these in turn have been widely used in industrial applications. These are *Corynebacterium callunae* (environment), *Corynebacterium glutamicum* (environment, production of glutamic acid), *Corynebacterium flavescens* (dairy products) (Collins and Cummins, 1986), *Corynebacterium casei* (soft cheeses) (Brennan et al., 2001), *Corynebacterium doosanense* (activated sludge) (Lee et al., 2009), *Corynebacterium efficiens* (environment, production of glutamic acid) (Fudou et al., 2002), *Corynebacterium glaucum* (cosmetic dye) (Yassin et al., 2003), *Corynebacterium halotolerans* (saline soil) (Chen et al., 2004), *Corynebacterium lubricantis* (coolant lubricant) (Kampfer et al., 2009), *Corynebacterium maris* (mucus of sea coral) (Ben-Dov et al., 2009), *Corynebacterium marinum* (sea sediment) (Du et al., 2010), *Corynebacterium terpenotabidum* (environment, capable of degrading squalene) (Takeuchi et al., 1999) and *Corynebacterium variabile* (formerly *Arthrobacter variabilis*, later *Corynebacterium variabilis*, from animal fodder) (Collins, 1987a).

Enrichment and isolation procedures

Members of this genus require one or more vitamins, amino acids, purines, and pyrimidines in culture medium to grow (Collins and Cummins, 1986; Funke and Bernard, 2007). Growth for some species is enhanced by the addition of lipids or by using an extended incubation period (detailed below, *Corynebacterium urealyticum* when recovered from urine). Growth is generally achieved using a temperature range from 30–37°C, particularly under a 5% CO_2 atmosphere with other ranges described as appropriate by species, below. No species described to date will grow on MacConkey agar except *Corynebacterium lubricantis* (described below). Isolation of *Corynebacterium diphtheriae*, *Corynebacterium ulcerans*, or *Corynebacterium pseudotuberculosis* from non-sterile sites is outlined below.

Maintenance procedures

Corynebacterium species in general do not require special procedures for storage. Short-term (weeks or months) storage has historically been done using enriched media such as PAIs, Loeffler's, or blood agar slants (von Graevenitz and Bernard, 2003). Medium-term storage may be done by storage of bacteria on beads in cryopreservative at −80°C (available commercially), and long-term preservation may be done by standard lyophilization procedures.

Procedures for testing special characters

Contemporary biochemical procedures for testing medically relevant species of this genus have been outlined previously (Funke and Bernard, 2007; von Graevenitz and Bernard, 2003). Rapid identification panels such as the API Coryne strip (BioMérieux) have been described for characterization of many of these species and are especially useful for those which grow well within the allotted 48 h incubation period and are reactive with the slate of substrates. Users are cautioned that the underlying database is only infrequently updated and does not provide enough discrimination to delineate among newly described species outlined here. The API Coryne strip does not

include starch utilization, a feature which historically was used in part to differentiate among biovars (= biotypes) of *Corynebacterium diphtheriae*. A web-based query to decode API data exists; it provides possible adjunct tests to assist with identification (https://apiweb.bioMerieux.com). Ancillary strips such as BioMérieux's API ZYM enzyme strip, API CH50 with API 20 E and panels manufactured by other companies, are also frequently used. The automated Biolog system has been used to provide phenotypic characteristics for various *Corynebacterium* species including the screening of nutritional and other physiological properties (Fernandez-Natal et al., 2009). Enzyme testing has been done as single test assays, such as described for pyrazinamidase or phosphatase (synonym of alkaline phosphatase) in a previous version of this chapter (Collins and Cummins, 1986). The characterization of species with no medical relevance that were derived from the environment has been carried out, either manually or by automated means (e.g. by use of the Biolog system) by testing the ability of the organism to utilize different carbon compounds, by defining optimal or preferred salinity, temperature, atmosphere, and pH, and by considering chemotaxonomic and genetic features; this has been outlined recently for *Corynebacterium lubricantis* (below) (Kampfer et al., 2009).

Metachromatic granules (synonym polymetaphosphate), the presence of which is described for some species in this text, are observed as bluish-purple after staining with methylene blue stain (Macfaddin, 2000).

Many species in this genus are lipophilic, i.e. they grow poorly at 35–37°C in 24 h or longer on standard laboratory media, but show enhanced growth in 48–72 h on sheep blood or brain heart infusion broth enriched with a lipid, such as 0.1–1.0% Tween 80, as detailed below for *Corynebacterium macginleyi* (Riegel et al., 1995e).

Special selective agars exist for *Corynebacterium* species, but the most widely used are those which select for *Corynebacterium diphtheriae* or *Corynebacterium ulcerans* from respiratory specimens, e.g. Tellerite or modified Tinsdale agars (Efstratiou and George, 1999).

The entire gene or fragments of the gene associated with the production of diphtheria toxin by *Corynebacterium diphtheriae*, *Corynebacterium ulcerans*, and *Corynebacterium pseudotuberculosis* can be detected by conventional (Efstratiou et al., 1998; Efstratiou et al., 2000) or real-time PCR (Mothershed et al., 2002; Schuhegger et al., 2008a, 2008b; Soriano et al., 2009). However, definitive toxigenic status is achieved only by detecting expression of diphtheria toxin, which is generally done using the modified Elek test (Engler et al., 1997). CAMP reaction and expression of phospholipase D by the CAMP inhibition assay for *Corynebacterium ulcerans* and *Corynebacterium pseudotuberculosis* strains (Barksdale et al., 1981) are useful tests for these species.

Differentiation of the genus *Corynebacterium* from other genera

Of the 60-plus *Corynebacterium* species described since publication of the previous edition of the *Manual*, many but not all authors describe testing for the presence of corynemycolates, types of cell-wall sugars and/or types of diaminopimelic acid, types and quantities of long-chain cellular fatty acids, and G+C mol% (Table 31). Testing for other features such as for other types of cell-wall lipids or phospholipids, polyamines, or acyl types are now only infrequently done. Staining for the presence of metachromatic granules is now almost never described.

Taxonomic comments

The genus *Corynebacterium* belongs to the phylum *Actinobacteria*, class *Actinobacteria*, order *Corynebacteriales*, family *Corynebacteriaceae*. Specific 16S rRNA signature nucleotides for these bacteria have been outlined (Zhi et al., 2009).

It has become increasingly difficult to distinguish *Corynebacterium* species based on phenotypic testing alone, as shown from some of the data outlined in Table 32. Additional identification approaches, such as the use of 16S rRNA and *rpoB* gene sequencing, are being used more routinely as a significant adjunct to characterization. However, assignment of species nova to this genus requires testing for as many genetic and chemotaxonomic properties as are available to researchers, and, increasingly, full genome sequencing is highly recommended.

Many of the genera described in the previous edition of this *Manual* as being closely related to the genus *Corynebacterium* have subsequently been found to be unrelated or qualitatively distinguishable by genetic and chemotaxonomic methods. All *Corynebacterium* species previously attributed to causing disease in plants have been reassigned, and none of the species described here have been documented to be phytopathogens. *Corynebacterium* species reassigned since the previous edition to other genera include: *Corynebacterium betae*, *Corynebacterium oortii*, *Corynebacterium flaccumfaciens* Hedges 1922 and *Corynebacterium poinsettiae* to *Curtobacterum flaccumfaciens* comb. nov. Collins and Jones 1984 (Effective publication: Collins and Jones 1983b.); *Corynebacterium equi* and *Corynebacterium hoagii* to *Rhodococcus equi* (Magnusson 1928) Goodfellow and Alderson 1977[AL]; *Corynebacterium fascians* Tilford 1936 to *Rhodococcus fascians* comb. nov. Goodfellow 1984b (Effective publication: Goodfellow 1984a.); *Corynebacterium insidiosum*, *Corynebacterium michiganense*, *Corynebacterium nebraskense*, and *Corynebacterium sepedonicum* to the genus *Clavibacter* as subspecies of *Clavibacter michiganensis* (Davis et al. 1984); *Corynebacterium iranicum* to *Rathayibacter iranicus* comb. nov. Zgurskaya et al. 1993; *Corynebacterium paurometabolum* Steinhaus 1941 to *Tsukamurella paurometabolum* comb. nov. Collins et al. 1988c, later corrected to *Tsukamurella paurometabola* by Euzéby (1998); *Corynebacterium pyogenes* Glage 1903 to *Actinomyces pyogenes* Reddy et al. 1982 to *Arcanobacterium pyogenes* comb. nov. (Glage 1903) Pascual Ramos et al. 1997 and then to *Trueperella pyogenes* comb. nov. (Glage 1903) Yassin et al. 2011; *Corynebacterium rathayi* Smith 1913 to *Clavibacter rathayi* comb. nov. Davis et al. 1984 then to *Rathayibacter rathayi* comb. nov. (Smith 1913) Zgurskaya et al. 1993, and lastly, *Corynebacterium tritici* Carlson and Vidaver 1982 to *Clavibacter tritici* comb. nov. Davis et al. 1984 then to *Rathayibacter tritici* comb. nov. (Carlson and Vidaver 1982) Zgurskaya et al. 1993.

Corynebacterium ilicis was proposed as the name for a bacterial pathogen of American holly that caused a blight of foliage and twigs (Mandel et al., 1961). The type strain (DSM 20138 = ATCC 14264 = NCPPB 1228) was belatedly designated in 1977 (Dye and Kemp, 1977). Assignment of *Corynebacterium ilicis* to the genus *Corynebacterium* was controversial and subsequently it was reclassified as *Arthrobacter ilicis* Collins et al. 1981. Upon further review, the *Corynebacterium ilicis* type strain was found to

TABLE 31. Features differentiating the genus *Corynebacterium* from closely related genera[a,b]

Characteristic	*Corynebacterium*	*Dietzia*	*Gordonia*	*Hoyosella*	*Millisia*	*Mycobacterium*	*Nocardia*	*Rhodococcus*	*Segniliparus*	*Skermania*	*Smaragdicoccus*	*Tsukamurella*	*Turicella*	*Williamsia*
Mycolates detected	Most species +	+	+	–	+	+	+	+	+	+	+	+	–	+
No. carbons in mycolates	22–36 or NA	34–38	46–66	na	44–52	70–90	50–62	34–64	No data	58–64	43–49	62–78	Not found	50–56
Predominant menaquinone(s)	MK-8(H_2); MK-9(H_2)[c]	MK-8 (H_2)	MK-9 (H_2)	MK-8	MK-8(H_2)	MK-9(H_2)	MK-8(H_4, ω-cycl)	MK-8(H_2)	No data	MK-8(H_4, ω-cycl)	SQA-8 (H_4, ω-cycl); SQB-8 (H_4, dicycl)	MK-9	MK-10, MK-11	MK-9(H_2)
DNA G+C content (mol%)	51–74	66–73	63–69	49	65	60–73	64–72		68–72	68	64	68–78	65–72	65
Modified acid-fast	–	–	Weak +	Weak +	+	+	Weak +	Weak +	Strong +	–	Not described	Weak +	–	–
Aerial hyphae observed	–	–	–	–	+	Variable	+ occasional –	–	–	– except if viewed microscopically	–	–	–	–

[a]Symbols: +, >85% positive; –, 0–15% positive; na, not applicable; some small discrepancies exist among references describing no. of carbons in mycolates, so broadest range used (data from Chun et al., 1997; Kämpfer et al., 1999; Soddell et al., 2006; Adachi et al., 2007; Jurado et al., 2009; Koerner et al., 2009).

[b]Genera of the order *Corynebacteriales*; all genera have cell-wall type IV, that is with *meso*-diaminopimelic acid, arabinose, and galactose with the caveat that CW sugars were not determined for the genus *Segniliparus* (Butler et al., 2005); *Corynebacterium*, *Dietzia*, and *Hoyosella* have an acetylated acyl type; the remaining genera have glycolated acyl types except for *Segniliparvus* and *Turicella*, where acyl type has not been determined.

[c]MK-7 (H_2) also detected in *Corynebacterium lubricantis* (Kampfer et al., 2009) and MK-10(H_2) found in *Corynebacterium thomssenii* (Zimmermann et al., 1998).

TABLE 32. Phenotypic reactions of species of the genus *Corynebacterium*[a]

Characteristic	*C. diphtheriae* biovar gravis[b]	*C. diphtheriae* biovar intermedius[b]	*C. diphtheriae* biovarmitis and belfanti[b]	*C. accolens*	*C. afermentans* subsp. *afermentans*	*C. afermentans* subsp. *lipophilum*	*C. ammoniagenes*[c]	*C. amycolatum*
Metabolic process	F	F	F	F	O	O	F	F
Lipophilism	–	+	–	+	–	+	–	–
Nitrate reduction	+	+	+/–	+	–	–	+	d
Urease	–	–	–	–	–	–	+	d
Esculin hydrolysis	–	–	–	–	–	–	–	–
PYZ	–	–	–	d	+	+	–	+
PAL	–	–	–	–	+	+	–	+
Acid production from:								
Glucose	+	+	+	+	–	–	+	+
Maltose	+	+	+	–	–	–	–	d
Mannitol	–	–	–	d	–	–	–	–
Sucrose	–	–	–	D	–	–	–	D
Xylose	–	–	–	–	–	–	–	–
CAMP Reaction	–	–	–	–	d	d	–	–
MDR[f]	–	–	–	–	+	–	–	+
Other traits	Glycogen +	Lipophilic; glycogen +	Glycogen –				Tyrosine +; citrate alkalinized	Lacks corynemycolates; grows at 42°C, not 20°C

Characteristic	*C. appendicis*	*C. aquilae*	*C. argentoratense*	*C. atypicum*	*C. aurimucosum*	*C. auris*	*C. auriscanis*	*C. bovis*
Metabolic process	F	F	F	F	F	O	O	F
Lipophilism	+	–	–	–	–	–	–	+
Nitrate reduction	–	–	–	–	–	–	–	–
Urease	+	–	–	–	–	–	–	–
Esculin hydrolysis	–	–	–	–	d	–	d	–
PYZ	+	+	+	–	+	+	–	–
PAL	+	+	d	–	+	+	+	+
Acid production from:								
Glucose	(+)	+	+	+	+	–	+	+
Maltose	(+)	–	–	+	+	–	–	–
Mannitol	–	–	–	–	d	–	–	–
Sucrose	–	–	–	+	+	–	–	–
Xylose	–	–	–	–	–	–	–	–
CAMP Reaction	nd	–	–	nd	–	+	–	–
MDR[f]	–	–	–	–	+	–	–	–
Other traits	Slowly reactive with sugars; TBSA detected	Chymotrypsin +	Chymotrypsin may be +; fructose +	Lacks corynemycolates	Yellowish or black adherent colonies; 1 strain oxidase positive	Dry, slightly adherent to agar		TBSA positive; can be oxidase +

(continued)

TABLE 32. (continued)

Characteristic	*C. callunae*	*C. camporealensis*	*C. canis*	*C. capitovis*	*C. casei*	*C. caspium*	*C. ciconiae*	*C. confusum*
Metabolic process	F	F	F	F	F	F	F	F
Lipophilism	–	–	–	–	–	–	–	–
Nitrate reduction	–	–	+	–	+	–	–	+
Urease	+	–	–	–	–	+	–	–
Esculin hydrolysis	–	–	nd	–	–	–	–	–
PYZ	nd	+	+	–	–	–	+	+
PAL	nd	+	+	+	+	–	+	+
Acid production from:								
Glucose	+	+	+	+	+	+	+	(+)
Maltose	+	–	+	–	–	–	+	–
Mannitol	nd	–	–	–	–	–	–	–
Sucrose	+	–	+	–	–	–	–	–
Xylose	–	–	–	–	–	–	–	–
CAMP Reaction	nd	+	–	nd	–	–	–	–
MDR[f]	–	–	–	–	–	–	–	+
Other traits			Dry, adherent, convoluted colony			Lacks corynemycolates	Lacks corynemycolates	Tyrosine –, TBSA +

Characteristic	*C. coyleae*	*C. cystitidis*	*C. doosanense*	*C. durum*	*C. efficiens*	*C. falsenii*	*C. felinum*	*C. flavescens*
Metabolic process	F	F	F	F	F	F	F	F
Lipophilism	–	–	nd	–	–	–	–	–
Nitrate reduction	–	–	+	+	+	–	–	–
Urease	–	+	–	(d)	d	(+)	–	–
Esculin hydrolysis	–	–	–	(d)	d	–	–	nd
PYZ	+	+	+	+	+	(+)	+	–
PAL	+	nd	–	–	–	+	–	–
Acid production from:								
Glucose	(+)	+	+	+	+	(+)	+	+
Maltose	–	+	–	+	+	d	+	–
Mannitol	–	nd	–	d	–	–	–	nd
Sucrose	–	–	–	+	nd	–	–	–
Xylose	–	+	–	–	–	–	–	–
CAMP Reaction	+	nd	–	–	nd	–	nd	nd
MDR[f]	+	–	nd	–	–	–	–	–
Other traits	Slowly reactive with glucose, occasionally other sugars		Yellowish colonies; oxidase +	Sticky colonies; bulges in Gram		Yellowish colonies		Yellowish colonies

(continued)

TABLE 32. (continued)

Characteristic	*C. freiburgense*	*C. freneyi*	*C. glaucum*	*C. glucuronolyticum*	*C. glutamicum*	*C. halotolerans*	*C. hansenii*	*C. imitans*
Metabolic process	F	F	F	F	F	O	F	F
Lipophilism	–	–	–	–	–	nd	–	–
Nitrate reduction	+	d	–	d	+	+	–	–
Urease	–	–	–	d	+	–	–	–
Esculin hydrolysis	(+)	–	–	d	–	nd	–	–
PYZ	–	+	+	+	nd	nd	–	(+)
PAL	nd	+	+	d	nd	nd	–	+
Acid production from:								
Glucose	+	+	+	+	+	+	+	+
Maltose	+	+	–	d	+	–	+	+
Mannitol	–	d	–	–	nd	–	–	–
Sucrose	+	+	+	+	+	–	+	(+)
Xylose	–	–	–	d	–	–	–	–
CAMP Reaction	–	–	nd	+	nd	nd	nd	+
MDR[f]	–	–	–	+	–	nd	–	–
Other traits	Unusual "spoke wheel" colony after 3 d	Grows at 20°C and 42°C; distinctive wrinkled colonies	'Bluish' light grey colonies		Yellowish colonies	Yellow colonies; optimal growth 28°C and in 10% salt	Yellow pigmented and dry colony	Tyrosine –

Characteristic	*C. jeikeium*	*C. kroppenstedtii*	*C. kutscheri*	*C. lipophiloflavum*	*C. lubricantis*	*C. macginleyi*	*C. marinum*	*C. maris*
Metabolic process	O	F	F	O	O	F	F	O
Lipophilism	+	–	–	(+)	nd	+	–	nd
Nitrate reduction	–	d	+	–	–	+	+	–
Urease	–	–	+	(+)	–	–	–	–
Esculin hydrolysis	–	+	–	–	+	–	–	–
PYZ	+	+	+	+	nd	–	+	+
PAL	+	–	–	+	nd	+	–	+
Acid production from:								
Glucose	+	+	+	–	+	+	+	–
Maltose	d	d	+	–	–	–	+	–
Mannitol	–	–	nd	–	–	d	–	–
Sucrose	–	+	+	–	–	+	+	–
Xylose	–	–	–	–	–	–	–	–
CAMP Reaction	–	–	nd	–	nd	–	nd	nd
MDR[f]	+	–	–	–	nd	+	–	–
Other traits	Fructose –; ng anaerobically	Lacks corynemycolates, TBSA +		Yellow colonies	Only species which grows on MacConkey agar; has MK-7(H_2)		Yellow, creamy colonies; growth best at 30–32°C, 1% NaCl	TBSA +; gelatin +, oxidase +; yellowish

(continued)

TABLE 32. (continued)

Characteristic	*C. massiliense*	*C. mastitidis*	*C. matruchotii*	*C. minutissimum*	*C. mucifaciens*	*C. mustelae*	*C. mycetoides*	*C. phocae*
Metabolic process	O	O	F	F	O	F	O	F
Lipophilism	nd	+	−	−	−	−	−	−
Nitrate reduction	−	−	+	−	−	−	−	−
Urease	−	d	−	−	−	−	−	d
Esculin hydrolysis	−	−	d	−	−	+	−	−
PYZ	+	+	+	+	+	−	nd	+
PAL	+	+	+	+	+	−	nd	+
Acid production from:								
Glucose	−	−	+	+	+	+	+	+
Maltose	−	−	+	+	−	+	−	+
Mannitol	−	−	−	−	−	−	−	−
Sucrose	−	−	+	d	d	+	−	d
Xylose	−	−	−	−	−	−	−	−
CAMP Reaction	nd	−	−	−	−	−	nd	nd
MDR[f]	nd	−	−	+	−	−	−	−
Other traits	TBSA +; Poor growth anaerobically		Whip handle in Gram stain; PA detected	Tyrosine +	Distinctive, mucoid-brown yellowish colonies	Greenish-beige adherent colony exudes odor	Yellowish colonies	

Characteristic	*C. pilbarense*	*C. pilosum*	*C. propinquum*	*C. pseudodiphtheriticum*	*C. pseudotuberculosis*[b]	*C. pyruviciproducens*	*C. renale*	*C. resistens*
Metabolic process	F	F	O	O	F	F	F	F
Lipophilism	−	−	−	−	−	−	−	+
Nitrate reduction	−	+	+	+	D	−	−	−
Urease	−	+	−	+	+	−	+	−
Esculin hydrolysis	−	−	−	−	−	−	−	−
PYZ	+	+	d	+	−	+	+	−
PAL	+	−	d	d	d	−	−	+
Acid production from:								
Glucose	+	+	−	−	+	+	+	+
Maltose	−	+	−	−	+	+	d	−
Mannitol	−	nd	−	−	−	−	nd	−
Sucrose	+	−	−	−	d	+	−	−
Xylose	−	−	−	−	−	+	−	−
CAMP Reaction	nd	nd	−	−	REV	−	nd	−
MDR[f]	−	−	−	+	−	−	−	+
Other traits			Tyrosine +; formerly ANF-3			Produces pyruvic acid	Yellowish colony	

(continued)

TABLE 32. (continued)

Characteristic	*C. riegelii*	*C. simulans*[d]	*C. singulare*	*C. sphenisci*	*C spheniscorum*	*C. sputi*	*C. stationis*	*Corynebacterium striatum*[e]
Metabolic process	F	F	F	F	F	F	F	F
Lipophilism	–	–	–	–	–	+	–	–
Nitrate reduction	–	+	–	+	–	–	+	D
Urease	+	–	+	–	–	+	+	–
Esculin hydrolysis	–	–	–	–	–	–	–	–
PYZ	d	d	+	+	+	+	(+)	+
PAL	d	+	+	–	–	–	–	+
Acid production from:								
Glucose	–	+	+	+	+	+	(+)	+
Maltose	(+)	–	+	+	+	–	–	–
Mannitol	–	–	–	–	–	–	–	–
Sucrose	–	+	+	–	–	–	–	d
Xylose	–	–	–	–	–	–	–	–
CAMP Reaction	–	–	–	–	–	nd	–	d
MDR[f]	–	–	–	–	–	nd	–	+
Other traits	Urease positive in 5 min	Reduces nitrite; tyrosine +	Tyrosine +	Ferments glucose at 42°C, grows at 20°C		Cream-yellow, dry colony	Citrate alkalinized	Tyrosine +

Characteristic	*C. suicordis*	*C. sundsvallense*	*C. terpenotabidum*	*C testudinoris*	*C. thomssenii*	*C. timonense*	*C. tuberculostearicum*	*C. tuscaniense*
Metabolic process	F	F	O	F	F	F	F	O
Lipophilism	–	–	–	–	–	nd	+	–
Nitrate reduction	–	–	–	+	–	–	D	–
Urease	+	+	+	–	+	–	–	–
Esculin hydrolysis	–	–	–	+	–	+w	–	–
PYZ	+	d	nd	–	+	+	+	+
PAL	+	d	nd	–	+	+	+	+
Acid production from:								
Glucose	–	+	–	+	+	+	+	+
Maltose	–	+	nd	+	+	–	d	+
Mannitol	–	–	nd	–	–	–	–	–
Sucrose	–	+	nd	+	+	–	d	–
Xylose	–	–	nd	–	–	–	–	–
CAMP Reaction	–	–	nd	nd	–	nd	–	–
MDR[f]	–	–	nd	–	–	–	+	–
Other traits		Sticky yellowish colonies, >bulges = in Gram stain	Squalene degrader from soil; large vol. TBSA detected		*N*-Acetyl-β-glucosaminidase +, adherent; MK-10(H_2) detected	Yellow colony;	TBSA+, includes many CDC group G -2 strains	

(continued)

TABLE 32. (continued)

Characteristic	*C. ulcerans*[b]	*C. ulceribovis*	*C. urealyticum*	*C. ureicelerivorans*	*C. variabile*	*C. vitaeruminis*	*C. xerosis*
Metabolic process	F	F	O	F	O	F	F
Lipophilism	–	–	+	+	–	–	–
Nitrate reduction	–	–	–	–	?–	+	D
Urease	+	–	+	+	?–	+	–
Esculin hydrolysis	–	–	–	–	?	+	–
PYZ	–	+	+	+	nd	+	+
PAL	+	+	d	+	nd	+	+
Acid production from:							
Glucose	+	+	–	+	?–	+	+
Maltose	+	–	–	–	–	+	+
Mannitol	d	–	–	–	–	nd	–
Sucrose	–	–	–	–	–	+	+
Xylose	–	–	–	(+)	–	nd	–
CAMP Reaction	REV	nd	–	–	nd	nd	–
MDR[f]	–	nd	+	+	–	–	–
Other traits	Glycogen +	Large colony	Strong, rapid urease production	Rapid urea + (~ 60 s)	Optimal growth 25–30°C; significant TBSA		

[a]Species recovered from human clinical material except as described in comment. Abbreviations and symbols: F, fermentative; O, oxidative; +, positive; –, negative; d, variable; ( ), delayed or weak reaction; nd, no data for test described; ng, no growth; REV, CAMP inhibition reaction; PYZ, pyrazinamidase; PAL, alkaline phosphatase and in older texts, was also called phosphatase; unk, unknown; TBSA, cellular fatty acid tuberculosteric acid (10-methyl 18:0) detected.

[b]*Corynebacterium diphtheriae* biovar mitis reduces nitrate but biovar belfanti is nitrate negative; all biovars of *Corynebacterium diphtheriae*, *Corynebacterium pseudotuberculosis*, and *Corynebacterium ulcerans* produce cysteinase which is detected by producing a brown-black colony with a brown black halo on cysteine-containing agar such as Tinsdale medium. These species can be lyogenized by the bacteriophage that confers ability to produce diphtheria toxin.

[c]*Corynebacterium ammoniagenes* (as *Brevibacterium ammoniagenes*) described as being completely non-reactive in sugars, and that reactivity was media dependant (Jones and Keddie, 1986).

[d]*Corynebacterium simulans*, a strong nitrite reducer at low and high concentrations may appear to be nitrate reduction negative unless further tested using zinc dust (Wattiau et al., 2000). One strain of this species was catalase negative (Bernard et al., 2002).

[e]For *Corynebacterium striatum*, the CAMP reaction, if done, has been described as negative (Martinez-Martinez et al., 1995b) or occasionally positive (Leonard et al., 1994).

[f]MDR, Multidrug resistance described at least once in the literature as being resistant to three or more drug classes using CLSI guidelines and current data (Clinical Laboratory Standards Institute, 2006, 2009).

be apathogenic for plants and a member of a different taxon group with respect to *Corynebacterium ilicis* reference strains ICMP 2608 and ICMP 2609, described at the same time as the type strain, which were pathogenic for American holly. Following Request for an Opinion no. 87 (Young et al., 2004), the Judicial Commission ruled that features associated with the name *Corynebacterium ilicis* Mandel et al. 1961 should be represented by the type strain ICMP 2608 = ICPB CI144 (later reassigned to *Curtobacterium flaccumfaciens*), and that *Arthrobacter ilicis* would be typified by strain DSM 20138 = ATCC 14264 = NCPPB 1228 (Effective publication: Collins et al., 1981.) and validated (Collins et al., 1982c; Young et al., 2004; Judicial Commission of the International Committee on the Systematics of Prokaryotes, 2008).

Brevibacterium liquefaciens Okabayashi and Masuo 1960[AL] was reclassified as *Corynebacterium liquefaciens* (Okabayashi and Masuo 1960) Lanéelle et al. 1980 based on the study of the type strain, ATCC 14929 (Lanéelle et al., 1980). This isolate was later found to have significant differences when compared to a new copy of ATCC 14929 and, as a result, all recommendations for reassignment were withdrawn in an erratum. *Brevibacterium liquefaciens* strain ATCC 14929 was subsequently reassigned to *Arthrobacter nicotianae* Giovannozzi-Sermanni 1959 by Gelsomino et al. (2004).

It is not anticipated that further changes in taxonomic classification of members of the genus *Corynebacterium* will occur except by the emendation or augmentation by new species.

List of species of the genus *Corynebacterium*

Information on this list was corroborated with by information compiled by Euzéby (2010). Accession numbers for *rpoB* gene sequences describe either complete gene (3100–3450 bp) or partial gene sequences (with ~400–425 bp).

1. **Corynebacterium diphtheriae** (Kruse 1886) Lehmann and Neumann 1896, 350[AL] ("*Bacillus diphtheria*" Kruse *in* Flügge 1886, 225)

diph.the.ri′a.e. Gr. n. *diphthera* leather, skin; N.L. fem. n. *diphtheria* a disease in which a leathery membrane forms in the throat; N.L. gen. n. *diphtheriae* of diphtheria.

(This description is largely based on that of Collins and Cummins, 1986.)

Straight or slightly curved rods, frequently swollen at one or both ends, 0.3–0.8 × 1.0–8.0 µm. Usually stains unevenly and

often contains metachromatic granules (polymetaphosphate) which stain bluish purple with methylene blue. Gram-stain-positive, but easily decolorized, especially in older cultures.

Descriptive cultural types or biovars (= biotypes) of *Corynebacterium diphtheriae* strains are still commonly applied and are designated gravis, intermedius, mitis, and belfanti. Designations for gravis, intermedius, and mitis were originally given in accordance with the clinical severity of cases from which the different strains were most frequently isolated. On blood agar, colonies vary in size and appearance but generally are 1–3 mm at 24 h (except intermedius, described below) and may demonstrate a narrow band of hemolysis, but no soluble hemolysin is produced. Colonies on blood tellurite (with 0.04% potassium tellurite) are gray to black, with appearance dependant on type. Colonies on modified Tinsdale agar are 1–2 mm, black or charcoal gray, with each colony having a brown-black halo visible in the agar as cysteinase produced by the organism reacts with the cysteine in the medium. Although less frequently used now, growth on Loeffler's medium is abundant, with grayish to cream colored colonies and no liquefaction. The use of historical descriptions of colonies (such as gravis being large, radially striated brittle colonies and mitis being smooth, shiny, and butyrous) or Gram stain details (gravis having short regular rods, intermedius with long rods with marked cross-striations, mitis with long curved irregular rods) as a means to subdivide strains into gravis, intermedius, and mitis are now rarely used.

Biovars are usually reported for clinical isolates and are generally differentiated by phenotype: biovar gravis reduces nitrate and utilizes both glycogen and starch; biovar intermedius is somewhat lipophilic in that colonies are 0.5–1 mm after 24–48 h growth on blood agar, but otherwise reduces nitrate and may utilize glycogen (rapid strip API Coryne usually negative but conventional tube sugar positive) and starch; biovar mitis reduces nitrate but does not utilize glycogen and rarely starch; biovar belfanti neither reduces nitrate nor utilizes glycogen or starch. CAMP and CAMP inhibition reactions negative. All strains exhibit the same reactions except for occasional sucrose-fermenting isolates described primarily in South America (de Mattos-Guaraldi and Formiga, 1998) or *Corynebacterium diphtheriae* biovar belfanti like isolates that were derived from symptomatic cats and did not ferment maltose (Hall et al., 2010). Optimal growth temperature 30–37°C, and complex media is required for growth.

All *Corynebacterium diphtheriae* strains, regardless of biovar, conform to the genus description chemotaxonomically. All contain corynemycolates, the polar lipids phosphatidylglycerol, phosphatidylinositol, and monoacylated phosphatidylinositol dimannoside, and MK-8 (H2) as major menaquinone. Cell wall contains *meso*-diaminopimelic acid and the sugars arabinose and galactose. Straight-chain saturated fatty acids are mainly palmitic and stearic acids with minimal branched saturated fatty acids; significant quantities of $C_{16:1}$ ω7*c* isomer is characteristically detected (Bernard et al., 1991). All produce propionic as a product of fermentation (Bernard et al., 2002).

Source: clinical specimens from humans or animals.

DNA G+C content (mol%): 52–55 (method unknown); 53.48 (full genome sequencing).

Type strain: ATCC 27010, CIP 100721, DSM 44123, NCTC 11397.

Sequence accession no. (16S rRNA gene): GQ118341, X82059, X84248.

Further comments: additional GenBank accession numbers include (complete genome sequence) BX248353 and (complete *rpoB* gene) AY492230. All biovars of *Corynebacterium diphtheriae* may produce diphtheria toxin which clinically should be tested for as described elsewhere in this chapter. Non-toxigenic strains otherwise have typical features. Full genome sequence of *Corynebacterium diphtheriae* strain NCTC 13129 has been published containing 2,488,635 bp (Cerdeno-Tarraga et al., 2003).

2. **Corynebacterium accolens** Neubauer, Sourek, Ryc, Bohacek, Mara and Mnukova 1991a, 331[VP] (Effective publication: Neubauer, Sourek, Ryc, Bohacek, Mara and Mnukova 1991b, 50.)

ac′co.lens. L. v. *accolere* live by; L. part. adj. *accolens* living close by.

Cells are Gram-stain-positive rods, 0.6–0.9 × 1.0–2.0 µm, nonmotile, occurring singly or in clusters typical for corynebacteria. Colonies are roundish, slightly convex, grayish, and show the phenomenon of satellitism, i.e., enhanced growth in the vicinity of *Staphylococcus aureus* indicating that the strains are lipophilic. Facultatively anaerobic. Oxidase negative. Growth occurs at 30–40°C with optimal growth at 37°C, pH 7.2. Acid is produced from D-glucose, galactose, fructose, D-mannose, and maltose. Fermentation of sucrose is variable. Acid is not produced from lactose, D-xylose, trehalose, L-arabinose, raffinose, L-rhamnose, dextrin, mannitol, dulcitol, D-sorbitol, glycerol, *myo*-inositol, salicin, and inulin. Esculin, urea, gelatin, casein, and starch are not hydrolyzed. No production of indol, H_2S, acetoin, and lipase. The methyl red test is negative. CAMP reaction negative. Cell wall contains *meso*-diaminopimelic acid; corynemycolic acids made up of 22–36 carbon atoms and arabinose and galactose sugars. Cellular fatty acids are as described for the genus. Menaquinone or polar lipid types not extant. Does not produce propionic acid as metabolic product (Bernard et al., 2002).

Source: human clinical specimens, in particular specimens from ear, nose, throat, and eyes.

DNA G+C content (mol%): 53.2 (method unknown).

Type strain: CNCTC Th1/57, ATCC 49725, CCUG 28779, CIP 104783, DSM 44278, JCM 8331.

Sequence accession no. (16S rRNA gene): AJ439346, X80500.

Further comments: additional GenBank accession numbers include (complete *rpoB* gene) AY492242. *rpoB* gene sequencing should be done to distinguish strains from *Corynebacterium macginleyi*, as those species can not be easily distinguished by 16S rRNA gene sequencing.

3. **Corynebacterium afermentans** Riegel, de Briel, Prévost, Jehl, Monteil and Minck 1993b, 291[VP]

a.fer.men′tans. Gr. pref. *a* not; L. part. adj. *fermentans* leavening; N.L. part. adj. *afermentans* nonfermenting; i.e. nonfermenting carbohydrates.

The bacteria are Gram-stain-positive irregular rods or coccobacilli that sometimes contain metachromatic granules. Cells are arranged in typical V-shaped forms or palisades. Nonlipophilic. Good growth under aerobic conditions, and very slight growth under anaerobic conditions.

Smooth, grayish-white colonies with entire margins. Colonies are 1–2 mm in diameter. Acid is not produced from glucose, glycogen, lactose, sucrose, ribose, D-xylose, D-mannose, D-galactose, trehalose, and D-mannitol. Cells utilize acetate and lactate. Propionate, D-glucose, L-arabinose, D-mannose, D-mannitol, *N*-acetylglucosamine, maltose, gluconate, caprate, adipate, malate, citrate, and phenylacetate are not utilized. Nitrate is not reduced. Urea and esculin not hydrolyzed. Tyrosine, gelatin, DNA, and starch are not degraded. The methyl red test is negative, and acetoin, indole, and H_2S (on triple-sugar iron agar) are not produced. Hydrolysis of hippurate is variable. Growth is visible in 6.5% NaCl. Alkaline phosphatase, esterase, lipase, and acid phosphatase are produced. α-Galactosidase, β-galactosidase, β-glucuronidase, α-glucosidase, and β-glucosidase are not produced. All strains are resistant to fosfomycin. CAMP reaction variable (Funke and Bernard, 2007).

The cell wall contains *meso*-diaminopimelic acid, arabinose, and galactose. Cellular fatty acids are consistent with those described for the genus. Corynemycolates are present with $C_{30:0}$, $C_{30:1}$, $C_{32:1}$, $C_{33:1}$, $C_{34:2}$, and $C_{36:2}$ predominating. Does not produce propionic acid as metabolic product (Bernard et al., 2002).

Source: human clinical specimens.

DNA G+C content (mol%): 66 (HPLC).

Type strain: ATCC 51403, CCUG 32103, CIP 103499, DSM 44280, JCM 10390, LCDC 88199.

Sequence accession no. (16S rRNA gene): X82054.

Further comments: Corynebacterium afermentans Riegel et al. (1993b) was previously known as CDC coryneform ANF-1. By 16S rRNA gene sequencing, *Corynebacterium afermentans* is closely related to (98% identity with) to *Corynebacterium coyleae* and *Corynebacterium mucifaciens* but differs (~94% identity with) when *rpoB* gene sequences are compared (Khamis et al., 2004) and by phenotypic testing (Table 32).

3a. **Corynebacterium afermentans subsp. afermentans** Riegel, de Briel, Prévost, Jehl, Monteil and Minck 1993b, 291[VP]

a.fer.men′tans. Gr. pref. *a* not; L. part. adj. *fermentans* leavening; N.L. part. adj. *afermentans* nonfermenting; i.e. nonfermenting carbohydrates.

Colonies are 1–2 mm in diameter after 24 h on blood agar and are not lipophiilc.

Source: human clinical specimens.

DNA G+C content (mol%): 66 (HPLC).

Type strain: ATCC 51403, CCUG 32103, CIP 103499, DSM 44280, JCM 10390, LCDC 88199.

Sequence accession no. (16S rRNA gene): X82054.

Further comments: additional GenBank accession numbers include (complete *rpoB* gene) AY492265.

3b. **Corynebacterium afermentans subsp. lipophilum** Riegel, de Briel, Prévost, Jehl, Monteil and Minck 1993b, 291[VP]

li.po′phi.lum. Gr. n. *lipos* animal fat; Gr. neut. adj. *philon* loving; N.L. neut. adj. *lipophilum* fat loving.

The characteristics of *Corynebacterium afermentans* subsp. *lipophilum* are similar to those of *Corynebacterium afermentans* subsp. *afermentans* except that very small colonies (<0.5 mm in diameter) appear after 48 h on sheep blood agar. Strains are lipophilic. Hippurate is not hydrolyzed.

Chemotaxonomic features are like those of *Corynebacterium afermentans* subsp. *afermentans* except that branched saturated fatty acids are heptadecanoic acid, and straight-chain unsaturated fatty acids are mainly oleic acid, linoleic acid, and arachidonic acid.

Source: human clinical specimens.

DNA G+C content (mol%): 68 (HPLC).

Type strain: T18502, ATCC 51404, CCUG 32105, CIP 103500, DSM 44282, JCM 10391.

Sequence accession no. (16S rRNA gene): X82055.

Further comments: additional GenBank accession numbers include (complete *rpoB* gene) AY492266.

4. **Corynebacterium ammoniagenes** (Cooke and Keith 1927) Collins 1987b, 442[VP] ("*Bacterium ammoniagenes*" Cooke and Keith 1927)

am.mo.ni.a′ge.nes. N.L. n. *ammonia* ammonia; N.L. suff. *-genes* (from Gr. v. *gennaô* to produce) producing; N.L. part. adj. *ammoniagenes* ammonia-producing.

Gram-stain-positive, nonsporeforming, nonmotile rod-shaped cells (1–4.5 × 0.6 –1.2 µm in diameter). Cells are irregular and occur singly or in pairs and may exhibit V forms. Colonies are circular, low convex with an entire edge, and are gray-white or yellow. Facultatively anaerobic. Optimum growth temperature is approximately 30°C. Growth occurs at 10°C and 37°C but not at 45°C. Growth occurs in the presence of 10% NaCl. Fermentation of carbohydrates has been described as being equivocal (Collins, 1987b). In a recent study, the following reactions were found for ATCC 6871^T: glucose, ribose, and fructose were slowly fermented, but xylose, mannitol, lactose, maltose, galactose, glycerol, glycogen, raffionse, salicin, trehalose, and mannose were not. Nitrate reduced to nitrite. Urease is produced. Hippurate and tyrosine are hydrolyzed. Gelatin, casein, DNase, and starch are not hydrolyzed. Citrate is alkalinized. CAMP and reverse CAMP reactions are negative. Leucine arylamidase positive, but pyrazinamidase, pyrrolidonyl arylamidase, alkaline phosphatase, esterase, esterase lipase, lipase, valine arylamidase, cystine arylamidase, trypsin, alpha chymotrypsin, acid phosphatase, naphthol-AS-Bl-phosphohydrolase, α-galactosidase, β-galactosidase, β-glucuronidase, β-glucuronidase, α-glucosidase, β-glucosidase, *N*-acetyl-β-glucosaminidase, α-mannosidase, and α-fucosidase not detected (Bernard et al., 2010). Some organic acids may be assimilated.

Cell wall contains *meso*-DAP. The glycan moiety of murein contains only acetyl residues. Cell wall contains arabinogalactan polymer. Corynomycolic acids are present. Long-chain fatty acids are of the saturated, monounsaturated, and 10-methyl-branched types with hexadecanoic, octadecanoic, octadecenoic, and 10-methyl-octadecanoic acids predominating. TBSA detected but does not produce propionic acid as metabolic product (Bernard et al., 2010). The major phospholipids are diphosphatidylglycerol, phosphatidylglycerol, phosphatidylinositol, and phosphatidylinositol dimannoside. The major menaquinone is MK-9(H_2).

Source: feces of infants and piggery waste.

DNA G+C content (mol%): 53.7–55.8 (T_m).

Type strain: ATCC 6871, CCUG 38796, CIP 101283, DSM 20306, JCM 1305, NBRC 12612, NCCB 60030, NCIMB 8143, VKM B-672.

Sequence accession no. (16S rRNA gene): X84440.

Further comments: additional GenBank accession numbers include (complete *rpoB* gene) AY492243 and (partial *rpoB* gene) FJ648511.

5. **Corynebacterium amycolatum** Collins, Burton and Jones 1988b, 449[VP] (Effective publication: Collins, Burton and Jones 1988a, 351.)

a.my.co′la.tum. N.L. neut. adj. *amycolatum* wanting in mycolates.

Cells are Gram-stain-positive, nonmotile, nonsporeforming, pleomorphic rods. Colonies are whitish-grayish, dry, rough, and with uneven edges. Facultatively anaerobic. Nonlipophilic. Grows at 37°C but not at 10°C or 20°C at 3 d (Wauters et al., 1998); some strains grow at 40°C. Grows in 5% NaCl; some strains grow in 10% NaCl. Acid is usually produced from D-fructose, D-glucose, glycerol, D-mannose, and ribose. Acid production from maltose, sucrose, galactose (Wauters et al., 1996), and trehalose is variable. Acid is not produced from *N*-acetylglucosamine, starch, amygdalin, D-arabinose, L-arabinose, D-arabitol, L-arabitol, arbutin, cellobiose, D-fucose, L-fucose, b-gentiobiose, glycogen, gluconate, 2-keto-gluconate, 5-keto-gluconate, inositol, inulin, lactose, D-lyxose, mannitol, melezitose, melibiose, α-methyl-D-glucoside, α-methyl-D-mannoside, β-methyl xyloside, D-raffinose, rhamnose, salicin, sorbitol, L-sorbose, xylitol, D-xylose, and L-xylose. Glucose may be fermented at 42°C and ethylene glycol may be acidified at 48 h (Wauters et al., 1998). CAMP reaction is negative. Nitrate reduction and urea hydrolysis are variable. Esculin, arginine, casein, cellulose, gelatin, ornithine, tyrosine, and xanthine are not hydrolyzed. Starch is hydrolyzed (Funke et al., 1996a). Some strains hydrolyze hippurate. Pyrazinamidase, alkaline phosphatase, esterase, esterase lipase, cystine arylamidase, and acid phosphatase are detected; lipase, leucine arylamidase, and phosphoamidase are variable, but valine arylamidase, trypsin, chymotrypsin, α-galactosidase, β-galactosidase, β-glucuronidase, α-glucosidase, β-glucosidase, *N*-acetyl-β-glucosaminidase, α-mannosidase, and α-fucosidase are not detected (Funke et al., 1996a). Voges–Proskauer and methyl red positive.

Cell-wall peptidoglycan contains *meso*-diamimopimelic acid, alanine, and glutamic acid. Arabinose and galactose are wall sugars. Mycolic acids are not present. The cellular fatty acids are consistent with those described for the genus. The principle menaquinone is MK-9(H_2) with significant quantities of MK-8(H_2). Polar lipid types include acyl phosphatidylglycerol, phosphatidylinositol, phosphatidylinositol dimannoside, and several unknown phospholipids (Collins et al., 1988a; Yague et al., 1997). Strains produce propionic acid as a product of fermentation (Funke et al., 1996a).

Source: human clinical specimens; also recovered from animals (Hommez et al., 1999).

DNA G+C content (mol%): 61 (T_m).

Type strain: S160, ATCC 49368, CCUG 35685, CIP 103452, DSM 6922, JCM 7447, NBRC 15207, NCIMB 13130.

Sequence accession no. (16S rRNA gene): X82057.

Further comments: additional GenBank accession numbers include (complete *rpoB* gene) AY492241. When identification is done based solely on the use of phenotypic methods, *Corynebacterium amycolatum* isolates have been misidentified as *Corynebacterium xerosis*, *Corynebacterium minutissimum*, *Corynebacterium striatum*, and other closely related taxa (Funke et al., 1996a; Wauters et al., 1996; Zinkernagel et al., 1996). Most strains identified as *Corynebacterium amycolatum* using a polyphasic approach are MDR due in part to the presence of the *ermX* gene and other mechanisms as yet not fully elaborated (Yague et al., 1997). Older publications where identification of *Corynebacterium xerosis* and *Corynebacterium minutissimum* was based on biochemical, not genetic, traits should be interpreted with caution, as strains described as being multidrug resistant may, in fact, be misidentified *Corynebacterium amycolatum* isolates. The *Corynebacterium amycolatum* type strain (CCUG 35685[T]) and at least one other reference strain, CIP 100836 ("*Corynebacterium asperum*"), differ from most clinical isolates as they are fully sensitive to all drug classes tested by microbroth dilution (K. Bernard, personal communication). Strains formerly designated CDC group I2 or CDC group F2 have been assigned to *Corynebacterium amycolatum* (Wauters et al., 1996).

6. **Corynebacterium appendicis** Yassin, Steiner and Ludwig 2002b, 1168[VP]

ap.pen′di.cis. L. fem. gen. n. *appendicis* of an appendage, intended to mean pertaining to appendicitis, from which the patient from whom the clinical material was taken for isolation of the organism was suffering.

Cells are Gram-stain-positive, thin, nonmotile, nonsporeforming, and pleomorphic coryneforms. Form small (<0.5 mm in diameter), grayish, and nonhemolytic colonies on Columbia sheep blood agar after 2 d at 37°C. Lipophilic. Facultatively anaerobic. Nitrate is not reduced. Acid is produced from glucose, maltose, and glycerol but not from arabinose, arabitol, cellobiose, glycogen, inulin, lactose, mannitol, melibiose, melzitose, pullulan, salicin, sorbitol, sucrose, tagatose, trehalose, raffinose, rhamnose, ribose, or xylose. Does not hydrolyze esculin, gelatin, hippurate, or starch. Displays urease, alkaline phosphatase, and pyrazinamidase activities but not acid phosphatase, arginine dihydrolase, esterase, esterase lipase, lipase, α-glucosidase, β-glucosidase, α-galactosidase, β-galactosidase, β-glucuronidase, *N*-acetyl-β-glucosaminidase, α-mannosidase, α-fucosidase, phosphoamidase, alanine-phenylalanine-proline arylamidase, glycyl-tryptophan arylamidase, pyroglutamic acid arylamidase, pyrrolidonyl arylamidase, valine arylamidase, cystine arylamidase, leucine arylamidase, trypsin, and chymotrypsin. Acetoin production is positive, but indole production is negative. Produces lactate, but not propionate, as the major product of glucose fermentation.

Contains *meso*-DAP as wall diamino acid in addition to galactose and arabinose in whole-cell hydrolysates (i.e. cell-wall chemotype IV). Corynomycolic acids are present, and the fatty acid profile contains saturated, unsaturated, and huge amounts of tuberculostearic acids. Type PI phospholipids pattern with no nitrogen-containing compounds.

Source: abdominal swab of a patient with appendicitis with abscess formation.

DNA G+C content (mol%): 65.8 (HPLC).

Type strain: CCUG 48298, DSM 44531, IMMIB R-3491, JCM 11765, NRRL B-24151.

Sequence accession no. (16S rRNA gene): AJ314919.

Further comments: additional GenBank accession numbers include (partial *rpoB* gene) EU004066.

7. **Corynebacterium aquilae** Fernández-Garayzábal, Egido, Vela, Briones, Collins, Mateos, Hutson, Domínguez and Goyache 2003, 1137[VP]

a.qui′la.e. L. gen. n. *aquilae* of an eagle.

Cells are Gram-stain-positive, nonmotile, nonsporeforming rods. Colonies are whitish, low convex, nonhemolytic, dry, and rough, 1–2 mm in diameter after 48 h incubation at 37°C. Facultatively anaerobic. Oxidase negative. Nonlipophilic. CAMP reaction is negative. Nitrate is not reduced. Acid is produced from D-glucose, D-fructose, D-mannose, glycerol, ribose, *N*-acetylglucosamine, and galactose, but not from maltose, trehalose, D-xylose, L-xylose, mannitol, lactose, sucrose, erythritol, D-arabinose, L-arabinose, adonitol, methyl β-xyloside, L-sorbose, rhamnose, inositol, sorbitol, methyl α-D-mannoside, methyl α-D-glucoside, amygdalin, arbutin, salicin, cellobiose, melibiose, inulin, melezitose, D-raffinose, xylitol, b-gentiobiose, D-turanose, D-lyxose, D-tagatose, D-fucose, L-fucose, D-arabitol, L-arabitol, 2-keto-gluconate, 5-keto-gluconate, and glycogen. Esculin, urea, and gelatin are not hydrolyzed. Alkaline and acid phosphatases, esterase, esterase lipase, leucine arylamidase, chymotrypsin, and phosphoamidase activities are detected. No activity is detected for lipase, α-glucosidase, β-glucosidase, β-glucuronidase, α-mannosidase, α-galactosidase, β-galactosidase, α-fucosidase, *N*-acetyl- β-glucosaminidase, valine arylamidase, cystine arylamidase, and trypsin.

The cell wall contains *meso*-DAP. Mycolic acids (C_{30}-C_{36}) are present. Fatty acids are of the straight-chain saturated ($C_{14:0}$, $C_{16:0}$, and $C_{18:0}$) and monounsaturated ($C_{16:1}$ ω9*c* and $C_{18:1}$ ω9*c*) types.

Source: eagles.

DNA G+C content (mol%): not available.

Type strain: S-613, CCUG 46511, CECT 5993, JCM 12268.

Sequence accession no. (16S rRNA gene): AJ496733.

8. **Corynebacterium argentoratense** Riegel, Ruimy, de Briel, Prévost, Jehl, Bimet, Christen and Monteil 1995a, 537[VP]

ar.gen.to.ra.ten′se. L. neut. adj. argentoratense of or pertaining to *Argentoratus*, Latin name of the city of Strasbourg, where the organism was isolated.

Cells are Gram-stain-positive, nonmotile, nonsporeforming pleomorphic rods. They grow on blood agar as circular acuminated creamy colonies which are not hemolytic. Facultatively anaerobic. Nonlipophilic. Nitrate is not reduced. Esculin, urea, gelatin, tyrosine, and DNA are not hydrolyzed or degraded. Hydrolyses of starch and hippurate occur variably. Acid is produced from D-glucose and fructose but not from sucrose, maltose, lactose, galactose, D-xylose, trehalose, glycogen, or D-mannitol. Acidification from ribose occurs variably. Pyrazinamidase, esterase lipase, cystine arylamidase, and, characteristically, α-chymotrypsin are present. Production of esterase, leucine arylamidase, and acid phosphatase is variable, and alkaline phosphatase is usually not produced. Pyrrolidonyl arylamidase, β-glucuronidase, β-galactosidase, α-glucosidase, and *N*-acetyl- β-glucosaminidase are not produced.

The cell wall contains *meso*-DAP and the sugars arabinose and galactose. Mycolic acids of short-chain lengths (C_{26}–C_{36}) are present. Strains produce propionic acid as a metabolic product (Bernard et al., 2002).

Source: human clinical specimens, in particular throat specimens.

DNA G+C content (mol%): 60–61 (HPLC).

Type strain: IBS B10697, ATCC 51927, CCUG 34893, CIP 104296, DSM 44202, JCM 10392.

Sequence accession no. (16S rRNA gene): X83955.

Further comments: additional GenBank accession numbers include (complete *rpoB* gene) AY492249.

9. **Corynebacterium atypicum** Hall, Collins, Hutson, Lawson, Falsen and Duerden 2003, 1067[VP]

a.ty′pi.cum. N.L. neut. adj. *atypicum* not typical, referring to the absence of corynomycolic acids, a feature normally present in corynebacteria.

Cells are Gram-stain-positive, short to filamentous rods. Colonies on Columbia agar with 5% horse blood, after incubation at 37°C for 48 h, are pinpoint, convex, entire-edged, shiny, white, and nonhemolytic. Facultatively anaerobic. Nonlipophilic. Growth occurs in broth containing 7.5% NaCl but not in 10% NaCl. Nitrate is not reduced to nitrite. Esculin, urea, gelatin, and starch are not hydrolyzed. Acid is produced from D-glucose, maltose, ribose, and sucrose but not from lactose, mannitol, glycogen, or D-xylose. Activity is detected for β-glucuronidase, cystine arylamidase, leucine arylamidase, and valine arylamidase. Alkaline and acid phosphatases, chymotrypsin, esterase, esterase lipase, α-fucosidase, α-galactosidase, β-galactosidase, a-glucosidase, β-glucosidase, lipase, α-mannosidase, *N*-acetyl-β-glucosaminidase, phosphoamidase, pyrrolidonyl arylamidase, pyrazinamidase, and trypsin are not produced. Acetoin is not produced.

The cell-wall murein contains *meso*-DAP. Cellular fatty acids are consistent with those described for the genus, but tuberculostearic acid is not present. Mycolic acids are not detected.

Source: an unknown human clinical source; habitat is not known.

DNA G+C content (mol%): not available.

Type strain: R2070, CCUG 45804, CIP 107431, JCM 12368.

Sequence accession no. (16S rRNA gene): AJ441057.

10. **Corynebacterium aurimucosum** Yassin, Steiner and Ludwig 2002a, 1004[VP] emend. Daneshvar, Hollis, Weyant, Jordan, MacGregor, Morey, Whitney, Brenner, Steigerwalt, Helsel, Raney, Patel, Levett and Brown 2005, 7 (Effective publication: Daneshvar, Hollis, Weyant, Jordan, MacGregor, Morey, Whitney, Brenner, Steigerwalt, Helsel, Raney, Patel, Levett and Brown 2004, 4197.)

au.ri.mu.co′sum. L. n. *aurum* gold; L. neut. adj. *mucosum* slimy; N.L. neut. adj. *aurimucosum* slimy and gold-colored, pertaining to the appearance of colonies.

Cells are Gram-stain-positive and non-acid-fast. They are thin, nonmotile, nonsporeforming, and pleomorphic coryneforms. On Columbia blood agar supplemented with 5% sheep blood, the colonies are sticky and slightly yellow or charcoal black in color. On trypticase soy agar, they appear colorless or charcoal black and slimy. On brain heart infusion agar supplemented with 1% Tween 80, some strains are able to form a corraloid precipitin in agar when grown in the presence of CO_2. Facultatively anaerobic. Oxidase very rarely positive. Acid is produced from fructose, D-glucose, maltose, sucrose, and occasionally D-mannitol but not from adonitol, amygdalin, arabinose, cellobiose, glycerol, glycogen, inulin, lactose, mannose, melezitose,

raffinose, rhamnose, ribose, salicin, sorbitol, trehalose, or xylose. Hydrolyzes hippurate and occasionally gelatin and urea but not starch. Esculin hydrolysis is variable, and nitrate reductase is rare. Positive for alkaline phosphatase, leucine arylamidase, and pyrazinamidase activities, but negative for acid phosphatase, arginine dihydrolyse, esterase, ester lipase, lipase, α-glucosidase, β-glucosidase, α-galactosidase, β-galactosidase, β-glucuronidase, *N*-acetyl-β-glucosaminidase, α-mannosidase, α-fucosidase, phosphoamidase, pyrrolidonyl arylamidase, valine arylamidase, cystine arylamidase, trypsin, and chymotrypsin. Acetoin positive and indole negative. Lactate, not propionate, is the major end product of glucose fermentation.

Cells contain *meso*-DAP with galactose and arabinose sugars in whole-cell hydrolysates (i.e. the cell-wall chemotype is chemotype IV). Short-chain corynomycolic acids are present. The fatty acid profile contains saturated, unsaturated, and tuberculostearic acids. It has type PI phospholopid pattern with no nitrogen-containing compounds.

Source: human clinical specimens; black-pigmented colony types are from female urogenital sites, some of which are associated with complications in pregnancy.

DNA G+C content (mol%): 63.7 (HPLC); 60.6 by complete genome sequence analysis of strain CN-1.

Type strain: CCUG 47449, DSM 44532, IMMIB D-1488, JCM 11766, NRRL B-24143.

Sequence accession no. (16S rRNA gene): AJ309207.

Further comments: additional GenBank accession numbers include (complete *rpoB* gene) AY492282. *Corynebacterium nigricans* (Shukla et al. 2004) (Effective publication: Shukla et al. 2003.) was reassigned as a later heterotypic synonym of *Corynebacterium aurimucosum* (Yassin et al. 2002a), Daneshvar 2005 (Effective publication: Daneshvar et al. 2004.). The complete genome of a black-pigmented *Corynebacterium aurimucosum* strain CN-1 (= ATCC 700975, type strain of *Corynebacterium nigricans*) with 2,790,189 bp has been deposited under GenBank accession no. CP001601 (Trost et al., 2010). By 16S rRNA gene sequencing alone, this species can not be readily discerned from *Corynebacterium minutissimum* and *Corynebacterium singulare*, but these species can be resolved using *rpoB* gene sequencing (Khamis et al., 2004).

11. **Corynebacterium auris** Funke, Lawson and Collins 1995c, 738[VP]

au′ris. L. gen. fem. n. *auris* of the ear.

Gram-stain-positive, nonmotile, nonsporeforming diphtheroids. Colonies are circular, convex, and dry, becoming slightly yellowish with time; slightly adhere to agar. Weakly adherent if subcultured on agar plates. Nonlipophilic. Oxidative metabolism. Acid is not produced from glucose, maltose, sucrose, mannitol, xylose, ribose, lactose, and glycogen. The following substrates are utilized: γ-hydroxybutyric acid, L-malic acid, pyruvic acid, succinamic acid, *N*-acetyl-L-glutamic acid, L-asparagine, L-glutamic acid, glycyl-L-glutamic acid, and L-pyroglutamic acid. Nitrate is not reduced. Urea and esculin are not hydrolyzed. The CAMP reaction is positive. Pyrazinamidase, alkaline and acid phosphatases, esterase, esterase lipase, lipase, leucine arylamidase, and phosphoamidase are produced.

The cell wall contains *meso*-DAP. Mycolic acids are produced but are cleaved at the temperature (300°C) produced in the injection port of the commercial MIDI system, resulting in fatty acids which are identified as, e.g. $C_{17:1}$ ω6*c*–$C_{17:1}$ ω9*c* (Funke and Bernard, 2007). Cellular fatty acids are consistent with those described for the genus. Propionic acid not detected as metabolic product (Bernard et al., 2002).

Source: outer ear channel of humans.

DNA G+C content (mol%): 68–74 (HPLC).

Type strain: strain ATCC 51966, CCUG 33426, CIP 104632, DMMZ 328, DSM 44122, JCM 11946, LMG 19072.

Sequence accession no. (16S rRNA gene): X81873.

Further comments: additional GenBank accession numbers include (complete *rpoB* gene) AY492234. *Corynebacterium auris* was originally described as Coryneform CDC group ANF-1 like.

12. **Corynebacterium auriscanis** Collins, Hoyles, Lawson, Falsen, Robson and Foster 2000, 423[VP] (Effective publication: Collins, Hoyles, Lawson, Falsen, Robson and Foster 1999b, 3446.)

au.ris.ca′nis. L. fem. n. *auris* ear; L. masc. n. *canis* dog; N.L. gen. n. *auriscanis* of the ear of the dog.

Cells are Gram-stain-positive typically club-shaped rods, which appear as single cells, in pairs, or in clusters. Obligately aerobic. Nonlipophilic. CAMP reaction negative. Acid is produced from glucose but not from glycogen, lactose, maltose, mannitol, sucrose, ribose, or D-xylose. Nitrate is not reduced, and the Voges–Proskauer test is negative. Hippurate is hydrolyzed. Esculin hydrolysis is variable. Urea, gelatin, and starch are not hydrolyzed. Alkaline and acid phosphatases, esterase (weak reaction), ester lipase (weak reaction), leucine arylamidase, phosphoamidase, and pyrrolidonyl arylamidase activities are detected. *N*-Acetyl-β-glucosaminidase, chymotrypsin, cystine arylamidase, α-fucosidase, α-galactosidase, β-galactosidase, α-glucosidase, β-glucosidase, β-glucuronidase, α-mannosidase, pyrazinamidase, trypsin, and valine arylamidase activities are not detected. Lipase activity variable.

The cell wall contains *meso*-DAP. Short-chain mycolic acids (C_{28}–C_{34}) are present, with $C_{30:0}$, $C_{32:0}$, and $C_{34:0}$ as the main components. Cellular fatty acids are consistent with those described for the genus, but tuberculostearic acid is not present.

Source: clinical specimens from dogs; habitat is not known.

DNA G+C content (mol%): 61 (T_m).

Type strain: M598/96/1, CCUG 39938, CIP 106629, DSM 44609, JCM 12369.

Sequence accession no. (16S rRNA gene): AJ243819.

Further comments: additional GenBank accession numbers include (complete *rpoB* gene) AY492244.

13. **Corynebacterium bovis** Bergey, Harrison, Breed, Hammer and Huntoon 1923, 388[AL]

bo′vis. L. n. *bos* a cow; L. gen. n. *bovis* of a cow.

(This description is largely based on that of Collins and Cummins, 1986.)

Irregular rods 0.5–0.7 × 2.5–3.0 μm, often clubbed shaped; coccobacillary forms may occur. Colonies on nutrient agar supplemented with Tween 80 (0.1%) are white to cream, circular, entire, slightly shiny, ~1–2 mm in diameter. Aerobic. Lipophilic. Most strains ferment glucose, fructose, galactose, maltose, and glycerol. Oxidase, arabinose, lactose, trehalose, and dextrin variable. Xylose, rhamose,

lactose, mannitol, mannose, sucrose, raffinose, salicin, and starch not utilized. Esculin, starch, and casein not hydrolyzed. Hippurate hydrolyzed; phosphatase and pyrazinamidase positive, but variable for β-galactosidase. Nitrate not reduced; urease not produced (Collins and Cummins, 1986; Hollis and Weaver, 1981).

The cell wall contains *meso*-DAP (variation A1γ), with sugars arabinose and galactose. Low-molecular-weight mycolic acids (~C_{22}–C_{36}) found, with the α-alkyl group side-chain consisting of C_6H_{13} and C_8H_{17}. MK-9 (H2) detected. Cellular fatty acids are consistent with those described for the genus (Bernard et al., 1991), with TBSA being detected. Propionic acid not detected as metabolic product (Bernard et al., 2002).

Source: aseptically drawn milk, as a commensal on cow's udder or as cause of bovine mastitis and occasionally as cause of infection in humans (Achermann et al., 2009; Bernard et al., 2002; Dutly et al., 2003; Vale and Scott, 1977).

DNA G+C content (mol%): 67.8–69.7 (T_m).

Type strain: ATCC 7715, CCUG 2705, CIP 54.80, DSM 20582, JCM 11947, NCTC 3224.

Sequence accession no. (16S rRNA gene): X84444.

Further comments: additional GenBank accession numbers include (complete *rpoB* gene) AY492236.

14. **Corynebacterium callunae** (Lee and Good, 1963) Yamada and Komagata 1972, 412[AL] ("*Corynebacterium callunae*" Lee and Good 1963, 1349.)

cal.lu′na.e. N.L. n. *calluna* generic name of heather; N.L. gen. n. *callunae* of heather.

(This description is largely based on that of Collins and Cummins, 1986.)

Short, strongly Gram-stain-positive rods, metachromatic granules present. Moderate growth on nutrient agar. Glucose, fructose, mannose, maltose, sucrose, trehalose, salicin, and methyl red positive. Hippurate hydrolyzed and urease produced. Negative for arabinose, xylose, rhamnose, galactose, lactose, raffinose, dextrin, and starch fermentation. Esculin, casein, and gelatin hydrolysis negative, and nitrate not reduced. Grows at pH 6 and in 30% glucose but not at 45°C (Fudou et al., 2002).

Cell wall contains *meso*-DAP. Arabinose and galactose are major sugars. Mycolates are present. Cellular fatty acids are consistent with those of the genus. Menaquinone is MK-9(H_2).

Source: heather; habitat remains unknown.

DNA G+C content (mol%): 51.2 (T_m).

Type strain: ATCC 15991, CCUG 28793, CIP 104277, DSM 20147, HAMBI 2053, JCM 9489, NBRC 15359.

Sequence accession no. (16S rRNA gene): X84251.

Further comments: additional GenBank accession numbers include (complete *rpoB* gene) AY492245.

15. **Corynebacterium camporealensis** Fernández-Garayzábal, Collins, Hutson, Gonzalez, Fernández and Domínguez 1998, 466[VP]

cam.po.re.al.en′sis. N.L. adj. *camporealensis* pertaining to Campo Real, Madrid, Spain.

Gram-stain-positive pleomorphic rods occurring singly or are arranged in palisades or V-shaped forms. Colonies are nonhemolytic, circular, slightly convex, smooth, and with a creamy consistency. Facultatively anaerobic. Nonlipophilic. CAMP reaction is strongly positive. Nitrate is not reduced. Esculin, urea, and gelatin are not hydrolyzed. Acetoin and indole are not produced. Acid is produced from glucose, but not from ribose, xylose, mannitol, lactose, maltose, sucrose, and glycogen. Pyrazinamidase, alkaline phosphatase, esterase, esterase lipase, lipase, leucine arylamidase, valine arylamidase, and cystine arylamidase are produced. Pyrrolidonyl arylamidase, acid phosphatase, β-glucuronidase, α-galactosidase, β-galactosidase, α-glucosidase, β-glucosidase, α-mannosoidase, α-fucosidase, *N*-acetyl-β-glucosaminidase, trypsin, and chymotrypsin are not produced.

The cell wall contains *meso*-diaminopimelic acid. Short-chain mycolic acids are present.

Source: milk of sheep affected with subclinical mastitis.

DNA G+C content (mol%): not available.

Type strain: CRS-51, ATCC BAA-77, CCUG 39412, CECT 4897, CIP 105508, DSM 44610, JCM 11664.

Sequence accession no. (16S rRNA gene): Y09569.

Further comments: additional GenBank accession numbers include (complete *rpoB* gene) AY492246.

16. **Corynebacterium canis** Funke, Englert, Frodl, Bernard and Stenger 2010a, 2546[VP]

ca′nis. L. gen. n. *canis* of a dog.

Cells are Gram-stain-positive, nonsporeforming, and nonmotile. Some cells are typically club-shaped, rods but some are filamentous, and some even show branching. Colonies are beige-whitish, dryish, with irregular edges, convoluted, up to 1–2 mm in diameter after 48 h incubation, and adherent to sheep blood agar. Facultatively anaerobic. Catalase positive. Acid is produced from glycerol, galactose, glucose, fructose, mannose, arbutin, salicin, maltose, sucrose, trehalose, starch, glycogen, tagatose, and 5-keto-gluconate but not from erythritol, arabinose, ribose, xylose, adonitol, methyl-β-D-xylopyranoside, L-sorbose, inositol, D-mannitol, D-sorbitol, methyl-α-D-mannopyranoside, methyl-α-D-glucopyranoside, *N*-acetylglucosamine, amygdalin, D-cellobiose, D-lactose, D-melibiose, inulin, D-melezitose, xylitol, gentiobiose, D-turanose, D-lyxose, fucose, arabitol, gluconate, and 2-keto-gluconate. Activities of the following enzymes can be detected: nitrate reductase, pyrazinamidase, β-glucosidase, α-glucosidase, alkaline phosphatase, esterase, esterase lipase, leucine arylamidase, trypsin, and phosphoamidase. Activities of urease, pyrrolidonyl arylamidase, gelatinase, lipase, cystine arylamidase, α-chymotrypsin, α-galactosidase, β-galactosidase, β-glucuronidase, *N*-acetyl-β-glucosaminidase, α-mannosidase, and α-fucosidase are not detected. The CAMP reaction is negative.

The cell wall contains *meso*-DAP. Mycolic acids are present. Cellular fatty acids are consistent with those described for the genus.

Source: a patient's wound after a dog bite.

DNA G+C content (mol%): not available.

Type strain: 1170, CCUG 58627, DSM 45402.

Sequence accession no. (16S rRNA gene): GQ871934.

Further comments: additional GenBank accession numbers include (partial *rpoB* gene) GQ871935.

17. **Corynebacterium capitovis** Collins, Hoyles, Foster, Sjödén and Falsen 2001a, 858[VP]

ca.pit.o′vis. L. gen. n. *capitis* of a head; L. gen. n. *ovis* of a sheep; N.L. gen. n. capitovis of a sheep's head.

Cells are Gram-stain-positive diphtheroids. Colonies are circular, entire, convex, nonhemolytic, and lemon-pigmented. Facultatively anaerobic. Nonlipophilic. Acid is produced from glucose but not from lactose, maltose, mannitol, ribose, sucrose, and D-xylose. Nitrate is not reduced. Esculin, urea, and gelatin are not hydrolyzed. Activity for alkaline and acid phosphatases, esterase (weak), esterase lipase, lipase (weak), and leucine arylamidase is detected. No activity is detected for cystine arylamidase, chymotrypsin, α-fucosidase, α-galactosidase, β-galactosidase, α-glucosidase, β-glucosidase, β-glucuronidase, α-mannosidase, *N*-acetyl-β-glucosaminidase, valine arylamidase, pyrazinamidase, phosphoamidase, pyrrolidonyl arylamidase, and trypsin.

Cell wall contains *meso*-DAP. Long-chain fatty acids are of the straight-chain saturated and monounsaturated types. Tuberculostearic acid is not present. Mycolic acids are present (C_{32}–C_{36}).

Source: infected head of a sheep; habitat is unknown.

DNA G+C content (mol%): not available.

Type strain: S108/98/2, CCUG 39779, CIP 106739, DSM 44611, JCM 12101.

Sequence accession no. (16S rRNA gene): AJ297402.

Further comments: additional GenBank accession numbers include (complete *rpoB* gene) AY492247.

18. **Corynebacterium casei** Brennan, Brown, Goodfellow, Ward, Beresford, Simpson, Fox and Cogan 2001, 850[VP]

ca′se.i. L. gen. n. *casei* of cheese, named because the organism was isolated from cheese.

Cells are Gram-stain-positive irregularly shaped rods of 1–3 μm in length. Colonies are cream, circular, matt, entire, low-concave, and 1 mm in diameter. Facultatively anaerobic. Nitrate is reduced to nitrite. Esculin, urea, gelatin, tyrosine, and ONPG are not hydrolyzed. CAMP reaction is negative. Acid is produced from glucose, ribose, mannose, and fructose but not from glycogen, glycerol, erythritol, D-arabinose, L-arabinose, D-xylose, L-xylose, adonitol, methyl-β-xyloside, galactose, sorbose, rhamnose, dulcitol, inositol, mannitol, sorbitol, methyl-α-D-mannoside, methyl-α-D-glucoside, amygdalin, arbutin, salicin, cellobiose, maltose, lactose, melibiose, sucrose, inulin, melezitose, raffinose, xylitol, gentiobiose, D-turanose, D-lyxose, D-tagatose, D-fucose, L-fucose, D-arabitol, L-arabitol, gluconate, or 2-ketogluconate. Alkaline and acid phosphatases, esterase, esterase lipase, leucine arylamidase, cystine arylamidase, and pyrazinamidase activities are present. The isolates do not produce lipase, trypsin, chymotrypsin, α-galactosidase, α-mannosidase, β-galactosidase, β-glucuronidase, α-glucosidase, β-glucosidase, and *N*-acetyl-β-glucosaminidase.

The cell wall was found to be type IV, containing *meso*-diaminopimelic acid, arabinose, galactose, and short-chain mycolic acids (C_{22}–C_{36}). The menaquinones in the cell wall were of the MK-9(H_2) type. Analysis of the polar lipid of the cell wall revealed the presence of diphosphatidylglycerol, phosphatidylglycerol, phosphotidylinositol, and phosphatidylglycerol mannosides.

Source: surface of smear-ripened cheese.

DNA G+C content (mol%): 51 (HPLC).

Type strain: CIP 107182, DPC 5298, DSM 44701, JCM 12072, LMG S-19264, NCIMB 30130.

Sequence accession no. (16S rRNA gene): AF267152.

Further comments: additional GenBank accession numbers include (complete *rpoB* gene) EU616817.

19. **Corynebacterium caspium** Collins, Hoyles, Foster and Falsen 2004, 926[VP]

cas.pi′um. L. neut. adj. *caspium* belonging to the Caspian Sea, referring to the isolation of the type strain from a Caspian seal.

Cells are Gram-stain-positive, irregular-shaped tapered rods and clubs. Nonlipophilic. Colonies are circular, convex, entire, opaque, dull, nonhemolytic and can be moved across the plate while retaining their integrity. Facultatively anaerobic. Nitrate is not reduced. CAMP reaction negative. Acid is produced from D-glucose and D-ribose, but not from glycogen, lactose, maltose, mannitol, sucrose, or D-xylose. Urea is hydrolyzed but esculin and gelatin are not. Activity is detected for esterase, esterase lipase, pyrazinamidase, and trypsin. No activity is detected for acid phosphatase, alkaline phosphatase, chymotrypsin, cystine arylamidase, α-fucosidase, α-galactosidase, β-galactosidase, β-glucuronidase, α-glucosidase, β-glucosidase, *N*-acetyl-β-glucosaminidase, lipase, leucine arylamidase, α-mannosidase, pyrrolidonyl arylamidase, phosphoamidase, or valine arylamidase.

Cell-wall murein is based on *meso*-diaminopimelic acid. Corynomycolic acids are not present. Cellular fatty acids are consistent with those described for the genus; tuberculostearic acid is not present.

Source: a Caspian seal; habitat is not known.

DNA G+C content (mol%): not available.

Type strain: M/106/00/5, CCUG 44566, CIP 107965, JCM 13387.

Sequence accession no. (16S rRNA gene): AJ566641.

20. **Corynebacterium ciconiae** Fernández-Garayzábal, Vela, Egido, Hutson, Lanzarot, Fernández-García and Collins 2004, 2194[VP]

ci.co.ni′a.e. L. gen. fem. n. *ciconiae* of a (black) stork.

Cells are Gram-stain-positive rods. Nonlipophilic. Colonies are creamy-white, circular, convex, dry, and nonhemolytic on Columbia blood agar. Facultatively anaerobic. CAMP reaction negative. Nitrate is not reduced. Esculin, urea, and gelatin are not hydrolyzed. Acid is produced from glucose, ribose, glycerol, D-fructose, D-mannose, *N*-acetyl-β-glucosamine, and maltose, but not from D-xylose, mannitol, lactose, sucrose, glycogen, erythritol, L-arabinose, L-xylose, adonitol, methyl β-xyloside, galactose, sorbose, L-rhamnose, dulcitol, inositol, sorbitol, methyl α-D-mannoside, methyl α-D-glucoside, amygdalin, arbutin, salicin, cellobiose, melibiose, inulin, melezitose, D-raffinose, xylitol, b-gentiobiose, D-turanose, D-lyxose, D-tagatose, D-fucose, D-arabitol, L-arabitol, 2-keto-gluconate, or 5-keto-gluconate. Acidification of D-arabinose, L-fucose, trehalose, starch, and gentiobiose is variable. Activity of alkaline and acid phosphatases, esterase, ester lipase, lipase, leucine arylamidase, phosphoamidase, β-glucosidase, and pyrazinamidase is detected. Pyrrolidonyl arylamidase, β-glucuronidase, β-galactosidase, α-glucosidase, *N*-acetyl-β-glucosaminidase, α-mannosidase,

α-galactosidase, α-fucosidase, chymotrypsin, trypsin, valine arylamidase, and cystine arylamidase are not produced. Mycolic acids are absent. Cellular fatty acids are consistent with those described for the genus.

Source: the trachea of apparently healthy wild nesting black storks (*Ciconia nigra*).

DNA G+C content (mol%): not available.

Type strain: BS13, CCUG 47525, CECT 5779, JCM 13388.

Sequence accession no. (16S rRNA gene): AJ555193.

21. **Corynebacterium confusum** Funke, Osorio, Frei, Riegel and Collins 1998c, 1294[VP]

con.fu′sum. L. past part. *confusum* confused, to indicate that this bacterium might be phenotypically confused with many other *Corynebacterium* species.

Cells are Gram-stain-positive, typically occurring as club-shaped rods as single cells, in pairs, or in small clusters. Colonies are whitish, glistening, convex, and creamy, up to 1.5 mm in diameter after 48 h incubation. Facultatively anaerobic, but weak anaerobic growth. Nonlipophilic. CAMP reaction is negative. Nitrate is reduced. Esculin, urea, and tyrosine are not hydrolyzed. Acid is produced from D-glucose, ribose, D-fructose, tagatose, and 5-ketogluconate, but acid is not produced from maltose, sucrose, mannitol, D-xylose, glycerol, erythritol, arabinose, adonitol, β-methylxyloside, galactose, L-sorbose, rhamnose, dulcitol, inositol, sorbitol, methyl-α-D-mannoside, methyl-α-D-glucoside, *N*-acetylglucosamine, amygdalin, arbutin, salicin, cellobiose, lactose, melibiose, trehalose, inulin, melezitose, D-raffinose, glycogen, xylitol, D-turanose, D-lyxose, fucose, arabitol, gluconate, or 2-ketogluconate. Acid production from β-gentiobiose is variable. Activities of pyrazinamidase, alkaline phosphatase, esterase, and esterase lipase are detected, but pyrrolidonyl arylamidase, valine arylamidase, trypsin, chymotrypsin, α-galactosidase, β-galactosidase, β-glucuronidase, α-glucosidase, β-glucosidase, *N*-acetyl-β-glucosaminidase, α-mannosidase, and α-fucosidase are not detected. Activities of leucine arylamidase and phosphoamidase are variable.

The cell wall contains *meso*-diaminopimelic acid. Mycolic acids are present. Main straight-chain saturated fatty acids are palmitic and stearic acids; oleic acid is the predominant unsaturated fatty acid. Minor amounts of tuberculostearic acid are present. A clinical strain was CAMP positive, and propionic acid was detected as a metabolic product (Bernard et al., 2002).

Source: human clinical specimens.

DNA G+C content (mol%): not available.

Type strain: CCUG 38267, CIP 105403, DMMZ 2439, DSM 44384, JCM 12102.

Sequence accession no. (16S rRNA gene): Y15886.

Further comments: additional GenBank accession numbers include (complete *rpoB* gene) AY492248.

22. **Corynebacterium coyleae** Funke, Pascual Ramos and Collins 1997e, 94[VP]

coy.le′a.e. N.L. gen. fem. n. *coyleae* of Coyle, to honor the American microbiologist Marie B. Coyle for her contributions to the clinical microbiology of coryneform bacteria.

Cells are Gram-stain-positive diphtheroids. Colonies are whitish, circular, convex, and slightly glistening; some colonies have a creamy consistency, and others have a sticky consistency and are ~1 mm after 24 h incubation at 37°C on blood agar. Facultatively anaerobic, but very weak anaerobic growth. Nonlipophilic. Acid is slowly produced from glucose, ribose, D-fructose, D-mannose, and 5-keto-gluconate but not from maltose, sucrose, mannitol, xylose, glycerol, erythritol, D-arabinose, L-arabinose, adonitol, β-methylxyloside, galactose, L-sorbose, rhamnose, dulcitol, inositol, sorbitol, α-methyl-D-mannoside, α-methyl-D-glucoside, *N*-acetylglucosamine, amygdalin, arbutin, salicin, cellobiose, lactose, melibiose, trehalose, inulin, melezitose, D-raffinose, starch, glycogen, xylitol, b-gentiobiose, D-turanose, D-lyxose, D-tagatose, D-fucose, L-fucose, D-arabitol, L-arabitol, gluconate, and 2-keto-gluconate. Nitrate is not reduced. Urea, esculin, casein, tyrosin, and xanthine are not hydrolyzed. The CAMP reaction is strongly positive. Pyrazinamidase, alkaline and acid phosphatases, esterase, esterase lipae, leucine aryamidase, cystine arylamidase, and phosphoamidase activities are detected, but valine arylamidase, chymotrypsin, α-galactosidase, β-galactosidase, β-glucuronidase, α-glucosidase, β-glucosidase, *N*-acetyl-β-glucosaminidase, α-mannosidase, and α-fucosidase activities are not detected. Pyrrolidonyl arylamidase activity is variable.

The cell wall contains *meso*-DAP. Mycolic acids are present. Cellular fatty acids are consistent with those described for the genus. Does not produce propionic acid as metabolic product (Bernard et al., 2002).

Source: human clinical specimens.

DNA G+C content (mol%): 62–64 (T_m).

Type strain: ATCC 700219, CCUG 38194, CIP 104919, DMMZ 214, DSM 44184, JCM 10381.

Sequence accession no. (16S rRNA gene): X96497.

Further comments: additional GenBank accession numbers include (complete *rpoB* gene) AY492250. Six *Corynebacterium coyleae* strains associated with neonatal bacteremias were found to possess *ermX* and express resistance to MLS antibiotics (Fernandez-Natal et al., 2008). By 16S rRNA gene sequencing, *Corynebacterium coyleae* is closely related to (98% identity with) *Corynebacterium mucifaciens* and *Corynebacterium afermentans* but differs from them (~94% identity with) when *rpoB* gene sequences are compared (Khamis et al., 2004) as well as by phenotypic means (Table 32).

23. **Corynebacterium cystitidis** Yanagawa and Honda 1978, 215[AL]

cys.ti′ti.dis. Gr. n. *kustis* bladder; N.L. n. *cystitis* cystitis; N.L. gen. n. *cystitidis* of cystitis.

(This description is largely based on that of Collins and Cummins, 1986.)

Gram-stain-positive rods, 0.5–1.3 µm singly, in pairs often at angles, or in irregular masses. Fimbriae (pili) visible using electron microscopy. Colonies on nutrient agar and serum agar are creamy to pale yellow, entire, circular, opaque, and 1 mm in diameter at 24 h. No hemolysis on various blood agars. Pellicle and granular sediment formed in broth. No growth at 5°C; grows at 41.5°C, and cells remain viable for 30 min at 56°C. Aerobic and facultatively anaerobic. Complex vitamin and amino acid requirements. Positive for glucose, xylose, fructose, maltose, trehalose, dextrin, and

starch; negative for arabinose, rhamnose, galactose, mannose, lactose, sucrose, raffinose, and salicin. Nitrate is not reduced. Urease is produced, hippurate and starch are hydrolyzed, and pyrazinamidase is detected. Tyrosine, esculin, and gelatin are not hydrolyzed; casein is not digested; methyl red, and phosphatase are negative.

Meso-DAP and sugars arabinose and galactose are present. MK-8(H_2) detected.

Source: cows with severe hemorrhagic cystitidis and from the prepuce of healthy bulls.

DNA G+C content (mol%): 52.6–53.9 (T_m).

Type strain: ATCC 29593, CCUG 28794, CIP 103424, DSM 20524, JCM 3715, NBRC 15284, NCTC 11863.

Sequence accession no. (16S rRNA gene): X84252.

Further comments: additional GenBank accession numbers include (complete *rpoB* gene) AY492251.

24. **Corynebacterium doosanense** Lee, Cho, Jung, Van Nguyen, Jung, Park, Le and Kim 2009, 2736[VP]

do.o.san.en′se. N.L. neut. adj. *doosanense* belonging to Doosan, named after the Doosan group, a foundation of Chung-Ang University, Seoul, Korea where taxonomic studies on this species were performed.

Gram-stain-positive, nonmotile, nonsporeforming irregular and club-shaped rods that are 0.8~1.0 × 1.0~1.2 µm in size. Colonies are 1~2 mm in diameter, yellow, low convex on GYEA media, and yellow, opaque, low-convex on sheep blood agar after 48 h incubation at 30°C. Aerobic. Activities for sodium pyruvate, pyrazinamidase, ester lipase, leucine arylamidase, and naphthol-AS-BI-phosphohydrolase are detected. Reduction of nitrate and hippurate hydrolysis are positive. No activity is detected for pyrrolidonyl arylamidase, alkaline phosphatase, esterase, lipase, valine arylamidase, cystine arylamidase, trypsin, acid phospatase, α-chymotrypsin, β-glucuronidase, α-galactosidase, β-galactosidase, α-glucosidase, β-glucosidase, α-mannosidase, α-fucosidase, *N*-acetyl-β-glucosaminidase, leucine aminopeptidase, urease, gelatin, and esculin hydrolysis. Acid production occurs only from glucose.

The cell wall contains *meso*-DAP. Whole cell hydrolysates contain mainly galactose and arabinose. Mycolic acids are detected. Cellular fatty acids are consistent with those described for the genus.

Source: activated sludge, taken from a wastewater treatment plant in Yeongdeuk-gun, Republic of Korea.

DNA G+C content (mol%): 53.5 (T_m).

Type strain: CAU 212, CCUG 57284, KCTC 19568.

Sequence accession no. (16S rRNA gene): EU998655.

25. **Corynebacterium durum** Riegel, Heller, Prévost, Jehl and Monteil 1997a, 1110[VP]

du′rum. L. neut. adj. *durum* hard, tough.

The bacteria are Gram-stain-positive pleomorphic rods which can be long and sometimes filamentous. After aerobic incubation at 37°C for 72 h, colonies are beige, convex, and rough, with convolutions and an irregular margin, and strongly adhere to the agar. Colonies incubated in an aerobic atmosphere supplemented with 10% CO_2 have a dense center and nearly regular margins; they adhere weakly to the agar. Formation of filaments under these conditions is rare. Facultatively anaerobic, but weak anaerobic growth. Nitrate reduction positive, but urea hydrolysis is variable. A weak hydrolysis of esculin can be observed sometimes after at least 72 h of incubation. Gelatin and tyrosine are not degraded. Acid is produced from glucose, maltose, fructose, sucrose, galactose, and mannitol but not from ribose, lactose, trehalose, glycogen, or xylose. Pyrazinamidase is produced, but pyrrolidonyl arylamidase, alkaline phosphatase, β-glucuronidase, β-galactosidase, α-glucosidase, and *N*-acetyl-β-glucosaminidase are not. Propionic acid, but not succinic acid is produced as end product of anaerobic metabolism of glucose.

The cell wall contains *meso*-DAP, with sugars arabinose and galactose. Mycolic acids with short chain lengths (C_{26}–C_{36}) are present (HPLC method). Cellular fatty acids are consistent with those described for the genus (Rassoulian-Barrett et al., 2001).

Source: human respiratory tract specimens and occasionally other normally sterile sites.

DNA G+C content (mol%): 55 (capillary electrophoresis).

Type strain: IBS G15036, CCUG 37331, CIP 105490, DSM 44351, JCM 11948.

Sequence accession no. (16S rRNA gene): Z97069.

Further comments: additional GenBank accession numbers include (complete *rpoB* gene) AY492252. *Corynebacterium durum* is the most prominent *Corynebacterium* recovered from throat swabs of healthy adults (von Graevenitz et al., 1998).

26. **Corynebacterium efficiens** Fudou, Jojima, Seto, Yamada, Kimra, Nakamatsu, Hiraishi and Yamanaka 2002, 1130[VP]

ef.fi.ci′ens. L. part. adj. *efficiens* effecting, effective, efficient.

Cells are Gram-stain-positive club-shaped rods 0.8–1.1 × 1.0–4.5 µm in size. Colonies on nutrient agar are smooth, entire, circular, dull to slightly glistening, and generally yellow. Facultatively anaerobic. All strains require biotin for growth. Good growth at 30–40°C; growth occurs up to 45°C. No growth occurs when the pH is below 6 or in the presence of 30% glucose. Nitrate is reduced to nitrite. Hydrolysis of esculin and urea is variable. Acid is formed from glucose, fructose, mannose, ribose, maltose, and dextrin, but not from xylose, mannitol, lactose, salicin, galactose, starch, or glycogen. All strains assimilate acetate, pyruvate, and L-lactate, but they do not assimilate D-lactate, succinate, 2-oxoglutarate, citrate, adipicate, pimelate, glycolate, or glyoxylate. Pyrazinamidase is detected but pyrrolidonyl arylamidase, alkaline phosphatase, β-galactosidase, α-glucosidase, *N*-acetyl-β-glucosaminidase, and tyrosinase are not detected. Produces large amounts of L-glutamic acid under aerobic conditions.

The cell wall contains *meso*-DAP. Mycolic acids are present. MK-9(H_2) is the major menaquinone. Cellular fatty acids are consistent with those described for the genus. Polar lipids include phosphatidylinositol and its mannoside.

Source: soil and vegetables.

DNA G+C content (mol%): 59–60.2 (T_m), 63.4 (complete genome sequencing).

Type strain: YS-314, AJ 12310, CCUG 47130, CCUG 48037, DSM 44549, JCM 11189, NBRC 100395.

Sequence accession no. (16S rRNA gene): AB055963.

Further comments: additional GenBank accession numbers include (complete *rpoB* gene) AP005215. The complete genome of *Corynebacterium efficiens* YS-314^{T}, deposited under GenBank accession no. NC_004369 with 3,147,090 bp, as well as discussion of how amino acid substitutions positively affect thermostability, have been reported (Brune et al., 2005; Nishio et al., 2003).

27. **Corynebacterium falsenii** Sjödén, Funke, Izquierdo, Akervall and Collins 1998, 73^{VP}

fal.se′ni.i. N.L. gen. masc. n. *falsenii* of Falsen, to honor the Swedish microbiologist and taxonomist Enevold Falsen curator of the CCUG, for his lifelong contributions to bacterial taxonomy as well as for the systematic collection and characterization of prokaryotes.

Gram-stain-positive, typically club-shaped rods which appear as single cells, pairs, or in small clusters. Nonlipophilic. Colonies are whitish, circular with entire edges, convex, glistening, of creamy consistency, and start to develop a yellowish pigment after 72 h incubation. Weak anaerobic growth. Acid is produced from glucose, galactose, 5-keto-gluconate, glycerol, ribose, and trehalose, but not from sucrose, mannitol, xylose, adonitol, amygdalin, arabinose, arabitol, arbutin, cellobiose, dulcitol, erythritol, D-fucose, β-gentiobiose, gluconate, 2-keto-gluconate, *N*-acetylglucosamine, methyl α-D-glucoside, glycogen, inositol, inulin, D-lyxose, methyl α-D-mannoside, melezitose, melibiose, D-raffinose, salicin, sorbitol, sorbose, D-turanose, xylitol, and β-methyl-xyloside. Acid production from maltose, fructose, lactose, rhamnose, and D-tagatose is variable. Nitrate is not reduced. Urea hydrolysis is positive but delayed. Esculin, casein, tyrosine, and xanthine are not hydrolyzed. The CAMP reaction is negative. Pyrazinamidase, alkaline and acid phosphatase, esterase, esterase lipase, and phosphoamidase activities are detected, but pyrrolidonyl arylamidase, valine arylamidase, trypsin, chymotrypsin, α-galactosidase, β-glucosidase, *N*-acetylglucosaminidase, α-mannosidase, and α-fucosidase activities are not detected. Lipase, leucine arylamidase, and cystine arylamidase activities are variable.

The cell wall contains *meso*-DAP. Mycolic acids are present. Cellular fatty acids are consistent with those described for the genus. Does not produce propionic acid as metabolic product (Bernard et al., 2002).

Source: human clinical specimens.

DNA G+C content (mol%): not available.

Type strain: Y13024, CCUG 33651, CIP 105466, DSM 44353, JCM 11949.

Sequence accession no. (16S rRNA gene): Y13024.

Further comments: additional GenBank accession numbers include (complete *rpoB* gene) AY492253. *Corynebacterium falsenii* was previously known as EF (Enevold Falsen) group 42 bacteria.

28. **Corynebacterium felinum** Collins, Hoyles, Hutson, Foster and Falsen 2001b, 1351^{VP}

fe.li′num. L. neut. adj. *felinum* pertaining to cats.

Cells are Gram-stain-positive diphtheroids. Colonies are nonhemolytic. Facultatively anaerobic. Nonlipophilic. Acid is produced from glucose, maltose, and ribose but not from lactose, mannitol, sucrose, *N*-acetyl-β-glucosamine, and D-xylose. Activity is detected for α-glucosidase, pyrazinamidase, pyrrolidonyl arylamidase, and leucine arylamidase. No activity is observed for alkaline and acid phosphatases, chymotrypsin, esterase, ester lipase, lipase, α-galactosidase, β-galactosidase, β-glucosidase, β-glucuronidase, α-fucosidase, α-mannosidase, phosphoamidase, valine arylamidase, and trypsin. Nitrate is not reduced. Esculin, urea, and gelatin are not hydrolyzed.

The cell wall contains *meso*-DAP. Mycolic acids (C_{32}–C_{36}) detected. MK-8(H_2) is the predominant menaquinone. Cellular fatty acids are consistent with those described for the genus, but tuberculostearic acid is not present.

Source: a dead Scottish wild cat (*Felis sylvestris*); habitat is unknown.

DNA G+C content (mol%): not available.

Type strain: M714/95/5, CCUG 39943, CIP 106740, DSM 44508, JCM 12103.

Sequence accession no. (16S rRNA gene): AJ401282.

Further comments: additional GenBank accession numbers include (complete *rpoB* gene) AY492254.

29. **Corynebacterium flavescens** Barksdale, Lanéelle, Pollice, Asselineau, Welby and Norgard 1979, 222^{AL} (*Microbacterium flavum* Orla-Jensen 1919, 181)

fla.ves′cens. L. v. *flavescere* to become yellow; L. part. adj. *flavescens* becoming yellow.

(This description is largely based on that of Collins and Cummins, 1986.)

Gram-stain-positive to Gram-stain-variable pleomorphic rods, often with tapered ends, showing metachromatic granules. Growth is yellow on Loeffler's agar; on BHI agar, colonies are smooth, butyrous, and cream-colored. Colonies on tellurite agar are grayish black, later developing grayish white centers. Aerobic and facultatively anaerobic. Complex nutritional requirements.

Glucose, fructose, galactose, mannose, and methyl red are positive. Negative for arabinose, xylose, rhamnose, lactose, maltose, sucrose, trehalose, raffinose, salicin, dextrin, and starch fermentation. Hippurate and gelatin hydrolysis negative. Nitrate not reduced, urease not detected, and pyrazinamidase negative. Reactions for esculin, tyrosine, and casein hydrolysis unknown.

Information regarding DAP species, menaquinones, presence of mycolates unknown. Cellular fatty acids are consistent with those of the genus (Bernard et al., 1991).

Source: dairy products.

DNA G+C content (mol%): 58.3 (T_m).

Type strain: 8 of Orla-Jensen, ATCC 10340, CCUG 28791, CIP 69.5, DSM 20296, JCM 1317, LMG 4046, NBRC 14136, NCCB 42012, NCIMB 8707, VKM Ac-1956.

Sequence accession no. (16S rRNA gene): X84441.

Further comments: additional GenBank accession numbers include (complete *rpoB* gene) AY492255.

30. **Corynebacterium freiburgense** Funke, Frodl, Bernard and Englert 2009, 2056^{VP}

frei.bur.gen′se. N.L. neut. adj. *freiburgense* from Freiburg/Breisgau, Germany, named after the city where the bacterium was first isolated.

Gram-stain-positive typically club-shaped rods that occur as single cells, in pairs, or in small clusters. Colonies are beige-whitish, dryish, with irregular edges, adherent to agar, convoluted, and up to 1–2 mm in diameter after 48 h. In 5-d-old colonies, a "spoke-wheel" macroscopic morphology may be observed. Facultatively anaerobic, but anaerobic growth is weak. Nonlipophilic. Acid is produced from glucose, maltose, sucrose, fructose, galactose, 5-keto-gluconate, lactose, mannose, ribose, and tagatose, but acid is not produced from *N*-acetylglucosamine, adonitol, amygdalin, arabinose, arabitol, arbutin, cellobiose, dulcitol, erythritol, fucose, gentiobiose, gluconate, glycerol, inositol, inulin, 2-keto-gluconate, lyxose, mannitol, melezitose, melibiose, methyl-α-D-glucoside, methyl-α-D-mannoside, β-methyl-xyloside, D-raffinose, rhamnose, salicin, sorbitol, sorbose, starch, trehalose, D-turanose, xylitol, and xylose. Activity of the following enzymes can be detected: nitrate reductase, β-galactosidase, β-glucosidase, esterase, esterase lipase, leucine arylamidase, cystine arylamidase, acid phosphatase, and phosphoamidase. Activities of pyrazinamidase, urease, pyrrolidonyl arylamidase, gelatinase, lipase, valine arylamidase, trypsin, chymotrypsin, α-galactosidase, β-glucuronidase, α-glucosidase, *N*-acetyl-β-glucosaminidase, α-mannosidase, and α-fucosidase are not detected. The CAMP reaction is negative.

The cell wall contains *meso*-DAP. Mycolic acids are present. Cellular fatty acids are consistent with those described for the genus.

Source: a patient's wound after a dog bite.

DNA G+C content (mol%): not available.

Type strain: 1045, CCUG 56874, DSM 45254.

Sequence accession no. (16S rRNA gene): FJ157329.

Further comments: additional GenBank accession numbers include (partial *rpoB* gene) GQ913437.

31. **Corynebacterium freneyi** (Renaud, Aubel, Riegel, Meugnier and Bollet 2001) emend. Funke and Frodl 2008a, 1515 (Effective publication: Funke and Frodl 2008a, 642.)

fre′ney.i. N.L. gen. masc. n. *freneyi* of Freney, to honor the French microbiologist Jean Freney.

Gram-stain-positive typically club-shaped rods. Colonies are whitish-grayish, but a minority of strains is yellowish, dry, rough, with a distinct wrinkled morphology and irregular edges. Colonies are 1–2 mm in diameter after 48 h of incubation on blood agar. Nonlipophilic. Fermentative metabolism. Reduction of nitrates is variable. The strains express α-glucosidase, pyrazinamidase, alkaline phosphatase, esterase, esterase lipase, leucine arylamidase (20 of 21 strains), and phosphoamidase. They do not produce pyrrolidonyl arylamidase, β-glucuronidase, β-galactosidase, *N*-acetyl-β-glucosaminidase, valine arylamidase, cystine arylamidase, trypsin, chymotrypsin, α-galactosidase, α-mannosidase, or α-fucosidase. The strains do not hydrolyze esculin, gelatin, or urea. The CAMP reaction is negative. Produces acid from glucose, maltose, sucrose, galactose (20 of 21 strains), D-fructose, D-mannose, trehalose, D-turanose, and 5-keto-gluconate. Ribose and D-tagatose acidifications are slow and weak. The results of fermentation of lactose and glycogen are variable, and very few strains (2 of 21) ferment mannitol. Xylose, glycerol, erythritol, D-arabinose, and 2-keto-gluconate are not fermented. Glucose is also fermented at 42°C, and strains may grow at 20°C.

The cell wall contains *meso*-DAP, sugars arabinose and galactose, and mycolic acids. Cellular fatty acids are consistent with those described for the genus.

Source: human clinical specimens.

DNA G+C content (mol%): not available.

Type strain: CCUG 45704, CIP 106767, DSM 44506, ISPB 6695110, JCM 12104.

Sequence accession no. (16S rRNA gene): AJ292762.

Further comments: additional GenBank accession numbers include (complete *rpoB* gene) AY492237. Some strains are resistant to erythromycin, clindamycin, penicillin, and cefotaxime. *Corynebacterium freneyi* cannot be easily discerned from *Corynebacterium xerosis* and *Corynebacterium hansenii* by 16S rRNA, *rpoB*, or 16S–23S spacer region gene sequence analyses; these are best separated by DNA–DNA hybridization or phenotypic means.

32. **Corynebacterium glaucum** Yassin, Kroppenstedt and Ludwig 2003, 707[VP]

glau′cum. L. neut. adj. *glaucum* bluish, light-gray, pertaining to the appearance of colonies.

Cells are Gram-stain-positive, dumbbell-shaped (when examined after 18 h growth), or showing typical coryneform morphology (when examined after 1 week of growth). On Columbia blood agar supplemented with 5% sheep blood, BHI agar and trypticase soy agar, colonies are light-gray in color. Facultatively anaerobic. Nitrate is not reduced. Hydrolyzes hippurate but not esculin, urea, gelatin, and starch. Produces acid from glucose and sucrose but not from arabinose, cellobiose, glycerol, glycogen, inulin, lactose, maltose, mannitol, raffinose, rhamnose, ribose, salicin, sorbitol, trehalose, and xylose. Displays alkaline phosphatase, pyrazinamidase, ester lipase, leucine arylamidase, and phosphoamidase activities but is negative for acid phosphatase, arginine dihydrolase, cystine arylamidase, esterase, lipase, α-glucosidase, β-glucosidase, α-galactosidase, β-galactosidase, β-glucuronidase, *N*-acetyl-β-glucosaminidase, α-mannosidase, α-fucosidase, pyrrolidonyl arylamidase, valine arylamidase, trypsin, and chymotrypsin. Acetoin production is positive, but indole production is negative. Produces lactate but not propionate as the major product of glucose fermentation.

Contains *meso*-DAP in addition to sugars galactose and arabinose (i.e. cell-wall chemotype IV). Contains corynemycolic acids, and fatty acid profile contains saturated and unsaturated fatty acids. Tuberculostearic acid is absent. Contains MK-7(H_2), MK-8(H_2), and MK-9(H_2) as respiratory menaquinones, with MK-8(H_2) as the major component. Phospholipid pattern type PI, with no nitrogen-containing compounds.

Source: a cosmetic dye.

DNA G+C content (mol%): 64.3 (HPLC).

Type strain: IMMIB R-5091, DSM 44530, JCM 12208, NRRL B-24142.

Sequence accession no. (16S rRNA gene): AJ431634.

33. **Corynebacterium glucuronolyticum** Funke, Bernard, Bucher, Pfyffer and Collins 1995b, 879[VP] (Effective publication: Funke, Bernard, Bucher, Pfyffer and Collins 1995a, 214.)

glu.cu.ro.no.ly'ti.cum. N.L. n. *acidum glucuronicum* glucuronic acid; N.L. neut. adj. *lyticum* (from Gr. neut. adj. *lutikon*) able to loosen, able to dissolve; N.L. neut. adj. *glucuronolyticum* cleaving β-glucuronic acid.

Gram-stain-positive coryneforms, about 1–3 µm in length. Colonies are white-yellowish, circular, convex, and nonhemolytic on sheep blood agar. Colonies are 1–1.5 mm after 24 h of incubation in 5% CO_2 on blood agar. Nonlipophilic. Facultatively anaerobic. Acid is produced from glucose and sucrose; some strains produce acid from maltose, xylose, and ribose. Acid is not produced from mannitol and glycogen. Pyrazinamidase, esterase, esterase lipase, leucine arylamidase, cystine arylamidase, acid phosphatase, and, β-glucuronidase are present; some strains exhibit activity of alkaline phosphatase, phosphoamidase, α-glucosidase, and β-glucosidase. Hydrolysis of urea and esculin are variable. Some strains reduce nitrate.

Cellular fatty acids are consistent with those described for the genus.

Source: genitourinary specimens of human males, but can also be isolated from the urogenital tract of pigs (Devriese et al., 2000) or human body fluids that are normally sterile (Bernard et al., 2002).

DNA G+C content (mol%): 52–58 (HPLC).

Type strain: 6, ATCC 51860, CCUG 35055, CIP 104577, DMMZ 838, DSM 44120, JCM 11612, LMG 19047.

Sequence accession no. (16S rRNA gene): X86688.

Further comments: additional GenBank accession numbers include (complete *rpoB* gene) AY492256. *Corynebacterium glucuronolyticum* strains may be resistant to erythromycin, clindamycin, and tetracycline (Funke et al., 1996b). *Corynebacterium seminale* (Riegel et al., 1996b) (Effective publication: Riegel et al. 1995d.) is deemed to be a later heterotypic synonym of *Corynebacterium glucuronolyticum* (Devriese et al., 2000). The DNA G+C content (mol%) of *Corynebacterium seminale* was found to be 60% (capillary electrophoresis method), but that of *Corynebacterium glucuronolyticum* sp. nov. was not extant in 1995. *Corynebacterium seminale* type strain has been curated under ATCC 51866, CCUG 34780, CCUG 34888, CIP 104297, DSM 44288, IBS B12915, JCM 10394, with GenBank accession number *(16S rRNA gene)* X84375.

34. **Corynebacterium glutamicum** (Kinoshita, Nakayama and Akita 1958) Abe, Takayama and Kinoshita 1967, 299[AL] (*Micrococcus glutamicus* Kinoshita, Nakayama and Akita 1958, 176)

glu.ta'mi.c.um. N.L. neut. adj. *glutamicum* pertaining to glutamic acid.

(This description is largely based on that of Collins and Cummins, 1986.)

Short Gram-stain-positive rods or ellipsoids 0.7–1.0 × 1.0–3.0 µm, occurring singly, in pairs, or in irregular masses. In lag phase cultures, quite long branching cells, but in mid- to late exponential phase cultures the cells are very short, ellipsoidal to almost coccal. Colonies on nutrient agar are smooth, entire, circular, dull to slightly glistening, and generally pale yellow to yellow. Gray to black colonies on tellurite agar. Moderate turbidity with floccular sediment in broth. Optimal growth at 25–37°C, with only slight growth at 42°C. Aerobic and facultatively anaerobic. Glucose, fructose, mannose, maltose, sucrose, trehalose, and methyl red positive. Negative for arabinose, xylose, rhamnose, galactose, lactose, raffinose, salicin, dextrin, and starch fermentation. Hippurate hydrolyzed, urease is produced, but gelatin is negative. Nitrate is reduced but phosphatase and pyrazinamidase negative. Esculin and casein negative.

Cell wall contains *meso*-DAP and the sugars arabinose and galactose. Mycolates are present. MK-9(H_2) is the major menaquinone. Cellular fatty acids consistent with those of the genus. All strains produce large amounts of L-glutamic acid under aerobic conditions.

Source: sewage.

DNA G+C content (mol%): 55–57.7 (T_m), 53.8 (complete genome sequencing) (Ikeda and Nakagawa, 2003; Kalinowski et al., 2003).

Type strain: ATCC 13032, CCUG 27702, CIP 82.8, DSM 20300, HAMBI 2052, JCM 1318, LMG 3730, NBRC 12168, NRRL B-2784.

Sequence accession no. (16S rRNA gene): AF314192.

Further comments: additional GenBank accession numbers include (complete *rpoB* gene) NC_003450. *Brevibacterium divaricatum* Su and Yamada 1960[AL], *Corynebacterium lilium* Lee and Good 1963, 1349[AL], "*Brevibacterium flavum*" DSM 20411, and "*Brevibacterium lactofermentum*" DSM 20412 and DSM 1412 are all considered as later heterotypic synonyms of *Corynebacterium glutamicum* (Liebl et al., 1991). Complete genome sequence of *Corynebacterium glutamicum* ATCC 13032[T] was deposited under GenBank accession no. NC_006958 with 3,282,708 bp, from which various transcriptional functions were conjectured (Brune et al., 2005; Kalinowski et al., 2003). Concurrently, a second genome sequencing project of the same strain, deposited under GenBank accession no. NC_004350, was described as having 3,309,401 bp (Ikeda and Nakagawa, 2003).

35. **Corynebacterium halotolerans** Chen, Li, Tang, Kroppenstedt, Stackebrandt, Xu and Jiang 2004, 781[VP]

ha.lo.to'le.rans. Gr. n. *hals halos* salt; L. part. adj. *tolerans* tolerating; N.L. pres. part. *halotolerans* referring to the ability to tolerate high salt concentrations.

Gram-stain-positive diphtheroid and irregular rods. Colonies on modified ISP 5 medium, trypticase/soy agar medium, and Mueller–Hinton agar medium are moderately yellow, circular, entire, somewhat convex, and opaque after 24 h at 28°C. Optimum growth temperature is 28°C. Obligately aerobic. Nonlipophilic. Optimum gowth concentration of KCl, NaCl, and $MgCl_2{\cdot}6H_2O$ is 10%. Positive for nitrate reduction, but negative for milk peptonization and coagulation, gelatin liquefaction, growth in cellulose, production of H_2S and melanin, starch hydrolysis, and urease production. Activities for lipase and β-glucuronidase are positive. Ornithine decarboxylase, arginine dihydrolase, lysine decarboxylase, α- and β-galactosidase, *N*-acetyl-β-glucosaminidase, and β-glucosidase activities are negative. The following substrates are utilized: glucose, galactose, sucrose, arabinose, mannose, mannitol, maltose, starch, xylose, ribose, salicin, and dextrin. Cellobiose, fructose, amygdalin, and lactose are not utilized. Acid production occurs only from glucose.

The cell wall contains *meso*-DAP with sugars mainly galactose and arabinose. Menaquinones are MK-8(H_2) (35.5%)

and MK-9(H_2) (64.5%); phospholipids are diphosphatidylglycerol, phosphatidylglycerol, phosphatidylinositol, glycolipid, and phosphatidylinositol mannosides. Cellular fatty acids are consistent with those described for the genus, with significant quantities (7.4% of total) TBSA detected. Predominant mycolic acids include $C_{32:0}$ (36.0%), $C_{34:0}$ (20.8%), $C_{34:1}$ (25.1%), $C_{36:0}$ (3.6%), $C_{36:1}$ (8.4%), and $C_{36:2}$ (5.1%).

Source: saline soil collected in Xinjiang Province, west China.

DNA G+C content (mol%): 63 (T_m).

Type strain: YIM 70093, CCTCC AA 001024, DSM 44683, JCM 12676.

Sequence accession no. (16S rRNA gene): AY226509.

Further comments: additional GenBank accession numbers include (partial *rpoB* gene) EU004065.

36. **Corynebacterium hansenii** Renaud, Le Coustumier, Wilhem, Aubel, Riegel, Bollet and Freney 2007, 1115[VP]

han.sen′i.i. N.L. gen. *masc.* n. *hansenii* of Hansen, to honor the Belgian microbiologist Willy Hansen.

Gram-stain-positive, typically club-shaped rods. Colonies are yellow-pigmented, dry, rough, and with irregular edges, roughly 0.5–1.0 mm in diameter. Nonlipophilic. Facultatively anaerobic. Produces acid from glucose, ribose, maltose, and sucrose. Does not reduce nitrates. No hydrolysis of esculin, urea, or gelatin. Pyrazinamidase positive. Does not produce alkaline phsophatase, pyrrolidonyl arylamidase, β-glucuronidase, β-galactosidase, α-glucosidase, or *N*-acetyl-β-glucosaminidase. D-Ribose, D-galactose, D-glucose, and D-fructose are used as carbon substrates, but D-maltose and D-turanose are not. Glucose is not fermented at 42°C but growth will occur at 20°C.

Source: human liposarcoma pus; the pathogenic role is not known.

DNA G+C content (mol%): not available.

Type strain: C-138, CCUG 53252, CIP 108444, DSM 45109, JCM 15293.

Sequence accession no. (16S rRNA gene): AM946639.

Further comments: additional GenBank accession numbers include (partial *rpoB* gene) AY684044 and FJ268580. *Corynebacterium hansenii* cannot be easily discerned from *Corynebacterium xerosis* and *Corynebacterium freneyi* by 16S rRNA, *rpoB*, or 16S–23S spacer region gene sequence analyses; these are best separated by DNA–DNA hybridization or phenotypic means.

37. **Corynebacterium imitans** Funke, Efstratiou, Kuklinska, Hutson, De Zoysa, Engler and Collins, 1997a, 1274 (Effective publication: Funke, Efstratiou, Kuklinska, Hutson, De Zoysa, Engler and Collins 1997b, 1982.)

i′mi.tans. L. part. adj. *imitans* imitating, copying, indicating that this bacterium was isolated from patients with an illness that was imitating the clinical picture of pharyngeal diphtheria and also indicating that the biochemical profile of the newly described bacterium imitates the biochemical profiles of other *Corynebacterium* species.

Gram-stain-positive diphtheroids. Nonlipophilic. Colonies are white-grayish, glistening, circular, convex, creamy, and have entire edges. Acid is produced from D-arabinose, ribose, D-glucose, D-fructose, D-mannose, maltose, lactose, and L-fucose and weakly from sucrose but not from glycerol, erythritol, L-arabinose, xylose, adonitol, β-methyl-xyloside, galactose, L-sorbose, rhamnose, dulcitol, inositol, mannitol, sorbitol, α-methyl-mannoside, α-methyl-glucoside, *N*-acetyl-glucosamine, amygdalin, arbutin, salicin, cellobiose, melibiose, trehalose, inulin, melezitose, D-raffinose, glycogen, xylitol, b-gentiobiose, D-turanose, D-lyxose, D-tagatose, D-fucose, D-arabitol, L-arabitol, gluconate, and 2-keto-gluconate. Nitrate reduction is variable (Funke, unpublished). Urea, esculin, and tyrosine are not hydrolyzed. The CAMP reaction is positive. Pyrazinamidase (weak), alkaline phosphatase, esterase, esterase lipase, acid phosphatase, and phosphoamidase activities are detected, but lipase, pyrrolidonyl arylamidase, leucine arylamidase, valine arylamidase, trypsin, chymotrypsin, α-galactosidase, β-galactosidase, β-glucuronidase, α-glucosidase, β-glucosidase, *N*-acetyl-β-glucosaminidase, α-mannosidase, and α-fucosidase activities are not detected.

Meso-DAP and mycolic acids are present. Cellular fatty acids are consistent with those described for the genus. Does not produce propionic acid as metabolic product (Bernard et al., 2002).

Source: human clinical specimens.

DNA G+C content (mol%): 62 (T_m).

Type strain: ATCC 700354, CCUG 36877, CIP 105130, DSM 44264, IFO (now NBRC) 16163, JCM 10386, NBRC 100416, NCTC 13015.

Sequence accession no. (16S rRNA gene): Y09044.

Further comments: additional GenBank accession numbers include (complete *rpoB* gene) AY492259.

38. **Corynebacterium jeikeium** Jackman, Pitcher, Pelczynska and Borman 1988, 136[VP] (Effective publication: Jackman, Pitcher, Pelczynska and Borman 1987, 88.)

jei.kei′um. N.L. neut. adj. *jeikeium* formed from the initial letters of the surnames of W. D. Johnson and D. Kaye, doctors who described infections attributed to the taxon in 1970.

Gram-stain-positive rods, ~0.5 × 2 μm, pleomorphic, occasionally club-shaped, arranged in V-forms or palisades. Cells contain metachromatic granules. Colonies are small (1–2 mm), entire, grayish-white, and nonhemolytic. Obligate aerobic. Lipophilic. Good growth occurs at 30–42°C but poorly at 22°C. Grows on 0.03% tellurite agar and bile salt agar, hydrolyzes Tweens 20 and 80 but not Tweens 40 and 60. Acid is produced from glucose and galactose; some strains produce acid from maltose. Acid is not produced from adonitol, arabinose, cellobiose, dulcitol, dextrin, erythritol, fructose, glycerol, glycogen, inositol, lactose, mannitol, mannose, melibiose, raffinose, rhamnose, salicin, sorbose, sucrose, and trehalose. Methyl red test negative; nitrate is not reduced. H_2S, acetoin, and indole are not produced. Tyrosine, casein, gelatin, and urea are not degraded. Citrate is not utilized. Esculin and starch are not hydrolyzed, gluconate is not converted to 2-ketogluconate. Arginine dihydrolase, and lysine and ornithine decarboxylase tests are negative.

Cell wall contains *meso*-DAP and sugars arabinose and galactose. Mycolic acids are present and are principally of carbon-chain length C_{32}–C_{36}. Cellular fatty acids are con-

sistent with those described for the genus with only trace amounts of 10-methyl-octadecanoic acid (tuberculostearic acid) occurring. Does not produce propionic acid as metabolic product (Bernard et al., 2002).

Source: human body surfaces and human clinical specimens.

DNA G+C content (mol%): 58–61 (T_m).

Type strain: ATCC 43734, CCUG 27192, CIP 103337, DSM 7171, JCM 9384, NCTC 11913.

Sequence accession no. (16S rRNA gene): X84250.

Further comments: additional GenBank accession numbers include (complete *rpoB* gene) AY492231.

Corynebacterium jeikeium Jackman et al. 1988 was previously known as CDC coryneform group JK. Four genomospecies among *Corynebacterium jeikeium* strains have been described (Riegel et al., 1994b). *Corynebacterium jeikeium* strains are often multidrug-resistant (Riegel et al., 1996a; Sanchez et al., 2003). The complete genome of *Corynebacterium jeikeium* strain K411 deposited under GenBank accession no. CR931997 has 2,462,499 bp (Tauch et al., 2005).

39. **Corynebacterium kroppenstedtii** Collins, Falsen, Åkervall, Sjödén and Alvarez 1998, 1453[VP]

krop.pen.sted′ti.i. N.L. gen. masc. n. *kroppenstedtii* of Kroppenstedt, to honor the German microbiologist Reiner M. Kroppenstedt for his many contributions to the microbiology of actinomycetes.

Gram-stain-positive diphtheroids that occur as single cells or are arranged in V-shaped forms or palisades. Colonies on blood agar are nonpigmented, small (<0.5 in diameter after 24 h incubation), smooth, convex, and nonhemolytic. Facultatively anaerobic. Grows in 10% NaCl and at 42°C. Nitrate is not reduced to nitrite. Esculin is hydrolyzed but urea and gelatin are not. Acid is produced from glucose, maltose (weak reaction), and sucrose. Acid is not produced from lactose, ribose, D-xylose, mannitol, and glycogen. Leucine arylamidase, esterase, esterase lipase, and pyrazinamidase are produced. Alkaline and acid phosphatases, *N*-acetyl-β-glucosaminidase, cystine arylamidase, chymotrypsin, α-fucosidase, α-galactosidase, β-galactosidase, β-glucosidase, β-glucuronidase, lipase, α-mannosidase, pyrrolidonyl arylamidase, trypsin, and valine arylamidase are not detected.

Cell wall contains *meso*-DAP and the sugars arabinose and galactose Cellular fatty acids are consistent with those described for the genus, with tuberculostearic acid being detected. Mycolic acids are not present. Some clinical isolates produce propionic acid as a metabolic product (Bernard et al., 2002).

Source: human clinical specimens, in particular, in cases of lobular granulomatous mastitis (Kieffer et al., 2006; Paviour et al., 2002; Riegel et al., 2004).

DNA G+C content (mol%): 62 (T_m).

Type strain: CCUG 35717, CIP 105744, DSM 44385, JCM 11950.

Sequence accession no. (16S rRNA gene): Y10077.

Further comments: additional GenBank accession numbers include (complete *rpoB* gene) AY492274. Although *Corynebacterium kroppenstedtii* was not described as being lipophilic by Collins et al., this feature has been observed by other authors (Riegel et al., 2004; Tauch et al., 2008a). The complete genome of DSM 44385[T], with 2,446,804 bp, has been deposited under GenBank accession no. NC_012704 (Tauch et al., 2008a).

40. **Corynebacterium kutscheri** (Migula 1900) Bergey, Harrison, Breed, Hammer and Huntoon 1925, 395[AL] (*Bacterium kutscheri* Migula 1900, 372)

kut′sche.ri. N.L. gen. masc. n. *kutscheri* of Kutscher, the bacteriologist who first isolated the species.

(This description is largely based on that of Collins and Cummins, 1986.)

Irregularly staining slender rods, often clubbed, sometimes with pointed ends; metachromatic granules present. Large numbers of fimbriae present. Small, thin, yellowish or grayish white serrate colonies on nutrient agar. Facultatively anaerobic. Reduces potassium tellurite. Positive for glucose, fructose, mannose, maltose, sucrose, salicin, dextrin, and starch; negative for arabinose, xylose, rhamnose, galactose, lactose, and raffinose. Trehalose variable. Nitrate is reduced, urease is produced, hippurate is hydrolyzed and pyrazinamidase is detected. Tyrosine, esculin, and gelatin are not hydrolyzed; casein is not digested; methyl red and phosphatase are negative.

Cell wall contains *meso*-DAP and the sugars arabinose, galactose, mannose, and rhamnose. This species appears to be the only organism in the genus *Corynebacterium* which may have rhamnose as a cell-wall sugar in addition to arabinose and galactose. Metabolic products include propionate and lactate, with lesser amounts of acetate, pyruvate, and oxalacetate.

Source: a frequent parasite of mice and rats, but also occurs in other small rodents such as voles. An infection in a human has been reported after the patient had been bitten by a rat (Holmes and Korman, 2007).

DNA G+C content (mol%): ~46 (T_m).

Type strain: ATCC 15677, CCUG 27535, CIP 103423, DSM 20755, JCM 9385, NBRC 15288, NCTC 11138.

Sequence accession no. (16S rRNA gene): X81871.

Further comments: additional GenBank accession numbers include (complete *rpoB* gene) AY492257.

41. **Corynebacterium lipophiloflavum** Funke, Hutson, Hilleringmann, Heizmann and Collins 1997a, 1274[VP] (Effective publication: Funke, Hutson, Hilleringmann, Heizmann and Collins 1997c, 223.)

li.po.phi.lo.fla′vum. Gr. n. *lipos* fat; Gr. neut. adj. *philon* loving; L. adj. *flavus -a -um* yellow; N.L. neut. adj. *lipophiloflavum* fat loving and yellow.

Gram-stain-positive club-shaped rods, 1–3 μm in length. Slightly lipophilic. Colonies are yellowish, circular, convex, and slightly dry; tiny colonies (0.2 mm in diameter) after 24 h incubation on blood agar, but ~1 mm if grown on blood agar enriched with 1% Tween 80. Acid is not produced from glucose, maltose, sucrose, mannitol, xylose, lactose, and glycogen. Nitrate is not reduced. Urea is slowly hydrolyzed, but esculin is not hydrolyzed. The CAMP reaction is negative. Pyrazinamidase, alkaline phosphatase, esterase, esterase lipase, lipase, leucine arylamidase, acid phosphatase, and phosphoamidase activities are detected but pyrrolidonyl arylamidase, valine arylamidase, cystine arylamidase,

trypsin, chymotrypsin, α-galactosidase, β-galactosidase, β-glucuronidase, α-glucosidase, β-glucosidase, *N*-acetyl-β-glucosaminidase, α-mannosidase, and α-fucosidase activities are not detected.

Cell wall contains *meso*-DAP. Mycolic acids are present. Cellular fatty acids are consistent with those described for the genus.

Source: human clinical specimens.

DNA G+C content (mol%): 65 (T_m).

Type strain: ATCC 700352, CCUG 37336, CIP 105127, DMMZ 1944, DSM 44291, JCM 10383.

Sequence accession no. (16S rRNA gene): Y09045.

Further comments: additional GenBank accession numbers include (complete *rpoB* gene) AY492260.

42. **Corynebacterium lubricantis** Kämpfer, Lodders, Warfolomeow, Falsen and Busse 2009, 1114[VP]

lu.bri.can′tis. L. v. *lubricare* to lubricate; N.L. *lubricans – antis* (from L. part. adj. *lubricans*); N.L. gen. n. *lubricantis* of/from a (coolant) lubricant.

Gram-stain-positive rods ~1 µm long, with yellow, shiny, translucent colonies 0.5 mm in diameter after 24 h. Grows from 10–45°C but most testing was done at 28°C. Unique, as this species can grow on MacConkey agar. The following compounds are used as sole sources of carbon: D-fructose, D-glucose, acetate, fumarate, and DL-lactate; the following compounds are not utilized: *N*-acetylgalactosamine, *N*-acetylglucosamine, L-arabinose, L-arbutin, cellobiose, D-galactose, maltose, D-mannose, L-rhamnose, D-ribose, salicin, trehalose, D-xylose, adipate, 2-oxoglutarate, D-gluconate, melibiose, sucrose, adonitol, *myo*-inositol, maltitol, D-mannitol, D-sorbitol, propionate, *cis*- and *trans*-aconitate, 4-aminobutyrate, citrate, glutarate, DL-3-hydroxybutyrate, itaconate, L-malate, mesaconate, pyruvate, L-alanine, D-alanine, L-aspartate, L-leucine, L-ornithine, L-proline, L-serine, putrescine, azelate, suberate, L-histidine, L-phenylalanine, L-serine, L-tryptophan, 3-hydroxybenzoate, and phenylacetate. Acid is produced from glucose and trehalose. No acids are produced from sucrose, D-mannitol, dulcitol, lactose, rhamnose, maltose, galactose, salicin, adonitol, inositol, sorbitol, L-arabinose, raffinose, D-xylose, trehalose, cellobiose, methyl-D-glucoside, erythritol, melibiose, D-arabitol, or D-mannose. *p*-Nitrophenyl (pNP)-β-D-glucopyranoside, *p*-NP-β-D-glucuronide, bis-*p*-NP-phosphate, and bis-*p*-NP-phenylphosphonate are hydrolyzed. The following compounds are not hydrolyzed: *p*-NP-α-D-glucopyranoside, *p*-NP-β-D-galactopyranoside, *p*-NP-β-D-xylopyranoside, bis- *p*-NP-phosphorylcholine, L-alanine-*p*-nitroanilide (pNA), L-proline pNA, and C-L-glutamate pNA.

Chemotaxonomic properties as described for genus except that in addition to MK-9(H_2) and MK-8(H_2), MK-7(H_2), is also detected. The lipid profile consists of the major compounds phosphatidylglycerol and an unknown glycolipid, moderate amounts of phosphatidylinositol, diphosphatidylglycerol, two unknown glycolipids, three unknown aminolipids and an unknown polar lipid, and minor amounts of two glycolipids, an aminolipid and a polar lipid. Polyamines are present in small amounts; the major compounds are spermidine and spermine. The characteristic peptidoglycan diamino acid is *meso*-DAP. Testing for corynemycolates was not done. Cellular fatty acids are consistent with those described for the genus except that significant (11–31% of total vol.) of tuberculostearic acid is detected.

Source: coolant lubricant.

DNA G+C content (mol%): 66.6 (HPLC).

Type strain: KSS-3Se, CCM 7546, CCUG 56567, DSM 45231, JCM 16607.

Sequence accession no. (16S rRNA gene): FM173119.

43. **Corynebacterium macginleyi** Riegel, Ruimy, De Briel, Prévost, Jehl, Christen and Monteil 1995e, 132[VP]

mac.gin′ley.i. N.L. gen. masc. n. *macginleyi* of McGinley, named in honor of Kenneth John McGinley who made important contributions to the knowledge of lipid-requiring diphtheroids.

Gram-stain-positive pleomorphic rods occasionally with metachromatic granules, arranged in palisades or V-shaped forms. Facultatively anaerobic. Strains are lipophilic in that no growth is seen in brain heart infusion broth after 72 h at 37°C, but growth is visible after 24 h in the same broth supplemented with 0.01–1% Tween 80. Very small (less than 1 mm in diameter) and non-β-hemolytic colonies appear after 48 h on sheep blood agar, but on 1% Tween 80-supplemented sheep blood agar reddish-beige colonies are larger (2–4 mm in diameter) and Tween esterase activity is noted. Nitrate is reduced to nitrite. Acid is produced from D-glucose, ribose, and sucrose but not from maltose, lactose, D-xylose, trehalose, or glycogen. Fermentation of D-mannitol occurs variably. Tyrosine, gelatin, starch, and urea are not degraded or hydrolyzed. Esculin is not hydrolyzed. DNA is degraded. Hydrolysis of hippurate is variable. Arginine is not degraded. Cells produce alkaline phosphatase, but pyrazinamidase, pyrrolidonyl, arylamidase, β-glucuronidase, β-galactosidase, α-glucosidase, and *N*-acetyl-β-glucosaminidase are not produced.

Cell wall contains *meso*-DAP and the sugars arabinose and galactose. Mycolic acids of short chain lengths (C_{26}–C_{36}) are present cellular fatty acid composition consistent with those of the genus, but tuberculostearic acid was not detected. Propionic acid is not detected as metabolic product (Bernard et al., 2002).

Source: predominately recovered from human eye specimens, particularly the conjunctiva (Funke et al., 1998d; Joussen et al., 2000) and from a case of endophthalmitis (Ferrer et al., 2004).

DNA G+C content (mol%): 58 (HPLC).

Type strain: JCL-2, ATCC 51787, CCUG 32361, CIP 104099, DSM 44284, JCM 11684.

Sequence accession no. (16S rRNA gene): AJ439345, X80499.

Further comments: additional GenBank accession numbers include (complete *rpoB* gene) AY492276. *Corynebacterium macginleyi* Riegel et al. 1995d was previously known as CDC coryneform group G-1, the nitrate reducing form of CDC coryneform group G as well as a lipid-requiring diphtheroid, genomospecies II. Opportunistic infections in humans by *Corynebacterium macginleyi* associated with other sites have been described, including intravenous catheter-related infections (Dobler and Braveny, 2003; Villanueva et al., 2002) and endocarditis (Pubill Sucarrat et al., 2003). An outbreak involving quinolone-resistant strains has been reported (Eguchi et al., 2008). *rpoB* Gene sequencing

should be done to differentiate strains from *Corynebacterium accolens*, as these species can not be easily distinguished by 16S rRNA gene sequencing.

44. **Corynebacterium marinum** Du, Jordan, Rooney, Chen and Austin 2010, 1946[VP]

ma.ri′num. L. neut. adj. *marinum* of the sea, marine.

Cells are short Gram-stain-positive diphtheroid rods; some of the cells are arranged in a V formation. Colonies on Marine 2216E agar medium are circular, erose, convex, yellow, of a creamy consistency, and ~0.5–1.5 mm after 48 h at 28°C. Facultatively anaerobic; methyl red negative. Voges–Proskauer positive, nitrate is reduced, and horse blood cells are lyzed. Esculin and urea are not hydrolyzed, but casein is digested, and starch and pullulan are hydrolyzed. Gelatin is not liquefied. Tween 20–80 are hydrolyzed. Cells grow in the presence of 0–8% (w/v) NaCl and at 4–37°C. Prolific growth occurs at 30–32°C in media that contains 1% (w/v) NaCl. Using the API 50CH system, esculin ferric, citrate, salicin, D-maltose, and glycogen are utilized. Acid is produced from glucose, maltose, sucrose, and glycogen, but not from ribose, xylose, mannitol, or lactose. Esterase lipase, leucine arylamidase, α-chymotrypsin, naphthol-AS-BI-phosphohydrolase, pyrazinamidase, and β-glucuronidase are positive. Esterase, lipase, valine arylamidase, cystine arylamidase, trypsin, alkaline phosphatase, pyrrolidonyl arylamidase, acid phosphatase, α-galactosidase, β-galactosidase, α-glucosidase, β-glucosidase, *N*-acetyl-β-glucosaminidase, α-mannosidase, and α-fucosidase are negative. The antibiotic susceptibility of the isolate was tested using antimicrobial discs. The type strain is resistant to nalidixic acid (30 μg), nitrofurantoin (50 μg), sulfamethizole (200 μg), tetracycline (100 μg), and cotrimoxazole (25 μg), but sensitive to ampicillin (25 μg), chloramphenicol (50 μg), gentamicin (10 μg), kanamycin (30 μg), carbenicillin 143 (100 μg), and streptomycin (25 μg).

Cellular fatty acid composition is consistent with those of the genus. Detection of corynemycolates and other chemotaxonomic information not described.

Source: coastal sediment close to a coal-fired power station in Qingdao, China.

DNA G+C content (mol%): 65.0 (HPLC).

Type strain: D7015, CGMCC 1.6998, NRRL B-24779.

Sequence accession no. (16S rRNA gene): DQ219354.

45. **Corynebacterium maris** Ben-Dov, Ben Yosef, Pavlov and Kushmaro 2009, 2461[VP]

ma′ris. L. gen. n. *maris* of the sea.

Gram-stain-positive coccobacilli approximately 0.5–0.8 μm × 0.8–1.5 μm. Nonhemolytic. Forms small colonies (approximately 1 mm and 2.5 mm in diameter after 48 and 72 h, respectively) after incubation at 30°C. Colonies are yellowish to yellow, circular, convex, smooth, and opaque. Grows well at 0.5–4.0% salinity, at pH 7.2–9.0, and at 30–37°C. Aerobic. Oxidase positive. Alkaline phosphatase, esterase, esterase lipase, lipase, leucine arylamidase, α-glucosidase, pyrazinamidase, pyrrolidonyl arylamidase, and gelatin hydrolysis detected. No activity is observed for nitrate reduction, valine and cystine arylamidases, trypsin, α-chymotrypsin, acid phosphatase, naphthol-AS-BI-phosphohydrolase, α- and β-galactosidases, β-glucuronidase, β-glucosidase, urease, *N*-acetyl-β-glucosaminidase, α-mannosidase, or α-fucosidase. The strain oxidizes the following carbon compounds as sole energy sources: maltose, lactulose, β-hydroxybutyric acid, α-ketovaleric acid, Tween 40, phenylethylamine, *N*-acetyl-D-galactosamine, malonic acid, L-threonine, L-glutamic acid, L-fucose, L-alanyl glycine, inosine and, less efficiently, raffinose, D-arabitol, L-asparagine, and citric acid, as determined with the Biolog GN system.

Cellular fatty acids are consistent with those described for the genus, with tuberculostearic acid detected. Mycolic acids (C_{30}–C_{36}) are present.

Source: the mucus of the coral *Fungia granulosa*, Gulf of Eilat, Red Sea.

DNA G+C content (mol%): 66.6 (HPLC).

Type strain: Coryn-1, DSM 45190, JCM 17108, LMG 24561.

Sequence accession no. (16S rRNA gene): FJ423600.

46. **Corynebacterium massiliense** Merhej, Falsen, Raoult and Roux 2009, 1958[VP]

mas.si.li.en′se. L. neut. adj. *massiliense* of or pertaining to *Massilia*, the old Roman name for Marseille from where the type strain was isolated.

Gram-stain-positive club-shaped rods, occurring as single cells, in pairs, or small clusters, 0.2–1.8 μm in length and 0.3–0.7 μm in diameter after 48 h growth in TSB medium. Colonies are circular, grayish, glistening, and 0.5–1 mm in diameter after growth on blood agar for 48 h. Capable of aerobic growth with only weak growth anaerobically and microaerophilically. Temperature range 30–44°C (optimum, 37°C). With the API Coryne system, acid is not produced from D-glucose, D-ribose, D-xylose, D-mannitol, maltose, D-lactose, sucrose, or glycogen. Nitrate is not reduced. Urea, gelatin, and esculin are not hydrolyzed. With API Coryne and API ZYM strips, positive for leucine arylamidase, weakly positive for pyrazinamidase, alkaline phosphatase, esterase and esterase lipase, but negative for pyrrolidonyl arylamidase, lipase, valine arylamidase, cystine arylamidase, trypsin, a-chymotrypsin, acid phosphatase, naphthol-AS-BI-phosphohydrolase, α-galactosidase, β-galactosidase, β-glucuronidase, α-glucosidase, β-glucosidase, *N*-acetyl-β-glucosaminidase, α-mannosidase, and α-fucosidase.

Cellular fatty acids are consistent with those described for the genus, and tuberculostearic acid may be detected. Testing for corynemycolates or other cell wall markers not done.

Source: human clinical materials.

DNA G+C content (mol%): not available.

Type strain: 5402485, CCUG 53857, CIP 109423, CSUR P19.

Sequence accession no. (16S rRNA gene): EF217056.

Further comments: additional GenBank accession numbers include (partial *rpoB* gene) EF217057.

47. **Corynebacterium mastitidis** Fernández-Garayzábal, Collins, Hutson, Fernandez, Monasterio, Marco and Dominguez 1997, 1084[VP]

mas.ti′ti.dis. N.L. gen. n. *mastitidis* of an inflammation of the mammary gland.

Gram-stain-positive rods occurring singly, in palisades, or V-shaped forms. On blood agar, small (diameter less than 1 mm), rough, whitish, low convex colonies formed after 3 d of incubation at 37°C. Nonhemolytic. Esculin and gelatin not hydrolyzed, and nitrate not reduced. Hydrolysis of urea variable. Acid is not produced from glucose, ribose, xylose, mannitol, lactose, maltose, sucrose, and glycogen. Pyrazinamidase, alkaline phosphatase, acid phosphatase, esterase, esterase lipase, lipase, leucine arylamidase, valine arylamidase, and cystine arylamidase are produced. Pyrrolidonylarylamidase, β-glucuronidase, α-galactosidase, β-galactosidase, α-glucosidase, β-glucosidase, α-mannosidase, α-fucosidase, *N*-acetyl-*β*-glucosaminidase, trypsin, and chymotrypsin are not produced. The strains do not grow in the presence of 6.5% NaCl. The type strain has characteristics of the species described above except that it is urease and naphthol-AS-BI-phosphate positive.

The cell wall contains *meso*-DAP. Short-chain mycolic acids are present. The major long-chain fatty acids are $C_{16:0}$, $C_{18:0}$, and $C_{18:1}$ ω9*c*. Tuberculostearic acid is not produced.

Source: milk and udder of infected sheep, but strains genetically most like *Corynebacterium mastitidis* have been found to cause human ocular infections (Eguchi et al., 2008).

DNA G+C content (mol%): not available.

Type strain: S-8, CCUG 38654, CECT 4843, CIP 105509, DSM 44356, JCM 12269, LMG 19040, NBRC 16160.

Sequence accession no. (16S rRNA gene): Y09806.

Further comments: additional GenBank accession numbers include (complete *rpoB* gene) AY492281. Sheep strains are susceptible to penicillin G, ampicillin, amoxycillin-clavulanic acid, gentamicin, cephalothin, and nalidixic acid. However, the strains described by Eguchi, which by 16S rRNA gene sequencing were closest to (98.2% identity) *Corynebacterium mastitidis* from human ocular infections, were generally susceptible to antimicrobics tested (Eguchi et al., 2008).

48. **Corynebacterium matruchotii** (Mendel 1919) Collins 1983, 438[VP] (Effective publication: Collins 1982a, 365.) (*Cladothrix matruchoti* Mendel 1919, 584; *Bacterionema matruchotii* Gilmour, Howell and Bibby 1961, 139)

ma.tru.cho'ti.i. N.L. gen. masc. n. *matruchotii* of Matruchot; named after Professor Matruchot, a French mycologist.

(This description also includes information from Collins and Cummins, 1986.)

Cells are pleomorphic, comprising nonseptate and septate filaments and bacilli. Characteristic morphology is a bacillus attached to a filament ("whip-handle"). Branching is frequent with aerobic and/or acidic conditions. Metachromatic granules formed. Colony appearance is variable; aerobically incubated colonies are 0.5–1.5 mm in diameter, circular, convex, rough with entire or filamentous margin or irregular "molar-toothed" rough colonies. May be 1–2 mm and adherent if grown anaerobically. *Corynebacterium matruchotii* colonies at 37°C on TBSA have also been described as being 1 mm in diameter, white, creamy or gray, with smooth matte surface and creamy texture; some strains have a crinkled surface and can be lifted as a single dry colony when touched by a loop (Rassoulian-Barrett et al., 2001). Facultatively anaerobic. Optimal temperature 37°C. Complex nutritional requirements. Positive for glucose, fructose, mannose, maltose, sucrose, salicin, and dextrin. Negative for arabinose, xylose, rhamnose, galactose, lactose, and trehalose. Raffinose and starch fermentation variable. Nitrate is reduced and hippurate hydrolyzed. Gelatin is hydrolyzed, and casein is not digested; methyl red, and phosphatase are negative. Esculin and urea hydrolysis described originally as being positive or occasionally positive. Rassoulian-Barrett et al., based on study of genetically characterized strains, found that *Corynebacterium matruchotii* isolates were conventional tube esculin test negative but could be esculin positive when using the API CORYNE strip; strains were urease and mannitol negative by both methods (Rassoulian-Barrett et al., 2001). The production of pyrrolidonyl arylamidase was the only trait that consistently distinguished *Corynebacterium matruchotii* strains from *Corynebacterium durum* strains which are otherwise very similar to each other biochemically (Rassoulian-Barrett et al., 2001).

meso-DAP is detected, and arabinose and galactose are cell-wall sugars. Cellular fatty acids were consistent with those described for the genus, but tuberculostearic acid is not detected. Species contains corynemycolates. Principal menaquinones are MK-9(H_2) and MK-8(H_2). Polar lipids include diphosphatidylglycerol, phosphatidylglycerol, phosphatidylinositol, phosphatidylinositol dimannosides, and unidentified glycolipids. Propionic acid is produced as a metabolic product (Riegel et al., 1997a).

Source: the oral cavity, particularly from calculus and plaque deposits; is otherwise an occasional pathogen of humans or primates.

DNA G+C content (mol%): 55–58 (T_m).

Type strain: ATCC 14266, CCUG 27545, CCUG 46620, CIP 81.82, DSM 20635, JCM 9386, NBRC 15360, NCTC 10254.

Sequence accession no. (16S rRNA gene): X82065, X84443.

Further comments: additional GenBank accession numbers include (complete *rpoB* gene) AY492238.

49. **Corynebacterium minutissimum** (*ex* Sarkany, Taplin and Blank 1962) Collins and Jones 1983a, 870[VP] emend. Yassin, Steiner and Ludwig 2002a, 1004

mi.nu.tis'si.mum. L. sup. neut. adj. *minutissimum* very small.

Colonies on blood agar are circular, about 1 mm in diameter after 24 h, circular, slightly convex, shiny, moist, and not pigmented. When grown in the presence of rich media (e.g. 20% fetal calf serum), colonies may demonstrate a coral-red to orange fluorescence when illuminated by a Wood's lamp (365 nm). A coralloid precipitin in agar is formed after growth on BHI agar supplemented with 1% Tween 80 whether in air or in CO_2. No hemolysis on blood agar. Cells described as short, straight, or slightly curved rods (1–2 × 0.3–0.6 µm) arranged at right angles to give V-formations. Metachromatic granules may be observed. Facultatively anaerobic. Optimal temperature 37°C. Positive for glucose, fructose, maltose, mannose, hippurate, alkaline phosphatase, pyrazinamidase, and leucine arylamidase. Negative for xylose, lactose, raffinose, trehalose, starch, adonitol, amygdalin, arabinose, cellobiose, glycerol, glycogen, inulin, mannitol, melezitose, rhamnose, ribose, salicin, and sorbitol. Hippurate, sucrose, and mannose described

as positive or variable but later as negative, positive, and negative, respectively. Acetoin positive, but indole negative; nitrate is not reduced, and urease is not produced. Tyrosine hydrolyzed (Funke and Bernard, 2007), but esculin and gelatin are not. Casein is not digested and methyl red is negative. Acid phosphatase, arginine dihydrolase, esterase, ester lipase, lipase, α-glucosidase, β-glucosidase, α-galactosidase, β-galactosidase, β-glucuronidase, *N*-acetyl-β-glucosaminidase, α-mannosidase, α-fucosidase, naphthol-AS-BL-phosphoydrolase, pyrrolidonyl arylamidase, valine arylamidase, cystine arylamidase, trypsin, and chymotrypsin were negative.

Cells contain *meso*-DAP with arabinose and galactose as cell-wall sugars. Menaquinones MK-8(H_2) and MK-9(H_2) are detected. Diphosphatidylglycerol, phosphatidylinositol, phosphatidylinositol mannoside, and unidentified glycolipids are found. Corynemycolates are present (De Briel et al., 1992). Cellular fatty acids are consistent with those of the genus, and tuberculostearic acid is detected (Bernard et al., 1991). Lactic, but not propionic, acid is produced as metabolic product.

Source: a rare opportunistic human pathogen.

DNA G+C content (mol%): 56–58 (HPLC).

Type strain: ATCC 23348, CCUG 541, CIP 100652, DSM 20651, JCM 9387, NBRC 15361, NCTC 10288.

Sequence accession no. (16S rRNA gene): X82064, X84678, X84679.

Further comments: additional GenBank accession numbers include (complete *rpoB* gene) AY492235. Accession number for NCTC 10288 X84678 was described by the depositor as *Corynebacterium minutissimum* clone 1 and NCTC 10288 X84679 as clone 2, respectively. By 16S rRNA gene sequencing, *Corynebacterium minutissimum* is closely related to (>98.9–99% identity) *Corynebacterium aurimucosum* and *Corynebacterium singulare* (Riegel et al., 1997b), but differs from these (~93.8–94.6% identity, respectively) when partial or complete sequences of the *rpoB* gene are compared (Khamis et al., 2004). *Corynebacterium minutissimum* had long been attributed as a cause of erythrasma, a skin infection, but this inference has been challenged, as most publications citing this association did so in the absence of laboratory-based evidence (Coyle and Lipsky, 1990). It has been thought that some published *Corynebacterium minutissimum* reports in the past may actually represent misidentified *Corynebacterium amycolatum* strains (Zinkernagel et al., 1996).

50. **Corynebacterium mucifaciens** Funke, Lawson and Collins 1997d, 956[VP]

mu.ci.fa′ci.ens. L. n. *mucus* slime; L. part. adj. *faciens* producing; N.L. part. adj. *mucifaciens* slime producing.

Colonies on blood agar are highly unusual with respect to members of this genus, as they are circular, convex, glistening, yellowish, uniquely mucoid, and about 1–1.5 mm in diameter after 24 h incubation at 37°C in 5% CO_2. Facultatively anaerobic and nonhemolytic. Acid produced from glycerol, glucose, fructose, and mannose but not from erythritol, arabinose, xylose, adonitol, *p*-methyl-xyloside, galactose, L-sorbose, rhamnose, dulcitol, inositol, mannitol, sorbitol, α-methyl-D-mannoside, α-methyl-D-glucoside, *N*-acetylglucosamine, amygdalin, arbutin, salicin, maltose, lactose, melibiose, trehalose, melezitose, D-raffinose, glycogen, *p*-gentiobiose, D-turanose, D-lyxose, D-tagatose, fucose, arabitol, gluconate, and 2-ketogluconate. Acid production from ribose and sucrose variable. Nitrate is not reduced. Urea and esculin are not hydrolyzed. Not lipophilic. CAMP reaction negative. Pyrazinamidase, alkaline phosphatase, esterase, esterase-lipase, cystine arylamidase, and acid phosphatase are positive, but pyrrolidonyl arylamidase and phosphoamidase are variable. Lipase, valine arylamidase, trypsin, chymotrypsin, α-galactosidase, β-galactosidase, β-glucuronidase, α-glucosidase, β-glucosidase, *N*-acetyl-β-glucosaminidase, α-mannosidase, and α-fucosidase activities are not detected.

Cell wall contains *meso*-diaminopimelic acid. Mycolic acids are present. Propionic acid is not produced as a metabolic product (Bernard et al., 2002).

Source: human disease from various clinical materials.

DNA G+C content (mol%): 63–65 (HPLC).

Type strain: ATCC 700355, CCUG 36878, CIP 105129, DMMZ 2278, DSM 44265, JCM 10384.

Sequence accession no. (16S rRNA gene): Y11200.

Further comments: additional GenBank accession numbers include (complete *rpoB* gene) AY492261. By 16S rRNA gene sequencing, *Corynebacterium mucifaciens* is closely related to (98% identity with) *Corynebacterium coyleae* and *Corynebacterium afermentans*, but differs from them (~94% identity with) when *rpoB* gene sequences are compared (Khamis et al., 2004).

51. **Corynebacterium mustelae** Funke, Frodl and Bernard 2010b, 873[VP]

mus.te′la.e. L. n. *mustela* a weasel, also a scientific zoological name; L. gen. *mustelae* of a weasel, of *Mustela*, indicating that the type strain was isolated from a ferret (*Mustela putorius furo*).

Gram-stain-positive club shaped rods. Unusual greenish-beige adherent colonies with irregular edges, ~1.0– 2 mm in diameter observed after 48 h. Has odor, described as "humid-cellar like". Nonlipophilic. Facultatively anaerobic. Acid is produced from ribose, glucose, fructose, mannose, *N*-acetylglucosamine, arbutin, maltose, sucrose, trehalose, gentiobiose, tagatose, L-fucose, and 5-ketogluconate, but not from glycerol, erythritol, L-arabinose, xylose, adonitol, methyl-β-xyloside, galactose, sorbose, rhamnose, dulcitol, inositol, mannitol, sorbitol, methyl-α-D-mannoside, methyl-α-D-glucoside, amygdalin, salicin, cellobiose, lactose, melibiose, inulin, melezitose, raffinose, starch, glycogen, xylitol, turanose, lyxose, D-fucose, arabitol, gluconate, or 2-ketogluconate. The following enzymes are detected: pyrazinamidase, α-glucosidase, esculinase, esterase, esterase lipase, leucine and cystine arylamidases, and phosphoamidase but nitrate reductase, pyrrolidonyl arylamidase, urease, alkaline and acid phosphatases, lipase, valine arylamidase, trypsin, chymotrypsin, α- and β- galactosidases, β-glucuronidase, *N*-acetyl-β-glucosaminidase, α-mannosidase, and α-fucosidase are not. CAMP reaction negative.

The cell wall contains *meso*-DAP and mycolic acids. Cellular fatty acids are consistent with those of the genus, but tuberculostearic acid was not detected. The type strain was susceptible to all antibiotic classes tested.

Source: lung tissue at necropsy of a ferret with lethal sepsis.

DNA G+C content (mol%): not available.

Type strain: 3105, CCUG 57279, DSM 45274.

Sequence accession no. (16S rRNA gene): FJ374773.

Further comments: additional GenBank accession numbers include (partial *rpoB* gene) FJ467330.

52. **Corynebacterium mycetoides** (*ex* Castellani 1942) Collins 1983, 438[VP] (Effective publication: Collins 1982b, 399.) (*Corynebacterium mycetoides* (Castellani) Ortali and Capocaccia 1956, 490)

my.ce.to′i.des. Gr. n. *mukês -êtos* fungus; L. suff. *-oides* (from Gr. suff. *-eides* from Gr. n. *eidos* that which is seen, form, shape, figure) ressembling, similar; N.L. adj. *mycetoides* similar to fungi, referring to ulcers caused by fungi.

Surface colonies on blood or nutrient agar are ~1 mm in diameter after 2–3 d, circular, convex, entire margin, shiny, and yellow pigmented. Straight to slightly curved Gram-stain-positive rods 1–3 × 0.3–0.5 μm; V-forms and coccoidal forms may be observed. May stain unevenly, and metachromatic granules may be present.

Optimal growth temperature 37°C. Facultatively anaerobic. Glucose is fermented; fructose and trehalose are variable, but maltose, mannose, sucrose, arabinose, lactose, dextrin, cellobiose, galactose, raffinose, ribose, sorbose, xylose, melibiose, glycogen, adonitol, arbutin, inositol, and erythritol are not fermented. Gelatin, cellulose, and esculin not hydrolyzed. Indole, methyl red, and Voges–Proskauer negative. Nitrates not reduced, and urease not produced. Phosphatase positive.

Cell wall contains *meso*-DAP, with alanine and glutamic acid also being present. Arabinose and galactose are the cell-wall sugars. Corynemycolates (30–36 carbon atoms) present. Significant quantities of unusual, α-alkylbranches with odd numbers of carbon atoms such as $C_{15}H_{31}$ are detected. Metabolic products are not extant. Cellular fatty acids typical of the genus are detected including the presence of tuberculostearic acid (Bernard et al., 1991). Menaquinones MK-8(H_2) with small amounts of MK-9(H_2) found. Diphosphatidylglycerol, phosphatidylglycerol, phosphatidylinositol, and phosphatidylinositol mannoside, as well as unknown glycolipids and some unknown glycolipids and phospholipids are found.

Source: a type of ulcer (tropicaloid ulcer) on the legs of soldiers in the desert regions of North Africa in 1942; not reported in recent literature.

DNA G+C content (mol%): 59 (T_m).

Type strain: ATCC 43995, CCUG 27538, CIP 55.51, DSM 20632, JCM 9388, NBRC 15289, NCTC 9864.

Sequence accession no. (16S rRNA gene): X82066, X84241.

Further comments: additional GenBank accession numbers include (complete *rpoB* gene) AY492262.

53. **Corynebacterium phocae** Pascual, Foster, Alvarez and Collins 1998, 603[VP]

pho′ca′e. L. n. *phoca* a seal and also a scientific genus name (*Phoca*); L. gen. n. *phocae* of a seal, of *Phoca*, named because the organism was isolated from the common seal, *Phoca vitulina.*

Cells are Gram-stain-positive short diphtheroids. Colonies are circular, convex, and slightly glistening. The colony diameter is ~1 mm after 24 h incubation on sheep blood agar at 37°C. Facultatively anaerobic. Acid is produced from D-glucose, D-mannose, and maltose, but not from adonitol, D-arabitol, L-arabitol, D-arabinose, L-arabinose, amygdalin, arbutin, cellobiose, dulcitol, erythritol, D-fucose, L-fucose, D-fructose, galactose, β-gentiobiose, gluconate, glycerol, glycogen, inositol, inulin, 2-ketogluconate, D-lyxose, mannitol, melibiose, methyl α-D-glucoside, methyl α-D-mannoside, β-methylxyloside, D-raffinose, rhamnose, salicin, sorbitol, L-sorbose, starch, D-tagatose, trehalose, D-turanose, xylitol, and xylose. Acid production from galactose, *N*-acetylglucosamine, lactose, sucrose, and D-raffinose is variable. Nitrate not reduced to nitrite, and urea hydrolysis variable. Esculin and gelatin are not hydrolyzed. Pyrazinamidase, alkaline phosphatase, esterase-lipase, acid phosphatase, and α-glucosidase are detected, but valine arylamidase, chymotrypsin, α-galactosidase, β-galactosidase, β-glucuronidase, β-glucosidase, *N*-acetyl-β-glucosaminidase, α-mannosidase, esterase, lipase, cystine arylamidase, trypsin, and α-fucosidase activities are not detected. Leucine arylamidase activity variable.

The cell wall contains *meso*-DAP, and mycolic acids (C_{30}–C_{34}) are present. Cellular fatty acids are consistent with those found for the genus, but tuberculostearic acid is not produced.

Source: the common seal (*Phoca vitulina*).

DNA G+C content (mol%): 58 (method unknown).

Type strain: M408/89/1, CCUG 38205, CIP 105741, DSM 44612, JCM 12105.

Sequence accession no. (16S rRNA gene): Y10076.

Further comments: additional GenBank accession numbers include (complete *rpoB* gene) AY492277. Strain M408/89/1 has the characteristics described for the species except that it produces urease and has a negative leucine arylamidase reaction. M408/89/1 produces acid from galactose but not from D-fructose, *N*-acetylglucosamine, lactose, sucrose, and D-raffinose.

54. **Corynebacterium pilbarense** Aravena-Roman, Spröer, Sträubler, Inglis and Yassin 2010, 1486[VP]

pil.ba.ren′se. N.L. neut. adj. *pilbarense* pertaining to Pilbara, Western Australia, the region from which the strain was isolated.

Gram-stain-positive pleomorphic to short rods. Colonies are creamy, circular, and approximately 0.5–2.0 mm in diameter on Columbia blood agar after 24 h incubation at 37°C. Nonhemolytic. Facultatively anaerobic. Nonlipophilic. Esculin, gelatin, and hippurate are not hydrolyzed. Urease and nitrate reduction are negative. Acid is produced from D-glucose, D-ribose, and sucrose. Acid is not produced from L-arabinose, glycogen, inulin, lactose, maltose, mannitol, D-raffinose, sorbitol, trehalose, or D-xylose. Activity for alkaline and acid phosphatases, leucine arylamidase, pyrazinamidase, pyrrolidonyl arylamidase, and naphthol-AS-BI-phosphohydrolase are detected. No activity is detected for arginine dihydrolase, esterase, ester lipase, α-glucosidase, β-glucosidase, α-galactosidase, β-galactosidase, β-glucuronidase, *N*-acetyl-β-glucosaminidase, α-mannosidase,

α-fucosidase, chymotrypsin, trypsin, valine arylamidase, and cystine arylamidase. Acetoin production was negative.

Mycolic acids are present. Long-chain fatty acids are typical for those of the genus, but tuberculostearic acid is absent.

Source: an anaerobic Bactec vial inoculated with ankle aspirate from a man who was thought to be suffering from gout.

DNA G+C content (mol%): not available.

Type strain: IMMIB WACC 658, CCUG 57942, DSM 45350.

Sequence accession no. (16S rRNA gene): FN295567.

55. **Corynebacterium pilosum** Yanagawa and Honda 1978, 209[AL]

pi.lo′sum. L. neut. adj. *pilosum* having much hair; intended to mean having many fimbriae (pili).

(This description also contains information from Collins and Cummins, 1986.)

Gram-stain-positive rods, 0.5 × 1.3 µm, arranged as singly, pairs often at angles, or in irregular masses. Metachromatic granules present. Numerous fimbriae or pili observed using electron microscopy. Colonies on nutrient agar and serum agar are cream to pale yellow, entire, circular, opaque, and about 1 mm in diameter after 24 h incubation at 37°C. No hemolysis on sheep, guinea pig, or rabbit blood agar. Facultatively anaerobic. Pellicle and granular sediment formed in broth. No growth at 5°C or 41.5°C, but cells remain viable for 30 min at 56°C. Complex amino acid requirements. Positive for glucose, fructose, mannose, maltose, trehalose, dextrin, and starch; negative for arabinose, xylose, rhamnose, galactose, lactose, sucrose, raffinose, and salicin. Nitrate is reduced, urease is produced, hippurate is hydrolyzed, and pyrazinamidase is detected. Esculin, tyrosine, and gelatin are not hydrolyzed; casein is not digested; methyl red and phosphatase are negative.

meso-DAP and the sugars arabinose and galactose are present. Short-chain mycolates are present (De Briel et al., 1992). Menaquinone MK-8(H_2) detected. Cellular fatty acids are consistent with those found for the genus, and tuberculostearic acid is detected (Bernard et al., 1991).

Source: the urine and vagina of healthy cows, but occasionally causes cystitis and pyelonephritis.

DNA G+C content (mol%): 57.9–60.9 (T_m).

Type strain: 46 Hara, ATCC 29592, CCUG 27193, CIP 103422, DSM 20521, JCM 3714, NBRC 15285, NCTC 11862.

Sequence accession no. (16S rRNA gene): X81908, X84246.

Further comments: additional GenBank accession numbers include (complete *rpoB* gene) AY492258.

56. **Corynebacterium propinquum** Riegel, de Briel, Prévost, Jehl and Monteil 1994a, 370[VP] (Effective publication: Riegel, de Briel, Prévost, Jehl and Monteil 1993a, 232.)

pro.pin′qu.um. L. neut. adj. *propinquum* near, close.

Gram-stain-positive with occasional metachromatic granules and arranged in palisades or V-shaped forms. Colonies (1–2 mm in diameter) on 5% sheep blood agar, nonhemolytic, gray-white with a matted surface. Facultatively anaerobic. Characteristically "ANF", i.e. an absolute nonfermenter, but reduces nitrate to nitrite. Acid is not produced from D-glucose, glycogen, lactose, sucrose, ribose, D-xylose, D-mannose, D-galactose, trehalose, and D-mannitol or on triple-sugar iron agar. Tyrosine degraded but gelatin, starch, and urea are not. Esculin not hydrolyzed. The methyl red test is negative; acetoin not produced. Growth is visible in 6.5% NaCl. Hydrolysis of hippurate is variable. Production of pyrazinamidase, pyrrolidonyl arylamidase, alkaline phosphatase is variable, but β-glucuronidase, β-galactosidase, α-glucosidase, *N*-acetyl-glucosaminidase are not produced. Cells utilize acetate, lactate, glyconate, and malate. Utilization of adipate and phenyl acetate is variable. Propionate, glucose, arabinose, mannose, mannitol, *N*-acetylglucosamine, maltose, caprate, and citrate are not utilized.

Cell wall contains *meso*-DAP and the sugars arabinose and galactose. The predominant types of mycolic acids are isomers with 30–36 carbons. Cellular fatty acids are consistent with those of the genus.

Source: human sources, mainly from the respiratory tract, but also from other sites.

DNA G+C content (mol%): 57–59 (HPLC).

Type strain: B 77159, ATCC 51488, CCUG 33048, CIP 103792, DSM 44285, JCM 12106.

Sequence accession no. (16S rRNA gene): X81917, X84438.

Further comments: additional GenBank accession numbers include (complete *rpoB* gene) AY492279.

Corynebacterium propinquum was formerly called *Corynebacterium* group ANF-3. Species has <2% variance by 16S rRNA gene sequencing analysis from *Corynebacterium pseudodiphtheriticum*, but may be distinguished by *rpoB* analysis (Khamis et al., 2004).

57. **Corynebacterium pseudodiphtheriticum** Lehmann and Neumann 1896, 361[AL]

Historically, this species has been described by a number of synonyms, including "*Bacillus pseudodiphtheriticus*" (Lehmann and Neumann 1896) Kruse 1886, "*Bacterium pseudodiphtheriticum*" (Lehmann and Neumann 1896) Migula 1900, "*Mycobacterium pseudodiphthericum*" (*sic*) (Lehmann and Neumann 1896) Chester 1901, "*Corynebacterium pseudodiphthericum*" (*sic*) (Lehmann and Neumann 1896) Bergey et al. 1925 and had also been referred to as "*Corynebacterium hofmannii*" Holland 1920, a name which was never validly published.

pseu.do.diph.the.ri′ti.cum. Gr. adj. *pseudes* false; N.L. fem. n. *diphtheria* diphtheria; N.L. adj. *diphtheriticus -a -um* diphtheric; N.L. neut. adj. *pseudodiphtheriticum* relating to false diphtheria.

(This description is largely based on that of Collins and Cummins, 1986.)

Short (0.5–2.0 × 0.3–0.5 µm), regular Gram-stain-positive rods which stain evenly except for a transverse medial unstained septum; club forms seen. Metachromatic granules absent or minimally observed. Organisms said to lie in rows with long axes parallel. Grows well on all media. When grown on blood agar, colonies are white to cream colored, regular and smooth, butyrous consistency, and are nonhemolytic. Facultatively anaerobic. Characteristically does not attack commonly used carbohydrates, although it

can utilize a wide variety of amides, esters, amino acids, and other organic compounds. Organisms reduce nitrate and hydrolyze urea. Hippurate and pyrazinamidase positive but negative for phosphatase. Optimal temperature 37°C.

Cell-wall sugars are arabinose, galactose, and glucose, and contains *meso*-DAP. Corynemycolates are present. Cellular fatty acids are consistent with those found for the genus (Bernard et al., 1991). Propionic acid is not produced as metabolic product (Bernard et al., 2002).

Source: nasal mucosa and various human clinical materials.

DNA G+C content (mol%): 54.9–56.8 (T_m).

Type strain: ATCC 10700, CCUG 27539, CIP 103420, DSM 44287, JCM 11665, NBRC 15362, NCTC 11136.

Sequence accession no. (16S rRNA gene): AJ439343, X81918, X84258.

Further comments: additional GenBank accession numbers include (complete *rpoB* gene) AY492232. Species has <2% variance by 16S rRNA gene sequencing analysis from *Corynebacterium propinquum*, but may be distinguished by *rpoB* analysis (Khamis et al., 2004). Although occasionally present in flora of the upper respiratory tract in healthy individuals, this agent has been associated with exudative pharyngitis mimicking diphtheria, occasionally provoking public health response (Izurieta et al., 1997); recognized as cause of pneumonia, endocarditis, lymphadenitis, keratitis, conjunctivitis, and septic arthritis. Diseases caused by this agent and clinical sites of recovery have been reviewed (Camello et al., 2009). *Corynebacterium pseudodiphtheriticum* strains can be multidrug resistant, which has been linked to the presence of the *ermX* gene (Olender and Niemcewicz, 2010).

58. **Corynebacterium pseudotuberculosis** (Buchanan 1911) Eberson 1918, 294[AL]

Synonyms: "*Bacillus pseudotuberculosis-ovis*" Lehmann and Neumann 1896, "*Bacillus pseudotuberculosis*" Buchanan 1911, "*Corynebacterium ovis*" Bergey et al. 1923, "*Corynebacterium pseudotuberculosis-ovis*" (Lehmann and Neumann 1896) Hauduroy et al. 1937, "*Corynebacterium preisz-nocardi*" Hauduroy et al. 1937, "*Mycobacterium tuberculosis-ovis*" Krasil'nikov 1941.

pseu.do.tu.ber.cu.lo′sis. Gr. adj. *pseudes* false; N.L. fem. n. *tuberculosis* tuberculosis; N.L. gen. n. *pseudotuberculosis* of false tuberculosis.

(This description is largely based on that of Collins and Cummins, 1986.)

In stained smears, described as being similar to *Corynebacterium diphtheriae*, especially the gravis type, with small irregular rods, 0.5–0.6 × 1.0–3.0 µm, with club forms and metachromatic granules. Fimbriae (pili) may be observed. Colonies on blood agar are yellowish-white, opaque, convex with a matt surface, about 1 mm after 24 h incubation at 37°C. Hemolysis may be observed on horse blood agar (Peel et al., 1997). Like *Corynebacterium diphtheriae* and *Corynebacterium ulcerans*, *Corynebacterium pseudotuberculosis* produces cystinase, as demonstrated by production of brown colonies and haloes when grown on modified Tinsdale medium. Facultatively anaerobic. Positive for glucose, fructose, galactose, mannose, and maltose. Urease production and methyl red are positive. Variable for arabinose, sucrose, dextrin, and trehalose; variable for gelatin, nitrate reduction, and starch hydrolysis. Negative for xylose, rhamnose, lactose, raffinose, and salicin. Negative for esculin and hippurate hydrolysis. Neither pyrazinamidase nor phosphatase is detected. Tyrosine is not hydrolyzed, and casein is not digested. Biochemically, this species is most similar to urease-producing *Corynebacterium ulcerans* but differs by occasionally reducing nitrate and being negative or rarely reactive with starch, glycogen, or trehalose (Dorella et al., 2006; Hollis and Weaver, 1981; Peel et al., 1997).

Cell walls contain arabinose, galactose, glucose, and mannose and *meso*-DAP. Menaquinone MK-8(H_2) is present. Polar lipids detected include diphosphatidylglycerol, phosphatidylinositol, and monoacylated phosphatidylinositol dimannoside. Corynemycolates are present. Cellular fatty acids are those described for the genus except, as for cysteinase-producing species *Corynebacterium diphtheriae*, *Corynebacterium pseudotuberculosis*, and *Corynebacterium ulcerans*, has significant quantities of a $C_{16:1}$ isomer. Tuberculostearic acid is not detected (Bernard et al., 1991). Propionic acid is detected as a fermentation product (Bernard et al., 2002).

Source: infections in sheep, goats, horses, and other warm-blooded animals; occasionally linked to disease in humans.

DNA G+C content (mol%): 51.8–52.5 (T_m).

Type strain: ATCC 19410, CCUG 2806, CIP 102968, DSM 20689, JCM 9389, NBRC 15363, NCTC 3450.

Sequence accession no. (16S rRNA gene): X81916, X84255.

Further comments: additional GenBank accession numbers include (complete *rpoB* gene) AY492239. *Corynebacterium pseudotuberculosis* and *Corynebacterium ulcerans* are the only *Corynebacterium* species to produce phospholipase D (PLD), with expression of the *pld* gene product being detected by inhibition of the CAMP reaction (Barksdale et al., 1981). PLD is believed to be a major virulence determinant for spread of caseous lymphadenitis, a disease primarily of sheep and goats. PLD spreads the bacterium from the site of infection to the lymph nodes followed by caseation by means of a complex regulatory process (McKean et al., 2007). Disease in humans is usually occupationally linked to those who handle infected animals (McKean et al., 2007; Peel et al., 1997). Detection of the *pld* gene has been used clinically to diagnose diseased animals (Pacheco et al., 2007). *Corynebacterium pseudotuberculosis* isolates may produce diphtheria toxin with the potential to cause diphtherial-like disease, which clinically should be tested for (as described elsewhere in this chapter). Nontoxigenic strains otherwise have typical features. By 16S rRNA gene sequencing, *Corynebacterium pseudotuberculosis* is most closely related to (>99% identity with) *Corynebacterium ulcerans* and to (>97% identity with) *Corynebacterium diphtheriae*, but *Corynebacterium ulcerans* and *Corynebacterium pseudotuberculosis* are only distantly related (93% level) when *rpoB* gene sequences are compared (Khamis et al., 2004).

59. **Corynebacterium pyruviciproducens** Tong, Liu, Summanen, Xu and Finegold 2010, 1139[VP]

py.ru.vi.ci.pro.du′cens. N.L. n. *acidum pyruvicum* pyruvic acid; L. part. adj. *producens* producing; N.L. part. adj. *pyruviciproducens* pyruvic acid-producing.

Gram-stain-positive coryneform rods. Lipophilic. Can grow aerobically or facultatively anaerobically at 37°C and

42°C. Optimum growth at 37°C under aerobic conditions. Colonies are small, i.e., 0.3–0.5 mm in diameter, circular, entire, convex, and translucent after 2 d of incubation on blood agar. Urease negative. Cannot reduce nitrates. CAMP negative. Acid is produced from D-ribose, D-xylose, D-glucose, maltose, and sucrose, but not from fructose, D-mannitol, lactose, or glycogen. Cells produce esterase, esterase lipase, leucine arylamidase, pyrazinamidase, a small amount of naphthol-AS-BI-phosphohydrolase, and pyrrolidonyl arylamidase, but not acid phosphatase, β-galactosidase, α-glucosidase, *N*-acetyl-β-glucosaminidase, lipase, valine arylamidase, cystine arylamidase, α-chymotrypsin, α-galactosidase, α-fucosidase, alkaline phosphatase, α-mannosidase, or β-glucosidase. Trypsin and gelatin are not hydrolyzed. β-Lactamase negative. Susceptible to the vibriostatic agent O/129 and highly sensitive to ampicillin, ceftriaxone, clindamycin, and erythromycin.

Cellular fatty acids are consistent for those of the genus, but no tuberculostearic acid is detected. Short-chain mycolic acids (C_{22}–C_{36}) are present. Metabolic products include acetic and pyruvic acids but not propionic.

Source: a human groin abscess.

DNA G+C content (mol%): 62 (HPLC).

Type strain: 06–17730, ATCC BAA-1742, CCUG 57046, WAL 19168.

Sequence accession no. (16S rRNA gene): FJ185225.

Further comments: additional GenBank accession numbers include (partial *rpoB* gene) FJ899747.

60. **Corynebacterium renale** (Migula 1900) Ernst 1906, 89[AL] (*Bacterium renale* Migula 1900, 504)

re.na′le. L. neut. adj. *renale* pertaining to the kidneys.

(This description is largely based on that of Collins and Cummins, 1986.)

Large, irregularly staining bacillus, 0.7 × 3.0 µm, often with pointed ends; fimbriae (pili) may be present after examination by electron microscopy. Colonies on blood agar are yellow pigmented after 24 h incubation at 37°C. Grows in broth at pH 5.4. Facultatively anaerobic. Positive for glucose, fructose, mannose, and dextrin. Variable for maltose and trehalose. Negative for arabinose, xylose, rhamnose, galactose, lactose, sucrose, raffinose, starch, and salicin. Urease production and methyl red are positive. Hippurate hydrolysis positive. Pyrazinamidase positive, but phosphatase negative. Esculin, tyrosine, and gelatin are not hydrolyzed. Casein is digested. Nitrate is not reduced.

Corynemycolates (30–36 carbons) and menaquinone MK-8(H_2) are present. Cellular fatty acids consistent with those for the genus are found, but tuberculostearic acid is not detected. Propionic acid is not detected as fermentation product (Bernard et al., 2002).

Source: cystitis and pyelitis in cattle and occasionally in other animals. Disease in humans described historically, but no genetic confirmation.

DNA G+C content (mol%): 53–58 (T_m).

Type strain: ATCC 19412, CCUG 27542, CIP 103421, DSM 20688, HAMBI 2321, JCM 9391, NBRC 15290, NCTC 7448.

Sequence accession no. (16S rRNA gene): X81909, X84249.

Further comments: additional GenBank accession numbers include (complete *rpoB* gene) Y492240.

61. **Corynebacterium resistens** Otsuka, Kawamura, Koyama, Iihara, Ohkusu and Ezaki 2005b, 2235[VP] (Effective publication: Otsuka, Kawamura, Koyama, Iihara, Ohkusu and Ezaki 2005a, 3716.)

re.sis.tens. L. part. adj. *resistens* enduring, resistant (multidrug-resistant).

Cells are Gram-stain-positive, typically club-shaped rods, 1–3 µm in length, and arranged as single cells, in pairs, or in small clusters. Growth on TSA with 5% sheep blood produces nonpigmented, grayish-white, glistening, pearly colonies up to 1.0 mm in diameter. Colonies are nonhemolytic and very slow-growing under anaerobic conditions. Tween 80 enhances growth, resulting in colonies 2–4 mm in diameter, i.e. strains are lipophilic. CAMP negative. Nitrate not reduced. Ferments D-tagatose, 5-ketogluconate, ribose, and D-glucose with trehalose and L-sorbose being variable. Negative for glycerol, erythritol, D-arabinose, L-arabinose, D-xylose, L-xylose, adonitol, β-methylxyloside, galactose, D-fructose, D-mannose, rhamnose, dulcitol, inositol, mannitol, sorbitol, α-methyl-D-mannoside, α-methyl-D-glucoside, *N*-acetylglucosamine, amygdalin, arbutin, esculin, salicin, cellobiose, maltose, lactose, melibiose, sucrose, inulin, melezitose, D-raffinose, starch, glycogen, xylitol, β-gentiobiose, D-turanose, D-lyxose, D-fucose, L-fucose, D-arabitol, L-arabitol, gluconate, and 2-ketogluconate. Alkaline phosphatase, esterase, esterase lipase, leucine arylamidase, acid phosphatase, and naphthol-AS-BI-phosphohydrolase strongly positive, but lipase, cysteine, and arylamidase are weakly positive. Reactions for pyrazinamidase, valine arylamidase, trypsin, chymotrypsin, α-galactosidase, β-galactosidase, β-glucuronidase, α-glucosidase, β-glucosidase, *N*-acetyl-β-glucosaminidase, α-mannosidase, and α-fucosidase are negative.

Cellular fatty acids are consistent for those described for the genus; other chemotaxonomic information is not extant.

Source: human disease from bacteremias of leukemic patients, bronchial aspirates of patients with lymphoma or subarachnoid hemorrhaging, and a patient with cellulitis.

DNA G+C content (mol%): 54.643 (standard deviation, 0.03%) (HPLC).

Type strain: CCUG 50093, GTC 2026, JCM 12819, SICGH 158.

Sequence accession no. (16S rRNA gene): AB128981.

Further comments: Corynebacterium resistens is resistant to multiple drug classes, but all strains are susceptible to vancomycin.

62. **Corynebacterium riegelii** Funke, Lawson and Collins 1998b, 627[VP] (Effective publication: Funke, Lawson and Collins 1998a, 626.)

ri.e.gel′i.i. N.L. gen. masc. n. *riegelii* of Riegel, to honor contemporary French microbiologist Philippe Riegel for his contributions to the taxonomy of the genus *Corynebacterium* as well as to the clinical microbiology of coryneform bacteria.

Gram-stain-positive typically club-shaped rods which appear as single cells, in pairs, or in small clusters. Colonies are whitish with a creamy or a slightly sticky consistency, circular with entire edges, convex, glistening, and up to 1.5 mm

in diameter after 48 h of incubation. Weak growth anaerobically. Biochemically unusual, as acid is produced from maltose, ribose, trehalose, D-tagatose, and 5-ketogluconate, but not produced from glucose, sucrose, mannitol, xylose, glycerol, erythritol, arabinose, adonitol, β-methylxyloside, galactose, D-fructose, D-mannose, L-sorbose, rhamnose, dulcitol, inositol, sorbitol, α-methyl-D-mannoside, α-methyl-D-glucoside, *N*-acetylglucosamine, amygdalin, arbutin, salicin, cellobiose, lactose, melibiose, inulin, melezitose, D-raffinose, starch, glycogen, xylitol, β-gentiobiose, D-turanose, D-lyxose, fucose, arabitol, gluconate, or 2-ketogluconate. Nitrate is not reduced. Urea hydrolysis strongly positive, but esculin not hydrolyzed. CAMP reaction negative. Esterase, esterase lipase, leucine arylamidase, and cystine arylamidase detected, but pyrazinamidase and alkaline and acid phosphatases are variable. Pyrrolidonylarylamidase, lipase, valine arylamidase, trypsin, chymotrypsin, α-galactosidase, β-galactosidase, β-glucuronidase, α-glucosidase, β-glucosidase, *N*-acetyl-β-glucosaminidase, α-mannosidase, and α-fucosidase are not detected. The type strain has features as described above except that the activity of alkaline phosphatase but not that of pyrazinamidase or acid phosphatase is detected.

The cell wall contains *meso*-DAP. Mycolic acids are present. Cellular fatty acids are consistent with those described for the genus, but tuberculostearic acid is not detected. Propionic acid is not produced as a metabolic product (Bernard et al., 2002).

Source: human clinical specimens.

DNA G+C content (mol%): not available.

Type strain: ATCC 700782, CCUG 38180, CIP 105310, DMMZ 2415, DSM 44326, JCM 10389.

Sequence accession no. (16S rRNA gene): Y14651.

Further comments: additional GenBank accession numbers include (complete *rpoB* gene) AY492278.

63. **Corynebacterium simulans** Wattiau, Janssens and Wauters 2000, 351[VP]

si′mu.lans. L. part. adj. *simulans* simulating, because it resembles *Corynebacterium striatum.*

Cells are Gram-stain-positive showing a diphtheroid arrangement. Colonies are grayish-white, glistening, and 1–2 mm in diameter on blood agar after 48 h incubation at 37°C. Facultatively anaerobic. Fermentative. Growth does not occur or is very weak at 20°C within 3 d. Acid is produced from glucose, sucrose, fructose, and mannose. Acid production from *N*-acetylglucosamine, galactose, and ribose is variable. Acid is not produced from mannitol, maltose, D- and L-xylose, glycerol, erythritol, D- and L-arabinose, adonitol, methyl-β-D-xyloside, sorbose, rhamnose, dulcitol, inositol, sorbitol, methyl-α,D-mannoside, methyl-α,D-glucoside, amygdalin, arbutin, esculin, salicin, cellobiose, lactose, melibiose, trehalose, inulin, melezitose, raffinose, starch, glycogen, xylitol, gentiobiose, D-turanose, D-lyxose, D-tagatose, D- and L-fucose, D- and L-arabitol, gluconate, 2-ketogluconate, and 5-keto-gluconate. Urea, esculin, and gelatin not hydrolyzed. Nitrate and nitrite reduction positive, a feature unique among all *Corynebacterium* species with nitrite reduction occurring at concentrations of 0.001–0.01%.

Alkalinization of buffered formate is positive. No acid produced from ethylene glycol. Tween esterase and tyrosine clearing positive. Pyrazinamidase is variable. CAMP reaction negative. Alkaline phosphatase, esterase, esterase-lipase, leucine arylamidase, trypsin, and naphthol-AS-BI-phosphohydrolase positive but lipase, valine arylamidase, cystine arylamidase, chymotrypsin, acid phosphatase, α- and β-galactosidase, β-glucuronidase, α- and β-glucosidase, *N*-acetyl-β-glucosaminidase, α-mannosidase, and α-fucosidase negative. The type strain of *Corynebacterium simulans* is as described here with ribose, *N*-acetylglucosamine, galactose, and pyrazinamidase being positive. Cell wall contains the sugars arabinose and galactose as well as *meso*-DAP. Cellular fatty acids are consistent with those described for the genus. Short-chain mycolic acids (C_{22}–C_{36}) are present. Propionic acid is not detected as a metabolic product (Bernard et al., 2002).

Source: human clinical materials including bile (Bernard et al., 2002; Wattiau et al., 2000).

DNA G+C content (mol%): not available.

Type strain: Co 553, ATCC BAA-15, CCUG 43305, CIP 106488, DSM 44415, JCM 12107, UCL 553.

Sequence accession no. (16S rRNA gene): AJ012837.

Further comments: additional GenBank accession numbers include (complete *rpoB* gene) AY492264.

64. **Corynebacterium singulare** Riegel, Ruimy, Renaud, Freney, Prévost, Jehl, Christen and Monteil 1997b, 1095[VP]

sin.gu.la′re. L. neut. adj. *singulare* single, unique.

Gram-stain-positive irregular rods arranged in typical V-shaped forms or palisades. Facultatively anaerobic. Nonhemolytic colonies (1–2 mm diameter) grayish and smooth after 24 h at 37°C on sheep blood agar. Colonies circular and slightly convex with entire margins. Nitrate is not reduced to nitrite. Urea and tyrosine degraded, but gelatin and esculin are not hydrolyzed. Acid is produced from glucose, maltose, and sucrose but not from lactose, glycogen, ribose, trehalose, mannitol, and D-xylose. Alkaline phosphatase, pyrrolidonylarylamidase, and pyrazinamidase are produced but α-glucosidase, β-glucuronidase, β-galactosidase, and *N*-acetyl-β-glucosaminidase are not. Cells utilize D-glucose, D-fructose, D-trehalose, D-mannose, sucrose, maltose, glycerol, D-turanose, L-malate, phenylacetate, putrescine, DL-lactate, caprylate, L-histidine, succinate, fumarate, 3-hydroxybutyrate, L-aspartate, L-glutamate, D-alanine, L-serine, propionate, and L-tyrosine as sole carbon sources. D-Ribose, *N*-acetyl-D-glucosamine, and D-gluconate are not utilized.

Cell wall contains *meso*-DAP, the sugars arabinose and galactose, and short-chain mycolic acids (C_{26}–C_{36}). Cellular fatty acids are consistent with those described for the genus. Propionic acid is not produced by anaerobic metabolism of glucose.

Source: human semen.

DNA G+C content (mol%): 62 (capillary electrophoresis).

Type strain: CCUG 37330, CIP 105491, DSM 44357, IBS B52218, JCM 10385, NBRC 16162.

Sequence accession no. (16S rRNA gene): Y10999.

Further comments: additional GenBank accession numbers include (complete *rpoB* gene) AY492280.

By 16S rRNA gene sequencing alone, this species can not be readily discerned from *Corynebacterium aurimucosum* and *Corynebacterium minutissimum*, but these can be resolved using *rpoB* gene sequencing (Khamis et al., 2004).

65. **Corynebacterium sphenisci** Goyache, Ballesteros, Vela, Collins, Briones, Hutson, Potti, García-Borboroglu, Domínguez and Fernández-Garayzábal 2003a, 1011[VP]

sphe.nis′ci. N.L. masc. gen. n. *sphenisci* of *Spheniscus*, a systematic genus of penguins, source of the type strain.

Gram-stain-positive rods. Colonies are whitish, low-convex, dry and rough, ~1–2 mm in diameter after 48 h incubation at 37°C on sheep blood agar. Facultatively anaerobic. Nonhemolytic, nonlipophilic, and CAMP negative. Nitrates are reduced. Acid is produced from glucose, maltose, galactose, fructose, mannose, and trehalose, but not from ribose, sucrose, glycogen, xylose, mannitol, lactose, erythritol, D-arabinose, L-arabinose, adonitol, galactose, L-sorbose, rhamnose, inositol, sorbitol, methyl-α-D-mannoside, methyl-α-D-glucoside, amygdalin, dulcitol, arbutin, salicin, cellobiose, melibiose, inulin, melezitose, D-raffinose, xylitol, β-gentiobiose, D-turanose, D-lyxose, D-tagatose, D-fucose, L-fucose, D-arabitol, L-arabitol, *N*-acetylglucosamine, 2-ketogluconate, 5-ketogluconate, or glycerol. Gelatin, urea, and esculin are not hydrolyzed. Pyrazinamidase, esterase, ester lipase, lipase, leucine arylamidase, valine arylamidase, cystine arylamidase, chymotrypsin, acid phosphatase, and naphthol-AS-BI-phosphohydrolase detected, but alkaline phosphatase, pyrrolidonyl arylamidase, α-glucosidase, β-glucosidase, β-glucuronidase, *N*-acetyl-β-glucosaminidase, α-mannosidase, α-galactosidase, β-galactosidase, α-fucosidase, and trypsin are not.

Cell-wall murein is based on *meso*-DAP. Cellular fatty acids are consistent with those described for the genus, but tuberculostearic acid is not present. Corynomycolates detected in small amounts.

Source: the cloaca of an apparently healthy wild magellanic penguin (*Spheniscus magellanicus*).

DNA G+C content (mol%): not available.

Type strain: CCUG 46398, CECT 5990, JCM 12270.

Sequence accession no. (16S rRNA gene): AJ440964.

Further comments: additional GenBank accession numbers include (partial *rpoB* gene) EU004067 for DSM 44792.

66. **Corynebacterium spheniscorum** Goyache, Vela, Collins, Ballesteros, Briones, Moreno, Yorio, Domínguez, Hutson and Fernández-Garayzábal 2003b, 45[VP]

sphe.nis.co′rum. N.L. masc. n. *spheniscus* a genus of penguin; N.L. masc. pl. gen. n. *spheniscorum* of penguins.

Gram-stain-positive rods. Colonies are whitish, circular, smooth, entire, and ~ 1–2 mm diameter on Columbia blood agar after 24 h incubation at 37°C. Nonhemolytic.

Facultatively anaerobic; grows slightly under anaerobic conditions. Nonlipophilic, and CAMP-positive using *Staphylococcus aureus* in the test assay. Esculin, gelatin, and urea are not hydrolyzed. Nitrate not reduced. Acid is produced from glucose, ribose, D-fructose, D-mannose, trehalose, *N*-acetyl-β-glucosamine, and maltose, but not from D-xylose, L-xylose, mannitol, lactose, rhamnose, galactose, adonitol, inositol, D- or L-arabinose, sucrose, melibiose, melezitose, or glycogen. Acidification of dulcitol is variable. Enzyme activity for ester lipase, esterase, pyrazinamidase, and leucine arylamidase (weak) detected but not for alkaline and acid phosphatases, pyrrolidonyl arylamidase, α-glucosidase, β-glucosidase, β-glucuronidase, α-galactosidase, β-galactosidase, α-mannosidase, α-fucosidase, chymotrypsin, trypsin, valine arylamidase, cystine arylamidase, and naphthol-AS-BI-phosphohydrolase.

Cell wall contains *meso*-DAP, and Cellular fatty acids are consistent with those described for the genus, but tuberculostearic acid is not present. Mycolic acids are present (C_{32}–C_{36}).

Source: the cloacae of apparently healthy penguins (*Spheniscus magellanicus*).

DNA G+C content (mol%): not available.

Type strain: PG 39, CCUG 45512, CECT 5986, JCM 12271.

Sequence accession no. (16S rRNA gene): AJ429234.

Further comments: additional GenBank accession numbers include (complete *rpoB* gene) AY492283.

67. **Corynebacterium sputi** Yassin and Siering 2008, 2878[VP]

spu′ti. L. gen. n. *sputi* of sputum.

Gram-stain-positive rods. Colonies are cream-yellow, circular, convex, dry, and ~ 0.2–2 mm in diameter on Columbia blood agar after 48 h incubation at 37°C. Nonhemolytic. Lipophilic, as Tween 80 encourages growth of the organism. Facultatively anaerobic. Urea hydrolyzed, but esculin, gelatin, and hippurate are not. Nitrate not reduced. Acid produced from D-glucose but not from L-arabinose, glycogen, inulin, D-lactose, maltose, D-mannitol, raffinose, D-ribose, D-sorbitol, starch, sucrose, trehalose, or D-xylose. Activity detected for α-glucosidase, lipase, lipase, leucine arylamidase, pyrazinamidase, and naphthol-AS-BI-phosphohydrolase but not for acid and alkaline phosphatases, arginine dihydrolase, chymotrypsin, cysteine arylamidase, esterase, α-fucosidase, α-galactosidase, β-galactosidase, β-glucuronidase, β-glucosidase, *N*-acetyl-β-glucosaminidase, α-mannosidase, pyrrolidonyl arylamidase, trypsin, or valine arylamidase. Acetoin production negative.

Corynemycolic acids are present. Cellular fatty acids are consistent with those described for the genus, and tuberculostearic acid is present.

Source: the sputum of a patient with pneumonia.

DNA G+C content (mol%): not available.

Type strain: IMMIB L-999, CCUG 55795, DSM 45148.

Sequence accession no. (16S rRNA gene): AM930556.

68. **Corynebacterium stationis** (ZoBell and Upham 1944) Bernard, Wiebe, Burdz, Reimer, Ng, Singh, Schindle and Pacheco 2010, 877[VP] ("*Achromobacter stationis*" Zobell and Upham 1944, 273; *Brevibacterium stationis* Breed 1953, 14)

sta.ti.o′nis. L. gen. n. *stationis* of a fixed position.

Gram-stain-positive short rods, 0.6–1.0 µm in diameter, occurring singly, in pairs, in "V" forms, and can be club shaped. Colonies on blood agar after 24 h are gray-white but become yellowish with age, ~1 mm in diameter, raised, with no hemolysis observed. Facultatively anaerobic, but grows better under aerobic conditions. Poor or no growth under strictly anaerobic conditions. Grows at 25, 35, and 42°C in air. Nonlipophilic. Acid is produced, albeit slowly, from glucose, fructose, and ribose. Mannose and fucose found in the API 50CH gallery also weakly reactive. Xylose, mannitol, lactose, sucrose, maltose, galactose, glycerol, glycogen, raffinose, salicin, and trehalose are not reactive. Triple-sugar iron remains neutral or alkaline/neutral. Grows

in the presence of 6–10% NaCl. Urease produced. Nitrate reduced to nitrite, but nitrite is not reduced to nitrogen using either conventional or API Coryne panel methods. Simmons' citrate is alkalinized. Tyrosine is hydrolyzed, but gelatin, esculin, casein, and starch are not. Not reactive in tests for lysine, arginine, or ornithine decarboxylases. CAMP and CAMP-inhibition reactions not observed. DNase and indole are not produced. Pyrazinamidase may be produced, but otherwise most enzymes of the API ZYM panel are not detected. Strains are generally susceptible to a wide variety of antimicrobials.

Cell wall contains *meso*-DAP and the sugars are arabinose and galactose. Corynemycolates are present. Menaquinones MK-8(H_2) and MK-9(H_2) detected. Cellular fatty acids are consistent with those described for the genus. Propionic acid is not detected as metabolic product.

Source: sea water. ATCC 6872, previously identified as *Corynebacterium ammoniagenes* but now assigned to *Corynebacterium stationis*, was isolated from human infant stools. Additional strains were recovered from human blood cultures (Bernard et al., 2010).

DNA G+C content (mol%): 53.9 (T_m).

Type strain: ATCC 14403, CCUG 43497, CIP 104228, DSM 20302, JCM 11611, NBRC 12144, VKM B-1228.

Sequence accession no. (16S rRNA gene): FJ172667.

Further comments: additional GenBank accession numbers include (partial *rpoB* gene) FJ172671.

69. **Corynebacterium striatum** (Chester 1901) Eberson 1918, 22[AL] (*Bacterium striatum* Chester 1901, 171)

stri.a′tum. L. part. neut. adj. *striatum* grooved.

Note: None of the reference strains contained in two major culture collections (ATCC, NCTC) fit the descriptions found in the original publications and the wrong strains appear to have been deposited for this species. Therefore, there is a case for declaring *Corynebacterium striatum* a *nomen dubium* (Rule 56a) exists. However, if a careful search reveals a strain or strains used in the original description agreeing in its characteristics, then a neotype strain can be proposed (Rule 18c). The description here is based primarily on characteristics (now widely accepted for this species) linked to reactions for ATCC 6940 (Coyle et al., 1993a; Funke and Bernard, 2007; Hollis and Weaver, 1981; Leonard et al., 1994; Martinez-Martinez et al., 1995b). The description shown here outlines several biochemical differences from those outlined in the previous edition of this *Manual* (Collins and Cummins, 1986).

Pleomorphic Gram-stain-positive rods, often club-shaped, 0.25–0.5 × 2.0–3.0 µm. Coccoidal forms and long filaments can be found among older cultures. Metachromatic granules may be observed. Colonies may be slow-growing, white, smooth, entire, about 1 mm in diameter after 48 h of incubation at 37°C on sheep blood agar (Collins), but some are creamy, pale-yellow colonies. A strain which caused multiple cases of pneumonia in an ICU was brown pigmented (Leonard et al., 1994). Nonhemolytic, but slight hemolysis may be seen around deep cultures. Nonlipophilic. Facultatively anaerobic. Positive for glucose, fructose, mannose, starch, and dextrin. Variable for galactose and trehalose. Nitrate is reduced, maltose and lactose are negative, and reaction for sucrose is variable. Arabinose, xylose, mannitol, rhamnose, raffinose, and salicin are negative. This is in contrast to a negative reaction for nitrate reduction, a positive reaction for maltose fermentation, and variable reactions for lactose and sucrose as described in the previous version of *Bergey's Manual* (Collins and Cummins, 1986). Urease is not produced, and methyl red is variable (Coyle et al., 1993a). Pyrazinamidase and alkaline phosphatase positive. Esculin not hydrolyzed. Gelatin is described as variable (Collins and Cummins, 1986) or negative (Coyle et al., 1993a). Tyrosine hydrolyzed (Funke and Bernard, 2007; Martinez-Martinez et al., 1995b).

ATCC 6940[T] cell wall contains arabinose and galactose as well as mycolic acids. Menaquinone MK-8(H_2) detected. Cellular fatty acids are like those described for the genus, but tuberculostearic acid is not detected (Bernard et al., 1991). Propionic acid is not detected as a metabolic product of glucose fermentation (Bernard et al., 2002).

Source: human nasopharynx; strains recovered from a variety of human materials, but historically also recovered from the milk of cows with mastitis.

DNA G+C content (mol%): 57.6 (T_m).

Type strain: ATCC 6940, CCUG 27949, CIP 81.15, DSM 20668, JCM 9390, NBRC 15291, NCTC 764.

Sequence accession no. (16S rRNA gene): X81910, X84442.

Further comments: additional GenBank accession numbers include (complete *rpoB* gene) AY492267. Multidrug resistant strains have been increasingly documented among patients with *Corynebacterium striatum* infections (Tarr et al., 2003) and it may cause potentially fatal disease among compromised patients (Lee et al., 2005; Otsuka et al., 2006). Person to person transmissions among ICU patients have been documented (Brandenburg et al., 1996; Leonard et al., 1994). Also associated with bovine mastitis (Coyle et al., 1993a).

70. **Corynebacterium suicordis** Vela, Mateos, Collins, Briones, Hutson, Domínguez and Fernández-Garayzábal 2003, 2029[VP]

su.i.cor′dis. L. n. *sus suis* pig; L. n. *cor cordis* heart; N.L. gen. n. *suicordis* of/from pig heart.

Gram-stain-positive rods. Colonies are whitish, circular, smooth, entire, and 1–2 mm in diameter after 48 h incubation at 37°C on sheep blood agar. Facultatively anaerobic. Nonhemolytic. CAMP negative and nonlipophilic. Nitrate is not reduced. Acid is not produced from D-glucose, maltose, lactose, ribose, sucrose, glycogen, mannitol, glycerol, erythritol, D-arabinose, L-arabinose, D-xylose, L-xylose, adonitol, inositol, methyl-β-xyloside, galactose, D-fructose, D-mannose, L-sorbose, rhamnose, methyl-α-D-mannoside, methyl α-D-glucoside, sorbitol, *N*-acetyl-β-glucosamine, amygdalin, arbutin, salicin, cellobiose, melibiose, trehalose, inulin, melezitose, D-raffinose, xylitol, b-gentiobiose, D-turanose, D-lyxose, D-tagatose, D-fucose, L-fucose, D-arabitol, L-arabitol, 5-ketogluconate, or 2-ketogluconate. Urea hydrolyzed, but esculin and gelatin are not. Pyrazinamidase, esterase, ester lipase, alkaline phosphatase, acid phosphatase, and naphthol-AS-BI-phosphohydrolase detected, but pyrrolidonyl arylamidase, β-glucuronidase, β-galactosidase, α-glucosidase, lipase, leucine arylamidase, valine arylamidase,

cystine arylamidase, trypsin, chymotrypsin, α-galactosidase, β-glucosidase, α-mannosidase, and α-fucosidase, are not.

Cell wall contains *meso*-DAP, and corynomycolic acids are present (C_{28}–C_{36}). Cellular fatty acids are consistent with those described for the genus.

Source: the heart, pleural cavity, lymph nodes, and lungs of diseased pigs.

DNA G+C content (mol%): not available.

Type strain: P81/02, CCUG 46963, CECT 5724, JCM 12370.

Sequence accession no. (16S rRNA gene): AJ504424.

Further comments: additional GenBank accession numbers include (partial *rpoB* gene) EU004068 for CIP 108201.

71. **Corynebacterium sundsvallense** Collins, Bernard, Hutson, Sjödén, Nyberg and Falsen 1999a, 364[VP]

sunds.val.len′se. N.L. neut. adj. *sundsvallense* of or belonging to Sundsvall, Sweden, named after the city from where the bacterium was first isolated.

Gram-stain-positive rods; some branching and bulges/knobs at the ends of some cells may be observed. Colonies are buff or yellowish, opaque, shiny, heaped, and adherent to medium. Nonhemolytic. Nonlipophilic and CAMP negative. Growth possible in 6% NaCl but not in 10% NaCl. Acid produced from fructose, glucose, maltose, and sucrose but not from amygdalin, *N*-acetylglucosamine, galactose, glycogen, lactose, mannitol, ribose, raffinose, salicin, trehalose, or D-xylose. Hippurate is hydrolyzed but esculin, gelatin, and starch are not. Nitrate not reduced. Urease positive and phosphoamidase weakly detected. Variable for α-glucosidase, as 2 strains were positive but 2 are negative (Bernard et al., 2002). Some strains are positive for alkaline phosphatase, leucine arylamidase, pyrazinamidase, ester lipase, and esterase. Acid phosphatase, cystine arylamidase, chymotrypsin, α-fucosidase, α-galactosidase, β-galactosidase, β-glucosidase, β-glucuronidase, lipase, α-mannosidase, pyrrolidonyl arylamidase, trypsin, and valine arylamidase are not detected. Type strain has features as described above except weak reactions for pyrazinamidase and alkaline phosphatase are observed and leucine arylamidase, ester lipase, and esterase activities are not detected.

The cell wall contains *meso*-DAP, and mycolic acids are present. Cellular fatty acids consistent with those described for the genus, but tuberculostearic acid is not present. Lactate and succinate but not propionate are major products of glucose fermentation.

Source: human clinical specimens.

DNA G+C content (mol%): 64 (T_m).

Type strain: CCUG 36622, CIP 105936, DSM 44613, JCM 12401.

Sequence accession no. (16S rRNA gene): Y09655.

Further comments: additional GenBank accession numbers include (complete *rpoB* gene) AY492268. By 16S rRNA gene sequencing, closely related to (~99.0% identity) to *Corynebacterium thomssenii*, but differs from that species (~90.0% identity) when complete sequences of the *rpoB* gene are compared (Khamis et al., 2004).

72. **Corynebacterium terpenotabidum** Takeuchi, Sakane, Nihira, Yamada and Imai 1999, 228[VP]

ter.pen.o.ta′bi.dum. N.L. n. *terpenum* terpene; L. neut. adj. *tabidum* dissolving; N.L. neut. adj. *terpenotabidum* terpene-dissolving.

Gram-stain-positive irregular rods, 0.5–0.7 × 19–15 μm long in young cultures; some cells are arranged at a V angle. In older cultures, cells are 0.5–0.7 × 0.6–1.0 μm. Colonies on PY-BHI agar are circular, have a rough surface, and are grayish white. Growth occurs under aerobic, but not anaerobic, conditions. Methyl red test negative. Nitrate not reduced to nitrite. Hugh–Leifson's test in glucose oxidation-fermentation (OF) medium is oxidative. Voges–Proskauer test positive. Urease produced, but arginine dihydrolase, lysine decarboxylase, and ornithine decarboxylase are not. Tween 80 is hydrolyzed, but starch, gelatin, casein, cellulose, esculin, and tyrosine are not. Fructose, glucose, mannose, lactate, and ethanol are utilized as carbon sources, but galactose, lactose, maltose, sucrose, glycerol, sorbitol, mannitol, inositol, citrate, succinate, malonate, pimelate, *m*-hydroxybenzoate, *p*-hydroxybenzoate, arginine, aspartate, histidine, methylamine, ethylamine, and methanol are not. Thiamin and biotin required for growth. Growth is observed in PY-BHI broth containing 8% but not 10% NaCl.

Cell wall contains *meso*-DAP with arabinose, galactose, and mannose, as major cell-wall sugars. The muramic acids of peptidoglycan occur in the *N*-acetyl form. Menaquinone MK-9(H_2) predominates. Mycolic acids are present. Cellular fatty acids are consistent with those described for the genus, and high levels of tuberculostearic acid are present.

Source: soil.

DNA G+C content (mol%): 67.5 (HPLC).

Type strain: Y -11, CIP 105927, JCM 10555, NBRC 14764, VKM Ac-2071.

Sequence accession no. (16S rRNA gene): AB004730.

Further comments: additional GenBank accession numbers include (complete *rpoB* gene) AY492269.

73. **Corynebacterium testudinoris** Collins, Hoyles, Hutson, Foster and Falsen 2001b, 1351[VP]

tes.tu.din.o′ris. L. n. *testudo -inis* tortoise; L. gen. neut. n. *oris* of the mouth; N. L. gen. neut. n. *testudinoris* of the mouth of a tortoise.

Gram-stain-positive rods. Yellow pigmented. Facultatively anaerobic. Nonlipophilic. Acid is produced from glucose, maltose, ribose, and sucrose but not from glycogen, lactose, mannitol, or D-xylose. Activity detected for acid phosphatase (weak), esterase (weak), ester lipase (weak), β-glucosidase, and leucine arylamidase. No activity detected for alkaline phosphatase, cystine arylamidase, chymotrypsin, α-fucosidase, α-galactosidase, β-galactosidase, β-glucuronidase, *N*-acetyl- β-glucosaminidase, lipase, α-mannosidase, valine arylamidase, pyrazinamidase, phosphoamidase, pyrrolidonyl arylamidase, urease, or trypsin. α-Glucosidase variable. Esculin is hydrolyzed, but gelatin is not. Nitrate is reduced.

Cell wall contains *meso*-DAP. Cellular fatty acids are consistent with those described for the genus, but tuberculostearic acid is not present. Mycolic acids are present (C_{30}–C_{36}).

Source: necrotic lesions in mouth of a tortoise; habitat is unknown.

DNA G+C content (mol%): not available.

Type strain: M935/96/4, CCUG 41823, CIP 106763, DSM 44614, JCM 12108.

Sequence accession no. (16S rRNA gene): AJ295841.

Further comments: additional GenBank accession numbers include (complete *rpoB* gene) AY492284.

74. **Corynebacterium thomssenii** Zimmermann, Spröer, Kroppenstedt, Fuchs, Köchel and Funke 1998, 491[VP]

thoms.se′ni.i. N.L. gen. masc. n. *thomssenii* of Thomssen, to honor Reiner Thomssen, a prominent German virologist and medical microbiologist.

Gram-stain-positive diphtheroid-like rods. Colonies of this fastidious slow-growing bacterium are whitish, circular, mucoid, and sticky. After 24 h incubation on Columbia agar supplemented with 5% sheep blood, the colony diameter is less than 0 ± 5 mm. Fermentative. Acid is produced from glucose, maltose, sucrose, D-fructose, D-mannose, trehalose, and 5-keto-gluconate, but not from mannitol, xylose, glycerol, erythritol, arabinose, adonitol, β-methyl-xyloside, L-sorbose, rhamnose, dulcitol, inositol, sorbitol, α-methyl-D-mannoside, α-methyl-D-glucoside, *N*-acetyl-β-glucosamine, amygdalin, arbutin, salicin, cellobiose, melibiose, inulin, melezitose, D-raffinose, glycogen, xylitol, β-gentiobiose, D-lyxose, L-arabitol, gluconate, and 2-keto-gluconate. Pyrazinamidase, alkaline phosphatase, esterase, esterase lipase, leucine arylamidase, cystine arylamidase, acid phosphatase, and *N*-acetyl-β-glucosaminidase present, but lipase, valine arylamidase, chymotrypsin, α-galactosidase, β-glucuronidase, α-glucosidase, β-glucosidase, α-mannosidase, and α-fucosidase are not. One isolate was *N*-acetyl-β-glucosamine negative (Bernard et al., 2002). Urea hydrolyzed, but nitrate not reduced. Esculin hydrolysis negative. Nonlipophilic and CAMP reaction negative. DNase activity observed after 48 h. Tyrosine not hydrolyzed.

Cell wall contains arabinose and galactose as well as *meso*-DAP. Corynemycolates (C_{32}–C_{36}) are present. Cellular fatty acids are consistent with those described for the genus. Polar lipids are composed of diphosphatidylglycerol, phosphatidylglycerol, phosphatidylinositol, and phosphatidylinositol mannosides. MK-8(H2) and MK-9(H2) found; small amounts of MK-10(H2) also found. Propionic acid not detected as a fermentation product (Bernard et al., 2002).

Source: pleural fluid from a patient with chronic renal failure, stroke, and pneumonia and from air (Bernard et al., 2002).

DNA G+C content (mol%): not available.

Type strain: CCUG 38516, CIP 105597, DSM 44276, JCM 12109.

Sequence accession no. (16S rRNA gene): AF010474.

Further comments: additional GenBank accession numbers include (complete *rpoB* gene) AY492270. By 16S rRNA gene sequencing, closely related to (~99.0% identity) *Corynebacterium sundsvallense*, but differs from that species (~90.0% identity) when sequences of the *rpoB* gene are compared (Khamis et al., 2004).

75. **Corynebacterium timonense** Merhej, Falsen, Raoult and Roux 2009, 1956[VP]

ti.mo.nen′se. N.L. neut. adj. *timonense* of or pertaining to Hôpital de la Timone, the name of a hospital in Marseille France where the type strain was isolated.

Gram-stain-positive typically club-shaped rods that occur as single cells, in pairs, or in small clusters. Rods are 0.6–2.1 × 0.4–0.6 µm after 48 h growth in TSB medium (as determined by electron microscopy). After 24 h growth on sheep blood agar at 37°C, surface colonies are circular, yellow, shiny, and 1–2 mm in diameter. Capable of aerobic and anaerobic growth. Temperature range for growth is 25–50°C (optimum, 37°C). With the API Coryne system, acid produced from D-glucose and D-ribose, but not from D-xylose, D-mannitol, maltose, D-lactose, sucrose, or glycogen. Nitrate not reduced. Esculin is weakly hydrolyzed, but urea and gelatin are not. With API Coryne and API ZYM strips, positive for pyrazinamidase, alkaline phosphatase, esterase, esterase lipase, lipase (weakly), leucine arylamidase, valine arylamidase, and acid phosphatase, but negative for pyrrolidonyl arylamidase, cystine arylamidase, trypsin, achymotrypsin, naphthol-AS-BI-phosphohydrolase, α-galactosidase, β-galactosidase, β-glucuronidase, α-glucosidase, β-glucosidase, *N*-acetyl-β-glucosaminidase, α-mannosidase, and α-fucosidase.

Cellular fatty acids are found to be consistent with those described for the genus, but tuberculostearic acid is not detected. Other chemotaxonomic properties were not extant.

Source: multiple blood cultures of a septic patient post-surgically after a pacemaker had been implanted.

DNA G+C content (mol%): not available.

Type strain: 5401744, CCUG 53856, CIP 109424, CSUR P20.

Sequence accession no. (16S rRNA gene): EF217055.

Further comments: additional GenBank accession numbers include (partial *rpoB* gene) EF217058.

76. **Corynebacterium tuberculostearicum** Feurer, Clermont, Bimet, Candréa, Jackson, Glaser, Bizet and Dauga 2004, 1059[VP] ("*Corynebacterium tuberculostearicum*" Brown, Lanéelle, Asselineau and Barksdale 1984, 251)

tu.ber.cu.lo.ste.a′ri.cum. N.L. neut. adj. *tuberculostearicum* pertaining to tuberculostearic acid which is contained in the cells.

Pleomorphic Gram-stain-positive rods which can develop coccoid forms in stationary cultures. Colonies on trypto casein soy agar supplemented with Tween 80 are circular, convex, glistening, and 1 mm in diameter. Aerobic to facultatively anaerobic. Lipophilic. Acid is produced from galactose, glucose, glycerol, fructose, mannose, ribose, and 5-ketogluconate but not from mannitol, glycogen, starch, sorbitol, lactose, inulin, or xylose. Production of acid from trehalose, maltose, gluconate, sucrose, and *N*-acetylglucosamine is variable. Urea, esculin and gelatin are not hydrolyzed. Presence of nitrate reductase is variable. DNase is absent. Esterase and naphthol-AS-BI-phosphohydrolase activities are detected, and esterase lipase, leucine arylamidase, valine arylamidase, cystine arylamidase, acid phosphatase, and alkaline phosphatase production is variable. No activity is detected for α-galactosidase, β-galactosidase, α-glucosidase, β-glucosidase, α-fucosidase, lipase (C14), trypsin, or α-chymotrypsin. The following substrates are utilized in 2 or 4 d: D-glucose, sucrose, D-ribose, glycerol, L-malate, 2-ketogluconate, succinate, fumarate, L-aspartate, L-glutamate, L-proline, and L -serine. The following substrates are not utilized in 6 d: caprate, citrate, and DL-glycerate. The type strain has all the

properties given for the species except that it also assimilates D-mannose.

Corynemycolic acids are present, and the Cellular fatty acids are consistent with those described for the genus as well as for CDC group G (Bernard et al., 1991); strains usually contain small volumes of tuberculostearic acid.

Source: a case of lepromatous leprosy in the Phillipines and a wide variety of human clinical specimens.

DNA G+C content (mol%): not available.

Type strain: Medalle X, LDC-20, ATCC 35692, CCUG 45418, CIP 107291.

Sequence accession no. (16S rRNA gene): AJ438050, X84247.

Further comments: additional GenBank accession numbers include (partial *rpoB* gene) AY581869.

ATCC 35529, listed as the type strain of *Corynebacterium tuberculostearicum* sp. nov., was deaccessioned by the ATCC and replaced with ATCC 35692 (Feurer et al., 2005).

By 16S rRNA gene sequencing, some strains previously identified as CDC group G or as "*Corynebacterium pseudogenitalium*", including CIP 106714, are closely related (have >99% identity by 16S rRNA gene sequencing) to *Corynebacterium tuberculostearicum* (K. Bernard and G. Funke, personal communication). In 1984, "*Corynebacterium tuberculostearicum*" was first described as a novel species but not validly named, with the type strain being recovered from bone marrow of a patient with lepromatous leprosy in the Philippines (Brown et al., 1984). This taxon was re-evaluated, with additional clinical strains described from inguinal node, lymph node, blood culture, urethra, skin, peritoneum, urine, and a healthy urogenital tract. Also recovered from tuna and an industrial environment.

77. **Corynebacterium tuscaniense** corrig. Riegel, Creti, Mattei, Nieri and von Hunolstein 2006b, 2025[VP] (Effective publication: Riegel, Creti, Mattei, Nieri and von Hunolstein 2006a, 311.)

tus.ca.ni.en′se. L. neut. adj. *tuscaniense* pertaining to *Tuscania*, Latin name of the Italian region, Tuscany, where the type strain was isolated.

Note: "Corynebacterium tuscaniae" epithet was corrected to *Corynebacterium tuscaniense* (Euzéby, 2006).

Gram-stain-positive rods. Colonies are whitish, circular with entire edges, nonhemolytic, grow aerobically and in the presence of a 5% CO_2-enriched atmosphere. Acid produced from glucose and maltose but not from ribose, xylose, mannitol, lactose, sucrose, or glycogen. Nitrate not reduced. Produces pyrazinamidase and alkaline phosphatase but not pyrrolidonyl arylamidase, β-glucuronidase, β-galactosidase, α-glucosidase, β-*N*-acetyl-glucosaminidase, urease, or esterase activity. Hydrolyzes hippurate but not tyrosine, gelatin, or esculin. CAMP test and reverse CAMP test negative.

Contains mycolic acids with short chain lengths (C_{26}–C_{36}), but other chemotaxonomic features such as Cellular fatty acids not extant.

Source: the blood of a patient with endocarditis.

DNA G+C content (mol%): not available.

Type strain: ATCC BAA-1141, CCUG 51321, ISS-5309, JCM 15294.

Sequence accession no. (16S rRNA gene): AY677186.

Further comments: additional GenBank accession numbers include (partial *rpoB* gene) FJ268581.

78. **Corynebacterium ulcerans** (*ex* Gilbert and Stewart 1927) Riegel, Ruimy, De Briel, Prévost, Jehl, Christen and Monteil 1995b, 619[VP] (Effective publication: Riegel, Ruimy, De Briel, Prévost, Jehl, Christen and Monteil 1995c, 275.) ("*Corynebacterium ulcerans*" Gilbert and Stewart 1927)

ul′ce.rans. L. part. adj. *ulcerans* making sore, causing to ulcerate.

Gram-stain-positive pleomorphic rods arranged in palisades or V-shaped forms. May contain metachromatic granules. Facultatively anaerobic. Colonies (1–2 mm in diameter) on 5% sheep blood agar are gray-white, exhibit a light hemolysis, and may have a dry and waxy consistency. Circular, slightly convex with an entire margin. Nitrate is not reduced to nitrite. Acid is produced from D-glucose, glycogen, maltose, and fructose but not from lactose, sucrose, D-xylose, and trehalose. Some strains produce acid from mannitol. Starch, DNA, and urea are degraded but tyrosine is not. Gelatin is hydrolyzed at 25°C and at 37°C by some strains. Esculin is not hydrolyzed. Methyl red is positive, and acetoin is not produced. Some strains grow in 6.5% NaCl. Hippurate is hydrolyzed. Alkaline phosphatase and α-glucosidase are produced, but pyrazinamidase, pyrrolidonyl arylamidase, β-glucuronidase, β-galactosidase, *N*-acetyl-β-glucosaminidase are not. As sole carbon sources, cells utilize glucose, arabinose, mannose, maltose and malate, with utilization of mannitol and gluconate occurring variably. Acetate, lactate, propionate, caprate, adipate, and phenylacetate are not ulitilzed. Fermentation of glycogen and starch plus urease is unique among species described here. The type strain is as described for the species but ferments mannitol and not gluconate.

Cell wall contains *meso*-DAP and the sugars arabinose and galactose. Short chain length mycolic acids (C_{26}–C_{36}) are present. Cellular fatty acids are consistent with those described for the genus, including characteristically (for *Corynebacterium diphtheriae*, *Corynebacterium pseudotuberculosis*, and *Corynebacterium ulcerans*), a large volume of a $C_{16:1}$ isomer (Bernard et al., 1991). Propionic acid is produced as a metabolic product (Bernard et al., 2002).

Source: animals and humans. Infection of farm animals or their milk can result in the transmission of infection to humans. Infections from companion pets have been documented to be transferred to and cause disease in humans (De Zoysa et al., 2005b; Hogg et al., 2009).

DNA G+C content (mol%): 53 (T_m).

Type strain: ATCC 51799, CCUG 2708, CIP 106504, DSM 46325, JCM 10387, NCTC 7910.

Sequence accession no. (16S rRNA gene): X84256.

Further comments: additional GenBank accession numbers include (complete *rpoB* gene) AY492271. *Corynebacterium pseudotuberculosis* and *Corynebacterium ulcerans* are the only *Corynebacterium* species to produce phospholipase D (PLD), with expression of the *pld* gene product being detected by inhibition of the CAMP reaction (Barksdale et al., 1981) or by PCR detection of the gene (Pacheco et al., 2007). Like *Corynebacterium diphtheriae* and *Corynebacterium pseudotuberculosis*, *Corynebacterium ulcerans* produces cystinase, as demonstrated by production of brown colonies and haloes when grown on modified Tinsdale medium, and fails to produce pyrazinamidase (PYRa). By 16S rRNA gene sequencing,

this species is most closely related to (>99% identity with) *Corynebacterium pseudotuberculosis* and to (>97% identity with) *Corynebacterium diphtheriae* but can be readily distinguished when *rpoB* gene sequences are compared (Khamis et al., 2004). *Corynebacterium ulcerans* is able to harbor the bacteriophage which bears diphtheria toxin gene and also express the toxin, causing diphtherial like disease. If recovered from pseudomembranous materials, patient must be treated as for diphtheria.

79. **Corynebacterium ulceribovis** Yassin 2009, 36[VP]

ul.ce.ri.bo'vis. L. n. *ulcus*, *-eris* ulcer; L. n. *bos*, *bovis* a cow a bull; N.L. gen. n. *ulceribovis* of an ulcer of a cow.

Gram-stain-positive rods. Colonies are creamy, circular, smooth, entire, and 2–4 mm in diameter after 48 h incubation at 37°C on Columbia agar supplemented with 5% sheep blood. Facultatively anaerobic. Colonies are nonhemolytic. Hippurate and Tween 80 are hydrolyzed, but esculin, gelatin, and urea are not. Activity is detected for acid phosphatase, alkaline phosphatase, esterase lipase, leucine arylamidase, pyrazinamidase, and naphthol-AS-BI-phosphohydrolase but not for arginine dihydrolase, chymotrypsin, cysteine arylamidase, esterase, α-fucosidase, α-galactosidase, β-galactosidase, β-glucuronidase, α-glucosidase, β-glucosidase, *N*-acetyl-β-glucosaminidase, lipase, α-mannosidase, pyrrolidonyl arylamidase, trypsin, or valine arylamidase. Nitrate is not reduced. Acetoin production is positive.

Corynemycolic acids are present. Cellular fatty acids are consistent with those described for the genus, but tuberculostearic acid is not detected.

Source: the skin of the udder of a cow with a profound ulceration in Schleswig Holstein, Germany.

DNA G+C content (mol%): not available.

Type strain: IMMIB L-1395, CCUG 55727, DSM 45146.

Sequence accession no. (16S rRNA gene): AM922112.

80. **Corynebacterium urealyticum** Pitcher, Soto, Soriano and Valero-Guillén 1992, 180[VP]

u.re.a.ly'ti.cum. N.L. fem. n. *urea* urea; N.L. neut. adj. *lyticum* (from Gr. neut. adj. *lutikon*) able to loosen, able to dissolve; N.L. neut. adj. *urealyticum* urea dissolving.

Gram-stain-positive rods which may become coccoidal after prolonged culture, are 0.5–1 μm arranged in palisades and V shapes, with no tendency to branch. Bacteria grow on blood agar as pinpoint colonies after 48 h of incubation at 25, 37, and 42°C. Colonies are whitish, opaque, smooth, convex, and nonhemolytic. Respiratory metabolism, i.e. does not grow on blood agar incubated anaerobically for 48 h or on MacConkey agar. Nonreactive with usual sugars, i.e. acid is not produced from glucose, sucrose, maltose, mannitol, xylose, ribose, L-arabinose, sorbitol, lactose, trehalose, inulin, raffinose, starch, and glycogen. Does not hydrolyze gelatin or esculin. Nitrate is not reduced. Does not degrade DNA. Possesses strong urease activity. Hydrolyzes Tween 80. Lipophilic, i.e. growth is stimulated by Tween 80. Some strains hydrolyze hippurate or give a positive Voges–Proskauer reaction.

Cell wall contains *meso*-DAP and the sugars arabinose and galactose. Cellular fatty acids are consistent with those described for the genus and 10-methyloctadecanoic (tuberculostearic) acid is detected. Mycolic acids (C_{26}–C_{36}) are present. Menaquinone MK-9(H_2) is found. Contains diphosphatidylglycerol, phosphatidylglycerol, phosphatidylethanolamine, phosphatidylinositol, and phosphatidylinositol mannosides. Propionic acid is not produced by anaerobic metabolism of glucose.

Source: urine, particularly in humans with lithiasis and alkaline-encrusted cystitis as complications, but also recovered from other normally sterile sites and human skin. Occasionally recovered from animals with urosepsis (Bailiff et al., 2005).

DNA G+C content (mol%): 61–62 (T_m; Pitcher et al., 1992), 65–66 (HPLC, Riegel et al., 1992), and 64.2 (complete genome sequence, Tauch et al., 2008b).

Type strain: ATCC 43042, CCUG 18158, CIP 103524, DSM 7109, JCM 10395, LMG 19041, NCTC 12011.

Sequence accession no. (16S rRNA gene): X81913, X84439.

Further comments: additional GenBank accession numbers include (complete *rpoB* gene) AY492275. *Corynebacterium urealyticum* Pitcher et al. 1992 was previously known as CDC coryneform group D-2 (Riegel et al., 1992). The complete genome of *Corynebacterium urealyticum* strain DSM 7109T, with 2,369,219 bp has been deposited in GenBank under accession no. NC_010545 (Tauch et al., 2008b). Clinical features with respect to data inferred from complete genomic analyses have been reviewed (Soriano and Tauch, 2008). Strains can be multidrug resistant (Fernandez-Roblas et al., 2009; Philippon and Bimet, 1990) and nosocomially acquired (Famularo et al., 2008).

81. **Corynebacterium ureicelerivorans** Yassin 2007, 1202[VP]

u.re.i.ce.le.ri.vo'rans. N.L. fem. n. *urea* urea; L. adj. *celer*, *-eris* fast; L. part. adj. *vorans* devouring; N.L. part. adj. *ureicelerivorans* fast urea-devouring, referring to the rapid utilization of urea.

Gram-stain-positive rods. Colonies are creamy, circular, dry, and approximately 0.1–0.3 mm in diameter on Columbia blood agar after 48 h incubation at 37°C. Colonies are nonhemolytic. Facultatively anaerobic. Lipophilic. Urea rapidly positive (reaction in approximately 60 sec). Hippurate hydrolyzed, but esculin and gelatin are not. Nitrate not reduced. Acid produced from glucose. Weak acid production is observed from ribose and D-xylose in the API Coryne and API 20 Strep systems after 3 d. Acid is not produced from L-arabinose, glycerol, glycogen, inulin, lactose, maltose, mannitol, sorbitol, sucrose, trehalose, or D-raffinose. Alkaline and acid phosphatases, ester lipase, naphthol- AS-BI-phosphohydrolase, leucine arylamidase, pyrrolidonyl arylamidase, and pyrazinamidase are detected, but arginine dihydrolase, esterase, α-glucosidase, β-glucosidase, α-galactosidase, β-galactosidase, β-glucuronidase, *N*-acetyl-β-glucosaminidase, α-mannosidase, α-fucosidase, chymotrypsin, trypsin, valine arylamidase, cystine arylamidase, and leucine arylamidase, are not. Acetoin produced.

Mycolic acids are present. Cellular fatty acids are consistent with those described for the genus, and tuberculostearic acid is present.

Source: blood culture of patient with septicemia (Yassin, 2007), but also recovered from other normally sterile body fluids (Fernandez-Natal et al., 2009).

DNA G+C content (mol%): not available.

Type strain: IMMID RIV-2301, CCUG 53377, DSM 45051, JCM 15295.

Sequence accession no. (16S rRNA gene): AM397636.

82. **Corynebacterium variabile** corrig. (Müller 1961) Collins 1987a, 287 [VP] (*Arthrobacter variabilis* Müller 1961, 524)
Note: original spelling of the specific epithet, *Corynebacterium variabilis* (*sic*), was later corrected to *Corynebacterium variabile*.

va.ri.a′bi.le. L. neut. adj. *variabile* changeable, variable.

Gram-stain-positive rod-shaped cells (0.8–1.1 × 1.4–3.5 μm), irregularly shaped (club-shaped or tapered), and occurring singly, in pairs with typical V forms, or clumps; ovoid forms occur in older cultures. Colonies are small (~2–4 mm), circular (sometimes irregular), convex, and gray-white (occasionally slight pink) with a dry appearance. Optimum temperature is 25–30°C. Grows in 7% NaCl. Strictly aerobic. Acetate, propionate, capronate, 4-aminobutyrate, caprylate, succinate, DL-malate, levulinate, and some other compounds may be used as sole carbon sources. Xanthine, tyrosine, and starch are not hydrolyzed.

Cell wall contains *meso*-DAP, with the glycan moiety containing only acetyl residues. The cell wall also contains an arabinogalactan polymer. Short-chain mycolic acids (C_{30}–C_{36} carbon atoms) are present. Cellular fatty acids are consistent with those described for the genus, including high levels of tuberculostearic acid. The major menaquinones are MK-9(H_2) and MK-8(H_2).

Source: the environment and the surface of soft cheese (Gelsomino et al., 2005).

DNA G+C content (mol%): 65 (T_m).

Type strain: ATCC 15753, CCUG 45246, CIP 102112, DSM 20132, HAMBI 1872, JCM 2154, NBRC 15286, NCIMB 9455, NRRL B-4201, VKM Ac-1122.

Sequence accession no. (16S rRNA gene): AJ222815.

Further comments: additional GenBank accession numbers include (complete *rpoB* gene) AY492272. *Arthrobacter variabilis* Müller 1961[AL] *and Caseobacter polymorphus* Crombach 1978[AL] are both considered as later synonyms of *Corynebacterium variabile* (Collins 1987a, 1989). *Corynebacterium moorparkense* (Brennan et al., 2001) is also considered as a later heterotypic synonym of *Corynebacterium variabile* corrig. (Müller 1961) Collins 1987a, 287 (Gelsomino, Vancanneyt, Snauwaert, Vandemeulebroecke, Hoste, Cogan and Swings 2005, 1129).

83. **Corynebacterium vitaeruminis** corrig. (Bechdel et al. 1928) Lanéelle, Asselineau, Welby, Norgard, Imaeda, Pollice and Barksdale 1980, 544[VP] ["*Flavobacterium vitarumen*" Bechdel, Honeywell, Dutcher and Knutsen 1928, 234; *Brevibacterium vitarumen* (Bechdel et al. 1928) Breed, Murray and Smith 1957, 495]

vi.ta.e.ru.mi′nis. L. n. *vitae* life; L. n. *rumen -inis* throat, gullet, rumen; N.L. gen. n. *vitaeruminis* of rumen life.

Note: spelling of the epithet, *vitarumen* (*sic*), was corrected by Trüper and De'Clari 1997, 908.

(Some information from this description is based on that of Collins and Cummins, 1986.)

Pleomorphic Gram-stain-positive rod-shaped bacteria wth moderately tapered ends. May contain metachromatic granules when grown on phosphate rich media. Lemon yellow colonies on Loeffler slants with raised, smooth, butyrous yellow colonies on chocolate agar, and black colonies with chocolate agar containing 0.03% tellurite. Facultatively anaerobic. Positive for glucose, fructose, galactose, mannose, maltose, sucrose, salicin, and trehalose. Negative for glycerol, inositol, mannitol, lactose, and starch. Esculin hydrolyzed, nitrate is reduced to nitrite, acetoin and urease are produced, and methyl red is positive. Pyrazinamidase positive, but phosphatase negative. Hippurate and gelatin are not hydrolyzed.

Cell wall contains *meso*-DAP and the sugars arabionse and galactose. Short-chain corynemycolates and menaquinone MK-8(H_2) are present.

Source: the rumen of a cow.

DNA G+C content (mol%): 64.8 (T_m).

Type strain: ATCC 10234, CCUG 28792, CIP 82.7, DSM 20294, JCM 1323, NBRC 12143, NCIMB 9291, VKM B-1211.

Sequence accession no. (16S rRNA gene): AY438066.

Further comments: additional GenBank accession numbers include (complete *rpoB* gene) AY492273.

84. **Corynebacterium xerosis** (Lehmann and Neumann 1896) Lehmann and Neumann 1899, 385[AL] (*Bacillus xerosis* Lehmann and Neumann 1896, 361)

xe.ro′sis. N.L. gen. n. *xerosis* (from Gr. adj. *xeros* dry) of xerosis.

Note: description below is based on ATCC 373[T], as some commercially available reference strains were shown to be misidentified after contemporary characterization methods were applied, with some being identifiable as *Corynebacterium striatum* (Coyle et al., 1993b) or *Corynebacterium amycolatum* (Funke et al., 1996a; Wauters et al., 1996).

Gram-stain-positive rods with irregular staining, barred rods with occasional granules and club forms. Colonies are small, circular ~0.2–1.0 mm in diameter after growth on blood agar at 24 h. Colonies dryish and slightly yellow colored (Coyle et al., 1993b). In broth, forms a large pellicle over a clear broth, with settled clumps observed. Nonhemolytic. Not lipophilic. Facultatively anaerobic. Grows anaerobically. CAMP reaction negative. Nitrate either reduced or considered as variable (Funke and Bernard, 2007). Glucose, maltose, and sucrose fermented, but xylose and mannitol are not (Coyle et al., 1993b; Funke and Bernard, 2007). Also described as usually being negative for arabinose, rhamnose, lactose, trehalose, raffinose, dextrin, and starch, but positive for salicin (Collins and Cummins, 1986). ATCC 373[T] has been described as containing cell-wall sugars of arabinose, galactose, and glucose with smaller amounts of rhamnose. Corynemycolates are present. Cellular fatty acids consistent with those described for the genus are present, but tuberculostearic acid is not detected (Bernard et al., 1991). Propionic acid is not detected as a fermentation product (Funke et al., 1996a).

Source: the ear discharge of a child.

DNA G+C content (mol%): 67.3 (method unknown).

Type strain: ATCC 373, CCUG 27544, CIP 100653, DSM 20743, JCM 1971, NBRC 16721, NCTC 11861.

Sequence accession no. (16S rRNA gene): X81914, X84446.

Further comments: additional GenBank accession numbers include (complete *rpoB* gene) AY492233. Once described as a relatively common human pathogen, *Corynebacterium xerosis* infections are now considered to occur only very rarely, and diseases attributed to this agent should be considered as definitive only if polyphasic, including genetic identification methods, are used. In particular, older publications implicating *Corynebacterium xerosis* as a pathogen, especially those where microbiological descriptions were based solely on phenotypic testing, may be actually describing disease caused by *Corynebacterium amycolatum* Funke et al. 1996a or other *Corynebacterium* species. Reference strains other than ATCC 373^T designated *Corynebacterium xerosis* must be carefully evaluated prior to use in research. ATCC 7711, with a DNA G+C content of 68.5 mol% (Collins and Cummins, 1986), has been found to be closely related to ATCC 373^T by 16SrRNA and partial *rpoB* gene sequence analyses, but NCTC 7243 is closely related to (>99% identity with) *Corynebacterium amycolatum* (K. Bernard, personal communication). *Corynebacterium xerosis* cannot be easily discerned from *Corynebacterium freneyi* and *Corynebacterium hansenii* by 16S rRNA, *rpoB*, or 16S–23S spacer region gene sequence analyses; these are best separated by DNA–DNA hybridization or phenotypic means (Renaud et al., 2007).

Genus II. **Turicella** Funke, Stubbs, Altwegg, Carlotti and Collins 1994, 272^{VP}

Hans-Jürgen Busse

Tu.ri.cel′la. *Turicum* proper name Turicum; L. fem. dim. suff. *-ella*; N.L. fem. dim. n. *Turicella* pertaining to Turicum, the Latin name of Zurich, Switzerland, where the first isolates were collected.

Cells are Gram-stain positive, nonmotile, nonsporeforming diphtheroids that occur as single cells or are arranged in V-shaped forms or palisades. Colonies are circular, convex, and creamy in color and range from 1.0–2.0 mm in diameter after 48 h of incubation at 37°C on sheep blood agar. **Catalase positive and oxidase negative**. **Metabolism is respiratory**. **Cell wall contains *meso*-diaminopimelic acid, arabinose, and galactose**. **Mycolic acids are not produced**. **The principal menaquinones** are **MK-10 and MK-11**. Major fatty acids are **$C_{16:0}$, $C_{18:0}$, and $C_{18:1}$; tuberculostearic acid is produced**.

DNA G+C content (mol%): 65–72.

Type species: **Turicella otitidis** Funke, Stubbs, Altwegg, Carlotti and Collins 1994, 272^{VP}.

Further descriptive information

In (1993) Simonet et al. reported on the isolation and characterization of facultatively anaerobic, Gram-stain-positive bacteria from middle ear fluid of children suffering from acute otitis media. In routine diagnosis, 16 isolates shared numerous traits with *Corynebacterium afermentans* subsp. *afermentans* (CDC *Corynebacterium* group ANF-1; Riegel et al., 1993b). Like *Corynebacterium afermentans* subsp. *afermentans*, these isolates grew well under aerobic and microaerobic conditions and weakly under anaerobic conditions, but showed clear differences in colony morphology and, unlike *Corynebacterium afermentans*, lacked mycolic acid. Despite the presence of *meso*-diaminopimelic acid and arabinogalactan in the cell wall, the authors concluded that the 16 isolates could not be members of the genus *Corynebacterium* and tentatively designated them ANF-1 like. In the following year, Funke et al. (1994) reported on the classification of three Gram-stain-positive, coryneform rods which had been isolated from middle ear fluids from 1–5-year-old patients treated for otitis media in Zürich, Switzerland. The three strains shared 100% identity in a 500-base fragment of the 16S rRNA gene and the selected reference strain exhibited in almost complete 16S rRNA gene highest sequence similarity with species of the genus *Corynebacterium* (90.7–91.6%). The strain was shown to contain a cell wall with *meso*-diaminopimelic acid, arabinose, and galactose, but, unlike the majority of *Corynebacterium* species, it lacked mycolic acids and exhibited a completely unsaturated quinone system with the major compounds MK-10 and MK-11. Funke et al. (1994), mainly on the basis of phylogenetic distinctness, lack of mycolic acids, and the quinone system, concluded that these strains were representatives of a novel genus and novel species for which they proposed the genus *Turicella* with the single species *Turicella otitidis*. Since then, no new species has been added to the genus.

Phylogenetically, *Turicella otidis* has been reported to occupy a separate line of descent within the 16S rRNA *Corynebacterium* gene tree (Funke et al., 1994; Pascual et al., 1995; Ruimy et al., 1995); *rpoB* sequence data are in line with these findings (Khamis et al., 2004). However, 16S rRNA gene sequence similarities below 92.0% (by applying FASTA; Pearson and Lipman, 1988) and not higher than 93.1% with recognized *Corynebacterium* species by applying EzTaxon (Chun et al., 2007) support separation from *Corynebacterium*. Interestingly, in gene banks the partial 16S rRNA gene sequence (888 nucleotides; accession number Z33617) of a single strain (CDC group ANF-1-like ATCC 51308) shows a high similarity (98.1%) with the type of *Turicella otitidis*, suggesting it originated from a *Turicella* species.

A culture-independent approach showed that *Turicella otitidis*-like organisms were among the most abundant colonizers of the human ear canal of healthy individuals (Frank et al., 2003; erroneously these authors designated the reference species *Corynebacterium otitidis*). These cloned 16S rRNA gene sequences exhibited 97.1–99.6% with *Turicella otitidis* indicating that they originated from *Turicella otitidis*-like organisms. In another study, the human microbial skin community was analyzed on the basis of cloned 16S rRNA gene sequences (Grice et al., 2009). These authors detected *Turicella otitidis*-like sequences (>98% similarity) predominantly in samples from the external auditory canal but also from the manubrium, elbow, and retroauricular crease. Other *Turicella otitidis*-like sequences deposited in gene banks were recovered from skin samples of the antecubital and popliteal fossa, volar forearm, and also from floor and mattress dusts. Another *Turicella otitidis*

strain with a similar 16S rRNA gene sequence (>99% similarity in 551 nucleotides) was identified in a clone library from a deep-sea sediment core (Stach et al., 2003).

Lack of mycolic acids distinguishes *Turicella otitidis* from most members of the order *Corynebacteriales*, and only few species of the genus *Corynebacterium* have been reported to lack mycolic acids. These include *Corynebacterium amycolatum* (Collins et al., 1988a), *Corynebacterium atypicum* (Hall et al., 2003), *Corynebacterium kroppenstedtii* (Collins et al., 1998), and *Corynebacterium ciconiae* (Fernández-Garayzábal et al., 2004). Neither 16S rRNA gene sequences nor *rpoB* sequences suggest a closer relationship between *Turicella otitidis* and the mycolateless *Corynebacterium* species than with mycolic acid containing species. Quinone systems with completely unsaturated menaquinones are rare and the presence of menaquinones MK-10 and MK-11 is so far unique within the family *Corynebacteriaceae*. The lipoglycan of *Turicella otitidis* is a truncated lipoarabinomannan consisting of a mannosyl phosphatidylinositol anchor unit which carries an (1→6)-linked mannan core and substituted terminal-arabinosyl-branches (Gilleron et al., 2005).

However, other taxa classified in the order *Corynebacteriales* lack mycolic acids and have a quinone system with completely unsaturated menaquinones, such as *Hoyosella altamirensis* (MK-8; Jurado et al., 2009) and *Amycolicicoccus subflavus* (MK-8; Wang et al., 2010). In addition, *Tsukamurella* species contain completely unsaturated menaquinones with nine isoprenoic units in the side chain (MK-9) though they do contain mycolic acids (Collins et al., 1988c; Yassin et al., 1996, 1997).

For treatment of *Turicella otitidis* infections, linezolid, teicoplanin, and vancomycin are usually effective and often *Turicella otitidis* strains also tend to show low MICs to β-lactams (ampicillin, imipenem, penicillin G), fluoroquinolones (ciprofloxacin, levofloxacin), macrolides (azithromycin, erythromycin, miocamycin), chloramphenicol, clindamycin, gentamicin, rifampin, and tetracycline (Funke et al., 1996b; Gómez-Garcés et al., 2007). Troxler et al. (2001) showed that *Turicella otitidis* strains are sensitive to numerous antibiotics though a significant proportion of *Turicella otitidis* strains show high MICs against macrolides and clindamycin (Funke et al., 1996b). Cross-resistance between erythromycin and clindamycin is frequently observed in *Turicella otitidis* (Funke et al., 1996b). It seems likely that resistance to azithromycin, clarithromycin, erythromycin, and spiramycin is conferred by mutations in the 23S rRNA gene at position 2058 and/or 2059 (*Escherichia coli* numbering), whereas these mutations do not influence the susceptibility to pristinamycin (Boumghar-Bourtchai et al., 2009).

Pathogenicity. *Turicella otitidis* is frequently isolated from children suffering from different variants of otitis media. Its role in this disease is controversial as it is also isolated from the external auditory canal of healthy children suggesting that it is part of the normal flora, though it seems that the frequency of *Turicella otitidis* is higher in patients with otitis media with effusions than in controls (Funke et al., 1994; Gómez-Garcés et al., 2004; Holzmann et al., 2002; Pierot et al., 2005). However, *Turicella otitidis* has also been found to be associated with otitis externa, mastoiditis, febrile bacteremia, and to be the causative agent of an auricolar and a cervical abscess (Dana et al., 2001; Fernández Pérez et al., 1999; Jeziorski et al., 2009; Loïez et al., 2002; Poulter and Hinnebusch, 2005; Reynolds et al., 2001). Hence, *Turicella otitidis* must be considered at least to be an opportunistic pathogen.

Enrichment and isolation procedures

Susceptibility testing of *Turicella otitidis* (Troxler et al., 2001) has shown that this species exhibits natural resistance to aztreonam, cotrimoxazole, fosfomycin, nitrofurantoin, pipemidic acid, and sulfamethoxazole, hence, these antibiotics might be useful for selective enrichment. *Turicella otitidis* can be cultivated on Columbia agar base supplemented with 5% sheep blood in a 5% CO_2 atmosphere (Funke et al., 1994), trypticase soy agar with 5% sheep blood, chocolate agar supplemented with pyridoxal in a 5% CO_2 atmosphere (Poulter and Hinnebusch, 2005), cooked-meat broth (Reynolds et al., 2001), and brain-heart infusion broth supplemented with 2% yeast extract (Gilleron et al., 2005).

Differentiation of the genus *Turicella* from other genera

Identification of novel *Turicella* strains other than *Turicella otitidis* is hampered by the fact, that so far only one species has been described; hence it is not clear which phenotypic traits are shared by other representatives of the genus. Species of the neighboring genus *Corynebacterium* show some variability in phenotypic traits usually considered to be stable within a genus, including the quinone system and the presence of mycolic acids. Hence, it is possible that undescribed species of the genus *Turicella* may not lack mycolic acids or may exhibit a quinone system different from that found in *Turicella otitidis*. Novel *Turicella* strains can be reliably differentitated from *Corynebacterium* by high similarities in the sequences of the 16S rRNA gene and *rpoB* since *Turicella otitidis* shares <94% and <84% similarity, respectively with species of the genus *Corynebacterium* and even less with species of other genera of the *Corynebacteriales*. Detection of a quinone system with completely unsaturated menaquinones with ten and eleven isoprenoic units in the side chain (MK-10, MK-11) and lack of mycolic acids distinguishes *Turicella otitidis* from *Corynebacterium* species. Furthermore, the non-fermentative metabolism is another characteristic distinguishing *Turicella otitidis* from the majority of *Corynebacterium* species. Traits useful for the differentiation of *Turicella otitidis* from *Corynebacterium auris* (CDC coryneform group ANF-1-like bacteria) and *Corynebacterium afermentans* (CDC coryneform ANF-1 bacteria) are listed in Table 33.

TABLE 33. Differentiation of *Turicella otitidis* from two other absolute non-fermenter species, *Corynebacterium afermentans* and *Corynebacterium aurum*[a,b]

	T. otitidis	*C. afermentans*	*C. aurum*
Mycolic acids	−	+	+
Leucine arylamidase	+	−	+
Esterase lipase C8	+	−	+
Lipase C14	−	+	+
DNase	+	−	+
Assimilation of:			
D-Alanine	+	−	−
Caprylate	−	−	+
L-Glutamate	+	−	+
3-Hydroxybutyrate	−	−	+
2-Keto-D-gluconate	+	−	−
α-Ketoglutarate	+	−	−
L-Proline	+	−	−
L-Serine	+	−	+
Succinate	+	−	−

[a] Symbols: +, >85% positive; −, 0–15% positive.

[b] Data from Riegel et al. (1993b), Funke et al. (1994), and Renaud et al. (1996).

Taxonomic comments

Many published phylogenetic trees indicate that the monospecific genus *Turicella* forms a deeply branching lineage within and not separate from the clade comprising the species of the genus *Corynebacterium* (Brennan et al., 2001; Funke et al., 1997e, 1998c; Jurado et al., 2009; Ludwig et al., 2009a; Renaud et al., 2001; Riegel et al., 1995e, 1997b, 2004; Ruimy et al., 1995; Wattiau et al., 2000). Hence, the phylogenetic placement of *Turicella otitidis* among *Corynebacterium* species creates a non-monophyletic genus *Corynebacterium* which in the taxonomy of today is undesirable. However, recognition of the genus *Turicella* is supported by a quinone system (MK-10, MK-11) which is not found in *Corynebacterium* species and by the lack of mycolic acids which distinguishes the species from the vast majority of *Corynebacterium* species.

List of species of the genus *Turicella*

1. **Turicella otitidis** Funke, Stubbs, Altwegg, Carlotti and Collins 1994, 272[VP]

o.ti′ti.dis. Gr. n. *ous*, *otos* ear; N.L. suff. *-itis*, *-idis* suffix used in names of inflammations; N.L. gen. n. *otitidis* of inflammation of the ear.

Cells are Gram-stain positive, nonmotile, nonsporeforming diphtheroids which occur as single cells or are arranged in V-shaped forms or palisades. Colonies are circular, convex, and creamy in color. Colony diameters range from 1.0–2.0 mm after 48 h of incubation at 37°C on sheep blood agar. Growth is visible in the presence of 6.5% NaC1. Catalase positive and oxidase negative. Metabolism is respiratory. Acid is not produced from D-glucose, glycogen, lactose, maltose, D-mannitol, ribose, sucrose, and D-xylose or on triple-sugar iron agar. Nitrate is not reduced. Does not produce indole or hydrolyze urea or esculin. Growth is visible in the presence of 6.5% NaCl. Acid phosphatase, alkaline phosphatase, and leucine arylamidase are produced. The cell wall contains *meso*-diaminopimelic acid, arabinose, and galactose. Mycolic acids are not produced. The major menaquinones are MK-10 and MK-11. Straight-chain saturated fatty acids are mainly palmitic and stearic acids; oleic acid is the predominant unsaturated fatty acid. Tuberculostearic acid is produced.

Source: middle-ear effusions of patients with otitis media.

DNA G+C content (mol%): 65–72 (T_m).

Type strain: 234/92, ATCC 51513, CCUG 32254, CIP 104075, DSM 8821, JCM12146, LMG 19071.

Sequence accession no. (16S rRNA gene): X73976.

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Family II. **Dietziaceae** Rainey, Ward-Rainey and Stackebrandt 1997, 486[VP] emend. Zhi, Li and Stackebrandt 2009, 595[VP]

Fred A. Rainey

Diet.zi.a.ce′a.e. N.L. fem. n. *Dietzia* type genus of the family; suff. *-aceae* ending to denote a family. N.L. fem. pl. n. *Dietziaceae* the *Dietzia* family.

Aerobic, **Gram-stain-positive**, cocci/short rods, **chemo-organotrophs**. **Peptidoglycan of the A1γ type** containing ***meso*-diaminopimelic acid**. Short-chain **mycolic acids**. **MK-8(H_2)** is the predominant menaquinone. Member of the order *Corynebacteriales*, of the class *Actinobacteria* based on 16S rRNA gene based phylogeny. The pattern of 16S rRNA signatures defining the family consists of nucleotides at positions 241:285 (U–G), 250 (U), 316:337 (C–G), 418:425 (U–A), 599:639 (C–G), 662:743 (C–G), 987:1218 (A–U), 1000:1040 (A–U), 1059:1198 (U–A), and 1115:1185 (C–G). The family contains the type genus *Dietzia* Rainey et al. (1995b).

DNA G+C content (mol%): 65.5–73.

Type genus: **Dietzia** Rainey, Klatte, Kroppenstedt and Stackebrandt 1995b, 33[VP].

Genus I. **Dietzia** Rainey, Klatte, Kroppenstedt and Stackebrandt 1995b, 33[VP] emend. Kämpfer, Langer, Martin, Jäckel and Busse 2010, 394[VP]

Fred A. Rainey

Diet′zi.a. N.L. fem. n. *Dietzia* honoring Alma Dietz, an American microbiologist.

Gram-stain-positive, non-spore-forming cocci that germinate into short rods. Rod-shaped cells exhibit a morphogenetic cycle. **Aerobic** and **chemo-organotrophic**. **Mesophilic**. **Catalase-positive**. The diagnostic amino acid of the peptidoglycan is ***meso*-diaminopimelic acid**; the glycan moiety of cell wall contains *N*-acetyl residues (*N*-acetylmuramic acid). The major cell-wall sugars are arabinose and galactose. **Short-chain mycolic acids** with 33–39 carbon atoms are present. The long-chain cellular fatty acids are predominantly straight-chain saturated and monounsaturated fatty acids. **Tuberculostearic acid** is present. The polar lipids can include phosphatidylethanolamine, phosphatidylglycerol, diphosphatidylglycerol, phosphatidylinositol, and phosphatidylinnositol mannosides. **All species studied contain phosphatidylglycerol**. **Menaquinone MK-8 (H_2)** is the major menaquinone. Species of the genus have been isolated from air, soil, lake sediments, deep-sea mud, deep-sea sediments, plant tissues, skin, and intestines of carp, fish-egg processing plant water, human perianal swab, human bone biopsy, and human skin.

Based on 16S rRNA gene sequence phylogeny the species of the genus form a distinct family level lineage within the order *Corynebacteriales* of the class *Actinobacteria*.

DNA G+C content (mol%): 65.5–73.

Type species: **Dietzia maris** (Nesterenko, Nogina, Kasumova, Kvasnikow and Batrako 1982) Rainey, Klatte, Kroppenstedt and Stackebrandt 1995b, 33[VP].

Further descriptive information

Phylogeny. 16S rRNA gene sequence phylogenetic analyses of the species of the genus *Rhodococcus* have led to the formation of the genus *Dietzia* as a home for the misclassified *Rhodococcus* species, *Rhodococcus maris* (Rainey et al., 1995a, 1995b). In the 16S rRNA gene sequence analysis of the sequences of the type strains of type species of all actinomycete genera, the species *Dietzia maris* was found to represent a distinct lineage within the radiation of mycolic acid-containing bacteria at the family level, and the family *Dietziaceae* was described (Stackebrandt et al., 1997). This single-genus family was described solely on the basis of phylogenetic position and the following set of defined 16S rRNA gene nucleotide signatures (Stackebrandt et al., 1997): 70–98 (U–A), 293:304 (G–U), 307 (U), 418:425 (U–A), 508 (U), 614:626 (U–G), 631 (G), 661:744 (A–U), 771:808 (A–U), 824:876 (C–G), 825:875 (G–C), 843 (C), 1049:1198 (U–A), and 1122:1151 (A–U). Li et al. (2009) emended the description of the family *Dietziaceae* by providing a new set of nucleotide signatures as follows: 241:285 (U–G), 250 (U), 316:337 (C–G), 418:425 (U–A), 599:639 (C–G), 662:743 (C–G), 987:1218 (A–U), 1000:1040 (A–U), 1059:1198 (U–A) and 1115:1185 (C–G). Notably, with the addition of new species to the genus *Dietzia* as well as the addition of new genera to and species to the order *Corynebacteriales*, all but one of the original nucleotide signatures in the set defining the family no longer hold true. With the exception of the signature 418:425 (U–A), all of the nucleotide signatures included in the emended family are new (Li et al., 2009).

16S rRNA gene sequence-based phylogenetic analyses show the species of the genus *Dietzia* to form a monophyletic branch within the radiation of the genera of the order *Corynebacteriales* (Figure 84). The type strains of the 11 species of the genus *Dietzia* share 16S rRNA gene sequence pairwise similarities in the range 95.5–99.4% which in turn is reflected in the pattern of clustering displayed in Figure 85. Eight of the 11 species fall into three clusters that are supported by bootstrap values of at least 99%. Cluster *A* contains the species *Dietzia cercidiphylli*, *Dietzia natronolimnaea*, and *Dietzia psychralcaliphila* which share 99.1–99.3% pairwise sequence similarity (Figure 85). Another three species, *Dietzia kunjamensis*, *Dietzia maris*, and *Dietzia schimae*, comprise a cluster designated *B* (Figure 85) and share 99.8–99.1% pairwise sequence similarity. Cluster *C* is a two species cluster with *Dietzia cinnamea* and *Dietzia papillomatosis* (Figure 85) sharing 98.5% pairwise sequence similarity. The species *Dietzia aerolata* falls between the species of clusters *A* and *B* and its position is supported by an 80% bootstrap value (Figure 85). The position of *Dietzia lutea* between the *A*/*B* cluster and cluster *C* is supported by an 88% bootstrap value. The deepest branching species of the *Dietzia* lineage is *Dietzia timorensis* which shares 95.5–98.5% pairwise sequence similarity with other *Dietzia* species (Figure 85). It should be noted that in the case of a number of highly similar 16S rRNA gene sequences, extended branching is observed (Figure 84 and Figure 85). These extended branches are due to sequence quality with very small differences between the sequences

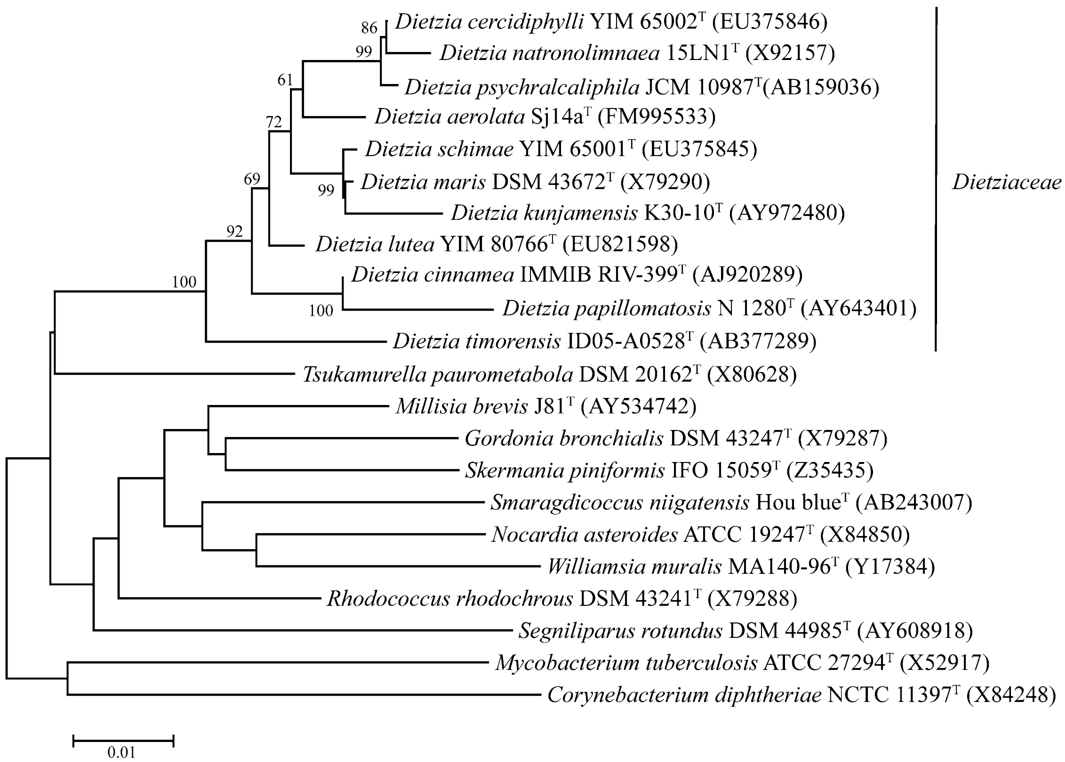

FIGURE 84. 16S rRNA gene sequence based phylogeny showing the position of the species of the genus *Dietzia* within the radiation of the type species of the genera of the order *Corynebacteriales*. The scale bar infers one nucleotide substitution per 100 nucleotides. The phylogeny was reconstructed from distance matrices using the neighbor-joining method.

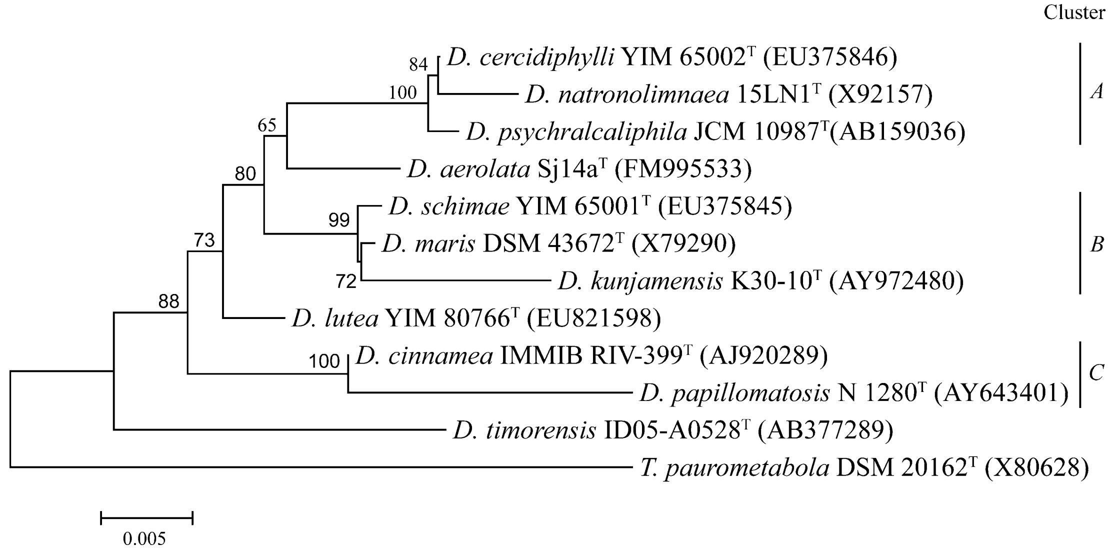

FIGURE 85. 16S rRNA gene sequence based phylogeny showing the relationships of the species of the genus *Dietzia*. The scale bar infers 0.5 nucleotide substitution per 100 nucleotides. The phylogeny was reconstructed from distance matrices using the neighbor-joining method. The outgroup species used in the analyses was *Tsukamurella paurometabola*.

being compared. It is recommended that new 16S rRNA gene sequences be obtained for the type strains of species described before high quality sequencing became routine.

The high 16S rRNA gene sequence similarity values obtained between a number of species have required DNA–DNA hybridization studies to determine the species status of a number of new isolates. The majority of the DNA–DNA hybridization values reported in the literature are in the 40–50% range. There is little correlation between the higher 16S rRNA gene sequence similarities and the respective DNA–DNA hybridization values.

For the genus *Dietzia* there is only one report of a DNA–DNA hybridization study between two strains of the same species (i.e. strains 14LN1 and 15LN1^T of the species *Dietzia natronolimnaea*) which had a value of 83% (Duckworth et al., 1998).

Chemotaxonomic properties. The cell-wall structure has been determined for the type strains of all described species of the genus *Dietzia*, with the exception of *Dietzia aerolata* (Kämpfer et al., 2010). The cell-wall chemotype of the species of the genus *Dietzia* is type IV (Lechevalier and Lechevalier, 1970). The diagnostic amino acid of the peptidoglycan is *meso*-diaminopimelic acid and the peptidoglycan is of the A1γ type (Schleifer and Kandler, 1972). The cell-wall sugars are arabinose and galactose. The major isoprenologue detected in species of the genus *Dietzia* is the dihydrogenated menaquinone with eight isoprene units, i.e. MK-8(H_2). The presence of the isoprenologue MK-7(H_2) as a minor menaquinone component has been reported for the species *Dietzia cinnamea* (Yassin et al., 2006) and *Dietzia papillomatosis* (Jones et al., 2008). In the case of *Dietzia aerolata*, Kampfer et al. (2010) reported the detection of MK-8(H_2), MK-7(H_2) (14%), MK-9(H_2) (2%), MK-7 (<1%), and MK-8 (<1%).

The whole-cell fatty acids of the species of the genus *Dietzia* consist of predominantly straight-chain saturated and unsaturated fatty acids. The following fatty acids have been detected in all described species: $C_{14:0}$; $C_{16:1}$ ω6*c* and $C_{16:1}$ ω7*c*; $C_{16:0}$; 10-methyl $C_{17:0}$, and 10-methyl $C_{18:0}$. The small differences that exist between fatty acid profiles to date have not been used for the differentiation of *Dietzia*. This is partly due to the fact that the majority of species of the genus are represented by a single strain, the type strain, and so nothing is known about intraspecies fatty acid profile variation. For the species of the genus *Dietzia*, various combinations of the polar lipids (phosphatidylethanolamine, phosphatidylglycerol, diphosphatidylglycerol, and phosphatidylinositol) and phosphatidylinositol mannosides are found. Phosphatidylglycerol is the only polar lipid present in all strains analyzed. Five different polar lipid profiles have been detected for the ten species of the genus for which polar lipid profiles have been determined. No polar lipid profile has been determined for the species *Dietzia psychralcaliphila* (Yumoto et al., 2002). A profile consisting of phosphatidylethanolamine, phosphatidylglycerol, and diphosphatidylglycerol is found for *Dietzia maris* (Rainey et al., 1995b), *Dietzia cinnamea* (Yassin et al., 2006), *Dietzia papillomatosis* (Jones et al., 2008), and *Dietzia natronolimnaea* (Duckworth et al., 1998). The species *Dietzia aerolata* (Kämpfer et al., 2010) and *Dietzia cercidiphylli* (Li et al., 2008) have the polar lipids (phosphatidylglycerol, diphosphatidylglycerol, and phosphatidylinositol) and phosphatidylinositol mannosides. A polar lipid profile containing phosphatidylglycerol, diphosphatidylglycerol, and phosphatidylinositol is found for *Dietzia kunjamensis* (Mayilraj et al., 2006) and *Dietzia schimae* (Li et al., 2008). A combination of all of the polar lipids (phosphatidylethanolamine, phosphatidylglycerol, diphosphatidylglycerol, and phosphatidylinositol) and phosphatidylinositol mannosides known for the genus is found in *Dietzia lutea* (Li et al., 2009). The most recently described species *Dietzia timorensis* (Yamamura et al., 2010) has a unique polar lipid profile containing phosphatidylglycerol and phosphatidylinositol.

Ecology and habitats. Strains of the genus *Dietzia* have been isolated from a wide variety of habitats including animal and human sources. Most species are represented by a single strain, the type strain, and would seem to have been isolated as part of overall diversity studies rather than targeted or specific enrichment approaches. *Dietzia maris* has been isolated from halibut (Harrison, 1929), soils, and the skin and intestinal contents of carp (*Cyprinus carpio*) (Nesterenko et al., 1982). There are reports in the literature of the isolation of *Dietzia maris* strains from deep-sea muds (Takami et al., 1997), deep-sea trenches in the Pacific Ocean (Colquhoun et al., 1998), the dinoflagellate *Pyrodinium bahamense* (Azanza et al., 2006), and from clinical samples (Pidoux et al., 2001; Reyes et al., 2006). *Dietzia aerolata* was isolated from a bioaerosol collected in a duck barn in Germany (Kämpfer et al., 2010). The species *Dietzia cercidiphylli* and *Dietzia schimae* were isolated from surface sterilized plant material of *Schima* sp. and *Cercidiphyllum japonicum* using tap water yeast extract agar (Li et al., 2008). *Dietzia cinnamea* (Yassin et al., 2006) and *Dietzia papillomatosis* (Jones et al., 2008) are species of human origin. Soil was the source of the species *Dietzia lutea* (Li et al., 2009), *Dietzia kunjamensis* (Mayilraj et al., 2006), and *Dietzia timorensis* (Yamamura et al., 2010) while an additional environmental isolate *Dietzia natronolimnaea* (Duckworth et al., 1998) was recovered from littoral sediments of a soda lake in Kenya. In the search for organisms capable of degradation of petroleum products at low temperatures, the species *Dietzia psychralcaliphila* was isolated from water from a drain pool in a fish egg processing plant (Yumoto et al., 2002). A search of the public databases demonstrates that large numbers of 16S rRNA gene sequences recovered from various habitats have been shown to fall within the radiation of the species of the genus *Dietzia*. Koerner et al. (2009) speculated on the role of species of the genus *Dietzia* as emerging opportunistic pathogens.

Nutrition and growth conditions

The species of the genus *Dietzia* are chemo-organotrophs with optimal temperatures for growth in the mesophilic range and pH optima in the neutral/alkaline range. The species *Dietzia psychralcaliphila* has been described as a psychrophilic, alkaliphile capable of growing at 5°C and optimally at pH values of 9–10 (Yumoto et al., 2002). *Dietzia natronolimnaea* is also an alkaliphile having an optimum pH for growth of 9.0 (Duckworth et al., 1998). Of those tested for growth at high NaCl concentrations the species *Dietzia maris* (Rainey et al., 1995b), *Dietzia lutea* (Li et al., 2009), and *Dietzia schimae* (Li et al., 2008) have been shown to grow at a NaCl concentration of 15% (w/v).

The substrates reported in the literature to be used as sole carbon sources are given in Table 34. The data presented in Table 34 are from all papers describing new species of the genus *Dietzia* and not just the paper describing the species in question. As additional species have been added to the genus, comparative studies have been made and new data on existing species have been accumulated. For some species, namely *Dietzia kunjamensis* (Mayilraj et al., 2006) and *Dietzia psychralcaliphila* (Yumoto et al., 2002), there are many discrepancies between the substrate utilization data presented in the original publication and that presented in subsequent publications even though the same type strains have been used as reference material in comparative studies. The substrates for which conflicting data exist are recorded as +/− in Table 34. The high number of nd (not determined) entries in Table 34 indicate that no formal standards for the description of species of the genus *Dietzia* have been proposed and so it continues to be difficult to carry out comparative studies and differentiate species on the basis of phenotype.

The information provided in the species descriptions below is a compilation of data from the original publication of the particular species and subsequent publications in which data for the type strains of other previously described species were determined for comparative purposes.

TABLE 34. Characteristics of the species of the genus *Dietzia*[a]

Characteristic	*D. maris*	*D. aerolata*	*D. cercidiphylli*	*D. cinnamea*	*D. kunjamensis*	*D. lutea*	*D. natronolimnaea*	*D. papillomatosis*	*D. psychralcaliphila*	*D. schimae*	*D. timorensis*
Cell size (in µm)	0.6–1.0 × 1.0–2.0	1.0–1.5	nd	nd	1.0–1.2 × 1.1–2.0	1.0–1.2 × 1.1–2.4	0.9–1.1 × 1.2–2.3	1.0–1.4 × 0.2–0.4	0.8–1.0 × 1.0–2.2	nd	nd
Cell shape	Short ovoids	Coccoid	Short rods	Rods	Rods/cocci	Short rods/cocci	Rods	Rods/cocci	Rods	Short rods	Rods/cocci
Motile	–	nd	–	nd	–	–	–	–	–	–	–
Cell division	Snapping and V-forms	nd	Snapping and V-forms	Snapping and V-forms	nd	Snapping and V-forms	Snapping and V-forms	Snapping and V-forms	Snapping and V-forms	Snapping and V-forms	nd
Colony morphology	Raised, butyrous, cir, entire edge	nd	Cir, smooth, opaque[b]	Smooth	Smooth, glistening, convex	Cir, smooth, opaque, convex[b]	Cir, convex, glistening	Cir, convex, shiny	Cir, convex, glistening	Cir, smooth, opaque	Cir, convex, glistening
Colony color	Orange	Deep orange	Reddish orange	Yellow	Coral red	Orange-yellow[b]	Soft pink/coral red	Orange	Coral red	Deep pink	Orange-yellow
Oxidase	nd	+	–	–	+	–	–	–	+	–	nd
Catalase	+	+	+	+	+	+	+	+	+	+	nd
Temperature range for growth (°C)	10–45	10–30	10–37	22–45	10–37	10–45	20–40	10–37	5–30	10–45	10–37
Temperature optimum (°C)	nd	25–30	28	nd	25	28	30	30	nd	28	nd
pH range for growth	nd	nd	6–9	nd	7–10	5–9	6–10	7–10	7–10	6–9	nd
pH optimum	nd	nd	7	nd	8	7	9	nd	9–10	7	nd
NaCl range for growth	to 15%	nd	nd	to 12%	to 7%	to 15%	to 10%	to 8%	to 12%	to 15%	to 7%
Growth at 5°C	nd	–	–	–	–	–	–	–	+	–	–
Growth at 10°C	nd	+	+	–	+	+	–	+/–	+	+	+
Growth at 45°C	–	–	–	+	–	+	–	–	–	+	–
Growth at 5% NaCl	+	nd	+	+	+	+	+	+	+	+	nd
Growth at 7% NaCl	+	nd	+	+	w	+	+	+	+	+	nd
Voges–Proskauer	nd	nd	–	nd	–	–	nd	nd	–	–	nd
Methyl red	–	nd	–	nd	–	–	nd	nd	–	–	nd
H_2S production	–	nd	–	nd	–	–	nd	nd	–	–	nd
Indole production	–	nd	–	nd	–	–	+	nd	–	–	nd
Nitrate reduction	+	nd	–	+	+	–	+/–	+	–	+	–
Milk coagulation	–	nd	–	nd	nd	–	nd	nd	nd	–	nd
Hydrolysis of:											
Casein	–	nd	nd	–	–	–	nd	–	–	nd	–
Cellulose	–	nd	nd	+	+	nd	+	+	nd	nd	nd
Guanine	–	nd	nd	–	nd	nd	nd	nd	nd	nd	nd
Gelatin	v	nd	–	–	–	–	–	–	–	–	nd
Hypoxanthine	–	nd	nd	–	nd	nd	nd	–	nd	nd	–
Starch	–	nd	–	nd	–	–	+	+	–	–	nd
Tween 20	+	nd	+	nd	nd	+	nd	+	+	+	nd

Tween 40	+	nd	+	nd	+	+	nd	nd	+	+	nd
Tween 80	+	nd	+	nd	+	+	nd	+	+	+	nd
Tyrosine	–	nd	nd	–	nd	nd	nd	nd	nd	nd	nd
Urea	v	nd	+	+	–	nd	+	+	–	–	–
Xanthine	–	nd	nd	–	nd	nd	nd	nd	nd	nd	nd
Utilization as sole carbon source:											
Acetate	+	nd	nd	+	+	nd	nd	nd	+	nd	nd
D-Adonitol	–	–	–	–	–	–	–	+	+	–	+
Esculin	–	nd	–	nd	–	+	–	nd	–	+	+
Amygdalin	+	nd	–	nd	–	–	+	nd	+	–	+
D-Arabinose	–	nd	–	nd	–	+	–	nd	–	–	+
L-Arabinose	–	–	+	–	+/–	+	–	+	–	–	+
D-Arabitol	–	–	–	nd	–	–	–	nd	+/–	–	+
Arbutin	–	–	+	nd	–	–	–	+	–	–	+
Butyrate	+	nd	nd	nd	nd	nd	nd	nd	+	nd	nd
D-Cellobiose	–	–	–	–	+/–	–	+	+	+/–	+	+
Citrate	v	nd	nd	–	nd	nd	nd	nd	–	nd	nd
Ducitol	–	nd	–	nd	nd	nd	nd	nd	nd	–	+
meso-Erythritol	nd	nd	–	–	nd	nd	nd	+	–	–	+
D-Fructose	+	nd	+	+	+	nd	–	+	+	+	+
D-Fucose	nd	nd	–	nd	nd	nd	nd	+	nd	–	+
L-Fucose	–	nd	–	nd	+	–	–	+	–	–	+
D-Galactose	–	nd	–	–	–	+	–	+	–	–	+
Gentiobiose	–	nd	–	–	+	–	–	+	+	–	+
D-Glucose	+	+	+	+	+	+	+	+	+/–	+	+
Glycerol	+/–	nd	–	nd	–	+	+	+	w/–	+	+
Glycogen	–	nd	–	nd	+/–	–	–	nd	–	–	+
Inositol	–	–	–	–	–	+	–	+	+/–	–	+
Inulin	–	–	–	nd	+/–	–	–	nd	–	–	+
D-Lactose	–	nd	+	–	+/–	–	+	+	+/–	+	+
D-Lyxose	–	nd	+	–	+	+	–	nd	–	–	+
Malate	+	nd	nd	nd	–	nd	nd	nd	–	nd	nd
D-Maltose	+/–	–	+	+	–	–	+	+	+/–	–	+
D-Mannitol	–	nd	–	–	–	+	–	+	–	–	+
D-Mannose	v	+	+	nd	+	+	+	+	+/–	+	+
D-Melezitose	+	nd	–	–	w/–	–	+	+	+	–	+
D-Melibiose	–	–	–	nd	+/–	–	+	+	+/–	–	+
Methyl-α-D-glucopyranoside	–	nd	–	nd	–	–	+	nd	+	–	+
Methyl-α-D-mannopyranoside	+	nd	–	nd	–	+	–	nd	+	–	+
Methyl-β-D-xylpyranoside	–	nd	–	nd	–	+	–	nd	+	–	+
N-Acetylglucosamine	–	–	–	nd	–	–	+	nd	+	–	+
Potassium gluconate	nd	nd	–	nd	nd	nd	nd	nd	nd	–	+
Potassium 2-ketogluconate	nd	nd	–	nd	nd	nd	nd	nd	nd	–	+

(continued)

TABLE 34. (continued)

Characteristic	*D. maris*	*D. aerolata*	*D. cercidiphylli*	*D. cinnamea*	*D. kunjamensis*	*D. lutea*	*D. natronolimnaea*	*D. papillomatosis*	*D. psychralcaliphila*	*D. schimae*	*D. timorensis*
Potassium 5-ketogluconate	+	nd	+	nd	w	–	–	nd	+	–	+
Pyruvate	+	nd	nd	nd	+	nd	nd	nd	nd	nd	nd
D-Raffinose	–	–	–	–	+/–	–	+	+	+/–	–	+
L-Rhamnose	–	–	–	–	+	+	–	nd	–	–	+
D-Ribose	–	nd	–	nd	+/–	+	–	+	–	–	+
Salicin	–	–	–	+	–	nd	–	+	–	–	+
Sorbitol	–	nd	–	–	–	nd	–	+	–	–	+
L-Sorbose	–	nd	–	nd	–	+	–	nd	–	–	+
Sucrose	v	–	+	–	+/–	–	+	+	+/–	+	+
Succinate	+	nd	nd	nd	–	nd	–	nd	–	nd	nd
D-Tagatose	–	nd	+	+	–	–	–	+	–	–	+
D-Trehalose	–	–	–	–	+	–	+	+	+/–	–	+
D-Turanose	–	nd	+	nd	+/–	–	–	+	–	–	+
Xylitol	–	nd	–	nd	–	–	–	+	+	–	+
D-Xylose	w/–	–	–	–	+/–	+	–	+	–	–	+
L-Xylose	–	nd	–	nd	–	+	–	nd	–	–	+
DNA G+C content (mol%)	70.4	nd	72.6	72.3	67.0	70.5	66.1	nd	69.6	71.9	65.5
Polar lipids	PE, PG, DPG	PG, DPG, PI, PIM	PG, DPG, PI, PIM	PE, PG, DPG	PG, DPG, PI	PE, PG, DPG, PI, PIM	PE, PG, DPG	PE, PG, DPG	nd	PG, DPG, PI	PG, PI
Mycolic acid chain length	33–38	nd	33–40	nd	33–40	nd	34–38	nd	34–39	33–40	nd

[a]Symbols and abbreviations: nd, not determined; v, variable between strains of same species; +, positive; –, negative; +/–, results differ between publications using the same strains; w, weak positive; cir, circular; TSA, trypticase soy agar; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; DPG, diphosphatidylglycerol; PI, phosphatidylinositol; PIM, phosphatidylinositol mannosides.

[b]On TSA.

List of species of the genus *Dietzia*

1. **Dietzia maris** Rainey, Klatte, Kroppenstedt and Stackebrandt 1995b, 33[VP] (*Rhodococcus maris* Nesterenko, Nogina, Kasumova, Kvasnikow and Batrako 1982, 11)

ma′ris. L. gen. n. *maris* of the sea.

Aerobic, Gram-stain-positive staining, nonmotile, non-spore-forming, non-acid-fast, V-forms and coccoid to rod–shaped cells, which exhibit snapping division. Circular, raised, butyrous, glistening, orange pigmented colonies with entire margins on nutrient agar (NA) plates. Catalase-positive. The cell-wall amino acid is *meso*-diaminopimelic acid and the major cell-wall sugars are arabinose and galactose. Whole-cell fatty acids consist of predominantly straight-chain saturated and unsaturated components, namely $C_{14:0}$ (0.8%), $C_{15:1}$ ω8*c* (0.15%), $C_{15:1}$ ω5*c* (0.42%), $C_{16:1}$ ω6*c* and $C_{16:1}$ ω7*c* (10.6%), 10-methyl $C_{16:0}$ (0.6%), $C_{16:0}$ (15.3%), $C_{17:1}$ ω8*c* (17.2%), $C_{10:0}$ (13.3%), 10-methyl $C_{17:0}$ (5.3%), $C_{18:1}$ ω9*c* (13.9%), $C_{18:0}$ (12.3%), and 10-methyl $C_{18:0}$ (10.3%). Mycolic acids containing 33–38 carbon atoms are present. The polar lipid profile consists of phosphatidylethanolamine, phosphatidylglycerol, and diphosphatidylglycerol. MK-8(H_2) is the major menaquinone. Growth occurs between 10 and 45°C, with optimal growth occurring at 28°C. Growth occurs in the presence of 15% (w/v) NaCl. Nitrate is reduced. Methyl red is negative. H_2S and indole are not produced. Negative for milk coagulation. Degrades gelatin, Tweens 20, 40, 60, and 80, but not casein, cellulose, guanine, hypoxanthine, L-tyrosine, or xanthine. Urea is hydrolyzed. Acetate, amygdalin, butyrate, citrate (some strains), D-fructose, D-glucose, glycerol (variable results), malate, D-maltose (variable results), D-mannose (some strains), D-melezitose, methyl-α-D-mannopyranoside, potassium 5-ketogluconate, pyruvate, sucrose (some strains), succinate, and D-xylose (weak and variable) are used as sole carbon sources. The following substrates are not used as sole carbon sources: D-adonitol, esculin, D-arabinose, L-arabinose, D-arabitol, arbutin, D-cellobiose, dulcitol, L-fucose, D-galactose, gentiobiose, glycogen, inositol, inulin, D-lactose, D-lyxose, D-mannitol, D-melibiose, methyl-α-D-glucopyranoside, methyl-β-D-xylopyranoside, *N*-acetylglucosamine, D-raffinose, L-rhamnose, D-ribose, salicin, sorbitol, L-sorbose, D-tagatose, D-trehalose, D-turanose, xylitol, and L-xylose.

Source: soil; skin, and intestinal tracts of carp (*Cyprinus carpio*).

DNA G+C content (mol%): 70.4 (HPLC).

Type strain: ATCC 35013, AUCNM A-593, CCUG 44488, CIP 104188, DSM 43672, IEGM 55, IFO (now NBRC) 15801, IMV 195, JCM 6166, LMG 5361, NRRL B-16941, VKM Ac-593.

Sequence accession no. (16S rRNA gene): X79290.

2. **Dietzia aerolata** Kämpfer, Langer, Martin, Jackel and Busse 2010, 395[VP]

a.e.ro.la′ta. Gr. n. *aer* air; L. fem. part. adj. *lata* carried; N.L. fem. part. adj. *aerolata* airborne.

Aerobic, Gram-stain-positive staining, nonmotile, coccoid shaped cells. Deep orange pigmented colonies on TSA plates. Catalase- and oxidase-positive. Whole-cell fatty acids consist of predominantly straight-chain saturated and unsaturated components, namely: $C_{14:0}$ (0.5%), $C_{16:1}$ ω6*c* and $C_{16:1}$ ω7*c* (5.6%), $C_{16:0}$ (22.7%), $C_{17:1}$ ω8*c* (5.0%), $C_{17:0}$ (14.0%), 10-methyl $C_{17:0}$ (0.7%), $C_{18:1}$ ω9*c* (19.7%), $C_{18:0}$ (6.9%), 10-methyl $C_{18:0}$ (13.2%), $C_{19:0}$ (8.4%), $C_{20:4}$ ω6,9,12,15*c* (1.4%), and $C_{20:0}$ (0.4%). The polar lipid profile consists of phosphatidylglycerol, diphosphatidylglycerol, phophatidylinositol, phosphatidylinositol mannoside, two unknown glycolipids, and three unknown polar lipids. Mycolic acids are present. The major polyamines are spermine and spermidine. MK-8(H_2) is the major menaquinone. Growth occurs between 10 and 30°C, with optimal growth occurring at 25–30°C. D-Glucose and D-mannose are utilized as sole carbon sources. D-Adonitol, L-arabinose, D-arabitol, arbutin, cellobiose, inositol, inulin, maltose, melibiose, *N*-acetylglucosamine, raffinose, L-rhamnose, salicin, sucrose, trehalose, and D-xylose are not utilized.

Source: air of a duck barn.

DNA G+C content (mol%): not determined.

Type strain: strain Sj14a, CCM 7659, DSM 45334.

Sequence accession no. (16S rRNA gene): FM995533.

3. **Dietzia cercidiphylli** Li, Zhao, Zhang, Klenk, Pukall, Qin, Xu and Li 2008, 2552[VP]

cer.ci.di.phyl′li. N.L. gen. n. *cercidiphylli* of the plant *Cercidiphyllum*, isolated from root sample of *Cercidiphyllum japonicum.*

Aerobic, Gram-stain-positive staining, nonmotile, non-spore-forming, non-acid–alcohol-fast, short rods (which exhibit snapping division), and V-forms. Circular, smooth, opaque reddish orange pigmented colonies on trypticase soy agar (TSA) plates. Catalase-positive; oxidase-negative. The cell-wall amino acid is *meso*-diaminopimelic acid and the major cell-wall sugars are arabinose and galactose. Whole-cell fatty acids consist of predominantly straight-chain saturated and unsaturated components, namely $C_{14:0}$ (1.1%), $C_{15:1}$ iso (0.4%), $C_{16:0}$ iso (1.6%), $C_{16:1}$ ω9*c* (0.5%), $C_{16:1}$ ω6*c* and $C_{16:1}$ ω7*c* (18.7%), 10-methyl $C_{16:0}$ (1.1%), $C_{16:0}$ (18.9%), $C_{17:1}$ anteiso ω9*c* (0.9%), $C_{17:1}$ ω8*c* (3.7%), $C_{17:1}$ ω7*c* (2.2%), $C_{17:0}$ iso (0.2%), $C_{17:0}$ anteiso (0.9%), 10-methyl $C_{17:0}$ (0.4%), $C_{18:1}$ ω7*c* (3.4%), $C_{18:1}$ ω9*c* (27.9%), and 10-methyl $C_{18:0}$ (18.0%). Mycolic acids containing 33–40 carbon atoms are present. The polar lipid profile consists of phosphatidylglycerol, diphosphatidylglycerol, phosphatidylinositol, and phosphatidylinositol mannoside. MK-8(H_2) is the major menaquinone. Growth occurs between 10 and 37°C, with optimal growth occurring at 28°C. Growth occurs between pH 6 and 9, with optimal growth at pH 7. Growth occurs in the presence of 10% (w/v) NaCl. Nitrate is not reduced. Voges–Proskauer and methyl red tests are negative. H_2S and indole are not produced. Negative for milk coagulation and peptonization. Gelatin and starch are not hydrolyzed. Urea, Tweens 20, 40, and 80 are hydrolyzed. L-Arabinose, arbutin, D-fructose, D-glucose, D-lactose, D-lyxose, maltose, D-mannose, potassium 5-ketogluconate, sucrose (weakly), D-tagatose, and turanose are used as sole carbon sources. The following substrates are not used as sole carbon sources: D-adonitol, esculin, amygdalin, D-arabinose, L-arabitol, D-arabitol, cellobiose, dulcitol, L-fucose, D-fucose, gentiobiose, glycerol, glycogen, inositol, inulin, D-mannitol, melezitose, melibiose, methyl-α-D-glucopyranoside, methyl-α-D-mannopyranoside, methyl-β-D-xylopyranoside, *N*-acetylglucosamine, potassium gluconate, potassium 2-ketogluconate, raffinose, L-rhamnose, D-ribose, salicin, D-sorbitol, starch, trehalose, xylitol, L-xylose, and D-xylose.

Source: surface sterilized root sample of *Cercidiphyllum japonicum* collected from Yunnan Province, southwest China.

DNA G+C content (mol%): 72.6 (HPLC).

Type strain: YIM 65002, CCTCC AA 207016, DSM 45140, JCM 16002.

Sequence accession no. (16S rRNA gene): EU375846.

4. **Dietzia cinnamea** Yassin, Hupfer and Schaal 2006, 644[VP]

cin.na′me.a. L. fem. adj. *cinnamea* of or from cinnamon referring to the color of the cellular biomass.

Aerobic, Gram-stain-positive staining, nonmotile, non-spore-forming, non-acid-fast, V-forms and rod–shaped cells, which exhibit snapping division. Circular, convex, shiny, yellow-pigmented colonies on Columbia agar supplemented with 5% sheep blood, and orange-pigmented colonies on modified Bennett's agar after growth for 5d at 30°C. Catalase-positive; oxidase-negative. The cell-wall amino acid is *meso*-diaminopimelic acid and the major cell-wall sugars are arabinose and galactose. The glycan moiety of the cell wall contains N-acetyl residues (*N*-acetylmuramic acid). Whole-cell fatty acids consist of predominantly straight-chain saturated and unsaturated components, namely $C_{14:0}$ (0.65%), $C_{15:1}$ *cis*-10 (0.5%), $C_{15:0}$ (4.66%), $C_{16:1}$ *cis*-9 (2.35%), $C_{16:1}$ *cis*-10 (12.2%), $C_{16:0}$ (22.97%), 10-methyl $C_{16:0}$ (0.56%), $C_{17:0}$ *cis*-9 (11.43%), $C_{17:0}$ (4.0%), 10-methyl $C_{17:0}$ (5.23%), $C_{18:0}$ *cis*-9 (13.49%), $C_{18:0}$ (0.32%), 10-methyl $C_{18:0}$ (20.65%), and $C_{19:1}$ *cis*-9 (1.1%). The polar lipid profile consists of phosphatidylethanolamine, phosphatidylglycerol, and diphosphatidylglycerol. Pyrolysis of mycolic acids releases the following fatty acids: $C_{14:0}$, $C_{15:0}$, $C_{16:0}$, $C_{17:0}$, $C_{18:1}$, and $C_{18:0}$. MK-8(H_2) is the major menaquinone and MK-7(H_2) is the minor one. Growth occurs between 22 and 45°C. No growth at 5°C. Growth occurs pH 7 and 10 but not at pH 5. Growth occurs in the presence of 12% (w/v) NaCl. Nitrate is reduced. Degrades cellulose, testosterone, and urea but not adenine, casein, elastin, esculin, gelatin, guanine, hypoxanthine, tyrosine, or xanthine. Acetate, D-fructose, D-glucose, D-maltose, 1,2-propanediol, salicin, and D-tagatose are used as sole carbon sources. The following substrates were not used as sole carbon sources: adipate, D-adonitol, isoamyl alcohol, L-arabinose, 2, 3-butanediol, D-cellobiose, citrate, *meso*-erythritol, D-galactose, gluconate, gentiobiose, *m*-hydroxybenzoate, *p*-hydroxybenzoate, inositol, lactate, D-lactose, L-lyxose, D-mannitol, melezitose, D-raffinose, L-rhamnose, sorbitol, sucrose, D-trehalose, and D-xylose. Unable to use acetamide, L-alanine, arginine, gelatin, ornithine, proline, or serine as simultaneous carbon and nitrogen sources. The utilization of the following carbon sources is reported to be both positive and negative in different studies: adonitol, L-arabinose, butane-2, 3-diol, D-cellobiose, *meso*-erythritol, D-galactose, *myo*-inositol, D-lactose, D-mannitol, D-melezitose, D-raffinose, sucrose, trehalose, and D-xylose.

Source: perianal swab of a patient with a bone marrow transplant.

DNA G+C content (mol%): 72.3 (HPLC).

Type strain: IMMIB RIV-399, CCUG 50875, DSM 44904, JCM 13663, NBRC 102147.

Sequence accession no. (16S rRNA gene): AJ920289.

5. **Dietzia kunjamensis** Mayilraj, Suresh, Kroppenstedt and Saini 2006, 1670[VP]

kun.ja.men′sis. N.L. fem. adj. *kunjamensis* pertaining to Kunjam Pass of the cold desert of the Indian Himalayas.

Aerobic, Gram-stain-positive staining, nonmotile, non-spore-forming, rod–coccus shaped cells, occurring in groups. Colonies are small, smooth, glistening, convex, and coral red-pigmented on TSA. Catalase- and oxidase-positive. Negative for H_2S production, indole, methyl red, and Voges–Proskauer tests. The cell-wall amino acid is *meso*-diaminopimelic acid and the major cell-wall sugars are arabinose and galactose. The glycan moiety of the cell wall contains N-acetyl residues (*N*-acetylmuramic acid). Whole-cell fatty acids consist of predominantly straight-chain saturated and unsaturated components, namely $C_{16:0}$ (14.43%), $C_{16:1}$ *cis*-10 (6.0%), $C_{17:0}$ (12.95%), $C_{17:1}$ *cis*-9 (8.85%), $C_{17:1}$ *cis*-10 (7.41), $C_{18:1}$ *cis*-9 (30.39%), and tuberculostearic acid (10.51%). Mycolic acids containing 33–40 carbon atoms are present. The polar lipid profile consists of phosphatidylglycerol, diphosphatidylglycerol, and phosphatidylinositol. MK-8(H_2) is the major menaquinone. Nitrate is reduced. Degrades cellulose, Tweens 40 and 60, but not casein, DNA, and starch. Acetic acid, L-arabinose, L-asparagine, dextrin, D-fructose, D-fructose 6-phosphate, L-fucose, gentiobiose, D-glucose, L-glutamic acid, glycerol, β-hydroxybutyric acid, γ-hydroxybutyric acid, α-ketovaleric acid, maltotriose, D-mannose, palatinose, D-psicose, D-ribose, succinic acid monomethyl ester, thymidine, D-trehalose, turanose, D-xylose, propionic acid, and pyruvic acid are used as sole carbon sources. Adenosine, 2′-deoxyadenosine, L-alaninamide, D- and L-alanine, AMP, 2, 3-butanediol, α-cyclodextrin, β-cyclodextrin, *N*-acetyl-D-glucosamine, *N*-acetyl-β-D-mannosamine, amygdalin, D-arabitol, arbutin, citrate, D-cellobiose, D-galactose, D-galacturonic acid, D-gluconic acid, α-D-glucose 1-phosphate, D-glucose 6-phosphate, methyl-α-D-glucoside, *N*-acetyl-L-glutamic acid, DL-α-glycerol phosphate, glycyl L-glutamic acid, L-alanyl glycine, glycogen, α-hydroxybutyric acid, *p*-hydroxyphenylacetic acid, inosine, *myo*-inositol, inulin, α-ketoglutaric acid, lactamide, D-lactic acid methyl ester, L-lactic acid, α-D-lactose, lactulose, D- and L-malic acid, maltose, mannan, D-mannitol, D-melezitose, D-melibiose, methyl-α-D-galactoside, methyl-β-D-galactoside, methyl-D-glucose, methyl-β-D-glucoside, methyl-α-D-mannoside, putrescine, L-pyroglutamic acid, pyruvic acid methyl ester, D-raffinose, L-rhamnose, salicin, sedoheptulosan, serine, D-sorbitol, stachyose, succinic acid, succinamic acid, sucrose, D-tagatose, TMP, UMP, uridine, and xylitol are not utilized. Acid is produced from mannitol and sucrose but not from arabinose, cellobiose, fructose, galactose, glucose, lactose, maltose, mannose, raffinose, rhamnose, salicin, sorbitol, or xylose. Growth occurs between 20 and 37°C with optimal growth at 25°C. Growth does not occur at 5, 10, or 45°C. Growth occurs between pH 7 and 10 with optimal growth at pH 8. Growth does not occur at pH 5. Weak growth occurs in the presence of 7% (w/v) NaCl.

Source: soil 45 cm below an ice glacier, at 4200 m above sea level, Kunjam Pass, Himachal Pradesh, India.

DNA G+C content (mol%): 67 (HPLC).

Type strain: K30-10, DSM 44907, JCM 13325, MTCC 7007.

Sequence accession no. (16S rRNA gene): AY972480.

6. **Dietzia lutea** Li, Chen, Zhao, Klenk, Pukall, Zhang, Tang and Li 2011, 1[VP] (Effective publication: Li, Chen, Zhao, Klenk, Pukall, Zhang, Tang and Li 2009, 122.)

lu′te.a. L. fem. adj. *lutea* orange-yellow colored.

Aerobic, Gram-stain-positive, nonmotile, non-spore-forming, non-acid-fast, V-forms and short rod–coccus shaped cells, which exhibit snapping division. Circular, convex, smooth, opaque, orange-yellow colonies on TSA plates. Catalase-positive; oxidase-negative. The cell wall amino acid

is *meso*-diaminopimelic acid and the major cell sugars are arabinose and galactose. The major whole-cell fatty acids are $C_{14:0}$ (1.03%), $C_{15:0}$ (3.71%), $C_{16:0}$ (15.42%), $C_{16:1}$ *cis*-9 (3.86%), $C_{17:0}$ (22.42%), $C_{17:1}$ *cis*-9 (12.29%), 10-methyl $C_{17:0}$ (2.95%), $C_{18:0}$ (8.09%), $C_{18:1}$ *cis*-9 (10.24%), 10-methyl $C_{18:0}$ (tuberculostearic acid) (7.86%), $C_{19:0}$ (2.56%), $C_{16:1}$ *trans* 9/15i2-OH (6.70%), and $C_{18:1}$ *trans* 9/t6/c11 (1.77%). Mycolic acids are present. The polar lipid profile consists of phosphatidylethanolamine, phosphatidylglycerol, diphosphatidylglycerol, phosphatidylinositol, and phosphatidylinositol mannoside. MK-8(H_2) is the major menaquinone. Growth occurs between 10 and 45°C, with optimal growth at 28°C. Growth occurs between pH 5 and 9, with optimal growth at pH 7. Growth occurs in the presence of 15% (w/v) NaCl. Nitrate is not reduced. Voges–Proskauer and methyl red tests are negative. H_2S and indole are not produced. Negative for milk coagulation and peptonization. Cellulose, gelatin, starch, and urea are not hydrolyzed. Tweens 20, 40, and 80 are hydrolyzed. Alkaline phosphatase, α-galactosidase, β-glucuronidase and α-glucosidase enzymic activities are detected. *N*-acetyl-β-glucosaminidase, α-mannosidase, β-fucosidase, trypsin, α-chymotrypsin, β-galactosidase, and β-glucosidase are present, but not esterase (C4), esterase lipase (C8), lipase (C14), leucine arylamidase, valine arylamidase, cysteine arylamidase, acid phosphatase, and naphthol-AS-BI-phosphohydrolase when using the API ZYM test strips. Utilizes L-arabinose, D-arabinose, esculin, D-fructose, D-galactose, D-glucose, glycerol, inositol, D-lyxose, D-mannitol, D-mannose, methyl-α-D-manno-pyranoside, methyl-β-D-xylopyranoside, L-rhamnose, D-ribose, L-sorbose, D-xylose, and L-xylose. D-Adonitol, starch, amygdalin, L-arabitol, D-arabitol, arbutin, D-cellobiose, dulcitol, erythritol, L-fucose, gentiobiose, glycogen, inulin, D-lactose, D-maltose, D-melezitose, D-melibiose, methyl-α-D-glucopyranoside, *N*-acetylglucosamine, potassium gluconate, potassium 2-keto-gluconate, potassium 5-keto-gluconate, D-raffinose, salicin, D-sorbitol, sucrose, D-tagatose, D-trehalose, D-turanose, or xylitol are not utilized as sole carbon sources.

Source: desert soil in Egypt.

DNA G+C content (mol%): 70.5 (HPLC).

Type strain: YIM 80766, KCTC 19232, DSM 45074, CCTCC AA 207008.

Sequence accession no. (16S rRNA gene): EU821598.

7. **Dietzia natronolimnaea** Duckworth, Grant, Grant, Jones, Meijer 1999, 1[VP] (Effective publication: Duckworth, Grant, Grant, Jones, Meijer 1998, 365.)

na.tro.no.lim.na′e. a. N.L. n. *natron* (arbitrarily derived from the Arabic n. natrun or natron), soda, sodium carbonate; N.L. fem. adj. *limnaea* (from Gr. fem. adj. limnaia) of or from the marsh; N.L. fem. adj. *natronolimnaea*, of or from a soda lake (marsh).

Aerobic, Gram-stain-positive, nonmotile, non-spore-forming, non-acid-fast, V-forms and short rod-shaped cells, which exhibit snapping division. Circular, convex, glistening soft pink to coral red colonies. Catalase-positive; oxidase-negative. The cell wall amino acid is *meso*-diaminopimelic acid and the major cell sugars are arabinose and galactose. Whole-cell fatty acids consist of predominantly straight-chain saturated and unsaturated components, namely $C_{14:0}$ (0.66%), $C_{15:0}$ (0.79%), $C_{16:1}$ *cis*-9 (5.37%), $C_{16:1}$ *cis*-10 (8.29%), $C_{16:0}$ (28.50%), $C_{17:0}$ *cis*-9 (3.49%), $C_{17:0}$ (2.1%), $C_{18:0}$ *cis*-9 (31.1%), $C_{18:0}$ (0.75%), 10-methyl $C_{18:0}$ (18.26%), and $C_{19:1}$ *cis*-9 (0.73%). Mycolic acids containing 34–38 carbon atoms are present. The polar lipid profile consists of phosphatidylethanolamine, phosphatidylglycerol, and diphosphatidylglycerol. MK-8(H_2) is the major menaquinone. Growth occurs between 20 and 40°C, with optimal growth at 30°C. Growth occurs between pH 6 and 10, with optimal growth at pH 9. Growth occurs in the presence of 10% (w/v) NaCl. Nitrate reduction has been reported as both positive and negative. Indole is produced. Cellulose, starch, and urea are hydrolyzed. Gelatin is not hydrolyzed. Alkaline phosphatase, esterase (C4), esterase/lipase (C8), leucine arylamidase, cysteine arylamidase, acid phosphatase, naphthol-AS-BI-phosphohydrolase, and α-glucosidase enzymic activities are detected. Lipase (C14), valine arylamidase, trypsin, α-chymotrypsin, α-galactosidase, β-galactosidase, β-glucuronidase, β-glucosidase, *N*-acetyl-β-glucosaminidase, α-mannosidase, and β-fucosidase were not detected using the API ZYM test strips. Utilizes L-arginine, L-asparagine, glycine, L-methionine, L-phenylalanine, L-proline, L-serine, starch, succinate, and L-valine under alkaline conditions, but not L-alanine, formate, fumarate, D-galactose, D-lactose, L-lysine, pyruvate, or D-xylose. Under neutral conditions acetate, *N*-acetylglucosamine, citrate, D-fructose, fumarate, D-glucose, glutamate, glycogen, 3-hydroxybutyrate, mannitol, L-proline, propionate, succinate, suberate, L-serine, and valerate are utilized but not L-alanine, L-arabinose, caprate, L-fucose, D-galactose, histidine, 4-hydroxybenzoate, inositol, itaconate, 2-ketogluconate, 3-hydroxybenzoate, 5-ketogluconate, DL-lactate, lactose, malonate, maltose, D-melibiose, rhamnose, D-ribose, salicin, D-sorbitol, or sucrose.

Source: littoral sediments of Lake Oloidien (Little Lake Naivasha), Kenya.

DNA G+C content (mol%): 66.1 (T_m).

Type strain: 15LN1, CBS 107. 95, DSM 44860, JCM 11417.

Sequence accession no. (16S rRNA gene): X92157.

8. **Dietzia papillomatosis** Jones, Koerner, Natarajan, Perry and Goodfellow 2008, 71[VP]

pa.pil.lo.ma.to′sis. N.L. gen. n. *papillomatosis* of papillomatosis.

Aerobic, Gram-stain-positive staining, nonmotile, non-spore-forming, non-acid–alcohol-fast, V-forms with a rod–coccus life cycle, and reproduction by snapping division. Resistant to lysozyme. Circular, convex, shiny, orange-pigmented colonies on modified Bennett's agar after growth for 5 days at 30°C. Neither aerial hyphae nor diffusible pigments are formed. Catalase-positive; oxidase-negative. The cell-wall amino acid is *meso*-diaminopimelic acid and the major cell-wall sugars are arabinose and galactose. The glycan moiety of the cell wall contains N-acetyl residues (*N*-acetylmuramic acid). Whole-cell fatty acids consist of predominantly straight-chain saturated and unsaturated components, namely pentadecanoic acid ($C_{15:0}$; 5.4%), hexadecanoic acid ($C_{16:0}$; 21.1%), monounsaturated hexadecenoic acid ($C_{16:1}$; 3.0%), septadecanoic acid ($C_{17:0}$; 6.1%), monounsaturated septadecenoic acid ($C_{17:1}$; 2.7%), monounsaturated octadecenoic acid ($C_{18:1}$; 9.0%), tuberculostearic acid 10-methyl $C_{18:0}$ (22.1 %), nonadecanoic acid ($C_{19:0}$; 2.6%), and unidentified peaks with the retention times of 19.99 (10.6%), 21.61 (5.6%), and 21.88 (5.7%). The polar lipid profile consists of phosphatidylethanolamine, phosphatidylglycerol, and diphosphatidylglycerol. MK-8(H_2) is the major menaquinone and MK-7(H_2) is the minor one. Nitrate is reduced to nitrite, but nitrite is not reduced. Degrades cellulose, chitin, starch, L-tyrosine, Tweens 20, 40, 60, and 80, but

not adenine, casein, DNA, elastin, gelatin, hypoxanthine, pectin, RNA, tributyrin, uric acid, xanthine, or xylan. Allantoin, arbutin, and urea are hydrolyzed but not esculin. Adonitol, isoamyl alcohol, L-arabinose, arbutin, *m*-hydroxybenzoic acid, butane-1,3-diol, butane-1, DL-β-hydroxybutyric acid, 4-diol, butane-1-ol, butane-2, 3-diol, D-cellobiose, dextrin, ethanol, *meso*-erythritol, D-fructose, D-fucose, L-fucose, fumaric acid, D-galactose, D-gentiobiose, D-glucose, glycerol, *myo*-inositol, D-lactose, maltose, D-mannitol, D-mannose, D-melezitose, D-melibiose, propane-1, 2-diol, propane-1,3-diol, D-raffinose, D-ribose, D-salicin, sodium acetate, sodium benzoate, sodium *n*-butyrate, sodium propionate, sodium pyruvate, sodium DL-malate, D-sorbitol, sucrose, D-tagatose, trehalose, turanose, D-xylose, and D-xylitol were used as sole carbon sources. The following substrates were not used as sole carbon sources: 3, 3-dimethylglutaric acid, sodium adipate, sodium azelate, sodium citrate, sodium gluconate, sodium malonate, sodium oleate, sodium pimelate, sodium oxalate, sodium sebacate, sodium suberate, or sodium succinate. Acetamide, L-alanine, L-asparagine, L-aspartic acid, L-cysteine, L-glutamic acid, L-glycine, L-histidine, L-leucine, L-isoleucine, DL-norleucine, L-norvaline, L-ornithine, DL-phenylalanine, L-proline, L-serine, L-thymidine, L-valine, and urea were used as sole carbon and nitrogen sources. L-arginine is not used. Acid is produced from D-fructose, but not from D-glucose, D-mannose, D-raffinose, or sucrose. Growth occurs at 37°C but not at 5, 10, or 45°C. Growth occurs pH 7 and 10, but not at pH 5. Growth occurs in the presence of 8% (w/v) NaCl. Growth occurs in the presence of filter paper discs soaked in cephalexin (30 μg/ml), clindamycin hydrochloride (2 μg/ml), colistin (25 μg/ml), erythromycin (5 μg/ml), nalidixic acid (30 μg/ml), novobiocin (5 μg/ml), and tetracycline hydrochloride (10 μg/ml), but not in the presence of bacitracin (10 U), ciprofloxacin (5 μg/ml), cotrimoxazole (25 μg/ml), fusidic acid (10 μg/ml), or penicillin (1 μg/ml).

Source: skin of an immunocompetent patient with confluent and reticulated papillomatosis.

DNA G+C content (mol%): not determined.

Type strain: N 1280, DSM 44961, JCM 15317, NCIMB 14145.

Sequence accession no. (16S rRNA gene): AY643401.

9. **Dietzia psychralcaliphila** Yumoto, Nakamura, Iwata, Kojima, Kusumoto, Nodasaka and Matsuyama 2002, 89[VP]

psy.chral.ca.li′phi.la. Gr. adj. *psychros* cold; N.L. *alcali* alkali, from Arabic *alqali* potash soil; Gr. adj. *philos* friendly to; N.L. fem. adj. *psychralcaliphila* loving cold, alkaline environments.

Aerobic, Gram-stain-positive, nonmotile, non-spore-forming, non-acid-fast, V-forms and rod-shaped cells exhibiting snapping division. Circular, convex, glistening, coral red colonies on TSA plates. Catalase- and oxidase-positive. The cell wall amino acid is *meso*-diaminopimelic acid and the major cell sugars are arabinose and galactose. Whole-cell fatty acids consist of predominantly straight-chain saturated and unsaturated components, namely $C_{14:0}$ (0.44%), $C_{15:0}$ (1.63%), $C_{16:1}$ *cis*-9 (5.38%), $C_{16:1}$ *cis*-10 (3.78%), $C_{16:0}$ (25.65%), $C_{17:0}$ *cis*-9 (9.26%), $C_{17:0}$ (4.78%), $C_{18:0}$ *cis*-9 (35.55%), 10-methyl $C_{18:0}$ (13.52%). Mycolic acids containing 34–39 carbon atoms are present. The polar lipid profile has not been determined. MK-8(H_2) is the major menaquinone. Growth occurs between 5 and 30°C and between pH 7 and 10, with optimal growth occurring at pH 9–10. Growth occurs in the presence of 12% (w/v) NaCl. Nitrate is not reduced. Voges–Proskauer and methyl red tests are negative. H_2S and indole are not produced. Casein, DNA, gelatin, starch, and urea are not hydrolyzed. Tweens 20, 40, 60, and 80 are hydrolyzed. Utilizes acetate, *n*-butyrate, *n*-eicosane, ethanol, D-fructose, D-glucose, *n*-hexadecane, 3-hydroxybutyrate, isobutyrate, *n*-pentadecane, pristine, propionate, pyruvate, *n*-tetracosane, *n*-tridecane, and valerate, but not *N*-acetylglucosamine, aconitate, L-α-alanine, D-α-alanine, anthracene, D-arabinose, L-arginine, arabitol, L-aspartate, D-cellobiose, caprate, citrate, cyclododecane, *n*-decane, *n*-dotriacontane, *myo*-erythritol, fluorene, fumarate, D-galactose, α-D-galacturonate, glutarate, gluconate, glucuronate, glutamate, 2-oxoglutarate, DL-glycerate, D-glycerol, *p*-histidine, hydroxybenzoate, inositol, DL-lactate, lactose, L-leucine, L-lysine, DL-malate, maltose, mannitol, D-mannose, melibiose, L-ornithine, DL-phenylalanine, L-proline, pyrene, raffinose, rhamnose, D-ribose, salicin, D-sorbitol, succinate, sucrose, L-tartrate, trehalose, L-tyrosine, or D-xylose.

Source: the drain of a fish product processing plant.

DNA G+C content (mol%): 89.6 (HPLC).

Type strain: ILA-1, CCUG 47128, IAM 14896, JCM 10987, NBRC 103065, NCIMB 13777.

Sequence accession no. (16S rRNA gene): AB159036

10. **Dietzia schimae** Li, Zhao, Zhang, Klenk, Pukall, Qin, Xu and Li 2008, 2552[VP]

schi′ma.e. N.L. gen. n. *schimae* of the plant genus *Schima*, isolated from a stem sample of *Schima* sp.

Aerobic, Gram-stain-positive staining, nonmotile, non-spore-forming, non-acid–alcohol-fast, short rods, exhibiting snapping division, and V-forms. Circular, smooth, opaque, deep pink pigmented colonies on TSA plates. Catalase-positive; oxidase-negative. The cell-wall amino acid is *meso*-diaminopimelic acid and the major cell-wall sugars are arabinose and galactose. Whole-cell fatty acids consist of predominantly straight-chain saturated and unsaturated components, namely $C_{13:0}$ (0.2%), $C_{13:0}$ 3-OH and $C_{15:1}$ iH (0.5%), $C_{14:1}$ ω5*c* (0.2%), $C_{14:0}$ (2.9%), $C_{13:0}$ 2-OH (0.5%), $C_{15:1}$ iso G (0.9%), $C_{15:1}$ A (0.4%), $C_{15:1}$ ω8*c* (0.8%), $C_{15:1}$ ω5*c* (0.4%), $C_{16:1}$ ω6*c*/$C_{16:1}$ ω7*c* (25.8%), 10-methyl $C_{16:0}$ (0.8%), $C_{16:0}$ (22.2%), C17:1 ω7*c* (19.2%), $C_{17:1}$ anteiso B/$C_{17:1}$ iso I (6.8%), 10-methyl $C_{17:0}$ (2.0%), 10-methyl $C_{18:0}$ (13.6%), $C_{19:0}$ iso (1.7%), and $C_{20:0}$ (0.9%). Mycolic acids containing 33–40 carbon atoms are present. The polar lipid profile consists of phosphatidylglycerol, diphosphatidylglycerol, and phosphatidylinositol. MK-8 (H_2) is the major menaquinone. Growth occurs between 10 and 37°C, with optimal growth at 28°C, and between pH 6 and 9, with optimal growth at pH 7. Growth occurs in the presence of 15% (w/v) NaCl. Nitrate is reduced. Voges–Proskauer and methyl red tests are negative. H_2S and indole are not produced. Negative for milk coagulation and peptonization. Gelatin, starch, and urea are not hydrolyzed. Tweens 20, 40, and 80 are hydrolyzed. Esculin, cellobiose, D-fructose, D-glucose, glycerol, D-lactose, D-mannose, and sucrose are used as sole carbon sources. The following substrates are not used as sole carbon sources: D-adonitol, amygdalin, L-arabinose, D-arabinose, D-arabitol, L-arabitol, arbutin, dulcitol, erythritol, D-fucose, L-fucose, D-galactose, gentiobiose, glycogen, inositol, inulin, D-lyxose, maltose, D-mannitol, melezitose, melibiose, methyl-α-D-glucopyranoside, methyl-α-D-mannopyranoside, methyl-β-D-xylopyranoside, *N*-acetylglucosamine, potassium gluconate, potassium 2-ketgluconate,

potassium 5-ketogluconate, raffinose, L-rhamnose, D-ribose, salicin, D-sorbitol, L-sorbose, starch, D-tagatose, trehalose, turanose, xylitol, D-xylose, or L-xylose.

Source: surface-sterilized stem sample of *Schima* sp. collected from Yunnan Province, southwest China.

DNA G+C content (mol%): 71.9 (HPLC).

Type strain: YIM 65001, CCTCC AA 207015, DSM 45139, JCM 16003.

Sequence accession no. (16S rRNA gene): EU375845.

11. **Dietzia timorensis** Yamamura, Lisdiyanti, Ridwan, Ratnakomala, Sarawati, Lestari, Triana, Kartina, Widyastuti and Ando 2010, 452[VP]

ti.mo.ren′sis. N.L. fem. adj. *timorensis* pertaining to West Timor, Indonesia, from where the organism was first isolated.

Aerobic, Gram-stain-positive staining, nonmotile, rod–coccoid shaped cells. Circular, convex, glistening moderately orange-yellow pigmented colonies. The cell-wall amino acid is *meso*-diaminopimelic acid and the major cell-wall sugars are arabinose and galactose. The major cellular fatty acids are $C_{16:0}$ (48%), $C_{18:1}$ ω9*c*, and 10-methyl $C_{18:0}$ (8%). Mycolic acids containing 34–38 carbon atoms are present. The polar lipid profile consists of phosphatidylglycerol, and trace amounts of phosphatidylinositol. Growth occurs between 10 and 37°C, but not at 5 or 45°C. Growth occurs in the presence of 7% (w/v) NaCl. Esculin is hydrolyzed. Nitrate is not reduced. Arbutin and urea are not hydrolyzed. Does not degrade adenine, casein, elastin, hypoxanthine, testosterone, tyrosine, uric acid, or xanthine. Utilizes esculin, cellobiose, D-fructose, D-glucose, glycerol, lactose, D-mannose, sucrose, D-adonitol, amygdalin, L-arabinose, D-arabinose, L-arabitol, D-arabitol, arbutin, dulcitol, erythritol, L-fucose, D-fucose, D-galactose, gentiobiose, glycogen, inositol, inulin, D-lyxose, maltose, D-mannitol, melezitose, melibiose, methyl-α-D-glucopyranoside, methyl-α-D-mannopyranoside, methyl-β-D-xylopyranoside, *N*-acetylglucosamine, potassium gluconate, potassium 2-ketogluconate, potassium 5-ketogluconate, raffinose, L-rhamnose, D-ribose, salicin, D-sorbitol, L-sorbose, starch, D-tagatose, trehalose, turanose, xylitol, L-xylose, and D-xylose.

Source: soil sample collected from under mahogany (*Swietenia mahogani*) trees on West Timor in Indonesia.

DNA G+C content (mol%): 65.5 (HPLC).

Type strain: ID05-A0528, BTCC B-560, NBRC 104184.

Sequence accession no. (16S rRNA gene): AB377289.

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Family III. **Mycobacteriaceae** Chester 1897, 63[AL]

John G. Magee and Alan C. Ward

My.co.bac.te.ri.a.ce′a.e. N.L. neut. n. *Mycobacterium* type genus of the family; suff. *-aceae* ending to denote a family; N.L. fem. pl. n. *Mycobacteriaceae* the *Mycobacterium* family.

The family contains the type genus *Mycobacterium*. The phylogenetic position of the family *Mycobacteriaceae*, as determined by 16S rRNA gene sequence analysis, is in the order *Corynebacteriales*.

Genus I. **Mycobacterium** Lehmann and Neumann 1896, 363[AL]

John G. Magee and Alan C. Ward

My.co.bac.te′ri.um. Gr. n. *mukês* a fungus; L. neut. n. *bacterium*, a small rod; N.L. neut. n. *Mycobacterium* a fungus rodlet.

Aerobic to microaerophilic, slightly curved or straight rods (0.2–0.6 × 1.0–10 µm), **which are acid–alcohol-fast at some stage of growth**. Most species are difficult to stain by Gram's method, but are usually considered Gram-stain-positive. Branching may sometimes occur and a filamentous or mycelium-like growth may appear which, on slight disturbance, becomes fragmented into rods or coccoid elements. **Cells are nonmotile and asporogenous; conidia or capsules are not produced; there are no grossly visible aerial hyphae. Colonies may be white- to cream-colored; some strains produce yellow- or orange-pigmented colonies with or without light stimulation. Whole-organism hydrolysates are rich in *meso*-diaminopimelic acid, arabinose, and galactose. The peptidoglycan is of the A1γ type. Muramic acid moieties are *N*-glycolated. Cells and cell walls are rich in lipids. These include waxes which have characteristic, chloroform-soluble, mycolic acids with long (60–90 carbon atoms) branched chains. The fatty acid esters released on pyrolysis MS of mycolic acid esters have 22–26 carbon atoms. Cells contain diphosphatidylglycerol, phosphatidylethanolamine, phosphatidylinositol, and phospatidylinositol mannosides as predominant polar lipids, straight-chain saturated, unsaturated, and 10-methyloctadecanoic (tuberculostearic) fatty acids as major fatty acid components, and dihydrogenated menaquinones with nine isoprene units as the predominant isoprenolog.** The genus includes obligate parasites, saprophytes, and opportunistic forms.

DNA G+C content (mol%): 57–73 (T_m; HPLC; whole-genome sequencing).

Type species: **Mycobacterium tuberculosis** (Zopf 1883) Lehmann and Neumann 1896, 363[AL].

Further descriptive information

Phylogeny. The family *Mycobacteriaceae* contains the type genus *Mycobacterium* Lehmann and Neumann (1896). Initially, the genus was proposed to include the tubercle and leprosy bacilli. Subsequently, many more mycobacterial species were proposed, although only 41 species were included in the *Approved Lists of Bacterial Names* (Skerman et al., 1980). At the time of writing, there were 129 species and subspecies with validly published names classified in the genus (Table 35). Most mycobacterial species can be assigned to two taxonomic groups based on growth rate. For practical determinative purposes, slow-growing species require 7 or more days of incubation at optimal temperature to produce easily seen colonies from highly dilute inocula. Conversely, rapid-growing species are seen in less than 7 d under comparable conditions (Wayne and Kubica, 1986). Clinically relevant species are more likely, although not exclusively, to occur in the slow-growing group (Goodfellow and Magee, 1998). Phylogenies based on 16S rRNA gene similarity studies suggest that this division is a natural one (Fox and Stackebrandt, 1987; Goodfellow and Magee, 1998; Rogall et al., 1990; Stahl and Urbance, 1990).

There is some association between the number of ribosomal operons and the growth rate of mycobacterial strains. Bercovier et al. (1986) showed that fast-growing strains commonly have two operons (*rrnA* and *rrnB*), whereas slow-growing strains usually have one. The mycobacterial operon is composed of a leader region, a 16S rRNA gene, intergenic spacer-1 (ITS-1), a 23S rRNA gene, ITS-2, and a 5S rRNA gene (Ji et al., 1994a). The sizes of the leader and spacer regions vary between fast- and slow-growing mycobacteria (Ji et al., 1994b). This latter study led to the proposal that slow-growing species separated from the main phyletic line following the deletion of a DNA segment which spanned an *rrnB*-like operon, leaving an *rrnA*-like operon as the sole source of rRNA. The leader and spacer regions of rrn operons are highly conserved and have occasionally proved useful in further assessing phylogenetic relationships between closely related mycobacterial species.

Tsukamura (1967c) proposed the separation of fast-growing mycobacteria into a new subgenus *Mycomycobacterium*. However, rapid- and slow-growing mycobacteria share too many properties in common, as exemplified by their antigenic composition (Chaparas et al., 1978a, 1978b; Stanford and Grange, 1974), bacteriophage susceptibility (Froman et al., 1954), DNA homology (Baess and Bentzon, 1978; Bradley, 1973; Gross and Wayne, 1970), and lipid composition (Minnikin and Goodfellow, 1980a, 1980b; Minnikin et al., 1980) to justify their separation into two subgenera.

Phylogenetic analyses of 16S rRNA gene sequence data show that mycolic acid-containing actinomycetes form a well-defined clade within the order *Corynebacteriales* (formerly suborder *Corynebacterineae* (Stackebrandt et al., 1997). Mycobacteria form a distinct phyletic line within this clade. Using these data, the constituent genera have been assigned to the families *Corynebacteriaceae* (encompassing *Corynebacterium* and *Dietzia*),

TABLE 35. Full *Mycobacterium* species list

Species	Description no.
M. abscessus	117
M. africanum	2
M. agri	75
M. aichiense	61
M. alvei	102
M. arupense	44
M. asiaticum	22
M. aubagnense	114
M. aurum	68
M. austroafricanum	65
M. avium subsp. *avium*	13a
M. avium subsp. *paratuberculosis*	13b
M. avium subsp. *silvaticum*	13c
M. boenickei	104
M. bohemicum	9
M. bolletii	119
M. botniense	40
M. bovis	3
M. branderi	37
M. brisbanense	107
M. brumae	80
M. canariasense	111
M. caprae	4
M. celatum	36
M. chelonae	116
M. chimaera	15
M. chitae	58
M. chlorophenolicum	88
M. chubuense	89
M. colombiense	16
M. conceptionense	98
M. confluentis	81
M. conspicuum	51
M. cookii	54
M. cosmeticum	112
M. diernhoferi	110
M. doricum	70
M. duvalii	74
M. elephantis	82
M. fallax	59
M. farcinogenes	100
M. flavescens	72
M. florentinum	29
M. fluoranthenivorans	109
M. fortuitum subsp. *acetamidolyticum*	95b
M. fortuitum subsp. *fortuitum*	95a
M. frederiksbergense	108
M. gadium	125
M. gastri	11
M. genavense	33
M. gilvum	93
M. goodii	78
M. gordonae	21
M. haemophilum	7
M. hassiacum	122
M. heckeshornense	39
M. heidelbergense	30
M. hiberniae	43
M. hodleri	126
M. holsaticum	62
M. houstonense	97
M. immunogenum	118
M. interjectum	23
M. intermedium	53
M. intracellulare	14
M. kansasii	10
M. komossense	60
M. kubicae	27
M. kumamotonense	42
M. lacus	52
M. lentiflavum	35
M. leprae	46
M. lepraemurium	47
M. madagascariense	123
M. mageritense	86
M. malmoense	8
M. marinum	18
M. massiliense	120
M. microti	5
M. monacense	71
M. montefiorense	32
M. moriokaense	124
M. mucogenicum	113
M. murale	63
M. nebraskense	12
M. neoaurum	106
M. neworleansense	105
M. nonchromogenicum	45
M. novocastrense	73
M. obuense	94
M. palustre	25
M. parafortuitum	92
M. parascrofulaceum	26
M. parmense	34
M. peregrinum	84
M. phlei	79
M. phocaicum	115
M. pinnipedii	6
M. porcinum	103
M. poriferae	90
M. pseudoshottsii	20
M. psychrotolerans	91
M. pulveris	83
M. pyrenivorans	69
M. rhodesiae	96
M. salmoniphilum	121
M. saskatchewanense	24
M. scrofulaceum	50
M. senegalense	99
M. seoulense	49
M. septicum	101
M. shimoidei	57
M. shottsii	19
M. simiae	28
M. smegmatis	77
M. sphagni	87
M. szulgai	48
M. terrae	41
M. thermoresistibile	76
M. tokaiense	64
M. triplex	31
M. triviale	55
M. tuberculosis	1
M. tusciae	56
M. ulcerans	17
M. vaccae	67
M. vanbaalenii	66
M. wolinskyi	85
M. xenopi	38

Mycobacteriaceae (*Mycobacterium*), and *Nocardiaceae* (encompassing *Gordonia*, *Nocardia*, *Rhodococcus*, and *Tsukamurella*) (Goodfellow and Magee, 1998). However, Stackebrandt et al. (1997) proposed a new hierarchic classification based on the presence of key signature nucleotides, a concept that was further developed by Zhi et al. (2009). Though again placing the mycolic acid-containing actinomycetes within the order *Corynebacteriales*, each of the constituent taxa, namely the families *Corynebacteriaceae* (encompassing *Corynebacterium* and *Turicella*), *Dietziaceae*, *Mycobacteriaceae*, *Nocardiaceae* (encompassing *Gordonia*, *Nocardia*, *Millisia*, *Rhodococcus*, *Skermania*, and *Williamsia*), *Segniliparaceae*, and *Tsukamurellaceae*, were circumscribed by the presence of a characteristic pattern of nucleotides at defined positions on the 16S rRNA gene. The family *Mycobacteriaceae* was defined by a pattern of 16S rRNA gene signatures which consisted of nucleotides at positions 128:233 (G–C), 250 (U), 316:337 (C–G), 418:425 (C–G), 586:755 (U–G), 599:639 (U–G), 662:743 (C–G), 987:1218 (G–C), 1000:1040 (A–U), and 1026:1035 (U–G).

Cell morphology. Cell growth is slow or very slow with cell generation times ranging from 2 h to more than 20 h (David, 1973). Incubation periods of only a few days or over 8 weeks may be needed for a dilute inoculum to produce easily visible colonies on solid media at optimal temperatures. Optimal temperatures vary widely according to the species, ranging from well below ambient to over 45°C. Most species adapt readily to growth on very simple substrates, using amino acids or ammonia as nitrogen sources and glycerol as a carbon source in the presence of mineral salts. Some fastidious organisms require supplements, such as hemin (*Mycobacterium haemophilum*), mycobactins (*Mycobacterium avium* subsp. *paratuberculosis*), or other iron transport compounds. *Mycobacterium leprae* has not been cultivated outside living cells.

Cell-wall composition. The cell-wall peptidoglycolipid contains *meso*-diaminopimelic acid, alanine, glutamic acid, glucosamine, muramic acid, arabinose, and galactose (wall chemotype type IV *sensu* Lechevalier and Lechevalier, 1970). Resistance to decolorization by an acid–alcohol mixture following staining with basic fuchsin is a characteristic feature of mycobacteria and some closely related actinomycetes. This property may be related to the lipid barrier of the wall mycolyl-arabinogalactan impeding the penetration of the decolorizing agent. Variations in wall lipid content may account for differences in the degree of acid-fastness between mycobacterial species.

The lipid content of mycobacterial cells and cell walls is high (Minnikin and Goodfellow, 1980b). Wall chemotype IV actinomycetes, including mycobacteria, contain mycolic acids, which are high-molecular-mass, long chain, 3-hydroxy fatty acids with an aliphatic side chain at position 2. Non-mycobacterial mycolic acids vary in chain length (22–74 carbons) and in the number of double bonds (0–5). Mycobacterial mycolates have between 60 and 90 carbon atoms, occur in a variety of structural types, and do not have more than two points of unsaturation (Minnikin and Goodfellow, 1980b). Mycobacterial species can be assigned to one of several groups based on the presence or absence of different functional groups, as detected by TLC of methanolysates (Table 36; Goodfellow and Magee, 1998). Pyrolysis of mycobacterial mycolic acid methyl esters yields long-chain

TABLE 36. Grouping of mycobacteria based on mycolic acid patterns[a]

Mycolate types	Mycobacterial species
α	*M. brumae*, *M. fallax*, *M. triviale*
α, α′	*M. abscessus*, *M. canariasense*, *M. chelonae*, *M. salmoniphilum*
α, wax esters	*M. bohemicum*, *M. hassiacum*
α, α′, wax esters	*M. cookii*
α, keto	*M. confluentis*, *M. leprae*
α, α′, keto	*M. genavense*, *M. heidelbergense*, *M. intermedium*, *M. lentiflavum*, *M. malmoense*, *M. simiae*
α, keto, wax esters[b]	*M. aichiense*, *M. aurum*, *M. austroafricanum*, *M. avium*, *M. branderi*, *M. celatum*, *M. chimaera*, *M. chlorophenolicum*, *M. conspicuum*, *M. diernhoferi*, *M. elephantis*, *M. flavescens*, *M. frederiksbergense*, *M. gadium*, "*M. gallinarum*", *M. heckeshornense*, *M. hiberniae*, *M. hodleri*, *M. intracellulare*, *M. kumamotonense*, *M. lepraemurium*, *M. madagascariense*, *M. monacense*, *M. moriokaense*, *M. murale*, *M. nonchromogenicum*, *M. neoaurum*, "*M. novum*", *M. novocastrense*, *M. obuense*, *M. paratuberculosis*, *M. parmense*, *M. phlei*, *M. poriferae*, *M. psychrotolerans*, *M. pulveris*, *M. rhodesiae*, *M. scrofulaceum*, *M. sphagni*, *M. terrae*, *M. tokaiense*, *M. tusciae*, *M. xenopi*
α, α′, keto, wax esters	*M. chubuense*, *M. duvalii*, *M. gilvum*, *M. obuense*, *M. parafortuitum*, *M. shimoidei*, *M. vaccae*
α, ω-1-methoxy	*M. alvei*
α, α′, methoxy	*M. agri*
α, methoxy, wax esters	*M. doricum*
α, keto, methoxy	*M. asiaticum*, *M. bovis*, *M. bovis* BCG, *M. florentinum*, *M. gastri*, *M. gordonae*, *M. holsaticum*, *M. kansasii*, *M. kubicae*, *M. marinum*, *M. microti*, *M. szulgai*, *M. tuberculosis*, *M. ulcerans*
α, α′, keto, methoxy	*M. thermoresistibile*
α, keto, methoxy, wax esters	*M. komossense*
α, α′, epoxy	*M. chitae*, *M. farcinogenes*, *M. fluoranthenivorans*, *M. fortuitum* subsp. *fortuitum*, *M. fortuitum* subsp. *acetamidolyticum*, *M. mageritense*, *M. peregrinum*, *M. porcinum*, *M. senegalense*, *M. smegmatis*
α, epoxy, wax esters	*M. pyrenivorans*
α, keto, epoxy, wax esters	*M. interjectum*

[a]Adapted from Dobson et al. (1985) and Goodfellow and Magee (1998) to include data from: Apajalahti et al. (1986), Ausina et al. (1992), Böttger et al. (1993), Butler et al. (1993), Derz et al. (2004), Domenech et al. (1997), Fanti et al. (2004), Floyd et al. (2000), Haas et al. (1997), Häggblom et al. (1994), Jiménez et al. (2004), Kazda et al. (1990, 1992, 1993), Kirschner et al. (1992), Kleespies et al. (1996), Koukila-Kähkölä et al. (1995), Lévy-Frébault et al. (1986a), Luquin et al. (1993), Meier et al. (1993), Padgitt and Moshier (1987), Reischl et al. (1998, 2006), Richter et al. (2002), Roth et al. (2000), Schröder et al. (1997), Shojaei et al. (1997, 2000), Springer et al. (1993, 1995a, 1995b, 1996c), Tortoli et al. (1999, 2001, 2004, 2005), Trujillo et al. (2004), Tsukamura et al. (1986c), Valero-Guillén et al. (1988), Vuorio et al. (1999), Willumsen et al. (2001), and Yassin et al. (1993).

[b]Wax ester mycolates are composed of ω-carboxymycolates esterified to 2-eicosanol and homologs.

fatty acid methyl esters (C_{22} to C_{26}); shorter chain methyl esters are derived from other mycolic acid-containing actinomycetes (Minnikin and Goodfellow, 1980b).

Polar lipid composition can yield valuable information for the classification and identification of actinomycetes (Goodfellow, 1989). The most common polar lipid types are the phospholipids. Lechevalier et al. (1981) classified actinomycetes into five phospholipid groups based on semi-quantitative analyses of the major lipid markers found in whole-organism extracts. The most common phospholipids found in mycobacteria are derivatives of phosphatidic acid. Mycobacteria typically contain diphosphatidylglycerol, phosphatidylethanolamine, phosphatidylinositol, and phosphatidylinositol mannosides (Goodfellow and Magee, 1998; Minnikin, 1982).

Menaquinones are the most common type of isoprenoid quinone found in actinomycetes (Collins, 1994). These compounds are classified according to the length of their isoprenyl side chain, which can vary from one up to 15 isoprene units, and in the degree of saturation or hydrogenation of the C_5 isoprenyl side chain. *Corynebacterium*, *Gordonia*, and *Mycobacterium* strains contain dihydrogenated menaquinones with nine isoprene units as the main component [abbreviated as MK-9(H_2); Collins, 1994] and can thereby be differentiated from the other mycolic acid-containing taxa (see section under the order *Corynebacteriales*).

Colony morphology. Most mycobacteria form more than one kind of colony, but colonies of some species, such as *Mycobacterium intracellulare*, are usually smooth on primary isolation from clinical specimens. Cells of rough strains are usually compacted in curving strands; those of smooth strains are not visibly oriented in any pattern. Some colonies, such as those of *Mycobacterium fortuitum* and *Mycobacterium xenopi*, may be mycelial in early stages of growth, but older colonies exhibit branching filamentous extensions on and into media such as cornmeal glycerol agar; fragmentation to bacillary forms usually occurs as a result of smear preparation. However, *Mycobacterium farcinogenes* and *Mycobacterium senegalense* differ from other mycobacteria in the formation of a stable mycelium (Chamoiseau, 1973, 1979). Aerial filamentous extensions are never visible without magnification (×30–100). Many strains produce white- to cream-colored colonies. However, the presence of carotenoid pigments in some strains leads to the formation of yellow- or orange-colored (rarely pink) colonies. Such pigmentation may only be produced when growth is exposed to light (photochromogenic species) or may develop in darkness without light stimulation (scotochromogenic species). Diffusible pigments are rarely found.

Nutrition, growth conditions and metabolism. Most mycobacteria are obligate aerobes, although some species tolerate reduced oxygen levels. Carbon dioxide is also necessary for growth and is obtained either from the atmosphere or from growth supplements. Mycobacteria show considerable variation in substrates used as sources of carbon and nitrogen. Such variations can be used as an aid to species identification (see below). Additional growth requirements include iron salts, magnesium, potassium, sodium, and sulfur. Though the growth of many species is supported by simple media, such as modified Sauton's agar (Mordarska et al., 1972), cultivation of clinically significant mycobacteria is better with egg-based media, notably Löwenstein–Jensen (Jensen, 1932; Löwenstein, 1931) or Middlebrook media (Lorian, 1968). Some species need complex supplements; for instance, *Mycobacterium haemophilum* requires growth media to be supplemented with 0.4% hemoglobin or 60 µM hemin (Sompolinsky et al., 1978).

In most respects, the metabolism of mycobacteria is common to that of other bacteria, although some features are more typical of this genus than others. Glycerol is a common constituent of growth media since most mycobacterial species efficiently metabolize it to pyruvate; one exception is *Mycobacterium bovis*, which grows better in media containing pyruvate (Stonebrink, 1958). Similarly, asparagine is a valuable nitrogen source and is included as a constituent in most standard media used to cultivate mycobacteria. Acquisition of iron is vital to most bacteria and, in living tissues, successful competition with iron-binding proteins of the host may be a marker of pathogenicity. Unusually, mycobacteria possess two types of siderophores (iron-chelating agents): exochelins, which are secreted from the cell; and mycobactins, which are cell-wall-bound (Ratledge, 1984). Mycobactins are hydroxamate compounds produced by all mycobacteria, except *Mycobacterium avium* subsp. *paratuberculosis* (Bergey et al., 1923).

Genetics and whole-genome sequence analyses. One of the first micro-organisms subjected to whole-genome sequencing was *Mycobacterium tuberculosis* H_{37}Rv (Cole et al., 1998). Subsequently, the genomes of other members of the *Mycobacterium tuberculosis* complex and representatives of several other mycobacterial species have been, or are being, sequenced. However, despite this, the impact of whole-genome sequencing on the taxonomy of the genus *Mycobacterium* is currently limited and somewhat biased given the choice of strains. Strains have been selected for whole-genome sequencing in response to biological questions (Cole et al., 1998), pathogenicity determinants (Fleischmann et al., 2002), vaccine efficacy (Brosch et al., 2007), and in the search for novel antibiotic targets (Cole, 2002). The expectation that whole-genome sequences would provide clear-cut data for the construction of a taxonomic framework for mycobacteria has been undermined by the sheer complexity of the acquired data. Nevertheless, the availability of mycobacterial whole-genome sequences highlights changes which are shaping the diversity of these organisms. Rearrangement of gene content occurs due to the acquisition or loss of DNA through gene duplication and horizontal gene transfer, and the elimination of genes that do not give any selective advantage (Kuo and Ochman, 2009). These factors influence mycobacterial speciation (Cole et al., 2001; Garnier et al., 2003).

The importance of gene loss in virulence and host specificity is revealed in the genome of *Mycobacterium leprae* which, compared to that of *Mycobacterium tuberculosis*, has lost over 2000 genes with over 1000 being identified as pseudo-genes, that is, defective genes not yet completely deleted (Cole et al., 2001). Gene loss seems to be a factor in the evolution of *Mycobacterium tuberculosis* and in the ongoing evolution of host specificity in other members of the *Mycobacterium tuberculosis* complex (Garnier et al., 2003; Stinear et al., 2004, 2007).

The evolutionary bottleneck leading to *Mycobacterium tuberculosis* from an ancestral "*Mycobacterium canettii*" has been largely reconstructed from gene deletion analysis (Brosch et al., 2002). Progress in whole-genome sequencing of members of

the *Mycobacterium tuberculosis* complex and of "*Mycobacterium canettii*" will "add grist to the mill", as it has for unraveling the evolution of *Mycobacterium bovis* (Smith et al., 2006). Similarly, whole-genome sequence analysis suggests that *Mycobacterium avium* subsp. *avium*, *Mycobacterium avium* subsp. *paratuberculosis*, and *Mycobacterium avium* subsp. *silvaticum* are undergoing parallel evolutionary development from a different environmental/pathogenic precursor, "*Mycobacterium avium* subsp. *hominissuis*" (Li et al., 2005; Turenne et al., 2007). The latter is an environmental organism with a large genome and evidence of much genetic diversity. Two groups of specialist pathogens are evolving from this genetic pool, namely those of birds (*Mycobacterium avium* subsp. *avium*, *Mycobacterium avium* subsp. *silvaticum*) and of ruminants (*Mycobacterium avium* subsp. *paratuberculosis*). These organisms, like members of the *Mycobacterium tuberculosis* complex, show a pattern of phenotypic diversity and genome reduction, but high clonality and strong homology with the ancestral genome (Turenne et al., 2007).

A notable example of such genomic changes is provided by *Mycobacterium ulcerans*. The recent evolution and changing ecological niche and host specificity of this species is being driven by massive gene loss as the organism diverges from *Mycobacterium marinum* (Stinear et al., 2004; Stinear et al., 2007). *Mycobacterium ulcerans* has acquired the plasmid *p*mum001, the insertion sequences IS*2404* and IS*2606*, and the plasmid-encoded polyketide synthases required to synthesize the mycotoxin mycolactone (Stinear et al., 2004). These genomic elements are driving gene rearrangements, inactivation, and deletions so that the genome of *Mycobacterium marinum* contains 6.63 Mb and that of *Mycobacterium ulcerans* 5.63 Mb (Stinear et al. 2007, 2008). The novel genetic trait of mycotoxin biosynthesis is still being exploited and diversified in different lineages of *Mycobacterium marinum* and *Mycobacterium ulcerans* (Kaser et al., 2007; Stinear et al., 2005). About 90% of the protein-coding genes in *Mycobacterium ulcerans* are syntenic orthologs of the corresponding genes in *Mycobacterium marinum* with a mean identity of >98%; the 16S rRNA gene has a single base difference present in all strains and one other base differing in some strains (>99.8% similarity). Nevertheless, the two species are phenotypically distinct and give <40% similarity in DNA–DNA pairing analyses (Stinear et al., 2004; Stinear et al., 2007).

It can be concluded that whole-genome analyses are revealing taxonomic inconsistencies and the complexity of mycobacterial species. In general, the increasing availability of whole-genome sequences can be considered as a step towards a genomic taxonomy (Achtman and Wagner, 2008; Coenye et al., 2005).

Phage susceptibility. Bacteriophages lytic for many mycobacteria, including *Mycobacterium tuberculosis* strains, have been isolated by using soil enrichment techniques (Froman et al., 1954). Lysogenic (Russell et al., 1964) and plasmid-bearing (Crawford et al., 1981a) mycobacteria have been found in nature. Many investigators have tried to use phage typing as a taxonomic tool, but poor inter-laboratory reproducibility and the ability of phages to cross species lines have limited its value. However, phage patterns within the species *Mycobacterium tuberculosis* have been found useful for epidemiological studies (Baess, 1969; Bates and Mitchison, 1969; Jones et al., 1982). Bacteriocins have been detected in mycobacteria and used in the classification of some species (Takeya and Tokiwa, 1972).

Antigenic structure. Although now seldom used, antigenic analyses have proven valuable at several hierarchical levels. Ribosomal proteins are conserved within the genus and immunodiffusion analyses have been used to compare members of the genus *Mycobacterium* with those of closely allied taxa (Ridell et al., 1979). Analyses of immunodiffusion and immunoelectrophoretic patterns of whole-cell filtrates and extracts have provided useful data for defining species within the genus (Chaparas et al., 1978a; Norlin, 1965; Stanford and Grange, 1974), as have quantitative comparisons of skin hypersensitivity reactions of sensitized guinea pigs to crude culture filtrates of homologous and heterologous mycobacteria (Magnusson, 1980). Seroprecipitation studies of mycobacterial catalase have been used to establish immunological distances between species, as an indirect measure of evolutionary divergence (Wayne and Diaz, 1982). Whole-cell seroagglutination reactions, based on peptidoglycolipid surface antigens, usually differentiate strains at the infra-subspecific level (Brennan, 1981; Schaefer, 1967), though some species appear to comprise single serovars (Wayne, 1971).

Antibiotic or drug susceptibility. In the absence of genomic mutations conferring resistance, *Mycobacterium tuberculosis* is susceptible to a range of chemotherapeutic agents. Though all populations of *Mycobacterium tuberculosis* contain some mutant organisms, it is generally held that a strain is susceptible to a particular chemotherapeutic agent when such mutants comprise less than 1% of all cells present in an *in vitro* culture. Conversely, when the proportion of mutants is found to be greater than 1%, these strains will become dominant if the drug concerned is used in therapy (Middlebrook and Cohn, 1953). In order to ensure effectiveness of therapy and to reduce the duration of treatment, chemotherapy of tuberculosis is based on the use of combinations of several drugs, to which the strain is known to be susceptible, usually delivered in a two-phase strategy (Fox, 1968; Mitchinson and Dickinson, 1978).

Standard treatment will commonly involve the chemotherapeutic agents isoniazid and rifampin, supplemented with pyrazinamide and either ethambutol or streptomycin during the initial intensive phase. As a result, *in vitro* susceptibility testing of isolated strains is usually focused on these agents. However, a number of issues may force modifications of drug regimes in resource-poor countries (International Union Against Tuberculosis and Lung Disease, 1988, 1993). These factors, together with the development of drug resistance in some strains, mean that other chemotherapeutic agents (e.g. WHO quinolones and thiacetazone) may occasionally be tested (Shimao, 1998). Additionally, species of mycobacteria other than those encompassed in the *Mycobacterium tuberculosis* complex (non-tuberculous mycobacteria) may be implicated in disease and require specific drug treatments. Although standard anti-tuberculosis chemotherapeutic agents are still used in the treatment of infections due to non-tuberculous mycobacteria, when tested *in vitro* against these agents these species are often found to be resistant. However, synergistic activity can sometimes be detected between combinations of agents (Banks and Jenkins, 1987). Furthermore, conventional antibiotics such as macrolides may prove to have activity against some non-tuberculous mycobacteria, notably rapid-growing species (Campbell et al., 1998).

Pathogenicity. The genus includes obligate and opportunistic parasites and saprophytes. All mycobacteria produce

granulomatous lesions in experimental animals if a sufficiently large inoculum is used (Wayne and Kubica, 1986). However, only some species grow in host tissues and produce progressive or self-limiting disease. Individual species may be pathogenic for cold-blooded animals, mammals, and/or birds. The site of infection varies with the mycobacterial species, some show a predilection for internal organs, especially lungs, and others are less invasive and cause skin surface lesions. Major disease syndromes include leprosy and tuberculosis.

Ecology. Most mycobacterial species are free-living in soil and water. However, the major ecological niche for some mycobacteria is diseased tissue of warm-blooded hosts. Although most human infections are due to either *Mycobacterium leprae* or *Mycobacterium tuberculosis*, it has long been recognized that members of other mycobacterial species may be opportunistic pathogens of humans. This is particularly so in immunocompromised individuals. Many animals, including aquatic, bird, and surface-dwelling species, may be hosts to mycobacterial species.

Enrichment and isolation procedures

The presence of a mixed flora in clinical samples, soil, water, and some other environmental sources means that the isolation of mycobacteria requires sample decontamination. This is attempted with agents that selectively kill non-acid-fast contaminants. Suitable agents include dilute acids or alkalis, with or without quaternary ammonium compounds, followed by neutralization. Time of exposure to decontaminating agents must be carefully controlled, as resistance of mycobacteria is not absolute (Krasnow and Wayne, 1966). Decontaminated specimens are inoculated onto agar media, inspissated egg media, or into liquid culture media; media may also be supplemented with selective antimicrobial agents. Although now rarely applied, an alternative method is to inject liquefied specimens into guinea pigs or mice; pure cultures can be obtained from their internal organs after 2–3 weeks.

Maintenance procedures

Strains will remain viable on egg medium held at 5°C for many months and will survive indefinitely at −70°C or after lyophilization. Most species are readily subcultured on inspissated egg medium, Dubos' oleic acid-albumin, or Middlebrook 7H10 or 7H11 agar, and in liquid media containing Tween 80 for dispersal and bovine serum albumin to neutralize traces of oleic acid. Laboratory-adapted strains often grow well on synthetic media containing asparagine, glycerol, and mineral salts.

Taxonomic comments

The taxonomic history of the genus *Mycobacterium* is difficult to disentangle from that of related taxa, notably from the genera *Corynebacterium*, *Nocardia*, and *Rhodococcus* (Bousfield and Goodfellow, 1976). The intricacies of the pioneering studies on these genera can be gleaned from one or more comprehensive review articles (Barksdale and Kim, 1977; Goodfellow and Magee, 1998; Goodfellow and Minnikin, 1977, 1984).

Early work on the taxonomy of mycobacteria was dominated by clinical interest, which promoted a tendency to see different types of mycobacteria in terms of their relationships with *Mycobacterium tuberculosis*. This unfortunate, but understandable, skew was reflected in the common use of the term "atypical mycobacteria" for strains that could not be identified as either *Mycobacterium bovis* or *Mycobacterium tuberculosis*. Indeed, it was not until the mid-1950s that a much-needed classification was introduced for mycobacteria by Ernest Runyon and his colleagues (Runyon, 1958, 1959; Timpe and Runyon, 1954). In Runyon's classification, mycobacteria, with the exception of those in the *Mycobacterium tuberculosis* complex and members of uncultivable taxa, were divided into four overtly artificial groups based on growth rates and pigmentation properties. Runyon's Groups I, II, and III were composed of slow-growing strains of mycobacteria, with Group IV containing the rapid growers. The acceptance of Runyon's Groups by the clinical community was a defining moment in the development of mycobacterial systematics, one brilliantly captured in a review article by Wayne (2000).

The seminal studies of Runyon and his colleagues paved the way for more exacting taxonomic investigations as it was soon realized that Runyon's Groups were heterogeneous. Ruth Gordon and her colleagues (Gordon and Mihm, 1959; Gordon and Smith, 1953, 1955) classified rapid-growing mycobacteria not on the basis of a few subjectively weighted phenotypic properties but on overall similarities, in essence, a numerical taxonomic approach without the use of computers. However, initial attempts to classify slow-growing mycobacteria using standard phenotypic tests merely reaffirmed the limited differences afforded by Runyon's Groups I to III.

Wayne (1967) broke the impasse outlined above by introducing a portfolio of phenotypic tests which were suitable for the classification of slow-growing mycobacteria. He went on to be the motive force behind a series of studies designed to clarify relationships between both established and novel mycobacterial species, and to highlight properties for the recognition of clinically significant slow-growing species. A coordinated series of well-planned taxonomic studies carried out under the auspices of the International Working Group on Mycobacterial Taxonomy (IWGMT) led to significant improvements in the classification of fast-growing (Goodfellow et al., 1974; Kubica et al., 1972) and slow-growing mycobacteria (Meissner et al., 1974; Wayne et al., 1971, 1978, 1981, 1983, 1989, 1991). Phenotypic data acquired in some of these investigations were used in subsequent IWGMT studies to produce a panel of reproducible tests (Wayne et al., 1974, 1976) for the generation of frequency matrices designed to facilitate the accurate identification of clinically significant slow-growing mycobacteria (Wayne et al., 1980).

The use of a combination of taxonomic criteria in the IWGMT studies was instrumental in placing mycobacterial systematics on a sound footing, notably for the benefit of the clinical community (Wayne, 2000). Nevertheless, several slow-growing mycobacterial taxa remained ill-defined, a problem which was addressed in the final IWGMT study (Wayne et al., 1996). In this investigation, slow-growing mycobacteria that did not fit into any of the species that had been adequately characterized in earlier IWGMT studies were analyzed using a combination of chemotaxonomic and molecular systematic procedures, including 16S rRNA gene sequencing and DNA–DNA pairing studies. In general, good overall agreement was

found between the resultant phenotypic clusters and groups of organisms circumscribed using the molecular procedures. Wayne and his colleagues concluded that polyphasic taxonomy provided a satisfactory way to resolve relationships between members of the genus *Mycobacterium*.

Polyphasic taxonomy is now universally practiced in the delineation of novel *Mycobacterium* species. The taxonomic relationships of both putatively novel and poorly classified mycobacteria can be determined readily by comparing their 16S rRNA gene sequences with those of corresponding mycobacterial species held in 16S rRNA gene databases. Indeed, many recent novel *Mycobacterium* species have been delineated using 16S rRNA gene sequence and associated phenotypic data, as exemplified by proposals for *Mycobacterium bohemicum* (Reischl et al., 1998), *Mycobacterium elephantis* (Shojaei et al., 2000), and *Mycobacterium novocastrense* (Shojaei et al., 1997).

16S rRNA molecules from members of closely related mycobacterial taxa may be so conserved that they cannot be used to differentiate between them at the species level. In such instances, DNA–DNA pairing remains the method of choice as it gives a higher resolution than corresponding 16S rRNA gene sequencing data (see Adékambi et al., 2006a). Kusunoki and Ezaki (1992) used a number of DNA–DNA pairing techniques to clarify the taxonomy of members of the *Mycobacterium fortuitum* complex. These and associated studies led to the proposal to reinstate *Mycobacterium peregrinum* as a distinct species and for *Mycobacterium chelonae* subsp. *abscessus* to be given species status as *Mycobacterium abscessus*.

The fact that DNA–DNA pairing studies tend to be hampered by technical difficulties led some workers to clarify relationships between closely related taxa by using sequence data generated from genes less well conserved than the 16S rRNA gene. The description of *Mycobacterium conceptionense*, for example, was to a considerable extent based on the results of a partial *rpoB* gene sequence analysis which allowed this species to be clearly separated from *Mycobacterium porcinum*, its nearest phylogenetic neighbor (Adékambi et al., 2006c). Similarly, *rpoB* sequence data proved to be invaluable in distinguishing *Mycobacterium aubagnense* and *Mycobacterium phocaicum* from one another and from their phylogenetic neighbor *Mycobacterium mucogenicum* (Adékambi et al., 2006a), as well as delineating *Mycobacterium salmoniphilum* from *Mycobacterium chelonae* (Whipps et al., 2007). Closely related taxa have also been distinguished using ITS sequences, as in the case of proposals for the recognition of *Mycobacterium heckeshornense* (Roth et al., 2000) and *Mycobacterium monacense* (Reischl et al., 2006), and by using a multigene approach as applied to the classification of *Mycobacterium doricum* by Devulder et al. (2005).

The increasingly varied phylogenetic approaches that are being adopted for the circumspection of mycobacterial species makes comparison between them difficult, especially in instances where inadequate phenotypic data are provided. Such *ad hoc* approaches, which run counter to the IWGMT philosophy, risk eroding the road to taxonomic consensus that has prevailed in recent times. One way forward would be to update the minimal standards for the description of novel slow-growing *Mycobacterium* species (Lévy-Frébault and Portaels, 1992) and for the introduction of corresponding minimal standards for the recognition of fast-growing *Mycobacterium* species. To such ends, key phenotypic characteristics should be acquired from procedures for determining growth rate, growth temperature (both range and optimum), colony morphology, pigmentation (type and color), and, for rapid-growers, NaCl tolerance and growth on MacConkey agar. Analyses of mycolic acids by HPLC and/or TLC also provide valuable taxonomic data, as do fatty acid analyses (Goodfellow and Magee, 1998). Guidance is also needed on which molecular systematic approaches should be recommended in addition to 16S rRNA gene sequencing.

Differentiation of the genus *Mycobacterium* from other genera

The genus *Mycobacterium* can be differentiated from other mycolic acid-containing genera using a combination of chemotaxonomic and morphological markers (see section on the order *Corynebacteriales*) and by the possession of unique 16S rRNA nucleotide signatures (see below). In addition, the type strains of most *Mycobacterium* species can be distinguished by their 16S rRNA gene sequences and phylogeny as seen in Figure 86 and Figure 87.

Differentiation of species within the genus *Mycobacterium*

Very few phenotypic characters underpin the separation of rapid- and slow-growing mycobacteria. Differences in resistance to decolorization by alkali of bacilli stained with neutral red suggest variation in lipid composition between rapid- and slow-growing species; slow-growers showing greater resistance (Wayne, 1959). Similarly, acid–alcohol-fastness after staining with basic fuchsin (see below) tends to be a stronger attribute in slow-growing mycobacteria. The uptake of iron from an iron-rich medium is more likely to be a property of rapid-growing species (Wayne, 1967), as is growth in the presence of 5% (w/v) NaCl (Kubica et al., 1970) and 0.2% (w/v) picrate (Wayne and Kubica, 1986). None of these characteristics are exclusive markers of growth rate, but practical experience has demonstrated that different sets of tests are necessary for characterizing rapid- and slow-growing mycobacterial species. Members within the two groups can be distinguished using a combination of phenotypic properties (Table 37 and Table 38).

Phylogenetic trees were constructed using 16S rRNA gene sequence data (Figure 86 and Figure 87). Wherever possible, sequence data were taken from original species descriptions. However, as emphasized by Stackebrandt et al. (2002), high quality rRNA gene sequence data are needed to determine phylogenetic relationships; hence, in some cases, high quality sequence data were taken not from the original study but from a subsequent one. Nevertheless, sequences derived from type strains were used for all but a few species. For the species, *Mycobacterium haemophilum*, *Mycobacterium sphagni*, and *Mycobacterium ulcerans*, type strain sequences were all of short lengths. In these cases longer sequences derived from representative strains were validated by matching against the available sequence data of the type strain, and then used in the generation of phylogenetic trees. Two sequences were available for the type strain of *Mycobacterium peregrinum*; the sequence selected was that which best matched the phylogeny to the phenotypic characteristics. The sequence data were aligned using CLUSTAL X (Thompson

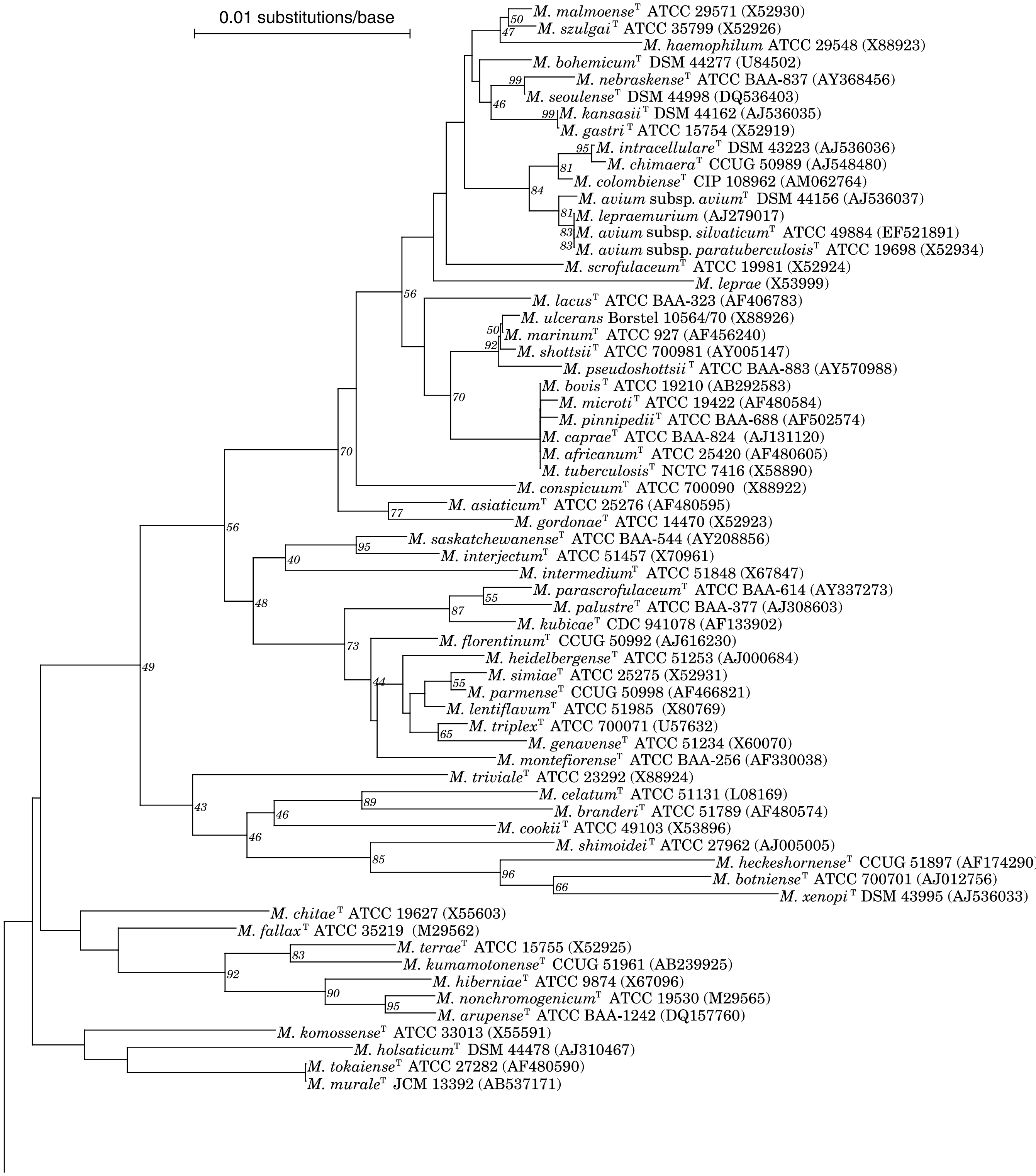

FIGURE 86. Phylogenetic tree generated using Jukes and Cantor (1969) similarities and the neighbor-joining algorithm (Saitou and Nei, 1987) using SEAView v4 (Gouy et al., 2010) and Dendroscope v2.74 (Huson et al., 2007); regions with gaps excluded. Bootstrap values were calculated from 1000 iterations.

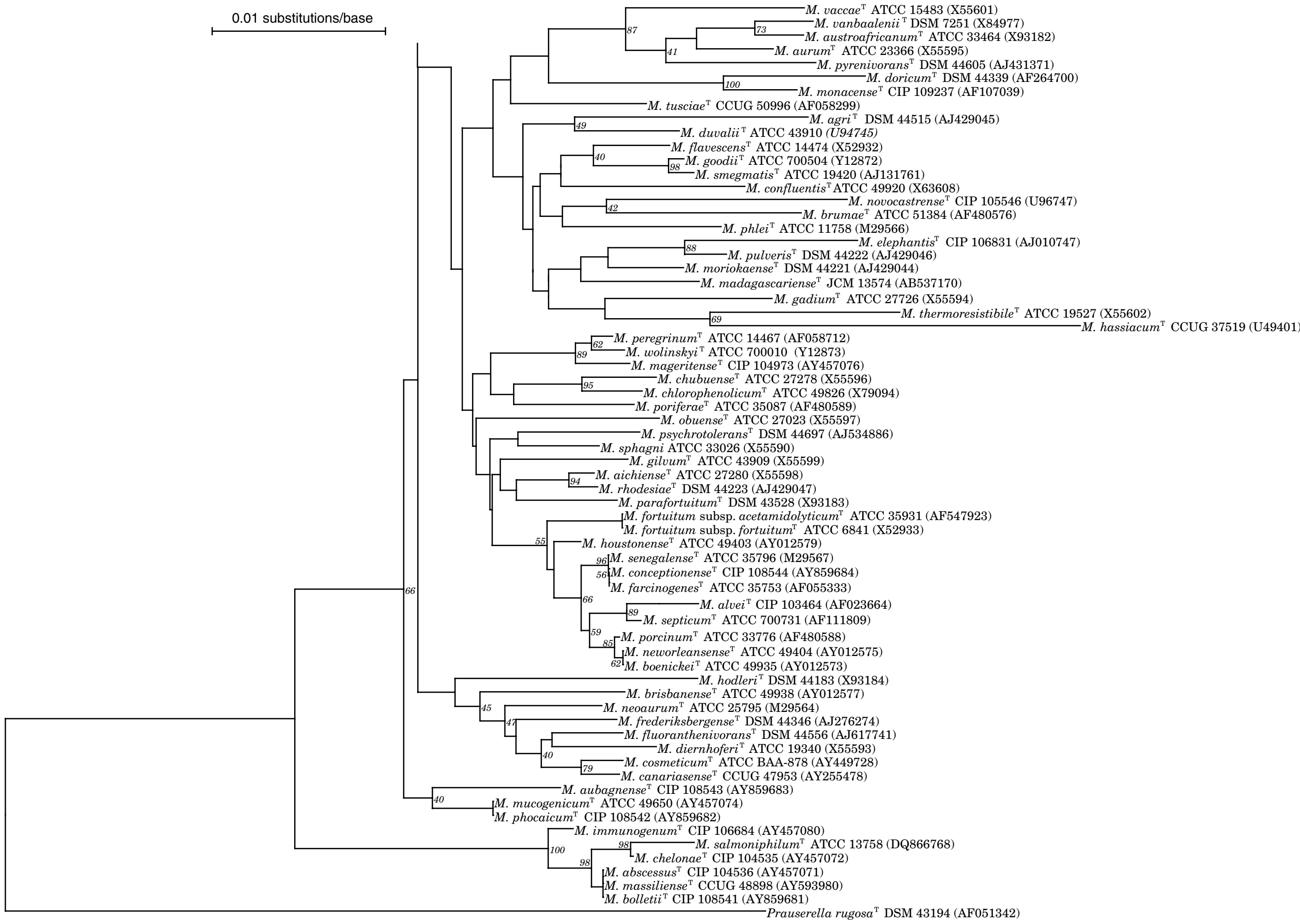

FIGURE 86. (continued)

et al., 1997) and MUSCLE (Edgar, 2004) software and the alignments compared and manually edited taking secondary structure into account by using SEAView v4 (Gouy et al., 2010). Jukes and Cantor (1969) similarities were calculated and phylogenetic trees were generated using the neighbor-joining algorithm (Saitou and Nei, 1987) in SEAView (Gouy et al., 2010); regions with gaps were excluded. Bootstrap values were calculated from 1000 iterations.

Accession numbers for the sequences used in generating the phylogenetic trees are shown in the species descriptions and in Figure 86 and Figure 87 with the exception of those species named above (where type strain sequences are given in the species descriptions but the sequence used is noted in the Figures). A series of phylogenetic trees were generated using different treeing algorithms; a high level of agreement being found between these analyses. 16S rRNA gene sequence data are almost invariably presented as linear dendrograms thereby making it difficult to see detached relationships between species.

The species descriptions which follow are organized into clades based on a consensus of distance (Jukes and Cantor, 1969; Kimura, 1980), parsimony (Fitch, 1977) and maximum likelihood (Felsenstein and Churchill, 1996) trees and phenotypic properties. This allowed the lists of phenotypic characters in Table 37 and Table 38 to be displayed in related groups.

Acknowledgements

A great debt is due to Laurence G. Wayne and George P. Kubica for their classification of the family *Mycobacteriaceae* in the last edition of *Bergey's Manual*. It is testimony to their expertise and forethought that many aspects of their work have survived in the current treatment of the genus.

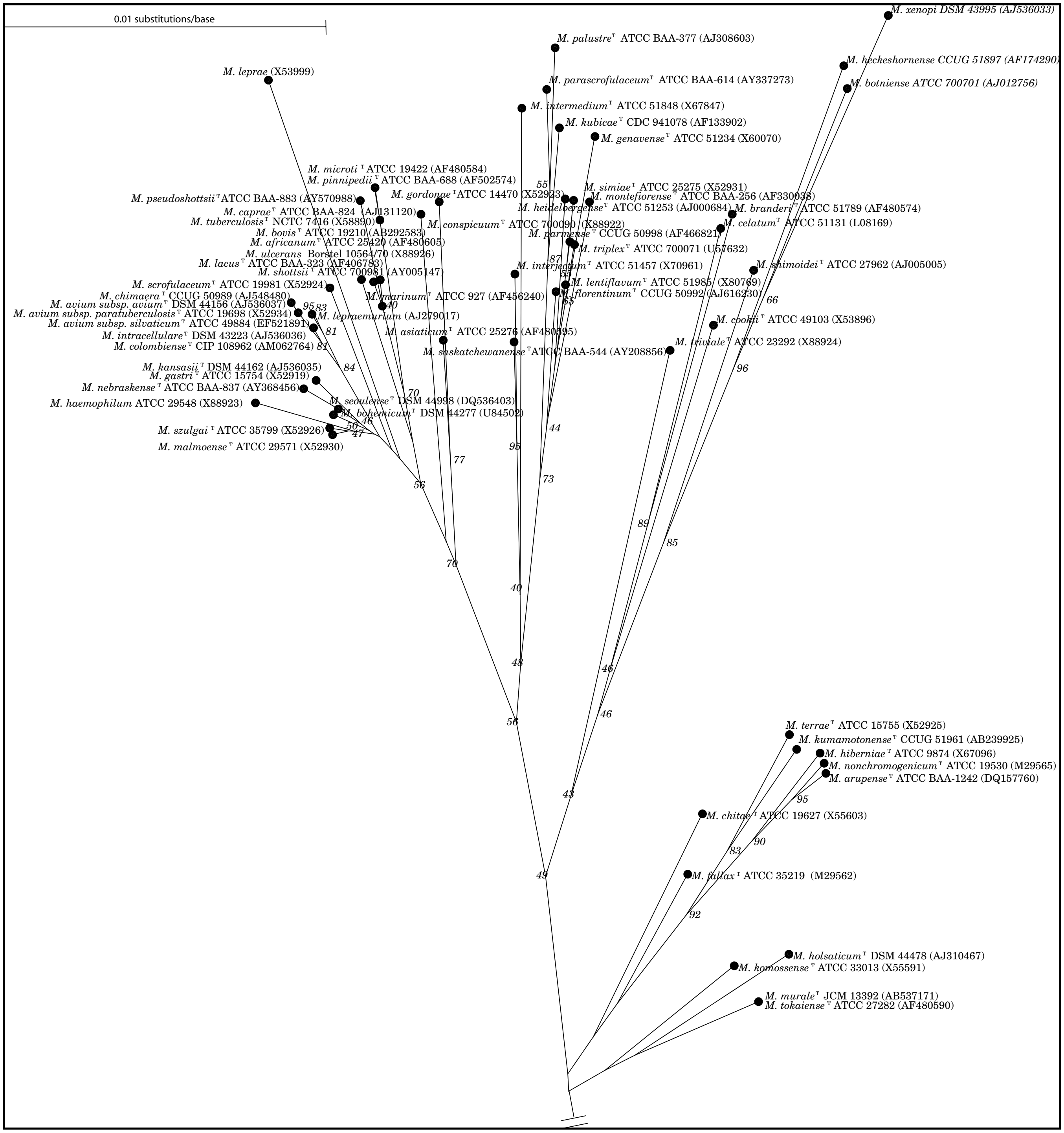

FIGURE 87. Phylogenetic tree generated as for Figure 86, but displayed as a radial tree.

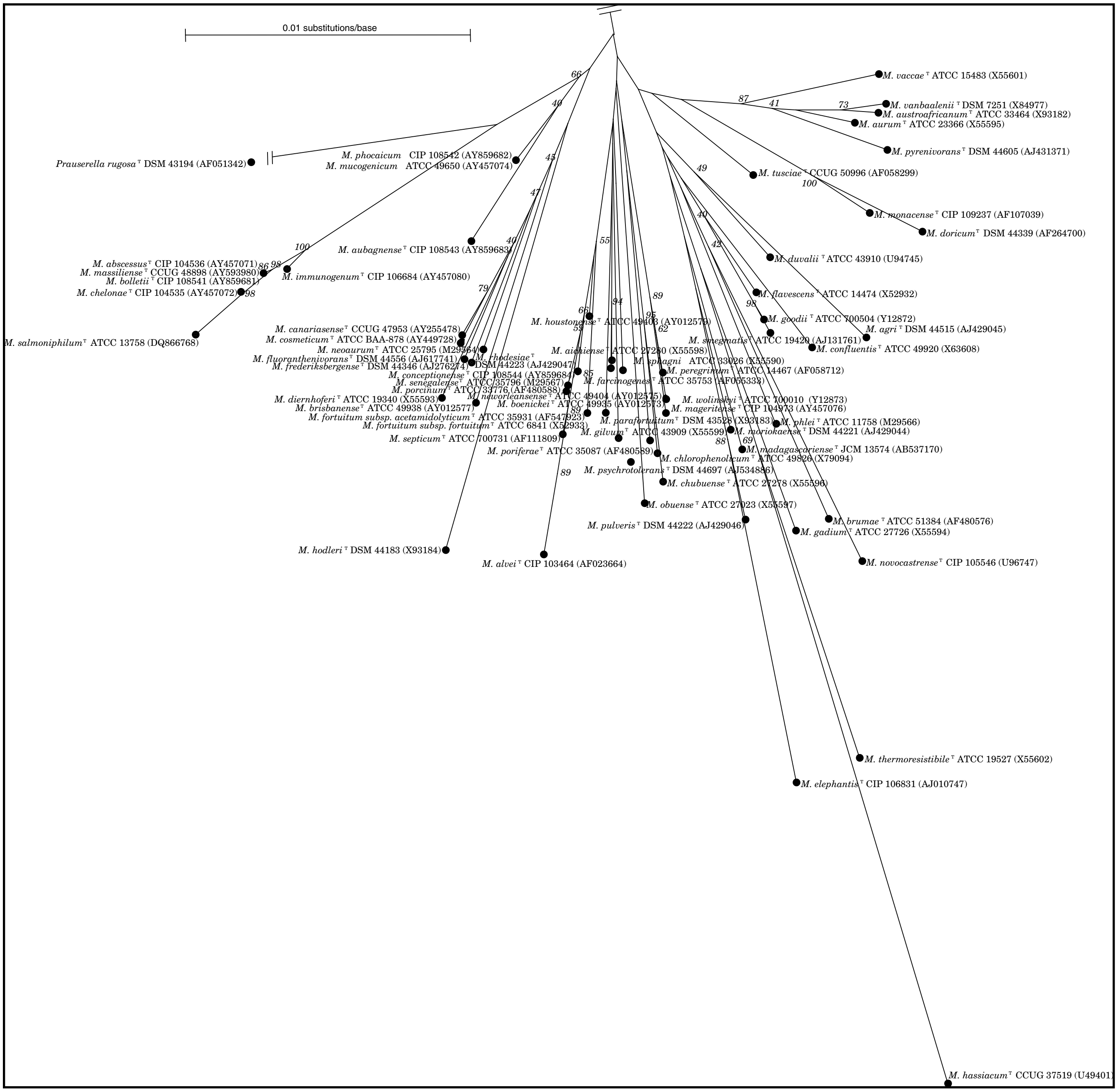

FIGURE 87. (continued)

Further reading

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TABLE 37. Differential properties of slowly growing *Mycobacterium* species[a]

Character	*M. tuberculosis*	*M. bovis*	*M. africanum* subtype I	*M. africanum* subtype II	*M. caprae*	*M. microti*	*M. pinnipedii*	*M. haemophilum*	*M. malmoense*	*M. bohemicum*	*M. kansasii*	*M. gastri*	*M. nebraskense*	*M. avium* subsp. *avium*	*M. avium* subsp. *paratuberculosis*	*M. avium* subsp. *silvaticum*	*M. intracellulare*	*M. chimaera*	*M. colombiense*	*M. ulcerans*	*M. marinum*	*M. shottsii*	*M. pseudoshottsii*	*M. gordonae*	*M. asiaticum*	*M. interjectum*	*M. saskatchewanense*	*M. palustre*	*M. parascrofulaceum*	*M. kubicae*
Test:																														
Growth at 22°C	–	–	–	–	–	–	–	nd	v	+	+	–	nd	v	–	–	+	–	+	nd	nd	+	+	+	–	–	nd	–	nd	–
Growth at 25°C	–	–	–	–	–	–	–	+	v	+	+	+	+	v	–	–	+	+	+	w	+	+	+	+	nd	–	+	–	+	–
Growth at 42°C	–	–	–	–	–	–	–	–	–	–	v	–	–	+	+	+	v	–	–	–	–	–	–	–	nd	–	–	+	–	–
Growth at 45°C	–	–	–	–	–	–	–	–	–	–	–	–	–	v	nd	nd	v	–	–	–	–	–	–	–	nd	–	–	d	–	–
Pigmentation	N	N	N	N	N	N	N	N	N	S	P	N	S	N	N	N	N	N	N	–/S	P	N	P	S	P	S	S	S	S	S
Growth on 5% (w/v) NaCl	–	–	–	–	–	–	nd	–	nd	–	–	–	–	d	–	–	–	–	–	–	nd	–	–	–	–	nd	–	nd	–	nd
Nitrate reduction	+	–	+	–	–	d	–	–	–	–	+	–	–	–	–	–	–	–	v	–	–	–	–	–	–	–	–	d	–	–
Niacin	+	–	+	–	–	+	v	–	–	–	–	–	nd	–	–	–	–	–	–	d	–	+	+	–	–	–	nd	nd	–	nd
Tellurite reduction (9 d)	+	+	nd	nd	nd	nd	nd	–	+	nd	–	–	nd	+	nd	+	+	nd	nd	nd	v	nd	nd	nd	nd	nd	d	nd	nd	nd
Growth stimulation by pyruvate	–	+	–	+	+	nd	+	nd	nd	nd	nd	nd	nd	d	+	–	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Arylsulfatase (3 d)	–	–	nd	nd	–	nd	nd	–	–	–	nd	nd	–	–	–	–	nd	–	v	–	–	–	–	–	nd	–	–	–	–	–
Arylsulfatase (7 d)	–	–	nd	nd	–	nd	nd	nd	–	–	nd	nd	nd	–	–	–	nd	nd	v	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Arylsulfatase (10 d)	–	d	nd	nd	–	nd	nd	nd	–	–	+	+	nd	d	–	–	+	nd	v	–	+	nd	nd	d	+	w	nd	d	nd	nd
Acid phosphatase	+	+	+	d	+	nd	nd	–	–	–	–	+	nd	–	–	–	–	nd	–	d	+	d	d	d	nd	–	nd	+	–	nd
Catalase	nd	nd	nd	nd	+	nd	nd	–	+	+	+	nd	nd	nd	nd	nd	nd	nd	+	nd	nd	nd	nd	nd	+	+	nd	nd	nd	nd
Catalase (68°C)	–	–	–	–	–	–	nd	–	d	+	+	–	+	d	d	+	+	+	+	+	–	d	d	+	+	+	+	nd	+	nd
Catalase (semi-quantitative)	–	–	–	–	nd	–	nd	–	–	–	+	–	–	–	–	–	–	–	nd	nd	d	–	–	+	+	nd	d	–	nd	+
Tween 80 hydrolysis	d	–	nd	nd	w	nd	nd	–	+	–	+	+	v	–	v	–	–	–	–	–	+	–	–	+	+	–	+	+	–	+
Urea hydrolysis	+	+	+	–	v	+	nd	–	v	+	+	+	–	–	–	–	–	–	+	–	+	+	+	d	–	+	–	+	+	–
Pyrazinamidase	+	–	+	–	–	+	nd	+	+	d	d	–	–	+	nd	nd	+	nd	nd	–	+	–	–	–	d	+	–	+	+	nd
α-Esterase	+	+	d	d	nd	+	nd	nd	w	–	–	–	nd	+	nd	nd	+	nd	nd	–	–	nd	nd	+	+	+	nd	nd	nd	nd
β-Esterase	nd	nd	nd	nd	nd	nd	nd	nd	w	–	–	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	+	+	–	nd	nd	nd	nd
β-Galactosidase	nd	nd	nd	nd	nd	nd	nd	–	nd	–	–	nd	nd	–	nd	nd	–	nd	nd	–	–	nd	nd	–	–	v	nd	–	nd	nd
Resistance to:																														
Hydroxylamine (500 mg/l)	–	–	–	–	–	–	nd	nd	nd	nd	–	–	nd	nd	nd	nd	nd	nd	nd	–	+	–	–	nd	+	nd	nd	nd	nd	nd
Ethambutol (2 mg/l)	–	–	–	–	nd	nd	nd	+	–	nd	–	–	nd	+	+	+	+	+	nd	nd	nd	nd	nd	nd	nd	+	d	nd	d	nd
Isoniazid (1 mg/l)	–	–	–	–	nd	–	nd	+	+	nd	+	–	nd	+	+	+	+	nd	nd	+	d	+	+	nd	nd	+	+	nd	+	–
Rifampin (32 mg/l)	nd	nd	nd	nd	nd	nd	nd	nd	+	nd	–	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	–	–	nd	–	nd
Streptomycin (8 mg/l)	nd	nd	nd	nd	nd	nd	nd	+	+	nd	+	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	+	–	nd	d	+

(continued)

TABLE 37. (continued)

Character	*M. simiae*	*M. florentinum*	*M. heidelbergense*	*M. triplex*	*M. montefiorense*	*M. genavense*	*M. parmense*	*M. lentiflavum*	*M. celatum*	*M. branderi*	*M. xenopi*	*M. heckeshornense*	*M. botniense*	*M. terrae*	*M. kumamotonense*	*M. hiberniae*	*M. arupense*	*M. nonchromogenicum*	*M. lepraemurium*	*M. szulgai*	*M. seoulense*	*M. scrofulaceum*	*M. conspicuum*	*M. lacus*	*M. intermedium*	*M. cookii*	*M. triviale*	*M. tusciae*	*M. shimoidei*
Test:																													
Growth at 22°C	+	nd	–	–	nd	nd	nd	+	–	nd	–	–	–	+	nd	+	+	+	nd	+	nd	+	+	nd	+	+	nd	nd	nd
Growth at 25°C	+	+	–	–	+	w	+	+	–	+	–	–	–	+	+	+	+	+	nd	+	+	+	+	w	+	+	+	+	nd
Growth at 42°C	d	–	–	–	–	+	–	–	+	+	+	+	+	–	+	–	–	d	–	–	nd	+	–	+	nd	–	–	–	+
Growth at 45°C	–	–	–	–	–	nd	–	–	+	+	+	+	+	–	nd	–	–	–	–	–	–	–	–	nd	–	–	–	–	d
Pigmentation	P	N	N	N	N	N	S	S	N	N	S	S	S	N	N	S	N	N	N	S/P	S	S	S	N	P	S	N	S	N
Growth on 5% (w/v) NaCl	–	–	–	–	–	nd	–	nd	–	nd	–	–	nd	–	–	nd	–	–	–	–	–	–	–	–	nd	nd	+	–	–
Nitrate reduction	–	v	–	+	–	–	–	–	–	–	–	–	–	d	+	+	–	–	–	+	+	–	–	+	–	–	+	+	–
Niacin	+	–	–	–	–	–	–	–	nd	–	–	–	nd	–	nd	nd	–	–	–	–	nd	–	–	–	–	nd	–	–	–
Tellurite reduction (9 d)	nd	+	nd	nd	nd	nd	–	nd	+	nd	nd	nd	nd	+	nd	nd	nd	nd	nd	nd	+	nd	nd	+	nd	nd	–	v	nd
Growth stimulation by pyruvate	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Arylsulfatase (3 d)	–	–	–	–	–	–	–	–	+	nd	v	–	–	nd	–	nd	–	nd	–	nd	–	–	+	w	+	+	v	–	nd
Arylsulfatase (7 d)	–	–	nd	nd	–	–	nd	–	+	nd	nd	–	nd	nd	nd	nd	nd	nd	–	nd	–	nd	+	w	+	+	v	nd	nd
Arylsulfatase (10 d)	–	–	nd	nd	–	–	nd	–	+	nd	+	–	+	d	nd	+	nd	+	–	d	–	d	+	w	+	+	d	nd	nd
Acid phosphatase	–	nd	–	nd	nd	nd	nd	nd	nd	nd	+	nd	–	+	+	+	nd	+	–	+	nd	–	d	w	+	+	nd	+	+
Catalase	+	v	–	nd	nd	nd	nd	nd	nd	nd	+	nd	nd	nd	nd	nd	nd	nd	+	nd	nd	+	nd	nd	+	+	nd	nd	nd
Catalase (68°C)	+	nd	+	+	+	+	+	+	+	nd	+	+	–	+	nd	nd	+	+	–	+	+	+	+	–	+	nd	+	+	+
Catalase (semi-quantitative)	–	v	nd	+	–	+	–	v	+	–	–	–	–	+	nd	nd	nd	+	–	+	nd	+	–	–	nd	nd	+	–	–
Tween 80 hydrolysis	–	–	+	–	–	–	+	–	–	–	–	–	–	+	+	d	+	+	–	d	–	–	+	w	+	–	+	+	+
Urea hydrolysis	+	+	+	+	–	+	+	–	–	–	–	–	–	–	–	–	–	–	–	d	–	+	–	+	+	–	–	+	–
Pyrazinamidase	d	nd	+	nd	nd	+	nd	v	+	nd	+	–	+	–	nd	–	–	+	+	+	+	d	–	nd	–	–	–	nd	+
α-Esterase	+	nd	nd	nd	nd	nd	nd	nd	nd	nd	+	nd	nd	d	nd	nd	nd	–	nd	d	nd	d	nd	nd	+	nd	nd	nd	nd
β-Esterase	+	nd	nd	nd	nd	nd	nd	nd	nd	nd	+	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	d	nd	nd	d	nd	nd	nd	nd
β-Galactosidase	–	nd	nd	nd	nd	nd	nd	nd	nd	nd	–	nd	–	+	nd	nd	nd	+	nd	nd	nd	–	–	nd	+	nd	–	–	nd
Resistance to:																													
Hydroxylamine (500 mg/l)	+	+	nd	nd	nd	nd	–	nd	nd	nd	–	nd	nd	+	nd	nd	nd	+	–	–	nd	+	+	nd	nd	nd	nd	nd	–
Ethambutol (2 mg/l)	+	nd	–	+	nd	+	–	+	nd	–	+	nd	nd	–	nd	–	–	–	nd	nd	nd	–	–	–	–	–	nd	+	nd
Isoniazid (1 mg/l)	+	+	–	+	nd	+	+	+	+	+	–	+	nd	+	nd	+	nd	+	nd	nd	nd	+	+	–	+	–	+	nd	+
Rifampin (32 mg/l)	v	nd	–	nd	nd	–	–	+	nd	+	nd	nd	nd	nd	nd	+	+	nd	nd	nd	nd	–	–	–	–	–	nd	–	nd
Streptomycin (8 mg/l)	+	nd	–	+	nd	–	–	+	–	–	–	nd	nd	nd	nd	+	+	nd	nd	nd	nd	+	–	d	+	–	nd	–	nd

[a]Symbols: +, 90% or more of strains are positive; –, 90% or more of strains are negative; d, 11–89% of strains are positive; v, variability of reaction of strains within a given species; w, weak reaction; N, non-chromogenic; S, scotochromogenic; P, photochromogenic. nd, Not data available.

TABLE 38. Differential properties of rapidly growing *Mycobacterium* species[a]

Character	*M. chitae*	*M. fallax*	*M. komossense*	*M. aichiense*	*M. holsaticum*	*M. murale*	*M. tokaiense*	*M. austroafricanum*	*M. vanbaalenii*	*M. vaccae*	*M. aurum*	*M. pyrenivorans*	*M. doricum*	*M. monacense*	*M. flavescens*	*M. novocastrense*	*M. duvalii*	*M. agri*	*M. thermoresistibile*	*M. smegmatis*	*M. goodii*	*M. phlei*	*M. brumae*	*M. confluentis*	*M. elephantis*	*M. pulveris*	*M. peregrinum*	*M. wolinskyi*	*M. mageritense*	*M. sphagni*	*M. chlorophenolicum*	*M. chubuense*	*M. poriferae*	*M. psychrotolerans*
Test:																																		
Growth at 42°C	–	nd	nd	nd	–	nd	nd	–	d	+	nd	–	–	+	+	+	nd	+	nd	+	nd	+	nd	–	+	+	–	nd	+	–	–	nd	–	–
Growth at 45°C	–	–	–	–	–	–	–	–	–	–	–	–	–	+	–	–	–	+	+	+	+	+	–	–	+	d	–	+	d	–	–	–	–	–
Pigmentation	N	N	S	S	v	S	S	S	S	P	S	S	S	S	S	P	S	N	N	N	N*	S	N	N	N	N	N	N	N	S	S	S	S	S
Growth on MacConkey	–	–	–	nd	–	–	nd	nd	nd	–	nd	nd	nd	–	nd	+	nd	–	nd	–	+	–	–	nd	–	nd	+	+	+	–	nd	nd	nd	–
Growth on 5% (w/v) NaCl	+	–	–	nd	+	–	nd	nd	nd	d	nd	nd	–	+	nd	+	nd	nd	nd	+	+	+	nd	–	+	nd	+	+	+	–	nd	nd	+	+
Nitrate reduction	+	+	–	–	+	–	–	+	+	+	–	d	+	+	nd	+	+	+	+	+	+	+	+	+	+	+	+	+	d	+	–	+	–	+
Iron uptake	v	–	+	nd	–	nd	nd	nd	nd	+	nd	nd	nd	nd	nd	–	nd	–	nd	+	+	+	+	–	nd	nd	+	+	+	–	nd	nd	+	nd
Arylsulfatase (3 d)	–	–	–	+	–	+	+	+	+	d	–	nd	–	–	nd	–	–	d	–	–	–	–	–	–	–	nd	+	–	+	–	d	–	–	nd
Arylsulfatase (7 d)	+	nd	nd	+	nd	+	+	+	+	+	nd	nd	nd	nd	nd	–	–	nd	nd	nd	nd	nd	nd	–	–	nd	+	nd	+	nd	nd	+	–	nd
Acid phosphatase	+	nd	+	+	+	nd	–	–	nd	–	nd	nd	nd	nd	nd	nd	nd	–	nd	–	nd	+	nd	nd	+	+	+	nd	nd	+	d	–	nd	+
Catalase	+	+	nd	–	–	+	–	nd	+	nd	+	+	nd	nd	nd	+	+	+	+	nd	+	nd	+	nd	+	nd	+	+	nd	nd	nd	–	+	+
Catalase (68°C)	+	–	nd	nd	–	d	nd	nd	nd	nd	nd	nd	nd	+	nd	nd	nd	+	nd	nd	–	nd	+	+	+	+	+	+	–	nd	nd	nd	+	–
Tween 80 hydrolysis	–	+	+	–	d	+	–	d	+	–	+	–	–	+	nd	–	+	+	nd	nd	nd	nd	+	–	+	+	v	nd	–	+	nd	–	+	nd
Urea hydrolysis	+	–	+	+	+	+	+	+	+	nd	+	nd	+	v	nd	nd	nd	+	+	nd	nd	nd	+	+	+	–	+	nd	+	+	nd	+	+	W
Pyrazinamidase	+	nd	–	–	+	nd	+	+	nd	nd	+	nd	nd	nd	nd	nd	nd	+	+	nd	nd	nd	nd	nd	nd	nd	+	nd	+	d	nd	+	nd	nd
α-Esterase	+	nd	nd	–	nd	nd	+	d	nd	–	nd	nd	nd	nd	nd	nd	nd	+	nd	nd	nd	nd	nd	d	nd	+	nd	nd	nd	nd	nd	–	nd	nd
β-Esterase	+	nd	nd	–	nd	nd	+	+	nd	v	nd	nd	nd	nd	nd	nd	nd	+	nd	nd	nd	nd	nd	d	nd	nd	nd	nd	nd	nd	nd	+	nd	nd
β-Galactosidase	nd	nd	nd	+	nd	nd	+	–	nd	nd	nd	nd	nd	nd	nd	nd	–	+	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	+	nd	–
Utilization of:																																		
Acetamide	+	nd	+	–	–	nd	+	–	nd	nd	–	nd	nd	nd	nd	nd	nd	–	–	nd	nd	nd	nd	nd	nd	nd	nd	nd	+	nd	nd	–	nd	nd
Citrate	–	–	+	–	nd	nd	–	+	–	+	–	nd	nd	nd	+	nd	–	–	–	+	–	+	+	nd	nd	nd	–	+	d	–	nd	–	+	nd
Xylose	–	nd	nd	–	nd	nd	+	+	–	v	–	–	nd	nd	nd	nd	nd	+	–	nd	+	nd	nd	nd	nd	nd	–	+	–	nd	+	+	nd	+
Mannitol	–	–	nd	nd	nd	nd	nd	+	nd	+	+	+	nd	nd	nd	nd	+	–	–	+	+	+	–	nd	nd	nd	+	+	+	nd	nd	nd	nd	+
Sorbitol	–	nd	nd	nd	nd	nd	nd	–	–	–	–	–	nd	nd	nd	nd	nd	–	–	nd	nd	nd	nd	nd	nd	nd	–	nd	nd	nd	+	nd	nd	+
Trehalose	–	nd	nd	+	nd	nd	+	–	–	v	+	–	nd	nd	nd	nd	nd	–	–	nd	–	nd	nd	nd	nd	nd	+	+	+	nd	+	–	+	+
i-*myo*-Inositol	–	–	nd	+	nd	nd	+	+	nd	nd	+	nd	nd	nd	nd	nd	nd	+	–	nd	+	nd	+	nd	nd	nd	nd	+	d	nd	+	–	–	+
Acid from:																																		
L-Arabinose	–	nd	–	nd	nd	nd	nd	nd	nd	+	–	nd	nd	nd	nd	nd	nd	nd	–	+	nd	+	–	nd	nd	nd	–	nd	nd	–	nd	nd	–	nd
i-*myo*-Inositol	–	nd	d	nd	nd	nd	nd	nd	nd	nd	–	nd	nd	nd	nd	nd	–	nd	–	nd	nd	nd	+	nd	–	nd	–	nd	+	+	nd	nd	nd	nd
D-Mannitol	–	nd	+	nd	nd	nd	nd	nd	nd	nd	–	nd	nd	nd	+	nd	+	nd	–	nd	nd	nd	–	nd	–	nd	+	nd	+	+	nd	nd	+	nd
L-Rhamnose	–	nd	+	nd	nd	nd	nd	nd	nd	nd	–	nd	nd	nd	nd	nd	–	nd	–	nd	nd	nd	nd	nd	nd	nd	–	nd	nd	–	nd	nd	–	nd
D-Sorbitol	–	nd	+	nd	nd	nd	nd	nd	nd	nd	–	nd	nd	nd	+	nd	+	nd	–	nd	nd	nd	nd	nd	–	nd	–	nd	nd	–	nd	nd	–	nd
Sucrose	+	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	–	nd	nd	nd	nd	nd	+	nd
D-Xylose	–	nd	–	nd	nd	nd	nd	nd	nd	+	–	nd	nd	nd	nd	nd	–	nd	–	d	nd	+	–	nd	nd	nd	–	nd	nd	–	nd	nd	+	nd
Trehalose	–	nd	+	nd	nd	nd	nd	nd	nd	nd	+	nd	nd	nd	+	nd	+	nd	–	nd	nd	nd	+	nd	nd	nd	nd	nd	nd	+	nd	nd	nd	nd
Resistance to:																																		
Hydroxylamine (500 mg/l)	–	nd	–	–	nd	–	–	–	nd	–	nd	nd	–	nd	nd	+	–	–	–	–	nd	–	–	nd	nd	–	+	nd	nd	–	nd	–	–	nd
Amikacin (32 mg/ml)	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	–	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	–	nd	nd	nd	nd	nd	nd	nd
Doxycycline (16 mg/ml)	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	–	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	+	nd	nd	nd	nd	nd	nd	+
Ethambutol (5 mg/ml)	+	nd	nd	–	nd	nd	–	–	nd	nd	nd	nd	nd	nd	nd	–	nd	–	nd	nd	–	nd	–	–	–	+	nd	nd	+	nd	nd	nd	nd	nd

(continued)

TABLE 38. (continued)

Character	*M. parafortuitum*	*M. gilvum*	*M. obuense*	*M. fortuitum* subsp. *fortuitum*	*M. fortuitum* subsp. *acetamidolyticum*	*M. rhodesiae*	*M. houstonense*	*M. conceptionense*	*M. senegalense*	*M. farcinogenes*	*M. septicum*	*M. alvei*	*M. porcinum*	*M. boenickei*	*M. neworleansense*	*M. neoaurum*	*M. brisbanense*	*M. frederiksbergense*	*M. fluoranthenivorans*	*M. diernhoferi*	*M. canariasense*	*M. cosmeticum*	*M. mucogenicum*	*M. aubagnense*	*M. phocaicum*	*M. chelonae*	*M. abscessus*	*M. immunogenum*	*M. bolletii*	*M. massiliense*	*M. salmoniphilum*	*M. hassiacum*	*M. madagascariense*	*M. moriokaense*	*M. gadium*	*M. hodleri*
Test:																																				
Growth at 42°C	v	nd	nd	v	+	nd	+	–	nd	nd	–	nd	+	–	–	nd	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	+	–	+	–	–
Growth at 45°C	v	–	–	–	–	–	nd	–	–	nd	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	+	–	–	–	–
Pigmentation	P	S	S	N	N	S	N	N	N	N	N	–	N	N	N	S	N	S	N	N	N	S	N	N	N	N	N	N	N	N	N	S	S	N	S	S
Growth on MacConkey	–	nd	nd	+	+	nd	+	nd	–	nd	+	–	nd	+	+	nd	+	–	nd	nd	+	+	+	nd	nd	+	+	+	nd	+	+	nd	–	nd	nd	nd
Growth on 5% (w/v) NaCl	nd	nd	nd	+	–	nd	+	+	+	nd	+	nd	+	+	+	nd	+	nd	nd	nd	–	–	–	–	–	–	+	–	–	+		+	–	d	nd	nd
Nitrate reduction	d	+	–	+	+	–	+	+	+	+	+	+	–	+	+	–	+	+	–	+	–	+	d	–	+	–	–	–	–	–	–	–	–	+	+	–
Iron uptake	nd	nd	nd	+	+	nd	nd	+	–	–	nd	–		nd	nd	nd	nd	nd	nd	nd	–	+	–	–	–	–	–	–	–	–	nd	–	+	nd	nd	nd
Arylsulfatase (3 d)	–	+	+	+	+	+	+	+	+	nd	–	+	+	d	+	–	+	nd	nd	–	+	–	+	+	+	+	+	+	+	+	+	nd	+	+	–	nd
Arylsulfatase (7 d)	+	+	+	+	+	+	+	+	+	nd	nd	+	+	nd	+	nd	+	nd	nd	d	+	nd	+	+	+	+	+	+	+	+	+	nd	+	+	–	nd
Acid phosphatase	–	nd	–	+	–	+	nd	nd	nd	nd	nd	nd	–	nd	nd	nd	nd	nd	nd	d	nd	nd	nd	nd	–	d	–	nd	nd	nd	nd	w	d	–	nd	nd
Catalase	nd	+	–	nd	nd	+	nd	+	+	–	nd	+	+	nd	nd	nd	nd	+	+	–	nd	nd	–	nd	d	d	nd	nd	nd	+	nd	+	nd	–	+	+
Catalase (68°C)	nd	nd	nd	nd	nd	nd	+	nd	nd	nd		+	nd	+	+	nd	–	nd	nd	–	+	nd	–	nd	nd	nd	nd	nd	nd	nd		+	nd	nd	nd	nd
Tween 80 hydrolysis	nd	+	–	nd	nd	+	nd	nd	nd	nd	nd	+	–	nd	nd	+	nd	+	–	–	+	nd	+	nd	nd	–	–	nd	nd	nd		–	+	nd	–	+
Urea hydrolysis	nd	nd	+	+	+	+	nd	–	+	d	nd	+	+	nd	+	+	+	–	–	+	nd	nd	+	–	–	+	+	nd	–	–		+	+	nd	nd	+
Pyrazinamidase	nd	nd	+	d	+	+	nd	+	nd	nd	nd	nd	+	nd	nd	nd	nd	nd	nd	+	nd	nd	nd	–	+	+	+	nd	nd	+	nd	+	+	nd	nd	nd
α-Esterase	–	nd	+	d	–	–	nd	nd	nd	nd	nd	nd	d	nd	nd	–	nd	nd	nd	–	nd	nd	nd	nd	nd	d	d	nd	nd	nd	nd	nd	nd	nd	nd	nd
β-Esterase	v	nd	+	d	+	–	nd	nd	nd	nd	nd	nd	+	nd	nd	+	nd	nd	nd	+	nd	nd	nd	nd	nd	+	+	nd	nd	nd	nd	nd	nd	nd	nd	nd
β-Galactosidase	nd	–	+	–	nd	–	nd	–	nd	nd	nd	+	–	nd	nd	nd	nd	nd	nd	–	nd	nd	nd	nd	nd	nd	nd	nd	nd	+	nd	nd	nd	nd	–	nd
Utilization of:																																				
Acetamide	nd	nd	–	d	+	–	+	nd	nd	nd		nd	+	+	+	nd	+	–	nd	–	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	–	–	nd	–
Citrate	+	–	–	–	–	–	–	nd	+	+	–	–	+	–	–	nd	–	nd	nd	d	–	+	+	–	+	+	–	–	–	–	nd	+	–	nd	–	–
Xylose	v	nd	–	–	–	+	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	+	+	+	nd	+	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	–	nd	+
Mannitol	+	+	nd	d	–	nd	nd	–	nd	nd	nd	–	+	nd	nd	nd	nd	+	+	+	+	+	+	nd	nd	–	–	–	–	nd	nd	nd	nd	+	nd	+
Sorbitol	nd	nd	nd	–	–	nd	nd	–	nd	nd	nd	nd	nd	nd	nd	nd	nd	–	+	–	nd	–	–	nd	nd	–	–	–	–	nd	nd	nd	nd	+	nd	+
Trehalose	nd	nd	+	d	–	–	nd	nd	nd	nd	nd	nd	+	nd	nd	nd	nd	+	+	–	nd	+	nd	nd	nd	d	d	nd	nd	nd	nd	nd	nd	d	nd	–
i-*myo*-Inositol	nd	nd	–	nd	nd	+	nd	+	nd	nd	nd	–	+	nd	nd	nd	nd	+	+	+	+	+	–	nd	nd	–	–	–	nd	nd	nd	–	nd	d	nd	nd
Acid from:																																				
L-Arabinose	+	nd	nd	nd	nd	nd	–	nd	nd	nd	–	nd	–	–	–	nd	+	nd	nd	nd	+	nd	nd	nd	nd	–	–	nd	nd	nd	nd	nd	+	nd	–	nd
i-*myo*-Inositol	+	+	nd	–	–	nd	+	nd	nd	nd	+	–	+	+	+	nd	+	nd	nd	nd		nd	nd	nd	nd	–	–	nd	nd	–	nd	nd	d	nd	d	nd
D-Mannitol	+	+	nd	–	–	nd	+	nd	nd	nd	+	–	+	+	+	nd	+	nd	nd	nd	nd	nd	nd	nd	nd	–	–	nd	nd	–	nd	–	+	nd	–	nd
L-Rhamnose	v	–	nd	–	–	nd	–	nd	nd	nd	–	–	–	–	–	nd	+	nd	nd	nd	nd	nd	nd	nd	nd	–	–	nd	nd	–	nd	nd	+	nd	–	nd
D-Sorbitol	–	+	nd	–	–	nd	+	nd	nd	nd	–	nd	–	–	–	nd	+	nd	nd	nd	nd	nd	nd	nd	nd	–	–	nd	nd	–	nd	nd	d	nd	+	nd
Sucrose	nd	nd	nd		nd	nd	–	nd	nd	nd	–	nd	–	–	–	nd	–	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	–	nd
D-Xylose	+	–	nd	–	–	nd	–	nd	nd	nd	+	–	–	–	+	nd	–	nd	nd	nd	+	nd	nd	nd	nd	–	–	nd	nd	–	nd	nd	+	nd	–	nd
Trehalose	+	+	nd	nd	nd	nd	nd	nd	nd	nd	nd	+	+	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	–	–	nd	nd	nd	nd	nd	+	nd	–	nd
Resistance to:																																				
Hydroxylamine (500 mg/l)	–	–	–	+	+	–	nd	nd	nd	nd	nd	+	+	nd	nd	–	nd	nd	nd	–	nd	nd	nd	nd	nd	+	+	nd	nd	nd	nd	nd	–	–	nd	nd
Amikacin (32 mg/ml)	nd	nd	nd	–	nd	nd	–	nd	nd	nd	–	nd	–	–	–	nd	–	nd	nd	nd	nd	+	nd	nd	nd	nd	nd	–	nd	+		nd	nd	nd	nd	nd
Doxycycline (16 mg/ml)	nd	nd	nd	–	nd	nd	+	nd	nd	nd	–	nd	nd	d	–	nd	+	nd	nd	nd	nd	+	nd	nd	nd	nd	nd	+	nd	–		nd	nd	nd	nd	nd
Ethambutol (5 mg/ml)	nd	nd	+	+	+	–	nd	nd	nd	nd	nd	–	+	nd	nd	nd	nd	nd	nd	–	nd	nd	nd	nd	nd	+	+	nd	nd	nd	nd	–	nd	+	–	nd

[a]Symbols: +, 90% or more of strains are positive; –, 90% or more of strains are negative; d, 11–89% of strains are positive; v, variability of reaction of strains within a given species; w, weak reaction; N, non-chromogenic; N*, pigmentation may develop after 10–14 d incubation; S, scotochromogenic; P, photochromogenic. nd, Not data available.

List of slow-growing species of the genus *Mycobacterium*

Multimembered 16S rRNA gene clades

The *Mycobacterium tuberculosis* clade

1. **Mycobacterium tuberculosis** (Zopf 1883) Lehmann and Neumann 1896, 363[AL] (*Bacterium tuberculosis* Zopf 1883, 67)

tu.ber.cu.lo′sis. L. dim. n. *tuberculum* a small swelling, tubercle; Gr. suff. *-osis* suffix expressing state or condition, in medical terminology denoting a state of disease; N.L. gen. n. *tuberculosis* of tuberculosis.

Strongly acid–alcohol-fast rods (0.3–0.6 × 1–4 μm), straight or slightly curved, occurring singly and in occasional threads. Staining may be uniform or irregular, often showing banded or beaded forms. On most solid media, colonies are rough, raised, and thick, with a nodular or wrinkled surface and an irregular thin margin; may become somewhat pigmented (off-white to faint buff, or even yellow). Growth tends to be in serpentine, cordlike masses in which the bacilli show a parallel orientation. Colonies of avirulent forms are less compact. Colonies on oleic acid-albumin agar are flat, rough, corded, dry, and usually non-pigmented. In liquid media lacking a dispersing agent, strains form a pellicle which, with age, becomes thick and wrinkled. In Dubos' Tween albumin medium, growth is diffuse, settling if undisturbed, but readily dispersed. Generation time *in vitro* under optimal conditions is 14–15 h.

The optimal temperature for growth is 37°C some growth occurs at 30–34°C. Optimum pH is 6.4–7.0. Growth is stimulated by incubation in air with 5–10% added CO_2, and by inclusion of glycerol [0.5% (w/v)] in the medium. Bacilli grown under highly aerobic conditions die rapidly on abrupt shift to anaerobiosis, but when allowed to grow and settle slowly through a self-generated oxygen gradient they adapt a tolerance to oxygen deprivation and exhibit synchronized growth on resuspension (Wayne and Lin, 1982). TLC of methanolysates shows α-, keto-, and methoxy-mycolates. The Differential characteristics of slowly growing species are shown in Table 37.

Strain-to-strain differences in tubercle bacilli have been demonstrated by their different phage susceptibility patterns, but three major patterns are recognized (Bates and Mitchison, 1969; Rado et al., 1975). In cross-sensitivity studies using experimentally sensitized animals, extracts (sensitins) of *Mycobacterium tuberculosis* cannot be differentiated from those of *Mycobacterium africanum*, *Mycobacterium bovis*, or *Mycobacterium microti*, although they are readily distinguished from those of other species (Magnusson, 1980). Similarly, immunodiffusion analysis of bacillary extracts shows a pattern common to *Mycobacterium africanum*, *Mycobacterium bovis*, *Mycobacterium microti*, and *Mycobacterium tuberculosis* (Stanford and Grange, 1974). Monoclonal antibodies have been prepared that appear to distinguish between *Mycobacterium bovis* and *Mycobacterium tuberculosis*, although the specificity of at least one such preparation appears to be at the strain rather than the species level (Coates et al., 1981; Daniel and Janicki, 1978). Immunological analysis of T-catalase extracted from *Mycobacterium tuberculosis* indicates structural identity with that of *Mycobacterium africanum* and *Mycobacterium bovis* and marked divergence from that of other *Mycobacterium* species (Wayne and Diaz, 1979).

Infected animals, including man, exhibit delayed hypersensitivity to crude or purified *Mycobacterium tuberculosis* culture filtrates (tuberculins) and less sensitivity to tuberculin-like preparations from other mycobacteria. Disease caused by *Mycobacterium bovis* cannot be distinguished from that due to *Mycobacterium tuberculosis* by use of commonly available tuberculins. Infections by other mycobacterial species result in much lower degrees of sensitivity to tuberculin, although if "second strength" (250 tuberculin units) PPD tuberculin is used, skin tests may be interpreted erroneously as indicating *Mycobacterium tuberculosis.* Discovery of the role of T-lymphocytes and interferon-gamma in the immune system offered an alternative to tuberculin skin tests (TSTs; Rothel et al., 1990) and led to the development of assays for cell-mediated immune reactivity (Lalvani et al., 2001; Mazurek et al., 2001). By using artificially produced *Mycobacterium tuberculosis*-specific antigens, notably ESAT-6 (early secretory antigen target-6) and CFP-10 (culture filtrate protein-10), which are present in PPD but absent from the BCG strain and from most non-tuberculous mycobacteria, cross-reactivity is largely eliminated and assays correlate with high specificity to *Mycobacterium tuberculosis* exposure (Ewer et al., 2003; Mori et al., 2004).

Experimentally, from an inoculum of 0.01 mg, *Mycobacterium tuberculosis* is highly pathogenic for guinea pigs and hamsters, but relatively non-pathogenic for bovines, cats, domestic fowls, goats, and rabbits. Inocula of 0.001–1 mg produce experimental disease in mice. Attenuation of virulence may occur spontaneously upon subculture in artificial media. Virulence can be maintained by selection of appropriate portions of growth in suitable media or by animal passage. Strains of *Mycobacterium tuberculosis* isolated from patients from southern India may cause only localized lesions in guinea pigs and these tend to regress. These strains produce catalase and are susceptible to both hydrogen peroxide and isoniazid (Mitchinson et al., 1960). Resistance to isoniazid is frequently accompanied by changes in other properties, such as loss of peroxidase and catalase activity and attenuation of virulence for guinea pigs (Middlebrook, 1954; Middlebrook and Cohn, 1953).

Source: isolated from cases of tuberculosis in man, other primates, dogs and some other animals which have contact with man.

DNA G+C content (mol%): 65.6 (whole-genome sequencing).

Type strain: ATCC 27294.

Sequence accession no. (16S rRNA gene): X58890.

Additional remarks: phenotypic distinctions can be made between *Mycobacterium tuberculosis* and *Mycobacterium africanum*, *Mycobacterium bovis*, and *Mycobacterium microti*, but numerical taxonomic data places them all in a "macro cluster", distinct from other slowly growing mycobacteria. DNA–DNA homology between *Mycobacterium bovis* and *Mycobacterium tuberculosis* is about 100% (Baess, 1979). All members of the *Mycobacterium tuberculosis* complex share identical 16S rRNA and ITS sequences. However, *gyrB* polymorphism analysis can help to separate some species (Kasai et al., 2000).

2. **Mycobacterium africanum** Castets, Rist and Boisvert 1969, 321[AL]

a.fri.ca′num. L. neut. adj. *africanum* of Africa, African.

Rods averaging 3 μm in length. Colonies are flat, dull, and rough when grown on egg medium at 37°C. Sodium pyruvate stimulates growth in egg medium. Growth is homogeneous in Dubos' medium supplemented with Tween 80 and granular in Youman's medium supplemented with bovine serum. Growth in Lebek agar-deeps occurs 15 mm below the surface. Differential characteristics of slowly growing species are shown in Table 37.

Source: isolated from sputum of tuberculosis patients in Senegal and a cause of human tuberculosis in tropical Africa.

DNA G+C content (mol%): not determined.

Type strain: ATCC 25420, CIP 105147.

Sequence accession no. (16S rRNA gene): AF480605.

Additional remarks: David et al. (1978) reported that many African strains exhibited more phenotypic heterogeneity than those of *Mycobacterium tuberculosis* and *Mycobacterium bovis* isolated from other geographical areas, but noted that individual *Mycobacterium africanum* strains clustered with one or the other of these species. They also reported that subclustering behavior reflected the geographic region from which they were isolated on the African continent. This observation led to some workers to assign *Mycobacterium africanum* strains to West African subtype I or East African subtype II. The former, which consist of strains isolated from Dakar, Mauritania, and Yaounde, are similar in their phenotypic properties to *Mycobacterium tuberculosis* and the latter, from Burundi and Rwanda, have similar phenotypic features to *Mycobacterium bovis*. Clinical isolates of *Mycobacterium africanum* were found to be indistinguishable from *Mycobacterium bovis* on the basis of pyrolysis MS data (Sisson et al., 1991).

3. **Mycobacterium bovis** Karlson and Lessel 1970, 280[AL]

bo′vis. L. n. *bos* the ox; L. gen. n. *bovis* of the ox.

Short to moderately long acid-fast, Gram-stain-positive, nonmotile rods. On primary isolation, growth is very poor on glycerol-containing media, although repeated subculture permits adaptation to growth on such media. Growth is improved by the addition of pyruvate to media (Stonebrink, 1958). Freshly isolated cultures of *Mycobacterium bovis* are microaerophilic; inocula dispersed into liquid, semisolid, or solid agar media grow within the medium but not on the surface, thereby distinguishing *Mycobacterium bovis* from *Mycobacterium tuberculosis* which is highly aerobic (Schmeidel and Gerloff, 1965). On repeated subculture, *Mycobacterium bovis* will adapt to aerobic growth. Dilute inocula on egg media yield small, rounded, white colonies, with irregular edges and a granular surface after incubation at 37°C for 21 d or more. Colonies on transparent oleic acid-albumin agar are thin, flat, generally corded, and not easily emulsified in the absence of a detergent. Loss of virulence for guinea pigs and rabbits and of catalase activity accompanies a loss of sensitivity to isoniazid, as for *Mycobacterium tuberculosis*. TLC of methanolysates shows a pattern of α-, keto-, and methoxy-mycolates. Differential characteristics of slowly growing species are shown in Table 37.

Experimentally, highly pathogenic for calves, guinea pigs, and rabbits; at least moderately pathogenic for hamsters and mice; slightly pathogenic for cats, dogs, horses, and rats; not pathogenic for most fowl. Certain strains isolated from cases of lupus and scrofuloderma in man have low pathogenicity for animals (Griffith, 1957).

Source: originally isolated from tubercles in cattle, and generally more pathogenic for animals than *Mycobacterium tuberculosis*. Causes tuberculosis in cattle, both domestic and wild ruminants; in man and other primates; in carnivores including cats and dogs; and in swine, parrots and possibly some birds of prey.

DNA G+C content (mol%): 65.6 (whole-genome sequencing).

Type strain: ATCC 19210, CIP 105234, NCTC 10772.

Sequence accession no. (16S rRNA gene): AB292583.

Additional remarks: All members of the *Mycobacterium tuberculosis* complex share identical 16S rRNA gene and ITS sequences, but *gyrB* polymorphism analysis can aid separation of some species (Kasai et al., 2000). Some differences in phage susceptibility between *Mycobacterium bovis* and *Mycobacterium tuberculosis* have been described (Baess, 1969). *Mycobacterium bovis* has a very close relationship to other species of the *Mycobacterium tuberculosis* complex. The bacillus of Calmette–Guérin (BCG; Calmette and Guérin, 1908) originally conformed to the properties described for *Mycobacterium bovis* apart from much attenuated pathogenicity and anaerobic growth on glycerinated media. However, since its first description, the BCG strain has been maintained and used independently in a number of laboratories throughout the world. Re-examination of some of these isolates showed that they formed a cluster with no greater matching affinity to *Mycobacterium bovis* than to *Mycobacterium tuberculosis* (Wayne et al., 1980). In addition, samples of BCG isolates have been distinguished from *Mycobacterium bovis* and *Mycobacterium tuberculosis* by their mycolic acid patterns (Minnikin et al., 1983). Pyrolysis MS analysis of BCG isolates showed them to be heterogeneous and more closely related to laboratory-adapted strains of *Mycobacterium tuberculosis* than to more recent isolates of either *Mycobacterium bovis* or *Mycobacterium tuberculosis* (Sisson et al., 1991).

4. **Mycobacterium caprae** (Aranaz, Liébana, Gómez-Mampaso, Galán, Cousins, Ortega, Blázquez, Baquero, Mateos, Súarez and Domínguez 1999) Aranaz, Cousins, Mateos and Domínguez 2003, 1788[VP] comb. nov. [*Mycobacterium tuberculosis* subsp. *caprae* Aranaz, Liébana, Gómez-Mampaso, Galán, Cousins, Ortega, Blázquez, Baquero, Mateos, Súarez and Domínguez 1999, 1271; *Mycobacterium bovis* subsp. *caprae* (Aranaz et al. 1999) Niemann, Richter and Rüsch-Gerdes 2002, 435]

ca′pra.e. L. fem. n. *capra* goat; L. fem. gen. n. *caprae* of a goat, the host animal from which the bacterium was first isolated.

Acid–alcohol-fast, asporogenous, nonmotile rods, with weak cord formation. Colonies are smooth and non-chromogenic after incubation at 36°C for 4–6 weeks. Growth is enhanced by pyruvate. Does not grow at 25, 30, or 43°C, or on media supplemented with 5% (w/v) NaCl. Differential characteristics of slowly growing species are shown in Table 37.

Source: isolated from the lymph nodes and lungs of tuberculous goats and from cattle, deer, pigs, sheep, and wild boars and from a captive Siberian tiger (Aranaz et al., 2003; Aranaz et al., 1999; Erler et al., 2004; Lantos et al., 2003; Prodinger et al., 2002). Has also been found as a pathogen in humans linked with goat farming (Gutierrez et al., 1997).

DNA G+C content (mol%): not determined.

Type strain: CIP 105776, ATCC BAA-824.

Sequence accession no. (16S rRNA gene): AJ131120.

Additional remarks: Mycobacterium caprae was initially described as a subspecies of *Mycobacterium tuberculosis* (Aranaz et al., 1999), and then was transferred to *Mycobacterium bovis* as a subspecies (Niemann et al., 2002). This designation was challenged by Aranaz et al. (2003) who argued that *Mycobacterium caprae* was an evolutionary precursor to *Mycobacterium bovis* (see also Brosch et al., 2002) and, largely on public health grounds, proposed that *Mycobacterium caprae* be elevated to species rank. However, *Mycobacterium caprae* has a very close relationship to other species classified in the *Mycobacterium tuberculosis* complex and has a 16S rRNA gene profile which is characteristic of all members of the *Mycobacterium tuberculosis* complex. It can be separated from *Mycobacterium africanum*, *Mycobacterium bovis*, and *Mycobacterium tuberculosis* strains by spoligotyping (Niemann et al., 2002).

5. **Mycobacterium microti** Reed *in* Breed, Murray and Smith 1957, 703[AL]

mi.cro′ti. N.L. masc. n. *Microtus* a genus that includes the vole; N.L. gen. n. *microti* of *Microtus*.

Acid-fast, Gram-stain-positive, nonmotile rods. Primary growth on glycerol-free egg media in 28–60 d; may adapt to tolerance to glycerol. Colonial morphology is variable. Optimum temperature is 37°C. TLC of methanolysates yields α-, keto-, and methoxy-mycolates. Differential characteristics of the species are shown in Table 37.

Pathogenicity is lost on repeated subculture. The organism is not considered to be a human pathogen, though infections in immune-suppressed individuals have been reported (van Soolingen et al., 1998). Local lesions are produced in calves, rabbits, and guinea pigs. This species causes naturally acquired generalized tuberculosis in voles.

Source: originally isolated from a vole (*Microtus agrestis*) by Wells (1937).

DNA G+C content (mol%): not determined.

Type strain: ATCC 19422, CIP 104256, NCTC 8710.

Sequence accession no. (16S rRNA gene): AF480584.

6. **Mycobacterium pinnipedii** Cousins, Bastida, Cataldi, Quse, Redrobe, Dow, Duignan, Murray, Dupont, Ahmed, Collins, Butler, Dawson, Rodríguez, Loureiro, Romano, Alito, Zumarraga and Bernardelli 2003, 1312[VP]

pin.ni.pe′di.i. N.L. gen. n. *pinnipedii* of a pinniped, referring to the host animal from which the organism was first isolated.

Acid–alcohol-fast, non-spore-forming, nonmotile bacilli which show loose cord formation. Colonies on egg-based medium after incubation for 3–6 weeks are dysgonic, rough, flat, and non-photochromogenic. Growth is generally enhanced by sodium pyruvate. Optimal temperature for growth is 36–37°C. Differential characteristics of slowly growing species are shown in Table 37.

Isolates can be recovered from the lung and associated lymph nodes of tuberculous pinnipeds and occasionally from mesenteric lymph nodes and organs such as the liver. Strains are pathogenic for guinea pigs and rabbits. Incidental infections in bovines, humans, and a tapir suggest the species may have a wide host range and that inter-species transmission is possible (Moser et al., 2008). The ability of this species to cause disease in humans was reported by Thompson et al. (1993).

Source: isolated from captive and wild seals from diverse geographical areas (Cousins et al., 2003).

DNA G+C content (mol%): not determined.

Type strain: ATCC BAA-688, NCTC 13288.

Sequence accession no. (16S rRNA gene): AF502574.

Additional remarks: Mycobacterium pinnipedii is closely related to other species classified as *Mycobacterium tuberculosis* complex; it shares a 16S rRNA gene sequence similarity of 99.9% with *Mycobacterium tuberculosis*. Cousins et al. (2003) showed that spoligotypes of *Mycobacterium pinnipedii* formed a distinct and homogeneous cluster within the *Mycobacterium tuberculosis* complex even though strains were from diverse geographical sources. Nevertheless, they recognized four different spoligotypes; all of the isolates from Australia and all but one of those from Argentina had a unique pattern, designated SS1. The pattern of the remaining Argentinian isolate was designated SS2, that of some strains from Great Britain and New Zealand was SS3, and others from New Zealand were SS4.

The *Mycobacterium haemophilum* clade

7. **Mycobacterium haemophilum** Sompolinsky, Lagziel, Naveh and Yankilevitz 1978, 74[AL]

ha.e.mo′phi.lum. Gr. n. *haima* (Latin transliteration *haema*) blood; Gr. adj. *philos* loving; N.L. neut. adj. *haemophilum* blood loving.

Strongly acid–alcohol-fast, short (1.4–3.2 × 0.4–0.7 µm), occasionally curved, nonmotile rods. Non-pigmented, rough colonies appear after incubation for 2–4 weeks on Middlebrook 7H10 agar or Löwenstein–Jensen medium, but only if supplemented with 0.4% hemoglobin or 60 µM hemin, but not with $FeCl_3$ or catalase. Small numbers of smooth colonies often occur. Does not grow on media supplemented with 5% (w/v) NaCl. Dawson and Jennis (1980) report that 15 mg/ml ferric ammonium citrate can be substituted for hemin. Growth is strictly intracellular in tissue culture in fibroblasts (Sompolinsky et al., 1978). Temperature range for growth is 25–35°C and optimal growth is at 30–32°C; does not grow at 37°C. Differential characteristics of slowly growing species are shown in Table 37.

Guinea pigs do not develop any obvious pathology after intravenous, intramuscular, or subcutaneous inoculation with dense suspensions of the organisms. Some mice die 2–4 weeks after inoculation; numerous intracellular bacilli are found in monocytes and macrophages of kidney, liver, and spleen, but gross lesions are not formed.

Source: Isolated in Israel from subcutaneous granulomata of a patient undergoing treatment for Hodgkin's disease. Subsequently found in skin lesions of patients undergoing

immunosuppressive treatment in Australia (Dawson and Jennis, 1980).

DNA G+C content (mol%): not determined.

Type strain: ATCC 29548, CCUG 47452, CIP 105049, DSM 44634, NCTC 11185.

Sequence accession no. (16S rRNA gene): X88923.

Additional remarks: seroagglutination studies of whole bacilli specific for *Mycobacterium haemophilum*, both of Australian and Israeli origin, show little if any cross-reaction with unabsorbed sera and none using absorbed sera, with any of the serovars of *Mycobacterium avium/Mycobacterium intracellulare* complex or *Mycobacterium asiaticum*, *Mycobacterium malmoense*, *Mycobacterium marinum*, *Mycobacterium scrofulaceum*, *Mycobacterium simiae*, *Mycobacterium szulgai*, or *Mycobacterium ulcerans* (Dawson and Jennis, 1980; Sompolinsky et al., 1978).

8. **Mycobacterium malmoense** Schröder and Juhlin 1977, 245[AL]

mal.mo.en′se. N.L. neut. adj. *malmoense* of or belonging to Malmö, Sweden, the source of the strains on which the original description is based.

Acid–alcohol-fast, Gram-stain-positive, coccoid to short rods are formed on Löwenstein–Jensen medium; longer rods occur when grown on Middlebrook 7H10 agar. Neither cording nor cross-barring occurs. Cells are non-capsulate and asporogenous. Does not produce aerial hyphae. Colonies are smooth, grayish white, non-pigmented, usually domed, and circular. Some umbonate colonies occur with compact, raised centers and flattened irregular edges on Middlebrook and cornmeal agars. Growth requires incubation for over 1 week and may take up to 6 weeks. Temperature range for growth is 22–37°C; growth does not occur at 42°C. Strains are micro-aerophilic and grow beneath the surface of semi-solid agar medium after deep inoculation. TLC of methanolysates shows the presence of α-, α′-, and keto-mycolates. Differential characteristics of slowly growing species are shown in Table 37.

Subcutaneous inoculation of guinea pigs with 0.1 mg bacilli produces local, but not generalized, lesions. Intravenous inoculation of chickens with 0.1 mg bacilli caused macroscopic lesions in the liver and spleen in about half of the birds, some of which died. Intravenous inoculation of rabbits with 0.1 mg bacilli caused rare minimal lesions that contained rare viable bacilli.

Source: isolated from sputum and biopsy specimens from patients with pulmonary disease and considered to be the etiologic agent of the disease.

DNA G+C content (mol%): not determined.

Type strain: ATCC 29571, CCUG 37761, CIP 105775, DSM 44163, JCM 13391, NCTC 11298.

Sequence accession no. (16S rRNA gene): X52930.

Additional remarks: seroagglutination studies have demonstrated the presence of a single serovar in *Mycobacterium malmoense* that is distinct from those of other *Mycobacterium* species. Similarly, strains exhibit a single unique TLC pattern of surface lipids (Wayne et al., 1983), while dermal hypersensitivity studies show distinction from other *Mycobacterium* species (Schröder and Juhlin, 1977). In numerical taxonomy studies, Wayne et al. (1983) demonstrated that *Mycobacterium malmoense* formed a discrete cluster with 85% internal matching similarity, but with corresponding low similarities to other mycobacterial clusters.

9. **Mycobacterium bohemicum** Reischl, Emler, Horak, Kaustova, Kroppenstedt, Lehn and Naumann 1998, 1354[VP]

bo.he′mi.cum. N.L. neut. adj. *bohemicum* referring to the Czech Republic where the organism was first isolated.

Acid-fast, rod-shaped cells. Smooth, scotochromogenic colonies that are 1–2 mm in diameter are formed on Löwenstein–Jensen medium and Middlebrook 12B agar following incubation for 4–6 weeks. Does not grow on media containing 5% (w/v) NaCl. Temperature range for growth is 25–40°C; optimal growth temperature is 37°C; does not grow at 42 or 45°C. TLC of methanolysates shows the presence of α-mycolates, and wax esters. Differential characteristics of slowly growing species are shown in Table 37.

Source: originally isolated from three consecutive sputum samples from a patient with Down's syndrome suffering from tuberculosis. Subsequently, the species has been considered to be a cause of lymphadenitis in immunocompetent children (Tortoli et al., 2000) and has been isolated from veterinary and environmental sources (Torkko et al., 2001).

DNA G+C content (mol%): 63.5 (HPLC).

Type strain: DSM 44277, JCM 12402, CIP 105808/105811.

Sequence accession no. (16S rRNA gene): U84502.

The *Mycobacterium kansasii* clade

10. **Mycobacterium kansasii** Hauduroy 1955, 73[AL]

kan.sas′i.i. N.L. gen. n. *kansasii* of Kansas, USA.

Moderately long to long acid-fast rods which broaden and exhibit cross-barring when grown in the presence of fatty acids. Dilute inocula on agar or inspissated egg media yield smooth to rough colonies after incubation at 37°C for 7 or more days. Colonies usually appear somewhat rough microscopically, but are readily emulsified in water; some strains resist emulsification. Colonies grown in the dark are non-pigmented; when grown in light, or when exposed briefly to light young colonies become brilliant yellow (photochromogenic). Rarely, strains produce a deep orange pigment when grown in the dark or do not form pigments. Most strains, when grown in a lighted incubator, form dark red crystals of β-carotene on the surface and inside colonies (Runyon, 1965). Temperature range for growth is 32–42°C; does not grow at 45°C. TLC of methanolysates shows α-, keto-, and methoxy-mycolates. Differential characteristics of slowly growing species are shown in Table 37.

Causes chronic human pulmonary disease resembling tuberculosis although not normally considered contagious from man to man (Wolinsky, 1979a). Pathogenicity may be related to catalase activity; isolates with strong catalase activity are thought to be more virulent (Steadham, 1980). Most strains are strongly catalase-positive, but some are weakly positive and inactivated at 68°C for 20 min, i.e. lacking M-catalase (Wayne and Diaz, 1982); these variants appear to be less pathogenic for man (Wayne, 1962).

Source: isolated from human pulmonary lesions. Occasionally associated with lesions of lungs or lymph nodes of cattle, deer, and swine (Pattyn et al., 1967; Worthington and Kleeberg, 1964). Extensive soil sampling has failed to yield isolates of *Mycobacterium kansasii* (Wolinsky and Rynearson, 1968), but strains have been isolated from domestic water sources (Bailey et al., 1970; Engel et al., 1980; Fisheder

et al., 1991; Kaustova et al., 1981; Steadham, 1980) suggesting that the organism may be concentrated within water distribution systems. *Mycobacterium kansasii* is one of several species frequently incriminated in nosocomial and pseudo-outbreaks (Phillips and von Reyn, 2001).

DNA G+C content (mol%): not determined.

Type strain: ATCC 12478, CIP 104589, DSM 44162, JCM 6379, NCTC 13024.

Sequence accession no. (16S rRNA gene): AJ536035.

Additional remarks: Magnusson (1967) distinguished between *Mycobacterium kansasii* and 10 other *Mycobacterium* species, including *Mycobacterium marinum*, by dermal hypersensitivity testing. Dermal desensitization is effected only by homologous antigens although some degree of cross-reactivity does occur (Worthington and Kleeberg, 1967). One agglutinating serovar characteristic of *Mycobacterium kansasii* was used to identify 154 out of 155 tested smooth strains, the identities of which were confirmed by biochemical means (Hobby et al., 1967). It was not possible to type rough strains due to spontaneous agglutination. High and low catalase variants exhibit the same agglutinating serovar (Wayne, 1966). Cross-reactivity does not occur between the surface antigens of *Mycobacterium kansasii*, *Mycobacterium gastri*, and *Mycobacterium marinum* (Wayne et al., 1978). Immunodiffusion analysis of cell extracts of *Mycobacterium kansasii* showed four antigens that were not shared with other mycobacterial species with the exception of *Mycobacterium gastri* (Stanford and Grange, 1974). The T-catalase of *Mycobacterium gastri* shows a very close structural relationship to that of *Mycobacterium kansasii* (Wayne and Diaz, 1982).

11. **Mycobacterium gastri** Wayne 1962, 923[AL]

gas′tri. L. n. *gaster -tri* belly, stomach; L. gen. n. *gastri* of the stomach.

Acid–alcohol-fast moderately long rods which frequently show cross-barring. Smooth to rough and pale buff to white colonies are formed on inspissated egg medium. On oleic acid-albumin agar, smooth or somewhat granular colonies are formed after incubation at 37°C for 7 or more days. Temperature range for growth is 25–40°C.

Closely related to *Mycobacterium kansasii* biochemically, but is not agglutinated by *Mycobacterium kansasii*-typing serum (Wayne, 1966) nor does the phenol-soluble antigen cross-react with this serum (Wayne, 1971). Immunodiffusion of cell extracts or culture filtrates does not permit differentiation between *Mycobacterium gastri* and *Mycobacterium kansasii* (Norlin et al., 1969; Stanford and Grange, 1974). Similarly, serological analysis of T-catalases does not demonstrate any significant difference between *Mycobacterium gastri* and *Mycobacterium kansasii*; only strains of the latter produce an M-catalase (Wayne and Diaz, 1982). *Mycobacterium gastri* forms a homogeneous group by reciprocal intradermal skin testing and is thereby distinguishable from *Mycobacterium kansasii* (Magnusson, 1971). TLC of methanolysates shows the presence of α-, keto-, and methoxy-mycolates. Differential characteristics of slowly growing species are shown in Table 37.

Fails to produce progressive disease in guinea pigs, but is usually capable of producing local ulceration at sites with intradermal inoculation of 10^2–10^3 mg bacilli (Wayne, 1966).

Source: isolated from a specimen of human gastric lavage. Found in human gastric lavage or sputum specimens as casual residents, but is not considered to be a cause of disease (Kestle et al., 1967; Wayne, 1966). Also found in soil (Wolinsky and Rynearson, 1968).

DNA G+C content (mol%): not determined.

Type strain: ATCC 15754, CCUG 20995, CIP 104530, DSM 43505, JCM 12407.

Sequence accession no. (16S rRNA gene): X52919.

Additional remarks: in the 1st (1986) edition of *Bergey's Manual of Systematic Bacteriology*, Wayne and Kubica noted that "the similarities of cytoplasmic antigens of *Mycobacterium gastri* to those of *Mycobacterium kansasii*, as well as a number of shared biochemical properties, raised the question of whether *Mycobacterium gastri* represents a distinctly separate species. The distinctions based on M-catalase, nitrate reduction, pigment, drug susceptibility, surface antigens and clinical significance have tended to support separation of the species. However, the 16S rRNA gene phylogeny does not distinguish between these two species." Further studies are needed.

12. **Mycobacterium nebraskense** Mohamed, Iwen, Tarantolo and Hinrichs 2004, 2060[VP]

ne.bras.ken′se. N.L. neut. adj. *nebraskense* of or belonging to the State of Nebraska, USA.

Acid-fast, non-sporing rods. Growth occurs in 3 weeks on Middlebrook 7H11 agar and in 4 or more weeks on Löwenstein–Jensen medium. Colonies are rough with an elevated center and produce strong yellow pigmentation in the dark. Growth is inhibited on MacConkey agar (without crystal violet) and on 5% (w/v) NaCl agar. Temperature range for growth is 25–35°C; optimal temperature is 30–35°C; does not grow at 42°C. Differential characteristics of slowly growing species are shown in Table 37.

Source: description of this organism is based on five strains isolated from the sputum of patients living in different areas of Nebraska.

DNA G+C content (mol%): not determined.

Type strain: ATCC BAA-837, DSM 44803.

Sequence accession no. (16S rRNA gene): AY368456.

The *Mycobacterium avium* clade

13. **Mycobacterium avium** Chester 1901, 356[AL]

13a. **Mycobacterium avium subsp. avium** Chester 1901, 356[AL] (nom. cons. Opin. 47, Jud. Comm. 1973) Thorel, Krichevsky and Lévy-Frébault 1990, 258[VP]

a′vi.um. L. n. *avis* a bird; L. gen. pl. n. *avium* of birds.

Cells are short to long rods with some filaments, but no evidence of cord formation. Dilute inocula on inspissated egg or oleic acid-albumin media usually yield smooth, but occasionally rough, non-pigmented colonies after 7 or more days of incubation at 37°C; on ageing, colonies may become yellow. Temperature range for growth is 37–42°C; human/porcine isolates may have an extended temperature range (25–45°C). Some strains may be scotochromogenic exhibiting a bright yellow pigment (Thorel et al., 1990). Certain strains are tolerant of 5% (w/v) NaCl when incorporated in Middlebrook 7H10 medium. When

first isolated from lesions, colonies on agar are flat and translucent, and exhibit the characteristic pathogenicity in experimental animals described below. On repeated passage, this colony form is replaced by a domed opaque type; the latter shows greatly diminished pathogenicity (Meissner et al., 1974).

Schaefer (1965) established two agglutinating serovars for *Mycobacterium avium*. Bacilli isolated from diseased birds almost always fall into one of these serovars, with serovar II as the most frequent cause of natural bird infection. Subsequently, Marks et al. (1969) showed that serovar II could be divided into two subtypes which showed some cross-reaction. The serovar designations I and II have since been replaced by serovars 1, 2, and 3 (Wolinsky and Schaefer, 1973). Serovar 2 is the most consistently pathogenic in experimental infection of chickens. Stanford and Grange (1974) found that, in addition to common genus antigens, immunodiffusion analysis of extracts revealed at least six antigens which were shared by *Mycobacterium avium*, *Mycobacterium intracellulare*, and *Mycobacterium lepraemurium*, but not by other mycobacterial species. Reciprocal intradermal testing in most cases permits discrimination between *Mycobacterium avium* and *Mycobacterium intracellulare* (Magnusson, 1980; Meissner et al., 1974). Strains of *Mycobacterium avium* may be susceptible to several phages, but no pattern is characteristic of any given serovar (Crawford et al., 1981b). TLC of methanolysates shows the presence of α- and keto-mycolates, and wax esters. Differential characteristics of slowly growing species are shown in Table 37.

Avian serovars 1 and 2 are widely distributed over at least three continents (Wolinsky and Rynearson, 1968), although type 3 is seen mainly in Europe (Marks et al., 1969). Experimentally, in the rabbit and mouse, the organism usually proliferates without macroscopic tubercles. Not pathogenic for guinea pigs or rats. Lesions in cattle may be caused by either *Mycobacterium avium* or *Mycobacterium intracellulare* serovars (Schaefer, 1968). Chickens infected intravenously with 5 mg moist bacilli die within 2 months, showing gross lesions in the spleen and microscopic lesions in lungs and spleen (Engbaek et al., 1968). An inoculum of 0.01 mg kills rabbits, but not mice. Rabbits inoculated intravenously with 5 mg moist bacilli usually die within 40 d showing macroscopic lesions in the spleen, and occasionally lungs, and microscopic lesions in the spleen and lungs. Animals surviving 3 months show lesions in joints and tendon sheaths (Engbaek et al., 1964; Engbaek et al., 1968).

Mycobacterium avium serotypes have been implicated in human pulmonary disease, although *Mycobacterium avium*-like organisms causing human disease are usually more similar to *Mycobacterium intracellulare* and fit one of the serovars of that species (Hobby et al., 1967). *Mycobacterium avium* may cause disseminated disease in immunocompromised individuals and lymphadenitis in immunecompetent children (Thorel et al., 1990). Other properties of the organisms can be found elsewhere (Thorel et al., 1990; Wayne and Kubica, 1986).

Source: isolated from tubercles in fowls. Widely distributed as the causal agent of tuberculosis in birds and less frequently found in lesions or lymph nodes of cattle, swine, or other animals. Rarely found in soil.

DNA G+C content (mol%): 69.0 (whole-genome sequencing).

Type strain: ATCC 25291, CCUG 20992, CIP 104244, DSM 44156, NCTC 13034.

Sequence accession no. (16S rRNA gene): AJ536037.

Additional remarks: the subspecies name *Mycobacterium avium* subsp. *avium* Chester 1901 was automatically created by the valid publications of *Mycobacterium avium* subsp. *paratuberculosis* (Bergey et al. 1923; Thorel et al. 1990), and of *Mycobacterium avium* subsp. *silvaticum* Thorel et al. 1990. Separation of *Mycobacterium avium* into three subspecies is supported by numerical taxonomic data (Thorel et al., 1990) and by analysis of DNA fragments by field inversion gel electrophoresis (Lévy-Frébault et al., 1989). Based on differences in *IS*1245 RFLP, the 16S–23S rRNA gene ITS sequences, and growth temperature, it has been suggested, but not formally proposed, that the designation *Mycobacterium avium* subsp. *avium* be reserved for bird-type isolates and that of "*Mycobacterium avium* subsp. *hominissuis*" be applied to strains isolated from humans or pigs (Mijs et al., 2002).

In cooperative taxonomy studies based on biochemical tests, growth characteristics, and drug susceptibilities, *Mycobacterium avium* and *Mycobacterium intracellulare* present as clusters with extensive overlap, whereas *Mycobacterium scrofulaceum* appears as a discrete cluster (Meissner et al., 1974; Wayne, 1982). Hawkins (1977) proposed that strains which appear to belong to *Mycobacterium avium*, *Mycobacterium intracellulare*, or *Mycobacterium scrofulaceum*, but are inconsistent in phenotypic properties, be treated as *Mycobacterium avium/intracellulare/scrofulaceum* (MAIS) intermediates until their taxonomic standing could be clarified. This proposal has been misinterpreted by some workers who refer to the "MAIS complex", a taxon which includes well-defined strains of these three species, implying that they bear a close taxonomic relationship to one another. There is ample evidence, based on numerical taxonomic data (Meissner et al., 1974), reciprocal intradermal skin testing (Magnusson, 1980), immunodiffusion studies (Stanford and Grange, 1974), immunologic distance of catalases (Wayne and Diaz, 1979, 1982), and DNA homology data (Baess, 1979), that *Mycobacterium scrofulaceum* is distinct from *Mycobacterium avium* and *Mycobacterium intracellulare*. These studies provide more equivocal evidence on relationships between *Mycobacterium avium* and *Mycobacterium intracellulare*, as reflected in two conflicting recommendations, presented as majority and minority opinions, on the advisability of reducing *Mycobacterium intracellulare* to synonymy of *Mycobacterium avium* (Meissner et al., 1974). DNA–DNA hybridization studies have confirmed that *Mycobacterium avium*, *Mycobacterium intracellulare*, and *Mycobacterium scrofulaceum* belong to distinct genomic species and indicate that strains belonging to serovars 4, 5, 6, and 8 appear to be more closely related to *Mycobacterium avium* than to *Mycobacterium intracellulare* (Baess, 1983). For convenience in clinical mycobacteriology, strains phenotypically resembling *Mycobacterium avium* (including *Mycobacterium intracellulare*) and not distinguished by molecular hybridization probes are said to belong to the *Mycobacterium avium* complex (MAC). Several strains formerly assigned to this complex have now been described as separate species.

13b. **Mycobacterium avium subsp. paratuberculosis** (Bergey et al. 1923) Thorel, Krichevsky and Lévy-Frébault 1990, 259[VP] (*Mycobacterium paratuberculosis* Bergey, Harrison, Breed, Hammer and Huntoon 1923, 374)

pa.ra.tu.ber.cu.lo′sis. Gr. pref. *para* beside, related; N.L. n. *tuberculosis* tuberculosis; N.L. gen. n. *paratuberculosis* of tuberculosis-like, of paratuberculosis.

Cells are cocco-bacillary, 1–2 μm in length. They stain uniformly, but occasionally longer forms may show alternately stained and unstained segments. Cord formation does not occur. This organism is difficult to cultivate. Originally, growth in primary cultures was only possible in media containing heat-killed acid-fast bacilli (Twort and Ingram, 1913). It is now apparent that this requirement can be overcome by supplementing media with mycobactin (Snow, 1970). Mycobactins are iron-binding hydroxamate compounds produced by all mycobacteria, except *Mycobacterium avium* subsp. *paratuberculosis*. On subculture strains can be adapted to produce a mycobactin (Merkal and McCullough, 1982). Growth may be further stimulated by pyruvate, but is not enhanced by culture at pH 5.5 (Thorel et al., 1990). Growth is inhibited by 5% (w/v) NaCl. Strains are characteristically tolerant to cycloserine (50 μg/ml). Despite the presence of growth stimulants, 3–4 months are necessary for primary isolation. On subculture, rough, non-pigmented colonies appear after incubation for 3–6 weeks. TLC of methanolysates shows the presence of α- and keto-mycolates, and wax esters. Differential characteristics of slowly growing species are shown in Table 37.

Source: isolated from cattle with chronic hypertrophic enteritis or Johne's disease by Johne and Frothingham (1895). The organism also causes enteritis in other ruminants and has been implicated in the pathogenesis of Crohn's disease in humans (McFadden et al., 1992; McFadden et al., 1987). Strains have not been isolated from the environment.

DNA G+C content (mol%): 69.3 (whole-genome sequencing).

Type strain: ATCC 19698, CIP 103963, DSM 44133.

Sequence accession no. (16S rRNA gene): X52934.

Additional remarks: Mycobacterium avium subsp. *paratuberculosis* can be distinguished from other members of the MAC by its stable mycobactin dependence (Thorel et al., 1990) and by the presence of multiple copies of the unique insertion element *IS*900 (Green et al., 1989; McFadden et al., 1992; McFadden et al., 1987). Three RFLP types have been recognized in animal isolates of the organism using an *IS*900 probe. Bovine and ovine strains shared the same RFLP types (types A and B), whereas a single caprine isolate had a unique RFLP pattern (type C).

13c. **Mycobacterium avium subsp. silvaticum** Thorel, Krichevsky and Lévy-Frébault 1990, 259[VP]

sil.va′ti.cum. L. neut. adj. *silvaticum* of or belonging to a wood or to trees.

Acid-fast short to long rods with no cord formation. Rough colonies occur after incubation for 2 weeks or more on oleic acid medium when enhanced by low pH (pH 5.5). Growth is not stimulated by pyruvate and does not occur on Löwenstein–Jensen or other egg-based media. Growth is inhibited by 5% (w/v) NaCl and by cycloserine (50 μg/ml). Strains do not require mycobactin for growth. Differential characteristics of slowly growing species are shown in Table 37.

Source: isolated from birds, especially wood pigeons and cranes, and from mammals, especially deer. Strains have not been isolated from the environment and are obligate pathogens for animals, causing tuberculosis in birds and paratuberculosis in mammals (Collins et al., 1985; Mathews and McDiarmid, 1979; McDiarmid, 1948).

DNA G+C content (mol%): not determined.

Type strain: CIP 103317, ATCC 49884, CCUG 47446, DSM 44175.

Sequence accession no. (16S rRNA gene): EF521891.

14. **Mycobacterium intracellulare** (Cuttino and McCabe 1949) Runyon 1965, 258[AL] [*Nocardia intracellularis* Cuttino and McCabe 1949, 16; *Mycobacterium intracellularis* (*sic*) Runyon 1965, 258]

in.tra.cel.lu′la.re. L. prep. *intra* within; L. n. *cellula* small room and in biology a cell; L. neut. suff. *-are* suffix denoting pertaining to; N.L. neut. adj. *intracellulare* intracellular.

Acid-fast, short to long rods; transiently filamentous in new growth, eventually becoming coccobacillary. Dilute inocula on inspissated egg and oleic acid agar media usually yield smooth, rarely rough, non-pigmented colonies after incubation at 37°C for 7 or more days; on ageing, colonies may become yellow. TLC of methanolysates shows α- and keto-mycolates, and wax esters. Differential characteristics of slowly growing species are shown in Table 37.

Source: isolated from fatal systemic disease in a child. Most frequently encountered in pulmonary secretions from individuals suffering from tuberculosis-like disease and from surgical specimens of such patients. When isolated from human secretions, it often represents the etiologic agent of pulmonary disease, although it is frequently isolated as an apparent casual resident (Wolinsky, 1979a). Also isolated from limited disease processes in cattle and swine. Occasionally found in soil or water (Wendt et al., 1980; Wolinsky and Rynearson, 1968).

DNA G+C content (mol%): not determined.

Type strain: ATCC 13950, CCUG 28005, CIP 104243, DSM 43223, JCM 6384, NCTC 13025.

Sequence accession no. (16S rRNA gene): AJ536036.

Additional remarks: Experimentally, causes limited disease in chickens and mice though much less severe than *Mycobacterium avium*, and rarely fatal. In general, chicken pathogenicity corresponds to serovar, with *Mycobacterium avium* serovar II more consistently pathogenic than the *Mycobacterium intracellulare* serovars (Meissner et al., 1974). Growth at 42°C has been reported to increase pathogenicity (Scammon et al., 1964). Lesions in guinea pigs are usually limited to the site of inoculation. Hamsters inoculated intra-testicularly show extensive local lesions and, frequently, secondary focal lesions in liver, spleen, and abdominal lymph nodes. Rabbits receiving 10^{-3} mg intravenously do not develop demonstrable lesions (Feldman and Ritts, 1963) but 5 mg, although not causing death,

produces rare macroscopic lesions in visceral organs and usually moderate-to-severe lesions in joints and tendon sheaths (Engbaek et al., 1964).

At least 17 agglutinating serovars have been established among cultures identified as *Mycobacterium intracellulare* (Wolinsky and Schaefer, 1973), but have been labeled "*avium* complex" (serovars 4–20). Members of these serovars are predominantly non-pathogenic for chickens and have been isolated mainly from man, cattle, and swine, as opposed to three distinct serovars of *Mycobacterium avium*, which are virulent for chickens, and less frequently associated with mammalian disease. Strains of "*avium* complex" serovar 8, once proposed as the separate species "*Mycobacterium brunense*" (Kazda, 1967) and later considered to belong to *Mycobacterium intracellulare* (Kubin et al., 1969) appear intermediate between *Mycobacterium avium* and *Mycobacterium intracellulare* in virulence for experimental animals. Many, if not most strains of "*avium* complex" serovar 18 more closely resemble *Mycobacterium simiae* than *Mycobacterium intracellulare* (Wayne et al., 1983). No single phage typing pattern is characteristic of this species or of individual serovars within it (Crawford et al., 1981b). See also discussion of the antigenic structure of *Mycobacterium avium*.

15. **Mycobacterium chimaera** Tortoli, Rindi, Garcia, Chiaradonna, Dei, Garzelli, Kroppenstedt, Lari, Mattei, Mariottini, Mazzarelli, Murcia, Nanetti, Piccoli and Scarparo 2004, 1283[VP]

chi.ma.e′ra. L. fem. n. *chimaera* the chimaera, the mythological being composed of parts of three different animals, referring to the apparent mix of genetic features characterizing the strains.

Acid-fast, nonmotile, non-spore-forming cocco-bacilli. Colonies are slow-growing and non-pigmented. Growth does not occur on MacConkey agar or on media supplemented with 5% (w/v) NaCl. Temperature range for growth is 25–37°C; does not grow at 45°C. In the widely used Accuprobe identification system, *Mycobacterium chimaera* strains hybridize with probes for the *Mycobacterium avium* complex and for *Mycobacterium intracellulare*, but not with the specific probe for *Mycobacterium avium*. TLC of methanolysates shows the presence of α- and keto-mycolates, and wax esters. Differential characteristics of slowly growing species are shown in Table 37.

Source: isolated from either sputum or bronchial aspirates obtained from elderly patients. Seven of the twelve strains studied by Tortoli et al. (2004) were considered to be of clinical significance.

DNA G+C content (mol%): not determined.

Type strain: CIP 107892, CCUG 50989, DSM 44623.

Sequence accession no. (16S rRNA gene): AJ548480.

Additional remarks: the 16S rRNA gene sequence of this species shows only one nucleotide mismatch with that of *Mycobacterium intracellulare* compared with 20 or more mismatches in sequences in the 16S–23S rRNA gene-ITS region. In contrast, the ITS sequence showed only one mismatch in comparison to that of sequevar *Mycobacterium avium* complex-A. However, *Mycobacterium chimaera* has a HPLC profile that distinguishes it from that of other members of the *Mycobacterium avium* complex.

16. **Mycobacterium colombiense** Murcia, Tortoli, Menendez, Palenque and Garcia 2006, 2053[VP]

co.lom.bi.en′se. N.L. neut. adj. *colombiense* of or belonging to Colombia, the South American country where the strains were first isolated.

Acid-fast nonmotile rods. Visible growth of non-pigmented, rough colonies appears on Löwenstein–Jensen medium and on Ogawa Kudoh and Sauton agars in 3 weeks. Some colonies may develop yellowish pigmentation with age. Does not grow on MacConkey agar or on media supplemented with 5% (w/v) NaCl. Temperature range for growth is 20–37°C. Strains show a relationship to members of the *Mycobacterium avium* complex. They hybridize with probes for the *Mycobacterium avium* complex in the widely used Accuprobe identification system. However, *Mycobacterium colombiense* differs from other members of the *Mycobacterium avium* complex in producing urease. Differential characteristics of slowly growing species are shown in Table 37.

Source: the type strain was isolated from the blood of an HIV-positive patient in Colombia.

DNA G+C content (mol%): not determined.

Type strain: CIP 108962.

Sequence accession nos: AM062764 (16S rRNA gene), AM062764 (ITS-1), AM062765 (*hsp65*).

Additional remarks: Mycobacterium colombiense can be distinguished from members of the *Mycobacterium avium* complex by its unique 16S rRNA gene sequence and by DNA–DNA relatedness data. Additionally, although *Mycobacterium colombiense* shows an *hsp*65 sequence identical to that of *Mycobacterium avium* variant I (av-I), it also displays a unique ITS-1 sequence (MAC-X).

The *Mycobacterium ulcerans* clade

17. **Mycobacterium ulcerans** MacCallum, Tolhurst and Buckle *in* Fenner 1950, 817[AL]

ul′ce.rans. L. part. adj. *ulcerans* making sore, causing to ulcerate.

Acid-fast, Gram-stain-positive, moderately long rods. Growth on inspissated egg medium is evident after incubation for 4 weeks at 30–33°C as minute, transparent, domed colonies. On ageing, colonies become convex to flat, with irregular outlines, and have a rough, yellow surface. Rough, corded colonies develop on oleic acid-albumin agar. Weak growth is observed at 25°C, but does not grow at 37°C. In contrast to its close relative *Mycobacterium marinum*, *Mycobacterium ulcerans* does not adapt to growth at 37°C on repeated subculture. TLC of methanolysates shows the presence of α-, keto-, and methoxy-mycolates. Differential characteristics of slowly growing species are shown in Table 37.

Mycobacterium ulcerans has been recovered from ulcerative skin infections of humans in Malaya, Mexico, New Guinea, and on the African continent (Boisvert, 1977). Skin ulcers in humans are characterized by indolent extensions from areas of inconspicuous induration to involvement of large areas with undermining edges. Mice and rats can be infected experimentally; but not fowls, guinea pigs, lizards, or rabbits. Experimentally inoculated rats develop hemorrhagic necrotic lesions surrounded by zones of cellular

accumulations consisting of leukocytes, lymphocytes, and macrophages, but without giant cells. The necrotic and cellular zones show large clumps of acid-fast bacilli in the extracellular spaces and in macrophages. In experimental animals, lesions develop only in cooler parts of the body. Inoculation of mouse footpads consistently causes local lesions. Inoculation by the intranasal, intraperitoneal, or intravenous route does not cause visceral lesions, but a long incubation results in ulcerating lesions in hairless peripheral parts of the body and on the scrotum (Fenner, 1956). The characteristic necrosis is associated with an extracellular heat-labile toxin with a molecular mass of approximately 105 (Read et al., 1974). Human lesions do not show inflammatory responses, but consist of areas of lipid necrosis and tissue breakdown.

Source: originally isolated from a human skin lesion in Australia.

DNA G+C content (mol%): 65.4 (whole-genome sequencing).

Type strain: ATCC 19423, CIP 105425, NCTC 10417.

Sequence accession no. (16S rRNA gene): X88926.

Additional remarks: Mycobacterium ulcerans appears to be closely related to *Mycobacterium marinum* as both species have identical 16S rRNA gene and ITS sequences (Portaels et al., 1996; Roth et al., 1998). However, only the *Mycobacterium ulcerans* genome contains the insertion sequence *IS*2404 (Stinear et al., 1999). Additionally, *Mycobacterium ulcerans* causes a more progressive, malignant disease than *Mycobacterium marinum* and is found in tropical regions whereas *Mycobacterium marinum* is common in temperate climates. The name "*Mycobacterium buruli*", which has no formal standing in nomenclature, was applied to strains isolated in Uganda (Clancey, 1964). "*Mycobacterium buruli*" strains are considered to be members of *Mycobacterium ulcerans* on the basis of biochemical (Schröder, 1975) and immunological (Stanford and Grange, 1974) data.

18. **Mycobacterium marinum** Aronson 1926, 320[AL]

ma.ri'num. L. neut. adj. *marinum* of the sea, marine.

Acid-fast, moderate to long rods with frequent cross barring. Colonies are smooth to rough on Löwenstein–Jensen medium and Middlebrook agar after incubation for 7 or more days at 30°C. On primary isolation, growth is restricted to the temperature range 25–35°C, but adaptation to 37°C may occur after several subcultures. Colonies are photochromogenic; they show no pigmentation in the dark, but when grown in light or exposed to light when colonies are young, they develop a brilliant yellow color. TLC of methanolysates shows α-, keto-, and methoxy-mycolates. Differential characteristics of slowly growing species are shown in Table 37.

In humans, *Mycobacterium marinum* may cause skin lesions resulting from infection of abrasions incurred in swimming pools or fish tanks. Causes cutaneous granulomas ("swimming pool granuloma"), usually on elbows, fingers, forearms, or wrists, but also can occur on feet, knees, and toes. Papules or nodules occur which may ulcerate. Healing may be spontaneous over a period of some months (Norden and Linell, 1951; Schaefer and Davis, 1961). However, chemotherapy may often be required.

Mice receiving a large inoculum intraperitoneally develop ulcerations on paws, scrotum, and tail; visceral lesions and death sometimes occur. Following intravenous inoculation, lesions are limited to the tail. Footpad inoculations lead to local swelling and some ulceration (Fenner, 1956). Guinea pigs inoculated subcutaneously or by inhalation do not exhibit disease; intraperitoneal inoculation occasionally leads to scrotal lesions. Rats inoculated intraperitoneally do not develop disease, but nodules occur in the omentum and hilar lymph nodes. Rabbits develop local lesions when inoculum is applied to abraded skin sites and they may develop granuloma with caseous necrosis in the scrotum after intraperitoneal or intravenous inoculation. Representatives of 50 poikilothermic species (amphibians, fish, and reptiles) have been found susceptible to fatal systemic infection when maintained at 30°C (Clark and Shepard, 1963).

Source: isolated from diseased fish and aquaria.

DNA G+C content (mol%): 65.7 (whole-genome sequencing).

Type strain: ATCC 927, CCUG 20998/27843, CIP 104528, DSM 44344, JCM 12275, NCTC 2275.

Sequence accession no. (16S rRNA gene): AF456240.

Additional remarks: Mycobacterium marinum strains have also been classified under the invalid names "*Mycobacterium balnei*" and "*Mycobacterium platypoecilus*". However, Castelnuovo and Morellini (1962), employing immunoelectrophoretic analysis, concluded that these organisms represented a single species. Magnusson (1967) distinguished *Mycobacterium marinum* from 10 other mycobacterial species, including *Mycobacterium kansasii*, by dermal hypersensitivity. One agglutinating serovar was originally recognized and cross-reaction is not seen with *Mycobacterium kansasii* by this technique, by immunodiffusion with phenol-soluble antigen (Wayne, 1971), or by immunofluorescence (Jones and Kubica, 1968). A second serovar has been proposed (Goslee et al., 1976). Stanford and Grange (1974) detected three antigens by immunodiffusion that were shared by "*Mycobacterium balnei*" and *Mycobacterium marinum*, but not by other mycobacterial species. A large structural divergence between the T-catalase of *Mycobacterium marinum* and those of *Mycobacterium avium* and *Mycobacterium kansasii* has been detected serologically (Wayne and Diaz, 1982). Numerical taxonomic studies concluded that members of *Mycobacterium marinum* form a discrete cluster (Wayne et al., 1978; Wayne et al., 1991). *Mycobacterium marinum* appears to be closely related to *Mycobacterium ulcerans*; both species show only minor variations in 16S rRNA gene and ITS sequences (Portaels et al., 1996; Roth et al., 1998). However, only the *Mycobacterium ulcerans* genome contains the insertion sequence *IS*2404 (Stinear et al., 1999).

19. **Mycobacterium shottsii** Rhodes, Kator, Kotob, van Berkum, Kaattari, Vogelbein, Quinn, Floyd, Butler and Ottinger 2003, 423[VP]

shot'tsi.i. N.L. gen. masc. n. *shottsii* of Shotts, named after Emmett Shotts, an American fish bacteriologist.

Acid-fast, coccobacilli (0.8–1 × 0.4–0.6 μm), which may form cell aggregates in culture. Visible growth is observed from dilute inocula after incubation at 23°C for 4–6 weeks. Little or no growth occurs at 30°C and none occurs at 37°C

or above. Colonies on Middlebrook 7H10 agar are small (0.5–1.0 mm), dysgonic, rough, non-pigmented, and typically flat with an irregular margin, becoming umbonate on ageing. Smooth colonies with an entire margin are seen less frequently. Growth does not occur on MacConkey agar (without crystal violet) or on 5% (w/v) NaCl agar. Differential characteristics of slowly growing species are shown in Table 37.

Source: isolated from striped bass (*Morone saxatilis*) during an epizootic of mycobacteriosis in Chesapeake Bay, Maryland, USA.

DNA G+C content (mol%): not determined.

Type strain: ATCC 700981, JCM 12657, NCTC 13215.

Sequence accession no. (16S rRNA gene): AY005147.

Additional remarks: this species shares a high 16S rRNA gene sequence similarity to *Mycobacterium marinum* and *Mycobacterium ulcerans* (99.2%), but differs from it in some phenotypic characteristics and is unusual in displaying a positive niacin test. See also comments under the description of *Mycobacterium pseudoshottsii*.

20. **Mycobacterium pseudoshottsii** Rhodes, Kator, McNabb, Deschayes, Reyrat, Brown-Elliot, Wallace, Trott, Parker, Lifland, Osterhout, Kaattari, Reece, Vogelbein and Ottinger 2005, 1144[VP]

pseu.do.shot′tsi.i. Gr. adj. *pseudês* false; N.L. gen. n. *shottsii* name of a species; N.L. gen. n. *pseudoshottsii* a false *Mycobacterium shottsii*, not the true *Mycobacterium shottsii*.

Acid-fast coccobacilli (0.8–1.0 × 0.4–0.6 µm) which form cell aggregates in culture. Visible growth is observed after 4–6 weeks incubation at 23°C from dilute inocula. Little or no growth occurs at 30°C and none occurs at 37°C or above. Colonies on Middlebrook 7H10 agar are small (1–3 mm), rough, photochromogenic, and typically flat with an irregular margin, becoming umbonate on ageing. A pale yellow to gold pigment develops following exposure to light and intensifies with age. Does not grow on MacConkey agar (without crystal violet) or on 5% (w/v) NaCl agar. Differential characteristics of slowly growing species are shown in Table 37.

Source: isolated from granulomatous lesions in splenic tissue from a striped bass (*Morone saxatilis*) during an epizootic of mycobacteriosis in Chesapeake Bay, Maryland, USA.

DNA G+C content (mol%): not determined.

Type strain: ATCC BAA-883, NCTC 13318.

Sequence accession nos: AY570988 (16S rRNA gene); AY571788 (*hsp65*).

Additional remarks: despite sharing some phenotypic characters and a common host, this species can be clearly differentiated from *Mycobacterium shottsii* on the basis of key phenotypic characters and on 16S rRNA gene, *erp*, and *hsp65* gene sequencing. However, on the basis of 16S rRNA and *hsp65* gene sequences, it is very similar to scotochromogenic species isolated from Mediterranean fish and described as strains of *Mycobacterium marinum* by Ucko et al. (2002).

The *Mycobacterium gordonae* clade

21. **Mycobacterium gordonae** Bojalil, Cerbón and Trujillo 1962, 344[AL]

gor.do′na.e. N.L. gen. fem. n. *gordonae* of Gordon, named after the American bacteriologist Ruth E. Gordon.

Cells are moderate to long rods. Colonies on Löwenstein–Jensen medium and Middlebrook agar are usually smooth, soft, and yellow- or orange-pigmented after incubation for 7 d. Pigment is produced when grown in the dark, but is often intensified when grown in continuous light. Temperature range for growth is 28–37°C; optimal temperature is 35°C; does not grow at 45°C. TLC of methanolysates shows the presence of α-, keto-, and methoxy-mycolates. Differential characteristics of slowly growing species are shown in Table 37.

Rarely, if ever, implicated in human disease but it has been considered to cause infections in severely immunocompromised individuals (Lessnau et al., 1993; Wolinsky, 1979a).

Source: frequently encountered as a casual resident in human sputum and gastric lavage specimens; also found in water taps and soil.

DNA G+C content (mol%): not determined.

Type strain: ATCC 14470, CCUG 21801/21811, CIP 104529, DSM 44160, JCM 6382, NCTC 10267.

Sequence accession no. (16S rRNA gene): X52923.

Additional remarks: at one time, many *Mycobacterium gordonae* strains were referred to by the illegitimate name "*Mycobacterium aquae*" though the "type strain" of "*Mycobacterium aquae*" had the properties of *Mycobacterium smegmatis* (Wayne, 1970). The name "*Mycobacterium aquae*" was then applied to strains that resembled *Mycobacterium gordonae*, but yielded a different TLC pattern (Jenkins et al., 1972). In Opinion 55, the Judicial Commission placed the name "*Mycobacterium aquae*" on the list of *nomina rejicienda* (Judicial Commission of the International Committee on Systematic Bacteriology, 1982).

Immunodiffusion analysis of bacillary extracts demonstrates five precipitin lines unique to *Mycobacterium gordonae* (Stanford and Grange, 1974). Members of the species were not agglutinated by sera produced against *Mycobacterium avium*, *Mycobacterium intracellulare*, *Mycobacterium kansasii*, *Mycobacterium marinum*, or *Mycobacterium scrofulaceum* (Hobby et al., 1967). Seven agglutinating serovars of *Mycobacterium gordonae* were proposed by Goslee et al. (1976). Their surface lipid antigens yielded characteristic chromatographic patterns (Jenkins et al., 1972), which are of the alkali-labile type associated with *Mycobacterium kansasii* rather than the alkali-stable type seen with *Mycobacterium scrofulaceum* (Brennan, 1981).

22. **Mycobacterium asiaticum** Weiszfeiler, Karasseva and Karczag 1971, 247[AL]

a.si.a′ti.cum. L. neut. adj. *asiaticum* Asiatic, of Asia.

Acid-fast coccoid rods. Growth on inspissated egg medium is dysgonic after incubation for 15–21 d at 37°C. Strains are usually photochromogenic, but occasionally fail to develop pigment after exposure to light; pigment is not produced in the dark (Wayne et al., 1981). TLC of methanolysates shows the presence of α-, keto-, and methoxy-mycolates. Differential characteristics of slowly growing species are shown in Table 37.

Produces focal lung lesions after intravenous inoculation of mice; may kill mice within 30–60 d (Weiszfeiler et al., 1971).

Source: original strains were isolated from monkeys, but one strain has been considered to be the cause of human lung disease (Wayne et al., 1981).

DNA G+C content (mol%): not determined.

Type strain: ATCC 25276, CCUG 29115, CIP 106809, DSM 44297, JCM 6409.

Sequence accession no. (16S rRNA gene): AF480595.

Additional remarks: Mycobacterium asiaticum strains group in a cluster that is distinct from that of *Mycobacterium gordonae* on numerical taxonomic analysis, but only pigmentation provides a simple criterion for distinguishing between these species (Wayne et al., 1981). Intradermal skin testing demonstrated homogeneity of the species and confirmed separation from five other mycobacterial species including *Mycobacterium simiae*, but no comparison was made with *Mycobacterium gordonae* (Baess and Magnusson, 1982). *Mycobacterium asiaticum* has a very low DNA homology to *Mycobacterium simiae*, but has not been compared with *Mycobacterium gordonae* (Baess and Magnusson, 1982).

The *Mycobacterium interjectum* clade

23. **Mycobacterium interjectum** Springer, Kirschner, Rost-Meyer, Schröder, Kroppenstedt and Böttger 1993, 3088[VP]

in.ter.jec′tum. L. v. *interjacio* to set, place, or put between; L. neut. part. adj. *interjectum* placed between, corresponding to the phylogenetic position between rapid and slow-growing mycobacteria.

Acid–alcohol-fast, rod-shaped coccobacilli (0.7–2.0 × 0.6–1.0 μm) which are often pleomorphic and may exhibit filaments (up to 6.0 μm). Spores, capsules, and aerial hyphae do not occur. Visible growth from dilute inocula requires incubation for 3–4 weeks. Colonies on Löwenstein–Jensen medium are dysgonic, smooth, scotochromogenic, yellowish in color, and 1–2 mm in diameter. Temperature range for growth is 31–37°C; does not grow at 22 or 41°C. TLC of methanolysates shows the presence of α-, keto-, and epoxy-mycolates, and wax esters. Differential characteristics of slowly growing species are shown in Table 37.

Source: isolated from an 18-month-old child with cervical lymphadenitis.

DNA G+C content (mol%): not determined.

Type strain: DSM 44064, CCUG 37514, ATCC 51457.

Sequence accession no. (16S rRNA gene): X70961.

24. **Mycobacterium saskatchewanense** Turenne, Thibert, Williams, Burdz, Cook, Wolfe, Cockcroft and Kabani 2004b, 665[VP]

sas.kat.che.wa.nen′se. N.L. neut. adj. *saskatchewanense* of or pertaining to Saskatchewan.

Acid–alcohol-fast, long, beaded rods. Colonies on Middlebrook 7H10 agar develop after incubation for 2–4 weeks; they are initially transparent, but mature colonies show a bright yellow scotochromogenic pigmentation in a raised center, and a transparent irregular apron. Growth on Löwenstein–Jensen medium is rough and dry in texture. Some strains show a thin film of growth on MacConkey agar (without crystal violet), but growth does not occur on media supplemented with 5% (w/v) NaCl. Temperature range for growth is 25–37°C; optimal temperature for growth is 37°C; does not grow at 42°C. Differential characteristics of slowly growing species are shown in Table 37.

Strains have been isolated from respiratory samples, but without proven disease involvement, suggesting that this species may be an opportunistic pathogen for humans.

Source: isolated from sputum samples from an elderly female patient with bronchiectasis and a recurrent chest infection.

DNA G+C content (mol%): not determined.

Type strain: ATCC BAA-544, CIP 108114, DSM 44616, JCM 13016.

Sequence accession no. (16S rRNA gene): AY208856.

Additional remarks: PCR/restriction enzyme sequence analysis of the *hsp65* gene revealed a close relationship between *Mycobacterium saskatchewanense* and *Mycobacterium interjectum*; a relationship supported by similarity of HPLC profiles of some strains assigned to *Mycobacterium interjectum* (although not the type strain). *Mycobacterium saskatchewanense* strains hybridize with the commonly used *Mycobacterium avium* complex hybridization probe (AccuProbe), but not with specific probes, they are also phenotypically distinct from *Mycobacterium avium* and *Mycobacterium intracellulare*. Additionally, HPLC profiling suggests a similarity to *Mycobacterium palustre*. It is clear that without sequence-based identification *Mycobacterium saskatchewanense* may be difficult to recognize (Turenne et al., 2004b).

The *Mycobacterium palustre* clade

25. **Mycobacterium palustre** Torkko, Suomalainen, Livanainen, Tortoli, Suutari, Seppänen, Paulin and Katila 2002, 1524[VP]

pa.lus′tre. L. neut. adj. *palustre* living in swamps, referring to the isolation of the organisms from peat land run-off water.

Acid–alcohol-fast bacilli. Visible growth from dilute inocula occurs following incubation for 2–3 weeks; mature colonies require incubation for 4–5 weeks. Colonies on Löwenstein–Jensen medium and on Middlebrook agar are yellow to pale yellow, smooth and scotochromogenic at 36°C, and photo- or scotochromogenic at 42°C. Temperature range for growth is 30–42°C; optimal growth is at 36°C; growth at 45°C requires heavy inocula and incubation for 5–6 weeks. Differential characteristics of slowly growing species are shown in Table 37.

Source: the description of the species is based on 13 isolates, eight of which were cultured from water samples collected from Finnish streams (Livanainen et al., 1993), two from sputum samples, one from a submandibular lymph node biopsy of a child, and two from submandibular tissue of pigs.

DNA G+C content (mol%): not determined.

Type strain: DSM 44572, ATCC BAA-377.

Sequence accession no. (16S rRNA gene): AJ308603.

Additional remarks: all clinical, environmental, and veterinary strains of this species hybridize with the commonly used *Mycobacterium avium* complex hybridization probe (AccuProbe), but not with the species-specific probes for *Mycobacterium avium* and *Mycobacterium intracellulare*.

26. **Mycobacterium parascrofulaceum** Turenne, Cook, Burdz, Pauls, Thibert, Wolfe and Kabani 2004b, 1550[VP]

pa.ra.scro.fu.la′ce.um. Gr. prep. *para* like; N.L. neut. adj. *scrofulaceum* specific epithet of a bacterial species; N.L. neut.

adj. *parascrofulaceum* beside *Mycobacterium parascrofulaceum* (referring to the phenotypic resemblance of the isolate to *Mycobacterium scrofulaceum*).

Pleomorphic acid-fast bacilli which show moderate beading. Colonies on Löwenstein–Jensen medium and on Middlebrook 7H10 agar grow within 2–4 weeks and are scotochromogenic with yellow to orange pigmentation. Most strains form smooth and domed colonies, but dry-wrinkled and mucoid forms can occur. Does not grow on MacConkey agar or on 5% (w/v) NaCl agar. Temperature range for growth is 25–37°C; optimal growth is at 37°C; does not grow at 42°C. Differential characteristics of slowly growing species are shown in Table 37.

Source: most isolates are derived from human respiratory samples and in at least one case the organism was believed to be clinically significant.

DNA G+C content (mol%): not determined.

Type strain: ATCC BAA-614, DSM 44648, JCM 13015.

Sequence accession no. (16S rRNA gene): AY337273.

Additional remarks: this species was described after further characterization of strains initially assigned to *Mycobacterium scrofulaceum*. The organism is biochemically and morphologically indistinguishable from *Mycobacterium scrofulaceum*; HPLC of lipids shows that the species is almost identical to organisms of the *Mycobacterium avium* complex and some strains of *Mycobacterium scrofulaceum*. *Mycobacterium parascrofulaceum* is misidentified as *Mycobacterium scrofulaceum* with some commercial genotyping systems. Turenne et al. (2004a) found 16S rRNA gene sequence analysis established *Mycobacterium parascrofulaceum* as a distinct species related to *Mycobacterium palustre* and *Mycobacterium simiae*. The 16S rRNA gene phylogenetic tree indicates a relationship to *Mycobacterium palustre*, but a somewhat tenuous one to *Mycobacterium simiae* (Figure 86).

27. **Mycobacterium kubicae** Floyd, Gross, Bonato, Silcox, Smithwick, Metchock, Crawford and Butler 2000, 1814[VP]

ku.bi′ca.e. N.L. gen. masc. n. *kubicae* of Kubica, to honor the contributions of George P. Kubica, an exceptional American mycobacteriologist, mentor, and teacher.

Acid–alcohol-fast, generally rod-shaped cells that frequently bent with occasional coccoid forms (0.25–7.0 × 0.25–0.5 μm). Does not show branching or the formation of aerial mycelia. Colonies are yellow-colored and scotochromogenic. Smooth, domed growth occurs on Middlebrook 7H11 agar and film-like growth is seen on Löwenstein–Jensen medium. Temperature range for growth is 28–37°C on Middlebrook agar, but for most isolates is confined to 33–37°C on Löwenstein–Jensen medium; does not grow at 42°C. TLC of methanolysates shows the presence of α-, keto- and methoxy-mycolates. Differential characteristics of slowly growing species are shown in Table 37.

Source: fifteen isolates were obtained from throughout the United States. Eight were from sputum, three were from bronchial washings, and four were from unspecified sources.

DNA G+C content (mol%): not determined.

Type strain: ATCC 700732, CIP 106428, DSM 44627, JCM 13573.

Sequence accession no. (16S rRNA gene): AF133902.

The *Mycobacterium simiae* clade

28. **Mycobacterium simiae** Karassova, Weiszfeiler and Kraznay 1965, 282[AL]

si.mi′a.e. L. n. *simia* the ape; L. gen. *simiae* of the ape.

Short acid-fast rods. Smooth colonies develop on inspissated egg medium after incubation for 2–3 weeks. Usually photochromogenic, but sometimes fails to produce pigment on exposure to light. Temperature range for growth is 25–37°C; optimal growth is at 37°C; does not grow at 45°C. TLC of methanolysates shows the presence of α-, α′-, and keto-mycolates. Differential characteristics of slowly growing species are shown in Table 37.

Three agglutinating serovars, labeled simiae 1, simiae 2 and avian 18, have been recognized in strains identified as *Mycobacterium simiae* (Meissner and Schröder, 1975; Wayne et al., 1983). Reciprocal intradermal skin testing demonstrated a single cluster for the species, with subtle distinctions between strains of simiae serovars 1 and 2; avian serovar 18 was not included in the study (Baess and Magnusson, 1982). Immunoelectrophoretic analysis of bacillary extracts demonstrated many antigens shared between simiae 1 and 2 serovars and with *Mycobacterium szulgai* (Wayne et al., 1981).

Source: initially isolated from lymph nodes of apparently healthy monkeys (Karassova et al., 1965); the species has since been implicated in cases of human pulmonary disease (Wolinsky, 1979a). The organisms multiply extensively in organs of mice.

DNA G+C content (mol%): not determined.

Type strain: ATCC 25275, CCUG 29114/42427, CIP 104531, DSM 44165, JCM 12377.

Sequence accession no. (16S rRNA gene): X52931.

Additional remarks: numerical taxonomic analysis shows *Mycobacterium simiae* to be comprised of a highly homogeneous cluster of strains of serovar 18 and a less tightly linked subcluster of the other two serovars (Wayne et al., 1983). Serovar 18 strains yield a negative niacin test, whereas the others give a positive one. Serovar 1 and 2 strains have been distinguished from one another in DNA–DNA relatedness studies, but not at a level sufficient to justify the establishment of another species (Baess and Magnusson, 1982). Strains previously designated "*Mycobacterium habana*" (Valdivia et al., 1971) are included in *Mycobacterium simiae* serovar 1 (Meissner and Schröder, 1975).

29. **Mycobacterium florentinum** Tortoli, Rindi, Goh, Katila, Mariottini, Mattei, Mazzarelli, Suomalainen, Torkko and Rastogi 2005, 1105[VP]

flo.ren.ti′num. L. neut. adj. *florentinum* of or belonging to *Florentia*, the Italian city of Florence, from where the majority of the strains were collected and investigated.

Acid-fast, nonmotile, non-capsulated, asporogenous rods. Colonies on Löwenstein–Jensen medium are smooth, creamy, and non-chromogenic and appear after incubation for 2 weeks. Does not grow on MacConkey agar or on media supplemented with 5% (w/v) NaCl. Temperature range for growth is 25–37°C; does not grow at 45°C. TLC of methanolysates shows the presence of α-, keto-, and methoxy-mycolates. Differential characteristics of slowly growing species are shown in Table 37.

Source: description is based on eight strains isolated from human clinical samples; seven from sputa of different patients and the type strain, which was isolated from an excised lymph node of a young girl. One strain, isolated from a severe pulmonary infection in Finland, was considered to be a sequevar of *Mycobacterium triplex* (Suomalainen et al., 2001).

DNA G+C content (mol%): not determined.

Type strain: DSM 44852, CCUG 50992, CIP 108409.

Sequence accession no. (16S rRNA gene): AJ616230.

30. **Mycobacterium heidelbergense** Haas, Butler, Kirschner, Plikaytis, Coyle, Amthor, Steigerwalt, Brenner, Salfinger, Crawford, Böttger and Bremer 1998, 627[VP] (Effective publication: Haas, Butler, Kirschner, Plikaytis, Coyle, Amthor, Steigerwalt, Brenner, Salfinger, Crawford, Böttger and Bremer 1997, 3208.)

hei.del.ber.gen′se. N.L. neut. adj. *heidelbergense* of or belonging to Heidelberg, Germany, the source of the strain on which the description of the species is based.

Acid–alcohol-fast coccobacilli (2.0–3.0 × 0.5–0.8 μm) though pleomorphic forms may occur (up to 8.0 μm in length). Aerial hyphae, capsules, and spores are absent. Initial isolation was made in Middlebrook 7H12 liquid medium. Visible growth from dilute inocula appears on Löwenstein–Jensen medium in 3–4 weeks. Colonies are eugonic, dome-shaped, smooth, non-pigmented, and 0.5–1.0 mm in diameter. Temperature range for growth is 30–37°C; optimal growth is at 33–35°C; does not grow at 25 or 45°C. However, colonies may be observed on Middlebrook 7H11 agar at temperatures ranging between 25 and 45°C. TLC of methanolysates shows α-, α′-, and keto-mycolates. Differential characteristics of slowly growing species are shown in Table 37.

Source: originally isolated from cervical lymph node tissue of a child suffering from lymphadenitis.

DNA G+C content (mol%): not determined.

Type strain: ATCC 51253, CIP 105424, DSM 44471.

Sequence accession no. (16S rRNA gene): AJ000684.

31. **Mycobacterium triplex** Floyd, Guthertz, Silcox, Duffey, Jang, Desmond, Crawford and Butler 1996, 2966[VP]

tri′plex. L. neut. adj. *triplex* threefold, triple, referring to something consisting of three parts, specifically, the triple-cluster HPLC pattern shown by these isolates.

Acid–alcohol-fast, short rods to coccoid forms. Colonies on Löwenstein–Jensen medium and Middlebrook 7H10 agar after 14–21 d are cream to buff, non-pigmented, usually smooth, sometimes appearing rough with age. Does not grow on media containing 5% (w/v) NaCl. Temperature range for growth is 30–37°C; does not grow at 25°C or 42°C. HPLC analysis of mycolic acids results in a three-cluster mycolate pattern, resembling that of *Mycobacterium simiae.* Differential characteristics of slowly growing species are shown in Table 37.

Source: isolated from cerebrospinal fluid, lymph nodes, and sputum of 10 patients from various locations within the USA.

DNA G+C content (mol%): not determined.

Type strain: ATCC 700071, CIP 106108, DSM 44626.

Sequence accession no. (16S rRNA gene): U57632.

Additional remarks: the incorrect accession number quoted in the effective publication (ATCC 70071) was corrected in Validation List no. 61, footnote d [Int. J. Syst. Bacteriol. *47*: 601–602 (1997)].

32. **Mycobacterium montefiorense** Levi, Bartell, Gandolfo, Smole, Costa, Weiss, Johnson, Osterhout and Herbst 2003, 2151[VP]

mon.te.fio.ren′se. N.L. neut. adj. *montefiorense* of or pertaining to Montefiore, referring to the Montefiore Medical Center, Bronx, New York, where the organism was isolated.

Acid-fast, beaded cells which are rod-shaped on Middlebrook 7H10 agar, though cocco-bacillary forms occur after long incubation (20 weeks) on sheep blood agar. Small, transparent, non-chromogenic colonies are formed on Middlebrook agar which are biochemically inactive. Colonies on sheep blood agar are rough and α-hemolytic. Growth occurs at 22–25°C, but not at 30°C or above. Does not grow on media containing 5% (w/v) NaCl. Differential characteristics of slowly growing species are shown in Table 37.

Source: isolated from a granulomatous skin lesion of a moray eel. Considered to be the etiological agent of granulomatous skin disease in moray eels as it produced a fatal outbreak in a captive population (Herbst et al., 2001).

DNA G+C content (mol%): not determined.

Type strain: DSM 44602, CCUG 51898, ATCC BAA-256.

Sequence accession no. (16S rRNA gene): AF330038.

33. **Mycobacterium genavense** Böttger, Hirschel and Coyle 1993, 842[VP]

ge.na.ven′se. L. neut. adj. *genavense* of or belonging to Geneva, the source of the first isolate.

Cells in tissues and in broth are small, clumped, acid-fast coccobacilli (2.0 × 1.0 μm). The organism is asporogenous, non-capsulate, and lacks aerial hyphae. It is highly fastidious; growth does not occur on standard solid media used for the isolation of mycobacteria, such as Löwenstein–Jensen medium, Middlebrook 7H10 agar, and unsupplemented Middlebrook 7H11 agar. Primary isolation requires broth media (Middlebrook 7H9 or 7H12) and incubation for 3–12 weeks. Growth is facilitated in acid broth media (Hoop et al., 1993). When inoculated with a broth culture suspension, visible growth on Middlebrook 7H11 agar supplemented with mycobactin J requires 3–9 weeks. Growth consists of tiny, transparent, non-photochromogenic, dysgonic colonies. Variant eugonic colonies which are either dense and creamy or flat and dry may be formed in older cultures. Cells from creamy colonies are pleomorphic ranging in length from 1.0 to 6.0 μm; the majority are approximately 2.0 μm long and may occur in clumps. Cells from dry colonies are similar but are more variable in length, somewhat curved, and show more clumping. After incubation for more than 3 months, the predominant cells are coccoid, short rods (1.0–2.0 μm in length), which frequently occur in clumps. Temperature range for growth is 25–42°C; optimal growth is at 42°C; does not grow at 45°C. TLC of methanolysates shows α-, α′-, and keto-mycolates. Differential characteristics of slowly growing species are shown in Table 37.

The species is considered to be an opportunistic pathogen of severely immune-suppressed individuals.

Source: isolated from a variety of specimen types (blood, bone marrow, liver and intestine, lymph nodes, and spleen) from patients with AIDS. Most patients exhibit diarrhea, fever, and weight loss. Strains have been isolated from pet birds (Hoop et al., 1993).

DNA G+C content (mol%): not determined.

Type strain: ATCC 51234.

Sequence accession no. (16S rRNA gene): X60070.

34. **Mycobacterium parmense** Fanti, Tortoli, Hall, Roberts, Kroppenstedt, Dodi, Conti, Polonelli and Chezzi 2004, 1126[VP]

par.men′se. L. neut. adj. *parmense* of or belonging to *Parma*, the Italian city of Parma, where the strain was isolated.

Small, acid–alcohol-fast, asporogenous, non-capsulate, nonmotile, rod-shaped bacteria. Small (≤1 mm), round, smooth, scotochromogenic colonies raised with round or lobate regular margins are formed on Löwenstein–Jensen medium after incubation for 2 weeks. Does not grow on MacConkey agar (without crystal violet) or on 5% (w/v) NaCl agar. Temperature range for growth is 25–37°C; does not grow at 42°C. TLC of methanolysates shows the presence of α- and keto-mycolates, and wax esters. Differential characteristics of slowly growing species are shown in Table 37.

Source: isolated from a cervical lymph node of a 3-year-old child with bilateral lymphadenitis.

DNA G+C content (mol%): not determined.

Type strain: CIP 107385, DSM 44553, CCUG 50998.

Sequence accession no. (16S rRNA gene): AF466821.

35. **Mycobacterium lentiflavum** Springer, Wu, Bodmer, Haase, Pfyffer, Kroppenstedt, Schröder, Emler, Kilburn, Kirschner, Telenti, Coyle and Böttger 1996b, 836[VP] (Effective publication: Springer, Wu, Bodmer, Haase, Pfyffer, Kroppenstedt, Schröder, Emler, Kilburn, Kirschner, Telenti, Coyle and Böttger 1996c, 1104.)

len.ti.fla′vum. L. adj. *lentus -a -um* slow; L. adj. *flavus -a -um* yellow; N.L. neut. adj. *lentiflavum* slow and yellow, two characteristic features of this species.

Acid–alcohol-fast coccobacilli. The organism does not produce aerial hyphae, capsules, or spores. Visible growth from dilute inocula appears in 3–4 weeks. Colonies on Löwenstein–Jensen medium are smooth, 1–2 mm in diameter, and show bright yellow scotochromogenic pigmentation. Temperature range for growth is 22–37°C; does not grow at 42–45°C. TLC of methanolysates shows the presence of α-, α′-, and keto-mycolates. Differential characteristics of slowly growing species are shown in Table 37.

Source: isolated from a variety of clinical samples, including an isolate from a patient with spondylodiscitis (type strain), four from gastric juice, four from sputum, and two from urine. Eleven isolates were identified as contaminants of a flexible fiber optic bronchoscope. Most isolates are fortuitous and probably represent colonization, but the source of the type strain indicates a potential for pathogenicity. Furthermore, the species has been identified as the cause of cervical lymphadenitis in a child (Haas et al., 1993).

DNA G+C content (mol%): 66.8 (HPLC).

Type strain: ATCC 51985, CCUG 42422/42559, CIP 105465, DSM 44418, JCM 13390.

Sequence accession no. (16S rRNA gene): X80769.

The *Mycobacterium celatum* clade

36. **Mycobacterium celatum** Butler, O'Connor, Yakrus, Smithwick, Plikaytis, Moss, Floyd, Woodley, Kilburn, Vadney and Gross 1993, 547[VP]

ce.la′tum. L. neut. part. adj. *celatum* hidden or concealed, referring to the concealed nature of these organisms among recognized *Mycobacterium* species.

Acid–alcohol-fast, slender, and predominantly rod-shaped cells with occasional coccoid forms (0.5–13.0 × 0.25–0.5 µm). May be somewhat filamentous, but does not exhibit cording or branching. Eugonic growth occurs on Löwenstein–Jensen medium at 33–42°C after incubation for 3–5 weeks. Dysgonic growth occurs at 27–30°C and notably at 45°C. Comparable results are found on Middlebrook 7H10 medium though growth is visible in 1 week. Mature colonies are polymorphic, usually small, smooth, dome-shaped, and either non-pigmented or pale yellow in older cultures. Flat and transparent colonies occur infrequently. Does not grow on MacConkey agar or on media containing 5% (w/v) NaCl. RFLP analysis of the *hsp65* gene gives two patterns, one of seven bands and one of six. TLC of methanolysates shows the presence of α- and keto-mycolates, and wax esters. Differential characteristics of slowly growing species are shown in Table 37.

Clinically significant infections are being reported more frequently in immune-compromised individuals, such as AIDS patients (Piersimoni et al., 1997). Isolates may be mistaken for the phenotypically similar species *Mycobacterium xenopi* (Zurawski et al., 1997).

Source: most of the original 24 strains were isolated either from sputum, including the type strain, or from bronchial washings. Additional strains have been isolated from blood, spine, and stool samples. HIV status was known for 11 of the patients of whom seven were positive (Butler et al., 1993).

DNA G+C content (mol%): not determined.

Type strain: ATCC 51131, CCUG 39185, CIP 106109, DSM 44243, JCM 12373.

Sequence accession no. (16S rRNA gene): L08169.

37. **Mycobacterium branderi** Koukila-Kähkölä, Springer, Böttger, Paulin, Jantzen and Katila 1995, 552[VP]

bran′de.ri. N.L. gen. masc. n. *branderi* of Brander, referring to Eljas Brander, the former head of the Tuberculosis Laboratory of the National Public Health Institute, Finland, who collected the strains.

Delicate, slender, and often slightly curved (1.2–3.0 µm in length) acid-fast rods. Growth occurs on Middlebrook 7H11 agar after incubation for 2–3 weeks. Colonies are smooth, often umbonate, non-chromogenic, and off-white to grayish in color. Temperature range for growth is 25–45°C. TLC of methanolysates shows the presence of α- and keto-mycolates, and wax esters. Differential characteristics of slowly growing species are shown in Table 37.

Source: the original description was based on respiratory

isolates obtained from nine patients, some of whom had cavitary mycobacteriosis of the lungs and were resistant to available drugs. In most cases, repeat samples from patients were positive for acid-fast bacilli and the only cultivable species was *Mycobacterium branderi*. Hence, this species must be considered as a potential human pathogen.

DNA G+C content (mol%): not determined.

Type strain: ATCC 51789, CIP 104592, DSM 44624, JCM 12687.

Sequence accession no. (16S rRNA gene): X82234.

The *Mycobacterium xenopi* clade

38. **Mycobacterium xenopi** Schwabacher 1959, 59[AL] [*Mycobacterium xenopei* (*sic*) Schwabacher 1959, 59]

xe.no′pi. N.L. n. *Xenopus* a genus of toad; N.L. gen. n. *xenopi* of *Xenopus*.

Acid-fast, long to filamentous rods, vary from 4–15 μm from *in vitro* cultures but are shorter in lesions; beading and palisade arrangements occur. Dilute inocula on Löwenstein–Jensen medium yield smooth, non-pigmented colonies after incubation at 37°C for 14 or more days; on ageing, most colonies become yellow. On Middlebrook 7H10 agar, uniquely characteristic colonies have compact centers, surrounded by a fringe of microscopically branching filaments on the agar surface. Colonies become adherent to the medium by a button-like growth into the agar (Runyon, 1968). Does not grow on MacConkey agar or on media supplemented with 5% (w/v) NaCl. Growth is optimal at 40–45°C. TLC of methanolysates shows the presence of α- and keto-mycolates, and wax esters. Differential characteristics of slowly growing species are shown in Table 37.

The species is distinguished from other mycobacteria by reciprocal intradermal hypersensitivity reactions (Magnusson, 1980) and shows four species-specific antigens by immunodiffusion tests (Stanford and Grange, 1974). One agglutinating serovar has been reported (Goslee et al., 1976); the surface lipid antigen is of the alkali-labile type seen in *Mycobacterium kansasii*, not the stable type of *Mycobacterium avium* (Brennan, 1981).

Occasionally associated with chronic pulmonary disease, but often isolated from human secretions without associated disease; infrequently found in disease of the genitourinary tract (Wolinsky, 1979a). The organism has been associated with waterborne nosocomial outbreaks of disease (Gross et al., 1976). Experimental intraperitoneal inoculation of different strains in mice produces a variable response with limited numbers of macroscopic lesions appearing in the kidney, liver, lungs, or spleen. Guinea pigs receiving 4 mg intramuscularly develop caseous abscesses at the site of inoculation; 0.1 mg intra-cutaneously administered inocula cause swelling and ulceration at the inoculation site. Rabbits receiving 4 mg intramuscularly develop caseous abscesses at the site of inoculation (Engbaek et al., 1967; Schwabacher, 1959).

Source: first isolated from skin granulomas of the toad *Xenopus laevis* (Marks and Schwabacher, 1965).

DNA G+C content (mol%): not determined.

Type strain: NCTC 10042, ATCC 19250, DSM 43995, CCUG 28011/31306, CIP 104035.

Sequence accession no. (16S rRNA gene): AJ536033.

Additional remarks: Mycobacterium xenopi forms a discrete, compact cluster in numerical taxonomic studies (Meissner et al., 1974) and it is well separated from other species on the basis of immunological distances of T-catalase (Wayne and Diaz, 1982). DNA–DNA relatedness values are less than 30% against *Mycobacterium avium*, *Mycobacterium scrofulaceum*, and *Mycobacterium tuberculosis* (Baess, 1979).

39. **Mycobacterium heckeshornense** Roth, Reischl, Schönfeld, Naumann, Emler, Fischer, Mauch, Loddenkemper and Kroppenstedt 2001, 264[VP] (Effective publication: Roth, Reischl, Schönfeld, Naumann, Emler, Fischer, Mauch, Loddenkemper and Kroppenstedt 2000, 4106.)

hec.kes.hor.nen′se. N.L. neut. adj. *heckeshornense* of or belonging to Heckeshorn, a peninsula in Berlin, Germany, the location of the hospital in which the strain was found.

Gram-stain-positive, acid-fast, non-spore-forming, non-motile, short rods, occasionally pleomorphic, but without branching. Colonies are small, smooth, round, and scotochromogenic with a yellow color, appearing after incubation for 4 weeks. Better growth is observed on Middlebrook agar and broth media than on Löwenstein–Jensen medium. Temperature range for growth is 37–45°C; optimal growth is at 42°C; does not grow at 50°C. TLC of methanolysates shows the presence of α- and keto-mycolates, and wax esters. Differential characteristics of slowly growing species are shown in Table 37.

Source: isolated from sputum and bronchoscopy samples from an immunocompetent young woman and was the cause of bilateral cavitary lung disease.

DNA G+C content (mol%): not determined.

Type strain: DSM 44428, CCUG 51897.

Sequence accession no. (16S rRNA gene): AF174290.

40. **Mycobacterium botniense** Torkko, Suomalainen, Livanainen, Suutari, Tortoli, Paulin and Katila 2000, 288[VP]

bot.ni.en′se. N.L. neut. adj. *botniense* of or belonging to *Botnia Occidentalis*, referring to the Latin name of the Västerbotten province (West Bothnia) of Finland where the organism was isolated.

Visible growth is observed from dilute inocula on egg media and on Middlebrook 7H11 agar following incubation for 5–8 weeks. Growth is enhanced on egg media and at lower pH values (5.5–6.5). Colonies are small, dysgonic, and scotochromogenic; a yellow pigment is produced. Temperature range for growth is 36–50°C; does not grow at 30°C. *Mycobacterium botniense* shows similarity in growth characteristics and biochemical properties to *Mycobacterium xenopi* and may have been misidentified for this species. GLC-MS analysis showed two unusual fatty acids, 2,4,6,x-tetramethyl-eicosanoic acid and 2,4,6,x,x-pentamethyl-docosanoic acid. Clear differentiation requires detailed GLC-MS analysis of fatty acids or 16S rRNA gene sequencing. Differential characteristics of slowly growing species are shown in Table 37.

Source: the original strains were isolated from stream water that had a high organic matter content and low pH and drained a peat land area. The organism has not been isolated from clinical samples.

DNA G+C content (mol%): not determined.

Type strain: ATCC 700701, CCUG 47976, CIP 106753, DSM 44537.

Sequence accession no. (16S rRNA gene): AJ012756.

The *Mycobacterium terrae* clade

41. **Mycobacterium terrae** Wayne 1966, 922[AL]

ter′ra.e. L. n. *terra* earth. L. gen. n. *terrae* of the earth.

Acid–alcohol-fast bacilli. Visible growth from dilute inocula occurs on Löwenstein–Jensen medium after 7 d or more if required. Colonies are nonchromogenic, pale buff to white. Temperature range for growth is 22–37°C; does not grow at 42°C. TLC of methanolysates shows the presence of α- and keto-mycolates, and wax esters. Differential characteristics of slowly growing species are shown in Table 37.

Produces neither local nor systemic lesions after intradermal inoculation of 10^{-1} mg in guinea pigs.

Source: isolated from human gastric lavage and sputum specimens; considered casual residents rather than pathogens. The species has also been isolated from soil (Wolinsky and Rynearson, 1968).

DNA G+C content (mol%): not determined.

Type strain: ATCC 15755, CCUG 27847, CIP 104321, DSM 43227, JCM 12143.

Sequence accession no. (16S rRNA gene): X52925.

Additional remarks: "*Mycobacterium novum*" Tsukamura 1967b, 163 is regarded as a synonym of *Mycobacterium terrae* Wayne (Meissner et al., 1974). "*Mycobacterium terrae*" Tsukamura 1967b is an invalidly proposed synonym for *Mycobacterium nonchromogenicum* Tsukamura 1965 and is not to be confused with *Mycobacterium terrae* Wayne 1966. See also discussion on *Mycobacterium nonchromogenicum. Mycobacterium triviale* shares many diagnostic features with *Mycobacterium nonchromogenicum* and *Mycobacterium terrae* (see below), but can be separated from them, notably by its consistent ability to grow in the presence of 5% (w/v) NaCl (Kubica et al., 1970).

42. **Mycobacterium kumamotonense** Masaki, Ohkusu, Hata, Fujiwara, Iihara, Yamada-Noda, Nhung, Hayashi, Asano, Kawamura and Ezaki 2007, 433[VP]

ku.ma.mo.to.nen′se. N.L. neut. adj. *kumamotonense* of or pertaining to Kumamoto Prefecture in Japan, where the type strain was isolated.

Acid–alcohol-fast, short rods. Colonies (1–2 mm diameter) are smooth, nonchromogenic, and raised with round or lobate regular margins on Middlebrook 7H9 agar and on 2% Ogawa medium after incubation for 7–14 d. Neither spores nor capsules are observed. Temperature range for growth is 25–42°C. Does not grow on media supplemented with 5% (w/v) NaCl. TLC of methanolysates shows α- and keto-mycolates, and wax esters. Differential characteristics of slowly growing species are shown in Table 37.

Source: the type strain was isolated from the sputum of a 57-year-old female patient in Japan.

DNA G+C content (mol%): 67.4 (HPLC).

Type strain: CCUG 51961, JCM 13453.

Sequence accession nos: AB239925 (16S rRNA gene); AB239920 (*hsp65*); AB239919 (*rpoB*).

Additional remarks: initially proposed by Masaki et al. (2006) and the name was subsequently validly published in Validation List no. 114 (Masaki et al., 2007). Two further isolates were considered to belong to the species on the basis of *rpoB* sequence analysis, but showed some variation in phenotypic properties and in 16S rRNA gene and *hsp65* sequence analyses (Masaki et al., 2006).

43. **Mycobacterium hiberniae** Kazda, Cooney, Monaghan, Quinn, Stackebrandt, Dorsch, Daffé, Müller, Cook and Tarnok 1993, 355[VP]

hi.ber.ni′a.e. L. gen. n. *hiberniae* of *Hibernia*, the Latin name for Ireland, the source of the strains.

Acid–alcohol-fast, beaded rods (0.9 × 1.2–1.5 μm) which frequently form clumps, but not cords. Aerial hyphae, capsules, spores, and true branching do not occur. Smooth, compact, and glistening rose-pink colonies (1–1.5 mm in diameter) are formed on Löwenstein–Jensen medium and Middlebrook 7H10 agar after incubation for 20 d. Strains are eugonic in the first phase of growth, but later become dysgonic and assume a rough, dry appearance after longer incubation (8 weeks). Temperature range for growth is 22–37°C, optimal growth is at 37°C; does not grow at 42 or 45°C. TLC of methanolysates shows α- and keto-mycolates, and wax esters. Differential characteristics of slowly growing species are shown in Table 37.

Cells provoke a non-specific hypersensitivity reaction to bovine tuberculin. The organism is not pathogenic for guinea pigs, mice, or rabbits.

Source: the original description is based on 13 strains isolated from moss, sphagnum vegetation, and soil collected in counties Clare and Donegal in Ireland.

DNA G+C content (mol%): not determined.

Type strain: ATCC 49874, CIP 104537, DSM 44241, JCM 13571.

Sequence accession no. (16S rRNA gene): X67096.

44. **Mycobacterium arupense** Cloud, Meyer, Pounder, Jost, Sweeney, Carroll and Woods 2006, 1417[VP]

a.rup.en′se. N.L. neut. adj. *arupense* pertaining to the ARUP Institute for Clinical and Experimental Pathology, where the type strain was characterized.

Acid-fast, non-chromogenic bacilli. The organism grows rapidly (5–7 d) on Löwenstein–Jensen medium at 30°C and slowly (10–12 d) at 37°C. It does not grow at 42°C on MacConkey agar lacking crystal violet or on media supplemented with 5% (w/v) NaCl. Differential characteristics of slowly growing species are shown in Table 37.

Source: the type strain was isolated from a human tendon. Additional strains have been obtained from bronchial washings, a finger wound, and sputum. Clinical significance may vary with site of isolation.

DNA G+C content (mol%): not determined.

Type strain: ATCC BAA-1242, DSM 44942.

Sequence accession nos: DQ157760 (16S rRNA gene); DQ168662 (*hsp65*); DQ168663 (ITS).

Additional remarks: the 16S rRNA gene sequence of *Mycobacterium arupense* has a long helix 18 in the hyper-variable region V3, a feature seen in slow-growing *Mycobacterium* species. However, phenotypic assessment of the growth rate shows more rapid growth at lower temperature. Hence, this species is considered to have an intermediate growth rate.

45. **Mycobacterium nonchromogenicum** Tsukamura 1965, 110[AL]

non.chrom.o.gen′i.cum. L. adv. *non* not; Gr. n. *chroma* color; Gr. v. *gennaio* to produce; L. suff. *-icum* suffix used with the sense of pertaining to; N.L. neut. adj. *nonchromogenicum* intended to mean not producing color.

Gram-stain-positive, acid-fast, moderately long to long rods. Colonies on Löwenstein–Jensen medium and Middlebrook agar are smooth to rough and white to buff after incubation for 7 or more days at 37°C. TLC of methanolysates shows α- and keto-mycolates, and wax esters. Differential characteristics of slowly growing species are shown in Table 37.

Source: isolated from mice injected with soil; bacilli persist in tissues of mice without evidence of pathogenicity (Tsukamura, 1967b). Usually appears in clinical specimens as an environmental contaminant. A few cases are documented in which *Mycobacterium nonchromogenicum* or *Mycobacterium terrae* have been considered as the cause of human disease (Wolinsky, 1979a).

DNA G+C content (mol%): not determined.

Type strain: ATCC 19530, CCUG 28009, CIP 106811, DSM 44164, JCM 6364, NCTC 10424.

Sequence accession no. (16S rRNA gene): M29565.

Additional remarks: in numerical taxonomic analyses, two clusters corresponding to *Mycobacterium nonchromogenicum* and *Mycobacterium terrae* are seen. However, these clusters link to one another at a higher similarity level than they do to other species (Meissner et al., 1974). Positive reactions for nicotinamidase and pyrazinamidase and negative nitrate reduction provide the most definitive means of distinguishing *Mycobacterium nonchromogenicum* from *Mycobacterium terrae*.

Dermal hypersensitivity testing shows a tenuous distinction between *Mycobacterium nonchromogenicum* and *Mycobacterium terrae* (Meissner et al., 1974), but immunodiffusion analysis does not permit their differentiation (Stanford and Grange, 1974). The overall similarities between *Mycobacterium nonchromogenicum*, *Mycobacterium terrae*, and *Mycobacterium triviale* raise questions about their status as separate species. Since all of them are generally considered clinically insignificant, it is common practice to identify such strains as members of the "*Mycobacterium terrae* complex" without further taxonomic resolution.

Single-membered 16S rRNA gene clades

46. **Mycobacterium leprae** (Hansen 1880) Lehmann and Neumann 1896, 372[AL] (*Bacillus leprae* Hansen 1880, 32)

le′pra.e. L. n. *lepra* leprosy; L. gen. n. *leprae* of leprosy.

Common name: Leprosy bacillus or Hansen's bacillus.

Strongly acid-fast rods (1.0–8.0 × 0.3–0.5 μm) with parallel sides and rounded ends which stain evenly or at times are beaded. When numerous, as from lepromatous cases, cells are generally arranged in clumps, rounded masses (globi) or in groups of bacilli aligned side by side. Fisher and Barksdale (1973) reported that bacilli in leprosy lesions were distinctive in losing their acid-fastness on extraction with pyridine. However, clinical specimens usually contain a high proportion of non-viable bacilli characterized by poor and uneven staining (McRae and Shepard, 1971). Hence, the pyridine effect may reflect either a species characteristic or merely the state of viability.

The organism has not been cultured in artificial media despite periodic claims to the contrary. Bacilli from human leprous tissue multiply with an apparent generation time of 20–30 d when inoculated into footpads of healthy mice (Shepard, 1960; Shepard and Chang, 1962). When corrected for the presence of "non-viable" (i.e. non-solid-staining) bacilli, generation times as low as 10 d are seen (Shepard and McRae, 1971). Generation times in mouse footpads appear to be strain-specific (Shepard and McRae, 1971). During *in vivo* cultivation in mice, the leprosy bacilli do not invade deep tissues and their multiplication can be inhibited by cycloserine, diaminodiphenyl sulfone, isoniazid, ρ-aminosalicylic acid, and rifampin. Experimental transmission to immunosuppressed mice and rats causes lepromatous-like model infections (Fieldsteel and McIntosh, 1971; Rees et al., 1967).

The discovery that *Mycobacterium leprae* can multiply extensively in tissues of the nine-banded armadillo (*Dasypus novemcinctus*) led to the use of this animal to produce large quantities of bacilli for subsequent study (Kirchheimer and Storrs, 1971). However, care must be taken to distinguish biological products of mycobacterial origin from those that may have been adsorbed onto the bacilli from host tissues.

Mycolic acids have been identified in *Mycobacterium leprae* and these resemble those of *Mycobacterium gordonae* more than those of any other *Mycobacterium* species (Daffé et al., 1981; Kusaka et al., 1981; Minnikin et al., 1985; Young, 1980). Little, if any, tuberculostearic acid is produced; in this regard, *Mycobacterium leprae* again resembles *Mycobacterium gordonae* (Asselineau, 1981). A phenolic glycolipid resembling mycoside A of *Mycobacterium kansasii* but bearing a different trisaccharide component has been isolated from *Mycobacterium leprae* (Hunter and Brennan, 1981).

Causes leprosy in man. In the lepromatous form of the disease, bacilli are so abundant in the tissue that they produce stuffed-cell granulomas; in tuberculoid and neural lesions, organisms are rare.

Source: obligate intracellular parasite; confined largely to the skin (especially to convex and exposed surfaces), testes, and to peripheral nerves. Probably does not grow in the internal organs.

DNA G+C content (mol%): 57.8 (whole-genome sequencing).

Type strain: not cultivated; none designated.

Sequence accession no. (16S rRNA gene): X53999.

Additional remarks: Stanford et al. (1975) detected 12 antigens in *Mycobacterium leprae* extracts by immunodiffusion and found that six were common to all mycobacteria and nocardiae tested with four appearing to be specific to *Mycobacterium leprae*. Sera from leprosy patients contain antibodies that react with antigens from a number of mycobacterial species by immunodiffusion and immunoelectrophoresis

analyses (Kronvall et al., 1976; Norlin et al., 1966). At least 20 distinct antigenic components have been detected in *Mycobacterium leprae* by immunoelectrophoresis (Closs et al., 1979), seven of which reacted with sera from lepromatous patients. Gillis and Buchanan (1982) found that out of 11 monoclonal antibodies raised against *Mycobacterium leprae* extracts only two reacted against *Mycobacterium leprae*; the others showed various patterns of reaction with the other 18 species tested. Brennan and Barrow (Brennan and Barrow, 1980) partially purified a lipid from *Mycobacterium leprae* which appeared to be analogous to the C-mycosidic peptidoglycolipids responsible for specific agglutination of the serovars of the *Mycobacterium avium* complex. This lipid is serologically active and may be specific for this *Mycobacterium leprae* strain. Heated suspensions of bacilli (obtained from nodules) produce a positive lepromin reactin in 75–97% of normal persons and of tuberculoid cases of leprosy, but usually do not produce a reaction in lepromatous individuals (Hayashi, 1932).

47. **Mycobacterium lepraemurium** Marchoux and Sorel 1912, 700[AL]

lep.rae.mu′ri.um. L. gen. n. *leprae* of leprosy; L. gen. pl. n. *murium* of mice; N.L. gen. pl. n. *lepraemurium* of leprosy of mice.

Strongly acid-fast rods (3–5 μm in length) with slightly rounded ends. Cells often show an irregular appearance when stained. Only the densely and uniformly stained forms appear to be infective for animals, in contrast to the "degenerate" unevenly stained forms (Rees et al., 1960). The bacilli from lesions are not bound together in clumps, rounded masses, or palisades as in human leprosy.

Following many years of unsuccessful attempts to grow this organism *in vitro*, Ogawa and Motomura (1970) succeeded by inoculating many cells onto an inspissated 1% egg yolk medium. This approach was used by Saito et al. (1976) to determine the biochemical and biological characteristics of *Mycobacterium lepraemurium* strain Keishicho. Dense compact inocula on Ogawa egg medium leads to the growth of rough, non-chromogenic colonies after incubation for 4–5 weeks at 30–37°C. Growth also occurs on agar-based media supplemented with cytochrome *c* and α-ketoglutaric acid. Slow growth has been recorded in an enriched Kirchner liquid medium, though this seems to be dependent upon the maintenance of reducing conditions (Dhople and Hanks, 1981). TLC of methanolysates shows α- and keto-mycolates, and wax esters. Differential characteristics of slowly growing species are shown in Table 37.

A cause of endemic disease of rats in various parts of the world. The natural disease occurs chiefly in the lymph nodes and on skin, causing induration, alopecia, and eventual ulceration. Nodular diseases of the skin of other animals have been associated with similar acid-fast bacilli.

Source: a primary isolate has never been recorded.

DNA G+C content (mol%): not determined.

Type strain: non-specified due to difficulties in cultivation.

Sequence accession no. (16S rRNA gene): AJ279017.

Additional remarks: cell walls of *Mycobacterium lepraemurium* contain arabinose and galactose as principal cell-wall sugars, alanine, glutamic acid, and *meso*-diaminopimelic acid as mucopeptide amino acids, and a high proportion of lipid, which is typical of other, cultivable mycobacteria (Cummins et al., 1967). DNA from *Mycobacterium lepraemurium* shows a higher homology to that of *Mycobacterium avium* than to any other mycobacterial species (Imaeda et al., 1982). Using immunodiffusion analysis, Stanford (1973) revealed three antigens from *Mycobacterium lepraemurium* that were previously considered unique to *Mycobacterium avium*. Additionally, *Mycobacterium lepraemurium* produced two antigens that were not shared with any other mycobacterial species tested. The T-catalase of *Mycobacterium lepraemurium* occupies a unique position on the diagram of divergence of T-catalase lying between those of *Mycobacterium avium* and *Mycobacterium tuberculosis* (Katoch et al., 1982).

48. **Mycobacterium szulgai** Marks, Jenkins and Tsukamura 1972, 211[AL]

szul′gai. N.L. gen. masc. n. *szulgai* of Szulga, named after T. Szulga, a Polish microbiologist.

Acid-fast, moderately long rods with some patchy staining or cross-barring. Colonies on egg medium are usually smooth and easily emulsified following incubation for 2 weeks at 37°C, but rough forms may appear after prolonged subculture. The production of an orange pigment is enhanced by growth in continuous light. *Mycobacterium szulgai* is scotochromogenic when grown at 37°C, but at 25°C it tends to be photochromogenic (Schaefer et al., 1973). Growth is inhibited in media supplemented with 5% (w/v) NaCl. Strains exhibit specific agglutination with *Mycobacterium szulgai* antiserum and a unique TLC pattern of a surface lipid antigen (Marks et al., 1972; Schaefer et al., 1973). This antigen is of the alkali-labile type (Brennan, 1981). TLC of methanolysates shows α-, keto-, and methoxy-mycolates. Differential characteristics of slowly growing species are shown in Table 37.

Source: isolated from patients with cervical adenitis, olecranon bursitis, and pulmonary disease.

DNA G+C content (mol%): not determined.

Type strain: ATCC 35799, CCUG 37675, CIP 104532, DSM 44166, JCM 6383, NCTC 10831.

Sequence accession no. (16S rRNA gene): X52926.

Additional remarks: in routine diagnostic clinical practice, *Mycobacterium szulgai* may be mistaken for *Mycobacterium flavescens*, nominally a rapid grower. Since, unlike *Mycobacterium szulgai*, *Mycobacterium flavescens* is not a pathogen, it is essential that appropriate tests be conducted to distinguish between them. In spite of superficial similarities, these species form distinct clusters in numerical taxonomic studies (Selva-Sutter et al., 1976; Wayne et al., 1981).

49. **Mycobacterium seoulense** Mun, Kim, Oh, Kim, Bai, Yu, Park, Cha, Kook and Kim 2007, 597[VP]

se.oul.en′se. N.L. neut. adj. *seoulense* of or pertaining to Seoul, Republic of Korea, the geographical origin of the type strain.

Acid–alcohol-fast, rod-shaped cells that are frequently bent and occasionally coccoid. Spores and filaments are not present. Micro-colonies may be visible in 2 weeks on Middlebrook 7H10 agar; smooth, orange-pigmented; mature

colonies require incubation for 3 weeks. Growth may be slower (4 or more weeks) on Löwenstein–Jensen medium and appear as an orange-colored surface film. Temperature range for growth is 25–37°C, optimal growth is at 37°C; does not grow at 45°C. Does not grow on MacConkey agar or on media supplemented with 5% (w/v) NaCl. Differential characteristics of slowly growing species are shown in Table 37.

Source: isolated on three occasions from sputum samples of a 52-year-old female patient experiencing generalized pulmonary symptoms.

DNA G+C content (mol%): not determined.

Type strain: DSM 44998, KCTC 19146.

Sequence accession no. (16S rRNA gene): DQ536403.

50. **Mycobacterium scrofulaceum** Prissick and Masson 1956, 802[AL]

scro.fu.la′ce.um. L. pl. n. *scrofulae* a swelling of the glands of the neck, scrofula; L. neut. suff. *-aceum* suffix used with various meanings; N.L. neut. adj. *scrofulaceum* of or pertaining to scrofula.

Acid-fast, short to long rods or filaments. Colonies on Middlebrook medium and Löwenstein–Jensen medium are usually smooth and yellow to orange in color, but occasional strains may produce rough growth. Growth occurs in 7 or more days at 37°C. Temperature range for growth is 22–42°C; optimal growth is at 35°C. TLC of methanolysates shows α- and keto-mycolates. Differential characteristics of slowly growing species are shown in Table 37.

Mycobacterium scrofulaceum does not cause extensive generalized disease in chickens, hamsters, or rats, but may produce some lymph node involvement and rarely localized lesions in the liver or spleen (Penso et al., 1957; Prissick and Masson, 1957). Subcutaneous inoculation of guinea pigs produces abscesses at the site of inoculation and enlargement of regional nodes. Intraperitoneal inoculation causes enlargement and suppuration of regional lymph nodes and, consistently, peritonitis of various degrees and, rarely, lesions of the liver or spleen, but not generalized disease or death (Penso et al., 1957; Prissick and Masson, 1957).

Source: isolated from a closed lesion of cervical lymphadenitis in a child (Prissick and Masson, 1956). Also found in pus from suppurating cervical lymph nodes, especially in children, and considered to be the etiologic agent of the lesions (Prissick and Masson, 1957). Although *Mycobacterium scrofulaceum* is still occasionally encountered in such conditions, it is now a less likely cause of lymphadenitis in children than other mycobacterial species, notably organisms of the *Mycobacterium avium* complex (Falkinham, 1996). *Mycobacterium scrofulaceum* is also found in human sputum and gastric lavage samples, and is occasionally associated with pulmonary disease (Wolinsky, 1979a). The species has been found in the environment (Kestle et al., 1967; Wolinsky and Rynearson, 1968) and in swine (Schaefer, 1968).

DNA G+C content (mol%): not determined.

Type strain: ATCC 19981, CCUG 29045, CIP 105416, DSM 43992, JCM 6381, NCTC 10803.

Sequence accession no. (16S rRNA gene): X52924.

Additional remarks: Mycobacterium scrofulaceum was conserved over the specific epithet *Mycobacterium marianum*, a senior subjective synonym, in Opinion 53 of the Judicial Commission. *Mycobacterium marianum* was placed on the list of *nomina rejicienda* because of its orthographic similarity to the valid epithet *Mycobacterium marinum* (Judicial Commission of the International Committee on Systematic Bacteriology, 1978).

Schaefer (1965, 1968) established three serovars within this species by seroagglutination. These correspond to TLC patterns seen with surface lipid antigens (Jenkins et al., 1972; Wayne et al., 1971) of the alkali-stable type (Brennan, 1981). A fourth serovar, designated 44 (Goslee et al., 1976), has since been redesignated *avium* complex serovar 27 (Wolinsky, 1979b). *Mycobacterium scrofulaceum* forms a distinct species on the basis of reciprocal dermal skin testing (Baess and Magnusson, 1982; Magnusson, 1962, 1980; Runyon and Dietz, 1971; Wayne et al., 1971), immunoelectrophoresis (Castelnuovo and Morellini, 1962), and immunodiffusion (Stanford and Grange, 1974).

Mycobacterium scrofulaceum is sometimes assigned to the so-called MAIS complex. The data from many taxonomic studies using several different procedures make it inappropriate to include this species in a complex with *Mycobacterium avium* and *Mycobacterium intracellulare* (see discussion under *Mycobacterium avium* subsp. *avium*).

51. **Mycobacterium conspicuum** Springer, Tortoli, Richter, Grünewald, Rüsch-Gerdes, Uschmann, Suter, Collins, Kroppenstedt and Böttger 1996a, 362[VP] (Effective publication: Springer, Tortoli, Richter, Grünewald, Rüsch-Gerdes, Uschmann, Suter, Collins, Kroppenstedt and Böttger 1995b, 2810.)

con.spi′cu.um. L. neut. adj. *conspicuum* in view, visible, apparent, obvious, referring to the unique profile of this species.

Acid–alcohol-fast coccobacilli. Does not form aerial hyphae, capsules, or spores. Visible growth occurs in 2–3 weeks from dilute inocula on Löwenstein–Jensen medium. Colonies are dysgonic, smooth, yellowish, and non-photochromogenic. Temperature range for growth is 22–31°C; does not grow at 37°C under routine conditions. However, growth at 37°C was achieved in BACTEC 12B medium. Does not grow on MacConkey agar or in the presence of 5% (w/v) NaCl. TLC of methanolysates shows α- and keto-mycolates, and wax esters. Differential characteristics of slowly growing species are shown in Table 37.

Source: isolated from two patients with immune deficiency and disseminated mycobacterial infection.

DNA G+C content (mol%): not determined.

Type strain: DSM 44136, CIP 105165, ATCC 700090.

Sequence accession no. (16S rRNA gene): X88922.

52. **Mycobacterium lacus** Turenne, Chedore, Wolfe, Jamieson, Broukhanski, May and Kabani 2002a, 2138[VP]

la′cus. L. gen. n. *lacus* of the lake, where the organism was acquired.

Large, dispersed acid-fast bacilli with prominent beading. Neither aerial hyphae or spores are produced. Visible growth appears in 2 weeks at 37–42°C from dilute inocula, but requires 3–4 weeks at 25–30°C. Colonies on Löwenstein–Jensen medium are small, dry, and

non-chromogenic. Colonies on Middlebrook 7H10 agar are small, non-pigmented, and smooth to rough with a slightly irregular edge. Younger colonies appear slightly transparent. Temperature range for growth is 25–42°C, optimal growth at 37°C. Does not grow on MacConkey agar (without crystal violet) or in the presence of 5% (w/v) NaCl. Differential characteristics of slowly growing species are shown in Table 37.

Source: a single isolate was obtained from synovial tissue of a 68-year-old female patient with bursitis of the right elbow thought to be due to trauma whilst swimming in a lake.

DNA G+C content (mol%): not determined.

Type strain: ATCC BAA-323, DSM 44577.

Sequence accession no. (16S rRNA gene): AF406783.

53. **Mycobacterium intermedium** Meier, Kirschner, Schröder, Wolters, Kroppenstedt and Böttger 1993, 207[VP]

in.ter.me′di.um. L. neut. adj. *intermedium* intermediate, referring to the phylogenetic position of this organism between the rapidly and slowly growing mycobacteria.

Acid–alcohol-fast coccobacilli (2.0 × 2.6 µm). Does not form aerial hyphae, capsules, or spores. Visible growth appears in 2–3 weeks from dilute inocula on Löwenstein–Jensen medium. Colonies are 3–5 mm in diameter, eugonic, smooth, and photochromogenic. Temperature range for growth is 22–41°C; optimal growth is between 31 and 37°C; does not grow at 45°C. TLC of methanolysates shows α-, α′-, and keto-mycolates. Differential characteristics of slowly growing species are shown in Table 37.

Source: three isolates were obtained from the sputum of a patient with pulmonary disease.

DNA G+C content (mol%): not determined.

Type strain: DSM 44049, ATCC 51848, CCUG 37583, CIP 104542, JCM 13572.

Sequence accession no. (16S rRNA gene): X67847.

54. **Mycobacterium cookii** Kazda, Stackebrandt, Smida, Minnikin, Daffé, Parlett and Pitulle 1990, 221[VP]

cook′i.i. N.L. gen. masc. n. *cookii* of Cook, named for Bertram Cook, who discovered the natural reservoir of this species.

Acid–alcohol-fast, polymorphic rods (0.8 × 1.4–1.9 µm) which often form clumps but not cords, nor is cross banding evident. Does not produce aerial hyphae, capsules, spores, or true branching. Colonies on Löwenstein–Jensen medium and Middlebrook 7H10 agar are 0.5–1.0 mm in diameter and smooth, glistening with yellow-orange scotochromogenic pigmentation. Visible growth from dilute inocula occurs after incubation for 4 weeks. Optimal temperature for growth is 31°C; many strains grow at 22°C, but growth does not occur at or above 37°C. The organism shows limited enzymic activity and does not grow on media containing single carbon sources or single sources of carbon plus nitrogen. TLC of methanolysates shows α- and α′-mycolates, and wax esters. Differential characteristics of slowly growing species are shown in Table 37.

The strains were not pathogenic for guinea pigs, mice, or rabbits, but they did stimulate non-specific tuberculin reactions in sensitized guinea pigs and were regarded as a source of non-specific tuberculin reactions in cattle in some regions of New Zealand (Cook and Kazda, 1988; Kazda and Cook, 1987).

Source: seventeen strains were isolated from sphagnum vegetation and pond water collected from sites throughout the North and South Islands of New Zealand.

DNA G+C content (mol%): 63.7 ± 3 (T_m).

Type strain: ATCC 49103, CIP 105396, DSM 43922, JCM 12404.

Sequence accession no. (16S rRNA gene): X53896.

55. **Mycobacterium triviale** Kubica *in* Kubica, Silcox, Kilburn, Smithwick, Beam, Jones and Stottmeier 1970, 162[AL]

tri.vi.a′le. L. neut. adj. *triviale* common, commonplace, vulgar, ordinary, of little importance.

Acid-fast rods. From dilute inocula, mature colonies do not appear on solid media for over a week. Colonies on egg medium are rough, dry, heaped, and non-chromogenic. Characteristic rough R colonies are seen on oleic acid agar that are easily confused with those of *Mycobacterium tuberculosis*. Grows poorly, if at all, on cornmeal agar. *Mycobacterium triviale* shares many diagnostic features with *Mycobacterium nonchromogenicum* and *Mycobacterium terrae*, but differs from these taxa and from most other slow-growing mycobacteria in its consistent ability to grow in the presence of 5% (w/v) NaCl (Kubica et al., 1970). TLC of methanolysates shows α-mycolates. Differential characteristics of slowly growing species are shown in Table 37.

Source: isolated from sputum, but is not considered to be a pathogen.

DNA G+C content (mol%): not determined.

Type strain: ATCC 23292, CCUG 42431, DSM 44153.

Sequence accession no. (16S rRNA gene): X88924.

56. **Mycobacterium tusciae** Tortoli, Kroppenstedt, Bartoloni, Caroli, Jan, Pawlowski and Emler 1999, 1843[VP]

tus.ci′a.e. L. gen. n. *tusciae* of *Tuscia*, now Tuscany, the Italian region where all the organisms were isolated.

Acid–alcohol-fast, nonmotile, non-spore forming rods. Colonial growth occurs in 4 weeks on Löwenstein–Jensen medium at temperatures from 25 to 32°C. At 37°C, growth on Löwenstein–Jensen is inconsistent and scanty, but is better on Middlebrook 7H11 agar and in Bactec 12B liquid medium. Colonies are rough and scotochromogenic with an elevated center surrounded by a flat and uneven fringe. Does not grow on MacConkey agar or on media supplemented with 5% (w/v) NaCl. TLC of methanolysates shows α- and keto-mycolates, and wax esters. Differential characteristics of slowly growing species are shown in Table 37.

Source: isolated from a lymph node of an immunocompromised child. Additional isolates have been obtained from tap water and from a respiratory sample from a patient with chronic fibrosis; however, the organism is not to be considered to be a pathogen.

DNA G+C content (mol%): 66.4 (HPLC).

Sequence accession no. (16S rRNA gene): AF058299.

Additional remarks: although this organism displays slow growth it appears to cluster near to the rapidly growing species in the phylogeny shown in Figure 86.

57. **Mycobacterium shimoidei** Tsukamura 1982, 67[VP]

shi.moid′e.i. N.L. gen. masc. n. *shimoidei* of Shimoide, named for H. Shimoide, a Japanese microbiologist who first isolated a strain of this species.

Acid-fast rods (3–5 × 0.6 µm) with frequent cross barring, but does not exhibit cord formation or a mycelium. Rough, non-pigmented colonies appear after incubation for 14–21 d on Löwenstein–Jensen medium. Does not grow on media supplemented with 5% (w/v) NaCl. Temperature range for growth is 28–45°C; optimal growth is at 37–45°C. TLC of methanolysates shows α-, α′-, and keto-mycolates, and wax esters. Differential characteristics of slowly growing species are shown in Table 37.

Source: isolated from sputum and considered to be a cause of pulmonary disease.

DNA G+C content (mol%): not determined.

Type strain: ATCC 27962, CCUG 37517, DSM 44152, JCM 12376.

Sequence accession no. (16S rRNA gene): AJ005005.

Additional remarks: the four cultures upon which the original description of this species was based were isolated from the same patient in Japan (Tsukamura, 1982). However, another culture exhibiting similar properties was isolated from a patient in Australia. All of the strains differed markedly from those of other known species on the basis of numerical taxonomic data (Wayne et al., 1971).

List of rapid-growing species of the genus *Mycobacterium*

Multimembered 16S rRNA gene clades

The *Mycobacterium chitae* clade

58. **Mycobacterium chitae** Tsukamura 1967a, 44[AL]

chi′ta.e. N.L. gen. n. *chitae* of Chita, a place in Japan.

Acid-fast, coccoid-like elements, which do not show branching, cord, or mycelium formation. Acid-fastness is strong in younger cultures, but may become partial in older cultures (Wayne and Kubica, 1986). White or cream colored, smooth, wet-looking, non-pigmented colonies are formed on most media after 3–5 d following the addition of dilute inocula. The temperature range for growth is 28–37°C; does not grow at 45°C. *Mycobacterium chitae* is tolerant to 5% (w/v) NaCl, but does not grow on MacConkey agar (without crystal violet). TLC of methanolysates shows α-, α′-, and epoxy-mycolates. Differential characteristics of the rapidly growing species are shown in Table 38.

Virulence studies revealed *Mycobacterium chitae* to be avirulent for chickens, guinea pigs, mice, and rabbits injected with 2–10 mg of the organism. Not known to be associated with human disease.

Source: four strains of this species were originally isolated from soil samples collected from manure heaps.

DNA G+C content (mol%): not determined.

Type strain: ATCC 19627, CCUG 39504, CIP 105383, DSM 44633, JCM 12403, NCTC 10485.

Sequence accession no. (16S rRNA gene): X55603.

Additional remarks: initially described in Japanese (Tsukamura, 1966c), and later in English (Tsukamura, 1967a).

59. **Mycobacterium fallax** Lévy-Frébault, Rafidinarivo, Promé, Grandry, Boisvert and David 1983, 342[VP]

fal′lax. L. neut. adj. *fallax* deceptive, in the sense that the colonies resemble those of *Mycobacterium tuberculosis*.

Acid-fast, short rods (0.5 × 1.0 µm long), except for a small number (less than 20%) of cyanophil forms. Smears prepared from Youman's medium (without Tween 80) show clumps of rods or cords. Growth occurs within 5 d at 30°C on Löwenstein–Jensen, Middlebrook 7H10, and nutrient agar media. Larger colonies are produced by incubation at 37°C, but colonies grow more slowly (12–21 d or more); significant numbers of isolates fail to grow on nutrient agar at this temperature. At both temperatures, colonies are eugonic, rough, and buff-colored, and resemble those of *Mycobacterium tuberculosis*. Cord formation is evident at the edges of colonies on Middlebrook 7H10 medium. In addition to colonial characteristics, this organism shares nitrate reductase production and thermolabile catalase activity with *Mycobacterium tuberculosis*, but differs from the latter as it is niacin-negative and grows rapidly at 30°C. TLC of methanolysates shows α-mycolates. Differential characteristics of the rapidly growing species are shown in Table 38.

Source: all but three of the initial 22 strains were isolated from river and lake water. The remaining three strains were isolated from sputum though this organism is not known to be a cause of disease.

DNA G+C content (mol%): not determined.

Type strain: CIP 81.39, ATCC 35219, CCUG 37584, DSM 44179, JCM 6405.

Sequence accession no. (16S rRNA gene): M29562.

The *Mycobacterium komossense* clade

60. **Mycobacterium komossense** Kazda and Müller 1979, 364[AL]

ko.mos.sen′se. N.L. neut. adj. *komossense* of or belonging to Komosse sphagnum bog in south Sweden.

Short to moderately long rods, often clumped, but never corded or cross-barred. Dilute inocula on both inspissated egg and oleic acid-albumin media yield eugonic, smooth, glistening, yellow-beige, scotochromogenic colonies in less than 7 d. Temperature range for growth is 22–37°C, with optimal growth at 31°C; does not grow at 45°C. TLC of methanolysates shows α-, keto-, and methoxy-mycolates, and wax esters. Differential characteristics of the rapidly growing species are shown in Table 38.

Injection of guinea pigs, mice, and rabbits with large inocula (1–10 mg wet weight of cells) produced neither local nor disseminated disease.

Source: isolated from sphagnum vegetation of intact sphagnum bogs in southern Sweden and the Atlantic coastal area of Norway; not recovered from partially cultivated moors.

DNA G+C content (mol%): not determined.

Type strain: ATCC 33013, CIP 105293, DSM 44078.

Sequence accession no. (16S rRNA gene): X55591.

61. **Mycobacterium aichiense** Tsukamura, Mizuno and Tsukamura 1981, 274[VP]

ai.chi.en′se. N.L. neut. adj. *aichiense* of or belonging to Aichi prefecture, Japan.

Rods, less than 2 μm in length. Acid-fast in young cultures, but may lose some acid-fastness on prolonged culture. Dilute inocula on egg and agar media yield yellow-orange, smooth colonies in 3–4 d or less. The temperature range for growth is 25–37°C; does not grow at 45°C. TLC of methanolysates shows α- and keto-mycolates, and wax esters. Differential characteristics of the rapidly growing species are shown in Table 38.

Source: isolated from soil and from sputum of humans but is not associated with disease.

DNA G+C content (mol%): not determined.

Type strain: ATCC 27280, CIP 106808, DSM 44147, JCM 6376, NCTC 10820.

Sequence accession no. (16S rRNA gene): X55598.

Additional remarks: this species was originally described in Japanese (Tsukamura, 1973) and was not cited in the *Approved Lists of Bacterial Names* (Skerman et al., 1980), but was revived by Tsukamura et al. (1981).

62. **Mycobacterium holsaticum** Richter, Niemann, Gloeckner, Pfyffer and Rüsch-Gerdes 2002, 1995[VP]

hol.sa′ti.cum. M.L. neut. adj. *holsaticum* of or belonging to *Holsatia*, the German region of Holstein, the location of the institute in which the strains were first analyzed.

Acid–alcohol-fast rods and cocci. Colony morphology is dependent on the temperature of incubation; colonies are dysgonic and transparent at 22°C, but smooth, moist, shiny, and off-white- to yellow-pigmented at higher temperatures. Growth occurs in 7 d within the temperature range 22–40°C. Strains tolerate 5% (w/v) NaCl, but do not grow on MacConkey agar. TLC of methanolysates shows α-, keto-, and methoxy-mycolates. Differential characteristics of the rapidly growing species are shown in Table 38.

Source: nine strains were isolated from a variety of clinical specimens from different patients in various regions of Germany; the species is of doubtful pathogenicity for humans.

DNA G+C content (mol%): 68.4 (HPLC).

Type strain: DSM 44478, CCUG 46266, JCM 12374.

Sequence accession nos: AJ310467 (16S rRNA gene); AJ310468 (16S–23S rRNA ITS); AJ310469 (*hsp65*).

63. **Mycobacterium murale** Vuorio, Andersson, Rainey, Kroppenstedt, Kämpfer, Busse, Viljanen and Salkinja-Salonen 1999, 34[VP]

mu.ra′le. L. neut. adj. *murale* of or belonging to a wall.

Acid–alcohol-fast rods or coccoid cells (0.6–1.4 × 0.4–0.5 μm). Growth occurs as smooth, saffron yellow, scotochromogenic colonies within 5 d on tryptone soya blood agar. Does not grow on MacConkey agar (without crystal violet) or on 5% (w/v) NaCl agar. The temperature range for growth is 10–37°C, with optimal growth at 30°C; does not grow at 45°C. TLC of methanolysates shows α- and keto-mycolates, and wax esters. Differential characteristics of the rapidly growing species are shown in Table 38.

Source: five strains were isolated from water-damaged indoor building material in Finland, mycobacteria were not found in non-water damaged parts of the same building materials (Andersson et al., 1997).

DNA G+C content (mol%): 72.9 (HPLC).

Type strain: DSM 44340, CCUG 39728, CIP 105980, JCM 13392.

Sequence accession no. (16S rRNA gene): AB537171.

64. **Mycobacterium tokaiense** Tsukamura, Mizuno and Tsukamura 1981, 274[VP]

to.kai.en′se. N.L. neut. adj. *tokaiense* of or belonging to Tokai district of Japan.

Rods (1–7 μm long) which are sometimes cross-barred. Acid-fast in young cultures but may lose acid-fastness on prolonged incubation. Dilute inocula on egg media yield smooth, scotochromogenic colonies in less than 5 d. The temperature range for growth is 25–37°C, does not grow at 45°C. TLC of methanolysates shows α- and keto-mycolates, and wax esters. Differential characteristics of the rapidly growing species are shown in Table 38.

Source: isolated from soil.

DNA G+C content (mol%): not determined.

Type strain: ATCC 27282, CIP 106807, DSM 44635, JCM 6373, NCTC 10821.

Sequence accession no. (16S rRNA gene): AF480590.

Additional remarks: this species was initially described in Japanese (Tsukamura, 1973), was not cited on the *Approved Lists of Bacterial Names* (Skerman et al., 1980), and was revived by Tsukamura et al. (1981).

The *Mycobacterium austroafricanum* clade

65. **Mycobacterium austroafricanum** Tsukamura, van der Meulen and Grabow 1983c, 467[VP]

aus.tro.a.fri.ca′num. L. adj. *australis* southern; L. adj. *africanus -a -um* pertaining to Africa; N.L. neut. adj. *austroafricanum* of or pertaining to South Africa, the source of the isolates.

Acid-fast rods (2–6 × 0.5 μm). Growth occurs within 3 d at 28 and 37°C, but not at 42°C. Colonies are mucoid, yellowish in the dark, with pigment intensifying after exposure to light. The organism is susceptible to ethambutol (5 μg/ml), isoniazid (10 μg/ml), and $NH_2OH.HCl$ (250 μg/ml), but is resistant to rifampin (25 μg/ml) and thiophene-2-carboxylic acid hydrazide (1 μg/ml). Grows in the presence of 5% (w/v) NaCl. TLC of methanolysates shows α- and keto-mycolates, and wax esters. Differential characteristics of the rapidly growing species are shown in Table 38.

Source: all strains were recovered from South African waters and are not known to be associated with human disease.

DNA G+C content (mol%): not determined.

Type strain: ATCC 33464, CCUG 37667, CIP 105395, DSM 44191, JCM 6369.

Sequence accession no. (16S rRNA gene): X93182.

66. **Mycobacterium vanbaalenii** Khan, Kim, Paine and Cerniglia 2002, 2001[VP]

van.ba.a.len′i.i. N.L. gen. masc. n. *vanbaalenii* of Van Baalen, in memory of Chase Van Baalen (1925–1986), a professor at The University of Texas Marine Science Institute, Port Aransas Marine Laboratory, Port Aransas, Texas, USA.

Gram-stain-positive, acid-fast rods (1.4 × 0.7 µm). Colonies are smooth and scotochromogenic with a saffron yellow pigment on Middlebrook 7H10 medium. Grows well on brain heart infusion, trypticase soy, tryptone-yeast extract, and minimal balanced salt media. Pyrene, a polycyclic aromatic hydrocarbon, is degraded. The temperature range for growth is 24–37°C; at best, minimal growth is seen at 42°C; does not grow at 45°C. Differential characteristics of the rapidly growing species are shown in Table 38.

Source: isolated from sediments in an oil-contaminated site in Redfish Bay, Texas, USA (Heitkamp et al., 1988).

DNA G+C content (mol%): 67.8 (whole-genome sequencing).

Type strain: DSM 7251, JCM 13017.

Sequence accession no. (16S rRNA gene): X84977.

Additional remarks: this species is closely related phylogenetically to *Mycobacterium austroafricanum*; the two type strains share a 16S rRNA gene sequence similarity value of 99.8%. However, in dot-blot DNA–DNA hybridization studies, the organisms showed less than 40% hybridization (Khan et al., 2002).

67. **Mycobacterium vaccae** Bönicke and Juhasz 1964, 133[AL]

vac′ca.e. L. n. *vacca* a cow; L. gen. n. *vaccae* of the cow.

Short acid-fast rods (1–4 × 0.5–0.8 µm) which may be curved with rounded or thickened ends; occasionally Y-shaped cells are seen. Acid-fastness is irregular in old cultures. Dilute inocula on Löwenstein–Jensen medium yield smooth, moist, yellow-orange, shiny, butyrous, domed colonies in less than 5 d. Most strains are very light sensitive; they are non-pigmented if grown in complete darkness, but become yellow after brief (minutes) exposure to light. Occasionally, rough or non-pigmented colonies are observed. The temperature range for growth is nominally 17–42°C, but growth is restricted and pigmentation is inhibited at 17 and 42°C; does not grow at 45°C. TLC of methanolysates shows α-, α′-, and keto-mycolates, and wax esters. Differential characteristics of the rapidly growing species are shown in Table 38.

Source: isolated from the lacteal glands and skin lesions of cattle and also from soil, watering ponds, and wells.

DNA G+C content (mol%): not determined.

Type strain: ATCC 15483, CCUG 21003, CIP 105934, DSM 43292, JCM 6389, NCTC 10916.

Sequence accession no. (16S rRNA gene): X55601.

Additional remarks: DNA–DNA relatedness studies (Baess, 1982) confirmed this as a distinct species and resolved some of the conflict in earlier international cooperative studies (Kubica et al., 1972; Saito et al., 1977).

68. **Mycobacterium aurum** Tsukamura 1966d, 266[AL]

au′rum. L. n. *aurum* gold, the color of gold, intended to mean gold-pigmented.

Acid-fast rods (1–6 µm in length). Smooth, yellow-orange colonies appear in less than 5 d on inspissated egg media. The temperature range for growth is 25–37°C; does not grow at 45°C. TLC of methanolysates shows α- and keto-mycolates, and wax esters; some strains contain α′-mycolates. Differential characteristics of the rapidly growing species are shown in Table 38.

Source: strains isolated from soil may occasionally be seen in sputum in humans, but they do not cause disease.

DNA G+C content (mol%): not determined.

Type strain: ATCC 23366, CCUG 37666, CIP 104465, DSM 43999, JCM 6366.

Sequence accession no. (16S rRNA gene): X55595.

Additional remarks: the species was originally described in Japanese (Tsukamura and Tsukamura, 1966) and was first thought to be a variety of *Mycobacterium parafortuitum*; however, it could be differentiated from this taxon principally on the basis of growth temperature and pigmentation (Tsukamura, 1966a).

69. **Mycobacterium pyrenivorans** Derz, Klinner, Schuphan, Stackebrandt and Kroppenstedt 2004, 2316[VP]

py.re.ni.vo′rans. N.L. n. *pyrenum* pyrene; L. part. adj. *vorans* devouring, destroying; N.L. part. adj. *pyrenivorans* destroying pyrene.

Acid-fast rods. Rough, yellow scotochromogenic colonies are formed within 7 d at 35°C. Colonial pigmentation intensifies after exposure to light. In liquid media, cells clump together or show biofilm formation on glass. The temperature range for growth is 24–37°C; does not grow at 42 or 45°C. TLC of methanolysates shows α- and epoxy-mycolates, and wax esters. Differential characteristics of the rapidly growing species are shown in Table 38.

Source: the type strain was isolated from soil that was highly contaminated with polycyclic aromatic hydrocarbons on the site of a former coking plant in Germany. It is named for its ability to use pyrene as a sole source of carbon and energy.

DNA G+C content (mol%): not determined.

Type strain: DSM 44605, NRRL B-24349.

Sequence accession no. (16S rRNA gene): AJ431371.

The *Mycobacterium doricum* clade

70. **Mycobacterium doricum** Tortoli, Piersimoni, Kroppenstedt, Montoya-Burgos, Reischl, Giacometti and Emler 2001, 2011[VP]

do′ri.cum. L. neut. adj. *doricum* of or belonging to *Dorica civitas*, the ancient name of the Italian city of Ancona, from where the organism was first isolated.

Acid–alcohol-fast, nonmotile, asporogenous rods. Colonies on Löwenstein–Jensen medium are smooth, scotochromogenic, and strongly yellow-pigmented. Growth occurs within 2 weeks at 25–37°C. Does not grow at 45°C, on MacConkey agar (without crystal violet), or on media supplemented with 5% (w/v) NaCl. TLC of methanolysates shows α- and methoxy-mycolates, and wax esters. Differential characteristics of the rapidly growing species are shown in Table 38.

Source: isolated from cerebrospinal fluid of a severely immunocompromised AIDS patient who was being treated for *Cryptococcus neoformans* infection.

DNA G+C content (mol%): 66.5 (HPLC).

Type strain: DSM 44339, CCUG 46352, CIP 106867, JCM 12405.

Sequence accession no. (16S rRNA gene): AF264700.

Additional remarks: Tortoli et al. (2001) describe strains as having α- and keto-mycolates, but Tortoli (2003) found the mycolate pattern to be α- and methoxy-mycolates, and wax esters; this latter finding was supported by Reischl et al. (2006) who used the mycolates pattern cited above to resolve the phenotypic similarities found between *Mycobacterium doricum* and *Mycobacterium monacense.* Despite slow growth on artificial media, the 16S rRNA gene nucleotide sequence of *Mycobacterium doricum* is characterized by a short helix 18, a feature usually associated with rapidly growing mycobacteria. Devulder et al. (2005) placed *Mycobacterium doricum* amongst rapidly growing species following 16S rRNA gene, *hsp65*, *rpoB*, and *sod* gene sequencing studies. Reischl et al. (2006) found that the 16S rRNA gene sequences of *Mycobacterium doricum* and *Mycobacterium monacense* differed by 14 bp, but recovered both taxa with rapidly growing species.

71. **Mycobacterium monacense** Reischl, Melzl, Kroppenstedt, Miethke, Naumann, Mariottini, Mazzarelli and Tortoli 2006, 2578[VP]

mo.na.cen′se. M.L. neut. adj. *monacense* of or pertaining to *Monacum*, the Latin name of the German city of Munich where the strain was first isolated.

Gram-stain-positive, acid-fast, asporogenous, nonmotile rods. Smooth, yellow, scotochromogenic colonies are produced within 7 d of incubation. Temperature for growth is 25–45°C; optimal growth is between 25–37°C. Grows on Löwenstein–Jensen medium supplemented with 5% (w/v) NaCl, but not on MacConkey medium (without crystal violet). TLC of methanolysates shows α- and keto-mycolates, and wax esters. Differential characteristics of the rapidly growing species are shown in Table 38.

Source: initially isolated from a bronchial lavage sample obtained from an 80-year-old patient with carcinoma. The species is of uncertain clinical significance, but one strain was isolated from a biopsy sample taken from a penetrating wound in an 11-year-old boy.

DNA G+C content (mol%): not determined.

Type strain: DSM 44395, CIP 109237.

Sequence accession nos: AF107039 (16S rRNA gene); DQ473393 (16S–23S rRNA ITS).

Additional remarks: the close relationship between *Mycobacterium monacense* and *Mycobacterium doricum* was borne out by comparative sequencing studies on the *hsp65* gene. See also comments following the description of *Mycobacterium doricum.*

The *Mycobacterium flavescens* clade

72. **Mycobacterium flavescens** Bojalil, Cerbón and Trujillo 1962, 344[AL]

fla.ves′cens. L. v. *flavesco* to become golden yellow; L. part. adj. *flavescens* becoming yellow.

Rod-shaped organism. Dilute inocula on Löwenstein–Jensen medium usually produce soft, yellow-orange, butyrous colonies after incubation for 7–10 d at 25–37°C; the colonies may adhere to the medium. The temperature range for growth is 25–42°C; does not grow at 45°C. Metabolic and physiological properties are more like those of rapidly growing species, even though the growth rate is intermediate between rapid and slow growers. TLC of methanolysates shows α- and keto-mycolates, and wax esters. Differential characteristics of the rapidly growing species are shown in Table 38.

Source: isolated from drug-treated tuberculous guinea pig; other isolations as apparent normal flora of humans suggest an environmental habitat.

DNA G+C content (mol%): not determined.

Type strain: ATCC 14474, CCUG 29041, CIP 104533, DSM 43991, JCM 12274, NCTC 10271.

Sequence accession no. (16S rRNA gene): X52932.

73. **Mycobacterium novocastrense** Shojaei, Goodfellow, Magee, Freeman, Gould and Brignall 1997, 1206[VP]

no.vo.cas.tren′se. L. adj. *novus* new; L. neut. n. *castrum* castle; N.L. neut. adj. *novocastrense* of or pertaining to Newcastle, a city in the northeast of England.

Gram-stain-positive, asporogenous, nonmotile, weakly acid–alcohol-fast rods (3–4 μm long) with some longer rods and filamentous forms in older cultures. Dilute inocula on Löwenstein–Jensen medium and Middlebrook 7H10 agar produce moderately photochromogenic, yellow-pigmented growth when incubated for 3–7 d in the light at 30–42°C. Older cultures show a deeper yellow pigmentation. Good growth occurs on Columbia blood agar (3 d); weak growth is formed on MacConkey agar (without crystal violet) and 5% (w/v) NaCl agar after 14 d. TLC of methanolysates shows α- and keto-mycolates, and wax esters. Differential characteristics of the rapidly growing species are shown in Table 38.

Source: isolated from a slowly spreading skin granulation of the hand of a child.

DNA G+C content (mol%): not determined.

Type strain: DSM 44203, CIP 105546.

Sequence accession no. (16S rRNA gene): U96747.

Additional remarks: in clinical settings, it is possible that *Mycobacterium novocastrense* might be confused with *Mycobacterium marinum*, another photochromogenic organism which causes self-limiting granulomatous skin lesions. However, the two organisms can be distinguished readily as only *Mycobacterium novocastrense* reduces nitrate and grows at 42°C.

74. **Mycobacterium duvalii** Stanford and Gunthorpe 1971, 637[AL]

du.va′li.i. N.L. gen. masc. n. *duvalii* of Duval, named for Professor C.W. Duval who isolated two strains of the organism.

Pleomorphic bacilli producing rough or smooth, bright yellow colonies in less than 7 d on inspissated egg media at 25–37°C, but not at 45°C. TLC of methanolysates shows α-, α′-, and keto-mycolates, and wax esters. Serological specificity was demonstrated by immunodiffusion (Stanford and Gunthorpe, 1971) and by numerical taxonomic

data (Tsukamura et al., 1981). Differential characteristics of the rapidly growing species are shown in Table 38.

Source: the four original strains were isolated from cases of human leprosy. However, the species has not been incriminated as a human or animal pathogen.

DNA G+C content (mol%): not determined.

Type strain: ATCC 43910, CCUG 41352, CIP 104539, DSM 44244, JCM 6396, NCTC 358.

Sequence accession no. (16S rRNA gene): U94745.

The *Mycobacterium agri* clade

75. **Mycobacterium agri** Tsukamura 1981, 256[VP]

ag′ri. L. gen. n. *agri* of a field.

Acid-fast rods (3–7 μm long) which frequently join to form long threads; does not form a mycelium or show cord formation. Colonies are rough and non-pigmented, both in the dark and after exposure to light, when grown for less than 5 d on egg media. The temperature range for growth is 28–45°C; does not grow at 52°C. TLC of methanolysates shows α-, α′-, and methoxy-mycolates. Differential characteristics of the rapidly growing species are shown in Table 38.

Source: the original isolate was recovered from an alkali-treated soil sample inoculated onto egg medium and incubated at 42°C. *Mycobacterium agri* is not known to be associated with disease.

DNA G+C content (mol%): not determined.

Type strain: ATCC 27406, CCUG 37673 A, CIP 105391, DSM 44515, JCM 6377.

Sequence accession no. (16S rRNA gene): AJ429045.

Additional remarks: the organism was originally described by Tsukamura (1972b) but was not cited in the *Approved Lists of Bacterial Names* (Skerman et al., 1980); the epithet was revived by Tsukamura (1981).

76. **Mycobacterium thermoresistibile** Tsukamura 1966b, 266[AL]

ther.mo.re.sis.ti′bi.le. Gr. n. *thermê* heat; L. verb. *resisto* to stand back, remain standing, endure; L. neut. suff. *-ile* suffix denoting an active quality, able to; N.L. neut. adj. *thermoresistibile* resistant to high temperature.

Acid-fast rods (3–6 μm long). On primary isolation, growth may be slow and the species may be confused with slowly growing scotochromogens. However, subculture on inspissated egg media yields smooth or rough, yellow colonies in 3–5 d. The temperature range for growth is 37–52°C. *Mycobacterium thermoresistibile* lacks many properties common to rapidly growing mycobacteria such as growth on fumarate, malate, mannose, or succinate as sole carbon sources, and gives a negative aryl sulfatase reaction. TLC of methanolysates shows α-, α′-, methoxy, and keto-mycolates. Differential characteristics of the rapidly growing species are shown in Table 38.

Source: isolates have been recovered from soil and may occasionally be found in sputum samples from humans, but the organism is not known to be pathogenic.

DNA G+C content (mol%): not determined.

Type strain: ATCC 19527, CCUG 28008/41353, CIP 105390, DSM 44167, JCM 6362.

Sequence accession no. (16S rRNA gene): X55602.

Additional remarks: this organism was casually mentioned by Tsukamura (1965, in Japanese), then the name was validly published by Tsukamura (1966d) in English and by Tsukamura (1966b) in Japanese. The species was further described by Tsukamura (1971), with more definitive features presented by Tsukamura et al. (1981).

The *Mycobacterium smegmatis* clade

77. **Mycobacterium smegmatis** (Trevisan 1889) Lehmann and Neumann 1899, 403[AL] (*Bacillus smegmatis* Trevisan 1889, 14)

smeg.ma′tis. L. n. *smegma -atis* an unguent (for making the skin smooth), a detergent, a cleansing medicine, and in biology the sebaceous humor; L. gen. n. *smegmatis* of smegma.

Acid-fast rods (3–5 μm long), occasionally curved with branching or Y-shaped cells. Cells are sometimes swollen and may appear as deeper staining beaded or ovoid forms. Acid-fastness is irregular after incubation for 5 d (10–80%). Colonies that appear on Löwenstein–Jensen medium in 2–4 d are usually rough, wrinkled or coarsely folded, and non-pigmented or creamy white. Smooth, glistening, butyrous colonies are seen, but pigmentation is rare, though it may be seen in older cultures. On Middlebrook agar, the rough colonial form appears smooth textured over a rugose, but non-corded, granular colony; the smooth form is domed, smooth textured, and granular (Jones and Kubica, 1965). Temperature range for growth is 25–45°C. TLC of methanolysates shows α-, α′-, and epoxy-mycolates. Differential characteristics of the rapidly growing species are shown in Table 38.

Not pathogenic for chickens, guinea pigs, hamsters, or mice, but pathogenic cultures may be obtained from the spleens of guinea pigs and/or mice (Durr et al., 1959). *Mycobacterium smegmatis* is usually associated with secretions of the normal genitalia and with soft lesions following accidental or surgical trauma (Wallace et al., 1988).

Source: isolated from smegma tissue.

DNA G+C content (mol%): 67.4 (whole-genome sequencing).

Type strain: ATCC 19420, CCUG 21002/21815, CIP 104444, DSM 43756, JCM 5866/6386, NCTC 8159.

Sequence accession no. (16S rRNA gene): AJ131761.

Additional remarks: Mycobacterium smegmatis was named by Trevisan (1889) for the Smegma Bacillus of Alvarez and Tavel (1885) and was redescribed by Lehmann and Neumann (1899). Gordon and Mimh (1959) showed the taxon to be a good species, a conclusion supported by other workers (Baess, 1982; Kubica et al., 1972; Tsukamura, 1966d). Members of the species formed homogeneous groups based on species-specific sensitins (Magnusson, 1962), and immunodiffusion and immunological techniques (Castelnuovo et al., 1960; Gimpl and Lanyi, 1965; Lind, 1960; Norlin, 1965).

78. **Mycobacterium goodii** Brown, Springer, Steingrube, Wilson, Pfyffer, Garcia, Menendez, Rodriguez-Salgado, Jost, Chiu, Onyi, Böttger and Wallace 1999, 1509[VP]

go.od′i.i. N.L. gen. masc. n. *goodii* of Good, named for Robert Good who made significant contributions to the study of mycobacteria.

Acid- and alcohol-fast bacilli that produce smooth to mucoid off-white- to cream-colored colonies after incubation for 2–4 d on Middlebrook 7H10 and trypticase soy

agars. Yellow to orange pigment is produced by 78% of isolates after incubation for 10–14 d. Temperature range for growth is 30–45°C. Growth occurs on MacConkey agar (without crystal violet) and in the presence of 5% (w/v) NaCl. Differential characteristics of the rapidly growing species are shown in Table 38.

Many of the 28 strains investigated were considered to be the causative agents of osteomyelitis or of respiratory infections.

Source: the type strain was isolated from a patient with a post-traumatic osteomyelitis of the heel.

DNA G+C content (mol%): 66 (HPLC).

Type strain: ATCC 700504, CIP 106349, DSM 44492, JCM 12689.

Sequence accession no. (16S rRNA gene): Y12872.

Additional remarks: this species was identified in a cooperative IWGMT study designed to determine the heterogeneity within the species *Mycobacterium smegmatis* (Brown et al., 1999). *Mycobacterium goodii* was previously known as *Mycobacterium smegmatis* Group 2. As with *Mycobacterium smegmatis* and strains of the *Mycobacterium fortuitum* complex, *Mycobacterium goodii* and *Mycobacterium wolinskyi* (see below) have been implicated in post-traumatic wound infections.

The *Mycobacterium phlei* clade

79. **Mycobacterium phlei** Lehmann and Neumann 1899, 411[AL]

phle′i. N.L. neut. n. *Phleum* a genus of grass, timothy; N.L. gen. n. *phlei* of *Phleum*, of timothy.

Short rods (1.0–2.0 µm long), acid-fast in young cultures, but staining irregularly after incubation for 5–7 d (5–100% acid-fast). Colonies are usually rough, coarsely wrinkled, and deep yellow- to orange-colored after incubation for 2–5 d on Löwenstein–Jensen medium. Smooth, butyrous colonial forms are occasionally seen. The rough colonial form on Middlebrook agar is flat, granular, loosely corded with irregular edges, and has dark granules near the center (Jones and Kubica, 1965). Temperature range for growth is 22–52°C; only *Mycobacterium phlei* and *Mycobacterium thermoresistibile* are known to grow at 52°C and both remain viable after heating at 60°C for 4 h. *Mycobacterium phlei* is tolerant of 5% (w/v) NaCl, but does not grow on MacConkey agar. TLC of methanolysates shows α- and keto-mycolates, and wax esters. Differential characteristics of the rapidly growing species are shown in Table 38.

Source: once widely distributed in nature, especially hay and grass, but now rarely seen in environmental surveys (Wolinsky and Rynearson, 1968). Not pathogenic for carp, chicken, frog, guinea pig, mouse, rabbit, or rat (Durr et al., 1959; Penso et al., 1951).

DNA G+C content (mol%): not determined.

Type strain: ATCC 11758, CCUG 21000, CIP 105389, DSM 43239, JCM 5865/6385, NCTC 8151.

Sequence accession no. (16S rRNA gene): M29566.

Additional remarks: Mycobacterium phlei was proposed for the Timothy or Grass *Bacillus* of Moëller (1898). The taxon has been shown to be a good species using immunofluorescence (Jones and Kubica, 1968), species-specific sensitin (Magnusson, 1962), immunodiffusion and immunoelectrophoretic relatedness (Castelnuovo et al., 1960; Gimpl and Lanyi, 1965; Lind, 1960; Norlin, 1965), and DNA–DNA relatedness data (Baess, 1982).

80. **Mycobacterium brumae** Luquin, Ausina, Lévy-Frébault, Lanéelle, Belda, García-Barceló, Prats and Daffé 1993, 411[VP]

bru′ma.e. L. gen. n. *brumae* of winter, referring to the time of year at which the first strains were isolated.

Rods (2–2.5 × 0.3–0.5 µm). Most cells are strongly acid-fast but a few (less than 10%) show cyanophil forms. Clumping or cord formation may be observed on Middlebrook 7H12 medium. Colonies on Löwenstein–Jensen and on Middlebrook 7H12 agar are eugenic, rough, and non-pigmented in the dark and after exposure to light. Growth occurs within 5 d at 30 and 37°C, but not at 45°C. Growth does not occur on MacConkey agar (without crystal violet). TLC of methanolysates shows α-mycolates. Differential characteristics of the rapidly growing species are shown in Table 38.

Source: initially, 11 strains were isolated; eight were from samples of river water, two were from soil, and one was from a sputum sample.

DNA G+C content (mol%): not determined.

Type strain: CIP 103465, ATCC 51384, CCUG 37586, DSM 44177, JCM 12273.

Sequence accession no. (16S rRNA gene): AF480576.

81. **Mycobacterium confluentis** Kirschner, Teske, Schröder, Kroppenstedt, Wolters and Böttger 1992, 261[VP]

con.flu.en′tis. M.L. gen. n. *confluentis* of *Confluentes*, now Koblenz, the source of the strain on which the species description is based.

Acid–alcohol-fast cocco-bacilli (0.5–0.8 × 0.7–1.7 µm). Does not form aerial hyphae, capsules, or spores. Visible growth from dilute inocula occurs within 2–4 d. Colonies on Löwenstein–Jensen medium are colorless, smooth, and 3–5 mm in diameter. A dark pigmentation, not dependent on light, may be observed after prolonged culture (8–12 weeks). The temperature range for growth is 22–41°C; the optimal temperature is 31–37°C. Does not grow at 42 or 45°C. TLC of methanolysates shows α- and keto-mycolates. Differential characteristics of the rapidly growing species are shown in Table 38.

Source: a single isolate was obtained from the sputum of a healthy male.

DNA G+C content (mol%): not determined.

Type strain: DSM 44017, ATCC 49920, CIP 105510, JCM 13671.

Sequence accession no. (16S rRNA gene): X63608.

82. **Mycobacterium elephantis** Shojaei, Magee, Freeman, Yates, Horadagoda and Goodfellow 2000, 1819[VP]

e.le.phan′tis. L. n. *elephas -antis* elephant; L. gen. n. *elephantis* of the elephant.

Gram-stain-positive, aerobic, asporogenous, nonmotile, weakly acid–alcohol-fast cocco-bacilli (1.2–1.4 µm long). Colonies are smooth, domed, and non-chromogenic, although weak chromogenicity may appear in older cultures. The organism tolerates 5% (w/v) NaCl, but does not grow on MacConkey agar (without crystal violet). Good growth occurs within 7 d on Löwenstein–Jensen medium

and Middlebrook 7H10 agar at 25–45°C. TLC of methanolysates shows α- and keto-mycolates, and wax esters. Differential characteristics of the rapidly growing species are shown in Table 38.

Source: isolated from a lung abscess of an elephant that had died from chronic respiratory disease. Subsequently, isolates were obtained from human clinical samples, predominantly sputum (Turenne et al., 2002b) though pathogenicity for humans remains doubtful. However, a case of lymph node infection appears to be clinically significant (Turenne et al., 2002b).

DNA G+C content (mol%): not determined.

Type strain: DSM 44368, CIP 106831, JCM 12406.

Sequence accession no. (16S rRNA gene): AJ010747.

83. **Mycobacterium pulveris** Tsukamura, Mizuno and Toyama 1983a, 811[VP]

pul've.ris. L. gen. n. *pulveris* of dust, referring to the source, house dust.

Acid-fast short rods or coccoid forms (<2.0 by approx. 0.5 µm). Does not form mycelia or branching. Abundant growth appears in 5 d at 37°C from dilute inocula on Ogawa egg medium. Colonies are wet, smooth, creamy or slightly yellowish, and non-photochromogenic. Strains grow in the presence of 5% (w/v) NaCl. The temperature range for growth is 28–42°C; occasional strains will grow at 45°C. TLC of methanolysates shows α- and keto-mycolates, and wax esters. Differential characteristics of the rapidly growing species are shown in Table 38.

Source: eight isolates were recovered from house dust from five houses in Obu, Aichi Prefecture, Japan.

DNA G+C content (mol%): not determined.

Type strain: ATCC 35154, CCUG 37668, CIP 106804, DSM 44222, JCM 6370.

Sequence accession no. (16S rRNA gene): AJ429046.

Additional remarks: despite a rapid growth rate, this species demonstrates some characteristics typical of slow-growing mycobacteria and was considered to be intermediate between rapid and slow-growing species by Tsukamura et al. (1983a).

The *Mycobacterium peregrinum* clade

84. **Mycobacterium peregrinum** Kusunoki and Ezaki 1992, 244[VP]

pe.re.gri'num. L. neut. adj. *peregrinum* strange, foreign.

Acid-fast rods (1.5–4 × 0.5 µm). Colonies on egg medium appear in 7 d and are intermediate between smooth and rough, and white to slightly yellowish in color but non-photochromogenic. Temperature range for growth is 28–37°C; does not grow at 43°C. Growth occurs at 28 and 37°C on media supplemented with 5% (w/v) NaCl. However, on MacConkey agar growth occurs at 28°C, but not at 37°C. TLC of methanolysates shows the presence of α-, α'-, and epoxy-mycolates. Differential characteristics of the rapidly growing species are shown in Table 38.

This organism is a relatively rare cause of disease, but may do so in the same settings as *Mycobacterium fortuitum*.

Source: the type strain was isolated from bronchial aspirations from a Mexican child.

DNA G+C content (mol%): not determined.

Type strain: ATCC 14467, CCUG 27976, CIP 105382, DSM 43271, JCM 12142, NCTC 10264.

Sequence accession no. (16S rRNA gene): AF058712.

Additional remarks: this organism, proposed by Bojalil et al. (1962), was later considered to be synonymous with *Mycobacterium fortuitum* (Kubica et al., 1972; Minnikin et al., 1984; Pattyn et al., 1974; Stanford and Gunthorpe, 1969), hence its omission from the *Approved Lists of Bacterial Names* (Skerman et al., 1980). However, data from DNA–DNA relatedness studies (Baess, 1982; Lévy-Frébault et al., 1986b) supported the reintroduction of *Mycobacterium peregrinum* as a distinct species; Kusunoki and Ezaki (1992) formally proposed the reintroduction of the species. See also notes following the description of *Mycobacterium chelonae*.

85. **Mycobacterium wolinskyi** Brown, Springer, Steingrube, Wilson, Pfyffer, Garcia, Menendez, Rodriguez-Salgado, Jost, Chiu, Onyi, Böttger and Wallace 1999, 1508[VP]

wo.lins'ky.i. N.L. masc. gen. n. *wolinskyi* of Wolinsky, named for Emanuel Wolinsky for his significant contributions to the study of non-tuberculous mycobacteria.

Acid–alcohol-fast bacilli that produce smooth to mucoid off-white- to cream-colored colonies on Middlebrook 7H10 and trypticase soy agar after incubation for 2–4 d. Yellow/orange pigment is not produced. Growth occurs on MacConkey agar (without crystal violet) and in the presence of 5% (w/v) NaCl. The temperature range for growth is 30–45°C. Differential characteristics of the rapidly growing species are shown in Table 38.

Source: initially isolated from a post-surgical facial abscess in Switzerland and identified as *Mycobacterium smegmatis* Group 3 (Pennekamp et al., 1997). An additional strain was isolated from a sternal wound infection following open heart surgery in Texas.

DNA G+C content (mol%): 68 ± 2 (HPLC).

Type strain: ATCC 700010, CCUG 47168, CIP 106348, DSM 44493, JCM 13393.

Sequence accession no. (16S rRNA gene): Y12873.

Additional remarks: this species was classified in an International Working Group on Mycobacterial Taxonomy (IWGMT) cooperative study designed to determine the degree of heterogeneity in the species *Mycobacterium smegmatis* (Brown et al., 1999). *Mycobacterium wolinskyi* has been implicated in post-traumatic wound infections.

86. **Mycobacterium mageritense** Domenech, Jimenez, Menendez, Bull, Samper, Manrique and Garcia 1997, 539[VP]

ma.ge.ri.ten'se. N.L. neut. adj. *mageritense* of or pertaining to *Magerit*, old (first) Arabic name of Madrid, the source of most of the isolates.

Strongly acid–alcohol-fast rods. Smooth, mucoid nonchromogenic colonies appear in 2–4 d on Löwenstein–Jensen medium. Strains grow on MacConkey agar (without crystal violet) and most, but not all, will tolerate 5% (w/v) NaCl. Temperature range for growth is 22–45°C; optimal growth range is 30–37°C. TLC of methanolysates shows the presence of α-, α'-, and epoxy-mycolates. Differential characteristics of the rapidly growing species are shown in Table 38.

Source: the original description was based on five strains isolated from sputum of different patients over a 2 year period. Subsequently, isolates were described from blood, bronchial washings, sinus drainage, and wound infections (Wallace et al., 2002). Two cases of furunculosis associated with contaminated footbaths attributed to the organism were described by Gira et al. (2004).

DNA G+C content (mol%): 67 ± 3 (HPLC).

Type strain: CIP 104973, ATCC 700351, CCUG 37984, DSM 44476, JCM 12375.

Sequence accession no. (16S rRNA gene): AY457076.

Additional remarks: see also description of *Mycobacterium cosmeticum*.

The *Mycobacterium sphagni* clade

87. **Mycobacterium sphagni** Kazda 1980, 81[VP]

sphag′ni. N.L. n. *Sphagnum* generic name of the moss of sphagnum bogs; N.L. gen. n. *sphagni* of *Sphagnum*.

Short, thick, often pleomorphic rods; usually clumped but not corded. Eugonic, smooth, glistening, orange-yellow scotochromogenic colonies are produced from dilute inocula on Löwenstein–Jensen and Middlebrook 7H10 agar media. Does not grow on MacConkey agar (without crystal violet) or in the presence of 3% (w/v) NaCl. The temperature range for growth is 22–37°C; optimal growth occurs at 31°C in 3 d and in less than 7 d at temperature extremes; does not grow at 45°C. TLC of methanolysates shows the presence of α- and keto-mycolates, and wax esters. Differential characteristics of the rapidly growing species are shown in Table 38.

Source: isolated from sphagnum vegetation in moors of Germany and Scandinavia. No demonstrable virulence for rabbits, guinea pigs, or mice injected with 1–10 mg wet weight of cells.

DNA G+C content (mol%): not determined.

Type strain: ATCC 33027, DSM 44076.

Sequence accession no. (16S rRNA gene): X55590.

Additional remarks: the uniqueness of the taxon is supported by skin testing of guinea pigs hyper-sensitized by injections of mycobacteria, by characteristic lipid patterns, and by immunodiffusion in agar (Kazda, 1980).

88. **Mycobacterium chlorophenolicum** (Apajalahti, Kärpänoja and Salkinoja-Salonen 1986) Häggblom, Nohynek, Palleroni, Kronqvist, Nurmiaho-Lassila, Salkinoja-Salonen, Klatte and Kroppenstedt 1994, 491[VP] (*Rhodococcus chlorophenolicus* Apajalahti, Kärpänoja and Salkinoja-Salonen 1986, 248)

chlo.r.o.phe.nol′i.cum. N.L. n. *chlorophenol* chlorophenol; L. neut. suffix *-icum* suffix used with the sense of pertaining to; N.L. neut. adj. *chlorophenolicum* related to chlorophenols.

Acid-fast rods in young cultures fragment into coccoid forms on further incubation. Optimum growth occurs at 28°C with the production of yellow to orange scotochromogenic colonies in 4–8 d. Temperature range for growth is 18–37°C; does not grow at 45°C. TLC of methanolysates shows the presence of α- and keto-mycolates, and wax esters. Differential characteristics of the rapidly growing species are shown in Table 38.

Source: three strains of this species were independently isolated from chlorophenol-contaminated soil and sludge collected from different geographic locations in Finland.

DNA G+C content (mol%): 67.5–69.2 (HPLC).

Type strain: DSM 43826, ATCC 49826, CIP 104189, JCM 7439.

Sequence accession no. (16S rRNA gene): X79094.

Additional remarks: strains of this species were previously assigned to the genus *Rhodococcus* as *Rhodococcus chlorophenolicus* (Apajalahti et al., 1986). The species was transferred to the genus *Mycobacterium* (Häggblom et al., 1994) based on mycolic acid composition (Dobson et al., 1985), on the presence of *meso*-diaminopimelic acid, arabinose, and galactose in whole-organism hydrolysates, and 16S rRNA gene sequence data (Briglia et al., 1994)

89. **Mycobacterium chubuense** Tsukamura, Mizuna and Tsukamura 1981, 274[VP]

chu.bu.en′se. N.L. neut. adj. *chubuense* of or belonging to Chubu, coming from soil of Chubu hospital, Japan.

Acid-fast cocci in early stages of growth but acid-fastness may be lost on prolonged incubation. Smooth yellow colonies formed in 3 d on egg-based media. Temperature range for growth is 25–37°C; does not grow at 45°C. TLC of methanolysates shows the presence of α-, α′-, and keto-mycolates, and wax esters. Differential characteristics of the rapidly growing species are shown in Table 38.

Source: isolated from garden soil.

DNA G+C content (mol%): not determined.

Type strain: ATCC 27278, CCUG 37670, CIP 106810, DSM 44219, JCM 6374, NCTC 10819.

Sequence accession no. (16S rRNA gene): X55596.

Additional remarks: the species was originally described by Tsukamura (1973) in Japanese, but was not cited in the *Approved Lists of Bacterial Names* (Skerman et al., 1980); the epithet was revived by Tsukamura et al. (1981).

90. **Mycobacterium poriferae** Padgitt and Moshier 1987, 189[VP]

po.ri.fe′ra.e. N.L. gen. *poriferae* of the Porifera, the phylum of sponges.

Gram-stain-positive, strongly acid–alcohol-fast rods (1.1–4.9 × 0.7–2.5 µm) which frequently appear coccoid. Non-dividing cells in cultures are typically 2.7–3.1 × 2.2–2.3 µm. Cells may become enlarged at one end and display an ovoid, non-acid-fast body, but neither branching nor Y-shaped cells occur. Visible growth appears on various media in 4 d, including Löwenstein–Jensen medium and Middlebrook 7H11 agar. Colonies are smooth, moist, shiny, and dome-shaped with a near-translucent apron which disappears on continued growth. Strains are scotochromogenic producing a strongly orange coloration. They tolerate a wide pH range (5.0–8.5) and will grow in the presence of 5% (w/v) NaCl. Temperature range for growth is 20–37°C; optimal growth occurs at 28–30°C; does not grow at 42°C. TLC of methanolysates shows the presence of α- and keto-mycolates, and wax esters. Differential characteristics of the rapidly growing species are shown in Table 38.

Source: isolated from cell suspensions of a marine sponge.

DNA G+C content (mol%): not determined.

Type strain: ATCC 35087, CIP 105394, JCM 12603.
Sequence accession no. (16S rRNA gene): AF480589.

91. **Mycobacterium psychrotolerans** Trujillo, Velázquez, Kroppenstedt, Shumann, Rivas, Mateos and Martínez-Molina 2004, 1461[VP]

psy.chro.to′le.rans. Gr. adj. *psuchros* cold; L. part. adj. *tolerans* tolerating; N.L. part. adj. *psychrotolerans* cold-tolerating.

Acid-fast, nonmotile, non-spore-forming, short rods. Smooth, entire, bright orange, scotochromogenic colonies are formed on glucose-yeast extract and nutrient agars after incubation for 2 d. Growth occurs on 5–7% (w/v) NaCl agar; is moderate on Löwenstein–Jensen medium, but growth does not occur on MacConkey agar. The temperature range for growth is 4–37°C. TLC of methanolysates shows the presence of α- and keto-mycolates, and wax esters. Differential characteristics of the rapidly growing species are shown in Table 38.

Source: the type strain was isolated from pond water near a uranium mine in Spain.
DNA G+C content (mol%): 64 (T_m).
Type strain: DSM 44697, JCM 13323.
Sequence accession no. (16S rRNA gene): AJ534886.

The *Mycobacterium parafortuitum* clade

92. **Mycobacterium parafortuitum** Tsukamura, Toyama and Mizuno 1965, 232[AL]

pa.ra.for.tui′tum. Gr. prep. *para* alongside of or near; L. neut. adj. *fortuitum* casual, accidental, and also a specific epithet; N.L. neut. adj. *parafortuitum* alongside of (*Mycobacterium) fortuitum*.

Rods (2–3 µm long). Creamy or pale yellow, smooth, moist colonies are formed from dilute inocula on Löwenstein–Jensen media and on agar media usually within 3–4 d. Pigment increases markedly in most strains with further incubation and after exposure to light. Tolerance to 5% (w/v) NaCl is variable. Temperature range for growth is 25–37°C; some strains grow well at 45°C. TLC of methanolysates shows α-, α′-, and keto-mycolates, and wax esters. Differential characteristics of the rapidly growing species are shown in Table 38.

Injection of mice with 2 mg wet weight of cells did not cause any pathology and the organisms were rapidly eliminated.

Source: isolated from soil.
DNA G+C content (mol%): not determined.
Type strain: ATCC 19686, CCUG 20999, CIP 106802, DSM 43528, JCM 6367, NCTC 10411.
Sequence accession no. (16S rRNA gene): X93183.
Additional remarks: DNA–DNA homology studies (Baess, 1982) shows that *Mycobacterium parafortuitum* forms a distinct genospecies. It is most closely related to *Mycobacterium aurum* and *Mycobacterium vaccae*.

The taxon was first described by Tsukamura et al. (1965) and subsequently by Tsukamura (1966a). It was incorrectly cited in the *Approved Lists of Bacterial Names* (Skerman et al., 1980) as *Mycobacterium parafortuitum* Tsukamura (1966a) [see Hill et al. (1984)].

93. **Mycobacterium gilvum** Stanford and Gunthorpe 1971, 636[AL]

gil′vum. L. neut. adj. *gilvum* pale yellow.

Pleomorphic bacilli producing pale yellow, smooth colonies in less than 7 d on inspissated egg media. Grows at 25–37°C, but not at 45°C. The species was further characterized using numerical taxonomic data (Tsukamura et al., 1981). TLC of methanolysates shows α-, α′-, and keto-mycolates, and wax esters. Differential characteristics of the rapidly growing species are shown in Table 38.

Source: isolated from sputum and pleural fluid but has not been isolated more than once from the same patient. It is not thought to be pathogenic.
DNA G+C content (mol%): 67.9 (whole-genome sequencing).
Type strain: ATCC 43909, CCUG 37676, CIP 106743, DSM 44503, JCM 6395, NCTC 10742.
Sequence accession no. (16S rRNA gene): X55599.

94. **Mycobacterium obuense** Tsukamura, Mizuno and Tsukamura 1981, 274[VP]

o.bu.en′se. N.L. neut. adj. *obuense* of or belonging to Obu, Japan.

Rods (2–6 µm), acid-fast in young cultures, but may lose some acid-fastness on prolonged culture. Dilute inocula on inspissated egg media yield smooth yellow-orange colonies in 5 d or less. The temperature range for growth is 25–27°C; does not grow at 45°C. TLC of methanolysates shows α- and keto-mycolates, and wax esters. Differential features of the species are shown in Table 38.

Source: isolated from soil and from the sputum of one patient; not thought to be associated with disease.
DNA G+C content (mol%): not determined.
Type strain: ATCC 27023, CCUG 37669, CIP 106803, DSM 44075, JCM 6372, NCTC 10778.
Sequence accession no. (16S rRNA gene): X55597.
Additional remarks: this species was originally described by Tsukamura and Mizuno (1971), was not cited in the *Approved Lists of Bacterial Names* (Skerman et al., 1980) and was revived by Tsukamura et al. (1981).

The *Mycobacterium fortuitum* clade

95. **Mycobacterium fortuitum** da Costa Cruz 1938, 299[AL]

95a. **Mycobacterium fortuitum subsp. fortuitum** da Costa Cruz 1938, 299[AL]

for.tui′tum. L. neut. adj. *fortuitum* casual, accidental.

Acid-fast rods, 1–3 µm long; coccoid and short forms and some longer rods are seen, as are occasional beaded or swollen cells with non-acid-fast ovoid bodies at one end. Long filamentous branching forms are seen in pus. Acid-fastness varies from 10 to 100% of cells after incubation for 5 d at 28°C. Dilute inocula on Löwenstein–Jensen medium yield smooth, hemispheric colonies after incubation for 2–4 d. Colonies may be butyrous, waxy, multilobate, or even rosette-clustered; dull, waxy, rough colonies are also common. Colonies are usually off-white- or cream-colored, but when grown on malachite green-containing media, they may absorb the

green dye (Hartwig et al., 1962). Temperature range for growth is 22–42°C; does not grow at 45°C. Organisms grow at 28°C on MacConkey agar (without crystal violet) and in the presence of 5% (w/v) NaCl. TLC of methanolysates shows α-, α′-, and epoxy-mycolates. Differential characteristics of the rapidly growing species are shown in Table 38.

Generalized disease is rarely seen in most experimental animals, although localized kidney lesions are common in calves, guinea pigs, mice, monkeys, and rabbits; middle ear lesions lead to a characteristic "spinning disease" in mice (Penso et al., 1952; Wells et al., 1955). Some strains have been isolated from the lymph glands of cattle and systemic, nodular infections of frogs.

Source: strains were originally recovered from an injection site abscess and have been isolated from augmentation mammaplasties, endocarditis, local abscesses, meningitis, osteomyelitis, post-operative sternal wound infections, and pulmonary disease in humans. Also found in soil.

DNA G+C content (mol%): not determined.

Type strain: ATCC 6841, CCUG 20994, CIP 104534, DSM 46621, JCM 6387, NCTC 10394.

Sequence accession no. (16S rRNA gene): X52933.

Additional remarks: the valid publication of *Mycobacterium fortuitum* subsp. *acetamidolyticum* (Tsukamura et al., 1986c) automatically created *Mycobacterium fortuitum* subsp. *fortuitum* as a subspecies novum under Rule 40b of the Bacteriological Code (Tindall, 1999). Stanford and Gunthorpe (1969) found *Mycobacterium fortuitum* to be identical to Kuster's frog tubercle bacillus which was raised to species status as *Mycobacterium ranae* by Bergey et al. (1923) and proposed that by priority, *Mycobacterium ranae* should be the official name of the taxon. Runyon (1972) challenged the former name as a *nomen ambiguum* and, for stability in taxonomy, requested conservation of the epithet *fortuitum*, a request acceded to by the Judicial Commission of the International Committee on Systematic Bacteriology (1974).

Three subgroups or biovars of *Mycobacterium fortuitum* were described by Bönicke (1966) and further studied by Wallace et al. (1991). Subsequently, two biovars were assigned to distinct species status as *Mycobacterium fortuitum* and *Mycobacterium peregrinum*; the third biovariant complex, containing two unnamed taxa (sorbitol-positive and sorbitol-negative) remained unnamed (Wallace et al., 1991). The third biovar was subsequently found to contain significant heterogeneity and was the subject of polyphasic studies which resulted in proposals for four novel species, namely *Mycobacterium boenickei*, *Mycobacterium brisbanense*, *Mycobacterium houstonense*, and *Mycobacterium neworleansense* (see below; Schinsky et al., 2004).

95b. **Mycobacterium fortuitum subsp. acetamidolyticum** Tsukamura, Yano and Imaeda 1986a, 489[VP] (Effective publication: Tsukamura, Yano and Imaeda 1986c, 108.)

a.ce.ta.mi.do.ly′ti.cum. N.L. n. *acetamidum* acetamide; N.L. neut. adj. *lyticum* (from Gr. neut. adj. *lutikon*) able to loosen, able to dissolve; N.L. neut. adj. *acetamidolyticum* digesting acetamide.

Acid-fast rods of a short or intermediate length (2–6 × 0.5–1 μm), which do not form cords. Colonies are rough, non-photochromogenic, and white in color after incubation at 37°C for 5 d on Löwenstein–Jensen or Ogawa egg media. The temperature range for growth is 22–42°C; does not grow at 45°C. Strains do not grow on MacConkey agar and do not tolerate 5% (w/v) NaCl. TLC of methanolysates shows α-, α′-, and epoxy-mycolates. Differential characteristics of the rapidly growing species are shown in Table 38.

Source: isolated from the sputum of a human patient; probably a lung pathogen.

DNA G+C content (mol%): not determined.

Type strain: ATCC 35931, CIP 105423, DSM 44220, JCM 6368.

Sequence accession no. (16S rRNA gene): AF547923.

96. **Mycobacterium rhodesiae** Tsukamura, Mizuno and Tsukamura 1981, 274[VP]

rho.de.si′a.e. N.L. gen. n. *rhodesiae* of Rhodesia.

Very short rods (<2 μm). May lose acid-fastness on prolonged culture. Dilute inocula on egg and agar media yield brilliant yellow, smooth, moist colonies after incubation for less than 5 d. The temperature range for growth is 25–37°C; does not grow at 45°C. TLC of methanolysates shows α- and keto-mycolates, and wax esters. Differential characteristics of the rapidly growing species are shown in Table 38.

No demonstrable virulence for chickens, guinea pigs, mice, or rabbits.

Source: isolated from sputum samples from Rhodesian patients suspected to have tuberculosis. However, no positive evidence has emerged that the species is pathogenic for humans.

DNA G+C content (mol%): not determined.

Type strain: ATCC 27024, CIP 106806, DSM 44223, JCM 6363, NCTC 10779.

Sequence accession no. (16S rRNA gene): AJ429047.

Additional remarks: originally described by Tsukamura et al. (1971), this species was not cited in the *Approved Lists of Bacterial Names* (Skerman et al., 1980), but was revived by Tsukamura et al. (1981).

97. **Mycobacterium houstonense** Schinsky, Morey, Steigerwalt, Douglas, Wilson, Floyd, Butler, Daneshvar, Brown-Elliot, Wallace, McNeil, Brenner and Brown 2004, 1664[VP]

hous.ton.en′se. N.L. neut. adj. *houstonense* of or pertaining to Houston, Texas, USA, where the first isolate of the *Mycobacterium fortuitum* third biovariant (sorbitol-positive) was identified.

Pleomorphic acid-fast bacilli, often with long filamentous forms. Colonies on heart infusion agar supplemented with 5% (v/v) rabbit blood are white to slightly beige, mucoid, convex, round, and entire-edged. The temperature range for growth is 28–42°C. Growth occurs on Löwenstein–Jensen medium at 35 and 42°C after incubation for less than 7 d, but is microscopically visible as small (approx. 1 mm diameter) colonies after incubation for 2 d at 35°C. Growth occurs on MacConkey agar (without crystal violet) and 5% (w/v) NaCl agar at 28°C. Differential characteristics of the rapidly growing species are shown in Table 38.

Source: the type strain was isolated from a face wound.

DNA G+C content (mol%): 64 (T_m).

Type strain: ATCC 49403, DSM 44676.

Sequence accession no. (16S rRNA gene): AY012579.

Additional remarks: see remarks following the description of *Mycobacterium boenickei* and *Mycobacterium fortuitum*.

98. **Mycobacterium conceptionense** Adékambi, Stein, Carvajal, Raoult and Drancourt 2006b, 2025[VP] (Effective publication: Adékambi, Stein, Carvajal, Raoult and Drancourt 2006c, 1272.)

con.cep.tio.nen′se. N.L. neut. adj. *conceptionense* of or pertaining to Hôpital la Conception, the hospital where the first strain was isolated.

Gram-stain-positive, acid-fast bacilli. Colonies are nonpigmented and appear on 5% sheep blood agar, Middlebrook 7H10 agar, and Löwenstein–Jensen slants after incubation for 2–5 d. The temperature range for growth is 25–37°C; optimal growth is at 30°C; does not grow at 42°C. Tolerant of 5% (w/v) NaCl. Differential characteristics of the rapidly growing species are shown in Table 38.

Source: the species was isolated from tissue associated with post-traumatic osteitis, subsequent to an open tibia fracture in a 31-year-old woman who had endured an extended period in river water following an accident.

DNA G+C content (mol%): 64 ± 2 (HPLC).

Type strain: CIP 108544, CCUG 50187.

Sequence accession no. (16S rRNA gene): AY859684.

Additional remarks: in 16S rRNA gene sequencing studies, *Mycobacterium conceptionense* shows 99.7% similarity (4 bp difference) to *Mycobacterium porcinum* and 99.4% similarity (8 bp difference) to *Mycobacterium fortuitum*. The species has a similarities of 96.4% to *Mycobacterium porcinum* (15 bp difference) and 98.3% (7 bp difference) to *Mycobacterium fortuitum* based on *hsp65* gene sequences; similarities of 96.0 and 94.8%, respectively, based on *sodA* gene sequences and 95 and 94.8%, respectively, based on *recA* gene sequences. Sequencing of a 723 bp region of the *rpoB* gene shows *Mycobacterium conceptionense* to have 95.7% similarity to *Mycobacterium fortuitum* and 97% similarity to *Mycobacterium porcinum*.

99. **Mycobacterium senegalense** (Chamoiseau 1973) Chamoiseau 1979, 407[AL] (*Mycobacterium farcinogenes* subsp. *senegalense* Chamoiseau 1973, 220)

se.ne.gal.en′se. N.L. neut. adj. *senegalense* of or belonging to the West African Republic of Senegal.

Gram-stain-positive, strongly acid–alcohol-fast cells which form short or long, bent, and branched filaments. The latter are found in clumps or in a tangled lacy network and do not fragment into bacillary forms. This appearance does not change whether the organisms are seen in pus from lesions or smears from cultures. Colonies on Löwenstein–Jensen medium are non-chromogenic or ochre in color, rough and convoluted, firmly attached to the medium, and surrounded by an iridescent halo. In broth medium (tryptose broth with serum), a thick, rough, dry growth develops with a whitish surface veil, but the broth remains clear. Growth occurs in 1–2 d in liquid or on solid medium. TLC of methanolysates shows α-, α′-, and epoxy-mycolates. Differential characteristics of the rapidly growing species are shown in Table 38.

Source: isolated from lesions of farcy in African bovines. Strains produce a massive, generalized peritonitis in guinea pigs.

DNA G+C content (mol%): not determined.

Type strain: ATCC 35796, CCUG 21001, CIP 104941, DSM 43656, NCTC 10956.

Sequence accession no. (16S rRNA gene): M29567.

Additional remarks: originally described as a subspecies of *Mycobacterium farcinogenes* Chamoiseau 1973 but was later recognized to be a totally different species on the basis of marked differences in growth rate, metabolic activities, lipid composition, and DNA homology (Baess, 1982; Chamoiseau, 1979). *Mycobacterium senegalense* is one of two acid-fast causative agents of farcy, a disease of the skin and superficial lymphatics in African bovines, the other being *Mycobacterum farcinogenes*. See also comments under the description of *Mycobacterium farcinogenes*.

100. **Mycobacterium farcinogenes** (Chamoiseau 1973) Chamoiseau 1979, 407[AL] (*Mycobacterium farcinogenes* subsp. *tchadense* Chamoiseau 1973, 220)

far.ci.no′ge.nes. Fr. n. *farcin* farcy or glanders; Gr. v. *gennaio* produce; N.L. adj. *farcinogenes* producing farcy.

Gram-stain-positive, strongly acid–alcohol-fast cells which form short or long, bent, and branched filaments. The latter are in clumps or in a tangled lacy network and do not fragment into bacillary forms. This appearance is shown in pus from lesions or smears from cultures. Colonies on Löwenstein–Jensen medium are honey yellow, rough and convoluted, firmly attached to the medium, and surrounded by an iridescent halo. In broth medium such as tryptose broth with serum, a thick rough dry growth develops with a whitish surface veil, but the broth remains clear. Growth occurs in 15–20 d in liquid or on solid medium. TLC of methanolysates shows α-, α′-, and epoxy-mycolates. Differential characteristics of the rapidly growing species are shown in Table 38.

Subcutaneous inoculation of the organism into guinea pigs produces draining abscesses after 8 d, which heal slowly. Six to 7 d after intraperitoneal injection of guinea pigs, abscesses are seen in the testes, seminal vesicles, or vagina; large abscesses are seen rarely in peritoneal walls and viscera; most animals die after prolonged infection.

Source: isolated from lesions of farcy in African bovines.

DNA G+C content (mol%): not determined.

Type strain: NCTC 10955, ATCC 35753, CCUG 21047, DSM 43637.

Sequence accession no. (16S rRNA gene): AF055333.

Additional remarks: a number of organisms isolated from lesions of bovine farcy were gathered into a group and designated *Nocardia farcinica*. Chamoiseau (1973) established that the agents of farcy in African bovines belonged in the genus *Mycobacterium*. At that time, he proposed the name *Mycobacterium farcinogenes*, subdividing the species into two subspecies, subsp. *tchadense* and subsp. *senegalense*. Subsequently, on the basis of marked differences in growth rate, metabolic activities, lipid composition, and DNA homology (Baess, 1982), he concluded that these taxa represented two distinct species, *Mycobacterium farcinogenes* and *Mycobacterium senegalense* (Chamoiseau, 1979). These species show a close relationship in 16S rRNA gene sequencing studies and differ from all other known mycobacteria in their occurrence in typical lesions in zebu cattle. See also the description of *Mycobacterium senegalense*.

101. **Mycobacterium septicum** Schinsky, McNeil, Witney, Steigerwait, Lasker, Floyd, Hogg, Brenner and Brown 2000, 580[VP]

sep′ti.cum. L. neut. adj. *septicum* producing a putrefaction, putrefying, septic, referring to the isolation of the organism from blood.

Gram-stain-positive, acid-fast, asporogenous, non-capsulated, pleomorphic coccobacilli. Longer filamentous forms are often observed, but true branching and aerial hyphae do not occur. Growth on Löwenstein–Jensen medium at 35°C produces cerebriform, slightly beige colonies with an irregular edge. Organisms grow at 28°C on MacConkey agar (without crystal violet) and on 5% (w/v) NaCl agar, but do not grow at 42 or 45°C. Differential characteristics of the rapidly growing species are shown in Table 38.

Source: isolated from a catheter tip and from the blood of a 2-year-old child with metastatic hepatoblastoma.

DNA G+C content (mol%): 64 (T_m).

Type strain: ATCC 700731, CCUG 43574, CIP 106642, DSM 44393.

Sequence accession no. (16S rRNA gene): AF111809.

102. **Mycobacterium alvei** Ausina, Luquin, García Barceló, Lanéelle, Lévy-Frébault, Belda and Prats 1992, 531[VP]

al′ve.i. L. gen. n. *alvei* of the bed of a river, referring to the place where this species was first isolated.

Short rods (1–3 × 0.5–0.7 µm), which frequently appear to be coccoid; strongly acid-fast, except for a small number (less than 10%) of cyanophil forms. Clumping of cells may occur, but there is no cord formation. Colonies on Löwenstein–Jensen medium and on Middlebrook 7H10 agar are eugonic, rough, and non-pigmented. The temperature range for growth is 25–37°C, with optimum growth at 30°C; does not grow at 45°C. Growth occurs after incubation for 5 d at 30°C, but at 37°C the organisms grow more slowly (10–15 d). Does not grow on MacConkey agar (without crystal violet) or on 5% (w/v) NaCl agar. TLC of methanolysates shows α- and ω-1 methoxy-mycolates. Differential characteristics of the rapidly growing species are shown in Table 38.

Source: the species description was based on six strains, three of which were isolated from water samples from the River Llobregat in Barcelona, two from human sputum samples, and the remaining one from soil.

DNA G+C content (mol%): not determined.

Type strain: CIP 103464, ATCC 51304, DSM 44176, JCM 12272.

Sequence accession no. (16S rRNA gene): AF023664.

103. **Mycobacterium porcinum** Tsukamura, Nemoto and Yugi 1983b, 164[VP]

por′ci.num. L. neut. adj. *porcinum* pertaining to swine.

Acid-fast rods (1.5–6 × 0.5 µm) with no cross-barring, cord formation, or mycelium production. Growth occurs after 3 d on egg-based media. Colonies are non-photochromogenic and on egg media are smooth to rough, but on agar media are dry and rough. The temperature range for growth is 28–42°C; does not grow at 45°C. The species is phenotypically similar to *Mycobacterium fortuitum* but lacks nitrate reductase, exhibits a positive succinamidase activity, and utilizes benzoate as a sole source of carbon. TLC of methanolysates shows α-, α′-, and epoxy-mycolates. Differential characteristics of the rapidly growing species are shown in Table 38.

Source: ten strains were recovered from the lymph nodes of swine having a tuberculosis-like lymphadenitis.

DNA G+C content (mol%): not determined.

Type strain: ATCC 33776, CCUG 37674, CIP 105392, DSM 44242, JCM 6378.

Sequence accession no. (16S rRNA gene): AF480588.

104. **Mycobacterium boenickei** Schinsky, Morey, Steigerwalt, Douglas, Wilson, Floyd, Butler, Daneshvar, Brown-Elliot, Wallace, McNeil, Brenner and Brown 2004, 1664[VP]

bo.e.nic′ke.i. N.L. gen. masc. n. *boenickei* of Bönicke, in honor of the contribution of Rudolf Bönicke, a German microbiologist, who first recognized the heterogeneity within the *Mycobacterium fortuitum* complex.

Acid-fast pleomorphic bacilli, often with long filamentous forms. On heat infusion agar with 5% (v/v) rabbit blood, colonies are matt, white to slightly beige, domed, and scallop-edged. Aerial hyphae are not present. Small colonies (approx. 1 mm diameter) are visible microscopically after 2 d incubation at 35°C. Mature colonies on Löwenstein–Jensen medium at 35°C are visible in less than 7 d incubation. The temperature range for growth is 28–35°C; does not grow at 42°C. Growth occurs on MacConkey agar (without crystal violet) and 5% (w/v) NaCl agar at 28°C. Differential characteristics of the rapidly growing species are shown in Table 38.

Source: isolated from a leg wound.

DNA G+C content (mol%): 64 (T_m).

Type strain: ATCC 49935, DSM 44677.

Sequence accession no. (16S rRNA gene): AY012573.

Additional remarks: the unnamed third *Mycobacterium fortuitum* biovariant complex, containing two unnamed taxa (sorbitol-positive and sorbitol-negative), was first described by Wallace et al. (1991). Members of this biovariant complex frequently occur as human pathogens which cause skin and soft tissue abscesses with associated systemic involvement (Wallace et al., 1991; Wallace et al., 1983). Schinsky et al. (2004) carried out detailed genotypic and phenotypic analyses of clinical isolates formerly classified as the *Mycobacterium fortuitum* third biovariant complex, demonstrated significant heterogeneity, and proposed four novel species, namely *Mycobacterium boenickei*, *Mycobacterium brisbanense*, *Mycobacterium houstonense*, and *Mycobacterium neworleansense*.

105. **Mycobacterium neworleansense** Schinsky, Morey, Steigerwalt, Douglas, Wilson, Floyd, Butler, Daneshvar, Brown-Elliot, Wallace, McNeil, Brenner and Brown 2004, 1665[VP]

new.or.le.an.sen′se. N.L. neut. adj. *neworleansense* of or pertaining to New Orleans, Louisiana, USA, the source of the type strain.

Acid-fast pleomorphic bacilli, often with long filamentous forms. Colonies are white to slightly beige, rough, wrinkled, and irregular-edged on heart infusion agar supplemented with 5% (v/v) rabbit blood. The temperature range for

growth is 28–37°C; growth does not occur at 42°C. Growth occurs on Löwenstein–Jensen medium at 35°C after incubation for less than 7 d and is visible as small (approx. 1 mm diameter) colonies after incubation at 35°C for 2 d; it also grows on MacConkey agar (without crystal violet) and 5% (w/v) NaCl agar at 28°C. Differential characteristics of the rapidly growing species are shown in Table 38.

Source: this species was described on the basis of a single strain isolated from a scalp wound.

DNA G+C content (mol%): 60 (T_m).

Type strain: ATCC 49404, DSM 44679.

Sequence accession no. (16S rRNA gene): AY012575.

Additional remarks: see comments following the descriptions of *Mycobacterium boenickei* and *Mycobacterium fortuitum.*

The *Mycobacterium neoaurum* clade

106. **Mycobacterium neoaurum** Tsukamura 1972a, 229[AL]

ne.o.au′rum. Gr. adj. *neos* new; L. n. *aurum* gold; N.L. n. *neoaurum* a new gold, intended to mean a new gold-pigmented organism.

Intermediate to long rods. Golden yellow colonies are visible after incubation for less than 5 d on inspissated egg media. The temperature range for growth is 25–37°C; does not grow at 45°C. TLC of methanolysates shows α- and keto-mycolates, and wax esters. Differential characteristics of the rapidly growing species are shown in Table 38.

Source: isolated from soil and not known to be related to human disease.

DNA G+C content (mol%): not determined.

Type strain: ATCC 25795, CCUG 37665, CIP 105387, DSM 44074, JCM 6365, NCTC 10818.

Sequence accession no. (16S rRNA gene): M29564.

Additional remarks: an original description in Japanese (Tsukamura, 1972a) was followed by a more detailed description in English (Tsukamura et al., 1981).

107. **Mycobacterium brisbanense** Schinsky, Morey, Steigerwalt, Douglas, Wilson, Floyd, Butler, Daneshvar, Brown-Elliot, Wallace, McNeil, Brenner and Brown 2004, 1665[VP]

bris.ban.en′se. N.L. neut. adj. *brisbanense* of or pertaining to Brisbane, Queensland, Australia, the source of the type strain.

Acid-fast, pleomorphic bacilli, often with long filamentous forms. Colonies are white to slightly beige, mucoid, convex, round, and entire-edged on heart infusion agar supplemented with 5% (v/v) rabbit blood; growth is visible as small (approx. 1 mm diameter) colonies after incubation for 2 d at 35°C. Growth occurs on MacConkey agar (without crystal violet), on 5% (w/v) NaCl agar at 28°C, and on Löwenstein–Jensen medium at 35°C after incubation for less than 7 d. The temperature range for growth is 28–37°C; does not grow at 42°C. Differential characteristics of the rapidly growing species are shown in Table 38.

Source: the species was described on the basis of a single strain isolated from an antral sinus.

DNA G+C content (mol%): 62 (T_m).

Type strain: ATCC 49938, CCUG 47584, DSM 44680.

Sequence accession no. (16S rRNA gene): AY012577.

Additional remarks: see comments following the descriptions of *Mycobacterium boenickei* and *Mycobacterium fortuitum.*

108. **Mycobacterium frederiksbergense** Willumsen, Karlson, Stackebrandt and Kroppenstedt 2001, 1719[VP]

fre.de.riks.ber.gen′se. N.L. neut. adj. *frederiksbergense* of or belonging to Frederiksberg, Denmark, referring to the place of isolation.

Gram-stain-positive, acid-fast, nonmotile, short rods; coccoid elements occur. Colonies are smooth, cadmium yellow, and scotochromogenic after incubation for 5 d on trypticase soy broth and Middlebrook 7H10 agar. The temperature range for growth is 15–37°C; optimal growth occurs at 30°C. Does not grow on MacConkey agar. Degrades polycyclic aromatic hydrocarbons. TLC of methanolysates shows α- and keto-mycolates, and wax esters. Differential characteristics of the rapidly growing species are shown in Table 38.

Source: isolated from the site of a former gas works at Frederiksberg, Denmark.

DNA G+C content (mol%): not determined

Type strain: DSM 44346, CIP 107205.

Sequence accession no. (16S rRNA gene): AJ276274.

109. **Mycobacterium fluoranthenivorans** Hormisch, Brost, Kohring, Giffhorn, Kroppenstedt, Stackebrandt, Farber and Holzapfel 2006, 1459[VP] (Effective publication: Hormisch, Brost, Kohring, Giffhorn, Kroppenstedt, Stackebrandt, Farber and Holzapfel 2004, 659.)

flu.o.ran.the.ni.vo′rans. N.L. n. *fluoranthenum* fluoranthene; L. part. adj. *vorans* devouring, digesting; N.L. part. adj. *fluoranthenivorans* fluoranthene devouring, a property of the organism.

Gram-stain-positive, acid–alcohol-fast, nonmotile rods (2 × 1 μm), which are non-chromogenic. Grows rapidly within a temperature range of 20–37°C, but does not grow at 42°C. Metabolizes fluoranthene but not other polycyclic aromatic hydrocarbons. TLC of methanolysates shows α-, α′-, and epoxy-mycolates. Differential characteristics of the rapidly growing species are shown in Table 38.

Source: isolated from polycyclic aromatic hydrocarbon-polluted soil from a former coal gas plant, using a complex liquid medium.

DNA G+C content (mol%): not determined.

Type strain: DSM 44556, CIP 108203.

Sequence accession no. (16S rRNA gene): AJ617741.

110. **Mycobacterium diernhoferi** Tsukamura, van der Meulen and Grabow 1983c, 468[VP]

di.ern.ho′fer.i. N.L. gen. masc. n. *diernhoferi* of Diernhofer, who originally isolated the organisms.

Acid-fast rods (2–6 × 0.5 μm). Colonies are smooth, white, and nonphotochromogenic after incubation for 3 d at 28 and 37°C. Does not grow at 42°C. Susceptible to $NH_2OH.HCl$ (250 μg/ml) and ethambutol (5 μg/ml), but is resistant to isoniazid (10 μg/ml), rifampin (25 μg/ml), and thiophene-2-carboxylic acid hydrazide (1 μg/ml). Reduces nitrate but is negative in the 3 d arylsulfatase test. Grows in media supplemented with 5% (w/v) NaCl.

TLC of methanolysates shows α- and keto-mycolates, and wax esters. Differential characteristics of the rapidly growing species are shown in Table 38.

Source: strains were recovered from soil in a cattle field and are not known to be associated with human disease.

DNA G+C content (mol%): not determined.

Type strain: ATCC 19340, CIP 105384, DSM 43524, JCM 6371.

Sequence accession no. (16S rRNA gene): X55593.

Additional remarks: the species was originally described by Bönicke and Juhasz (1965), was not cited in the *Approved Lists of Bacterial Names* (Skerman et al., 1980), but was revived by Tsukamura et al. (1983c).

111. **Mycobacterium canariasense** Jiménez, Campos-Herrero, García, Luquin, Herrera and García 2004, 1733[VP]

ca.na.ri.a.sen′se. N.L. neut. adj. *canariasense* of or belonging to the Canarias (the Spanish name of the Canary Islands), where all strains were isolated.

Partially acid-fast rods. Colonies are smooth, moist, shiny, and non-pigmented after incubation for 2–3 d on Löwenstein–Jensen medium. The temperature range for growth is 30–37°C; does not grow at 22, 42, or 45°C. Growth occurs on MacConkey agar (without crystal violet), but not on agar supplemented with 5% (w/v) NaCl.

Strains are positive for arylsulfatase activity (3 d) and Tween 80 hydrolysis, produce a low level of heat-stable catalase, and are negative for nitrate reduction. TLC of methanolysates shows α- and α′-mycolates. Differential characteristics of the rapidly growing species are shown in Table 38.

Source: isolated from blood samples of patients suffering from a febrile illness considered to be nosocomially acquired, possibly as a result of venous catheterization.

DNA G+C content (mol%): not determined.

Type strain: CCUG 47953, CIP 107998.

Sequence accession nos: AY255478 (16S rRNA gene); AY255477 (*hsp65*).

Additional remarks: on the basis of the presence of two operons in the genome and a short helix 18, this species belongs to the II-s mycobacterial class proposed by Menendez et al. (2002).

112. **Mycobacterium cosmeticum** Cooksey, de Waard, Yakrus, Rivera, Chopite, Toney, Morlock and Butler 2004, 2390[VP]

cos.me′ti.cum. N.L. neut. adj. *cosmeticum* referring to cosmetics.

Acid-fast rods (approx. 1.5 × 0.55 μm) which rarely form cell aggregates in liquid culture. Colonies have smooth surfaces and edges, and are domed and scotochromogenic, with a pale yellow coloration on Löwenstein–Jensen medium and on Middlebrook 7H10 agar. Growth from dilute inocula appears after 3 d incubation on Löwenstein–Jensen medium at 28–37°C; does not grow at 45°C. Grows on MacConkey agar (without crystal violet), but not on media supplemented with 5% (w/v) NaCl. Differential characteristics of the rapidly growing species are shown in Table 38.

Source: the type strain was isolated from a granulomatous lesion of a female patient in Venezuela who was undergoing mesotherapy. Three further strains were isolated from footbath drains and a sink at a nail salon located in Atlanta, Georgia, USA (Gira et al., 2004).

DNA G+C content (mol%): not determined.

Type strain: ATCC BAA-878, CIP 108170.

Sequence accession nos: AY449728 (16S rRNA gene); AY449730 (*hsp65*).

Additional remarks: the association of rapidly growing mycobacteria with furunculosis resulting from cosmetic nail treatments has been repeatedly reported; see also the description of *Mycobacterium mageritense* and Withrop et al. (2002).

The *Mycobacterium mucogenicum* clade

113. **Mycobacterium mucogenicum** Springer, Böttger, Kirschner and Wallace 1995a, 266[VP]

mu.co.gen′i.cum. L. n. *mucus* mucus, Gr. v. *gennaio* to produce; L. neut. suff. *-icum* suffix used with the sense of pertaining to; N.L. neut. adj. *mucogenicum* intended to mean producing mucus, referring to the highly mucoid character of most strains on solid agar.

Gram-stain-positive, acid–alcohol-fast, asporogenous, curved bacilli. Visible growth appears on Middlebrook 7H10 and trypticase soy agars in 2–4 d. Colonies are usually smooth, off-white in color, mucoid, and non-pigmented. Growth occurs (although poorly at times) on MacConkey agar (without crystal violet), but not in the presence of 5% (w/v) NaCl. The temperature range for growth is 28–37°C; does not grow at 42°C. Differential characteristics of the rapidly growing species are shown in Table 38.

Source: originally recovered from outbreaks of peritonitis associated with automated peritoneal dialysis machines and the tap water used to supply the machines. Subsequently, isolates of this species have commonly been involved in water-borne nosocomial outbreaks. *Mycobacterium mucogenicum* has been associated with some incidents of post-traumatic wound infection and catheter-related sepsis (Wallace et al., 1993). The type strain was recovered from a human neck abscess.

DNA G+C content (mol%): not determined.

Type strain: ATCC 49650, CCUG 47451, CIP 105223, DSM 44625, JCM 13575.

Sequence accession no. (16S rRNA gene): AY457074.

Additional remarks: since many characteristics of the isolates were similar to those of *Mycobacterium chelonae*, isolates of *Mycobacterium mucogenicum* were tentatively designated *Mycobacterium chelonae*-like organisms (MCLO) by Silcox et al. (1981).

114. **Mycobacterium aubagnense** Adékambi, Berger, Raoult and Drancourt 2006a, 140[VP]

au.bag.nen′se. N.L. neut. adj. *aubagnense* of or pertaining to Aubagne, the city from which the first patient originated.

Gram-stain-positive, acid-fast bacilli. Colonies are non-pigmented and appear on 5% sheep blood and Middlebrook 7H10 agars and on Löwenstein–Jensen medium after incubation for 2–5 d. The temperature range for growth is 24–37°C; optimal growth occurs at 30°C; does

not grow at 42°C. Does not grow on media supplemented with 5% (w/v) NaCl. Differential characteristics of the rapidly growing species are shown in Table 38.

Source: isolated from a bronchial aspirate of a patient with chronic pneumonia.

DNA G+C content (mol%): 63–65 (HPLC).

Type strain: CIP 108543, CCUG 50186.

Sequence accession nos: AY859683 (16S rRNA gene); AY859689 (*recA*); AY859677 (*hsp65*); AY859707 (*sodA*).

Additional remarks: in 16S rRNA and *rpoB* gene sequence studies, this species shows similarities of 99.1% and 92.7%, respectively to *Mycobacterium mucogenicum*. Other genetic regions (*hsp65*, *sodA*, and *recA*) show considerable diversity within the same species. The taxon is described as a distinct species on the basis of *rpoB* gene sequence variation. See also descriptions of *Mycobacterium bolletii* and *Mycobacterium phocaicum*.

115. **Mycobacterium phocaicum** Adékambi, Berger, Raoult and Drancourt 2006a, 140[VP]

pho.ca′i.cum. L. neut. adj. *phocaicum* Phocoean, referring to Phocaea, a maritime town of Ionia, a colony of the Athenians, whose inhabitants fled to escape from Persian domination and founded Massilia (Marseille), which was the source of the type strain.

Gram-stain-positive acid-fast bacilli. Colonies are nonpigmented and appear on 5% (v/v) sheep blood and Middlebrook 7H10 agars, and Löwenstein–Jensen medium after incubation for 2–5 d. The temperature range for growth 24–37°C; optimal growth occurs at 30°C; does not grow at 42°C. Does not grow in media supplemented with 5% (w/v) NaCl. Differential characteristics of the rapidly growing species are shown in Table 38.

Source: the type strain was isolated from a bronchial aspirate; two other strains have been isolated from patients with chronic pneumonia.

DNA G+C content (mol%): not determined.

Type strain: CIP 108542, CCUG 50185.

Sequence accession nos: AY859682 (16S rRNA gene); AY859688 (*recA*); AY859676 (*hsp65*); AY859706 (*sodA gene*).

Additional remarks: this species shows 100% 16S rRNA gene and 95.0% *rpoB* gene sequence similarity to *Mycobacterium mucogenicum*. The taxon was described as a distinct species on the basis of *rpoB* gene sequence data. See also descriptions of *Mycobacterium aubagnense* and *Mycobacterium bolletii*.

The *Mycobacterium chelonae* clade

116. **Mycobacterium chelonae** corrig. Bergey, Harrison, Breed, Hammer and Huntoon 1923, 376[AL]

che.lo′na.e. Gr. fem. n. *chelone* a tortoise; N.L. gen. n. *chelonae* of a tortoise.

Cells are pleomorphic rods, ranging from long and narrow to short and thick (1–6 × 0.2–0.5 µm), with coccoid forms (0.5 µm) also reported. In cultures less than 5-d-old, organisms are strongly acid-fast; thereafter, non-acid-fast forms begin to develop. Colonies may be smooth, moist, and shiny or rough. They are usually nonchromogenic to creamy buff in color and are produced from dilute inocula after incubation for 3–4 d on most media. Though the temperature range for growth is 22–40°C, some strains of *Mycobacterium chelonae* may not grow (or may grow poorly) at temperatures of 37°C or higher; does not grow at 42°C. TLC of methanolysates shows α- and α′-mycolates. Differential characteristics of the rapidly growing species are shown in Table 38.

Source: although rarely isolated from sputum, this species has caused cervical adenitis, corneal infections, prosthetic valve endocarditis, and wound infections (including post-operative infections). It is also found in soil.

DNA G+C content (mol%): not determined.

Type strain: ATCC 35752, CCUG 47445, CIP 104535, DSM 43804, JCM 6388, NCTC 946.

Sequence accession no. (16S rRNA gene): AF480594.

Additional remarks: originally proposed as *Mycobacterium chelonei* for Friedmann's turtle tubercle bacillus, but it was noted that, given the gender of the binomial name for the turtle, the correct name for this organism is *Mycobacterium chelonae* (Hill et al., 1984; von Graevenitz and Berger, 1980). Several studies have confirmed the distinction of this species from closely related rapid-growing mycobacterial species (Jenkins et al., 1971; Stanford and Beck, 1969; Stanford et al., 1972), while others have suggested that this species is synonymous with *Mycobacterium abscessus* and "*Mycobacterium borstelense*" (Kubica et al., 1972; Stanford and Beck, 1969; Stanford et al., 1972). In contrast, cooperative numerical taxonomic studies showed *Mycobacterium abscessus* to differ in some features from *Mycobacterium chelonae* and "*Mycobacterium borstelense*" (Kubica et al., 1972; Saito et al., 1977). Kubica et al. (1972) proposed two subspecies, the taxonomic integrity of which was supported by subsequent studies (Silcox et al., 1981; Tsukamura, 1981). Baess (1982) reported that *Mycobacterium chelonae* subsp. *abscessus* ATCC 19977[T] and *Mycobacterium chelonae* subsp. *chelonae* ATCC 19235 were closely related, as the two strains showed 99% DNA homology. However, strain ATCC 19235 is not the type strain of *Mycobacterium chelonae* subsp. *chelonae* and a more comprehensive DNA–DNA relatedness study, which included the type strain of *Mycobacterium chelonae* subsp. *chelonae* ATCC 35752[T], was carried out by Lévy-Frébault et al. (1986b) who concluded that the two taxa should be viewed as independent species. Kusunoki and Ezaki (1992) formally proposed the re-establishment of *Mycobacterium abscessus* as a distinct species.

117. **Mycobacterium abscessus** Kusunoki and Ezaki 1992, 244[VP]

abs.ces′sus. L. gen. n. *abscessus* of an abscess, referring to the ability of the organism to form abscesses.

Acid-fast rods (1.0–2.5 × 0.5 µm). Colonies on egg medium appear in 7 d, are intermediate between smooth and rough, and are white to gray in color and non-photochromogenic. The temperature range for growth is 28–37°C; does not grow at 43°C. On MacConkey agar and on media containing 5% (w/v) NaCl, growth occurs at 28°C and at 37°C. TLC of methanolysates shows α- and α′-mycolates. Differential characteristics of the rapidly growing species are shown in Table 38.

Source: originally isolated from the synovium of the knee and associated gluteal abscesses of a patient. Causes wound and soft tissue infections, but is also found in soil.

DNA G+C content (mol%): 64.1 (T_m).

Type strain: ATCC 19977, CCUG 20993, CIP 104536, DSM 44196, JCM 13569, NCTC 13031.

Sequence accession no. (16S rRNA gene): X82235.

Additional remarks: see notes following description of *Mycobacterium chelonae*. This species was described originally by Moore and Frerichs (1953), but was not cited on the *Approved Lists of Bacterial Names* (Skerman et al., 1980).

118. **Mycobacterium immunogenum** Wilson, Steingrube, Böttger, Springer, Brown-Elliot, Vincent, Jost, Zhang, Garcia, Chiu, Onyi, Rossmoore, Nash and Wallace 2001, 1762[VP]

im.mu.no′gen.um. N.L. neut. adj. *immunogenum* eliciting an immune response.

Gram-stain-positive, acid–alcohol-fast, asporogenous, curved bacilli. Visible growth appears on Middlebrook 7H10 and trypticase soy agars in less than 7 d. Colonies are non-pigmented, off-white in color, and rough, although smooth forms may occur. Growth occurs on MacConkey agar (without crystal violet), but not in media supplemented with 5% (w/v) NaCl. The temperature range for growth is 30–35°C; optimal growth is at 30°C; does not grow at 45°C. Differential characteristics of the rapidly growing species are shown in Table 38.

Isolates of this species, along with strains of *Mycobacterium abscessus* and *Mycobacterium chelonae*, have commonly been involved in water-borne nosocomial outbreaks and pseudo-outbreaks. However, *Mycobacterium immunogenum* is clearly incriminated in hypersensitivity pneumonitis associated with metalworking fluid (Fraser et al., 1992; Kreiss and Cox-Ganser, 1997; Shelton et al., 1999). The species has also been identified as a cause of keratitis following laser *in situ* keratomileusis (Sampaio et al., 2006).

Source: the type strain was isolated from a sample of bronchoscope wash water.

DNA G+C content (mol%): not determined.

Type strain: ATCC 700505, CCUG 47286, CIP 106684.

Sequence accession no. (16S rRNA gene): AY457080.

Additional remarks: phenotypic separation of *Mycobacterium immunogenum* from *Mycobacterium abscessus* and *Mycobacterium chelonae* is difficult and identification of this species is primarily based on 16S rRNA gene sequence data. Additionally, *Mycobacterium immunogenum*, unlike *Mycobacterium abscessus* and *Mycobacterium chelonae*, does not contain a single rRNA operon.

119. **Mycobacterium bolletii** Adékambi, Berger, Raoult and Drancourt 2006a, 140[VP]

bol.let′i.i. N.L. gen. masc. n. *bolletii* of Bollet, to honor Claude Bollet, a famous clinical microbiologist and taxonomist.

Gram-stain-positive, acid-fast bacilli. Colonies are non-pigmented and appear on 5% (v/v) sheep blood and Middlebrook 7H10 agars, and on Löwenstein–Jensen medium after incubation for 2–5 d. The temperature range for growth is 24–37°C; optimal growth occurs at 30°C; does not grow at 42°C. Strains do not grow on media supplemented with 5% (w/v) NaCl. Differential characteristics of the rapidly growing species are shown in Table 38.

Source: isolated from sputum.

DNA G+C content (mol%): not determined.

Type strain: CIP 108541, CCUG 50184.

Sequence accession nos: AY859681 (16S rRNA gene); AY859687 (*recA*); AY859675 (*hsp65*); AY862403 (*sodA*).

Additional remarks: this species shows 100% 16S rRNA gene and 95.6% *rpoB* gene sequence similarity to *Mycobacterium abscessus*. The taxon is described as a distinct species on the basis of *rpoB* gene sequence data. See also descriptions for *Mycobacterium aubagnense* and *Mycobacterium phocaicum*.

120. **Mycobacterium massiliense** Adékambi, Reynaud-Gaubert, Greub, Gevaudan, La Scola, Raoult and Drancourt 2006b, 2025[VP]

mas.si.li.en′se. L. neut. adj. *massiliense* pertaining to *Massilia*, Latin name of Marseille, where the organism was isolated.

Gram-stain-positive, acid-fast, nonmotile, and asporogenous rods. Strictly aerobic. Colonies on 5% (v/v) sheep blood agar are non-photochromogenic and intermediate between smooth and rough. Growth occurs after incubation for 2–4 d on 5% (v/v) sheep blood and Middlebrook 7H10 agars, and on Löwenstein–Jensen medium. The temperature range for growth is 24–37°C; optimal growth occurs at 30°C; does not grow at 42°C. Growth occurs on MacConkey agar (without crystal violet) and on Löwenstein–Jensen medium supplemented with 5% (w/v) NaCl. Differential characteristics of the rapidly growing species are shown in Table 38.

Source: isolated from the sputum and bronchoalveolar lavage of a patient with hemoptoic pneumonia.

DNA G+C content (mol%): 65 ± 3 (HPLC).

Type strain: CIP 108297, CCUG 48898.

Sequence accession nos: AY593980 (16S rRNA gene); AY596465 (*hsp65*); AY593975 (*sodA*); AY593979 (*recA*); AY593981 (*rpoB*); AY593978 (ITS).

Additional remarks: this species was initially proposed by Adékambi et al. (2004) and the name was validly published in Validation List no. 111 (Adékambi et al., 2006b). The 16S rRNA gene sequence of *Mycobacterium massiliense* shows complete identity with that of *Mycobacterium abscessus* (1483 bp). The 441-bp region of the *hsp65* gene described by Telenti et al. (1993), and a partial 441-bp region of the *sodA* gene differed by 5 and 3 nucleotides, respectively, from the corresponding sequences of *Mycobacterium abscessus*. Partial *recA* and *rpoB* gene sequence analyses showed 98% and 96% similarity, respectively, to *Mycobacterium abscessus*. 16S–23S rRNA ITS sequence analysis showed that *Mycobacterium massiliense* differed from *Mycobacterium abscessus* by one substitution and a single insertion.

121. **Mycobacterium salmoniphilum** Whipps, Butler, Pourahmad, Watral and Kent 2007, 2529[VP]

sal.mo.ni′phi.lum. L. n. *salmo -onis* a salmon; N.L. adj. *philus -a -um* (from Gr. adj. *philos -ê -on*) friend, loving; N.L. neut. adj. *salmoniphilum* salmon-loving.

Acid-fast bacilli, slender, straight or slightly curved (1–4 × 0.25–0.6 µm), with occasional short and thicker forms. Colonies are cream-colored, smooth, and shiny after incubation for 4–6 d on Middlebrook 7H10 agar and on Löwenstein–Jensen slants. Growth also occurs on MacConkey and blood agars. After prolonged incubation (more than 10 d), colonies may be waxy, with an irregular border and a "fried egg" appearance. The temperature range for growth is 20–30°C, with weak or delayed growth at 10°C; does not grow at 37°C. Differential characteristics of the rapidly growing species are shown in Table 38.

Source: isolates were recovered from the viscera (predominantly the kidney) of infected salmonid fish.

DNA G+C content (mol%): not determined.

Type strain: ATCC 13758, DSM 43276.

Sequence accession nos: DQ866768 (16S rRNA gene); DQ866777 (*hsp*65); DQ866790 (*rpoB*).

Additional remarks: originally described by Ross (1960), this species was not cited in the *Approved Lists of Bacterial Names* (Skerman et al., 1980), but was revived by Whipps et al. (2007). Gene sequence analyses show a close relationship between *Mycobacterium salmoniphilum* and members of the *Mycobacterium chelonae* complex.

Single-membered 16S rRNA gene clades

122. **Mycobacterium hassiacum** Schröder, Naumann, Kroppenstedt and Reischl 1997, 90[VP]

has.si.a′cum. M.L. neut. adj. *hassiacum* of or belonging to *Hassia*, the German province of Hesse, where the organism was first isolated.

Partially acid-fast rods which are longer and thinner following growth at higher temperatures. Colonies are 2–5 mm in diameter, smooth, and scotochromogenic after incubation for 2–3 d on Löwenstein–Jensen medium. Growth occurs on MacConkey agar (without crystal violet) and on media supplemented with 5% (w/v) NaCl. In dense growth, colonies are slimy, but when grown at 40–65°C, they are distinct and drier. The temperature range for growth is 30–65°C; higher temperatures seem to favor growth. TLC of methanolysates shows α-mycolates, and wax esters. Differential characteristics of the rapidly growing species are shown in Table 38.

Source: isolated from urine on a single occasion; not thought to be pathogenic.

DNA G+C content (mol%): 65.8 (HPLC).

Type strain: DSM 44199, CCUG 37519, CIP 105218, JCM 12690.

Sequence accession no. (16S rRNA gene): U49401.

Additional remarks: Schröder et al. (1997) noted that while this organism displayed rapid growth and has the molecular signature of a rapidly growing species, it appeared to be phylogenetically nearer to the slow-growing species *Mycobacterium xenopi*. This conclusion is borne out by 16S rRNA gene sequence data (Figure 86).

123. **Mycobacterium madagascariense** Kazda, Müller, Stackebrandt, Daffé, Müller and Pitulle 1992, 526[VP]

mad.a.gas.car.i.en′se. N.L. neut. adj. *madagascariense* of or belonging to the island of Madagascar, the source of the strains.

Gram-stain-positive, acid–alcohol-fast, polymorphic rods (1.2–1.5 × 0.6 µm) which often form clumps, but not cords or cross bands. Colonies from dilute inocula on Löwenstein–Jensen medium and Middlebrook 7H10 agar are 1–2 mm in diameter, eugonic, smooth, and glistening with yellow or orange pigmentation. Growth occurs after 3 d at 31°C and after 7 d at 22°C; does not grow at 37°C. Does not grow on MacConkey agar or on media supplemented with 5% (w/v) NaCl. TLC of methanolysates shows α- and keto-mycolates, and wax esters. Differential characteristics of the rapidly growing species are shown in Table 38.

Strains are not pathogenic for guinea pigs, mice, or rabbits.

Source: isolated from sphagnum vegetation in Madagascar.

DNA G+C content (mol%): not determined.

Type strain: ATCC 49865, CIP 104538, JCM 13574.

Sequence accession no. (16S rRNA gene): X55600.

124. **Mycobacterium moriokaense** Tsukamura, Yano and Imaeda 1986b, 335[VP]

mo.ri.o.ka.en′se. N.L. neut. adj. *moriokaense* of or belonging to Morioka, the locality where the species was first isolated.

Acid-fast, asporogenous, nonmotile rods (2–6 × 0.5 µm), which do not form cords. Colonies on Löwenstein–Jensen medium and on Ogawa egg medium are dry, rough, and non-pigmented after incubation for 3 d at 28–42°C. Does not grow at 45°C. TLC of methanolysates shows α- and keto-mycolates, and wax esters. Differential characteristics of the rapidly growing species are shown in Table 38.

Source: isolated from the sputum of a patient with tuberculosis. However, this species is common in soil and is unlikely to be a human pathogen.

DNA G+C content (mol%): not determined.

Type strain: ATCC 43059, CCUG 37671, CIP 105393, DSM 44221, JCM 6375.

Sequence accession no. (16S rRNA gene): AJ429044.

125. **Mycobacterium gadium** Casal and Rey Calero 1974, 306[AL]

ga′di.um. L. gen. pl. n. *gadium*, of *Gades* (the modern Cadiz, a town on the Atlantic coast of Spain).

Gram-stain-positive, acid–alcohol-fast, short rods. Colonies on Löwenstein–Jensen medium are small, butyrous, and spherical after incubation for 3–4 d; yellow, scotochromogenic pigmentation increases slightly on exposure to light. Colonies become dry, rugged, and flat in older cultures. The temperature range for growth is 28–37°C; does not grow at 45°C. TLC of methanolysates shows α- and keto-mycolates, and wax esters. Differential characteristics of the rapidly growing species are shown in Table 38.

Intraperitoneal injection of large numbers of the organism into guinea pigs and mice produced local

lymphadenopathy in 2 weeks, with some dissemination into nearby organs, but this process resolved in 8 weeks. Not likely to be pathogenic for humans.

Source: isolated from the sputum of a patient in whom an *Mycobacterium tuberculosis* infection was later diagnosed.

DNA G+C content (mol%): not determined.

Type strain: ATCC 27726, CCUG 37515, CIP 105388, DSM 44077, JCM 12688, NCTC 10942.

Sequence accession no. (16S rRNA gene): X55594.

126. **Mycobacterium hodleri** Kleespies, Kroppenstedt, Rainey, Webb and Stackebrandt 1996, 686[VP]

hod'le. ri. N.L. gen. masc. n. *hodleri* of Hodler, referring to Christian Hodler, director of the Ministry of Science and Culture of the State of Lower Saxony, Germany, a strong supporter of natural sciences.

Gram-stain-positive, acid-fast, asporogenous, nonmotile rods (1.8–2.3 × 1 μm). Some cells appear as V-forms and in palisades. Colonies are smooth, but with some rough variants, and are saffron yellow and scotochromogenic after incubation for 4–5 d on trypticase soy broth and Middlebrook 7H10 agars. The temperature range for growth is 18–28°C; optimal growth is at 25°C. Degrades polycyclic aromatic hydrocarbons. TLC of methanolysates shows α- and keto-mycolates, and wax esters. Differential characteristics of the rapidly growing species are shown in Table 38.

Source: isolated from a fluoranthene-contaminated site near Jülich, Germany.

DNA G+C content (mol%): 73 (HPLC).

Type strain: DSM 44183, CIP 104909, JCM 12141.

Sequence accession no. (16S rRNA gene): X93184.

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Family IV. **Nocardiaceae** (Castellani and Chalmers 1919) emend. Zhi, Li and Stackebrandt 2009

MICHAEL GOODFELLOW

No.car.di.a.ce′a.e. N.L. fem. n. *Nocardia* type genus of the family, suff. *-aceae*, ending to denote a family; N.L. fem. pl. n. *Nocardiaceae* the *Nocardia* family.

Aerobic, Gram-stain-positive to Gram-stain-variable, nonmotile actinomycetes which are typically acid–alcohol- or partially acid–alcohol-fast at some stages of the growth cycle. Some strains form an extensively branched substrate mycelium which fragments into coccoid- and rod-shaped elements, others are coccoid or show a rod–coccus/rod–coccus-mycelial growth cycle. Some strains from aerial hyphae. **Chemo-organotrophs with an oxidative metabolism**. Whole-cell hydrolysates are rich in *meso*-diaminopimelic acid, arabinose and galactose. The peptidoglycan is of the A1γ type. **Muramic acid moieties are *N*-glycolated. Cells contain diphosphatidylglycerol, phosphatidylethanolamine, phosphatidylinositol and phosphatidylinositol mannosides as predominant polar lipids, major proportions of straight-chain saturated, unsaturated and 10-methyloctadecanoic (tuberculostearic) fatty acids though the latter may be found in smaller amounts, and cyclized menaquinones with eight isoprene units. Mycolic acids have 30–64 carbon atoms and up to four double bonds. The fatty acids released on pyrolysis gas chromatography of mycolic acid methyl esters have 12–18 carbon atoms.** The pattern of 16S rRNA signatures consist of nucleotides at positions 250 (U), 316:337 (C–G), 418:425 (C–G), 580:761 (U–A), 559:639 (C–G), 662:743 (C–G), 987:1218 (G–C), and 1000:1040 (A–U). The phylogenetic position of the family *Nocardiaceae*, as determined by 16S rRNA gene sequence analysis, is in the order *Corynebacteriales*.

Members of the family *Nocardiaceae* are widely distributed in aquatic and terrestrial habitats, notably in soil, marine sediments, herbivorous dung and wastewater systems. Some species are opportunistic pathogens for animals, including humans, others are plant pathogens.

DNA G+C content (mol%): 63–73.

Type genus: **Nocardia** Trevisan 1889, 9[AL].

Further descriptive information

Phylogeny. The family *Nocardiaceae* as emended by Rainey et al. (1997) encompassed the genera *Nocardia* and *Rhodococcus*. The subsequent emendation of the family by Zhi et al. (2009) also includes the genera *Gordonia, Millisia, Skermania,* and *Williamsia*; the genus *Smaragdicoccus* Adachi et al. 2007 can be added to this taxon. This broader grouping of genera is essentially based on taxon-specific signatures in 16S rRNA genes. Nevertheless, a case can be made for the continued recognition of the family *Gordoniaceae* Rainey et al. 1997 to encompass the genera *Gordonia, Millisia, Skermania,* and *Williamsia* (see section on the family *Gordoniaceae*), a move which leaves the family *Nocardiaceae* as a home for the genera *Nocardia, Rhodococcus,* and *Smaragdicoccus*. However, the family *Gordoniaceae* may be heterogeneous as the genera *Millisia* and *Skermania* are only loosely associated with the genera *Gordonia* and *Williamsia* based on 16S rRNA gene sequence data. The difficulty in interpreting such phylogenetic trees is a timely reminder that the position of some taxa in actinobacterial 16S rRNA gene trees can be influenced by the strategies employed to construct trees and by the different sequences included in analyses (e.g. Ludwig and Klenk, 2005; Zhi et al., 2009). In such instances there is no sound basis for accepting one tree over another as experience has shown that ambiguities remain in most phylogenetic trees, irrespective of the genes used to construct them. Despite such problems current assignments of genera to families in the order *Corynebacteriales*, like those which preceded them, need to be seen as staging posts leading to better classifications in the future. Indeed, it can be anticipated that suprageneric relationships within the order *Corynebacteriales* based on 16S rRNA gene sequences will need to be reassessed as sequences of novel taxa are added to the existing tree.

Chemotaxonomy. Members of the family *Nocardiaceae* in the sense outlined above share several key chemotaxonomic properties. They have walls rich in *meso*-diaminopimelic acid, arabinose and galactose (wall chemotype IV *sensu* Lechevalier and Lechevalier, 1970), *N*-glycolated muramic acid, a phospholipid pattern containing major amounts of diphosphatidylglycerol, phosphatidylethanolamine (taxonomically significant phospholipid), phosphatidylinositol, phosphatidylinositol mannosides (phospholipid type 2; Lechevalier et al., 1977, 1981c), a fatty acid profile containing straight chain saturated, unsaturated and 10-methyloctadecanoic (tuberculostearic) acids (fatty acid type 1b; Kroppenstedt, 1985), mycolic acids that fall within the range 34–78 carbon atoms and cyclized menaquinones with eight isoprene units (Adachi et al., 2007; Goodfellow and Maldonado, 2006). This pattern of properties serves to distinguish the family *Nocardiaceae* from corresponding families classified in the order *Corynebacteriales* (see Table 29, in the order *Corynebacteriales*, above). It can also be seen from this table that the constituent genera of the family *Nocardiaceae* can be distinguished by using a combination of chemotaxonomic and micromorphological features, notably on the basis of fatty acid, menaquinone, and mycolic acid composition. Reliable procedures which can be used to detect chemical markers of value in the systematics of mycolic acid-containing genera are highlighted in the section on the order *Corynebacteriales*.

Genus I. **Nocardia** Trevisan 1889[AL]

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No.card′ia. N.L. fem. n. *Nocardia* named after Edmond Nocard (1850–1903), a French veterinarian who first isolated members of this taxon.

Aerobic, Gram-stain-positive to Gram-stain-variable, nonmotile catalase-positive actinomycetes which are typically acid–alcohol-fast at some stages of the growth cycle. Rudimentary to extensively branched, substrate hyphae that often fragment *in situ* or on mechanical disruption into coccoid to rod-shaped, nonmotile elements. Aerial hyphae, at times visible only microscopically,

are almost always present. Colonial appearance is variable; colonies may be smooth to granular and irregular, wrinkled or heaped. Carotenoid-like pigments confer various shades of orange, pink, red, or yellow to colonies growing on solid culture media. Soluble brown or yellowish diffusible pigments may be produced. **Chemo-organotrophic with an oxidative type of carbohydrate metabolism. Arylsulfatase-negative.** Most strains grow well from 20°C up to 45°C.

Whole-organism hydrolysates are rich in *meso*-2,6 diaminopimelic acid, arabinose, and galactose, and have an A1γ type peptidoglycan. Muramic acid moieties are *N*-glycolated. Cells contain diphosphatidylglycerol, phosphatidylethanolamine, phosphatidylinositol, and phosphatidylinositol mannosides as major polar lipids, a hexahydrogenated menaquinone with eight isoprene units where the two end units are cyclized [MK-8(H_4, ω cyclo] as the predominant isoprenologue, and major amounts of straight-chain, saturated, unsaturated. and 10-methyl (tuberculostearic) fatty acids. Mycolic acids have 46–64 carbon atoms and up to four double bonds. The fatty acid esters released on pyrolysis gas chromatography of mycolic acid methyl esters contain 12–18 carbon atoms and can be saturated or unsaturated. *Nocardia*, as determined by 16S rRNA gene sequence analysis, is in the order *Corynebacteriales*.

Nocardiae are widely distributed in aquatic and terrestrial habitats, notably in soils, marine sediments, and wastewater systems. Some species are opportunistic pathogens for animals, including humans.

DNA G+C content (mol%): 63–72% (HPLC, T_m).

Type species: **Nocardia asteroides** (Eppinger 1891) Blanchard 1896, 856[VP].

Further descriptive information

Phylogeny. The genus *Nocardia* is classified in the order *Corynebacteriales*, formerly suborder *Corynebacterineae* (Stackebrandt et al. 1997) Zhi et al. 2009, based on 16S rRNA signatures and shares similar chemotaxonomic characteristics with genera assigned to the order, notably the presence of mycolic acids (Adachi et al., 2007; Goodfellow et al., 1999; Goodfellow and Maldonado, 2006). The taxon is the type genus of the family *Nocardiaceae* (Castellani and Chalmers, 1913) emended Stackebrandt et al., (1997) which also contains the genus *Rhodococcus*, and according to Zhi et al. (2009), the genera *Gordonia* (*ex* Tsukamura, 1971) Stackebrandt et al., (1989) (effective publication Stackebrandt et al., 1988), *Millisia* (Soddell et al., 2006a), *Skermania* (Chun et al., 1997), *Smaragdicoccus* Adachi et al. 2007, and *Williamsia* (Kämpfer et al., 1999).

The genus contains 75 validly published species, most of which have been delineated over the past 10 years using a combination of genotypic and phenotypic criteria (Jurado et al., 2008; Kaewkla and Franco, 2010; Yassin et al., 2001a, 2001b; Zhang et al., 2003). Most of these novel species were associated with human infections (Conville et al., 2008; Kageyama et al., 2004b, 2004c, 2004d, 2004e, 2004f, 2004g, 2004h, 2004i; Rodriguez-Nava et al., 2004) with nearly all of the remaining ones isolated from soil collected from different geographical locations (Kämpfer et al., 2007; Maldonado et al., 2000; Saintpierre-Bonaccio et al., 2004). It is evident from the 16S rRNA *Nocardia* gene tree (Figure 88) that the type strains of well established pathogenic species, including *Nocardia asteroides, Nocardia crassostreae, Nocardia farcinica, Nocardia nova*, and *Nocardia otitidiscaviarium* (formerly *Nocardia caviae*), are scattered across the clade and in some cases are most closely related to soil isolates.

The importance of high quality nocardial 16S rRNA gene sequences was highlighted by Roth et al. (2003) who provided a molecular basis for accurate species identification, including taxa assigned to the *Nocardia asteroides* complex. They confirmed that the interspecies heterogeneity between closely related species can be low; found that sequence diversity is less than 5 bases in 8 out of 11 species where more than one strain had been studied; showed that at least 10 taxa merit description as new species, and noted that some subclades contain strains with distinct antimicrobial profiles based on the six drug patterns recognized by Wallace et al. (1988). Strains previously classified as *Nocardia asteroides* were assigned to four distinct phylogenetic groups, namely, the cluster containing the type strain (*Nocardia sensu stricto*), *Nocardia abscessus, Nocardia cyriacigeorgica* and to taxa closely related to either *Nocardia carnea* or *Nocardia flavorosea*. The *Nocardia abscessus, Nocardia cyriacigeorgica*, and the latter two subclades were equivalent to drug patterns I, VI, and II of Wallace and his colleagues. Drug patterns III and IV were shown by strains belonging to the *Nocardia nova* and *Nocardia farcinica* subclades, respectively. However, the discovery that some *Nocardia nova* isolates have different 16S rRNA genes emphasizes the need to carefully interpret 16S rRNA gene sequence data especially between closely related species (Conville and Witebsky, 2005).

The type strains of the two most closely related species, *Nocardia kruczakiae* and *Nocardia veterana*, share a 16S rRNA gene sequence similarity of 99.8% and a DNA–DNA relatedness value of 55±8.5% (Conville and Witebsky, 2005), a figure below the 70% threshold recommended for the circumscription of bacterial species (Wayne et al., 1987). In contrast, the type strains of the most distantly related species, *Nocardia coubleae, Nocardia cummidelens*, and *Nocardia pigrifrangens* each share a 16S rRNA gene sequence similarity of 93.1%. Several multimembered taxa supported by high bootstrap values can be seen in the nocardial tree (Figure 88). These include the *Nocardia africana, Nocardia beijingensis, Nocardia carnea*, and *Nocardia salmonicida* subclades, all of which include strains assigned to species isolated from clinical material and from soil. DNA–DNA relatedness data have played a pivotal role in distinguishing between closely related *Nocardia* species (Conville et al., 2008; Kageyama et al., 2004b, 2004c, 2004d, 2004e, 2004f, 2004g, 2004h, 2004i; Kinoshita et al., 2001; Kudo et al., 1988; Lamm et al., 2009).

Conville et al. (2006) have shown that sequence analysis and alignment of a 468-bp region of the *secA1* gene gives greater resolution between closely related *Nocardia* species than does the corresponding 16S rRNA gene sequence data. They found that the sequence similarities of type and reference strains to their nearest species were in the range 85.0–98.7% for the *secA1* gene compared with 94.4–99.8% for the corresponding 16S rRNA gene sequences. The type strains of *Nocardia kruczakiae* and *Nocardia veterana*, for instance, showed a sequence diversity with the *secA1* gene of 91.9% compared with a 16S rRNA gene sequence similarity of 99.8%. Similarly, alignment of deduced amino acid sequences (composed of 156 amino acid residues) of the 468-bp *secA1* gene region showed good separation of type and reference *Nocardia* strains. Indeed, each type or reference strain shared a unique sequence with similarities between the

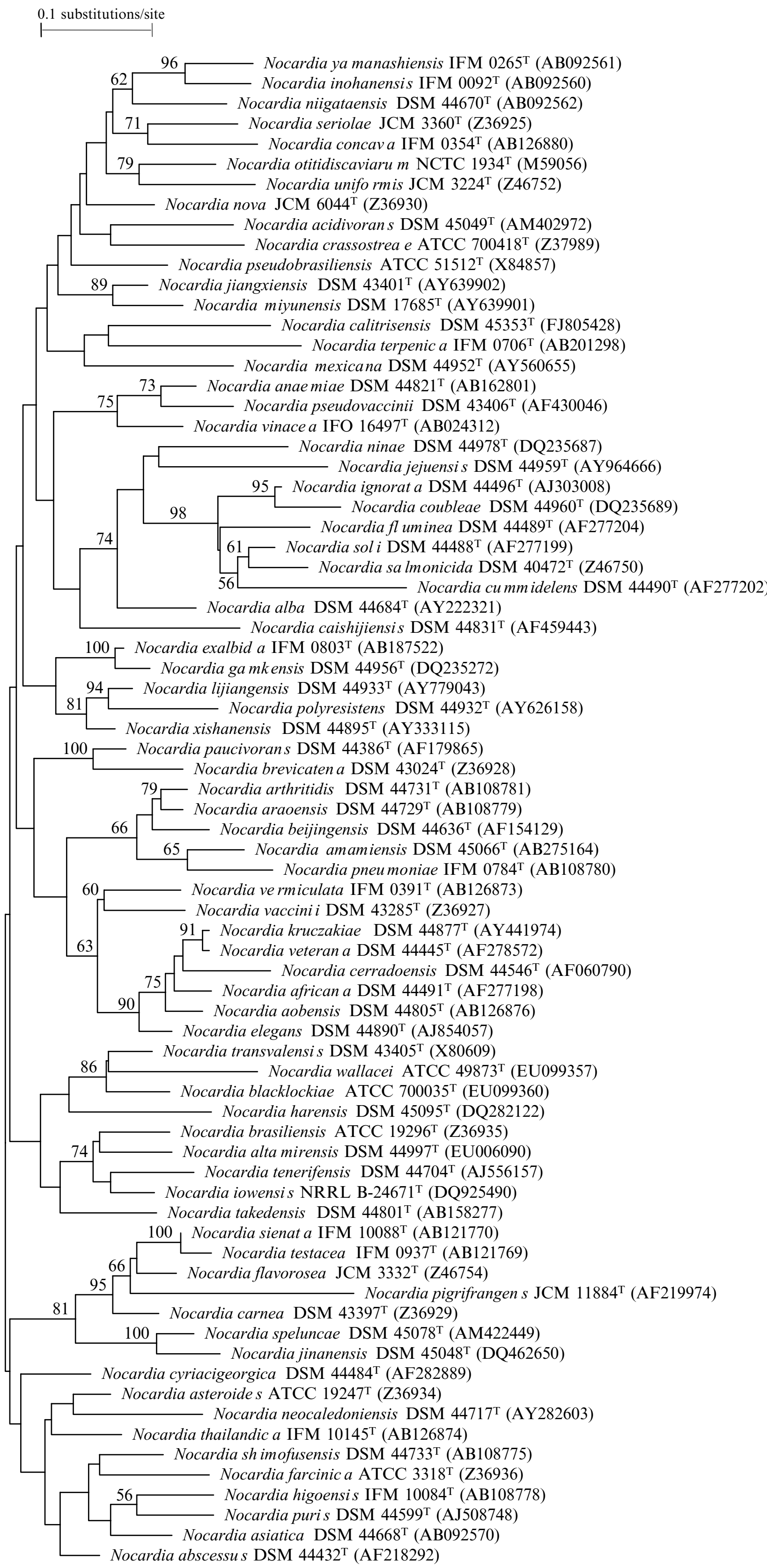

FIGURE 88. Neighbor-joining tree (Saitou and Nei, 1987) based on nearly complete 16S rRNA gene sequences showing relationships between *Nocardia* species. Numbers at the nodes indicate levels of bootstrap support (Felsenstein, 1985) based on an analysis of 1000 resampled datasets. Only values over 55% are given. Bar = 0.1 substitutions per nucleotide position.

strains ranging from 91% (14 amino acid differences) to 99.4% (single amino acid difference). The *secA1* protein is an essential component of the preprotein translocase ATPase which is involved in the export of proteins across bacterial cytoplasmic membranes (Schmidt and Kiser, 1999).

Cell morphology. Nocardiae do not have any distinctive morphological features other than the ability to form aerial hyphae which fragment into coccoid and rod-like elements (Locci, 1976; Nesterenko et al., 1978b; Williams et al., 1976). These workers found that the development and stability of aerial and substrate mycelia is influenced by the organisms and the conditions used to cultivate them. It is clear that micromorphological observations are sensitive to the techniques chosen for specimen preparation and the methods chosen to examine them (Locci, 1976).

The most detailed morphological studies were carried by Locci (1976) who showed that the growth cycle of nocardiae is an intricate process with stages that are difficult to decipher. Generally cocci (Plate 1, parts 1 and 2) and rods (Plate 1, parts 3–7) germinate by forming one or two germ tubes at the extremities of rods and at points along the long axis of the hypha that give rise to cocci. However, in each case, the germ tubes tend to converge almost immediately from the main axis of the parent hypha (Plate 1, parts 1–7). A true mycelial stage is now formed albeit for varying periods of time depending on the strain, substrates, and a number of environmental factors (Plate 1, parts 8 and 9). At this stage, hyphal tips are flexible and tend to change directions thereby determining the configuration of the microcolony (Plate 1, parts 9 and 11). Next, hyphae lose their cytoplasmic continuity and divide transversely giving rise to segments. This process usually takes place quite rapidly and yields rods that are more or less regular in size (Plate 1, parts 10–12). Rods may divide further giving rise to cocci. Each rod and coccoid-like element that is formed from a fragmenting microcolony has the potential to germinate thereby ensuring the further development of the microcolony into a macroscopic colony.

Aerial mycelia are formed from both individual hyphae and from independent fragments (Plate 2, part 1). The extent to which aerial hyphae are formed ranges from sparse to abundant. In *Nocardia asteroides* and *Nocardia farcinica* aerial hyphae tend to form synnemata which coalesce even when borne in relatively distant regions with the consequent appearance of hyphal bridges at the base of many synnemata (Plate 2, parts 2–4). Aerial hyphae whether isolated or grouped together may fragment into spore-like structures. The fragments usually retain a rod-like appearance, but are generally irregular in shape and size (Plate 2, parts 5 and 6). However, a few nocardiae form well-developed spores, as exemplified in Figure 89 and Figure 90. Short chains of spores are produced on the aerial mycelium of *Nocardia aobensis* (Kageyama et al., 2004d), *Nocardia asiatica* (Kageyama et al., 2004b), *Nocardia callitridis* (Kaewkla and Franco, 2010), *Nocardia inohanensis* (Kageyama et al., 2004i), *Nocardia niigatensis* (Kageyama et al., 2004i), and *Nocardia takedensis* (Yamamura et al., 2005). *Nocardia brevicatena* is unusual as it forms distinctive oval to round spores in short chains on both aerial and substrate mycelia (Lechevalier et al., 1961).

Colony morphology. Nocardiae grow well on standard agar media used to cultivate filamentous actinomycetes. Smooth to rough, convex to irregular colonies with filamentous margins are formed on glucose-yeast extract agar (Gordon and Mihm, 1962; Maldonado et al., 2000). Colonies are 0.3–3.0 mm in diameter and show a range of colors, including beige, brown, buff, orange, pink, red, and yellow. Soluble pigments, when present, are usually brown or yellow.

Chemotaxonomy. Nocardiae have cell walls rich in *meso*-diaminopimelic acid, arabinose, and galactose (Goodfellow et al., 1999; Michel and Bordet, 1976; Sun et al., 2009; Yamamura et al., 2007), that is, they have a wall chemotype IV *sensu* Lechevalier and Lechevalier (1970), an A1γ peptidoglycan (Schleifer and Kandler, 1972), and *N*-glycolated muramic acid moieties (Gürtler and Mayall, 2001; Uchida and Aida, 1979b, 1979a; Uchida et al., 1999; Uchida and Seino, 1997). They contain major amounts of diphosphatidylglycerol, phosphatidylethanolamine (a taxonomically significant nitrogenous phospholipid), phosphatidylinositol, and phosphatidylinositol mannosides and hence have a phospholipid pattern II after Lechevalier et al. (1977, 1981c) with a variable distribution of phosphatidylglycerol (Kämpfer et al., 2007; Minnikin et al., 1977), predominant amounts of hexahydrogenated menaquinones with eight isoprene units where the two terminal units are cyclized (Collins et al., 1987; Howarth et al., 1986; Sun et al., 2009; Yassin et al., 2000a), and a DNA G+C content of 63–72 mol%. Nocardiae also contain characteristic lipid soluble, iron binding compounds, the nocobactins (Ratledge and Patel, 1976).

Simple fatty acids and mycolic acids of *Nocardia* strains have been used extensively as taxonomic markers (Goodfellow and Maldonado, 2006; Minnikin and Goodfellow, 1976, 1980). Nocardiae typically contain major amounts of straight-chain saturated and unsaturated fatty acids and significant amounts of tuberculostearic acid (i.e., they have a type II fatty acid profile as defined by Kroppenstedt, 1985) and mycolic acids with 46–64 carbon atoms and up to four double bonds (Alshamaony et al., 1976a; Hoshino et al., 2007; Minnikin and Goodfellow, 1980; Yassin et al., 2000a; Yassin et al., 2000b). In general, nocardiae have similar qualitative fatty acid profiles though quantitative differences in cellular fatty acid composition has been used to distinguish between closely related species (Hoshino et al., 2007; Kageyama et al., 2004b, 2004c, 2004d, 2004e, 2004f, 2004g, 2004h, 2004i; Kämpfer et al., 2007). *Nocardia asteroides* can adapt to changes in temperature by altering the structure of mycolic acids in the cell wall (Tomiyasu, 1982).

One dimensional thin-layer-chromatography of nocardial whole-organism methanolysates usually reveals two lipid spots: one corresponds to mycolic acids (R_f value around 0.47) and the other (R_f 0.91) to nonhydroxylated fatty acids (Minnikin et al., 1975; Yassin and Brenner, 2005; Yassin et al., 2000a, 2000b, 2003). However, a second mycolic acid spot has been detected in a few *Nocardia* species, including *Nocardia abscessus, Nocardia asiatica, Nocardia beijingensis,* and *Nocardia exalbida* (Iida et al., 2006; Kageyama et al., 2001, 2004b; Poonwan et al., 2005).

The cell envelopes of nocardiae are complex; they contain a thick peptidoglycan, sugars, and are rich in lipids, notably mycolic acids (Minnikin, 1982). Ester bonds connect mycolic acids with an arabinogalactan which is attached to the peptidoglycan. The outer mycolic acid layer probably has the same function as the outer membrane of Gram-stain-negative bacteria, which contain channel-forming proteins, the porins, for the passage of hydrophilic solutes. Very little is known about the molecular basis of the permeability of nocardial cell walls, although porins have been detected in the mycolic acid layer

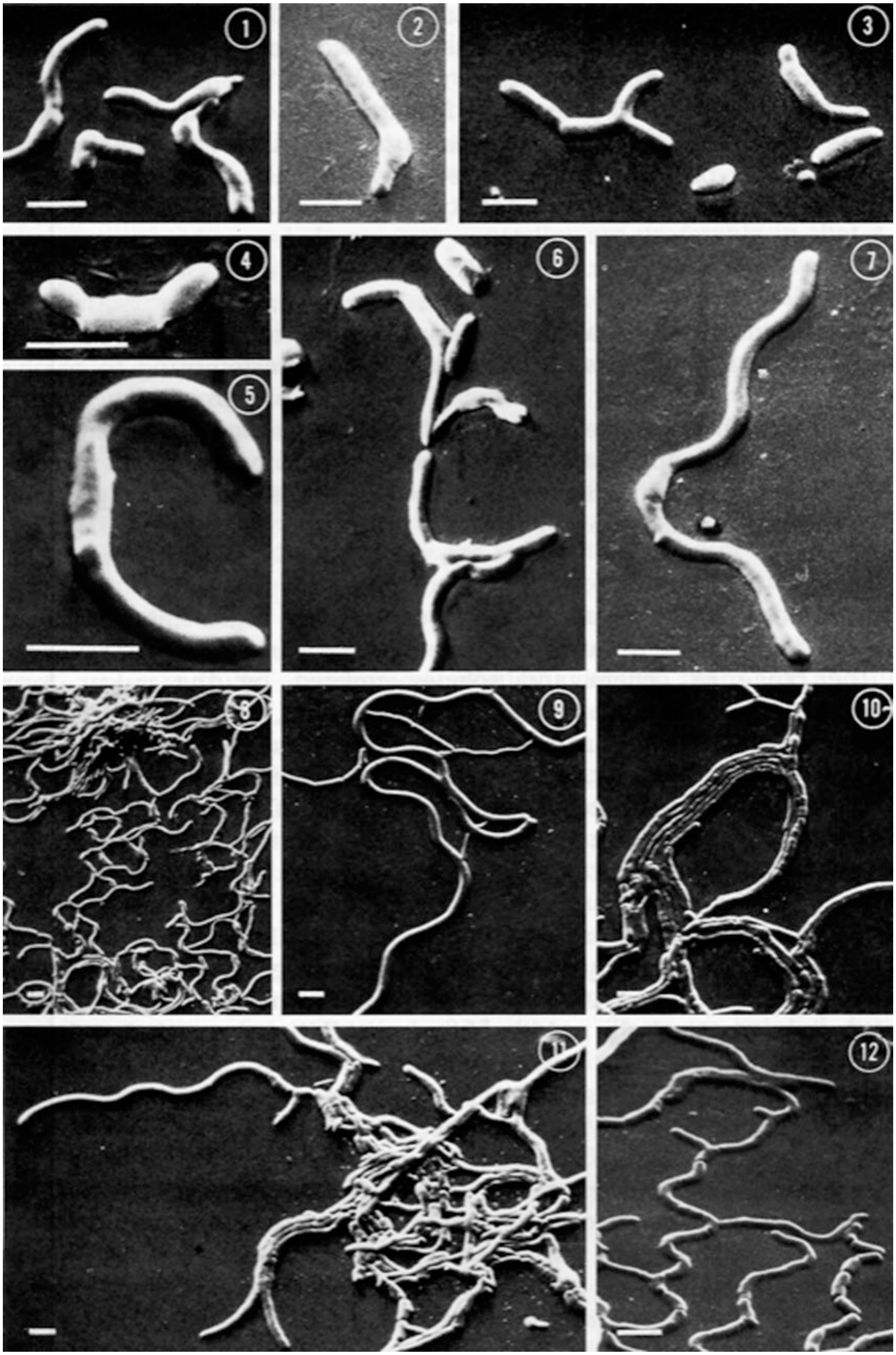

PLATE 1. *Nocardia farcinica* IPV 2119: propagule germination (parts 1–7), microcolonies (parts 8–11) and initial fragmentation (parts 11–12); bars = 2 μm. (Reproduced with permission from Locci 1976. *Actinomycetes: the Boundary Microorganisms*. Toppan, Tokyo.)

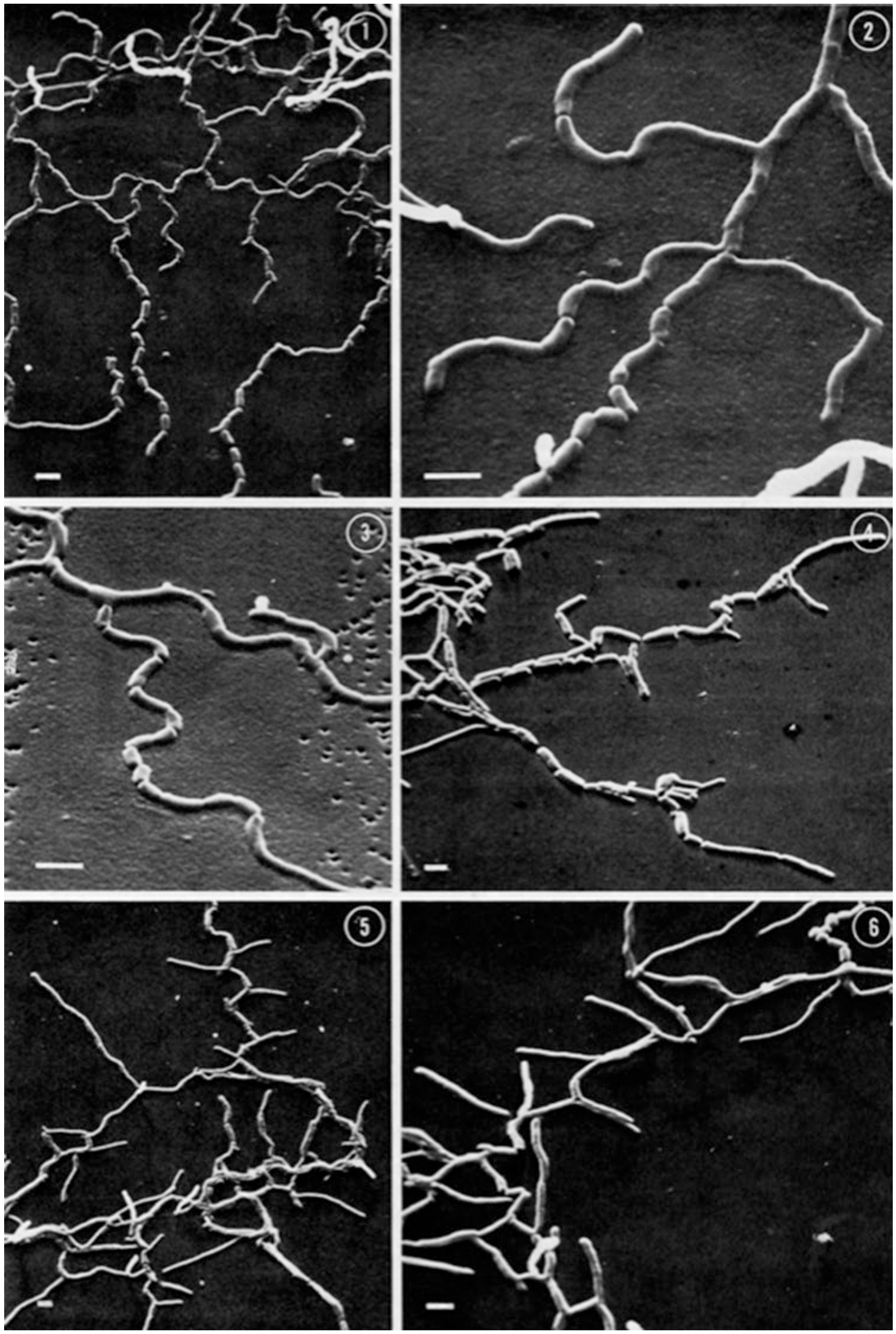

PLATE 2. Hyphal fragmentation (parts 1–3) and germination of fragments (parts 4–6) in *Nocardia farcinica* IPV 2110; bar = 2 µm. (Reproduced with permission from Locci. 1976. *Actinomycetes: the Boundary Microorganisms*. Toppan, Tokyo.)

of *Nocardia asteroides* and *Nocardia farcinica* (Riess et al., 1998; Riess et al., 1999). The cell wall of the *Nocardia asteroides* strain contains an 84-kDa channel-forming protein. It was shown that the cell wall activity of this strain was controlled by negatively charged groups at the channel mouths, indicating that porins are preferentially permeable to cations. The channels were also shown to exhibit asymmetric voltage dependence.

Fine structure. As mentioned earlier, nocardiae typically form branched filaments which fragment into rods and cocci. There have been innumerable light microscope observations of the developmental process (McClung, 1949, 1954a, 1955; McClung and Uesaka, 1961), but very few ultrastructural investigations since the pioneering work of Kawata and Inoue (1965) and Farshtchi and McClung (1967), apart from the extensive

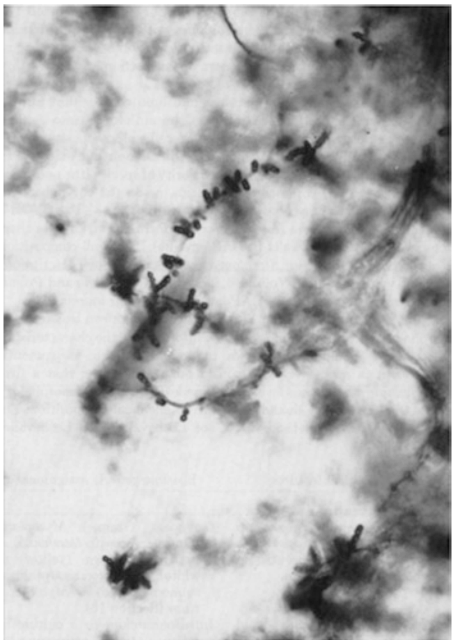

FIGURE 89. Aerial spore chains of *Nocardia brevicatena* (× 1250). (Reprinted from the 1st edition of *Bergey's Manual of Determinative Bacteriology*.)

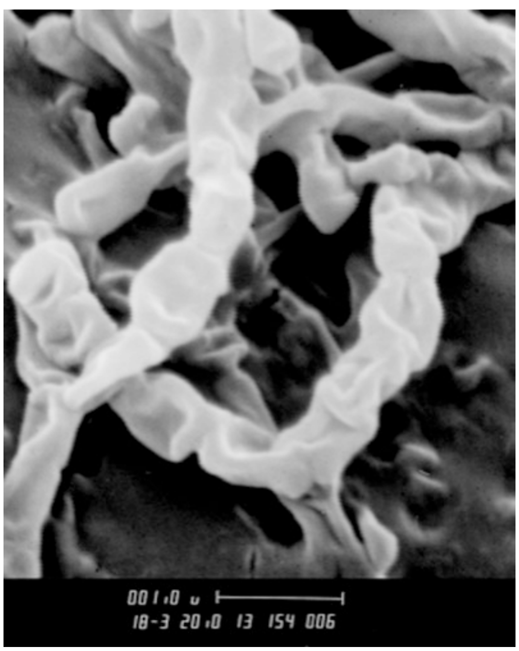

FIGURE 90. Scanning electron micrograph of *Nocardia callitridis* strain CAP 290^T grown on yeast extract-malt extract agar (ISP medium 2) for 21 d at 37°C. (Reproduced with permission from Kaewkla and Franco. 2010. Int. J. Syst. Evol. Microbiol. *50*: 1532–1536.)

work of Blaine Beaman and his colleagues (Beaman, 1968, 1973, 1975, 1976, 1982, 1984, 1993; Beaman and Beaman, 1994; Beaman and Ogata, 1993; Beaman et al., 1978). Transmission electron microscopy has shown that some nocardiae have a trilaminar cell-wall structure (Beadles et al., 1980; Beaman, 1975; Friedman et al., 1998), as exemplified in Figure 91.

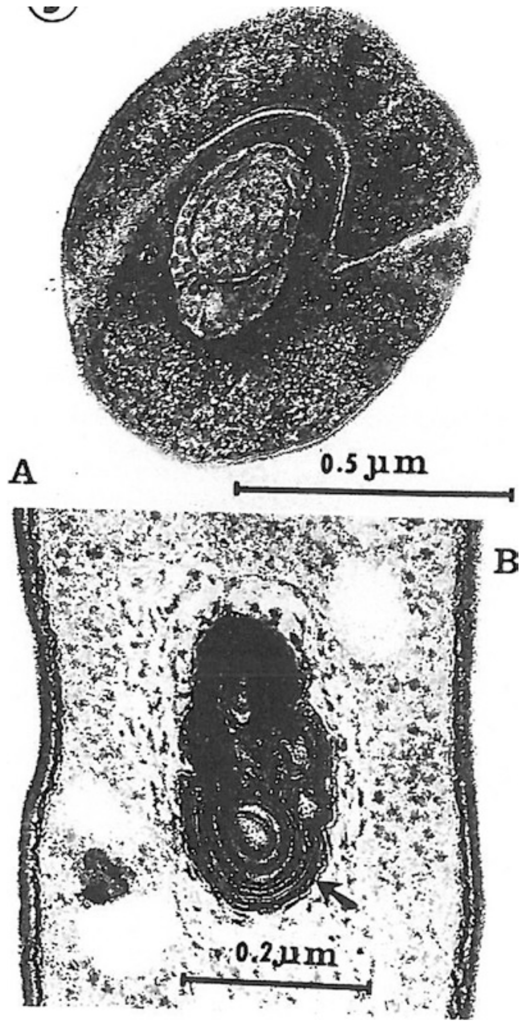

FIGURE 91. Comparative ultrastructural appearance of *Nocardia asteroides* GUH.2 (Beaman) grown *in vitro* (brain heart infusion broth [BHI]) and *in vivo* (mouse tissues). A. Cell grown to mid-exponential phase in BHI broth showing the characteristic tri-layered cell wall (short arrow) and a large laminar mesosome (*arrow*). B. Cell 12 d post-inoculation into the peritoneal cavity of a mouse showing large lipid inclusions (V) and simple vesicular membrane inclusions (*arrow*). A and B are at the same magnification. (Reproduced with permission from Beaman. 1978. Zentralbl. Bakteriol. Parasitenkd. Infektionskr. Hyg. 1. Abt. Suppl. 6, 201–220.)

Beaman and Shankel (1969) showed that as *Nocardia* strain 721-A aged a dark staining inner layer of peptidoglycan and neutral sugars thickened while a light staining outer layer rich in lipids and proteins became more diffuse. Likewise, the amount of peptidoglycan in the cell wall has been shown to increase and the lipid content decrease (Beaman, 1975). Beadles et al. (1980) showed that *Nocardia asteroides* and *Nocardia brasiliensis* strains contained cell membranes, as well as trilaminar cell walls. They showed that the thickness of the cell wall layers, especially the inner peptidoglycan layer, varied between strains, a phenomenon that could be correlated with their virulence and diverse pathogenicities. Beaman (1973) demonstrated a similar relationship *in vivo* between the inner cell walls of a strongly virulent strain, *Nocardia asteroides* Mahve, and a weakly virulent organism, *Nocardia asteroides* 10905, when these

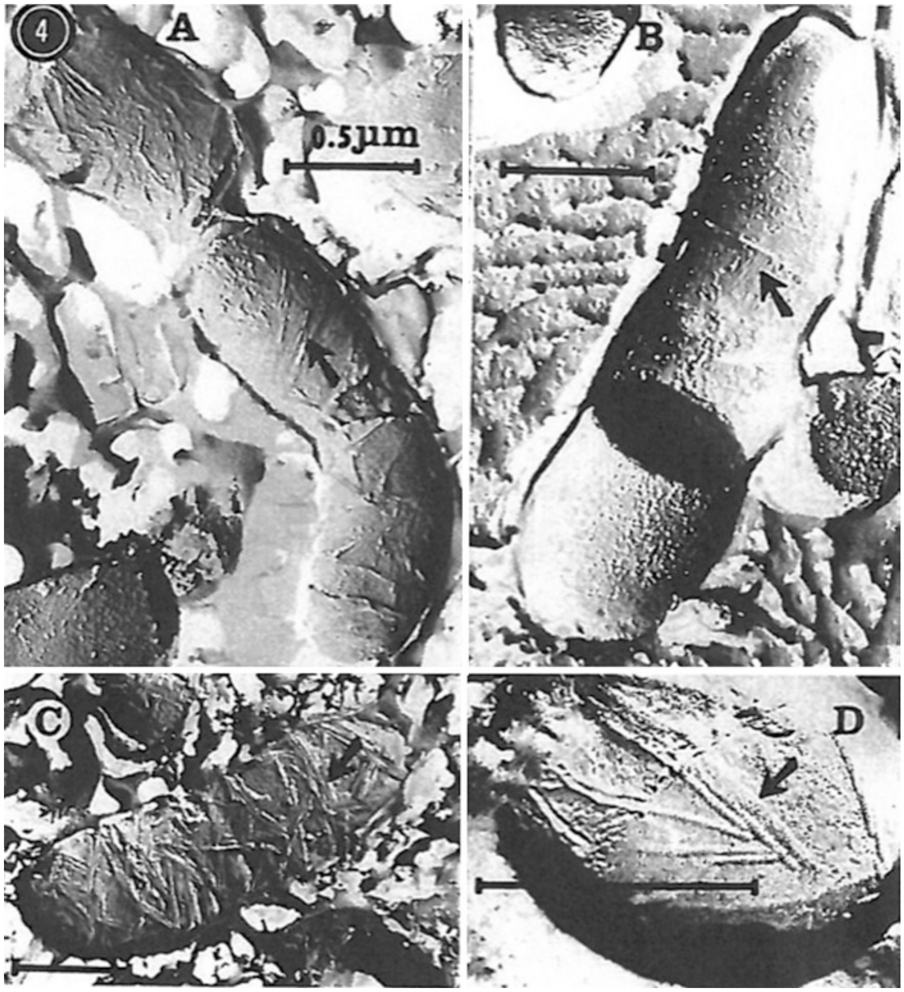

FIGURE 92. Thin section of a exponential phase cell of *Nocardia otitidis-caviarium* (formerly *Nocardia caviae*) strain 260 grown in brain heart infusion broth at 34°C showing the presence of a tubulovesicular mesosome. B. *Nocardia asteroides* strain 10905 grown in brain heart infusion broth showing a large lamellar mesosome embedded in the nuclear material. The cells shown in A and B were grown under the same conditions and fixed using the same chemicals. (Reproduced with permission from Beaman. 1978. Zentralbl. Bakteriol. Parasitenkd. Infektionskr. Hyg. 1. Abt. Suppl. 6, 201–220.)

strains were observed in infected mouse tissue (Figure 91). It was concluded that these quantitative differences in peptidoglycan might govern the ability of strains to resist degradation by phagosomal hydrolases, especially lysozyme.

L-Forms have been isolated from several pathogenic nocardiae *in vitro* by inducing protoplasts and sphaeroplasts with glycine plus lysozyme (Beaman, 1976). Some nocardiae appear to be more susceptible to L-form conversion than others, thereby suggesting chemical differences in the cell-wall structure between the various strains tested (Bourgeois, 1975; Bourgeois and Beaman, 1975). Some cell wall deficient nocardiae may be either pathogenic or persist within a host in a latent form for up to a year, thereby showing that intact cell-wall structure is not essential for pathogenicity (Beaman, 1980).

Serrano et al. (1971) used shadowing and negative staining methods to show that the outer surface of *Nocardia asteroides* strains, including ATCC 14957, was covered by many irregular staining fibers; freeze-dried replicas confirmed these observations (Beaman et al., 1978; Williams et al., 1976). Serrano and his colleagues also observed coccoid, bacillary, and filamentous forms in ultrathin sections of *Nocardia asteroides* ATCC 14957; the walls of the bacillary forms were 10–20 mm thick and contained mesosomes. *Nocardia asteroides* strains generally contain lamellar mesosomes, and *Nocardia brasiliensis* and *Nocardia otitidiscaviarum* tuberculovesicular mesosomes (Beaman et al., 1978; Figure 92).

Nocardiae typically contain lipid inclusions and metachromatic granules (Farshtchi and McClung, 1967; Serrano et al., 1974; Williams et al., 1976) though the accumulation of these materials is influenced by the strain, the age of the culture, and the growth medium (Dispersio and Deal, 1974). Lipid inclusions are readily observed by light microscopy following staining with Sudan Black B and metachromatic granules by light microscopy following staining with methylene blue or toluidine blue. Transmission electron microscopy of thin sectioned preparations show lipid inclusions as electron-lucent, spherical

regions within cells (Beaman et al., 1978), as shown in Figure 91. *In vivo* grown cells of *Nocardia asteroides* usually contain large lipid inclusions which give cells a pronounced beaded appearance when stained for light microscopy (Beaman, 1973).

Nutritional and growth conditions. *Nocardia* strains grow well on most standard media, such as glucose-yeast extract (Gordon and Mihm, 1962), brain heart infusion (Difco), modified Bennett's (Jones, 1949), modified Sauton's (Mordarska et al., 1972), Sabouraud glucose and yeast extract-malt extract (Shirling and Gottlieb, 1966) agars, and on Mueller–Hinton II medium supplemented with glucose (Kageyama et al., 2004b, 2004c, 2004d, 2004e, 2004f, 2004g, 2004h, 2004i). Acetate, glucose, and propionate are used as sole carbon sources, and amino acids, ammonia, and nitrate as simple nitrogen sources; more complex nitrogen sources, such as meat, soy, or yeast peptones and hydrolysates, are also utilized. Doubling times, as for most actinomycetes, are longer than for other bacteria: a generation time of 5.5 h has been reported for *Nocardia asteroides* and *Nocardia brasiliensis* strains (Beadles et al., 1980). Some strains grow to stationery phase in 3–7 d, others grow more slowly.

Metabolism. Nocardiae are aerobic, catalase-positive, chemo-organotrophic actinomycetes which have an oxidative metabolism. They degrade a broad range of organic substrates, and metabolize many sole carbon compounds and carbon and nitrogen compounds (Conville et al., 2008; Goodfellow, 1971; Gordon and Mihm, 1957; Gordon et al., 1978; Kämpfer et al., 2004; Kim et al., 2002b; McClung, 1954b; Orchard and Goodfellow, 1980). However, relatively little is known about their metabolic properties, partly due to the early difficulties in establishing the taxonomic status of strains. Indeed, it is now clear that organisms belonging to other taxa possess many of the metabolic properties attributed to "nocardiae", notably members of the genera *Amycolatopsis* (Arai et al., 1988; Cain, 1981; Matsushima et al., 1987; Schupp et al., 1975; Tárnok, 1976), and *Gordonia* and *Rhodococcus* (Bradley, 1978; Brownell and Denniston, 1984; Peczynska-Czoch and Mordarski, 1988; Raymond and Jamison, 1971; Tárnok, 1976).

The complete genome sequence of *Nocardia farcinica* IFM 10152, a clinical isolate, shows that it has extensive metabolic capabilities (Ishikawa et al., 2004); it contains at least 103 oxygenases, 27 of which are putative cytochrome P450 monoxygenases. This strain was considered to have a higher metabolic potential than *Pseudomonas putida* KT2240, an organism which contains 37 oxygenases (Nelson et al., 2002).

Some nocardiae, including clinically significant isolates, produce industrially significant enzymes (Coco et al., 2001; Lamm et al., 2009) and bioactive metabolites (Hoshino et al., 2004a, 2004b; Mikami et al., 1992). The type strain of *Nocardia iowensis* (formerly *Nocardia* strain NRRL 5646) was initially identified as an antibiotic-producing organism (Hlavka and Bitha, 1977; Martin et al., 1977) but was subsequently shown to be remarkably metabolically versatile. It converts many natural and synthetic compounds into valuable products, including carnosic acid derivatives (Hosny et al., 2002), flavanoids (Herath et al., 2006; Maatoog and Rosazza, 2005), vanillic acid (Dhar et al., 2007), and 4-vinylphenol (Lee and Rosazza, 2004). It transforms quinovic acid glycosides (Cheng et al., 2004) and is the source of a novel ATP/NADPH-dependent carboxylic acid reductase (Li and Rosazza, 1997); the gene encoding the latter has been cloned (He et al., 2004a). The reduction system requires a phosphopantetheinyl transferase (Venkitasubramanian et al., 2007).

Strain NRRL 5646^T contains the first nitric oxide synthase (NOS) system characterized in prokaryotes (Chen and Rosazza, 1994, 1995). As part of this system, it produces guanylate cyclase for cyclic GMP synthesis (He and Rosazza, 2003; Son and Rosazza, 2000) and GTP cyclohydrolase I (He et al., 2004b) for tetrahydrobiopterin biosynthesis. Thiol formation and the detection of mycothiol-dependent dehydrogenase activity in cell extracts of strain NRRL 5646^T are relevant to the possible modulation of nitric oxide (Lee et al., 2007).

Some nocardiae are the source of interesting secondary metabolites such as anti-coumacin B, which targets bone morphogenetic protein-2, from *Nocardia jinanensis* (Sun et al., 2009), nocardicins from *Nocardia uniformis* (Aoki et al., 1976; Hosoda et al., 1977), and tubelactomicin A, a new 16-membered lactose antibiotic from *Nocardia vinacea* (Kinoshita et al., 2001). Several new bioactive compounds have been detected from clinically significant *Nocardia* strains. These include asterobactin, a new siderophore group, from *Nocardia asteroides* (Nemoto et al., 2002), a toxic substance from *Nocardia otitidiscaviarum* (Mikami et al., 1990), novel terpenoid immunosuppressive antibiotics from *Nocardia terpenica* (Hoshino et al., 2007; Tanaka et al., 1997a), transvalecin A, a new thiazolidine-type antibiotic from *Nocardia transvalensis* (Hoshino et al., 2004a; Hoshino et al., 2004b), and the nocathiacins, new thiazolyl peptide antibiotics from a *Nocardia* strain (Leet et al., 2003; Li et al., 2003).

Nocardia brasiliensis has been shown to be a particularly rich source of bioactive compounds, as exemplified by the discovery of brasilidine A, a cytotoxic indole alkaloid (Imai et al., 1997; Kobayashi et al., 1997), brasilicardin A, a new terpenoid antibiotic (Komaki et al., 1998; Shigemori et al., 1998), brasilinolide A, a new immunosuppressive macrolide (Shigemori et al., 1996; Tanaka et al., 1997b), brasiliquinones A ~ C, novel cytotoxic benz [a] anthroquinones (Nemoto et al., 1997; Tsuda et al., 1996), a new mutactinomycin derivative (Mikami et al., 1992), nocardicyclines A and B, new anthracyline antibiotics (Tanaka et al., 1997a), and brasilibactin A, a novel cytotoxic compound (Tsuda et al., 2005).

The anthraquinone chrysophanol is the first secondary metabolite known to be synthesized in an organism-specific way, namely through different folding mechanisms (Bringmann et al., 2006). These workers found that *Nocardia* strain Acta 1057, an isolate from a hay meadow soil, produced chrysophanol through folding mode S, whereas eukaryotes (insects, fungi, and plants) use folding mechanism F. A third folding mode, S′, was discovered in *Streptomyces* strain AK 671 (Bringmann et al., 2009).

Genetics. Genetic recombination and plasmids have been detected in *Nocardia asteroides* (Kasweck and Little, 1982; Kasweck et al., 1981, 1982). Plasmids have also been detected in pathogenic strains of *Nocardia* (Ishikawa et al., 2004; Provost et al., 1996). The inability of Provost and his colleagues to detect plasmids in most of the strains they studied suggests that these elements are not directly involved in virulence. They were able to demonstrate a statistically significant correlation between the localization of cutaneous infections and the incidence of plasmid-bearing strains, but were unable to relate

the presence of plasmids to individual phenotypic traits. Xia et al. (2006) characterized one of the smallest circular plasmids found in *Nocardia* strains, namely plasmid PXT 107 from *Nocardia* strain 107. The complete sequence of this plasmid consists of 4335 bp and encodes a replication extragenic palindromic (Rep) protein and six hypothetical proteins. It was also shown that the *Escherichia coli–Nocardia* shuttle vector pHAQ22, which contains the rep gene of PXT107, propagates in *Nocardia*, but not in *Streptomyces*.

Nocardiophages have been reported for *Nocardia asteroides* (Andrejzewski et al., 1978; Andrzejewski and Pietkiewicz, 1972; Prauser, 1976, 1981a; Pulverer et al., 1975), *Nocardia brasiliensis* (Pulverer et al., 1974), *Nocardia carnea* (Williams et al., 1980), and for *Nocardia otitidiscaviarum* and *Nocardia vaccinii* (Prauser, 1976). Members of the family *Nocardiaceae* are usually susceptible to nocardiophage, unlike strains assigned to the families *Mycobacteriaceae* and *Pseudonocardiaceae* (Prauser, 1981a; Williams et al., 1980). Conversely, phages that lyse *Amycolatopsis mediterranei* are inactive against nocardiae (Thiemann et al., 1964).

The complete genome of *Nocardia farcinica* IFM 10152 (Ishikawa et al., 2004) consists of a single circular chromosome (6,021,225 bp) with a mean G+C content of 70.8 mol%, and plasmids pNF 1 (184,027 bp) and pNF2 (87,093 bp) with mean G+C contents of 67.2 and 68.4 mol%, respectively. The chromosome, which encodes 5,674 putative protein-coding sequences, 53 tRNA genes, and 3 copies of rRNA operons, contains many candidate genes for virulence, multidrug resistance, and secondary metabolites. In addition, plasmids pNF1 and pNF2 encode 160 and 90 predicted protein-coding sequences, respectively. An analysis of paralogous protein families suggests that gene duplication gives the organism the capacity to survive both in the soil ecosystem and in animal tissues. Further analysis of the genome sequence can be expected to provide other insights into the molecular basis of the versatility of *Nocardia farcinica* IFM 10152.

Antibiotic sensitivity patterns. Studies on the antibiotic sensitivity patterns of nocardiae have been driven by clinical (Brown-Elliott et al., 2001; Septimus and Wallace, 1986; Vera-Cabrera et al., 2004; Welsh and Vera-Calera, 2003) and taxonomic considerations (Boiron and Provost, 1988b, 1988a; Goodfellow and Orchard, 1974; Gutmann et al., 1983; Wallace and Steele, 1988; Wallace et al., 1983). In general, nocardiae are sensitive to antibacterial agents such as aminoglycosides, sulfonamides, and tetracyclines, less so to cephalosporins, penicillins, and peptide antibiotics (Goodfellow and Lechevalier, 1989). Sulfonamides, notably the combination of sulfamethoxazole and trimethoprim, are often the agents of choice for the treatment of actinomycetoma and nocardiosis (Wallace et al., 1983), though optimal antimicrobial therapy for patients with nocardiosis has yet to be established (Brown-Elliott et al., 2006; McNeil et al., 1990). Other antimicrobial regimes used to treat nocardial infections have been reviewed by Brown-Elliott et al. (2006).

The susceptibility of 78 geographically diverse, clinical isolates of *Nocardia asteroides* to 12 antimicrobial agents, including aminoglycosides, ciprofloxacin, erythromycin, and β-lactams, was determined by Wallace et al. (1988) using a microdilution procedure. A limited number of patterns of susceptibility to all drug classes was identified, with 95% of isolates exhibiting one of five patterns (types 1–5). The largest group (35% of isolates) contained strains resistant to ampicillin, but susceptible to imipenem and broad-spectrum cephalosporins. Other groups encompassed isolates resistant to ampicillin and erythromycin (18%), to the cephalosporins (17%), and to ampicillin and carbenicillin with intermediate sensitivity to imipenem (17%). The most active parenteral agents were amikacin (95%), imipenem (88%), ceftriaxone (82%), and cefotaxime (88%) and the most effective oral agents were the sulfonamides (100%), minocycline (100%), and ampicillin (40%).

The taxonomic significance of the work outlined above was subsequently found to be in line with the taxonomic significance of previous studies in which pathogenic strains of *Nocardia asteroides, Nocardia brasiliensis, Nocardia farcinica*, and *Nocardia otitidiscaviarum* were found to have different drug susceptibility patterns (Boiron and Provost, 1988b, 1988a; Goodfellow and Orchard, 1974; Wallace et al., 1987; Wallace et al., 1983). Thus, *Nocardia asteroides* complex strains resistant to ceftriaxone, cefotaxime, and ceframandole (type 5) were equated with *Nocardia farcinica* (Wallace et al., 1990), and those resistant to ampicillin and erythromycin (type 3) with *Nocardia nova* (Wallace et al., 1991). Members of these species contain serious pathogens (Farina et al., 2007; Poonwan et al., 2005; Schaal and Lee, 1992). Indeed, the high mortality associated with disseminated *Nocardia farcinica* infections in immunosuppressed patients may be compounded by the resistance of some strains to multiple antimicrobial agents (Hitti and Wolff, 2005; Torres et al., 2000). The antibiotic sensitivity profiles of individual *Nocardia* species are given in the species descriptions.

Pathogenicity. *Nocardia* species are being isolated with increasing frequency from clinical material, especially from specimens taken from immunocompromised patients (Boiron et al., 1998; Farina et al., 2001; Poonwan et al., 2005; Saubolle and Sussland, 2003; Serrano et al., 2007). The best known nocardial pathogens are agents of suppurative and granulomatous diseases in humans and animals, notably mycetoma and nocardiosis (Beaman and Beaman, 1994; Boiron et al., 1993; Brown-Elliott et al., 2006; Goodfellow, 1998; McNeil and Brown, 1994).

Mycetoma, a localized chronic, granulomatous, progressive inflammatory disease that involves subcutaneous tissue and sometimes bone (Brown-Elliott et al., 2006; Fahal, 2004; Fahal, 2006; Schaal and Beaman, 1984), is endemic in certain tropical and subtropical regions where it has a devastating effect on patients (and rural communities) as it often leads to deformities and amputations with a consequent loss of their livelihood. Characteristic features of the disease are one or more painless subcutaneous masses of inflammatory cells and sinus tracts, which may discharge granules (small colonies) of the infective agent. The disease process usually begins at the site of a localized injury, such as puncture wounds caused by thorns or splinters.

Mycetoma may be caused by fungi (eumycetes) or by aerobic, filamentous actinomycetes and hence is classified into eumycetoma and actinomycetoma, respectively. Approximately 60% of mycetoma cases worldwide are caused by actinomycetes, the rest by fungi (Vera-Cabrera et al., 2004). *Nocardia asteroides, Nocardia brasiliensis, Nocardia otitidiscaviarum*, and *Nocardia transvalensis*, and more recently recognized species such as *Nocardia abscessus, Nocardia africana, Nocardia mexicana*, and *Nocardia veterana*, have been reported to cause human mycetoma (Beaman and Beaman, 1994; Buot et al., 1987; Fahal, 2006; Horre et al., 2002; Kano et al., 2002; Lum and Vadmal, 2003; Mirza and Campbell, 1994). The major causal agent remains *Nocardia brasiliensis*; this

organism is responsible for the vast majority of mycetoma cases in Mexico (Lopez Martinez et al., 1992) though other *Nocardia* species, such as *Nocardia mexicana*, may be overlooked since the identification of *Nocardia brasiliensis* strains is often based on a small number of phenotypic properties (Rodriguez-Nava et al., 2004). Mycetoma has been simulated in a mouse model (González-Ochoa, 1973; Salinas-Carmona et al., 1999; Zlotnik, 2007; Zlotnik and Buckley, 1980), which has been used to study host–parasite relationships (Ortiz-Ortiz et al., 1984).

In contrast to actinomycetoma, nocardiosis has a worldwide distribution. It usually develops as an opportunistic or disseminated infection which complicates primary diseases, such as leukemia, lymphoma, and other neoplasms (Brown-Elliott et al., 2006; McNeil and Brown, 1994). Other predisposing factors include immunosuppressive treatments, particularly steroid therapy, chronic bronchopulmonary disease, organ transplantations, and acquired immunodeficiency syndrome (Choucino et al., 1996; Diego et al., 2005; Gallant and Ko, 1996; Patel and Paya, 1997). However, in immunocompetent hosts, infection usually appears after traumatic inoculation into the skin and remains localized (Beaman and Beaman, 1994; Maraki et al., 2004). Nocardia–host interactions have been studied using mice as the experimental animal (Beaman, 1973, 1992, 1993; Beaman and Ogata, 1993; Beaman and Beaman, 1993).

Accurate diagnosis and treatment of actinomycetoma and nocardiosis depend upon the isolation and identification of the causal organisms (Brown-Elliott et al., 2006; Goodfellow, 1998; McNeil and Brown, 1994). The procedures involved are laborious and somewhat cumbersome hence the true incidences of these diseases are underestimated, a problem sometimes compounded by poor documentation and misidentification of the causal agents. The recent dramatic increases in the reported frequency of human nocardial infections can be attributed to the widespread use of immunosuppressive drugs, improved selective isolation procedures, and raised clinical and microbiological awareness (Brown-Elliott et al., 2006; Goodfellow, 1998; Poonwan et al., 2005). However, in countries where chronic lung diseases (particularly tuberculosis) are prevalent, nocardiosis cases may be missed (Baily et al., 1988; Idigbe et al., 1999; Koffi et al., 1998; McLeod et al., 1989; Osoagbaka, 1984; Osoagbaka and Njoku-Obi, 1985). This situation is unsatisfactory as identification of clinically significant nocardiae to the species level is needed to determine the spectrum of diseases caused by members of individual *Nocardia* species and for predicting effective antimicrobial therapy (Brown-Elliott et al., 2006; Conville et al., 2000; Hamid et al., 2001b).

Human cases of nocardiosis can be distinguished clinically as skin infections (cutaneous, subcutaneous, and lymphocutaneous), pulmonary and extrapulmonary nocardioses, and systemic infections which involve two or more body sites (Schaal, 1998; Schaal and Beaman, 1984). In general, cutaneous and lymphocutaneous nocardioses are caused by *Nocardia asteroides, Nocardia brasiliensis*, and *Nocardia otitidiscaviarum*, and sporadically by *Nocardia farcinica* and *Nocardia transvalensis*. Most pulmonary and systemic infections in temperate regions are due to *Nocardia asteroides, Nocardia brasiliensis, Nocardia farcinica*, and *Nocardia nova*, and fewer to *Nocardia brasiliensis, Nocardia otitidiscaviarum, Nocardia pseudobrasiliensis*, and *Nocardia transvalensis* (Farina et al., 2001, 2007; Palmer et al., 1974; Pottumarthy et al., 2003; Torres et al., 2000).

Additional agents of pulmonary nocardiosis include *Nocardia africana*, an organism described by Hamid et al. (2001b) for strains isolated from sputum of patients with chronic pulmonary infections, and *Nocardia veterina* (Conville et al., 2003; Pottumarthy et al., 2003). The most prevalent agents of nocardiosis in Thailand were reported to be *Nocardia beijingensis, Nocardia cyriacigeorgica*, and *Nocardia farcinica* (Poonwan et al., 2005). *Nocardia farcinica*, in particular, is a serious and versatile pathogen which is known to cause cerebral, cutaneous, and pulmonary abscesses (Farina et al., 2007; Hitti and Wolff, 2005; Kageyama et al., 2001; Schaal and Lee, 1992). Other strains isolated from abscesses have been described as *Nocardia abscessus* (Yassin et al., 2001b), *Nocardia niigatensis* (Kageyama et al., 2004i), *Nocardia nova* (Hamdad et al., 2007), *Nocardia puris* (Yassin et al., 2003), and *Nocardia yamanashiensis* (Kageyama et al., 2004i). *Nocardia abscessus* has been reported as the cause of disseminated nocardiosis in an HIV patient (Diego et al., 2005).

At one time, nocardiosis was considered to be a late presenting, community acquired infection but it is now known that the disease is transmissible (Cox and Hughes, 1975; Exmelin et al., 1996; Young et al., 1971). Clusters of patients with *Nocardia asteroides* infections have been reported from liver (Sahathevan et al., 1991) and renal transplant units (Baddour et al., 1986; Houang et al., 1980; Palmer et al., 1974). Schaal (1991) considered that nosocomial airborne transmission, possibly in the operating room environment, was responsible for a cluster of *Nocardia farcinica* postoperative wound infections in patients undergoing cardiac and other vascular surgeries at a university hospital. The isolation of immunosuppressed patients from ones with nocardiosis has been recommended (Houang et al., 1980; Stevens et al., 1981). Nocardial infections also occur in HIV patients (Javaly et al., 1992; Kim et al., 1991; Poonwan et al., 1995).

Blaine Beaman and his colleagues have carried out extensive studies on the mechanisms involved in pathogenicity as well as on host immunity to nocardial infections (Beaman and Beaman, 1992, 1993, 1994, 1998, 2000; Beaman and Moring, 1988). They showed that virulent strains of *Nocardia asteroides* were facultative intracellular pathogens that grew in a variety of cells from humans and experimental animals. The virulence of *Nocardia asteroides* appears to be associated with several factors, such as the stage of the growth cycle, its ability to inhibit phagosome–lysozyme fusion, neutralize phagosomal acidification, resist oxidative killing mechanisms of phagocytes, alter lysosomal enzymes within phagocytes, and invade and grow within the brains of experimental animals. However, the mechanisms of host resistance to nocardial infections are complex and poorly understood (Beaman, 1992) and the role of L forms in nocardial infections is not known (Beaman, 1982).

Nocardiae cause infections in animals (Beaman and Beaman, 1994; Beaman and Sugar, 1983; Ramos-Vara et al., 2007). Animals susceptible to nocardial infections include antelopes, armadillos, birds, cats, cattle, chickens, deer, dogs, dolphins, ducks, fish, foxes, goats, guinea pigs, horses, orangutans, sheep, and whales. The most frequently recognized conditions are systemic and pulmonary nocardioses, including infections of the brain. In dairy animals, notably cows, nocardial mastitis can be a major problem (Battig et al., 1990; Bushnell et al., 1979; Da Costa et al., 1996; Manninen et al., 1993; Stark and Anderson, 1990); strains involved in an epizootic in Canada

were provisionally classified as *Nocardia farcinica* (Manninen et al., 1993). This organism has been shown to be a significant cause of mastitis in goats in the Sudan (Maldonado et al., 2004). The most frequently recognized nocardial pathogens of animals are *Nocardia asteroides, Nocardia brasiliensis*, and *Nocardia otitidiscaviarum. Nocardia crassostreae* causes nocardioses in Pacific oysters (Friedman et al., 1998); *Nocardia salmonicida* and *Nocardia seriolae* are fish pathogens (Isik et al., 1999b; Kudo et al., 1988; Rucker, 1949). *Nocardia africana* has been isolated from a feline mycetoma (Hattori et al., 2003).

Ecology. Nocardiae are widely distributed in aquatic and terrestrial habitats and form mutualistic associations with blood-sucking arthropods (Cross et al., 1976; Goodfellow and Williams, 1983; Xu et al., 1996; Yamamura et al., 2003b), though they are known best as causal agents of mycetoma and nocardiosis (Brown-Elliott et al., 2006; McNeil and Brown, 1994). However, their primary habitat is probably soil as populations of up to 1.4×10^5 colony forming units per gram dry weight have been reported from diverse temperate and tropical soils (Orchard et al., 1977; Xu et al., 1996). Large nocardial populations have been reported from sediment and water samples collected from lakes in Yunnan Province, South-west China (Jiang and Xu, 1996; Jiang and Xu, 1985), from soil amended with municipal water, sewage effluent, or dried sludge derived from domestic and industrial sources (Orchard, 1978, 1979, 1981), and from oil contaminated soil (Khan et al., 1997).

The lack of knowledge about the functional activities of nocardiae in natural habitats is lamentable though some evidence shows that they have a role in the turnover of organic matter (Goodfellow and Williams, 1983; Orchard, 1979, 1981). They have been implicated in the biodeterioration of natural rubber joints in water and sewage pipes (Hookey, 1984; Hutchinson et al., 1975) and in the formation of foam in activated sludge plants (Seviour and Nielsen, 2010; Soddell and Seviour, 1990). Indeed, nocardiae have been considered to be the major causal agents of foaming (Jenkins et al., 1993), but it is now evident that diverse mycolic acid-containing taxa are involved, notably members of the genera *Gordonia, Skermania*, and *Tsukamurella* (Blackall et al., 1989; Chun et al., 1997; Goodfellow et al., 1996; Soddell et al., 1992; Soddell and Seviour, 1998; Stainsby et al., 2002).

Improvements in the classification of the genus *Nocardia* provide a sound framework for the circumscription of new species isolated from man-made and natural habitats. Novel taxa isolated from terrestrial habitats include *Nocardia amamiensis* from a sugar-cane field (Yamamura et al., 2007), *Nocardia cerradoensis* from a Cerrado soil (Alburquerque de Barros et al., 2003), *Nocardia coubleae* from oil-contaminated soil (Rodriguez-Nava et al., 2007), *Nocardia jiangxiensis* from an acid pine forest soil (Cui et al., 2005), and *Nocardia neocaledoniensis* from a brown hypermagnesian ultramafic soil (Saintpierre-Bonaccio et al., 2004). Similarly, *Nocardia harenae* was isolated from beach sand (Seo and Lee, 2006), *Nocardia jejuensis* and *Nocardia speluncae* from a natural cave (Lee, 2006; Seo et al., 2007), and *Nocardia takedensis* from scumming activated sludge (Yamamura et al., 2005). Such studies are important as they provide a basis for determining the distribution and activities of individual *Nocardia* species in the environment. *Nocardia pseudovaccinii* was proposed to accommodate two strains previously assigned to the plant pathogen *Nocardia vaccinii* (Kim et al., 2002b).

Isolation procedures

Traditional isolation procedures were based on the ability of nocardiae to metabolize hydrocarbons as sole sources of carbon and energy for growth (Cross et al., 1976; Nesterenko et al., 1978a; Tárnok, 1976). Modifications of Söhngen's paraffin baiting technique (Sőhngen, 1913) have been used to isolate nocardiae from clinical specimens and soil (Ashdown, 1990; Khan et al., 1997; Ollar, 1976; Portaels, 1976; Schaal and Bickenbach, 1978), but they merely indicate the presence or absence of the organisms without providing any quantitative data. Similarly, alternative isolation methods, such as inoculating hamster or guinea pig testicles with soil suspensions supplemented with penicillin and streptomycin (Conti-Diaz et al., 1971), or plating suspensions onto media which contain cholesterol acetate and sodium azide (Farmer, 1962), underestimate nocardial populations in natural habitats.

It is now common practice to isolate *Nocardia* strains from pretreated or non-pretreated clinical or environmental samples followed by plating serial dilutions onto either general purpose or selective media supplemented with antifungal antibiotics (e.g., actidione and nystatin at 50 μg/ml). Inoculated media are incubated at 25, 30, or 37°C for up to 3 weeks followed by colony selection and characterization. It is surprising given the biotechnological, clinical, and ecological importance of nocardiae that an agreed, effective selective isolation procedure is not available. However, large numbers of nocardiae have been isolated by plating heat pretreated soil suspensions (55°C for 6 min) onto Diagnostic Sensitivity Test (DST) agar supplemented with antifungal antibiotics and various combinations of tetracycline antibiotics (Orchard and Goodfellow, 1974; Orchard et al., 1977). Characteristic colonies with pink to red substrate mycelium and sparse to abundant white aerial hyphae are evident following incubation at 25°C for up to 3 weeks. Such colonies have been provisionally classified as *Nocardia asteroides* (Orchard and Goodfellow, 1974; Orchard et al., 1977) though *Nocardia cummidelens, Nocardia fluminea*, and *Nocardia soli* have been isolated from soil and water samples (Maldonado et al., 2000). It seems likely that at least some strains of *Nocardia asteroides* will be inhibited by the antibacterial antibiotics in DST agar (Schaal and Heimerzhein, 1974). A selective medium free of such antibiotics has been used to isolate *Nocardia asteroides* from clinical material (Schaal, 1972).

Small numbers of nocardiae have been isolated from soil on nutrient rich media, as exemplified by the isolation of *Nocardia alba* and *Nocardia lijianensis* on HV agar (Li et al., 2004a; Xu et al., 2005), *Nocardia beijingensis* on glucose-asparagine agar (Wang et al., 2001), *Nocardia caishjiensis* on Bennett's agar (Zhang et al., 2003), *Nocardia cerradoensis* on R5 agar (Alburquerque de Barros et al., 2003), *Nocardia neocaledoniensis* on oatmeal agar (Saintpierre-Bonaccio et al., 2004), *Nocardia polyresistens* on proline-tap water agar (Xu et al., 2005), *Nocardia tenerifensis* on soil extract agar (Kämpfer et al., 2004), and *Nocardia xishanensis* on modified Sauton's agar (Zhang et al., 2004). *Nocardia amamiensis* was isolated from soil using the SDS/yeast extract pretreatment method (Hayakawa and Nonomura, 1989) and HV agar supplemented with nalidixic acid (Yamamura et al., 2007), and *Nocardia jiangensis* and *Nocardia miyunensis* by plating soil suspensions, prepared using the dispersion and differential centrifugation procedure (Wang et al., 2003), onto acidified selective isolation medium (Cui et al., 2005). Large numbers

of nocardiae have been isolated by spreading dilutions of sediment, soil, and water samples onto rich media, such as colloidal chitin agar, glycerol-asparagine agar, and starch-casein agar (Jiang and Xu, 1996; Xu et al., 1996).

Nonselective media such as brain heart infusion and Sabouraud's glucose agars have been recommended for the isolation of clinically significant nocardiae (Schaal, 1977). Sabouraud's glucose agar supplemented with chloramphenicol has been used to recover nocardiae from sputum (Ajello and Roberts, 1981) though many nocardiae are inhibited by this antibiotic (Gutmann et al., 1983). Similarly, decontamination of respiratory specimens using procedures applied to the isolation of mycobacteria have been shown to kill nocardiae (Murray et al., 1987). However, Hamid et al. (2001b) isolated *Nocardia africana* strains on Lowenstein-Jensen slopes which had been inoculated with sputum samples taken from patients with pulmonary disease then treated using the digestion-decontamination technique of Roberts et al. (1991); most of the patients had either failed to respond to anti-tubercular drug treatment or had responded then relapsed. The use of chemically defined media containing paraffin agar may prove to be an effective way of selectively isolating nocardiae from clinical specimens (Shawar et al., 1990).

Specific *Nocardia* species have been isolated from clinical material using rich nutrient media, as illustrated by the isolation of *Nocardia araoensis* on Ogawa agar (Kageyama et al., 2004g) and *Nocardia ignorata* from brain heart infusion agar (Yassin et al., 2001b). *Nocardia asteroides* and *Nocardia otitidiscaviarum* have been isolated by plating foam from activated sludge plants onto either Czapek's agar supplemented with yeast extract (Higgins and Lechevalier, 1969) or glycerol agar (Gordon and Smith, 1953) and incubating plates for 5–7 d at 28°C. *Nocardia takedensis* was isolated from scumming activated sludge using a *Nocardia* isolation method based on sucrose-gradient centrifugation (Yamamura et al., 2003a, 2005). Stratton et al. (1996) isolated strains using a micromanipulator to pick up cocco-bacillary elements prior to plating them onto R2A agar.

Maintenance procedures

A convenient method for short-term storage involves serial transfer every 2 months from appropriate media, such as modified Bennett's (Jones, 1949) and glucose-yeast extract agar slopes (Gordon and Mihm, 1962) with storage between transfers at 4°C. Long-term preservation can be achieved by lyophilization, storage in liquid nitrogen, or by using frozen glycerol suspensions. For lyophilization, biomass is suspended in a suitable fluid such as glucose serum (7.5%, w/v) or skimmed milk supplemented with glucose (7.5%, w/v). For preservation in liquid nitrogen, biomass is inoculated into a suitable medium held in small tubes until sufficient growth is visible. Such preparations are sealed with cotton wool plugs dipped in liquid paraffin wax and placed in a liquid nitrogen container. Glycerol suspensions are prepared by scraping growth from heavily inoculated agar plates and making heavy suspensions in aqueous glycerol (3 ml) held in small vials which are stored at −20°C (Wellington and Williams, 1978).

Differentiation of the genus *Nocardia* from other genera

The genus *Nocardia* can be difficult to distinguish from other mycolic acid-containing taxa and from other filamentous actinomycetes using standard staining and morphological properties. *Nocardia* strains that lack aerial hyphae, for instance, can be difficult to distinguish from mycobacteria, rhodococci, and "bald" streptomycetes whereas those with abundant aerial hyphae can be confused with genera classified in the families *Pseudonocardiaceae, Streptosporangiaceae*, and *Thermomonosporaceae*. Until recently, reliable differentiation of mycolic acid-containing genera depended on the use of a combination of morphological and chemical markers (see Table 29 in the *Corynebacteriales* chapter).

Simplified procedures are available for the detection of staining and morphological properties (Goodfellow, 1996), cell wall constituents (Hancock, 1994), including the acyl type of muramic acid (Uchida et al., 1999), fatty acids (McNabb et al., 1997; Suzuki et al., 1993), and mycolic acids (Butler et al., 1986; Embley and Wait, 1994; Nishiuchi et al., 2000), menaquinones (Collins et al., 1985), and polar lipids (Suzuki et al., 1993). Such procedures are being complemented, and to some extent replaced, by molecular systematic methods (Gürtler and Mayall, 2001), notably by 16S rRNA gene sequencing studies (Stackebrandt et al., 1997; Zhi et al., 2009). The methods used to distinguish mycolic acid-containing genera from one another and from other filamentous actinomycete taxa are considered in greater detail in the section on the order *Corynebacteriales*.

Taxonomic comments

The long and intricate taxonomic history of the genus has been the subject of several comprehensive reviews (Goodfellow, 1992, 1996; Goodfellow et al., 1998a, 1999; Goodfellow and Minnikin, 1977; Lechevalier, 1976; Minnikin and Goodfellow, 1980) and hence will not be considered in detail here. The genus was proposed by Trevisan (1889) to accommodate five species, one of which *Nocardia farcinica*, contained an aerobic filamentous organism which Edmond Nocard had isolated in Guadeloupe in 1888 from lesions in cattle suffering from farcy. *Nocardia farcinica*, which, because it was cited first, was subsequently assumed to be the type species while a second species, *Nocardia foersteri*, was the first actinomycete to be described (Cohn, 1875). One year later, in 1896, Eppinger isolated an aerobic, branching filamentous organism from a human with a fatal brain abscess and named it *Cladothrix asteroides*, but soon thereafter it was renamed *Nocardia asteroides* Blanchard 1896. Early additions to the genus *Nocardia* included several agents of disease, namely *Nocardia asteroides* (Eppinger 1891) Blanchard 1896, *Nocardia brasiliensis* (Lindenberg 1909) Pinoy 1913, *Nocardia otitidiscaviarum* corrig. Snijders 1924, and *Nocardia transvalensis* Pijper and Pullinger 1927. Subsequently, the genus became a dumping ground for a heterogeneous assortment of nocardioform actinomycetes, i.e., strains grouped around *Nocardia*, which were considered to form a mycelium that fragmented into coccoid and rod-like elements (see Lechevalier, 1976).

It was only with the application of new taxonomic methods, notably chemotaxonomy, molecular systematics and numerical phenetic taxonomy, that dramatic improvements were made in the classification of nocardioform actinomycetes. Species previously assigned to the genus *Nocardia* were classified directly or indirectly into either established or novel mycolic acid-containing genera (*Gordonia, Rhodococcus*, and *Skermania*) or into corresponding taxa that lacked mycolic acids (*Actinomadura, Amycolatopsis, Cellulomonas, Lechevalieria, Nocardiopsis, Oerskovia, Prauserella, Pseudonocardia*, and *Rothia*). The redefined

genus contained only 9 of the 20 species listed under *Nocardia* in the *Approved Lists of Bacterial Names* (Skerman et al., 1980), namely *Nocardia amarae, Nocardia asteroides, Nocardia brasiliensis, Nocardia brevicatena, Nocardia carnea, Nocardia farcinica, Nocardia otitidiscaviarum, Nocardia transvalensis,* and *Nocardia vaccinii.*

Gordon and her colleagues brought considerable clarity into the taxonomy of the species *Nocardia asteroides, Nocardia brasiliensis, Nocardia carnea, Nocardia otitidiscaviarium, Nocardia transvalensis,* and *Nocardia vaccinii* based on pioneering studies in which they used a broad range of phenotypic properties (Gordon and Mihm, 1957, 1959, 1962a, 1962b; Gordon et al., 1978; Mishra et al., 1980). Data summarized from these publications together with corresponding results from studies on *Nocardia amarae* and *Nocardia brevicatena* (Goodfellow et al., 1982b, 1982c; Goodfellow and Pirouz, 1982a; Lechevalier, 1968; Lechevalier and Lechevalier, 1974) featured prominently in the section on *Nocardia* in the last edition of *Bergey's Manual of Systematic Bacteriology* (Goodfellow and Lechevalier, 1989).

Goodfellow and his coworkers considered *Nocardia amarae, Nocardia brasiliensis, Nocardia farcinica,* and *Nocardia otitidiscaviarum* to be sound taxospecies, but, like other workers, stressed that *Nocardia asteroides*, as defined by Gordon, was markedly heterogeneous (Bradley et al., 1978; Goodfellow, 1971; Kurup et al., 1983; Kurup and Scribner, 1981; Magnusson, 1976; Mordarski et al., 1977, 1978; Pier and Fichtner, 1971, 1981; Ridell, 1981; Schaal and Reutersberg, 1978; Tsukamura, 1977). The subsequent recognition of *Gordonia amarae* (Lechevalier and Lechevalier 1974) Klatte et al. 1994b for actinomycetes previously classified as nocardiae was a key development in nocardial systematics as the genus *Nocardia* was left as a well-delineated taxon for the first time since its inception. The remaining *Nocardia* species cited on the *Approved Lists of Bacterial Names* (Skerman et al., 1980) were assigned to several established and novel genera as outlined in the *Nocardia* chapter in the last edition of *Bergey's Manual of Systematic Bacteriology* (Goodfellow and Lechevalier, 1989).

The principal nomenclatural problem in the redescribed genus *Nocardia* was the status of the original type species, *Nocardia farcinica.* The strain, originally isolated by Nocard (1888) from a case of bovine farcy was, by the decision of the Judicial Commission in 1954, made the type species of the genus *Nocardia.* It subsequently became known that Nocard's original isolate was represented by two supposedly identical, but actually very different strains, ATCC 3318 and NCTC 4524. The former contained mycolic acids characteristic of nocardiae, and the latter mycolic acids and mycosides similar to those of mycobacteria (Lanéelle et al., 1971; Lechevalier et al., 1971). Orchard and Goodfellow (1980) found that these strains fell into different clusters defined in their numerical taxonomic study. Schaal and Reutersberg (1978) found that ATCC 3318 clustered with *Nocardia asteroides* strains, but they did not examine NCTC 4524. Using serological and physiological data, Ridell (1975) noted that the latter strain grouped with mycobacteria and ATCC 3318 with *Nocardia asteroides,* thereby confirming her previous results using immunodiffusion; the latter showed that some *Nocardia farcinica* strains were closer to mycobacteria than to nocardiae (Ridell and Norlin, 1973). Base composition studies also showed that strains ATCC 3318 and NCTC 4524 were different, the mol% G+C values being 68.0 and 71.6, respectively (Mordarski et al., 1978), as did phage sensitivity studies (Prauser, 1981b). In view of the uncertain status of *Nocardia farcinica* (Lechevalier, 1976), the type species of the genus was changed to *Nocardia asteroides,* with ATCC 19247 as the type strain (Judicial Commission of the International Committee on Systematic Bacteriology, 1985; Skerman et al., 1980; Sneath, 1982) and *Nocardia farcinica* retained, with ATCC 3318 as the type. An appeal to the Judicial Commission to reject *Nocardia farcinica* as a *nomen dubium* was published (Tsukamura, 1982a); however, the commission voted to retain the *farcinica* epithet.

The improved classification of the genus has provided a sound framework for the recognition of novel pathogenic *Nocardia* species and for the reclassification of misclassified strains (Goodfellow et al., 1999). Species encompassing human and animal pathogens, such as *Nocardia brasiliensis, Nocardia crassrostreae,* and *Nocardia otitidiscaviarum,* were considered to be homogeneous whereas others, notably *Nocardia asteroides,* were seen to be heterogeneous. *Nocardia asteroides* contained a mish mash of strains which included *Nocardia asteroides sensu stricto, Nocardia farcinica,* and *Nocardia nova* (Tsukamura, 1982a; Wallace et al., 1991; Yano et al., 1990) and several biotypes and serotypes (Kurup et al., 1983; Kurup and Scribner, 1981; Wallace et al., 1988, 1990). However, regardless of their designation, members of what became known as the *Nocardia asteroides* complex have been frequently isolated from humans and animals with serious, often fatal, infections (Beaman and Sugar, 1983; Wallace et al., 1990, 1991).

The confusion over the taxonomic status of *Nocardia asteroides* and related strains led to additional clinically significant isolates being assigned to the *Nocardia asteroides* complex (Ambaye et al., 1997; Poonwan et al., 1995), a practice which has continued until quite recently (Farina et al., 2001; Pintado et al., 2002; Saubolle and Sussland, 2003). This situation is unsatisfactory as rapid and accurate identification of clinically significant nocardiae to the species level is at a premium for predicting antimicrobial susceptibilities, especially given the potential of nocardiae to cause infections in the growing immunocompromised patient community (Patel et al., 2004; Wauters et al., 2005). It is also unfortunate that the type strain of *Nocardia asteroides* does not represent any of the common nocardiae associated with clinical nocardiosis (Baba et al., 1997; Patel et al., 2004).

Additional improvements in nocardial systematics (Goodfellow and Maldonado, 2006) have clarified relationships between isolates assigned to the *Nocardia asteroides* complex, as exemplified by the recognition of several new species, such as *Nocardia abscessus* (Yassin et al., 2000b), *Nocardia anemiae* (Kageyama et al., 2005d), *Nocardia araoensis* (Kageyama et al., 2004g), *Nocardia cyriacigeorgica* (Yassin et al., 2001a), and *Nocardia higoensis* (Kageyama et al., 2004f). Similarly, isolates assigned to the *Nocardia transvalensis* 16S rRNA gene clade have been given species status as *Nocardia blacklockiae* and *Nocardia wallacei* (Conville et al., 2008, 2009), and strains misclassified as *Nocardia brasiliensis* have been assigned to two species, *Nocardia mexicana* (Rodriguez-Nava et al., 2004; Rodriguez-Nava, 2006) and *Nocardia pseudobrasiliensis* (Ruimy et al., 1996). Additional new species of human origin include *Nocardia asiatica* (Kageyama et al., 2004b), *Nocardia kruczakiae* (Conville et al., 2004, 2005), and *Nocardia testaceae* corrig Kageyama et al. (2004h).

The improved procedures available for the circumscription of *Nocardia* species have led to the recognition of new species

isolated from natural habitats, including *Nocardia acidovorans* (Kämpfer et al., 2007), *Nocardia alba* (Li et al., 2004b), *Nocardia amamiensis* (Yamamura et al., 2007), *Nocardia coubleae* (Rodriguez-Nava et al., 2007), *Nocardia lijianensis* (Xu et al., 2006b), *Nocardia neocaledoniensis* (Saintpierre-Bonaccio et al., 2004), and *Nocardia xishanensis* (Zhang et al., 2004). In addition, strains assigned to the *Nocardia vaccinii* 16S rRNA gene clade have been reclassified as two new species, *Nocardia elegans* (Yassin and Brenner, 2005) and *Nocardia pseudovaccinii* (Kim et al., 2002b).

The genus *Nocardia* encompasses 75 validly published species the majority of which have been delineated using a broad range of genotypic and phenotypic properties, including DNA–DNA relatedness data. However, despite the recent explosion in the number of *Nocardia* species, the genus is still underspeciated (Liu et al., 1983; Maldonado et al., 2000; Orchard and Goodfellow, 1980; Patel et al., 2004; Roth et al., 2003; Wang et al., 1999). It also seems likely that strains assigned to the *Nocardia asteroides* complex will be found to merit recognition as new species (Goodfellow et al., 1999; Roth et al., 2003). The type strain of *Nocardia asteroides* forms a distinct phyletic line in the 16S rRNA *Nocardia* gene tree and can be readily distinguished from most other pathogenic nocardiae based on numerical phenotypic data (Goodfellow et al., 1999) and on the composition of mycolic acid molecular species determined by gas-chromatography-mass spectrometry (Baba et al., 1997).

Differentiation of the species *Nocardia*

Nocardia species remain difficult to identify even though a multiplicity of diagnostic procedures have been recommended for this purpose (Baba et al., 1997; Boiron et al., 1993). Nevertheless, in practice, unknown nocardiae can be assigned to established or novel species by first establishing their position in the 16S rRNA gene tree (Figure 88) followed, where necessary, by DNA–DNA relatedness studies and finally by the application of biochemical, degradation and nutritional tests (Table 39). This approach is simple in theory but in practice it is beset by difficulties, not only because the time taken from the acquisition of specimens to identification can take several weeks but also because the process is labor intensive. These problems are compounded by the lack of an agreed set of diagnostic phenotypic tests and by the fact that the latter can give unreliable data, especially since many recently proposed *Nocardia* species are based on descriptions of single strains. To compensate for such problems, supplementary tests grounded on antibiotic sensitivity and enzyme activity have been recommended for the delineation of some *Nocardia* species (Biehle et al., 1996; Boiron and Provost, 1988a, 1988b, 1990b, 1990c; Boiron et al., 1993; Wilson et al., 1998), as has the use of carbon utilization profiles (Kämpfer et al., 2004, 2007; Kim et al., 2002b). An abbreviated set of phenotypic tests have been proposed for the identification of medically significant nocardiae (Kiska et al., 2002). In addition, laboratory specialists need to be aware of the occasional occurrence of unusual strains of *Nocardia asteroides* (Boiron et al., 1990).

The difficulties associated with the identification of *Nocardia* species using phenotypic tests led to the introduction of a plethora of molecular-based procedures, particularly for the identification of clinically significant nocardiae. The application of 16S rRNA gene sequencing was instrumental in the delineation of numerous new *Nocardia* species and, as noted earlier, many of these were clinically significant. Methods specifically developed for the identification of clinically significant nocardiae included the polymerase chain reaction (PCR) coupled with restriction endonuclease analysis of PCR products (Isik et al., 2002; Rodriguez-Nava et al., 2006; Steingrübe et al., 1995, 1997; Wilson et al., 1998), PCR-randomly amplified polymorphic DNA fingerprinting (Isik et al., 2002), partial 16S rRNA gene sequencing (Cloud et al., 2004; Patel et al., 2004), and sequence analysis of a portion of the *secA1* gene (Conville et al., 2006). However, when using sequence-based identification procedures it is important to be aware of potential complications which may be caused by the presence of differing 16S rRNA genes in the same isolates (Conville and Witebsky, 2005). DNA probes have been developed for the identification of *Nocardia asteroides* using genomic based libraries generated from selected members of the species (Brownell and Belcher, 1990), and a digoxigenen-labeled cDNA library for the recognition of *Nocardia farcinica* (McNeil et al., 1997).

The long-term practical value of diagnostic molecular approaches to the identification of pathogenic nocardiae, such as those outlined above, is difficult to forsee due to the need to apply them to the expanding number of pathogenic *Nocardia* species (Conville et al., 2006). This problem is exacerbated by the discovery that key pathogenic species, such as *Nocardia farcinica, Nocardia nova, Nocardia otitidiscaviarum*, and *Nocardia transvalensis*, are heterogeneous (Baba et al., 1997; Patel et al., 2004)—an observation that is a timely reminder of the need to build reliable identification procedures on sound classifications (Goodfellow, 2000). However, direct molecular diagnosis of nocardiosis has proved decisive in culture-negative cases of the disease (Couble et al., 2005; Marchandin et al., 2006).

Serological procedures and skin testing for cutaneous hypersensitivity were applied for many years for the early diagnosis of nocardial infections in animals and humans (Boiron et al., 1993; Bojalil and Zamora, 1963; Ortiz-Ortiz et al., 1976; Pier, 1984; Shainhouse et al., 1978). An enzyme-linked immunosorbent assay (ELISA) technique based on a 55-kDa protein specific for *Nocardia* allowed the detection of antibodies in over 90% of patients afflicted with either cutaneous or pulmonary nocardiosis (Angeles and Sugar, 1987). Similarly, a conventional solid-phase ELISA, based on two immunodominant antigens, was used for the diagnosis of *Nocardia brasiliensis* infections in mycetoma patients (Salinas-Carmona et al., 1993) and a partially purified 54-kDa antigen introduced for the diagnosis of nocardiosis using an immunoblot technique (Boiron and Provost, 1990a; Boiron and Stynen, 1992). Polysaccharide antigens, extracted and purified from *Nocardia asteroides* and *Nocardia brasiliensis* cells, have been used to test for cutaneous hypersensitivity (Zamora et al., 1963). Antigens in culture filtrates have been employed to detect the distribution of *Nocardia asteroides* serotypes (Pier and Fichtner, 1971, 1981) and to determine cutaneous hypersensitivity in animals and humans infected with *Nocardia asteroides, Nocardia brasiliensis*, and *Nocardia otitidiscaviarum* (Pier et al., 1968; Salman et al., 1982). In recent times, serological procedures have fallen out of favor, partly because of their low sensitivity and lack of specificity, partly because of the popularity of diagnostic molecular procedures, but also because of the limited value of individual serological techniques in detecting nocardial diseases which are now known to be caused by members of several *Nocardia* species.

TABLE 39. Phenotypic tests distinguishing between *Nocardia* species

	1. *N. asteroides* ATCC 19247^{T}	2. *N. abscessus* DSM 44432^{T}	3. *N. acidivorans* DSM 45049^{T}	4. *N. africana* DSM 44491^{T}	5. *N. alba* DSM 44684^{T}	6. *N. altamirensis* DSM 44997^{T}	7. *N. amamiensis* DSM 45066^{T}	8. *N. anemiae* DSM 44821^{T}	9. *N. aobensis* DSM 44805^{T}	10. *N. araoensis* DSM 44729^{T}	11. *N. arthritidis* DSM 44731^{T}	12. *N. asiatica* DSM 44668^{T}	13. *N. beijingensis* DSM 44636^{T}	14. *N. blackockiae* ATCC 700035^{T}	15. *N. brasiliensis* ATCC 19296^{T}	16. *N. brevicatena* DSM 43024^{T}	17. *N. caishijiensis* DSM 44831^{T}	18. *N. callitrisensis* DSM 45353^{T}	19. *N. carnea* DSM 43397^{T}	20. *N. cerradoensis* DSM 44546^{T}	21. *N. concava* IFM 0354^{T}	22. *N. coubleae* DSM 44960^{T}	23. *N. crassostreae* ATCC 700418^{T}	24. *N. cummidelens* DSM 44490^{T}	25. *N. cyriacigeorgica* DSM 44484^{T}	26. *N. elegans* DSM 44890^{T}	27. *N. exalbida* IFM 0803^{T}	28. *N. farcinica* ATCC 3318^{T}
Biochemical tests:																												
Allantoin	+	+	nr	+	nr	nr	+	nr	nr	−	+	+	+	nr	+	−	+	nr	+	+	nr	nr	−	+	−	−	+	+
Arbutin	+	+	nr	−	+	nr	−	+	+	+	+	+	+	+	+	−	−	+	−	−	+	nr	−	+	−	−	+	+
Degradation tests:																												
Esculin	+	−	+	−	nr	nr	nr	nr	nr	−	w	+	+	+	+	+	−	−	+	+	nr	nr	+	+	+	+	+	+
Adenine	−	−	nr	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	−	−	−	−	−	−	−
Arbutin	+	+	nr	−	nr	nr	−	nr	nr	−	+	+	−	nr	+	+	−	nr	−	nr	nr	nr	nr	+	nr	nr	−	+
Casein	−	−	nr	+	−	nr	−	−	−	−	−	−	−	−	+	−	−	nr	−	−	−	−	−	−	−	−	+	−
Elastin	−	−	nr	−	nr	nr	−	nr	nr	−	−	−	−	nr	+	−	−	nr	−	−	nr	nr	−	−	−	−	−	−
Hypoxanthine	−	−	nr	−	−	w	−	+	−	−	−	−	−	+	+	−	−	−	−	−	+	−	−	−	−	−	−	−
Testosterone	+	+	nr	+	+	+	+	nr	nr	+	+	+	−	nr	−	+	nr	nr	+	+	nr	+	nr	+	+	+	+	+
Tyrosine	−	−	nr	−	−	w	−	−	−	−	−	−	−	−	+	−	−	−	−	−	−	−	−	−	−	−	−	−
Uric acid	−	−	nr	−	−	+	+	+	+	+	+	w	−	+	−	−	+	nr	−	−	nr	−	−	−	+	+	+	−
Xanthine	−	−	nr	−	−	nr	−	−	−	−	−	−	+	−	−	−	−	−	−	−	+	−	−	−	−	−	−	−
Growth on sole carbon sources (1%, w/v):																												
Adonitol	−	nr	nr	nr	nr	nr	nr	+	nr	−	−	−	−	+	−	−	−	nr	−	−	nr	nr	nr	nr	−	−	nr	−
L-(+)-Arabinose	−	−	nr	−	−	−	−	+	−	−	−	−	+	−	−	−	−	nr	+	+	+	−	−	−	−	−	−	−
D-(+)-Cellobiose	−	−	−	−	nr	nr	−	nr	nr	nr	nr	nr	+	−	−	−	+	nr	−	−	nr	nr	nr	nr	−	−	nr	−
Dulcitol	nr	nr	nr	nr	nr	nr	−	nr	nr	nr	nr	nr	nr	−	nr	nr	nr	nr	−	nr	nr	nr	nr	nr	nr	nr	nr	nr
meso-Erythritol	−	−	nr	−	nr	nr	−	−	−	−	−	−	+	+	−	−	−	nr	−	−	−	nr	nr	nr	−	−	−	−
D-(−)-Fructose	+	nr	−	−	nr	+	nr	nr	nr	nr	nr	nr	+	+	+	nr	+	w	nr	+	nr	+	nr	+	nr	nr	nr	+
D-(+)-Galactose	−	−	+	−	−	+	−	+	−	−	−	−	+	−	+	−	+	+	+	−	+	+	−	−	−	−	−	−
Gluconate	+	−	nr	−	nr	nr	−	nr	nr	−	−	nr	+	nr	−	−	nr	nr	−	−	nr	nr	nr	+	+	−	−	−
D-(+)-Glucose	+	+	nr	+	+	+	+	+	+	+	+	+	+	+	+	−	+	nr	+	+	+	+	+	+	−	+	+	+
Inositol	−	−	nr	−	+	nr	−	+	−	−	−	−	−	−	+	−	−	nr	−	−	+	nr	nr	−	−	−	−	−
Inulin	nr	nr	nr	nr	nr	nr	−	nr	nr	nr	nr	nr	+	nr	nr	nr	+	nr	−	+	nr	nr	nr	nr	nr	nr	nr	nr
Lactose	−	nr	nr	nr	nr	nr	−	nr	nr	−	−	nr	+	−	−	−	−	w	−	−	nr	nr	nr	nr	−	−	nr	−
D-(+)-Maltose	−	+	−	−	+	nr	+	−	−	−	−	+	+	+	+	−	+	nr	−	+	−	+	−	−	−	−	−	+
Mannitol	−	−	+	−	+	+	−	nr	nr	+	+	w	+	+	+	−	−	−	+	−	nr	+	−	−	−	−	nr	−
D-(+)-Mannose	−	nr	+	−	+	nr	+	−	−	−	−	−	+	+	−	nr	+	nr	−	+	−	+	nr	+	nr	nr	−	+
D-(+)-Melibiose	nr	nr	−	nr	nr	nr	−	nr	nr	nr	nr	nr	−	−	nr	nr	+	nr	+	+	nr	nr	nr	nr	nr	nr	nr	−
D-(+)-Melezitose	−	nr	nr	nr	nr	nr	−	nr	nr	nr	nr	nr	−	nr	−	−	+	nr	−	−	nr	nr	nr	nr	−	−	nr	−
D-(+)-Raffinose	−	−	nr	−	−	−	−	nr	nr	nr	nr	nr	+	−	−	−	+	+	−	−	nr	+	nr	−	−	−	nr	−
L-(+)-Rhamnose	−	+	−	−	+	−	−	−	−	−	−	+	+	−	−	+	+	nr	−	+	−	−	−	−	−	−	−	+
Sorbitol	−	−	−	−	−	−	−	+	−	−	−	−	+	+	−	−	−	w	+	+	−	−	−	+	−	−	−	−
D-(+)-Sucrose	+	+	−	−	nr	−	nr	nr	nr	nr	nr	nr	+	−	w	−	+	+	−	+	nr	+	+	−	+	−	nr	+
D-(+)-Trehalose	+	+	−	−	nr	nr	+	nr	nr	nr	nr	nr	+	+	+	+	+	w	+	+	nr	nr	+	nr	−	−	nr	+
Xylitol	nr	nr	nr	nr	nr	nr	−	nr	nr	nr	nr	nr	−	nr	nr	nr	−	nr	−	−	nr	nr	nr	nr	nr	nr	nr	nr
D-(+)-Xylose	−	−	nr	−	nr	nr	−	nr	nr	−	+	nr	+	−	−	−	+	w	−	−	nr	nr	−	+	−	−	nr	−
2,3-Butanediol	−	−	nr	−	nr	nr	+	nr	nr	nr	nr	nr	−	nr	−	−	nr	nr	−	−	nr	nr	nr	nr	−	−	nr	+
Isoamyl alcohol	−	−	nr	−	nr	nr	+	nr	nr	nr	nr	nr	−	nr	w	+	nr	nr	−	−	nr	nr	nr	nr	−	−	nr	+
1,2-Propanediol	−	−	nr	−	nr	nr	+	nr	nr	nr	nr	nr	−	nr	−	+	nr	nr	−	w	nr	nr	nr	nr	−	−	nr	+
Growth on sole carbon sources at 0.1% (w/v):																												
Acetamide	nr	nr	nr	nr	nr	nr	nr	nr	nr	−	+	nr	nr	−	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	−	nr
m-Hydroxybenzoate	−	−	nr	+	nr	nr	−	nr	nr	nr	nr	nr	−	nr	−	−	nr	nr	−	+	nr	nr	nr	nr	−	−	nr	+
p-Hydroxybenzoate	−	−	nr	−	nr	nr	−	nr	nr	nr	nr	nr	+	nr	−	−	nr	nr	w	−	nr	nr	nr	nr	−	−	nr	−
Sodium acetate	+	+	nr	−	+	nr	−	nr	nr	+	+	+	+	nr	+	+	+	nr	+	+	nr	nr	−	+	+	+	nr	+
Sodium adipate	−	−	nr	nr	nr	nr	−	nr	nr	−	−	−	nr	nr	−	−	−	nr	−	nr	nr	nr	nr	nr	nr	nr	−	−
Sodium citrate	−	+	nr	−	−	nr	+	+	−	+	+	+	+	+	+	−	−	+	−	−	+	nr	−	−	−	−	+	−
Sodium pimelate	−	−	nr	nr	nr	nr	−	nr	nr	nr	nr	nr	nr	nr	−	−	nr	nr	−	nr	nr	nr	nr	+	nr	nr	nr	+

(continued)

TABLE 39. (continued)

	1. *N. asteroides* ATCC 19247^{T}	2. *N. abscessus* DSM 44432^{T}	3. *N. acidivorans* DSM 45049^{T}	4. *N. africana* DSM 44491^{T}	5. *N. alba* DSM 44684^{T}	6. *N. altamirensis* DSM 44997^{T}	7. *N. amamiensis* DSM 45066^{T}	8. *N. anemiae* DSM 44821^{T}	9. *N. aobensis* DSM 44805^{T}	10. *N. araoensis* DSM 44729^{T}	11. *N. arthritidis* DSM 44731^{T}	12. *N. asiatica* DSM 44668^{T}	13. *N. beijingensis* DSM 44636^{T}	14. *N. blackockiae* ATCC 700035^{T}	15. *N. brasiliensis* ATCC 19296^{T}	16. *N. brevicatena* DSM 43024^{T}	17. *N. caishijiensis* DSM 44831^{T}	18. *N. callitrisensis* DSM 45353^{T}	19. *N. carnea* DSM 43397^{T}	20. *N. cerradoensis* DSM 44546^{T}	21. *N. concava* IFM 0354^{T}	22. *N. coubleae* DSM 44960^{T}	23. *N. crassostreae* ATCC 700418^{T}	24. *N. cummidelens* DSM 44490^{T}	25. *N. cyriacigeorgica* DSM 44484^{T}	26. *N. elegans* DSM 44890^{T}	27. *N. exalbida* IFM 0803^{T}	28. *N. farcinica* ATCC 3318^{T}
Growth on sole nitrogen sources (0.1%, w/v):																												
L-Alanine	−	−	+	+	nr	nr	nr	nr	nr	nr	nr	nr	w	nr	+	−	+	nr	−	−	nr	nr	nr	+	−	−	nr	−
Gelatin	−	−	nr	−	nr	nr	nr	nr	nr	nr	nr	nr	−	nr	+	−	−	nr	−	−	nr	nr	nr	nr	−	−	nr	−
L-Histidine	nr	nr	+	nr	nr	nr	nr	nr	nr	nr	nr	nr	−	nr	+	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	+
L-Leucine	−	+	+	nr	nr	nr	nr	nr	nr	nr	nr	nr	−	nr	+	+	−	nr	+	+	nr	nr	nr	+	nr	nr	nr	−
L-Ornithine	nr	nr	−	nr	nr	nr	nr	nr	nr	nr	nr	nr	−	nr	−	nr	nr	nr	nr	nr	nr	nr	−	nr	nr	−	nr	−
L-Phenylalanine	nr	nr	−	nr	nr	nr	nr	nr	nr	nr	nr	nr	−	nr	−	nr	−	nr	−	−	nr	nr	nr	nr	nr	nr	nr	−
L-Proline	+	−	+	−	nr	nr	nr	nr	nr	nr	nr	nr	+	nr	+	−	+	nr	−	+	nr	nr	nr	+	−	−	nr	−
L-Serine	−	−	+	−	nr	nr	nr	nr	nr	nr	nr	nr	−	nr	w	−	+	nr	−	−	nr	nr	nr	+	−	−	nr	−
L-Tryptophan	nr	nr	−	nr	nr	nr	nr	nr	nr	nr	nr	nr	−	nr	−	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	−
L-Valine	−	−	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	+	nr	+	+	−	nr	+	+	nr	nr	−	+	nr	nr	nr	−

	29. *N. flavorosea* JCM 3332^{T}	30. *N. fluminea* DSM 44489^{T}	31. *N. gamkensis* DSM 44956^{T}	32. *N. harensis* DSM 45095^{T}	33. *N. higoensis* IFM 10084^{T}	34. *N. ignorata* DSM 44496^{T}	35. *N. inohanensis* IFM 0092^{T}	36. *N. iowensis* NRRL B-24671^{T}	37. *N. jejuensis* DSM 44959^{T}	38. *N. jiangxiensis* DSM 43401^{T}	39. *N. jinanensis* DSM 45048^{T}	40. *N. kruczakiae* DSM 44877^{T}	41. *N. lijiangensis* DSM 44933^{T}	42. *N. mexicana* DSM 44952^{T}	43. *N. miyunensis* DSM 17685^{T}	44. *N. neocaledoniensis* DSM 44717^{T}	45. *N. niigatensis* DSM 44670^{T}	46. *N. ninae* DSM 44978^{T}	47. *N. nova* JCM 6044^{T}	48. *N. otitidiscaviarum* NCTC 1934^{T}	49. *N. paucivorans* DSM 44386^{T}	50. *N. pigrifrangens* JCM 11884^{T}	51. *N. pneumoniae* IFM 0784^{T}	52. *N. polyresistens* DSM 44932^{T}	53. *N. pseudobrasiliensis* ATCC 51512^{T}	54. *N. pseudovaccinii* DSM 43406^{T}	55. *N. puris* DSM 44599^{T}	56. *N. salmonicida* DSM 40472^{T}
Biochemical tests:																												
Allantoin	−	+	nr	−	+	+	nr	nr	+	+	−	nr	nr	nr	+	+	nr	nr	+	+	+	+	nr	−	−	nr	nr	+
Arbutin	−	−	+	+	+	+	+	nr	+	−	−	+	−	nr	−	+	nr	+	+	+	+	+	−	+	+	nr	+	+
Degradation tests:																												
Esculin	−	+	+	+	nr	+	nr	+	+	+	+	+	−	nr	+	+	nr	nr	−	+	−	−	−	−	+	nr	+	+
Adenine	−	−	−	nr	−	−	−	nr	−	+	nr	−	−	+	−	nr	−	+	−	−	−	−	−	−	+	nr	−	−
Arbutin	+	+	w	nr	nr	nr	nr	nr	nr	+	nr	nr	nr	nr	−	nr	nr	nr	+	+	−	+	+	nr	−	nr	nr	+
Casein	−	−	+	−	−	−	−	−	+	−	nr	−	nr	−	+	−	−	−	−	−	−	−	−	−	+	nr	−	−
Elastin	−	−	nr	−	nr	−	nr	nr	−	−	−	nr	nr	nr	−	−	nr	nr	−	−	−	−	−	+	+	nr	−	−
Hypoxanthine	−	−	−	−	−	−	+	+	+	−	nr	−	+	+	−	−	w	+	−	+	−	−	−	−	+	nr	−	−
Testosterone	+	+	nr	nr	nr	+	nr	nr	+	nr	nr	nr	nr	−	nr	nr	nr	+	−	+	−	nr	+	nr	−	nr	+	+
Tyrosine	−	+	+	−	−	−	−	+	+	−	nr	−	+	−	−	+	−	−	−	−	−	−	−	−	+	nr	−	+
Uric acid	+	−	nr	+	nr	−	+	+	+	nr	−	nr	nr	+	nr	−	nr	+	−	+	−	+	−	nr	+	nr	+	−
Xanthine	−	−	−	−	−	−	−	+	−	−	nr	−	+	−	−	−	−	nr	−	+	−	−	−	−	−	nr	−	−
Growth on sole carbon sources (1%, w/v):																												
Adonitol	−	nr	−	−	nr	−	−	−	−	−	nr	−	+	+	−	−	−	nr	−	−	−	−	−	+	nr	nr	−	nr
L-(+)-Arabinose	−	nr	−	−	−	−	−	−	+	+	−	−	+	+	+	−	−	+	−	−	−	+	−	+	−	nr	−	−
D-(+)-Cellobiose	−	nr	w	w	nr	−	nr	nr	−	+	+	−	+	nr	+	−	−	nr	−	−	−	+	nr	+	−	nr	−	−
Dulcitol	+	nr	nr	w	nr	nr	nr	nr	−	nr	nr	−	+	nr	nr	nr	nr	nr	nr	nr	nr	+	nr	+	nr	nr	nr	−
meso-Erythritol	+	nr	−	−	−	−	−	nr	−	w	nr	−	nr	nr	w	nr	−	nr	−	−	−	+	−	nr	−	nr	−	−
D-(−)-Fructose	−	nr	+	w	nr	+	nr	+	+	+	+	−	+	+	+	+	−	nr	−	nr	nr	+	nr	+	+	nr	nr	+
D-(+)-Galactose	−	+	w	nr	−	−	−	nr	−	+	+	−	+	+	+	−	−	+	+	−	−	−	−	+	+	−	−	−

(continued)

TABLE 39. (continued)

	29. *N. flavorosea* JCM 3332^T	30. *N. fluminea* DSM 44489^T	31. *N. gamkensis* DSM 44956^T	32. *N. harensis* DSM 45095^T	33. *N. higoensis* IFM 10084^T	34. *N. ignorata* DSM 44496^T	35. *N. inohanensis* IFM 0092^T	36. *N. iowensis* NRRL B-24671^T	37. *N. jejuensis* DSM 44959^T	38. *N. jiangxiensis* DSM 43401^T	39. *N. jinanensis* DSM 45048^T	40. *N. kruczakiae* DSM 44877^T	41. *N. lijiangensis* DSM 44933^T	42. *N. mexicana* DSM 44952^T	43. *N. miyunensis* DSM 17685^T	44. *N. neocaledoniensis* DSM 44717^T	45. *N. niigatensis* DSM 44670^T	46. *N. ninae* DSM 44978^T	47. *N. nova* JCM 6044^T	48. *N. otitidiscaviarum* NCTC 1934^T	49. *N. paucivorans* DSM 44386^T	50. *N. pigrifrangens* JCM 11884^T	51. *N. pneumoniae* IFM 0784^T	52. *N. polyresistens* DSM 44932^T	53. *N. pseudobrasiliensis* ATCC 51512^T	54. *N. pseudovaccinii* DSM 43406^T	55. *N. puris* DSM 44599^T	56. *N. salmonicida* DSM 40472^T
Gluconate	−	+	nr	nr	nr	+	−	nr	nr	nr	nr	nr	nr	nr	nr	nr	−	nr	−	w	−	+	−	nr	−	−	−	+
D-(+)-Glucose	−	+	+	+	−	+	+	+	+	nr	+	+	+	+	+	+	+	+	+	+	−	+	+	+	+	nr	+	+
Inositol	−	+	−	−	−	−	+	+	+	nr	−	−	+	+	+	nr	nr	nr	−	+	−	nr	−	+	+	+	+	−
Inulin	−	nr	w	−	nr	nr	nr	nr	+	nr	+	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	+	nr	nr	nr	nr	nr	−
Lactose	−	nr	w	−	nr	−	nr	−	+	+	nr	−	+	nr	+	−	nr	nr	−	−	−	−	−	+	−	nr	−	−
D-(+)-Maltose	−	nr	nr	w	−	+	−	+	−	+	nr	+	+	−	+	−	−	w	−	−	−	−	−	+	−	nr	+	−
Mannitol	−	−	−	w	nr	+	nr	−	−	+	w	−	+	+	−	+	−	−	−	+	−	−	−	+	+	nr	+	+
D-(+)-Mannose	−	nr	w	−	−	+	+	nr	−	+	+	−	+	+	−	+	−	−	−	nr	nr	+	−	+	+	nr	nr	nr
D-(+)-Melibiose	−	nr	w	−	nr	nr	nr	−	+	+	nr	−	+	nr	+	−	−	nr	nr	nr	nr	−	nr	+	−	nr	nr	nr
D-(+)-Melezitose	−	nr	−	−	nr	−	nr	nr	+	+	+	nr	+	nr	+	nr	nr	nr	−	−	−	−	nr	+	−	nr	−	−
D-(+)-Raffinose	−	nr	−	−	nr	−	nr	nr	−	+	+	−	+	−	+	+	nr	−	−	−	−	−	nr	+	−	nr	−	−
L-(+)-Rhamnose	−	+	−	−	−	−	−	−	−	+	w	−	−	+	+	−	−	−	−	−	−	−	−	−	−	−	−	−
Sorbitol	−	−	nr	−	−	−	−	nr	+	+	+	−	−	+	+	−	−	−	+	−	−	−	−	−	+	nr	+	+
D-(+)-Sucrose	−	+	−	+	nr	+	nr	−	+	+	nr	−	+	nr	w	−	−	nr	−	−	−	+	nr	+	+	−	+	−
D-(+)-Trehalose	−	nr	w	+	nr	+	nr	+	−	+	+	−	−	nr	+	+	−	nr	−	w	+	+	nr	−	+	nr	−	+
Xylitol	−	nr	w	−	nr	nr	nr	nr	−	nr	nr	nr	+	nr	nr	nr	nr	nr	nr	nr	nr	−	nr	+	nr	nr	nr	nr
D-(+)-Xylose	−	+	−	+	nr	−	nr	−	−	+	−	−	+	−	+	−	nr	nr	−	−	−	+	−	+	−	nr	−	−
2,3-Butanediol	+	nr	nr	−	nr	−	nr	nr	−	nr	nr	nr	nr	nr	nr	nr	nr	nr	−	−	−	−	nr	nr	nr	nr	−	−
Isoamyl alcohol	nr	nr	nr	nr	nr	−	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	+	−	−	−	nr	nr	nr	nr	−	+
1,2-Propanediol	nr	nr	nr	−	nr	−	nr	nr	−	nr	nr	nr	nr	nr	nr	nr	nr	nr	−	−	−	−	nr	nr	nr	nr	−	−
Growth on sole carbon sources at 0.1% (w/v):																												
Acetamide	nr	nr	nr	nr	nr	−	nr	nr	nr	nr	nr	−	nr	nr	nr	nr	nr	nr	nr	nr	−	nr	−	nr	nr	nr	−	−
m-Hydroxybenzoate	−	−	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	−	−	−	−	nr	nr	+	−	−	−
p-Hydroxybenzoate	−	−	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	−	−	−	+	nr	nr	−	+	−	−
Sodium acetate	+	−	+	−	nr	+	nr	nr	nr	−	−	nr	−	nr	−	−	−	nr	+	+	+	+	+	−	+	nr	+	−
Sodium adipate	nr	nr	nr	nr	nr	−	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	−	−	−	nr	−	nr	nr	nr	−	nr
Sodium citrate	−	+	+	−	−	−	+	nr	nr	+	−	−	−	nr	+	−	−	nr	−	−	−	−	+	−	+	−	+	+
Sodium pimelate	−	−	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	+	+	nr	nr	nr	−	nr	nr	−
Growth on sole nitrogen sources (0.1%, w/v):																												
L-Alanine	+	+	nr	nr	nr	−	nr	nr	nr	+	nr	nr	+	nr	+	nr	−	nr	−	+	−	+	nr	+	+	+	+	−
Gelatin	nr	nr	nr	nr	nr	+	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	−	−	−	−	nr	nr	nr	nr	−	−
L-Histidine	nr	nr	+	nr	nr	nr	nr	nr	nr	−	nr	nr	+	nr	−	nr	−	nr	nr	nr	nr	nr	nr	+	nr	nr	nr	nr
L-Leucine	−	+	nr	nr	nr	nr	nr	nr	nr	−	nr	nr	nr	nr	+	nr	−	nr	nr	+	+	−	nr	nr	−	+	nr	+
L-Ornithine	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	−	nr
L-Phenylalanine	−	nr	+	nr	nr	nr	nr	nr	nr	nr	+	nr	+	nr	nr	nr	nr	nr	nr	nr	nr	−	nr	−	nr	nr	nr	−
L-Proline	+	+	−	nr	nr	+	nr	nr	nr	−	nr	nr	+	nr	+	nr	−	nr	−	−	−	+	nr	w	+	−	−	+
L-Serine	+	+	+	nr	nr	−	nr	nr	nr	−	nr	nr	nr	nr	−	nr	−	nr	−	w	−	nr	nr	nr	+	−	−	+
L-Tryptophan	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	+	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	−	nr	nr	nr	nr
L-Valine	−	+	+	nr	nr	nr	nr	nr	nr	nr	−	nr	+	nr	nr	nr	nr	nr	nr	−	+	+	nr	+	+	+	nr	+

(continued)

TABLE 39. (continued)

	57. *N. seriolae* JCM 3360^T	58. *N. shimofusensis* DSM 44733^T	59. *N. sienata* IFM 10088^T	60. *N. soli* DSM 44488^T	61. *N. speluncae* DSM 45078^T	62. *N. takedensis* DSM 44801^T	63. *N. tenerifensis* DSM 44704^T	64. *N. terpenica* IFM 0706^T	65. *N. testacea* IFM 0937^T	66. *N. thailandica* IFM 10145^T	67. *N. transvalensis* DSM 43405^T	68. *N. uniformis* JCM 3224^T	69. *N. vaccinii* DSM 43285^T	70. *N. vermiculata* IFM 0391^T	71. *N. veterana* DSM 44445^T	72. *N. vinacea* IFO 16497^T	73. *N. wallacei* ATCC 49873^T	74. *N. xishanensis* DSM 44895^T	75. *N. yamanashiensis* IFM 0265^T
Biochemical tests:																			
Allantoin	+	nr	+	+	+	+	nr	−	+	nr	+	+	+	nr	−	+	nr	+	nr
Arbutin	−	+	−	+	−	+	+	+	−	−	+	+	+	−	+	+	nr	+	+
Degradation tests:																			
Esculin	+	nr	+	+	+	+	+	nr	+	nr	+	+	−	nr	w	w	+	+	nr
Adenine	−	−	−	−	nr	−	−	+	−	−	−	−	−	−	−	−	−	−	−
Arbutin	+	nr	nr	+	nr	nr	nr	nr	nr	nr	+	+	−	nr	nr	nr	nr	+	nr
Casein	−	−	−	−	+	−	+	+	−	−	−	−	−	−	−	−	−	−	−
Elastin	−	nr	−	−	−	−	nr	nr	w	−	+	+	−	nr	−	−	nr	−	nr
Hypoxanthine	−	−	−	−	−	−	+	+	−	−	+	+	−	−	−	+	+	−	+
Testosterone	−	nr	nr	+	nr	nr	nr	nr	nr	nr	+	+	−	nr	+	+	nr	nr	nr
Tyrosine	−	−	−	−	−	−	+	+	−	−	−	+	−	−	−	−	−	−	−
Uric acid	−	nr	−	−	+	+	+	nr	−	−	+	+	−	nr	+	+	+	+	+
Xanthine	−	−	−	−	−	−	−	−	−	−	−	+	−	−	−	−	−	−	−
Growth on sole carbon sources (1%, w/v):																			
Adonitol	−	nr	−	nr	−	−	−	+	−	nr	+	−	−	nr	nr	+	−	−	−
L-(+)-Arabinose	−	−	−	−	−	−	−	−	−	−	−	−	+	−	−	+	−	−	−
D-(+)-Cellobiose	nr	nr	−	nr	−	−	nr	nr	+	nr	−	−	−	nr	−	nr	−	+	nr
Dulcitol	nr	nr	−	nr	−	nr	nr	nr	−	nr	nr	−	nr	nr	nr	nr	−	−	nr
meso-Erythritol	−	−	−	nr	−	−	nr	−	−	−	+	nr	−	−	−	−	−	−	−
D-(−)-Fructose	nr	nr	nr	+	−	−	−	nr	nr	nr	+	+	+	nr	−	nr	−	+	nr
D-(+)-Galactose	−	−	−	−	−	+	+	+	−	−	+	−	+	−	−	+	+	+	−
Gluconate	−	nr	−	+	nr	−	−	nr	−	−	+	−	+	−	−	nr	nr	nr	+
D-(+)-Glucose	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Inositol	−	−	−	−	−	−	+	+	−	−	−	+	−	−	+	+	−	w	+
Inulin	nr	nr	−	nr	−	nr	nr	nr	−	nr	nr	−	nr	nr	nr	nr	nr	−	nr
Lactose	nr	nr	nr	nr	−	nr	−	nr	nr	nr	+	−	−	nr	nr	nr	−	−	nr
D-(+)-Maltose	−	−	−	−	+	nr	−	−	−	−	+	−	−	−	−	−	+	+	−
Mannitol	−	nr	−	−	+	−	+	nr	−	nr	+	−	+	nr	−	+	−	−	nr
D-(+)-Mannose	−	−	+	+	−	+	−	−	−	−	+	+	+	−	−	−	−	w	−
D-(+)-Melibiose	nr	nr	+	nr	−	−	−	nr	−	nr	nr	−	nr	nr	nr	nr	−	+	nr
D-(+)-Melezitose	nr	nr	−	nr	−	−	nr	nr	−	nr	nr	−	−	nr	nr	nr	nr	−	nr
D-(+)-Raffinose	nr	nr	nr	−	nr	−	nr	nr	nr	nr	−	−	−	nr	−	nr	−	−	nr
L-(+)-Rhamnose	−	−	−	+	−	−	−	−	+	−	+	−	+	−	+	−	−	+	−
Sorbitol	−	−	−	−	−	−	−	+	−	−	+	−	−	−	−	+	−	w	−
D-(+)-Sucrose	−	nr	nr	+	+	+	−	nr	nr	nr	+	−	−	nr	−	nr	+	+	nr
D-(+)-Trehalose	−	nr	nr	nr	+	−	+	nr	nr	nr	+	−	+	nr	−	nr	+	+	nr
Xylitol	nr	nr	−	nr	−	nr	nr	nr	−	nr	nr	−	nr	nr	nr	nr	nr	−	nr
D-(+)-Xylose	nr	nr	+	+	−	−	nr	nr	+	−	−	−	+	nr	−	nr	−	−	nr
2,3-Butanediol	nr	nr	+	nr	+	+	nr	nr	+	nr	−	nr	−	nr	+	nr	nr	nr	nr
Isoamyl alcohol	nr	nr	nr	nr	nr	−	nr	nr	nr	nr	+	nr	−	nr	−	nr	nr	nr	nr
1,2-Propanediol	nr	nr	nr	nr	−	−	nr	nr	nr	nr	−	nr	−	nr	+	nr	nr	nr	nr
Growth on sole carbon sources at 0.1% (w/v):																			
Acetamide	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	−	nr	nr
m-Hydroxybenzoate	+	nr	nr	nr	nr	−	nr	nr	nr	nr	w	−	−	nr	−	nr	nr	nr	nr
p-Hydroxybenzoate	−	nr	nr	nr	nr	−	+	nr	nr	nr	−	−	w	nr	−	nr	nr	nr	nr
Sodium acetate	+	nr	+	−	+	−	nr	nr	+	nr	+	−	−	nr	−	−	nr	+	nr
Sodium adipate	nr	nr	nr	nr	nr	−	−	nr	nr	nr	nr	nr	−	nr	nr	nr	nr	+	nr
Sodium citrate	+	−	−	−	−	−	+	+	−	−	−	−	+	−	−	+	+	−	+
Sodium pimelate	−	nr	nr	−	nr	nr	nr	nr	nr	nr	−	−	−	nr	−	nr	nr	nr	nr

(continued)

TABLE 39. (continued)

	57. *N. seriolae* JCM 3360^T	58. *N. shimofusensis* DSM 44733^T	59. *N. sienata* IFM 10088^T	60. *N. soli* DSM 44488^T	61. *N. speluncae* DSM 45078^T	62. *N. takedensis* DSM 44801^T	63. *N. tenerifensis* DSM 44704^T	64. *N. terpenica* IFM 0706^T	65. *N. testacea* IFM 0937^T	66. *N. thailandica* IFM 10145^T	67. *N. transvalensis* DSM 43405^T	68. *N. uniformis* JCM 3224^T	69. *N. vaccinii* DSM 43285^T	70. *N. vermiculata* IFM 0391^T	71. *N. veterana* DSM 44445^T	72. *N. vinacea* IFO 16497^T	73. *N. wallacei* ATCC 49873^T	74. *N. xishanensis* DSM 44895^T	75. *N. yamanashiensis* IFM 0265^T
Growth on sole nitrogen sources (0.1%, w/v):																			
L-Alanine	+	nr	nr	+	nr	nr	+	nr	nr	nr	–	+	–	nr	+	nr	nr	+	nr
Gelatin	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	–	nr	–	nr	–	nr	nr	nr	nr
L-Histidine	nr	nr	nr	nr	nr	nr	+	nr	nr	nr	–	–	nr	nr	nr	nr	nr	nr	nr
L-Leucine	+	nr	nr	+	nr	nr	+	nr	nr	nr	–	–	–	nr	–	nr	nr	–	nr
L-Ornithine	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	–	nr	nr	nr	nr	nr	nr	nr	nr
L-Phenylalanine	nr	nr	–	nr	–	nr	nr	nr	–	nr	–	nr	nr	nr	nr	nr	nr	–	nr
L-Proline	+	nr	nr	+	nr	nr	+	nr	nr	nr	+	+	–	nr	+	nr	nr	+	nr
L-Serine	–	nr	nr	+	nr	nr	+	nr	nr	nr	+	–	–	nr	–	nr	nr	–	nr
L-Tryptophan	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	–	–	nr	nr	nr	nr	nr	nr	nr
L-Valine	–	nr	–	+	+	nr	nr	nr	–	nr	–	+	–	nr	–	nr	nr	+	nr

Symbols: +, positive reaction; –, negative reaction; w, weak reaction; nr, not reported.

Reliable typing methods are needed to establish the infection source and mode of transmission of clinically significant nocardiae, notably with respect to hospital acquired infections (Brown-Elliott et al., 2006; McNeil and Brown, 1994; Schaal and Lee, 1992). Serological typing of *Nocardia asteroides* is a useful epidemiological tool, as shown by investigations on group infections. Studies on herds of dairy cattle where cases of mastitis were evident showed that the infecting causal agents were usually of the same serotype implying animal-to-animal transmission (Pier and Fichtner, 1981). Similarly, an outbreak of nocardiosis in immunocompromised patients in a renal unit was attributed to a specific *Nocardia asteroides* serotype (Stevens et al., 1981). All of the strains isolated from the environment of the renal unit were of the same serotype whereas corresponding serotypes from different areas of the hospital and from nocardiosis patients from other hospitals were a more random mix. These results were indicative of a patient-to-environment-to patient cycle of infection in the renal unit.

Nocardia asteroides strains have been typed using plasmid (Jonsson et al., 1986) and restriction fragment length polymorphism (RFLP; Patterson et al., 1992) profiles. These latter workers showed that 18 out of 19 clinical isolates shared an identical RFLP pattern using the restriction enzyme *Pvu*III. Pulsed field gel electrophoresis has been used to distinguish between *Nocardia asteroides* strains implicated in a suspected outbreak of nocardiosis (Louie et al., 1997). This technique was used to show that a *Nocardia farcinica* strain was responsible for postoperative wound infections in a hospital surgical ward in Germany (Blümel et al., 1998). These investigators found that organisms with the same genotype were present in air samples taken from the same location and were the causal agents of additional pulmonary infections in an area close to the surgical ward.

List of species of the genus *Nocardia*

1. **Nocardia asteroides** (Eppinger 1891) Blanchard 1896, 856 (Approved Lists 1980) ("*Cladothrix asteroides*" Eppinger 1891: "*Streptothrix eppingerii*" (*sic*) Rossi-Doria 1891; "*Streptothrix asteroids*" (*sic*) (Eppinger 1891) Gasperini 1892): "*Oospora asteroides*" (Eppinger 1891) Sauvageau and Radais 1892; "*Actinomyces asteroides*" (Eppinger 1891) Gasperini 1894; "*Actinomyces eppingeri*" (Rossi-Doria 1891) Berestnev 1897; *Discomyces asteroides* (Eppinger 1891) Gedoelst 1902; "*Actinomyces eppinger*" (*sic*) (Rossi-Doria 1891) Namyslowski 1912; "*Asteroides asteroides*" (Eppinger 1891) Puntoni and Leonardi 1936; "*Proactinomyces asteroids*" (Eppinger 1891) Baldacci 1937.

as′te.ro′i.des. N.L. fem. adj. *asteroides* (from Gr. adj. *asteroeides -es*), star-like.

Acid–alcohol-fast actinomycetes which form a branched substrate mycelium that fragments into coccoid to rod-shaped elements. An orange to red substrate mycelium carries sparse to white aerial hyphae. Grows at 30°C but not at 45°C. Proline is used as simultaneous carbon and nitrogen source, but not acetamide, L-alanine, gelatin, or serine. 2-Deoxythymidine-5′-*p*-nitroanilide (*p*NA) phosphate, *p*-nitrophenyl (pNP) phosphorylcholine, and *p*NP-β-D-xyloside are hydrolyzed. Susceptible to amikacin, and amoxicillin-clavulanic acid, but

is resistant to ampicillin, cefulaxime, cefuroxime, chloramphenicol, clindamycin, erythromycin, gentamicin, kanamycin, minocycline, perfloxacin, tetracycline, sulfonamides, trimethoprim, and trimethoprim-sulfamethoxazole (Boiron and Provost, 1988b). The type strain is sensitive (in μg/ml) to bacitracin (100) and polymyxin (5), but is resistant to amoxicillin (10), ampicillin (5), cephaloridine (10), doxycycline (10), erythromycin (10), kanamycin (5), lincomycin (10), lividomycin (10), neomycin (10), novobiocin (100), paromomycin (100), penicillin (10), streptomycin (10), and sulfamethoxazole (100) (Hamid et al., 2001b) and is sensitive to (in μg/ml per disc) imipenem (2.5) and tobramycin (2.5), slightly sensitive to kanamycin (30), but resistant to 5-fluorouracil (Kageyama et al., 2005d). Grows in the presence (%, w/v) of sodium chloride (5), but is inhibited by pyronin G (0.1), sodium azide (0.01), sodium chloride (7), sodium nitrate (0.1), tetrazolium salt (0.1) and thallous acetate (0.001).

Additional phenotypic properties are cited in Table 39. Whole-cell hydrolysates contain arabinose and galactose as characteristic sugars and *meso*-diaminopimelic acid as the principal diamino acid. Muramic acid moieties are *N*-glycolated. Cellular fatty acids contain major proportions of $C_{16:0}$, $C_{16:1}$ *cis*9, $C_{18:1}$ *cis*9, and $C_{18:0}$ 10-methyl, smaller proportions (<10%) of $C_{15:0}$, $C_{17:1}$ *cis*9, $C_{17:0}$, $C_{18:0}$, and -$C_{19:1}$ *cis*-10, and traces of $C_{14:0}$, -$C_{15:1}$ *cis*10, and $C_{20:0}$. Mycolic acids have 46–60 carbon atoms with 0–3 double bonds. Pyrolysis gas chromatography of purified mycolic acid methyl esters release fatty acid methyl esters ($C_{12:0}$–$C_{18:0}$). Menaquinone and polar lipid profiles are typical of the genus. DNA–DNA relatedness values between the type strain and corresponding strains of related species are as follows: (i) *Nocardia farcinica* (25%), *Nocardia nova* (27%), *Nocardia salmonicida* (57%), and *Nocardia seriolae* (57%) (Kudo et al., 1988), and (ii) *Nocardia abscessus* (8%), *Nocardia nova* (10%), *Nocardia paucivorans* (9), *Nocardia pseudobrasiliensis* (11%), *Nocardia transvalensis* (12%), *Nocardia vaccinii* (9%), and *Nocardia vinacea* (10%) (Kinoshita et al., 2001).

DNA G+C content (mol%): 63–69 (T_m).

Type strain: ATCC 19247, CCUG 10073, CIP 104503, DSM 43757, IFO (now NBRC) 15531, IMET 7547, JCM 3384, NCTC 11293, NRRL B-3828.

Sequence accession no. (16S rRNA gene): AF430019.

Additional remarks: a range of additional phenotypic properties are cited in extensive numerical taxonomic studies (Goodfellow, 1971; Orchard and Goodfellow, 1980; Tsukamura, 1982a).

2. **Nocardia abscessus** Yassin, Rainey, Mendrock, Brzezinka and Schaal 2000b, 1492[VP]

abs.ces′sus. L. gen. masc. n. *abscessus* of or from an abscess, referring to the characteristic clinical conditions from which the organisms were isolated.

Slightly acid–alcohol-fast actinomycetes which form an extensive, orange colored substrate mycelium that bears abundant white aerial hyphae. Towards the end of the growth cycle filaments fragment into rod-shaped elements. Mature colonies measure 0.5–0.1 mm in diameter. Does not grow at 45°C. Does not hydrolyze 2-deoxythymidine-5-*p*-nitrophenyl (*p*NP) phosphate, *p*NP-phosphorylcholine, or *p*NP-β-D-xyloside. Does not use acetamide, L-alanine, gelatin, proline, or serine as simultaneous carbon and nitrogen sources. Susceptible (μg per disc) to tobramycin (2.5), slightly susceptible to imipenem (10), moderately susceptible to kanamycin (10), but resistant to 5-fluorouracil (30) (Kageyama et al., 2005d). Additional phenotypic properties are cited in Table 39.

Whole-cell hydrolysates contain arabinose and galactose as characteristic sugars and *meso*-diaminopimelic acid as the principal diamino acid. Cellular fatty acids contain major proportions of $C_{16:0}$, $C_{18:0}$, and $C_{18:0}$ 10-methyl, and smaller proportions (<10%) of $C_{16:1}$, $C_{18:1}$ *cis*9, $C_{18:0}$, and traces of $C_{14:0}$, $C_{15:0}$, $C_{16:0}$10-methyl, $C_{17:0}$, $C_{20:0}$, $C_{20:1}$, and $C_{21:1}$. Mycolic acids have 46–56 carbon atoms with 0–4 double bonds. Pyrolysis gas chromatography of purified mycolic acid methyl esters release fatty acid methyl esters ($C_{14:0}$ and $C_{16:0}$). The polar lipid and menaquinone profiles are typical for the genus. DNA–DNA relatedness values between *Nocardia abscessus* strains and the type strains of *Nocardia asteroides* and *Nocardia paucivorans* are in the range 23.7–56.8 and 32.0–60.0%, respectively (Yassin et al., 2000b). Similarly, the values between the type strain and corresponding strains of closely related species, namely *Nocardia asteroides, Nocardia nova, Nocardia paucivorans, Nocardia pseudobrasiliensis, Nocardia transvalensis, Nocardia vaccinii*, and *Nocardia vinacea*, fall within the range 4–8% (Kinoshita et al., 2001).

Source (type strain): a joint abscess of a patient with a complete endoprosthesis of one of his knees.

DNA G+C content (mol%): 67.5–69.8.1 (HPLC).

Type strain: ATCC BAA-279, CCUG 45938, CIP 106822, DSM 44432, IMMIB D-1592, JCM 10984, NBRC 100374.

Sequence accession no. (16S rRNA gene): AF218292.

3. **Nocardia acidivorans** Kämpfer, Huber, Buczolits, Thummes, Grün-Wollny and Busse 2007, 1187[VP]

a.cid.i.vo′rans. N.L. n. *acidum* (from L. adj. *acidus* sour) an acid; L. v. *vorare* to devour; N.L. part. adj. *acidivorans* acid-devouring.

A branched substrate mycelium fragments into rod-shaped elements. A light orange substrate mycelium bears yellowish-white aerial hyphae. Grows well on nutrient agar at 25–30°C. L-Alanine-*p*-nitroanilide (*p*NA), *p*-nitrophenyl (*p*NP)-β-D-glucopyranoside, *bis*-*p*NP-phosphate and *p*NP-β-D-xylanopyranoside are hydrolyzed, but not 2-deoxythimidine-5′-*p*NP phosphate, *p*NP-β-D-galactopyranoside, *p*NP-α-D-glucopyranoside, *p*NP-β-D-glucuronide, L-glutamate, L-glutamate-γ-3 carbonyl *p*NA, *p*NP-phosphorylcholine, or L-proline *p*NA. Additional phenotypic properties are cited in Table 39.

Cellular fatty acids contain major proportions of $C_{15:0}$, $C_{16:0}$, $C_{17:1}$ ω8*c* cis8 and 10-methyl $C_{17:0}$, and smaller proportions (<10%) of $C_{14:0}$, $C_{17:0}$, $C_{15:1}$ *cis*5, $C_{18:1}$ *cis*9, 10-methyl $C_{18:0}$, and 10-methyl $C_{19:0}$. The polar lipid and menaquinone profiles are typical for the genus. DNA–DNA relatedness values between the type strain and the corresponding strains of related species are as follows: *Nocardia jiangsiensis* (50.9%), *Nocardia miyunensis* (41.7%), *Nocardia niigatensis* (11.0%), *Nocardia nova* (62.6%), *Nocardia pseudobrasiliensis*

(46.1%), and *Nocardia uniformis* (21.0%) (Kämpfer et al., 2007).

Source: soil collected from the island of Stramboli, Italy.

DNA G+C content (mol%): not determined.

Type strain: CCUG 53410, CIP 109315, DSM 45049, GW4–1778, JCM 14671.

Sequence accession no. (16S rRNA gene): AM402972.

Additional remarks: the carbon source utilization profile of the type strain and corresponding strains of related species have been reported (Kämpfer et al., 2007).

4. **Nocardia africana** Hamid, Maldonado, Sharaf Eldin, Mohamed, Saeed and Goodfellow 2001b, 1229[VP] (Effective publication: Hamid, Maldonado, Sharaf Eldin, Mohamed, Saeed and Goodfellow 2001a, 627.)

a.fri.ca′na. L. fem. adj. *africana* African, of Africa, the source of the isolates.

Acid–alcohol-fast actinomycetes which form a branched substrate mycelium that fragments into irregular rod-shaped elements. Sparse white aerial hyphae are occasionally seen. Orange wrinkled colonies are formed on glucose-yeast extract agar. Does not produce diffusible pigments. Grows at 20–45°C. L-Alanine is used as a simultaneous carbon and nitrogen source, but not acetamide, gelatin, proline, or serine. Susceptible (μg/ml) to bacitracin (100), cephaloridine (10), gentamicin (10), kanamycin (5), lincomycin (10), lividomycin (100), paromomycin (100), polymyxin (5), streptomycin (100), and sulfamethoxazole (100), but not to amoxicillin (10), ampicillin (5), cephaloridine (100), doxycycline (10), erythromycin (100), gentamicin (100), lincomycin (100), neomycin (100), novobiocin (10), penicillin (100), and spiramycin (5) (Hamid et al., 2001b), and susceptible to (μg per disc) 5-fluorouracil (30), imipenem (2.5), kanamycin (5.0), and tobramycin (10) (Kageyama et al., 2005d). Grows in the presence (%, w/v) of sodium nitrate (0.1), tetrazolium salt (0.1) and thallous acetate (0.001), but is inhibited by pyronin G (0.1), sodium azide (0.01), and sodium chloride (7). Additional phenotypic properties are cited in Table 39.

Whole-cell hydrolysates contain arabinose and galactose as characteristic sugars and *meso*-diaminopimelic acid as the principal diamino acid. Cellular fatty acids contain major proportions of $C_{16:0}$, $C_{18:1}$ *cis*9, $C_{18:0}$, and $C_{18:0}$ 10-methyl, and smaller proportions (<10%) of $C_{16:1}$ *cis*9, and $C_{17:0}$. Mycolic acids co-migrate on one-dimensional thin-layer-chromatography of whole-organism methanolysates with those of reference *Nocardia* strains. The menaquinone profile is typical for the genus. DNA–DNA relatedness values between the type strain and corresponding strains of related species are as follows: (i). *Nocardia vinacea* (Alburquerque de Barros et al., 2003), and (ii) *Nocardia cerradoensis* (12%), *Nocardia nova* (2%), *Nocardia vaccinii* (10%), *Nocardia vermiculata* (4%), *Nocardia veterana* (4%), and *Nocardia vinacea* (2) (Kageyama et al., 2004c).

Source: sputa of patients with pulmonary disease.

DNA G+C content (mol%): not determined.

Type strain: SD 769, ATCC BAA-280, CCUG 46122, DSM 44491, JCM 11438, NBRC 100379, NCTC 13181.

Sequence accession no. (16S rRNA gene): AF430054.

5. **Nocardia alba** Li, Jiang, Kroppenstedt, Xu and Jiang 2004b (Effective publication: Li, Jiang, Kroppenstedt, Xu and Jiang, 2004a, 310.)

al′ba. L. fem. adj. *alba* white color.

Substrate and aerial hyphae fragment irregularly into rod-shaped elements. Grows well on glucose-asparagine, inorganic salts-starch, oatmeal, and yeast extract-malt extract agars (ISP media 5, 4, 3, and 2, respectively); diffusible pigments are not produced on any of these media. Pinkish white aerial mycelium and yellow-white substrate mycelium are formed on ISP media 3 and 4. Optimal growth temperature and pH are 28°C and pH 7.0, respectively. Susceptible (μg per disc) to 5-fluorouracil (30), impenem (2.5), kanamycin (5.0), and tobramycin (2.5), and 5-fluorouracil (Hoshino et al., 2007). Additional phenotypic properties are cited in Table 39.

Whole-cell hydrolysates contain arabinose and galactose as characteristic sugars and *meso*-diaminopimelic acid as the principal diamino acid. The predominant fatty acids are $C_{16:0}$, $C_{16:1}$ *cis*9, $C_{18:1}$ *cis*9, and $C_{18:0}$ 10-methyl. Mycolic acids have 48–56 carbon atoms with C52 as the major component. The polar lipid and menaquinone profiles are typical of the genus. The DNA–DNA relatedness value between the type strain and the corresponding strain of *Nocardia ninae*, a closely related species, is below 20% (Laurent et al., 2007).

Source: soil collected in Yunnan Province, China.

DNA G+C content (mol%): 74 (T_m).

Type strain: CCTCC AA 001030, DSM 44684, JCM 13373, YIM 30243.

Sequence accession no. (16S rRNA gene): AY222321.

Additional remarks: the type strain CCTCC AA 001030 was cited as CCTCC AA001030 in the effective publication (Lists Editor, 2004b).

6. **Nocardia altamirensis** Jurado, Boiron, Kroppenstedt, Laurent, Couble. Laiz Klenk, González, Saiz-Jimenez, Mouniée, Bergeron and Rodriguez-Nava 2008, 2212[VP]

al.ta.mi.ren′sis. N.L. fem. adj. *altamirensis* of or belonging to the Altamira cave, Cantabria, Spain, where the type strain was isolated.

Slightly acid–alcohol-fast actinomycete. Forms a branched substrate mycelium that fragments into irregular coccoid to rod-shaped elements. The substrate mycelium is yellowish-orange and the aerial mycelium white but with a patchy distribution. Colonies are 2–3 mm in diameter on Bennett's agar. Grows well at 30°C, but does not grow at 45°C. Additional phenotypic properties are cited in Table 39. Whole-cell hydrolysates contain arabinose and galactose as characteristic sugars and *meso*-diaminopimelic acid as the principal diamino acid. Cellular fatty acids contain major proportions of $C_{16:0}$, $C_{18:1}$, and $C_{18:0}$ 10-methyl. Mycolic acids have 52–58 carbon atoms. The menaquinone profile is typical of the genus. The type strain and the corresponding strain of *Nocardia tenerifensis* share a DNA–DNA relatedness of 29.0% (Jurado et al., 2008).

Source (type strain): The Altamira cave, Cantabria, Spain.

DNA G+C content (mol%): 64.4 (T_m).

Type strain: CIP 109606, DSM 44997, JCM 14670, OFM S17.

Sequence accession no. (16S rRNA gene): EU006090.

7. **Nocardia amamiensis** Yamamura, Tamura, Sakiyama and Harayama 2007, 1601[VP]

a.ma.mi.en′sis. N.L. fem. adj. *amamiensis* of or pertaining to Amami Island, the source of the isolate.

Partially acid–alcohol-fast actinomycete which forms moderate white aerial hyphae on modified Bennett's agar. Does not produce diffusible pigments. Grows between 15 and 37°C, but not at 10 or 45°C. Additional phenotypic properties are cited in Table 39. Whole-cell hydrolysates contain arabinose and galactose as characteristic sugars and *meso*-diaminopimelic acid as the principal diamino acid. Cellular fatty acids contain major proportions of $C_{16:0}$, $C_{16:1}$, $C_{18:1}$ *cis*9, and 10-methyl-$C_{17:0}$. Mycolic acids co-migrate on one-dimensional thin-layer-chromatography of whole-organism methanolysates with those of reference *Nocardia* strains. The polar lipid and menaquinone profiles are typical for the genus.

DNA–DNA relatedness values between the type strain and corresponding strains of related species are: *Nocardia araoensis* (18.3%), *Nocardia arthritidis* (20.3%), *Nocardia beijingensis* (6.9%), and *Nocardia pneumoniae* (20.3%) (Yamamura et al., 2007).

Source: a soil sample collected from a sugar-cane field on Amami Island, Japan.

DNA G+C content (mol%): 67.4 (HPLC).

Type strain: DSM 45066, JCM 14877, KCTC 19208, NBRC 102102, TT 00–78.

Sequence accession no. (16S rRNA gene): AB275164.

8. **Nocardia anaemiae** Kageyama, Yazawa, Nishimura and Mikami 2005b, 1396[VP] (Effective publication: Kageyama, Yazawa, Nishimura and Mikami 2005c, 25.)

a.na.e.mi′a.e. N.L. fem. n. anemiae, from Gr. fem. n. *anaimia* want of blood, N.L. gen. n. *anaemiae* of anaemia, referring to the disease of the patient with this condition.

Acid-fast actinomycetes which form a branched, pale substrate mycelium that fragments into irregular rod-shaped elements. White aerial hyphae are formed on brain heart infusion agar. Colonies are 0.2–0.3 mm in diameter on Mueller–Hinton II agar supplemented with glucose (0.2%, w/v) after 7 d at 30°C. Grows at 37°C, but not at 45°C. Susceptible (μg per disc) to impenem (2.5) and tobramycin (2.5), but not to 5-fluorouracil (30) (Kageyama et al., 2005c). Additional phenotypic properties are shown in Table 39. Whole-cell hydrolysates contain arabinose and galactose as characteristic sugars and *meso*-diaminopimelic acid as the principal diamino acid. Mycolic acids co-migrate on one-dimensional thin-layer chromatography of whole-organism methanolysates with those of reference *Nocardia* strains. The menaquinone profile is typical of the genus. The DNA–DNA relatedness value between the type strain and the corresponding strain of *Nocardia vinacea*, a closely related species, is 56% (Kageyama et al., 2005d).

Source (type strain): a patient with a history of autoimmune hemolytic anemia and steroid therapy.

DNA G+C content (mol%): 66.2 (HPLC).

Type strain: DSM 44821, IFM 0323, JCM 12396, NBRC 100462.

Sequence accession no. (16S rRNA gene): AB162801.

9. **Nocardia aobensis** Kageyama, Suzuki, Yazawa, Nishimura, Kroppenstedt and Mikami 2005b, 547[VP] (Effective publication: Kageyama, Suzuki, Yazawa, Nishimura, Kroppenstedt and Mikami 2004d, 821.)

a.o.ba.en′sis. N.L. fem. adj. *aobensis* of or pertaining to Aobacho, Chiba, Japan, where the type strain was isolated.

Partially acid–alcohol-fast actinomycetes which produce an orange to dull-orange substrate mycelium. Does not form visible hyphae on most standard media, though short spore chains can be seen. Colonies are 0.3–3.0 mm in diameter on Mueller–Hinton II agar supplemented with glucose and glycerol (each at 1%, w/v) after 7 d at 30°C. Grows at 37°C, but not at 45°C. Moderately susceptible (μg per disc) to impenem (5.0), slightly susceptible to kanamycin (30), but is resistant to 5-fluorouracil (10) and tobramycin (10) (Kageyama et al., 2004d). Additional phenotypic properties are shown in Table 39. Whole-cell hydrolysates contain arabinose and galactose as characteristic sugars and *meso*-diaminopimelic acid as the principal diamino acid. Cellular fatty acids contain major proportions of $C_{16:0}$, $C_{16:1}$ *cis*9, $C_{18:1}$ *cis*9, $C_{18:0}$, and 10-methyl $C_{18:0}$, and smaller proportions (<10%) of $C_{14:0}$ and $C_{17:0}$. Mycolic acids co-migrate on one-dimensional thin-layer chromatography of whole-organism methanolysates with those of reference *Nocardia* strains. The menaquinone profile is typical of the genus. DNA–DNA relatedness values between *Nocardia aobensis* strains and the type strains of related species are as follows: *Nocardia africana* (44–48%), *Nocardia cerradoensis* (58–65%), and *Nocardia veterana* (12–24%) (Kageyama et al., 2004d).

Source: clinical material.

DNA G+C content (mol%): 67–68 (HPLC).

Type strain: DSM 44805, IFM 0372, JCM 12352, NBRC 100429.

Sequence accession no. (16S rRNA gene): AB126876.

Additional remarks: the specific epithet, *aobensis*, is a N.L. fem. adj., not a N.L. fem. n. as stated in the effective publication.

10. **Nocardia araoensis** Kageyama, Yazawa, Mukai, Kohara, Nishimura, Kroppenstedt and Mikami 2004g, 2028[VP]

a.ra.o.en′sis. N.L. fem. adj. *araoensis* of or belonging to Arao City, where the bacterium was isolated.

Partially acid-fast actinomycetes which produce a branched, faint yellow substrate mycelium that fragments into rod-shaped elements (0.3–0.5 × 1.0–1.7 μm). Forms a cotton white to grayish white aerial mycelium but does not produce diffusible pigments. Colonies 0.5–1.4 mm in diameter are formed on Mueller–Hinton II medium supplemented with glycerol (0.2% w/v) after 7 d at 30°C. Grows at 37 and 45°C. Slightly susceptible (mg per disc) to impenem (10.0 and kanamycin (30) (Kageyama et al., 2004g). Additional phenotypic properties are cited in Table 39. Cellular fatty acids contain major proportions of $C_{16:0}$, $C_{16:1}$ *cis*7, $C_{16:1}$ *cis*7, and $C_{18:1}$ *cis*9, smaller proportions (<10%) of $C_{14:0}$, $C_{18:0}$, $C_{18:0}$ 10-methyl, and $C_{18:1}$ *cis*9, and traces of $C_{15:0}$, $C_{16:1}$ *cis*9, $C_{19:1}$ *cis*11, $C_{19:0}$, and $C_{20:1}$ *cis*11. Mycolic acids co-migrate on one-dimensional thin-layer chromatography of whole-organism methanolysates with those of reference *Nocardia* strains. The menaquinone profile is typical of the genus.

DNA–DNA relatedness values between the type strain and the corresponding strains of related species are as follows: *Nocardia beijingensis* (<46%), and *Nocardia pneumoniae* (35%) (Kageyama et al., 2004g).

Source (type strain): sputum of a patient with a history of lung cancer, diabetes, emphysema, and radiation pneumonia.

DNA G+C content (mol%): 69 (HPLC).

Type strain: DSM 44729, IFM 0575, JCM 12118, NBRC 100135.

Sequence accession no. (16S rRNA gene): AB108779.

11. **Nocardia arthritidis** Kageyama, Torikoe, Iwamoto, Masuyama, Shibuya, Okazaki, Yazawa, Minota, Kroppenstedt and Mikami 2005a, 1[VP] (Effective publication: Kageyama, Torikoe, Iwamoto, Masuyama, Shibuya, Okazaki, Yazawa, Minota, Kroppenstedt and Mikami 2004e, 2370.)

ar.thri′ti.dis. Gr. n. *arthritis -idos* gout, arthritis; N.L. gen. n. *arthritidis* of arthritis.

Acid–alcohol-fast actinomycete. Forms an orange, branched substrate mycelium that fragments into irregular rod-shaped elements (0.5–0.6 × 1.4 × 1.7 µm). Pale orange aerial hyphae are formed on brain heart infusion agar. Does not produce melanin pigments on standard agar media. Colonies 0.5–1.2 mm in diameter are formed on Mueller–Hinton II medium supplemented with glucose (0.2%, w/v) after 7 d at 30°C. Grows best at 45°C. Susceptible (mg per disc) to tobramycin (2.5), moderately susceptible to impenem (5.0) and kanamycin (10), and resistant to 5-fluorouracil (10) (Hoshino et al., 2007). Additional phenotypic properties are cited in Table 39. Whole-cell hydrolysates contain arabinose and galactose as characteristic sugars and *meso*-diaminopimelic acid as the principal diamino acid. Cellular fatty acids contain major proportions of $C_{16:0}$, $C_{18:1}$ *cis*9, $C_{18:0}$, and $C_{18:0}$ 10-methyl, smaller proportions (<10%) of $C_{14:0}$, $C_{16:1}$ *cis*8, and $C_{16:1}$ *cis*9, and traces of $C_{15:0}$, $C_{16:1}$ *cis*7, and 10-methyl-$C_{16:0}$. The menaquinone profile is typical of the genus, as is the presence of mycolic acids. The DNA–DNA relatedness values between the type strain and the corresponding strain of *Nocardia beijingensis*, a closely related species, is within the range of 14–17% (Kageyama et al., 2004e).

Source (type strain): sputum of a patient with rheumatoid arthritis and lung nocardiosis.

DNA G+C content (mol%): 68 (HPLC).

Type strain: DSM 44731, IFM 10035, JCM 12120, NBRC 100137.

Sequence accession no. (16S rRNA gene): AB108781.

Additional remarks: the strains DSM 44731 and JCM 12120 were only cited in the abstract of the effective publication, but the authors did provide certificates of deposition from these collections (Lists Editor, 2005).

12. **Nocardia asiatica** Kageyama, Poonwan, Yazawa, Mikami and Nishimura 2004b, 127[VP]

a.si.a′ti.ca. L. fem. adj. *asiatica* of Asia, the source of the isolates

Partially acid-fast actinomycetes. Form a beige substrate mycelium which undergoes fragmentation. Straight to flexuous aerial hyphae differentiate into chains of 2–20 cylindrical spores (0.3–0.5 × 1.5–1.7 µm). Colonies 0.3–1 mm in diameter are formed on Mueller–Hinton II medium supplemented with glucose (0.2%, w/v). Grows at 37°C, but not at 45°C. Susceptible (µg per disc) to imipenem (2.5) and tobramycin (2.5); moderately susceptible to kanamycin (10), but resistant to 5-fluorouracil (Kageyama et al., 2005d). Additional phenotypic properties are cited in Table 39. Whole-cell hydrolysates contain arabinose and galactose as characteristic sugars and *meso*-diaminopimelic acid as the principal diamino acid. Two kinds of mycolic acids co-migrate on one-dimensional thin-layer chromatography of whole-cell methanolysates with those of reference *Nocardia* strains. The menaquinone profile is typical of the genus. DNA–DNA relatedness values between the type strains of *Nocardia asiatica* and *Nocardia beijingensis*, a closely related species, is within the range of 33–39% (Kageyama et al., 2004b).

Source: clinical material.

Source (type strain): sputum of a patient with nocardiosis.

DNA G+C content (mol%): 68.4–69.9 (HPLC).

Type strain: CCUG 48826, DSM 44668, IFM 0245, JCM 11892, NBRC 100129.

Sequence accession no. (16S rRNA gene): AB092566.

Additional remarks: the specific epithet *asiatica* is a N.L. fem. adj., not a L. gen. masc. n. as cited in the paper by Kageyama et al. (2004b).

13. **Nocardia beijingensis** Wang, Zhang, Lu, Shi, Liu, Maldonado and Goodfellow 2001, 1785[VP]

bei.jing.en′sis. N.L. fem. adj. *beijingensis* of or pertaining to Beijing, the source of the isolate.

Slightly acid–alcohol-fast actinomycete. Forms an orange, branched substrate mycelium that fragments *in situ* into irregular rod-shaped elements and carries sparse to abundant aerial hyphae on modified Sauton's agar. Short chains of up to four spores are formed on aerial and substrate hyphae. Does not produce diffusible pigments. Colony elevation is convex to irregular and colony margins filamentous. Does not grow at 45°C. L-Alanine-*para*-nitroanilide (*p*NA), 2-deoxythymidine-5-*p*-nitrophenyl (*p*NP) phosphate, *bis*-*p*NP-phosphate, and *p*NP-phenylphosphonate are hydrolyzed, but not *p*NP-α-D-glucopyranoside, *p*NP-β-D-glucopyranoside, *p*NP-glucuronide, L-glutamate-γ-3-carboxyl, *o*-nitrophenyl-β-D-galactopyranoside, *p*NP-phosphorylcholine, L-proline *p*NA, and *p*NP-β-D-xylopyranoside. L-Alanine, L-aspartate, L-glutamate, monoethanolamine, L-proline, uric acid, and L-valine are used as simultaneous carbon and nitrogen sources, but not acetamide, gelatin, L-leucine, L-phenylalanine, or urea. Susceptible (µg/ml per disc) to imipenem (2.5), kanamycin (5.0), and tobramycin (2.5), but not to 5-fluorouracil (30) (Kageyama et al., 2005d). Additional phenotypic properties are cited in Table 39. Whole-cell hydrolysates contain arabinose and galactose as characteristic sugars and *meso*-diaminopimelic acid as the principal diamino acid. Cellular fatty acids contain major proportions of $C_{16:0}$, $C_{18:1}$ *cis*9, and $C_{18:0}$ 10-methyl, smaller proportions (<10%) of $C_{14:0}$, $C_{16:1}$ *cis*9, and $C_{17:1}$ *cis*9, and traces of $C_{16:1}$ *cis*7, $C_{16:1}$ *cis*8, $C_{16:1}$ *cis*11, $C_{19:1}$ *cis*9, $C_{19:0}$, and C_{20} *cis*11. Two types of mycolic acids co-migrate on one-dimensional thin-layer-chromatography of whole-organism methanolysates with those of reference *Nocardia*

strains. The menaquinone profile is typical of the genus. The DNA–DNA relatedness value between the type strain and corresponding strain of *Nocardia tenerifensis*, a closely related species, is 16.8% (Kämpfer et al., 2004).

Source (type strain): a mud sample taken from a sewage ditch at Xishain mountain in Beijing.

DNA G+C content (mol%): 69 (T_m).

Type strain: O2, AS4.1521, CCUG 46096, DSM 44636, IFO (now NBRC) 16342, JCM 10666.

Sequence accession no. (16S rRNA gene): AF154129.

Additional remarks: the specific epithet is a N.L. feminine adjective, not a N.L. masculine adjective as cited in the paper by Wang et al. (2001).

14. **Nocardia blacklockiae** Conville, Brown, Steigerwalt, Brown-Elliott and Witebsky 2009, 1 (Effective publication: Conville, Brown, Steigerwalt, Brown-Elliott and Witebsky 2008, 1183.)

black.loc.ki′a.e. N.L. fem. gen. n. *blacklockiae* of Blacklock, in memory of Zeta M. Blacklock, in honor of and in recognition of her contributions to the study of *Nocardia* taxonomy.

Acid-fast actinomycetes. Form a branched substrate mycelium which carries aerial hyphae. Grows at 25°C and 35°C, but not at 45°C. The type strain is susceptible to amoxicillin-clavulanic acid, cefriaxine and linezolid, but is resistant to amikacin and clarithromycin. Does not use acetamide as a simultaneous carbon and nitrogen source. Additional phenotypic properties are cited in Table 39. DNA–DNA relatedness values between the type strain and those of corresponding strains of closely related species are as follows: *Nocardia transvalensis* (34%+8.5% divergence) and *Nocardia wallacei* (45%+7.5% divergence) (Conville et al., 2008). The organism is a pathogen of immunocompromised patients.

DNA G+C content (mol%): not determined.

Type strain: ATCC 700035, DSM 45135.

Sequence accession no. (16S rRNA gene): EU099360.

Additional remarks: the taxonomic status of *Nocardia blacklockiae* is supported by DNA–DNA homology data and by results from 65-kDa heat-shock protein and *secA1* gene sequence analyses (Conville et al., 2008).

15. **Nocardia brasiliensis** (Lindenberg 1909) Pinoy 1913, 337[AL]. ["*Discomyces brasiliensis* (Lindenberg 1909) Sartory 1920; "*Actinomyces brasiliensis*" (Lindenberg 1909) Gomes 1923: "*Actinomyces violaceus* subsp. *brasiliensis*" Krasil'nikov 1941.]

bra.si.li.en′sis. N.L. fem. adj. *brasiliensis* of or pertaining to Brazil.

Weakly acid-fast actinomycetes. Form pinkish or orange-tan to tan or brown substrate mycelia which carry moderate to abundant, nonfragmenting aerial hyphae that are usually off-white to pink-gray in color. Soluble dark pigments are usually formed. Grows well at 30°C and 45°C. L-Alanine-*p*-nitroanilide (*p*NA), 2-deoxythymidine-5′-*p*-nitrophenyl (*p*NA) phosphate, *p*NP-α-D-glucopyranoside, *p*NP-β-D-glucopyranoside, L-glutamate-γ-3 carbonyl *p*NA, *bis-p*NP-phosphate, *p*NP-phenylphosphonate, *p*NP-phosphorylchloine, L-proline *p*NA and *p*NP-β-D-xylopyranoside are hydrolyzed, but not *p*NP-β-D-glucuronide or *o*-nitrophenyl-β-D-galactosidase. L-Alanine, gelatin, proline, and serine are used as simultaneous carbon and nitrogen sources, but not acetamide. Susceptible to amikacin, amoxicillin + clavulanic acid, gentamicin and minocycline, but resistant to ampicillin, cefuroxime, cefotaxime, chloramphenicol, clindomycin, erythromycin, kanamycin, pefloxacin, sulfonomides, tetracycline, trimethoprim, and trimethoprim-sulfamethoxazole (Boiron and Provost, 1988b). The type strain is sensitive (μg/ml) to amoxicillin (100), bacitracin (100), gentamicin (10), kanamycin (5), lincomycin (10), novobiocin (10), polymyxin (5), and sulfamethoxazole (100), but resistant to ampicillin (5), cephaloridine (100), doxycycline (10), erythromycin (100), gentamicin (100), kanamycin (50), lincomycin (100), lividomycin (100), neomycin (100), novobiocin (100), paromomycin (100), penicillin (10), spiramycin (50), and streptomycin (100) (Hamid et al., 2001b), and sensitive to (μg ml per disc) tobramycin (2.5), but not to 5-fluorouracil (30), imipenem (10), and kanamycin (30) (Kageyama et al., 2005d). Grows in the presence (%, w/v) of sodium chloride (5), sodium nitrate (0.1), tetrazolium salt (0.1) and thallous acetate (0.001), but is inhibited by pyronin G (0.1), sodium azide (0.01), and sodium chloride (7). Additional phenotypic properties are shown in Table 39.

Whole-cell hydrolysates contain arabinose and galactose as characteristic sugars and *meso*-diaminopimelic acid as the principal diamino acid. Cellular fatty acids contain major proportions of $C_{16:0}$, $C_{18:1}$ *cis*9 and $C_{18:0}$ 10-methyl, and smaller proportions (<10%) of $C_{14:0}$, $C_{15:0}$, $C_{17:0}$, and $C_{18:0}$, and traces of $C_{10:0}$, $C_{12:0}$, $C_{16:1}$ *cis*9, $C_{17:1}$ *cis*9, and $C_{15:1}$ anteiso. Muramic acid moieties are *N*-glycolated. Mycolic acids have 46–60 carbon atoms with 0–3 double bonds. Pyrolysis gas chromatography of purified mycolic acid methyl esters release fatty acid methyl esters ($C_{12:0}$–$C_{18:0}$). Menaquinone and polar lipid profiles are typical of the genus. Grows in the presence (%, w/v) of sodium chloride (5), sodium nitrate (0.1), tetrazolium salt (0.1), and thallus acetate (0.001). Growth is inhibited by pyrosin G (0.1), sodium azide (0.01), and sodium chloride (7). The DNA–DNA relatedness value between the type strain and the corresponding strain of *Nocardia tenerifensis*, a closely related species, is 26.2% (Kämpfer et al., 2004).

Source: patients with nocardioses and mycetoma, and occasionally from soil.

DNA G+C content (mol%): 67–68 (T_m).

Type strain: ATCC 19296, CCUG 10098, CIP 104502, DSM 43758, JCM 3374, IFO (now NBRC) 14402, NCTC 11294, VKM Ac-863.

Sequence accession no. (16S rRNA gene): AF430038.

16. **Nocarnudia brevicatena** (Lechevalier, Solotorovsky and McDurmont 1961) Goodfellow and Pirouz 1982a, 384[VP] (Effective publication: Goodfellow and Pirouz 1982b, 523; basonym: *Micropolyspora brevicatena* Lechevalier, Solotorovsky and McDurmont 1961, 13[AL])

bre.vi.ca.te′na. L. adj. *brevis* short; L. n. *catena* chain; N.L. n. *brevicatena* short chain (of spores).

White to yellowish substrate mycelium carry aerial hyphae. Single or short chains of spores (2–10) are formed either directly or on short sporophores on both substrate and aerial hyphae. Spores are oblong, sometimes pyriform,

and are about 1.5 μm in diameter. Aerial hyphae are often aggregated into long filamentous sheaths. Abundant, slightly wrinkled, pale orange growth occurs on casein hydrolysate-glycerol agar. Growth on yeast extract agar is thin, pale orange with white powdery aerial hyphae. Grows well at 36°C and pH 6.3, but not at 45°C or pH 5.1. Susceptible (μg ml per disc) to chloramphenicol (5), neomycin (5), and streptomycin (100) (Lechevalier et al., 1961). Susceptible (μg ml per disc) to kanamycin (5.0) and tobramycin (2.5), slightly susceptible to imipenem (10), but not to 5-fluorouracil (30) (Kageyama et al., 2005d). Additional phenotypic properties are cited in Table 39. Whole-cell hydrolysates contain arabinose and galactose as characteristic sugars and *meso*-diaminopimelic acid as the principal diamino acid. Muramic acid moieties are *N*-glycolated. Mycolic acids have 46–60 carbon atoms with 0–3 double bonds. Pyrolysis gas chromatography of purified mycolic acid methyl esters release fatty acid methyl esters. Menaquinone and polar lipid profiles are typical of the genus. The DNA–DNA relatedness value between the type strain and the corresponding strain of *Nocardia paucivorans*, a closely related species, is 61.9% (Yassin et al., 2000a).

DNA G+C content (mol%): not determined.

Type strain: ATCC 15333, CCUG 46115, CIP 104508, DSM 43024, IFO (now NRBC) 12119, IMET 9542, IMRU 1086W, JCM 3029, NRRL B-2896, RIA 709, VKM Ac-936.

Sequence accession no. (16S rRNA gene): AF430040.

17. **Nocardia caishijiensis** Zhang, Liu and Goodfellow 2003, 1003[VP]

cai.shi.ji.en′sis. N.L. fem. adj. *caishijiensis* of or belonging to Caishiji, the source of the soil from which the type strain was isolated.

Slightly acid–alcohol-fast actinomycete. Forms an extensively branched substrate mycelium that fragments *in situ* into rod-shaped elements. An orange to brown substrate mycelium carries sparse to abundant white to pinkish aerial hyphae on modified Sauton's agar. A brown substrate mycelium and white to grayish aerial hyphae are formed on modified Bennett's agar. Does not produce diffusible pigments. Colony elevation is convex to irregular and colony margins are filamentous. Grows at 17–37°C, but not at 45°C, and at pH 5.2–10.0. L-Alanine, L-aspartate, D-glucosamine, L-proline, and L-serine are used as simultaneous carbon and nitrogen sources, but not acetamide, L-asparagine, gelatin, L-leucine, L-phenylalanine, or L-valine. Susceptible (μg ml per disc) to chloramphenicol (20), erythromycin (15), midecamycin (15), minocycline (30), rifampin (5), tobramycin (10), and vancomycin (30), but not to gentamicin (10), penicillin (G), and streptomycin (10) (Zhang et al., 2003). Susceptible (μg per disc) to 5-fluorouracil (30) and imipenem (2.5) but not to kanamycin (30) and tobramycin (2.5) (Kageyama et al., 2005d). Additional phenotypic properties are cited in Table 39. Whole-cell hydrolysates contain arabinose and galactose as characteristic sugars and *meso*-diaminopimelic acid as the principal diamino acid. Cellular fatty acids contain major proportions of $C_{16:0}$, $C_{18:0}$, *C16:1 cis9* and $C_{18:1}$ *cis*9, and smaller proportions (<10%) of $C_{15:0}$, $C_{17:0}$, $C_{17:1}$ *cis*9, $C_{20:2}$ *cis*11,14, and $C_{18:0}$ 10-methyl. Menaquinone and polar lipid profiles are typical of the genus, as is the presence of mycolic acids in whole-organism methanolysates.

Source (type strain): a soil sample collected in Caishiji, Anshii Province, China.

DNA G+C content (mol%): 69.4 (T_m).

Type strain: AS 4.1728, F829, JCM 11508.

Sequence accession no. (16S rRNA gene): AF459443.

18. **Nocardia callitridis** Kaewkla and Franco 2010, 1534[VP]

cal.li′tri.dis. N.L. gen. n. *callitridis* of *Callitris*, the botanical name of a genus of pine, named after the source plant of the type strain, the Australian native pine *Callitris preissii*.

Non-acid–alcohol-fast actinomycete which forms an extensive substrate mycelium which fragments into rod-shaped elements. A pale orange substrate mycelium bears white to orange white aerial hyphae on Bennett's, half strength potato-dextrose, and yeast extract-malt extract (ISP medium 2) agars. A brownish-orange diffusible pigment is produced on glucose-asparagine agar (ISP medium 5). Grows at 15–32°C and pH 5.0–10.0, but not at 37°C or pH 4.0. Grows in the presence of 5%, w/v sodium chloride. Additional phenotypic properties are shown in Table 39. Whole-cell hydrolysates contain arabinose and galactose as characteristic sugars and *meso*-diaminopimelic acid as the principal diamino acid. Cellular fatty acids contain major proportions of $C_{16:0}$, $C_{18:1}$ *cis*9, and $C_{18:0}$ 10-methyl, smaller proportions (<10%) of $C_{14:0}$, $C_{16:1}$ *cis*7, $C_{16:1}$ *cis*6, and $C_{18:0}$, and traces of *iso*- and $C_{17:1}$ *cis*10, and *anteiso*- and $C_{17:1}$ *cis*9. The menaquinone profile is typical of the genus, as is the presence of mycolic acids in whole-organism methanolysates.

Source (type strain): internal tissues of a surface sterilized root of an Australian pine tree, *Callistris preisii.*

DNA G+C content (mol%): 68.7 (HPLC).

Type strain: ACM 5287, CAP 290, DSM 45353.

Sequence accession no. (16S rRNA gene): FJ805428.

19. **Nocardia carnea** (Rossi-Doria 1891) Castellani and Chalmers 1913, 337[VP] ("*Streptothrix carnea*" Rossi-Doria 1891)

car′ne.a. L. fem. adj. *carnea* of flesh.

Rarely acid-fast actinomycete. Forms a peach colored substrate mycelium that carries sparse to abundant pinkish aerial hyphae. Some strains have been reported to bear rudimentary spores on the aerial mycelium. Soluble pigments may be produced. Grows at 30°C, but not at 45°C. 2-Deoxythymidine-5′-*para*-nitrophenyl (*p*NP) phosphate, *p*NP-phosphorylcholine, and *p*NP-β-D-xyloside are hydrolyzed. Susceptible (μg per disc) to imipenem (2.5), kanamycin (5.0), and tobramycin (2.5), but not to 5-fluorouracil (30) (Kageyama et al., 2005d). Does not use L-phenylalanine or L-valine as simultaneous carbon and nitrogen sources. Additional phenotypic properties are shown in Table 39. Whole-cell hydrolysates contain arabinose and galactose as characteristic sugars and *meso*-diaminopimelic acid as the principal diamino acid. Muramic acid moieties are *N*-glycolated.

Source: soil and air.

DNA G+C content (mol%): 64–68 (T_m).

Type strain: ATCC 6847, CCUG 48177, CIP 104509, DSM 43397, IFO (now NRBC) 14403, IMET 7504, JCM 3375, NCTC 3527, NRRL B-1336, NRRL WC-3809, VKM Ac-814.

Sequence accession no. (16S rRNA gene): AF430035.

20. **Nocardia cerradoensis** Albuquerque de Barros, Manfio, Ribeiro Maitan, Mendes Bataus, Kim, Maldonado and Goodfellow 2003, 32[VP]

cer.ra.do.en′sis. N.L. fem. adj. *cerradoensis* of or pertaining to Cerrado soil, the source of the organism.

Slightly acid–alcohol-fast actinomycete. Forms an extensively branched substrate mycelium that fragments *in situ* into rod-shaped elements. Pinkish white aerial hyphae are formed. Orange to tan colonies are produced on modified Bennett's agar. Does not produce diffusible pigments. Colony elevation is convex to regular and colony margins are filamentous. Grows well at 30°C but does not grow at 45°C. L-Glutamate, L-leucine, L-proline, and L-valine are used as simultaneous carbon and nitrogen sources, but not acetamide, L-aspartic acid, gelatin, monoethanolamine, L-phenylalanine, uric acid, or urea. Susceptible (μg/ml per disc) to imipenem (2.5), kanamycin (5.0), but not to 5-fluorouracil (30) and tobramycin (10) (Kageyama et al., 2005d). Additional phenotypic properties are cited in Table 39. Whole-cell hydrolysates contain arabinose and galactose as characteristic sugars and *meso*-diaminopimelic acid as the principal diamino acid. Cellular fatty acids contain major proportions of $C_{16:0}$, $C_{16:1}$ *cis9*, and $C_{18:0}$ 10-methyl, smaller proportions (<10%) of $C_{17:0}$, $C_{18:0}$, $C_{19:1}$ *cis*10, $C_{20:1}$, and $C_{20:0}$, and traces of $C_{14:0}$, $C_{15:0}$, $C_{17:1}$ *cis*9, and 10-methyl $C_{17:0}$. Mycolic acids co-migrate on one-dimensional thin-layer-chromatography of whole-organism methanolysates with those of reference *Nocardia* strains. Menaquinones and polar lipid profiles are typical of the genus. DNA–DNA relatedness values between the type strain and corresponding strains of closely related species are as follows: *Nocardia africana* (11%), *Nocardia nova* (10%), *Nocardia vaccinii* (41%), *Nocardia veterana* (37%), *Nocardia vinacea* (13), and *Nocardia vermiculata* (9%) (Kageyama et al., 2004c).

Source (type strain): cerrado soil in Brazil.

DNA G+C content (mol%): 68.8 (HPLC).

Type strain: CCT 5601, DSM 44546, JCM 12209, NBRC 101014, Y9.

Sequence accession no. (16S rRNA gene): AF060790.

Additional remarks: the first authors name (Albuquerque de Barros) is incorrectly cited as Alburquerque de Barros in the Author Index (IJSEM, 2003, 53, part 1, p. VII). In addition, the author's name Ribeiro Maitan is incorrectly cited as V. Ribiero Maitan on page 29 but cited correctly in the table of contents (IJSEM, 2003, 53; part 1, p. 1).

21. **Nocardia concava** Kageyama, Yazawa, Taniguchi, Chibana, Nishimura, Kroppenstedt and Mikami 2005d, 2083[VP]

con.ca′va. L. fem. adj. *concava* hollow, concave, referring to the colony morphology on agar plates.

Acid–alcohol-fast actinomycetes. Form a grayish-orange to faint-orange substrate mycelium that fragments into irregular rod-shaped elements. Aerial hyphae, when present, are sparse. Colonies 1.0–3.5 mm in diameter are produced on Mueller–Hinton agar supplemented with glucose (0.2%, w/v) after 7 d at 30°C. Grows at 37°C, but not at 45°C. Susceptible (μg ml per disc) to 5-fluorouracil (30), kanamycin (2.5), and tobramycin (2.5), but not to imipenem (10) (Kageyama et al., 2005d). Additional phenotypic properties are cited in Table 39. Whole-cell hydrolysates contain arabinose and galactose as characteristic sugars and *meso*-diaminopimelic acid as the principal diamino acid. Cellular fatty acids contain major proportions of $C_{16:0}$, $C_{16:1}$ *cis*9, and $C_{18:0}$ 10-methyl, smaller proportions (<10%) of $C_{14:0}$, $C_{15:1}$ *cis*7, $C_{15:1}$ *cis*10, $C_{15:0}$, $C_{16:1}$ *cis*7, $C_{17:1}$ *cis*9, $C_{17:0}$, 10-methyl-$C_{17:0}$, $C_{18:1}$ *cis*9, and -$C_{19:1}$ *cis*9, and traces of $C_{18:0}$ 10-methyl, -$C_{18:1}$ *cis*11, $C_{18:0}$, $C_{19:0}$, 10-methyl-$C_{19:0}$, and $C_{20:1}$. Mycolic acids have 52–62 carbon atoms with C54 and C56 as major components. The menaquinone profile is typical of the genus. DNA–DNA relatedness values between *Nocardia concava* strains and the type strain of *Nocardia seriolae*, a closely related species, is within the range of 12–16% (Kageyama et al., 2005c).

Source: Japanese patients.

DNA G+C content (mol%): 67–68 (HPLC).

Type strain: DSM 44804, IFM 0354, JCM 12351, NBRC 100430.

Sequence accession no. (16S rRNA gene): AB126880.

22. **Nocardia coubleae** Rodríguez-Nava, Khan, Pötter, Kroppenstedt, Boiron and Laurent 2007, 1485[VP]

cou′ble.ae. N.L. gen. fem. n. *coubleae* of Couble, named after Andrée Couble, in recognition of her contribution to the French Nocardiosis Observatory, Lyon, France.

Acid–alcohol-fast actinomycetes. Produce a white substrate mycelium which bears sparse to abundant white aerial hyphae on Bennett's agar. Colonies 1–2 mm in diameter are formed. Grows at 25–37°C, but not at 45°C. Whole-cell hydrolysates contain arabinose and galactose as characteristic sugars and *meso*-diaminopimelic acid as the principal diamino acid. Cellular fatty acids contain major proportions of $C_{16:0}$, $C_{16:1}$, $C_{18:0}$, and $C_{18:0}$ 10-methyl, smaller proportions (<10%) of $C_{14:0}$, $C_{15:0}$, $C_{17:0}$, $C_{17:1}$, and $C_{18:1}$, and traces of $C_{10:0}$ and $C_{15:1}$. Mycolic acids have 52–58 carbons with C56 as the major component. The menaquinone profile is typical of the genus. The DNA–DNA relatedness value between the type strain and the corresponding strain of *Nocardia ignorata*, a closely related species, is 26% (Rodriguez-Nava et al., 2007).

Source: oil contaminated soil collected in Kuwait.

DNA G+C content (mol%): not determined.

Type strain: CIP 108996, DSM 44960, JCM 15318, OFN N12.

Sequence accession no. (16S rRNA gene): DQ235688.

23. **Nocardia crassostreae** Friedman, Beaman, Chun, Goodfellow, Gee and Hedrick 1998, 244[VP]

cras.sos.tre′a.e. N.L. n. *Crassostrea* generic name of Pacific oyster; N.L. gen. n. *crassostreae* of *Crassostrea*, referring to the source of the organism.

Acid-fast actinomycetes. Form an extensive branched substrate mycelium which fragments into irregular rod-shaped elements. Colonies on brain heart infusion agar are dry, waxy, and wrinkled, and do not carry aerial hyphae. Grows at 22–30°C, at pH 9, but not at 5 or 45°C. L-Valine is used as a simultaneous carbon and nitrogen source, but not L-arginine, lysine, or ornithine. Susceptible to (μg ml^{-1}) 5-fluorouracil (20), mitomycin (57), and rifampin (20), but not to isoniazid (200) (Friedman et al., 1998). Does not grow in the presence of 5%, w/v sodium chloride.

Additional phenotypic properties are cited in Table 39. Whole-cell hydrolysates contain arabinose and galactose as characteristic sugars and *meso*-diaminopimelic acid as the principal diamino acid. Mycolic acids have 46–58 carbons. Pyrolysis gas chromatography of purified mycolic acid methyl esters release both odd and even numbered methyl mycolates within the range of C_{12}–C_{18}. The menaquinone profile is typical of the genus.

Source: diseased, cultured oysters (*Crassostreae gigas*).

DNA G+C content (mol%): 68.6–69.3 (T_m).

Type strain: ATCC 700418, CIP 105895, DSM 44597, JCM 10500, NBRC 100342, NB4H.

Sequence accession no. (16S rRNA gene): AF430049.

24. **Nocardia cummidelens** Maldonado, Hookey, Ward and Goodfellow 2001, 1619[VP] (Effective publication: Maldonado, Hookey, Ward and Goodfellow 2000, 371.)

cum.mi.de′lens. L. n. *cummi* rubber; L. partic. adj. *delens* destroying; N.L. partic. adj. *cummidelens* rubber destroying.

Acid–alcohol-fast actinomycetes. Form an extensively branched substrate mycelium that fragments into coccoid to rod-shaped elements. Pale pink colonies carry sparse aerial hyphae on glucose-yeast extract agar. Does not form diffusible pigments. Colony elevation is convex to irregular and colony margins filamentous. Does not grow at 45°C. 2-Deoxythymidine-5-*p*-nitrophenyl (*p*NP), *p*NP-phosphorylcholine, and *p*NP-β-D-xyloside are hydrolyzed. Sensitive (μg/ml) to cephaloridine (16), demethylchlortetracycline (16), gentamicin (8), rifampin (1), streptomycin (16), and tobramycin (1) (Maldonado et al., 2001). Sensitive to (μg per disc) imipenem (2.5), tobramycin (2.5), but not to 5-fluorouracil (30) and kanamycin (30) (Kageyama et al., 2005d). Resistant to lysozyme. Additional phenotypic properties are cited in Table 39. Whole-cell hydrolysates contain arabinose and galactose as characteristic sugars and *meso*-diaminopimelic acid as the principal diamino acid.

DNA G+C content (mol%): not determined.

Type strain: CCUG 46121, CCUG 46295, CIP 10725, DSM 44490, JCM 11439, NBRC 100378, NCIMB 13758, R89.

Sequence accession no. (16S rRNA gene): AF430052.

25. **Nocardia cyriacigeorgica** Yassin, Rainey and Steiner 2008, 1760[VP] (Effective publication: Yassin, Rainey and Steiner 2001a, 1422.)

cy.ri.a.ci.ge.or′gi.ca. Gr. neut. n. *kuriakon* house of the Lord, church; N.L. neut. n. *cyriacum, cyriaci* church; N.L. fem. adj. *georgica* of or related to St George; N.L. fem. adj. *cyriacigeorgica* related to St George's church, referring to the origin of the name of the German town Gelsenkirchen where the type strain was isolated.

Slightly acid–alcohol-fast actinomycete. Forms an extensive branched substrate mycelium that fragments into rod-shaped elements at a late stage of growth. The substrate mycelium bears white aerial hyphae. Acetamide is used as a simultaneous carbon and nitrogen source, but not L-alanine, L-proline, or L-serine. Susceptible (μg per disc) to imipenem (2.5) and tobramycin (2.5), but not to 5-fluorouracil (30) and kanamycin (30) (Kageyama et al., 2005d). Additional phenotypic properties are cited in Table 39. Whole-cell hydrolysates contain arabinose and galactose as characteristic sugars and *meso*-diaminopimelic acid as the principal diamino acid. Cellular fatty acids contain major proportions of $C_{16:1}$ *cis*7, $C_{18:0}$, and $C_{18:0}$ 10-methyl, smaller proportions (<10%) of $C_{16:0}$ *cis*7, $C_{17:0}$, $C_{18:1}$ *cis*9, $C_{21:0}$, and traces of $C_{14:0}$, $C_{15:0}$, $C_{16:0}$, $C_{16:1}$ *cis*, $C_{17:0}$ 10-methyl, $C_{19:0}$, and $C_{21:1}$. Mycolic acids have 46–54 carbon atoms with 0–3 double bonds. Pyrolysis gas chromatography of the purified methyl mycolates of the type strain release fatty acid methyl esters with C_{14}–C_{18} carbons. Menaquinone and polar lipid profiles are typical of the genus. DNA–DNA relatedness values between the type strain and corresponding closely related strains are as follows: *Nocardia abscessus* (45%) and *Nocardia paucivorans* (32%) (Yassin et al., 2001a).

Source (type strain): the bronchial secretion of a patient with chronic bronchitis.

DNA G+C content (mol%): 68.1 (HPLC).

Type strain: CCUG 48295, DSM 44484, IMMIB D-1627, JCM 11763, NBRC 100375.

Sequence accession no. (16S rRNA gene): AF430027.

Additional remarks: the original spelling of the specific epithet *cyriacigeorgici* (*sic*) was corrected by the Lists Editor, IJSEM (2001). In addition, according to Rules 27(3) and 30 of the Bacteriological Code, this name is not validly published in the effective publication; it only documents the deposit of the type strain in a single recognized culture collection (Euzéby and Tindall, 2004). However, according to Judicial Opinion 81 (2008), *Nocardia cyriacigeorgica* corrig. Yassin et al. 2001a is considered to be validly published.

26. **Nocardia elegans** Yassin and Brenner 2005, 1508[VP]

e′le.gans. L. fem. adj. *elegans* fastidious (with respect to utilization of nutrients).

Partially acid–alcohol-fast actinomycetes. Form an extensive irregularly branched orange substrate mycelium that bears white aerial hyphae. At a late stage of growth hyphae fragment into rod-shaped elements. Grows from 22–42°C. Does not use acetamide, arginine, gelatin, ornithine, proline, or serine as simultaneous carbon and nitrogen sources. Susceptible (μg/ml) to arbekacin (<2), ampicillin (16), clarithomycin (2), cefoticin (8), and erythromycin (4) (Iida et al., 2006). Additional phenotypic properties are cited in Table 39. Whole-cell hydrolysates contain arabinose and galactose as characteristic sugars and *meso*-diaminopimelic acid as the principal diamino acid. Cellular fatty acids contain major proportions of $C_{16:0}$, $C_{18:0}$, and $C_{18:0}$ 10-methyl, smaller proportions (<10%) of $C_{17:0}$, $C_{18:1}$ *cis*9, and $C_{21:0}$, and traces of $C_{14:0}$, $C_{15:0}$, $C_{16:1}$ *cis*7, 10-methyl-$C_{16:0}$, 10-methyl $C_{17:0}$, $C_{18:0}$, $C_{19:0}$, $C_{20:0}$, and $C_{21:1}$. Mycolic acids co-migrate on one-dimensional thin-layer chromatography of whole-organism methanolysates with those of reference *Nocardia* strains. Pyrolysis gas chromatography of purified mycolic acid methyl esters release $C_{16:0}$, $C_{18:1}$, and $C_{18:0}$ components. Menaquinone and polar lipid profiles are typical of the genus. DNA–DNA relatedness values between *Nocardia elegans* strains and the type strains of closely related species are as follows: *Nocardia africana* (30.2–33.6%) and *Nocardia veterana* (33.6%) (Yassin and Brenner, 2005).

Source: sputa of patients with pulmonary infections.

DNA G+C content (mol%): not determined.

Type strain: CCUG 50200, CIP 108553, IMMIB N-402, JCM 13374.

Sequence accession no. (16S rRNA gene): AJ854057.

27. **Nocardia exalbida** Iida, Kageyama, Yazawa, Uchiyama, Toyohara, Chohnabayashi, Suzuki, Nomura, Kroppenstedt and Mikami 2006, 1195[VP]

ex.al′bi.da. L. fem. adj. *exalbida* whitish or white, referring to the color of the aerial mycelium.

Partially acid–alcohol-fast actinomycetes which produce an extensively branched substrate mycelium that fragments into rod-shaped elements (0.4–0.8 × 0.8–1.7 μm). Orange-tan to tan colonies carry abundant white to off white aerial hyphae. Does not produce soluble pigments. Colonies 1.0–2.8 mm in diameter are formed on Mueller–Hinton medium supplemented with glucose (0.2%, w/v) after 7 d at 37°C. May show weak growth at 45°C. Susceptible (μg/ml) to arbekacin (<2), ampicillin (16), clarithromycin, cefetamet pivoxil (0.5), cefotiam (8), erythromycin (2), and sulfamethoxazole-trimethoprim (152:8) (Iida et al., 2006), and to (μg ml per disc) imipenem (2.5) and tobramycin (2.5). Moderately susceptible to kanamycin (10) but resistant to 5-fluorouracil (Iida et al., 2006). Additional phenotypic properties are shown in Table 39. Whole-cell hydrolysates contain arabinose and galactose as characteristic sugars and *meso*-diaminopimelic acid as the principal diamino acid. Two types of mycolic acids co-migrate on one-dimensional thin-layer chromatography of whole-cell methanolysates with reference *Nocardia* strains. The major mycolic acids have 48–56 carbon atoms. Fatty acid and menaquinone profiles are typical of the genus. DNA–DNA relatedness values between *Nocardia exalbida* strains and the type strain of *Nocardia xishanensis*, a closely related species, is within the range of 57–59% (Iida et al., 2006).

Source (type strain): the bronchoalveolar lavage of a patient with lung nocardiosis in Japan.

DNA G+C content (mol%): 68 (HPLC).

Type strain: DSM 44883, IFM 0803, JCM 12667, NBRC 100660.

Sequence accession no. (16S rRNA gene): AB187522.

28. **Nocardia farcinica** Trevisan 1889[AL] ["*Streptothrix farcinica*" (Trevisan 1889) Rossi-Doria 1891; "*Bacillus farcinicus*" (Trevisan 1889) Gasperini 1892; "*Actinomyces farcinicus*" (Trevisan 1889) Gasperini 1892; "*Oospora farcinica*" (Trevisan 1889) Sauvageau and Radais 1892; "*Actinomyces bovis farcinicus*" Gasperini 1894; "*Streptothrix farcini bovis*" Kitt 1899, "*Bacterium nocardi*" (*sic*) Migula 1900; "*Streptothrix nocardii*" (Migula 1900) Foulerton and Jones 1901; "*Cladothrix farcinica*" (Trevisan 1889) Macé 1901; "*Bacillus nocardi*" (*sic*) (Migula 1900) Matzuschita 1902; "*Discomyces farcinicus*" (Trevisan 1889) Krasil'nikov 1941]

far. ci′ni.ca. L. n. *farcinimum* a disease of horses; Fr. n. *farcin* farcy or glanders; L. fem. suff. *-ica*, suffix used with the sense of pertaining to; N.L. fem. adj. *farcinica* relating to farcy.

Acid-fast actinomycetes. Form an extensively branched substrate mycelium which fragments into coccoid and rod-shaped elements. An orange to red substrate mycelium carries sparse, grayish aerial hyphae.Grows well at 25–45°C, but not at 10°C, and at pH 6.0–10.0. L-Alanine- *p*-nitroanilide (*p*NA), 2-deoxythymidine-5′*p*-nitrophenyl phosphate (*p*NP), *p*-nitrophenyl (*p*NP)-α-D-glucopyranoside, *p*NP-β-D-glucopyranoside, L-glutamate-γ-3-carbonyl *p*NA, *bis*-*p*NP-phosphate, *p*NP-phenylphosphonate, *p*NP- phosphorylcholiine, L-proline *p*NA and *p*NP-β-D-xylopyranoside are hydrolyzed, but not *p*NP-β-D-glucuronide or *o*-nitrophenyl-β-D-galactopyranoside. Acetamide is used as a simultaneous carbon and nitrogen source, but not L-alanine, gelatin, proline, or serine. Resistant to ampicillin, cefotaxime, chloramphenicol, clindamycin, sulfonamides, trimethroprim, sulfamethoxazole, but gives variable responses to amikacin, amoxicillin, clavulanic acid, cefuroxime, erythromycin, gentamicin, kanamycin, minocycline, pefloxocin, and tetracycline (Boiron and Provost, 1988b). The type strain is sensitive (μg/ml) to amoxicillin (100), bacitracin (100), cephaloridine (100), erythromycin (100), kanamycin (50), lincomycin (100), novobiocin (10), penicillin (100), polymyxin (5), and streptomycin (100), but is resistant to amoxicillin (10), ampicillin (50), cephaloridine (10), doxycycline (10), erythromycin (10), gentamicin (10), lividomycin (100), neomycin (100), novobiocin (100), paromomycin (10), spiramycin (50), and sulfamethoxazole (100) (Hamid et al., 2001b), and sensitive to (μg per disc) imipenem (2.5), but not to 5-fluorouracil (30), kanamycin (30), and tobramycin (10) (Kageyama et al., 2005d). Grows in the presence (%, w/v) of sodium nitrate (0.1), but does not grow in the presence of pyronin G (0.1), sodium azide (0.01), sodium chloride (7), tetrazolium salt (0.1), and thallous acetate (0.001). Additional phenotypic properties are cited in Table 39. Whole-cell hydrolysates contain arabinose and galactose as characteristic sugars and *meso*-diaminopimelic acid as the principal diamino acid. Cellular fatty acids contain major proportions of $C_{16:0}$, $C_{16:1}$ *cis*9, $C_{18:1}$ *cis*9, and $C_{18:0}$ 10-methyl, smaller proportions (<10%) of $C_{17:0}$, $C_{18:0}$, and *cis*11$C_{20:1}$, and traces of $C_{16:0}$ and $C_{20:0}$. Mycolic acids have 50–58 carbon atoms with 0–3 double bonds. Pyrolysis mass chromatography of purified mycolic acid methyl esters release fatty acid methyl esters ($C_{13:0}$–$C_{16:0}$). Menaquinone and polar lipid profiles are typical of the genus. DNA–DNA relatedness values between the type strain and those of corresponding strains of closely related species are as follows: (i) *Nocardia asteroides* (31%), *Nocardia nova* (29%), *Nocardia salmonicida* (31%), and *Nocardia seriolae* (18%) (Kudo et al., 1988), and (ii) *Nocardia tenerifensis* (15.2%) (Kämpfer et al., 2004).

DNA G+C content (mol%): 66–71 (T_m).

Type strain: ATCC 3318, CCUG 10109, CCUG 27778, CCUG 48656, CIP104775, DSM 43665, IEGM 621, IFO (now NBRC) 15532, JCM 3088, NCTC 11134, NRRL B-2089.

Sequence accession no. (16S rRNA gene): Z36936.

Additional remarks: Tsukamura (1982b) proposed the rejection of the name *Nocardia farcinica* as a *nomen dubium* as some strains bearing this name were members of the genus *Mycobacterium*. However, Chamoiseau (1979) previously established two species, *Mycobacterium senegalense* (Approved Lists 1980) and *Mycobacterium farcinogenes* (Approved Lists 1980), to accommodate the farcy-associated strains of mycobacteria thereby eliminating the source of confusion. Thereafter, the Judicial Commission voted to deny the action proposed by Tsukamura (Wayne, 1982). Additional phenotypic properties are cited in extensive numerical taxonomic studies (Goodfellow, 1971; Orchard and Goodfellow, 1980; Tsukamura, 1982a).

29. **Nocardia flavorosea** Chun, Seong, Bae, Lee, Kang, Goodfellow and Hah 1998, 904[VP] (synonym: "*Nocardia flavoronea*" Liu, Ruan and Yan 1983)

fla.vo.ro′se.a. L. adj. *flavus* yellow; L. fem. adj. *rosea* rose-colored; N.L. fem. adj. *flavorosea* yellow rose.

Slightly acid-fast actinomycete. Forms an extensive branched substrate mycelium which fragments *in situ* into coccoid to rod-shaped elements. An orange substrate mycelium carries white to pinkish aerial hyphae. Does not form diffusible pigments. Colony elevation is convex to irregular and colony margins are filamentous. Growth occurs at 25–50°C, but not at 10°C or 45°C. 2-Deoxythymidine-5′-*p*-nitrophenyl (*p*NP), *p*NP-phosphorylcholine, and *p*NP-β-D-xyloside are hydrolyzed. Does not use L-phenylalanine or L-valine as simultaneous carbon and nitrogen sources. Sensitive (μg per Oxoid disc) to gentamicin (10), streptomycin (10), and tobramycin (10), but not to bacitracin (10 U) and penicillin (10) (Chun et al., 1998). Sensitive to (μg per disc) imipenem (2.5), kanamycin (5.0), and tobramycin (2.5), but not to 5-fluorouracil (30) (Kageyama et al., 2005d). Additional phenotypic properties are cited in Table 39.

Whole-cell hydrolysates contain arabinose and galactose as characteristic sugars, and *meso*-diaminopimelic acid as the principal diamino acid. Cellular fatty acids contain major proportions of $C_{16:0}$, $C_{18:1}$ *cis*9, and $C_{18:0}$ 10-methyl. Mycolic acids have 50–56 carbon atoms with between 2–4 double bonds. The menaquinone profile is typical of the genus. DNA–DNA relatedness values between the type strain and the corresponding strain of *Nocardia carnea*, a closely related species, in the range 2–5% (Chun et al., 1998).

Source (type strain): soil collected in Yunnan Province, China.

DNA G+C content (mol%): 64–72 (T_m).

Type strain: CCUG 46925, CIP 104511, DSM 44480, IFO (now NRBC) 14341, JCM 3332, NRRL B-16176.

Sequence accession no. (16S rRNA gene): Z46754.

30. **Nocardia fluminea** Maldonado, Hookey, Ward and Goodfellow 2001, 1619[VP] (Effective publication: Maldonado, Hookey, Ward and Goodfellow 2000, 371.)

flu.mi′ne.a.. L. fem. adj. *fluminea* of, in, or belonging to a river.

Acid–alcohol-fast actinomycetes. Form an extensive branched substrate mycelium which fragments into coccoid to rod-shaped elements. Brownish colonies bear sparse aerial hyphae and produce a brown diffusible pigment on glucose-yeast extract agar. Colony elevation is convex to irregular and margins are filamentous. Does not grow at 45°C. 2-Deoxythymidine-5′-*p*-nitrophenyl (*p*NP), *p*NP-phosphorylcholine, and *p*NP-β-D-xyloside are hydrolyzed. Sensitive (μg/ml) to cephaloridine (16), demethylchlortetracycline (16), gentamicin (8), neomycin (8), rifampin (1), streptomycin (16), and tobramycin (1) (Maldonado et al., 2001). Sensitive to (μg per disc) imipenem (2.5) and tobramycin (2.5); moderately sensitive to kanamycin (10.0); resistant to 5-fluorouracil (30) (Kageyama et al., 2005d). Resistant to lysozyme. Additional phenotypic properties are cited in Table 39. Whole-cell hydrolysates contain arabinose and galactose as characteristic sugars and *meso*-diaminopimelic acid as the principal diamino acid.

Source: soil collected adjacent to the River Thames at Medmenham, England.

DNA G+C content (mol%): not determined.

Type strain: CCUG 46120, CCUG 46296, CIP 107226, DSM 44489, JCM 11440, NBRC 100377, NCIMB 13759, S1.

Sequence accession no. (16S rRNA gene): AF277204.

31. **Nocardia gamkensis** LeRoes and Meyers 2007, 1[VP] (Effective publication: *Nocardia gamkensis* Le Roes and Meyers 2006, 294.)

gam.ken′sis. N.L. fem. adj. *gamkensis* pertaining to the River Gamka in South Africa.

Slightly acid–alcohol-fast actinomycete. Forms a light orange substrate mycelium that carries white aerial hyphae on Bennett's agar. Fragmentation into rod-shaped elements occurs after 5 d growth on agar media. Grows well on Bennett's and yeast extract-malt extract agars. Additional phenotypic properties are cited in Table 39. Whole-cell hydrolysates contain arabinose and galactose as characteristic sugars, and *meso*-diaminopimelic acid as the principal diamino acid. The polar lipid pattern is typical of the genus, as is the presence of mycolic acids on one-dimensional thin-layer chromatography of whole-organism methanolysates. The DNA–DNA relatedness value between the type strain and the corresponding strain of *Nocardia xishanensis*, a closely related species, is 10.35±3.15% (Le Roes and Meyers, 2006).

Source: a soil sample taken from the banks of the River Gamka in the Swartberg Nature Reserve, Western Cape Province, South Africa.

DNA G+C content (mol%): not determined.

Type strain: CZH20, DSM 44956, JCM 14299, NRRL B-24450.

Sequence accession no. (16S rRNA gene): DQ235272.

32. **Nocardia harenae** Seo and Lee 2006, 2206[VP]

ha.re′na.e L. gen. n. *harenae* of sand, referring to the isolation of the type strain from beach sand.

Slightly acid-fast actinomycete. Forms an extensively branched, reddish-orange substrate mycelium that fragments into irregular rod-shaped elements. Moderate amounts of white, fragmenting aerial hyphae are formed on oatmeal and inorganic salts-starch agars (ISP media 3 and 4), but aerial hyphae formation is only sparse on tryptic soy and yeast extract-malt extract (ISP medium 2) agars. Does not produce diffusible pigments. Grows at 10–40°C, but not at 45°C, and at pH 4.1–10.1. Additional phenotypic properties are shown in Table 39. Whole-cell hydrolysates contain arabinose and galactose as characteristic sugars and *meso*-diaminopimelic acid as the principal diamino acid. Cellular fatty acids contain major proportions of $C_{16:0}$, $C_{18:1}$ *cis*9, and $C_{18:0}$ 10-methyl, smaller proportions (<10%) of $C_{18:0}$ iso, $C_{18:0}$, and 2-OH-$C_{14:0}$, and traces of other saturated, iso and anteiso-branched chain fatty acids. Menaquinone and polar lipid profiles are typical of the genus, as is the presence of mycolic acids in one-dimensional thin-layer-chromatography of whole-cell methanolysates.

Source (type strain): beach sand collected on the coast of Jeju Island, Republic of Korea.

DNA G+C content (mol%): 68.9 (HPLC).

Type strain: JCM 14548, KCCM 42317, NRRL, B-24459, WS-20.

Sequence accession no. (16S rRNA gene): DQ282122.

33. **Nocardia higoensis** Kageyama, Yazawa, Mukai, Kinoshita, Takata, Nishimura, Kroppenstedt and Mikami 2004f, 1930[VP]

hi.go.en′sis. N.L. fem. adj. *higoensis* pertaining to Higo, a traditional geographical name for Kumamoto Prefecture in Japan, the source of the type strain.

Partially acid-fast actinomycete. Forms an extensive branched substrate mycelium that fragments *in situ* into rod-shaped elements (0.6–0.8 × 0.9–2.1 μm). Orange to reddish orange substrate mycelium bears white to reddish white aerial hyphae. Colonies lacking aerial hyphae may be produced. Colonies 0.3–0.6 mm in diameter are formed on Mueller–Hinton II medium supplemented with glucose (0.2%, w/v) after 7 d at 30°C. Susceptible (μg per disc) to imipenem (2.5), kanamycin (5.0), and tobramycin (2.5), and is slightly susceptible to 5-fluorouracil (30) (Kageyama et al., 2004f). Additional phenotypic properties are shown in Table 39. Whole-cell hydrolysates contain arabinose and galactose as characteristic sugars and *meso*-diaminopimelic acid as the principal diamino acid. Cellular fatty acids contain major proportions of $C_{16:0}$, $C_{18:1}$ *cis*9, and $C_{18:0}$ 10-methyl, smaller proportions (<10%) of $C_{14:0}$, $C_{17:1}$ *cis*9, $C_{17:0}$, $C_{18:0}$, $C_{19:1}$ *cis*9, and $C_{20:1}$ *cis*11, and traces of $C_{15:0}$ and 10-methyl $C_{17:0}$. Mycolic acids co-migrate on one-dimensional thin-layer chromatography of whole-organism methanolysates with those of representative *Nocardia* strains. The menaquinone profile is typical of the genus. DNA–DNA relatedness values between the type strain and corresponding strains of closely related species are as follows: *Nocardia farcinica* (9%) and *Nocardia shimofusensis* (28%) (Kageyama et al., 2004f).

Source (type strain): a patient with lung nocardiosis.

DNA G+C content (mol%): 69 (HPLC)

Type strain: NBRC 100133, DSM 44732, IFM 10084, JCM 12121.

Sequence accession no. (16S rRNA gene): AB108778.

34. **Nocardia ignorata** Yassin, Rainey and Steiner 2001b, 2130[VP]

ig.no.ra′ta. L. fem. part. adj. *ignorata* unrecognized, unknown, pertaining to the fact that the type strain was mistakenly used as a member of the genus *Mycobacterium* in a mycobacterial quality control test.

Slightly acid–alcohol-fast actinomycete. Forms a well developed orange substrate mycelium that bears white aerial hyphae. At a late stage of growth hyphae fragment into rod-shaped elements. Grows at 22–45°C. Gelatin and proline are used as simultaneous carbon and nitrogen sources, but not acetamide, alanine, or serine. Susceptible (μg per disc) to imipenem (2.5), and tobramycin (2.5) but not to 5-fluorouracil (30) and kanamycin (30) (Kageyama et al., 2005d). Additional phenotypic properties are cited in Table 39. Whole-cell hydrolysates contain arabinose and galactose as characteristic sugars and *meso*-diaminopimelic acid as the principal diamino acid. Cellular fatty acids contain major proportions of $C_{16:0}$, $C_{18:0}$, $C_{18:1}$, $C_{20:1}$, $C_{22:0}$, $C_{22:1}$, $C_{18:0}$ 10-methyl, smaller proportions (<10%) of $C_{16:1}$ *cis*7, $C_{17:0}$, and $C_{18:1}$ *cis*9, and traces of $C_{14:0}$, $C_{15:0}$, $C_{19:0}$, and $C_{20:0}$. Mycolic acids have 46–57 carbon atoms with 0–3 double bonds. Pyrolysis gas chromatography of purified fatty acid methyl esters release fatty acid methyl esters ($C_{16:0}$–$C_{18:0}$). Menaquinone and polar lipid profiles are typical of the genus. The DNA–DNA relatedness values between the type strain and the corresponding strain of *Nocardia salmonicida*, a closely related species, is 19.8% (Yassin et al., 2001b).

DNA G+C content (mol%): 68.1 (HPLC).

Type strain: CCUG 48296, DSM 44496, IMMIB R-1434, JCM 11764, NRRL B-24141.

Sequence accession no. (16S rRNA gene): AJ303008.

35. **Nocardia inohanensis** Kageyama, Yazawa, Nishimura and Mikami 2004i, 568g[VP]

in.o.han.en′sis. N.L. fem. adj. *inohanensis* of or pertaining to Inohana, Chiba, Japan, where the type strain was isolated.

Partially acid-fast actinomycete. Forms an extensively branched grayish tan to tan substrate mycelium which fragments into rod-shaped elements. Aerial hyphae are only visible microscopically. Short chains of spores are formed at the tips of aerial hyphae. Colonies are 0.3–1.3 mm in diameter on Mueller–Hinton II medium supplemented with glucose (0.2%, w/v) after 7 d at 30°C. Grows at 37°C, but not at 45°C. Resistant (μg per disc) to impenem (10), kanamycin (30), and tobramycin (10) (Kageyama et al., 2004i). Additional phenotypic properties are cited in Table 39. Whole-cell hydrolysates contain arabinose and galactose as characteristic sugars, and *meso*-diaminopimelic acid as the principal diamino acid. Mycolic acids co-migrate on one-dimensional thin-layer chromatography of whole-organism methanolysates with those of reference *Nocardia* strains. The menaquinone profile is typical of the genus. DNA–DNA relatedness values between the type strain and corresponding strains of closely related species are as follows: *Nocardia nugatensis* (10%), *Nocardia otitidiscaviarium* (7.7%), *Nocardia seriolae* (13%), *Nocardia uniformis* (17%), and *Nocardia yamanashiensis* (44%) (Kageyama et al., 2004i).

Source (type strain): clinical material in Mexico.

DNA G+C content (mol%): 68.8–69.5 (HPLC).

Type strain: DSM 44667, IFM 0092, JCM 11891, NBRC 100128.

Sequence accession no. (16S rRNA gene): AB092560.

36. **Nocardia iowensis** Lamm, Khare, Conville, Lau, Bergeron and Rosazza 2009, 2411[VP]

i.o.wen′sis. N.L. fem. adj. *iowensis* of or belonging to the State of Iowa, the source of the isolate.

Acid-fast actinomycete. Forms a substrate mycelium that fragments into irregular elements. A whitish substrate mycelium bears whitish aerial hyphae on sporulating agar after a few days at 29°C. Moderate growth occurs on asparagine-glucose, Benedict's, Bennett's potato starch, Weinstein's and yeast extract agars, light growth on Hickey and Tresner's, oatmeal and tomato paste agars, but poor growth on Czapek's, inorganic salts-starch, Kustner's oatflake and rice agars. Optimal growth temperature is 29°C. Additional phenotypic properties are cited in Table 39. Cellular fatty acids contain major proportions of $C_{16:0}$, $C_{18:1}$ *cis*9, and $C_{18:0}$ 10-methyl, smaller proportions (<10%) of $C_{17:1}$ *cis*8 and $C_{17:0}$,

and traces of $C_{10:0}$, $C_{14:0}$, $C_{15:1}$ *cis*5, $C_{17:0}$ 10-methyl, $C_{18:0}$, $C_{19:1}$ *cis*9, $C_{20:1}$ *cis*9, $C_{18:0}$ 10-methyl, and $C_{19:0}$ 10-methyl. Menaquinone and polar lipid profiles are typical of the genus, as is the presence of mycolic acids. DNA–DNA relatedness values of the type strain to corresponding strains of *Nocardia brasiliensis* and *Nocardia tenerefensis*, closely related species, are between 15.8 and 19.9% and between 5.4 and 7.4%, respectively (Lamm et al., 2009).

Source: a garden soil sample collected in Osceola, Iowa, USA.

DNA G+C content (mol%): not determined.

Type strain: DSM 45197, NRRL 5646, NRRL B-24671, UI 122540.

Sequence accession no. (16S rRNA gene): DQ925490.

Additional remarks: the taxonomic status of the species is supported by the results of 65-kDa heat-shock protein (*hsp 65*) and preprotein translocase (*secA1*) gene sequence analyses (Lamm et al., 2009). The type strain is a rich source of biotransforming enzymes and nitric oxide synthase.

37. **Nocardia jejuensis** Lee 2006, 561[VP]

je.ju.en′sis. N.L. fem. adj. *jejuensis* of or belonging to Jeju Island, Republic of Korea, from where the type strain was isolated.

Slightly acid–alcohol-fast actinomycete. Forms an extensive branched substrate mycelium that fragments into rod-shaped elements. An orange substrate mycelium carries a white to pinkish aerial mycelium which fragments into rod-shaped elements. Grows at 10–37°C, and at pH 4.1–10.1. Susceptible (μg per disc) to 5-fluorouracil (30) and tobramycin (2.5) and slightly susceptible to impenem (10) and kanamycin (30) (Hoshino et al., 2007). Additional phenotypic properties are shown in Table 39. Whole-cell hydrolysates contain arabinose and galactose as characteristic sugars and *meso*-diaminopimelic acid as the principal diamino acid. Cellular fatty acids contain major proportions of $C_{16:0}$, $C_{18:1}$ *cis*9, $C_{18:0}$, and $C_{18:0}$ 10-methyl, and smaller proportions (<10%) of $C_{14:0}$, $C_{15:0}$, $C_{16:1}$, and $C_{17:0}$. Mycolic acids co-migrate on one-dimensional thin-layer chromatography of whole-organism methanolysates with those of reference *Nocardia* strains. Menaquinone and polar lipid profiles are typical of the genus.

Source: a natural cave on Jeju Island, Republic of Korea.

DNA G+C content (mol%): 69.6 (HPLC).

Type strain: JCM 13281, N3–2, NBRC 103114, NRRL B-24430.

Sequence accession no. (16S rRNA gene): AY964666.

38. **Nocardia jiangxiensis** Cui, Wang, Huang, Liu and Goodfellow 2005, 1924[VP]

jiang.xi.en′sis. N.L. fem. adj. *jiangxiensis* of or belonging to Jiangxi Province, southern China, the source of the type strain.

Partially acid–alcohol-fast actinomycete. Forms an extensive branched substrate mycelium that fragments into rod-shaped elements. A pinkish white to orange substrate mycelium bears sparse to abundant white to pink aerial hyphae on modified Sauton's agar. A yellowish brown substrate mycelium carries white aerial hyphae on oatmeal agar (ISP medium 3) at an initial pH of 5.5. Does not form diffusible pigments. Grows at 17–37°C, but not at 10 or 45°C, and at an initial pH of 3.5 and 9.5. L-Alanine-*p*-nitroanilide (*p*NA), *p*-nitrophenyl (*p*NP)-β-D-galactopyranoside, *p*NP-β-D-glucopyranoside, *bis*-*p*NP-phosphate, *p*NP-phenylphosphonate, and *p*NP-β-D-xylopyranoside are hydrolyzed, but not 2-deoxythymidine-5′-*p*NP phosphate, *p*NP-glucopyranoside, *p*NP-β-D-glucuronide, L-glutamate-γ-3 carbonyl *p*NA, *p*NP-phosphorylcholine, or L-proline *p*NA. Susceptible (μg per disc) to 5-fluorouracil (30) and impenem (2.5), and moderately susceptible to kanamycin (10) and tobramycin (5.0) (Hoshino et al., 2007). Additional phenotypic properties are cited in Table 39. Whole-cell hydrolysates contain arabinose and galactose as characteristic sugars and *meso*-diaminopimelic acid as the principal diamino acid. Cellular fatty acids contain major proportions of $C_{16:0}$, $C_{16:1}$ *cis*7, $C_{18:1}$ *cis*9, $C_{18:0}$, and $C_{18:0}$ 10-methyl, smaller proportions (<10%) of $C_{17:0}$, $C_{15:1}$ *cis*5, $C_{17:1}$ *cis*8, $C_{17:1}$ *cis*5, $C_{17:0}$, and traces of $C_{14:0}$, $C_{20:0}$, and $C_{16:1}$ *cis*9. Mycolic acids co-migrate on one-dimensional thin-layer chromatography of whole-organism methanolysates with those of reference *Nocardia* strains. Menaquinone and polar lipid profiles are typical of the genus. DNA–DNA relatedness values between the type strain and corresponding strains of related species are as follows: *Nocardia miyunensis* (53%), *Nocardia nova* (46%), and *Nocardia pseudobrasiliensis* (39%) (Cui et al., 2005).

Source: the rhizosphere of goose grass (*Eleusine indica*) that was growing next to a copper mine in Wushan, Jiangxi Province.

DNA G+C content (mol%): not determined.

Type strain: 43401, CGMCC 4.1905, DSM 43401, JCM 12861, NBRC 101359.

Sequence accession no. (16S rRNA gene): AY639902.

39. **Nocardia jinanensis** Sun, Zhang, Huang, Zhang, Yang and Liu 2009, 419[VP]

ji.nan.en′sis. N.L. fem. adj. *jinanensis* of or pertaining to Jinan, capital city of Shandong Province, China, soil of which was the source of the type strain.

Partially acid–alcohol-fast actinomycete. Forms a branched substrate mycelium that fragments *in situ* into irregular rod-shaped elements. White to yellow substrate hyphae bear sparse to abundant, white to yellowish aerial hyphae on standard agar media such as yeast extract-malt extract agar (ISP medium 2). Does not produce diffusible pigments. Grows at 15–37°C and pH 5.5–10.5, but not at 45°C and pH 4.5. L-Phenylalanine is used as a simultaneous carbon and nitrogen source. Sensitive (μg per disc) to filter-paper soaked in kanamycin (30) and tobramycin (10). Additional phenotypic properties are cited in Table 39. Whole-cell hydrolysates contain arabinose and galactose as characteristic sugars and *meso*-diaminopimelic acid as the principal diamino acid. Cellular fatty acids contain major proportions of $C_{16:0}$, $C_{16:1}$ *cis*7, $C_{18:1}$ *cis*9, and $C_{15:0}$ iso 2-OH. Menaquinone and polar lipid profiles are characteristic of the genus, as is the presence of mycolic acids in whole-cell methanolysates. The DNA–DNA relatedness value between the type strain and the corresponding strain of *Nocardia speluncae*, a closely related species, is 22.5±2.1% (Sun et al., 2009).

Source: a soil sample collected from Jinan City, Shandong Province, Northern China.

DNA G+C content (mol%): 65.0 (T_m).

Type strain: 04-5195, CGMCC 4.3508, DSM 45048, JCM 14879.

Sequence accession no. (16S rRNA gene): DQ 462650.

40. **Nocardia kruczakiae** Conville, Brown, Steigerwalt, Lee, Anderson, Fishbain, Holland and Witebsky 2005, 547[VP] (Effective publication: Conville, Brown, Steigerwalt, Lee, Anderson, Fishbain, Holland and Witebsky 2004, 5144.)

kruc.zak′e.i. N.L. gen. fem. n. *kruczakiae* of Kruczak, in memory of Patricia Kruczak-Filipov (1953–1997), in honor of and recognition for her contributions to the Mycology and Mycobacteriology Sections of the Microbiology Service, Department of Laboratory Medicine, Warren G. Magnuson Clinical Center, National Institutes of Health, USA.

Weakly acid-fast actinomycetes. Form a branched substrate mycelium that carries aerial hyphae. Grows at 25–45°C, with best growth at 45°C. Susceptible to amikacin, ceftriazone, clarithromycin, imipenem, linezolid, minocycline, and trimethoprim-sulfamethoxazole, but not to amoxicillin-clavulanic acid, ciprofloxacin, and sulfamethoxazole (Conville et al., 2004). Susceptible to (µg per disc) 5-fluorouracil (30) and impenem (2.5), moderately susceptible to kanamycin (10.0), but resistant to tobramycin (10) (Hoshino et al., 2007). DNA–DNA relatedness values between the type strain and the corresponding strains of closely related species are as follows: *Nocardia africana* (58±4.5%), *Nocardia nova* (60±5.5%), and *Nocardia veterana* (55±8.5%) (Conville et al., 2004).

Source: a respiratory pathogen isolated from immunocompromised patients.

DNA G+C content (mol%): not determined.

Type strain: ATCC BAA-948, DSM 44877, JCM 13022, MB2876, NBRC 101016.

Sequence accession no. (16S rRNA gene): AY441974.

41. **Nocardia lijiangensis** Xu, Li, Tang, Jiang, Gao, Xu and Jiang 2006b, 2025[VP] (Effective publication: *Nocardia lijiangensis* Xu, Li, Yang, Jiang, Gao, Xu and Jiang 2006a, 312.)

li.jiang.en′sis. N.L. fem. adj. *lijiangensis* of or pertaining to the city of Lijiang, in Yunnan Province, southwest China, the source of the type strain.

Slightly acid–alcohol-fast actinomycete which forms an extensively branched substrate mycelium which fragments into rod-shaped elements. Aerial hyphae fragment into short to elongated rod-like elements. Grows well on yeast extract-malt extract agar (ISP medium 2) forming a white aerial mycelium and a deep orange yellow substrate mycelium. A yellow white aerial mycelium and a light to pale yellow substrate mycelium is formed on oatmeal, inorganic salt-starch, and glycerol-asparagine agars (ISP media 3–5). Grows at 28–37°C and pH 7.0–9.0. Susceptible (µg per disc) to amikacin (30), aureomycin (30), chloramphenicol (30), erythromycin (15), gentamicin (10), netilimicin (10), streptomycin (10), terramycin (30), tetracycline (30), and tobramycin (10) but not to ciprofloxacin (10), kanamycin (10), novobiocin (30), oleandomycin (10), penicillin-G (10 U), polymyxin B (300 U), and vancomycin (10) (Xu et al., 2006a). Susceptible (µg per disc) to impenem (2.5) and tobramycin (2.5) but not to 5-fluorouracil (10) and kanamycin (30) (Hoshino et al., 2007). Resistant to lysozyme. Grows in the presence of phenol (0.01%, w/v) but not of sodium chloride. Additional phenotypic properties are cited in Table 39. Whole-cell hydrolysates contain arabinose and galactose as characteristic sugars and *meso*-diaminopimelic acid as the principal diamino acid. Cellular fatty acids contain major proportions of $C_{16:0}$, $C_{18:1}$ *cis*9, $C_{18:0}$, and $C_{18:0}$ 10-methyl, and lesser proportions of $C_{15:0}$ anteiso, $C_{16:0}$ iso, $C_{16:1}$ *cis*7, iso- $C_{15:0}$ 2-OH, $C_{17:0}$ anteiso, $C_{18:2}$ *cis*6–9, and $C_{20:2}$ *cis*6–9. Mycolic acids co-migrate on one-dimensional thin-layer chromatography of whole-organism methanolysates with those of reference *Nocardia* strains. Menaquinone and polar lipid profiles are typical of the genus. DNA–DNA relatedness values between the type strain and corresponding strains of closely related species are as follows: *Nocardia polyresistens* (45.4%) and *Nocardia xishanensis* (28.6%) (Xu et al., 2006a).

Source (type strain): soil collected in Lijiang, Yunnan Province, Southwest China.

DNA G+C content (mol%): 65.4 (T_m).

Type strain: CCTCC AA 204005, DSM 44933, JCM 13592, KCTC 19028, YIM 33378.

Sequence accession no. (16S rRNA gene): AY779043.

Additional remarks: the culture collection number DSM 44933 was provided on request for validation (Lists Editor, 2006b).

42. **Nocardia mexicana** Rodríguez-Nava, Couble, Molinard, Sandoval, Boiron and Laurent 2006, 925[VP] (Effective publication: *Nocardia mexicana* Rodriguez-Nava, Couble, Molinard, Sandoval, Boiron and Laurent 2004, 4534.)

me.xi.ca′na. N.L. fem. adj. *mexicana* of or belonging to Mexico City, the geographical area from which the isolates were collected.

Acid–alcohol-fast actinomycete. Forms extensively branched hyphae with a tendency to fragment in early stages of growth. Rough brownish purple colonies 1–3 mm in diameter are formed on Bennett's agar. Grows at 25–37°C, but not at 45°C. Susceptible (median inhibitory concentrations (µg/ml)) to amikacin (32), amoxycillin (>128), cefotaxime (8), ceftriaxone (3), ciprofloxacin (2), gentamicin (128), imipenem (16), sulfamethoxazole (128), trimethoprim-sulfamethoxazole (3.2/64), and tetracycline (64) (Rodriguez-Nava et al., 2004). Additional phenotypic properties are cited in Table 39. Whole-cell hydrolysates contain *meso*-diaminopimelic acid as the principal diamino acid. Cellular fatty acids contain major proportions of $C_{16:0}$, $C_{16:1}$ *cis*7, $C_{18:1}$, and $C_{18:0}$, smaller proportions (<10%) of $C_{13:1}$, $C_{14:0}$, $C_{15:1}$, and $C_{17:1}$, and traces of $C_{15:0}$. Mycolic acids have 54–60 carbon atoms with C56 as the major component. The menaquinone profile is typical of the genus. DNA–DNA relatedness values between the type strain and corresponding strains of closely related species are as follows: *Nocardia asteroides* (26.3%) and *Nocardia pseudobrasiliensis* (32.1%) (Rodriguez-Nava et al., 2004).

Source: human mycetomas.

DNA G+C content (mol%): not determined.

Type strain: CIP 108295, DSM 44952, JCM 14590, OFN 1325.

Sequence accession no. (16S rRNA gene): AY903610.

Additional remarks: the culture collection accession number DSM 44952 was provided on request for validation (Lists Editor, 2006a).

43. **Nocardia miyunensis** Cui, Wang, Huang, Liu and Goodfellow 2005, 1924[VP]

mi.yun.en′sis. N.L. fem. adj. *miyunensis* of or belonging to Miyun County in Beijing, the source of the type strain.

Partially acid–alcohol-fast actinomycete which produces an extensively branched substrate mycelium that fragments into rod-shaped elements. A white to yellowish white substrate mycelium bears sparse to abundant, white to pink aerial hyphae on modified Sauton's agar. A yellowish-brown substrate mycelium carries white aerial hyphae on oatmeal agar (ISP medium 3) at an initial pH of 5.5. Does not form diffusible pigments. Grows at 17–37°C, but not at 10 or 45°C. Growth occurs at initial pH 4.5–9.5. Does not hydrolyze L-alanine (*p*NA), 2-deoxythymidine-5′ *p*-nitrophenyl (*p*NP) phosphate, *p*NP-β-D-galactopyranoside, *p*NP-α-D-glucopyranoside, *p*NP-β-D-glucopyranoside, *p*NP-β-D-glucuronide, L-glutamate-γ-3-carbonyl *p*NA, *bis*-*p*NP-phosphate, *p*NP-phenylphosphonate, *p*NP-phosphorylcholine, L-proline *p*NA, or *p*NP-β-D-xylopyranoside. Susceptible (μg per disc) to 5-fluorouracil (30), impenem (2.5), kanamycin (5.0), and tobramycin (2.5) (Hoshino et al., 2007). Additional phenotypic properties are shown in Table 39. Whole-cell hydrolysates contain arabinose and galactose as characteristic sugars and *meso*-diaminopimelic acid as the principal diamino acid. Cellular fatty acids contain major proportions of $C_{16:0}$, $C_{16:1}$ *cis*7, and $C_{18:0}$ 10-methyl, and smaller proportions (<10%) of $C_{18:1}$ *cis*9 and $C_{18:0}$. Mycolic acids co-migrate on one-dimensional thin-layer chromatography of whole-organism methanolysates with those of reference *Nocardia* strains. Menaquinone and polar lipid profiles are typical of the genus. DNA–DNA relatedness values between the type strain and corresponding strains of closely related species are as follows: *Nocardia jiangiensis* (58%), *Nocardia nova* (54%), and *Nocardia pseudobrasiliensis* (42%) (Cui et al., 2005).

Source (type strain): a pine forest soil sample collected in Miyun County, Beijing, China.

DNA G+C content (mol%): not determined.

Type strain: 117, CGMCC 4.1904, DSM 17685, JCM 12860.

Sequence accession no. (16S rRNA gene): AY639901.

44. **Nocardia neocaledoniensis** Saintpierre-Bonaccio, Maldonado, Amir, Pineau and Goodfellow 2004, 602[VP]

ne.o.ca.le.do.ni.en′sis. N.L. fem. adj. *neocaledoniensis* of or pertaining to New Caledonia, the source of the isolate.

Slightly acid–alcohol-fast actinomycete. Forms an extensively branched substrate mycelium that fragments *in situ* into irregular rod-shaped elements. An orange substrate mycelium bears abundant pale orange aerial hyphae on modified Bennett's agar. Melanin pigments are produced on peptone-yeast extract-iron agar (ISP medium 6). Grows at 10–45°C and pH 4–12. Sensitive (μg/ml) to tetracycline (30) but not to erythromycin (4), gentamicin (10), penicillin G (25), rifamipicin (6), streptomycin (5), and vancomycin (10) (Saintpierre-Bonaccio et al., 2004). Sensitive to (μg per disc) impenem (2.5) and tobramycin (2.5), slightly susceptible to kanamycin (30) and weakly susceptible to 5-fluorouracil (30) (Hoshino et al., 2007). Grows in the presence (%, w/v) of crystal violet (0.0002), phenol (0.01), and sodium chloride (3) but not in the presence of potassium tellurite (0.005) and sodium chloride (5). Additional phenotypic properties are cited in Table 39. Whole-cell hydrolysates contain arabinose and galactose as characteristic sugars and *meso*-diaminopimelic acid as the principal diamino acid. Mycolic acids co-migrate on one-dimensional thin-layer chromatography of whole-organism methanolysates with those of reference *Nocardia* strains. Menaquinone and polar lipid profiles are typical of the genus. The DNA–DNA relatedness value between the type strain and the corresponding strain of *Nocardia asteroides*, a closely related species, is 40.8% (Saintpierre-Bonaccio et al., 2004).

Source (type strain): a brown hypermagnesian ultramafic soil at the Southern end of the main island of New Caledonia.

DNA G+C content (mol%): not determined.

Type strain: DSM 44717, JCM 12604, NCIMB 13955, SBH_R OA6.

Sequence accession no. (16S rRNA gene): AY282603.

45. **Nocardia niigatensis** Kageyama, Yazawa, Nishimura and Mikami 2004i, 568[VP]

ni.i.gat.en′sis. N.L. fem. adj. *niigatensis* of or pertaining to Niigata, Japan, the source of the isolate.

Partially acid-fast actinomycete. Forms branched substrate hyphae that fragment into oval to rod-shaped elements. Tan to grayish-tan colonies bear sparse, white, patchy distributed aerial hyphae. Short chains of spores may occur on the aerial mycelium. A faint brown diffusible pigment is produced. Colonies 1.5–3.2 mm in diameter are produced on Mueller–Hinton medium supplemented with glucose (0.2%, w/v) after 7 d at 30°C. Grows at 37°C, but not at 45°C. L-Alanine *p*-nitroanilide (*p*NA), 2-deoxythymidine-5′ *p*-nitrophenyl (*p*NP), *p*NP-α-D-glucopyranoside, *p*NP-β-D-glucopyranoside, *bis*-*p*NP-phosphate, *p*NP-phenylphosphonate, and *p*NP-xylopyranoside are hydrolyzed, but not *p*NP-α-D-galactopyranoside, *p*NP-β-D-glucuronide, L-glutamate-γ-carboxyl *p*NA, *p*NP-phosphorylcholine, or L-proline *p*NA. Susceptible (μg per disc) to tobramycin (2.5) and kanamycin (5), but not to impenem (10) (Kageyama et al., 2004i). Whole-cell hydrolysates contain arabinose and galactose as characteristic sugars and *meso*-diaminopimelic acid as the principal diamino acid. Cellular fatty acids contain major proportions of $C_{16:0}$, $C_{18:1}$ *cis*9, and/or $C_{16:1}$ *cis*7/$C_{15:0}$ iso 2-OH and $C_{18:0}$ 10-methyl; smaller proportions (<10%) of $C_{14:0}$, $C_{15:0}$, $C_{17:0}$, $C_{18:0}$, $C_{15:1}$ *cis*5, $C_{17:1}$ *cis*8, and $C_{17:0}$ 10-methyl, and traces of $C_{16:0}$ 10-methyl. Muramic acid moieties are *N*-glycolated. Mycolic acids co-migrate on one-dimensional thin-layer chromatography with those of whole-organism methanolysates of representative *Nocardia* strains. The menaquinone profile is typical of the genus. DNA–DNA relatedness values between the type strain and corresponding strains of closely related species are as follows: *Nocardia inohanensis* (21%), *Nocardia otitidiscaviarum* (20%), *Nocardia*

seriolae (41%), *Nocardia uniformis* (22%), and *Nocardia yamanashiensis* (29%) (Kageyama et al., 2004i).

Source: skin abscesses.

DNA G+C content (mol%): 68.8–69.5 (HPLC).

Type strain: DSM 44670, IFM 0330, JCM 11894, NBRC 100131.

Sequence accession no. (16S rRNA gene): DQ659910.

46. **Nocardia ninae** Laurent, Rodríguez-Nava, Noussair, Couble, Nicolas-Chanoine and Boiron 2007, 664[VP]

ni′na.e. N.L. fem. gen. n. *ninae* of Nina, the first name of the patient from whom the type strain was isolated.

Acid–alcohol-fast actinomycete. Produces branched hyphae which tend to fragment into coccoid and rod-like elements. An orange substrate mycelium bears sparse to moderate amounts of white aerial hyphae. Rough colonies 1–3 mm in diameter are formed on Bennett's agar. Grows at 37°C, but not at 45°C. Additional phenotypic properties are shown in Table 39. Whole-cell hydrolysates contain *meso*-diaminopimelic acid as the only peptidoglycan diamino acid. Cellular fatty acids contain major proportions of $C_{16:0}$, $C_{16:1}$, and $C_{18:0}$ 10-methyl, and a smaller proportions (<10%) of $C_{18:0}$. Mycolic acids have 52–58 carbon atoms. The menaquinone profile is typical of the genus. The DNA–DNA relatedness values between the type strain and the corresponding strain of *Nocardia alba*, a closely related species, is below 20% (Laurent et al., 2007). These workers were also able to separate the type strain from corresponding strains of other *Nocardia* species, including *Nocardia alba* and *Nocardia jejuensis*, on the basis of *hsp65* gene sequences.

Source: a human respiratory sample.

DNA G+C content (mol%): not determined.

Type strain: CIP 108955, DSM 44978, JCM 14667, OFN 02.72.

Sequence accession no. (16S rRNA gene): DQ235687.

47. **Nocardia nova** Tsukamura 1983, 896[VP] (Effective publication: Tsukamura 1982a, 1115.)

no′va. L. fem. adj. *nova* new.

Weakly or partially acid-fast actinomycete. Forms a mycelium that fragments into coccoid and rod-shaped elements. Forms rough orange colonies. Grows at 28–42°C, but not at 45°C. L-Alanine-*p*-nitroanilide (*p*NA), *p*-nitrophenyl-α-D-glucopyranoside, (*p*NP)-β-D-glucopyranoside, *bis*-*p*NP-phosphate, *p*NP-phenylphosphonate, and *p*NP-xylopyranoside are hydrolyzed, but not deoxythymidine-5′-*p*NP-phosphate, *p*NP-α-D-galactopyranoside, *p*NP-β-D-glucuronide, L-glutamate-γ-3-carboxyl *p*NA, *p*NP-phosphorylcholine, or L-proline, *p*NA. Does not use acetamide, L-alanine, gelatin, proline, or serine as simultaneous carbon and nitrogen sources. The type strain is sensitive (μg/ml) to kanamycin (5), lincomycin (10), novobiocin (10), paromomycin (100), polymyxin (5), spiramycin (5), and sulfamethoxazole (10) but resistant to ampicillin (5), cephaloridine (100), erythromycin (10), gentamicin (10), kanamycin (50), lividomycin (10), neomycin (10), novobiocin (100), penicillin (10), polymyxin (50), spiramycin (50), and streptomycin (10) (Hamid et al., 2001b). Sensitive to (μg per disc) imipenem (2.5) and 5-fluorouracil (30) but not to kanamycin (30) and tobramycin (10) (Kageyama et al., 2005d). Grows in the presence (%, w/v) of pyronin G (0.1) but not in the presence of sodium azide (0.01), sodium chloride (7), sodium nitrate (0.1), tetrazolium salt (0.1), and thallous acetate (0.001). Additional phenotypic properties are shown in Table 39. Whole-cell hydrolysates contain arabinose and galactose as characteristic sugars and *meso*-diaminopimelic acid as the principal diamino acid. Cellular fatty acids contain major proportions of $C_{16:0}$, $C_{18:1}$ *cis*9, and/or $C_{16:1}$ *cis*7/$C_{15:0}$ iso 2-OH and $C_{18:0}$ 10-methyl, and smaller proportions (<10%) of $C_{14:0}$, $C_{15:0}$, $C_{17:0}$, and $C_{18:0}$. Mycolic acids contain 52–64 carbon atoms with 1–4 double bonds, including one in the α-alkyl position. DNA–DNA relatedness values between the type strain and the corresponding strains of closely related species are as follows: (i) *Nocardia asteroides* (19%), *Nocardia farcinica* (16%), *Nocardia salmonicida* (24%), and *Nocardia seriolae* (8%) (Kudo et al., 1988); (ii) *Nocardia asteroides* (39%) and *Nocardia farcinica* (20%) (Yano et al., 1990); (iii) *Nocardia abscessus* (9%), *Nocardia asteroides* (14%), *Nocardia paucivorans* (11%), *Nocardia pseudobrasiliensis* (16%), *Nocardia transvalensis* (23%), *Nocardia vaccinii* (16%), and *Nocardia vinacea* (9%) (Kinoshita et al., 2001); (iv) *Nocardia africana* (3%), *Nocardia cerradoensis* (11%), *Nocardia vaccinii* (13%), *Nocardia vermiculata* (10%), *Nocardia veterana* (12%), and *Nocardia vinacea* (3%) (Kageyama et al., 2004c).

Source: sputa of patients with lung disease.

DNA G+C content (mol%): not determined.

Type strain: ATCC 33726, CCUG 45939, CIP 104777, DSM 44481, R.E. Gordon R443, IFO (now NBRC) 15556, JCM 6044, VKM Ac-1971, Tsukamura 23095.

Sequence accession no. (16S rRNA gene): AF430028.

48. **Nocardia otitidiscaviarum** corrig. Snijders 1924, 338[AL] ["*Actinomyces caviae*" Erikson 1935; *Nocardia caviae* (Erikson 1935) Erikson 1935]

o.ti.ti.dis.ca.vi.a′rum. N.L. n. *otitis*, *-idis* inflammation of the ear; N.L. n. *Cavia* (gen. plur. *caviarum*) generic name for cavy, another name for guinea pig; N.L. gen. pl. n. *otitidiscaviarium* of ear disease of guinea pigs.

Weakly acid–alcohol-fast actinomycete. Forms an extensively branched substrate mycelium which fragment into coccoid to rod-shaped elements. Cream grayish to peach tan to purplish substrate hyphae carry very sparse, off-white aerial hyphae. The latter are always present but may only be visible microscopically. Soluble pigments variably present. Grows at 30–45°C. 2-Deoxythymidine-5′ *p*-nitrophenyl (*p*NP), *p*NP-phosphorylcholine, and *p*NP-β-D- xyloside are hydrolyzed. L-Alanine and serine are used as simultaneous carbon and nitrogen sources, but not acetamide, gelatin, or proline. Susceptible to amikacin, chloramphenicol, clindamycin, gentamicin, kanamycin, and minocycline but not to ampicillin, amoxicillin-clavulanic acid, perfloxacin, trimethoprim, trimethoprin-sulfamethoxazole; variable responses to cefotaxime, cefuroxime, and tetracycline (Boiron and Provost, 1988b). The type strain is sensitive (μg/ml) to amoxicillin (10), ampicillin (50), bacitracin (10), cephaloridine (100), erythromycin (10), gentamicin (10), kanamycin (5), lincomycin (10), lividomycin (10), neomycin (100), penicillin (100), polymyxin (50), streptomycin (100), and sulfamethoxazole 100, but resistant to ampicillin (100), deoxycycline (10), erythromycin (100),

novobiocin (10), and spiramycin (50) (Hamid et al., 2001b). Sensitive to (µg per disc) kanamycin (5.0); slightly susceptible to tobramycin (10.0), but resistant to 5-fluorouracil (30) and imipenem (10) (Kageyama et al., 2005d). Grows in the presence (%, w/v) of sodium chloride (5), sodium nitrate (0.1) but not in the presence of pyronin G (0.1), sodium azide (0.01), sodium chloride (7), tetrazolium salt (0.1), and thallous acetate (0.001). Additional phenotypic properties are shown in Table 39. Whole-cell hydrolysates contain arabinose and galactose as characteristic sugars and *meso*-diaminopimelic acid as the principal diamino acid. Cellular fatty acids contain major proportions $C_{16:1}$ *cis*9, $C_{16:0}$, and $C_{18:0}$ 10-methyl, smaller proportions (<10%) of $C_{5:0}$, $C_{17:1}$ *cis*9, $C_{17:0}$, 10-methyl-$C_{17:0}$, $C_{18:1}$ *cis*9, $C_{19:1}$ *cis*9, and 10-methyl-$C_{19:0}$, and traces of $C_{14:0}$ and $C_{15:1}$ *cis*10. Mycolic acids have 40–60 carbon atoms with 0–3 double bonds. Pyrolysis gas chromatography of purified mycolic acid methyl esters release fatty and methyl esters ($C_{12:0}$–$C_{18:0}$). Menaquinone and polar lipid profiles are typical of the genus. DNA–DNA relatedness values between the type strain and corresponding strains of related species are as follows: *Nocardia inohanensis* (23%), *Nocardia niigatensis* (20%), *Nocardia seriolae* (19%), *Nocardia uniformis* (19%), and *Nocardia yamanashiensis* (25%) (Kageyama et al., 2004i).

Source: soil, but some strains are pathogenic for animals, including humans.

DNA G+C content (mol%): 66–67 (T_m).

Type strain: ATCC 14629, CCUG 10075, CIP 104514, DSM 43242, IFO (now NBRC) 14405, JCM 3377, JCM 3393, NCTC 1934, VKM Ac-869.

Sequence accession no. (16S rRNA gene): AF430067.

Additional remarks: the original spelling, namely *Nocardia otidis-caviarum* (*sic*), was corrected by Farmer (1983) and Hill et al. (1984). Additional phenotypic properties are cited in extensive numerical taxonomic studies (Goodfellow, 1971; Tsukamura, 1982a).

49. **Nocardia paucivorans** Yassin, Rainey, Burghardt, Brzezinka, Mauch and Schaal 2000a, 807[VP]

pau.ci.vo′rans. L. adj. *paucus* little; L. v. *vorare* to eat, to devour; L. pres. part. *vorans* eating; N.L. part. adj. *paucivorans*, eating little, referring to the few compounds that are utilized as sole sources of carbon and energy.

Slightly acid–alcohol-fast actinomycete. Forms a well developed substrate mycelium that penetrates agar media and bears white aerial hyphae. At a late stage of growth, hyphae fragment into rod-shaped elements. Does not hydrolyze 2-deoxythymidine-5′-*p*-nitrophenyl (*p*NP) phosphate, *p*NP-phosphorylcholine, or *p*NP-β-xyloside. Does not use acetamide, alanine, gelatin, proline, or serine as simultaneous carbon and nitrogen sources. Susceptible (µg per disc) to imipenem (2.5), kanamycin (5.0), and tobramycin (2.5) but not to 5-fluorouracil (30) (Kageyama et al., 2005d). Additional phenotypic properties are shown in Table 39. Whole-cell hydrolysates contain arabinose and galactose as characteristic sugars, and *meso*-diaminopimelic acid as the principal diamino acid. Cellular fatty acids contain major proportions of $C_{16:1}$ *cis*9 and $C_{18:0}$ 10-methyl, and smaller proportions (<10%) of $C_{15:0}$, $C_{16:0}$, $C_{18:1}$ *cis*9, and $C_{18:0}$. Mycolic acids have 48–54 carbon atoms with 2–4 double bonds. Pyrolysis gas chromatography of mycolic acids methyl esters release fatty acid methyl esters ($C_{14:0}$–$C_{18:0}$) with $C_{16:0}$ as the major component. Menaquinone and polar lipid profiles are typical of the genus. DNA–DNA relatedness values between the type strain and the corresponding strains of related species are as follows: (i) *Nocardia brevicatena* (61.9%) (Yassin et al., 2001a), and (ii) *Nocardia abscessus* (5%), *Nocardia asteroides* (7%), *Nocardia nova* (6%), *Nocardia pseudobrasiliensis* (6%), *Nocardia transvalensis* (6%), *Nocardia vaccinii* (6%), and *Nocardia vinacea* (4%) (Kinoshita et al., 2001).

Source (type strain): sputa and bronchial secretions of a patient with chronic lung disease.

DNA G+C content (mol%): 65.9 (HPLC).

Type strain: ATCC BAA-278, CCUG 46117, CIP 10698, DSM 44386, IMMIB D-1632, JCM 10919, NBRC 100373.

Sequence accession no. (16S rRNA gene): AF430041.

50. **Nocardia pigrifrangens** Wang, Zhang, Huang, Maldonado, Liu and Goodfellow 2004, 1685[VP]

pi.gri.frang′ens. L. adj. *piger* slow; L. part. adj. *frangens* (from L. v. *frango*) breaking up, small; N.L. part. adj. *pigrifrangens* slow to break up, referring to the fact that the substrate mycelium remains stable for up to 14 d before undergoing fragmentation.

Slightly acid–alcohol-fast actinomycete. Forms a branched substrate mycelium that fragments *in situ* into rod-shaped elements. An orange substrate mycelium bears sparse white to pinkish aerial hyphae on modified Bennett's, modified Sauton's, and oatmeal agars. Colonies are convex with filamentous margins. Grows at 18–35°C, but not at 45°C. L-Alanine, L-aspartate, L-glutamate, monoethanolamine, L-proline, uric acid, and L-valine are used as simultaneous carbon and nitrogen sources, but not acetamide, gelatin, L-leucine, L-phenylalanine, or urea. Susceptible (µg per disc) to 5-fluorouracil (30), impenem (2.5), kanamycin (5.0), and tobramycin (2.5) (Hoshino et al., 2007). Additional phenotypic properties are shown in Table 39. Whole-cell hydrolysates contain arabinose and galactose as characteristic sugars and *meso*-diaminopimelic acid as the principal diamino acid. Cellular fatty acids contain major proportions of $C_{16:0}$, $C_{18:1}$ *cis*9, $C_{18:0}$, and $C_{18:0}$ 10-methyl, and smaller proportions (<10%) of $C_{16:1}$ *cis*7 and *cis*9-$C_{21:1}$. Menaquinone and polar lipid profiles are typical of the genus.

Source (type strain): a contaminated plate of a clinically significant strain in Shanghai, China.

DNA G+C content (mol%): 68.7 (T_m).

Type strain: 7031, AS 4.1808, DSM 44957, JCM 11884.

Sequence accession no. (16S rRNA gene): AF219974.

51. **Nocardia pneumoniae** Kageyama, Yazawa, Mukai, Kohara, Nishimura, Kroppenstedt and Mikami 2004g, 2028[VP]

pneu.mo.ni′a.e. Gr. fem n. *pneumonia* disease of the lungs; N.L. fem. n. *pneumonia* disease of the lungs N.L. gen. n. *pneumoniae* of a disease of the lungs, of pneumonia.

Partially acid–alcohol-fast actinomycete. Forms a branched substrate mycelium that fragments into rod-shaped elements (0.3–0.5 × 0.6–1.1 µm). A faint yellow substrate mycelium bears white to grayish white hyphae. Does not produce soluble pigments. Colonies 2.0–3.0 mm

in diameter are formed on Mueller–Hinton II medium supplemented with glucose (0.2 mm) after 7 d at 30°C. Grows at 37 and 45°C. Susceptible (μg per disc) to impenem (2.5) and slightly susceptible to kanamycin (Kageyama et al., 2004g). Additional phenotypic properties are cited in Table 39. Whole-cell hydrolysates contain arabinose and galactose as characteristic sugars, and *meso*-diaminopimelic acid as the principal diamino acid. Cellular fatty acids contain major proportions of $C_{16:0}$, $C_{16:1}$ *cis*7, $C_{19:1}$ *cis*9, and $C_{18:0}$ 10-methyl, smaller proportions (<10%) of $C_{14:0}$, $C_{18:0}$ and $C_{19:1}$ *cis*, and traces of $C_{15:0}$, $C_{16:1}$ *cis*9, $C_{16:1}$ *cis*8, $C_{16:0}$ 10-methyl, $C_{17:1}$ *cis*9, 10-methyl-$C_{17:0}$, $C_{19:1}$ *cis*11, and $C_{19:0}$. Mycolic acids co-migrate on one-dimensional thin-layer chromatography of whole-organism methanolysates with those of reference *Nocardia* strains. The menaquinone profile is typical of the genus. DNA–DNA relatedness values between the type strain and the corresponding strains of related species are as follows: *Nocardia araonensis* (35%) and *Nocardia beijingensis* (<46%) (Kageyama et al., 2004g).

Source (type strain): a patient with a history of lung cancer, emphysema, and radiation pneumonia.

DNA G+C content (mol%): 68 (HPLC).

Type strain: DSM 44730, IFM 0784, JCM 12119, NBRC 100136.

Sequence accession no. (16S rRNA gene): AB108780.

52. **Nocardia polyresistens** Xu, Li, Tang, Jiang, Chen, Xu and Jiang 2005, 1469[VP]

po.ly.re.sis′tens. Gr. adj. *polus* many; L. part. adj. *resistens* resisting; N.L. part. adj. *polyresistens* resisting many (antibiotics).

Slightly acid–alcohol-fast actinomycete. Forms extensively branched aerial and substrate hyphae that fragment into irregular rod-shaped elements. A pale-yellow to moderate orange-yellow substrate mycelium bears sparse to abundant, white aerial hyphae on modified Bennett's. Czapek's, nutrient, potato, modified Sauton's, and yeast extract-malt extract agars. A pale yellow to yellow-white substrate mycelium carries pale yellow to yellow-white aerial hyphae on inorganic salts-starch and glycerol-asparagine agars (ISP media 4 and 5). Does not form diffusible pigments. Grows at 28–37°C, but not at 45°C, and grows at pH 7–9. L-Asparagine, L-histidine, and L-tyrosine are used as sole nitrogen sources, but not acetamide, L-arginine, L-cysteine, L-glutamic acid, glycine, L-hydroxyproline, L-lysine, L-methionine, or L-threonine. Susceptible (μg per disc) to chloramphenicol (30), streptomycin (10), and tetracycline (30) but not to amikacin (30), aureomycin (30), ciprofloxacin (10), erythromycin (15), gentamicin (10), kanamycin (5), netilimicin (10), novobiocin (30), oleandomycin (10), penicillin G (10 U), polymycin-B (300 U), terramycin (30), tobramycin (10) and vancomycin (10) (Xu et al., 2005). Susceptible (μg per disc) to impenem (2.5) and tobramycin 2.5 but not to 5-fluorouracil (30) and kanamycin (30) (Hoshino et al., 2007). Resistant to lysozyme. Grows in the presence (%, w/v) of phenol (0.1) but not in the presence of sodium chloride (4). Additional phenotypic properties are cited in Table 39. Whole-cell hydrolysates contain arabinose and galactose as characteristic sugars, and *meso*-diaminopimelic acid as the principal diamino acid. Cellular fatty acids contain major proportions of $C_{16:0}$, $C_{18:2}$ *cis*6–9, $C_{18:1}$ *cis*9, $C_{18:0}$, and $C_{18:0}$ 10-methyl, and smaller proportions (<10%) of $C_{15:0}$ iso, $C_{16:0}$ iso, $C_{16:1}$ *cis*7, $C_{15:0}$ iso 2-OH, $C_{17:0}$, $C_{17:0}$ anteiso, and $C_{17:0}$. Mycolic acids co-migrate on one-dimensional thin-layer chromatography of whole-organism methanolysates with those of reference *Nocardia* strains. Menaquinone and polar lipid profiles are typical of the genus.

Source: soil collected in Yunnan Province, southwest China.

DNA G+C content (mol%): 65.6 (T_m).

Type strain: CCTCC AA 204004, JCM 13593, KCTC 19027, YIM 33361.

Sequence accession no. (16S rRNA gene): AY626158.

53. **Nocardia pseudobrasiliensis** Ruimy, Riegel, Carlotti, Boiron, Bernardin, Monteil, Wallace and Christen 1996, 263[VP]

pseu.do.bra.si.li.en′sis. Gr. adj. *pseudês* false; N.L. fem. adj. *brasiliensis* a specific epithet; N.L. fem. adj. *pseudobrasiliensis* a false (*Nocardia*) *brasiliensis*.

Acid-fast to partially acid-fast actinomycete. Forms a branched substrate mycelium that fragments into bacteroid, coccoid or rod-shaped elements. An aerial mycelium is produced. Grows at 30–37°C, but not at 10 or 45°C. L-Alanine-*p*-nitroanilide (*p*NA), *p*-nitrophenyl (*p*NP)-β-D-galactopyranoside, *p*NP-α-D-glucopyranoside, *p*NP-β-D-glucopyranoside, *bis*-*p*NP-phosphate, *p*NP-phenylphosphonate, and *p*NP-β-D-xylopyranoside are hydrolyzed, but not 2-deoxythymidine-5′-*p*NP- phosphate, *p*NP-β-D-glucuronide, L-glutamate-γ-3-carboxyl *p*NA, *p*NP-phosphorylcholine, or L-proline *p*NA. Susceptible (μg per disc) to imipenem (2.5), tobramycin (2.5) but not to 5-fluorouracil (30) and kanamycin (30) (Kageyama et al., 2005d). Additional phenotypic properties are shown in Table 39. Whole-cell hydrolysates contain arabinose and galactose as characteristic sugars, and *meso*-diaminopimelic acid as the principal diamino acid. Cellular fatty acids contain major proportions of $C_{16:0}$ and/or $C_{16:1}$ *cis*7/$C_{15:0}$ iso 2-OH, and $C_{18:0}$ 10-methyl, smaller proportions (<10%) of $C_{17:0}$, $C_{18:0}$, and $C_{18:1}$ *cis*9, and traces of $C_{14:0}$, $C_{17:1}$ *cis*8, and $C_{17:0}$ 10-methyl. The menaquinone profile is typical of the genus, as is the presence of mycolic acids in whole-organism methanolysates. DNA–DNA relatedness values between the type strain and the corresponding strains of related *Nocardia* species are as follows: (i) *Nocardia brasiliensis* (11%), *Nocardia nova* (12%), *Nocardia otitidiscaviarum* (11%), *Nocardia seriolae* (7%), and *Nocardia vaccinii* (14%) (Ruimy et al., 1996), and (ii) *Nocardia abscessus* (7%), *Nocardia asteroides* (12%), *Nocardia nova* (13%), *Nocardia paucivorans* (8%), *Nocardia transvalensis* (17%), *Nocardia vaccinii* (13%), and *Nocardia vinacea* (9%) (Kinoshita et al., 2001).

Source (type strain): a leg abscess on a patient with ulcerative colitis.

DNA G+C content (mol%): 67–68 (chemical method).

Type strain: ATCC 51512, CCUG 35436, CIP 104600, DSM 44290, JCM 9894.

Sequence accession no. (16S rRNA gene): AF430042.

54. **Nocardia pseudovaccinii** Kim, Roth, Andrees, Lee and Kroppenstedt 2002b, 1828[VP]

pseu.do.vac.cin′i.i. Gr. adj. *pseudês* false; N.L. gen. n. *vaccinii* a specific epithet; N.L. gen. n. *pseudovaccinii* a false (*Nocardia*)

vaccinii, referring to the earlier misclassification of the type strain as a strain of *Nocardia vaccinii*.

A beige red substrate mycelium bears sparse white, branched aerial hyphae. The reverse side of the colony is yellow orange on yeast extract-malt extract agar. Deoxythymidine-5-*p*-nitrophenyl (*p*NP) phosphate, *p*NP-phosphorylcholine, and *p*NP-β-D-xyloside are hydrolyzed. Susceptible (μg per disc) to 5-fluorouracil (30), impenem (2.5), and tobramycin (2.5) but not to kanamycin (30) (Hoshino et al., 2007). Additional phenotypic properties are cited in Table 39. Whole-cell hydrolysates contain arabinose and galactose as characteristic sugars and *meso*-diaminopimelic acid as the principal diamino acid. Muramic acid moieties are *N*-glycolated. Cellular fatty acids contain major proportions of $C_{16:0}$, $C_{16:1}$, $C_{18:1}$, and $C_{18:0}$ 10-methyl, smaller proportions (<10%) of $C_{14:0}$, $C_{17:1}$, $C_{17:0}$, 10-methyl-$C_{17:0}$, $C_{18:0}$, and $C_{19:1}$, and a trace of $C_{15:0}$. Mycolic acids have 50–58 carbons with C52, C54, and C56 as major components. Menaquinone and polar lipid profiles are typical of the genus.

Source: unknown.

DNA G+C content (mol%): not determined.

Type strain: AR 368,38366-20, DSM 43406, JCM 11883, NBRC 100343, NRRL B-24154.

Sequence accession no. (16S rRNA gene): AF430046.

Additional remarks: the name *Nocardia pseudovaccinii* Kim et al. 2002b was proposed for strain DSM 43406 which had previously been classified as *Nocardia vaccinii*.

55. **Nocardia puris** Yassin, Sträubler, Schumann and Schaal 2003, 1598[VP]

pu′ris. L. gen. n. *puris* of corrupt matter, pus, pertaining to the abscess from which the type strain was isolated.

Partially acid–alcohol-fast actinomycete. Forms a well developed irregularly branched substrate mycelium which bears white aerial hyphae. At a late stage in the growth cycle, hyphae fragment into rod-shaped elements. Grows at 22–45°C. Alanine is used as a simultaneous carbon and nitrogen source, but not acetamide, arginine, gelatin, ornithine, proline, or serine. Susceptible (μg per disc) to imipenem (2.5), tobramycin (10) but not to 5-fluorouracil (30) and kanamycin (30) (Kageyama et al., 2005d). Additional phenotypic properties are cited in Table 39. Whole-cell hydrolysates contain arabinose and galactose as characteristic sugars, and *meso*-diaminopimelic acid as the principal diamino acid. Cellular fatty acids contain major proportions of $C_{16:0}$, $C_{18:0}$, $C_{18:1}$, $C_{18:1}$ *cis*9, and $C_{18:0}$ 10-methyl, smaller proportions (<10%) of $C_{16:1}$ *cis*7, $C_{17:0}$, $C_{20:0}$, $C_{20:1}$, $C_{21:0}$, and $C_{21:0}$, $C_{21:1}$, and traces of $C_{14:0}$, $C_{15:0}$, $C_{19:0}$, $C_{20:0}$, and $C_{21:0}$. Pyrolysis gas chromatography of purified mycolic acid methyl esters release fatty acid methyl esters ($C_{14.0}$–$C_{18:0}$) with $C_{16:0}$ as the major component. Menaquinone and polar lipid profiles are typical of the genus. DNA–DNA reassociation values between the type strain and corresponding strains of related species are as follows: *Nocardia abscessus* (20.6%), *Nocardia cyriacigeorgica* (37.6%), and *Nocardia farcinica* (42.3%) (Yassin et al., 2003).

Source (type strain): a human abscess.

DNA G+C content (mol%): not determined.

Type strain: CCUG 48752, DSM 44599, JCM 13031, IMMIB R-145, NRRL B-24204.

Sequence accession no. (16S rRNA gene): AJ508748.

56. **Nocardia salmonicida** (*ex* Rucker) Isik, Chun, Hah and Goodfellow 1999b, 834[VP]

sal.mo.ni′ci.da. L. n. *salmo, salmonis* salmon; L. suff. *cida* from L. v. *caedo* to cut or kill; N.L. n. *salmonicida* salmon-killer ("*Streptomyces salmonicida*" Rucker 1949, "*Nocardia salmonicida*" (Rucker 1949) Pridham 1970.

Acid–alcohol-fast actinomycete. Forms an extensive branched substrate mycelium that fragments *in situ* into coccoid to rod-shaped elements. An orange substrate mycelium bears white to pink aerial hyphae on glucose-yeast extract agar. Does not form diffusible pigments. Colony elevation is convex to irregular and colony margins are filamentous. Grows at 20 and 30°C, but not at 10 or 45°C. *p*-Nitroanilide (*p*NP)-β-D-xyloside is hydrolyzed, but not 2-deoxythymidine-5′-(*p*NP) phosphate, or *p*-nitrophenyl (*p*NP-) phosphorylcholine. Proline and serine are used as simultaneous carbon and nitrogen sources, but not acetamide, L-alanine, or gelatin. Susceptible (μg per disc) to imipenem (2.5) and tobramycin (2.5); slightly susceptible to kanamycin (30.0), but resistant to 5-fluorouracil (30) (Kageyama et al., 2005d). Resistant to lysozyme. Growth occurs in the presence (%, w/v) of bismuth citrate (0.0001), crystal violet (0.0,0001), phenol (0.01), and sodium chloride (3) but not in the presence of sodium azide (0.01) and sodium chloride (10). Additional phenotypic properties are cited in Table 39. Whole-cell hydrolysates contain arabinose and galactose as characteristic sugars, and *meso*-diaminopimelic acid as the principal diamino acid. Muramic acid moieties are *N*-glycolated. Cellular fatty acids contain major proportions of $C_{16:0}$, $C_{18:1}$, and $C_{18:0}$ 10-methyl. Mycolic acids co-migrate on one-dimensional thin-layer chromatography of whole-organism methanolysates with those of representative *Nocardia* strains and have 46–60 carbon atoms. The menaquinone profile is typical of the genus.

Source (type strain): diseased blueback salmon fingerlings (*Oncorhychus nerka*).

DNA G+C content (mol%): 66–67.0 (T_m).

Type strain: ATCC 27463, CBS 694.72, CIP 104517, DSM 40472, IFO (now NBRC) 13393, ISP 5472, JCM 4826, NRRL B-2778, NRRL B-12385.

Sequence accession no. (16S rRNA gene): AF430050.

57. **Nocardia seriolae** Kudo, Hatai and Seino 1988, 173[VP]

se.ri.o′ la.e. N.L. gen. n. *seriolae* of Seriola, named after *Seriola*, the genus of yellowtail fish from which the organism was isolated.

Branching substrate hyphae fragment into rod-like elements. Some strains form microscopically visible aerial hyphae. The substrate mycelium is yellowish-orange on yeast extract-malt extract agar (ISP medium 2). Grows at 15 and 30°C, but not above 35°C. *p*-Nitrophenyl (*p*NP)-β-D-xyloside is hydrolyzed, but not 2-deoxythymidine-5-*p*NP phosphate, or *p*NP-phosphorylcholine. Susceptible (μg per disc) to kanamycin (5.0) and tobramycin (2.5) but not to 5-fluorouracil (30) and imipenem (10) (Kageyama et al., 2005d). Grows in the presence of 5%, w/v sodium chloride. Additional phenotypic properties are cited in Table 39. Whole-cell hydrolysates contain arabinose and galactose as characteristic sugars and *meso*-diaminopimelic acid as the principal diamino acid. Cellular fatty acids contain major proportions of $C_{16:0}$, $C_{16:1}$, $C_{18:1}$, and 10-methyl-$C_{19:0}$, smaller

proportions (<10%) of $C_{15:0}$, $C_{15:1}$, $C_{17:0}$, $C_{17:1}$, $C_{19:1}$, and 10-methyl-$C_{19:0}$, and traces of $C_{14:0}$, $C_{18:0}$, $C_{19:1}$, and 10-methyl-$C_{17:0}$. Mycolic acids have 44–58 carbon atoms and include odd numbered components. DNA–DNA relatedness values between the type strain and corresponding strains of related species are as follows: (1) *Nocardia asteroides* (37%), *Nocardia farcinica* (18%), and *Nocardia nova* (17%) (Kudo et al., 1988), and (ii) *Nocardia inohanensis* (33%), *Nocardia niigatensis* (42%), *Nocardia otitidiscaviarum* (14%), *Nocardia uniformis* (16%), and *Nocardia yamanashiensis* (29%) (Kageyama et al., 2004i).

Source: the causal agent of nocardiosis from yellowtails (*Seriola quinqueradiata*) and Japanese flounder (*Paralichlhys olivaceus*).

DNA G+C content (mol%): 66.8–67.4 (HPLC).

Type strain: ATCC 43993, CCUG 46828, CIP 104778, DSM 44129, NBRC 15557, JCM 3360, VKM Ac-1967.

Sequence accession no. (16S rRNA gene): AF 430039.

58. **Nocardia shimofusensis** Kageyama, Yazawa, Mukai, Kinoshita, Nishimura, Kroppenstedt and Mikami 2004f, 1930[VP]

shi.mo.fus.en′sis. N.L. fem. adj. *shimofusensis* of or pertaining to Shimofusa, a traditional geographical name for a Northern part of Chiba Prefecture in Japan, the source of the isolates.

Partially acid-fast actinomycete. Forms an extensive branched substrate mycelium that fragments into rod-shaped elements (0.5–0.7 × 0.9–1.7 µm). An orange to reddish orange substrate mycelium bears white to reddish white aerial hyphae. Does not produce diffusible pigments. Colonies 0.2–1.0 mm in diameter are produced on Mueller–Hinton II medium supplemented with glucose (0.2%, w/v) after 7 d at 30°C. Does not grow at 45°C. Susceptible (µg per disc) to imipenem (2.5), kanamycin (5), and tobramycin (2.5), and slightly susceptible to 5-fluorouracil (30) (Kageyama et al., 2004f). Additional phenotypic properties are cited in Table 39. Whole-cell hydrolysates contain arabinose and galactose as characteristic sugars and *meso*-diaminopimelic acid as the principal diamino acid. Cellular fatty acids contain major proportions of $C_{16:0}$, $C_{16:1}$ *cis*9, $C_{18:1}$ *cis*9, and $C_{18:0}$ 10-methyl, and smaller proportions (<10%) of $C_{14:0}$, $C_{15:0}$, $C_{17:1}$ *cis*9, $C_{17:0}$, $C_{18:0}$, $C_{19:1}$ *cis*9, and $C_{20:1}$ *cis*11. Mycolic acids co-migrate on one-dimensional thin-layer chromatography of whole-organism methanolysates with those of representative *Nocardia* strains. The menaquinone profile is typical of the genus. DNA–DNA relatedness values between *Nocardia shimofuensis* strains and those of the type strains of related species are as follows: *Nocardia farcinica* (5–12%) and *Nocardia higoensis* (17–32%) (Kageyama et al., 2004f).

Source: soil samples collected in the city of Chonki and the village of Chyosei, Japan.

DNA G+C content (mol%): 68–69 (HPLC).

Type strain: DSM 44733, IFM 10311, JCM 12122, NBRC 100134, YZ 96.

Sequence accession no. (16S rRNA gene): AB108775.

59. **Nocardia sienata** corrig. Kageyama, Yazawa, Nishimura and Mikami 2004a, 1005[VP] (Effective publication: *Nocardia sienata* corrig Kageyama, Yazawa, Nishimura and Mikami 2004h, 275.)

si.e.na′ta. N.L. fem. adj. *sienata* sienna-colored, referring to the colony color of ochre-yellow of the strain.

Slightly acid–alcohol-fast actinomycete. Forms a pale yellow substrate mycelium that bears ochre-yellow aerial hyphae. At a late stage in the growth cycle, the hyphae fragment into rod-shaped elements. Does not form melanin pigments. Grows at 37°C but not at 45°C. Susceptible (µg per disc) to 5-fluorouracil (30), impenem (2.5), kanamycin (10), and tobramycin (2.5) (Kageyama et al., 2004h). Additional phenotypic properties are cited in Table 39. Whole-cell hydrolysates contain arabinose and galactose as characteristic sugars, and *meso*-diaminopimelic acid as the principal diamino acid. The menaquinone profile is typical of the genus, as is the presence of mycolic acids in whole-organism methanolysates. DNA–DNA relatedness values between the type strain and corresponding strains of related species are as follows: *Nocardia carnea* (12%), *Nocardia flavorosea* (7–8%), and *Nocardia testacea* (51%) (Kageyama et al., 2004h).

Source (type strain): sputum of a patient with acute myeloid leukemia in Japan.

DNA G+C content (mol%): 68.3 (HPLC).

Type strain: DSM 44766, IFM 10088, JCM 12236, NBRC 100364.

Sequence accession no. (16S rRNA gene): AB121770.

Additional remarks: the original spelling, *Nocardia senatus* (*sic*) was corrected on validation according to Rule 61 of the Bacteriological Code (Lists Editor, 2004a).

60. **Nocardia soli** Maldonado, Hookey, Ward and Goodfellow 2001, 1619[VP] (Effective publication: Maldonado, Hookey, Ward and Goodfellow 2000, 375.)

so′li. L. gen. n. *soli* from the soil.

Acid–alcohol-fast actinomycete. Forms an extensively branched substrate mycelium that fragments into coccoid to rod-shaped elements. Brown to pale orange colonies with sparse aerial hyphae are formed on glucose-yeast extract agar. Colony elevation is convex to irregular, and colony margins are filamentous. Does not form diffusible pigments or grow at 45°C. 2-Deoxythymidine-5-*p*-nitrophenyl (*p*NP)-phosphate, *p*NP-phosphorylcholine, and *p*NP-β-D-xyloside are hydrolyzed. Sensitive (µg/ml) to cephaloridine (16), demethylchlortetracycline (16), gentamicin (8), rifampin (1), streptomycin (16), and tobramycin (1) (Maldonado et al., 2000). Sensitive to (µg per disc) imipenem (2.5), tobramycin (2.5) but not 5-fluorouracil (30) and kanamycin (30.0) (Kageyama et al., 2005d). Additional phenotypic properties are cited in Table 39. Whole-cell hydrolysates contain arabinose and galactose as characteristic sugars, and *meso*-diaminopimelic acid as the principal diamino acid.

Source: water collected from the River Thames at Medmenham, England.

DNA G+C content (mol%): not determined.

Type strain: CIP 107223, DSM 44488, JCM 11441, NBRC 100376, NCIMB 13760, W30.

Sequence accession no. (16S rRNA gene): AF430051.

61. **Nocardia speluncae** Seo, Yun and Lee 2007, 2934[VP]

spe.lun′ca.e. L. gen. n. *speluncae* of a cave, grotto or hole, referring to the site of isolation of the type strain.

Abundant aerial and substrate mycelia fragment into irregular rod-shaped elements. Yellow to orange substrate hyphae bear white to pinkish aerial hyphae. Grows well on yeast-extract-malt extract and oatmeal agars (ISP media 2 and 3) and on nutrient and tryptic soy agars. Grows at 10–37°C but not at 45°C; the pH for growth is 6.1–12.1. L-Valine is used as a simultaneous carbon and nitrogen source, but not L-phenylalanine. Additional phenotypic properties are cited in Table 39. Whole-cell hydrolysates contain arabinose and galactose as characteristic sugars, and *meso*-diaminopimelic acid as the principal diamino acid. Cellular fatty acids contain major proportions of $C_{16:0}$, $C_{18:1}$ *cis*9, and *cyclo*-$C_{19:0}$. Mycolic acids co-migrate on one-dimensional thin-layer chromatography of whole-organism methanolysates with those of reference *Nocardia* strains. Menaquinone and polar lipid profiles are typical of the genus.

Source (type strain): a soil sample collected from a natural cave on Jeju Island, Republic of Korea.

DNA G+C content (mol%): 66.3±0.4 (HPLC).

Type strain: DSM 45078, JBRI 2006, JCM 14881, KCTC 19223, N2-11.

Sequence accession no. (16S rRNA gene): AM422449.

62. **Nocardia takedensis** Yamamura, Hayakawa, Nakagawa, Tamura, Kohno, Komatsu and Iimura 2005, 435[VP]

ta.ke.den′sis. N.L. fem. adj. *takedensis* of or pertaining to the Takeda Shrine, from where the organism was first isolated.

Branched substrate and aerial hyphae fragment into rod-like elements. An orange substrate mycelium bears a white aerial mycelium on which short aerial hyphae differentiate into chains of spores. Does not produce diffusible pigments. Grows well at 30°C, but does not grow at 45°C. Susceptible (µg per disc) to 5-fluorouracil (30), impenem (2.5), and tobramycin (2.5) but not kanamycin (30) (Hoshino et al., 2007). Additional phenotypic properties are cited in Table 39. Whole-cell hydrolysates contain arabinose and galactose as characteristic sugars, and *meso*-diaminopimelic acid as the principal diamino acid. Cellular fatty acids contain major proportions of $C_{16:0}$, $C_{18:1}$ *cis*9, and $C_{18:0}$ 10-methyl. Mycolic acids co-migrate on one-dimensional thin-layer chromatography of whole-organism methanolysates with those of representative *Nocardia* strains. Menaquinone and polar lipid profiles are typical of the genus. DNA–DNA relatedness values between the type strain and corresponding strains of *Nocardia beijingensis, Nocardia brasiliensis*, and *Nocardia tenerifensis* fall within the range 8.1–23.1% (Yamamura et al., 2005).

Source (type strain): a sediment sample collected from a moat surrounding the Takeda Shrine in Yamanashi Prefecture, Japan.

DNA G+C content (mol%): 68.6 (HPLC).

Type strain: DSM 44801, JCM 13313, MS1-3, NBRC 100417.

Sequence accession no. (16S rRNA gene): AB158277.

63. **Nocardia tenerifensis** Kämpfer, Buczolits, Jäckel, Grün-Wollny and Busse 2004, 383[VP]

te.ne.ri.fen′sis. N.L. fem. adj. *tenerifensis* of or belonging to Tenerife, from where the organism was isolated

An orange substrate mycelium fragments readily into irregular rod-shaped elements. Aerial hyphae are yellowish-white. Good growth occurs on nutrient agar and medium 65 at 25–30°C. L-Alanine-*p*-nitroanilide (*p*NA), 2-deoxythymidine 5′-*p*-nitrophenyl (*p*NP) phosphate, *p*NP-α-D- glucopyranoside, *p*NP-β-D-glucopyranoside, L-glutamate-γ-3-carboxyl *p*NA, *bis*-*p*NA phosphate, *p*NP-phenylphosphonate, *p*NP-phosphocholine, L-proline- *p*NA, and *p*NP-β-D-xylopyranoside are hydrolyzed, but not *p*NP-β-D-glucuronide or *o*-nitrophenyl-β-D-galactopyranoside. Susceptible (µg per disc) to impenem (2.5) and tobramycin (2.5) but not to 5-fluorouracil (30) and kanamycin (30) (Hoshino et al., 2007). Additional phenotypic properties are cited in Table 39. Cellular fatty acids contain major proportions of $C_{16:0}$, $C_{18:1}$ *cis*9, and 10-methyl-$C_{16:0}$, smaller proportions (<10%) of $C_{18:0}$, and traces of $C_{10:0}$, $C_{14:0}$, $C_{17:0}$, $C_{16:1}$ *cis*9, $C_{17:1}$ *cis*5, $C_{18:1}$ *cis*6, and $C_{20:2}$ *cis*9. Mycolic acids co-migrate on one-dimensional thin-layer chromatography of whole-organism methanolysates with those from reference *Nocardia* strains. Menaquinone and polar lipid profiles are typical of the genus, apart from the presence of significant amounts of phosphatidylglycerol. DNA–DNA relatedness values between the type strain and corresponding strains of closely related species are as follows: *Nocardia beijingensis* (25.4%), *Nocardia brasiliensis* (29.3%), *Nocardia farcinica* (15.8%), and *Nocardia transvalensis* (16.2%) (Kämpfer et al., 2004).

Source (type strain): soil collected in Tenerife, Spain.

DNA G+C content (mol%): not determined.

Type strain: CCUG 49019, CIP 107929, DSM 44704, GW39-1573, JCM 12693, NBRC 101015.

Sequence accession no. (16S rRNA gene): AJ556157.

64. **Nocardia terpenica** Hoshino, Watanabe, Iida, Suzuki, Kudo, Kogure, Yazawa, Ishikawa, Kroppenstedt and Mikami 2007, 1459[VP]

ter.pe′ni.ca. N.L. n. *terpenum* terpene; L. suff. *-icus -a -um* suffix used with various meanings; N.L. fem. adj. *terpenica* referring to the ability to produce terpenoid antibiotics.

Acid–alcohol-fast actinomycete. Forms a colorless to beige substrate mycelium that fragments into irregular, long rod-shaped elements (0.2–0.5 × 0.6–1.4 µm) towards the end of the growth cycle. If present, aerial hyphae are scanty on most media. Colonies 1.0–3.5 mm in diameter formed on Mueller–Hinton II agar supplemented with glucose (0.2%, w/v) after 7 d at 30°C. Grows at 37°C, but not at 45°C. Susceptible (µg per disc) to tobramycin (2.5) but not to 5-fluorouracil (30), impenem (10), and kanamycin (30) (Hoshino et al., 2007). Additional phenotypic properties are shown in Table 39. Whole-cell hydrolysates contain arabinose and galactose as characteristic sugars and *meso*-diaminopimelic acid as the principal diamino acid. Cellular fatty acids contain major proportions of $C_{16:0}$, $C_{18:1}$ *cis*9, and $C_{18:0}$ 10-methyl, smaller proportions (<10%) of $C_{18:1}$ *cis*9, $C_{16:1}$ *cis*11, $C_{17:1}$ *cis*9, $C_{17:0}$, and $C_{18:0}$, and traces of $C_{12:0}$, $C_{14:0}$, $C_{15:1}$ *cis*10, $C_{16:1}$ *cis*7, and 10-methyl-$C_{16:0}$. Mycolic acids have between 52–60 carbon atoms with C56 as the major component. The menaquinone profile is typical of the genus. The DNA–DNA relatedness value between the type strain and the corresponding strain of *Nocardia nova*, a closely related species, is 48% (Hoshino et al., 2007).

Source (type strain): sputum of a patient with lung nocardiosis at Okayama, Japan.

DNA G+C content (mol%): 65.4 (HPLC).

Type strain: DSM 44935, IFM 0706, JCM 13033, NBRC 100888.

Sequence accession no. (16S rRNA gene): AB201298.

65. **Nocardia testacea** corrig. Kageyama, Yazawa, Nishimura and Mikami 2004a, 1005[VP] (Effective publication: corrig. Kageyama, Yazawa, Nishimura and Mikami 2004h, 274.)

tes.ta′ce.a. L. fem. adj. *testacea* brick-colored, referring to the brick colony color.

Acid–alcohol-fast actinomycete. Forms a substrate mycelium that fragments into irregular, rod-shaped elements. A dark orange to brick colored substrate mycelium bears a whitish aerial mycelium which has a reddish tinge. Does not produce melanin pigments. Grows between 27 and 45°C. Does not use L-phenylalanine or L-valine as sole carbon and nitrogen sources. Susceptible (μg per disc) to impenem (2.5), kanamycin (5.0), tobramycin (2.5) but not to 5-fluorouracil (30) (Kageyama et al., 2004h). Additional phenotypic properties are cited in Table 39. Whole-cell hydrolysates contain arabinose and galactose as characteristic sugars, and *meso*-diaminopimelic acid as the principal diamino acid. The menaquinones are typical of the genus, as is the presence of mycolic acids in whole-organism methanolysates. DNA–DNA relatedness values between the type strain and corresponding strains of closely related species are as follows: *Nocardia carnea* (8.3%), *Nocardia flavorosea* (6.6%), and *Nocardia sienata* (51%) (Kageyama et al., 2004h).

Source (type strain): sputum of a patient with a nontuberculous mycobacterial infection in Japan.

DNA G+C content (mol%): 68.6 (HPLC).

Type strain: DSM 44765, IFM 0937, JCM 12235, NBRC 100365.

Sequence accession no. (16S rRNA gene): AB192415.

Additional remarks: the original spelling, *Nocardia testaceus* (*sic*) was corrected on validation according to Rule 61 of the Bacteriological Code (Lists Editor, 2004a). In addition, the specific epithet is a L. adj. not a N.L. gen. n. as cited in the paper by Kageyama et al. (2004h).

66. **Nocardia thailandica** Kageyama, Poonwan, Yazawa, Suzuki, Kroppenstedt and Mikami 2005b, 547[VP] (Effective publication: *Nocardia thailandica* Kageyama, Poonwan, Yazawa, Suzuki, Kroppenstedt and Mikami 2004c, 32.)

thai.lan′di.ca. N.L. fem. adj. *thailandica* of or pertaining to Thailand, the source of the strain.

Branched substrate hyphae fragment into bacteroid, rod-shaped elements (0.4–0.6 × 0.8–1.6 μm). Visible aerial hyphae occur on most standard media, including brain heart infusion agar. Colonies 0.2–1.2 mm in diameter are produced on Mueller–Hinton II agar supplemented with glucose (0.2%, w/v) after 7 d at 30°C. Grows at 37°C, but not at 45°C. Susceptible (μg per disc) to impenem (2.5), 5-fluorouracil (30), kanamycin (5.0), and tobramycin (2.5) (Hoshino et al., 2007).

Additional phenotypic properties are cited in Table 39. Whole-cell hydrolysates contain arabinose and galactose as characteristic sugars, and *meso*-diaminopimelic acid as the principal diamino acid. Cellular fatty acids contain major proportions of $C_{16:0}$, $C_{18:1}$ *cis*9, and $C_{18:0}$, smaller proportions (<10%) of $C_{15:0}$, $C_{17:1}$ *cis*9, $C_{17:0}$, 10-methyl-$C_{17:0}$, $C_{18:1}$ *cis*9, and $C_{19:1}$ *cis*9, and traces of $C_{14:0}$, $C_{15:1}$ *cis*9, 10-methyl-$C_{16:0}$, $C_{19:0}$, and $C_{20:0}$. Mycolic acids co-migrate on thin-layer chromatography of whole-organism methanolysates with those of reference *Nocardia* strains. The menaquinone profile is typical of the genus. The DNA–DNA relatedness value between the type strain and the corresponding strain of *Nocardia asteroides*, a closely related species, is below 12% (Kageyama et al., 2004c).

Source: pus of a Thai patient.

DNA G+C content (mol%): 72 (HPLC).

Type strain: DSM 44808, IFM 10145, JCM 12356, NBRC 100428.

Sequence accession no. (16S rRNA gene): AB126874.

67. **Nocardia transvalensis** Pijper and Pullinger 1927, 338[AL] ["*Actinomyces transvalensis*" Pijper and Pullinger 1927, "*Proactinomyces transvalensis*" (Pijper and Pullinger 1927) Krasil'nikov 1941.]

trans.val.en′sis. N.L. fem. adj. *transvalensis* of or pertaining to the Transvaal, South Africa.

Partially acid-fast actinomycete. Produces pale tannish cream or purplish colonies which bear moderate to abundant aerial hyphae. Does not form soluble pigments. Grows at 30–37°C but not at 45°C. L-Alanine-*p*-nitroanilide (*p*NA), 2-deoxythymidine 5′ *p*-nitrophenyl (*p*NP) phosphate, *p*NP-α-D-glucopyranoside, *p*NP-β-D-glucopyranoside, L-glutamate-γ-3-carbonyl-*p*NA, *o*-nitrophenyl-β-D-galactopyranoside, *bis*-*p*NP-phosphate, *p*NP-phenylphosphonate, *p*NP-phosphorylcholine, L-proline *p*NA, and *p*NP-β-D-xylopyranoside are hydrolyzed but not *p*NP-β-D-glucuronide. Proline and serine are used as simultaneous carbon and nitrogen sources, but not acetamide, L-alanine, or gelatin. Susceptible (μg per disc) to imipenem (2.5) but not to 5-fluorouracil (30), kanamycin (30.0), and tobramycin (10.0) (Kageyama et al., 2005d). Additional phenotypic properties are cited in Table 39. Whole-cell hydrolysates contain arabinose and galactose as characteristic sugars and *meso*-diaminopimelic acid as the principal diamino acid. Cellular fatty acids contain major proportions of $C_{16:0}$, $C_{18:1}$ *cis*9, and $C_{18:0}$ 10-methyl, smaller proportions (<10%) of $C_{14:0}$ and $C_{18:0}$, and traces of $C_{10:0}$, $C_{12:0}$, $C_{15:0}$, $C_{17:0}$, $C_{16:1}$ *cis*9, $C_{17:1}$ *cis*8, $C_{18:1}$ *cis*7, $C_{15:0}$ *cis* 3-OH, and $C_{17:0}$10-methyl. DNA–DNA relatedness values between the type strain and corresponding strains of closely related species are as follows: (i) *Nocardia abscessus* (6%), *Nocardia asteroides* (11%), *Nocardia nova* (6%), *Nocardia paucivorans* (6%), *Nocardia pseudobrasiliensis* (12%), *Nocardia vaccinii* (9%), and *Nocardia vinacea* (5%) (Kinoshita et al., 2001) and (ii) *Nocardia tenerifensis* (23.6%) (Kämpfer et al., 2004).

Source (type strain): mycetoma of the foot in South Africa.

DNA G+C content (mol%): 67.0 (T_m).

Type strain: ATCC 6865, CCUG 45937, CIP 104841, DSM 43405, NBRC 15921, IMET 7500, JCM 9099, NRRL B-16037, VKM Ac-867.

Sequence accession no. (16S rRNA gene): AF430047.

68. **Nocardia uniformis** (*ex* Marton and Szabó 1959) Isik, Chun, Hah and Goodfellow 1999a, 1229[VP] ("*Nocardia uniformis*" Marton and Szabó 1959)

u.ni.for′mis. L. fem. adj. *uniformis* having only one form, uniform.

Acid–alcohol-fast actinomycete. Forms an extensive branched substrate mycelium that fragments *in situ* into coccoid to rod-shaped elements (0.7–1.1 × 1.1–1.4 µm). A yellowish orange substrate mycelium bears whitish, sparse to abundant, aerial hyphae. Colony elevation is convex to irregular and colony margins are filamentous. Grows at 14–40°C but not at 10°C, and at pH 6.0–10.0 but not at pH 5.0. L-Alanine-*p*-nitroanilide (*p*NA), 2-deoxythymidine 5′-*p*-nitrophenyl (*p*NP) phosphate, *p*NP-β-D-galactopyranoside, *p*NP-α-D-glucopyranoside, *p*NP-glucopyranoside, *bis*-*p*NP-phosphate, *p*NP-β-D-xylopyranoside are hydrolyzed but not *p*NP-β-D-glucuronide, L-glutamate-γ-3-carbonyl-*p*NA, *p*NP-phosphorylcholine, or L-proline. Susceptible (µg per disc) to 5-fluorouracil (30) and tobramycin (2.5) (Kageyama et al., 2005d). Additional phenotypic properties are cited in Table 39. Whole-cell hydrolysates contain arabinose and galactose as characteristic sugars, and *meso*-diaminopimelic acid as the principal diamino acid. Cellular fatty acids contain major proportions of $C_{16:0}$, $C_{16:1}$ *cis*9, $C_{18:1}$ *cis*9, and $C_{18:0}$ 10-methyl, smaller proportions (<10%) of $C_{15:0}$, 10-methyl-$C_{16:0}$, $C_{17:1}$ *cis*9, $C_{17:0}$, 10-methyl-$C_{17:0}$, $C_{19:1}$ *cis*9, and traces of $C_{14:0}$, and $C_{15:1}$ *cis*10. The menaquinone profile is characteristic of the genus. DNA–DNA relatedness values between the type strain and corresponding strains of related species are as follows: *Nocardia inohanensis* (22%), *Nocardia niigatensis* (25%), *Nocardia otitidiscaviarum* (33%), *Nocardia seriolae* (33%), and *Nocardia yamanashiensis* (27%) (Kageyama et al., 2004i).

Source: a degraded solonchak solonets soil collected from the Hortobagy steppe in eastern Hungary.

DNA G+C content (mol%): not determined.

Type strain: CBS 224.60, CIP 104824, DSM 43136, IFO (now NRBC) 13702, JCM 3224, NCIB 963.

Sequence accession no. (16S rRNA gene): AF430044.

69. **Nocardia vaccinii** Demaree and Smith 1952, 338[VP]

vac.ci′ni.i. N.L. gen. n. *vaccinii* of *Vaccinium*, the generic name of blueberry; referring to the plant from which the type strain was isolated.

Partially acid-fast actinomycete. Forms cream to peach colonies which bear moderate to sparse white aerial hyphae. Aerial hyphae rarely differentiate into spores. Grows best at 25–28°C but poorly at 37°C. Does not hydrolyze 2-deoxythymidine-5′-*p*-nitrophenyl (*p*NP)-phosphate, *p*NP- phosphorylcholine, or *p*NP-β-D-xyloside. Does not use acetamide, L-alanine, gelatin, proline, or serine as simultaneous carbon and nitrogen sources. Susceptible (µg per disc) to 5-fluorouracil (30), imipenem (2.5), kanamycin (5.0), and tobramycin (2.5) (Kageyama et al., 2005d). Additional phenotypic properties are shown in Table 39. Whole-cell hydrolysates contain arabinose and galactose as characteristic sugars and *meso*-diaminopimelic acid as the principal diamino acid. Cellular fatty acids contain major proportions of $C_{16:0}$, $C_{18:1}$ *cis*9, and $C_{18:0}$ 10-methyl, smaller proportions (<10%) of $C_{16:1}$ *cis*9 and $C_{17:1}$ *cis*10, and traces of $C_{14:0}$, $C_{15:0}$, $C_{16:1}$ *cis*7, $C_{17:0}$, $C_{18:0}$, $C_{19:1}$, and $C_{19:0}$. DNA–DNA relatedness values between the type strain and corresponding strains of related species are as follows: (i) *Nocardia abscessus* (5%), *Nocardia asteroides* (8%), *Nocardia nova* (8%), *Nocardia paucivorans* (5%), *Nocardia pseudobrasiliensis* (8%), *Nocardia transvalensis* (11%), and *Nocardia vinacea* (4%) (Kinoshita et al., 2001), and (ii) *Nocardia africana* (10%), *Nocardia cerradoensis* (43%), *Nocardia nova* (10%), *Nocardia veterana* (42%), *Nocardia vinacea* (5%), and *Nocardia vermiculata* (6%) (Kageyama et al., 2004c).

Source (type strain): bud-proliferating galls on blueberry (*Vaccinium* spp.).

DNA G+C content (mol%): not determined.

Type strain: ATCC 11092, CIP 104899, DSM 43285, ICMP 15782, IMET 7503, JCM 3395, NBRC 15922, NCPPB 954, NRRL WC-3500, VKM Ac-856.

Sequence accession no. (16S rRNA gene): AF430045.

Additional remarks: the strain ICMP 5814, previously cited as the type strain of *Nocardia vaccinii*, is a *Staphylococcus* sp. Consequently, the strain ICMP 5814 has been replaced by strain ICMP 15782 (List of Prokaryotic Names with Standing in Nomenclature, http://www.bacterio.cict.fr).

70. **Nocardia vermiculata** Kageyama, Poonwan, Yazawa, Suzuki, Kroppenstedt and Mikami 2005b, 547[VP] (Effective publication: *Nocardia vermiculata* Kageyama, Poonwan, Yazawa, Suzuki, Kroppenstedt and Mikami 2004c, 30.)

ver.mi.cu.la′ta. L. fem. part. adj. *vermiculata* in the form of worms, referring to the morphology of the aerial mycelium.

Partially acid-fast actinomycete. Produces branched substrate hyphae that fragment into oval to rod-shaped elements (0.4–0.7 × 0.7–1.0 µm). A beige substrate mycelium bears a moderate orange to pale orange brown aerial mycelium. The aerial mycelium shows primitive spiral-like structures (worm-like structures). Colonies 0.3–1.0 µm in diameter are formed on Mueller–Hinton II agar supplemented with glucose (0.2%, w/v). Aerial hyphae differentiate into short chains of spores. A faint diffusible pigment is formed. Grows at 37°C but not at 45°C. Susceptible (µg per disc) to 5-fluorouracil (30), imipenem (2.5), kanamycin (5), and tobramycin (2.5) (Hoshino et al., 2007). Additional phenotypic properties are shown in Table 39. Whole-cell hydrolysates contain arabinose and galactose as characteristic sugars and *meso*-diaminopimelic acid as the principal diamino acid. Cellular fatty acids contain major proportions of $C_{16:0}$, $C_{18:1}$ *cis*9, and $C_{18:0}$, smaller proportions (<10%) of $C_{14:0}$, $C_{15:0}$, $C_{16:1}$ *cis*8, $C_{16:1}$ *cis*9, $C_{17:1}$ *cis*9, $C_{17:0}$, and $C_{18:0}$ 10-methyl, and traces of $C_{12:0}$, $C_{13:1}$, $C_{17:1}$ *cis*10, 10-methyl-$C_{17:0}$, $C_{18:1}$ *cis*11, and $C_{19:1}$ *cis*9. Mycolic acids co-migrate on one dimensional thin-layer chromatography with those of reference *Nocardia* strains. The menaquinone profile is typical of the genus. DNA–DNA relatedness values between the type strain and corresponding strains of related species are as follows: *Nocardia africana* (10%), *Nocardia cerradoensis* (9%), *Nocardia nova* (11%), *Nocardia vaccinii* (10%), *Nocardia veterana* (1%), and *Nocardia vinacea* (2%) (Kageyama et al., 2004c).

Source: sputum of a Japanese patient in 1985.

DNA G+C content (mol%): 67 (HPLC).

Type strain: DSM 44807, IFM 0391, JCM 12354, NBRC 100427.

Sequence accession no. (16S rRNA gene): AB126873.

71. **Nocardia veterana** Gürtler, Smith, Mayall, Pötter-Reinemann, Stackebrandt and Kroppenstedt 2001, 935[VP] (Effective publication: Gürtler, Smith, Mayall, Põtter-Reinemann, Stackebrandt and Kroppenstedt 2001, 935.)

ve.te.ra′na. L. fem. adj. *veterana* old in service (as soldiers), referring to the veteran's hospital where the organism was isolated.

A beige substrate mycelium bears scant, dirty white, aerial hyphae. The highly branched substrate and aerial mycelium do not fragment. The reverse side of colonies is yellowish. Grows at 25 and 45°C. *p*-Nitrophenyl (*p*NP) phosphorylcholine is hydrolyzed, but not 2-deoxythymidine-5-*p*NP phosphate, or *p*NP-β-D-xyloside. Does not use acetamide, L-alanine, gelatin, proline, or serine as simultaneous carbon and nitrogen sources. Susceptible (μg per disc) to 5-fluorouracil (30) and imipenem (2.5); moderately susceptible to kanamycin (10); resistant to tobramycin (10) (Kageyama et al., 2005d). Additional phenotypic properties are shown in Table 39. Whole-cell hydrolysates contain arabinose as the characteristic sugar, and *meso*-diaminopimelic acid as the only diamino acid. Muramic acid moieties are *N*-glycolated. Cellular fatty acids contain major proportions of $C_{16:0}$, $C_{16:1}$ *cis*9, $C_{18:1}$ *cis*9, and $C_{18:0}$ 10-methyl, smaller proportions (<10%) of $C_{14:0}$, $C_{15:0}$, $C_{17:0}$, $C_{18:0}$, and traces of $C_{16:1}$ *cis*7, $C_{17:1}$, and $C_{19:1}$. Mycolic acids have 54–64 carbon atoms with C56, C58, and C60 as major components. Menaquinone and polar lipid profiles are typical of the genus. DNA–DNA relatedness values between the type strain and corresponding strains of related species are as follows: (1) *Nocardia vaccinii* (31%), (Gurtler et al., 2001), and (ii) *Nocardia africana* (7%), *Nocardia cerradoensis* (3.8%), *Nocardia nova* (7%), *Nocardia vaccinii* (46%), *Nocardia vermiculata* (7%), and *Nocardia vinacea* (3%), (Kageyama et al., 2004c). A causative agent of pulmonary disease in immunocompromised patients.

Source (type strain): a bronchial lavage specimen of a patient with a past history of tubercular pleurisy who presented with bilateral upper lobe lesions in a hospital at Heidelberg, Australia.

DNA G+C content (mol%): not determined.

Type strain: CCUG 46118, CIP 107095, DSM 44445, JCM 11307, M157222, NBRC 100344, NRRL B-24136.

Sequence accession no. (16S rRNA gene): AF430055.

72. **Nocardia vinacea** Kinoshita, Homma, Igarashi, Ikeno, Hori and Hamada 2002, 3[VP] (Effective publication: Kinoshita, Homma, Igarashi, Ikeno, Hori and Hameda 2001, 4.)

vi.na′ce.a. L. fem. adj. *vinacea* of or belonging to wine or to the grape, referring to the pale reddish purple diffusible pigment.

Colorless pale yellow to pale brown substrate mycelium undergoes fragmentation. Straight, flexuous to spiral aerial hyphae differentiate into cylindrical and ellipsoidal spore-like elements (0.4–0.6 × 0.8–1.8 μm). Pale reddish purple soluble pigments may be produced. Does not form melanin pigments. Grows at 20–37°C but not at 45°C. Susceptible (μg per disc) to imipenem (10), tobramycin (2.5) but not to 5-fluorouracil and kanamycin (30) (Kageyama et al., 2005d). Additional phenotypic properties are cited in Table 39. Whole-cell hydrolysates contain arabinose and galactose as characteristic sugars and *meso*-diaminopimelic acid as the principal diamino acid. Cellular fatty acids contain major proportions of $C_{16:0}$, $C_{18:1}$ *cis*9, $C_{18:0}$ 10-methyl, and $C_{16:1}$ *cis*9. Mycolic acids have 48–56 carbon atoms. Menaquinone and polar lipid profiles are typical of the genus. DNA–DNA relatedness values between the type strain and corresponding strains of related species, namely *Nocardia abscessus, Nocardia africana, Nocardia asteroides, Nocardia cerradoensis, Nocardia nova, Nocardia paucivorans, Nocardia pseudobrasiliensis, Nocardia transvalensis, Nocardia vaccinii, Nocardia veterana*, and *Nocardia vermiculata* range from 5 to 13% (Kageyama et al., 2001, 2004c).

Source (type strain): a soil sample collected at Suwa-shi, Nagano, Japan.

DNA G+C content (mol%): 65 (HPLC).

Type strain: DSM 44638, NBRC 16497, JCM 10988, MK703-102F1.

Sequence accession no. (16S rRNA gene): AB024312.

Additional remarks: according to Rules 27(3) and 30 of the Bacteriological Code, this name is not validly published inasmuch as the effective publication only documents the deposit of the type strain in a single recognized culture collection (Euzéby and Tindall, 2004). However, according to Judicial Opinion 81, *Nocardia vinacea* Kinoshita et al. 2002 should be considered to be validly published (Judicial Commission of the International Committee on the Systematics of Prokaryotes, 2008).

73. **Nocardia wallacei** Conville, Brown, Steigerwalt, Brown-Elliott and Witebsky 2009, 1183[VP] (effective publication)

wal.la′ce.i. N.L. masc. gen. n. *wallacei* of Wallace, named in honor of Richard J. Wallace, Jr, in recognition of his contribution to the understanding of the taxonomy and drug susceptibility of *Nocardia* species.

Acid-fast positive actinomycete. Form a branched substrate mycelium that bears aerial hyphae. Grows at 25–45°C and optimally at 35°C. Does not use acetamide as a simultaneous carbon and nitrogen source. The type strain is susceptible to ceftriaxone, ciprofloxacin, and linezolid but not to amikacin and clarithromycin (Conville et al., 2008). Grows in the presence of lysozyme. Does not use acetamide as a simultaneous carbon and nitrogen source. Additional phenotypic properties are cited in Table 39. DNA–DNA relatedness values between the type strain and corresponding strains of related species are as follows: *Nocardia blacklockiae* (53+ 8.5%) and *Nocardia transvalensis* (44±10.5%) (Conville et al., 2008).

Source (type strain): pleural fluid and sputum of a patient with underlying health problems.

DNA G+C content (mol%): 65 (T_m).

Type strain: ATCC 49873, DSM 45136.

Sequence accession no. (16S rRNA gene): EU099357.

Additional remarks: the taxonomic integrity of *Nocardia wallacei* is supported by results from 65-kDa heat-shock protein and s*ecA1* gene sequence analyses (Conville et al., 2008).

74. **Nocardia xishanensis** Zhang, Liu and Goodfellow 2004, 2304[VP]

xi.shan.en′sis. N.L. fem. adj. *xishanensis* of or belonging to Xishan Mountain, the source of the soil from which the type strain was isolated.

Slightly acid–alcohol-fast actinomycete which forms an extensive branched substrate mycelium that fragments into coccoid and rod-shaped elements. A yellow to orange substrate mycelium bears sparse to abundant, white aerial hyphae on modified Sauton's agar. Does not form diffusible pigments. Colony elevation is convex to irregular and colony margins filamentous. Grows at 22–38°C but not at 45°C, and from pH 5.5 to 10. L-Alanine, L-proline, and L-valine are used as simultaneous carbon and nitrogen sources, but not acetamide, L-asparagine, L-aspartate, gelatin, D-glucosamine, L-leucine, L-phenylalanine, or L-serine. Sensitive (µg per disc) to chloramphenicol (30), erythromycin (15), midecamycin (15), minocycline (50), rifampin (5), streptomycin (10), tobramycin (10), and vancomycin (30) but not to gentamicin (10) (Zhang et al., 2004). Sensitive (µg/ml) to arbekacin (4), ampicillin (4), clarithromycin (<0.006), cefotiam (2), and erythromycin (<0.006) (Iida et al., 2006). Resistant to lysozyme. Grows in the presence of NaCl at 3%, w/v, but not at 5%, w/v. Additional phenotypic properties are cited in Table 39. Whole-cell hydrolysates contain arabinose and galactose as characteristic sugars, and *meso*-diaminopimelic acid as the principal diamino acid. Cellular fatty acids contain major proportions of $C_{16:0}$, $C_{18:1}$ *cis*9, and $C_{18:0}$ 10-methyl, and a smaller proportion (<10%) of $C_{18:0}$. Mycolic acids co-migrate on one-dimensional thin-layer chromatography of whole-organism methanolysates with those of reference *Nocardia* strains. The menaquinone profile and polar lipid profiles are typical of the genus. DNA–DNA relatedness values between the type strain and the corresponding strains of related species are as follows: *Nocardia abscessus* (39%) and *Nocardia asteroides* (35%) (Zhang et al., 2004).

Source (type strain): a soil sample collected from Xishan mountain, Beijing, China.

DNA G+C content (mol%): 68.8 (T_m).

Type strain: 276 AS 4.1860, CGMCC 4.1860, DSM 44895, JCM 12160.

Sequence accession no. (16S rRNA gene): AY333115.

75. **Nocardia yamanashiensis** Kageyama, Yazawa, Nishimura and Mikami 2004i, 568[VP]

ya.ma.na.shi.en′sis. N.L. fem. adj. *yamanashiensis* of or pertaining to Yamanashi prefecture of Japan, the source of the type strain.

Partially acid–alcohol-fast actinomycete. Forms a grayish-tan to tan branched substrate mycelium that fragments into bacteroid, coccoid, and rod-shaped elements. Aerial hyphae are sparse on most media and are only visible microscopically. Colonies 1.0–1.9 mm in diameter are formed on Mueller–Hinton II medium supplemented with glucose (0.2%, w/v) after 7 d at 30°C. Susceptible (µg per disc) to impenem (2.5), kanamycin (2.5), and tobramycin (2.5) and slightly susceptible to 5-fluorouracil (30) (Kageyama et al., 2004c). Whole-cell hydrolysates contain arabinose and galactose as characteristic sugars, and *meso*-diaminopimelic acid as the principal diamino acid. Muramic acids co-migrate on one-dimensional thin-layer chromatography of whole-organism methanolysates with those of reference *Nocardia* strains. The menaquinone profile is typical of the genus. DNA–DNA relatedness values between the type strain and corresponding strains of related species are as follows: *Nocardia inohanensis* (40%), *Nocardia niigatensis* (21%), *Nocardia otitidiscaviarum* (13%), *Nocardia seriolae* (20%), and *Nocardia uniformis* (8.2%) (Kageyama et al., 2004i).

Source (type strain): clinical material.

DNA G+C content (mol%): 69.2 (HPLC).

Type strain: DSM 44669, IFM 0265, JCM 11893, NBRC 100130.

Sequence accession no. (16S rRNA gene): AB092561.

Genus II. **Gordonia** (Tsukamura 1971) Stackebrandt, Smida and Collins 1988, 345[VP]

Michael Goodfellow, Yashawant Kumar and Luis A. Maldonado

Gor.do′ni.a. N.L. fem. n. *Gordonia* named after Ruth E. Gordon, a celebrated bacterial systematist.

Aerobic, Gram-stain-positive to Gram-stain-variable, nonspore-forming actinomycetes that are **usually partially acid–alcohol-fast**. **Nonmotile, short rods and cocci** (0.5–1.0 × 1.0–2.5 µm) **occur singly, in pairs, in V-shaped arrangements, or as short chains**. **Elementary branched hyphae which fragment into rod- and coccoid-like elements are formed by some species.** Colonial appearance ranges from convex, shiny and smooth to rough, matt and folded with irregular margins. Colonies may be cream, beige, light yellow, or tan through to apricot, orange, pink, or red. Chemo-organotrophic with an oxidative type of metabolism. Mycobactins are formed. Catalase-positive, arylsulfatase-negative, and sensitive to lysozyme. Most strains grow well between 20 and 37°C.

Whole-organism hydrolysates are rich in *meso*-diaminopimelic acid, arabinose and galactose. The peptidoglycan is of the A1γ type. Muramic acid moieties are *N*-glycolated. **Cells contain diphosphatidylglycerol, phosphatidylethanolamine, phosphatidylinositol, and phosphatidylinositol mannosides as major phospholipids, major proportions of straight chain saturated, unsaturated and 10-methyloctadecanoic (tuberculostearic) fatty acids, and dihydrogenated menaquinones with nine isoprene units as the predominant isoprenologue. Mycolic acids have 46–70 carbon atoms and 1–6 double bonds. The fatty acid esters released on pyrolysis gas chromatography of mycolic acid esters have 16–18 carbon atoms.** The phylogenetic position of *Gordonia*, as determined by 16S rRNA gene sequence analysis, is in the family *Nocardiaceae*.

Gordoniae are widely distributed in aquatic and terrestrial habitats, notably in soils, marine sediments, and wastewater systems. Members of some species are opportunistic pathogens for man and animals.

DNA G+C content (mol%): 63–69 (HPLC, T_m).

Type species: **Gordonia bronchialis** (Tsukamura 1971) Stackebrandt, Smida and Collins 1988, 345[VP].

Further descriptive information

Phylogeny. *Gordonia* is the type genus of the family *Gordoniaceae* Stackebrandt et al. (1997) which also includes the genera *Millisia* Soddell et al. 2006a, *Skermania* Chun et al., (1997), and *Williamsia* Kämpfer et al. (1999). These taxa were proposed to form a branch in the *Corynebacteriales* 16S rRNA gene tree and distinct phyletic lines in the *Gordoniaceae* 16S rRNA gene tree (Figure 93a). However, further analyses with more comprehensive collections of outgroups suggest that these genera should be combined within the family *Nocardiaceae* (Zhi et al., 2009; Ludwig et al. – the roadmap to this volume). While the family *Gordoniaceae* is not used in the current work, the classification of *Gordonia* is far from certain based upon the current evidence.

In contrast to the 16S rRNA gene, greater resolution is found between members of the genus *Gordonia* in phylogenetic trees based on GyrB amino acid sequences (Figure 93b; Shen et al., 2006). Shen and his co-workers have also shown that gordoniae have much lower interspecies *gyrB* substitution rates than members of the genera *Corynebacterium*, *Nocardia*, and *Rhodococcus*.

Chemotaxonomic and 16S rRNA gene sequence data have been instrumental in clarifying the taxonomic positions of misclassified mycolic acid-containing taxa (Blackall et al., 1994; Goodfellow et al., 1982b), as exemplified by the transfer of *Nocardia amarae* and *Rhodococcus aichiensis* to the genus *Gordonia* as *Gordonia amarae* and *Gordonia aichiensis* (Klatte et al., 1994b). These species also fall within the evolutionary radiation occupied by the genus *Gordonia* in the *gyrB* tree (Figure 93b).

Cell morphology. Most gordoniae form short rods and cocci. Cells in early growth phase are rods and those in exponential phase are cocci; such strains exhibit a typical rod–coccus life cycle. Some species, including *Gordonia lacunae*, *Gordonia polyisoprenivorans, Gordonia shandongensis*, and *Gordonia soli*, produce elementary branching hyphae which fragment into rod- and coccoid-like elements (Le Roes et al., 2008; Linos et al., 1999; Shen et al., 2006a). *Gordonia defluvii* shows acute-angled and right-angled branching (Soddell et al., 2006a), a cellular morphology that is intermediate between the right-angled branching pattern typical of *Gordonia amarae* (Klatte et al., 1994b; Lechevalier and Lechevalier, 1974) and the "pine-tree-like" morphology of *Skermania piniformis* (Blackall et al., 1989; Chun et al., 1997). *Gordonia amarae* strains form moderately branched, non-fragmenting, vegetative hyphae in undisturbed culture; the hyphae usually show branching both in the natural environment (foam on the surface of aeration tanks in activated-sludge sewage treatment plants) and *in situ* under phase-contrast microscopy (Lechevalier and Lechevalier, 1974). These workers also noted that many newly isolated strains showed banded hyphae, a characteristic which was lost on prolonged cultivation. Sections through banded hyphae showed numerous cross walls, laminated walls, irregularly thickened cells and many mesosomes.

Cell envelope composition. Gordoniae have (a) a peptidoglycan composed of *N*-acetylglucosamine, D-alanine, L-alanine, and D-glutamic acid with *meso*-diaminopimelic acid (*meso*-A_2pm) as the diamino acid, and muramic acid in the *N*-glycolated form; (b) arabinose and galactose as diagnostic wall sugars (i.e. whole-organism sugar pattern type A *sensu* Lechevalier and Lechevalier (1970); (c) a phospholipid pattern consisting of diphosphatidylglycerol, phosphatidylethanolamine (taxonomically significant phospholipid), phosphatidylinositol, and phosphatidylinositol mannosides (i.e. phospholipid pattern type II *sensu* Lechevalier et al., 1977, 1981b); (d) a fatty acid profile consisting of major amounts of straight-chain, unsaturated and tuberculostearic fatty acids (i.e. a type IV fatty acid pattern *sensu* Lechevalier et al., 1977); (e) mycolic acids with 46–70 carbon atoms (Alshamaony et al., 1976b), and (f) dihydrogenated menaquinones with nine isoprene units as the predominant isoprenologue (Collins et al., 1985). Gordoniae have fatty acid profiles rich in oleic, palmitic, palmitoleic, and tuberculostearic acids though quantitative and qualitative species-specific and intraspecific differences have been reported (Kim et al., 2003; Klatte et al., 1994b; Shen et al., 2006a). A lipoglycan structurally related to mycobacterial lipoarabinomannan has been detected in *Gordonia rubripertincta* (*sic*) (Flaherty and Sutcliffe, 1999).

Selective ion monitoring gas chromatography-mass spectrometry (SIM GC-MS) analysis provided a more detailed profile than GC-MS of the mycolic acid composition of a *Gordonia amarae* strain isolated from activated sludge foam (Stratton et al., 1999). This organism was shown to contain many shorter chain lengths and more fully saturated mycolic acids than corresponding data from previous studies on *Gordonia* strains. Stratton and her colleagues suggested that gordoniae isolated from activated sludge foams might have a different mycolic acid composition than *Gordonia* type strains.

Colony morphology. Gordoniae grow well on standard agar media used to cultivate actinomycetes. Smooth to slightly wrinkled, flat to raised or umbonate colonies with irregular margins are formed on glucose-yeast extract agar (Gordon and Mihm, 1962a). Colonies range from 2 to 10 mm in diameter, and may be opaque, beige to peach, cream, pale gray, orange, red, white, yellow, or deep pink in color. *Gordonia amarae* and *Gordonia defluvii* form microscopically visible aerial hyphae. Diffusible pigments are not produced. Strains growing on glucose-yeast extract isolation plates may show pale yellow or orange central papilla which are lost on transfer to fresh plates. Some strains, such as the type strains of *Gordonia alkanivorans* and *Gordonia westfalica*, form smooth and rough colonies (Kummer et al., 1999; Linos et al., 2002); cultures showing smooth colonies can generate rough colonies, a change which seems to be irreversible. There is evidence that glycosylated peptidolipids influence the colony morphology of *Gordonia hydrophobica* (Moorman et al., 1997).

Nutritional and growth conditions. *Gordonia* strains grow well on standard nutrient media, such as modified Bennett's (Jones, 1949), modified Sauton's (Mordarska et al., 1972), and glucose-yeast extract (Gordon and Mihm, 1962a) agars. In general, they grow well between 20°C and 37°C, but not at 5°C or 45°C. The pH growth range is 5–10.

Metabolism. Gordoniae are aerobic, catalase-positive, chemo-organotrophic actinomycetes which have an oxidative metabolism. They degrade a broad range of organic substrates, and use a wide range of sole carbon sources, and sole carbon and nitrogen compounds (Goodfellow et al., 1982b, 1982c, 1996; Kotani et al., 2003; Santos et al., 2006). They also cleave diverse 7-amino-4-methylcoumarin (7AMC) and 4-methylumbelliferone (4MU-) conjugated fluorogenic substrates (Goodfellow et al., 1991a).

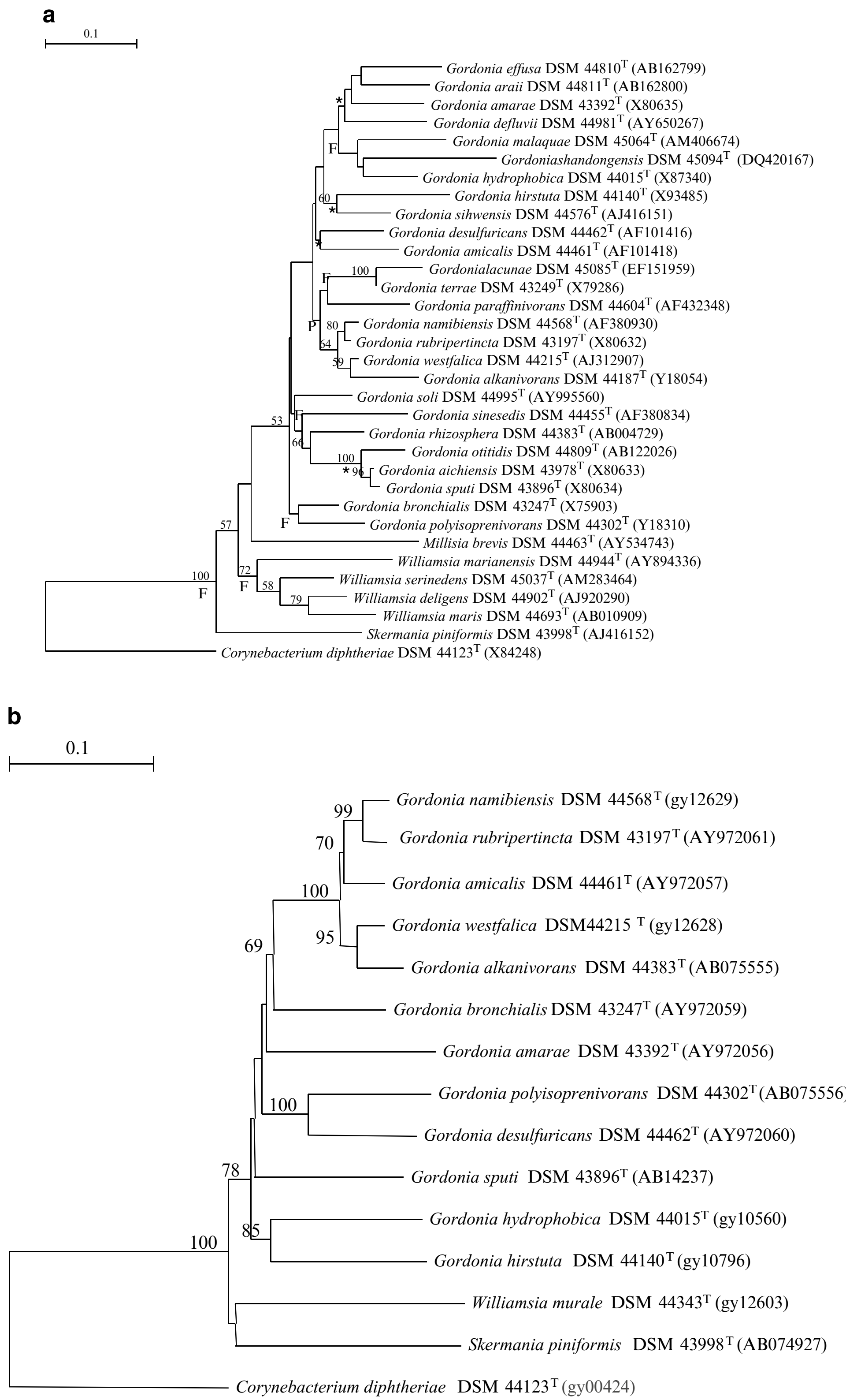

FIGURE 93. (a) Neighbor-joining tree based on nearly complete 16S rRNA gene sequences showing relationships between type strains of species in *Gordonia, Millisia, Skermania* and *Williamsia*; *Corynebacterium diphtheriae* was the out-group. Asterisks indicate branches of the tree that were found using the least-squares and maximum-parsimony tree-making algorithms. F indicates branches that were recovered using the least-squares method. The numbers at the nodes indicate the levels of bootstrap support derived from a neighbor-joining analysis of 1000 resampled datasets; only values above 50% are given. Bar = 0.1 substitutions per nucleotide position. (b) Neighbour-joining tree based on *gyrB* amino acid sequences showing relationships between genera classified in the family *Gordoniaceae* and some representative members of other genera belonging to the order *Corynebacteriales.*

Gordoniae encompass a rich reservoir of metabolic diversity which makes them attractive candidates for the bioremediation of toxic wastes, as biocatalysts, and for commercially useful industrial processes (Arenskötter et al., 2004). The presence of the dibenzothiophene-desulfurization pathway in *Gordonia amicalis* DSM 44461^{T} is consistent with their emerging status as a source of metabolic diversity rivalling that of rhodococci (Kim et al., 2000). Other gordoniae that have desulfurization pathways include *Gordonia aichiensis* strain 51 (Finkel'shtein et al., 1999), *Gordonia rubropertincta* (*sic*) strain TO8 (Matsui et al., 2001), *Gordonia* strain F.5.25.8 (Santos et al., 2006), and *Gordonia* strain GYSK1 (Rhee et al., 1998), all of which remove sulfur from dibenzothiophene, and *Gordonia desulfuricans* NCIB 40817 and NCIB 40616^{T} which have a unique sulfur-scavenging pathway whereby benzothiophene is desulfurized to 2-(2′ hydroxyphenyl)-ethan-1-al and an inorganic compound, possibly sulfite (Gilbert et al., 1998; Kim et al., 1999). This reaction corresponds to the dibenzothiophene desulfurization pathway discovered in *Rhodococcus erythropolis* IGTS8 (Oldfield et al., 1997, 1998).

The metabolic activity of *Gordonia* strains includes the ability of *Gordonia alkanivorans* to metabolize hexadecane (Kummer et al., 1999), *Gordonia nitida* (now a synonym of *Gordonia alkanivorans*) to utilize alkylpyridines, 3-ethylpyridine and 3-methylpyridine (Yoon et al., 2000c), and *Gordonia polyisoprenivorans* and *Gordonia westfalica* to degrade natural rubber substrates (Linos et al., 1999, 2000, 2002). Other poorly accessible carbon sources that can be degraded by gordoniae include *t*-butyl ether, methyl *t*-butyl either, *t*-amyl methyl ether, cyclic alkanes, and polycyclic aromatic hydrocarbons (Hernandez-Perez et al., 2001; Kästner et al., 1998; Koma et al., 2003). Members of the genus have been shown to metabolize butyl benzyl phthalates (Chaterjee and Dutta, 2003), fluoroanthene (Brito et al., 2006), and hazardous nitro compounds such as hexahydro-1,3,5-trinitro-1,3,5-triazine (hexogen), an explosive that is difficult to degrade (Gorontzy et al., 1994; Thompson et al., 2005).

An interesting soil isolate, designated *Gordonia* sp. strain TY-5, was found to utilize propane as a sole carbon and energy source (Kotani et al., 2003). These workers showed that the propane was oxidized to 2-propanol via subterminal oxidation by propane monoxygenase and further oxidized to acetone by secondary alcohol dehydrogenases. In a continuation of these studies, Kotani et al. (2007) found that the acetone was oxidized to methyl acetate by a novel Baeyer-Villiger monoxygenase (*acmA* gene product) and that the resultant methyl acetate was hydrolyzed to acetate and methanol by an esterase (*acmB* gene product). This study provided the first evidence for monoxygenase-dependent acetone oxidation and shed light on the poorly understood microbial pathway of acetone oxidation.

Gordoniae are a source of useful compounds such as carotenoids (De Miguel et al., 2000, 2001), lysine and some of its analogs (Kurimura et al., 1975), and gordonan, an acid, cell-aggregation-inducing polysaccharide (Kondo et al., 2000). "*Gordonia jacobaea*" MV-1 accumulates several carotenoids which include the ketocarotenoid *trans*-canthaxanthine and *trans*-astaxanthin. These compounds are currently produced by Hoffman-La Roche and have been approved by the Food and Drug Administration as food additives in poultry and fish feed. The generation of carotenoid-overproducing *Gordonia* strains could provide a valuable new biological source of these commercially important products. Such organisms may prove to be a valuable source of biosurfactants (Fusconi and Godinho, 2002; Nazina et al., 2003).

Genetics and molecular data. Gordonial genetics is in its infancy. However, the increasing interest in the metabolic activities of members of the genus has led to the generation of cloning vectors which allow the transfer of genes between different *Gordonia* species, the transfer of foreign genes from *Escherichia coli* to gordoniae, and the expression of these genes (Arenskötter et al., 2003; Arenskötter et al., 2004; Bröker et al., 2004). Gene transfer based on electroporation and conjugation has been demonstrated for *Gordonia alkanivorans* DSM 44187, "*Gordonia jacobaea"* MV-1 and MV-26, "*Gordonia nitida*" DSM 44499, *Gordonia rubripertincta* DSM 43197^{T} and DSM 46038, and *Gordonia terrae* DSM 43249^{T} (Arenskötter et al., 2003; Veiga-Crespo et al., 2006).

16S rRNA gene sequence similarities of *Gordonia* species range from 92.7% relatedness between the type strains of *Gordonia hydrophobica* and *Gordonia sinesedis* to 99.7% relatedness between those of *Gordonia aichiensis* and *Gordonia sputi*. The differences in the 16S rRNA gene sequences of *Gordonia* strains occur mainly in two hypervariable regions, i.e., between nucleotide positions 136 and 229, and 996 and 1028 using the numbering of the *Escherichia coli* K-12 16S rRNA gene sequence (Arenskötter et al., 2004). It is evident from comparative DNA–DNA relatedness data that the *Gordonia* species form distinct genomic species (Table 40). DNA–DNA relatedness data provided powerful support for the proposal that *Gordonia nitida* Yoon et al. 2000c be recognized as a later synonym of *Gordonia alkalinovorans* Arenskötter et al., 2005, as anticipated by Maldonado et al. (2003).

Greater resolution is found between *Gordonia* species in comparative analyses of *gyrB* sequences than in equivalent 16S rRNA gene sequences (Shen et al., 2006a, 2008). A comparison of *gyrB* protein sequences and corresponding 16S rRNA nucleotide sequences between *Gordonia* strains and representatives of *Corynebacterium xerosis* (66–70% against 71–72%), *Dietzia maris* (70–74% against 92–94%), *Nocardia brasiliensis* (75–79% against 94–95%), *Rhodococcus rhodochrous* (76–80% against 94–95%), *Skermania piniformis* (76–80% against 93–95%), *Tsukamurella paurometabola* (72–76% against 94–95%), and *Williamsia murale* (73–79% against 94–96%) illustrates this point (Shen et al., 2006b). It is evident, therefore, that *Gordonia* strains form a well-delineated phyletic line in the *gyrB* tree and can thereby be separated from other genera classified in the order *Corynebacteriales*.

Antibiotic sensitivity. Few studies have addressed the antibiotic susceptibility patterns of *Gordonia* strains and even then such work have been driven mainly by taxonomic considerations (Goodfellow et al., 1994; Goodfellow and Orchard, 1974; Kummer et al., 1999). However, this subject is of growing importance as some gordoniae are opportunistic human pathogens that are being isolated with increased frequency from patients and clinical materials. There is evidence that clinically significant gordoniae are highly susceptible to imidazole antifungal agents (Dabbs et al., 2003), and that bacteremias caused by *Gordonia* species can be effectively treated using a combination of aminoglycosides and penicillins (Riegel et al., 1996). The antibiotic sensitivity profiles of some *Gordonia* species are included in the species descriptions.

TABLE 40. DNA–DNA relatedness values between *Gordonia* species[a]

	1. *G. bronchialis*	2. *G. aichiensis*	3. *G. alkanivorans*	4. *G. amarae*	5. *G. amicalis*	6. *G. araii*	8. *G. desulfuricans*	9. *G. effusa*	10. *G. hirsuta*	11. *G. hydrophobica*	12. *G. lacunae*	13. *G. malaquae*	14. *G. namibiensis*	15. *G. otitidis*	16. *G. paraffinivorans*	18. *G. rhizosphera*	19. *G. rubripertincta*	24. *G. sputi*	25. *G. terrae*	26. *G. westfalica*
1. *G. bronchialis*	100		16													31	16	7		
2. *G. aichiensis*	28		8											31		30	7	38		
3. *G. alkanivorans*			100														40			46,61
4. *G. amarae*	23		4	100		4		3	2	3						10	4			
5. *G. amicalis*					100															
6. *G. araii*				5				6	5	5										
8. *G. desulfuricans*					32															
9. *G. effusa*				3		7		100	5	5										
10. *G. hirsuta*			8	1		4		4	100	4							7			
11. *G. hydrophobica*																				
12. *G. lacunae*											100									
13. *G. malaquae*																				
14. *G. namibiensis*													100							
15. *G. otitidis*														100						
16. *G. paraffinivorans*																				
18. *G. rhizosphera*	10,30		13													100	12			
19. *G. rubripertincta*	22–25		45,52		37						56		14			11	100	7,18		
24. *G. sputi*	12	38–40	5											4		10	4	100		
25. *G. terrae*	7,16,21		10													13	10	9		
26. *G. westfalica*																				100

[a] Data taken from Brandão et al. (2001), Iida et al. (2005), Kageyama et al. (2006), Kim et al. (1999, 2000), Kummer et al. (1999), Le Roes et al. (2008), Linos et al. (2002), Mordarski et al. (1981), Riegel et al. (1994), Takeuchi and Hatano (1998), Yoon et al. (2000a), and Zakrzewska-Czerwinska et al. (1988).

Pathogenicity. Gordoniae are being isolated increasingly from clinical material (Blaschke et al., 2007; Iida et al., 2005; Kageyama et al., 2006; Klatte et al., 1994b; Tsukamura, 1971, 1978). *Gordonia bronchialis* and *Gordonia sputi* have been isolated from sputa of patients with pulmonary disease, bronchiectasis, and cavitary pulmonary tuberculosis (Gugnani et al., 1998; Klatte et al., 1994b; Tsukamura, 1971, 1978; Tsukamura and Yano, 1985). A nosocomial outbreak of sternal wound infections in patients following coronary artery bypass surgery (Herve et al., 1991) was attributed to *Gordonia bronchialis*, as were cases of bacteremia in patients with pulmonary sequestration (Sng et al., 2004). *Gordonia bronchialis* has been reported to cause breast abscesses (Werno et al., 2005) and *Gordonia sputi*, bacteremia (Riegel et al., 1996) and mediastinitis (Kuwahara et al., 1999); a *Gordonia* strain related to *Gordonia sputi* was implicated in a case of endocarditis of a young woman fitted with a central venous catheter (Lesens et al., 2000). *Gordonia polyisoprenivorans* has been reported to cause endocarditis (Verma et al., 2006) and septicemia in a bone marrow transplant patient (Kempf et al., 2004). The first reported case involving *Gordonia rubripertincta* was a lung infection of a young immunocompromised female where an initial diagnosis was confused with tuberculosis (Hart et al., 1988). Gordoniae, especially *Gordonia terrae*, have been reported to cause central venous catheter-related bacteremia (Buchman et al., 1992; Lesens et al., 2000; Pham et al., 2003), brain abscesses (Drancourt et al., 1994, 1997), and cutaneous infections (Lasker et al., 1992; Lesens et al., 2000; Martin et al., 1991; Zardawi et al., 2004), including a case of mycetoma of the hand (Bakker et al., 2004).

Infections caused by gordoniae are probably underestimated as a result of oversight or misidentification in diagnostic laboratories (Blanc et al., 2007; Gil-Sande et al., 2006). Gordoniae are relatively slow-growing bacteria hence isolation plates need to be incubated beyond the usual 48 h period used in diagnostic settings. Precise identification of *Gordonia* strains requires the application of chemotaxonomic and molecular systematic procedures (Goodfellow et al., 1998a, 1999; Nishiuchi et al., 2000; Patel et al., 2004; Steingrübe et al., 1997), as reliance on biochemical tests can lead to the assignment of gordoniae to other mycolic acid-containing genera, notably the genus *Rhodococcus* (Gil-Sande et al., 2006; Pham et al., 2003; Sng et al., 2004).

Improvements in the classification of the genus *Gordonia* are contributing to the recognition of new opportunistic pathogens. *Gordonia arai* was described for an organism isolated from sputum of a 48-year-old man with bacterial pneumonia (Kageyama et al., 2006), *Gordonia effusa* from a 74-year-old man with kidney dysfunction (Kageyama et al., 2006), and *Gordonia otitidis* from the ear discharge of a young woman with external otitis, and from a 60-year-old man with bronchitis (Iida et al., 2005). Gordoniae have not been implicated as disease agents of animals, apart from being associated with a case of mesenteric lymphadenitis of the ileum in a 6-year-old pig (Tsukamura et al., 1988).

Ecology. Gordoniae are widely distributed in the environment possibly because of their ability to metabolize a wide range of pollutants, many of which are toxic (Brandão et al., 2001; Dabbs, 1998). They have been isolated from soil (Kim et al., 2000; Luo et al., 2007; Maldonado et al., 2003; Shen et al., 2006a), mangrove and marine sediments (Brito et al., 2006; Colquhoun et al., 1998b), packing material of a biofilter used for biological odor abatement (Bendinger et al., 1995), industrial wastewater (Yoon et al., 2000a), wastewater treatment bioreactors (Kim et al., 2003; Yassin et al., 2007a), an oil shale spoil heap (Kim et al., 1999), clinical material (Drancourt et al., 1994; Kageyama et al., 2006; Richet et al., 1991; Tsukamura, 1971), and the intestinal contents of mammals (Brown et al., 1999).

Improvements in gordonial systematics facilitated the design, evaluation, and use of an oligonucleotide primer set to determine the presence, distribution, and taxonomic diversity of members of the genus *Gordonia* in soil samples (Shen and Young, 2005). The primers, designated G268F/G1096R, amplified a 829-bp stretch of 16S rRNA genes from authenticated members of the genus *Gordonia*, but not from representatives of other mycolic acid containing genera. The primer set was used to detect the presence of gordoniae in diverse soil samples. In addition, BOX-PCR analysis revealed the presence of genetic polymorphisms amongst a few *Gordonia* wild-type isolates.

Large numbers of gordoniae have been isolated from foaming activated sludge plants in diverse geographical locations (Goodfellow et al., 1996, 1998b; Soddell and Seviour, 1990; Stainsby et al., 2002). It is generally accepted that mycolic acids render cells sufficiently hydrophobic to allow them to accumulate on the surfaces of aeration tanks (Seviour and Blackall, 1999). Initially, *Gordonia amarae* (formerly *Nocardia amarae*) was implicated in foam formation (Blackall et al., 1988; Dhaliwal, 1979; Hiraoka and Tsumura, 1984; Lechevalier and Lechevalier, 1974; Sakai et al., 1983; Sezgin et al., 1988; Soddell et al., 1992), but it is now evident that many different mycolic acid containing actinomycetes are involved, including *Gordonia defluvii* (Soddell et al., 2006b) and *Millisia brevis* (Soddell et al., 2006b).

Oligonucleotide hybridization and antibody probes have been designed to identify and quantify mycolic acid-containing actinomycetes, gordoniae and *Gordonia amarae* in order to determine whether foaming levels can be linked to the numbers of foam-causing organisms (de los Reyes et al., 1997, 1998a, 1998b, 1998c; Oerther et al., 1999). Subsequently these studies established a cause-effect relationship between *Gordonia amarae* and foaming (de los Reyes and Raskin, 2002). These workers also determined *Gordonia* levels for foam formation and foam stability, but pointed out that such thresholds might be treatment-plant specific. Davenport et al. (2000) used quantitative fluorescent *in situ* hybridization to examine the relationships between foaming and the concentration of mycolic acid-containing actinomycetes in a 20 m^3 completely mixed activated sludge reactor. The concentration of the organisms needed to induce foaming was about 2×10^6 cells/ml or 4×10^{12} cells/m^2. Pagilla et al. (2002) showed that foaming problems in activated sludge are due to the production of biosurfactants by *Gordonia amarae* when hydrophobic substances such as hexadecane are present.

Mycolic acid antibodies that react with *Gordonia amarae* and related taxa may provide an effective means of detecting mycolic acid-containing actinomycetes in activated sludge foam (Iwahori et al., 2001). Immunochemical detection of representatives of mycolic acid-containing genera in enzyme-linked immunosorbent assays (ELISAs) indicated that the extent of the antibody reactions was related to the length of the mycolic acid chains in the walls of the marker organisms. In contrast, reactivity against Gram-stain-positive bacteria that lacked mycolic acids was negligible.

Gordonia strains can survive in polluted habitats under near starvation conditions (Acharya and Desai, 1997; Warhurst and Fewson, 1994). They are able to metabolize diverse hydrocarbons, including halogenated compounds, as well as numerous substituted aromatic compounds (Peczynska-Czoch and Mordarski, 1988; Tárnok, 1976; Warhurst and Fewson, 1994). Their ability to grow in highly polluted environments makes them attractive candidates for the selective removal of contaminants from commodity products and in bioremediation.

Enrichment and isolation procedures

Large numbers of gordoniae have been isolated on glucose-yeast extract agar plates (Gordon and Mihm, 1962a) supplemented with cycloheximide and incubated at 30°C for 14 d following inoculation with serially diluted samples taken from foaming activated sludge plants (Goodfellow et al., 1996b, 1998a). The gordoniae were recognized by their ability to form rough, grayish-pink dry colonies on the isolation plates. Initial characterization studies showed that most of the isolates formed new centers of taxonomic variation in the genus.

Gordoniae can be isolated from sputa and soils by plating chemically treated samples onto a selective medium (Tsukamura, 1971; Tsukamura et al., 1988). Sputum samples suspended in distilled water (25 ml) are added to an equal volume of NaOH (4%) and liquefied either by shaking at room temperature for 15–20 min or by incubation at 37°C for 30 min then inoculated onto Ogawa egg medium (Tsukamura, 1962) and incubated for 4–8 weeks. Similarly, soils (5 g) suspended in distilled water (25 ml) are shaken vigorously in a 300-ml Erlenmeyer flask at room temperature on a reciprocal shaker for 30 min; the resultant suspension is allowed to settle for 10 min when 15 ml of the supernatant is added to an equal volume of NaOH (8%), and the preparation shaken for 10 min prior to centrifugation at 500 × *g* for 15 min. The residue is suspended in 10 ml of a 1% NaH_2PO_4 solution, and 0.002 ml is added to Ogawa egg medium slants that are incubated for 4–8 weeks.

Colonies growing on Ogawa egg medium slants are subcultured to fresh slopes supplemented with sodium salicylate (0.5 mg/ml), which inhibits *Mycobacterium tuberculosis* (Tsukamura, 1962). Smears, prepared from colonies growing on the slants supplemented with sodium salicylate after incubation at 37°C for 3 weeks, are stained by the Ziehl–Neelsen procedure and examined by light microscopy. Slightly acid-fast, rod-shaped bacteria are typical of gordoniae; those strongly acid-fast can be taken to be mycobacteria. Gordoniae form rough pinkish or reddish colonies on Ogawa egg slants plugged with cotton wool.

Suitable media for the isolation of *Gordonia amarae* include Czapek's agar supplemented with yeast extract (Higgins and Lechevalier, 1969) and glycerol agar (Gordon and Smith, 1953); macroscopically visible colonies are evident within 5–7 d at 30°C (Lechevalier et al., 1976; Lechevalier and Lechevalier, 1974). Similarly, *Gordonia rubripertincta* has been isolated on Munz and Winogradsky's nitrate agars supplemented with paraffin (Nesterenko et al., 1978a), *Gordonia lacunae* on yeast

extract-malt extract agar following incubation for 5 d at 30°C (Le Roes et al., 2008), *Gordonia otitidis* on blood agar plates incubated for 7 d at 37°C (Iida et al., 2005), *Gordonia hirsuta* on antibiotic sulfonamide sensitivity test agar (Klatte et al., 1996), and *Gordonia sihwensis* on succinate mineral agar plates incubated at 28°C for 7 d (Kim et al., 2003). *Gordonia defluvii* strains were isolated, by micromanipulation, from activated sludge foams, as described by Soddell and Seviour (1998).

A range of novel gordoniae have been recovered from diverse environmental samples by enrichment culture, as exemplified by the isolation of *Gordonia alkanivorans* (Kummer et al., 1999; Yoon et al., 2000c), *Gordonia amicalis* (Kim et al., 2000), *Gordonia desulfuricans* (Kim et al., 1999), *Gordonia namibiensis* (Brandão et al., 2001), and *Gordonia paraffinivorans* (Xue et al., 2003).

Maintenance procedures

A convenient method for short-term storage can be achieved by serial transfer every 2 months from appropriate media, such as modified Bennett's (Jones, 1949) and glucose-yeast extract (Gordon and Mihm, 1962a) agar slopes with storage between transfers at 4°C. Long-term storage involves lyophilization, or storage in liquid nitrogen or frozen glycerol suspensions. For lyophilization, biomass is suspended in a medium such as glucose serum (7.5%, w/v) or skimmed milk supplemented with glucose (7.5%, w/v). For preservation in liquid nitrogen, organisms are inoculated onto a suitable medium in small tubes until sufficient growth is visible. These preparations are sealed with cotton wool plugs dipped in liquid paraffin wax and placed in a liquid nitrogen container. Glycerol suspensions prepared by scraping growth from heavily inoculated agar plates are used to make heavy suspensions in aqueous glycerol (3 ml), held in small vials, which are stored at –20°C (Wellington and Williams, 1978).

Differentiation of the genus *Gordonia* from other genera

Gordonia strains can be differentiated from members of other genera classified in the order *Corynebacteriales* by using a combination of chemotaxonomic and morphological markers (Table 29) and by partial sequencing of ribosomal protein AT-L30 (Ochi, 1995). Carbon utilization tests (Biotype 100 strips) may also be of value in distinguishing between members of the genera *Dietzia, Gordonia*, and *Rhodococcus* (Bizet et al., 1997). Representatives of these taxa have been separated by mycolic acid profiles generated by capillary gas chromatography and mass spectrometry (Nishiuchi et al., 2000). Partial 16S rRNA gene sequences have been used for the identification of clinically significant aerobic actinomycetes belonging to taxa that include the genera *Gordonia, Nocardia*, and *Tsukamurella* (Patel et al., 2004), as has DNA amplification and restriction endonuclease analysis (Steingrübe et al., 1997).

Taxonomic comments

Michio Tsukamura proposed the genus *Gordona* for slightly acid-fast actinomycetes isolated from soil and sputa of patients with pulmonary disease. The founder members of the genus, *Gordonia bronchialis, Gordonia rubra*, and *Gordonia terrae*, were subsequently reclassified in the revised (Tsukamura, 1974) and redescribed genus *Rhodococcus* (Goodfellow and Alderson, 1977) though it soon became apparent that this taxon was markedly heterogeneous. The etymologically correct name, *Gordonia*, was introduced by Stackebrandt et al. (1997).

Rhodococcal species were eventually assigned to two aggregate groups based on the discontinuous distribution of key chemical markers (Goodfellow, 1998). Strains previously classified in the genus *Gordonia* contained mycolic acids with between 48 and 66 carbon atoms and major amounts of dihydrogenated menaquinones with nine isoprene units, the remaining organisms were characterized by shorter chain mycolic acids (34–52 carbon atoms) and dihydrogenated menaquinones with eight isoprene units as the predominant isoprenologue (Alshamaony et al., 1976b; Collins et al., 1977, 1985). The validity of these taxa was underpinned by results from antibiotic sensitivity tests (Goodfellow and Orchard, 1974), delayed skin reactions in sensitized guinea pigs, and by polyacrylamide gel electrophoresis of cell extracts (Hyman and Chaparas, 1977). The subsequent discovery that the two aggregate taxa were distinct phylogenetically led Stackebrandt et al. (1988) to revive the genus *Gordona* for organisms classified as *Rhodococcus bronchialis, Rhodococcus rubropertincta, Rhodococcus sputi*, and *Rhodococcus terrae. Rhodococcus obuensis* Tsukamura (1982a) was reduced to a subjective synonym of *Rhodococcus sputi* primarily on the basis of DNA–DNA relatedness data (Zakrzewska-Czerwinska et al., 1988).

The genus *Gordonia* contains 26 validly published species which form a well delineated clade within the evolutionary radiation encompassed by mycolic acid-containing actinomycetes (Goodfellow and Maldonado, 2006; Le Roes et al., 2008). The status of most of these species is supported by a wealth of genotypic and phenotypic data (Brandão et al., 2001; Goodfellow and Alderson, 1977; Goodfellow et al., 1991a; Luo et al., 2007; Soddell et al., 2006b; Tsukamura, 1974). Indeed, DNA–DNA relatedness data show that most *Gordonia* species, including *Gordonia amarae, Gordonia araii, Gordonia bronchialis, Gordonia desulfuricans, Gordonia namibiensis*, and *Gordonia westfalica* are well delineated genomic species (Brandão et al., 2001; Kageyama et al., 2006; Kim et al., 1999; Linos et al., 2002; Mordarski et al., 1976, 1980; Zakrzewska-Czerwinska et al., 1988). It seems likely that additional gordonial species will be delineated as there is evidence that the genus is underspeciated (Goodfellow et al., 1996, 1998a; Schuppler et al., 1995).

Differentiation of species of the genus *Gordonia*

Gordonia species can be distinguished using a combination of molecular sequence (Figure 93), DNA–DNA relatedness (Table 40), and phenotypic data (Table 41). Preliminary evidence suggests that fatty acid analyses (McNabb et al., 1997), Curie-point pyrolysis mass spectrometry (Goodfellow et al., 1996, 1998a) and rapid enzyme tests based on the fluorophores 7-amino-4-methylcoumarin and 4-methylumbelliferone (Goodfellow et al., 1991a) may provide a reliable means of distinguishing between *Gordonia* species. PCR-restriction enzyme analysis of the 439-bp Telenti fragment of the 65 *hsp* gene and carbon utilization tests have provided reliable data for assigning clinically significant gordoniae to the species level (Patel et al., 2004).

TABLE 41. Characteristics differentiating the species of the genus *Gordonia*[a]

Characteristic	*G. bronchialis*	*G. aichiensis*	*G. alkanivorans*	*G. amarae*	*G. amicalis*	*G. araii*	*G. defluvii*	*G. desulfuricans*	*G. effusa*	*G. hirsuta*	*G. hydrophobica*	*G. lacunae*	*G. malaquae*	*G. namibiensis*	*G. otitidis*	*G. paraffinivorans*	*G. polyisoprenivorans*	*G. rhizosphera*	*G. rubripertincta*	*G. shandongensis*	*G. sihwensis*	*G. sinesedis*	*G. soli*	*G. sputi*	*G. terrae*	*G. westfalica*
Biochemical tests:																										
Allantoin hydrolysis	–	–	–	+	–	nd	–	–	nd	–	+	–	nd	+	nd	+	–	–	–	nd	nd	–	nd	–	–	nd
Arbutin hydrolysis	–	+	–	+	–	nd	nd	–	nd	–	+	+	nd	–	nd	–	–	–	–	nd	nd	–	–	+	+	–
Esculin hydrolysis	–	–	–	+	–	nd	–	–	nd	–	+	+	–	+	nd	+	–	–	+	+	nd	–	–	+	+	–
Nitrate reduction	+	+	+	–	–	nd	+	+	nd	+	+	+	nd	–	nd	–	–	–	–	+	+	+	–	+	+	+
Urea hydrolysis	+	+	+	+	–	–	–	+	nd	–	–	+	+	+	nd	+	+	–	+	+	nd	+	+	+	+	+
Decomposition of:																										
Adenine	+	nd	–	+	–	–	–	–	nd	–	+	nd	–	–	nd	nd	+	–	–	nd	nd	+	nd	+	+	–
Hypoxanthine	–	–	–	+	–	–	–	+	nd	–	+	–	–	+	nd	–	–	–	+	nd	nd	+	–	+	+	–
Starch	+	–	+	+	+	nd	–	+	nd	+	–	–	nd	–	nd	–	+	+	+	–	nd	+	nd	+	+	+
Tributyrin	–	–	+	–	–	nd	+	–	nd	–	–	nd	nd	+	nd	nd	+	–	+	nd	nd	+	+	–	–	–
Tween 80	–	+	+	–	–	nd	+	+	nd	+	+	nd	nd	–	nd	+	+	–	–	nd	nd	+	+	–	–	+
Tyrosine	–	–	–	+	–	–	–	–	nd	–	–	nd	–	–	nd	–	–	–	–	–	nd	+	–	–	–	–
Uric acid	+	+	–	+	–	nd	+	+	nd	–	+	nd	nd	+	nd	–	+	+	+	nd	nd	+	–	+	+	–
Xanthine	–	–	–	–	–	–	–	–	nd	–	–	nd	–	–	nd	–	–	–	–	–	nd	–	–	+	+	–
Growth on sole carbon sources:																										
Arbutin	–	–	–	+	+	nd	+	+	nd	+	+	nd	nd	–	nd	nd	–	+	–	nd	nd	–	nd	–	–	nd
Cellobiose	–	–	–	–	–	nd	–	–	nd	+	–	+	–	+	nd	–	–	+	–	+	nd	–	nd	–	–	nd
Glycerol	+	+	+	+	+	nd	–	+	nd	+	+	nd	nd	–	nd	+	–	–	+	nd	nd	–	nd	+	+	nd
N-Acetyl-D-glucosamine	–	–	+	+	–	nd	+	–	nd	+	+	nd	nd	+	nd	nd	+	+	+	nd	nd	+	nd	–	–	nd
Betaine	–	–	–	–	–	nd	nd	–	nd	–	–	nd	nd	–	nd	nd	–	+	–	nd	nd	–	nd	+	+	nd
Propan-l-ol	–	–	–	+	–	nd	nd	+	nd	–	+	nd	nd	+	nd	nd	–	+	–	nd	nd	+	nd	–	+	nd
Sodium adipate	–	+	–	–	+	nd	nd	+	nd	–	+	nd	nd	+	nd	nd	+	+	–	nd	nd	–	nd	+	–	nd
Sodium fumarate	+	–	+	–	+	nd	nd	–	nd	–	–	nd	nd	+	nd	nd	–	+	+	nd	nd	+	nd	–	+	nd
Sodium oxalate	–	–	–	–	–	nd	nd	–	nd	–	–	nd	nd	–	nd	nd	–	–	–	nd	nd	–	nd	+	+	nd
Mycolic acid (no. carbons)	54–66	56–66	≤47–58	46–54	48–58	64–70	nd	56–64	56–64	60–64	54–62	nd	nd	54–62	58–64	52–62	58–64	56–64	48–62	nd	48–56	58–62	nd	56–66	52–64	54–62
G+C content (mol%)	63–65	65	67–68	60–66	64	nd	63	63–65	nd	69	69	nd	nd	nd	65	66	nd	67	67–69	nd	nd	nd	nd	65	64–69	nd

[a]Symbols and abbreviations: +, positive; –, negative; nd, not determined.

List of species of the genus *Gordonia*

1. **Gordonia bronchialis** (Tsukamura 1971) Stackebrandt, Smida and Collins 1989, 371[VP] (*Gordonia bronchialis* Tsukamura 1971, 22; basonym: *Rhodococcus bronchialis* Tsukamura 1971, 1974)

bron. chi′a.lis. L. pl. n. *bronchia* the bronchial tubes; L. fem. suff. -*alis* suffix used with the sense of pertaining to; N.L. fem. adj. *bronchialis* pertaining to the bronchi, coming from the bronchi.

Rod–coccus life cycle. Rough brownish colonies formed on egg media, glucose-yeast extract agar, and Sauton's agar. Synnemata composed of vertically arranged coalescing filaments are formed on the surface of colonies after 12–18 h of incubation. Grows between 20°C and 40°C, but not 10°C or 45°C.

Acetamidase, nicotinamidase, pyrazinamidase, and urease-positive, but negative for allantoinase, benzamidase, isonicotinamidase, malonamidase, salicylamidase, and succinamidase. Acid phosphatase and β-esterase-positive, but negative for α-esterase and β-galactosidase.

Acid is produced from glucose, inositol, maltose, mannose, and trehalose, but not from arabinose, galactose, raffinose, rhamnose, sorbitol, or xylose.

Grows on acetamide, *iso*-butanol, *p*-cresol, ethanol, glycerol, *p*-hydroxybenzoic acid, inositol, maltose, mannose, propanol, propylene glycol, benzoate, sodium gluconate, sodium lactate, sodium malate, sodium pyruvate, sodium sebacate, sorbitol, sodium succinate, sucrose, testosterone, and trehalose as sole carbon sources, but not on *m*-hydroxybenzoic acid, rhamnose, or L-tyrosine. L-glutamate, isonicotinamide, L-methionine, nicotinamide, pyrazinamide, L-serine, succinamide, and urea are used as sole nitrogen sources, but not benzamide or nitrite. Acetamide, D-alanine, and L-glutamate are used as sole sources of carbon and nitrogen, but not L-alanine, L-asparagine, benzamide, monoethanolamine, L-serine, L-threonine, or trimethylenediamine.

Resistant to crystal violet (0.001%, w/v), phenol (0.1%, w/v), phenyl ethanol, (0.3%, v/v), picric acid (0.2%, w/v), sodium aminosalicylate (0.2%, w/v), sodium azide (0.02%, w/v), and sodium chloride (7%, w/v). Resistant to (µg per ml) neomycin (4), oleandomycin (32), and vancomycin (4), but sensitive to chlortetracycline (4), fluorouracil (5), gentamicin (8), mitomycin C (5), and rifampin (8).

Mycolic acids have 54–66 carbon atoms. The major fatty acids are $C_{16:0}$ (19.6%), $C_{18:1}$ ω9*c* (13.3%) and 10-methyl $C_{18:0}$ ω9*c* (25.4%). The predominant menaquinone is MK-9(H_2).

Source: sputum of patients with pulmonary disease.

DNA G+C content (mol%): 63–65 (T_m).

Type strain: strain Tsukamura 3410, ATCC 25592, CCUG 22989, CCUG 34956, CIP 100847, DSM 43247, NBRC 16047, JCM 3198, JCM 3231, LMG 5355, KCC A-0198, NCTC 10667, VKM Ac-956.

Sequence accession no. (16S rRNA gene): X79287, X75903.

Sequence accession no. (gyrB): AY972059.

2. **Gordonia aichiensis** (Tsukamura 1982a) Klatte, Rainey and Kroppenstedt 1994b, 772[VP] (*Rhodococcus aichiensis* Tsukamura 1983, 896)

ai.chi.en′sis. N.L. fem. adj. *aichiensis* belonging to Aichi Prefecture, Japan from where the organism was isolated.

Rod–coccus life cycle. Rough pinkish or orange colonies are formed on egg media. Grows at 28°C and 42°C, but not at 45°C.

Positive for acetamidase, nicotinamidase, pyrazinamidase, urease, and valine arylamidase, but negative for allantoinase, benzamidase, isonicotinamidase, salicylamidase, and succinamidase. Positive for acid phosphatase, but negative for arylsulfatase, α- and β-esterase, and β-galactosidase.

Grows on fructose, glucose, mannose, sucrose, trehalose, *n*-butanol, ethanol, *iso*-butanol, *n*-propanol, sodium acetate, sodium citrate, sodium fumarate, sodium malate, sodium pyruvate, and sodium succinate as sole carbon sources, but not on arabinose, galactose, *meso*-inositol, mannitol, rhamnose, sorbitol, xylose, 1,3-, 1,4-, or 2,3-butylene glycol, propylene glycol, sodium benzoate, or sodium malate. Uses acetamide, glutamate, and monoethanolamine as sole sources of carbon and nitrogen.

Resistant to picric acid (0.2%, w/v), sodium nitrite (0.1%, w/v), and sodium salicylate (0.1%, w/v), but not to 5-fluorouracil (20 µg per ml), or mitomycin C (5 µg per ml).

Mycolic acids have 56–66 carbon atoms and up to 6 double bonds. Major fatty acids are $C_{16:1}$ *cis*9 (17.7%); $C_{16:0}$ (36.0%), $C_{18:1}$ *cis*9 (5.5%), and 10-methyl $C_{18:0}$ (23.0%). The predominant menaquinone is MK-8(H_2); minor amounts of MK-8(H_2) are also produced. The cellular polar lipid composition includes diphosphatidylglycerol, phosphatidylglycerol, phosphatidylinositol, and phosphatidylinositol mannosides.

Source: human sputum.

DNA G+C content (mol%): 65 (T_m).

Type strain: Tsukamura E 9028, ATCC 33611, CIP104184, DSM 43978, NBRC 160146, JCM 6046, NRRL B-16934.

Sequence accession no. (16S rRNA gene): X80633.

Sequence accession no. (gyrB): gy10795.

3. **Gordonia alkanivorans** Kummer, Schumann and Stackebrandt 1999, 1521[VP] (*Gordonia nitida* Yoon, Lee, Kang, Takeuchi, Shin, Lee, Kang and Park 2000c, 1209[VP])

al.ka.ni.vo′rans. N.L. n. *alkanum* saturated aliphatic hydrocarbon; L. v. *vorare* to eat; N.L. part. adj. *alkanivorans*, alkane-devouring.

Non-acid-fast, regular rod-shaped cells (0.5–0.9 × 1.0–2.3 µm) occur singly, in pairs, small clusters, in V-shape arrangements, or as short chains. Rod–coccus life cycle. Colonies orange to orange red. Colony morphology either convex and smooth or rough and flat with irregular margins. Grows at 13–40°C, but not at 5° or 42°C.

Acid is formed from fructose, glycerol, mannitol, sucrose, and sorbitol, but not from galactose, glucose, mannose, rhamnose, or salicin.

Acetate, *cis*-aconitate, benzoate, citrate, formate, malate, succinate, propionate, and pyruvate are used as sole carbon sources, but not lactate, oxalate, or tartrate.

Susceptible to (µg per ml) ampicillin (10), chloramphenicol (30), ciprofloxacin (5), gentamicin (10), kanamycin (30), neomycin (30), nitrofuran (100), oxytetracycline

(30), rifampin (30), streptomycin (10), and sulfonamide (250); weakly susceptible to erythromycin (15), oxacillin (5), penicillin G (10 IU), and polymyxin B (300 IU), but resistant to lincomycin (2).

Mycolic acids have 47–58 carbon atoms. The major fatty acids are $C_{16:0}$ (44.0%), $C_{18:1}$ ω9*c* (13%), and 10-methyl $C_{18:0}$ (28.0%). The predominant isoprenologue is MK-9(H_2); minor amounts of MK-8(H_2) are also produced.

Source: contaminated soil sample taken from the site of a former tar factory in Rositz, Germany.

DNA G+C content (mol%): 67–68 (HPLC).

Type strain: HKI 0136, CIP 106363, DSM 44369, NBRC 16433, JCM 10677.

Sequence accession no. (16S rRNA gene): Y18054.

Sequence accession no. (gyrB): ABO75553.

4. **Gordonia amarae** (Lechevalier and Lechevalier 1974) Klatte, Rainey and Kroppenstedt 1994b, 771[VP]. (*Nocardia amarae* Lechevalier and Lechevalier 1974, 286).

a.ma′ra.e. Gr. n. *amara* trench, conduit, channel; here a sewage duct; N.L. gen. n. *amarae* of a sewage duct.

Partially acid-fast actinomycete which forms moderately branching substrate hyphae that grow into agar media and do not fragment in undisturbed cultures. Aerial hyphae visible microscopically. Convoluted white to tannish colonies with filamentous margins are formed on standard media. Grows at 23–37°C, but not at 10° or 40°C.

Degrades Tweens 20, 40, and 60, but not casein, elastin, or gelatin.

Acid is produced from fructose, glucose, glycerol, *meso*-inositol, maltose, mannitol, mannose, rhamnose, salicin, sucrose, and trehalose, but not from L-arabinose, cellobiose, galactilol, galactose, glucitol, lactose, melibiose, α-methyl-D-glucoside, β-methyl-D-xyloside, raffinose, or xylose.

Grows on ethanol, fructose, glycerol, maltose, mannitol, rhamnose, salicin, sucrose, trehalose, sodium acetate, sodium adipate, sodium fumarate, sodium gluconate, sodium lactate, sodium malate, sodium pimelate, sodium propionate, sodium pyruvate, sodium sebacate, and sodium succinate as sole carbon sources, but not on acetamide, L-arabinose, benzamide, *p*-cresol, *m*-hydroxybenzoate, lactose, glucitol, melezitose, raffinose, xylose, sodium benzoate, sodium citrate, sodium oxalate, sodium tartrate, testosterone, or L-tyrosine. Does not use acetamide, D-alanine, L-alanine, L-asparagine, L-serine, L-threonine, or trimethylenediamine as sole carbon and nitrogen sources.

Susceptible to lysozyme, penicillin (10 IU), phenol (0.1%, w/v), phenyl ethanol (0.3%, v/v), and sodium chloride (5% and 7%, w/v), but not to crystal violet (0.0001% and 0.001%, w/v), phenol (0.01%, w/v), or sodium azide (0.01%, w/v). Resistant to (μg per ml) rifampin (8), but is sensitive to chlortetracycline (4), gentamicin (16), neomycin (4), oleandomycin (32), penicillin G (32), and vancomycin (4).

Mycolic acids have 46–56 carbon atoms and up to 3 double bonds. The major fatty acids are $C_{16:0}$ (28.6%), $C_{18:1}$ ω9*c* (19.3%), and 10 methyl $C_{18:0}$ (21.8%). The predominant isoprenologue is MK-9(H_2). The cellular polar lipid composition includes diphosphatidylglycerol, phosphatidylethanolamine, phosphatidylglycerol, phosphatidylinositol, and phosphatidylinositol mannosides.

Source: foam formed on the surface of aeration tanks in activated-sludge sewage-treatment plants.

DNA G+C content (mol%): 60–66 (T_m).

Type strain: SE6, ATCC 27808, CIP 104501, DSM 43376, DSM 43392, DSM 43587, HAMBI 2282, NBRC 15530, JCM 3171, KCC A-0171, NCIMB 11222, NRRL B-8176, NRRL B-16281, VKM Ac-801.

Sequence accession no. (16S rRNA gene): X80635.

Sequence accession no. (gyrB): AB014105.

5. **Gordonia amicalis** Kim, Brown, Oldfield, Gilbert, Iliarionov and Goodfellow 2000, 2033[VP]

am.i.ca′lis. L. fem. adj. *amicalis* pertaining to friendship.

Slightly acid–alcohol-fast actinomycete which forms short rods and coccoid elements. Red colonies are formed on modified Bennett's and peptone-yeast extract agars. Grows at 10–40°C, and within the pH range 5.5–10.0.

Arbutin, glycerol, sodium adipate, sodium fumarate, sodium propionate, and sodium salicylate are used as sole carbon sources, but not D-arabinose, betaine, *N*-acetyl-D-glucosamine, sodium oxalate, or 1-propanol.

Grows in the presence of oleic acid (0.8%, v/v) and zinc chloride (0.001%, w/v).

Mycolic acids have 48–58 carbon atoms with C_{52}, C_{54}, and C_{56} as the main components. The predominant menaquinone is MK-9(H_2), but substantial amounts of MK-8(H_2) are also produced.

Source: garden soil, near Perm, Russia.

DNA G+C content (mol%): 64.2 (HPLC).

Type strain: IEGM, CCUG 48822, DSM 44461, KCTC 9899, KCTC 9940, JCM 11271, NBRC 100051.

Sequence accession no. (16S rRNA gene): AF101418.

Sequence accession no. (gyrB): AY972057.

6. **Gordonia araii** Kageyama, Iida, Yazawa, Kudo, Suzuki, Koga, Scuto, Inagawa, Wada, Kroppenstedt and Mikami 2006, 1820[VP]

a.rai′i. N.L. gen. masc. n. *araii* of Arai, to honor Tadashi Arai, a Japanese microbiologist, for his valuable contributions to microbial taxonomy and secondary metabolites research.

Slightly acid-fast, short elementary branching hyphae fragment into rod and coccoid-like elements. Rough colonies (1.5–3.5 mm) with irregular margins are formed after 7 d at 30°C on MMII medium. Colonies are white, becoming beige. Soluble pigments are not formed.

Glucose, ribose, and sucrose are used as sole carbon sources, but not *N*-acetyl-D-glucosamine, acetamide, aconitol, L-alanine, arabinose, L-aspartate, erythritol, galactose, 4-hydroxybenzoate, *myo*-inositol, L-leucine, maltose, mannose, 2-oxoglutarate, putrescine, sodium quinate, sodium glutarate, sodium succinate, sorbitol, turanose, or L-valine.

Mycolic acids with 64–70 carbon atoms. The major fatty acids are $C_{14:0}$, $C_{16:1}$ *cis*9, $C_{16:0}$, $C_{18:1}$ *cis*9, $C_{18:0}$, and 10-methyl-$C_{18:0}$. The predominant menaquinone is MK-9(H_2).

Source: a patient with kidney dysfunction.

DNA G+C content (mol%): not determined.

Type strain: IFM 10211, DSM 44811, JCM 12131, NBRC 100433.

Sequence accession no. (16S rRNA gene): AB162800.

7. **Gordonia defluvii** Soddell, Stainsby, Eales, Seviour and Goodfellow 2006b, 2267[VP]

de.flu'vi.i. L. gen. n. *defluvii* of sewage.

Slightly acid–alcohol-fast, slowly growing actinomycete which shows acute-angled and right-angled branching. Non-pigmented colonies with filamentous margins and abundant aerial hyphae are formed on glucose-yeast extract agar. Diffusible pigments are not produced. Grows at pH 5–8 and 15–30°C, albeit weakly at the higher end of this range.

Esterase lipase (C_8), leucine aminopeptidase, and β-glucosidase-positive, but negative for alkaline phosphatase, chymotrypsin, α-fucosidase, α- and β-galactosidase, β-glucuronidase, *N*-acetyl-β-glucosaminidase, α-mannosidase, trypsin, and valine aminopeptidase. Positive for catalase and phosphatase, but negative for *m*- and *p*- nitrophenol oxidases.

Degrades Tween 20, but not casein or elastin.

Pyruvate is used as a sole carbon source, but not arabinose, fructose, galactose, maltose, mannose, melezitose, rhamnose, salicin, or sorbitol (all at 0.1% w/v), or acetamide, benzamide, sodium benzoate, sodium butyrate, sodium citrate, *p*-cresol, gluconate, *m*- and *p*-hydroxybenzoic acid, sodium lactate, sodium malate, sodium octanoate, sodium oxalate, sodium pimelate, sodium propionate, sodium sebacate, sodium succinate, sodium tartrate, testosterone, or tyrosine (all at 0.01% w/v).

Does not grow in the presence of crystal violet (0.001%, w/v), phenol (0.01%, w/v), phenol ethanol (0.03%, v/v), sodium azide (0.01%, w/v), sodium chloride (5%, w/v), or penicillin (10 IU).

The methyl esters of the mycolic acids have the same Rf value on one-dimensional thin-layer-chromatography as those of *Gordonia bronchialis* DSM 43247[T]. The major fatty acid components are oleic ($C_{18:1}$; 35%), palmitic ($C_{16:0}$; 21%), palmitoleic ($C_{16:1}$; 18%), and tuberculostearic (9%) acids. The predominant menaquinone is MK-9(H_2), and smaller amounts of MK-8(H_2) are also produced. The cellular polar lipid composition includes diphosphatidylglycerol, phosphatidylethanolamine, phosphatidylglycerol, phosphatidylinositol, and phosphatidylinositol mannosides.

Source: a foam sample taken from an activated sludge plant at Brimbank Park, Victoria, Australia.

DNA G+C content (mol%): 63.1 (HPLC).

Type strain: J4, DSM 44981, NCIMB 14149.

Sequence accession no. (16S rRNA gene): AY650267.

8. **Gordonia desulfuricans** Kim, Brown, Oldfield, Gilbert and Goodfellow 1999, 1847[VP]

de.sul.fu'ri.cans. L. pref. *de* from; L. n. *sulfur* sulfur; N.L. part. adj. *desulfuricans* reducing sulfur compounds.

Slightly acid–alcohol-fast actinomycete which forms short rods and coccoid elements. Rough pinkish colonies are formed on modified Bennett's and peptone-glucose-yeast extract agars. Neither aerial hyphae nor diffusible pigments are produced.

D-Arabinose, glycerol, sodium propionate, and sodium salicylate are used as sole carbon sources.

Grows in the presence of adenine (0.25%, w/v), oleic acid (0.8%, w/v), and zinc chloride (0.001%, w/v), but is sensitive to picric acid (0.3%, w/v).

Mycolic acids have 56–64 carbon atoms with up to 3 double bonds. The predominant menaquinone is MK-9(H_2), and minor amounts of MK-8(H_2) are also produced.

Source: a soil sample collected adjacent to an oil shale spoil heap near a disused mine located at West Calder, West Lothian, Scotland.

DNA G+C content (mol%): 63–65 (HPLC).

Type strain: 213E, DSM 44462, JCM 11762, NBRC 100010, NCIMB 40816.

Sequence accession no. (16S rRNA gene): AF 101416.

Sequence accession no. (gyrB): gy12627.

9. **Gordonia effusa** Kageyama, Iida, Yazawa, Kudo, Suzuki, Koga, Saito, Inagawa, Wada, Kroppenstedt and Mikami 2006, 1820[VP]

ef.fu'sa. L. fem. adj. *effusa*, poured out, extensive, vast, broad, wide, referring to the spreading colonial growth.

Slightly acid-fast, short elementary hyphae fragment into rod- and coccoid-like elements. Rough colonies (3.0–5.0 mm) with irregular margins are formed after 7 d at 30°C on MMII medium. Colonies are white, becoming beige. Soluble pigments are not formed.

N-Acetyl-*N*-glucosamine, L-alanine, L-aminobutyrate, L-aspartate, putrescine, sodium citrate, sodium succinate, turanose, and L-valine are used as sole carbon sources, but not acetamide, arabitol, galactose, sodium glutarate, 4-hydroxybenzoate, *myo*-inositol, L-leucine, 2-oxoglutarate, sodium quinate, L-rhamnose, or D-ribose.

Mycolic acids have 56–64 carbon atoms. The major fatty acids are $C_{14:0}$, $C_{15:0}$, $C_{16:0}$, $C_{17:0}$, $C_{18:0}$, and 10-methyl $C_{18:0}$. The predominant menaquinones are MK-9(H_8) and MK-9(H_6), and a small amount of MK-9(H_4) is also produced.

Source: a patient with pulmonary disease.

DNA G+C content (mol%): not determined.

Type strain: IFM 10200, DSM 44810, JCM 12130, NBRC 100432.

Sequence accession no. (16S rRNA gene): AB162799.

10. **Gordonia hirsuta** Klatte, Kroppenstedt, Schumann, Altendorf and Rainey 1996, 879[VP]

hir.su'ta. L. fem. adj. *hirsuta* shaggy, rough, referring to the rough surfaces of colonies.

Non-acid-fast rods. White to light yellow colonies with irregular margins and flat, rough surfaces are formed on various media.

Catalase-positive and oxidase-negative. Hydrolyzes pNP-phosphorylcholine, but not 2-deoxythymidine -5′-pNP-phosphate, or pNP-β-D-xyloside.

Galactose, *meso*-inositol and 2-oxoglutarate are metabolized, but not acetamide, *N*-acetyl-D-glucosamine, L-alanine, 4-aminobenzoate, D-arabinose, L-aspartate, D-glucosamine acid, 3- and 4- hydroxybenzoate, 2-hydroxyvaline, phenylacetic acid, sodium benzoate, sodium citrate, sodium gluconate, sodium pimelate, sodium succinate, putrescine, sodium quinate, ribose, rhamnose, ribose, L-serine, sucrose, turanose, tyramine, L-proline, or L-valine.

Mycolic acids have 60–64 carbon atoms with C_{62} and C_{64} as the main components. The major fatty acids are $C_{16:1}$

*cis*10 (16%), $C_{16:0}$ (30%), $C_{16:1}$ *cis*9 (30%), and 10-methyl $C_{18:0}$ (17%). MK-9(H_2) is the predominant menaquinone. The cellular polar lipid composition includes diphosphatidylglycerol, phosphatidylethanolamine, phosphatidylinositol, and phosphatidylinositol mannosides.

Source: packing material (tree bark compost) of a large-scale biofiller used for deodorization of animal-rending emissions.

DNA G+C content (mol%): 69 (HPLC).

Type strain: K718a, ATCC 700255, CCUG 38498, CIP 105097, DSM 44140, NBRC 16056, JCM 10105.

Sequence accession no. (16S rRNA): X93485.

Sequence accession no. (gyrB): gy10796.

11. **Gordonia hydrophobica** Bendinger, Rainey, Kroppenstedt, Moorman and Klatte 1995, 547[VP]

hy.dro.pho′bi.ca. Gr. n. *hydro* water, Gr. n. *phobos* fear, dread; L. fem. suff. *-ica* suffix used with the sense of pertaining to; N.L. fem. adj. *hydrophobica* water avoiding, related to hydrophobic.

Non-acid-fast actinomycete which forms long rods (2–3 μm) and shows a rod–coccus life cycle. White to tannish colonies are formed on standard media. Colonies may be rough and flat with irregular margins. On primary isolation, colonies may be convex with entire or undulate margins and a smooth surface.

Cleaves pNP-phosphorylcholine and 2-deoxythymidine-5′-pNP-phosphate, but not pNP-β-D-xyloside. Does not degrade adenine, or produce acid from sucrose aerobically or anaerobically.

Galactose, leucine, sodium citrate, sodium propionate, and sodium succinate are used as sole carbon sources, but not 5-aminovaleric acid, arabinose, aspartic acid, asparagine, gluconic acid, *meso*-inositol, ribose, L-rhamnose, or sodium adipate. Grows on readily volatile carbonyl compounds: butanone, 2- and 3-methylbutanal, 4-methyl-2-pentanone, methylpropanal, and 2-pentanone when added as sole carbon sources into sealed agar slope cultures.

Mycolic acids have 54–62 carbon atoms with C_{58} and C_{60} as the main components. The major fatty acids are $C_{16:1}$ *cis*10 (13%), $C_{16:0}$ (27%), $C_{18:1}$ *cis*9 (14%), and 10-methyl $C_{18:0}$ (26%). The predominant menaquinone is MK-9(H_2); minor amounts of MK-8(H_2) are also produced.

Source: a packing material (tree bark compost) of biofillers for deodorization of animal rendering emissions.

DNA G+C content (mol%): 69 (HPLC).

Type strain: 1610/1b, ATCC 700089, CCUG 38497, CIP 104672, DSM 44015, NBRC 16057, JCM 10086.

Sequence accession no. (16S rRNA gene): X87340.

Sequence accession no. (gyrB): gy10560.

12. **Gordonia lacunae** Le Roes, Goodwin and Meyers (2008)

la. cu′ na.e. L. gen. fem. n. *lacunae* of a pool, pond, intended to mean of or from a lagoon, the source of the isolate.

Partially acid-fast actinomycete which forms elementary branching hyphae that fragment into rod-shaped elements. Pink orange colonies are formed on yeast extract-malt extract agar. Colonies are yellow orange when cultivated in the dark. Light yellow brown colonies are produced on inorganic salts-starch agar. Melanin pigments are not formed on either peptone-yeast-extract, or tyrosine agars. Grows at 10°C (weak) and 37°C, and at pH 4.3.

Lecithin activity is observed on egg yolk agar. Produces hydrogen sulfide. Degrades guanine (weak), but not gelatin, hippurate, pectin, or xylan.

Adonitol (weak), DL-α-amino-*n*-butyrate, L-arabinose, fructose, galactose (weak), glucose, L-hydroxyproline, *meso*-inositol (weak), lactose, mannitol, mannose, melezitose (weak), melibiose (weak), raffinose (weak), salicin (weak), sodium acetate (weak), sodium citrate, sucrose, trehalose, xylitol, and xylose are used as sole carbon sources but not inulin, or ribose. L-Arginine, L-cysteine, L-histidine, L-methionine, potassium nitrate, L-serine, and L-valine are used as sole nitrogen sources with weak utilization of DL-α-amino-*n*-butyric acid, L-hydroxyproline, and L-phenylalanine.

Grows in the presence of phenol (0.1%, w/v), phenyl ethanol (0.3%, v/v), sodium azide (0.02%, w/v), and sodium chloride (7%, w/v). Resistant to (μg per ml) cephaloridine (100), kanamycin (100), oleandomycin (100), penicillin G (10 i.u. per ml), rifampin (50), tobramycin (50), and vancomycin (50), but susceptible to gentamicin (100), lincomycin (100), neomycin (50), and streptomycin (100).

Bioautographic analysis of organic solvent extracts of the cell mass shows weak antibiosis against *Mycobacterium aurum* A+ and *Mycobacterium smegmatis* LR222, but no activity against *Mycobacterium tuberculosis* H37Rv[T].

Whole-organism hydrolysates contain arabinose, galactose, glucose, and ribose. Mycolic acids have been detected by one-dimensional thin layer chromatography.

Source: sand taken from an estuary in Plettenberg Bay, Western Cape Province, South Africa.

DNA G+C content (mol%): not determined.

Type strain: BS2, DSM 45085, JCM 14873, NRRL B-24551.

Sequence accession no. (16S rRNA gene): EF151959.

Sequence accession no. (gyrB): EF608480.

13. **Gordonia malaquae** Yassin, Shen, Hupfer, Arun, Lai, Rekha and Young 2007a, 1067[VP]

mal.a′qua.e. L. adj. *malus* bad; L. n. *aqua* water, N. L. gen. n. *malaquae* of bad water, effluent.

Non-acid–alcohol-fast actinomycete which forms rod- and coccoid-like elements. Smooth cream colored colonies formed on agar media. Grows between 22–37°C, but not at 42°C.

Degrades testosterone, but not casein, elastin, gelatin, or guanine. Assimilates acetate, 2, 3 butanediol, citrate, glucose, paraffin, sucrose, trehalose, and xylose as carbon sources, but not adonitol, adipate, *iso*-amyl alcohol, L-arabinose, cellobiose, *meso*-erythritol, galactose, gluconate, *m*- and *p*-hydroxybenzoate, *myo*-inositol, lactate, lactose, maltose, mannitol, melezitose, 1,2-propandiol, raffinose, rhamnose, or sorbitol. L-Alanine, L-proline and L-serine are used as sole carbon and nitrogen sources, but not acetamide, arginine, gelatin, or ornithine.

Mycolic acids have been detected by one-dimensional thin layer chromatography. The major fatty acids are $C_{16:0}$ (40.0%), $C_{17:0}$ (18.5%), and 10-methyl $C_{18:0}$ (13.2%). The predominant menaquinone is MK-9(H_2); minor amounts of MK-8(H_2) are also produced. The cellular polar lipid composition includes diphosphatidylglycerol, phos-

phatidylethanolamine, phosphatidylglycerol, phosphatidylinositol, and phosphatidylinositol mannosides.

Source: sludge taken from a wastewater sediment plant.

DNA G+C content (mol%): not determined.

Type strain: IMMIB WWCC-22, CCUG 53555, DSM 45064, JCM 14874.

Sequence accession no. (16S rRNA gene): AM406674.

14. **Gordonia namibiensis** Brandão, Maldonado, Ward, Bull and Goodfellow 2001, 514[VP]

na.mi.bi.en′sis. N.L. fem. adj. *namibiensis* of or belonging to Namibia, the origin of the soil sample from which the organism was isolated.

Slightly acid–alcohol-fast actinomycete which forms short rods and coccoid elements. Orange or red-pink colonies are formed on glucose-yeast extract agar. Neither aerial hyphae nor diffusible pigments are produced. Grows at 20–40°C, and pH 4.5–9.5.

D-Arabinose, dulcitol, *meso*-erythritol (weak), ethanol, D-fucose, fructose, galactose, glycerol (weak), glycogen, glucose, *meso*-inositol, lactose, maltose, mannose, melezitose, melibiose, α-methyl-D-glucoside, raffinose, ribose, trehalose, xylose, xylitol (all at 1% w/v), sodium acetate, sodium fumarate, sodium gluconate, *m*- and *p*-hydroxybenzoic acid, sodium H-malate, sodium pyruvate, and sodium succinate (0.1% w/v) are used as sole carbon sources, but not adonitol, inulin, salicin, sucrose (1.0%, w/v), benzoate, or butyrate (0.1%, w/v). Acetamide, gelatin, and L-glutamate are used as sole carbon and nitrogen sources, but not L-alanine, L-aspartic acid, L-leucine, monooleic ethanolamine, L-proline, L-phenylalanine, urea, uric acid, or L-valine (all at 0.1%, w/v).

Grows in the presence of oleic acid (0.1%, w/v) and zinc chloride (0.001%, w/v).

Mycolic acids have 54–62 carbon atoms with C_{58} and C_{60} as the main components. The predominant menaquinone is MK-9(H_2).

Source: Kalahari sand collected in the Walerberg area of central Namibia.

DNA G+C content (mol%): not determined.

Type strain: NAM-BN063A, DSM 44568, NCIMB 13780.

Sequence accession no. (16S rRNA gene): AF380930.

Sequence accession no. (gyrB): gy12629.

15. **Gordonia otitidis** Iida, Taniguchi, Kageyama, Yazawa, Chibana, Murata, Nomura, Kroppenstedt and Mikami 2005, 1874[VP]

o.ti′ti.dis. Gr. n. *ous otos* ear; L. suff. *itis*, *idis* suffix used in names of inflammations; N.L. gen. n. *otitidis* of inflammation of the ear.

Partially acid-fast, short elementary branching hyphae fragment into rod- and coccoid-like elements (0.6–0.8 × 2.5–3.1 μm). Colonies are white becoming apricot to pale orange.

Utilizes acetamide, *N*-acetyl-D-glucosamine, L-alanine, 4-aminobutyrate, benzoate, 4-hydroxybenzoate, 2-hydroxyvalerate, 2-oxoglutarate, phenylacetate, L-proline, putrescine, quinate, L-serine, and L-valine, but not L-aspartate, caprate, citrate, galactose, glucarate, gluconate, 3-hydroxybenzoate, *meso*-inositol, rhamnose, ribose, succinate, or tyramine.

Mycolic acids have 58–64 carbon atoms with C_{62} as the main component. The major fatty acids are $C_{16:0}$ (31.7%), 16:1 *cis*9 (18.9%), $C_{18:11}$ (18.2%), and 10-methyl $C_{18:0}$ (23.0%). The predominant menaquinone is MK-9(H_2); a substantial amount of MK-8(H_2) is also produced.

Source: ear discharge of a patient with external otitis, and from pleural fluid of a patient with bronchitis.

DNA G+C content (mol%): 64.9–65.2 (HPLC).

Type strain: IFM 10032, CCUG 52243, DSM 44809, JCM 12355, NBRC 100426.

Sequence accession no. (16S rRNA gene): AB122026.

16. **Gordonia paraffinivorans** Xue, Sun, Zhou, Liu, Liang and Ma 2003, 1645[VP]

pa.raf.fi.ni.vo′rans. N. L. n. *paraffina* paraffin; L. part. adj. *vorans* devouring; N. L. part. adj. *paraffinivorans* paraffin-devouring, referring to the ability to degrade paraffin.

Non-acid-fast rod-shaped actinomycete (0.3–0.5 × 1–2 μm). Cells occur singly or in typical coryneform V-shapes. Colonies orange-red. Grows well at 30–37°C, but slowly at 20° and 45°C. Grows at pH 5.5–9.5.

Catalase-positive, oxidase-negative. Does not degrade arbutin, gelatin, cellulose, or Tween 20.

Does not produce acid from galactose, glucose, glycerol, mannose, or sucrose. L-Alanine, dextrin, fructose, galactose, glucose, glutamate, glycerol, L-leucine, maltose, L-proline, and sucrose are used as sole carbon sources, but not L-arabinose, arabitol, citrate, esculin, lactate, L-lysine, L-malic acid, mannitol, raffinose, rhamnose, ribose, L-sorbose, sorbitol, tartrate, or xylose.

Grows in the presence of oleic acid (0.8%, w/v) and zinc chloride (0.001%, w/v).

Mycolic acids have 52–62 carbon atoms. The major fatty acids are $C_{16:0}$ (25.8%), $C_{18:1}$ ω9*c*, $C_{18:0}$ (9.3%), and 10-methyl $C_{18:0}$ (22.4%). The predominant menaquinone is MK-9(H_2).

Source: a producing-well water sample from Daqing oilfield, China.

DNA G+C content (mol%): 66 (T_m).

Type strain: HD 321, AS 4.1730, DSM 44604, JCM 12461.

Sequence accession no. (16S rRNA gene): AF432348.

17. **Gordonia polyisoprenivorans** Linos, Steinbücher, Spröer and Kroppenstedt 1999, 1789[VP]

po.ly.i.so.pre.ni.vo′rans. N.L. n. *polyisoprenum* polyisoprene; L. part. adj. *vorans* devouring; N.L. part. adj. *polyisoprenivorans* polyisoprene eating, referring to the ability to degrade polyisoprene.

Slightly acid-fast, elementary branching hyphae fragment into rod- and coccoid-like elements. Smooth creamy beige colonies become pastel orange when cultivated in the presence of light.

Does not hydrolyze 2-desoxythymidine-5-*p*-nitrophenyl phosphate, *p*-nitrophenylphosphoryl choline, or *p*-nitrophenyl-β-D-xyloside.

Utilizes acetamide, L-alanine, D-arabitol, L-aspartate, benzoate, 4-hydroxybenzoate, citrate, galactose, gluconate, D-glucosaminic acid, *meso*-inositol, L-proline, putrescine, quinate, rhamnose, sucrose, turanose, and valine by means of the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) reduction test, but not 4-aminobenzoate,

caprate, D-glucarate, 3-hydroxybenzoate, 2-hydroxyvalerate, L-leucine, 2-oxoglutarate, phenylacetate, pimelate, ribose, L-serine, or succinate.

Mycolic acids have 58–64 carbon atoms with C_{60} and C_{62} as the main components. The major fatty acids are $C_{16:1}$ (12%), $C_{16:0}$ (29%), $C_{18:1}$ (21%), and 10-methyl $C_{18:0}$ (29%). The predominant menaquinone is MK-9(H_2). The cellular polar lipid composition includes diphosphatidylglycerol, phosphatidylethanolamine, phosphatidylinositol, and phosphatidylinositol mannosides.

Source: fouling tire water inside a deteriorated automobile tire in Munster, Germany.

DNA G+C content (mol%): not determined.

Type strain: Kd 2, ATCC BAA-14, CIP 106350, DSM 44302, JCM 10675, NBRC 16320.

Sequence accession no. (16S rRNA gene): Y18310.

Sequence accession no. (gyrB): ABO75556.

18. **Gordonia rhizosphera** Takeuchi and Hatano 1998, 910[VP]

rhi.zo.sphe′ra. Gr. fem. n. *rhiza* root; Gr. fem. n. *sphaira* ball, sphere; L. (*sic*) fem. adj. (*sic*) *rhizosphera* (*sic*) within the sphere of the root (*sic*).

Rod-shaped actinomycete (0.2–0.4 × 0.4–1.0 μm) which forms pink to orange colonies with irregular margins and flat, rough surfaces on standard media, including HV and PY agars.

Catalase-positive and oxidase-negative.

Acid is produced from rhamnose (weakly), but not from galactose, *meso*-inositol, sorbitol, sucrose, or trehalose. Grows on L-alanine, citrate, gluconate, L-leucine (weak), L-proline (weak), and L-valine (weak) as sole carbon sources, but not on acetamide, aspartate, benzoate, galactose, *meso*-inositol, ribose, succinate, or sucrose.

Mycolic acids have 56–64 carbon atoms with C_{60} and C_{62} as the main components. The major fatty acids are $C_{16:0}$ (27%), $C_{17:0}$ (13%), $C_{18:1}$ 10-methyl $C_{18:0}$ (27%); only traces of tuberculostearic acid are present. The predominant menaquinone is MK-9(H_2).

Source: rhizophere of *Bruguiera gymnorrhiza* Lank, in the Shiira River estuary, Iriomote Island, Japan.

DNA G+C content (mol%): 66.8 (HPLC).

Type strain: 141, DSM 44383, IFO 16068, NBRC 16068.

Sequence accession no. (16S rRNA gene): AB004729.

Sequence accession no. (gyrB): ABO75552.

19. **Gordonia rubripertincta** corrig. (Hefferan 1904) Stackebrandt, Smida and Collins 1989[VP] (Effective publication: Stackebrandt, Smida and Collins 1988; basonym: *Rhodococcus rubropertinctus* (Hefferan 1904) Tsukamura 1974; *Rhodococcus corallinus* (Bergey, Harrison, Breed, Hammer and Huntoon 1923) Goodfellow and Alderson 1977, 115)

ru.bri.per.tinc′ta. L. adj. *ruber -bra -brum* red; L. pref. *per* very; L. fem. part. adj. *tincta* dyed, colored; N.L. fem. part. adj. *rubripertincta* heavily dyed red.

Rod–coccus life cycle. Rough, orange to red colonies formed on egg media, glucose-yeast extract agar and Sauton's agar. Grows at 25°C and 40°C, but not at 10°C or 45°C.

Positive for allantoinase, β-esterase, and urease, but negative for acetamidase, acid phosphatase, benzamidase, α-esterase, β-galacturonidase, isonicotinamidase, malonamidase, nicotinamidase, pyrazinamidase, salicylamidase, and succinamidase.

Acid is produced from glucose, mannitol, mannose, and sorbitol, but not from arabinose, galactose, inositol, raffinose, rhamnose, or xylose. Butan-2,3-diol, 2,3 butylene glycol, butane 2,3-diol, *p*-cresol, ethanol, glycerol, *m*-hydroxybenzoic acid, inulin, mannitol, propan-1-ol, sodium citrate, sodium gluconate, sodium malate, sodium octanoate, sodium succinate, sucrose, trehalose, and testosterone are used as sole carbon sources, but not acetamide, L-glutamate, *p*-hydroxybenzoic acid, inositol, propane -1,2-diol, propylene glycol, rhamnose, salicin, sorbitol, sodium benzoate, sodium malate, sodium lactate, sodium malonate, sodium pimelate, sodium pyruvate, sodium sebacate, or L-tyrosine. Glucosamine hydrochloride and monoethanolamine are used as sole carbon and nitrogen sources, but not acetamide, D-alanine, L-alanine, L-asparagine, benzamide, serine, L-threonine, or trimethylenediamine.

Resistant to crystal violet (0.001%, w/v), phenol (0.1%, w/v), phenyl ethanol (0.3%, v/v), picric acid (0.2%, w/v), sodium azide (0.01%, w/v), sodium-*p*-aminosalicylic acid (0.2%, w/v), sodium chloride (7%, w/v), and sodium nitrite (0.1%, w/v). Resistant to (μg per ml) chlortetracycline (4), ethambutol (5), gentamicin (16), neomycin (4), and penicillin G (32), but is sensitive to fluorouracil (20), mitomycin C (5), rifampin (25), and vancomycin (2).

Mycolic acids have 48–62 carbon atoms. The major fatty acids are $C_{16:1}$ ω9*c* (15%), $C_{16:0}$ (37%), $C_{18:1}$ (19%), and 10-methyl $C_{18:0}$ (19%). The predominant menaquinone is MK-9(H_2).

Source: soil.

DNA G+C content (mol%): 67–69 (T_m).

Type strain: strain N4, ATCC 14352, CCUG 34957, CIP 104661, DSM 43197, JCM 3204, LMG 5367, KCC A-0204, NCIMB 9664, NBRC 101908, VKM Ac-1016.

Sequence accession no. (16S rRNA gene): X80632.

Sequence accession no. (23S rRNA gene): AY956799.

Sequence accession no. (gyrB): AY972061.

20. **Gordonia shandongensis** Luo, Gu, Xie, Hu, Liu and Huang 2007, 607[VP]

shan.dong.en′sis. N.L. fem. adj. *shandongensis* of or belonging to Shandong Province, China, the source of the type strain.

Slightly acid-fast actinomycete which forms a short elementary mycelium that fragments into rod- and coccoid-like elements. Rough, light yellow colonies are formed on yeast extract-malt extract agar. Grows well at 20–37°C, and from pH 5.0–9.0.

Hydrolyzes urea. Degrades guanine, but not casein.

D-Arabinose and cellobiose are used as sole carbon sources, but not L-alanine, L-arginine, L-aspartate, L-hydroxyproline, L-proline, rhamnose, ribose, sodium benzoate, sodium citrate, sodium sebacate, sodium succinate, or L-valine.

Mycolic acids have been detected by one-dimensional thin-layer-chromatography. The major fatty acids are $C_{15:0}$ iso 2-OH (14.6%), $C_{16:0}$ (36%), $C_{18:1}$, $C_{18:1}$ω9*c* (21%), 10-methyl $C_{18:0}$ (15%), and $C_{15:0}$ iso 2-OH (15%). The predominant menaquinone is MK-9(H_2). The cellular polar lipid composition includes diphosphatidylglycerol, phosphatidylethanolamine, phosphatidylinositol, and phosphatidylinositol mannosides.

Source: farmyard soil in Shangdon Province, China.

DNA G+C content (mol%): not determined.

Type strain: SD29, CGMCC 4.3492, DSM 45094, JCM 13907.

Sequence accession no. (16S rRNA gene): DQ420167.

21. **Gordonia sihwensis** Kim, Lee, Kroppenstedt, Stackebrandt and Lee 2003, 1432^VP

sih.wen′sis. N.L. fem. adj. *sihwensis* of or belonging to Lake Sihwensis in Korea, the source of the type strain.

Non-acid-fast, branching hyphae fragment into rod- and coccoid-like elements. Rough, white to tan colonies with irregular margins are formed on standard nutrient media.

Hydrolyzes 2-deoxythymidine-5-*p*-nitrophenyl phosphate, *p*-nitrophenyl-phosphorylcholine, but not *p*-nitrophenyl-β-D-xyloside.

N-Acetyl-D-glucosamine, 4-aminobutyrate, D-arabitol, caprate, citrate, galactose, gluconate, D-glucosaminic acid, 2-hydroxyvalerate, L-leucine, 2- oxoglutarate, phenylacetate, pimelate, L-proline, L-serine, sucrose, turanose, and L-valine are used as sole carbon sources, but not acetamide, L-aspartate, benzoate, glutarate, 3- and 4-hydroxybenzoate, putrescine, quinate, rhamnose, ribose, succinate, or tyramine.

Mycolic acids have 48–56 carbon atoms with C_{52}, C_{53}, C_{54}, and C_{56} as the main components. The major fatty acids are $C_{16:1}$ *cis*9 (19%), $C_{16:0}$ (39%), $C_{18:1}$ (12%), and $C_{18:0}$ 10-methyl (22%). The predominant menaquinone is MK-9(H_2), a small amount of MK-8(H_2) is also produced. The cellular polar lipid composition includes diphosphatidylglycerol, phosphatidylethanolamine, phosphatidylinositol, and phosphatidylinositol mannosides.

Source: a sulfur-oxidizing autotrophic denitrification reactor used for advanced treatment of wastewater from Lake Sihwa, Korea.

DNA G+C content (mol%): not determined.

Type strain: SPR2, DSM 44576, JCM 13435, NRRL B-24155.

Sequence accession no. (16S rRNA gene): AJ416151.

22. **Gordonia sinesedis** Maldonado, Stainsby, Ward and Goodfellow 2003, 79^VP

sine.se′dis. L. prep. *sine* without; L. n. *sedes -is* dwelling place, residence, habitation; N.L. gen. (?) n. (?) *sinesedis* of homeless (?), intended to mean without home, reflecting the unstable position of the organism in the *Gordonia* 16S DNA tree.

Slightly acid–alcohol-fast actinomycete which forms long thin rods and coccoid elements. Dry, creamish colonies are formed on glucose-yeast extract agar. Grows well at 20–40°C, and pH 4.5–9.5.

Acetate, D-arabinose, *iso*-butanol (weak), cellobiose, dulcitol, *meso*-erythritol (weak), ethanol, fructose, D-fucose, fumarate, galactose, gluconate, glycerol (weak), glycogen, glucose, *m*- and *p*-hydroxybenzoic acid, *meso*-inositol, lactose, H-malate, mannose, melezitose, melibiose, α-methyl-D-glucoside, *iso*-propanol, pyruvate, raffinose, ribose, succinate, trehalose, xylitol, and xylose are used as sole carbon sources, but not L-alanine, L-aspartic acid, L-leucine, monoethanolamine, L-phenylalanine, L-proline, urea, uric acid, or L-valine.

Grows in the presence of oleic acid (0.1%, w/v) and zinc chloride (0.001% w/v).

Mycolic acids have 58–62 carbon atoms as the main components. The major fatty acids are $C_{17:0}$ (7.3%), $C_{16:0}$ (19.9%), $C_{16:0}$ *cis*7 (9.5%), $C_{18:0}$ (8.8%), and 10-methyl $C_{18:0}$ (11.7%). The predominant menaquinone is MK-9(H_2), but MK-8(H_2) is also produced.

Source: soil adjacent to the river Thames.

DNA G+C content (mol%): not determined.

Type strain: J72, DSM 44455, JCM 121126, NCIMB 13802.

Sequence accession no. (16S rRNA gene): AF380834.

23. **Gordonia soli** Shen, Goodfellow, Jones, Chen, Arun, Lai, Rekha and Young 2006a, 2599^VP

so′li. L. gen. n. *soli* of/from the soil.

Partially acid–alcohol-fast actinomycete which forms elementary branching hyphae that fragment into rods and cocci. Pale orange, circular colonies (about 2 mm in diameter) with filamentous margins are formed on tryptic soy agar after 2 d at 30°C. Grows at 28–35°C, but not at 4° or 40°C. Grows at pH 5.5–10.0.

Positive for acid and alkaline phosphatase, butyrate esterase, caprylate esterase, α- and β-glucosidase, naphthol-AS-BI phosphohydrolase, pyrazinamidase, and urease, but negative for *N*-acetyl-β-glucosamidase, α-chymotrypsin, α-fucosidase, α- or β-galactosidase, β-glucuronidase, leucine arylamidase, α-mannosidase, pyrrolidonylarylamidase, and trypsin.

N-Acetyl-L-glutamic acid, D-alanine (weak), L-alanine, L-alanyl glycine (weak), L-alaninamide, α-and β-cyclodextrin, D-fructose-6-phosphate (weak), α-D-glucose, L-glutamic acid, glycogen (weak), α-and β-hydroxybutyric acid, *p*-hydroxyphenylacetic acid, α-ketoglutaric acid, DL-lactic acid (weak), D-malic acid (weak), mannan, mannose, 3-methyl-D-glucose (weak), D-psicose, L-pyroglutamic acid, pyruvic acid (weak), putrescine, sorbitol (weak), succinate (weak), sucrose (weak), Tweens 20 and 40, and uridine 5-monophosphate are used as sole carbon sources.

Mycolic acids co-migrate with those extracted from the type strain of *Gordonia bronchialis*. The major fatty acids are $C_{16:0}$ (33.6%), $C_{16:1}$ (10.4%), $C_{18:1}$ (11.7%), and $C_{18:0}$ 10-methyl (29.4%). The predominant menaquinone is MK-9(H_2). The cellular polar lipid composition includes diphosphatidylglycerol, phosphatidylethanolamine, phosphatidylglycerol, phosphatidylinositol, and phosphatidylinositol mannosides.

Source: the campus of the National Chung Hsing University, Taiching, Taiwan.

DNA G+C content (mol%): not determined.

Type strain: CC-AB07, BCRC 16810, DSM 44995.

Sequence accession no. (16S rRNA gene): AY995560

Sequence accession no. (gyrB): gy10475.

24. **Gordonia sputi** (Tsukamura 1978) Stackebrandt, Smida and Collins 1989^VP emend. Riegel, Kamne-Fotso, De Briel, Prévost, Jehl, Piédmont and Monteil 1994, 766^VP (*Rhodococcus sputi* Tsukamura 1978, 181; *Gordonia sputi* Stackebrandt, Smida and Collins 1988, 371; basonym: *Rhodococcus sputi* Tsukamura and Yamo 1985, 365; *Rhodococcus chubuensis* Tsukamura 1982a, 1116)

spu′ti. L. gen. n. *sputi* of discharge from the respiratory tract.

Slightly acid-fast short rods and cocci. Rough pink colonies formed on egg media and Sauton's agar. Dry, raised, beige colonies that become salmon colored after several days growth on Mueller–Hinton medium. Grows at 28°C and 37°C, but not at 42°C.

Positive for acetamidase, acid phosphatase, α-and β-esterase, nicotinamidase, pyrazinamidase, and urease, but negative for benzamidase, β-galactosidase, isonicotinamidase, salicylamidase, and succinamidase.

Acid is formed from glucose, mannitol, mannose, sorbitol, and trehalose, but not from arabinose, galactose, *meso*-inositol, rhamnose, or xylose. Grows on acetate, *n*-butanol, citrate, ethanol, fructose, glucose, L-malate, mannitol, mannose, *n*-propanol, pyruvate, sorbitol, sucrose, succinate, and trehalose as sole carbon sources, but not on benzoate, 1,3- or 1, 4 – butylene glycol, *meso*-inositol, malonate, propylene glycol, or rhamnose. Acetamide, nicotinamide, nitrate, and succinamide are used as sole nitrogen sources, but not benzamide, isonicotinamide, or nitrate. Acetamide and monoethanolamine are used as sole carbon and nitrogen sources, but not D-alanine, L-alanine, L-asparagine, glucosamine, L-serine, or L-threonine.

Grows in the presence of *p*-nitrobenzoic acid (0.5 mg) and picric acid (0.2%, w/v), but not in the presence of sodium nitrite (0.1%, w/v) or sodium salicylate (0.1%, w/v). Resistant to (μg per ml) chlortetracycline (4), ethambutol (5), neomycin (4), oleandomycin (32), rifampin (25), and vancomycin (4), but sensitive to 5-fluorouracil (20), gentamicin (16), mitomycin C (5), penicillin G (32), and rifampin (8).

Mycolic acids have 56–66 carbon atoms with 2–6 double bonds. The major fatty acids are $C_{16:0}$ (44%), $C_{18:1}$ (29%), and $C_{18:0}$ 10-methyl (15%). The predominant menaquinone is MK-9(H_2); significant amounts of MK-8(H_2) are also produced.

Source: human sputum.

DNA G+C content (mol%): 65.2 (HPLC).

Type strain: ATCC 29627, CCUG 47138, CIP, 100849, DSM 43896, IMET 7569, JCM 3228, KCC A-0228, NBRC 16049, NBRC 100414.

Sequence accession no. (16S rRNA gene): X80634.

Sequencek accession no. (gyr B): AY972064.

25. **Gordonia terrae** (Tsukamura 1971) Stackebrandt, Smida and Collins 1989, 371[VP] [*Gordonia terrae* Tsukamura 1971, 22; basonym *Rhodococcus terrae* (Tsukamura 1971) Tsukamura 1974, 43[VP]; *Gordonia terrae* Stackebrandt, Smida and Collins 1989, 371[VP]]

ter′ra.e. L.n. *terra* earth, L. gen. n. *terrae* of the earth.

Rod–coccus life cycle. Rough, pink to orange colonies are formed on egg media, Sauton's agar, and yeast extract agar.

Positive for allantoinase, β-esterase, α-esterase, nicotinamidase, pyrazinamidase, and urease, but negative for acetamidase, acid phosphatase, benzamidase, β-galactosidase, isonicotinamidase, malonamidase, and salicylamidase.

Acid is produced from mannitol, rhamnose, sorbitol, and trehalose, but not from arabinose, galactose, inositol, *p*-cresol, or xylose. Grows on ethanol, glycerol, *p*-hydroxybenzoic acid, inulin, maltose, mannitol, propanol, rhamnose, sorbitol, sodium azide, sodium citrate, sodium lactate, sodium malate, sodium octanoate, sodium pimelate, sodium pyruvate, sodium sebacate, sodium succinate, sucrose, testosterone, trehalose, L-tyrosine as sole carbon sources, but not on acetamide, butan-2,3-diol, 1,3-, -1,4-, or 2,3-butylene glycol, inositol, *m*-hydroxybenzoic acid, propane-1, 2-diol, or sodium malonate. Acetamide, L-alanine, benzamide, isonicotinamide, nicotinamide, nitrate pyrazinamide, and urea are used as sole nitrogen sources, but not D-alanine, or nitrate. L-Glutamate and monoethanolamine are used as sole sources of carbon and nitrogen, but not acetamide, benzamide, L-serine, or trimethylenediamine.

Resistant to picric acid (0.2%, w/v), sodium *p*-aminosalicylate (0.2%, w/v), and sodium nitrate (0.1%, w/v), but is sensitive to sodium salicylate (0.1 % w/v). Resistant to (μg per ml) chlorotetracycline (4), ethambutol (5), gentamicin (16), neomycin (4), oleandomycin (32), penicillin G (32), rifampin (25), and vancomycin (4), but sensitive to 5-fluorouracil (40) and mitomycin C (5).

Mycolic acids have 52–64 carbon atoms. The major fatty acids are $C_{16:1}$ *cis*9 (16%), $C_{16:0}$ (32%), $C_{18:1}$ (26%), and methyl-$C_{18:0}$ (17%). The predominant menaquinone is MK-9(H_2).

Source: soil.

DNA G+C content (mol%): 64–69 (T_m).

Type strain: ATCC 25594, CCUG 34959, CIP 104295, DSM 43249, JCM 3206, JCM 3229, LMG 5369, NBRC 100016, NCTC 10669, NRRL B-16283, VKM Ac-1023.

Sequence accession no. (16S rRNA gene): X79286.

Sequence accession no. (gyrB): gy10475.

26. **Gordonia westfalica** Linos, Berekara, Steinbüchel, Kim, Spröer and Kroppenstedt 2002, 1137[VP]

west.fa′li. ca. N.L. fem. adj. *westfalica* of or belonging to Westfalia, referring to the origin of the species from a geographical area in Germany.

Slightly acid-fast, short, elementary branching hyphae fragment into rod- and coccoid-like elements. Smooth and rough, pastel orange colonies formed on nutrient agar.

Degrades and mineralizes natural rubber substrates and synthetic *cis*-1,4-polyisoprene.

Utilizes L-aspartate, benzoate, caprate, citrate, D-glucosaminic acid, 2-oxoglutarate, ribose, and sucrose by means of the MTT reduction test, but not acetamide, *N*-acetyl-D-glucosamine, L-alanine, 4-aminobutyrate, galactose, glucarate, gluconate, 3- and 4-hydroxybenzoate, 2-hydroxyvalerate, inositol, L-leucine, pimelate, phenylacetate, putrescine, L-proline, quinate, rhamnose, turanose, L-serine, succinate, tyramine, or L-valine.

Mycolic acids have 54–62 carbon atoms with C_{56}, C_{58}, and C_{60} as main components. The major fatty acids are $C_{16:1}$ *cis*9 (12%), $C_{16:0}$ (37%), $C_{18:1}$ (29%), and methyl $C_{18:0}$ (13%). The predominant menaquinone is MK-9(H_2). The cellular polar lipid composition includes diphosphatidylglycerol, phosphatidylethanolamine, phosphatidylinositol, and phosphatidylinositol mannosides.

Source: foul water from inside a deteriorated automobile tire found in a field near Munster, Westfalia, Germany.

DNA G+C content (mol%): not determined.

Type strain: Kb2, CCUG 46924, DSM 44215, JCM 11757, NRRL B-24152.

Sequence accession no. (16S rRNA gene): AJ312907.

Sequence accession no. (gyrB): gy12627.

Species *incertae sedis*

1a. **"Gordonia jacobaea"** de Miguel, Sieiro, Poza and Villa 2000, 108

Gram-stain-positive, non-sporing, rod-shaped cells with a tendency to bipolar staining. Grows at 25°C and 35°C. Metabolizes methyl pyruvate and Tweens 20 and 40, but gives negative or variable results for the other tests in the Biolog System. A rich source of carotenoids, including the ketocarotenoid *trans*-canthaxanthine.

Source: air.

DNA G+C content (mol%): 61 (thermal hyperchromisity method).

Sequence accession no. (16S rRNA gene): AF251791.

Additional remarks: this is probably a good species but it has not been validly published. "*Gordonia jacobaea*" MV-1 is most closely related to the type strain of *Gordonia sputi*; the two strains share a 16S rRNA gene sequence of 99.5%.

Genus III. **Millisia** Soddell, Stainsby, Eales, Kroppenstedt, Seviour and Goodfellow 2006a, 742[VP]

Jacques A. Soddell, Simon J. McIlroy and Robert J. Seviour

Mil.li′si.a N.L. fem. n. named after Nancy F. Millis, a celebrated microbiologist who promoted wastewater microbiology in Australia

Aerobic, Gram-stain-positive to Gram-stain-variable, acid–alcohol-fast, nonmotile, catalase-positive actinomycete that **forms non-sporing rods which show only rudimentary right-angled branching and which contain polyphosphate storage granules. In stationary phase, the rods fragment into spherical unicells** (Figure 94). Salmon pink, irregular colonies with filamentous margins and sparse unbranched aerial hyphae are formed on glucose-yeast extract agar. Colonies are matt and dry in appearance, soft in texture, and easy to emulsify. Diffusible pigments are not produced. **Whole-organism hydrolysates are rich in *meso*-diaminopimelic acid, arabinose, and galactose.** The organism contains *N*-glycolated muramic acid residues, **a predominant dihydrogenated menaquinone with eight isoprene units, and diphosphatidylglycerol, phosphatidylethanolamine, phosphatidylglycerol, phosphatidylinositol, and phosphatidylinositol mannosides as major polar lipids. Mycolic acids have 44–52 carbon atoms (principal components C_{48}, C_{50}, and C_{52}), and oleic, palmitic, and palmitoleic acids are the predominant fatty acids, with relatively small amounts of myristic, stearic, and tuberculostearic acids.** A member of the family *Nocardiaceae* in the order *Corynebacteriales*.

Isolated from activated sludge foam from Tamworth Sewage Treatment Plant, New South Wales, Australia.

DNA G+C content (mol%): 64.7 (HPLC).

Type species: **Millisia brevis** Soddell, Stainsby, Eales, Kroppenstedt, Seviour and Goodfellow 2006a, 742[VP].

Further descriptive information

The genus description is based on two isolates (J81[T] and J82) from a single source. Comparison of the 16S rRNA gene sequences of these strains show that they form a deep-rooted lineage in the *Corynebacteriales* tree (Figure 95) that is most closely related to *Gordonia* species (93.7–95.7% similarity) and *Skermania piniformis* (95.5% similarity) (Soddell et al., 2006a). The decision to place these isolates with a distinctive branched filament morphology (Figure 94) into a separate and novel genus was based on their phenotypic (Soddell and Seviour, 1998) and genotypic properties, and their menaquinone, polar lipid, cell wall, and mycolic acid compositions (Soddell et al., 2006a). High levels of oleic acid ($C_{18:1}$; 26.3%) and low levels of tuberculostearic acid (3%) distinguish them from members of other mycolic acid containing genera. Thin layer chromatography analysis of mycolic acids showed these were similar in their

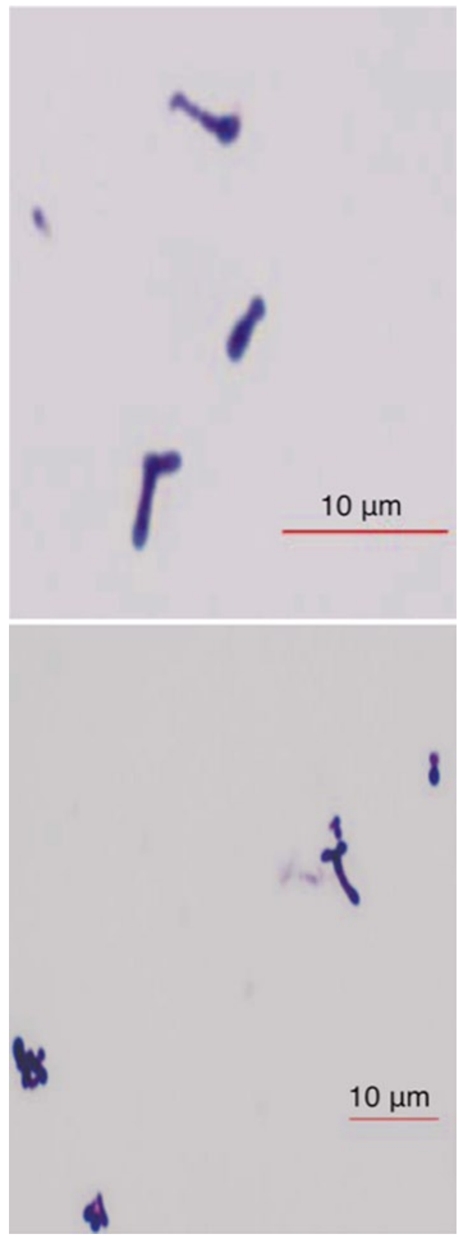

FIGURE 94. Micrographs showing distinctive rudimentary branching filamentous morphology of *Millisia brevis*, and the presence of unicellular elements thought to arise from filament fragmentation.

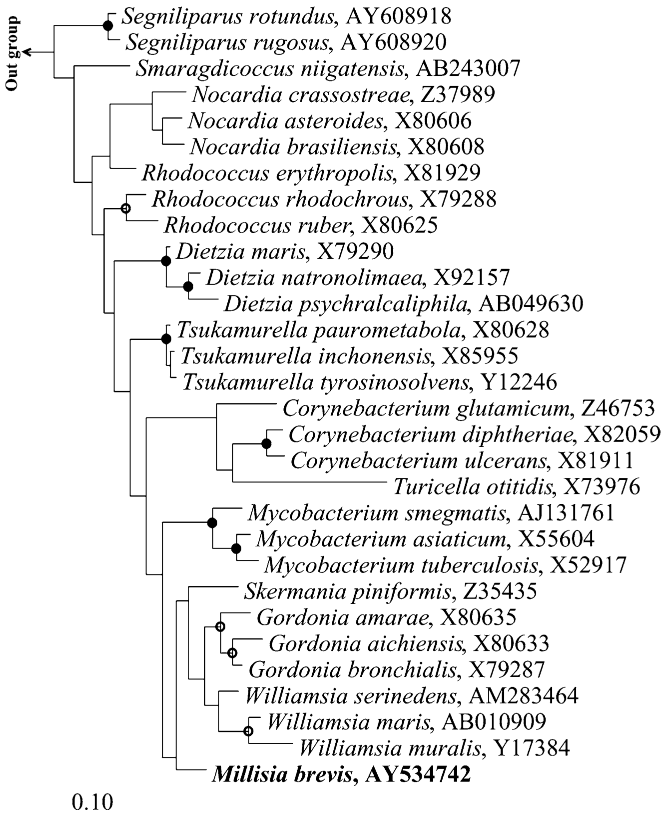

FIGURE 95. Maximum-likelihood tree (Felsenstein, 1981) constructed using ARB software, based on almost complete 16S rRNA gene sequences of *Millisia brevis* and selected type strain of species that are representative of genera classified in the order *Corynebacteriales*. Parsimony bootstrap values were calculated as a percentage of 1000 trials and are only indicated for values ≥75%. ○ Indicates a bootstrap value of ≥75% and ● indicates a bootstrap value of 95%. The sequence of *Bacillus thuringiensis* (X55062) was used to root the tree. The scale bar represents the number of substitutions per nucleotide position.

chromatographic behavior to those in representative *Gordonia* and *Rhodococcus* strains. However, Curie-point pyrolysis mass spectrometry (PyMS) analysis of strain J81^T clearly separated it from representatives of all other currently described mycolic acid containing genera (Soddell et al., 2006a). An organism (isolate J855) with an identical partial 16S rRNA gene sequence has been cultured from a marine invertebrate in Puerto Rico (Sfanos et al., 2005).

Enrichment and isolation procedures

Millisia was obtained by micromanipulation of rudimentary branched filaments with a Skerman micromanipulator onto standard methods agar (BBL SMAS medium, Becton-Dickinson) supplemented with 1% (v/v) horse serum from surface foam in an aerated reactor at a wastewater treatment plant in Tamworth, NSW, Australia (Soddell and Seviour, 1994). After incubation at 25°C, two strains (J81^T and J82) were obtained in pure culture. Both were shown to be members of the same species (Soddell and Seviour, 1998; Soddell et al., 2006a). They were preserved at −80°C.

Taxonomic comments

The two *Millisia* strains have 16S rRNA signature nucleotides that are characteristic of members of the order *Corynebacteriales*, but not characteristic of their closest phylogenetic neighbors in the family *Gordoniaceae* (Stackebrandt et al., 1997). The family *Gordoniaceae* is not used in the present volume, and the phylogenetic analysis used for the roadmap places *Millisia* within the family *Nocardiaceae* (Zhi et al., 2009; Ludwig et al., 2012 – the roadmap to this volume).

List of species of the genus *Millisia*

1. **Millisia brevis** Soddell, Stainsby, Eales, Kroppenstedt, Seviour and Goodfellow 2006a, 742^{VP}

bre′vis. L. fem. adj. short, denoting the formation of short branched rods.

In addition to the properties given under the genus description, the organism degrades Tweens 20, 40, and 60, but not adenine, casein, elastin, hypoxanthine, tyrosine, or xanthine. Hydrolyzes allantoin (weak) and urea (strong), but does not reduce nitrate or produce *m*- or *p*-nitrophenol oxidases. Grows between 15°C and 35°C, but not at 10°C or 37°C, and from pH 5.5 to 9.5. Cellobiose, ethanol, fructose, glucose, glycerol, inositol, mannitol, mannose, sucrose, and trehalose are used as sole sources of carbon for energy and growth, but not arabinose, galactose, inulin, lactose, maltose, melezitose, raffinose, rhamnose, salicin, sorbitol, or xylose (all at 0.1%, w/v). Similarly, *m*-hydroxybenzoic acid, sebacic acid, sodium butyrate, sodium gluconate, sodium lactate, sodium octanoate, and sodium pyruvate are used as sole carbon sources, but not acetamide, benzamide, *p*-hydroxybenzoic acid, pimelic acid, sodium benzoate, sodium succinate, sodium tartrate, testosterone, or tyrosine (0.01%, w/v); variable results are obtained with *p*-cresol, sodium acetate, sodium adipate, sodium citrate, sodium fumarate, sodium malate, and sodium propionate (all at 0.1%, w/v). Coconut oil, glycerol trioleate, kerosene, hexadexane, olive oil, paraffin oil, safflower oil, and xylene (weak) are used as sole carbon sources in shake flask liquid culture (all at 1%, v/v). Variable results were obtained with acetamide and serine as sole carbon and nitrogen sources, but trimethyldiamine does not support growth. Grows in the presence of crystal violet (0.0001%, w/v), phenol (0.01%, w/v), and phenyl ethanol (0.02 and 0.03%, v/v), but is sensitive to crystal violet (0.001%, w/v), phenol (0.1%, w/v), sodium azide (0.01 and 0.02%, w/v), sodium chloride (5 and 7%, w/v), and penicillin G (10 international units). Produces acid

phosphatase, alkaline phosphatase (weak), esterase (C_4), esterase lipase (C_8), α-and β-glucosidases, leucine arylamidase, α-mannosidase (weak), naphthol-AS-BI-phosphohydrolase, and valine arylamidase, but not chymotrypsin, lipase (C_{14}), α-fucosidase, α- and β-galactosidases, β-glucuronidase, *N*-acetyl-β-glucosaminidase, or trypsin; variable for cystine arylamidase.

DNA G+C content (mol%): 64.7 (HPLC).

Type strain: J81, DSM 44463, JCM 13999, NRRL B-24424.

Sequence accession no. (16S rRNA gene): AY534742.

Genus IV. **Rhodococcus** (Zopf 1891) emend. Goodfellow, Alderson and Chun 1998a

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Rho.do.coc′cus. Gr. n. *rhodon* the rose; N.L. masc. n. *coccus* (from Gr. masc. n. *kokkos* grain, seed) coccus; N.L. masc. n. *Rhodococcus* a red coccus.

Aerobic, Gram-stain-positive to Gram-stain-variable, nonmotile actinomycetes **that are usually partially acid–alcohol-fast at some stage of the growth cycle. Rods to extensively branched substrate mycelium may be formed.** In all strains the growth cycle begins with the coccus or short rod stage, with different organisms showing a succession of more or less complex morphological stages by which the completion of the cycle is achieved. Cocci may germinate into short rods, form filaments with side projections, show elementary branching, or, in the most differentiated forms, produce extensively branched hyphae. The next generation of cocci and short rods are formed by fragmentation of the rods, filaments, and hyphae. Some strains form sparse microscopically visible aerial hyphae, which may be branched, or aerial synnemata consisting of unbranched filaments that coalesce and project upwards. Colonies may be rough, smooth, or mucoid and pigmented buff, cream, orange, red, or yellow, although colorless variants occur. Chemo-organotrophic with an oxidative type of metabolism. Catalase-positive, arylsulfatase-negative, and sensitive to lysozyme. Most strains grow well on standard media between 15 and 40°C, and use a wide range of organic compounds as sole sources of carbon for energy and growth.

Whole-organism hydrolysates are rich in *meso*-2,6-diaminopimelic acid, arabinose, and galactose. The peptidoglycan is of the A1γ type. Muramic acid moieties are *N*-glycolated. **Cells contain diphosphatidylglycerol, phosphatidylethanolamine, phosphatidylinositol, and phosphatidylinositol mannosides as major phospholipids, and major proportions of straight-chain saturated, monounsaturated, and branched-chain fatty acids (e.g., 10-methyloctadecanoic acid), and dihydrogenated menaquinones with eight isoprene units as the predominant isoprenologue. Mycolic acids have 30–54 carbon atoms and up to 4 double bonds. The fatty acids released on pyrolysis gas chromatography of mycolic acid esters have 12–16 carbon atoms.** The genus *Rhodococcus*, as determined by 16S rRNA gene sequence analysis, is classified in the order *Corynebacteriales.*

Rhodococci are widely distributed in aquatic and terrestrial habitats, notably in herbivorous dung, soil and marine sediments. Some are opportunistic pathogens of animals, including humans, others are plant pathogens.

DNA G+C content (mol%): 63–73 (HPLC, T_m).

Type species: **Rhodococcus rhodochrous** (Zopf 1889) Tsukamura 1974, 43[VP].

Further descriptive information

Phylogeny. The genus *Rhodococcus* is a member of the family *Nocardiaceae* which also encompasses the genera *Nocardia* Trevisan 1889 and *Smaragdicoccus* Adachi et al., (2007). These taxa form a distinct phyletic branch in the *Corynebacteriales* 16S rRNA gene tree (Figure 96). Rhodococci are classified into 30 validly published species which can be assigned to three taxa in the *Rhodococcus* 16S rRNA gene tree, namely the *Rhodococcus equi, Rhodococcus erythropolis,* and *Rhodococcus rhodochrous* subclades; the taxonomic integrity of the latter are supported by relatively high bootstrap values (Figure 96). The position of *Rhodococcus equi* within the evolutionary radiation encompassed by the genera *Nocardia* and *Rhodococcus* is not stable. It is not clear whether *Rhodococcus equi* is more closely related to the genus *Nocardia* than to other members of the genus *Rhodococcus* or whether it should be recognized as a genus in its own right (Goodfellow et al., 1998a; McMinn et al., 2000; Rainey et al., 1995a; Ruimy et al., 1994).

Cell morphology. Rhodococci have no distinctive morphological features other than the ability of most strains to form hyphae that fragment into rods and cocci, but they do show considerable heterogeneity (Goodfellow et al., 2004; Helmke and Weyland, 1984; Locci, 1976, 1981; Locci and Sharples, 1984; Mayilraj et al., 2006; Nesterenko et al., 1982; Williams et al., 1976). *Rhodococcus aetherivorans* and *Rhodococcus kroppenstedtii* have been reported to be amycelial, but further morphological development is represented by *Rhodococcus erythropolis, Rhodococcus globerulus,* and *Rhodococcus rhodochrous,* as they show elementary branching prior to fragmentation. *Rhodococcus equi* shows traces of elementary branching at early stages of growth. *Rhodococcus coprophilus, Rhodococcus fascians, Rhodococcus marinonascens, Rhodococcus phenolicus,* and *Rhodococcus rhodnii* constitute a third group that forms well-branched substrate mycelia.

The time taken to complete the growth cycle ranges from 24 h in relatively undifferentiated forms, such as *Rhodococcus equi,* to several days for those like *Rhodococcus coprophilus* which show more pronounced morphological differentiation (Locci et al., 1982). The timing of the fragmentation process is influenced by environmental factors (Williams et al., 1976), which may act through their effects on growth rates. Rhodococci do not usually form aerial hyphae; an exception is *Rhodococcus coprophilus* which exhibits feeble aerial hyphae (Locci and Sharples, 1984).

Cell envelope composition. The genus *Rhodococcus* is defined primarily on the basis of wall envelope composition. Rhodococci have (a) a peptidoglycan consisting of *N*-acetylglucosamine, *N*-glycolylmuramic acid, D-and L-alanine, and D-glutamic acid with *meso*-diaminopimelic acid (*meso*-A_2pm) as the diamino acid; (b) arabinose and galactose as diagnostic wall

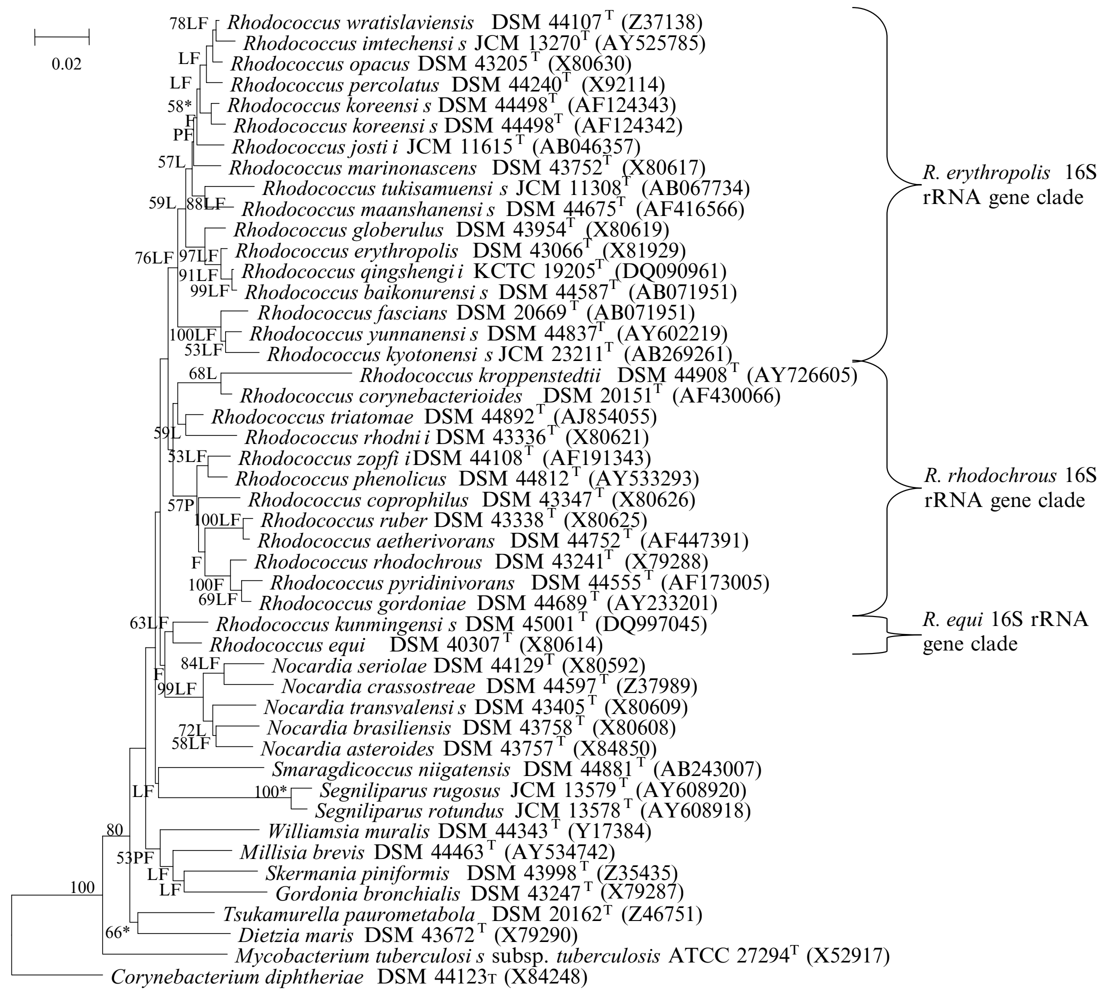


FIGURE 96. Neighbor-joining tree based on early complete 16S rRNA gene sequences showing relationships between *Rhodococcus* species and between them and representatives of other mycolic acid-containing genera classified in the order *Corynebacteriales*. Asterisks indicate branches of the tree that were also found using the least-squares and maximum-parsimony tree-making algorithms. F and L indicates branches that were recovered using the least-squares and maximum-parsimony methods, respectively. The numbers at the nodes indicate the levels of bootstrap support derived from a neighbor-joining analysis of 1000 resampled datasets; only values above 50% are given. The bar = 0.02 substitutions per nucleotide position.

sugars (i.e., rhodococci have a wall chemotype IV and whole-organism sugar pattern type A *sensu* Lechevalier and Lechevalier, 1970); (c) a phospholipid pattern that includes diphosphatidylglycerol, phosphatidylethanolamine (taxonomically significant phospholipid), phosphatidylinositol, and phosphatidylinositol mannosides as major components (i.e., phospholipid pattern type II *sensu* Lechevalier et al., 1977, 1981a); (d) a fatty acid profile consisting of major amounts of straight chain, unsaturated and tuberculostearic acids (i.e., a type IV fatty acid pattern *sensu* Lechevalier et al., 1977); (e) mycolic acids with 30–54 carbon atoms (Minnikin and Goodfellow, 1980, 1981); (f) dihydrogenated menaquinones with eight isoprene units (Collins et al., 1985; Goodfellow and Maldonado, 2006).

Selective ion monitoring gas chromatography-mass spectrometry (SIM GC-MS) analysis yields a much more detailed profile of the mycolic acid composition of rhodococci than GC-MS (Stratton et al., 1999). This structural diversity can be exemplified by the mycolic acids of *Rhodococcus rhodochrous* 11R which were assigned to 60 subgroupings based on α- and β-mycolic acid chain lengths and the degree of unsaturation. They also noted that mycolic acid composition was sensitive to cultural conditions, including carbon source and incubation temperature; hence, there is a need for standardized growth conditions for mycolic acid analyses. A model for the organization of the rhodococcal cell envelope has been proposed in which the mycolic acids (as in other mycolic acid-containing strains) represent an outer lipid permeability barrier (Sutcliffe, 1998). Consistent with this, channel forming proteins have been extracted from rhodococci (Lichtinger et al., 2000; Riess and Benz, 2000; Riess et al., 2003). Mycolic acids are either covalently bound to the peptidoglycan-arabinogalactan skeleton of the cell wall or are extractable (Goodfellow et al., 1976; Sutcliffe, 1997).

Lipoarabinomannan-type lipoglycans have been detected in the type strain of *Rhodococcus rhodnii* (Flaherty et al., 1996). The structure of a truncated lipoarabinomannan, novel lipoglycan, from *Rhodococcus equi* was reported by Garton et al.

(2002). The purified compound, termed ReqLAM, induced an immune response in equine macrophages that was equivalent to *Rhodococcus equi* whole cells suggesting that the early macrophage cytokine response to the bacteria can be attributed to ReqLAM. The structure of a related lipoglycan from *Rhodococcus ruber*, designated RruLAM, was found to be much simpler than that established for *Mycobacterium tuberculosis* lipoarabinomannan and different from that of *Rhodococcus equi* (Gibson et al., 2003). The RruLAm did not induce the production of pro-inflammatory cytokines in either human or murine macrophage cell lines. This suggests that some sophisticated structures, such as phosphoinositol capping motifs, are needed for such activity.

Colony morphology. Rhodococci grow well on standard laboratory media used to cultivate actinomycetes. Colonies may be rough, smooth, or mucoid. They are 0.25–2 mm in diameter, and may be opaque, buff, coral, cream, orange, pink, red, or yellow. Colorless variants occur. Pigmentation may be enhanced by light (Rowbotham and Cross, 1977b).

Nutritional and growth conditions. *Rhodococcus* strains grow well on media such as glucose-yeast extract agars (Gordon and Mihm, 1962a; Waksman, 1950), modified Bennett's agar (Jones, 1949), brain-heart infusion (Difco 0418), and modified Sauton's agar supplemented with thiamine (Mordarska et al., 1972), and Lowenstein-Jensen's medium (BBL 20908). Most strains grow between 15 and 40°C.

Metabolism. The remarkable metabolic diversity shown by rhodococci makes them ideal candidates for enhancing the bioremediation of contaminated sites, and as biocatalysts for a wide range of biotransformations (Beard and Page, 1998; Bell et al., 1998; Finnerty, 1992; Hughes et al., 1998; Warhurst and Fewson, 1994). Consequently, their metabolic diversity is of interest to the chemical, environmental, energy, and pharmaceutical sectors, as well as for applications in the desulfurization of fossil fuels (Matsui et al., 2002), and the industrial production of acrylamide (Hughes et al., 1998). Extensive reviews on the catabolic versatility and enzymic capabilities of rhodococci are available (De Carvalho and da Fonseca, 2005; Gürtler et al., 2004; Larkin et al., 2005, 2006; van der Geize and Dijkhuizen, 2004). Their metabolic diversity is related to the presence and mobilization of large linear plasmids and to multiple homologs of enzymes in catabolic pathways (McLeod et al., 2006; van der Geize and Dijkhuizen, 2004). There is evidence that catabolic genes are co-regulated in separate gene clusters arising from multiple adaptive recombinations (Larkin et al., 2005).

Rhodococcus strains assimilate a broad range of carbohydrates and proteins (Goodfellow and Alderson, 1977; Goodfellow et al., 1982a, 1982c) but also show an exceptional ability to degrade hydrophobic natural compounds and xenobiotics, including aliphatic hydrocarbons, halogenated aliphatic hydrocarbons, polycyclic aromatic hydrocarbons, and nitroaromatic compounds (Armfield et al., 1995; Cain, 1981; Peczynska-Czoch and Mordarski, 1984; Tárnok, 1976), as well as polychlorinated biphenyls (Seto et al., 1995; Warren et al., 2004), a particularly widespread and persistent class of environmental pollutants. Also, some persistent thiocarbonate and *s*-triazine herbicides (De Schrijver and De Mot, 1999) and 2-mercaptobenzothiazole (Haroune et al., 2004), which is used as a vulcanization accelerator in the rubber industry, are catabolized by rhodococci. *Rhodococcus* strains have been implicated in the degradation of lignin-related compounds (Eggeling and Sahm, 1980, 1981; Rast et al., 1980) and humic acid (Cross et al., 1976).

The metabolic versatility of *Rhodococcus* strains can be exemplified by the ability of *Rhodococcus aetherivorans* to degrade *t*-butyl ether (Goodfellow et al., 2004), *Rhodococcus erythropolis* to utilize 1-chlorobutane (Sallis et al., 1990), *Rhodococcus gordoniae* to metabolize very high concentrations of phenol (Jones et al., 2004), *Rhodococcus koreensis* to utilize 2,4-dinitrophenol (Yoon et al., 2000a), *Rhodococcus percolatus* to breakdown 2,4,6-trichlorophenol (Briglia et al., 1996), *Rhodococcus pyridinivorans* to metabolize pyridine (Yoon et al., 2000b), and *Rhodococcus zopfii* to degrade natural estrogens (Yoshimoto et al., 2004). In addition, *Rhodococcus imtechensis* utilizes toxic and recalcitrant compounds, such as *p*-nitrophenol and 2,4-dinitrophenol as sole carbon and energy sources (Ghosh et al., 2006), while *Rhodococcus qingshengii* degrades carbendiazine, a widely used benzimidazole fungicide (Xu et al., 2007), which harms the liver and endocrine system and has mutagenic and teratogenic effects on animals even at low concentrations (Mazellier et al., 2003). *Rhodococcus baikonurensis* metabolizes diesel oil (Lee et al., 2006).

Rhodococcus erythropolis cells and enzymes have been used extensively in bioremediation and biocatalysis studies as they carry out innumerable bioconversions and biodegradations (De Carvalho and da Fonseca, 2005). These organisms, for instance, produce hydrolases which catalyze useful oxidation reactions (Straathof et al., 2002); oxidases for the activation of chemically inert compounds (De Carvalho and da Fonseca, 2005); dehalogenases for the hydrolysis of halogenated compounds (Erable et al., 2005); dehydrogenases for the production of enantiomerically pure products (Schenkels et al., 2001); desulfurases**.** for the removal of sulfur from hydrocarbons, notably fossil fuels (Yu et al., 2006); nitrilases for the production of acrylamide (Yamada and Kobayashi, 1996).

Desulfurizing enzymes remove the sulfur moiety from organosulfur molecules leaving the carbon skeleton intact. Two kinds of desulfurization reactions are recognized (Oldfield et al., 1998). The dibenzothiophene (DBT)-specific pathway desulfurizes DBT to 2-hydroxybiphenyl (2-HBP) and inorganic sulfate, and the benzothiophene (BTH)-specific pathway desulfurizes BTH to 2-(2-hydroxyphenyl) ethan-1-al and probably inorganic sulfate. The DBT-desulfurization pathway was originally identified in "*Rhodococcus rhodochrous*" strain IGTS8 (Kayser et al., 1993), which is now known to be a strain of *Rhodococcus erythropolis* (Oldfield et al., 1998). Several mutant strains of *Rhodococcus erythropolis* ISTS8 have been genetically engineered to increase its ability to carry out desulfurization (Hirasawa et al., 2001; Holland et al., 2003). The influence of operational conditions during batch growth of strain IGTS8 on the desulfurizing capability of cells was studied by del Olmo et al. (2005), who also generated a kinetic model to describe the evolution of desulfurization ability during growth. Two additional *Rhodococcus erythropolis* isolates, strains A66 and A69, like strain IGTS8, desulfurized DBT to 2-HBP, following the 4S pathway of desulfurization (Santos et al., 2007). Extensive

desulfurization of diesel fuels has been achieved using a *Rhodococcus erythropolis* strain isolated from oil contaminated soil (Zhang et al., 2007).

Rhodococci produce enzymes that transform nitrile compounds (Beard and Page, 1998; Brandão et al., 2003; De Carvalho and da Fonseca, 2005; O'Mahony et al., 2005). Two types of enzyme are involved in nitrile biotransformations, nitrilases (which catalyze the direct cleavage of nitriles to the corresponding acids and ammonia) and nitrile hydratases (which catalyze the hydration of nitriles to amides) (Bunch, 1998). The use of enzymes as nitrile-transforming catalysts is attractive economically and environmentally for the production of acrylamide and acrylates, inasmuch as reactions are less severe than those of chemical processes and highly pure products are obtained without the formation of undesirable by-products (Beard and Page, 1998; Hughes et al., 1998; Nagasawa and Yamada, 1989). The nitrile hydratase catalyzed process for the production of acrylamide, which was developed by the Nitto Chemical Industrial Company in collaboration with Kyoto University, was the first successful commercial biocatalyst process involving the production of a commodity chemical. The important features of the early biocatalysts used by Nitto for acrylamide production have been described by Kobayashi et al. (1992).

Improved biocatalysts for acrylamide production sought through the Kyoto/Nitto collaboration led to *Rhodococcus* J1 becoming the third generation biocatalyst for the commercial production of acrylamide from acrylonitrile (Nagasawa et al., 1993). The nitrile hydratase of this organism is a robust and versatile enzyme that is induced when the culture medium is supplemented with cobalt and crotonamide (Nagasawa et al., 1988a, 1988b; Nagasawa and Yamada, 1990; Yamada and Kobayashi, 1996). The nucleotide sequence of the nitrile hydratase of this organism has been determined (Kobayashi et al., 1990). About 30,000 tons of acrylamide are produced using this enzyme every year (Kobayashi and Shimizu, 2000).

Rhodococcus rhodochrous strains have also been highlighted as potential biocatalysts for acrylamide synthesis (Raj et al., 2006; Sankhian et al., 2003), and the nitrile hydratase of a *Rhodococcus rhodochrous* strain has been shown to catalyze the conversion of butyronitrile to butyramide (Raj et al., 2007). *Rhodococcus erythropolis* BL1, an isolate from a marine sediment was found to grow exponentially in the presence of 900 mM acetonitrile. This tolerance of acetonitrile is the highest reported for a nitrile-hydrolyzing bacterium (Langdahl et al., 1996).

Nitrile-degrading rhodococci have been isolated from diverse aquatic and terrestrial habitats (Brandão and Bull, 2003; Brandão et al., 2002; Colquhoun et al., 1998a; Heald et al., 2001; O'Mahony et al., 2005), suggesting that nitrile transforming enzyme activity is geographically widely distributed in nature. Brandão and his colleagues found that most of their nitrile-metabolizing isolates were phylogenetically related to *Rhodococcus erythropolis*, and that the nitrile-transforming enzyme genes of some of their isolates were unique to the geographical region from which they were isolated. In a continuation of these studies, Brandão et al. (2003) found that amidase and nitrile hydratase genes present in geographically distinct *Rhodococcus erythropolis* strains were not globally mixed, thereby raising the prospect that the amino acid variation found for the nitrile hydratases might be used for molecular breeding (Ness et al., 2000; Raillard et al., 2001). They also provided evidence for the coevolution of amidase and nitrile hydratase genes in *Rhodococcus erythropolis*.

Rhodococci are a valuable source of novel bioflocculants and biosurfactants (De Carvalho and da Fonseca, 2005; Kurane et al., 1995; Lang and Philp, 1998; Rapp et al., 1979). *Rhodococcus* strains, for instance, respond to the presence of *n*-alkanes by producing biosurfactants which facilitate the assimilation of hydrophobic compounds as growth substrates (Kurane et al., 1995; Lang and Philp, 1998; Wagner et al., 1983). Particular attention has been paid to trehalose mycolates, notably those from *Rhodococcus erythropolis* (Kretschmer et al., 1982; Uchida et al., 1989). The demand for surfactants can be expected to increase (Desai and Banat, 1997), partly due to the environmental concern associated with the continued use of chemically synthesized components. Rhodococcal surface-active lipids are attractive for certain applications due to their biodegradability, lipophilicity, low toxicity, and nonionic nature, as well as their pH and temperature stability (Rapp et al., 1979; Rapp and Gabriel-Jurgens, 2003; Ristau and Wagner, 1983). Bioflocculants are used to flocculate a diverse range of solids (Kurane and Tomizuka, 1992).

Rhodococcus strains are a potential source of carotenoid pigments, which are used in the food industry as colorants. *Rhodococcus erythropolis* naturally synthesizes monocyclic carotenoids, namely 4-keto-γ-carotene and γ-carotene (Tao et al., 2004). In a continuation of this study, the β-carotene desaturase gene (*crtU*) from *Brevibacterium linens* was expressed in *Rhodococcus erythropolis* to produce chlorobactene, an asymmetric aryl carotenoid which has a similar structure to the food colorant isorenieratene (Tao et al., 2006). Growth of the engineered strain in eight media showed that nutrient broth-yeast extract medium supplemented with fructose gave the highest yield of chlorobactane. This compound has potential as a colorant that gives a different shade of color than isorenieratene and its hydroxyl derivatives (Dufossé et al., 2001).

Genetics. Initial developments in rhodococcal genetics have been the subject of several extensive reviews (Adams and Brownell, 1976; Brownell, 1978; Brownell and Denniston, 1984; Finnerty, 1992). The emphasis in these pioneering studies was on *Rhodococcus erythropolis*. Indeed, as early as 1963, a recombination system was demonstrated in strains now assigned to this taxon. Over 60 genetic traits were used to construct a *Rhodococcus erythropolis* linkage map, and temperate phages were introduced as cloning vectors for a gene cloning system (Adams, 1964; Adams and Bradley, 1963; Brownell and Denniston, 1984; Brownell et al., 1982). The flexibility of the *Rhodococcus* genome with respect to recombination was demonstrated in studies of plasmid integration in *Rhodococcus fascians* (Desomer et al., 1991).

Rhodococcus strains have been shown to contain plasmids which range from small cryptic closed circular molecules to large linear plasmids (Gürtler et al., 2004; Larkin et al., 1998, 2005; Letek et al., 2008; Matsui et al., 2007; van der Geize and Dijkhuizen, 2004). *Rhodococcus rhodochrous* B-276, which is used commercially for chiral epoxide production (Furuhashi, 1992) contains four circular cryptic plasmids (Saeki, 1998) and four-linear megaplasmids (Saeki et al., 1999). Well characterized circular plasmids are associated with the pathogenicity

determinants of *Rhodococcus equi* (Takai et al., 1991, 1993, 1995) and with the degradation of organic pollutants (Dabbs, 1998). The *Rhodococcus equi* virulence plasmids are representative of a new family of actinobacterial plasmids which combine plasmid-specific insertions with a conserved backbone structure linked to plasmid maintenance and transfer (Letek et al., 2008). Circular plasmids of rhodococci have been shown to code for diphenyl metabolism (Masai et al., 1997), chloroalkane degradation (Kulakov et al., 1997), dibenzothiophene desulfurization (Denis-Larose et al., 1997), 2-methylaniline metabolism (Schreiner et al., 1991) and propene degradation (Matsui et al., 2007).

The remarkable catabolic diversity of rhodococci can be partly attributed to the presence and mobility of large linear plasmids. The genome of the most effective degrader of polychlorinated biphenyls, *Rhodococcus* RHA1, consists of a chromosome and three large linear plasmids, pRHL1 (1100 kb), pRHL2 (450 kb), and pRHL3 (330 kb) (McLeod et al., 2006). Most of the genes of the upper biphenyl catabolic pathway are located on the two largest linear plasmids (Shimizu et al., 2001). Genes that encode related isozymes are scattered throughout the genome, as are those involved in the degradation of benzoates and 2-hydroxypenta-2,4-dienoate. The complete annotated sequence of pRHL3 indicates that it is a typical actinomycete invertron which contains large terminal inverted repeats with a tightly associated protein (Warren et al., 2004).

Other plasmid-borne catabolic plasmids have been detected in rhodococci. *Rhodococcus erythropolis* IGTS8 has a large plasmid (150 kb) that is involved in the desulfurization of organosulfur compounds (Denis-Larose et al., 1997) while the genes responsible for the oxidation of isopropylbenzene in *Rhodococcus erythropolis* are encoded on the linear plasmid pBD2 (Stecker et al., 2003). *Rhodococcus erythropolis* PR4, an alkane-degrading organism, contains one linear plasmid, pREL 1 (271 kb), and two circular plasmids, pREC1 and pREC2 (Sekine et al., 2006). The linear plasmid contains several regions homologous to plasmid pBD2 of *Rhodococcus erythropolis* BD2. Other large linear plasmids of rhodococci encode genes for the catabolism of alkylbenzene (Kim et al., 2002a), biphenyl (Taguichi et al., 2004), naphthalene (Kulakov et al., 2005), toluene (Priefert et al., 2004), and chloroaromatic compounds (Konig et al., 2004).

Several rhodococcal megaplasmids have been found to be conjugative (Dabrock et al., 1994; Desomer et al., 1988; Kalkus et al., 1993; Priefert et al., 2004; Shimizu et al., 2001). Sequences and gene annotations are available for megaplasmids, including p33701 from *Rhodococcus equi* ATCC 33701 (Takai et al., 2000b), pBD2 from *Rhodococcus erythropolis* BD2 (Stecker et al., 2003), pRHL from *Rhodococcus* RHA1 (Warren et al., 2004), and pREC1 and pREL2 from *Rhodococcus erythropolis* PR4 (Sekine et al., 2006). A single-strand DNA transfer system similar to other bacterial conjugative systems may function in the transfer of rhodococcal megaplasmids. Conjugative plasmids have been detected in *Rhodococcus erythropolis* AN12 (Yang et al., 2007).

Genetic tools have been developed to exploit the metabolic potential of rhodococci (Finnerty, 1992; Larkin et al., 1998): these include *Escherichia coli*–*Rhodococcus* shuttle vectors (De Mot et al., 1997; Hirasawa et al., 2001; Mangan et al., 2005; Matsui et al., 2006, 2007; Shao et al., 1995) and a gene description system (van der Geize et al., 2000). The pNC903-derived *Rhodococcus*–*Escherichia coli* shuttle vectors pNC9501 and pNC9503 have been used for the production of poly (3-hydroxyalkanoic acids) in *Rhodococcus opacus* PD630 (Kalscheuer et al., 1999). Integrative elements of the L1 mycobacteriophage have been used to transpose *Rhodococcus rhodnii*, a symbiont of the Chagas disease vector *Rhodnius prolixus* (Dotson et al., 2003). A transposon mutagenesis system has also been developed for *Rhodococcus equi* (Mangan and Meijer, 2001), along with targeted mutagenesis systems (Jian et al., 2003; Navas et al., 2001). Cloned and characterized amidase and nitrile hydratase genes from rhodococci have been expressed in *Escherichia coli* (Hashimoto et al., 1991; Ikehata et al., 1989; Kobayashi et al., 1991).

A multipurpose transposon-based vector system which mediates protein expression in *Rhodococcus erythropolis* was designed by Sallam et al. (2007). Inducible expression vectors operative in several *Rhodococcus* species have been constructed (Nakashima and Tamura, 2004a, 2004b). These workers reported expression yields of recombinant proteins up to 10 mg per liter of *Rhodococcus erythropolis* culture; they also found that some proteins were successfully expressed in *Rhodococcus erythropolis* but not in *Escherichia coli*. In addition, Tn*5* transposition complexes have been used to construct a *Rhodococcus rhodochrous* insertion library of 1500 mutants (Fernandez et al., 2001). Enhanced desulfurization has been reported in a transposon-mutant strain of *Rhodococcus erythropolis* (Watanabe et al., 2003).

It is apparent that rhodococci have large genomes, as exemplified by the complete genome sequences of *Rhodococcus aetherivorans* K4 (7 Mb), *Rhodococcus equi* (5 Mb), *Rhodococcus erythropolis* PR4 (9.7 Mb), and *Rhodococcus* RHA1 (McLeod et al., 2006; van der Geize and Dijkhuizen, 2004; http://www.sanger.ac.uk). Analyses of these and additional whole genome sequences will help unravel the basis of catabolic complexity and diversity shown by rhodococci and will facilitate the construction of *Rhodococcus* strains for biotechnological and environmental applications. Similarly, the application of effective genetic tools for rhodococci, such as unmarked gene deletion and transposon-complex based methods, will promote rational cell engineering. This, in turn, will lead to the exploitation of rhodococcal catabolic diversity by allowing the overexpression of key catabolic pathways and enzymes, and the optimization of biocatalytic properties through the inactivation of undesirable enzymes and pathways.

Equine *Rhodococcus equi* virulence is positively correlated with the expression of the *vapA* gene (Haites et al., 1997; Morton et al., 2001; Takai et al., 1991, 1993; Tkachuk-Saad and Prescott, 1991), which is located on a ca. 85-kb plasmid, the virulence-associated plasmid (Takai et al., 2000a). The *vap* operon of equine isolate ATCC 33701 is situated within a 27,500-bp pathogenicity island and encodes the functional genes, *vapA, vapC, vapD*, and *vapE*. Immunoblotting studies (Byrne et al., 2001) show that the gene products, VapA (a cell-surface lipoprotein) and VapC, VapD, and VapE (all secreted proteins) are expressed during growth at 37°C, and expression of these proteins at 37°C is consistent with their roles in virulence. The *vapA* gene from

Rhodococcus equi ATCC 33701 has been sequenced and encodes a 17.4-kDa polypeptide (Sekizaki et al., 1995). A variant of *vapA*, known as *vapB* (Byrne et al., 2001; Takai et al., 2000a), has been detected in some *Rhodococcus equi* isolates from pigs. The *vapB* gene from *Rhodococcus equi* A5, isolated from an AIDS patient, has been sequenced and encodes a 18.2-kDa polypeptide (Sekizaki et al., 1995). The sequence of a *vapB* associated plasmid has been determined and confirms that the Vap family of proteins is unique to *Rhodococcus equi* and is carried in a pathogenicity island inserted into a conserved plasmid backbone (Letek et al., 2008).

Until recently, a major bottleneck in *Rhodococcus equi* research was a dearth of suitable genetic tools. However, electroporation protocols are now available for introducing DNA into *Rhodococcus equi* strains (Sekizaki et al., 1998; Zheng et al., 1997), and procedures for random and targeted mutagenesis have been developed (Ashour and Hondalus, 2003; Jian et al., 2003; Mangan and Meijer, 2001; Navas et al., 2001). Several *Rhodococcus equi–Escherichia coli* shuttle vectors have been described, including a plasmid based on the origin of replication of the *Rhodococcus equi* virulence plasmid (Zheng et al., 1997), and a plasmid that has only been found in *Rhodococcus fascians* (Sekizaki et al., 1998). In addition, versatile *Rhodococcus equi–Escherichia coli* shuttle vectors are available for the analysis of gene function in *Rhodococcus equi* (Mangan et al., 2005). Even so, there is a need for integrative plasmids for the analysis of virulence of *Rhodococcus equi* in mice and foals.

Preliminary analysis of a partial genome sequence of *Rhodococcus equi* ATCC 33201 has revealed a number of interesting features, notably the extent of the homology of genes identified with those of *Mycobacterium tuberculosis* (Rahman et al., 2003). Striking similarities were found in the proportion of genes devoted to fatty acid degradation and to lipid biosynthesis. The *Rhodococcus equi* strain contained homologs of many of the genes of putative or proven importance in *Mycobacterium tuberculosis* thereby emphasizing the need to understand the basis of virulence in this organism. Comparative genomics will be greatly assisted by the completion of the 5-Mb genome sequence of *Rhodococcus equi* strain 103 by the Sanger Institute (http://www.sanger.ac.uk).

Antibiotic sensitivity. Most rhodococci are sensitive to antibacterial antibiotics such as aminoglycosides, cephalosporins, macrolides, penicillins, and tetracyclines, less sensitive to sulfonamides, and resistant to most antitubercular compounds (Goodfellow et al., 1982a; Goodfellow and Orchard, 1974; Helmke and Weyland, 1984; McNeil and Brown, 1994; Rowbotham and Cross, 1977b). They are also sensitive to lysozyme (Goodfellow, 1971; Mordarska et al., 1978). Conflicting results recorded for *in vitro* sensitivity studies on *Rhodococcus equi* can be attributed to factors such as variability in testing protocols and differences in tested populations of isolates. However, *Rhodococcus equi* strains are susceptible to amoxycillin–clavulanate, ampicillin–sulbactam, gentamicin, and imipenem, but tend to be resistant to amoxicillin, clindamycin, oxacillin, and penicillin (McNeil and Brown, 1992). They have also been reported to show a high degree of resistance to sulfonamides (Barton and Hughes, 1980; Woolcock and Mutimer, 1980).

Pathogenicity. *Rhodococcus equi* is a facultative intracellular pathogen which can infect a wide range of animals, but it is primarily a pathogen of foals (Barton and Hughes, 1980; Prescott, 1991). Indeed, it is considered to be the most significant pathogen of the equine breeding industry (Takai et al., 1995). The primary clinical manifestation of *Rhodococcus equi* infections in foals is severe suppurative bronchopneumonia. The prevalence and fatality rates of *Rhodococcus equi* pneumonia are high (Prescott, 1991; Takai et al., 1995), and there is evidence that pneumonia caused by this organism reduces the racing performance of foals (Ainsworth et al., 1998). The primary source of the organism is soil (Takai, 1997; Takai et al., 1986). Inhalation of *Rhodococcus equi* is probably the main route of exposure in foals (Barton and Hughes, 1980; Prescott, 1991).

The typical manifestation of *Rhodococcus equi* pneumonia is as a chronic suppurative bronchopneumonia with extensive abscess formation and associated lymphadenitis (Barton and Hughes, 1980; Prescott, 1991; Zink et al., 1986). Foals are typically affected within 1–5 months of birth. Although unknown, the predisposition of foals to infection within this age range is thought to be related to the relative immaturity of the immune system of individual foals (Chaffin et al., 2004; Darrah et al., 2004; Hooper-McGrevy et al., 2001). A more acute, but less common, form of *Rhodococcus equi* pneumonia involves the death of foals within a few hours or days of showing signs of respiratory distress. *Rhodococcus equi* has occasionally been implicated in equine abortion and placentitis (Patterson-Kane et al., 2002).

Nearly all strains isolated from affected foals contain the virulence 80–90-kb plasmid which carries the gene that encodes the 15–17-kDa protein, VapA (Takai, 1997; Takai et al., 1993). In non-equine animal hosts, the variant plasmid encoding VapB has been identified (Byrne et al., 2001; Letek et al., 2008; Oldfield et al., 2004; Takai et al., 2000a). Strains which lack VapA/B proteins are relatively common in non-equine isolates (Meijer and Prescott, 2004); such strains may carry other types of *Rhodococcus equi* virulence–extrachromosomal replicons (Ocampo-Sosa et al., 2007).

Rhodococcus equi infections of animals other than horses are rare. Nevertheless, such infections have been recorded in many animals, including buffaloes, cattle, cats, crocodiles, deer, dogs, goats, koala bears, marmosets, pigs, seals, sheep, and wild birds (Farias et al., 2007; Muscatello et al., 2007b; Walsh et al., 1993). An increasing number of *Rhodococcus equi* infections are being reported in cats and dogs (Takai et al., 2003) and goats (Kabongo et al., 2005; Tkachuk-Saad et al., 1998). In cats, pyrogranulomatous lesions are characteristic symptoms of *Rhodococcus equi* infections with primary involvement in the extremities (Patel, 2002). In cattle and pigs, the organism causes lymphadenitis (Karlson et al., 1940; Katsumi et al., 1991; Soedarmanto et al., 1997). The lesions observed in cases of lymphadenitis resemble those of tuberculosis, thereby causing a problem in the diagnosis of tuberculosis (Mutimer et al., 1982; Mutimer and Woolcock, 1980).

Rhodococcus equi is being increasingly recognized as a pathogen of immunocompromised humans, especially patients with AIDS (Harvey and Sunstrum, 1991; Lasky et al., 1991; Samies

et al., 1986; Scott et al., 1995). The clinical presentation of rhodococcal infections in humans is influenced by the immune status of the host and the virulence of the causal agent. The first documented human infection involved a patient suffering from *Rhodococcus equi* pneumonia who had been given corticosteroid therapy for chronic hepatitis (Golub et al., 1967). The organism is now typically seen as an agent of invasive pulmonary infections in severely immunosuppressed patients, particularly those with acquired immune deficiency syndrome (Arlotti et al., 1996; McNeil and Brown, 1994; Weinstock and Brown, 2002). Indeed, *Rhodococcus equi* pneumonia has become a disease of increasing significance in human medicine partly as a consequence of the AIDS pandemic (Kedlaya et al., 2001; Kwak et al., 2002; Linder, 1997). However, *Rhodococcus equi* infections are varied, ranking from localized infections (secondary to trauma) to fatal systemic infections (Kamboj et al., 2008; Kohl and Tillmanns, 2002; Napoleão et al., 2005; Nasser and Bizri, 2001; Verville et al., 1994). The first lethal case of *Rhodococcus equi* infection in a previously healthy person was reported by Gabriels et al. (2006). It seems likely that the incidence of human infection caused by *Rhodococcus equi* has been underestimated due to either misdiagnosis or unwarranted discarding of the organism as a contaminant diphtheroid (Weinstock and Brown, 2002).

The *Rhodococcus equi choE* gene, a chromosomal locus that encodes cholesterol oxidase (Navas et al., 2001), is considered to be a major virulence factor of *Rhodococcus equi* (Hondalus, 1997). ChoE is the membrane-damaging factor responsible for the characteristic shovel-shaped synergistic hemolysis [CAMP-like (acronym for "Christie–Atkins–Munch–Petersen")] reaction elicited by *Rhodococcus equi* in the presence of sphingomyelinase C-producing bacteria, such as *Bacillus cereus, Listeria ivanovii*, and *Staphylococcus aureus* (Navas et al., 2001). This CAMP-like reaction can be used as a phenotypic marker for the rapid identification of *Rhodococcus equi* (Ripio et al., 1995).

The basis of pathogenicity of *Rhodococcus equi* is its ability to replicate in alveolar macrophages (Hondalus and Mosser, 1994). This is dependent on its capacity to interfere with endosomal maturation following phagocytosis and to prevent acidification of the vacuole in which it resides (Fernandez-Mora et al., 2005; Toyooka et al., 2005; Zink et al., 1987). Intracellular proliferation of the pathogen eventually results in the death of the macrophage, and associated massive damage to lung tissue characterized by cavitation and granuloma formation (Lührmann et al., 2004; Meijer and Prescott, 2004). The availability of the complete genome sequence for *Rhodococcus equi* will significantly advance understanding of the molecular basis of its virulence.

A number of other rhodococcal species have been implicated as agents of human disease, including *Rhodococcus erythropolis, Rhodococcus globerulus, Rhodococcus luteus, Rhodococcus rhodnii, Rhodococcus rhodochrous*, and *Rhodococcus ruber* (Alture-Weber et al., 1968; Cuello et al., 2002; Gugnani et al., 1998; Haburchak et al., 1978; Lalitha et al., 2006; von Below et al., 1991). Information about the potential virulence of these organisms is limited, though it is known that immunosuppressed guinea pigs inoculated with *Rhodococcus* spp. develop visible granulomas (Haburchak et al., 1978). However, Osoagbaka (1989) found that *Rhodococcus ruber* isolated from human sputa and inoculated into immunosuppressed mice produced a pathological response in the lungs similar to that observed in humans. Mycolyl glycolipid fractions purified from *Rhodococcus ruber* have been shown to induce granuloma formation in immunosuppressed mice (Matsunaga et al., 1996; Yasuda, 1999). In addition, a *Rhodococcus* strain has been implicated as the cause of septic arthritis and osteomyelitis in a healthy, immunologically normal young girl (Broughton et al., 1981).

The type strain of *Rhodococcus corynebacteroides* was used as a substitute for *Mycobacterium tuberculosis* to identify the antigen responsible for adjuvant arthritis (Riess and Benz, 2000). This means that this organism contributes to an understanding of this disease, which shares some features common with rheumatoid arthritis of man (Paronetto, 1970, 1972). Thus, *Rhodococcus corynebacteroides* may provide a clue to the etiology of human arthritis (Lorentzen, 1999).

Rhodococcus fascians is a soil borne organism that infects numerous plants, inducing the formation of leafy galls, fasciation, and witch's broom (Goethals et al., 2001; Temmerman et al., 2000; Vereecke et al., 2000). It is a well adapted epiphyte that colonizes inner tissues and plant surfaces (Cornelis et al., 2001, 2002). One of the most severe symptoms of *Rhodococcus fascians* infections is the formation of leafy galls. These are proliferating masses of meristematic tissue that are covered with very short hypertrophied shoots that appear at the crown of infected plants (De O. Manes et al., 2001; Lacey, 1936; Vereecke et al., 2000). The severity of the disease symptoms induced by the organism depends upon the age and type of the plant and on the inoculation method (Lacey, 1939; Vereecke et al., 2000). Symptom persistence depends upon the continued presence of the bacteria (Lacey, 1936).

Extensive studies on the molecular basis of the *Rhodococcus fascians*-plant interaction have shown that the virulence determinants are located on a linear plasmid, pFi D188 (Crespi et al., 1992, 1994; Stange et al., 1996; Temmerman et al., 2000; Vereecke, 1997). Two bacterial loci, *att* and *fas*, are required for full virulence (Cornelis et al., 2002). The *att* operon is involved in the biosynthesis of regulatory compounds that are necessary to induce *att* and *fas* gene expression and are induced during the interaction with the plant (Maes et al., 2001). The *fas* operon encodes proteins, including an isopentenyl transferase (IPT) which is involved in the biosynthesis of a compound that shows cytokinin activity (Crespi et al., 1992, 1994; Temmerman, 2000); the IPT is homologous to isopentenyl transferases found in *Agrobacterium tumefaciens* and *Pseudomonas syringae* pv. *savastanoi* (Crespi et al., 1992). It is thought that novel compounds produced by *Rhodococcus fascians* help disrupt plant hormone balances eventually leading to disease (Vereecke et al., 2000). Temmerman et al. (2000) isolated an AraC-type regulatory gene, *fasR*, located on the linear plasmid pFID188, which was indispensible for pathogenesis and *fas* gene expression. They also found that expression of the *fas* genes is controlled at both transcriptional and the translation levels.

Ecology. Rhodococci are common in aquatic and terrestrial habitats (Brandão et al., 2002; Cross et al., 1976; Goodfellow and Williams, 1983; Jiang and Xu, 1996). They have been isolated from soil, freshwater, and marine sediments, and from

the gut contents of blood-sucking arthropods with which they may form mutualistic associations (Colquhoun et al., 1998b; Goodfellow and Aubert, 1980; Yassin, 2005; Zhang et al., 2002). However, relatively little is known about the numbers and activities of rhodococci in natural habitats, though it seems likely they are involved in the metabolism of recalcitrant compounds and xenobiotics (De Carvalho and da Fonseca, 2005; Larkin et al., 2005).

It seems likely that rhodococci contribute to foaming in activated sludge plants (Goodfellow et al., 1996; Lemmer and Kroppenstedt, 1984; Mori et al., 1988; Sezgin et al., 1988), especially in non-filamentous foams (Lemmer et al., 1998). Genus-specific 16S rRNA targeted oligonucleotide probes, Rco 1 and Rco 2, have been designed and used to detect rhodococci in activated sludge foam samples by confocal laser scanning microscopy (Davenport et al., 1998). Quantitative fluorescent *in situ* hybridization (FISH) has been used to examine the relationship between foaming and the concentration of mycolic acid-containing actinomycetes in a 20 m^3 completely mixed activated sludge plant (Davenport et al., 2000). The threshold foaming concentration of the organisms was about 2×10^6 cells/ml or 4×10^{12} cells/ml^2. Mycolic acid-containing bacteria are not readily permeabilized by conventional FISH procedures, but effective protocols have been devised for this purpose (Davenport et al., 2000; Macnaughton et al., 1994).

Rhodococcus coprophilus has been the subject of the most extensive ecological studies (Rowbotham and Cross, 1977a). This organism grows on herbivorous dung and high numbers have also been reported from grazed pastures and from streams, rivers, and lake muds that receive run-off from dairy farms. It seems that the coccoid survival stage contaminates grass in pastures or hay used for fodder, and remains viable after ingestion and passage through the rumen. A significant correlation found between the numbers of *Rhodococcus coprophilus* and fecal streptococci in polluted water led Al-Diwany and Cross (1978) to suggest that the organism might be an effective indicator of farm animal effluent, a view shared by other workers (Mara and Oragui, 1981; Oragui and Mara, 1985).

Little is known about the ecology of most other *Rhodococcus* species, though *Rhodococcus equi* has a worldwide distribution, notably in the feces and environment of horses (Barton and Hughes, 1980; Prescott, 1991; Takai et al., 2006). However, its natural habitat is soil, especially ones enriched with fecal material from domestic and wild animals (Takai et al., 1986). *Rhodococcus erythropolis, Rhodococcus rhodochrous,* and *Rhodococcus ruber* have also been isolated from soil (Goodfellow and Williams, 1983), *Rhodococcus gordoniae, Rhodococcus imtechensis,* and *Rhodococcus qingshengii* from contaminated land sites (Ghosh et al., 2006; Jones et al., 2004; Xu et al., 2007), *Rhodococcus koreensis* and *Rhodococcus pyridinivorans* from industrial wastewaters (Yoon et al., 2000a, 2000b), and *Rhodococcus marinonascens* from marine sediments (Helmke and Weyland, 1984). Other rhodococcal species have been recovered from more specialized locations, as exemplified by the isolation of *Rhodococcus baikonurensis* from the Mir space station (Li et al., 2004c), *Rhodococcus jostii* from a medieval grave (Takeuchi et al., 2002), *Rhodococcus kunmingensis* from the rhizosphere of *Taxus chinensis* (Wang et al., 2008), and *Rhodococcus coprophilus, Rhodococcus equi, Rhodococcus erythropolis, Rhodococcus rhodochrous,* and *Rhodococcus ruber* from activated sludge foam (Lemmer and Kroppenstedt, 1984; Sezgin et al., 1988).

Enrichment and isolation procedures

Rhodococci have been isolated from environmental samples, notably soil, using numerous nutrient media. These include Czapek's agar (Higgins and Lechevalier, 1969), glycerol agar (Gordon and Smith, 1953), glycerol-asparagine agar (Shirling and Gottlieb, 1966), modified Sauton's agar (Mordarska et al., 1972), and Winogradsky's nitrate medium (Winogradsky, 1949). A range of novel rhodococci were isolated by Colquhoun and her colleagues (1998b) from N.W. Pacific Ocean sediments using M3 agar (Rowbotham and Cross, 1977b) and Munz agar supplemented with 1%, v/v paraffin (Nesterenko et al., 1978a). Potato glucose agar has been used to isolate rhodococci from diseased sweet peas (Tilford, 1936). Micromanipulation procedures have been used to isolate rhodococci from activated sludge foams (Soddell et al., 1992).

Rhodococcus coprophilus has been isolated from aquatic and terrestrial habitats by plating heat pretreated environmental samples onto M3 agar and incubating at 30°C for 7 d (Rowbotham and Cross, 1977a). Samples (2 ml) of cream, milk, or water in 100 × 12 mm glass tubes sealed with silicon rubber bungs are heated in a water bath for 6 min at 55°C, then either further diluted or plated out; water samples are stored at 4°C before heating. Suspensions of dung, grass, soil (1:10), or hay (1:50) are homogenized in ¼ strength Ringer's solution containing gelatin (0.01%), pH 7.0, heat pretreated. They are then shaken on a Vortex mixer and 0.2 ml portions spread over M3 agar plates.

A broad range of nutrient media have been used to isolate members of individual rhodococcal species from diverse habitats. *Rhodococcus luteus* was isolated from soil and the skin and intestinal contents of carp on mineral salts agar supplemented with *n*-alkanes following incubation at 28°C (Nesterenko et al., 1982), *Rhodococcus imtechensis* from pesticide-contaminated soil using tryptone soy agar (Ghosh et al., 2006), and *Rhodococcus jostii* from skeletal remains using peptone-yeast extract agar (Takeuchi et al., 2002). *Rhodococcus marinonascens* was obtained by plating suspensions of marine sediments onto rich nutrient media supplemented with seawater and incubating for 8–12 weeks at 18°C (Helmke and Weyland, 1984; Weyland, 1969, 1981). *Rhodococcus kunmingensis* was obtained by suspending air-dried, rhizosphere soil in phosphate buffer containing sodium cholate, incubating at 45°C for an hour, centrifuging the preparation prior to plating out 0.1 ml of the supernatant in 9 ml of sterile buffer onto humic acid-vitamin-gellan gum, and incubating at 28°C for 30 d (Suzuki et al., 1999).

Several procedures have been recommended for the isolation of *Rhodococcus equi* from environmental samples and clinical material (Makrai et al., 2005; Muscatello et al., 2007a). A selective medium (NANAT) supplemented with cycloheximide, nalidixic acid, novobiocin, and potassium tellurite has been used to good effect for this purpose (Muscatello et al., 2007a; Mutimer and Woolcock, 1980; Woolcock et al., 1979). Similarly, *Rhodococcus equi* has been isolated from soil using selective enrichment broth (TANP broth) containing

cycloheximide, nalidixic acid, penicillin, and potassium tellurite prior to subculturing onto M3 medium supplemented with potassium tellurite (Barton and Hughes, 1981). An alternative medium for the isolation of *Rhodococcus equi* was developed by von Graevenitz and Punter-Streit (1995). This medium, (CAZ-NB), consisted of a Mueller-Hinton agar base supplemented with ceftazidine (20 µg per ml) and novobiocin (25 µg per ml). Compared to NANAT, a modified version of this medium supported the growth of significantly higher numbers of virulent *Rhodococcus equi* from soil (Muscatello et al., 2007a).

Numerous enrichment procedures have been used to isolate rhodococci from environmental samples. *Rhodococcus aetherivorans* was isolated from methyl *t*-butyl ether enrichments of petrochemical sludge obtained from a chemical effluent plant (Goodfellow et al., 2004; Salanitro et al., 1994), *Rhodococcus erythropolis* from a discharge point of an industrial site polluted with haloalkanes using a defined growth medium and 1-chlorobutane as sole carbon and energy source (Sallis et al., 1990), and *Rhodococcus percolatus* from a 2,4,6-trichlorophenol fed percolator using minimal salts medium K-N (Briglia et al., 1996; Sundman, 1964). Similarly, *Rhodococcus pyridinivorans* was isolated from industrial wastewater using a minimal salts medium supplemented with a trace element solution and pyridine. The culture was incubated at 30°C on a horizontal shaker for several days when a suspension was transferred to a batch of fresh medium and incubated as before; after three successive transfers, the suspension was plated onto solid media containing pyridine (Yoon et al., 2000c).

Maintenance procedures

Short-term storage can be achieved by serial transfer on standard media, such as modified Bennett's (Jones, 1949) and glucose-yeast extract (Gordon and Mihm, 1962a) agar slopes with storage between transfers at 4°C. Lyophilization, storage in liquid nitrogen, or frozen glycerol suspensions can be used for long-term storage. For lyophilization, cells are suspended in a suitable fluid such as glucose serum (7.5%, w/v) or skimmed milk plus glucose (7.5%, w/v). For storage in liquid nitrogen, the micro-organisms are inoculated into small test tubes containing an appropriate medium and incubated until good growth is visible. The tubes are then closed with cotton wool plugs, dipped in liquid paraffin wax, and placed in a liquid nitrogen container. Glycerol suspensions are prepared by scraping biomass from heavily inoculated agar plates and making suspensions in 3 ml of aqueous glycerol in small vials which are then stored at –20°C (Wellington and Williams, 1978). The frozen glycerol suspensions not only serve as a long-term means of preservation but as a quick source of inoculum. Working inocula are obtained by thawing suspensions at room temperature prior to treating. After use, glycerol suspensions are promptly frozen and stored again at –20°C.

Differentiation of the genus *Rhodococcus* from other genera

The genus *Rhodococcus* can be distinguished from other genera classified in the order *Corynebacteriales* by using a judicious selection of chemotaxonomic and morphological markers (Table 29, in the order *Corynebacteriales*, above), by 16S rRNA gene sequencing (Goodfellow and Maldonado, 2006), by partial sequencing of ribosomal protein AT-L30 (Ochi, 1995), and by quantitative fatty acid analysis (McNabb et al., 1997). Preliminary evidence suggests that carbon utilization tests (Biotype 100 strips) may be useful in separating members of the genera *Dietzia, Gordonia*, and *Rhodococcus* (Bizet et al., 1997). Representatives of these genera have also been distinguished by mycolic acid profiles generated by gas chromatography and mass spectrometry (Nishiuchi et al., 2000). DNA amplification and restriction endonuclease analysis has been used for the identification of clinically significant aerobic actinomycetes, including *Rhodococcus equi* strains (Steingrübe et al., 1997).

Taxonomic comments

The long and chequered taxonomic history of the genus *Rhodococcus* has been considered in several review articles (Bousfield and Goodfellow, 1976; Goodfellow et al., 1998a; Goodfellow and Cross, 1984; Goodfellow and Wayne, 1982). The genus was proposed by Zopf (1891) for two species of red bacteria described by Overbeck (1891) known as *Micrococcus erythromyxa* and *Micrococcus rhodochrous*. The genus, with *Rhodococcus rhodochrous* as the type species, was recognized in the first four editions of *Bergey's Manual of Determinative Bacteriology* (Bergey et al., 1923, 1925, 1930, 1934), but in the following two editions rhodococcal species were classified in the genus *Micrococcus* (Bergey et al., 1939; Breed et al., 1948). However, around this time rhodococcal-like strains were assigned to a plethora of morphologically defined genera. Indeed, only with dramatic improvements in the classification of Gram-stain-positive cocci, which stemmed from the application of chemotaxonomic and numerical taxonomic procedures, was the genus *Rhodococcus* seen to be a recognizable taxon of actinomycetes of agricultural, clinical, and industrial importance (Finnerty, 1992).

The epithet *rhodochrous* (Zopf 1891) was reintroduced by Gordon and Mihm (1957) for a group of actinomycetes that carried a multiplicity of generic and species names and which shared many properties in common with both mycobacteria and nocardiae. This presumptive new species was provisionally assigned to the genus *Mycobacterium* primarily on the basis of colony morphology and staining properties (Gordon, 1966). It soon became evident that *Mycobacterium rhodochrous* formed a recognizable, but heterogeneous taxon, which could be readily distinguished from the genera *Corynebacterium, Mycobacterium*, and *Nocardia*. Evidence to this effect came from several sources, including antibiotic sensitivity (Goodfellow and Orchard, 1974), chemotaxonomic (Alshamaony et al., 1976a, 1976b; Collins et al., 1977), and numerical taxonomic studies (Goodfellow, 1971; Goodfellow et al., 1974; Tsukamura et al., 1979).

The genus *Rhodococcus* was finally resurrected and redefined for rhodochrous strains by Tsukamura (1974) and Goodfellow and Alderson (1977), respectively. The reintroduction of the genus *Rhodococcus* represented a significant milestone in the systematics of mycolic acid-containing bacteria (Goodfellow et al., 1998a, 1999; Goodfellow and Maldonado, 2006).

The taxonomic status of most of the 16 *Rhodococcus* species recognized in the current edition of *Bergey's Manual of Systematic Bacteriology* (Goodfellow, 1989) is underpinned by a wealth of chemotaxonomic (Minnikin and Goodfellow, 1980), DNA–DNA pairing (Mordarski et al., 1980, 1981; Zakrzewska-Czerwinska et al., 1988), and numerical taxonomic data (Goodfellow and Alderson, 1977; Goodfellow et al., 1982c, 1990; Tsukamura, 1974, 1982a).

The genus *Rhodococcus* automatically became a more homogeneous taxon with the reintroduction of the genus *Gordonia* (Stackebrandt et al., 1988) and the proposal for the recognition of the genus *Dietzia* (Rainey et al., 1995c). The improved classification of the genus provided a sound framework for the recognition of additional *Rhodococcus* species. The genus encompasses 30 validly described species with most of the recent ones delineated using a rich mix of genotypic and phenotypic data (Goodfellow et al., 2004; Mayilraj et al., 2006; Wang et al., 2008; Xu et al., 2007). It can be anticipated that many additional rhodococcal species will be proposed in the future as there is evidence that the genus is underspeciated (Colquhoun et al., 1998a, 1998b; Goodfellow et al., 1990; Gürtler et al., 2004; Schuppler et al., 1995).

Improvements in the taxonomy of mycolic acid-containing actinomycetes has led to some species being reclassified and others becoming synonyms of previously described taxa. *Rhodococcus aichiensis* Tsukamura 1982a and *Rhodococcus chlorophenolicus* Apajalahti et al. 1986 have been reclassified as *Gordonia aichiensis* Klatte et al. 1994a and *Mycobacterium chlorophenolicum* Häggblom et al. 1994. In addition, *Rhodococcus chubuensis* Tsukamura 1982a, *Rhodococcus luteus* Nesterenko et al. 1982 and *Rhodococcus roseus* Tsukamura et al. 1991 have become synonyms of *Gordonia sputi* (Tsukamura 1978) Riegel et al. 1994, *Rhodococcus fascians* (Tilford 1936) Goodfellow 1984b, and *Rhodococcus rhodochrous* (Zopf 1891) Tsukamura 1974, respectively. In contrast, *Nocardia corynebacteroides* Serrano et al., 1972 and *Tsukamurella wratislaviensis* Goodfellow et al., 1995 have been reclassified as *Rhodococcus corynebacteroides* (Serrano et al., 1972) Yassin and Schaal 2005 and *Rhodococcus wratislaviensis* Goodfellow et al. 1995, 2002. Rainey et al., (1995b) confirmed that *Nocardia calcarea* Metcalfe and Brown 1957 should be seen as a synonym of *Rhodococcus erythropolis* (Gray and Thornton 1928) Goodfellow and Alderson 1977, and *Nocardia restricta* Turfitt 1944 as a synonym of *Rhodococcus equi* (Magnusson 1923) Goodfellow and Alderson 1977. It has also been shown that the well-known cholesterol oxidase-producing strain, *Brevibacterium sterolicum* ATCC 21387 (Fujishiro et al., 1990), is a strain of *Rhodococcus equi* (Ladrón et al., 2003).

The phylogenetic structure of the genus *Rhodococcus* is complex though several equidistant lineages have been recognized in the 16S rRNA gene tree (Goodfellow et al., 1998a; Goodfellow and Maldonado, 2006; Gürtler et al., 2004; Matsuyama et al., 2003; McMinn et al., 2000; Rainey et al., 1995a), notably those corresponding to the *Rhodococcus equi, Rhodococcus erythropolis*, and *Rhodococcus rhodochrous* subclades (Figure 96). It is particularly important to resolve the taxonomic status of *Rhodococcus equi* owing to its significance as a causal agent of bronchopneumonia in foals and its increasing role as a human pathogen. The results of 16S rRNA sequencing studies are equivocal in asmuch as the position of *Rhodococcus equi* within the evolutionary radiation occupied by the genera *Nocardia* and *Rhodococcus* is not stable. It is not clear, for instance, whether this taxon is more closely related to the genus *Nocardia* than to other members of the genus *Rhodococcus* (Goodfellow et al., 1998a; Matsuyama et al., 2003; Ruimy et al., 1994, 1995; Yoon et al., 2000a) or whether it merits recognition as a genus in its own right (Gürtler et al., 2004; McMinn et al., 2000; Rainey et al., 1995a). This situation is compounded by the fact that *Rhodococcus equi* is a heterogeneous species (Butler et al., 1987; Goodfellow et al., 1982a; Gotoh et al., 1991; McMinn et al., 2000; McNeil and Brown, 1994).

Differentiation of the species of the genus *Rhodococcus*

Rhodococcus species can be distinguished using a combination of 16S rRNA gene sequence (Figure 96) and DNA–DNA relatedness data (Table 42). It is difficult to assign unknown rhodococci to the *Rhodococcus equi, Rhodococcus erythropolis*, and *Rhodococcus rhodochrous* 16S rRNA clades using only phenotypic properties. In contrast, combinations of phenotypic features can be used to distinguish between species assigned to each of these taxa (Table 43, Table 44, and Table 45). There is evidence that differences in fatty acid profiles can be used for this purpose (Table 46, Table 47, and Table 48), and that rapid enzyme tests based on the fluorophores 7-amino-4-methylcoumarin and 4-methylumbelliferone provide a reliable means of distinguishing between some *Rhodococcus* species (Goodfellow et al., 1990), as does Curie-point pyrolysis mass spectrometry (PyMS; (McMinn et al., 2000). Curie-point PyMS also provides a rapid and effective way of dereplicating rhodococci for biotechnological purposes (Brandão et al., 2002; Colquhoun et al., 2000).

Prompt and accurate diagnosis of equine pneumonia due to *Rhodococcus equi* is necessary to foster improved clinical outcomes by early medical intervention given the insidious onset and development of the disease. Microbiological and serological methods have been recommended for this purpose (Giguere and Prescott, 1997; Takai et al., 1994) but they are not as rapid or sensitive as PCR assays (Sellon et al., 2001). Species-specific nonproprietary primers have been designed to detect a unique 700-bp fragment of *Rhodococcus equi* chromosomal DNA (Arriaga et al., 2002). Rapid identification of *Rhodococcus equi* can also be achieved by using a PCR assay that targets the *choE* gene (Ladrón et al., 2003). A study using a simple PCR typing system for *Rhodococcus equi* based on three virulent plasmids suggests that there is a clear association between specific plasmid types and animal hosts (Ocampo-Sosa et al., 2007).

Acknowledgements

The authors are indebted to Professor Ian Sutcliffe (University of Northumbria) for his helpful advice on several aspects of this work.

TABLE 42. DNA–DNA relatedness values (%) between type strains of *Rhodococcus* species[a]

	R. aetherivorans	*R. baikonurensis*	*R. coprophilus*	*R. corynebacterioides*	*R. equi*	*R. erythropolis*	*R. fascians*	*R. globerulus*	*R. gordoniae*	*R. imtechensis*	*R. jostii*	*R. koreensis*	*R. kroppenstedtii*	*R. kunmingensis*	*R. kyotonensis*	*R. maanshanensis*	*R. marinonascens*	*R. opacus*	*R. percolatus*	*R. phenolicus*	*R. pyridinivorans*	*R. qingshengii*	*R. rhodnii*	*R. rhodochrous*	*R. ruber*	*R. triatomae*	*R. tukisamuensis*	*R. wratislaviensis*	*R. yunnanensis*	*R. zopfii*
R. aetherivorans	100																								46					
R. baikonurensis		100				46		35											19		22									
R. coprophilus			100		13																			20	14					
R. corynebacterioides																														
R. equi					100																			16	13					
R. erythropolis						100																		16	11					
R. fascians																														
R. globerulus																														
R. gordoniae									100												60									
R. imtechensis										100		58						53	51									49		
R. jostii											100							26	19											
R. koreensis						35	32	27				100					19	29	30											
R. kroppenstedtii					62								100																	
R. kunmingensis														100				47												
R. kyotonensis							29								100													40	36	
R. maanshanensis						33		21				35				100	32	30	27							34				
R. marinonascens					35																									
R. opacus																														
R. percolatus																		71	100											
R. phenolicus																				100										11
R. pyridinivorans			11																		100		8	49	13					10
R. qingshengii		32				24		18														100								
R. rhodnii																														
R. rhodochrous			11																				8	100	39					6
R. ruber																														
R. triatomae				32																			43			100				
R. tukisamuensis						34	21	34				35				45	11	41	7								100	5		
R. wratislaviensis																		69	67									100		
R. yunnanensis							40																						100	
R. zopfii			48			41	58	11																21	27					100

[a]Percentages are rounded up to the nearest whole number.

TABLE 43. Characteristics differentiating *Rhodococcus* species classified in the *Rhodococcus equi* 16S rRNA gene clade[a,b]

	R. equi	*R. kunmingensis*
Biochemical tests:		
Esculin hydrolysis	−	+
Decomposition of:		
L-Tyrosine	−	+
Enzyme tests:		
Acid phosphatase	+	−
β-Glucuronidase	−	+
α-Mannosidase	−	+
Valine arylamidase	+	−
Growth on sole carbon sources:		
L-Arabinose	−	+
Inositol	−	+
Lactose	−	+
Mannitol	−	+
Raffinose	−	$+^{W}$
Rhamnose	−	+
Sorbitol	−	+
Trehalose	−	$+^{W}$
L-Xylose	−	+
Benzoate	−	+
Citrate	−	+
Lactate	+	−
Oxalate	−	−
Propionate	+	−
Tartrate	−	+
Antibiotic resistance:		
Penicillin (10 μg per ml)	+	−

[a]Symbols: +, positive; $+^{W}$, weak positive; −, negative.

[b]All strains produced alkaline phosphatase, esterase lipase (C4), leucine arylamidase, and urease, but not chymotrypsin; utilized *iso*-butanol (1%, v/v), fructose, mannose, and sucrose (1%, w/v) and acetate, malate, and succinate (0.1%, w/v) as sole carbon sources, but not maltose (1%, w/v), or oxalate (0.1%, w/v); degraded adenine, but not casein, elastin, or hypoxanthine and grew in the presence of sodium chloride (7.0%, w/v).

TABLE 44. Characteristics differentiating *Rhodococcus* species classified in the *Rhodococcus erythropolis* 16S rRNA gene clade[a,b]

	R. baikonurensis	*R. erythropolis*	*R. fascians*	*R. globerulus*	*R. imtechensis*	*R. jostii*	*R. koreensis*	*R. kyotonensis*	*R. maanshanensis*	*R. marinonascens*	*R. opacus*	*R. percolatus*	*R. qingshengii*	*R. tukisamuensis*	*R. wratislaviensis*	*R. yunnanensis*
Biochemical tests:																
Esculin hydrolysis	+	+	+	+	nd	−	−	+	+	+	−	−	nd	+	+	+
Urea hydrolysis	+	+	+	+	+	−	+	+	+	−	+	+	+	−	+	−
Decomposition of:																
Tween 80	+	+	+	+	+	−	+	+	+	nd	+	+	+	+	−	+
Growth on sole carbon sources:																
L-Arabinose	−	−	+	−	−	nd	$+^{w}$	−	−	−	−	−	nd	nd	+	+
Arabitol	−	−	+	+	−	nd	+	−	−	−	+	+	nd	−	+	+
Cellobiose	nd	−	−	−	−	nd	−	−	−	−	+	−	−	+	−	−
Galactose	−	−	+	−	nd	−	+	+	+	−	+	+	−	+	−	+
Glycerol	nd	+	+	+	nd	−	+	+	+	+	+	+	+	+	+	nd
Inositol	−	−	−	−	nd	−	+	+	−	+	+	+	−	−	+	−
Inulin	−	+	$+^{w}$	+	nd	nd	+	−	−	+	+	+	−	+	+	nd
Lactose	−	−	−	−	+	+	+	−	−	−	+	−	−	−	−	+
Maltose	−	+	−	+	+	+	+	−	−	−	+	−	−	+	−	+
Mannitol	−	+	+	+	nd	+	+	+	−	−	+	+	−	−	+	+
Mannose	$+^{w}$	+	+	+	nd	nd	+	−	$+^{w}$	+	+	+	+	+	+	+
Melezitose	−	−	−	−	−	nd	+	−	+	−	+	+	nd	+	−	nd
Melibiose	−	−	−	−	−	nd	+	−	+	−	+	+	−	+	$+^{w}$	nd
Raffinose	−	−	−	−	−	nd	+	−	−	−	+	+	−	+	−	nd
Rhamnose	nd	−	−	−	+	−	+	+	−	−	−	−	−	+	+	−
Ribose	+	+	+	+	−	−	+	+	$+^{w}$	−	+	+	+	$+^{w}$	−	+
Sorbitol	−	+	+	+	nd	−	+	+	−	+	+	+	−	−	+	+
Sucrose	−	+	+	+	nd	−	+	+	+	−	+	+	$+^{w}$	+	+	+
Trehalose	−	+	+	+	+	−	+	−	−	−	+	+	−	+	+	+
D-Xylose	−	−	+	−	−	nd	+	−	−	−	+	+	nd	−	+	nd
L-Xylose	−	−	+	+	−	+	+	−	−	$+^{w}$	−	+	nd	−	−	+

[a]Symbols: +, positive; $+^{W}$, weak positive; −, negative; nd, not determined.

[b]All of the strains used fructose and glucose as sole carbon sources.

TABLE 45. Characteristics differentiating the *Rhodococcus* species classified in the *Rhodococcus rhodochrous* 16S rRNA gene clade[a,b]

	R. aetherivorans	*R. coprophilus*	*R. corynebacterioides*	*R. gordoniae*	*R. kroppenstedtii*	*R. phenolicus*	*R. pyridinivorans*	*R. rhodnii*	*R. rhodochrous*	*R. ruber*	*R. triatomae*	*R. zopfii*
Acid production from:												
Cellobiose	nd	−	+	−	−	nd	−	−	−	−	nd	−
Biochemical tests:												
Esculin hydrolysis	−	−	−	−	−	−	+	−	+	−	−	+
Urea hydrolysis	−	+	−	−	−	−	+	+	+	+	−	+
Decomposition of:												
Starch	+	+	−	+	−	−	−	−	−	+	nd	+
Tween 80	nd	+	nd	nd	+	+	+	−	+	+	+	+
L-Tyrosine	nd	−	−	+	−	+	+	+	+	+	−	+
Growth on sole carbon sources:												
Cellobiose	+	−	−	+	nd	−	+	−	−	−	−	+
Fructose	+	+	+	+	nd	−	+	+	+	+	nd	+
Galactose	+	−	−	+	nd	−	+	−	−	−	−	+
Glucose	+	+	+	+	nd	−	−	+	+	+	+	+
Inositol	−	−	+	−	nd	nd	−	−	−	−	−	−
Maltose	+	+	−	+	nd	−	−	−	+	+	−	+
Mannitol	+	−	+	nd	nd	−	+	+	+	+	−	−
Melezitose	+	+	−	+	nd	nd	+	−	−	−	−	+
Rhamnose	nd	−	−	nd	+	nd	−	−	−	−	−	−
Ribose	+	+	nd	+	nd	−	+	−	+	+	nd	+
Salicin	+	−	+	+	−	nd	+	−	+	−	nd	+
Sorbitol	+	−	+	+	nd	−	+	+	+	+	−	−
Sucrose	+	+	+	+	nd	nd	+	+	+	+	−	−
Trehalose	+	+	+	+	nd	+	−	+	+	+	−	−
Citrate	nd	−	+	nd	−	nd	+	+	+	+	+	−
Gluconate	−	+	+	−	+	nd	−	+	+	+	−	+
m-Hydroxybenzoic acid	+	+	−	+	nd	nd	−	−	+	+	−	+
p-Hydroxybenzoic acid	+	−	−	+	+	nd	+	+	+	+	−	+
Pyruvate	+	+	+	+	nd	+	+	−	+	+	nd	+
Succinate	+	−	+	+	nd	+	+	+	+	+	nd	+
Growth on sole carbon and nitrogen sources:												
Acetamide	+	−	−	+	nd	+	+	+	+	+	+	+

[a]Symbols: +, positive; −, negative; nd, not determined.

[b]All of the strains utilized ethanol (1%, v/v) and acetate (0.1%, w/v) as sole carbon sources, but not adonitol (1%, w/v); none degraded casein, hypoxanthine, or xanthine.

TABLE 46. Fatty acid profiles of organisms classified in the *Rhodococcus equi* 16S rRNA gene clade[a]

Species	Strain	$C_{14:0}$	$C_{15:0}$	$C_{16:1}$ ω7*c*	$C_{16:1}$ ω9*c*	$C_{16:1}$ ω10*c*	$C_{16:0}$	10-methyl-$C_{16:0}$	$C_{17:1}$ ω9*c*	$C_{17:0}$	10-methyl-$C_{17:0}$	$C_{18:1}$ ω9*c*	$C_{18:0}$	$C_{18:0}$ 10-methyl	$C_{19:1}$ ω9*c*	$C_{19:1}$ ω11*c*	$C_{20:4}$
R. equi	ATCC 6939[T]	3.3	1.3				26.4	2.1		9.8	1.5	3.6	1.1	20.1			2.7
	DSM 20307[T]	3.0	1.0	1.0	12.0		32.0	3.0		2.0	1.0	5.0	1.0	34.0		1.0	
	DSM 20307[T]	10.0	2.0		6.0	14.0	30.0	7.0	2.0	2.0	2.0	8.0	1.0	12.0	2.0		
	KCTC 9082[T]	2.9	1.3		1.5		26.1	2.7	3.2	17.3	1.6		2.4	18.2			
R. kunmingensis	YIM 45607[T]			10.2			44.0					25.9					

[a]Abbreviations: $C_{14:0}$, tetradecanoic acid; $C_{15:0}$, pentadecanoic acid; $C_{16:1}$ ω7*c*, *cis*-7-hexadecenoic acid; $C_{16:1}$ ω9*c*, *cis*-9-hexadecenoic acid; $C_{16:1}$ ω10*c*, *cis*-10-hexadecenoic acid; $C_{16:0}$, hexadecanoic acid; 10-methyl-$C_{16:0}$, carbon 16 version of tuberculostearic acid; $C_{17:1}$ ω9*c*, *cis*-9-septadecenoic acid; $C_{17:0}$, septadecanoic acid; 10-methyl-$C_{17:0}$, carbon 17 version of tuberculostearic acid; $C_{18:1}$ ω9*c*, *cis*-9-octadecenoic acid; $C_{18:0}$, octadecanoic acid; $C_{18:0}$ 10-methyl, tuberculostearic acid; $C_{19:1}$ ω9*c*, *cis*-9-nonadecenoic acid; $C_{19:1}$ ω11*c*, *cis*-11-nonadecenoic acid; $C_{20:4}$, eicosatetraenoic acid.

TABLE 47. Fatty acid profiles for strains classified in the *Rhodococcus erythropolis* 16S rRNA gene clade[a]

Species	Strain	$C_{14:0}$	$C_{15:1}$ ω5*c*	$C_{15:0}$	$C_{16:1}$ ω7*c*	$C_{16:1}$ ω9*c*	$C_{16:1}$ ω10*c*	$C_{16:0}$	$C_{16:0}$ 10-methyl	$C_{17:1}$ ω5*c*	$C_{17:1}$ ω8*c*	$C_{17:1}$ ω9*c*	$C_{17:0}$	$C_{17:0}$ 10-methyl	$C_{18:1}$ ω9*c*	$C_{18:0}$	$C_{18:0}$ 10-methyl	$C_{19:1}$ ω9*c*	$C_{19:1}$ ω11*c*	$C_{19:0}$	$C_{20:1}$ ω9*c*	$C_{20:4}$	$C_{20:0}$
R. baikonurensis	DSM 44587[T]	5.0			9.0			41.0							18.0	4.0	22.0						
R. erythropolis	ATCC 4277[T]	6.9	0.7	3.5				25.2	2.9		1.7		1.8	2.8	4.5	0.8	16.0			7.4			3.6
	DSM 43066[T]	6.0		5.0		2.0	15.0	25.0	3.0			3.0	2.0	4.0	10.0		18.0			10.9			
	KCTC 9082[T]	7.3		5.0		2.8		22.0	2.1			3.0		2.4	9.7		11.0						5.1
R. fascians	DSM 20669[T]	4.0		20.0		10.0		12.0			8.0		2.0	4.0	14.0		9.0		11.0				
	DSM 20669[T]	5.0		20.0		10.0		9.0			11.0		2.0	4.0	15.0		8.0		12.0				
R. globerulus	ATCC 25714[T]	18.7		2.7				17.2	1.1		0.8		0.9		12.0	0.9	12.7			8.7			1.2
	DSM 43954[T]	4.0		2.0	1.0	14.0		30.0	1.0		2.0		1.0	2.0	12.0	2.0	23.0						
R. imtechensis	JCM 13270[T]				21.2			34.1	1.6		15.5			1.6	8.7		1.7	8.7					
R. jostii	NRB 16295[T]	4.0		8.0	13.0			29.0			13.0		9.0		16.0	4.0	4.0						
R. koreensis	KCTC 059BP[T]	2.8		11.6		7.0		28.4				16.7	11.4	1.5	10.6	2.7	1.3			0.5			
R. kyotonensis	IAM 15415[T]	7.2		2.1	5.5			40.8							18.1		17.5						
R. maanshanensis	JCM 11374[T]	4.5		5.6				29.7	3.3			5.3	4.1	3.3	10.9	4.1	16.7	10.9					
R. marinonascens	DSM 43752[T]	2.0		23.0		3.0		7.0			22.0		20.0	2.0	7.0	1.0	1.0						
R. opacus	DSM 43205[T]	2.0		11.0	8.0	5.0	22.0		1.0		13.0		7.0	6.0	11.0	2.0	8.0						
R. percolatus	MBS1[T]	2.8		11.7		8.8		24.0			19.1		9.8		10.9		0.9						
R. qingshengii	KCTC 19205[T]	8.1	8.5					26.0							7.3	7.0	19.8			4.3			3.4
R. tukisamuensis	JCM 11308[T]							33.7							18.6								
R. wratislaviensis	DSM 44107			12.9		5.1	8.5	22.6				12.6	6.5	6.3	10.4		6.1						
R. yunnanensis	YIM 70056[T]	6.3	2.0	2.8					1.6	1.2	2.4				11.4	18.3	15.6			3.2		1.8	1.3

[a]Abbreviations: $C_{14:0}$, tetradecanoic acid; $C_{15:1}$ ω5*c*, *cis*-5-pentadecenoic acid; $C_{15:0}$, pentadecanoic acid; $C_{16:1}$ ω7*c*, *cis*-7-hexadecenoic acid; $C_{16:1}$ ω9*c*, *cis*-9-hexadecenoic acid; $C_{16:1}$ ω10*c*, *cis*-10-hexadecenoic acid; $C_{16:0}$, hexadecanoic acid; 10-methyl-C16:0, carbon 16 version of tuberculostearic acid; $C_{17:1}$ ω5*c*, *cis*-5-septadecenoic acid; $C_{17:1}$ ω8*c*, *cis*-8-septadecenoic acid; $C_{17:1}$ ω9*c*, *cis*-9-septadecenoic acid; $C_{17:0}$, septadecanoic acid; 10-methyl-$C_{17:0}$, carbon 17 version tuberculostearic acid; $C_{18:1}$ ω9*c*, *cis*-9-octadecenoic acid; $C_{18:0}$, octadecanoic acid; $C_{18:0}$ 10-methyl, tuberculostearic acid; $C_{19:1}$ ω9*c*, *cis*-9-nonadecenoic acid; $C_{19:1}$ ω11*c*, *cis*-11-nonadecenoic acid; $C_{19:0}$, nonadecenoic acid; $C_{20:1}$ ω9*c*, *cis*-9-eicosanoic acid; $C_{20:4}$, eicosatetraenoic acid; $C_{20:0}$, eicosanoic acid.

TABLE 48. Fatty acid profiles of strains classified in the *Rhodococcus rhodochrous* 16S rRNA gene clade[a]

Species	Strain	$C_{14:0}$	$C_{15:0}$	$C_{16:1}$ ω7*c*	$C_{16:1}$ ω9*c*	$C_{16:1}$ ω10*c*	$C_{16:0}$	$C_{16:0}$ 10-methyl	$C_{17:1}$ ω7*c*	$C_{17:1}$ ω8*c*	$C_{17:1}$ ω9*c*	$C_{17:0}$	$C_{17:0}$ 10-methyl	$C_{18:1}$ ω7*c*	$C_{18:1}$ ω9*c*	$C_{18:0}$	$C_{18:0}$ 10-methyl	$C_{19:1}$ ω9*c*	$C_{19:1}$ ω11*c*	$C_{19:0}$	$C_{20:1}$ ω7*c*	$C_{20:1}$ ω9*c*	$C_{20:4}$	$C_{20:0}$	$C_{21:1}$ ω11*c*	$C_{22:0}$	$C_{24:0}$
R. coprophilus	ATCC 29080[T]	3.9	2.0	18.0			19.4	25.3		1.6		0.4	1.7		1.6		5.7										
	DSM 43347[T]	1.8	2.7		5.6		23.1	12.5			1.3	1.8	4.5		2.4		24.1										
	DSM 43347[T]	1.0	3.0	9.0	14.0		19.0	6.0		4.0		2.0	3.0		10.0	1.0	22.0		1.0								
R. corynebacterioides	DSM 20151[T]	4.6	3.5	3.3			35.7	2.8		2.5		2.8	0.6		16.7	3.4	8.4							1.2		8.5	5.5
	MTCC 699[T]	8.2		13.8			36.5			9.0					22.7		9.9										
R. kroppenstedtii	DSM 44908[T]	5.8		10.1			42.1			6.8					28.5		6.1										
R. phenolicus	DSM 44812[T]	3.5					33.9	2.7	0.9		1.3		0.5	1.5	6.4	2.3	16.9			0.8	0.4	1.6		0.5			
R. pyridinivorans	KCTC 0647BP[T]	1.8	1.0		5.8	0.5	34.0	1.4			1.6	3.0	0.7		21.6	5.2	11.5			2.4				1.2			
R. rhodnii	DSM 43336[T]	3.1	3.3				34.3				2.3	1.4	2.1		13.7	1.0	23.7										
	DSM 43336[T]	2.0	4.0	2.0	20.0		25.0	2.0		4.0		3.0	3.0		16.0	1.0	18.0		1.0								
	DSM 43336[T]		4.0	1.0	14.0		29.0			5.0		3.0	4.0		18.0	1.0	18.0		1.0								
R. rhodochrous	ATCC 13808[T]	2.1	1.6				26.3	7.2		1.8		2.5	3.1		4.6	1.4	20.6			2.6			0.4	3.9			
	KCTC 9086[T]	1.7	1.9		4.1	1.0	22.7	5.2			1.6	2.8	2.2			1.9	22.8			2.7			6.0	5.2	0.6		
	DSM 43241[T]	2.0	1.0		6.0	18.0	29.0	7.0			1.0	1.0	2.0		8.0	1.0	22.0										
R. ruber	DSM 43338[T]	1.5	2.9		1.2		27.4	0.6			4.3	4.3	1.8		20.7	2.4	15.6										
	DSM 43338[T]	2.0	4.0		2.0	20.0	25.0	2.0			4.0	3.0	3.0		16.0	1.0	18.0	1.0									
R. triatomae	DSM 44892[T]	2.3	0.5		6.8		35.5					1.9			3.6	7.8	36.5						3.4	1.2			
R. zopfii	DSM 44108[T]	1.7	1.0		6.6		32.7	2.9			1.2				16.5	8.8	13.1			0.6				2.1	1.7		

[a]Abbreviations: $C_{14:0}$, tetradecanoic acid; $C_{15:0}$, pentadecanoic acid; $C_{16:1}$ ω7*c*, *cis*-7-hexadecenoic acid; $C_{16:1}$ ω9*c*, *cis*-9-hexadecenoic acid; $C_{16:1}$ ω10*c*, *cis*-10-hexadecenoic acid; $C_{16:0}$, hexadecanoic acid; 10-methyl-$C_{16:0}$, carbon 16 version of tuberculostearic acid; $C_{17:1}$ ω7*c*, *cis*-7-septadecenoic acid; $C_{17:1}$ ω8*c*, *cis*-8-septadecenoic acid; $C_{17:1}$ ω9*c*, *cis*-9-septadecenoic acid; $C_{17:0}$, septadecanoic acid; 10-methyl-$C_{17:0}$, carbon 17 version of tuberculostearic acid; $C_{18:1}$ ω7*c*, *cis*-7-octadecenoic acid; $C_{18:1}$ ω9*c*, *cis*-9-octadecenoic acid; $C_{18:0}$, octadecanoic acid; $C_{18:0}$ 10-methyl, tuberculostearic acid; $C_{19:1}$ ω9*c*, *cis*-9-nonadecenoic acid; $C_{19:1}$ ω11*c*, *cis*-11-nonadecenoic acid; $C_{19:0}$, nonadecenoic acid; $C_{20:1}$ ω7*c*, *cis*-7-eicosanoic acid; $C_{20:1}$ ω9*c*, *cis*-9-eicosanoic acid; $C_{20:4}$, eicosatetraenoic acid; $C_{20:0}$, eicosanoic acid; $C_{21:1}$ ω11*c*, *cis*-11- heneicosanoic acid; $C_{22:0}$, docosenoic acid; $C_{24:0}$, tetracosenoic acid.

List of species of the genus *Rhodococcus*

1. **Rhodococcus rhodochrous** (Zopf 1891) Tsukamura 1974, 43[AL] [*Staphylococcus rhodochrous* Zopf 1889, 173; *Rhodococcus rubropertinctus* (Hefferan 1904) Tsukamura 1974, 43]

rho.do.ch′rous. Gr. n. *rhodon* the rose; Gr. n. *khoria* color; N.L. masc. adj. *rhodochrous* rose colored

Gram-stain-positive actinomycete. Forms a primary mycelium that fragments into rods and cocci. Rough, orange to red colonies are formed on glucose-yeast extract agar, Sauton's agar, and egg media. Grows between 10 and 40°C. Hydrolyzes arbutin, reduces nitrate to nitrite, but does not produce hydrogen sulfide. Positive for catalase, 2-deoxythymidine-5′-*p*NP-phosphate, β-esterase and *p*NP-β-D-xyloside, but negative for allantoinase, α-esterase, β-galactosidase, nicotinamidase, *p*NP-phosphoryl-choline, oxidase, and pyrazinamidase. Degrades adenine, DNA, and uric acid, but not cellulose, chitin, elastin, Tweens 20, 40, or 60, or xylan. Acid is produced from dextrin, fructose, glucose, glycerol, maltose, mannose, but not from adonitol, amygdalin, D- or L-arabinose, dulcitol, ethanol, galactose, glycogen, inositol, inulin, lactose, mannitol, melezitose, raffinose, rhamnose, sorbitol, sucrose, trehalose, or xylose. Grows on D- and L-arabitol, biphenyl (weak), *iso*-butanol, 2,3-butylene glycol, glycerol, inulin, mannose, propylene glycol, toluene, xylitol, adipate, benzoate, caprate, *p*-cresol, fumarate, glutarate, 3- and 4-hydroxybenzoate, γ-hydroxybutyric acid, lactate, malate, octanoate, 2-oxyglutarate, phenylacetate, pimelate, propionate, sebacate, tartrate, testosterone, and quinate as sole carbon sources, but not on amygdalin, D- or L-arabinose, arbutin, dulcitol, glycogen, lactose, α-methyl-D-galactoside, raffinose, starch, turanose, D- or L-xylose, *N*-acetyl-D-glucosamine, γ-aminobutyrate, hippurate, 2-hydroxyvalerate, D-lactic acid methyl ester, malonate, monoethanolamine, phenol. L-Alanine, L-leucine, DL-norleucine, tyramine, L-tyrosine, and valine are used as sole nitrogen sources, but not L-aspartate, betaine, D-glucosamine, L-proline, putrescine, D- or L-serine, L-tryptophan or trimethylenediamine. Strains utilize dodecane, gaseous hydrocarbons, degrade polychlorobenzoates, oxidize propane, convert acetylene to acetaldehyde, ethanol, acetate, and carbon dioxide and are a source of muconic acid. Resistant to crystal violet (0.001%, w/v), phenol (0.1%, w/v), phenyl ethanol (0.3%, v/v), picric acid (0.2%, w/v), sodium azide (0.01%, w/v), sodium chloride (7.0%, w/v), sodium nitrate (0.2%, w/v), and sodium salicylate (0.1%, w/v). Susceptible to 5-fluorouracil (5 µg per ml) and mitomycin (5 µg per ml), but not to ethambutol (5 µg per ml) or rifampin (25 µg per ml). Additional phenotypic features are shown in Table 45. Mycolic acids have 38–48 carbon atoms. The fatty acid profile is shown in Table 48. The major menaquinone is MK-8(H_2).

Source: soil.

DNA G+C content (mol%): 62–69 (T_m).

Type strain: ATCC 13808, ATCC 25592, CCUG 47165, CIP 104376, DSM 43241, HAMBI 1959, IEGM 62, NBRC 16069, JCM 3202, LMG 5365, NBRC 16069, NCTC 10210, NRRL B-2149, NRRL B-16536, VKM Ac-1227.

Sequence accession no. (5S rRNA): X55254.

Sequence accession no. (16S rRNA gene): X79288.

Sequence accession no. (gyrB): AB014173.

Additional remarks: Rhodococcus rhodochrous is used in the commercial production of acrylamide by the Nitto Chemistry Industry Company Ltd. in Japan.

2. **Rhodococcus aetherivorans** Goodfellow, Jones, Maldonado and Salanitro 2004, 64[AL]

a.e.the.ri.vo′rans. N.L. n. *aether aetheris* ether; L. part. adj. *vorans* eating, devouring; N.L. part. adj. *aetherivorans* devouring ether.

Gram-stain-positive, slightly acid–alcohol-fast actinomycete with a rod–coccus life cycle. Rough pinkish colonies are formed on modified Bennett's and peptone-yeast extract agars. Degrades methyl *t*-butyl ether, starch, but not DNA or uric acid. Reduces nitrate to nitrite, but does not hydrolyze esculin. Utilizes L-arabitol, *iso*-butanol, 2,3-butylene glycol, glycerol, inulin, mannose, xylitol, γ-aminobutyrate, benzoate, fumarate, γ-hydroxybutyric acid, and monoethanolamine as sole carbon sources, but not arbutin, dulcitol, methyl-α-D-glucoside, methyl-β-D-glucoside, or gluconate. L-Leucine is used as a sole nitrogen source. Additional phenotypic features are shown in Table 45. Mycolic acids co-migrate with those of the type strain of *Rhodococcus rhodochrous.* The major menaquinone is MK-8(H_2).

Source: enrichments of a petrochemical biotreater sludge obtained from a chemical effluent treatment plant.

DNA G+C content (mol%): not determined.

Type strain: 10bc312, DSM 44752, JCM 14343, NCIMB 13964.

Sequence accession no. (16S rRNA gene): AF447391.

3. **Rhodococcus baikonurensis** Li, Kawamura, Fujiwara, Naka, Liu, Huang, Kobayashi and Ezaki 2004c, 833[VP]

bai.ko.nur.en′sis. N.L. masc. adj. *baikonurensis* of or belonging to Baikonur, the town in Kazakhstan where the Mir space station was launched.

Gram-stain-positive actinomycete forms branched filaments which fragment into rods and cocci. Colonies are smooth, opaque and slightly pink on brain heart infusion agar. Degrades Tween 40, but not adenine, or arbutin. Resistant to lysozyme. Acid is not produced from D- or L-arabinose. Grows on 2,3-butylene glycol (weak), mannose (weak), acetate, L-glutamic acid, L-glutamic acid (weak), α-, β-, and γ-hydroxybutyric acid, α-ketovaleric acid, lactic acid, D-lactic acid methyl ester (weak), L-malic acid, methyl pyruvate, monomethyl succinate, propionic acid (weak), pyruvic acid, succinic acid (weak), and succinamic acid (weak), but not on arbutin, catechol, β-dextrin, lactulose, maltotriose, 3-methyl-glucose, methyl-α-D-glucoside, methyl-β-D-glucoside, palatinose, salicin, turanose, or xylitol. Alaninamide (weak), L-asparagine and putrescine are used as sole nitrogen sources, but not L-alanyl-glycine, carbendazim, uridine, or uridine 5′-monophosphate. Additional phenotypic features are shown in Table 44. A1γ cell-wall peptidoglycan. Mycolic acids have 46–54 carbon atoms. The fatty acid profile is shown in Table 47. The cellular polar lipid composition is cardiolipin, phosphatidylethanolamine, phosphatidylinositol, and

phosphatidylinositol mannosides. The major menaquinone is MK-8(H_2).

Source: air in the Russian space laboratory Mir.

DNA G+C content (mol%): 56 (T_m).

Type strain: A1-22, DSM 44587, GTC 1041, JCM 11411, NBRC 100611.

Sequence accession no. (16S rRNA gene): AB071951.

4. **Rhodococcus coprophilus** Rowbotham and Cross 1979, 80[VP] (Effective publication: Rowbotham and Cross 1977b, 136.)

co.pro.phi′lus. Gr. n. *kopros* dung; Gr. adj. *philos* loving; N.L. masc. adj. *coprophilus* dung loving.

Gram-stain-positive, non-acid-fast actinomycete. Forms a well-developed primary mycelium that fragments into rods and cocci after incubation for several days. Aerial hyphae are produced and may be branched. Rhizoid colonies (2 mm in diameter) with a central orange papilla are formed on Bennett's agar. Pigmentation is enhanced by light. Positive for catalase and nicotinamidase, but negative for acetamidase, acid phosphatase, 2-deoxythymidine-5′-*p*NP-phosphate, α- and β-esterase, oxidase, *p*NP-phosphorylcholine and *p*NP-β-D-xyloside. Degrades uric acid, but not adenine, casein, cellulose, chitin, elastin, guanine, hypoxanthine, xanthine, or xylan. Reduces nitrate to nitrite. Produces hydrogen sulfide, but does not hydrolyze arbutin. Acid is not produced from L-arabinose, galactose, inulin, lactose, raffinose, or rhamnose. Assimilates L-arabitol, arbutin, *iso*-butanol, cetyl alcohol, mannose, α-methyl-D-galactoside, raffinose, toluene (weak), xylitol, acetate, adipate, benzoate, isobutyrate, *p*-cresol, fumarate, 3-hydroxybenzoate, γ-hydroxybutyric acid, lactic acid, 2-oxyglutarate, pimelate, propionate, sebacate, testosterone, and valerate as sole carbon sources, but not L-arabinose, D-arabitol, biphenyl, butane-1,3-diol, butane-1,4-diol, dulcitol, glycerol, inulin, lactose, starch, turanose, D- or L-xylose, *N*-acetyl-D-glucosamine, γ-aminobutyrate, caprate, glutarate, glycollate, 4-hydroxybenzoate, 2-hydroxyvalerate, malate, D-mandelic acid, monoethanolamine, phenol, phenylacetate, tartrate, or quinate. L-Leucine is used as a sole nitrogen source, but not D- or L-alanine, L-asparagine, L-aspartate, D-glucosamine, L-glycine, DL-norleucine, L-phenylalanine, L-proline, putrescine, D- or L-serine, trimethylenediamine, tyramine, L-tyrosine, or valine. Grows in the presence of crystal violet (0.001%, w/v), phenol (0.1%, w/v), phenyl ethanol (0.3%, v/v), sodium azide (0.02%, w/v), and sodium chloride (7.0%, w/v), but is sensitive to lysozyme. Resistant (μg per ml) to capreomycin (100), cephaloridine (100), doxycycline 9100), erythromycin (50), fusidic acid (100), gentamicin (10), lincomycin (100), minocycline (50), neomycin (50), oleandomycin (50), polymyxin B (100), rifampin (50), streptomycin (100), tobramycin (50), and vancomycin (50). Additional phenotypic features are shown in Table 45. Mycolic acids have 38–48 carbon atoms. The fatty acid profile is shown in Table 48.

Source: dung of cows, sheep, goats, horses, and donkeys. It is common on grass and in the soil beneath grazed pastures, and is washed into streams and lakes where it can accumulate in the sediment.

DNA G+C content (mol%): 69–64 (T_m).

Type strain: CUB 687, ATCC 29080, CIP 104178, DSM 43347, IEGM 600, JCM 3200, LMG 5357, NBRC 100603, NCIMB 11211, NCTC 10994, NRRL B-16537, VKM Ac-571.

Sequence accession no. (16S rRNA gene): X80626.

Sequence accession no. (gyrB): AB014271.

5. **Rhodococcus corynebacterioides** (Serrano, Tablante, de Serrano, de San Blas and Imaeda 1972) Yassin and Schaal 2005, 1347[VP] (Basonym: *Nocardia corynebacterioides* Serrano, Tablante, de Serrano, de San Blas and Imaeda 1972, 347.)

co.ry.ne.bac.te.ri.oi′des. N.L. neut. n. *Corynebacterium* bacterial genus name; Gr. suff. *-oides* similar to; N.L. fem. adj. *corynebacterioides* similar to *Corynebacterium*.

Gram-stain-positive, partially acid–alcohol-fast actinomycete. Forms short rods, V-forms, clumps, and coccoid elements, and smooth, orange-red pigmented colonies. Grows at 22–45°C. Degrades adenine, but not casein, elastin, gelatin, guanine, xanthine, or xylan. Nitrate is not reduced to nitrite. Does not produce hydrogen sulfide or hydrolyze hippurate. Acid is produced from mannose and salicin, but not from adonitol, arabinose, dulcitol, inulin, lactose, or melibiose. Acetate, 2,3-butylene glycol, propylene glycol, malate, D- and L-xylose (weak), and testosterone are used as sole carbon sources, but not adipic acid, adonitol, *iso*-amylalcohol, L-arabinose, erythritol, lactose, raffinose, or lactic acid. L-Phenylalanine (weak), L-proline, D- and L-serine and ornithine are used as sole nitrogen sources, but not L-alanine, L-arginine, gelatin, L-histidine, methionine, or potassium nitrate. Resistant to sodium azide (0.01%, w/v) (weak), but susceptible to sodium chloride (10.0%, w/v), chloramphenicol (10 μg per ml), cephalothin (25 μg per ml), dihydrostreptomycin (2 μg per ml), novobiocin (30 μg per ml), and polymyxin (300 μg per ml). Additional phenotypic features are shown in Table 45. Fatty acid profile shown in Table 48. The cellular polar lipid composition is diphosphatidylglycerol, phosphatidylethanolamine, phosphatidylglycerol, phosphatidylglycerol, and phosphatidylinositol mannosides. The major menaquinone is MK-8(H_2).

Source: air contaminated culture media.

DNA G+C content (mol%): not determined.

Type strain: DSM 20151, ATCC 14898, CCUG 37877, CIP 104510, JCM 3376, JCM 3391, NBRC 14404, NRRL B-24037.

Sequence accession no. (16S rRNA gene): AF430066.

Sequence accession no. (gyrB): AB014109.

Additional remarks: in ultrathin sections the cell wall is composed of an outermost diffuse layer and a moderately dense layer 10 μm thick. The total thickness of the cell wall is about 18–20 μm.

6. **Rhodococcus equi** (Magnusson 1923) Goodfellow and Alderson 1977, 116[AL] [*Corynebacterium equi* Magnusson 1923, 36; *Corynebacterium hoagii* (*sic*) (Morse) Eberson 1918, 11; *Nocardia restricta* (Turfitt) McClung 1974, 743.]

e′qui. L. n. *equus* horse; L. gen. n. *equi* of the horse.

Rod–coccus life cycle though traces of elementary branching may be observed at early stages of growth. Smooth, shiny, orange to red colonies with entire margins are formed on glucose-yeast extract agar. Some strains

produce abundant slime which may drop onto the cover of inverted Petri dishes during incubation. Grows at 10–40°C. Positive for 2-deoxythymidine-5′-*p*NP-phosphate, *p*NP-phosphorylcholine, phosphoamidase and urease, but negative for acetamidase, allantoinase, nicotinamidase, and *p*NP-β-D-xyloside. Does not degrade arbutin, cellulose, chitin, Tweens 20, 40, or 60. Does not produce acid from L-arabinose, inositol, mannitol, rhamnose, or sorbitol. Amyl alcohol, butane-1,3-diol, butan-1-ol, ethanol, glycerol, propane 1, 2-diol, propan-1-ol, propylene glycol, caprate, fumarate, glutarate, 3- and 4-hydroxybenzoate, *m*- and *p*-hydroxybenzoic acid, 2-hydroxyvalerate, octanoate, 2-oxyglutarate, phenylacetate, pyruvate, and testosterone are used as sole carbon sources, but not adonitol, D-arabitol, butane-1,4-diol, 2,3-butylene glycol, cellobiose, erythritol, galactose, glycogen, inulin, melezitose, propan-2-ol, salicin, turanose, D-xylose, adipate, *N*-acetyl-D-glucosamine, γ-aminobutyrate, *p*-cresol, gluconate, hippurate, malonate, mucate, phenylacetate, pimelate, sebacate, or quinate. Acetamide, L-aspartate, D-glucosamine, L-leucine, and L-tyrosine are used as sole nitrogen sources, but not L-alanine, L-proline, putrescine, D- or L-serine, ornithine, trimethylenediamine, tyramine, or valine. Grows in the presence of crystal violet (0.001%, w/v), phenyl ethanol (0.3%, v/v), picric acid (0.2%, w/v), and sodium azide (0.02%, w/v). Susceptible to lysozyme, phenol (0.1, w/v), 5-fluorouracil (5 µg per ml) and mitomycin (5 µg per ml), but resistant (µg per ml) to ampicillin (20), erythromycin (4), gentamicin (8), lincomycin (64), minocycline (0.125), neomycin (8), novobiocin (4), polymyxin (256), rifampin (0.25), streptomycin (5), sulfadiazine (100), tetracycline (50), and tobramycin (10). Additional phenotypic features are shown in Table 43. Mycolic acids have 30–38 carbon atoms. The fatty acid profile is shown in Table 46. Causes bronchopneumonia in foals; occasionally infects other domestic animals such as cattle and pigs, and causes infections in human patients, notably ones compromised by immunosuppressive drug therapy, AIDS, or lymphoma.

Source: soil, herbwire dung, and the intestinal tract of cows, horses, pigs and sheep.

DNA G+C content (mol%): 58.5 (T_m).

Type strain: ATCC 25729, ATCC 6939, CCUG 892, CIP 54.72, DSM 20307, HAMBI 2061, NBRC 14956, JCM 1311, JCM 3209, LMG 18452, NBRC 101255, NCTC 1621, NRRL B-16538, VKM Ac-953.

Sequence accession no. (16S rRNA gene): X80614.

Sequence accession no. (gyrB): AB014110.

Taxonomic note: on the Approved Lists of Bacterial Names, this nomenclatural name is incorrectly cited as *Rhodococcus equi* (Magnusson 1923) Goodfellow and Alderson 1979 (*sic*). This has subsequently been corrected by Euzéby (1997).

7. **Rhodococcus erythropolis** (Gray and Thornton 1928) Goodfellow and Alderson 1979, 80[VP] (Effective publication: Goodfellow and Alderson 1977, 115; *Mycobacterium erythropolis* Gray and Thornton 1928, 87; *Nocardia calcarea* Metcalfe and Brown 1957, 568.)

e.ry.thro′po.lis. Gr. adj. *eruthros* red; Gr. n. *polis* a city. N.L. n. *erythropolis* red city.

Cocci give rise to branched filaments which fragment into cocci. Rough, orange to red colonies are formed on glucose-yeast extract and Sauton's agars. Grows at 4–40°C. Positive for 2-deoxythymidine-5′-*p*NP-phosphate, *p*NP-phosphorylcholine, *p*NP-β-D-xyloside, and phosphatase, but negative for allantoinase and oxidase. Degrades adenine, arbutin, and L-tyrosine, but not casein, cellulose, chitin, elastin, hypoxanthine, starch, Tweens 20, 40, or 60, or xylan. Acid is produced from glucose, glycerol, sorbitol, sucrose, and trehalose, but not from adonitol, D- and L-arabinose, cellobiose, galactose, glycogen, inulin, melezitose, rhamnose, or xylose. Grows on butane-1,3-diol, cetyl alcohol, ethanol, propylene glycol, salicin, adipate, acetate, *N*-acetyl-D-glucosamine, γ-aminobutyrate, caprate, citrate, *p*-cresol, fumarate, gluconate, glutarate, 4-hydroxybenzoate, *p*-hydroxybenzoic acid, α-hydroxybutyrate (weak), γ-hydroxybutyrate, 2-hydroxyvalerate, lactate, malate, methyl pyruvate, 2-oxyglutarate, phenylacetate, pimelate, propionate, pyruvate, sebacate, stearate, succinate, testosterone and quinate as sole carbon sources, but not amygdalin, D-arabitol, butane-1,4-diol, catechol, dextran, β-dextrin, dulcitol, glycogen, turanose, xylitol, benzoate, 3-hydroxybenzoate, *m*-hydroxybenzoate, β-hydroxybutyrate, malonate, or tartrate. Acetamide, D- and L-alanine, L-asparagine, D-glucosamine, L-leucine, DL-norleucine, L-phenylalanine, L-proline, putrescine, L-serine, and valine are used as sole nitrogen sources, but not L-alanine-glycine, L-aspartate, benomyl, carbendazim, 2,4-dinitrophenol, L-glycine, D-serine, trimethylenediamine, tyramine, or L-tyrosine. Resistant to crystal violet (0.001%, w/v), phenol (0.1%, w/v), phenyl ethanol (0.3%, v/v), sodium azide (0.02%, w/v), sodium chloride (7.0%, w/v), but susceptible to lysozyme. Additional phenotypic features are shown in Table 44. Mycolic acids have 36–48 carbon atoms. The fatty acid profile is shown in Table 47. The major menaquinone is MK-8(H_2).

Source: soil.

DNA G+C content (mol%): 61–67 (T_m).

Type strain: ATCC 4277, CIP 104179, DSM 43066, HAMBI 1953, IEGM 7, JCM 3201, JCM 20419, LMG 5359, NBRC 15567, NCIMB 9158, NCTC 13021, NRRL B-16025, VKM Ac-858).

Sequence accession no. (16S rRNA gene): X81929.

Sequence accession no. (5S rRNA): X05057.

Sequence accession no. (gyrB): AB014112.

Additional remarks: strains hydrolyze halogenated compounds, such as 1-chlorobutane, 1-chloropentane, 1-chlorohexane, 1-bromobutane, and 1-bromohexane in either aqueous or solid phase, produce *sec*-alcohol dehydrogenase by reducing ketones, can remove organic sulfur from fossil fuels and dibenzothiophenes, and degrades chloroaromatics and polychlorobiphenyls.

8. **Rhodococcus fascians** (Tilford 1936) Goodfellow 1984a, 503[VP] [Effective publication Goodfellow 1984b, 227; *Phytomonas fascians* Tilford 1936, 394; *Corynebacterium fascians* (Tilford) Dowson 1942, 313.]

fas′ci.ans. L. part. adj. *fascians* binding together, bundling.

Branched hyphae fragment into rods and cocci. Entire, convex, orange colonies formed on glucose-yeast extract agar. Thiamine is required for growth. Grows at 10–40°C. Positive for acid phosphatase, allantoinase, naphthol-AS-BI-phosphohydrolase, pyrazinamidase, urease, and valine

arylamidase, but negative for *N*-acetyl-β-glucosamine, cystine arylamidase, 2-deoxythymidine-5′-*p*NP-phosphate, esterase (C8), α- and β-galactosidase, β-glucosidase, β-glucuronidase, oxidase, *p*NP-phosphoryl-choline, and *p*NP-β-D-xyloside. Degrades adenine and L-tyrosine, but not arbutin, casein, cellulose, chitin, elastin, starch, or xylan. Acid is produced from dextrin, ethanol, fructose, galactose, glucose, glycerol, mannitol, mannose, ribose, sorbitol, sucrose, and trehalose, but not from adonitol, amygdalin, D- or L-arabinose, arbutin, cellobiose, dulcitol, glycogen, inositol, inulin, lactose, methyl-α-D-glucoside, methyl-β-D-glucoside, raffinose, rhamnose, salicin, or xylose. Assimilates D-arabitol, ethanol, xylitol, *N*-acetyl-D-glucosamine, citrate, *p*-cresol, fumarate, gluconate, glutarate, 4-hydroxybenzoate, *m*- or *p*-hydroxybenzoate acid, 2-hydroxyvalerate, lactate, malate, 2-oxyglutarate, pyruvate, sebacate, succinate, and quinate as carbon sources, but not adonitol, dulcitol, turanose, adipate, acetate, γ-aminobutyrate, benzoate, caprate, hippurate, 3-hydroxybenzoate, malonate, phenylacetate, pimelate, or testosterone. D- and L-Alanine, benomyl, D-glucosamine, L-proline, putrescine, D-serine, L-tyrosine, and valine are used as sole nitrogen sources, but not acetamide, L-aspartate, betaine, 2,4-dinitrophenol, L-leucine, DL-norleucine, L-serine, L-threonine, trimethylenediamine, or tyramine. Grows in the presence of crystal violet (0.0001%, w/v), phenol (0.1%, w/v), but is susceptible to lysozyme, phenyl ethanol (0.3%, v/v), sodium azide (0.01%, w/v), and sodium chloride (5.0%, w/v). Additional phenotypic features are shown in Table 44. Mycolic acids have 38–52 carbon atoms. The fatty acid profile is shown in Table 47.

Source: Chrysanthemum morifolium.

DNA G+C content (mol%): 63–68 (T_m).

Type strain: ATCC 12974, CFBP 2401, CIP 104713, DSM 20669, ICMP 5833, NBRC 12155, JCM 10002, LMG 3623, NCPPB 3067, NRRL B-16937, VKM Ac-1462.

Sequence accession no. (16S rRNA gene): X79186.

Sequence accession no. (gyrB): AB075563.

9. **Rhodococcus globerulus** Goodfellow, Weaver and Minnikin 1985, 224[VP] [Effective publication: Goodfellow, Weaver and Minnikin 1982c, 741; *Mycobacterium globerulum* Gray 1928, 265; *Nocardia globerula* (*sic*) (Gray 1928) Waksman and Henrici 1948, 903; *Nocardia corynebacterioides* (*sic*) Serrano, Tablante, Serrano, San Blas and Imaeda 1972, 348.]

glo.be′ru.lus. N.L. dim. masc. adj. *globerulus* globular.

Cocci give rise to branched filaments which fragment into rods and cocci. Entire, rough, pink to red colonies are formed on glucose-yeast extract agar. Positive for allantoinase, 2-deoxythymidine-5′-*p*NP-phosphate, *p*NP-phosphorylcholine and *p*NP-β-D-xyloside, but negative for benzamidase and oxidase. Arbutin is degraded, but not adenine, casein, cellulose, chitin, elastin, starch, L-tyrosine, or xylan. Acid is produced from dextrin, ethanol, fructose, glucose, glycerol, maltose, mannitol, mannose, sorbitol, sucrose, and trehalose, but not from adonitol, amygdalin, D- or L-arabinose, cellobiose, dulcitol, galactose, glycogen, inositol, lactose, melezitose, raffinose, rhamnose, or xylose. Grows on D-arabitol, β-dextrin (weak), ethanol, propylene glycol, xylitol, adipate, acetate, γ-aminobutyrate, benzoate, caprate, citrate, fumarate, gluconate, 3- and 4-hydroxybenzoate, *p*-hydroxybenzoate, γ-hydroxybutyrate acid, lactate, malate, methyl pyruvate, pimelate, pyruvate, sebacate, succinate and quinate as sole carbon sources, but not adonitol, 2,3-butylene glycol, catechol, dulcitol, lactose, salicin, turanose, *N*-acetyl-D-glucosamine, *p*-cresol, glutarate, hippurate, *m*-hydroxybenzoate, α- or β-hydroxybutyrate, 2-hydroxyvalerate, malonate, octanoate, 2-oxyglutarate, phenylacetate, or testosterone. Acetamide, D- or L-alanine, L-leucine, putrescine, D-serine and valine are used as sole nitrogen sources, but not L-alanyl-glycine, L-asparagine, L-aspartate, benomyl, betaine, carbendazim, 2,4-dinitrophenol, D-glucosamine, DL-norleucine, L-proline, L-serine, L-ornithine, L-threonine, L-tryptophan, trimethylenediamine, tyramine, or L-tyrosine. Resistant to crystal violet (0.001%, w/v), phenol (0.001%, w/v), phenyl ethanol (0.3%, v/v), sodium azide (0.02%, w/v), sodium chloride (7.0%, w/v), 5-fluorouracil (20 μg per ml), and mitomycin C (5 μg per ml), but susceptible to lysozyme. Additional phenotypic features are shown in Table 44. Mycolic acids have 30–42 carbon atoms. The fatty acid profile is shown in Table 47.

Source: soil.

DNA G+C content (mol%): 63–67 (T_m).

Type strain: R58, ATCC 25714, CIP 104174, DSM 43954, IEGM 591, NBRC 14531, JCM 7472, NRRL B-16938.

Sequence accession no. (16S rRNA gene): X80619.

Sequence accession no. (gyrB): AB014114.

10. **Rhodococcus gordoniae** Jones, Brown, Mishra, Perry, Steigerwalt and Goodfellow 2004, 409[VP]

gor.don.i′a.e. N.L. gen. fem. n. *gordoniae* of Gordon, named after Ruth Gordon, a celebrated microbial systematist.

Acid-fast actinomycete. Forms branched filaments that fragment into rod–coccus elements. Colonies are slightly raised, shiny and pigmented pink to coral with filamentous edges on blood and chocolate agar. Degrades DNA, but not casein, hypoxanthine, uric acid, or xanthine. Nitrate is reduced to nitrite. Degrades phenol at high concentrations (>25 mM). Acid is produced from L-arabinose, fructose, galactose, glucose, glycerol, mannitol, mannose, salicin, sorbitol, sucrose, trehalose, and xylose, but not from inositol, maltose, or rhamnose. Grows on L-arabitol, arbutin, mannose, xylitol, γ-aminobutyrate, benzoate, fumarate, γ-hydroxybutyrate, and monoethanolamine as sole carbon sources, but not dulcitol, glycerol, α-methyl-D-galactoside, or methyl-α-D-glucoside. Acetamide and L-leucine are used as sole nitrogen sources. Resistant to lysozyme, clindamycin, and norfloxacin, but susceptible to amikacin, amoxicillin/clavulinate, ampicillin, ampicillin/sulbactam, cefotaxime, cephalothin, ciprofloxacin, doxycycline, erythromycin, gentamicin, imipenem, minocycline, oxacillin, penicillin, rifampin, sulfamethoxazole, tetracycline, trimethoprim/sulfamethoxazole, and vancomycin. Additional phenotypic features are shown in Table 45. Mycolic acids co-migrate with those of *Rhodococcus rhodochrous.* The major menaquinone is MK-8(H_2).

Source: a blood culture of an immunoincompetent patient with fatal pneumonia associated with adult respiratory disease syndrome.

DNA G+C content (mol%): not determined.

Type strain: W 4937, DSM 44689, JCM 12658, NCTC 13296.

Sequence accession no. (16S rRNA gene): AY233201.

11. **Rhodococcus imtechensis** Ghosh, Paul, Prakash, Mayilraj and Jain 2006, 1968[VP]

im.tech.en′sis. N.L. masc. adj. *imtechensis* of or pertaining to the Institute of Microbial Technology (IMTECH), Chandigarh, India, where the type strain was characterized.

Gram-stain-positive actinomycete with a hyphal-rod–coccus life cycle. Circular, glistening, opaque, convex and creamish-pink colonies with smooth margins are produced on tryptic soy agar. Grows at 10–37°C and pH 5.0–10.0. Catalase-positive. Oxidase-negative. Does not produce hydrogen sulfide, or reduce nitrate. Degrades *p*-nitrophenol and 2,4-dinitrophenol, but not arbutin, casein, elastin, gelatin, or starch. Acid is produced from fructose, galactose, glucose, inositol, lactose, maltose, mannitol, raffinose, sorbitol, and sucrose, but not from adonitol, cellobiose, or dulcitol. α-Cyclodextrin, L-fucose, D-galacturonic acid, lactulose, methyl-α-D-mannoside, D-psicose, acetate, D-lactic acid methyl ester, propionate, and pyruvate are used as sole carbon sources, but not amygdalin, 2,3-butylene glycol, β-dextrin, D-fructose-6-phosphate, gentiobiose, α-D-glucose-1-phosphate, glycogen, inosine, mannan, methyl-β-D-galactoside, salicin, stachyose, *N*-acetyl-D-glucosamine, D-glucuronic acid, or malate. 2,4-Dinitrophenol, *p*-nitrophenol, L-proline, L-pyroglutamic acid, L-serine, and valine are used as sole nitrogen sources, but not adenosine-5′-monophosphate, 2′-deoxyadenosine, putrescine, thymidine, thymidine-5′-monophosphate, or uridine. Resistant to (µg per ml) ampicillin (10) and cephaloridin (30), but sensitive to chloramphenicol (10), chlortetracycline (30), gentamicin (10), neomycin (30), norfloxacin (10), oleandomycin (15), rifampin (5), streptomycin (10), tetracycline (30), tobramycin (10), and vancomycin (30). Additional phenotypic features are shown in Table 44. Mycolic acids are present. The fatty acid profile is shown in Table 47. The cellular polar lipid composition is phosphatidylethanolamine, diphosphatidylglycerol, phosphatidylinositol, phosphatidylinositol mannosides, and traces of diphosphatidylglycerol. The major menaquinone is MK-8(H_2); MK-9(H_2) is present in trace amounts.

Source: pesticide-contaminated site in Punjab State, India.

DNA G+C content (mol%): 72 (T_m).

Type strain: RKJ300, JCM 13270, MTCC 7085.

Sequence accession no. (16S rRNA gene): AY525785.

12. **Rhodococcus jostii** Takeuchi, Hatano, Sedlácek and Pácová 2002, 413[VP]

jos′ti.i. N.L. gen. masc. n. *jostii* of Jošt, in honor of Margrave Jošt Lucemburský (1351–1411), an important Czech ruler, from whose skeletal remains the strain was isolated.

Substrate mycelium fragments into rods or coccoid elements, which penetrate the agar. Form filaments (0.4–0.6 × 8–12 µm) in early growth phase and mostly cocci or short rods (0.2–0.5 × 1.7–3 µm) during the exponential growth phase. Colonies are light pink, opaque, convex with slightly irregular edges on peptone-yeast extract agar. Grows at 15–30°C, but not at 37°C. Positive for *N*-acetyl-β-glucosaminidase, catalase, β-galactosidase, and β-glucosidase, but negative for alkaline phosphatase, α-glucosidase, β-glucuronidase, lecithinase, pyrazinamidase, and pyrrolindonyl arylamidase. Does not reduce nitrate to nitrite. Degrades L-tyrosine, but not casein, DNA, gelatin, or starch. Acid is produced from fructose, glucose, lactose, mannitol, and xylose, but not from maltose. *N*-acetyl-D-glucosamine, fumarate, gluconate (weak), *m*- and *p*-hydroxybenzoic acid, succinate, tartrate (weak), and quinate are used as sole carbon sources, but not ethanol, citrate, lactate, or malate. Valine (weak) is used as a sole nitrogen source, but not L-leucine, L-proline, or L-serine. Additional phenotypic features are shown in Table 44. Mycolic acids are present. The fatty acid profile is shown in Table 47. The predominant menaquinone is MK-8(H_2).

Source: skeletal remains of Margrave Jošt Lucemburský, one of the most important Czech rulers.

DNA G+C content (mol%): 67.4 (HPLC).

Type strain: NBRC 16295, CCM 4760, JCM 11615.

Sequence accession no. (16S rRNA gene): AB046357.

Sequence accession no. (gyrB): AB088664.

13. **Rhodococcus koreensis** Yoon, Cho, Kang, Kim, Lee and Park 2000a, 1199[VP]

ko.re.en′sis. N.L. masc. adj. *koreensis* of or belonging to Korea, the country where the strain was isolated.

Gram-stain-positive actinomycete which shows elementary branching at early growth phase and cocci and rod-like elements in stationary phase. Cream colored, opaque, convex colonies with irregular edges are formed on tryptic soy agar. Grows at 25–30°C and pH 7.0–7.8. Catalase-positive. Oxidase-negative. Degrades 2,3-dinitrophenol, L-tyrosine, and uric acid, but not adenine, arbutin, casein, elastin, or starch.

Amygdalin, D-arabinose (weak), 2,3-butylene glycol, L-fucose, gentiobiose, D-galacturonic acid (weak), turanose, xylitol, acetate, citrate, malate, propionate, and succinate are used as sole carbon sources, but not adonitol, benzoate, α-cyclodextrin, dulcitol, lactulose, methyl-β-D-galactoside, methyl-α-D-mannoside, D-psicose, salicin, stachyose, *p*-hydroxybenzoic acid, or D-lactic acid methyl ester. 2,4-Dinitrophenol, *p*-nitrophenol, L-proline, L-serine, and uridine-5′-monophosphate (weak) are used as sole nitrogen sources, but not putrescine, or L-pyroglutamic acid. Resistant to sodium chloride (5.0%, w/v). Additional phenotypic features are shown in Table 44. Mycolic acids have 43–53 carbon atoms. The fatty acid profile is shown in Table 47. The predominant menaquinone is MK-8(H_2).

Source: industrial wastewater in Cheong-Ju.

DNA G+C content (mol%): 66 (HPLC).

Type strain: DNP505, CIP 106721, DSM 44498, JCM 10743, KCTC 0569BP, NBRC 100607.

Sequence accession no. (16S rRNA gene): AF124342, AF124343.

Sequence accession no. (gyrB): AB075566.

Additional remarks: 16S rRNA genes differ by 10 nucleotides (0.7% nucleotide sequence dissimilarity).

14. **Rhodococcus kroppenstedtii** Mayilraj, Krishnamurthi, Saha and Saini 2006, 981[VP]

krop.pen.sted′ti.i. N.L. gen. masc. n. *kroppenstedtii* of Kroppenstedt, to honor of Reiner Michael Kroppenstedt, a German microbiologist, for his many contributions to the taxonomy of actinomycetes.

Gram-stain-positive acid-fast actinomycete which forms rods and cocci that occur in groups. Colonies are small, smooth, glistening and convex with an orange-red pigmentation on tryptic soy agar. Grows well at 10–37°C, but not at 42°C. Grows at pH 5.0–11.0. Degrades adenine, Tweens 20, 40, and 60, but not elastin, gelatin, or guanine. Reduces nitrate to nitrite. Acid is produced from glucose, inositol, lactose, mannitol, raffinose, rhamnose and xylose, but not from adonitol, L-arabinose, cellobiose, dulcitol, fructose, galactose, inulin, maltose, mannose, melibiose, salicin, sucrose, or trehalose. Grows on lactic acid as a sole carbon source. L-Arginine, L-histidine, L-methionine, L-phenylalanine, potassium nitrate, L-proline, and D- and L-serine are used as sole nitrogen sources. Resistant to crystal violet (0.0001%, w/v), sodium azide (0.01%, w/v), sodium chloride (10.0%, w/v), and polymyxin (300 µg per ml). Additional phenotypic features are shown in Table 45. Mycolic acids have 42–50 carbon atoms and 1–3 double bonds. The fatty acid profile is shown in Table 48. The cellular polar lipid composition is diphosphatidylglycerol, phosphatidylethanolamine, phosphatidylinositol, and phosphatidylinositol mannosides. The major menaquinone is MK-8(H_2).

Source: a cold desert of the Himalayas, India.

DNA G+C content (mol%): 69.7 (HPLC).

Type strain: K07-23, DSM 44908, JCM 13011, NBRC 103113, MTCC 6634.

Sequence accession no. (16S rRNA gene): AY726605.

15. **Rhodococcus kunmingensis** Wang, Wang, Zhang, Xu, Jiang and Li 2008, 1469[VP]

kun.ming.en′sis. N.L. masc. adj. *kunmingensis* of or pertaining to Kunming, a city in Yunnan, south-west China.

Gram-stain-positive, acid-fast, actinomycete. Forms elementary branching filaments in early growth phase and coccoid-like elements at stationary phase. Circular, convex, smooth pink colonies are formed on yeast extract-malt extract agar. Grows at 10–37°C and pH 7.0–7.5.

Positive for *N*-acetyl-β-glucosamidase, catalase, esterase lipase (C8), fucosidase, α- and β-glucosidase, lipase C14, and naphthol-AS-BI-phosphohydrolase, but negative for cystine arylamidase, β-galactosidase, oxidase, and trypsin. Degrades L-arginine, L-asparagine, gelatin, L-histidine, and L-proline, but not starch. Reduces nitrate to nitrite, but does not produce hydrogen sulfide. Grows on amygdalin, butanediol, glucose, melibiose and xylitol as sole carbon sources, but not on chitin. Resistant to (µg per ml) clindamycin (2), norfloxacin (10), and trimethoprim (1.25), but susceptible to amikacin (30), amoxicillin/clavulinate, ampicillin (10), ciprofloxacin (5), erythromycin (15), gentamicin (10), rifampin (5), tobramycin (10), and vancomycin (30). Additional phenotypic features are shown in Table 43. Mycolic acids co-migrate with those of the type strain of *Rhodococcus equi*.The fatty acid pattern is shown in Table 46. The cellular polar lipid composition is diphosphatidylglycerol, phosphatidylethanolamine, phosphatidylinositol, and phosphatidylinositol mannosides. The major menaquinone is MK-8(H_2).

Source: a rhizosphere soil sample in Kunming, South-West China.

DNA G+C content (mol%): 64.9 (HPLC).

Type strain: YIM 45607, JCM 15626, KCTC 19149, DSM 45001.

Sequence accession no. (16S rRNA gene): DQ997045.

16. **Rhodococcus kyotonensis** Li, Furihata, Ding and Yokota 2007, 1957[VP]

kyo.to.nen′sis. N.L. masc. adj. *kyotonensis* of or pertaining to Kyoto, the source of soil from which the organism was isolated.

Gram-stain-positive, acid-fast actinomycete. Forms non-branching hyphae that fragment into short rod-to-coccus elements. Colonies are yellow-orange on tryptic soy agar. Grows at 27–30°C, but not at 40°C. Grows at pH 6.0–8.0. Positive for acid phosphatase, catalase, cystine arylamidase, esterase lipase (C8), leucine arylamidase, naphthol-AS-BI-phosphohydrolase, tryptophan deaminase, and valine, but negative for *N*-acetyl-β-glucosamidase, alkaline phosphatase, chymotrypsin, esterase lipase (C4), fucosidase, α- and β-galactosidase, β-glucosidase, β-glucuronidase, lipase, L-methionine-7-amino-methylcoumarin, *o*-nitro-phenyl-β-D-galactoside (ONPG)-arginine dihydrolase, ONPG-lysine decarboxylase, ONPG-ornithine decarboxylase, oxidase, and trypsin. Does not reduce nitrate or produce hydrogen sulfide. Degrades Tweens 20, 40, and 60, but not gelatin. D-Arabitol, sorbose, xylitol, and gluconate are used as sole carbon sources, but not adonitol, amygdalin, D-arabinose, arbutin, dulcitol, erythritol, D- or L-fucose, gentiobiose, glycogen, lyxose, methyl-α-D-glucoside, methyl-α-D-mannoside, methyl-β-D-xyloside, salicin, starch, tagatose, turanose, *N*-acetyl-D-glucosamine, citrate, 2-keto-gluconate, or 5-keto-gluconate. Grows in the presence of sodium chloride (9.0%, w/v). Additional phenotypic features are shown in Table 44. Mycolic acids co-migrate with those of the type strain of *Rhodococcus rhodochrous*.The fatty acid profile is shown in Table 47. The major menaquinone is MK-8(H_2); MK-7(H_2) and MK-9(H_2) are present in minor amounts.

Source: a soil sample in Kyoto City, Japan.

DNA G+C content (mol%): 64.5 (HPLC).

Type strain: DS472, CCTCC AB206088, IAM 15415, JCM 23211.

Sequence accession no. (16S rRNA gene): AB269261.

17. **Rhodococcus maanshanensis** Zhang, Zhang, Xiao, Liu and Goodfellow 2002, 2124[VP]

ma.an.shan.en′sis. N.L. masc. adj. *maanshanensis* of or belonging to Maanshan, the source of the soil from which the organism was isolated.

Gram-stain-positive, non-acid-fast actinomycete. Forms a branched substrate mycelium that fragments into rod- and coccoid-like elements. Cream colored, convex colonies with irregular edges are formed on Bennett's and tryptic soy agars. Grows well at 25–30°C and pH 6.0–8.5. Catalase-positive.

Hydrolyzes arbutin and reduces nitrate to nitrite. Oxidase-negative. Degrades adenine, Tweens 20 and 60, but not casein, elastin, guanine, hypoxanthine, starch, L-tyrosine, uric acid, or xanthine. Acid is produced from fructose, glucose, glycerol, mannose (weak), and ribose (weak), but not from L-arabinose, cellobiose, galactose, inulin, lactose, maltose, mannitol, raffinose, rhamnose, starch, sucrose, trehalose, or xylose. Salicin, turanose, acetate, fumarate, propionate, and succinate are used as sole carbon sources, but not adonitol, D-arabinose, dulcitol, erythritol, L-fucose, starch, xylitol, benzoate, citrate, formate, hippurate, phenol, tartrate, or 2,4,6-trichlorophenol. L-Alanine is used as a sole nitrogen source, but not acetamide, L-asparagine, L-aspartate, 2,4-dinitrophenol, D-glucosamine, or L-serine. Does not grow in the presence of lysozyme, or sodium chloride (4.0%, w/v). Additional phenotypic features are shown in Table 44. Mycolic acids co-migrate with those of the type strain of *Rhodococcus marinonascens*. The fatty acid profile is shown in Table 47. The cellular polar lipid composition is diphosphatidylglycerol, phosphatidylethanolamine, and phosphatidylinositol mannosides. The major menaquinone is MK-8(H_2).

Source: soil collected from Maanshan Mountain in Anhui Province, China.

DNA G+C content (mol%): 66.2 (T_m).

Type strain: M712, AS 4.1720, DSM 44675, JCM 11374, NBRC 100610.

Sequence accession no. (16S rRNA gene): AF416566.

Sequence accession no. (gyrB): AB262519.

18. **Rhodococcus marinonascens** Helmke and Weyland 1984, 137[VP]

ma.ri.no.nas′cens. L. adj. *marinus* of the sea; L. part. adj. *nascens* born. N.L. part. adj. *marinonascens* born of the sea.

Gram-stain-positive, partially acid-fast actinomycete. Forms a well developed branched primary mycelium that fragments into bacillary and coccoid elements on solid media. Irregularly wrinkled, cream-colored colonies, which are sometimes tinged with pink, are formed on yeast extract agar. Optimal growth occurs at 20°C and in media with 75–10% seawater content or an equivalent salt concentration. Degrades arbutin and L-tyrosine, but not adenine, casein, cellulose, chitin, elastin, or xylan. Nitrate is not reduced to nitrite. Acid is produced from fructose, glucose, inositol, inulin, and mannose, but not from cellobiose, dulcitol, ethanol, galactose, glycogen, lactose, maltose, mannitol, melezitose, raffinose, rhamnose, salicin, sucrose, trehalose, or xylose. Acetate, gluconate, malate, pyruvate, and succinate are used as sole carbon sources, but not ethanol, turanose, xylitol, benzoate, citrate, *p*-cresol, *p*-hydroxybenzoic acid, lactate, pimelate, sebacate, or testosterone. D- and L-Alanine are used as sole nitrogen sources, but not L-glycine, or L-proline. Resistant to crystal violet (0.01%, w/v), phenol (0.1%, w/v), phenyl ethanol (0.3%, v/v), sodium azide (0.02%, w/v), and sodium chloride (7.0%, w/v), but susceptible to lysozyme. Additional phenotypic features are shown in Table 44. Mycolic acids have 34–50 carbon atoms.The fatty acid profile is shown in Table 47. The major menaquinoine is MK-8(H_2).

Source: the uppermost layer of marine sediments from the North East Atlantic.

DNA G+C content (mol%): 65–66 (T_m).

Type strain: 3438W, ATCC 35653, CIP 104177, DSM 43752, NBRC 14363, JCM 6241, NRRL B-16940, VKM Ac-1182.

Sequence accession no. (16S rRNA gene): X80617.

Sequence accession no. (gyrB): AB014115.

19. **Rhodococcus opacus** Klatte, Kroppenstedt and Rainey 1994a, 357[AL]

o.pa′cus. L. masc. adj. *opacus* shady, nontransparent.

Gram-stain-positive, non-acid-fast actinomycete. Forms a branched substrate mycelium that fragments into bacillary and coccoid elements in stationary phase. Cream-gray colored colonies have an entire margin and a smooth to rough surface; they develop a depressed center after several days of growth. Positive for catalase and *p*NP-phosphoryl-choline, but negative for 2-deoxythymidine-5′-*p*NP-phosphate, oxidase, and *p*NP-β-D-xyloside. Degrades adenine, L-tyrosine, and uric acid, but not arbutin, casein, cellulose, elastin, gelatin, or starch. Nitrate is reduced to nitrite. Acid is produced from fructose, glucose, inositol, lactose, maltose, mannitol, and sorbitol, but not from mannose. D-Arabitol, 2,3-butylene glycol, *n*-hexadecane, D-psicose (weak), *n*-tetradecane, turanose, *n*-undecane, xylitol, acetate, *N*-acetyl-D-glucosamine, γ-aminobutyrate, benzoate, caprate, citrate, gluconate, 3- and 4-hydroxybenzoate, *p*-hydroxybenzoic acid, 2-hydroxyvalerate, 2-oxyglutarate, phenylacetate, pimelate, shikimate, succinate, and quinate are used as sole carbon sources, but not adonitol, amygdalin, D-arabinose, α-cyclodextrin, dulcitol, ethanol, L-fucose, gentiobiose, D-galacturonic acid, lactulose, methyl-β-D-galactoside, methyl-α-D-mannoside, salicin, stachyose, glutarate, lactate, D-lactic acid methyl ester, malate, or propionate. D- and L-Alanine, L-aspartate, barbituric acid, cytosine, D-glucosamine, L-leucine, L-proline, putrescine, L-serine, thymine, trimethylenediamine, tyramine, urea, uric acid, and valine are used as sole nitrogen sources, but not acetamide, 2,4-dinitrophenol, *p*-nitrophenol, orotic acid, L-phenylalanine, L-pyroglutamic acid, uracil, or uridine-5′-monophosphate. Resistant to lysozyme and sodium chloride (5.0%, w/v). Additional phenotypic features are shown in Table 44. Mycolic acids have 48–54 carbon atoms. The fatty acid profile is shown in Table 47. The cellular polar lipid composition is diphosphatidylglycerol, phosphatidylethanolamine, phosphatidylglycerol, phosphatidylinositol, and phosphatidylinositol mannosides. The major menaquinone is MK-8(H_2).

Source: soil.

DNA G+C content (mol%): 67.6 (HPLC).

Type strain: DSM 43205, ATCC 51881, CIP 104549, DSM 43205, NBRC 16217, JCM 9703, NBRC 100624.

Sequence accession no. (16S rRNA gene): X80630.

Additional remarks: strains can degrade aromatic compounds, notably aromatic carboxylic acids, catechols, and substituted phenols, phthalic acid esters, and pyridine. Some strains have the ability to utilize gaseous hydrocarbons as sole carbon sources resulting in the production of subterranean petroleum deposits.

20. **Rhodococcus percolatus** Briglia, Rainey, Stackebrandt, Schraa and Salkinoja-Salonen 1996, 29VP

per.co.la′tus. L. pref. *per* very, thoroughly, completely; L. part. masc. adj. *colatus* filtered; N.L. masc. adj. *percolatus* completely filtered, referring to the percolator system used for isolation.

Gram-stain-positive actinomycete. Forms a primary mycelium that fragments into rod–coccus elements, and shows snapping division and v-forms. Colonies are light pink. Positive for 2-deoxythymidine-5′-*p*NP-phosphate and *p*NP-β-D-xyloside, but negative for oxidase. Degrades L-tyrosine, Tweens 20 and 40, and uric acid, but not adenine, arbutin, casein, elastin, or starch. Amygdalin, D-arabitol, 2,3-butylene glycol, gentiobiose, methyl-β-D-galactoside, methyl-β-D-glucoside, D-psicose, salicin, turanose, xylitol, acetate, *N*-acetyl-D-glucosamine, γ-aminobutyrate, benzoate, caprate, citrate, fumarate, D-galacturonic acid, lactone, gluconate, L-glutamic acid, 3- and 4-hydroxybenzoate, *p*-hydroxybenzoic acid, α-hydroxybutyric acid, *m*- and *p*-hydroxyphenylacetic acid, 2-hydroxyvalerate, α-ketobutyric acid, lactate, malate, methyl pyruvate, monoethanolamine, monomethyl succinate, mucate, 2-oxyglutarate, phenylacetate, pimelate, propionate, pyruvate, D-saccharic acid, and succinate are used as sole carbon sources, but not *N*-acetyl-β-D-mannoside, adonitol, D-arabinose, α-cyclodextrin, dulcitol, ethanol, D-fructose-6-phosphate, L-fucose, D-galacturonic acid, α-D-glucose-1-phosphate, glucose-6-phosphate, DL-α-glycerol phosphate, inosine, lactulose, lyxose, maltotriose, α-methyl-D-galactoside, methyl-α-D-mannoside, propylene glycol, stachyose, acetoacetic acid, L-galacturonic acid lactone, D-glucuronic acid, glutarate, glycollate, glyoxylic acid, D-lactic acid methyl ester, tartrate, or tricarballylic acid. Acetamide, D-and L-alanine, L-asparagine, L-aspartate, L-aspartic acid, 2,4-dinitrophenol, D-glucosamine, L-glutamine, L-leucine, phenylethylamine, L-proline, putrescine (weak), D-threonine, and tyramine are used as sole nitrogen sources, but not adenosine, L-alanyl-glycine, D-aspartic acid, 2′-deoxyadenosine, glucuronamide, glycyl-L-aspartic acid, glycyl-L-glutamic acid, glycyl-L-proline, *p*-nitrophenol, L-pyroglutamic acid, D- or L-serine, L-threonine, thymidine, or uridine-5′-monophosphate. Grows in the presence of sodium chloride (5.0%, w/v). Additional phenotypic features are shown in Table 44. Mycolic acids have 46–54 carbon atoms. The fatty acid profile is shown in Table 47. The cellular polar lipid composition is diphosphatidylglycerol, phosphatidylethanolamine, phosphatidylglycerol, phosphatidylinositol, and phosphatidylinositol mannosides. The major menaquinone is MK-8(H_2).

Source: a 2,4,6- trichlorophenol enrichment culture in a percolator inoculated with contaminated sludge and sediment samples.

DNA G+C content (mol%): 67.4 (HPLC).

Type strain: MBS1, JCM 10087, NBRC 100626).

Sequence accession no. (16S rRNA gene): X92114.

Additional remarks: strains are able to produce extracellular surfactants, degrade pyridine and can grow on phenol and a range of xenobiotic compounds, including 2,3-dichlorophenol, 3,4-dichlorophenol, 2,4,5-trichlorophenol, 2,3,6-trichlorophenol, toluene, and 2,4,6-trichlorophenol.

21. **Rhodococcus phenolicus** Rehfuss and Urban 2005, 698AL

phe.nol.i′cus. N.L. n. *phenol* common name for the industrial solvent hydroxybenzene; L. suff. *-icus -a -um*, suffix used with the sense of belonging to; N.L. masc. adj. *phenolicus* pertaining to phenol.

Gram-stain-positive actinomycete which forms an extensive substrate mycelium that fragments into rods and cocci of variable size during later growth phases. Colonies develop a deep pink pigmentation during stationary phase with a varied morphological appearance that can be observed on tryptic soy agar. Most colonies are rugose and radiate in texture with an undulate margin and an umbonate elevation, a minority have a mucoid and smooth appearance. Grows well at pH 6.0–8.0 and moderately well at pH 4.0 and 10.0. Catalase-positive. Oxidase-negative. Nitrate is reduced to nitrite, but does not hydrolyze arbutin or produce hydrogen sulfide. Does not degrade DNA. Biphenyl (weak), chlorobenzene, chlorobenzoic acid, 1,4-dichlorobenzene, toluene (weak), and phenol are used as sole carbon sources, but not L-arabinose, inulin, lactose, raffinose, starch, or D- or L-xylose. Additional phenotypic features are shown in Table 45. The fatty acid profile is shown in Table 48.

Source: Johnson Space Center graywater bioprocessor.

DNA G+C content (mol%): 66.7 (HPLC).

Type strain: G2P, DSM 44812, JCM 14914, NRRL B-24323.

Sequence accession no. (16S rRNA gene): AY533293.

Additional remarks: aerial mycelia are formed when grown on chlorobenzene, chlorobenzoic acid, 1,4-dichlorobenzene, and phenol as sole carbon sources.

22. **Rhodococcus pyridinivorans** Yoon, Kang, Cho, Lee, Kho, Kim and Park 2000b, 2178VP

py.ri.di.ni.vo′rans. N.L. n. *pyridinum* pyridine; L. v. *vorare* to devour; N.L. part. adj. *pyridinivorans* pyridine-devouring.

Gram-stain-positive, branched filaments fragment into short rods and cocci. Colonies on tryptic soy agar are light orange, opaque, raised with slightly irregular edges. Grows at 10–45°C but not at 50°C. Grows at pH 6.0–9.0. Catalase-positive. Oxidase-negative. Hydrolyzes arbutin, produces hydrogen sulfide, reduces nitrate to nitrite, but does not degrade DNA or uric acid. Does not produce acid from L-arabinose, cellobiose, galactose, inulin, lactose, raffinose, or rhamnose. L-Arabitol, biphenyl, glycerol, mannose, starch, toluene, xylitol, γ-aminobutyrate, fumarate, and γ-hydroxybutyric acid are used as sole carbon sources, but not L-arabinose, arbutin, dulcitol, inulin, lactose, α-methyl-D-galactoside, raffinose, D- or L-xylose, benzoate, formate, hippurate, monoethanolamine, phenol, or tartrate. L-Leucine is used as a sole nitrogen source. Utilizes high concentrations (3500 mg l<MIN>1) of pyridine. Additional phenotypic features are shown in Table 45. Mycolic acids have 36–46 carbon atoms. The fatty acid pattern is shown in Table 48. The major menaquinone is MK-8(H_2).

Source: industrial wastewater in Korea.

DNA G+C content (mol%): 66 (HPLC).

Type strain: PDB9, DSM 44555, JCM 10940, KCC M80005, KCTC 0647BP, NBRC 100608.

Sequence accession no. (16S rRNA gene): AF173005.

Sequence accession no. (gyrB): AB088665.

23. **Rhodococcus qingshengii** Xu, He, Wang, Wang, Li, Tang and Li 2007, 2756[VP]

qing.shen′gi.i. N.L. gen. masc. n. *qingshengii* of Qing-Sheng, to honor Qing-Sheng Fan, a respected Chinese microbiologist, for his many contributions to the development of microbiology in China.

Gram-stain-positive actinomycete which forms an elementary mycelium that fragments into short rods and cocci. Colonies on Luria–Bertani agar are orange, opaque, and convex with irregular edges. Catalase-positive. Oxidase-negative. Does not reduce nitrate. Degrades carbendazim, Tween 40, but not starch. Catechol, dextrin, D-psicose, acetate, gluconate, α-ketovaleric acid, and malate are used as sole carbon sources, but not turanose, xylitol, α-, β-, or γ-hydroxybutyric acid, lactate, or methyl pyruvate. Alaninamide, L-alanine, L-alanyl-glycine (weak), benomyl, carbendazim, and urea are used as sole nitrogen sources, but not L-asparagine. Resistant to sodium nitrite (0.02%, w/v). Additional phenotypic features are shown in Table 44. Mycolic acids are present. The fatty acid profile is shown in Table 47. The cellular polar lipid composition is diphosphatidylglycerol, phosphatidylinositol, and phosphatidylmethylethanolamine. The major menaquinone is MK-8(H_2).

Source: carbendazim-contaminated soil from Jiangsu Province, China.

DNA G+C content (mol%): 59.1 (T_m).

Type strain: djl-6, CGMCC 1.6580, JCM 15477, KCTC 19205.

Sequence accession no. (16S rRNA gene): DQ090961.

24. **Rhodococcus rhodnii** Goodfellow and Alderson 1979, 80[VP] (Effective publication: Goodfellow and Alderson 1977, 117.)

rhod′ni.i. N.L. masc. n. *Rhodnius* generic name of the reduvid bud; N.L. gen. n. *rhodnii* of *Rhodnius*.

Gram-stain-positive actinomycete which forms a primary mycelium that fragments into short rods and cocci. Rough, red colonies are formed on glucose-yeast extract agar. Grows at 25–37°C. Catalase-positive, but does not hydrolyze allantoinase, 2-deoxythymidine-5′-*p*NP-phosphate, *p*NP-phosphoryl-choline, or *p*NP-xyloside. Oxidase-negative. Produces hydrogen sulfide, but does not hydrolyze arbutin or reduce nitrate to nitrite. Does not degrade adenine, cellulose, chitin, DNA, elastin, Tweens 20, 40, or 60, or xylan. Does not produce acid from L-arabinose, cellobiose, galactose, inulin, lactose, raffinose, or rhamnose. D-Arabitol, mannose, D-xylose (weak), adipate, benzoate, caprate, *p*-cresol, fumarate, 2-hydroxyvalerate, lactate, malate, octanoate, 2-oxyglutarate, pimelate, propionate, and sebacate are used as sole carbon sources, but not L-arabinose, biphenyl, 2,3-butylene glycol, glycerol, lactose, propylene glycol, raffinose, starch, toluene, turanose, L-xylose, *N*-acetyl-D-glucosamine, γ-aminobutyrate, glutarate, 3- and 4-hydroxybenzoate, phenol, phenylacetate, tartrate, testosterone, or quinate. L-Leucine, L-proline, and L-tyrosine are used as sole nitrogen sources, but not L-alanine, L-aspartate, D-glucosamine, putrescine, D- or L-serine, ornithine, trimethylenediamine, tyramine, or valine. Grows in the presence of crystal violet (0.001%, w/v), phenol (0.1%, w/v), sodium azide (0.02%, w/v), and sodium chloride (7.0%, w/v). Additional phenotypic features are shown in Table 45. Mycolic acids have 38–52 carbon atoms. The fatty acid profile is shown in Table 48.

Source: intestine of the reduvid bug, *Rhodnius prolixus*.

DNA G+C content (mol%): 66 (T_m).

Type strain: N445, ATCC 35071, CIP 104181, DSM 43336, IEGM 555, JCM 3203, KCC A-0203, LMG 5363, NBRC 100604, NCIB (now NCIMB) 11279, NRRL B-16535, VKM Ac-1187.

Sequence accession no. (16S rRNA gene): X80621.

Sequence accession no. (gyrB): AB014254.

25. **Rhodococcus ruber** (Kruse 1896) Goodfellow and Alderson 1979, 80[VP] (Effective publication: Goodfellow and Alderson 1977, 117; *Streptothrix rubra* Kruse 1896, 63.)

ru′ber. L. masc. adj. *ruber* red.

Gram-stain-positive actinomycete. Forms a primary mycelium that fragments into rods and cocci. Single unbranched aerial hyphae produced. Rough, pink to red colonies are formed on glucose-yeast extract agar, Sauton's agar, and egg media. Grows at 28–40°C. Positive for acetamidase, catalase, 2-deoxythymidine-5′-*p*NP-phosphate, and *p*NP-phosphoryl-choline, but negative for acid phosphatase, allantoinase, α- and β-esterase, β-galactosidase, and *p*NP-β-D-xyloside. Produces hydrogen sulfide, reduces nitrate to nitrite, but is oxidase-negative. Degrades adenine and uric acid, but not cellulose, chitin, DNA, elastin, Tweens 20, 40, and 60, or xylan. Unable to hydrolyze arbutin. Acid is produced from glucose, mannitol, and sorbitol, but not from L-arabinose, cellobiose, galactose, inositol, inulin, lactose, mannose, raffinose, rhamnose, or trehalose. D- and L-Arabitol, biphenyl (weak), *iso*-butanol, glycerol, inulin, mannose, propan-1-ol, propylene glycol, starch, toluene, xylitol, adipate, γ-aminobutyrate, benzoate, caprate, *p*-cresol, fumarate, L-glutamic acid, 3- and 4-hydroxybenzoate, γ-hydroxybutyric acid, lactate, malate, malonate, monoethanolamine, octanoate, 2-oxyglutarate, phenylacetate, pimelate, propionate, sebacate, testosterone, and quinate are used as sole carbon sources, but not L-arabinose, arbutin, dulcitol, lactose, α-methyl-D-galactoside, raffinose, turanose, D- and L-xylose, *N*-acetyl-D-glucosamine, glutarate, 2-hydroxyvalerate, or phenol. L-Alanine, D-glucosamine, L-leucine, putrescine, tyramine, L-tyrosine, and valine are used as sole nitrogen sources, but not L-aspartate, L-proline, D- and L-serine, or trimethylenediamine. Resistant to crystal violet (0.001%, w/v), phenol (0.1%, w/v), phenyl ethanol (0.3%, v/v), picric acid (0.2%, w/v), sodium azide (0.01%, w/v), sodium chloride (7.0%, w/v), sodium nitrate (0.1%, w/v), and sodium salicylate (0.1%, w/v), but susceptible to lysozyme, 5-fluorouracil (5 µg per ml), mitomycin (5 µg per ml), and rifampin (25 µg per ml). Additional phenotypic features are shown in Table 45. Mycolic acids have 38–50 carbon atoms. The fatty acid profile is shown in Table 47. Major menaquinone is MK-8(H_2).

Source: soil.

DNA G+C content (mol%): 65–69 (T_m).

Type strain: KCC A-0205, CIP 104180, DSM 43338, IEGM 70, NBRC 15591, JCM 3205, LMG 5366, VKM Ac-1021.

Sequence accession no. (16S rRNA gene): X80625.

Sequence accession no. (gyrB): AB014174.

Additional remarks: strains can use gaseous hydrocarbons as sole carbon sources, degrade aromatic compounds, form biosurfactants that facilitate oil removal from soil and produce poly(3-hydroxyalkanoic) acids of value in the production of biodegradable plastic.

26. **Rhodococcus triatomae** Yassin 2005, 1578[VP]

tri.a.to'ma.e. N.L. gen. n. *triatomae* of *Triatoma*, a genus of blood-sucking bug from which the micro-organism was isolated.

Gram-stain-positive, slightly acid–alcohol-fast actinomycete. Produces thin filamentous cells and shows a primary mycelium that fragments into rods and cocci. White to creamy colonies with slightly irregular edges are produced on Columbia blood agar. Grows at 22–42°C. Degrades adenine, but not elastin, gelatin, or guanine. Testosterone is used as a sole carbon source, but not adipate, amyl-alcohol, L-arabinose, 2,3-butylene glycol, erythritol, lactate, lactose, propylene glycol, raffinose, or L-xylose. Urea is used as a sole nitrogen source, but not L-alanine, L-arginine, gelatin, L-proline, L-serine, or ornithine. Additional phenotypic features are shown in Table 45. Mycolic acids present. The fatty acid pattern is shown in Table 48. The major menaquinone is MK-8(H_2).

Source: a blood-sucking bug of the genus *Triatoma*.

DNA G+C content (mol%): not determined.

Type strain: IMMIB RIV-085, CCUG 50202, DSM 44892, NBRC 103116.

Sequence accession no. (16S rRNA gene): AJ854055.

27. **Rhodococcus tukisamuensis** Matsuyama, Yumoto, Kudo and Shida 2003, 1334[VP]

tu.ki.sa.mu.en'sis. N.L. masc. adj. *tukisamuensis* of or belonging to Tukisamu, a town in Sapporo, Hokkaido, Japan where the type strain was isolated.

Gram-stain-positive, non-acid-fast actinomycete. Forms a primary mycelium that fragments into rods and cocci. Opaque cream colored colonies with irregular edges are produced on tryptic soy agar. Growth occurs at 15–45°C and pH 5.5–8.5. Catalase-positive. Oxidase-negative. Hydrolyzes arbutin, reduces nitrate, but does not produce hydrogen sulfide. Does not degrade adenine, casein, elastin, hypoxanthine, starch, L-tyrosine, uric acid, or xanthine. Does not produce acid from adonitol, L-arabinose, cellobiose, fructose, galactose, glucose, glycerol, inositol, lactose, maltose, mannitol, mannose, melibiose, raffinose, rhamnose, ribose, salicin, sorbitol, sucrose, trehalose, or xylose. Adonitol, dulcitol, turanose, xylitol, succinate, and testosterone are used as sole carbon sources, but not D-arabinose or citrate. Resistant to sodium chloride (4.0%, w/v). Additional phenotypic features are shown in Table 44. Mycolic acids have 44–52 carbon atoms. The fatty acid profile is shown in Table 47. The major menaquinone is MK-8(H_2).

Source: soil in Sapporo City, Japan.

DNA G+C content (mol%): 66 (HPLC).

Type strain: Mb8, JCM 11308, NBRC 100609, NCIMB 13903.

Sequence accession no. (16S rRNA gene): AB067734.

Sequence accession no. (gyrB): AB262518.

28. **Rhodococcus wratislaviensis** (Goodfellow, Zakrzewska-Czerwinska, Thomas, Mordarski, Ward and James 1995) Goodfellow, Chun, Stackebrandt and Kroppenstedt 2002, 752[VP] (*Tsukamurella wratislaviensis* Goodfellow, Zakrzewska-Czerwinska, Thomas, Mordarski, Ward and James 1995; *Tsukamurella wratislaviensis* Goodfellow, Zakrzewska-Czerwinska, Thomas, Mordarski, Ward and James 1991b)

wra.tis.la.vi.en'sis. N.L. masc. adj. *wratislaviensis* of or pertaining to Wratislavia (Wroclaw, Poland).

Gram-stain-positive, acid–alcohol-fast actinomycete. Forms straight to slightly curved rods. Colonies are white to creamy and irregular. Grows at 10–37°C and at pH 5–9. Cleaves 4-methylumbelliferyl (4MU)-2-acetoamido-2-deoxy-β-D-glucopyranoside, 4MU-α-L-arabinopyranoside, benzyloxycarbonyl-L-alanine-L-alanine-L-phenylalanine-7-amino-methylcoumarin (7AMC), benzyloxycarbonyl-L-arginine-7AMC, benzyloxycarbonyl-L-arginine-L-arginine-7AMC, benzyloxycarbonyl-L-phenylalanine-arginine-7AMC, 4MU-β-D-cellopyranoside, 4MU-elaidate, 4MU-heptanoate, *iso*-leucine-7AMC, L-lysine-7AMC, L-methionine-7AMC, 4MU-nonoate, 4MU-oleate, 4MU-palmitate, 4MU-phosphate, 4MU-*bis*-phosphate, 4MU-pyrophosphate, L-serine-7AMC, 4MU-stearate, succinyl-benzylcysteine-7AMC, 4MU-sulfate, L-tyrosine-7AMC, and L-valine-7AMC, but not 2-deoxythymidine-5′-*p*NP-phosphate, *p*NP-phosphoryl-choline, or *p*NP-β-D-xyloside. Degrades L-tyrosine and uric acid, but not adenine or arbutin. Amyl-alcohol, D-arabinose, *iso*-butanol, butane-1,3-diol, ethanol, L-fucose (weak), gentiobiose (weak), paraffin, D-psicose, salicin, stachyose, turanose, acetate, *N*-acetyl-D-glucosamine androsterone, benzoate, citraconic acid, citrate, fumarate, gluconate, 3- and 4-hydroxybenzoate, *m*- and *p*-hydroxybenzoic acid, 2-hydroxyvalerate, malate, D-mandelic acid, pimelate, propionate, pyruvate, sebacate, testosterone, and vanillin are used as sole carbon sources, but not adonitol, amygdalin, D-arabitol, 2,3-butylene glycol, α-cyclodextrin, dulcitol, D-galacturonic acid, lactulose, methanol, methyl-β-D-galactoside, methyl-α-D-mannoside, γ-aminobutyrate, anthranilic acid, caprate, glutarate, lactate, D-lactic acid methyl ester, 2-oxyglutarate, phenylacetate, succinate, or tartrate. D- and L-Alanine, L-asparagine, L-aspartate, betaine, D-glucosamine, L-leucine, DL-norleucine, L-phenylalanine, L-proline, putrescine, L-serine, spermine, and uridine-5′-monophosphate are used as sole nitrogen sources, but not acetamide, 2,4-dinitrophenol, *p*-nitrophenol, L-pyroglutamic acid, L-threonine, or tyramine. Grows in the presence of crystal violet (0.001%, w/v) and sodium chloride (5.0%, w/v), but not in the presence of phenol (0.001%, w/v), phenyl ethanol (0.3%, v/v), and sodium azide (0.001%, w/v). Resistant to (μg per ml) gentamicin (16), neomycin (9), novobiocin (2), oleandomycin (32), penicillin (32), polymyxin (32), rifampin (4), streptomycin (4), tobramycin (8), and vancomycin (4) but sensitive to chloramphenicol (32), chlorotetracycline (4), and vancomycin (4). Additional phenotypic features are shown in Table 44. Mycolic acids have 46–54 carbon atoms with up to three double bonds. The fatty acid profile is shown in Table 47. The major menaquinone is MK-8(H_2).

Source: soil.

DNA G+C content (mol%): 66–68 (T_m).

Type strain: N805, ATCC 51786, CCUG 38518, CIP 105033, DSM 44107, JCM 9689, NBRC 100605, NCIMB 13082, VKM Ac-1986.

Sequence accession no. (16S rRNA gene): Z37138.

Sequence accession no. (gyrB): AB014315.

29. **Rhodococcus yunnanensis** Zhang, Li, Kroppenstedt, Kim, Chen, Park, Xu and Jiang 2005, 1135[VP]

yun.nan.en′sis. N.L. masc. adj. *yunnanensis* of or pertaining to Yunnan, a province of south-west China.

Gram-stain-positive actinomycete. Forms hyphae that fragment into short-rods and coccobacilli. Smooth, opaque pale yellow to orange colonies (0.5–1.0 mm in diameter) are formed on yeast extract-malt extract, tryptic-soy, and peptone-yeast extract-glucose agars. Grows at 10–40°C and optimally at 28–30°C. Optimum pH for growth is 7.0–8.0. Positive for *N*-acetyl-β-glucosaminidase, alkaline phosphatase, α- and β-galactosidase, β-glucosidase, β-glucuronidase, and lipase, but negative for acid phosphatase, cystine arylamidase, esterase lipase (C8), naphthol-AS-BI-phosphohydrolase, and valine arylamidase. Degrades Tweens 20 and 40, but not gelatin, milk, or starch. Does not reduce nitrate to nitrite. Acid is produced from acetamide. Assimilates *N*-acetyl-D-glucosamine as a sole carbon source. Acetamide is used as a sole nitrogen source. Grows in the presence of sodium chloride (12.0%, w/v). Additional phenotypic features are shown in Table 44. Mycolic acids have 44–52 carbon atoms. The fatty acid profile is shown in Table 47. The predominant menaquinone is MK-8(H_2).

Source: forest soil collected from Yunnan Province, China.

DNA G+C content (mol%): 63.5 (T_m).

Type strain: YIM 70056, CCTCC AA 204007, DSM 44837, JCM 13366, KCTC 19021, NBRC 103083, NBRC 103115.

Sequence accession no. (16S rRNA gene): AY602219.

30. **Rhodococcus zopfii** Stoecker, Herwig and Staley 1994, 109[VP]

zop′fi. i. N.L. masc. gen. n. *zopfii* of Zopf, named in honor of Wilhelm Friedrich Zopf, who described the bacterium *Rhodococcus rhodochrous.*

Gram-stain-positive actinomycete. Forms an extensive branched mycelium that fragments into irregular rod-shaped to coccoid cells. Colonies on glucose-yeast extract agar are red-orange and wrinkled. Catalase-positive. Oxidase-negative. Nitrate is reduced to nitrite. Does not produce hydrogen sulfide. Degrades DNA and uric acid. Does not produce acid from L-arabinose, cellobiose, galactose, inulin, lactose, raffinose, or rhamnose. L-Arabitol, arbutin, biphenyl, glycerol, mannose, α-methyl-D-galactoside, toluene, xylitol, γ-aminobutyrate, benzoate, fumarate, γ-hydroxybutyric acid, monoethanolamine, and sebacate are used as sole carbon sources, but not dulcitol, starch, D-xylose, or phenol. L-Leucine is used as sole nitrogen source. Resistant to phenol (0.02%, w/v). Additional phenotypic features are shown in Table 45. Mycolic acids have 33–36 carbon atoms. The fatty acid profile is shown in Table 48.

Source: a toluene-phenol bioreactor operated by the Department of Civil Engineering, University of Washington, WA, USA.

DNA G+C content (mol%): 70 (T_m).

Type strain: T1, ATCC 51349, CIP 104275, DSM 44108, JCM 9919, NBRC 100606, NRRL B-16942.

Sequence accession no. (16S rRNA gene): AF191343.

Sequence accession no. (gyrB): AB014176.

Additional remarks: utilizes biphenyl, phenol, and toluene.

Genus V. **Skermania** Chun, Blackall, Kang, Hah and Goodfellow 1997, 129[VP]

Michael Goodfellow

Sker.man′i.a. N.L. n. *Skermania* named after Victor Bruce Darlington Skerman (1921–1993), a celebrated Australian bacterial systematist.

Gram-stain-positive, non-acid-fast, nonmotile, non-sporeforming, facultative aerobic actinomycetes which **form an extensive substrate mycelium that does not fragment in undisturbed cultures**; secondary branching is rare or absent. **Short branched and unbranched aerial hyphae are visible microscopically, but not to the naked eye**. **During early stages of growth the microscopic appearance of the organism resembles a pine tree**. Colonies are orange, opaque, macroscopically dry and friable. Chemo-organotrophic with an oxidative type of metabolism. Catalase-, oxidase-, and urease-positive. Grows between 15° and 31°C.

Whole-organism hydrolysates are rich in *meso*-diaminopimelic acid, arabinose, galactose, glucose, and ribose. The peptidoglycan is of the A1γ type. Muramic acid moieties are *N*-glycolated. **Cells contain diphosphatidylglycerol, phosphatidylethanolamine, phosphatidylinositol, and phosphatidylinositol mannosides as major phospholipids, large amounts of straight-chain saturated, unsaturated and 10-methyloctadecanoic (tuberculostearic) fatty acids, and predominant amounts of hexahydrogenated menaquinones with eight isoprene units where the last two are cyclicized**. **Mycolic acids have 58–64 carbon atoms, 2–6 double bonds, and monounsaturated side chains. The fatty acid esters released on pyrolysis gas chromatography of mycolic acid esters have 16–20 carbon atoms.** The phylogenetic position of *Skermania,* as determined by 16S rRNA gene sequence analysis, is in the family *Gordoniaceae.*

The organism grows as an extensive surface scum or foam on the surfaces of aeration tanks and in mixed liquors of activated sludge plants.

DNA G+C content (mol%): 67.5 (T_m).

Type species: **Skermania piniformis** Chun, Blackall, Kang, Hah and Goodfellow 1997, 129[VP].

Further descriptive information

Phylogeny. The genus *Skermania* contains a single species, *Skermania piniformis*, which falls towards the periphery of the 16S rRNA gene tree containing genera formerly classified in the family *Gordoniaceae* (see Figure 93 in *Gordonia*, above).

Cell morphology. *Skermania piniformis* was originally called the Pine-Tree Like Organism (PTLO) because of its distinctive tree-like microscopic morphology at the beginning of the growth cycle (Blackall et al., 1988, 1989). The branching angles of skermaniae are acute, rather than right-angled as in *Gordonia amarae*, and branches near the apex are shorter than those further away. The mycelium of *Skermania piniformis* does not fragment in undisturbed cultures and secondary branching is rare. Aerial mycelium is not visible to the naked eye, but short branched and unbranched aerial hyphae can be seen microscopically. Microcolonies show the presence of phase bright spherical regions at terminal and intercalary positions in the mycelium. The septate mycelia of skermaniae taper after branch points.

The original description of *Skermania piniformis* was based on a few strains and a small number of physiological properties (Blackall et al., 1989). Subsequently, additional strains which showed the branching morphology characteristic of the PTLO were found to have a distinctive API ZYM pattern (Soddell and Seviour, 1994). These strains exhibited considerable variations in branching angles, branching lengths, and interbranching distances, but formed a homogeneous cluster together with the type strain of *Skermania piniformis* in an extensive numerical taxonomic survey of actinomycetes isolated from activated sludge (Soddell and Seviour, 1998).

Cell wall composition. Skermaniae have (a) a peptidoglycan composed of *N*-acetylglucosamine, D-alanine, and D-glutamic acid with *meso*-diaminopimelic acid (*meso*-A_2pm) and muramic acid moieties that are *N*-glycolated; (b) arabinose and galactose as diagnostic sugars (i.e., whole-organism sugar pattern type A *sensu* Lechevalier and Lechevalier (1970); (c) a phospholipid pattern consisting of diphosphatidylglycerol, phosphatidylethanolamine (taxonomically significant phospholipid), phosphatidylinositol, and phosphatidylinositol mannosides (i.e., phospholipid pattern type II *sensu* Lechevalier et al., (1981b, 1977); (d) major amounts of straight-chain, unsaturated and methyloctadecanoic (tuberculostearic) fatty acids (i.e., a type IV fatty acid pattern *sensu* Lechevalier et al., 1977); (e) mycolic acids with 58–64 carbon units, 2–6 double bonds, and monounsaturated side chains (Blackall et al., 1989); and (f) predominant amounts of hexahydrogenated menaquinones with eight isoprene units where the last two are cyclicized (Blackall et al., 1989). The fatty acid profiles of skermaniae are particularly rich in hexadecanoic ($C_{16:0}$; 20–41%), hexadecenoic ($C_{16.1}$; 15–20%), octadecenoic ($C_{18:1}$; 20–32%), and 10-methyloctadecenoic (12–24%) acids (Blackall et al., 1989).

Colony morphology. Orange, opaque, circular colonies (1–2 mm in diameter) are formed on tryptone-yeast extract-glucose agar (Blackall et al., 1989). These workers found that the growth rate of colonies was variable for no apparent reason as on some occasions colonies were formed in 9–10 d, but in other instances the same strains took 21 d for colonies of the same size to develop. Colonies are macroscopically dry and friable, but are moist and shiny when observed under the microscope; they have a pasty texture, and are difficult to emulsify and culture. Whole colonies tend to remain intact when taken from the surface of agar plates.

Nutrition and growth conditions. Skermaniae have been cultivated on yeast extract, yeast extract-glucose, and tryptone-yeast extract-glucose agars (Blackall et al., 1989; Soddell and Seviour, 1998). They grow best on media containing glycerol as a carbon source and asparagine as a nitrogen source, features they have in common with mycobacteria (Ratledge, 1982). In tryptone-yeast extract-glucose broth, strains grow as macroscopically visible colonies in a slightly turbid liquid. They grow at 15° and 31°C, poorly above 28°C, but not at 10° or 40°C.

Metabolism. The classification of *Skermania piniformis* as a strict aerobe needs to be revisited in light of *in situ* microautoradiographic studies carried out by Eales et al. (2006). The organism does not behave as a strict aerobe in foam, as expected from pure culture studies (Chun et al., 1997), but has the metabolism of a facultative aerobe capable of anaerobic nitrate respiration, as all of the substrates were taken up under anoxic (NO_3^-) conditions. Also, uptake of substrates appears to be greater under aerobic than anoxic conditions. Eales and her colleagues also pointed out that an ability to take up a substrate in the absence of oxygen does not necessarily mean that it can grow in the absence of oxygen.

Skermaniae are positive for catalase, oxidase, and urease. In addition, they are positive for acid phosphatase, esterase, esterase lipase, α-glucosidase, leucine arylamidase, α-mannosidase, and phosphoamidase, but negative for the remaining API ZYM activities (Blackall et al., 1989). They have the capacity to cleave a broad range of 7-amino-4-methylcoumarin conjugated substrates (Chun et al., 1997), and can use a diverse range of compounds as sole carbon sources for energy and growth (Soddell and Seviour, 1998). There is evidence that skermaniae produce more biomass and grow much faster on hydrophobic substrates, such as oils, than on hydrophilic substrates like glucose (Soddell and Seviour, 1996).

Ecology. *Skermania piniformis* is abundant in foams on the surface of aeration tanks in activated sludge plants, a feature it has in common with *Gordonia amarae* (Blackall et al., 1988, 1989; Soddell and Seviour, 1994, 1998). Skermaniae are commonly associated with foaming in Australian activated sludge plants, particularly in the summer months (Blackall et al., 1988; Seviour et al., 1990; Soddell and Seviour, 1994, 1998), but there is evidence that they may be more widely distributed (de los Reyes et al., 2002; Eales et al., 2005; Jenkins et al., 1984, 1993; Soddell and Seviour, 1990; Soddell et al., 1993). Because of its slow growth (2–3 weeks compared with 4–7 d for *Gordonia amarae* and related mycolic acid-containing actinomycetes) it may have been overgrown, and hence overlooked, in earlier studies using dilution plating procedures. There is evidence that *Skermania piniformis* can have a very low growth rate in activated sludge (de los Reyes et al., 2002).

Eales et al. (2005) studied the *in situ* physiology of filamentous bacteria (Pine Tree Like Organisms) from two Danish activated sludge plants with a focus on their response to hydrophobic substrates. Microautoradiographic studies showed that

the PTLO were highly selective in their substrate utilization in foams as only oleic acid was assimilated out of eight tested substrates. Oleic acid was metabolized both aerobically and anaerobically, and a low uptake was detected under anoxic conditions with nitrate and nitrite as electron acceptors. Data acquired from a combination of *in situ* techniques indicated that a high proportion of the PTLO were metabolically inactive in the foam. Most showed low respiratory activity as detected with the redox dye CTC and low intensity signals from fluorescence *in situ* hybridization (FISH) probing with 16S rRNA targeted probes. A strong correlation was observed between the accumulation of polyhydroxyalkanoates in filaments and the intensity of their fluorescent FISH signals.

In a further investigation, Eales et al. (2006) studied the *in situ* physiology of *Skermania piniformis* in foam samples taken from two geographically distinct Australian activated sludge plants. A 16S rRNA probe, Spin 1449, designed for the identification of *Skermania piniformis* by FISH was validated using pure cultures and applied to the foam samples. Filaments of the target organism appeared to be comparatively hydrophobic though no clear preference was shown for hydrophobic or hydrophilic substrates. Microautoradiographic studies showed that filaments selectively took up substrates under aerobic and anoxic (NO_3^-) conditions, but not under anaerobic or anoxic (NO_2^-) regimes. Eales and her colleagues concluded from their substrate uptake data that an anaerobic selector might prove to be effective in the control of *Skermania piniformis* in activated sludge systems.

The results from the *in situ* physiology study of *Skermania piniformis* differ markedly from those obtained from the Danish PTLO scum FISH-MAR experiments, as the *Skermania piniformis* strains from the Australian plants used a more diverse range of substrates albeit only in the presence of oxygen and nitrate. In contrast, the Danish PTLO were able to take up substrates under anaerobic and anoxic conditions with nitrite as an electron acceptor. These results suggest either that the PTLO associated with the Danish scums were members of mycolic acid-containing taxa other than *Skermania piniformis* or that important physiological differences exist between strains in the two countries. In either case, developing universal control strategies for *Skermania piniformis* may prove to be difficult.

The aerobic metabolic capabilities of *Skermania piniformis* in pure culture studies (Soddell and Seviour, 1998) are broader than those found in the *in situ* studies of Eales et al. (2006). Neither the hydrophilic substrates, glucose nor acetate, were taken up by *Skermania piniformis* under *in situ* conditions though other bacteria in the foams were able to do so. The differences from the pure culture data are in line with studies which show that bacteria in laboratory culture do not necessarily behave the same way as they do in complex natural habitats.

Isolation procedures

The first successful isolation of *Skermania piniformis* from activated sludge foam and mixed liquor involved the use of a micromanipulatory technique (Blackall et al., 1989). A Skerman micromanipulator (Skerman, 1968) was used to transfer acute angled, branching filaments from activated sludge samples, collected from plants in Queensland, Australia, onto either yeast extract-glucose agar or tryptone-yeast extract-glucose agar plates, which were incubated at a range of temperatures. Visible colonies of *Skermania piniformis* (1–2 mm in diameter) grew on the isolation plates within 10–21 d. Additional strains have been isolated from mixed liquor and foam samples from activated sludge plants across Australia using the Skerman micromanipulator, as described by Soddell and Seviour (1994).

Maintenance procedures

Skermaniae can be maintained and preserved using the procedures described for *Gordonia* strains.

Differentiation of the genus *Skermania* from other genera

Identification of *Skermania piniformis* is based on its distinctive morphology whereby individual filaments show acute angled branching patterns. The organism is currently the only validly published member of the order *Corynebacteriales* with the PTLO morphotype. However, similar branching patterns have been observed in pure cultures of *Rhodococcus* and *Tsukamurella* strains (Soddell and Seviour, 1994; Stainsby et al., 2002) thereby raising the possibility that some PTLO organisms may be members of unclassified mycolic acid-containing taxa. Consequently, skermaniae cannot be unambiguously recognized solely on the basis of morphology, especially since they exhibit a range of branching variations (Soddell and Seviour, 1994, 1998).

Skermania piniformis can be separated from other genera classified in the order *Corynebacteriales* (which have an oxidative metabolism and *N*-glycolated muramic acid moieties in the wall peptidoglycan) using a combination of chemotaxonomic and morphological features (Table 29, in the order *Corynebacteriales*, above), and by using the oligonucleotide probe, Spin 1449 (Eales et al., 2006). It can be distinguished from the other three genera assigned to the family *Gordoniaceae* using the probe and by its distinctive menaquinone profile.

Taxonomic comments

The monospecific genus *Skermania* was proposed by Chun et al. (1997) for organisms which had been designated first as Pine-Tree Like Organisms because of their distinctive tree-like microscopic morphology (Blackall et al., 1988, 1989) and then as *Nocardia pinensis* Blackall et al. 1989. The organism was assigned to the genus *Nocardia* on the basis of chemotaxonomic, morphological, and physiological properties, but its taxonomic position was equivocal given the presence of mycolic acids monounsaturated in the 2-position, a distinctive antibiotic sensitivity pattern, and a relatively slow growth rate. Initial 16S rRNA gene sequence data cast doubt on the association with the genus *Nocardia* (Blackall et al., 1994).

Chun and his colleagues found that an almost complete 16S rRNA gene sequence of the type strain of *Nocardia pinensis* showed almost equal levels of relatedness to corresponding sequences of *Gordonia* strains (levels of similarity, 94.9–95.9%), *Nocardia* strains (94.5–95.9%), and *Rhodococcus* strains (94.5–95.9%), and lower levels of homology with *Corynebacterium* strains (91.4–92.6%), *Tsukamurella* strains (93.7–93.8%), and to the type strain of *Dietzia maris* (91.4–92.6%). The results of additional 16S rRNA gene sequence analyses taken together with the discontinuous distribution of key chemical markers confirm the taxonomic status of the genus *Skermania* (Adachi et al.,

2007; Goodfellow et al., 1998a; Goodfellow and Maldonado, 2006; Soddell et al., 2006a). However, further comparative taxonomic studies are needed on *Skermania piniformis* and related strains as there is evidence that the genus may be underspeciated (Soddell and Seviour, 1998).

Acknowledgements

The author is indebted to Professor Robert J. Seviour (La Trobe University) for his expert advice on the ecology of *Skermania piniformis*.

List of species of the genus *Skermania*

1. **Skermania piniformis** (Blackall, Parlett, Hayward, Minnikin, Greenfield and Harbers 1989) Chun, Blackall, Kang, Hah and Goodfellow 1997, 129[VP] *Nocardia pinensis* Blackall, Parlett, Hayward, Minnikin, Greenfield and Harbers 1989, 1555).

pi. ni. for′ mis. N.L. n. *pinus*, a pine, pine-tree; L. adj. suffix – *formis*, *-is*, *-e* in the form of; N.L. fem. adj. *piniformis*, pine-like, pertaining to the pine-like appearance of young microcolonies.

Aerobic, Gram-stain-positive, non-acid-fast actinomycetes which during the early stages of growth (24 h) have a microscopic appearance that resembles a pine tree. Colonies (1–2 mm in diameter) form on tryptone-yeast extract-glucose agar within 10–21 d though growth is variable. Cells from activated sludge plants and from artificial culture media contain intracellular sudanophilic and polyphosphate inclusions.

Positive for acid phosphatase, esterase (C4), esterase lipase (C8), α-glucosidase, leucine arylamidase, α-mannosidase, and phosphoamidase in API ZYM tests. Does not hydrolyze esculin. Nitrate is not reduced. Degrades Tweens 20, 40, and 60, but not casein, elastin, gelatin, hypoxanthine, tyrosine, or xanthine. Fructose, glucose, glycerol, mannose, sucrose, and trehalose are used as sole carbon sources, but not arabinose, cellobiose, ethanol, galactose, inositol, inulin, lactose, maltose, mannitol, raffinose, rhamnose, sorbitol, or xylose (all at 0.1%, v/v). Similarly, acetate, butyrate, propionate, and pyruvate are used as sole carbon sources at 0.01%, w/v, but not acetamide, adipate, benzamide, benzoate, citrate, *p*-cresol, fumarate, gluconate, *m*-or *p*-hydroxybenzoate, lactate, malate, octanoate, pimelate, succinate, tartrate, testosterone, or tyrosine. Does not use acetamide, serine, or trimethylamine as sole carbon and nitrogen sources.

Sensitive to (μg/ml) 5-fluorouracil (20), isoniazid (2), lysozyme (50), mitomycin C (5), or rifampin (20) when glycerol broth is used as the basal medium. Grows in the presence of sodium azide (0.02%, w/v), sodium chloride (7%, w/v), and penicillin (10 IU).

Forms an extensive surface scum or foam on the surface of aeration tanks of activated sludge plants.

DNA G+C content (mol%): 65.7 (T_m).

Type strain: ATCC 49497, CIP 104516, UQM 3063, DSM 43998, IFO (now NRBC) 15059.

Sequence accession no. (16S rRNA gene): Z35435.

Genus VI. **Smaragdicoccus** Adachi, Katsuta, Matsuda, Peng, Misawa, Shizuri, Kroppenstedt, Yokota and Kasai 2007, 300[VP]

Hiroaki Kasai

Sma.rag.di.coc′cus. L. n. *smaragdus* malachite; N.L. masc. n. *coccus* (from Gr. masc. n. *kokkos*) grain; N.L. masc. n. *Smaragdicoccus* malachite(-colored) coccus.

Coccoidal cells without branching (0.86 μm in diameter). Stain Gram-positive, do not form spores. Multiply by binary fission. **Nonmotile**. **Colonies are malachite green-like colored**, round, and 1–3 mm in diameter after 7–14 d on 1/10 TSA at 30°C. The cell wall contains *meso*-diaminopimelic acid and arabinose and galactose, corresponding to the chemotype IV-A of Lechevalier and Lechevalier (1970). Cell wall acyl type is glycolyl. Predominant cellular fatty acids are straight-chain saturated and monounsaturated acids. 10-methyl branched acids are in trace amounts. Mycolic acids are C_{43}–C_{49}. Polar lipids are diphosphatidylglycerol, phosphatidylethanolamine, phosphatidylinositol, phosphatidylinositol mannoside and some glycolipids. Two unusual menaquinones are present, MK-8(H_4, ω-methylenecycl) and MK-8(H_4, dicycl).

DNA G+C content (mol%): 63.7.

Type species: **Smaragdicoccus niigatensis** Adachi, Katsuta, Matsuda, Peng, Misawa, Shizuri, Kroppenstedt, Yokota and Kasai 2007, 300[VP].

Further descriptive information

Cells are regular cocci (Figure 97). The whole-cell hydrolysate contains *meso*-diaminopimelic acid, alanine, glutamic acid, as found in the cell-wall type A1γ of Schleifer and Kandler (1972). Following hydrolysis by the method of Uchida and Aida (1977), cell walls contain glycolyl residues. The components of the cell wall are similar to those of other members of the family *Nocardiaceae*. However, the cellular fatty acids are composed of

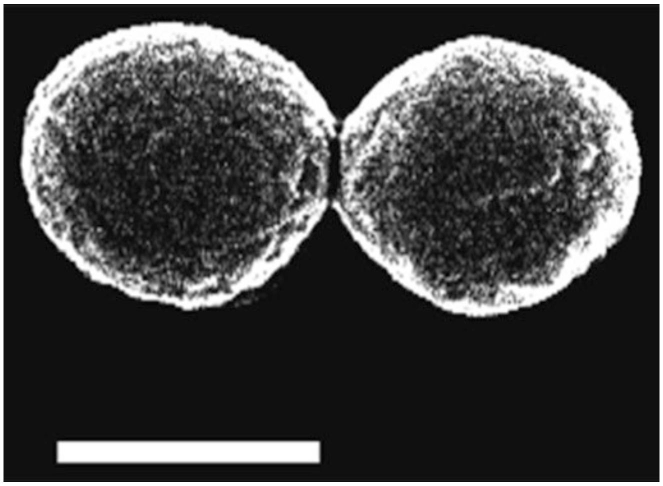


FIGURE 97. Scanning electron micrograph of cells of strain *Smaragdicoccus niigatensis* Hou_blue[T]. Cells grown for 14 d at 30°C on 1/10 TSA were fixed with 2.5 % glutaraldehyde. Bar, 1 µm.

straight-chain saturated and monounsaturated acids, and only traces of 10-methyl branched acids, which are common in the family *Nocardiaceae*, are present. Two cyclic menaquinones were detected by HPLC/PDA/MS/MS systems, one was MK-8(H_4, ω-methylenecycl) and the other was MK8 (H_4, dicycl) (Figure 98). MK-8(H_4, ω-methylenecycl) possessed the same UV and MS/MS profiles as MK-8(H_4, ω-cycl), which is diagnostic for *Nocardia* (Collins et al., 1987; Howarth et al., 1986). However, the ω-ring of the *Smaragdicoccus* menaquinone is 1,1-dimethyl-3-methylenecyclohexane and that of the *Nocardia* menaquinone is 1,5,5-trimethylcyclohex-1-ene (Figure 98). The 16S rRNA (AB243007) and *gyrB* (AB243008) gene sequences support the assignment of this genus to the family *Nocardiaceae*.

Enrichment and isolation procedures

Smaragdicoccus was isolated using hexadecane as the sole carbon source from an enrichment culture of petroleum-contaminated soil. The soil was found near a spurt of petroleum at Nishiyama-cho in Niigata, Japan. Malachite-green-like colored colonies appeared after 7 d on an inorganic medium (Peng et al., 2003) when incubated in a hexadecane-saturated atmosphere at 25°C.

Differentiation of the genus *Smaragdicoccus* from other genera

The results of 16S rRNA and *gyrB* gene sequence analyses (Figure 99), the cell-wall and peptidoglycan characterization, and the polar lipid analyses indicate that *Smaragdicoccus* belongs to the family *Nocardiaceae*. The profiles of quinones, fatty acids and mycolic acids indicate that *Smaragdicoccus* is an independent genus in this family.

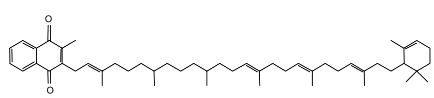
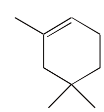
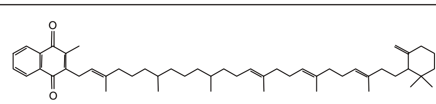
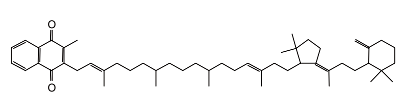
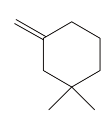
Genus	Structure of menaquinone	Structure of ω-ring
Nocardia	 MK-8(H_4, ω-cycl)	 1,5,5-trimethylcyclohex-1-ene
Smaragdicoccus	 MK-8(H_4, ω-methylenecycl)  MK-8(H_4, dicycl)	 1,1-dimethyl-3-methylenecyclohexane

FIGURE 98. Chemical structures of the menaquinones found in *Smaragdicoccus* and *Nocardia*.

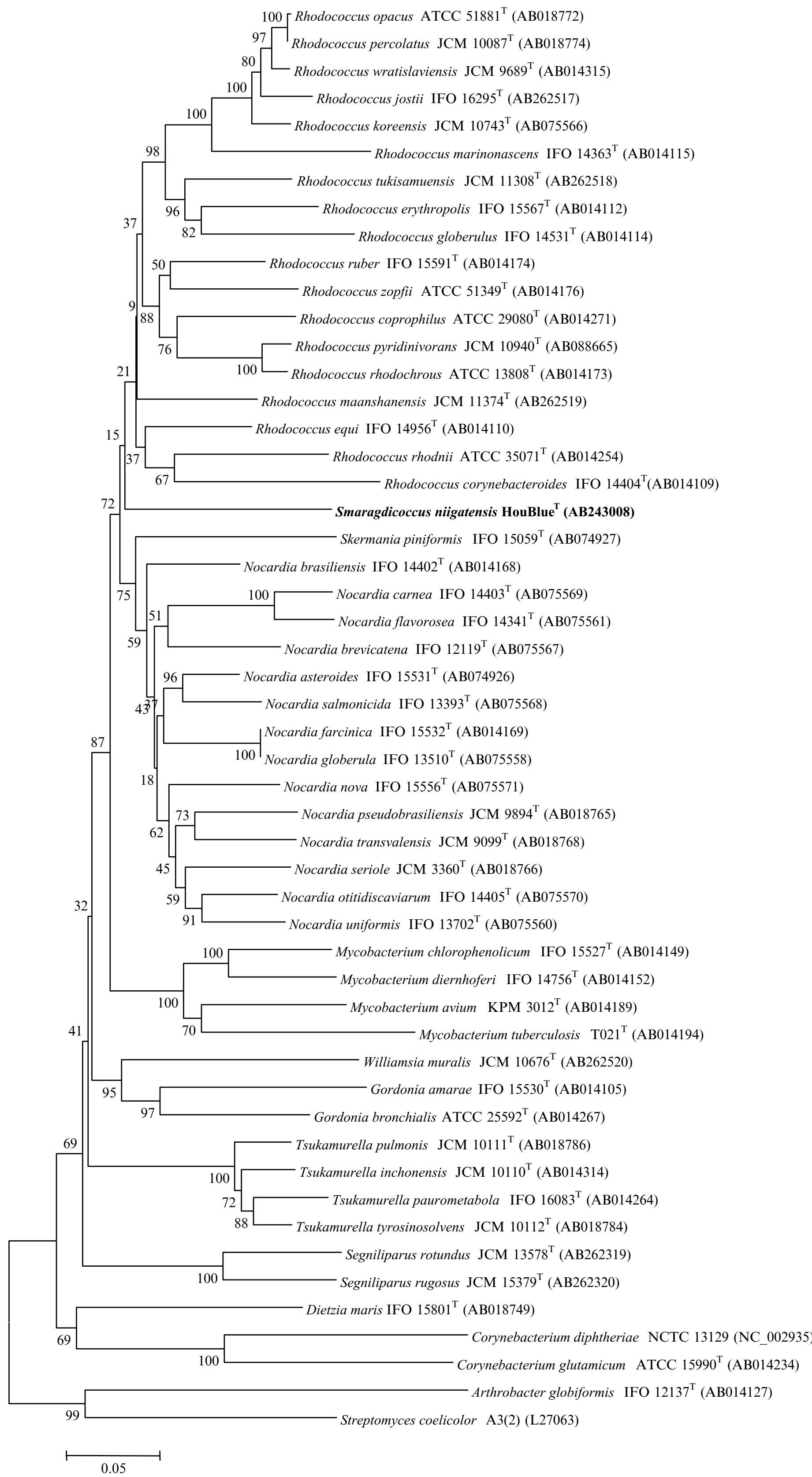


FIGURE 99. Neighbor-joining tree based on *gyrB* sequence comparisons showing the phylogenetic position of strain *Smaragdicoccus niigatensis* Hou_blue^{T}. 1260-nucleotide-long *gyrB* sequences were used for genetic distances calculation based on the Kimura two-parameter model. Bar, 5 nucleotide substitutions per 100 nucleotides.

List of species of the genus *Smaragdicoccus*

1. **Smaragdicoccus niigatensis** Adachi, Katsuta, Matsuda, Peng, Misawa, Shizuri, Kroppenstedt, Yokota and Kasai 2007, 300^{VP}

ni.i.ga.ten′sis. N.L. masc. adj. *niigatensis* of or belonging to Niigata Prefecture of Japan, the source of the soil from which the organism was isolated.

Coccoidal cells without branching (0.86 µm in diameter). The type strain Hou_blue^{T} utilizes the following carbon sources after 14 d of incubation at 30°C: D-fructose, D-glucose, sodium n-butyrate, and hexadecane. After prolonged incubation of 1 month, growth with sucrose is observed. Better growth is observed with 0.1% than 1% of these carbon sources. The following carbon sources were not used for growth: L-arabinose, *myo*-inositol, D-mannitol, L-rhamnose, raffinose, and D-xylose. Esterase activity (C4), esterase lipase (C8), lipase (C4), leucine arylamidase, naphthol-AS-BI-phosphohydrolase activities were detected by API ZYM enzyme assay.

The type strain was isolated from petroleum-contaminated soil at Nishiyama-cho, Niigata, Japan.

DNA G+C content (mol%): 63.7 (HPLC) for type strain.

Type strain: Hou_blue^{T}, CIP 109538, DSM 44881, IFM 10815, JCM 14666, MBIC 06267.

Sequence accession nos: AB243007 (16S rRNA gene) and (*gyrB*) AB243008.

Genus VII. **Williamsia** Kämpfer, Andersson, Rainey, Kroppenstedt and Salkinoja-Salonen 1999, 686^{VP}

Peter Kämpfer

Wil.li.am′si.a. N.L. fem. n. *Williamsia* named to honor Stanley Thomas Williams (1937–2004), a British microbiologist, for his numerous contributions to the taxonomy and ecology of actinomycetes.

Gram-stain-positive, non-sporing actinomycete which forms short rods. Aerobic and chemoheterotrophic. **The diagnostic amino acid** is ***meso*-diaminopimelic acid and the major cell-wall sugars are arabinose, galactose, mannose, and ribose.** Short chain mycolic acids are present (carbon chain length, C_{50}–C_{56}). The fatty acid pattern is mainly composed of straight-chain saturated and monounsaturated fatty acids. **Tuberculostearic acid is present in large amounts (>20% of the whole-cell fatty acids). The major polar lipids are phosphatidylethanolamine**, **phosphatidylinositol**, **phosphatidylglycerol, and diphosphatidylglycerol**. **Dihydrogenated menaquinone with nine isoprene units is the only isoprenologue**. On the basis of 16S rRNA gene sequence analysis, it is a member of the family *Gordoniaceae*.

DNA G+C content (mol%): 64–65 (HPLC).

Type species: **Williamsia muralis** Kämpfer, Andersson, Rainey, Kroppenstedt and Salkinoja-Salonen 1999, 686^{VP}.

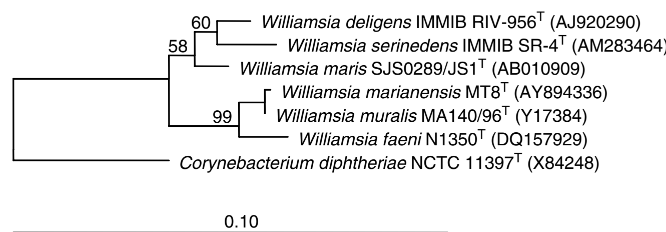


FIGURE 100. Phylogenetic analysis of *Williamsia* species based on 16S rRNA gene sequences available from the European Molecular Biology Laboratory data library (accession numbers in *parentheses*). The phylogenetic tree was constructed using the ARB software package (version December 2007; Ludwig et al., 2004) and the corresponding SILVA SSURef 95 database (release July 2008; Pruesse et al., 2007). Tree building was performed with the neighbor-joining method and without filters. Bootstrap values > 50% based on 1000 replications are listed as percentages at the branching points. Bar = 0.10 nucleotide substitutions per nucleotide position.

Further descriptive information

The genus *Williamsia* was proposed by Kämpfer et al. (1999) to accommodate an unusual mycolic-acid-containing actinomycete. Based on its mycolic acid profile, *Williamsia* occupies an intermediate position between *Rhodococcus* (mycolic acid chain length of 34–45 carbon atoms) and *Gordonia* (mycolic acid chain length 54–66 carbon atoms) (Kämpfer et al., 1999). The taxon currently contains six species with validly published names, *Williamsia deligens* (Yassin and Hupfer, 2006), *Williamsia faeni* (Jones et al., 2009), *Williamsia maris* (Stach et al., 2004), *Williamsia muralis* (Kämpfer et al., 1999), *Williamsia marianensis* (Pathom-aree et al., 2006), and *Williamsia serinedens* (Yassin et al., 2007b). Strains of these species were isolated from human blood, soil, meadow hay, deep-sea sediment, and indoor building materials. They form a distinct 16S rRNA group within the evolutionary radiation occupied by mycolic acid-containing actinomycetes, classified in the order *Corynebacteriales*, formerly suborder *Corynebacterineae* Stackebrandt et al. (1997). The range of 16S rRNA gene sequence similarities is 95.4–99.9%. A phylogenetic tree based on calculations with the maximum-likelihood algorithm is shown in Figure 100. Species of this genus are characterized by the presence of *meso*-A_2pm, arabinose, and galactose in whole-organism hydrolysates (wall chemotype IV *sensu* Lechevalier and Lechevalier, 1970), *N*-glycolyl muramic acid residues, dihydrogenated menaquinone with nine isoprene units as the sole isoprenologue, major proportions of straight chain, unsaturated, and tuberculostearic acids (fatty acid type 1b; (Kroppenstedt, 1985), phosphatidylethanol, diphosphatidylglycerol, phosphatidylglycerol, and phosphatidylinositol as major polar lipids (phospholipid type II *sensu* Lechevalier et al., 1977), and by mycolic acids that co-migrate with those of *Williamsia muralis* DSM 44343^{T} (Goodfellow and Maldonado,

2006; Pathom-aree et al., 2006; Yassin and Hupfer, 2006). The fatty acid profile is dominated by palmitic acid ($C_{16:0}$), hexadecenoic acid ($C_{16:1}$), oleic acid ($C_{18:1}$), and tuberculostearic acid (10-methyl octadecanoate). *Williamsia muralis* contains significant amounts of palmitoleic acid ($C_{16:1}$ *cis*9) whereas *Williamsia marianensis* has a fatty acid provisionally identified as hexadecenoic acid (Pathom-aree et al., 2006). In addition, *Williamsia muralis* contains a minor amount of $C_{16:1}$ *cis*11 (Kämpfer et al., 1999). The fatty acid profiles of *Williamsia marianensis* and *Williamsia muralis* are distinct from that found for the type strain of *Williamsia deligens* which was reported to contain only minor amounts of $C_{16:1}$ and significant quantities (40%) of the longer chain (C_{20}) saturated and unsaturated fatty acids (Yassin and Hupfer, 2006).

Enrichment and isolation procedures

No specific isolation medium has been described so far. Good growth occurs on nutrient rich media, like Brain heart Infusion agar, Tryptone Soy agar (TSA), and R2A agar (all from BBL Microbiology Systems), and glucose-yeast-extract agar (Gordon and Mihm, 1962) at 20–30°C.

Maintenance procedures

Williamsia cultures may be lyophilized by common procedures used for many bacteria (see also the chapter on *Gordonia*). In addition, cultures can be maintained by serial transfer on solid complex media. Growth on agar slants in screw-capped tubes can be kept at 4°C for about 2–4 weeks. Long-term preservation of liquid cultures supplemented with 5% dimethylsulfoxide is recommended in the vapor phase of liquid nitrogen (−140°C).

Differentiation of the genus *Williamsia* from other genera

Williamsia may be distinguished form other genera in the order *Corynebacterales* by the cell-wall peptidoglycan, menaquinone type, colony pigmentation, DNA G+C content, and fatty acid composition (Table 29 in the chapter on the *Corynebacteriales*). Differentiation from the genus *Gordonia* is mainly based on 16S rRNA gene sequence analysis.

List of species of the genus *Williamsia*

1. **Williamsia muralis** Kämpfer, Andersson, Rainey, Kroppenstedt and Salkinoja-Salonen 1999, 686[VP]

mu.ra′lis. L. fem. adj. *muralis* pertaining or belonging to wall(s).

Coccoid cells without branching (0.4–0.5 µm in width, 0.6–1.4 µm in length). Smooth, saffron yellow colored colonies formed on TSB agar (BBL). The temperature range for growth is 10–37°C; the optimum is 30°C, and no growth is detected at 4°C or 41°C. Good growth occurs on Nutrient Agar (Difco), R2A agar (Difco), and TSA (BBL). Acetate, adonitol, citrate, D-fructose, fumarate, D-gluconate, D-mannitol, D-mannose, propionate, putrescine, sorbitol, sucrose, and pyruvate are utilized (after 4 d of incubation at 30°C) as sole sources of carbon; L-alanine, D-glucose, DL-3-hydroxybutyrate, and L-proline are utilized after 10 d of incubation. pNP-α-D-glucopyranoside and *bis*-pNP-phosphate are hydrolyzed. Further physiological characteristics are given in Table 49.

Source: indoor building material, Finland.

DNA G+C content (mol%): 64.8 (HPLC).

Type strain: MA 140–96, CIP 106979, DSM 44343, IFM 10085, JCM 10676, KCTC 9830.

Sequence accession no. (16S rRNA gene): Y17384.

2. **Williamsia deligens** Yassin and Hupfer 2006, 196[VP]

de. li′gens L. part. adj. *deligens* choosing, selecting, referring to the preference of carbon source.

Gram-stain-positive, rod and coccoid-like cells, are partially acid–alcohol-fast. Smooth, orange to orange-red pigmented colonies are found on agar media. Grows over the temperature range 22–37°C, but not at 42°C. Its mycolic acids are cleaved on pyrolysis to release $C_{16:0}$ and $C_{18:0}$ fatty acids as the major cleavage products. Hydrolyzes urea, but not esculin. Does not degrade adenine, casein, elastin, gelatin, guanine, hypoxanthine, testosterone, tyrosine, or xanthine. Utilizes acetate, 2,3-butandiol, citrate, glucose, mannitol, paraffin, sucrose, sorbitol, and trehalose as carbon sources. Does not utilize adipate, iso-amyl alcohol, cellobiose, gluconate, lactate, lactose, melezitose, 1,2-propandiol, or raffinose. Utilizes L-alanine, but not acetamide, arginine, gelatin, ornithine, proline, or serine as carbon and nitrogen sources. Further physiological characteristics are given in Table 49.

Source: human blood.

DNA G+C content (mol%): not determined.

Type strain: IMMIB RIV-956, CCUG 50873, DSM 44902, JCM 13662.

Sequence accession no. (16S rRNA gene): AJ920290.

3. **Williamsia faeni** Jones, Payne and Goodfellow 2010, 2550[VP]

fa.e′ni. L. n. *faenum* hay; L. gen. n. *faeni* of hay, referring to its isolation from a hay meadow.

Forms coccoid elements. Irregular, convex, matt yellow pink pigmented colonies are produced on glucose-yeast extract agar after incubation for 5 d at 28°C. Grows between 10 and 30°C, but not at 37°C. Hydrolyzes allantoin and urea, but not arbutin. DNA, RNA, starch, and uric acid are degraded, but not adenine, chitin, elastin, xanthine, or xylan. D(−)-Amygdalin, D(−)-arabinose, D(+)-arabitol, arbutin, D(−)-fructose, D(−)-fucose, D(−)-glucose, inulin, D(+)-lactose, D(+)-mannose, D(+)-melibiose, α-methyl-D-glucoside, D(−)-ribose, and D(+)-turanose are used as sole carbon sources for energy and growth, but not dulcitol or salicin (all at 1%, w/v); butan-1,3-diol, butan-1,4-diol, butan-1-ol, butan-2,3-diol, ethanol, propan-1-ol, and propan-2-ol are also used as sole carbon sources (all at 1%, v/v); with *iso*-amyl alcohol, benzoic acid, fumaric acid, glycerol, glycogen, L-(+)-lactic acid, L-malic acid, oleic acid, propanoic acid, pyruvic acid, sodium acetate, sodium *n*-butyrate, L-(+)-tartaric acid, valeric acid, and xylitol used as carbon sources (all at 0.1%, w/v),

TABLE 49. Differential characteristics between the *Williamsia* species[a]

Characteristic	*W. muralis* DSM 44343[T]	*W. deligens* DSM 44902[T]	*W. faeni* N1350[T]	*W. marianensis* MT8[T]	*W. maris* DSM 44693[T]	*W. serinedens* DSM 45037[T]
Esculin hydrolysis	–	–	+	–	nd	–
Growth on sole carbon sources at 1 % (w/v):						
Adonitol	+	–	+	–	–	+
L(+)Arabinose/L(–)	+/–	–	+	+	–	+
D(+)Cellobiose	–	–	+	–	–	–
m-Erythritol	+	–	nd	+	–	nd
D(+)Galactose/D(–)	–	–	+	–	+/–	+
m-Hydoxybenzoate	+	–	nd	–	–	nd
p-Hydroxybenzoate	–	–	nd	–	+	nd
m-Inositol	–	–	+	–	+/–	–
D(+)Maltose	–	+	+	–	+/–	+
D(–)Mannitol	+	+	+	+	–	+
D(+)Melibiose	+	–	+	–	–	+
D(+)Raffinose	–	–	+	–	–	–
D(+)Rhamnose	+	–	+	+	+/–	–
D(–)Sorbitol	+	+	+	+	–	+
D(+)Sucrose	+	+	+	+	–	+
D(+)Trehalose	–	+	+	+	+	+
D(+)Xylose	–	+	+	–	+	+
Growth on sole carbon sources at 0.1%(w/v):						
m-Hydroxybenzoic acid	+	–	–	–	–	+
p-Hydroxybenzoic acid	–	–	+	–	–	–
Growth on:						
5%, w/v NaCl	–	–	nd	+	–	nd
7%, w/v NaCl	–	–	nd	+	–	nd
Growth at 1 %, v/v:						
1,2 Propanediol	–	–	+	–	+/–	+
Growth at:						
4°C	–	–	+	+	–	–
10°C	+	–	+	+	+	+
37°C	+	+	nd	–	+	nd
45°C	+	–	–	–	–	–

Symbols: +, positive; –, negative; nd, not determined; +/–, different results were reported by Stach et al. (2004) and Jones et al. (2009).

[a] Results for the reference strains taken from Kämpfer et al. (1999), Jones et al. (2009), Stach et al. (2004), Yassin and Hupfer (2006), and Pathom-aree et al. (2006).

but not adipic acid, citric acid, glutaric acid, malonic acid, D-mandelic acid, oxalic acid, sebacic acid, suberic acid, or succinic acid. Acetamide, L-alanine, L-aminobutyl, L-arginine, L-gelatin, D-gluconic acid, L-glycine, histidine, L-leucine, DL-methionine, monoethanolamine, DL-norleucine, L-norvaline, DL-phenylalanine, L-proline, serine, uric acid, urea, and L-valine are used as sole carbon and nitrogen sources (all at 0.1%, w/v), but not L-cysteine, L-glutamic acid, L-isoleucine, or L-ornithine. The fatty acid profile includes major amounts of hexadecanoic ($C_{16:0}$, 21% of total), monosaturated octadecanoic ($C_{18:1}$, 15%), tridecanoic ($C_{13:0}$, 11%), tuberculostearic (10-methyl octadecanoate [10-methyl $C_{18:0}$], 8%) and octadecanoic ($C_{18:0}$, 7%) acids; minor components include tetradecanoic ($C_{14:0}$), pentadecanoic ($C_{15:0}$), *iso*-hexadecanoic ($C_{16:0}$ iso), and eicosanoic acids ($C_{20:0}$). Further physiological characteristics are given in Table 49.

Source: a hay meadow plot at Cockle Park Experimental Farm, Northumberland, UK.

DNA G+C content (mol%): not determined.

Type strain: N1350, DSM 45372, NCIMB 15474, NRRL B-24794.

Sequence accession no. (16S rRNA gene): DQ157929.

4. **Williamsia marianensis** Pathom-aree, Nogi, Sutcliffe, Ward, Horikoshi, Bull and Goodfellow 2006, 1125[VP]

ma.ri.an′ensis. N.L. fem. adj. *marianensis* of or pertaining to the Mariana Trench, the source of the isolate.

Aerobic, Gram-stain-positive, non-acid–alcohol-fast, non-motile actinomycete which forms short rods and coccoid-like elements. Round, entire, convex, orange colonies with smooth matt surfaces are formed on glucose-yeast extract agar after 5 d incubation at 28°C. Good growth occurs on

TSA between 4 and 30°C, with an optimum temperature around 28°C. Neither arbutin nor esculin are hydrolyzed. Casein, cellulose, hypoxanthine, starch, and uric acid are degraded, but not gelatin, guanine, Tween 80, L-tyrosine, or xanthine. D-Fructose, D-mannitol, D-mannose, D-sucrose, D-sorbitol, and xylitol are used as sole carbon sources for energy and growth, but not L-arabitol, D-cellobiose, dextran, dextrin, D-glycerol, glycogen, D-melezitose, D-raffinose, D-salicin, or L-sorbose. Further physiological characteristics are given in Table 49.

Source: deep-sea sediment taken from the Mariana Trench.

DNA G+C content (mol%): 65.4 (HPLC).

Type strain: MT8, DSM 44944, JCM 14345, NCIMB 14085.

Sequence accession no. (16S rRNA gene): AY894336.

5. **Williamsia maris** Stach, Maldonado, Ward, Bull and Goodfellow 2004, 193[VP]

ma′ris. L. neut. n. *mare*, the sea; L. gen. n. *maris* of the sea.

Aerobic, Gram-stain-positive, non-acid–alcohol-fast, nonmotile actinomycete which forms short rods and coccoid-like elements. Round, convex, orange colonies are formed on glucose-yeast extract agar after 5 d incubation at 28°C; the colonies assume a pinkish hue after incubation for 5–10 d. Good growth on modified Bennett's and Tryptic Soy agars. Growth occurs between 10 and 37°C, with an optimum around 28°C. Utilizes glycerol, glycogen, *meso*-inositol, L-rhamnose, L-sorbose, and D-xylose as sole sources of carbon for energy and growth, but not D-arabitol, L-arabitol, dextran, dextrin, dulcitol, D-galactose, inulin, α-lactose, D-melezitose, α-D-methyl-glucopyranoside, D-raffinose, D-ribose, D-salicin, xylan, or xylitol. Further physiological characteristics are given in Table 49.

Source: sediment collected from the Sea of Japan at a depth of 289 m.

DNA G+C content (mol%): not determined.

Type strain: SJS0289/JS1, DSM 44693, JCM 12070, KCTC 9945, NCIMB 13945.

Sequence accession no. (16S rRNA gene): AB010909.

6. **Williamsia serinedens** Yassin, Young, Lai, Hupfer, Arun, Shen, Rekha and Ho 2007b, 560[VP]

se.ri.ne′dens. N.L. n. *serinum* serine; L. part. pres. *edens* eating; N.L. part. adj. *serinedens* eating serine.

Forms smooth, orange to orange-red pigmented colonies on agar media. Cells are Gram-stain-positive rods and are non-acid–alcohol-fast. Grows at 22–30°C, but not at higher temperatures. Contains the salient chemotaxonomic characteristics of the genus *Williamsia*. Its mycolic acids were cleaved on pyrolysis to release $C_{16:0}$ and $C_{18:0}$ fatty acids as the major products. The fatty acid profile consists mainly of straight-chain saturated, unsaturated, and 10-methyl branched fatty acids. Hydrolyzes gelatin and urea but not adenine, casein, elastin, esculin, guanine, hypoxanthine, testosterone, tyrosine, or xanthine. Assimilates acetate, adonitol, L-arabinose, 2,3-butandiol, citrate, *meso*-erythritol, galactose, glucose, *p*-hydroxybenzoate, lactate, maltose, mannitol, paraffin, 1,2-propandiol, sucrose, sorbitol, trehalose, and xylose as carbon sources, but not adipate, isoamyl alcohol, cellobiose, gluconate, *m*-hydroxybenzoate, *myo*-inositol, lactose, melezitose, raffinose, or rhamnose. Utilizes acetamide, L-alanine, and serine as simultaneous carbon and nitrogen sources, but not arginine, gelatin, ornithine, or proline.

Source (type strain): an oil-contaminated soil sample in Chyai County, Taiwan.

DNA G+C content (mol%): not determined.

Type strain: IMMIB SR-4, DSM 45037, CCUG 53151, JCM 14883.

Sequence accession no. (16S rRNA gene): AM283464.

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Family V. **Segniliparaceae** Butler, Floyd, Brown, Toney, Daneshvar, Cooksey, Carr, Steigerwalt and Charles 2005, 1621[VP] emend. Zhi, Li and Stackebrandt 2009, 595

W. Ray Butler

Seg.ni.li.pa.ra.ce′a.e. N.L. masc. n. *Segniliparus* type genus of the family; suff. *-aceae* ending to denote a family; N.L. fem. pl. n. *Segniliparaceae* the *Segniliparus* family.

Aerobic, acid–alcohol-fast, nonmotile, nonsporeforming, rod-shaped cells (0.4 µm in diameter). Does not show branching or produce an aerial mycelium. Strains form smooth and rough colony variants. **Mesophilic**, **with an optimum growth temperature of 33°C**. Visible growth in 3–4 d. **Branched β-hydroxy fatty acid in the cell wall is of an alpha-chemical mycolic acid structural type**. **The principal diamino acid of the peptidoglycan is *meso*-diaminopimelic acid**. Contains tuberculostearic acid. Found in the environment. The family is represented by a single genus, *Segniliparus.*

DNA G+C content (mol%): 68–72.

Type genus: **Segniliparus** Butler, Floyd, Brown, Toney, Daneshvar, Cooksey, Carr, Steigerwalt and Charles 2005, 1621[VP].

Genus I. **Segniliparus** Butler, Floyd, Brown, Toney, Daneshvar, Cooksey, Carr, Steigerwalt and Charles 2005, 1621[VP]

W. Ray Butler

Seg.ni.li.pa′rus. L. adj. *segnis* slow; Gr. adj. *liparos* fatty; N.L. masc. n.*Segniliparus* the slow fatty one, the one with slow fats, to indicate the possession of slowly reacting fatty acids, i.e. late-eluting mycolic acids detected with HPLC.

Aerobic, acid–alcohol-fast, nonmotile, actinomycete which forms straight, short to long rods. Cocci are not formed and branching is not evident. Does not form pili or flagella. Grows on media designed for the genus *Mycobacterium*, but not on heart infusion agar. Growth on chocolate agar is embedded in the surface and is mucoid-like. Division is by binary fission. **Cells contain non-oxygenated, non-polar, alpha-mycolic acids**. Growth occurs in 3–4 d at 22°C but is reduced at 45°C and at pH 6.6. **Optimal growth temperature is 33°C**. Colonies are nonpigmented, nonphotochromogenic, and odorless.

DNA G+C content (mol%): 68–72 (T_m).

Type species: **Segniliparus rotundus** Butler, Floyd, Brown, Toney, Daneshvar, Cooksey, Carr, Steigerwalt and Charles, 2005, 1622[VP].

Further descriptive information

Cells are rod shaped and of variable lengths (1.0–1.3 or 0.76–1.93 × 0.4 µm). The variety in rod length is responsible for the distinctive colony formation and appears to be species specific. Cells grow aerobically in Middlebrook and Cohn 7H9 broth with ADC enrichment, and in 7H10 and 7H11 agars with OADC enrichment. Grows on TGY agar at 25, 35, and 45°C and shows retarded, if any, growth on heart infusion agar. Colonies shine and glisten under the light microscope.

The peptidoglycan contains mycolic acids and *meso*-diaminopimelic acid. 10-Methyloctadecanoic acid (tuberculostearic acid) is part of the fatty acid profile, a characteristic of the order *Actinomycetales.* The presence of α-branched, β-hydroxy fatty acids (mycolic acids) places strains of the genus *Segniliparus* with the other mycolic acid-containing genera. The mycolic acids have long carbon chains and lack oxygen functions other than those of the hydroxyl group. Mycolic acid chemical functional groups are representative of a series of non-oxygenated, nonpolar mycolic acids designated α- and α′-mycolic acids. The α′-mycolic acids are indicative of the family *Segniliparaceae* and appear longer than the C_{90} carbon mycolic acids present in mycobacteria. Mass spectrometry confirms that a C_{24} fatty acid is derived from pyrolysis of the β-hydroxy, acyl side-chain of mycolic acids at 300°C, similar to some species of *Mycobacterium.*

High performance liquid chromatography (HPLC) of ρ-bromophenacyl esters of mycolic acids used to identify species in the genus *Mycobacterium* can be used to readily detect species in the genus *Segniliparus* (Butler et al., 1986). Many extremely long, α′-structural types of mycolic acids present a distinctive HPLC reference pattern (Butler et al., 2005). The late co-emergence of nonpolar, α′-mycolic acids as shoulder peaks with a nonpolar high-molecular-mass, 110 carbon-standard in the HPLC process, is an exclusive characteristic of the genus *Segniliparus.* Similar chemotaxonomic characteristics are noteworthy for rapidly growing *Mycobacterium* and for some species of the genus *Tsukamurella.*

Characteristic biochemical patterns of the species of the genus *Segniliparus* are given in Table 50. In addition, strains fail to hydrolyze acetamide, adenine, casein, esculin, hypoxanthine, tyrosine, and xanthine. L-Arabinose, cellobiose, citrate, dulcitol, iso-erythritol, galactose, iso-*myo*-inositol, lactose, mannose, melibiose, raffinose, L-rhamnose, salicin, and sodium citrate are used as sole carbon sources. Does not exhibit alkaline phosphatase, β-galactosidase, β-glucuronidase, α-glucosidase, *N*-acetyl-β-glucosaminidase, or pyrrolidonyl arylamidase activity.

Clinical strains have been isolated from non-sterile human specimens including sputum and nasal region and bronchial washings. MIC susceptibility breakpoints for antibacterial and antituberculosis drugs are determined after incubation of strains for 3 d at 30°C. Standardized testing conditions for the genus are unknown, hence performance standards for nontuberculosus mycobacteria and *Nocardia* are utilized

TABLE 50. Characteristics differentiating species of the genus *Segniliparus*[a]

Characteristic	*S. rotundus*	*S. rugosus*
Cell morphology	Rods of similar length	Rods of variable length
Cell length	1.0–1.3 × 0.4 μm	0.76–1.93 × 0.4 μm
Colony morphology (texture)	Smooth (dense)	Rough-wrinkled (creamy)
α-Mycolic acids	+	+
Acid–alcohol-fast	+	+
Complete growth swirl on MacConkey agar	−	+
Produces pinkish diffusible pigment >4 weeks	−	v
Growth on sodium chloride after 7/14 d	−/+	+/+
Develop >45 mm bubbles in catalase test	+	+
Growth in lysozyme	−	v
Arylsulfatase activity after 3/14 d	+/+	v/+
Tween hydrolysis	+	v
Tween opacity	v	+
Nitrate reduction	−	v
Tellurite reduction	−	+
Nitrate reduction	−	v
Iron uptake	−	v
Niacin production	−	−
Urea hydrolysis	+	+
Carbon used/acid produced:		
D-Fructose	+/+	−/−
D-Glucose	+/+	+/+
Glycerol	v/v	+/+
Maltose	+/+	+/+
Mannitol	−/−	v/v
D-Sorbitol	−/−	v/−
Sucrose	v/v	−/−
Trehalose	+/+	+/+
Presence of:		
β-Glucosidase	+	+
Pyrazinamidase	+	+

[a]Symbols: +, positive in most strains; −, negative in most strains; v, variable in different strains.

(National Committee on Clinical Laboratory Standards, 2003). Adequate growth requires a substitution of 7H9 broth for the recommended cation-adjusted Mueller–Hinton broth (Clinical Laboratory Standards Institute, 2006). Generally, both species are resistant to amikacin, clarithromycin, ethambutol, and tobramycin, but susceptible to imipenum, rifabutin, sulfamethoxazole, and trimethoprim-sulfamethoxazole. However, consideration must be given to drug results as efficacies may be ambiguous for the test conditions.

Segniliparus rugosus presents in infections of lungs of cystic fibrosis patients, although the comprehensive role in the disease is unknown. However, physicians consider the isolates to be opportunistic acid-fast pathogens and not colonization bacteria due to multiple isolations from sputum and bronchial washings, and to a rapidly progressive disease that is not typical of cystic fibrosis. Clinical strains analyzed with multilocus enzyme electrophoresis reveal dissimilar electrophoretic types indicative of environment contact and not human transmission. Misidentifications of clinical isolates occur as *Mycobacterium abscessus*, *Mycobacterium chelonae*, and uncharacterized nontuberculosus mycobacteria. Accurate species identification is made by comparison to 16S rRNA gene sequences deposited in GenBank for *Segniliparus* or by HPLC analysis of mycolic acids. The family consists of a single genus, *Segniliparus*, represented by two species, *Segniliparus rotundus* and *Segniliparus rugosus*. *Segniliparus rugosus* is the only reported species associated with disease.

Enrichment and isolation procedures

The methods employed for the isolation and growth of the *Segniliparus* species are the same as those used for the genus *Mycobacterium* (Kent and Kubica, 1985). Comprehensive growth requirements are undetermined and strains have been nonviable upon subculture with mycobacteria media.

Maintenance procedures

Stock cultures of *Segniliparus* can be stored in Middlebrook and Cohn 7H9 broth with added ADC enrichment at −70°C. Cultures have been kept in trypticase soy broth with 15% glycerol at −70°C. Recovery is in 7H9 broth or on 7H11 agar at 33–37°C. Subcultures have been maintained on LJ, 7H9, 7H10, and 7H11. Lyophilized cultures are preserved at −70°C.

Taxonomic comments

The taxonomic circumscription of the genus *Segniliparus* is supported by two publications, and a reassessment of its phylogenetic position is likely as more genetic data become available. The chemotaxonomic characteristic of α-, α′-mycolic acids analyzed by HPLC distinguishes the genus from other mycolic acid containing genera, and the longer carbon length suggests an ancestral evolutionary position within mycolic acid-containing taxa. 16S rRNA gene based comparisons confirm a close association between *Segniliparus* strains and provide genetic evidence for the circumscription of the genus. However, other genetic intra-relationships confirmed with multilocus enzyme electrophoresis typing with cellular enzymes reveals the separation of two strains with identical 16S rRNA gene sequences within the species *Segniliparus rugosus*. The different electrophoretic types suggest an uncharacterized species and demonstrate taxa delineation issues.

Evolutionary tree construction based on 16S rRNA gene sequences shows a distinct lineage within other genera containing mycolic acids. A 16S rRNA gene similarity of 98.9% (15 bp difference) between the type strains of the species is in line with a DNA–DNA hybridization (hydroxyapatite method with an optimum reassociation temperature of 70°C) value of <28% and underpins the separation of the two *Segniliparus* species. An overall 16S rRNA genetic similarity for gene sequences deposited in GenBank is 94.5% for *Segniliparus rotundus* and 94.8% for *Segniliparus rugosus* with a speculated genetic neighbor *Rhodococcus equi* ATCC 6939^{T}; values correspond to 78 and 79 bp differences, respectively. These divergent values also highlight the distinctiveness of the genus, but phylogenetic tree construction places *Segniliparus* in an illogical association with *Rhodococcus equi*. This association is not

supported by a DNA–DNA hybridization value of 2% with the *Rhodococcus equi* strain. Also, the absence of the *Rhodococcus equi*-specific cholesterol oxidase virulence gene is inconclusive for *Rhodococcus equi*.

The uncertainty of the genetic relationships of the genus *Segniliparus* is supported by the HPLC presentation of unique long chain mycolic acids and GLC detection of $C_{10:0}$ fatty acids, neither of which is found in *Rhodococcus equi*. These results suggest an apparent treeing artifact resulting from insufficient taxonomic data with respect to these taxa. The hypothetical evolutionary history position will likely vary when other representatives of the genus are examined or when interrelated families are discovered. This subject remains unsettled and will receive consideration as additional information is discovered for strains of the genus *Segniliparus*.

Differentiation of the genus *Segniliparus* from other genera

Classical biochemical differentiation of the genus *Segniliparus* is inadequate. The acid–alcohol-fast staining nature of *Segniliparus* strains immediately eliminates bacteria that lack this characteristic and associates strains with the suprageneric assemblage of mycolic acid-containing actinomycetes, namely the genera *Corynebacterium*, *Dietzia*, *Gordonia*, *Millisia*, *Mycobacterium*, *Nocardia*, *Rhodococcus*, *Skermania*, *Smaragdicoccus*, *Tsukamurella*, and *Williamsia*. HPLC chemotaxonomic analysis of mycolic acids provides a reliable separation of mycolic acid-containing actinomycetes. Comparative analysis of the first 500 bp or of the almost complete 16S rRNA gene easily differentiates the genus *Segniliparus* by comparison to reference sequences deposited in GenBank for other mycolic containing taxa.

List of species of the genus *Segniliparus*

1. **Segniliparus rotundus** Butler, Floyd, Brown, Toney, Daneshvar, Cooksey, Carr, Steigerwalt and Charles 2005, 1622[VP]

ro.tun′dus. L. masc. adj. *rotundus* rounded, referring to the smooth, round-domed colony forms.

Characteristics are the same as those described for the genus. Cells are of a similar size (1.0–1.3 × 0.4 μm). During cell division, rods are equally divided, producing V-forms. Cells distribute easily in broth medium. Short rods produce dense, smooth, round-domed colonies. Colonies are opaque, colorless (whitish), and can be pushed intact across the agar surface.

DNA G+C content (mol%): 68 (T_m).

Type strain: CDC 1076, ATCC BAA-972, CIP 108378, JCM 13578.

Sequence accession no. (16S rRNA gene): AY608918.

Further comments: an additional strain is ATCC BAA-973 = CIP 108379.

2. **Segniliparus rugosus** Butler, Floyd, Brown, Toney, Daneshvar, Cooksey, Carr, Steigerwalt and Charles 2005, 1622[VP]

ru.go′sus. L. masc. adj. *rugosus* wrinkled, in reference to the wrinkled-rough colony morphology.

Characteristics are the same as those described for the genus. Cells are 0.76–1.93 × 0.4 μm. Unequal cell division of the longer cells often results in the disproportional length of new cells. Cells aggregate in broth medium. Colony centers have a wrinkled-rough texture composed of older cell growth. The colony border is smooth with undulating edges, representative of new cell growth. Young colonies are colorless with a creamy texture and smear when disturbed. Colonies of the type strain >4 weeks old develop a pinkish, diffusible-pigment on 7H11 at <33°C, but produce greening on LJ. Infrequent colony conversions to smooth forms are seen after >4 week growth. Strains found thus far occur in non-sterile human clinical specimens.

DNA G+C content (mol%): 72 (T_m).

Type strain: CDC 945, ATCC BAA-974, CIP 108380, JCM 13579.

Sequence accession no. (16S rRNA gene): AY608920.

Further comments: an additional strain is ATCC BAA-975 = CIP 108381.

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Family VI. **Tsukamurellaceae** Rainey, Ward-Rainey and Stackebrandt 1997, 486[VP]

Michael Goodfellow

Tsu.ka.mu.rel.la.ce′a.e. N.L. fem. n. *Tsukamurella* the type genus of the family; suff. -*aceae* ending to denote a family; N.L. fem. pl. n. *Tsukamurellaceae* the *Tsukamurella* family.

The family *Tsukamurellaceae* Rainey et al. 1997 was proposed for members of the genus *Tsukamurella* as these organisms showed a distinctive pattern of 16S rRNA signatures, namely nucleotides at positions 70:98 (U–A), 293:304 (G–U), 307 (C), 631 (C), 661:744 (G–C), 824:876 (U–A), 825:875 (A–U), 843 (C), 1007:1022 (G–U), and 1122:1151 (A–U).

Type genus: **Tsukamurella** Collins, Smida, Dorsch and Stackebrandt 1988, 387[VP].

Genus I. **Tsukamurella** Collins, Smida, Dorsch and Stackebrandt 1988, 387[VP]

Michael Goodfellow and Yashawant Kumar

Tsu.ka.mu.rel′la. N.L. fem. dim. n. *Tsukamurella* named in honor of Michio Tsukamura, a celebrated Japanese microbiologist.

Aerobic, Gram-stain-positive, partially acid–alcohol-fast, nonmotile, nonsporeforming actinomycetes. **Straight to slightly curved rods occur in pairs or in masses though coccobacillary forms are also present.** Cells may give a pseudomycelial appearance. Chemoorganotrophic with an oxidative metabolism. Rough, dry, flat, or folded colonies form on Lowenstein-Jensen medium and on brain heart infusion agar. Colonies are normally white or yellow to tan or beige. **Aerial hyphae are not formed.** Catalase–positive, arylsulfatase–negative, and lysozyme–resistant.

Whole-organism hydrolysates are rich in *meso*-diaminopimelic acid, arabinose, and galactose. The peptidoglycan is of the A1γ type. Muramic acid moieties are *N*-glycolated. **Cells contain diphosphatidylglycerol, phosphatidylethanolamine, phosphatidylinositol, and phosphatidylinositol mannosides as predominant polar lipids, major amounts of straight chain saturated, unsaturated, and 10-methyloctadecanoic (tuberculostearic) fatty acids, and unsaturated menaquinones with nine isoprene units. Mycolic acids have 62–78 carbon atoms and 1–7 double bonds. The fatty acid esters released on pyrolysis mass spectrometry of mycolic acid esters have 22–26 carbon atoms.** The phylogenetic position of the family *Tsukamurellaceae*, as determined by 16S rRNA gene sequence analysis, is in the order *Corynebacteriales*.

Found in a diverse range of aquatic and terrestrial habitats, notably in activated sewage sludge foam and clinical material.

DNA G+C content (mol%): 68–78.

Type species: **Tsukamurella paurometabola** corrig. (Steinhaus 1941) Collins, Smida, Dorsch and Stackebrandt 1988, 387[VP] (*Corynebacterium paurometabolum* Steinhaus 1941, 783).

Further descriptive information

Phylogeny. The nine species of the genus *Tsukamurella* form a well-delineated clade within the evolutionary radiation occupied by genera classified in the order *Corynebacteriales* (Figure 101). The type strains of these taxa share identical or almost identical 16S rRNA gene similarity values but can be distinguished using DNA–DNA relatedness, ribotyping, and phenotypic data (Kattar et al., 2001; Nam et al., 2003a; Olson et al., 2007).

Cell morphology. Most tsukamurellae form straight to slightly curved rods (0.5–0.8 × 1.0–5.0 µm) that occur singly, in pairs, or in masses. Coccobacillary forms occur. In *Tsukamurella pulmonis* and *Tsukamurella tyrosinosolvens*, cells tend to be long rods which in later stages of growth fragment into three parts that separate and grow as independent rods (Yassin et al., 1996, 1997). Neither aerial hyphae, capsules, spores, nor true branching have been observed.

Cell envelope composition. Tsukamurellae have (a) a peptidoglycan composed of *N*-acetylglucosamine, D-alanine, L-alanine, and D-glutamic acid with *meso*-diaminopimelic acid (*meso*-A_2pm) as the diamino acid, and muramic acid in the *N*-glycolated form; (b) arabinose and galactose as diagnostic wall sugars (i.e. whole-organism sugar pattern type A *sensu* Lechevalier and Lechevalier, 1970), (c) a phospholipid pattern consisting of diphosphatidylglycerol, phosphatidylethanolamine (taxonomically significant phospholipid), phosphatidylinositol, and phosphatidylinositol mannosides (i.e. phospholipid pattern type II *sensu* Lechevalier et al., (1981, 1977), (d) a fatty acid profile consisting of major amounts of straight chain, unsaturated, and tuberculostearic acids (i.e. a type IV fatty acid pattern *sensu* Lechevalier et al., 1977), (e) mycolic acids with 62–78 carbon atoms (Collins and Jones, 1982; Collins et al., 1988; Olson et al., 2007), and (f) unsaturated menaquinones with nine isoprene units as the predominant isoprenologue (Collins et al., 1988; Nam, 2004).

Two dimensional thin-layer chromatography of whole-organism acid and alkaline methanolysates of *Tsukamurella* strains give a characteristic mycolic acid pattern consisting of α- and α′ mycolates in addition to the nonhydroxylated fatty acid spot (Yassin et al., 1995, 1996, 1997). The predominant fatty acids are hexadecanoic ($C_{16:0}$, 25–40%), oleic ($C_{18:1}$ ω9, 24–35%), and tuberculostearic ($C_{18:0}$ 10 methyl, 8–18%) acids (Nam et al., 2003a).

The type strain, *Tsukamurella paurometabola*, contains a new lipoarabinomannan variant, designated Tpa LAM, which shows pro-inflammatory activity when tested with either human or murine monocyte/macrophage cell lines (Gibson et al., 2004). This induction was completely annulled in the presence of an

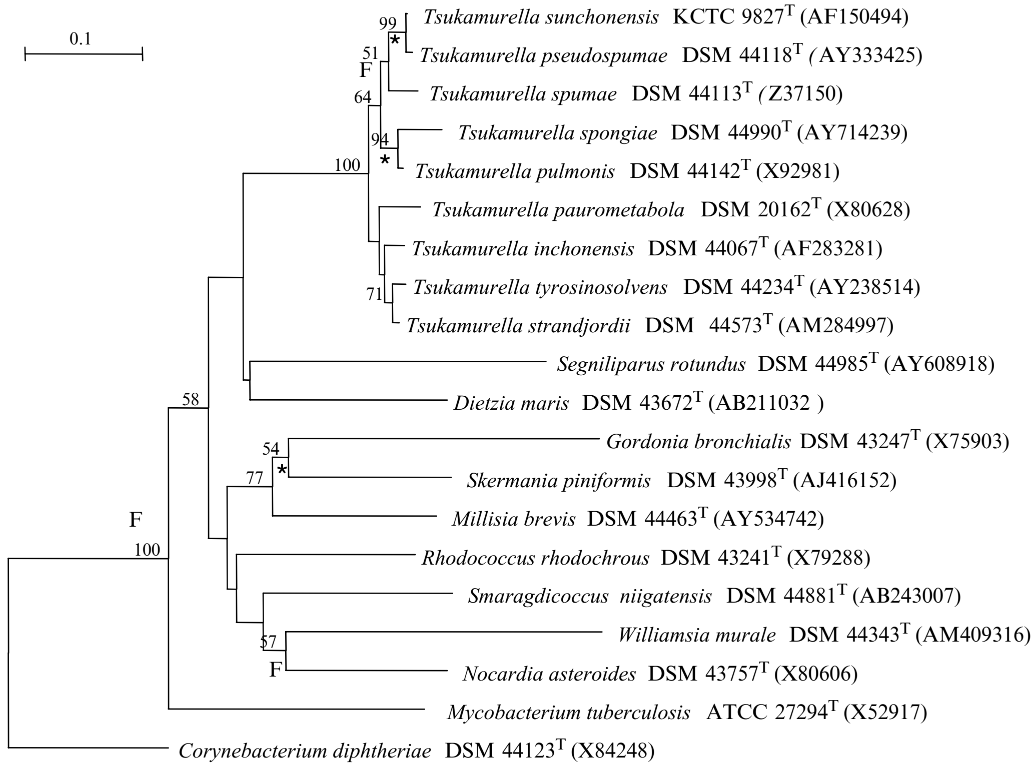

FIGURE 101. 16S rRNA neighbor-joining tree showing relationships between *Tsukamurella* species and representatives of other mycolic acid-containing genera classified in the order *Corynebacteriales*. Asterisks indicate branches of the tree that were also found using the least-squares and maximum-parsimony tree-making algorithms. F indicates a branch that was also recovered using the least-squares method. The numbers at the nodes indicate the level of bootstrap support derived from a neighbor-joining analysis of 1000 resampled datasets; only values above 50% are given. The bar indicates 0.1 substitutions per nucleotide position.

anti-toll-like receptor-2 (TLR-2) antibody which suggests that TLR-2 participates in the mediation of the pro-inflammatory cytokine tumor necrosis factor (TNF)-α in response to Tpa LAM. The lipomannan core of Tpa LAM is the primary moiety responsible for the observed TNF-α-inducing activity. The predominant motifs of the major cell wall arabinogalactan antigens of the type species *Tsukamurella paurometabola* have been structurally elucidated (Tropis et al., 2005).

A prospective member of a novel *Tsukamurella* species produced novel glycolipids (biosurfactants) when grown on sunflower oil as the sole carbon source (Vollbrecht et al., 1998). The glycolipids showed good surface–active behavior, and had antimicrobial properties. Dörner et al. (2004) detected a cation-specific channel (TipA) in the cell wall of a *Tsukamurella inchonensis* strain and went on to show that the gene of the channel-forming protein was identical to *mspA* of *Mycobacterium smegmatis* and *mppA* of *Mycobacterium phlei*.

Colony morphology. Rough, dry, flat, or folded, creamy white or yellow to tan or beige colonies are formed on Lowenstein-Jensen medium and brain heart infusion agar. Large colonies (3–5 mm) are produced on Middlebrook 7H11 and tryptic soy agar (Kattar et al., 2001). The colonies of *Tsukamurella inchonensis*, *Tsukamurella pulmonis*, *Tsukamurella strandjordii*, and *Tsukamurella tyrosinosolvens* are dry, rough, velvety, and flat but with a slightly raised center, and show a variety of colors. Those of *Tsukamurella inchonensis* and *Tsukamurella strandjordii* are yellow to orange whereas those of *Tsukamurella pulmonis* and *Tsukamurella tyrosinosolvens* are tan and buff. The type strain of *Tsukamurella paurometabola* produces smooth, creamy colonies with entire edges that have a distinctive fried egg appearance. *Tsukamurella spongiae* forms dry, matt, cream colonies with irregular spreading margins and raised, wrinkled, rough centers on marine agar (Olson et al., 2007). *Tsukamurella sunchonensis* forms rough, orange colonies (Seong et al., 2003).

Nutrition and growth conditions. Tsukamurellae grow well on most standard media, including modified Bennett's (Jones, 1949), brain heart infusion (Difco), modified Sauton's supplemented with thiamine (Mordarska et al., 1972), and nutrient (Oxoid) agars, and on Lowenstein-Jensen slants (BBL). Good growth occurs at 25–37°C.

Metabolism. Little is known about the metabolic properties of tsukamurellae though they are able to degrade a number of organic substrates and use a broad range of compounds as sole carbon and carbon and nitrogen sources (Erdlenbruch et al., 2001, Goodfellow et al., 1991, 1996; Nam et al., 2003a, 2004; Olson et al., 2007). They can also cleave a range of 7-amino-4-methylcoumarin (7-AMC-) and 4-methylumbelliferone (4MU-) conjugated fluorogenic substrates (Goodfellow et al., 1991; Nam et al., 2004). The results of a comprehensive numerical

taxonomic study based on such properties underpin the taxonomic integrity of *Tsukamurella* species (Nam, 2004).

Most, if not all, *Tsukamurella* strains degrade starch, Tweens 20, 40, 60, and 80, and uric acid, but not adenine, casein, elastin, keratin, guanine, testosterone, xanthine, or xylan; they utilize galactose, gentiobiose, glucose, *meso*-inositol, lactose, mannose, raffinose, rhamnose, ribose, sucrose, trehalose, turanose, xylitol, and xylose as sole carbon sources, but do not use adonitol or rhamnose; they use acetamide, alanine, asparagine, proline, and serine as sole carbon and nitrogen sources (Nam, 2004, 2003a), and cleave L-alanyl-7-AMC, benzyloxycarbonyl–glycyl–prolyl-7-AMC, L-glutamic acid-7-AMC, L-leucyl-7-AMC, lysyl-7-AMC, L-proline-7-AMC-L-phenylalanyl-7-AMC, 4MU-acetate, 4MU-butyrate, and 4MU-α-D-glucoside (Nam et al., 2003a). The type strains of *Tsukamurella inchonensis* and *Tsukamurella paurometabola*, like representatives of the genus *Corynebacterium*, produce similar polyamine patterns with spermadine as the predominant component (Altenburger et al., 1997).

Antibiotic sensitivity. The few comparative antibiotic sensitivity studies that have been carried out on *Tsukamurella* strains have been driven mainly by taxonomic considerations. *Tsukamurella* strains are resistant (μg/ml) to clindamycin (2), colistin (25), cotrimoxazole (25), fusidic acid (10), nalidixic acid (5), novobiocin (5), and penicillin (1 IU), but are sensitive to ciprofloxazine (5). Several antibiotic combinations have been proposed for the treatment of infections caused by *Tsukamurella* strains. The combination of a β-lactam and an aminoglycoside together with the removal of medical devices, would appear to be the treatment of choice (Alcaide et al., 2004), but optimal management is uncertain and should be based on susceptibility testing (Chong et al., 1997; Schwartz et al., 2002). A *Tsukamurella paurometabola* strain, like representatives of other mycolic acid-containing taxa, was found to be susceptible to imidazole antifungal drugs (Dabbs et al., 2003).

Pathogenicity. Information on human infections attributed to tsukamurellae is sparse and tends to be from reports on single strain histories (Rey et al., 1997). These suggest that *Tsukamurella* infections are sporadic, community acquired, and nosocomial in origin. However, infections caused by tsukamurellae may go unrecorded as the offending strains may be either missed or misidentified in diagnostic laboratories (Alcaide et al., 2004; Stanley et al., 2006). The most common clinical presentations caused by *Tsukamurella* species in humans are cutaneous infections, meningitis, pulmonary, and device related infections including catheter-related bacteremia, especially of the central venous catheter (Lai, 1993; Maertens et al., 1998; Schwartz et al., 2002; Sheridan et al., 2003). *Tsukamurella* strains have been shown to cause peritonitis associated with continuous ambulatory peritoneal dialysis (Shaer and Gadegbeku, 2001), a knee prosthesis infection (Larkin et al., 1999), and cavitary pneumonia in an AIDS patient (Alcaide et al., 2004). Most *Tsukamurella* infections occur in immunocompromised hosts, particularly those with underlying chronic lung disease. Most of the cases reported in the literature have been attributed to *Tsukamurella paurometabola*.

The first documented case of a *Tsukamurella* infection was made by Tsukamura and Kawakami (1982) who repeatedly isolated a *Tsukamurella paurometabola* strain from a lung infection in a nonimmunocompromised 50-year-old man. Subsequently, *Tsukamurella paurometabola* strains have been associated with cases of bacteremia (Jones et al., 1994; Lai, 1993; Shapiro et al., 1992), a chest disorder (Osoagbaka, 1989), lethal meningitis (Prinz et al., 1985), severe gangrenous tendosynovitis with multiple subcutaneous abscesses (Tsukamura et al., 1988), and with a cutaneous infection (Granel et al., 1996). Cases of catheter-related bacteremia have been attributed to *Tsukamurella pulmonis* and *Tsukamurella tyrosinosolvens* (Elshibly et al., 2005; Maertens et al., 1998; Schwartz et al., 2002; Sheridan et al., 2003). Members of these taxa have also been reported to cause lung infections, as has *Tsukamurella inchonensis* (Yassin et al., 1995, 1996, 1997). *Tsukamurella strandjordii* was isolated from blood cultures of a child with acute mycelogenous leukemia (Kattar et al., 2001). *Tsukamurella* strains with phenotypic properties similar to those of *Tsukamurella pulmonis* and *Tsukamurella tyrosinosolvens* have been considered to cause conjunctivitis (Woo et al., 2003). Similarly, a *Tsukamurella* strain has been implicated as the causal agent of an implantable cardioverter-defibrillator infection (Almehmi et al., 2004). A pseudoinfection caused by *Tsukamurella paurometabola* was traced back to laboratory contamination (Auerbach et al., 1992).

Ecology. Tsukamurellae have been isolated from a range of aquatic and terrestrial habitats, as well as from clinical material. The type strains of *Tsukamurella paurometabola* and *Tsukamurella spongiae* were isolated from mycetomes and ovaries of the bed bug (*Cimex lectularius*) and from a deep-water marine hexactinellid sponge, respectively (Olson et al., 2007; Steinhaus, 1941). Representative strains isolated from activated sewage sludge foam have been classified into three novel species, namely *Tsukamurella pseudospumae* Nam et al. (2004), *Tsukamurella spumae* Nam et al. (2003a) and *Tsukamurella sunchonensis* Seong et al. (2003). The remaining species, *Tsukamurella inchonensis*, *Tsukamurella pulmonis*, *Tsukamurella strandjordii*, and *Tsukamurella tyrosinosolvens*, were isolated from clinical specimens (Kattar et al., 2001; Yassin et al., 1995, 1996, 1997). Several *Tsukamurella* strains have been isolated from Challenger Deep sediment (10,898 m) collected from the Mariana Trench in the Pacific Ocean (Pathom-aree et al., 2006).

Tsukamurella strains are considered to be one of the causal agents of foaming in activated sludge plants (Goodfellow et al., 1996; Seong et al., 1999). Large numbers of strains presumptively identified as tsukamurellae have been detected in mixed liquor ($2.7 \times 10^5 \pm 0.9$ c.f.u./ml) and foam ($9.3 \times 10^6 \pm 4.2$ c.f.u./ml) samples collected from Stoke Bardolph Water Reclamation Works, Nottinghamshire, UK (Stainsby, 2002). Pyrolysis mass spectrometric analysis of representative isolates confirmed their assignment to the genus *Tsukamurella* (Goodfellow et al., 1998) and showed that many of them belonged to a taxon, members of which were subsequently given species status as *Tsukamurella spumae* (Nam et al., 2003a). The type strain of *Tsukamurella paurometabola* has been shown to give a positive result against anti-*Gordonia amarae* mycolic acid polyclonal antibodies in an enzyme-linked immunosorbent assay (Iwahori et al., 2001).

Enrichment and isolation procedures

A semisolid medium containing carbohydrates, gelatin, proteose peptone, rabbit serum, and mineral rabbit kidney, was used in the original isolation of *Tsukamurella paurometabola* (Steinhaus, 1941). When transferred to brain heart infusion agar the organism grew in 24–28 h. *Tsukamurella strandjordii* and *Tsukamurella tyrosinosolvens* were obtained from blood cultures (Kattar et al., 2001; Yassin et al., 1997), and *Tsukamurella pulmonis* from

sputum following decontamination with *N*-acetyl-L-cysteine, centrifugation and cultivation on Lowenstein–Jensen's medium (Yassin et al., 1997). *Tsukamurella spongiae* was isolated from a sponge suspension which had been heat pretreated (70°C for 15 min), plated onto maltose-sea water agar (Olson et al., 2000), and incubated for 28 d in the dark at ambient temperature. Colonies were transferred to fresh plates of the isolation medium and finally maintained on slants of marine agar 2216 (Becton Dickinson). The *Tsukamurella* strains from the Challenger Deep sediment were isolated on Difco marine agar 2216 (Pathom-aree et al., 2006).

Large numbers of *Tsukamurella* strains have been isolated on glucose-yeast extract agar plates (Gordon and Mihm, 1962) supplemented with cycloheximide, and incubated at 30°C for 14 d following inoculation with serially diluted samples taken from foaming activated sludge plants (Goodfellow et al., 1998, 1996). The tsukamurellae were recognized by their ability to produce characteristic deep orange, dry colonies. Preliminary characterization studies indicated that representative isolates belonged to several new centers of taxonomic variation within the genus *Tsukamurella*. More exacting taxonomic studies showed that some of these isolates belong to *Tsukamurella pseudospumae* and *Tsukamurella spumae* (Nam et al., 2003a, 2004). Tsukamurellae have also been isolated from foaming activated sludge plants by micromanipulation (Seong et al., 1999).

Maintenance procedures

Short term storage can be accomplished by serial transfer every two months using appropriate media, such as modified Bennett's agar (Jones, 1949) and glucose-yeast extract agar (Gordon and Mihm, 1962) slopes with storage at 4°C. Longer term preservation of strains can be achieved as frozen stocks in 20% (v/v) aqueous glycerol at −20°C or −80°C (liquid nitrogen vapor phase) or by using standard lyophilization techniques as described for *Gordonia* strains.

Differentiation of the genus *Tsukamurella* from other genera

The type strains of *Tsukamurella* species form a distinct monophyletic lineage in the *Corynebacteriales* 16S rRNA tree (Figure 101). *Tsukamurella* strains can be distinguished from all other genera containing mycolic acids using a combination of chemotaxonomic and morphological markers (Table 29, in the *Corynebacteriales* chapter, above). There is also evidence that they can be distinguished by diagnostic 16S rRNA signatures at positions 1007:1022 (G-U), partial sequencing of ribosomal protein AT-L30 (Ochi, 1995), and by DNA amplification and restriction analysis (Steingrübe et al., 1997).

Taxonomic comments

The genus *Tsukamurella* Collins et al. 1988 is the type and only genus of the family *Tsukamurellaceae*. The taxon was introduced for actinomycetes previously classified as *Corynebacterium paurometabolum* and *Rhodococcus aurantiacus*. *Corynebacterium paurometabolum* was proposed by Steinhaus (1941) for bacteria isolated from the mycetomes and ovaries of the bed bug (*Cimex lectularis*) though its inclusion in the genus *Corynebacterium* was questioned (Collins and Cummins, 1986; Collins and Jones, 1982; Jones, 1975). Like corynebacteria, *Corynebacterium paurometabolum* has a directly cross-linked peptidoglycan based on *meso*-diaminopimelic acid and an arabinogalactan polymer (Cummins, 1971; Schleifer and Kandler, 1972), but can be distinguished from them as it contains a series of very long (68–76 carbon atoms), highly unsaturated (2–6 double bonds) mycolic acids (Collins and Jones, 1982).

Rhodococcus aurantiacus was known to have a similar series of very long, highly unsaturated mycolic acids (Goodfellow et al., 1978; Tomoysau and Yano, 1984). This species was first described by Tsukamura and Mizuno (1971) as "*Gordonia aurantiaca*"; the type strain of this taxon was later found to fall outside the aggregate *Rhodococcus* cluster circumscribed by Goodfellow and Alderson (1977). Goodfellow et al. (1978) found that "*Gordonia aurantiaca*" strains formed a well-delineated group that was distinct from the genera *Mycobacterium*, *Nocardia*, and *Rhodococcus* and thereby considered that the taxon merited generic status. However, around the same time, "*Gordonia aurantiaca*" was transferred to the genus *Rhodococcus* as *Rhodococcus aurantiacus* (Tsukamura, 1974, 1978; Tsukamura and Yano, 1985). In addition to their distinctive mycolic acid profile, *Corynebacterium paurometabolum* and *Rhodococcus aurantiacus* strains differed from other mycolic-acid-containing taxa as they produce menaquinones with unsaturated multiprenyl side chains (Collins and Jones, 1982).

Collins et al. (1988) concluded, mainly from 16S rRNA gene similarity data, that *Corynebacterium paurometabolum* and *Rhodococcus aurantiacus* were closely related but distinct from the genera *Corynebacterium*, *Mycobacterium*, *Nocardia*, and *Rhodococcus*. On the basis of this and previous findings, they proposed that *Corynebacterium paurometabolum* and *Rhodococcus aurantiacus* be reduced to a single species and classified in a new genus, *Tsukamurella*, as *Tsukamurella paurometabolum* comb. nov. This species epithet was subsequently corrected to *paurometabola* as the noun *Tsukamurella* is feminine, hence the corresponding adjective should have a feminine ending (Euzéby, 1998). The original type strains of *Corynebacterium paurometabola* and *Rhodococcus aurantiacus* strains were shown to belong to different species (Auerbach et al., 1992; McNabb et al., 1997); the type strain of *Rhodococcus aurantiacus* was eventually found to be a typical strain of *Tsukamurella inchonensis* based on DNA–DNA and phenotypic data (Nam et al., 2003a). In contrast, *Tsukamurella wratislaviensis* Goodfellow et al. (1991) was transferred to the genus *Rhodococcus* as *Rhodococcus wratislaviensis* comb. nov. following a polyphasic taxonomic study (Goodfellow et al., 2002).

The genus *Tsukamurella* contains nine validly named species which form a distinct clade in the *Corynebacteriales* 16S rRNA gene tree (Adachi et al., 2007; Goodfellow and Maldonado, 2006). The type strains of these species share very high 16S rRNA gene nucleotide similarity values, but can be distinguished by DNA–DNA relatedness and phenotypic data (Kattar et al., 2001; Nam et al., 2003a, 2004; Olson et al., 2007). The type strains of *Tsukamurella paurometabola* and *Tsukamurella spumae* show distinct ribotype patterns (Nam et al., 2003a). However, the taxonomic positions of some strains classified as *Tsukamurella paurometabola* need to be resolved as they are misplaced in this species (Nam et al., 2003a).

Differentiation of the species of the genus *Tsukamurella*

Tsukamurella species can be distinguished from one another by using a combination of phenotypic properties (Table 51). Preliminary studies suggest that *groEL* gene sequencing may give sufficient resolution to distinguish between *Tsukamurella* species (Woo et al., 2003).

TABLE 51. Characteristics differentiating species of the genus *Tsukamurella*[a,b]

Characteristic	*T. paurometabola*	*T. inchonensis*	*T. pseudospumae*	*T. pulmonis*	*T. spongiae*	*T. spumae*	*T. strandjordii*	*T. sunchonensis*	*T. tyrosinosolvens*
Biochemical tests:									
Esculin hydrolysis	+	+	+	+	nd	–	+	+	+
Urea hydrolysis	+	+	–	+	nd	–	+	–	–
Color of colonies:									
Orange/red	–	–	+	–	–	+	–	+	–
White/cream	+	+	–	+	+	–	+	–	+
Degradation tests:									
Hypoxanthine	–	+	+	+	nd	+	–	+	+
Tyrosine	–	–	+	–	nd	+	–	+	+
Growth at 10°C	+	–	+	–	–		–	–	–
Growth on sole carbon sources (1%, w/v):									
D-Arabinose	–	+	+	+	–	–	–	–	+
L-Arabinose	–	–	+	+	+	+	–	–	+
Arabitol	–	–	+	+	+	+	+	nd	+
Cellobiose	–	–	–	+	+	–	–	nd	+
Dulcitol	–	–	–	+	–	+	–	–	+
meso-Erythritol	–	–	–	+	–	+	–	–	+
Fructose	+	–	+	+	+	+	–	+	+
Maltose	+	+	+	+	–	+	–	+	+
Mannitol	–	+	–	–	+	+	+	+	+
Melezitose	+	+	+	–	+	+	–	+	+
Melibiose	–	+	–	+	+	+	+	–	+
Ribose	+	+	+	+	+	+	–	nd	+
Salicin	+	+	–	+	+	–	+	nd	+
Sorbitol	–	+	–	–	+	+	+	nd	+
Xylose	–	+	–	+	+	+	–	–	+
Growth on sole carbon sources (0.1%, w/v):									
Butane-1,3-diol	+	+	+	+	nd	–	+	nd	+
Butane-1,4-diol	+	–	–	+	nd	–	+	nd	+
Propan-1,2-diol	+	+	+	+	nd	–	+	nd	+
Sodium adipate	+	–	–	+	nd	–	+	nd	+
Sodium benzoate	+	–	–	+	nd	–	+	–	+
Sodium citrate	+	+	+	+	nd	–	+	–	+
Sodium gluconate	+	+	+	+	nd	–	–	nd	+
Sodium lactate	+	+	–	+	nd	–	+	–	+

[a]Symbols: +, positive; –, negative; nd, not determined.

[b]Date taken from Nam et al. (2003a, 2004), Seong et al. (2003), Nam (2004) and Olson et al. (2007).

List of species of the genus *Tsukamurella*

1. **Tsukamurella paurometabola** corrig. (Steinhaus 1941) Collins, Smida, Dorsch and Stackebrandt 1988, 387[VP] (*Corynebacterium paurometabolum* Steinhaus 1941, 783)

pau.ro.me.ta'bo.la. Gr. adj. *pauros* little; Gr. adj. *metabolos* changeable; N.L. fem. adj. *paurometabola* little changeable.

Strictly aerobic, Gram-stain-positive, straight to slightly curved rods (0.5–0.8 × 1.0–5 µm) found singly, in pairs, or in masses. Coccobacillary forms occur. Small, white, to creamish colonies (0.5–2.0 mm in diameter) with entire edges are formed. Colonies are dry, but easy to emulsify. Metachromatic granules may be detected. Grows at 10 and 45°C. Does not survive heating at 60°C for 15 min.

Positive for β-galacturonidase, pyrazinamidase, and urease, but negative for arylsulfatase and α-esterase. Does not reduce nitrate. Acetamide and nicotinamide are used as sole nitrogen sources, but benzamide is not. Resistant (µg/ml) to ethambutol (5), 5-fluorouracil (20), mitomycin C (10), and picric acid (0.2%, w/v), but susceptible to bleomycin (5).

Additional phenotypic properties are shown in Table 51.

Mycolic acids have 62–78 carbon atoms. The major cellular fatty acids are hexadecanoic (25.4%), oleic (23.5%), and tuberculostearic (17.9%) acids. The cellular polar lipid composition is diphosphatidylglycerol, phosphatidylethanolamine, phosphatidylinositol, and phosphatidylinositol

mannosides. The predominant menaquinone is MK-9; minor amounts of MK-7, MK-8, and MK-10 are also present.

Source: clinical material and the mycetomes and ovaries of bed bugs (*Cimex letularius*).

DNA G+C content (mol%): 67–68 (T_m).

Type strain: JC7,ATCC 8368, CCUG 35730, CIP 100753, CECT 3055, DSM 20162, NBRC 16120, JCM 10117, NCTC 13040.

Sequence accession no. (16S rRNA gene): AF283280, FJ468341, X53206, X80628, Z46751.

2. **Tsukamurella inchonensis** Yassin, Rainey, Brzezinka, Burghardt, Lee and Schaal 1995, 526[VP]

in.cho.nen′sis. N.L. fem. adj. *inchonensis* of or belonging to Inchon, the city in South Korea where the type strain was isolated.

Aerobic, Gram-stain-positive, acid–alcohol-fast bacilli. Visible growth from dilute inocula occurs within 2 d. Eugonic, rough, brownish-orange colonies are formed on Lowenstein–Jensen medium. Grows at 24 and 45°C, but not at 10°C. Acetamidase, allantoinase, thermostable catalase, β-glucosidase, β-galactosidase, nicotinamidase, pyrazinamidase, and urease positive, but negative for arylsufatase, benzamidase, nitrate reductase, and succinamidase. Acid is formed from cellobiose, fructose, galactose, glucose, inositol, maltose, mannitol, mannose, melezitose, sorbitol, sucrose, and trehalose, but not from adonitol, inulin, lactose, raffinose, rhamnose, or xylose. Resistant to *p*-aminosalicylic acid, capreomycin, cycloserine, ethambutol, isoniazid, protionamide, and rifampin. Grows on MacConkey agar without crystal violet, and tolerates 5% (w/v) sodium chloride and *p*-nitrobenzoic acid.

Additional phenotypic properties are shown in Table 51.

Contains α- and α′ mycolates. The major cellular fatty acids are hexadecanoic (31.3%), oleic (27.4%), and tuberculostearic (14.6%) acids. The predominant menaquinone is MK-9; minor amounts of MK-8 and MK-10 are also present.

Source: clinical material.

DNA G+C content (mol%): 72 (HPLC).

Type strain: IMMIB D-771, ATCC 700082, CIP 104790, DSM 44067, JCM 10110.

Sequence accession no. (16S rRNA gene): AF 283281, X85955.

Additional comment: Tsukamurella paurometabola ATCC 25938 is a misnamed *Tsukamurella inchonensis* strain.

3. **Tsukamurella pseudospumae** Nam, Kim, Chun and Goodfellow 2004, 1211[VP]

pseu.do.spu′ma.e. Gr. adj. *pseudês* false; L. gen. n. *spumae* of foam and specific epithet of a bacterial species; N.L. gen. n. *pseudospumae* the false *spumae*, referring to the close relationship to *Tsukamurella spumae*.

Aerobic, Gram-stain-positive, partially acid–alcohol-fast actinomycetes which form straight to slightly curved rods and a few long filaments. Large orange to red colonies (<5 mm) with irregular margins are formed on glucose-yeast extract agar. Grows at 10 and 37°C, but not at 45°C. L-Phenylalanine is used as a sole carbon and nitrogen source, but histidine, lysine, succinamide, and valine are not. Resistant (µg/ml) to bekanamycin (64), 5-fluorouracil (20), gentamicin (32), kanamycin (32), neomycin (32), novobiocin (16), oleandomycin (64), rifamycin (2), tetracycline (10), and vancomycin (4), but is susceptible to chlortetracycline (8), erythromycin (8), novobiocin (64), penicillin (64), and rifampin (16). Grows in the presence of crystal violet (0.001%, w/v).

Additional phenotypic properties are shown in Table 51.

Mycolic acids have 68–76 carbon atoms and up to 7 double bonds; the major products from pyrolysis gas chromatography of methyl mycolates are straight-chain fatty acids $C_{20:1}$ and $C_{22:1}$. The major cellular fatty acids are hexadecanoic (23.3%), oleic (29.9%), and tuberculostearic (15.2%) acids. The predominant menaquinone is MK-9.

Source: activated sludge foam.

DNA G+C content (mol%): not determined.

Type strain: N1176, DSM 44118, JCM 13375, NCIMB 13963.

Sequence accession no. (16S rRNA gene): AY238513.

4. **Tsukamurella pulmonis** Yassin, Rainey, Brzezinka, Burghardt, Rifai, Seifert, Feldmann and Schaal 1996, 434[VP]

pul.mo′nis. L. gen. masc. n. *pulmonis* of the lung, referring to the organ from which the bacterium was isolated.

Aerobic, Gram-stain-positive, and slightly acid–alcohol-fast bacilli. Most cells are long rods which, at a later stage of growth fragment into three parts which separate and grow as independent rods. Visible growth from dilute inocula occurs within 2 d. Eugonic, rough, creamy colored colonies are formed on brain heart infusion agar and Löwenstein–Jensen medium. Grows at 24 and 37°C, but not at 10 or 45°C. Does not form melanin pigments on either peptone-yeast extract-iron (ISP medium 6) or tyrosine (ISP medium 7) agars. Acid is produced from fructose, galactose, glucose, mannose, mannitol, sorbitol, sucrose, and trehalose, but not from adonitol, arabinose, cellobiose, inositol, inulin, lactose, maltose, melezitose, raffinose, rhamnose, or xylose. Resistant to *p*-aminosalicylic acid, capreomicin, cycloserine, ethambutol, isoniazid, protionamide, rifampin, and streptomycin. Grows on MacConkey agar without crystal violet and tolerates 5% (w/v) sodium chloride and *p*-nitrobenzoic acid.

Additional phenotypic properties are shown in Table 51.

Contains α- and α′-mycolic acids. The major cellular fatty acids are hexadecanoic (29.7%), oleic (33.5%), and tuberculostearic (9.3%) acids. The cellular polar lipid composition is diphosphatidylglycerol, phosphatidylethanolamine, phosphatidylinositol, and phosphatidylinositol mannosides. The predominant menaquinone is MK-9; minor amounts of MK-8 and MK-10 are also present.

Source: clinical material.

DNA G+C content (mol%): 69.8 (HPLC).

Type strain: IMMIB D-1321, ATCC 700081, CCUG 35732, CIP 104791, DSM 44142, JCM 10111.

Sequence accession no. (16S rRNA gene): X92981.

5. **Tsukamurella spongiae** Olson, Harmody, Bej and McCarthy 2007, 1480[VP]

spon′gi.a.e. L. gen. n. *spongiae* of a sponge, referring to the source of isolation, a deep-water sponge.

Aerobic, Gram-stain-positive organism which forms straight to slightly curved rods (1 × 2.5 µm). Grows on brain heart infusion, nutrient, and marine agars after 24–48 h incubation at ambient temperature (about 25°C). Colonies are dry,

matt, and cream colored with irregular spreading margins and raised, wrinkled, rough centers; they range in size from 2–5 mm in diameter and show irregular variation. Optimal temperature for growth is 25–37°C; does not grow at 10°C or above 45°C. Galactose, glucose, mannose, rhamnose, sucrose, and trehalose are used as sole carbon sources, but amyl alcohol and methanol are not. Tolerates NaCl concentrations up to 4% (w/v), but growth is enhanced at lower concentrations.

Additional phenotypic properties are shown in Table 51.

Mycolic acids have 58–75 carbon atoms. The major cellular fatty acids are $C_{18:1}$ ω9*c* (40.7%), $C_{16:0}$ (27.7%), and $C_{16:1}$ ω7*c* and/or C_{15} iso 2-OH (10.3%); small proportions of tuberculostearic acid (1.5%) are also present.

Source: a deep-water sponge collected off the coast of Curaçao in the Netherlands Antilles, at a depth of 220 m.

DNA G+C content (mol%): 74.6 (T_m).

Type strain: K362, DSM 44990, JCM 14882, NRRL B-24467.

Sequence accession no. (16S rRNA gene): AY714239.

6. **Tsukamurella spumae** Nam, Chun, Kim, Kim, Zakrzewska-Czerwinska and Goodfellow 2003b, 1701[VP] (Effective publication: Nam, Chun, Kim, Kim, Zakrzewska-Czerwinska and Goodfellow 2003a, 373.)

spu′ma.e. L. gen. n. *spumae* of foam denoting the presence of the organism in the foam of activated sewage sludge plants.

Aerobic, Gram-stain-positive, partially acid–alcohol-fast organism which forms straight to slightly curved rods and a few long filaments. Large orange to red colonies (<5 mm) with irregular margins and elevation are formed on glucose–yeast extract agar. Grows at 10 and 37°C, but not at 45°C. Phenylalanine is used as a sole carbon and nitrogen source, but histidine, lysine, succinamide, and valine are not. Resistant (µg/ml) to bekanamycin (64), erythromycin (8), 5-fluorouracil (20), gentamicin (32), kanamycin (64), neomycin (32), novobiocin (16), oleanodomycin (64), rifampin (2), and vancomycin (4), but susceptible to chlortetracycline (8), erythromycin (16), novobiocin (64), penicillin G (64), and rifampin (16). Grows in the presence of crystal violet (0.001%, w/v).

Additional phenotypic properties are shown in Table 51.

Mycolic acids have 68–76 carbon atoms and up to 7 double bonds; the major products from pyrolysis gas chromatography of methyl mycolates are straight chain fatty acids $C_{20:1}$ and $C_{22:1}$. The major cellular fatty acids are hexadecanoic (26.5%), oleic (26.9%), and tuberculostearic (17.5%) acids. The predominant isoprenologue is MK-9.

Source: activated sludge foam.

DNA G+C content (mol%): 70 (T_m).

Type strain: N1171, CCUG 48751, DSM 44113, JCM 12608, NCIMB 13947.

Sequence accession no. (16S rRNA gene): Z37150.

7. **Tsukamurella strandjordii** corrig. Kattar, Cookson, Carlson, Stiglich, Schwartz, Nguyen, Daza, Wallis, Yarfitz and Coyle 2002, 1075[VP] (Effective publication: Kattar, Cookson, Carlson, Stiglich, Schwartz, Nguyen, Daza, Wallis, Yarfitz and Coyle 2001, 1474.)

strand.jor′di.i. N.L. gen. masc. n. *strandjordii* of Strandjord, named in honor of Paul Strandjord, founder and chair of the Department of Laboratory Medicine, University of Washington, from 1969 to 1994.

Strictly aerobic, Gram-stain-positive, slightly acid-fast organism which forms long rods. Grows well after incubation for 2 d on MacConkey agar without crystal violet and on Middlebrook 7H11 and tryptic soy agars. Colonies (2–5 mm) are rough and tan to yellow in color. Short, branching substrate hyphae and diphtheroid forms are produced on tap water agar. Grows at 28 and 35°C, but not at 10 or 42°C. Semi-quantitative catalase, 68°C heat-stable catalase, and iron uptake tests are positive. Nitrate is not reduced. Positive for L-leucyl-2-naphthylamide, bromo-2-naphthyl-β-D-glucopyranoside, 2-naphthylcaprylate, 2-naphthyl-α-D-glucopyranoside, and 2-naphthylphosphate (API ZYM system), and for pyrazinamidase and urease, but is arylsulfatase negative (3 and 14 d). Utilizes D- and L-arabitol, arbutin, fructose, fucose, galactose, glucose, gluconate, inositol, maltose, mannitol, mannose, melezitose, α-methyl-D-glucoside, sucrose, salicin, sorbitol, trehalose, and turanose (API 50 CH system). Susceptible to amikacin, clarithromycin, ciprofloxacin, imipenem, and trimethoprim sulfamethoxazole.

Additional phenotypic properties are shown in Table 51.

HPLC analyses of mycolic acids yield similar chromatographic profiles to those of other *Tsukamurella* species. The major cellular fatty acids are hexadecanoic (40.3%), oleic (23.7%), and tuberculostearic (16.5%) acids.

Source: blood of a 5-year-old girl with acute mycelogenous leukemia.

DNA G+C content (mol%): not determined.

Type strain: 32-92, ATCC BAA-173, DSM 44573, JCM 11487.

Sequence accession no. (16S rRNA gene): AF 283283.

8. **Tsukamurella sunchonensis** Seong, Kim, Baik, Choi, Kim, Kim and Goodfellow 2008, 1993[VP] (Effective publication: Seong, Kim, Baik, Choi, Kim, Kim and Goodfellow 2003, 88.)

sun.chon.en′sis. N.L. fem. adj. *sunchonensis* of or belonging to Sunchon, a city in South Korea where the organism was isolated.

Aerobic, Gram-stain-positive, slightly acid–alcohol-fast bacilli. Rough, orange colored colonies formed on yeast extract-malt extract agar. Dilute inocula on brain heart infusion agar yield large eugenic cream-colored colonies. Does not form melanin pigments. Grows at 28 and 37°C, but not at 10 or 45°C. Nitrate is not reduced. Does not degrade adenine, casein, elastin, gelatin, pectin, gelatin, starch, Tween 80, or xanthine. Acid is produced from fructose, glucose, maltose, melezitose, sucrose, trehalose, and xylose, but not from adonitol, maltose, mannose, or raffinose. Galactose, glucose, *meso*-inositol, and trehalose are used as sole carbon sources, but adonitol, dextran, lactose, melibiose, raffinose, and rhamnose are not. Sensitive (µg/ml) to gentamicin (10), rifampin (50), streptomycin (10), tobramycin (10), and vancomycin (30), but resistant to bacitracin (10 IU) and penicillin G (10 IU). Grows in the presence of phenol (0.1%, w/v), potassium tellurite (0.01%, w/v), and sodium chloride (7%, w/v), but is sensitive to crystal violet (0.0001, w/v), sodium azide (0.02%, w/v), and sodium chloride (10%, w/v).

Contains α- and α′-mycolates. The major cellular fatty acids are hexadecanoic (37.8%), oleic (32.6%), and tuberculostearic (11.5%) acids. The predominant menaquinone is MK-9.

Source: activated sludge foam.

DNA G+C content (mol%): 68.1 (slot dot method).

Type strain: SCNU5, JCM 15929, KCTC 9827, NRRL B-24668.

Sequence accession no. (16S rRNA gene): AF150494.

9. **Tsukamurella tyrosinosolvens** Yassin, Rainey, Burghardt, Brzezinka, Schmitt, Seifert, Zimmermann, Mauch, Gierth, Lux and Schaal 1997, 612[VP]

ty.ro.si.no.sol′vens. N.L. n. *tyrosinum* (from Gr. masc. n. *tyros* cheese) tyrosine an amino acid; L. pres. part. *solvens* dissolving; N.L. part. adj. *tyrosinosolvens* tyrosine dissolving, referring to the hydrolysis of tyrosine which is characteristic of this species.

Aerobic, Gram-stain-positive, and slightly acid–alcohol-fast bacilli. Most cells are long rods which, at a late stage of growth, fragment into three parts which separate and grow into independent rods. Visible growth from dilute inocula occurs within 2 d; colonies on brain heart infusion agar are yellowish, dry, and rough. Melanin pigments are not formed on either peptone-yeast extract-iron (ISP medium 6) or tyrosine (ISP medium 7) agars. Grows at 24 and 37°C, but not at 10 or 45°C. Acetamidase, allantoinase, catalase, β-galactosidase, nicotinamidase, phosphatase, pyrazinamidase, and urease positive, but negative for arylsulfatase (after 3 d), benzamidase, nitrate reductase, and succinamidase. Acid is produced from fructose, galactose, glucose, *meso*-inositol, maltose, mannitol, mannose, melezitose, sorbitol, sucrose, and trehalose, but not from adonitol, L-arabinose, cellobiose, inulin, lactose, raffinose, rhamnose, or xylose. Resistant to capreomycin, cycloserine, ethambutol, isoniazid, *p*-aminosalicylic acid, protionamide, rifampin, and streptomycin.

Additional phenotypic properties are shown in Table 51.

Contains α- and α′-mycolic acids. The major cellular fatty acids are hexadecanoic (34.2%), oleic (34.4%), and tuberculostearic (8.1%) acids. The cellular polar lipid composition is diphosphatidylglycerol, phosphatidylethanolamine, phosphatidylinositol and phosphatidylinositol mannosides. The predominant menaquinone is MK-9; minor amounts of MK-8 and MK-10 are also present.

Source: blood cultures.

DNA G+C content (mol%): 69–74 (HPLC).

Type strain: IMMIB D-1397, CCUG 38499, DSM 44234, JCM 10112.

Sequence accession no. (16S rRNA gene): AY 238514.

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Order VI. **Frankiales** ord. nov.

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Fran.ki′a.les. N.L. fem. n. *Frankia* type genus of the order; suff. *-ales* ending to denote an order; N.L. fem. pl. n. *Frankiales* the *Frankia* order.

The order *Frankiales* is formed by elevation of the suborder *Frankineae* (Stackebrandt et al., 1997). It is currently composed of bacteria that were treated differently in the last edition of the *Bergey's Manual of Systematic Bacteriology* (Williams, 1989). In that edition, section 27 was termed "Actinomycetes with multilocular sporangia" and contained the three genera *Frankia*, *Geodermatophilus*, and *Dermatophilus* (Lechevalier, 1989), members of which all produced spores in multilocular sporangia by septation in several planes. However, the taxa covered in this section turned out to be polyphyletic (Hahn et al., 1989). In addition to genera with a large number of species, like *Streptomyces* and *Streptoverticillium*, section 29 contained the formerly monospecific genus *Sporichthya*, which produces chains of spores, and the genus *Kineosporia* that had no features in common with the other genera except substrate hyphae (Locci, 1989). The current treatment clusters taxa on the basis of similarities in their 16S rRNA gene sequences, with the result that some additional genera are now included in the order *Frankiales*. Many of the taxa included in the *Frankiales* have little in common with the exception of similar 16S rRNA gene sequences (Table 52). This unusual situation is presumably the result of an adaptable ancestral bacterium that evolved to occupy markedly different ecological niches. These niches include root nodules of woody dicots, hot springs, rocky surfaces, gamma-irradiated substrates, activated sludge, compost, and, of course, soil. This range of ecological specialization is probably the most diverse in a single microbial order with only pathogens missing from the group now that *Dermatophilus* has been reassigned to family *Dermatophilaceae*, order *Micrococcales*, following the 16S rRNA gene sequencing analysis of Hahn et al. (1989). Understanding how the ancestor differentiated into the six known families is the subject of many genomics projects currently under way.

The current order is based on a number of 16S rRNA gene sequence analyses. While the original analyses suggested that *Kineosporia* was affiliated to this group in a novel family "*Kineosporiaceae*" (Lilburn and Garrity, 2004), subsequent analyses indicated that this genus represented a novel order, *Kineosporiales* (Zhi et al., 2009; Ludwig et al., this volume). In addition, the genus *Cryptosporangium* was transferred from the family "*Kineosporiaceae*" to a new family *Cryptosporangiaceae*, which remained with the order *Frankiales*. Even with these reclassifications, the order may be an artificial category in the sense that the groupings vary depending on the tree-reconstruction algorithm used. The family *Nakamurellaceae* (formerly *Microsphaeraceae*) is positioned here only artefactually, whereas the others constitute a more solid entity more likely to survive the rearrangements expected as a result of the whole-genome analyses, which are underway.

The order contains the family *Frankiaceae*, which contains symbiotic bacteria, as well as families containing non-symbiotic bacteria: *Geodermatophilaceae*, *Nakamurellaceae*, *Sporichthyaceae*, *Acidothermaceae*, and *Cryptosporangiaceae*. Phylogenetic analysis (neighbor-joining tree) based on 16S rRNA gene sequences shows the relationships between the six families, with *Nakamurellaceae* as the basal group, a large subgroup containing the *Frankiaceae*, the *Acidothermaceae*, the *Geodermatophilaceae*, the *Cryptosporangiaceae*, and the *Sporichthyaceae* (Figure 102).

Type genus: **Frankia** Brunchorst 1886, 174[AL].

TABLE 52. Distinguishing features of the six families of the order *Frankiales*[a]

Characteristic	*Frankiaceae*	*Acidothermaceae*	*Cryptosporangiaceae*	*Geodermatophilaceae*	*Nakamurellaceae*	*Sporichthyaceae*
Morphology:						
Hyphae	++	+/−	++	+/−	−	+/−
Rods	−	+/−	+/-	−	+	+/−
Sporangia	++	−	+/-	++	−	+/−
Vesicles	+	−	−	−	−	−
Physiology:						
Motility	−	−[b]	+/-	+	−	+
Temperature range (°C)	10–35[c]	37–70	10-37	10–37	10–35	10–42
Oxygen requirement	Microaerophilic	Aerobic	Aerobic	Aerobic	Aerobic	Aerobic
Growth rate	Slow (>15 d)	Fast (2 d)	Slow (>14 d)	Fast (2 d)	Slow (10 d)	Fast (24 h)
N_2 fixation	+	−	nd	−	−	−
Chemistry:						
Diamino acid	*meso*-DAP	−	*meso*-DAP	*meso*-DAP	*meso*-DAP	L-DAP
Menaquinone	$9(H_4)$, $9(H_6)$, $9(H_8)$	nd	$9(H_6)$, $9(H_4)$, $9(H_8)$	$9(H_4)$, $8(H_4)$	$8(H_4)$	$9(H_6)$, $9(H_8)$, $8(H_6)$
Cellular fatty acids	$C_{15:0}$, $C_{16:0\ iso}$,$C_{17:1}$	nd	$C_{16:0}$ iso, $C_{18:1}$, $C_{17:1}$, 10-Me$C_{17:0}$, 10-Me$C_{18:0}$	$C_{16:0}$ iso, $C_{16:1}$, $C_{18:1}$	$C_{15:0}$ iso, $C_{16:0}$ iso, $C_{18:1}$ iso	$C_{16:0}$ iso, $C_{18:0}$, $C_{17:1}$
DNA G+C content (mol%)	66–74	66.9	65–76	68–75	67.5	71
Source	Soil/plant root	Thermal springs	Soil, rock surfaces	Soil, rock surfaces, sea	Sludge	Soil

[a]Symbols: ++, strongly positive; +, positive; +/−, positive or negative; −, negative; nd, no data.

[b]Genes for flagellar apparatus are present in the *Acidothermus cellulolyticus* genome, but no motile phase has been observed (Barabote et al., 2009).

[c]Temperature range for most strains is 25–32°C.

[d]Time necessary for appearance of colony on solid medium.

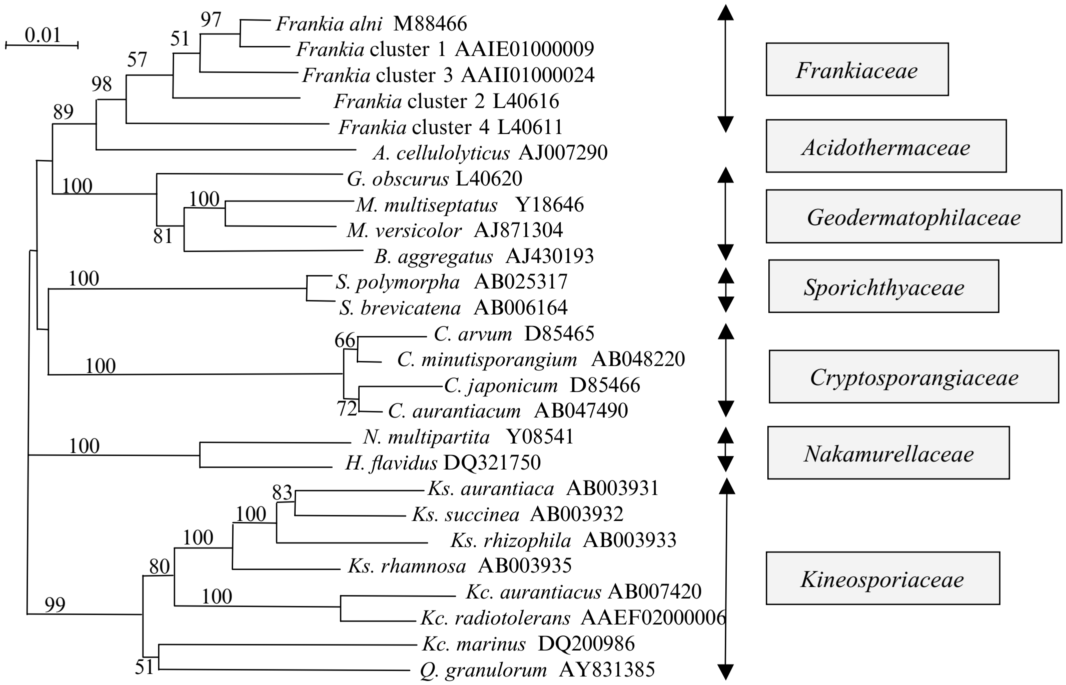

FIGURE 102. Phylogenetic tree of the families within the order *Frankiales*. Neighbor-joining tree (Saitou and Nei, 1987) of 16S rRNA gene sequences of all clusters (*Frankia*) and recognized species of the order *Frankiales*. Sequences were aligned using CLUSTAL X (Thompson et al., 1997), the distance matrix was calculated using Kimura's two-parameter method (1980), the robustness was estimated using the bootstrap method (Felsenstein, 1985), and the resulting topology is represented using NJPlot (Perrière and Gouy, 1996). Bootstrap values are shown at nodes. Bar = 0.01 substitutions per site. The tree is unrooted. The genus *Cryptosporangium* is representative of the family *Cryptosporangiaceae*. The family *Kineosporiaceae* is now classified in the order *Kineosporiales*. This topology is obtained with all tree reconstruction algorithms for these sequences (unpublished).

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Family I. **Frankiaceae** Becking 1970, 201^{AL} emend. Hahn, Lechevalier, Fischer and Stackebrandt 1989, 241 emend. Normand, Orso, Cournoyer, Jeannin, Chapelon, Dawson, Evtushenko and Misra 1996, 8 emend. Stackebrandt, Rainey and Ward-Rainey 1997, 487

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Fran.ki.a.ce′a.e. N.L. fem. n. *Frankia* type genus of the family; suff. -*aceae* ending to denote a family; N.L. fem. pl. n. *Frankiaceae* the *Frankia* family.

Circumscription, position and rank

The *Frankiaceae* as emended by Hahn et al. (1989) included, besides the genus *Frankia* containing plant symbionts, the genus *Geodermatophilus* with members occupying soil and rock surfaces, and the skin pathogen *Dermatophilus*. These genera have sporangia formed by cells dividing in three planes. This feature was judged insufficient to warrant placing them in the same family by Normand et al. (1996), particularly considering their many ecological and biochemical differences and the fact that members of the genus *Acidothermus*, although closer phylogenetically to *Frankia*, did not form the multilocular sporangia deemed to be the hallmark of the taxon. The *Frankiaceae* is thus currently a family with only one genus. This situation may change since it is now known that several related slow-growing soil-inhabiting actinomycetes exist (Normand and Chapelon, 1997).

The family *Frankiaceae* was first proposed by Becking (1970) to group microbes, unisolated at the time, that inhabit the root nodules of woody dicots. Becking relied heavily on morphological features of the bacteria as they occurred in plant tissues and on cross-inoculation studies using crushed root nodules as the inoculum. The first isolation was accomplished 8 years after Becking's publication (Callaham et al., 1978), paving the way for isolation of many additional strains. With strains in hand, it became clear that Becking's proposal was untenable. Cross-inoculation with pure cultures showed that isolated strains had broader host ranges than indicated by endophyte suspensions and that the *in planta* morphology was under control of the host plant (Lalonde, 1979). A signature sequence 5′-TGCAAGTCGAGCGAGGGGCTT-3′ has been proposed as being genus-specific (Normand and Chapelon, 1997), even though phylogenetically related uncharacterized soil actinomycetes have been obtained by PCR. Given that there is only one genus in the family, this signature may be appropriate for the family as a whole. A 16S rRNA gene sequence signature nucleotide pattern consisting of 139:224 (G–C), 148:174 (A–G), 155:166 (U–G), 839:847 (A–G), 987:1218 (G–C), 1059:1198 (C–G), and 1308:1329 (C–G) has been proposed (Stackebrandt et al., 1997).

Type genus: **Frankia** Brunchorst 1886, 174^{AL}.

Genus I. **Frankia** Brunchorst 1886, 174^{AL}

Philippe Normand and David R. Benson

Fran′ki.a. N.L. fem. n. *Frankia* named after Albert Bernhard Frank (1839–1900), a Swiss plant biologist, who studied extensively nitrogen nutrition in legumes and the micro-organisms causing root nodulation from 1877 to 1892 and who coined the term "symbiosis".

Vegetative hyphae with limited to extensive branching, 0.5–2.0 µm in diameter and occasionally wider in older cultures. **No aerial mycelium is formed** on solid media. Intra- and extracellular pigments may be formed. **Gram-stain-positive** in healthy cultures; Gram-stain-variable in older cultures. **Aerobic to microaerophilic** with a respiratory type of metabolism. No growth factors are required. Does not grow under anaerobic conditions. Catalase-positive. Mesophilic. Chemoorganotrophic. Usually very **slow-growing** with doubling times of 20 h to several days. Most strains are capable of fixing N_2 both *in vitro* and *in planta*. Nonpathogenic to humans and animals (Gordon et al., 1983).

Round to irregularly shaped **multilocular sporangia** are borne terminally, laterally, or in an intercalary position on the vegetative hyphae. Lateral sporangia are usually borne on sporangiophores; some are sessile. Sporangia are up to 100 µm in length formed by septation in three planes of the cytoplasm of pre-existing thin-walled swellings. Sporangiospores are nonmotile, irregular (often somewhat polygonal) in shape, 1–5 µm in size, usually colorless, sometimes black, showing multilaminar outer membrane-like layers in thin section. Spores are not thermally resistant. Sporangiospores usually do not develop and mature simultaneously resulting in the presence of spores of different maturity levels in the same sporangium.

Unique terminal or laterally formed "vesicles" or "diazovesicles" may be formed. These structures are terminal hyphal swellings that become increasingly septate with age (Figure 103). They possess envelopes with up to 90 laminated layers composed chiefly of bacteriohopane and its derivatives (Berry et al., 1993; Harriott et al., 1991). They are the site of nitrogen fixation in cells deprived of combined nitrogen in culture and, in symbioses, where vesicles are formed (Benson and Silvester, 1993). Vesicles

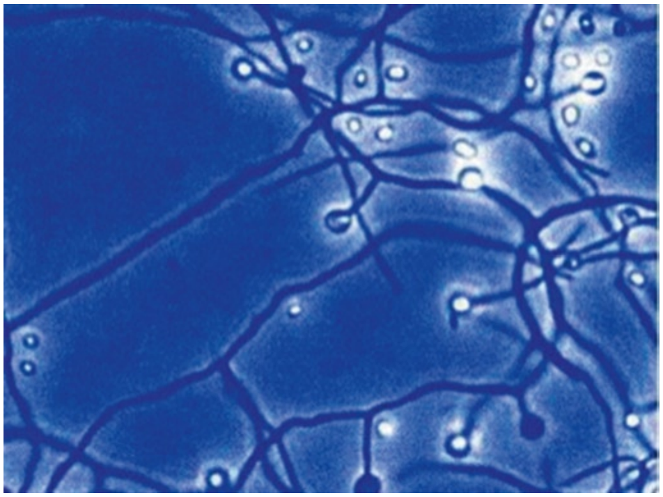

FIGURE 103. Culture of *Frankia alni* strain ACN14a showing vegetative branched septate hyphae as well as "diazovesicles", the thick-walled cells specializing in nitrogen-fixation (photo by Y. Hammad, Université Lyon 1-CNRS, France).

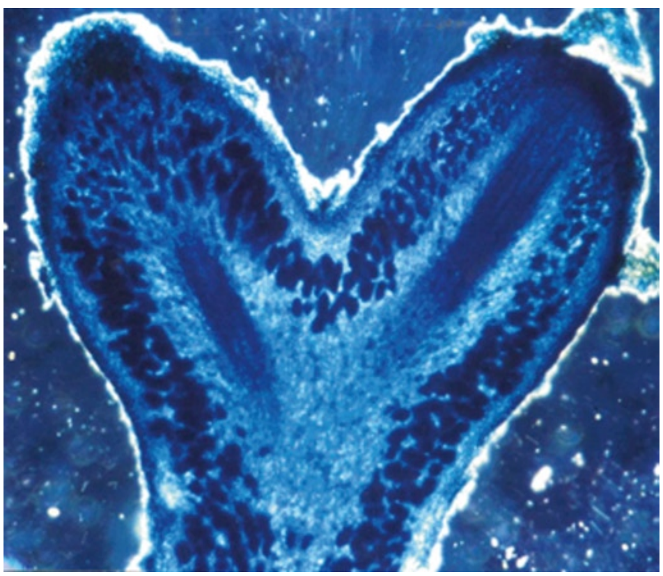

FIGURE 104. Longitudinal section of a dichotomous alder nodule stained to highlight the enlarged cortical cells packed with *Frankia* "diazovesicles". The basal diameter is about 2 mm (photo by P. Normand, Université Lyon 1-CNRS, France).

occasionally form under conditions where nitrogen fixation does not occur.

Cell walls contain *meso*-diaminopimelic acid (*meso*-DAP), glutamic acid, alanine, muramic acid, and glucosamine. No mycolates are present. Phospholipids comprise phosphatidylinositol mannosides, phosphatidylinositol, and diphosphatidylglycerol. Fatty acids are normal, branched-chain, and monounsaturated. Whole-cell sugar patterns show xylose (without arabinose), madurose, or fucose or cells may contain only glucose or galactose, sugars not previously found to have taxonomic significance in the *Actinomycetales.* **All strains tested contain 2-*O*-methyl-D-mannose** and most contain rhamnose. **Most strains are symbiotic with certain angiospermous plants**, inducing nodules on the roots of suitable hosts (Figure 104). May be found free in soil.

DNA G+C content (mol%): 66–73 (T_m).

Type species: **Frankia alni** (Woronin 1866) Von Tubeuf 1895, 118 (*Schinzia alni* Woronin 1866, 6).

Additional remarks: contains one recognized species, *Frankia alni*, and at least 12 genomospecies identified by DNA–DNA hybridization (Akimov and Dobritsa, 1992; An et al., 1985; Fernandez et al., 1989; Lumini et al., 1996). The other species described by Becking (1970) based on morphological descriptions of unisolated strains and on cross-inoculation data were later deemed to be invalid because it was found with the first isolates that the host plant determined the microbial *in planta* morphology (Lalonde, 1979).

Further descriptive information

Symbiotic relationships. Virtually all *Frankia* strains available in culture have been isolated or described in the context of the plant hosts from which they were isolated. Many additional strains have been identified by gene sequences obtained from field-grown root nodules during studies on strain diversity or population structure (Benson et al., 2004, 1996; Clawson et al., 1997, 1998, 1999, 2004; Clawson and Benson, 1999; Huguet et al., 2001, 2004, 2005a, 2005b; Jeong and Myrold, 1999; Oakley et al., 2004; Ritchie and Myrold, 1999a, 1999b; Simonet et al., 1999; Vanden Heuvel et al., 2004).

There are 23 genera of dicotyledonous plants, belonging to eight families, that have established symbiotic N_2-fixing root nodules with *Frankia* (Table 53; Benson and Silvester, 1993). The symbiosis is called "actinorhizal" and infected plants are referred to as "actinorhizal plants" (Torrey, 1983). Suitable plant hosts (Table 53) are readily infected by most frankiae, giving rise to nitrogen-fixing (effective) nodules on the roots. Some strains give rise to noneffective nodules and still others are not infective for the host plant from which they were isolated. The latter strains are often infective on plants belonging to the family Elaeagnaceae (Clawson et al., 2004; Gauthier et al., 1981). Infection in the Elaeagnaceae and in *Ceanothus* takes place by intercellular penetration into the root (Liu and Berry, 1991; Miller and Baker, 1985), whereas infection in other plants proceeds through root hairs (Berry and Sunell, 1990; Berry and Torrey, 1983; Callaham et al., 1979; Liu and Berry, 1991; Sunell and Berry, 1990). In cases where an "elaeagnus strain" infects plants from other families, the infection route is dictated by the plant as to whether it is via intercellular

TABLE 53. Actinorhizal plant genera and associated groups of *Frankia* strains[a]

Order[b]	Family[c]	Genus (no. species)	Geographical distribution[d]	Infective strain group[e]
Fagales	Betulaceae (1/6)	*Alnus* (30)	North temperate, higher elevations in SA, N. Africa, Asia	A, E
	Myricaceae (3/4)	*Comptonia* (1)	Eastern NA	A, E
		Morella (20)	Nearly cosmopolitan but not Australia or Mediterranean	A, E
		Myrica (2)	Circumpolar	A
	Casuarinaceae (4/4)	*Allocasuarina* (58)	Australia	C
		Casuarina (17)	Australia	C
		Ceuthostoma (1)	Malaysia	nd
		Gymnostoma (10)	Malaysia to W. Pacific	E
Rosales	Elaeagnaceae (3/3)	*Elaeagnus* (10)	Europe, Asia, NA	E
		Hippophae (2)	temperate Eurasia	E
		Shepherdia (2)	NA	E
	Rhamnaceae (6/55)	*Ceanothus* (55)	Western NA	R (E)
		Colletia (17)	Southern SA	E
		Discaria (15)	Southern SA, Australia, New Zealand	E
		Kentrothamnus (1)	Southern SA	E
		Retanilla (4)	Southern SA	E
		Trevoa (1)	Southern SA	E
	Rosaceae (4/100)	*Cercocarpus* (6–10)	Western NA	R
		Chamaebatia (2)	Western NA	R
		Dryas (2–3)	Circumboreal, arctic-alpine	R
		Purshia (8)	Western NA	R
Cucurbitales	Coriariaceae (1/1)	*Coriaria* (5–20)	Mexico to SA, Western Mediterranean, New Zealand, Papua New Guinea, Southeast Asia	R
	Datiscaceae (1/1)	*Datisca* (2)	Western NA, South Asia	R

[a] Compiled after Benson et al. (2004) and Swensen (1996). nd, Not determined.

[b] According to the classification of the Angiosperm Phylogeny Group (APG, 1998); all of these orders fall in the "Eurosid I" group of eudicots.

[c] Numbers in parentheses are the number of genera that are nodulated followed by the number of genera within the family. Not all genera within a family are capable of nodulation.

[d] NA, North America; SA, South America

[e] The infective strain group refers to the group of *Frankia* strains typically found in root nodules of each plant genus. A, alder strains; C, casuarina strains, E, elaeagnus strains, R, rosaceous strains. (E), Rare detection of an elaeagnus strain in a nodule or surface layers of a nodule (Benson et al., 2004).

penetration or the root hair (Miller and Baker, 1986; Racette and Torrey, 1989).

The molecular mechanisms whereby the plant recognizes the microbial symbiont and forms nodules are not currently known, except for the recently demonstrated role of SymRK kinase that is necessary for both nodule and mycorhiza formation (Gherbi et al., 2008). In *Frankia* genomes, canonical genes have not been identified (Normand et al., 2007a) and the factor that deforms *Alnus* root hairs has only been characterized as hydrophilic, thermostable, and sensitive to some enzymes such as pronase (Ceremonie et al., 1999).

Pure culture isolates have been obtained from most actinorhizal plant genera and families apart from the Rosaceae, the Coriariaceae, the Datiscaceae, and *Ceanothus* sp. in the Rhamnaceae (Table 53). Three clusters of symbiotic *Frankia* sp. strains are recognized in phylogenetic analyses of 16S rRNA genes and glutamine synthetases I and II (*glnA* and *glnII*) (Clawson et al., 2004; Cournoyer et al., 1993; Normand et al., 1996). Each cluster is characterized by the range of plants infected. Phylogenetic cluster 1 contains the species *Frankia alni*, as well as other strains infective and effective on *Alnus*, *Myrica*, and *Casuarina*. Phylogenetic cluster 2 is basal and includes strains that have been detected in nodules on the Rosaceae, Coriariaceae, Datiscaceae, and the Rhamnaceae of North America (*Ceanothus*), and have so far defied all isolation attempts. Phylogenetic cluster 3 contains strains infective on the Elaeagnaceae, *Gymnostoma* (Casuarinaceae), and the Rhamnaceae of South America. Strains from this group have also been isolated from nodules from other members of the Casuarinaceae; some of the latter strains are infective, but not effective (non-N_2-fixing) on *Alnus*. A fourth phylogenetic cluster, which contains noninfective strains as well as some strains that are infective but not effective on *Alnus*, appears to be basal to the clade.

Morphology. Some frankiae have loosely filamentous thalli with relatively little branching and hyphae that are about 1.0 µm in diameter. Others have finer hyphae (~0.5 µm), are more highly branched, and form compact, dense thalli. The hyphal filaments do not fragment, although in some isolates from *Casuarina*, the hyphae form elongated, narrow, sporangia-like structures with cross walls that break down into subunits under

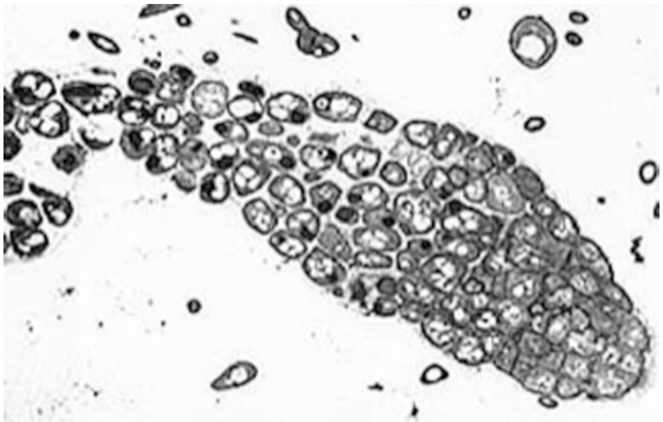

FIGURE 105. *Frankia* CeI5 sporangium (TEM) containing about 50 spores. The size is about 10 × 30 μm (photo by R.H. Berg, Integrated Microscopy Facility, Danforth Plant Science Center, St Louis, MO, USA).

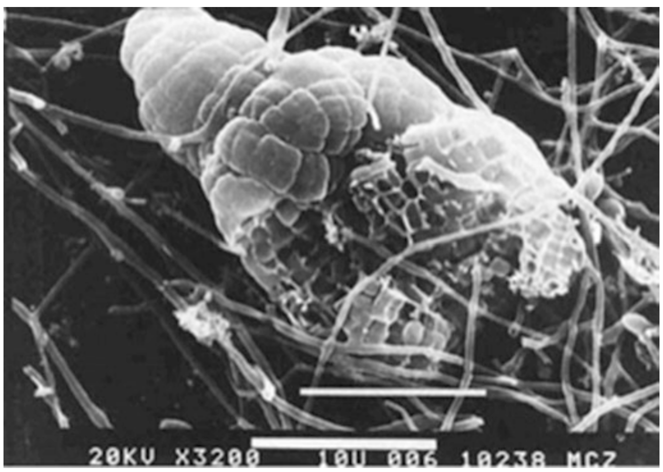

FIGURE 106. Multilocular sporangium and branched hyphae of *Frankia* sp. strain DDB 01020110 isolated from *Alnus viridis* subsp. *crispa* (photo by D.D. Baker and H.A. Lechevalier). Bar = 10 μm. [From the Digital Atlas of Actinomycetes (www.nih.go.jp/saj/DigitalAtlas/); copyright owned by The Society for Actinomycetes, Japan.]

pressure (Diem and Dommergues, 1985). Generally, the sporangia of the loosely filamentous type of strain are larger than in those that form compact colonies, and their mature spores are also larger (1.5–5 μm). Sporangial shapes are diverse, varying even within the same strain from round to highly irregular to elongate; most are multilocular (Figure 105). All spores examined to date are smooth-surfaced. Mature sporangiospores have thick walls and outer membrane-like layers that are similar to those associated with bacteriohopane layers on vesicles (Figure 106). Spores germinate at variable rates (usually very low) to give rise to 1–3 germ tubes for most strains. Sporangia are sometimes produced *in planta*, a characteristic that seems to be controlled genetically by the microorganism (Houwers and Akkermans, 1981; Normand and Lalonde, 1982; Van Dijk, 1978; VandenBosch and Torrey, 1984).

All *Frankia* strains tested make "vesicles" in N-deficient culture and often in symbiosis. Vesicles are lipid-encapsulated, roughly spherical cellular structures that measure between 2 and 6 μm in diameter. They are attached to hyphae by a short vesicle stalk that is also encapsulated. Vesicles contain the nitrogenase system and supporting enzymes. The lipid envelope consists of laminated layers of mainly bacteriohopanes that range from 10 to over 90 in number according to vesicle age and the pO_2 of the surrounding medium (Berry et al., 1993; Harriott et al., 1991; Lamont et al., 1988; Parsons et al., 1987; Torrey and Callaham, 1982). The layers are extracted during fixation for transmission electron microscopy, leaving a "void area" (Lalonde et al., 1976), but they can be visualized by permanganate fixation (Berg et al., 1999; Harriott et al., 1991). Each layer is about 4 nm thick. Bacteriohopanes play a role in membrane stability and fluidity (Poralla et al., 1980). In *Frankia* vesicles, they may limit oxygen diffusion into the O_2-labile nitrogenase (Berry et al., 1993). As vesicles senesce, septa become irregular, nitrogenase activity ceases, and the cytoplasm disappears. Some vesicles can, however, "germinate" and produce hyphae in culture (Schultz and Benson, 1989).

Physiology. In comparison with other actinobacteria, *Frankia* strains use a narrow range of carbon sources. Cluster 1 strains in particular seem limited to using organic acids such as short-chain fatty acids, tricarboxylic acid (TCA) cycle intermediates, or pyruvate as carbon sources. Sugars are not generally used, or are used slowly by strains in this group. Cluster 3 strains use a range of sugars or sugar alcohols including mannitol, fructose, and sorbitol. Growth on cellulose has been reported for one strain (Safo-Sampah and Torrey, 1988), but has not been confirmed by independent experiments. The genes for cellulase and pectinase are missing from the genomes of cluster 1 strains HFPCcI3 and ACN14a, but candidate genes for these enzymes are present in the cluster 3 strain EAN1pec (Normand et al., 2007b).

The most effective nitrogen source for growing *Frankia* is ammonia. It is assimilated primarily via the glutamine synthetase-glutamate synthase pathway (Tsai and Benson, 1989). Growth on individual amino acids is less effective unless a source of ammonia is provided either internally via N_2 fixation or externally in the medium. Cultures deprived of combined nitrogen, or cultivated in the presence of amino acids catabolized as glutamate rather than ammonia, develop vesicles and induce nitrogenase (Zhang and Benson, 1992). Thereupon, growth commences, but is slow relative to growth on ammonia.

As mentioned above, *Frankia* strains that currently have been cultured belong to clusters 1 and 3 of the major phylogenetic groups. No isolates from cluster 2 are available so far, despite numerous attempts on varied growth media. Cluster 1 strains appear similar to one another in culture with largely nonpigmented cells, except in older cultures, which tend to become yellow to red with extracellular pigments. These strains correspond to the group "B" *Frankia* described in a

previous treatment (Lechevalier and Lechevalier, 1989). Some tend to be microaerobic, growing into solid medium when plated and being relatively intolerant to aeration by shaking. As a group, they do not use carbohydrates up to 0.5% in the medium and instead prefer organic acids such as short-chain fatty acids (acetate, propionate), longer chain fatty acids supplied as Tweens or TCA cycle intermediates (succinate, malate), or pyruvate. Most of these strains induce effective (nitrogen-fixing) root nodules on their cognate hosts (Table 53). All strains tested to date also fix nitrogen in culture at atmospheric levels of oxygen or above and, when doing so, compartmentalize nitrogenase, nitrogenase reductase, and associated proteins in vesicles.

At present, two genomes are available from *Frankia* strains belonging to phylogenetic cluster 1. Analysis has confirmed the absence of the Entner–Doudoroff pathway and the presence of a complete Embden–Meyerhof–Parnas pathway, a pentose phosphate pathway, a TCA cycle, and all the components of oxidative phosphorylation. Strain HFPCcI3, a cluster 1 strain isolated from *Casuarina cunninghamiana* nodules, and EAN1pec, a cluster 3 strain isolated from *Elaeagnus angustifolia* root nodules, lack genes for isocitrate lyase in the glyoxylate pathway, but strain ACN14a, a cluster 1 strain, has the pathway genes, thus confirming its ability to grow on acetate or fatty acids. Since virtually all *Frankia* strains described to date have no growth factor requirements, biosynthetic pathways for vitamins, amino acids, nucleotides, and other essential metabolites are present in the genomes, including those for nitrogen fixation. The latter are clustered, in all cases, near the origin of replication.

Cluster 3 strains are typically pigmented, usually red in color, and tend to be more versatile physiologically than cluster 1 strains. They correspond to the group "A" *Frankia* strains of Lechevalier and Lechevalier (1989). On most media, they tend to grow faster than cluster 1 strains and can use carbohydrates such as fructose, mannitol, and sorbitol as sole carbon and energy sources. Strain EAN1pec possesses the components of the phosphotransferase system specific for fructose, sorbitol, and mannitol. In addition to sugars, most cluster 3 strains can also grow on short-chain fatty acids, especially propionate. One genome is available from a cluster 3 strain (EAN1pec). It can be distinguished from the cluster 1 genomes in being markedly larger with many gene duplicates and ancillary genes involving lipid and secondary metabolite metabolism not found in cluster 1 strains. Frankiae are sensitive to penicillin and streptomycin, but are naturally resistant to nalidixic acid.

Pigmentation and secondary metabolites. The capacity of *Frankia* strains to produce secondary metabolites has been highlighted by the recent availability of three genomes from cluster 1 (strains ACN14a and HFPCcI3) and cluster 3 (strain EAN1pec). Each genome contains several genes for polyketide synthases and non-ribosomal peptide synthetases; EAN1pec has the greatest number of such genes of any organism listed in the IMG database (version 1.3, December 2005; http://img.jgi.doe.gov/cgi-bin/pub/main.cgi). Each genome is also enriched with genes for lipid metabolism, similar to other actinobacteria. Secondary metabolites identified in *Frankia* have included red pigments identified as benzo[*a*]naphthacene quinones (Gerber and Lechevalier, 1984; Rickards, 1989) and calcimycins [demethyl (C-11) cezomycin] (Haansuu et al., 2001; Klika et al., 2001) that have some antibiotic activity. The number of biosynthetic genes identified in extant genomes suggests that many other secondary metabolites remain to be characterized from these organisms. *Frankia alni* has also been shown to produce phenylacetate in pure culture (Hammad et al., 2003), a compound known to have antifungal and antibacterial activity (Hwang et al., 2001).

Enrichment and isolation procedures

Pommer (1959) reported the first successful isolation of a *Frankia* strain from *Alnus glutinosa* root nodules using glucose-asparagine agar. The strain was subsequently lost, but drawings made by Pommer show typical *Frankia* morphology including sporangia and vesicles. Subsequently, Callaham et al. (1978) obtained a confirmed isolate (HFPCpI1) using microdissected vesicle clusters from nodules of *Comptonia peregrina* plated on a yeast extract-water agar medium. Once the slow-growing nature of the organism and its overall morphology were recognized, dozens of isolates from various laboratories became available over the next few years. Most available strains have no special growth requirements beyond a simple carbon source, ammonia, and mineral salts. Techniques for isolation have generally included pre-treating root nodules to separate or eliminate contaminants that otherwise quickly outgrow frankiae. The pre-treatments include soaking nodule pieces in surface-sterilizing agents such as mercuric chloride (Callaham et al., 1978), chloramine T (Diem et al., 1982), sodium hypochlorite (Baker and Torrey, 1979), and hydrogen peroxide (Zhongze et al., 1984). Isolation procedures have involved serial dilution of crushed nodule suspensions, microdissection of the nodule with or without pre-treatment with such cellulase or pectinase, and separation by sucrose gradient centrifugation followed by direct plating. The simplest and most effective method is that described by Benson (1982) involving the separation of vesicle clusters from plant material and contaminants by filtration through nylon screens followed by pour plating.

A variety of media has been used for isolation attempts including glucose-asparagine agar used initially by Pommer 1959, 0.5% yeast extract (Callaham et al., 1978), QMod (peptone, yeast extract, phospholipids, and salts; Lalonde and Calvert, 1979), *Frankia* broth (yeast extract, casein hydrolysate, salts and vitamins; Baker and Torrey, 1979), dilute Bennett "S" medium (casein hydrolysate, glucose, salts; Lechevalier et al., 1983), or defined media containing Tween 80, succinate, or pyruvate (Benson, 1982; Gauthier et al., 1981; Hafeez et al., 1984). In a time-course study of the outgrowth of frankiae from *Alnus incana* subsp. *rugosa* nodules, pyruvate was the best carbon source tested, followed by succinate and malate; propionate and acetate were less effective (Benson et al., 1984). Following plating, outgrowth may be observed microscopically

on inverted plates after 5 d for up to 8 weeks. The actual time involved depends to a great extent on the medium used. Some strains may require growth factors for initial outgrowth or for continued growth (Burggraaf, 1984; Normand and Lalonde, 1982; Quispel et al., 1983). However, such requirements are not universal and may reflect the composition of the medium used. Only one report of the isolation of *Frankia* strains using phenol-treated rhizosphere soil and the sucrose density technique exists (Baker and O'Keefe, 1984). Otherwise, isolations direct from the soil have not been reported.

Maintenance procedures

Depending on the strain used, cultures can be transferred as often as every 2 d for relatively fast-growing strains for up to every 4–6 months with storage at room temperature. A variety of media has been used for this purpose; liquid media used for routine cultivation are suitable for long-term on-the-shelf storage. For longer term storage and archival purposes, strains can be stored as heavy suspensions in 10% glycerol and frozen at −20°C or −80°C; −80°C is more effective for some strains. Upon thawing, glycerol should be removed with several washes of fresh medium as it inhibits growth of many strains. Outgrowth is usually rapid. Long-term preservation by lyophilization from broth or by storage in sterile soil also appears satisfactory. Nodules may be stored at −20°C for up to five or more years without loss of infectivity.

Differentiation of the genus *Frankia* from other genera

A proposed definition of the genus *Frankia* is essentially as proposed by Lechevalier and Lechevalier (1984):

1. Actinomycetic, nitrogen-fixing, root-nodule-forming endosymbionts or endoparasites that have been grown in pure culture *in vitro* and that:
   (a) induce effective (nitrogen-fixing) or ineffective (non-nitrogen-fixing) root nodules on a host plant and may be re-isolated from within the nodules of that plant; and
   (b) produce sporangia containing nonmotile spores in submerged liquid culture, and may also form vesicles.
2. Free-living actinomycetes having no known nodule-forming or nitrogen-fixing capacity, but that show the morphology described in 1(b) above.

List of species of the genus *Frankia*

1. **Frankia alni** (Woronin 1866) Von Tubeuf 1895, 118^{AL} (*Schinzia alni* Woronin 1866, 6)

al′ni. L. gen. n. *alni* of alder, the original source of isolation.

This is the only species designated in the genus; all other species proposed previously by Becking (1970) have been shown not to correspond to later descriptions of the genomic species (Akimov and Dobritsa, 1992; Akimov et al., 1991; An et al., 1983, 1985; Bloom et al., 1989; Fernandez et al., 1989; Lumini et al., 1996). Hence, the only species still valid is the first ever described that corresponds to the one described by Woronin (1866) as present in the roots of alder and shown later by Fernandez et al. (1989) to correspond to the numerically dominant group of strains infective on *Alnus*.

Aerial hyphae, chalky white, 0.5–1.2 µm in diameter.

There are probably about 20 genomic species among the isolates described so far and a similar number of unisolated strains not considering phyletic neighbors living in the soil (Normand and Chapelon, 1997), the rhizosphere of various plants, or other environments. However, given their slow growth rates and their tedious growth requirements, it is likely that a rigorous species designation is still a long way in the future. However, 16S rRNA gene sequence characterization work continues to be carried out (Clawson et al., 1998; Huguet et al., 2001; Jeong and Myrold, 1999; Lumini and Bosco, 1999; Navarro et al., 1999; Ritchie and Myrold, 1999a; Simonet et al., 1999; Wolters et al., 1997) and will be correlated with ecological work.

Source: root nodules of host plants (may also be found in soil).

DNA G+C content (mol%): 72.8 [as determined by complete genome sequencing (Normand et al., 2007b); see the Genoscope website for details (http://www.genoscope.cns.fr/externe/English/Projets/Projet_HF/HF.html)].

Type strain: since the publication of the Approved Lists of Bacterial Names, several strains of *Frankia alni* have been isolated. However, no type strain has been designated. The first strain isolated was HFPCpI1 (Callaham et al., 1978); it was deposited in the ATCC but, as is the case with several of the slow-growing *Frankia* strains, it could not be revived (although it is still available from diverse laboratories) and is no longer available there. Another strain, AvcI1 is available from the ATCC, but this strain has not been studied in detail. For these reasons, another strain, ACN14a isolated from *Alnus crispa* growing in Tadoussac (Normand and Lalonde, 1982), that has only two substitutions in the 16S rRNA gene (Normand et al., 1996) relative to HFPCpI1 has been widely distributed in different laboratories, and was chosen for genome sequencing.

Sequence accession nos: M88466 (16S rRNA gene; *Frankia alni* strain ACN14a), CT573213 (genome; *Frankia alni* strain ACN14a), CP000249 (genome; *Frankia* sp. strain HFPCcI3), and CP000820 (genome; *Frankia* sp. strain EAN1pec).

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Family II. **Acidothermaceae** Rainey, Ward-Rainey and Stackebrandt 1997, 487[VP]

Philippe Normand, Alison Berry and David R. Benson

A.ci.do.ther.ma.ce′a.e. N.L. masc. n. *Acidothermus* type genus of the family; suff. *-aceae* ending to denote a family; N.L. fem. pl. n. *Acidothermaceae* the *Acidothermus* family.

Rainey, Ward-Rainey and Stackebrandt (in Stackebrandt et al., 1997) created the one-genus family *Acidothermaceae*, which contains only the type genus *Acidothermus* (Mohagheghi et al., 1986). As its name implies, it grows in hot acid springs. Phylogenetic analyses have shown that members of the family are the closest neighbors to members of the genus *Frankia* (Normand et al., 1996).

Type genus: **Acidothermus** Mohagheghi, Grohmann, Himmel, Leighton and Updegraff 1986, 442[VP].

Genus I. **Acidothermus** Mohagheghi, Grohmann, Himmel, Leighton and Updegraff 1986, 442[VP]

Philippe Normand, Alison Berry and David R. Benson

A.ci.do.ther′mus. L. adj. *acidus* sour, acid; Gr. adj. *thermos* hot; N.L. masc. n. *Acidothermus* acid and hot (loving).

Slender rods and filaments, 0.4 × 5–20 µm, with rounded ends (Figure 107). No endospores are formed. No flagella have been reported (Figure 108) and no motility has been observed, although the genome of *Acidothermus cellulolyticus* contains coding sequences for a flagellar apparatus (Barabote et al., 2009). **Gram-stain-variable**, but generally Gram-stain-negative. Thin sections show no outer cell membranes. The main constituents of purified cell walls are DAP, glucosamine, muramic acid, serine, and alanine. On LPBM mineral salts agar (Mohagheghi et al., 1986), colonies are creamy white, smooth, circular, entire, and 1–3 mm in diameter. In liquid culture, moderate turbidity is observed and cells may tend to flocculate and sediment out after 3 d. Obligate aerobes, prototrophic; grow

on several carbon sources including d-glucose, cellobiose, and **cellulose**. **Thermophilic** with optimal temperature of 55°C (range 37–70°C) and acidophilic with **optimal pH of 5.5** (pH range 3–7). Isolated from 55–60°C acidic water and mud samples in Yellowstone National Park in the course of a screening program to obtain thermostable cellulases. Cells may be stored in 20% (v/v) glycerol in LPBM medium at −80°C, and thawed on ice.

DNA G+C content (mol%): 66.9 (determined by complete genome sequencing).

Type species: **Acidothermus cellulolyticus** Mohagheghi, Grohmann, Himmel, Leighton and Updegraff 1986, 442[VP].

List of species of the genus *Acidothermus*

1. **Acidothermus cellulolyticus** Mohagheghi, Grohmann, Himmel, Leighton and Updegraff 1986, 442[VP]

cell.u.lo.ly′ti.cus. N.L. n. *cellulosum* cellulose; N.L. masc. adj. *lyticus* (from Gr. masc. adj. *lutikos*) able to loosen, able to dissolve; N.L. masc. adj. *cellulolyticus* cellulose-dissolving.

Morphology is as described for the genus. Grows on D-glucose, cellobiose, cellulose, xylan, D-galactose, maltose, sucrose, raffinose, D-mannose, D-mannitol, or D-sorbitol as sole carbon and energy sources. Grows on Casamino acids (0.1%) plus tryptone (0.1%); no growth is observed on nutrient broth, acetate, lactate, citrate, or pectin. Citrate and acetate are inhibitory at 0.01 M. Resistant to penicillin G at 100 μg/ml; sensitive to vancomycin and lysozyme. Catalase-positive. Does not contain polyamines (Hamana et al., 1991). Actively digests cellulose. Contains several genes for cellulose degradation (McCarter et al., 2002). Contains a gene for *shc* (squalene hopene cyclase) that may be involved in the ability to grow at high temperatures (Alloisio et al., 2005).

Source: isolated from 55–60°C acidic water and mud samples in Yellowstone National Park in the course of a screening program to obtain thermostable cellulases.

DNA G+C content (mol%): 66.9 (determined by complete genome sequencing).

Type strain: ATCC 43068, 11B.

Sequence accession nos: AJ007290 (16S rRNA gene sequence of ATCC 43068[T]); CP000481 (complete genome sequence of strain 11B[T]).

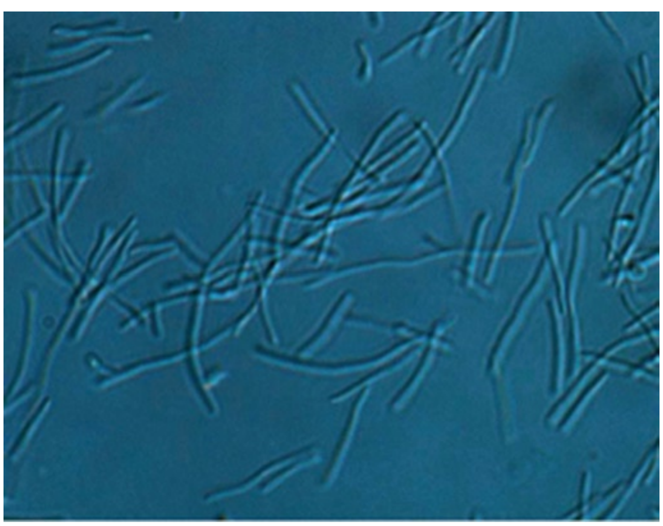

FIGURE 107. Light micrograph of cells of *Acidothermus cellulolyticus* grown in liquid medium at 55°C. (Photo by Petar Pujic, Universite Lyon 1-CNRS, France.)

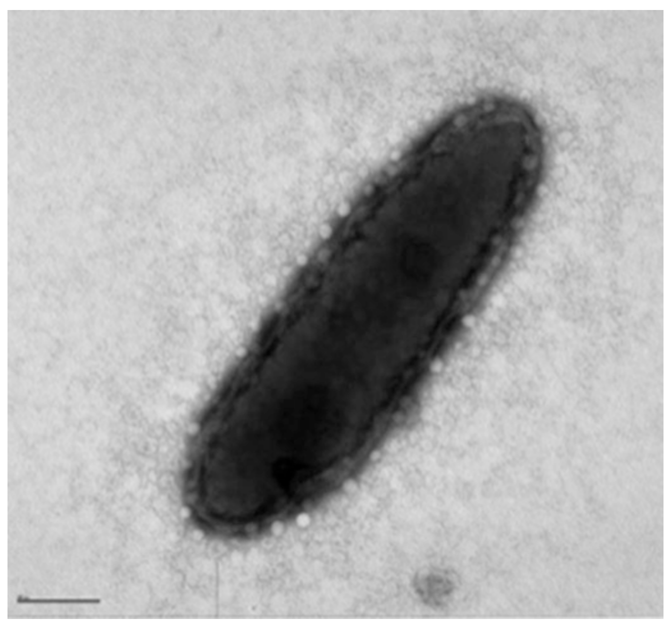

FIGURE 108. Transmission electron micrograph of cells of *Acidothermus cellulolyticus* grown in liquid medium at 55°C. Bar = 200 μm. (Photo by Alison Berry, University of Davis California, USA.)

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Family III. **Cryptosporangiaceae** Zhi, Li and Stackebrandt 2009, 596[VP]

Peter Kämpfer

Cryp.to.spo.ran.gi.a.ce′a.e. N.L. neut. n. *Cryptosporangium* type genus of the family; suff. *-aceae* ending to denote a family; N.L. fem. pl. n. *Cryptosporangiaceae* the *Cryptosporangium* family.

The delineation of the order is based on 16S rRNA gene sequence analysis and contains only the genus *Cryptosporangium* (Tamura et al., 1998). The pattern of 16S rRNA signatures consists of nucleotides at positions 195 (U), 196 (C), 601:637 (A–U), 602:636 (C–G), 841 (U), 952:1229 (C–G), 986:1219 (A–U), 1042 (U), 1059:1198 (U–A), 1251 (G), and 1003:1037 (A–C) according to Zhi et al. (2009). Phenotypic characters are that of the genus *Cryptosporangium*.

Members of the family are Gram-stain-positive, non-acid-fast, aerobic organisms with branching hyphae. The sporangia and aerial mycelia aggregate, and sporangiospores show motility when they are suspended in water. Strictly aerobic. Cell walls contain glutamic acid, glucosamine, glycine, alanine, and *meso*-diaminopimelic acid, with peptidoglycan type A1γ according to Schleifer and Kandler (1972). Glucose and acofriose are detected as whole-cell sugars. Major menaquinone is MK-9(H_6); small amounts of MK-9(H_4) and 9(H_8) are also present. Phosphatidylethanolamine and phosphatidylinositol are present as diagnostic phospholipids. The acyl type of cell wall polysaccharides is acetyl.

Type species: **Cryptosporangium** Tamura, Hayakawa and Hatano 1998, 1003[VP].

Genus I. **Cryptosporangium** Tamura, Hayakawa and Hatano 1998, 1003[VP]

Philippe Normand and David R. Benson

Cryp.to.spo.ran′gi.um. Gr. adj. *kruptos* hidden; N.L. n. *sporangium* [from Gr. n. *spora* a seed (and in biology a spore), and Gr. n. *angeion* (Latin transliteration *angium*) vessel], sporangium; N.L. neut. n. *Cryptosporangium* an organism with sporangia (spore containing vessels) covered or hidden by mycelium.

Gram-stain-positive, non-acid-fast, aerobic organisms with branching hyphae. Non-fragmentary substrate mycelia are present. The sporangia and aerial mycelia aggregate (Figure 109) and **sporangiospores show motility** when they are suspended in water. Morphology of a 2-week-old culture grown on inorganic salts-starch agar shows the presence of aerial mycelia. Strictly aerobic. Good growth occurs at 20–25°C. The organism shows good growth on oatmeal agar, inorganic salts-starch agar, and peptone-yeast extract iron agar. In general, the vegetative mycelia are yellow to orange and the aerial mycelia are white. Cell walls contain glutamic acid, glucosamine, glycine, alanine, and *meso*-DAP. Glucose and acofriose (3-*O*-methyl-rhamnose) are detected as whole-cell sugars. Major cellular fatty acids are $C_{17:1}$, $C_{18:1}$, and $C_{16:0}$ iso. The major menaquinone is MK-9(H_6); small amounts of MK-9(H_4) and MK-9(H_8) are also present. Phosphatidylethanolamine and phosphatidylinositol are present as diagnostic phospholipids. The acyl-type of the cell wall polysaccharides is acetyl (Tamura et al., 1998). Isolated from soil.

DNA G+C content (mol%): 70.

Type species: **Cryptosporangium arvum** Tamura, Hayakawa and Hatano 1998, 1003[VP].

FIGURE 109. Light micrograph (a) and scanning electron micrographs (b–d) of *Cryptosporangium arvum* YU 629-21[T] grown on humic acid-vitamin agar for 14 d at 28°C. Bars: (b) = 50 μm; (c) = 10 μm; (d) = 5 μm. The sporangia are multilocular, 3–7 μm in diameter, hidden under the hyphal mats (hence the genus name) and contain motile spores (Tamura et al., 1998). (Reprinted with permission from Tamura et al. 1998. Int. J. Syst. Bacteriol. *48*: 995–1005.)

Further descriptive information

The genus was created by Tamura et al. (1998) to accommodate soil isolates with a unique morphology and physiology and was composed of two species, *Cryptosporangium arvum* and *Cryptosporangium japonicum*. Two other species, comprising isolates initially described as "*Actinoplanes minutisporangius*" by Ruan et al. (1986) and "*Actinoplanes aurantiacus*", were added to the genus and renamed *Cryptosporangium minutisporangium* comb. nov. and *Cryptosporangium aurantiacum* comb. nov., respectively.

Differentiation of the species of the genus *Cryptosporangium*

Differential characteristics of the four *Cryptosporangium* species are given in Table 54.

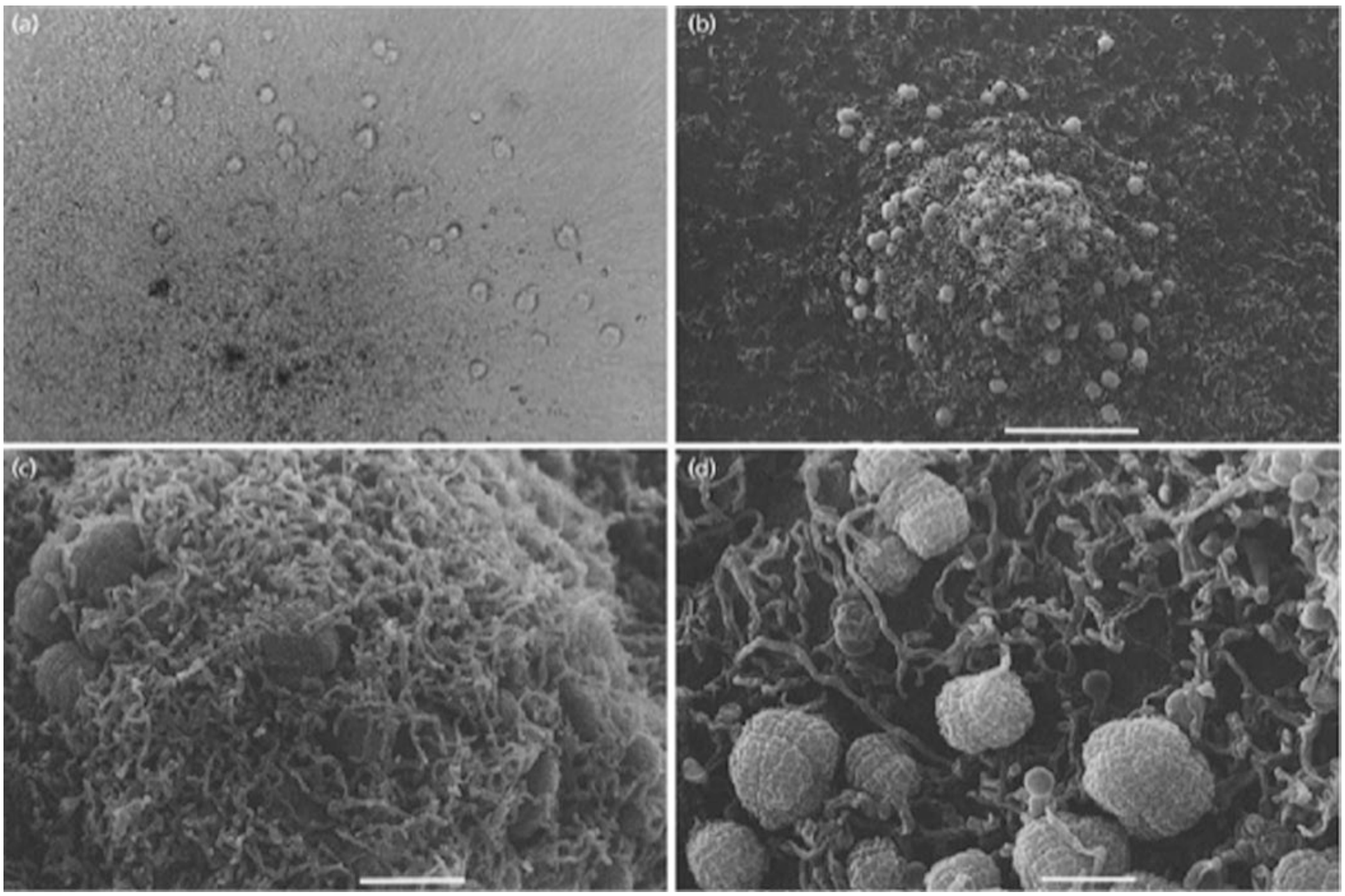

FIGURE 109. Light micrograph (a) and scanning electron micrographs (b–d) of *Cryptosporangium arvum* YU 629-21^T grown on humic acid-vitamin agar for 14 d at 28°C. Bars: (b) = 50 µm; (c) = 10 µm; (d) = 5 µm. The sporangia are multilocular, 3–7 µm in diameter, hidden under the hyphal mats (hence the genus name) and contain motile spores (Tamura et al., 1998). (Reprinted with permission from Tamura et al. 1998. Int. J. Syst. Bacteriol. *48*: 995–1005.)

TABLE 54. Differential characteristics of the four *Cryptosporangium* species[a,b]

Character	*C. arvum*	*C. aurantiacum*	*C. japonicum*	*C. minutisporangium*
Acid production from:				
Maltose	+	−	+	+
D-Lactose	+	−	+	+
i-Inositol	−	d	+	+
Methyl α-D-glucoside	+	−	+	+
Utilization of:				
i-Inositol	w	w	+	+
D-Sorbitol	−	w	−	d
Raffinose	w	−	+	d
Decomposition of urea	+	−	−	−
Hydrolysis of:				
Starch	+	+	−	−
Urea	+	−	−	−
Gelatin	+	−	−	−
Utilization of:				
Succinate	+	−	−	−
Oxalate	−	+	−	−
Malate	+	−	−	−
Nitrite from nitrate	+	+	−	−
Decomposition of calcium malate	−	−	+	+
Pigmentation in ISP7	−	Moderate red	Pale reddish-brown	Pale reddish-brown
Growth at:				
10°C	+	−	−	−
15°C	+	w	+	+
30°C	+	+	+	+
37°C	−	+	−	+

[a]Data from Ruan et al. (1985) and Tamura and Hatano (2001).

[b]d, Positive or negative; w, weak.

List of species of the genus *Cryptosporangium*

1. **Cryptosporangium arvum** Tamura, Hayakawa and Hatano 1998, 1003[VP]

ar′vum. L. n. *arvum* arable land, pertaining to an isolate obtained from arable land.

Morphological, chemotaxonomic, and general characteristics are as given above for the genus. Pale red-brown soluble pigment is produced on tyrosine agar (ISP medium 7). Gelatin liquefaction is negative. Does not hydrolyze starch. Decomposes calcium malate. Coagulates milk. Xylose, glucose, inositol, raffinose, rhamnose, mannitol, sucrose, arabinose, glycerol, lactose, maltose, and mannose are utilized, but sorbitol and inulin are not. No growth at 37°C. Major cellular fatty acids are $C_{16:0}$ iso, $C_{18:1}$, $C_{17:1}$, and $C_{17:0}$.

Source: isolated from cultivated soil.

DNA G+C content (mol%): 70.1 (HPLC; Mesbah and Whitman, 1989).

Type strain: YU629-21, IFO 15965.

Sequence accession no. (16S rRNA gene): D85465.

2. **Cryptosporangium aurantiacum** (Ruan, Zhang and Jiang 1976) Tamura and Hatano 2001, 2124[VP] (Basonym: *Actinoplanes aurantiacus* Ruan, Zhang and Jiang 1976, 291.)

au.ran.ti.a′cum. N.L. neut. adj. *aurantiacum* gold- or orange-colored.

Morphological, chemotaxonomic, and general characteristics are as given for the genus. Red soluble pigment is produced on tyrosine agar (ISP medium 7). Starch is hydrolyzed. Gelatin and esculin are not hydrolyzed. Nitrate is reduced. No growth in 4% NaCl. Acid is produced from mannitol, melibiose, rhamnose, arabinose, galactose, mannose, glucose, and xylose, but not from erythritol, adonitol, maltose, lactose, sorbitol, methyl α-D-glucoside, dulcitol, or raffinose. Mannitol, melibiose, maltose, rhamnose, lactose, inositol, sorbitol, arabinose, galactose, mannose, glucose, xylose, and oxalate are utilized, but erythritol, adonitol, methyl α-D-glucoside, dulcitol, raffinose, succinate, malate, and citrate are not (Ruan et al., 1985; Tamura and Hatano, 2001).

Source: isolated from cultivated soil.

DNA G+C content (mol%): not determined.

Type strain: IFO 13967, JCM 3241, 71-C38.

Sequence accession no. (16S rRNA gene): AB047490.

3. **Cryptosporangium japonicum** Tamura, Hayakawa and Hatano 1998, 1003[VP]

ja.po′ni.cum. N.L. neut. adj. *japonicum* pertaining to Japan where the organisms were isolated.

Morphological, chemotaxonomic, and general characteristics are as given above for the genus. Pale red–brown

soluble pigment is produced on tyrosine agar (ISP medium 7). Gelatin liquefaction is negative. Does not hydrolyze starch. Decomposes calcium malate. Coagulates milk. Xylose, glucose, inositol, raffinose, rhamnose, mannitol, sucrose, fructose, arabinose, lactose, galactose, maltose, and mannose are utilized, but sorbitol and inulin are not. Grows at 37°C.

Source: isolated from cultivated soil.

DNA G+C content (mol%): 70.2–70.4 (HPLC; Mesbah and Whitman, 1989).

Type strain: YU636-3, IFO 15966.

Sequence accession no. (16S rRNA gene): D85466.

4. **Cryptosorangium minutisporangium** (Ruan, Lechevalier, Jiang and Lechevalier 1986) Tamura and Hatano 2001, 2123[VP] (Basonym: *Actinoplanes minutisporangius* Ruan, Lechevalier, Jiang and Lechevalier 1986, 573.)

mi.nu.ti.spo.ran′gi.um. L. adj. *minutus* little, small, minute; N.L. n. *sporangium* [from Gr. n. *spora* a seed (and in biology a spore), and Gr. n. *angeion* (Latin transliteration *angium*) vessel] sporangium; N.L. n. *minutisporangium* (nominative in apposition) the small sporangium.

The description is based on data compiled by Ruan et al. (1985) and Tamura and Hatano (2001). Morphological, chemotaxonomic, and general characteristics are as given previously for the genus (Tamura et al., 1998). Spores are motile. Brown to brownish-black substrate mycelium is produced. Hydrolyzes casein, hypoxanthine, urea, starch, and gelatin. Does not hydrolyze adenine or xanthine. Reduces nitrate to nitrite. Phosphatase is negative. Acetate, malate, pyruvate, succinate, and propionate are utilized, but lactate, benzoate, citrate, mucate, oxalate, and tartrate are not utilized. Acid is produced from arabinose, cellobiose, fructose, galactose, glucose, glycerol, inositol, lactose, maltose, mannitol, mannose, melibiose, methyl α-D-glucoside, rhamnose, salicin, sucrose, trehalose, and xylose, but not from adonitol, dulcitol, erythritol, raffinose, sorbitol, or methyl α-D-xyloside.

Source: isolated from soil.

DNA G+C content (mol%): 76 (HPLC; Mesbah and Whitman, 1989).

Type strain: IFO 15962, JCM 9458, ATCC 49415, LL-A-60.

Sequence accession no. (16S rRNA gene): AB048220.

Genus *Incertae sedis* I. **Fodinicola** Carlsohn, Groth, Saluz, Schumann and Stackebrandt 2008, 1534[VP]

Peter Kämpfer and Peter Schumann

Fo.di.ni′co.la. L. n. *fodina* a pit, mine; L. suff. *-cola* (from L. n. *incola*) dweller; N.L. masc. n. *Fodinicola* a mine dweller.

Cells are filamentous. A branched substrate mycelium and sparse to abundant white aerial mycelium is produced. The aerial hyphae break up into irregular rod-like elements. Gram-stain-positive. **Spores are not formed. Nonmotile. Aerobic metabolism.** Good growth is observed on complex organic media at 20–30°C. **Oxidase-negative, catalase-positive.** The cell-wall peptidoglycan contains *meso*-diaminopimelic acid (*meso*-A_2pm), alanine, glycine, and glutamic acid. The muramic acid in the peptidoglycan is *N*-acetylated. The **cell-wall sugars are xylose and minor amounts of an unknown compound**. The predominant menaquinones are **MK-9(H_4), MK-9(H_6), and MK-9(H_8).** The polar lipids comprise **diphosphatidylglycerol, phosphatidylethanolamine, phosphatidylserine, phosphatidylinositol, and several unknown phospholipids and glycolipids** together with unknown ninhydrin-positive compounds. Mycolic acids are absent. The cellular fatty acid profile is characterized by the predominance of $C_{16:0}$ iso, 10-methyl-$C_{17:0}$, $C_{17:1}$ *cis*9, 10-methyl-$C_{18:0}$, and $C_{17:0}$. Based on 16S rRNA gene sequence similarities, the genus is closely related to the genus *Cryptosporangium* of the family *Cryptosporangiaceae*.

DNA G+C content (mol%): 65 (HPLC).

Type species: **Fodinicola feengrottensis** Carlsohn, Groth, Saluz Schumann and Stackebrandt 2008, 1534[VP].

Further descriptive information

Currently, the genus is represented by a single species, *Fodinicola feengrottensis*, which accommodates only one strain. 16S rRNA gene sequence comparisons showed that the type strain of *Fodinicola feengrottensis* shares 92.9–94.8% similarity with the type strains of species of the genus *Cryptosporangium*, and 93.9–94.5% similarity with the type strains of the genus *Sporichthya*. *Fodinicola feengrottensis* shares the 16S rRNA signature nucleotides of the families of the order *Frankiales* [as described for the *Frankineae* by Stackebrandt et al. (1997) with the exception of a U instead of a C at position 222 (Carlsohn et al., 2008)]. A maximum-likelihood tree is shown in Figure 110.

The peptidoglycan of *Fodinicola feengrottensis* contains *meso*-A_2pm, Ala, Gly, and Glu in a molar ratio of approximately 1.0:0.9:2.4:1.0. The presence of *meso*-A_2pm is shared by most genera of the order *Frankiales*, with the exception of *Sporichthya*, which contains LL-A_2pm (Carlsohn et al., 2008). The major menaquinones are MK-9(H_4), MK-9(H_6), and MK-9(H_8), in almost equal proportions, which is also in congruence with the quinones found in most of the closely related genera (Figure 110), with the exception of members of the genera *Nakamurella* and *Quadrisphaera*.

The major cellular fatty acids of the type strain of *Fodinicola feengrottensis* are $C_{16:0}$ iso (32.2%), 10-methyl-$C_{17:0}$ (10.7%), $C_{17:1}$ *cis*9 (8.9%), and 10-methyl-$C_{18:0}$ iso (8.5%); minor fatty acids are $C_{17:0}$ (7.4%), $C_{17:0}$ iso (5.8%), $C_{18:1}$ *cis*9 (4.8 %), 10-methyl-$C_{18:0}$ (4.7%), $C_{18:0}$ iso (3.2%), $C_{17:0}$ anteiso (3.0%), $C_{16:0}$ iso 2-OH (2.7%), and $C_{16:0}$ (2.6%). The phospholipids comprise diphosphatidylglycerol, phosphatidylethanolamine, phosphatidylserine, phosphatidylinositol, and several unknown phospholipids

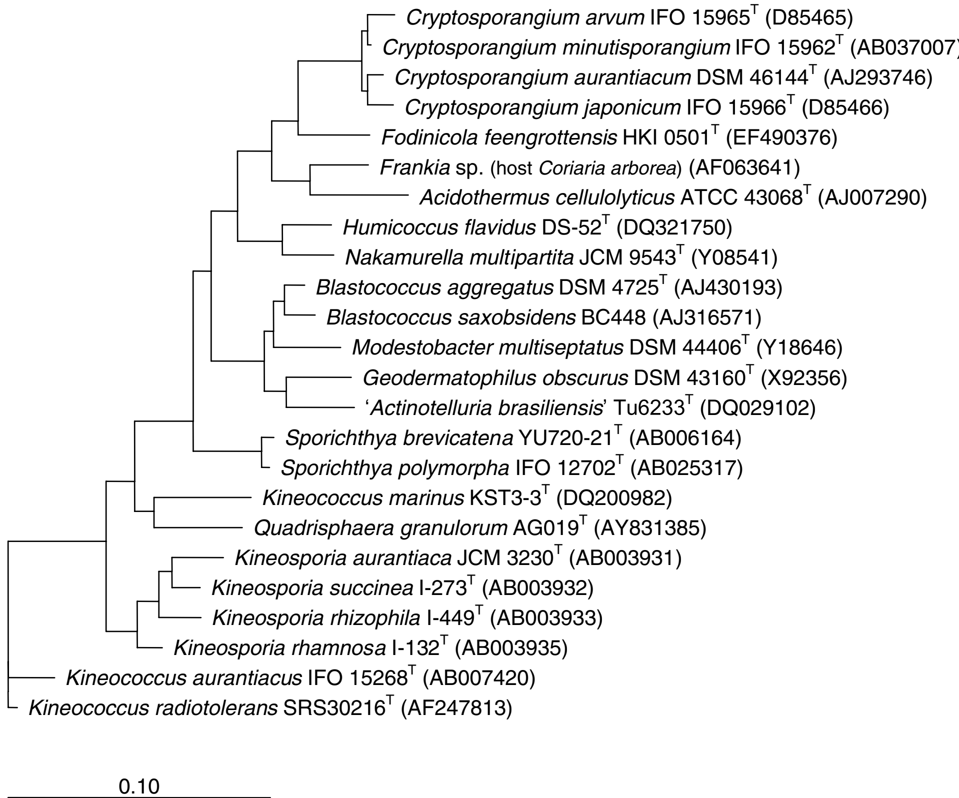

FIGURE 110. Phylogenetic analysis based on 16S rRNA gene sequences available from the EMBL database (accession numbers in parentheses), showing the position of *Fodinicola feengrottensis*. The phylogenetic tree was constructed using the ARB software package (version December 2007; Ludwig et al., 2004) and the corresponding SILVA SSURef 100 database (release August 2009; Pruesse et al., 2007). Tree building was performed using the maximum-likelihood method with fastDNAml (Olsen et al., 1994) and without filters. Bar = 0.10 nucleotide substitutions per nucleotide position.

and glycolipids, together with unknown ninhydrin-positive compounds (Carlsohn et al., 2008).

Enrichment and isolation procedures

Fodinicola feengrottensis grows readily in complex liquid or on solidified media, i.e., Bacto nutrient agar [(Difco) containing (g/l): Bacto beef extract, 3.0; Bacto peptone, 5.0; and Bacto agar, 15.0; pH 6.8] and tryptone soy agar (Oxoid). The type strain of *Fodinicola feengrottensis* was isolated from acidic and heavy metal-containing rocks in the "Barbara Grotto" of the Feengrotten medieval alum slate mine in Saalfeld, Thuringia, Germany, on starch-casein agar plates (Küster and Williams, 1964) supplemented with cycloheximide (50 mg/ml). The agar plates were incubated at 28°C for about 4 weeks. Subcultivation of the isolate was done on solidified organic medium 79 (Prauser and Falta, 1968; medium 426, http://www.dsmz.de/; 10.0 g glucose, 10.0 g peptone, 2.0 g casein peptone, 2.0 g yeast extract, 6.0 g NaCl, 15.0 g agar, 1000 ml distilled water; pH 7.8) and ISP 2 medium (Difco; Shirling and Gottlieb, 1966).

Maintenance procedures

Cultures of *Fodinicola feengrottensis* can be maintained by serial transfers on several complex media as listed above. Good growth is observed after 21 d of incubation at 28°C on casein agar plates (Küster and Williams, 1964), organic medium 79 (Prauser and Falta, 1968; medium 426, http://www.dsmz.de/), and ISP 2 medium (Difco; Shirling and Gottlieb, 1966).

Pure cultures can be preserved at 28°C as well-growing cultures in a mixture of organic medium 79 broth and glycerol medium that consisted of K_2HPO_4 (1.26%), KH_2PO_4 (0.36%), $MgSO_4$ (0.01%), sodium citrate (0.09%), $(NH_4)_2SO_4$ (0.18%), and glycerol (8.8%). Stock cultures of the novel isolate in liquid organic medium 79 supplemented with 5% DMSO can also be maintained in the vapor phase of liquid nitrogen.

Differentiation of the genus *Fodinicola* from other genera

Fodinicola feengrottensis forms branched substrate mycelium and sparse to abundant, short, white aerial hyphae that fragment into irregular rod-like elements. Spore chains and motility of the fragments, as reported for the coccoid to rod-shaped spores of the closely related *Sporichthya* strains (Lechevalier et al., 1968; Tamura et al., 1999), were not observed. Furthermore, the unique morphological properties of *Sporichthya* strains (lack of a substrate mycelium and the presence of a basal cell as a hold-fast in solid medium) can be useful to distinguish strain *Fodinicola feengrottensis* from members of that genus. Representatives

of the equally closely related genus *Cryptosporangium* (Tamura et al., 1998) can be also readily distinguished from *Fodinicola feengrottensis* because they produce spherical to irregularly shaped sporangia with spores that show motility when they are suspended in water. Differentiation from the only strain of the monospecific thermophilic genus *Acidothermus* is also possible on the basis of its typical morphological properties (slender rods, filaments).

List of species of the genus *Fodinicola*

1. **Fodinicola feengrottensis** Carlsohn, Groth, Saluz, Schumann and Stackebrandt 2008, 1534[VP]

fe.en.grot.ten′sis. N.L. masc. adj. *feengrottensis* pertaining to the Thuringian cave Feengrotten, the origin of the type strain.

Hyphal diameter is 0.35–0.52 μm. Diffusible pigments may be produced. Colonies are wrinkled and beige to orange in color. Good growth occurs between 20 and 28°C, but growth is not evident below 10°C or above 32°C. Grows well between pH 5.0 and 6.0, but does not grow at pH 4.0 or 8.0. Growth at pH 7.0 is delayed and reduced. A concentration of 1% NaCl in combination with organic medium 79 is tolerated, but 2% NaCl is not. Esculin, casein, gelatin, potato starch, and urea are hydrolyzed. Nitrate is not reduced to nitrite. Adenine, hypoxanthine, and tyrosine are not degraded. L-Arabinose, D-fructose, D-glucose (weakly), D-mannitol, raffinose, L-rhamnose, sucrose, and D-xylose are used as sole carbon sources for energy and growth, but *myo*-inositol and cellulose are not (all at 1%, w/v). Produces α-chymotrypsin (weakly), cystine arylamidase, leucine arylamidase, valine arylamidase, esterase (C4), esterase lipase (C8), α-galactosidase, β-galactosidase, *N*-acetyl-β-glucosaminidase, α-glucosidase, lipase (C14) (weakly), α-mannosidase, naphthol-AS-BI-phosphohydrolase, acid phosphatase, and alkaline phosphatase, but not α-fucosidase, β-glucosidase, or β-glucuronidase. Production of trypsin is variable (API ZYM tests). Susceptible to the following antibiotics (μg per disc): chloramphenicol (30), ciprofloxacin (5), imipenem (10), kanamycin sulfate (30), norfloxacin (10), novobiocin (5), oxytetracycline hydrochloride (30), streptomycin sulfate (10), sulfonamide (200), and vancomycin hydrochloride (30). Resistant to the following antibiotics (μg per disc, unless otherwise indicated): ampicillin (10), lincomycin hydrochloride (2), methicillin (5), nalidixic acid (30), penicillin G (10 IU), polymyxin B (300 IU), and rifampin (30).

Source: the type strain was isolated from acidic and heavy metal-containing rocks in the "Barbara Grotto" of the Feengrotten medieval alum slate mine in Saalfeld, Thuringia, Germany.

DNA G+C content (mol%): 65 (HPLC).

Type strain: HKI 0501, DSM 19247, JCM 14718.

Sequence accession no. (16S rRNA gene): EF490376.

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Family IV. **Geodermatophilaceae** Normand 2006, 2277[VP] (Effective publication: Normand, Orso, Cournoyer, Jeannin, Chapelon, Dawson, Evtushenko and Misra 1996, 8.)

Philippe Normand and David R. Benson

Ge.o.der.ma.to.phi.la.ce′a.e. N.L. masc. n. *Geodermatophilus* type genus of the family; suff. *-aceae* ending to denote a family; N.L. fem. pl. n. *Geodermatophilaceae* the *Geodermatophilus* family.

Normand et al. (1996) created the family *Geodermatophilaceae*, which was confirmed the following year (Stackebrandt et al., 1997); however, no type genus was designated at that time. The family status has been formalized recently and a formal description has been published (Normand, 2006). As proposed, this family contains the type genus *Geodermatophilus* (Luedemann, 1968) found in soils, the genus *Blastococcus* (Ahrens and Moll, 1970) found in seawater, and the genus *Modestobacter* (Mevs et al., 2000) found in polar "soil". Characteristics of the three genera are given in Table 55. A common morphological feature of the three genera is **rudimentary hyphae** and common physiological features are modest growth requirements and the **ability to grow as a pioneer on exiguous substrates**. A 16S rRNA gene sequence signature pattern has been proposed consisting of nucleotides at positions 139:224 (C–G), 157:164 (A–U), 158:163 (A–U), 186:191 (C–G), 263 (G), 293:304 (G–U), 986:1219 (U–A), 987:1218 (A–U), 1059:1198 (U–A), and 1308:1329 (U–A) (Stackebrandt et al., 1997). A further signature of the *Geodermatophilaceae* would appear to be 5′-TGGGATAACTCCAAG**AAATT**GG-3′ at coordinates 122–143 of the 16S rRNA gene sequence of *Blastococcus aggregatus* DSM 4725^{T} (accession no. AJ430193) with the underlined bold "AA" and "TT" being diagnostic for the family, including a proposed species "*Actinotelluria brasiliensis*" (accession no. DQ029102; M. Bertazzo and others, unpublished). A second signature of the *Geodermatophilaceae* would appear to be 5′-GTCGGTAAC**G**CCCGAAGCCGGTGGC-3′ at coordinates 1385–1409 of the 16S rRNA gene sequence of *Geodermatophilus obscurus* (accession no. L40620) with the underlined bold "G" being diagnostic for the family.

Type genus: **Geodermatophilus** Luedemann 1968, 1857[AL].

TABLE 55. Characteristics of the three genera of the family *Geodermatophilaceae*[a]

Characteristic	*Geodermatophilus*	*Blastococcus*	*Modestobacter*
Morphology:			
Hyphae	Rudimentary	–	–
Spores	Motile	–	–
Sporangia	+	–	–
Buds	–	+	+
Colony color	White, red, black	Pink, orange	White, pink, black
Physiology:			
O_2 requirements	Aerobic	Aerobic/microaerophilic	Aerobic
Temperature range (°C)	18–37	3–40	0–28
Temperature optimum (°C)	24–28	25	19–21
Tolerance to 3% NaCl	–	+	+
Hydrolysis of starch	+	–	–
Hydrolysis of D-glucose	+	–	+
Chemistry:			
Menaquinone	9(H_4)	9(H_4)	9(H_4)
DNA G+C content (mol%)	73–75	74	70
Source	Soil, rock surfaces	Rock surfaces, sea	Polar "soil"

[a]Data from Mevs et al. (2000), Urzì et al. (2004b), and Lechevalier (1989).

Genus I. **Geodermatophilus** Luedemann 1968, 1857[AL]

Philippe Normand and David R. Benson

Ge.o.der.ma.to.phi′lus. Gr. n. *ge* earth; Gr. n. *derma*, *atos* skin; Gr. adj. *philos* loving; N.L. masc. n. *Geodermatophilus* a group of microorganisms that live in the soil, yet that love the skin, by analogy to the genus *Dermatophilus*, the actinobacterial genus causing a skin disease, that has similar morphological features.

Morphology is similar to that of *Dermatophilus*, a group of actinobacteria that have **rudimentary hyphae** (Figure 111) that eventually develop into **complex sporangia** (Figure 112). *Geodermatophilus* strains have been isolated from relatively extreme environments such as desert or high altitude soils. They also appear to resist gamma irradiation and have been recovered from desert soils after exposure to 30 kGy, along with strains of *Deinococcus* that are more noted for radiation resistance (Rainey et al., 2005). They have been recovered during programs aimed at isolating bacteria, in particular motile ones (Hayakawa et al., 2000), that have atypical metabolic features such as **manganese-oxidizing ability**, and are frequently associated with "varnish" formation, the black to brown coatings found on rocks in a diverse range of environments (Hungate et al., 1987) and, more

surprisingly, in biofilm formation (Singh et al., 2003). Despite several trials, pathogenic lesions have not been produced by inoculation of *Geodermatophilus* isolates into rabbits (Gordon and Perrin, 1971). These trials had been attempted given the morphological and ontogenetic similarities with *Dermatophilus*. It is now known these morphological similarities constitute a case of convergent evolution of a few remarkable features rather than phylogenetic similarity.

DNA G+C content (mol%): 72.9–74.6.

Type species: **Geodermatophilus obscurus** Luedemann 1968, 1857[AL].

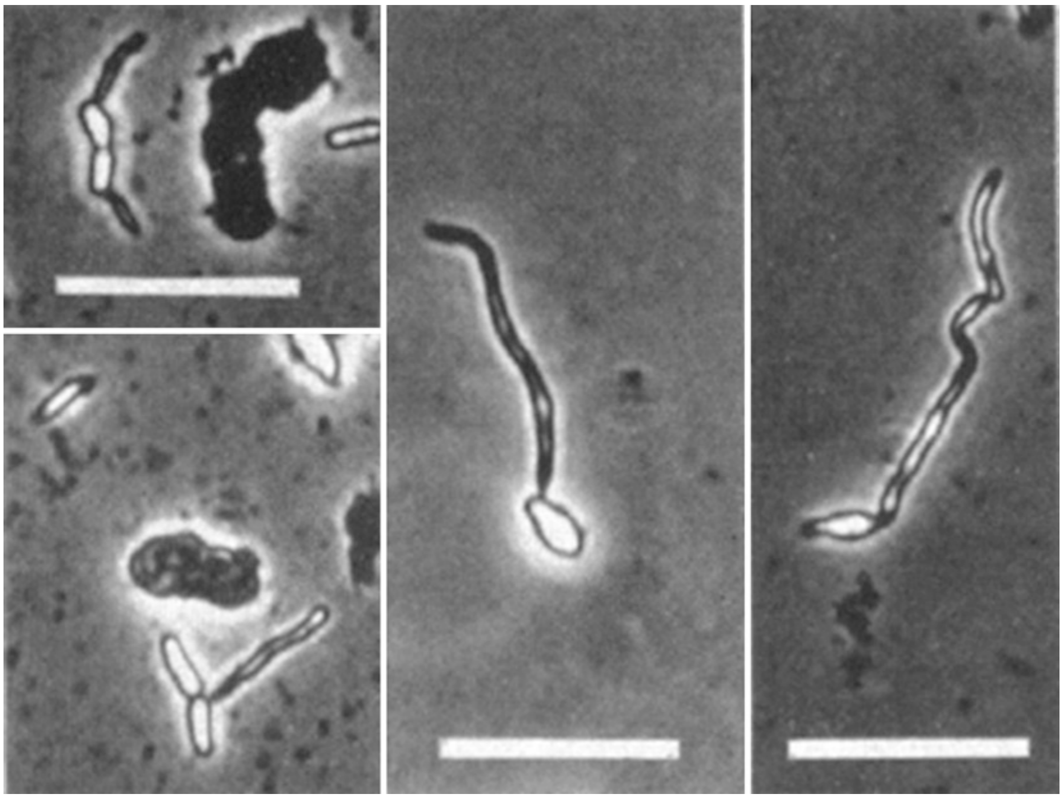

FIGURE 111. "*Geodermatophilus obscurus* subsp. *utahensis*" strain G-17 rudimentary hyphae in liquid cultures after 9–13 d (Luedemann, 1968). Bar = 1 µm. (Reprinted with permission from Luedemann, 1968. J. Bacteriol. *96:* 1848–1858.)

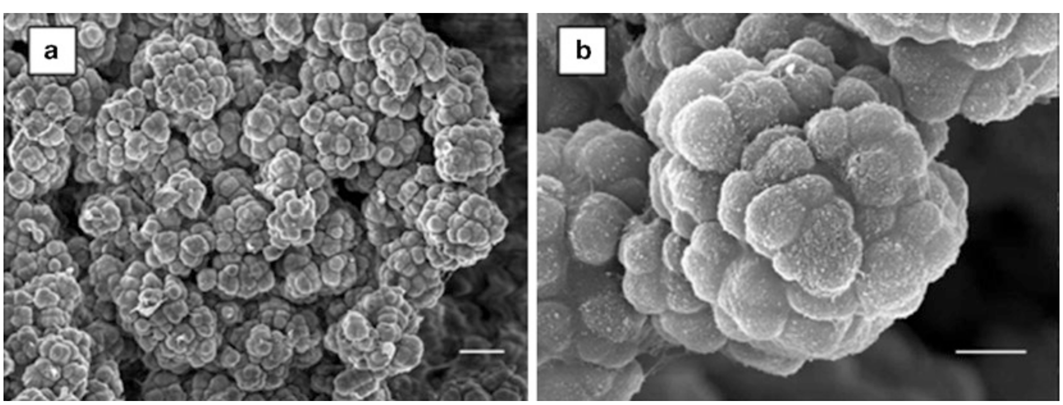

FIGURE 112. Multilocular sporangia of *Geodermatophilus obscurus* KCC A-0152^{T} (=ATCC 25078^{T}). Bars: (a) 5 µm; (b) 2 µm. (Photos courtesy of Shin-ichi Suzuki, Tanabe Seiyaku Co., Saitama, Japan.)

List of species of the genus *Geodermatophilus*

1. **Geodermatophilus obscurus** Luedemann 1968, 1857[AL]

ob.scur′us. L. masc. adj. *obscurus* dark, obscure, indistinct.

Pigmented. Gram-positive. Forms highly irregular tuber-shaped non-capsulated multilocular thalli. These structures contain highly irregular vibrioid or cuboid cells, 0.5–2.0 µm in diameter. Under favorable environmental conditions, the thalli will release individual non-motile cells. A proportion of these cells will develop into elliptical zoospores propelled by a terminal tuft of long flagella (Figure 113). Mycelium is rudimentary. No aerial mycelium is produced. Cell walls contain *meso*-DAP as well as glutamic acid, alanine, glucosamine and muramic acid. Aerobic. Chemoorganotrophic. Mesophilic. Thalli appear greenish-black and vary in size with the strain. Colonies after 30 d incubation at 28°C are flat, granular, dry, with a dank smell. Good growth on yeast extract-starch-sucrose-malt extract agar.

Source: the type strain was isolated from soil of the Amargosa Desert, Nevada, USA.

DNA G+C content (mol%): 72.9–74.6 (Luedemann and Fonseca, 1989).

FIGURE 113. "*Geodermatophilus obscurus* subsp. *everesti*" (16 h culture) with polar tuft of three flagella. Negatively stained with 0.02% potassium phosphotungstate (45,400×) (Ishiguro and Wolfe, 1970). (Reprinted with permission from Ishiguro and Wolfe, 1970. J. Bacteriol. *104*: 566–580.)

Type strain: ATCC 25078, DSM 43160, NBRC 13315, JCM 3152, NRRL B-3577, VKM Ac-658, CBS 237.69, IAM 14282, JCM 3152, KCC A-0152, NBRC 13315.

Sequence accession no. (16S rRNA): L40620.

Other organisms

Five subspecies (Ishiguro and Fletcher, 1975; Luedemann, 1968) have been proposed; discriminating features are given in Table 56.

1. **"Geodermatophilus obscurus subsp. obscurus"** Luedemann 1968, 1857

ob.scur′us. L. masc. adj. *obscurus* dark, obscure, indistinct.

Source: isolated from soil of the Amargosa Desert, Nevada, USA.

Type strain: ATCC 25078.

2. **"Geodermatophilus obscurus subsp. amargosae"** Luedemann 1968, 1857

a.mar.go′sae. N.L. gen. n. *amargosae* of the Amargosa Desert.

Source: isolated from soil of the Amargosa Desert, Nevada, USA.

Type strain: ATCC 25081.

3. **"Geodermatophilus obscurus subsp. utahensis"** Luedemann 1968, 1857

u.tah.en′sis. N.L. masc. adj. *utahenis* belonging to Utah (one of the United States).

Source: isolated from soil of Zion National Park, Utah, USA.

Type strain: ATCC 25079.

4. **"Geodermatophilus obscurus subsp. dictyosporus"** Luedemann 1968, 1858

dic.ty.o.spo′rus. Gr. n. *diktuon* a net; Gr. fem. n. *spora* a seed; N.L. n. *dyctiosporus* netted spores, referring to criss-crossings seen on spores.

Source: isolated from soil of Westgard Pass, California, USA.

Type strain: ATCC 25080.

5. **"Geodermatophilus obscurus subsp. everesti"** Ishiguro and Fletcher 1975, 106

ev.er.est′i. N.L. gen. n. *everesti* of Mount Everest, Nepal.

Source: isolated from West Ridge of Mount Everest at an elevation of 8300 m.

Type strain: 22-68.

TABLE 56. Discriminating features of the five proposed subspecies of *Geodermatophilus obscurus*[a]

Character	*Geodermatophilus obscurus* subsp.				
	"*obscurus*"	"*amargosae*"	"*utahensis*"	"*dictyosporus*"	"*everesti*"
Colony pigmentation	Dark brown-black	Black	Black	Black	Pale, orange, red
Nitrate reduction	–	–	++	–	+/–
Gelatin hydrolysis	–	+/–	–	++	–
Utilization of:					
D-Arabinose	–	–	+	–	–
L-Arabinose	++	++	+/–	++	nd
Glycerol	++	++	+/–	++	nd
Inositol	++	+	+	–	–
β-Lactose	–	+/–	–	++	–
Melezitose	–	+/–	+/–	++	nd

[a]++, Strongly positive; +, positive; +/–, weak; –, negative; nd not determined. From Lechevalier (1989).

Genus II. **Blastococcus** Ahrens and Moll 1970, 264[AL] emend. Urzì, Salamone, Schumann, Rohde and Stackebrandt 2004b, 257 emend. Lee 2006, 2394

Erko Stackebrandt and Peter Schumann

Blas.to.coc′cus. Gr. n. *blastos* bud, shoot; N.L. masc. n. *coccus* (from Gr. masc. n. *kokkos* grain, seed) coccus; N.L. masc. n. *Blastococcus* budding coccus.

Gram-stain-positive, coccoid, occurs singly or in pairs, **often reproducing by budding and multiple fission, giving rise to a variety of cell forms. Single cells may be motile rods and vibroid or non-motile cocci that tend to form aggregates. Strains may form motile zoospores.** Formation of buds on rods is common, but not universal. Membranous bodies, 1–4 per cell, which are linked to the cytoplasmic membrane, may be present. Oxidase-negative, catalase-positive, and aerobic; some strains may be microaerophilic. Strains from surfaces of marble and limestone utilize a broader spectrum of organic compounds than the only known marine strain. **Predominant fatty acids are $C_{16:0}$ iso, and, in some strains, $C_{16:1}$ iso, $C_{18:1}$ ω9*c*, $C_{17:1}$ ω8*c*, $C_{15:0}$ iso, and $C_{17:0}$. Peptidoglycan contains *meso*-DAP** as diagnostic diamino acid. **Predominant menaquinone is MK-9(H_4)**; MK-9 may also occur in high amounts. Polar lipids consist of diphosphatidylglycerol, phosphatidylglycerol, phosphatidylinositol, and phosphatidylethanolamine; phosphatidylcholine, phosphatidylethanolamine, and two unknown phospholipids may occur. Arabinose and galactose have been identified as whole-cell sugars in one species. Phylogenetically, a member of the family *Geodermatophilaceae*.

DNA G+C content (mol%): 72.3–74.

Type species: **Blastococcus aggregatus** Ahrens and Moll 1970, 264[AL] emend. Urzì, Salamone, Schumann, Rohde and Stackebrandt 2004b, 257.

Further descriptive information

The morphology of *Blastococcus aggregatus*, isolated from sediment of the Baltic Sea, is similar to that of *Geodermatophilus* as two forms occur: motile rods that multiply by budding and thallic aggregates of coccoid cells (Figure 114 and Figure 115). The original description of this species (Ahrens and Moll, 1970; in German) gives detailed information on morphological variations. The manifestation of the stages is influenced by environmental parameters: the formation of the motile rod stage is supported by low temperatures (5–10°C), low salt concentrations, and anaerobic conditions, whereas increased temperature and salt concentrations induce the formation of aggregates. Isolates from desert soil and altered stone surfaces from monuments and natural stones in the Mediterranean basin resemble members of the family *Geodermatophilaceae* morphologically (Figure 114 and Figure 115). Cells of *Blastococcus jejuensis* KST3-10[T] are coccoid and occur in pairs or are rod-shaped, flagellated, and motile (Figure 114 and Figure 115); only the rod-shaped cells show bud formation.

Phylogenetically, *Blastococcus* species (Eppard et al., 1996; Urzì et al., 2001), together with members of the genera *Geodermatophilus* and *Modestobacter*, constitute the family *Geodermatophilaceae* (Normand, 2006). Strains from arid environments are related to the genus *Geodermatophilus*, whereas those from the surface of calcareous stones and monuments, mainly from

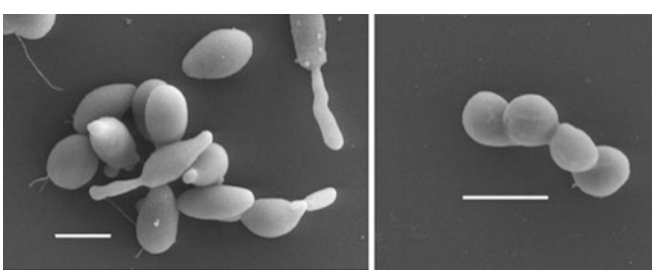

FIGURE 114. Electron micrographs of (left) *Blastococcus aggregatus* DSM 4725[T] and (right) *Blastococcus saxobsidens* BC444[T] showing single cells, pairs and tetrads. (Reprinted with permission from Urzì et al., 2004b. Int. J. Syst. Evol. Microbiol. *54*: 253–259.)

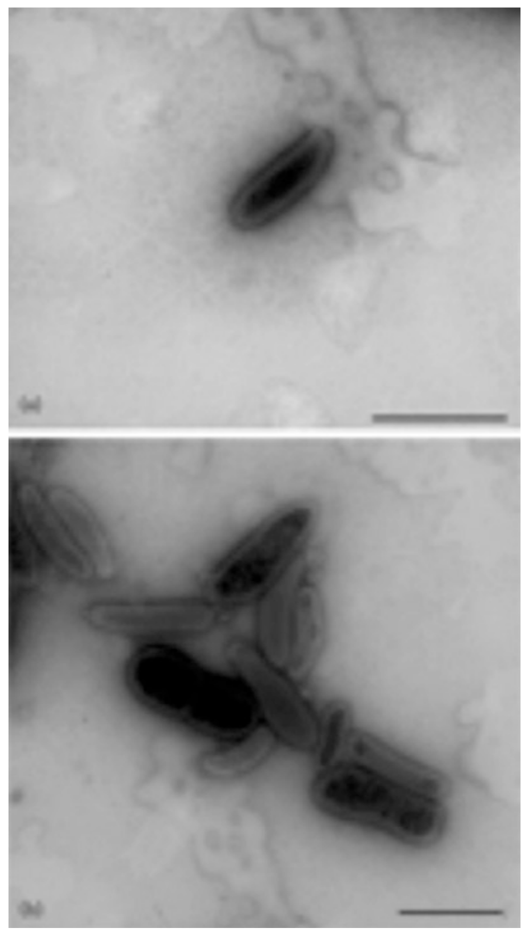

FIGURE 115. Transmission electron micrographs of *Blastococcus jejuensis* KST3-10[T] grown on YE-SW agar for 3 d at 30°C. (top) Rod-shaped cell with flagella; (bottom) coccoid cells in pairs and rod-shaped cells showing bud formation. Bars = 1.0 µm. (Reprinted with permission from Lee, 2006. Int. J. Syst. Evol. Microbiol. *56*: 2391–2396.)

Italy and Spain, exposed to rainy winters and hot, dry summers belong to the genera *Modestobacter* and *Blastococcus*. The type strain of *Blastococcus aggregatus* shared only a few characteristics in common with the stone surface strains such as the formation of pink–orange colonies, aerobic metabolism, and the presence of *meso*-DAP as diagnostic diamino acid and MK-9(H_4) as predominant menaquinone. Differences observed in the fatty acid composition and physiological properties led to the description of a novel species, *Blastococcus saxobsidens*, for a subset of these novel isolates. Strains of *Blastococcus saxobsidens* do not grow in concentrations of NaCl above 3%; the influence of external parameters on cell morphology has not been investigated for strains of this species (Urzì et al., 2004b). The third species, *Blastococcus jejuensis* (Lee, 2006), differs from the other two species of the genus by the absence of phosphatidylglycerol and the presence of phosphatidylcholine and phosphatidylmethylethanolamine; it differs from *Blastococcus aggregatus* in terms of the relative amounts of $C_{16:1}$ iso, $C_{17:1}$ ω8*c*, $C_{18:1}$ ω9*c*, and $C_{18:0}$ fatty acids (Urzì et al., 2004b) and from *Blastococcus saxobsidens* in possessing $C_{18:0}$ and $C_{12:0}$, and in terms of the relative amounts of $C_{16:0}$, $C_{17:1}$ ω8*c*, and $C_{18:1}$ ω9*c*.

Physiological properties that differentiate the three species are given in Table 57.

Enrichment and isolation procedures

Blastococcus aggregatus was isolated in peptone-yeast medium (5 g/l peptone, 1 g/l yeast extract, 0.1 g/l $FePO_4$ in 1 l tap water, pH 7.2) plus 0.8% NaCl. It grew as tiny, shiny, pink-colored colonies within 5 d at 20°C at NaCl concentrations between 0.8 and 2.4%. After 21 d, colonies were 1 mm in diameter and pink in color. Their surface showed an orange peel appearance under a microscope at low magnification.

For the isolation of strains and relatives of *Blastococcus saxobsidens*, material scratched from altered stones and monuments was dilution-plated on Bunt and Rovira medium (Bunt and Rovira, 1955), modified by the addition of 0.5% glucose, 0.5% NaCl and 0.03% Na_2CO_3, pH 8.6 (medium BRII) plus actidion (50 mg/l) and maintained in Luedemann medium (Luedemann, 1968). Various amounts (100 mg to 1 g) of rock samples or powder from scraped surfaces were taken depending on the artistic importance of the sampled surface (monument, building, or rock from quarry). In the laboratory, each sample was ground to a powder in a sterile mortar, suspended (ratio 1:10, w/v) in physiological saline (0.85% NaCl) supplemented with 0.001% (w/v) Tween 80, and vortexed for 60 min. Incubation was carried out at 28°C.

Blastococcus jejuensis KST3-10^T was isolated from sediment samples taken at a depth of 1 m from surface water of Gwakji beach on Jeju Island, Republic of Korea. Samples were placed into sterilized 50 ml Falcon tubes containing seawater. For bacterial isolation, 1 g sand sediment was placed into a sterile plastic tube containing 9 ml sterile distilled water and then mixed in a tube rotator for 30 min at a moderate speed. Aliquots (100 μl) of the serial dilution of the samples were transferred onto SC-SW

TABLE 57. Differentiation between the type strains of *Blastococcus* species using morphological and physiological parameters[a,b]

Characteristic	*B. aggregatus*	*B. saxobsidens*	*B. jejuensis*
Cell shape	Coccoid, rods, vibrios	Coccoid	Coccoid, rods
Bud formation	+	–	–
Germ tube	+	–	–
Oxygen requirement	Aerobic/microaerophilic	Aerobic	Aerobic
Pigmentation	Pink	Pink-orange	Apricot
$C_{15:0}$ iso fatty acid	<1%	>5%	>10%
$C_{17:1}$ ω8*c* fatty acid	<2%	>9%	>10%
API 20NE:			
Arginine dihydrolase	–	+	–
β-Galactosidase	–	+	–
Assimilation of arabinose	–	+	–
API ZYM:			
Valine arylamidase	–	–	w
Alkaline phosphatase	–	+	–
Cystine arylamidase	–	–	w
Naphthol-AS-BI-phosphohydrolase	–	w	–
α-Glucosidase	w	+	nr
β-Glucosidase	–	w	–
β-Glucuronidase	–	+	–
Biolog GP2:			
Adenosine	+	+	–
D-Malic acid	+	–	nr
Tween 40	+	+	–
Methylsuccinate	+	+	–
Acetic acid	+	–	+
α-Ketoglutaric acid	+	–	–

[a]+, >80% positive; –, <10% positive; w, weak reaction; nr, not recorded.

[b]Data from Urzì et al. (2004b) and Lee (2006).

agar plates supplemented with 60% (v/v) sterilized natural seawater. SC-SW agar comprised 1% soluble starch, 0.03% casein, 0.2% KNO_3, 0.2% NaCl, 0.002% $CaCO_3$, 0.005% $MgSO_4·7H_2O$, 0.001% $FeSO_4·7H_2O$, and 1.8% agar in 60% sterilized natural seawater and 40% distilled water (pH 7.2).

Maintenance procedures

Strains have been stored for some weeks on slants at 4°C and in 20% (w/v) glycerol suspensions at −20°C and −80°C. Long-term preservation methods include freeze-drying in skim milk and storage in liquid nitrogen at −196°C. *Blastococcus jejuensis* KST3-10^T was maintained on ISP 2 medium (Shirling and Gottlieb, 1966) supplemented with 60% sterilized natural seawater (YE-SW agar) and in a 20% (v/v) glycerol suspension supplemented with 60% (v/v) sterilized natural seawater at −20°C and −80°C.

Taxonomic comments

At the time of the first circumscription of the novel taxon "*Blastococcus*", the authors refrained from assigning strain B15 to a genus. They stated that in case strain B15 should merit species status, the name "*Blastococcus aggregatus*" should be created. Despite the lack of a proper description, *Blastococcus aggregatus* was placed on the Approved Lists of Bacterial Names (Skerman et al., 1980). The subsequent placement of the genus *Blastococcus* in the family *Geodermatophilaceae* was based on phylogenetic analysis of the type strains of *Blastococcus aggregatus* and *Geodermatophilus obscurus* as well as of two *Frankia* strains (Hahn et al., 1989). This study followed a phylogenetic survey of the family *Dermatophilaceae* (Stackebrandt et al., 1997), showing the separate position of the genera *Dermatophilus* and *Geodermatophilus* within the tree of members of the *Actinomycetales*. The taxonomic status of *Blastococcus* was confirmed with the description of the suborder *Frankineae* Stackebrandt et al. 1997, embracing the families *Frankiaceae* Becking 1970, *Microsphaeraceae* Rainey et al. 1997, *Sporichthyaceae* Rainey et al. 1997, *Acidothermaceae* Rainey et al. 1997 and *Geodermatophilaceae* Normand et al. 1996, Normand 2006. In the taxonomic roadmap to the present volume, the suborder *Frankineae* has been elevated to order status as *Frankiales*, in the class *Actinobacteria*. The family *Geodermatophilaceae* consists of the genera *Geodermatophilus*, *Blastococcus*, and *Modestobacter*. The latter genus is represented by beige-, pink-, and brown-pigmented organisms of multiple irregular shapes with motile buds (Mevs et al., 2000; Reddy et al., 2007).

Until recently, the description of *Blastococcus* has been based heavily on morphological properties of a single strain isolated from the Baltic Sea (Ahrens and Moll, 1970). Recently, many more strains from terrestrial, mainly arid environments have been reported (Eppard et al., 1996; Urzì et al., 2001, 2004b) and taxonomic studies pointed to the presence of a second species of *Blastococcus*. In due course, *Blastococcus saxobsidens* was described and the description of the type strain of *Blastococcus aggregatus* was also extended and brought up to present description standards (Urzì et al., 2004b).

Contradictory results have been published concerning the phylogenetic position of members of the order *Frankiales*. In the 16S rRNA gene dendrogram of Normand et al. (1996), the families *Frankiaceae* and *Geodermatophilaceae* were separated by members of the *Actinoplanes* group. The intra-class relatedness of *Actinobacteria* depicted by Stackebrandt et al. (1997) sees members of the *Frankiales* clustering together to the exclusion of *Actinoplanes* and related taxa of the *Micromonosporaceae*. A more recent dendrogram (Maszenan et al., 2005) supports the phylogenetic separation of the families, although the topology is in conflict with the higher taxonomic order. Obviously, the number of genera and species within the class *Actinobacteria* has now grown so large that the selection of a subset of reference organisms (number and affiliation) in the analysis, together with the different algorithms applied, will have a significant effect on the topology of phylogenetic trees. It is, however, encouraging that the relationship between members of the genera *Blastococcus* and *Geodermatophilus* remains unchanged (Lee, 2006; Mevs et al., 2000).

The genomic homogeneity of strains of *Blastococcus saxobsidens* has been demonstrated by RiboPrint analysis (Bruce, 1996; Urzì et al., 2004b) using the restriction enzymes *Pvu*II and *Pst*I (Figure 116).

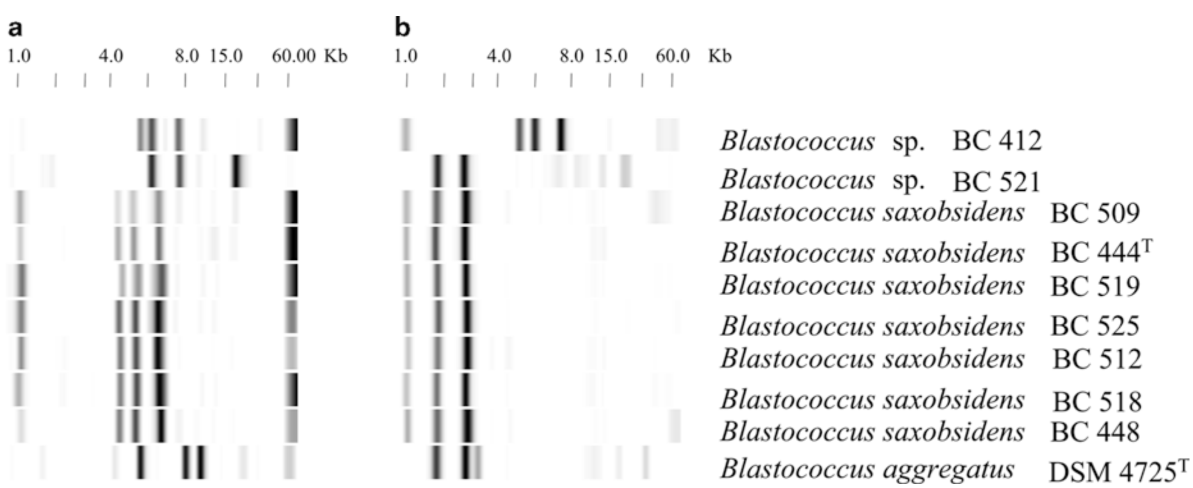

FIGURE 116. RiboPrint patterns of strains of *Blastococcus saxobsidens*, *Blastococcus aggregatus* DSM 4725^T and two phylogenetically moderately related *Blastococcus* isolates, generated with *Pvu*II (a) and *Pst*I (b).

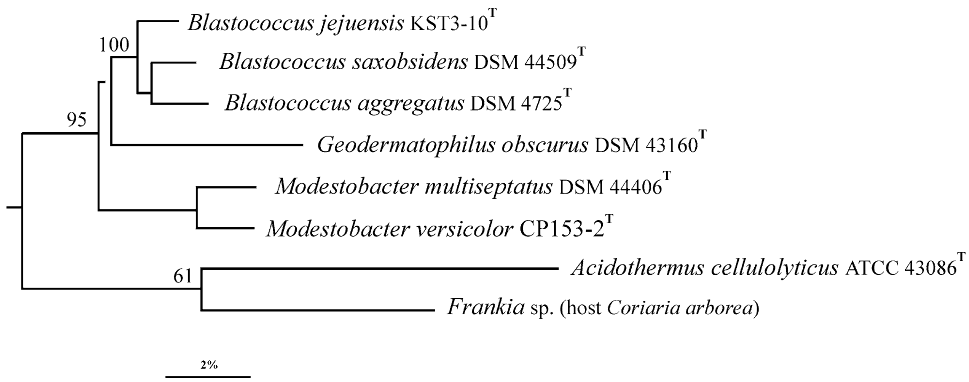

FIGURE 117. Distance matrix (DeSoete, 1983; Jukes and Cantor, 1969) 16S rRNA gene sequence dendrogram displaying the relatedness of *Blastococcus*-like organisms to the type strains of species of *Geodermatophilus*, *Modestobacter*, and *Blastococcus*. The sequences of members of the *Actinoplanaceae* served as an outside reference. Bootstrap values (Felsenstein, 1985) (1000 replicates, >70% confidence) are indicated at respective nodes. Sequence accession numbers used are as follows: *Blastococcus aggregatus* DSM 4725^T, AJ430193; *Blastococcus jejuensis* KST3-10^T, DQ200983; *Blastococcus saxobsidens* DSM 44509^T, AJ296061; *Geodermatophilus obscurus* DSM 43160^T, X92356; *Modestobacter multiseptatus* DSM 44406^T, Y18646; *Modestobacter versicolor* CP153-2^T, AJ871304; *Acidothermus cellulolyticus* ATCC 43068^T, AJ007290; *Frankia* sp. (host *Coriaria arborea*), AF063641. Bar = 2% sequence difference.

In addition to strains of *Blastococcus aggregatus* and *Blastococcus saxobsidens*, phylogenetic analysis of strains from rocks and ornaments pointed to the existence of two additional lineages within the genus, each represented by a single strain only [BC412 (=DSM 44517) and BC521 (=DSM 44518)]. These two strains (Figure 117), which shared 98.2% 16S rRNA gene sequence similarity, were not included in any *Blastococcus* species because of low DNA–DNA hybridization values with *Blastococcus saxobsidens* BC444^T (39% similarity with strain BC412 and 50% similarity with strain BC521). All three isolates were clearly unrelated to *Blastococcus aggregatus* DSM 4725^T (25–37% similarity). Based upon genomic differences, i.e. variations in ribopatterns (Figure 116) and morphological differences, i.e., formation of elongated cells, production of germ tubes and long filaments, as well as the formation of small motile zoospores, the single-strain taxa would merit a formal description. The rationale for refraining from describing novel species was based on the finding that *Blastococcus saxobsidens* showed a significant number of variable and weak physiological reactions. This indicates that the intraspecies metabolic diversity is higher than that expressed by the type strain.

Two 16S rRNA gene sequence oligonucleotides for differentiating members of the family *Geodermatophilaceae* have been described (Urzì et al., 2004a). One identifies members of the genus *Modestobacter* (TTCGCCGCTAGGGCA, position 96 of the *Escherichia coli* sequence), whereas the other is specific for members of the genera *Geodermatophilus* and *Blastococcus* (CCATCCCCAGCCGGAAACC, position 211; although not tested on *Blastococcus jejuensis*, the probe should also detect the type strain KST3-10^T). Differentiation between members of the latter two genera can be achieved on the basis of differences in pigmentation of colonies: those of *Geodermatophilus* are brown to black, whereas those of *Blastococcus* are pink to pinkish-orange (see Table 58). These oligonucleotides have been used in the affiliation of strains isolated from stone monuments (Urzì et al., 2001, 2004a)

Differentiation of the genus *Blastococcus* from other genera

Table 58 indicates diagnostic properties that are useful in the differentiation of the three genera of the family *Geodermatophilaceae*. A combination of the phylogenetic position of an isolate compared to the type strains of the family, together with colony pigmentation will allow assignment of species to genera.

Further reading

Akkermans, A.D.L., D. Hahn and D.D. Baker. 1992. The family *Frankiaceae*. *In* The Prokaryotes (edited by Balows, Trüper, Dworkin, Harder and Schleifer), Springer, New York, pp. 1069–1984.

Differentiation of the species of the genus *Blastococcus*

Data that differentiate the three *Blastococcus* species are given in Table 57. Results for additional strains of *Blastococcus saxobsidens* have been reported by Urzì et al. (2004b). All three type strains are positive for leucine arylamidase and glucose assimilation. *Blastococcus aggregatus* and *Blastococcus saxobsidens* are positive or weakly positive for esterase (C4), esterase (C8), Tween 40, Tween 80, and utilization of D-ribose and methyl succinate, whereas *Blastococcus jejuensis* KST3-10^T is negative for these properties. Biolog GP2 microplates give positive results for strain KST3-10^T and negative results for the type strains of *Blastococcus aggregatus* and *Blastococcus saxobsidens* for utilization of mannan, *N*-acetyl-β-D-mannosamine, amygdalin, cellobiose, D-fructose, D-galactose, D-galacturonic acid, gentiobiose, *myo*-inositol, maltose, D-mannitol, D-mannose, melibiose, methyl α-D-galactoside, methyl β-D-galactoside, 3-methyl-D-glucoside, palatinose, L-rhamnose, sedoheptulosan, D-sorbitol, sucrose, D-tagatose, turanose, xylitol, γ-hydroxybutyric acid, α-ketovaleric acid, D-lactic acid methyl ester, L-malic acid, succinic acid, L-asparagine, putrescine, 2,3-butanediol, and D-glucose 6-phosphate. All strains are negative for nitrate reduction, glucose fermentation, urease, and utilization of the majority of substrates in the Biolog GN substrate panel.

TABLE 58. Comparative phenotypic characteristics of members of the family *Geodermatophilaceae*[a]

Phenotypic characteristic	*Blastococcus*	*Geodermatophilus*	*Modestobacter*
Cell shape	Cocci, rods, vibrios	Cocci to cuboids, rods	Cocci, rods
Aggregation	v	+	+
Bud formation	v	v	+
Motility	v	v	+[d], v[e]
Colony pigmentation	Pink, pinkish-orange	Brown, black	Pale pink[d]/brown, pink, white[e]
Cell diameter (μm)	0.3–1.7	0.5–2.0	1.0–3.0
Flagellar insertion	Subpolar[b]	Polar	Polar[e]
Temperature range (°C)	3–40	18–37	0–37[d]/4–30[e]
NaCl tolerance:			
3%	v	−	+
5%	v	−	+[d]
6%	−	−	+[d]
Major menaquinone	MK-9(H_4)	MK-9(H_4)	MK-9(H_4)
NO_3 reduction to NO_2	w	w/v	v
Hydrolysis of gelatin	v	v	−
Hydrolysis of casein	v	−[f]	v
Carbon sources:			
Ethanol	w[b]	−[c]	−[d]
Glucose	v	+	+[d]
DNA G+C content (mol%)	70–75	73–75	68–73

[a]Abbreviations: w, weak; v, variable.

[b]Determined for *Blastococcus aggregatus*.

[c]Data for *Geodermatophilus obscurus* DSM 43160[T].

[d]Data for *Modestobacter multiseptatus*.

[e]Data for *Modestobacter versicolor* (Reddy et al., 2007).

List of species of the genus *Blastococcus*

1. **Blastococcus aggregatus** Ahrens and Moll 1970, 264[AL] emend. Urzì, Salamone, Schumann, Rohde and Stackebrandt 2004b, 257

ag.gre.ga′tus. L. masc. part. adj. *aggregatus* added to, joined together, referring to the tendency to form coccoid aggregates.

Gram-positive, highly irregular vibroid or rod-shaped cells occurring singly or in three-dimensional coccoid aggregates. The manifestation of these stages is influenced by environmental conditions; low salt concentrations, temperatures of 5–10°C, and microaerophilic conditions favor the motile rod stage. Single cells are either motile vibroids (0.3–1.5 × 0.4–3.0 μm) or rods or ellipsoid (1.2–1.5 × 1.5–3.0 μm). Rods are often separated by disk-like septa. These motile or non-motile rods carry 1–6 vibrioform buds, attached by a tapered pole onto the mother cell. Increased temperature and salt concentrations induce the formation of non-motile coccoid aggregates (1.2–2.5 μm in diameter), appearing as linear, band-like, or column-like three-dimensional forms. Larger aggregates (1 mm in diameter) are separated by rectangular and/or radial partitions. Cell types of different sizes occur mostly side by side. After 5 d on peptone-yeast extract agar at 20°C, colonies are pink, turbid, round, and convex with shiny surfaces. In liquid media, thin turbidity and formation of pink sediment are observed.

Catalase-positive, oxidase-negative. No growth is observed in mineral medium; weak growth is seen in the presence of ethanol, but not with glucose, acetate, citrate, methanol, ethylamine, or paraffin. Good growth is observed in mineral medium plus peptone (0.5%) and yeast extract (0.1%); in these media, addition of one of glucose, acetate, citrate, ethylamine, or paraffin (at 1%) reduces growth. Growth is enhanced in peptone-yeast extract medium by the addition of 1% NaCl. Increasing the salt concentration leads to increased formation of aggregates. No acid or gas is produced from glucose, galactose, fructose, xylose, sucrose, maltose, lactose, or glycerin. Methyl red and Voges–Proskauer reactions are negative. Does not hydrolyze starch, chitin, alginate, or cellulose. Reactions towards substrates provided by the API ZYM, API 20 NE, and Biolog GP2 panels are indicated in Table 57. Peptidoglycan diamino acid, menaquinone, and polar lipids are as described for the genus. Two unknown phospholipids may occur. Major fatty acids are 14-methylpentadecanoic acid ($C_{16:0}$ iso) and 14-methylpentadecenoic acid ($C_{16:1}$ iso); smaller amounts of $C_{18:0}$, $C_{16:1}$ ω7*c*, $C_{18:1}$ ω9*c*, $C_{16:0}$, and $C_{14:0}$ iso occur. Extinction maxima of carotenoids are at 470 and 500 nm.

Source: isolated from sediment of Station Breitengrund at 20 m depth, Western Baltic Sea.

DNA G+C content (mol%): 73.9 (HPLC).

Type strain: DSM 4725, ATCC 25902.

Sequence accession no. (16S rRNA gene): AJ430193.

2. **Blastococcus jejuensis** Lee 2006, 2395[VP]

je.ju.en′sis. N.L. masc. adj. *jejuensis* of or belonging to Jeju, Republic of Korea, the site from which the type strain was isolated.

Aerobic, motile, non-spore-forming, oxidase-negative, catalase-positive, Gram-stain-positive. Cells are cocci that occur in pairs or rods. Bud formation is observed for rod-shaped cells. Colonies are circular, smooth, transparent, and apricot in color. Starch and casein are hydrolyzed, but not elastin. Hypoxanthine, tyrosine, and xanthine are not decomposed. In API 20NE tests, glucose fermentation and indole production from tryptophan are not observed. Activities of arginine dihydrolase, urease, and β-galactosidase are not present. Nitrate is not reduced to nitrite. Esculin degradation and gelatin hydrolysis are not detected. Caprate, adipate, citrate, and phenylacetate are not assimilated. In API ZYM tests, results for leucine arylamidase and α-glucosidase are positive and weakly positive, respectively, whereas results for esterase lipase (C8), lipase (C14), trypsin, α-chymotrypsin, acid phosphatase, naphthol-AS-BI-phosphohydrolase, α-galactosidase, *N*-acetyl-β-glucosaminidase, α-mannosidase, and α-fucosidase are negative. The temperature range for growth is 10–37°C, with optimum growth at 30°C. Growth occurs at pH 6.1–10.1, with optimum growth at pH 7.1. Growth is observed in the presence of 0–1% NaCl, but not in 2% NaCl. The following substrates are used as sole carbon and energy sources: mannan, *N*-acetyl-β-D-mannosamine, amygdalin, cellobiose, D-fructose, D-galactose, D-galacturonic acid, gentiobiose, *myo*-inositol, melibiose, methyl α-D-galactoside, methyl β-D-galactoside, 3-methyl-D-glucoside, palatinose, L-rhamnose, sedoheptulosan, D-sorbitol, sucrose, D-tagatose, turanose, xylitol, acetic acid, γ-hydroxybutyric acid, α-ketovaleric acid, D-lactic acid methyl ester, succinic acid, L-asparagine, putrescine, 2,3-butanediol, adenosine, and D-glucose 6-phosphate. Tween 40, D-ribose, α-ketoglutaric acid, and methyl succinate are not utilized. The polar lipid profile contains phosphatidylcholine, diphosphatidylglycerol, phosphatidylethanolamine, phosphatidylmethylethanolamine, and phosphatidylinositol. Mycolic acids are not present. The major cellular fatty acids are $C_{16:0}$ iso, $C_{17:1}$ ω8*c*, and $C_{15:0}$ iso. The predominant menaquinone is MK-9(H_4). Whole-cell hydrolyzates contain *meso*-DAP as the diagnostic diamino acid and arabinose and galactose as diagnostic sugars.

Source: isolated from sand sediment from Gwakji beach on Jeju Island, Republic of Korea.

DNA G+C content (mol%): 72.3 (method not reported).

Type strain: KST3-10, NRRL B-24440, KCCM 42251.

Sequence accession no. (16S rRNA gene): DQ200983.

3. **Blastococcus saxobsidens** Urzì, Salamone, Schumann, Rohde and Stackebrandt 2004b, 258[VP]

sax.ob′si.dens. L. neut. n. *saxum* rock; L. part. adj. *obsidens* staying, remaining, occupying; N.L. masc. part. adj. *saxobsidens* rock-occupying.

Aerobic, Gram-stain-positive. Motile and nonmotile cells (1.0–1.7 µm in diameter) are coccoid, occurring in tetrads with a tendency to remain aggregated. Sparse growth on Luedemann medium, malt agar, and yeast extract-casein hydrolysates-starch-glucose agar. No growth on potato-dextrose agar or yeast extract-glucose-glycerol agar. Grows between 20 and 37°C and at pH 5–8.6; optimum growth is at 32°C and pH 6.8. No growth in 3% NaCl, except strain BC448, which can grow at 5% NaCl. Colonies are pink–orange-pigmented, irregular, and convex with a smooth to rough surface (2–3 mm in diameter). Catalase- and oxidase-positive. Nitrate is not reduced to nitrite. Carbon sources utilized for growth are: D-glucose, D-fructose, L-arabinose, ribose, *myo*-inositol, and lactose. No acid production is observed from D-fructose, *myo*-inositol, or lactose. Casein, gelatin, and starch are not hydrolyzed. DNA, tyrosine, xanthine, and hypoxanthine are not decomposed. Tweens 20, 40, 60, and 80 are not hydrolyzed. The peptidoglycan diamino acid, major menaquinone, and polar lipids are as described for the genus. Major fatty acids are 14-methyl-pentadecanoic acid ($C_{16:0}$ iso), *cis*-9-heptadecenoic acid ($C_{17:1}$ ω8*c*), and 13-methyl-tetradecanoic acid ($C_{15:0}$ iso), with smaller amounts of $C_{16:0}$, $C_{17:0}$ ante, $C_{16:1}$ iso, $C_{18:1}$ ω9*c*, and $C_{16:1}$ ω7*c*.

Source: the isolation site of the type strain was limestone sampled in Malta. Other strains were isolated from calcarenite and marble from Italy and Greece.

DNA G+C content (mol%): not reported.

Type strain: BC444, DSM 44509, NRRL B-24246.

Sequence accession no. (16S rRNA gene): AJ296061.

Genus III. **Modestobacter** Mevs, Stackebrandt, Schumann, Gallikowski and Hirsch 2000, 344[VP] emend. Reddy, Potrafka and Garcia-Pichel 2007, 2018

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Mo.des.to.bac′ter. L. adj. *modestus* modest, humble; N.L. masc. n. *bacter* a rod or staff; N.L. masc. n. *Modestobacter* a rod with modest growth requirements.

Gram-positive, non-spore-forming. Short rods or cocci with a tendency to remain aggregated and form short, **multiseptate filaments**. These produce slender **buds** (Figure 118). Aerobic heterotrophs **able to grow in oligotrophic** medium. Typically **psychrotolerant**. Major fatty acids include $C_{18:1}$, $C_{16:0}$ iso, and $C_{17:0}$ anteiso. The major respiratory quinone is MK-9(H_4). The cell wall peptidoglycan contains *meso*-DAP as diamino acid, with alanine, glutamate and *meso*-DAP present in a 2:1:1 stoichiometry.

DNA G+C content (mol%): around 70.

Type species: **Modestobacter multiseptatus** Mevs, Stackebrandt, Schumann, Gallikowski and Hirsch 2000, 344[VP].

Further descriptive information

The genus was created in 2000 to accommodate strains isolated from Antarctic "soil". The morphology of the strains was found to be sufficiently special to warrant the creation not only

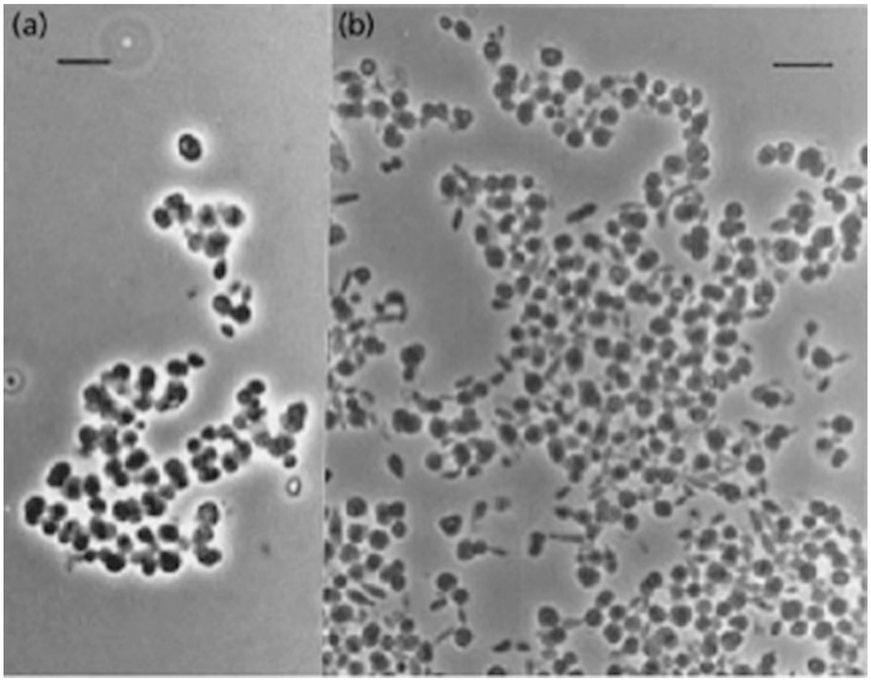

FIGURE 118. Phase-contrast light micrographs of *Modestobacter multiseptatus*. (a) Strain AA-802 grown for 4 d on PYGV agar (pH 8.0) at 16°C. (b) Strain AA-825 grown for 6 d on PYGV agar at 16°C. Bars = 5 µm. (Reprinted with permission from Mevs et al., 2000. Int. J. Syst. Evol. Microbiol. *50*: 337–346.)

TABLE 59. Distinguishing features of the two species of the genus *Modestobacter*[a]

Characteristic	*M. versicolor* CP153-2^T	*M. multiseptatus* AA-826^T
Temperature range (°C)	4–30	0–28
Temperature optimum (°C)	25	19–21
pH range	5–9	3–12
pH optimum	7	7.5–8.5
Oxidase	–	+
Lipase	+	–
Casein hydrolysis	+	–
Nitrate reduction	+	–
Starch hydrolysis	–	+
Utilization of:		
Arabinose	–	+
Acetate	–	+
Citrate	–	+
Galactose	–	+
Melibiose	+	–
Sorbitol	+	–
Trehalose	+	–
Inositol	+	–
Cellobiose	+	–
DNA G+C content (mol%)	73±2.5	68–70

[a]From Reddy et al. (2007).

of a novel species, but also of a new genus that was later positioned in the *Geodermatophilaceae* on the basis of its 16S rRNA gene sequence (Mevs et al., 2000). The word "soil" is debatable because the fine granular material found in the McMurdo dry valley being almost completely devoid of organic matter can better be called regolithic. The recent description of a second species, *Modestobacter versicolor* (Reddy et al., 2007), has permitted a more elaborate description. The two species are found in markedly different biotopes and thus have a number of distinguishing features (Table 59).

List of species of the genus *Modestobacter*

1. **Modestobacter multiseptatus** Mevs, Stackebrandt, Schumann, Gallikowski and Hirsch 2000, 344[VP] emend. Reddy, Potrafka and Garcia-Pichel 2007, 2018

mul.ti.sep.ta′tus. L. adj. *multus* much; L. adj. *septatus* fenced; N.L. masc. adj. *multiseptatus* much fenced, with many septa.

Colonies are irregularly shaped, shiny, and beige to pinkish. Cells are Gram-stain-positive, short rods or cocci with a tendency to remain aggregated. Cells show cross and longitudinal wall growth and multiply by budding and swarmer formation. Cell sizes vary (1.0–2.8 × 1.0–3.0 µm), with a mean size of 1.7 × 1.6–1.9 µm. Slender buds may become motile. Aerobic heterotroph; can grow on oligotrophic medium PYGV (Staley, 1968) or on DSMZ medium 65. Growth occurs between 0 and 28°C. Can tolerate pH 3–12, with optimum growth at pH 7.5–8.5. Positive for catalase, cytochrome oxidase, phosphatase, and amylase. Shows type II restriction endonuclease activity. H_2S is not formed from cystine or sulfate. Does not utilize fructose, xylose, or trehalose, but can utilize D-glucose, D-galactose, lactose, sucrose, mannitol, succinate, and malate. Utilization of maltose, mannose, melibiose, fucose, ribose, rhamnose, sorbitol, and *N*-acetylglucosamine is variable. Adenine, hypoxanthine, xanthine, hippurate, cellulose, chitin, dextrin, xylan, arbutin, and casein are not hydrolyzed. Utilizes peptone or yeast extract as nitrogen source and reduces nitrate aerobically or anaerobically. The main respiratory quinone is MK-9(H_4); MK-8(H_4) and MK-9(H_6) are present in small amounts. Main fatty acids are $C_{18:1}$ and $C_{16:0}$ iso. *meso*-DAP is present. Cell wall sugars are composed of galactose, glucose, and ribose.

Source: isolated from Antarctic surface "soil" from Linnaeus Terrace (1600 m) of the Asgard Range in the Transantarctic Mountains.

DNA G+C content (mol%): 68–70 (melting spectrometry; Mandel and Marmur, 1968).

Type strain: AA-826, DSM 44406, CIP 106529, JCM 12207.

Sequence accession no. (16S rRNA gene): Y18646.

2. **Modestobacter versicolor** Reddy, Potrafka and Garcia-Pichel 2007, 2018[VP]

ver.si′co.lor. L. masc. adj. *versicolor* that changes its color, of changeable color, of various colors, particolored.

Colonies are dark brown on oligotrophic medium and pink to white on copiotrophic medium, 1–4 mm in diameter, convex, entire, smooth to rugose, and slightly mucoid. Cells are short, small rods (straight, lightly curved, irregular, or even tapering), often developing into multiseptate cells, occurring singly or in pairs, sometimes remaining aggregated, and only rarely forming filaments longer than several cells. Single rods are 0.5–1.0 × 1.0–3.0 µm. Short filaments are up to 7.0 µm long. Septation is transverse (orthogonal to the long cellular axis) and apical cells may resemble buds. Motility is variable, by means of polar flagellation. Growth is observed at 4–30°C (but not at 37°C) and pH 5–9, with optimum growth at 25°C and pH 7. Produces copious melanins under oligotrophic conditions. Tolerates NaCl at concentrations less than 3%. Cells are positive for catalase, β-galactosidase, phosphatase, urease, and lipase, but negative for oxidase, gelatinase, arginine decarboxylase, lysine decarboxylase, ornithine decarboxylase, and phenylalanine deaminase. Negative for methyl red, Voges–Proskauer, and indole tests. Hydrolyzes casein and esculin, but not cellulose or starch. Reduces nitrate to nitrite. Does not produce H_2S gas and cannot grow on DNase or Simmons' citrate test plates. Utilizes a wide variety of sugars, low-molecular-mass organic acids, amino acids, and all four nitrogenous bases. Described as a nitrogen fixer, although this is debatable given that the evidence is based only on growth on nitrogen-free medium. The major fatty acids are $C_{15:0}$ iso, $C_{16:0}$ iso, $C_{15:0}$ anteiso, and $C_{18:1}$; MK-9(H_4) is the major respiratory quinone. Its peptidoglycan contains *meso*-DAP. Other features are given in Table 59.

Source: the type strain was isolated from a biological soil crust on the Colorado Plateau, USA (Reddy et al., 2007).

DNA G+C content (mol%): 73±2.5 (spectrophotometry; DeLey et al., 1970).

Type strain: CP153-2, ATCC BAA-1040, DSM 16678.

Sequence accession no. (16S rRNA gene): AJ871304.

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Family V. **Nakamurellaceae** Tao, Yue, Chen and Chen 2004, 999[VP]

Wen-Feng Chen and Tian-Shen Tao

Na.ka.mu.rel.la.ce′a.e. N.L. fem. n. *Nakamurella* type genus of the family; suff. *-aceae* ending to denote a family; N.L. fem. pl. n. *Nakamurellaceae* the *Nakamurella* family.

Yoshimi et al. (1996) proposed the bacterial genus *Microsphaera*. However, the genus name was the same as that of the fungal genus *Microsphaera* (Wallr.) Léveillé 1851, which is still used in the taxonomy of fungi and contains species that are common plant fungal pathogens that cause powdery mildew. According to Principle 2, Rule 51b(4) of the Bacteriological Code (Lapage et al., 1992), the name of the bacterial genus *Microsphaera* (Yoshimi et al., 1996) is illegitimate. Therefore, a new genus name, *Nakamurella*, was proposed for the bacterial genus. The type species *Microsphaera multipartita* Yoshimi et al. (1996) became *Nakamurella multipartita* gen. nov., comb. nov. Due to the illegitimacy of the genus name, the family *Microsphaeraceae* proposed by Stackebrandt et al. (1997) is also illegitimate and has been replaced by the new bacterial family name *Nakamurellaceae*.

The family *Nakamurellaceae* currently contains two genera, *Nakamurella* and *Humicoccus*. The family description is as given for the family *Microsphaeraceae* as proposed by Stackebrandt et al. (1997).

Type genus: **Nakamurella** Tao, Yue, Chen and Chen 2004, 999[VP].

Genus I. **Nakamurella** Tao, Yue, Chen and Chen 2004, 999[VP]

Wen-Feng Chen and Tian-Shen Tao

Na.ka.mu.rel′la. N.L. fem. dim. n. *Nakamurella* to honor the Japanese microbiologist Kazunori Nakamura.

Spherical cells, 0.8–3.0 µm; occur singly or sometimes in clusters. A cell wall structure is observed in the middle of each cell in early exponential growth phase and a number of septa are found in late exponential phase. Nonmotile. No spores are formed. Gram-stain-positive. Chemoorganotrophic and strictly aerobic metabolism with oxygen as the terminal electron acceptor. **Growth rate is low.** Visible colonies appear on agar media after 10 d of incubation. Catalase-positive. Oxidase-negative. Contains *meso*-DAP in cell wall peptidoglycan. **MK-8(H_4) is the major quinone.** Major fatty acids are $C_{16:0}$ iso, $C_{15:0}$ iso, and $C_{18:1}$.

DNA G+C content (mol%): 67.5 (HPLC).

Type species: **Nakamurella multipartita** Tao, Yue, Chen and Chen 2004, 999[VP].

Further descriptive information

Respiratory quinone profiles of activated sludge can provide valuable information on the bacterial population structure of the sludge. Analysis of an activated sludge loaded with sugar wastewater revealed that menaquinones accounted for more than half of the total quinone content of the sludge and that MK-8(H_4) was the predominant menaquinone (Yoshimi et al., 1996). Also, microscopic studies showed that the sludge contained large numbers of well-compacted flocs consisting of large coccus-shaped bacteria. The single species of the genus *Nakamurella*, *Nakamurella multipartita* (previously known as *Microsphaera multipartita*), a coccus-shaped bacterium, is one of the bacteria that predominated in the sludge studied. It produces menaquinones, with MK-8(H_4) as the major component.

Cells of *Nakamurella multipartita* are Gram-stain-positive, nonmotile, non-spore-forming, and coccus-shaped. Cells are 0.8–3.0 µm in diameter and occur singly, in pairs, or in small irregular clusters (Figure 119). Rod-shaped or filamentous cells have not been observed at any stage of growth. The cell size depends on the growth stage; large cells (more than 2.0 µm in diameter) predominate in the early stages of the exponential growth phase and the proportion of small cells increases during the late stages of the exponential growth phase (Figure 120). Thin-section electron microscopy revealed that a cell wall-like structure occurred in the middle of each cell at the early growth phase (Figure 121). Many septa were observed in the cells during the late exponential growth phase (Figure 121).

Growth rate is low under all growth conditions tested, even under optimal growth conditions. Doubling time is about 11 h in a liquid medium at pH 7.0 and 25°C. Visible colonies appear on agar media after 10 d of incubation. Colonies are circular, smooth, convex, and white at the early stage of growth. After about 2 weeks of incubation, the colonies become cream colored.

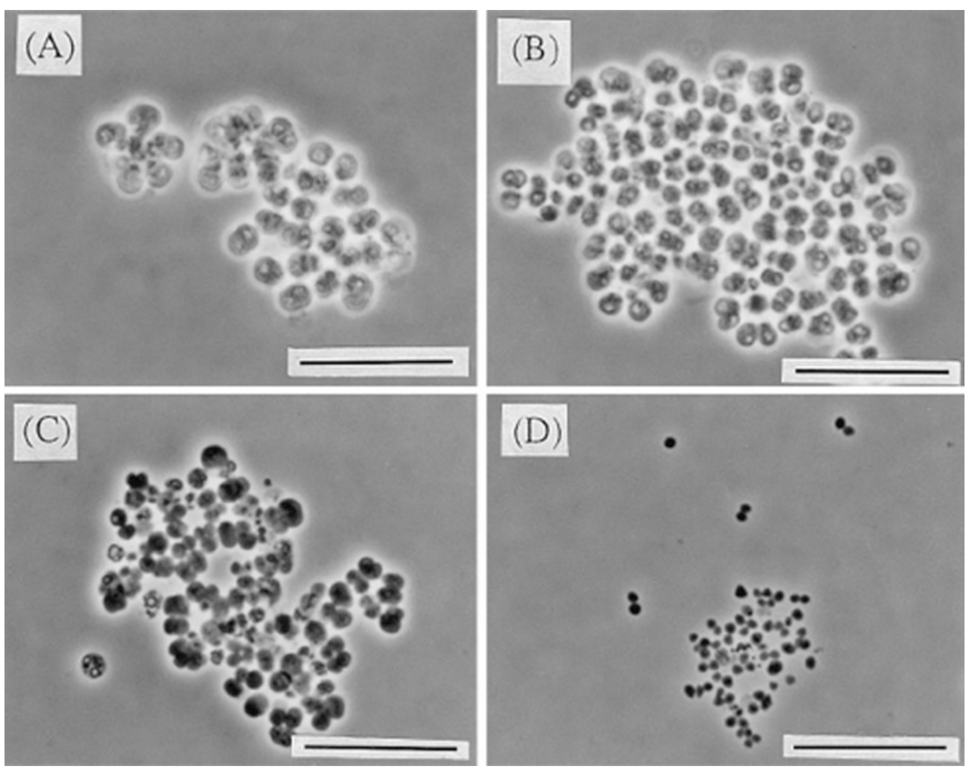

FIGURE 119. Phase-contrast photomicrographs of cells of *Nakamurella multipartita*. Cells were harvested in the early growth phase (A), in the mid-exponential phase (B), in the late exponential phase (C), and in the stationary phase (D). Bars = 10 µm. (Reprinted with permission from K. Nakamura.)

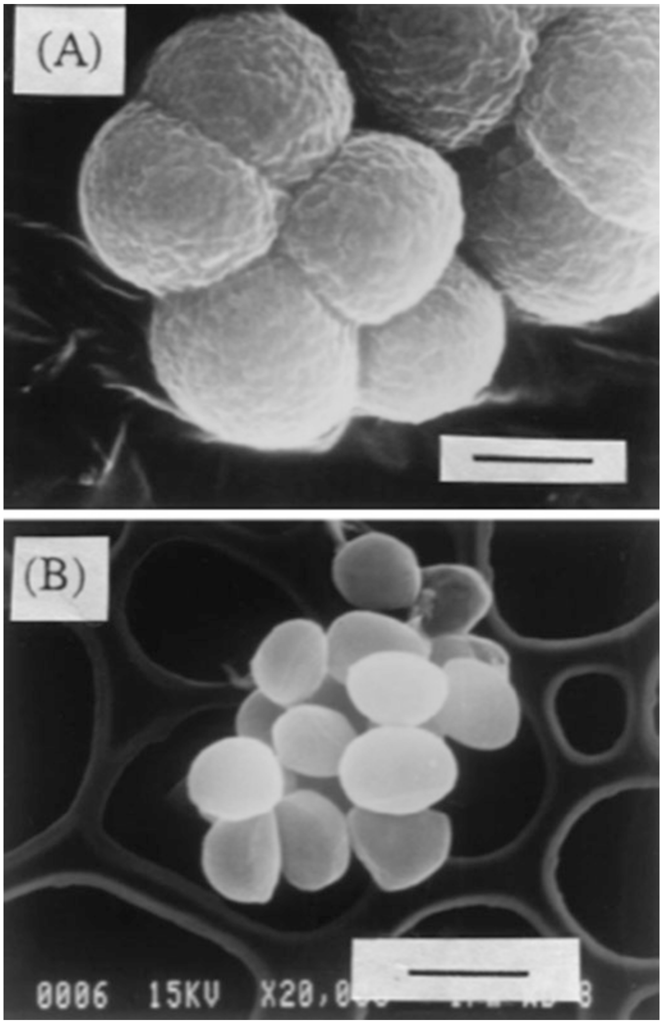

FIGURE 120. Scanning electron micrographs of cells of *Nakamurella multipartita*. Cells were harvested in the exponential phase (A) and in the stationary phase (B). Bars = 1 µm. (Reprinted with permission from K. Nakamura.)

Cells are able to uptake all sugars supplied, and large amounts of polysaccharide accumulate in cells in the absence of nitrogen and phosphate sources. A culture containing 1 g/l of the type strain (Y-104^T) of *Nakamurella multipartita* takes up 1 g/l glucose almost completely in 1 h. The polysaccharide content of the cells is about 50% (w/w, dry cells) after 1 h of incubation.

Obligately aerobic chemoorganotroph with oxygen as the terminal electron acceptor. It is unable to grow under strictly anaerobic conditions. Optimum growth conditions are 25°C and pH 7.0. Grows at NaCl concentrations of <6%.

Catalase is produced. Oxidase activity is negative. Alcohols and sugars including glucose, fructose, mannose, galactose, xylose, sucrose, maltose, lactose, mannitol, sorbitol, ethanol, propanol, glycerol, and starch are good carbon sources. Pyruvate, alanine, glutamate, glutamine, and histidine are also utilized. Acetate, malate, succinate, arginine, asparagine, methanol, and glycogen are not utilized.

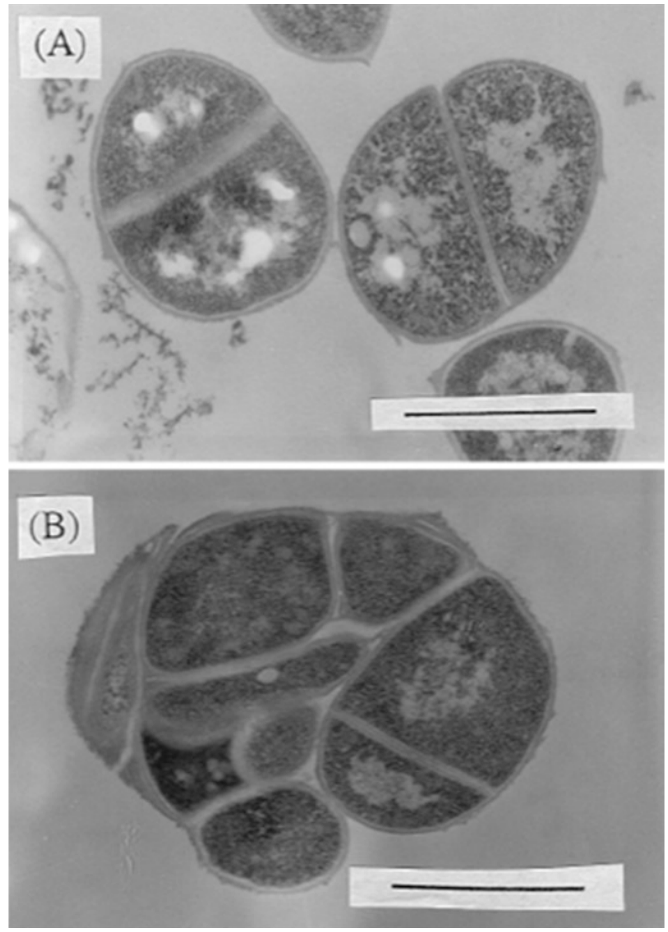

FIGURE 121. Transmission electron micrographs of thin sections of cells of *Nakamurella multipartita*. Cells were harvested in the early growth phase (A) and in the late exponential phase (B). Bars = 1 µm. (Reprinted with permission from K. Nakamura.)

The major quinone component is a menaquinone with eight tetrahydrogenated isoprene units [MK-8(H$_4$); 97.0%]. MK-7(H$_4$), MK-8(H$_2$), and MK-9(H$_4$) are minor components. Major fatty acids are $C_{16:0}$ iso (19.7%), $C_{15:0}$ iso (15.7%), and $C_{18:1}$ (14.0%); smaller, but substantial amounts of fatty acids are $C_{16:0}$ (10.3%), $C_{15:0}$ anteiso (9.2%), $C_{17:0}$ iso (8.5%), and $C_{17:0}$ anteiso (5.2%).

The cell wall peptidoglycan contains *meso*-DAP. Mycolic acids are not present.

Enrichment and isolation procedures

The only isolate of the only species described thus far was obtained from activated sludge acclimated with sugar-containing synthetic wastewater.

Activated sludge was cultured aerobically in a fed-batch reactor system (length of cycle, 8 h) with synthetic wastewater containing (per liter) 0.75 g glucose, 0.03 g peptone, 0.03 g yeast

extract, 0.1 g $(NH_4)_2SO_4$, and 0.07 g KH_2PO_4. At the end of each batch cycle, the sludge was sedimented, and two-thirds of the supernatant was replaced with the same volume of the synthetic medium. After 90 d of acclimation, bacteria were isolated from the sludge by the dilution plate method with GPY agar, which contained (per liter) 1.0 g glucose, 0.5 g peptone, 0.5 g yeast extract, 0.1 g KH_2PO_4, 0.1 g $(NH_4)_2SO_4$, and 0.1 g $MgSO_4 \cdot 7H_2O$ (pH 7.0). The plates were incubated at 25°C for 20 d. Colonies appearing on the plates were picked for standard purification.

Maintenance procedures

Nakamurella multipartita can be maintained on GPY agar slants and subcultured every month. For routine phenotypic tests, cells were grown aerobically at 25°C in GPY medium. The organism also can be maintained by lyophilization.

Acknowledgements

The authors would like to thank Professor Kazunori Nakamura for reading the manuscript of this chapter.

List of species of the genus *Nakamurella*

1. **Nakamurella multipartita** Tao, Yue, Chen and Chen 2004, 999[VP]

mul.ti.par.ti'ta. L. adj. *multus* much, great, many; L. fem. part. adj. *partita* (from L. v. *partio partire* to divide, part, distribute) divided, parted, distributed; N.L. fem. part. adj. *multipartita* micro-organisms having many divisions inside the cell.

The morphology of this species and its chemotaxonomic characteristics are the same as those described above for the genus. After about 2 weeks of incubation the colonies are cream colored. The optimum growth temperature is 25°C. The optimum pH is 7.0. No growth occurs at NaCl concentrations of 7% or more. The doubling time is about 11 h in liquid medium. The polysaccharide content of the cells may be high [sometimes more than 50% (w/w) depending on the culture conditions]. Catalase-positive. Oxidase-negative. Good carbon sources are sugars and alcohols and include D-xylose, D-lactose, mannitol, sorbitol, glycerol, ethanol, and propanol. Starch, pyruvate, alanine, glutamate, glutamine, and histidine are also utilized. Glycogen, methanol, acetate, malate, succinate, arginine, and asparagine are not utilized.

Source: occurs in activated sludge cultured in fed-batch reactors under aerobic conditions.

DNA G+C content (mol%): 67.5 (HPLC).

Type strain: ATCC 700099, JCM 9543, DSM 44233, CCM 4621, K. Nakamura Y-104.

Sequence accession no. (16S rRNA gene): Y08541.

Note: previously known by the illegitimate name *Microsphaera multipartita* (Yoshimi et al., 1996).

Genus II. **Humicoccus** Yoon, Kang, Jung and Oh 2007, 59[VP]

Jung-Hoon Yoon

Hu.mi.coc'cus. L. n. *humus* the soil; Gr. masc. n. *kokkos* a grain or berry; N.L. masc. n. *Humicoccus* coccus isolated from soil.

Cells are cocci, 0.6–1.2 µm in diameter. Gram-stain-positive. Non-spore-forming. **Nonmotile. Strictly aerobic.** Catalase-positive. Oxidase-negative. Nitrate is not reduced. Colonies are **light yellow** in color on nutrient agar. **The cell wall peptidoglycan contains *meso*-DAP. The whole-cell sugars are galactose, mannose, xylose, and rhamnose**. The major polar lipids are diphosphatidylglycerol, phosphatidylethanolamine, and phosphatidyldimethylethanolamine. **The predominant menaquinones are MK-8(H_4) and MK-9(H_4).** The cellular fatty acid profile consists of straight-chain and branched fatty acids.

DNA G+C content (mol%): 72.6 (HPLC).

Type species: **Humicoccus flavidus** Yoon, Kang, Jung and Oh 2007, 59[VP].

Further descriptive information

Humicoccus flavidus is the sole species of this genus. Cells may occur singly, in pairs, or in clusters when cultivated on nutrient agar. *Humicoccus flavidus* was isolated by using 10× diluted nutrient agar. However, it grows better on full-strength nutrient agar than on 2×, 5×, or 10× diluted nutrient agar. *Humicoccus flavidus* also grows better on nutrient agar than on trypticase soy agar and marine agar 2216. Growth occurs in the absence of NaCl. Characteristics are given in detail in the species description and are shown in Table 60. Phylogenetically, members of the genus *Humicoccus* are most closely related to *Nakamurella multipartita* (previously *Microsphaera multipartita*), family *Nakamurellaceae*, suborder *Frankineae* (Yoshimi et al., 1996; Stackebrandt et al., 1997; Tao et al., 2004), which has been elevated to order *Frankiales* in the taxonomic roadmap to the present volume. The clustering of the genera *Humicoccus* and *Nakamurella* is shown in phylogenetic trees constructed using three tree-making algorithms, i.e. the neighbor-joining, maximum-likelihood, and maximum-parsimony algorithms. The 16S rRNA gene sequence similarity value between the type strains of *Humicoccus flavidus* and *Nakamurella multipartita* is 96.5%. The 16S rRNA gene sequence similarity values between the type strain of *Humicoccus flavidus* and those of some other related genera are as follows: *Acidothermus* (92.4%), *Blastococcus* (93.5–93.9%), *Geodermatophilus* (92.0%), *Modestobacter* (92.8–93.0%), and *Sporichthya* (93.2–93.7%).

Enrichment and isolation procedures

Humicoccus flavidus was isolated from soil from Dokdo, an island of Korea. No selective media or enrichment procedures have

TABLE 60. Differential phenotypic characteristics of the genera *Humicoccus* and *Nakamurella*[a,b]

Characteristic	*Humicoccus*	*Nakamurella*
Cell diameter (μm)	0.6–1.2	0.8–3.0
Colony color	Light yellow	White
Maximum NaCl concentration for growth (%)	4	6
Growth at 4°C	+	–
Growth at 35°C	–	+
Hydrolysis of:		
Casein	–	+
Hypoxanthine	–	+
Utilization of:		
Succinate	+	–
L-Malate	+	–
Pyruvate	–	+
L-Glutamate	–	+
Antibiotic susceptibility:		
Polymyxin (100 U)	+	–
Gentamicin (30 μg)	+	–
Novobiocin (5 μg)	+	–
Tetracycline (30 μg)	+	–
Kanamycin (30 μg)	+	–
Lincomycin (15 μg)	+	–
Predominant menaquinone	8(H_4), 9(H_4)	8(H_4)
Cell-wall sugars	Gal, Man, Xyl, Rha	Glc, Man, Xyl, Rha
Dominant lipids	$C_{15:0}$ anteiso, $C_{15:0}$ iso, $C_{17:0}$	$C_{16:0}$ iso, $C_{18:1}$ ω9*c*
DNA G+C content (mol%)	72.6	67.5

[a]Symbols and abbreviations: +, positive; –, negative; Gal, galactose; Glc, glucose; Man, mannose; Xyl, xylose; Rha, rhamnose.

[b]Data taken from Yoshimi et al. (1996) and Yoon et al. (1993). Cells of the two genera are spherical and Gram-stain-positive. Both grow at 10°C and are positive for: catalase activity; hydrolysis of esculin, starch and urea; utilization of D-glucose, D-fructose, D-galactose, D-mannose, D-xylose, sucrose, and maltose; and susceptibility to streptomycin, chloramphenicol, neomycin, and oleandomycin. The two genera are negative for: motility; oxidase activity; anaerobic growth; hydrolysis of tyrosine and xanthine; utilization of acetate; and susceptibility to penicillin G, ampicillin, cephalothin, and carbenicillin.

yet been described that are specific for organisms of this genus. The genus *Humicoccus* may grow on a wide range of media. If identical samples are available, isolation of the genus *Humicoccus* may be achieved by the standard dilution-plating technique by using nutrient agar or 2–10× diluted nutrient agar.

Maintenance procedures

For short-term preservation, serial transfer from agar slants of appropriate media is recommended. Agar slants can be kept at 4°C for at least 2 months. For long-term preservation, lyophilization and storage in liquid nitrogen or in frozen glycerol suspensions are suitable. For lyophilization, the cell mass is suspended in an appropriate fluid, such as 20% (w/v) skim milk. For storage in liquid nitrogen, cell mass is inoculated into cryo-tubes containing an appropriate fluid such as 20% (w/v) glycerol. The glycerol suspension is prepared by making a suspension with cell mass in aqueous glycerol in the appropriate vial or tube. The vial or tube is stored at −20°C or −70°C.

Differentiation of the genus *Humicoccus* from other genera

Members of the genus *Humicoccus* have the same cellular morphology, peptidoglycan diamino acid type, and polar lipid profile as those of the genus *Nakamurella* (Yoon et al., 2007; Yoshimi et al., 1996). However, members of the genus *Humicoccus* can be distinguished from those of the genus *Nakamurella* by differences in the predominant menaquinone type, the fatty acid profile, and the whole-cell sugar profile (Table 60). Members of the genus *Humicoccus* contain similar amounts of MK-8(H_4) and MK-9(H_4) as the predominant menaquinones, whereas members of the genus *Nakamurella* contain only MK-8(H_4) as the predominant menaquinone. The genus *Humicoccus* contains $C_{15:0}$ anteiso, $C_{15:0}$ iso, and $C_{17:0}$ as the major fatty acids, whereas the genus *Nakamurella* contains $C_{16:0}$ iso and $C_{18:1}$ ω9*c* as the major fatty acids. Unsaturated and 10-methyl fatty acids, which are present in the genus *Nakamurella*, are not detected in the genus *Humicoccus*. There is a slight difference in whole-cell sugars between the genera *Humicoccus* and *Nakamurella*. The genus *Humicoccus* is clearly distinguishable from the genus *Nakamurella* based on differences in some phenotypic characteristics (listed in Table 60).

List of species of the genus *Humicoccus*

1. **Humicoccus flavidus** Yoon, Kang, Jung and Oh 2007, 59[VP]

fla′vi.dus. L. masc. adj. *flavidus* pale yellow.

Cells are cocci, 0.6–1.2 μm in diameter. Gram-stain-positive. Non-spore-forming. Nonmotile. Strictly aerobic. Colonies are circular, convex, smooth, glistening, light yellow in color, and 1.0–1.8 mm in diameter after incubation for 10 d at 25°C on nutrient agar. Growth occurs at 4 and 32°C, but not at 33°C. Optimal pH for growth is 6.0–7.0; growth occurs at pH 5.0 and 8.5, but not at pH 4.5 or 9.0. Growth occurs in 0–4% NaCl. Growth does not occur under anaerobic conditions on nutrient agar or on nutrient agar supplemented with nitrate. Nitrate is not reduced. Gelatin is hydrolyzed. Tweens 20, 40, 60, and 80 are not hydrolyzed. H_2S and indole are not produced. Arginine dihydrolase, lysine decarboxylase, ornithine decarboxylase, and tryptophan deaminase are absent. In assays with the API ZYM system, esterase (C4), esterase lipase (C8), leucine arylamidase, acid phosphatase, naphthol-AS-BI-phosphohydrolase, α-glucosidase, and β-glucosidase are present, but alkaline phosphatase, lipase (C14), valine arylamidase, cystine arylamidase, trypsin, α-chymotrypsin, α-galactosidase, β-galactosidase, β-glucuronidase, *N*-acetyl-β-glucosaminidase, α-mannosidase, and α-fucosidase are absent. Cellobiose, trehalose, L-arabinose, and salicin are utilized as sole carbon and energy sources. Benzoate, citrate, and formate are not utilized as sole carbon and energy sources. The whole-cell sugars are galactose, mannose, xylose, and rhamnose. The major polar lipids are diphosphatidylglycerol, phosphatidylethanolamine, and phosphatidyldimethylethanolamine. The major fatty acids (>10% of total fatty acids) are $C_{15:0}$ anteiso, $C_{15:0}$ iso, and $C_{17:0}$.

Source: isolated from soil.

DNA G+C content (mol%): 72.6 (HPLC).

Type strain: DS-52, KCTC 19127, CIP 108919.

Sequence accession no. (16S rRNA gene): DQ321750.

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Family VI. **Sporichthyaceae** Stackebrandt, Rainey and Ward-Rainey 1997, 487[VP]

Philippe Normand and David R. Benson

Spo.rich.thy.a.ce′a.e. N.L. fem. n. *Sporichthya* type genus of the family; L. suff. *-aceae* ending to denote a family; N.L. fem. pl. n. *Sporichthyaceae* the *Sporichthya* family.

A group of rare microorganisms isolated from cultivated soils that have a complex and unique morphology. The group is composed of a single bacterial genus, *Sporichthya* (Lechevalier et al., 1968). The two species are *Sporichthya brevicatena*, comprising a single isolate (Tamura et al., 1999), and *Sporichthya polymorpha*, only five isolates of which have been obtained over a course of 20 years. This rarity is presumably due to a lack of resistance to major antibiotics as well as to a slow growth rate using standard soil isolation procedures.

A pattern of 16S rRNA gene sequence signatures consisting of nucleotides at positions 139:224 (U–A), 186:191 (G–C), 600:638 (C–G), 839:847 (U–A), 987:1218 (A–U), 1059:1198 (U–A), and 1308:1329 (U–A) has been proposed (Stackebrandt et al., 1997). A signature of the *Sporichthyaceae* would appear to be 5′-CTTCGGGTGGGGATCAGTGGCG-3′ at coordinates 65–86 in the sequence of *Sporichthya polymorpha* DSM 46113 (sequence accession no. X72377).

Genus I. **Sporichthya** Lechevalier, Lechevalier and Holbert 1968, 279[AL]

Philippe Normand and David R. Benson

Spor.ich′thy.a. Gr. n. *spora* seed (and in biology, a spore); Gr. n. *ikhthus* fish; N.L. fem. n. *Sporichthya* an organism with fish-like spores.

Very short, aerial mycelium composed of hyphae, 0.5–1.0 µm in diameter, that grow on the surface of solid media (Figure 122). Aerial hyphae have a **unique upright posture maintained by holdfasts**, thickened cells that anchor the erect chains onto the solid surface (Figure 123 and Figure 124). The chains may be branched (rarely). **Cells in the chain look asymmetrical with constricted separations between cells.** No substrate mycelium is formed. The aerial mycelium forms rod-shaped to coccoid spores that may develop **polar flagella** when grown in liquid media. Under nitrogen-replete conditions, spores swell to yield various forms, some of which are vaguely fish-like, hence the name initially given to the genus. Cells are Gram-stain-variable, with young cells being Gram-stain-negative and older cells gradually acquiring a Gram-stain-positive reaction. The cell wall contains a large amount of LL-DAP. Facultatively anaerobic. Mesophilic; grows between 10 and 42°C. Chemoorganotrophic. Complex carbohydrates are utilized.

DNA G+C content (mol%): 71.0 (HPLC).

Type species: **Sporichthya polymorpha** Lechevalier, Lechevalier and Holbert 1968, 279[AL].

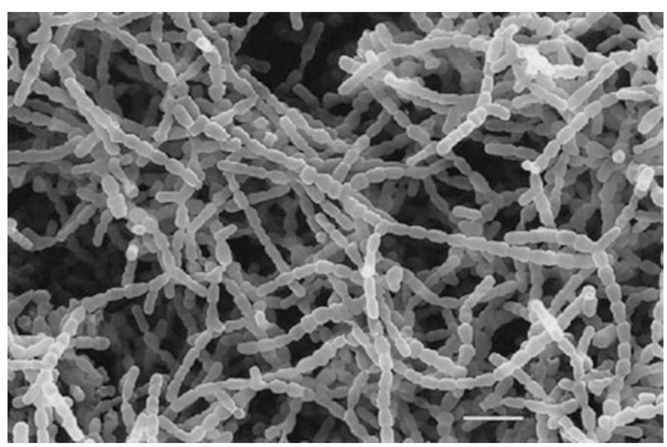

FIGURE 122. Aerial mycelia of *Sporichthya polymorpha* ATCC 23823[T] grown on humic acid-vitamin-gellan gum medium for 35 d at 27°C. Bar = 2 µm (Suzuki et al., 1999). (Reprinted with permission from Suzuki et al., 1999. Appl. Environ. Microbiol. *65*: 1930–1935.)

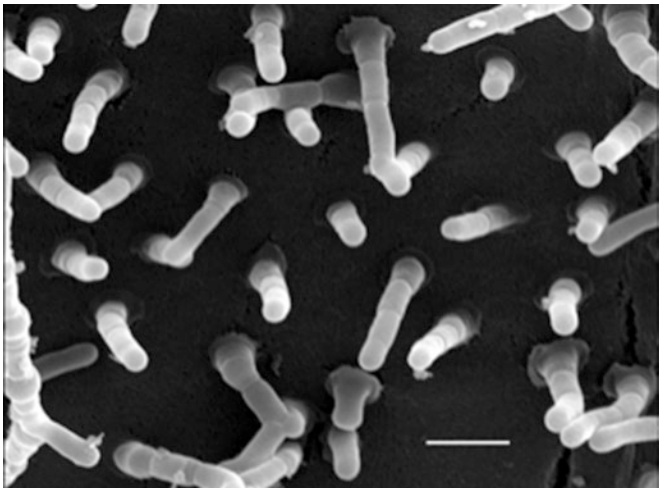

FIGURE 123. Scanning electron micrograph of *Sporichthya polymorpha* ATCC 23823[T] grown on HMG isolation medium (Suzuki, 2001) for 10 d at 27°C. Substrate mycelia are not observed. Bar = 1 μm. (Photo courtesy of Shin-ichi Suzuki, Tanabe Seiyaku Co., Saitama, Japan.)

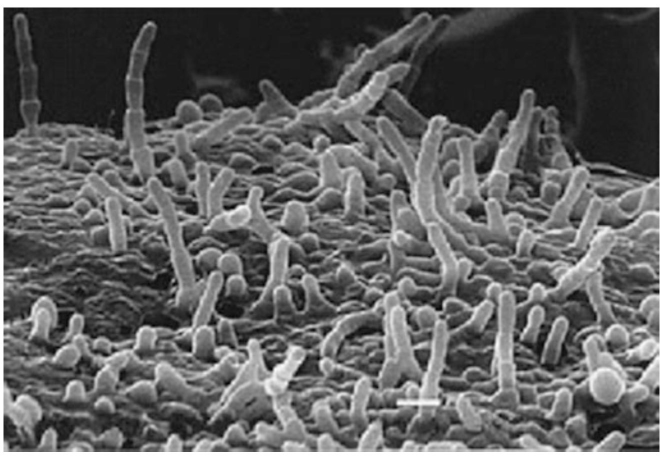

FIGURE 124. Scanning electron micrograph of *Sporichthya brevicatena* YU720-21[T] grown on yeast extract-starch agar for 4 d at 28°C. Bar = 1 μm (Tamura et al., 1999). (Reprinted with permission from Tamura et al., 1999. Int. J. Syst. Bacteriol. *49*: 1779–1784.)

TABLE 61. Differential characteristics of the two *Sporichthya* species[a]

Characteristic	*S. brevicatena*	*S. polymorpha*
Color of colony (on ISP 3 medium)	Yellowish-white	Colorless
Utilization of:		
D-Mannitol	+/−	−
L-Rhamnose	−	+
D-Xylose	+	−
Glycerol	+	−
Melibiose	+	−
Major menaquinone	MK-9(H_8)	MK-9(H_6)
Major cellular fatty acid	$C_{16:0}$ iso	$C_{16:0}$, $C_{17:1}$

[a]Data from Tamura et al. (1999).

Enrichment and isolation procedures

Isolation was obtained initially on tap water agar or on 1/10th diluted Czapek agar, but more recently Suzuki et al. (1999) used a procedure based on gellan gum and calcium chloride as growth stimulator and humic acid as sole carbon and nitrogen sources (Hayakawa and Nonomura, 1987) to assay about 30 soil samples from all continents. Many provenances yielded positive results, thus highlighting the fact there is still much progress to be made in the development of growth media for actinobacteria. All strains obtained so far have been isolated from cultivated soils. The colonies formed are, in general, very small and the help of a dissecting microscope is advised. Use of calcium chloride as a growth stimulator resulted in a marked increase in the size of colonies (Hayakawa and Nonomura, 1987).

Maintenance procedures

Transfer of slant-maintained cultures is advised every 3–4 months and storage at 4°C between transfers is recommended. Bennett or Czapek agars are used for maintenance. Long-term preservation by lyophilization from water suspension works well for most strains; use of milk is also possible, but inhibits some strains.

Differentiation of species of the genus *Sporichthya*

The genus comprises two species, *Sporichthya polymorpha* (Lechevalier et al., 1968) and *Sporichthya brevicatena* (Tamura et al., 1999), which have few discriminating features (Table 61).

List of species of the genus *Sporichthya*

1. **Sporichthya polymorpha** Lechevalier, Lechevalier and Holbert 1968, 279[AL]

po.ly.mor′pha. N.L. fem. adj. *polymorpha* (from Gr. adj. *polumorphos*, *-on*) multiform, polymorphic, microorganism having many shapes.

Aerial hyphae are chalky white, 0.5–1.2 μm in diameter, forming chains 10–25 μm in length. Division takes place by annular ingrowth of the cell wall, resulting in a sausage-string look. After division, the diameter of the growing hyphae increases, then stabilizes, and eventually decreases for a further division. Hyphae are hydrophobic. Sudan black reveals no lipid deposits. No production of pigment. Colony color ranges from chalky white on nitrogen-poor medium to slightly beige on nitrogen-rich medium.

Growth is rapid (24 h) on solid agar (Czapek, Bennett, yeast-extract-glucose agar). No growth is observed in shaken liquid culture, although without agitation, a pellicle forms on the surface of the medium and extends up the side of the container. Of the complex polymers tested, starch and esculin are hydrolyzed and some strains hydrolyze casein, but no hydrolysis of gelatin, adenine, hypoxanthine, or tyrosine is observed. No acid is produced from adonitol, arabinose, cellobiose, dextrin, fructose, galactose, glucose, glycerol, inositol, lactose, maltose, mannitol, mannose, melibiose, methyl α-D-mannoside, raffinose, rhamnose, trehalose, xylose, or methyl β-D-xyloside.

Acetate and pyruvate are utilized and lactate and succinate are used by some strains, whereas benzoate, citrate, malate, oxalate, propionate, and tartrate are not utilized. Nitrate is reduced. Phosphatase production varies. Urease-negative.

The pH range is 5–9, with optimum growth at pH 8 (Suzuki et al., 1999). The temperature range is 10–42°C, with optimum growth at 27°C.

Sporichthya polymorpha was found to be resistant to dry heating at 80°C for 1–2 h, a step recommended for its selective isolation (Suzuki et al., 1999). *Sporichthya polymorpha* strains show resistance to nalidixic acid (MIC, 5–25 μg/ml). Strains tested are susceptible to adriamycin, amikacin, bacitracin, daunorubicin, erythromycin, kanamycin, leucomycin, minocycline, nalidixic acid, novobiocin, paromomycin, rifampin, tunicamycin, and vancomycin. Use of non-sterilized 0.1% skim milk as flooding solution to recover spores was found to improve the isolation success rate.

Source: soil.

DNA G+C content (mol%): 71.0 (HPLC; Tamura et al., 1994).

Type strain: ATCC 23823, DSM 43042, IFO (now NBRC) 12702, JCM 3089, NRRL B-3709, VKM Ac-1863, KCC A-0089.

Sequence accession no. (16S rRNA gene): AB025317.

2. **Sporichthya brevicatena** Tamura, Hayakawa and Hatano 1999, 1783[VP]

bre.vi.ca.te′na. L. adj. *brevis* short; L. fem. n. *catena* chain; N.L. fem. n. *brevicatena* (nominative in apposition) a short chain, microorganism having short chains.

The name chosen by Tamura et al. (1999) implies that a feature distinguishing *Sporichthya brevicatena* from *Sporichthya polymorpha* would be the length of aerial mycelium. However, this does not appear to be striking since the photograph of *Sporichthya polymorpha* published by Lechevalier and Lechevalier (1989) shows chains of cells of 3 μm, whereas those published by Tamura et al. (1999) are even longer at 5 μm. That the two species are distinct is indeed the case given the low DNA–DNA hybridization result (30%) (Tamura et al., 1999), even though the genetic distance of 1.3% in the 16S rRNA gene sequence is not high. *Sporichthya brevicatena* comprises a single isolate, which would now be inadvisable according to Recommendation 30b of the Bacteriological Code (Lapage et al., 1992).

Source: soil.

DNA G+C content (mol%): 71.0 (HPLC; Tamura et al., 1994).

Type strain: YU720-21, IFO 16195.

Sequence accession no. (16S rRNA gene): AB006164.

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Order VII. **Glycomycetales** ord. nov

David P. Labeda

Gly.co.my.ce′ta.les. N.L. masc. n. *Glycomyces -etis*, type genus of the order; suff. *-ales*, ending to denote an order; N.L. fem. pl. n. *Glycomycetales* the *Glycomyces* order.

The order was formed by elevation of suborder *Glycomycineae* Rainey, Ward-Rainey and Stackebrandt 1997, 487[VP] (in Stackebrandt et al., 1997). **The description and signature nucleotides of the 16S rRNA gene are that of the family *Glycomycetaceae*.**

Type genus: **Glycomyces** Labeda, Testa, Lechevalier and Lechevalier 1985, 419[VP] emend. Labeda and Kroppenstedt 2004, 2345.

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Family I. **Glycomycetaceae** Rainey, Ward-Rainey and Stackebrandt 1997, 487^VP emend. Labeda and Kroppenstedt 2005, 1690

David P. Labeda

Gly.co.my.ce.ta.ce′a.e. N.L. masc. n. *Glycomyces -etis* type genus of the family; suff. *-aceae* ending to denote a family; N.L. fem. pl. n. *Glycomycetaceae* the *Glycomyces* family.

The family contains the genera *Glycomyces* and *Stackebrandtia.* Aerobic. Gram-stain-positive, non-acidfast, nonmotile actinomycetes comprising the genera *Glycomyces* and *Stackebrandtia.* Branched substrate mycelium (approximately 0.35–0.5 μm in diameter) and, on some media, aerial mycelia are produced. Mycolic acids are absent. Catalase-positive. **Contain *meso*-diaminopimelic acid as the diamino acid. Cell walls contain *N*-glycolylmuramic acid. Whole-cell sugar pattern contains ribose as a diagnostic sugar along with other genus-specific sugars. Phospholipid content includes phosphatidylglycerol and diphosphatidylglycerol in addition to other genus-specific phospholipids. The 16S rRNA gene pattern of 16S rRNA gene signature nucleotides contains 70:98 (A–U), 415 (C), 449 (C), 534 (G), 681:709 (A–U), 825:875 (G–C), 999:1041 (C–G), 1059:1198 (C–G), 1064:1192 (G–G), 1117:1183 (A–U), and 1309:1328 (C–G).**

DNA G+C content (mol%): 71–73.

Type genus: **Glycomyces** Labeda, Testa, Lechavalier and Lechevalier 1985, 419^VP emend. Labeda and Kroppenstedt 2004, 2345.

Key to the genera of the family *Glycomycetaceae*

1. The whole-cell sugar pattern consists of galactose, mannose, ribose, and xylose. Phospholipid pattern consists of significant amounts of phosphatidylinositol mannosides and phosphatidylinositol, phosphatidylglycerol, diphosphatidylglycerol, and phosphoglycolipids of unknown composition. The menaquines predominantly contain 10, 11, and/or 12 isoprene units, but the degree of unsaturation varies within each species. Fatty acid profile contains pentadecanoic, iso-hexadecanoic, and anteiso-heptadecanoic acids as the predominant components along with a slightly less iso-pentanoic acid.

   → Genus I. **Glycomyces**

2. The whole-cell sugar pattern consists of inositol and ribose, with traces of arabinose and mannose. The phospholipid pattern consists of phosphatidylglycerol, diphosphatidylglycerol, and two additional unknown phospholipids. The predominant menaquinones are MK-10(H_4), MK-10(H_6), MK-11(H_4), and MK-11(H_6). Fatty acid profile is rich in branched chain and saturated components including 10-methyl branched heptadecanoic acid and iso-branched 2-hydroxy fatty acids.

   → Genus II. **Stackebrandtia**

Genus I. **Glycomyces** Labeda, Testa, Lechavalier and Lechevalier 1985, 419^VP emend. Labeda and Kroppenstedt 2004, 2345

David P. Labeda

Gly.co.my′ces. Gr. adj. *glukus* sweet to the taste or smell; Gr. masc. n. *mukês* a mushroom; N.L. masc. n. *Glycomyces* a sweet (glycolipid-containing) mushroom.

Vegetative mycelia are branching (diameter approximately 0.35–0.40 μm); aerial mycelium may be produced on certain growth media. Oval, spherical, or rod-like spores may be formed on the vegetative hyphae in some species; chains of square-ended conidia may be produced on aerial hyphae. Gram-stain-positive. Lysozyme-sensitive. Catalase-positive and aerobic. **Type II cell-wall composition (*meso*-diaminopimelic acid and glycine) and whole-cell sugar pattern consisting of galactose, mannose, ribose, and xylose. Phospholipid pattern consists of significant amounts of phosphatidylinositol mannosides and diphosphatidylglycerol, phosphatidylglycerol, phosphatidylinositol, and phosphoglycolipids of unknown composition**. The menaquinones predominantly contain 10, 11, and/or 12 isoprene units, but the degree of unsaturation varies within each species.

DNA G+C content (mol%): 71–73.

Type species: **Glycomyces harbinensis** Labeda, Testa, Lechevalier and Lechevalier 1985, 420^VP.

Further descriptive information

The genus *Glycomyces* was defined by Labeda et al. (1985) for actinomycete species that produce chains of spores on aerial sporophores, have a peptidoglycan composed of D-glycine, L-alanine, D-alanine, D-glutamic acid, *meso*-diaminopimelic acid (*meso*-A_2pm), glucoseamine, and muramic acid, and with a whole-cell sugar pattern consisting of arabinose and xylose as diagnostic sugars. These observations correspond to a type II cell wall and whole-cell sugar pattern D *sensu* Lechevalier and Lechevalier (1970). The species in this genus do not contain nitrogenous phospholipids, but phosphatidylglycerol, phosphatidylinositol, and phosphatidylinositolmannosides are present, corresponding to a type PI phospholipid pattern *sensu* Lechevalier et al. (1977). The original genus description (Labeda et al., 1985) specified that the menaquinones generally present were those with 10 isoprene units which are tetra- to hexahydrogenated,

TABLE 62. Menaquinone content of *Glycomyces* species[a,b]

Menaquinone	*G. harbinensis*[c]	*G. algeriensis*	*G. arizonensis*	*G. lechevalierae*	*G. rutgersensis*[c]	*G. sambucus*	*G. tenuis*[c]
MK-9(H_6)[d]	–	–	–	–	–	–	+
MK-10(H_2)	–	23	–	17	–	–	–
MK-10(H_2)	–	–	18	21	+	–	–
MK-10(H_4)	–	–	52	11	–	–	–
MK-10(H_6)	+	–	tr	–	+		+
MK-11	–	65	–	24	–	69	–
MK-11(H_2)	–	–	9	19	–	–	–
MK-11($H_{4)}$	–	–	22	8	–	26	–
MK-11(H4)	–	–	–	–	–	–	+
MK11(H_6)	–	12	–	tr	–	–	–

[a]Symbols: +, >85% positive; -, 0–15% positive; tr, trace amount.

[b]Menaquinones listed as percentage of the total present. Quantitation is not presented for strains where menaquinone content has been determined qualitatively by mass spectroscopy.

[c]Determined by mass spectrometry.

[d]Abbreviations are exemplified by MK-9(H_4), a menaquinone which has two of the nine isoprene units hydrogenated.

but this has been recently emended (Labeda and Kroppenstedt, 2005) based on data from newly described species to include menaquinones that predominantly contain 10, 11, or 12 isoprene units with the degree of saturation being a species characteristic (see Table 62).

Enrichment and isolation procedures

Glycomyces strains can be selectively isolated from soil using the procedure described by Labeda and Kroppenstedt (2005). A 1:9 suspension of soil in sterile tap water is heated at 60°C for 5 minutes, and further dilutions are prepared in sterile tap water and spread on the surface of Czapek's sucrose agar (Waksman, 1950) supplemented with 50 μg/ml each of cycloheximide and nystatin, 10 μg/ml streptomycin, and 25 μg/ml novobiocin. After 3 weeks of incubation at 28°C, *Glycomyces*-like colonies will generally be observed growing on the surface of the plates if present in the samples. The identities of isolates can be confirmed most easily from comparison of their 16S rRNA gene sequences with those of authentic *Glycomyces* species, but observation of genus-specific chemotaxonomic characteristics, including presence of *meso*-A_2pm, arabinose and xylose as diagnostic whole-cell sugars, and lack of nitrogenous phospholipids, can also confirm their genus identity.

Recently, Gu et al. (2007) isolated a new endophytic species, *Glycomyces sambucus*, from the stem of *Sambucus adnata* Wall. The plant stems were surface-sterilized using the method of Coombs and Franco (2003) as modified by Gu et al. (2006), in which plant roots or stems are air-dried for 2 d, cut into segments, washed for 1 minute in 99% ethanol, 6 minutes in 3.125% sodium hypochlorite, 30 seconds in 99% ethanol, and finally rinsed in sterile reverse osmosis water. The stem samples were then aseptically sliced with a sterile blade and plated on BL-2 agar plates (glucose, 5 g; soluble starch, 5 g; acid hydrolysate of casein, 2 g; yeast extract 1 g; NaCl, 5 g; $CaCO_3$, 5 g; agar, 15 g; distilled water, 1 l; supplemented with 100 μg/ml penicillin), which were incubated for 2–4 weeks at 27°C. Growth of the microorganism around the stem material was observed with a stereomicroscope and subcultured onto yeast extract-malt extract agar.

Maintenance procedures

Working cultures of *Glycomyces* can be maintained as refrigerated (4°C) agar slants on appropriate media such as yeast extract-malt extract agar (Shirling and Gottlieb, 1966) or NZamine agar (DSMZ medium 554). The slants are generally incubated for 5–7 d at 28–30°C prior to refrigeration and then should be subcultured at monthly intervals. Longer term preservation of strains is best accomplished as frozen stocks in 20% (v/v) aqueous glycerol at −80°C (mechanical freezer) to −172°C (liquid nitrogen vapor phase) or by using traditional lyophilization techniques. Strains have also been successfully stored for shorter periods as quick-frozen stationary-phase broth cultures or mycelial suspensions in 20% aqueous glycerol at −20° to −72°C.

Procedures for testing special characters

Strains are routinely cultivated on NZamine medium (DSMZ medium no. 554 (DSMZ, 2001) at 28°C. Morphological observations are made on the media of Shirling and Gottlieb (1966) and NZamine agar. Chemotaxonomic analysis of strains for fatty acids, menaquinones, and polar lipids are performed using methods previously described by Grund and Kroppenstedt (1989), Minnikin et al. (1984), Sasser (1990) and Saddler et al. (1991).

Physiological tests, including production of acid from carbohydrates, utilization of organic acids, and hydrolysis and decomposition of adenine, casein, esculin, guanine, hippurate, hypoxanthine, tyrosine, xanthine, and urea are typically determined using the media of Gordon et al. (1974). Phosphatase activity is evaluated by using the method of Kurup and Schmitt (1973) substituting NZamine agar as the basal medium. Temperature range for growth is determined on slants of DSMZ medium no. 554 and salt tolerance on slants of the same medium supplemented with 4% and 5% NaCl.

Differentiation of the genus *Glycomyces* from other genera

The genus *Glycomyces* is distinct from other actinomycete genera on the basis of phylogeny as determined from 16S rRNA gene sequences (Figure 125), and this is doubtless the most valuable characteristic in differentiating *Glycomyces* species from

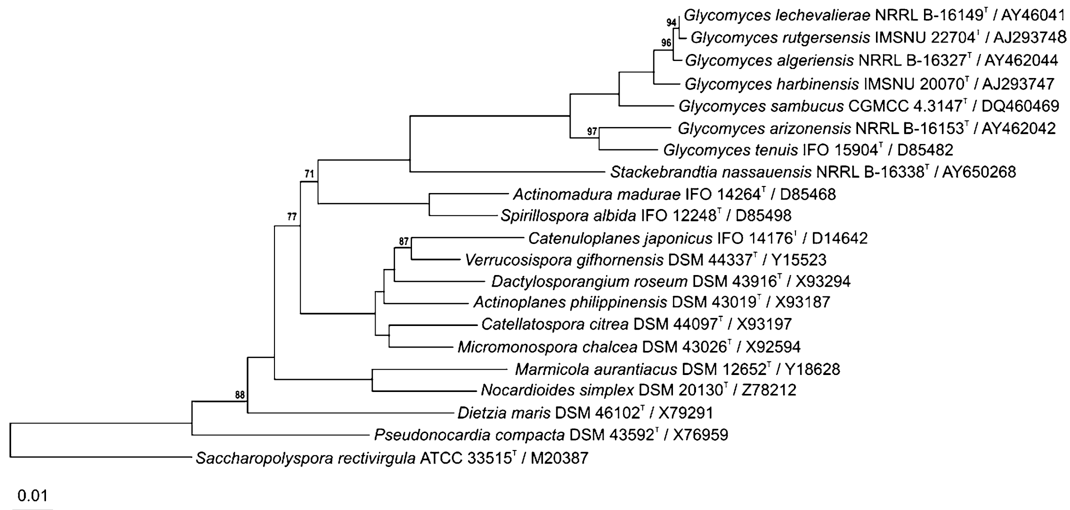

FIGURE 125. Phylogenetic tree for the genera *Glycomyces* and *Stackebrandtia* and near neighbors calculated from 16S rDNA sequences using Kimura's evolutionary distance method (1980) and the neighbor-joining method of Saitou and Nei (1987). Bar marker scale = 0.01 nucleotide substitutions per site.

TABLE 63. Comparison of chemotaxonomic profile of *Glycomyces* and *Stackebrandtia* with phylogenetically nearest taxa

Characteristic	*Glycomyces*	*Actinomadura*	*Spirillospora*	*Stackebrandtia*
A_2pm isomer	*meso*	*meso*	*meso*	*meso*
Whole-cell sugars	Galactose, mannose, ribose, xylose	Madurose	Madurose	Inositol, ribose
Polar lipids[a]	PIM, PI, PG, DPG, PGL_1, PGL_2	PI, PG, DPG	PI, PIM, DPG	PG, DPG, PL_1, PL_2
Predominant menaquinones	Species specific	MK-9(H_4), MK-9(H_6), MK-9(H_8)	MK-9(H_4), MK-9(H_6)	MK-10(H_4), MK-10(H_6), MK-11(H_4), MK-11(H_6)

[a]Abbreviations: DPG, diphosphatidylglycerol; PG, phosphatidylglycerol; $PGL_{1,2}$, unknown phosphoglycolipids; PI, phosphatidylinositol; PIM, phosphatidylinositolmannosides; $PL_{1,2}$, unknown phospholipids.

other actinobacterial genera that produce chains of spores. The chemotaxonomic profile of members of the genus is different from the nearest phylogenetic neighboring taxa, including *Stackebrandtia*, which is closest based on 16S rRNA phylogeny (Table 63). The whole-cell sugar pattern of galactose, mannose, ribose, and xylose is unique among actinobacteria as is the observation that the menaquinone content appears species-specific. It has been reported that *Glycomyces* species also contain teichoic acids (Potekhina et al., 1993) and that these profiles may also be species-specific (Potekhina et al., 1998).

Differentiation of the species of the genus *Glycomyces*

The physiological characteristics of *Glycomyces* species are summarized in Table 64 and can be used to differentiate between species. Gross colonial morphology provides some additional information for differentiation of the species based on the color of the substrate mycelium and production and color of soluble pigments, but many of the species appear quite similar when growing on agar media. Menaquinone content appears to be a species-specific feature (Table 62) and is also useful in distinguishing *Glycomyces* species. Fatty acid profiles are not grossly different among *Glycomyces* species (Table 65) but quantitative and minor component differences observed are useful in species discrimination. Potekhina et al. (1998) have reported that teichoic acid profiles permit distinction between *Glycomyces harbinensis*, *Glycomyces rutgersensis*, and *Glycomyces tenuis*, but these data are not available for more recently described species.

TABLE 64. Physiological characteristics of species of the genus *Glycomyces*[a]

Characteristic	*G. harbinensis*	*G. algeriensis*	*G. arizonensis*	*G. lechevalierae*	*G. rutgersensis*	*G. sambucus*	*G. tenuis*
Hydrolysis of:							
Adenine	+	+	–	+	+	nd	nd
Allantoin	nd	+	+	+	nd	nd	nd
Casein	+	+	+	+	+	nd	+
Esculin	+	+	+	+	+	+	+
Gelatin	–	–	–	–	+	–	nd
Hypoxanthine	+	–	–	+	+	–	+
Starch	+	–	–	+	+	+	+
Tyrosine	–	–	–	+	–	–	–
Urea	–	–	–	–	–	nd	–
Xanthine	–	–	–	–	–	nd	nd
Production of:							
Nitrate reductase	w	w	w	+	+	+	nd
Phosphatase	+	+	+	+	+	nd	nd
Growth in the presence of:							
4% NaCl	+	+	+	+	+	+	+
5% NaCl	+	+	+	+	+	–	+
Utilization of:							
Acetate	+	w	–	+	+	+	+
Benzoate	–	–	–	–	–	nd	–
Citrate	+	–	–	+	–	w	–
Lactate	–	–	–	–	+	w	+
Malate	+	w	–	+	+	+	–
Mucate	–	w	–	+	–	+	nd
Oxalate	–	w	–	+	–	–	nd
Propionate	+	w	–	–	+	–	nd
Succinate	+	–	–	+	–	+	–
Tartrate	–	–	–	–	–	+	–
Acid from:							
Adonitol	+	–	+	+	–	–	–
Arabinose	+	+	+	+	+	nd	+
Cellobiose	+	+	+	+	+	nd	nd
Dextrin	+	+	+	+	+	nd	nd
Dulcitol	–	–	–	–	–	nd	–
Erythritol	+	–	+	–	–	–	nd
Fructose	+	+	+	+	+	nd	+
Galactose	+	+	+	+	+	nd	+
Glucose	+	+	+	+	+	nd	+
Glycerol	+	+	+	+	+	nd	nd
Inositol	–	+	+	+	–	–	–
Lactose	+	+	+	+	w	+	+
Maltose	+	w	+	+	+	+	+
Mannitol	–	–	+	–	+	–	–
Mannose	+	+	+	+	+	nd	nd
Melezitose	–	–	+	–	w	–	nd
Melibiose	–	–	–	w	–	+	–
α-Methyl-D-glucoside	+	+	w	+	+	nd	nd
β-Methyl-D-xyloside	+	–	–	–	+	+	nd
Raffinose	+	–	–	–	+	+	+
Rhamnose	+	+	+	+	+	nd	+
Salicin	+	+	+	+	+	nd	nd
Sorbitol	+	–	+	w	+	–	–
Sucrose	–	–	+	+	–	+	+
Trehalose	+	–	+	+	+	–	nd
Xylose	+	+	+	+	+	nd	+

(continued)

TABLE 64. (continued)

Characteristic	*G. harbinensis*	*G. algeriensis*	*G. arizonensis*	*G. lechevalierae*	*G. rutgersensis*	*G. sambucus*	*G. tenuis*
Growth at:							
5°C	–	–	–	–	–	–	–
15°C	–	+	–	+	–	–	+
20°C	+	+	+	+	+	+	+
25°C	+	+	+	+	+	+	+
28°C	+	+	+	+	+	+	+
37°C	+	+	+	+	+	+	+
42°C	+	–	–	–	–	–	–
45°C	–	–	–	–	–	–	–

[a]Symbols: +, >85% positive; -, 0–15% positive; w, weak reaction; nd, not determined.

TABLE 65. Fatty acid profiles of *Glycomyces* and *Stackebrandtia* species[a,b]

Fatty acid	*G. harbinensis*	*G. algeriensis*	*G. arizonensis*	*G. lechevalierae*	*G. rutgersensis*	*G. tenuis*	*Stackebrandtia nassauensis*
$C_{14:0}$ iso	1.51	2.04	3.13	2.86	2.37	2.21	-
$C_{14:0}$	0.49	-	-	0.45	0.61	-	-
$C_{15:0}$ iso	11.26	14.41	7.18	14.77	19.9	13.95	8.7
$C_{15:0}$ anteiso	15.11	27.52	25.36	32.52	30.95	33.2	2.89
$C_{15:1}$	–	–	–	–	–	–	0.84
C_{15}	0.41	–	–	2.01	2.98	2.07	0.64
$C_{16:1}$ iso	10.34	4.37	11.53	4.14	4.37	2.59	2.11
$C1_{6:0}$ iso	17.83	10.9	22.69	16.19	12.52	16	8.72
$C_{16:1}$ 7*c*	–	–	–	–	–	–	0.53
$C_{16:1}$ 9*c*	0.62	–	–	–	–	–	3.17
$C_{16:0}$ iso	2.03	–	1.34	1.31	1.61	1.92	0.67
$C_{16:0}$ 10methyl	–	–	–	–	–	–	9.07
$C_{17:1}$ iso	–	–	–	–	–	–	0.58
$C_{17:1}$ anteiso	12.66	12.33	8.75	5.15	6.13	3.62	1.7
$C_{17:0}$ iso	1.34	2.85	1.01	1.36	0.98	1.58	9.02
$C_{17:0}$ anteiso	26.41	25.59	19.01	19.24	16.82	22.86	26.86
$C_{17:1}$ 9*c*	–	–	–	–	0.42	–	1.84
$C_{17:0}$	–	–	–	–	0.34	–	–
$C_{17:0}$ 10methyl	–	–	–	–	–	–	1.44
$C_{16:0}$ 2-OH	–	–	–	–	–	–	4.22
$C_{17:0}$ 2-OH	–	–	–	–	–	–	2.45
$C_{17:0}$ anteiso 2-OH	–	–	–	–	–	–	14.52

[a]Symbols: –, 0–15% positive.

[b]Fatty acids are listed as percentages of total fatty acids as determined by the Microbial Identification System software (MIDI) peak naming table.

List of species of the genus *Glycomyces*

1. **Glycomyces harbinensis** Labeda, Testa, Lechevalier and Lechevalier 1985, 420[VP]

har.bin.en′sis N.L. masc. adj. *harbinensis* of or belonging to Harbin, China (the source of the soil sample from which this organism was first isolated).

Pale-yellow to yellowish-white vegetative mycelium with very sparse production of white aerial mycelia on some media (e.g. Czapek sucrose agar). Ability to produce aerial mycelia appears to be lost on repeated transfer. Short chains of square-ended conidia produced on aerial hyphae. Soluble pigments are rarely produced, but are of yellowish shades when present. A fetid odor is produced during growth on some rich growth media. Physiological properties are shown in Table 64. Temperature range for growth is 20–42°C.

Source: soil.

DNA G+C content (mol%): 71.0 (T_m).

Type strain: LL-D05139, ATCC 43155, CGMCC 4.1364, DSM 46494, IAM 14283, IMET 43812, JCM 7347, KCTC 9362, KCTC 9655, NBRC 14487, NRRL 15337, VKM Ac-1247.

Sequence accession no. (16S rRNA gene): AJ293747, D85483.

2. **Glycomyces algeriensis** Labeda and Kroppenstedt 2004, 2345[VP]

al.ge.ri.en′sis. N.L. masc. adj. *algeriensis* from Algeria, named after the place of origin of the type strain, country of Algeria.

White to yellowish-white, waxy, plicate growth on most media. Sparse white aerial hyphae are produced on some media. Soluble pigments are not produced. Menaquinones present include MK-10, MK-11, and MK-12. Physiological properties shown in Table 64. Temperature for growth is 15–37°C.

Source: soil.

DNA G+C content (mol%): not determined.

Type strain: DSM 44727, JCM 14910, NBRL 103888, NRRL B-16327.

Sequence accession no. (16S rRNA gene): AY462044.

3. **Glycomyces arizonensis** Labeda and Kroppenstedt 2004, 2345[VP]

a.ri.zo.nen′sis. N.L. masc. adj. a*rizonensis* from Arizona, named after the place of origin of the type strain, state of Arizona, USA.

White to yellowish-white, waxy, plicate growth on most media. Aerial mycelia not produced. Faint pinkish-yellowish to yellow soluble pigment produced on some media. Menaquinones present include MK-10(H_2), MK-10(H_4), MK-11(H_2), and MK-11(H_4). Physiological properties shown in Table 64. Temperature for growth is 20–37°C.

Source: soil.

DNA G+C content (mol%): not determined.

Type strain: DSM 44726, JCM 14911, NBRC 103886, NRRL B-16153.

Sequence accession no. (16S rRNA gene): AY462042.

4. **Glycomyces lechevalierae** Labeda and Kroppenstedt 2004, 2346[VP]

le.che.val.i.e′ra.e. N. L. gen. fem. n. *lechevalierae* of Lechevalier, named for Mary Lechevalier, American microbiologist who isolated this strain and contributed substantially to the field of actinomycete biology during her career at the Waksman Institute of Microbiology.

White to yellowish-white waxy vegetative growth on most media. Light grayish-yellow plicate growth on NZamine medium (DSMZ medium no. 554). Sparse white aerial mycelia produced on several media. Menaquinones present include MK-10, MK-10(H_2), MK-10(H_4), MK-11, MK-11(H_2), and MK-11(H_4). Physiological properties shown in Table 64. Temperature for growth is 15–37°C.

Source: soil.

DNA G+C content (mol%): not determined.

Type strain: DSM 44724, JCM 14912, NBRC 103885, NRRL B-16149.

Sequence accession no. (16S rRNA gene): AY462041.

5. **Glycomyces rutgersensis** Labeda, Testa, Lechevalier and Lechevalier 1985, 420[VP]

rut.ger.sen′sis. N.L. masc. adj. *rutgersensis* of or belonging to Rutgers University where the organism was first isolated.

Yellowish-white to tan vegetative mycelium, dependent upon the growth medium. Moderate to abundant white aerial mycelium is formed on many media. Yellow to brown soluble pigments produced. A fetid odor is produced during growth on several rich growth media. Physiological properties shown in Table 64. Temperature for growth is 20–37°C.

Source: soil.

DNA G+C content (mol%): 73.0 (T_m).

Type strain: LL-I-20, ATCC 43156, CGMCC 4.1363, DSM 43812, IMET 43813, IMSNU 22074, JCM 6238, KCTC 9654, NBRC 14488, NBRC 15934, NRRL B-16106, VKM Ac-1248.

Sequence accession no. (16S rRNA gene): AJ293748, D85384.

6. **Glycomyces sambucus** Gu, Zeng and Huang 2007, 1996[VP]

sam′bu.cus. N.L. gen. n. *sambucus* of the plant genus *Sambucus.*

Yellowish-white to tan substrate mycelium, depending upon the growth medium. White aerial mycelia are produced and fragment into square-ended conidia. No soluble pigments are produced. Physiological properties shown in Table 64. Temperature for growth is 20–37°C.

Source: stems of *Sambucus adnata* Wall, a traditional Chinese medicinal plant.

DNA G+C content (mol%): 70 (T_m).

Type strain: E71, CGMCC 4.3147, DSM 45047.

Sequence accession no. (16S rRNA gene): DQ460469.

7. **Glycomyces tenuis** Evtushenko, Taptykhova, Akimov, Semyonova and Kalakoutskii 1991, 155[VP]

te′nu.is. L. masc. adj. *tenuis* thin, referring to the very thin vegetative hyphae of this strain.

The vegetative hyphae are very thin (diameter, 0.15–0.40 μm), long, densely branched, and white or ometimes cream or slightly pink. Aerial mycelium is not formed. Cultures grow well on rich organic medium and, when cultured on synthetic media, yeast extract or Casamino acids (0.3%) must be added. Physiological properties shown in Table 64. Temperature for growth is 10–40°C.

Source: an associate with *Streptomyces galilaeus* INA 5888, a soil isolate.

DNA G+C content (mol%): 72.0 (T_m).

Type strain: ATCC 49849, DSM 44171, IMSNU 22075, INA n-5888, JCM 9087, KCTC 9658, NBRC 15904, NRRL B-16895, VKM Ac-1250.

Sequence accession no. (16S rRNA gene): D85482.

Genus II. **Stackebrandtia** Labeda and Kroppenstedt 2005, 1690[VP]

David P. Labeda

Stack.e.brandt′i.a. N.L. fem. n. *Stackebrandtia* named for Erko Stackebrandt, a German microbiologist who contributed significantly to the molecular systematics of prokaryotes, including actinobacteria.

Aerobic. Gram-stain-positive, nonmotile actinomycetes. **Branched substrate mycelium (approximately 0.5 µm in diameter) and, on some media, aerial mycelia are produced.** Mycolic acids are absent. Catalase-positive. Contain *meso*-diaminopimelic acid as the diamino acid. **Cell walls contain *N*-glycolylmuramic acid. The whole-cell sugar pattern consists of inositol and ribose, with traces of arabinose and mannose. The phospholipid pattern consists of diphosphatidylglycerol, phosphatidylglycerol, and two additional unknown phospholipids. The predominant menaquinones are MK-10(H_4), MK-10(H_6), MK-11(H_4), and MK-11(H_6). Fatty-acid profile is rich in branched chain and saturated components including 10-methyl branched heptadecanoic acid and iso-branched 2-hydroxy fatty acids**. Phylogenetically nearest to the genus *Glycomyces*.

DNA G+C content (mol%): 72.4.

Type species: **Stackebrandtia nassauensis** Labeda and Kroppenstedt 2005, 1690[VP].

Further descriptive information

The fragmentation of aerial or substrate mycelium into spore-like elements has not been observed although observations of long-term growth on Czapek's sucrose agar (Figure 126) has revealed regions of growth that appear close to fragmentation. This genus could represent a new family within the order *Glycomycetales*, but this cannot be well supported based on a genus and species described for a single strain.

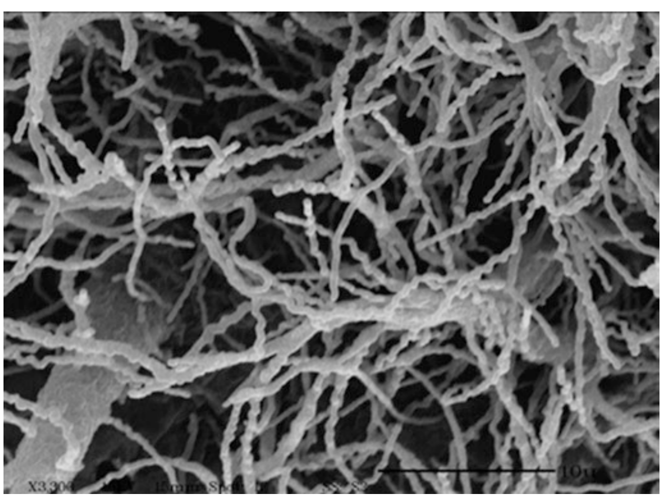

FIGURE 126. Scanning electron micrograph of 21-d growth of *Stackebrandtia nassauensis* NRRL B-16338[T] on yeast extract-malt extract (ISP-2) agar.

Maintenance procedures

Working cultures of *Stackebrandtia* can be maintained as refrigerated (4°C) agar slants on appropriate media such as yeast extract-malt extract medium (Shirling and Gottlieb, 1966) or NZamine medium (DSMZ medium 554). The slants are generally incubated for 5–7 d at 28–30°C prior to refrigeration, and then should be subcultured at monthly intervals. Longer term preservation of strains is best accomplished as frozen stocks in 20% (v/v) aqueous glycerol at −80°C (mechanical freezer) to −172°C (liquid nitrogen vapor phase) or by using traditional lyophilization techniques.

Procedures for testing special characters

Strains are routinely cultivated on NZamine medium (DSMZ medium no. 554 (DSMZ, 2001) at 28°C. Morphological observations are made on the media of Shirling and Gottlieb (1966) and on NZamine medium.

Chemotaxonomic analysis of strains for fatty acids, menaquinones, and polar lipids are performed using methods previously described by Minnikin et al. (1984), Grund and Kroppenstedt (1989), Sasser (1990), and Saddler et al. (1991).

Physiological tests, including production of acid from carbohydrates, utilization of organic acids, and hydrolysis and decomposition of adenine, casein, esculin, guanine, hippurate, hypoxanthine, tyrosine, urea, and xanthine are typically evaluated using the media of Gordon et al. (1974). Phosphatase activity is evaluated by using the method of Kurup and Schmitt (1973), substituting NZamine agar as the basal medium. Temperature range for growth is determined on slants of DSMZ medium no. 554 and salt tolerance on slants of the same medium supplemented with 4% and 5% NaCl.

Differentiation of the genus *Stackebrandtia* from other genera

The genus *Stackebrandtia* can be easily differentiated from *Glycomyces*, the phylogenetically nearest genus (Figure 125), based on chemotaxonomic characteristics. The whole-cell sugar pattern of *Stackebrandtia* consists of inositol and ribose with traces of arabinose and mannose, whereas *Glycomyces* typically exhibits galactose, mannose, ribose, and xylose as diagnostic sugars (Table 63). Moreover, *Stackebrandtia* and *Glycomyces* both contain diphosphatidylglycerol and phosphatidylglycerol, but *Stackebrandtia* contains two additional unknown phospholipids, whereas *Glycomyces* has significant quantities of sugar-containing phospholipids such as phosphatidylinositolmannosides and two phosphoglycolipids of unknown structure (Table 62). The fatty acid pattern of *Stackebrandtia* contains 10-methyl branched heptadecanoic acid and iso-branched 2-hydroxy fatty acids which are not found in *Glycomyces* (Table 65). The

menaquinone pattern of *Stackebrandtia*, in which MK-10(H_4), MK-10(H_6), MK-11(H_4), and MK-11(H_6) are predominant, is also different from that of the genus *Glycomyces* where the pattern appears almost species-specific but consists of menaquinones with 9, 10, 11, or 12 isoprene units with various degrees of hydrogenation (*Glycomyces* Table 64).

List of species of the genus *Stackebrandtia*

1. **Stackebrandtia nassauensis** Labeda and Kroppenstedt 2005, 1690[VP]

nas.sau.en′sis. N.L. fem. adj. *nassauensis* from Nassau, named after the place of origin of the type strain, Nassau, Providence, Bahamas.

Pale yellow to pale tan vegetative mycelium; white to yellowish-white aerial hyphae produced on most media, especially yeast extract-malt extract agar (ISP-2). Degrades or hydrolyzes allantoin, casein, esculin, gelatin, hypoxanthine, starch, and tyrosine. Neither adenine nor xanthine is degraded. Produces phosphatase. Weakly reduces nitrates. Weakly assimilates acetate and malate; does not assimilate benzoate, citrate, lactate, mucate, oxalate, propionate, succinate, or tartrate. Acid is produced from arabinose, cellobiose, dextrin, fructose, galactose, glucose, glycerol, lactose, maltose, mannose, melibiose, α-methyl-D-glucoside, raffinose, rhamnose, salicin, sorbitol, sucrose, trehalose, and xylose, but not from adonitol, dulcitol, erythritol, inositol, mannitol, melezitose, or β-methyl-xyloside. Temperature range for growth is 15–37°C. Grows in the presence of 4–9% NaCl. Temperature range for growth is 15–37°C.

Source: soil.

DNA G+C content (mol%): 72.4 (HPLC).

Type strain: LLR-40K-21, CIP 108903, DSM 44728, JCM 14905, NBRC 102104, NRRL B-16338.

Sequence accession no. (16S rRNA gene): AY650268.

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Order VIII. **Jiangellales** ord. nov.

Shu Kun Tang, Xiao-Yang Zhi and Wen-Jun Li

Ji.ang.el′la.les. N.L. fem. n. *Jiangella* type genus of the order; suff. -*ales* ending to denote an order; N.L. fem. pl. n. *Jiangellales* the *Jiangella* order.

The order *Jiangellales* was circumscribed for this volume mainly based on signature nucleotide patterns and phylogenetic criteria; the order contains the family *Jiangellaceae*. The order was created by elevation of suborder *Jiangellineae* of Tang et al. (2010).

Type genus: **Jiangella** Song, Li, Wang, Chen, Zhang and Xu 2005, 883[VP].

Taxonomic comments

The genus *Jiangella* was first proposed by Song et al. (2005) and assigned to the family *Nocardioidaceae* within the suborder *Propionibacterineae*, now the order *Propionibacteriales*. Upon the discovery of another novel genus, *Haloactinopolyspora* (Tang et al., 2011), phylogenetic analyses based on 16S rRNA gene sequences of the order *Propionibacteriales* revealed that the genera *Jiangella* and *Haloactinopolyspora* form a monophyletic branch at the periphery of the evolutionary radiation occupied by this order (Figure 127). It clearly shows that the genera *Jiangella* and *Haloactinopolyspora* do not belong to either the family *Nocardioidaceae* or the order *Propionibacteriales*. Further phylogenetic analyses including representatives of the entire phylum *Actinobacteria* reveal that the genera *Jiangella* and *Haloactinopolyspora* form a deep branch clearly delineated from other described orders of the class *Actinobacteria* (Figure 128). *Actinobacteria* outside these genera have 16S rRNA gene sequence similarities to the genera *Jiangella* and *Haloactinopolyspora* of less than 93.1%. Although the genera *Jiangella* and *Haloactinopolyspora* clearly belong to the class *Actinobacteria*, they do not belong to any of the described orders within that class. The 16S rRNA gene sequences of all orders of the class *Actinobacteria* (Zhi et al., 2009) and the genera *Jiangella* and *Haloactinopolyspora* were scanned for signature nucleotides. Both the genera *Jiangella* and *Haloactinopolyspora* have many unique 16S rRNA gene signature nucleotides compared with the described orders within the class *Actinobacteria*, particularly reflected in eleven different positions, namely, in 127:234 (G–C), 598:640 (C–G), 672:734 (G–C), 831:855 (U–A), 833:853 (G–C), 840:846 (A–U), 950:1231 (G–C), 952:1229 (G–C), 955:1225 (G–U), 986:1219 (U–G), and 987:1218 (C–G). Higher hierarchical taxa in the class *Actinobacteria* are mainly based on signature nucleotide patterns and phylogenetic criteria (Stackebrandt

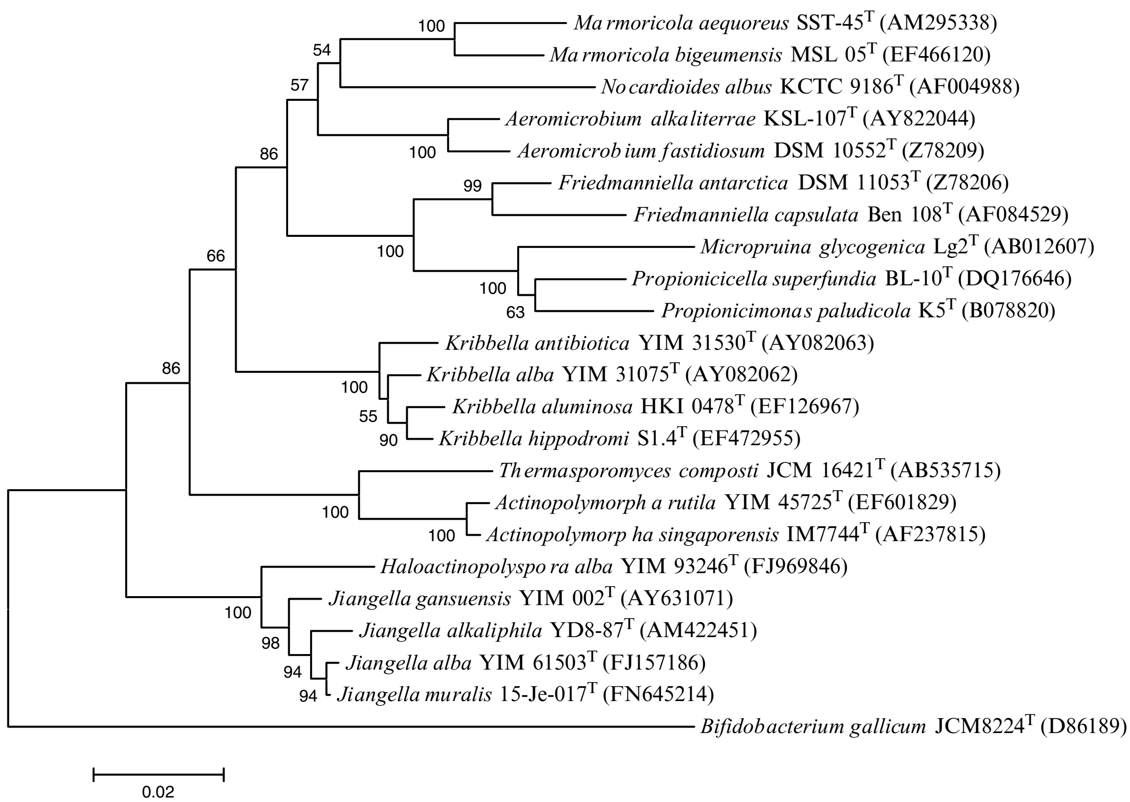

FIGURE 127. Phylogenetic dendrogram obtained by distance matrix analysis of 16S rRNA gene sequences showing the position of the genera *Jiangella* and *Haloactinopolyspora* and members of the order *Propionibacteriales*. Numbers on branch nodes are bootstrap values (1000 resamplings, only values over 50% are given). The sequence of *Bifidobacterium gallicum* JCM 8224[T] (D86189) was used as the outgroup. Bar = 0.02 substitutions per nucleotide position.

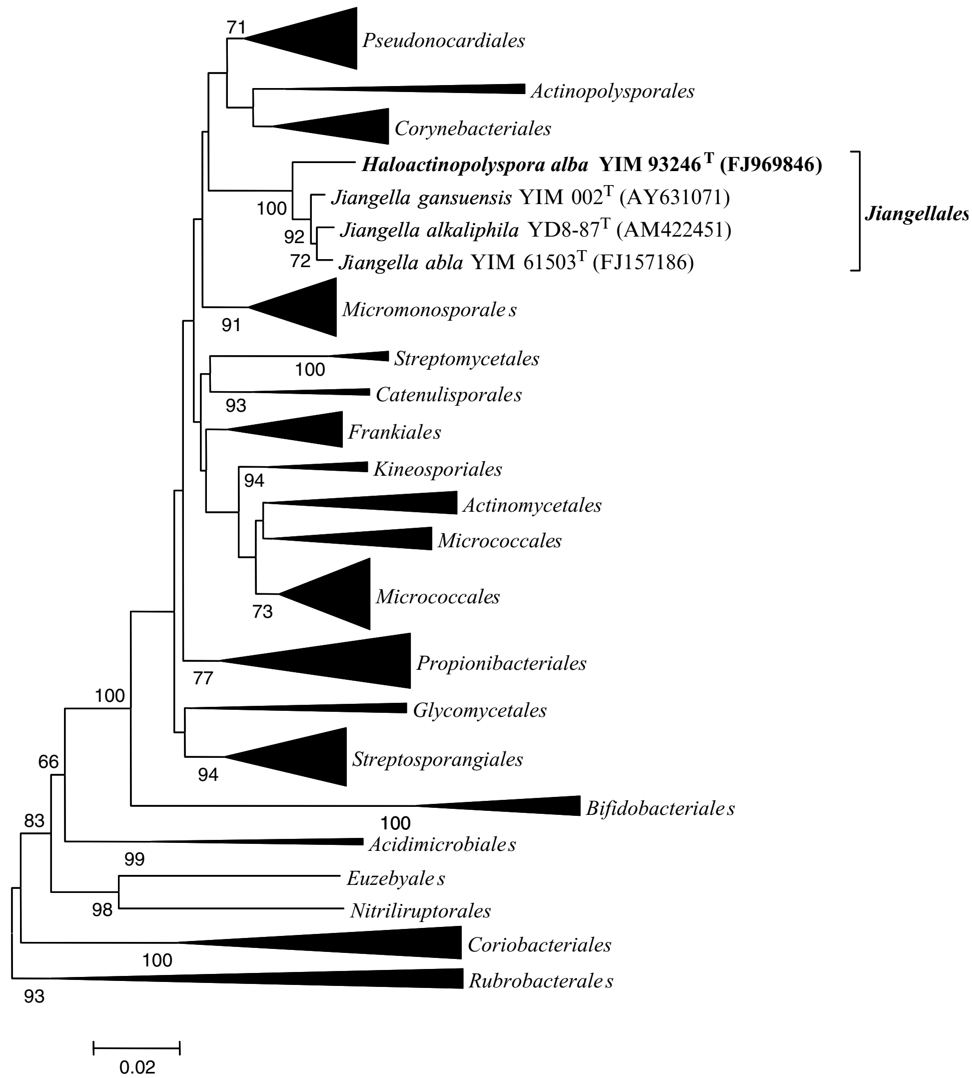

FIGURE 128. Phylogenetic position of members of the genera *Jiangella* and *Haloactinopolyspora* within the class *Actinobacteria* based on 16S rRNA gene sequence analysis. Tree topology and evolutionary distances were calculated by the neighbor-joining method (Saitou and Nei, 1987). Numbers at nodes are bootstrap percentages for the clade of each group based on 1000 replications; only values above 50% are shown. Bar = 0.02 substitutions per nucleotide position.

et al., 1997; Zhi et al., 2009). Accordingly, the genera *Jiangella* and *Haloactinopolyspora* are closely related phylogenetically and are clearly distinct from other orders in the class *Actinobacteria*. Thus, a novel suborder, *Jiangellineaes*, now the order *Jiangellales*, and a novel family *Jiangellaceae* were proposed to accommodate the genera *Jiangella* and *Haloactinopolyspora* (Tang et al., 2011).

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Family I. **Jiangellaceae** Tang, Zhi, Wang, Shi, Lou, Xu and Li 2011, 198[VP]

Shu-Kun Tang, Xiao-Yang Zhi and Wen-Jun Li

Ji.ang.el.la.ce′a.e. N.L. fem. n. *Jiangella* type genus of the family; suff. *-aceae* ending to denote a family; N.L. fem. pl. n. *Jiangellaceae* the family of the genus *Jiangella*.

The family *Jiangellaceae* was circumscribed for this volume mainly based on signature nucleotide patterns and phylogenetic criteria; the family contains the genera *Jiangella* and *Haloactinopolyspora*.

Members of the family *Jiangellaceae* are Gram-stain-positive, aerobic, halophilic or nonhalophilic filamentous actinomycetes.

DNA G+C content (mol%): 70–72.

Type genus: **Jiangella** Song, Li, Wang, Chen, Zhang and Xu 2005, 883[VP].

Taxonomic comments

Please refer to the order description, above.

Further descriptive information

Phylogenetic analysis of the 16S rRNA gene sequence places the family within the order *Jiangellales*. MK-9(H_4) is the predominant menaquinone, and LL-2,6-diamionpimelate is the cell-wall diamino acid. The major fatty acids are $C_{15:0}$ anteiso, $C_{16:0}$ iso, and $C_{17:0}$ anteiso. The pattern of 16S rRNA signature nucleotides is shown in 127:234 (G–C), 598:640 (C–G), 672:734 (G–C), 831:855 (U–A), 833:853 (G–C), 840:846 (A–U), 950:1231 (G–C), 952:1229 (G–C), 955:1225 (G–U), 986:1219 (U–G) and 987:1218 (C–G).

Genus I. **Jiangella** Song, Li, Wang, Chen, Zhang and Xu 2005, 883[VP]

Xiao-Yang Zhi, Shu-Kun Tang and Wen-Jun Li

Ji.ang.el′la. N.L. fem. dim. n. *Jiangella* named after the Chinese microbiologist Cheng-Lin Jiang in recognition of his work on actinomycete taxonomy.

Gram-stain-positive, filamentous actinomycete. Aerobic, with a strictly respiratory type of metabolism. Catalase positive.

DNA G+C content (mol%): 70–71.5.

Type species: **Jiangella gansuensis** Song, Li, Wang, Chen, Zhang and Xu 2005, 883[VP].

Further descriptive information

Phylogenetic analysis of the 16S rRNA gene sequence places the genus within the family *Jiangellaceae* and order *Jiangellales*. Its closest relative is the genus *Haloactinopolyspora*. Substrate mycelium fragments into short or elongated rods in the early stages of growth. Aerial mycelium differentiates well and no spores are formed (Figure 129). Cell wall contains LL-2,6-diaminopimelate as the diamino acid in the peptidoglycan. MK-9(H_4) is the predominant menaquinone. Major cellular fatty acids are $C_{15:0}$ anteiso, $C_{17:0}$ anteiso, $C_{15:0}$ iso, and $C_{16:0}$ iso. Polar lipids are diphosphatidylglycerol, phosphatidylglycerol, phosphatidylinositol, phosphatidylinositol mannosides, phosphatidylcholine, an unknown phospholipid, and an unknown phosphoglycolipid.

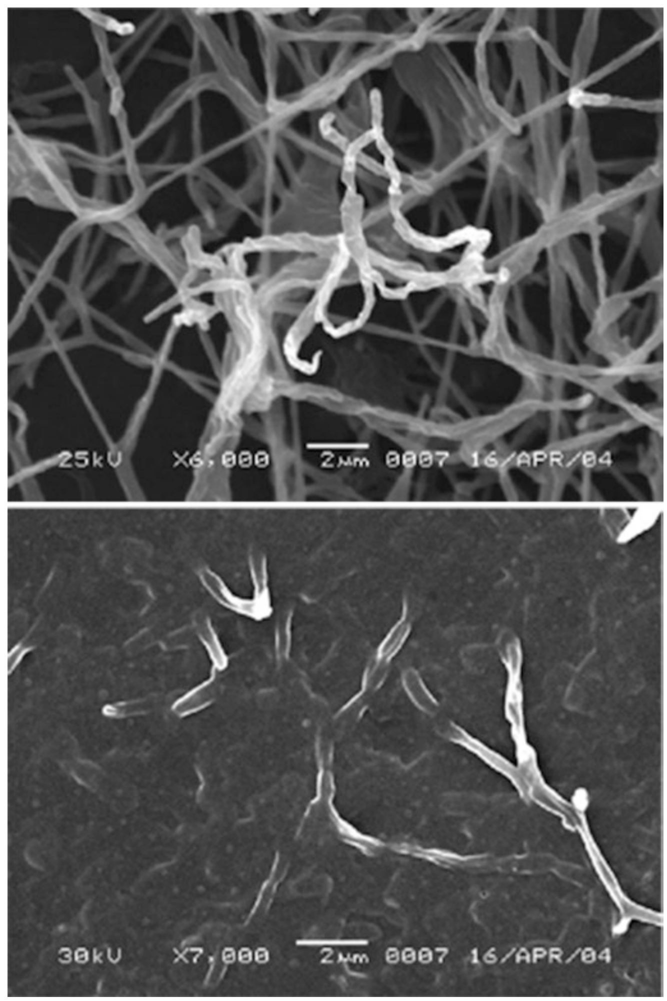

FIGURE 129. Scanning electron microscopy of *Jiangella gansuensis* grown on ISP medium 2 for 14 d at 28°C showing aerial mycelium (top) and substrate mycelium (bottom).

Enrichment and isolation procedures

The type species in the genus, *Jiangella gansuensis* YIM 002[T], was isolated from a desert soil sample collected from Sunan county, Gansu Province, northwest China, by using the dilution plating method. The medium used for selective isolation was glycerol-asparagine agar (ISP medium 5), which was incubated at 28°C for about 2 weeks. Strain *Jiangella alba* YIM 61503[T] was isolated from surface-sterilized stems of *Maytenus austroyunnanensis* collected from a tropical rainforest of Xishuangbanna, Yunnan Province, south-west China. Stem samples were air-dried at room temperature after being thoroughly washed under tap water and then surface sterilized according to the five-step sterilization procedure. Samples were then pulverized in a ceramic mortar and processed with a calcium carbonate enrichment method. The samples were serially diluted in sterile distilled water and spread-plated on glycerol-asparagine agar (ISP medium 5). Strain YIM 61503[T] was isolated after incubation at 28°C for 21 d.

TABLE 66. Characteristics differentiating the species of *Jiangella* and *Haloactinopolyspora*[a,b]

Characteristic	*J. gansuensis* YIM 002^T	*J. alba* YIM 61503^T	*J. alkaliphila* DSM 45079^T	*J. muralis* 15-Je-017^T	*H. alba* YIM 93246^T
Fragmentation of aerial mycelium	−	+	+	nd	−
Spore chain	−	−	−	nd	Long
Spores heap	−	−	−	nd	+ (Root-like)
Growth in:					
0% NaCl	+	+	+	+	−
20% NaCl	−	−	−	nd	+
Nitrate reduction	−	+	−	nd	−
Gelatin liquefaction	+	+	+	nd	−
Decomposition of:					
Adenine	nd	+	nd	nd	+
Casein	nd	+	+	nd	−
Hypoxanthine	−	+	+	nd	+
Xanthine	−	+	−	nd	−
Urea	+	−	−	nd	−
Utilization of:					
Cellobiose	+	−	+	+	+
Citrate	+	−	−	−	−
D-Galactose	+	−	−	w	−
D-Lactose	+	−	+	nd	+
D-Mannitol	−	+	+	−	−
myo-Erythritol	−	+	−	nd	−
myo-Inositol	+	−	−	−	+
Raffinose	−	+	−	nd	−
D-Ribose	−	+	−	+	−
D-Sorbitol	+	−	−	−	+
D-Xylitol	+	−	−	nd	−
Cell-wall sugars	rib, glu	glu, rib, gal	glu, rha, rib, man	glu, rha	GlcN, man, glc, ara, gal, rha, one unknown sugar
Phospholipids	DPG, PG, PGL, PL, PI, PIMs	DPG, PG, PI, PIM, PGL, PLs	DPG, PG, PC, PI, PIM, PL	DPG, PG, PC, PI, PIM	DPG, PGL, PL, PI, PIMs, GL
Major fatty acids (>10%)	$C_{15:0}$ anteiso (35.9%), $C_{17:0}$ anteiso (15.8%)	$C_{15:0}$ anteiso (26.1%), $C_{16:0}$ iso (20.6%)	$C_{15:0}$ anteiso (20.4%), $C_{16:0}$ iso (18.0%)	$C_{15:0}$ anteiso (30.8%), $C_{16:0}$ iso (12.1%), $C_{16:0}$ iso (11.5%),	$C_{16:0}$ iso (29.4%), $C_{15:0}$ anteiso (28.4%), $C_{17:0}$ anteiso (13.7%)
DNA G+C (mol%)	70	71.9	71.5	nd	70.5

[a]Symbols: +, positive; −, negative; nd, not determined. GlcN, glucosamine; man, mannose; gal, galactose; glc, glucose; ara, arabinose; gal, galactose; rha, rhamnose; rib, ribose.

[b]Strains: *Jiangella gansuensis* (Song et al., 2005; Kroppenstedt, unpublished data); *Jiangella alba* (Qin et al., 2009); *Jiangella alkaliphila* (Lee, 2008); *Jiangella muralis* (Kämpfer et al., 2011); *Haloactinopolyspora alba* YIM 93246^T (Tang et al., 2011).

Jiangella alkaliphila D8-87^T was isolated from soil in a natural cave in Jeju, Republic of Korea, by using a dilution-plating method on starch-casein agar (SCA) which was incubated at 30°C for 14 d. *Jiangella muralis* 15-Je-017^T was isolated in Jena, Germany, from the cellar wall of a house colonized with mold.

Maintenance procedures

The type strain is maintained on yeast extract-malt extract agar (ISP medium 2) and as mycelial fragments or spores in 20% (v/v) glycerol at −80°C. Lyophilized cultures are also used. *Jiangella alba* YIM 61503^T is maintained as mycelial fragments in a 20% (v/v) glycerol suspension at −80°C. *Jiangella alkaliphila* D8-87^T is maintained as mycelial fragments or spores in 20% (v/v) glycerol. *Jiangella muralis* 15-Je-017^T is maintained on organic medium M79 and preserved at −80°C.

Differentiation of the genus *Jiangella* from other genera

The characteristics differentiating *Jiangella* from the genus *Haloactinopolyspora* are shown in Table 66. *Jiangella* is a non-halophilic actinomycete, and this characteristic distinguishes it from *Haloactinopolyspora*. In addition, differences in the whole cell sugars, interpeptide bridge of peptidoglycan, polar lipids, and the major fatty acids further distinguish *Jiangella* from *Haloactinopolyspora*.

List of species of the genus *Jiangella*

1. **Jiangella gansuensis** Song, Li, Wang, Chen, Zhang and Xu 2005, 883[VP]

gan.su.en′sis. N.L. fem. adj. *gansuensis* pertaining to Gansu, a province of northwest China where the type strain was isolated.

The characteristics are as described for the genus. Optimal temperature and pH for growth are 28°C and 7.0–8.0, respectively. Utilizes arabinose, cellobiose, dextrin, fructose, galactose, glycerol glucose, inositol, lactose, maltose, mannitol, mannose, melibiose, raffinose, rhamnose, sucrose, xylitol, and

xylose as sole carbon sources, but not ribose. Positive for gelatin hydrolysis, urease, milk coagulation, and peptonization, but negative for cellulose hydrolysis, production of H_2S, starch hydrolysis, and nitrate reduction. Fatty acids present are $C_{15:0}$ anteiso (35.92%), $C_{17:0}$ anteiso (15.84%), $C_{15:0}$ iso (10.40%), $C_{17:1}$ ω8*c* (9.37%), $C_{16:0}$ iso (7.07%), $C_{17:0}$ (3.39%), $C_{17:0}$ iso (3.09%), $C_{16:1}$ iso G (2.90%), $C_{17:1}$ anteiso A (2.86%), $C_{15:0}$ 2-OH (2.28%), $C_{14:0}$ iso 2-OH (2.23%), $C_{15:1}$ anteiso A (1.31%), $C_{18:1}$ ω9*c* (1.24%), $C_{17:1}$ iso ω9*c* (1.05%), and $C_{15:0}$ (1.05%).

DNA G+C content (mol%): 70 (T_m).

Type strain: YIM 002, CCTCC AA 204001, DSM 44835, JCM 13367, KCTC 19044.

Sequence accession no. (16S rRNA gene): AY631071.

2. **Jiangella alba** Qin, Zhao, Li, Zhu, Xu and Li 2009, 2164[VP]

al′ba. L. fem. adj. *alba* white, referring to the white aerial hyphae.

Aerobic, Gram-stain-positive, non-acid–alcohol-fast actinomycete. Grows at 15–45 °C (optimum 28°C) and pH 6.5–9.0 (optimum pH 7.0). Grows in the presence of 10% NaCl. Forms well-developed white aerial mycelia and yellowish-white to orange–yellow substrate mycelia that fragment into short or elongated rods. No diffusible pigment is produced on any of the media tested. Adenine, casein, hypoxanthine, and xanthine are degraded, but chitin, starch, DL-tyrosine, and urea are not. Positive for catalase production, gelatin liquefaction, and nitrate reduction, but negative for milk coagulation and peptonization, cellulose hydrolysis, and H_2S production. Positive for utilization of DL-arabinose, *meso*-erythritol, D-fructose, D-glucose, glycerol, inulin, maltose, D-mannitol, D-mannose, melezitose, raffinose, L-rhamnose, D-ribose, salicin, sucrose, trehalose, and D-xylose as sole carbon and energy sources. Negative for utilization of acetate, adonitol, cellobiose, citrate, D-dulcitol, formate, D-galactose, myoinositol, lactose, malate, methyl α-D-mannoside, D-sorbitol, L-sorbose, and D-xylitol. The cell wall contains LL-DAP. The whole-cell sugars are glucose, ribose, and galactose. The predominant menaquinone is MK-9(H_4). Major fatty acids (>5%) are $C_{15:0}$ anteiso, $C_{16:0}$ iso, $C_{14:0}$ iso, $C_{17:1}$ω8c, $C_{17:0}$ anteiso, and $C_{15:0}$ iso.

DNA G+C content (mol%): 71.9 (HPLC).

Type strain: YIM 61503, CCTCC AA 208023, DSM 45237.

Sequence accession no. (16S rRNA gene): FJ157186.

3. **Jiangella alkaliphila** Lee 2008, 1178[VP]

al.ka.li.phi′la. Arabic article *al* the; Arabic n. *qaliy* ashes of saltwort; N.L. n. *alkali* alkali; N.L. adj. *philus -a -um* (from Gr. adj. *philos -ê -on*), friend, loving; N.L. fem. adj. *alkaliphila* loving alkaline conditions.

Aerobic, Gram-stain-positive, non-acid-alcohol-fast. Catalase positive. Forms well-developed, aerial and vegetative mycelia that fragment into short or elongated rods. The aerial mycelium is white or grayish-yellow brown. Mesophilic. The temperature for growth is in the range of 20–37°C, with good growth at 30°C. The initial pH range for growth is 6.1–10.1, with good growth at pH 8.1–10.1. Growth occurs in the presence of up to 3% NaCl. Utilizes D-arabinose, L-arabinose, D-cellobiose, dextran, D-fructose, D-glucose, inulin, D-lactose, maltose, D-mannose, L-rhamnose, D-xylose, adonitol (weak), glycerol, and D-mannitol as sole carbon sources, but not D-galactose, D-melezitose, melibiose, α-methyl-D-glucoside, α-methyl-D-mannoside, D-raffinose, D-ribose, salicin, L-sorbose, sucrose, D-trehalose, 2,3-butanediol, *meso*-erythritol, *meso*-inositol, 1,2-propanediol, D-sorbitol, and D-xylitol. Nitrate is not reduced to nitrite. H_2S is not produced. Gelatin is liquefied. Casein, DNA, elastin, esculin, and hypoxanthine are degraded, but chitin, starch, DL-tyrosine, urea, and xanthine are not. Assimilation of organic acids (acetate, benzoate, citrate, formate, malate, succinate, DL-tartrate) is not observed. Cell wall contains LL-A_2pm as the diagnostic diamino acid and glucose as the major sugar, respectively. The major menaquinone is MK-9(H_4). The phospholipid profile contains diphosphatidylglycerol, phosphatidylglycerol, phosphatidylcholine, phosphatidylinositol, phosphatidylinositol mannoside, and an unknown phospholipid. Mycolic acids are not present. The major cellular fatty acids are $C_{15:0}$ anteiso (20.4%), $C_{16:0}$ iso (18.0%), and $C_{14:0}$ iso (8.9%).

DNA G+C content (mol%): 71.5 (HPLC).

Type strain: D8-87, DSM 45079, JBRI 2008, KCTC 19222.

Sequence accession no. (16S rRNA gene): AM422451.

4. **Jiangella muralis** Kämpfer, Schäfer, Lodders and Martin 2011, 130[VP]

mu.ra′lis. L. fem. adj. *muralis* pertaining or belonging to wall(s).

Forms mycelia-like filaments, about 1.3 µm in width. Substrate mycelium on M79 agar is white to beige, aerial mycelium is white. Gram-positive, oxidase positive (weak reaction), showing an aerobic respiratory metabolism. Good growth occurs after 3 d of incubation on tryptone soy agar, M79 agar, and nutrient agar at 25–30°C. The quinone system of 15-Je-017[T] is composed of the predominant compound menaquinone MK-9(H_4). The polar lipid profile consists of diphosphatidylglycerol, phosphatidylglycerol, phosphatidylcholine, phosphaditylinositol, and phosphatidylinositol mannoside. Major fatty acids are $C_{15:0}$ iso, $C_{15:0}$ anteiso, $C_{16:0}$ iso, $C_{17:0}$ iso, and $C_{17:0}$ anteiso. Straight-chain fatty acids $C_{15:0}$, $C_{16:0}$, $C_{17:0}$, and $C_{18:0}$ are produced in minor amounts. Hydrolyzes esculin, pNP-phenyl-phosphonate, 2-deoxythymidine-5′-pNP phosphate, pNP-β-D-glucuronide, pNP-α-D-glucopyranoside, pNP-β-D-xylopyranoside, bis-pNP phosphate, pNP-phosphoryl-choline, L-alanine-pNA, and L-proline-pNA. Does not hydrolyze ONPG, pNP-β-D-glucopyranoside, and L-glutamate-gamma-3-carboxy-pNA. Utilizes (weakly) as sole source of carbon: *N*-acetyl-D-galactosamine, *N*-acetyl-D-glucosamine, L-arabinose, *p*-arbutin, D-cellobiose, D-fructose, D-galactose, D-glucose, D-mannose, D-maltose, L-rhamnose, D-ribose, sucrose, D-trehalose, D-xylose, adonitol, D-mannitol, acetate, fumarate, DL-3-hydroxybutyrate, DL-lactate, L-malate, 2-oxoglutarate, pyruvate, L-alanine, L-aspartate, L-histidine, L-proline, and L-phenylalanine. Cannot utilize gluconate, α-D-melibiose, D-mannitol, inositol, D-sorbitol, putrescine, propionate, *cis*-aconitate, *trans*-aconitate, adipate, 4-amninobutyrate, azelate, citrate, glutarate, itaconate, mesaconate, suberate, β-alanine, L-leucine, L-ornithine, L-serine, L-tryptophane, 3-hydroxybenzoate, 4-hydroxyenzoate, and phenylacetate.

DNA G+C content (mol%): not determined.

Type strain: 15-Je-017, DSM 45357, CCM 7680.

Sequence accession no. (16S rRNA gene): FN645214, GU574121.

Genus II. **Haloactinopolyspora** Tang, Zhi, Wang, Shi, Lou, Xu and Li 2011, 199^{VP}

Shun-Kun Tang, Xiao-Yang Zhi and Wen-Jun Li

ha.lo.ac.ti.no.po.ly.spo′ra. Gr. n. *hals halos* salt; Gr. n. *actis*, *actinos* a ray; Gr. adj. *poly* many; Gr. n. *spora* a seed and, in biology, a spore; N.L. fem. n. *Haloactinopolyspora* salt-loving and the many spored ray.

Gram-stain-positive, strictly aerobic, moderately halophilic filamentous actinomycete. The substrate mycelium fragments into rod-like elements, and the aerial mycelium has long spore chains and forms root-like spore heaps at maturity (Figure 130). The growth is good on GTY agar and potato agar, moderate on Czapek's agar, inorganic salts-starch agar (ISP 4), and oatmeal agar (ISP 3), weak on nutrient agar and glycerol/asparagine agar (ISP 5), but no growth is observed on yeast extract-malt extract agar (ISP 2). The color of the aerial mycelium is white and that of the substrate mycelium was white-yellow. No soluble pigments are produced.

DNA G+C content (mol%): 70–71.

Type species: **Haloactinopolyspora alba** Tang, Zhi, Wang, Shi, Lou, Xu and Li 2011, 199^{VP}.

Further descriptive information

Phylogenetic analysis of the 16S rRNA gene sequence places the genus within the family *Jiangellaceae* and order *Jiangellales*. The cell-wall hydrolysates contain LL-diaminopimelate, alanine, glycine, and glutamate as the cell-wall amino acids; glucosamine, glucose, galactose, mannose, and arabinose are the major cell-wall sugars. The phospholipids are diphosphatidylglycerol, phosphatidylinositol, phosphatidylinositol mannosides, one unknown phosphoglycolipid, one unknown phospholipid, and one unknown glycolipid. The predominant menaquinone is MK-9(H_4). The major fatty acids are $C_{16:0}$ iso, $C_{15:0}$ anteiso, and $C_{17:0}$ anteiso.

Isolation procedures

The only species was isolated from a soil sample collected from Qijiaojing Lake, which is a salt lake in Xinjiang Province, northwest China (GPS coordinates for the sampling site are 43°26′48″N 91°29′13″E), after 3 weeks incubation at 37°C on cellulose-casein multi-salt (CCMS) medium described by Tang et al. (2008).

Maintenance procedures

The type strain is maintained on GTY agar slants (Tang et al., 2011) containing 15% (w/v) NaCl at 4°C and in 20% (v/v) glycerol suspensions of well grown liquid cultures and stored frozen at –80°C or in liquid nitrogen.

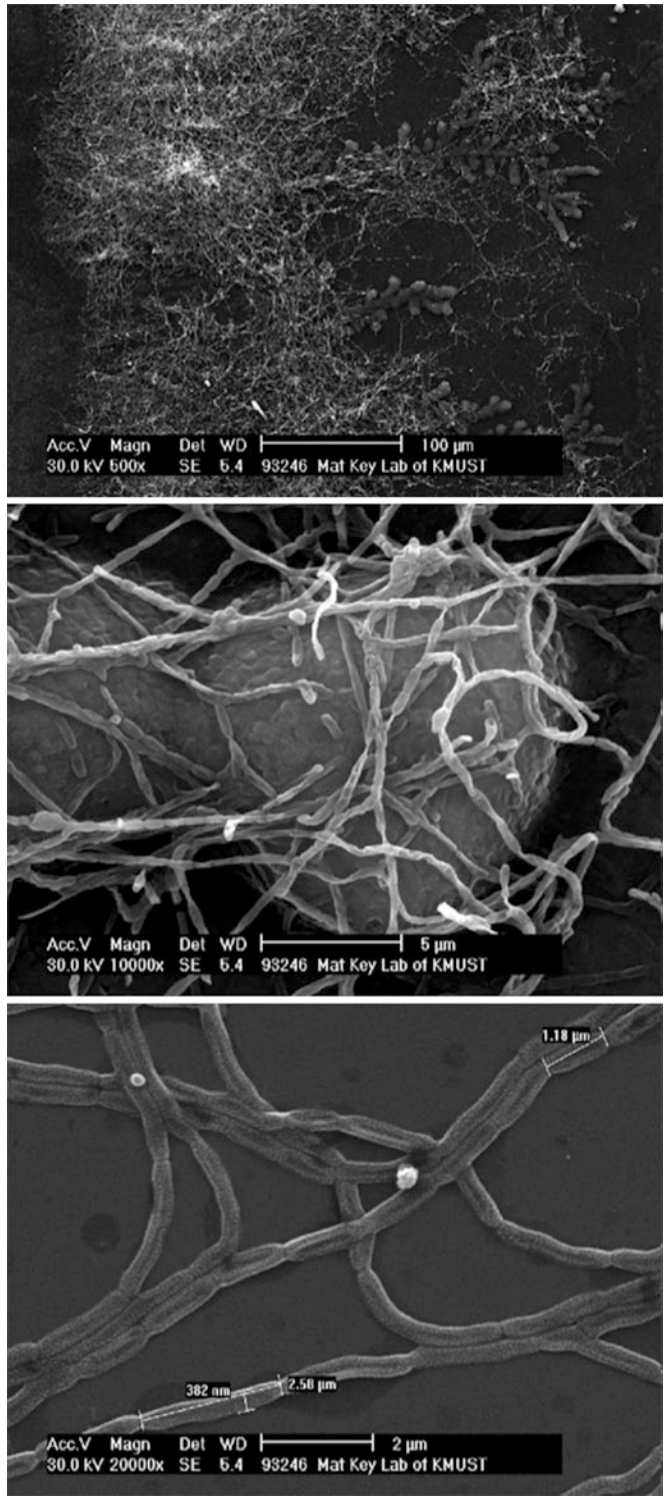

FIGURE 130. Scanning electron micrograph of spore chains of *Haloactinopolyspora alba* YIM 93246^{T} grown on GTY medium including 10% (w/v) NaCl for 28 d at 37°C; (top) aerial mycelia and root-like spore heap; (middle) long spore chains and spore heap; (bottom) fragmented substrate mycelia.

List of species of the genus *Haloactinopolyspora*

1. **Haloactinopolyspora alba** Tang, Zhi, Wang, Shi, Lou, Xu and Li 2011, 199[VP]

al′ba. L. fem. adj. *alba* white.

Displays the following properties in addition to those described for the genus. Growth occurs at 15–45°C, pH 4.0–9.0, and 7–23% (w/v) NaCl. Optimal growth occurs at 28–37°C, pH 7.0–8.0, and 10–15% (w/v) NaCl, and no growth occurs in the absence of NaCl. Esculin, Tweens 40, 60, and 80 are degraded, but casein, starch, dextrin, chitin, Tween 20, and urea are not. Tests for milk peptonization and coagulation are positive, but gelatin liquefaction, nitrate reduction, starch hydrolysis, H_2S, and melanin production are negative. Cellobiose, dulcitol, D-fructose, inositol, lactose, maltose, D-mannose, rhamnose, sucrose, sorbitol, and trehalose, are utilized as sole carbon sources, while erythritol, galactose, D-glucose, glycerol, glycine, mannitol, raffinose, D-ribose, sodium propionate, trisodium citrate, xylitol, and D-xylose are not. Adenine, L-arginine, L-histidine, hypoxanthine, L-lysine, L-methionine, L-proline, L-serine, and L-threonine are utilized as sole nitrogen sources, whereas growth on L-alanine, D-arabinose, L-asparagine, L-phenylalanine, L-tyrosine, and xanthine is not observed. In the API ZYM system, alkaline phosphatase, esterase (C4), esterase lipase (C8), lipase (C14), α- and β-galactosidase, α- and β-glucosidase, α-mannosidase, and *N*-acetyl-β-glucosaminidase are positive; acid phosphatase, α-chymotrypsin, cystine arylamidase, α-fucosidase, β-glucuronidase, leucine arylamidase, naphthol-AS-BI-phosphohydrolase, trypsin, and valine arylamidase are negative.

For all other characteristics, refer to the genus description.

DNA G+C content (mol%): 70.5 (HPLC).

Type strain: YIM 93246, DSM 45211, KCTC 19409.

Sequence accession no. (16S rRNA gene): FJ969846.

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Order IX. **Kineosporiales** ord. nov.

Peter Kämpfer

Ki.ne.o.spo.ri′a.les. N.L. fem. n. *Kineosporia* type genus of the order; suff. *-ales* ending to denote an order; N.L. fem. pl. n. *Kineosporales* the *Kineosporia* order.

The delineation of the order is based on 16S rRNA gene sequence analysis and contains only the family *Kineosporiaceae* (Zhi et al., 2009). The pattern of 16S rRNA signature nucleotides that defines the order is identical to that of the family *Kineosporiaceae*. The pattern of 16S rRNA signatures consists of nucleotides at positions 127:234 (A–U), 142:221 (C–U), 598:640 (U–G), 840:846 (A–C), 845 (A), 986:1218 (A–U), 1163:1173 (G–U), 1164:1172 (G–C), and 1165:1171 (G–A) according to Zhi et al. (2009) proposed for the *Kineosporiineae*.

Type genus: **Kineosporia** Pagani and Parenti 1978, 401[AL].

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Family I. **Kineosporiaceae** Zhi, Li and Stackebrandt 2009, 596[VP]

Philippe Normand and David R. Benson

Ki.ne.o.spo.ri.a.ce′a.e. N.L. fem. n. *Kineosporia* type genus of the family; suff. -*aceae* ending to denote a family; N.L. fem. pl. n. *Kineosporiaceae* the *Kineosporia* family.

The family *Kineosporiaceae* was proposed in the taxonomic outline to the 2nd edition of *Bergey's Manual of Systematic Bacteriology*, based on a heat-map analysis of aligned 16S rRNA sequences (Garrity et al., 2002; Lilburn and Garrity, 2004), to include initially three genera, *Kineosporia*, *Cryptosporangium*, and *Kineococcus*. However, this outline did not comprise a formal description. *Kineosporia* was included in the 1st edition of *Bergey's Manual of Systematic Bacteriology* in section 29 "Streptomycetes and related genera" (Locci, 1989) alongside *Sporichthya*. *Kineococcus* was described later in 1993 (Yokota et al., 1993), whereas *Cryptosporangium* was described in 1998 (Tamura et al., 1998). In the course of this work, it was noticed that the newly created genus *Quadrisphaera* (Maszenan et al., 2005) also belonged to this family; it is thus included here. Conversely, inclusion of the genus *Cryptosporangium* in the family *Kineosporiaceae* is debatable (Figure 102). Most recently, the family *Kineosporiaceae* was proposed to include only the genera *Kineosporia*, *Kineococcus*, and *Quadrisphaera*, and *Cryptosporangium* was transferred it to the suborder *Frankineae*, now the order *Frankiales* (Zhi et al., 2009). While the genus *Cryptosporangium* is now treated in the order Frankiales and family *Cryptosporangiaceae*, it does possess some chemotaxonomic similarities to the *Kineosporiaceae* (Table 67). The family comprises a group of diverse aerobic mesophilic microorganisms, isolated from soils, plant materials, and, in one case, from a radioactive work area, that have complex morphology. It includes the three bacterial genera *Kineosporia*, *Kineococcus*, and *Quadrisphaera*. Comparative phenotypic characteristics of members of the family *Kineosporiaceae* and the genus *Cryptosporangium* are given in Table 67. The DNA G+C content ranges from 68 to 76.6 mol%, making this taxon unusual. The cell wall contains mostly *meso*-DAP, menaquinonones are composed of 9(H_4), 9(H_2), 9(H_6), and 8(H_2), and fatty acids comprise $C_{16:0}$, $C_{18:1}$, and $C_{15:0}$ anteiso. With the removal of *Cryptosporangium*, the group forms a coherent clade upon 16S rRNA gene sequence analysis (Figure 102).

DNA G+C content (mol%): 68–76.6.

Type genus: **Kineosporia** Pagani and Parenti 1978, 401[AL] emend. Itoh, Kudo, Parenti and Seino 1989, 172 emend. Kudo, Matsushima, Itoh, Sasaki and Suzuki 1998, 1253.

TABLE 67. Comparative phenotypic characteristics of members of the family *Kineosporiaceae* and the genus *Cryptosporangium*[a]

Characteristic	*Kineosporia*	*Cryptosporangium*	*Kineococcus*	*Quadrisphaera*
Cell shape	Hyphae	Hyphae	Coccoid cells in clusters	Coccoid cells in tetrads
Motility	Motile spores	Motile spores	Motile	Non-motile
Physiology:				
O_2 requirements	Aerobic	Aerobic	Aerobic	Aerobic
Temperature range (°C)	20–37	10–37	11–41	15–40
Temperature optimum (°C)	28	15–30	32	37
pH range			5.1–10.1	5.0–9.0
Chemistry:				
Cell wall	LL-DAP, *meso*-DAP or both	*meso*-DAP	*meso*-DAP	*meso*-DAP
Menaquinone	9(H_4)	9(H_6), 9(H_8), 9(H_4)	9(H_2)	8(H_2)
Fatty acids	$C_{16:0}$, $C_{18:1}$, $C_{18:0}$ 10-Me	$C_{16:0}$, $C_{18:1}$, $C_{17:1}$	$C_{15:0}$ anteiso, $C_{16:0}$ iso	$C_{15:0}$ anteiso, $C_{16:0}$ iso
DNA G+C content (mol%)	68.3–70.8	70–76	73.3–76.6	75
Source	Plant samples, soil	Soil	Radioactive work area	Aerobic sludge

[a]Data from Maszenan et al. (2005), Kudo et al. (1998), Lee (2006), and Phillips et al. (2002).

Genus I. **Kineosporia** Pagani and Parenti 1978, 401[AL] emend. Itoh, Kudo, Parenti and Seino 1989, 172 emend. Kudo, Matsushima, Itoh, Sasaki and Suzuki 1998, 1253

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Ki.ne.o.spo′ri.a. Gr. n. *kinesis* motion; Gr. fem. n. *spora* a seed; N.L. fem. n. *spora* a spore; N.L. fem. n. *Kineosporia* an organism that has motile spores.

Aerobic, Gram-stain-positive, and non-acid-fast. Colonies on agar medium lack aerial mycelia, form central projections with radiating vegetative hyphae (Figure 131), and are occasionally accompanied by bunches of spore clusters in the agar. Mature colonies have a gelatinous matrix that confers a glossy appearance. Spores, which are spherical to ovoid or pyriform with a long axis of 1±2 µm, are **catenated** around the central projection or are located singly or in aggregates at the tips of hyphae. The **spores are motile** with polar tufts of flagella (Figure 132). The peptidoglycan contains either almost equivalent amounts of LL- and *meso*-DAP or mainly LL- or *meso*-DAP. The spores contain predominantly *meso*-DAP accompanied by glycine. The whole cultured organism contains galactose, glucose, mannose, and ribose. In addition to these sugars, rhamnose and 3-*O*-methylrhamnose may be present. Phosphatidylcholine is a diagnostic phospholipid and MK-9(II,III-H_4) is present as a major component. The cellular fatty acid profile is composed mainly of n-hexadecanoic acid and n-octadecenoic acid, and lacks iso- and anteiso-branched fatty acids. 10-Methyloctadecanoic acid and 2-hydroxyhexadecanoic acids are also present in some strains. Mycolic acids are absent.

DNA G+C content (mol%): 69–71.

Type species: **Kineosporia aurantiaca** Pagani and Parenti 1978, 401[AL].

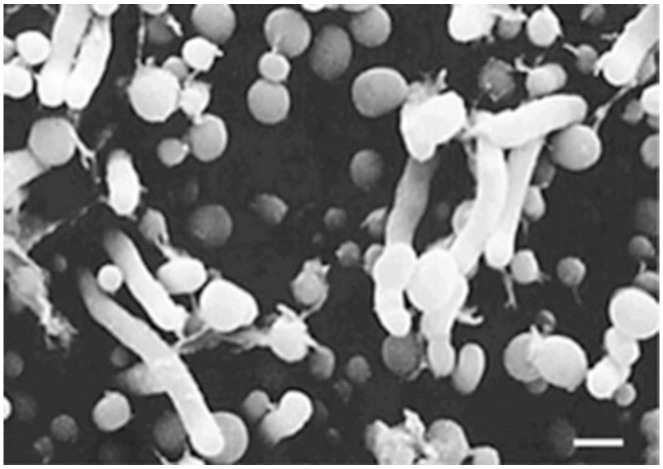

FIGURE 131. Cluster of cells of *Kineosporia* sp. strain MA-16. [From the Digital Atlas of Actinomycetes; photo by Y. Kawamura and K. Matsushima, Japan Collection of Microorganisms, Institute of Physical and Chemical Research (RIKEN), Saitama, Japan, www.nih.go.jp/saj/DigitalAtlas/; copyright owned by The Society for Actinomycetes, Japan.]

Differentiation of the species of the genus *Kineosporia*

Differential characteristics of the five recognized species are given in Table 68.

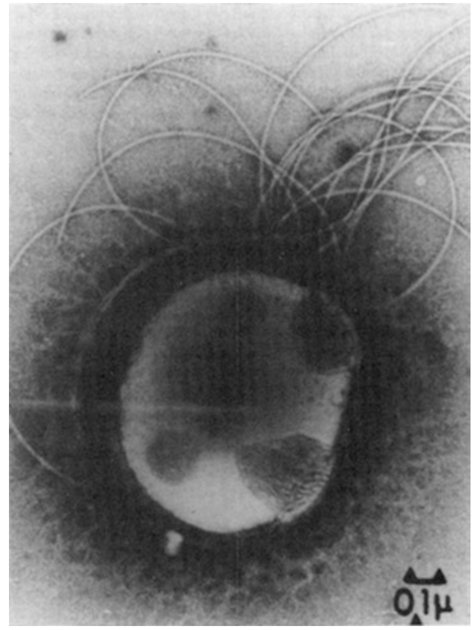

FIGURE 132. Electron micrograph of a motile spore of *Kineosporia* (Parenti, 1989). (Reproduced from the 1st edition of *Bergey's Manual of Systematic Bacteriology*. Originally published in S.D. Bernard and F. Parenti, 1983. Curr. Microbiol. *81*: 173–176.)

TABLE 68. Differential characteristics of the five *Kineosporia* species[a]

Characteristic	*K. aurantiaca*	*K. mikuniensis*	*K. rhamnosa*	*K. rhizophila*	*K. succinea*
Decomposition of:					
Esculin	+	−	−	−	−
Hypoxanthine	−	−	−	−	+
Testosterone	+	+/−	+/−	+	+
Utilization of:					
L-Arabinose	+	+	+/−	+	+
D-Arabitol	+	+	−	+	+
Dulcitol	−	+/−	+/−	−	+
myo-Inositol	+	+	+	−	+/−
D-Lactose	+	+	−	+	+
Melezitose	+	+	−	+	+
Melibiose	+	+	−	+	+
Methyl α-D-glucoside	−	−	+	−	−
Raffinose	−	−	+/−	+	−
D-Ribose	+	+	−	+	+
Salicin	−	−	+	−	−
D-Sorbitol	−	−	+	−	+
Sucrose	+	+/−	+/−	+	+
Trehalose	+	+	−	+	+
Citrate	+	−	−	−	−
Acid production from:					
myo-Inositol	−	+	+	−	−
Melezitose	+	−	−	+	−
D-Sorbitol	−	−	+	−	−
NaCl tolerance, % (w/v)	<3	<1	<1	<5	<5
Dominant DAP isomer	LL/*meso*	*meso*	*meso*	*meso*	LL
3-*O*-Methyl-rhamnose and rhamnose in whole-cell sugars	−	−	+	−	−

[a]Data from Kudo et al. (1998).

List of species of the genus *Kineosporia*

1. **Kineosporia aurantiaca** Pagani and Parenti 1978, 401[AL]

au.ran.ti.a′ca. N.L. fem. adj. *aurantiaca* of an orange color.

This species was the first species of the genus described and remained the only one for several years.

Colonies are pleomorphic on all media, particularly on yeast extract-malt extract agar. The wet, glossy appearance, evident after 7 d, is due to abundant formation of sporangia. Growth occurs by the spreading of the vegetative mycelium into the agar and by thickening of the layer formed by the hyphae. Small colonies of 0.5–2 mm in diameter are formed and are colorless for the first few days of growth, turning cream to orange thereafter. Colonial morphology is variable even on the same agar plate, ranging from conical-crateriform to cerebriform. Vegetative mycelium consists of small (1 µm in diameter), slightly branched hyphae. Aerial mycelium has not been observed on any medium tested. Sporangia appear as elongated, club-shaped vesicles, 1–2 µm in diameter, borne at the terminal end of the vegetative hyphae. Each sporangium contains a single spore. The spores are pleomorphic, their shape ranging from nearly spherical to oval or pyriform. The long axes of the spores vary between 1 and 2 µm. Upon sporangium dehiscence, spores are motile for several hours, with a polar tuft of flagella. Gram-stain-positive and non-acid-fast. The peptidoglycan of the cell wall contains varying amounts of LL-DAP, a very small amount of *meso*-DAP, and glycine. Traces of lysine are also found. Aerobic. Chemoorganotrophic, using a range of simple sugars. Optimum temperature for growth and sporulation is 20–30°C, with no growth at or above 37°C. Optimum pH 7.0. Nonpathogenic to mice.

Source: occurs free-living in soil.

DNA G+C content (mol%): 68–71 (reverse HPLC; Tamaoka and Komagata, 1984).

Type strain: ATCC 29727, DSM 43858, NBRC 14067, JCM 3230, NRRL B-16913, VKM Ac-702.

Sequence accession no. (16S rRNA gene): AB003931.

2. **Kineosporia mikuniensis** Kudo, Matsushima, Itoh, Sasaki and Suzuki 1998, 1253[VP]

mi.ku.ni.en′sis. N.L. fem. adj. *mikuniensis* of or belonging to Mt Mikuni, Gumma Prefecture, Japan, the location from which the type strain was isolated.

Substrate mycelia are orange to light brown (especially on yeast extract-starch agar). Produces faint yellowish diffusible pigments in oatmeal agar. Decomposes casein and urea, but not esculin, hypoxanthine, tyrosine, or xanthine. Utilizes L-arabinose, D-arabitol, cellobiose, D-fructose, D-galactose, D-glucose, *myo*-inositol, D-lactose, maltose, D-mannitol, melezitose, melibiose, methyl α-D-glucoside, L-rhamnose, D-ribose, salicin, starch, trehalose, and D-xylose as sole carbon sources, but not adonitol, i-erythritol, raffinose, D-sorbitol, L-sorbose, or xylitol. Utilizes fumaric acid, L-malic acid, and succinic acid, but not benzoic acid, citric acid, mucic acid, oxalic acid, or L-tartaric acid. Produces acid from L-arabinose, cellobiose, D-fructose, D-galactose, D-glucose, *myo*-inositol, L-rhamnose, sucrose, and D-xylose, but not adonitol, i-erythritol, melezitose, or D-sorbitol. Does not grow in the presence of 1% (w/v) NaCl. Mesophilic. Contains a major amount of *meso*-DAP in the cell wall. Contains neither rhamnose nor 3-*O*-methylrhamnose in the whole-cell hydrolyzate. Monotypic.

Source: sphagnum, Mt Mikuni, Gumma Prefecture, Japan.

DNA G+C content (mol%): 69 (reverse HPLC; Tamaoka and Komagata, 1984).

Type strain: I-463, JCM 9961.

Sequence accession no. (16S rRNA gene): not available.

3. **Kineosporia rhamnosa** Kudo, Matsushima, Itoh, Sasaki and Suzuki 1998, 1254[VP]

rham.no′sa. N.L. fem. adj. *rhamnosa* (from the Greek *rhamnos* a branch) pertaining to rhamnose.

Substrate mycelia are orange to light brown (especially on yeast extract-starch agar). Does not produce any diffusible pigments in yeast extract-starch or oatmeal agar media. Decomposes casein, DNA, and urea, but not esculin, hypoxanthine, tyrosine, or xanthine. Utilizes cellobiose, D-fructose, D-galactose, D-glucose, *myo*-inositol, maltose, D-mannitol, L-rhamnose, D-sorbitol, starch, and D-xylose as sole carbon sources, but not adonitol, D-arabitol, i-erythritol, D-lactose, melezitose, melibiose, methyl α-D-glucoside, D-ribose, salicin, L-sorbose, trehalose, or xylitol. Utilizes fumaric acid, L-malic acid, and succinic acid, but not benzoic acid, citric acid, mucic acid, oxalic acid, or L-tartaric acid. Produces acid from L-arabinose, cellobiose, D-fructose, D-galactose, D-glucose, *myo*-inositol, L-rhamnose, D-sorbitol, sucrose, and D-xylose, but not from adonitol, i-erythritol, or melezitose. Does not grow in the presence of 2% (w/v) NaCl. Mesophilic. Contains a major amount of *meso*-DAP in the cell wall. Contains rhamnose and 3-*O*-methyl-rhamnose in the whole-cell hydrolyzate.

Source: leaves of cat-tail (*Typha latifolia*), pond in Saitama, Japan.

DNA G+C content (mol%): 68–69 (reverse HPLC; Tamaoka and Komagata, 1984).

Type strain: I-132, JCM 9954.

Sequence accession no. (16S rRNA gene): AB003935.

4. **Kineosporia rhizophila** Kudo, Matsushima, Itoh, Sasaki and Suzuki 1998, 1253[VP]

rhi.zo′phi.la. Gr. n. *rhiza* root; N.L. fem. adj. *phila* (from Gr. fem. adj. *philê*) friend, loving; N.L. fem. adj. *rhizophila* root-loving.

Substrate mycelia are orange to light brown (especially on yeast extract-starch agar). Produces faint red diffusible pigments on yeast extract-starch agar. Decomposes casein, DNA, testosterone, and urea, but not adenine, esculin, hypoxanthine, tyrosine, or xanthine. Utilizes L-arabinose, D-arabitol, cellobiose, D-fructose, D-galactose, D-glucose, D-lactose, maltose, D-mannitol, melezitose, melibiose, raffinose, L-rhamnose, D-ribose, salicin, starch, sucrose, trehalose, and D-xylose as sole carbon sources, but not adonitol, dulcitol, i-erythritol, *myo*-inositol, methyl α-D-glucoside, D-sorbitol, L-sorbose, or xylitol. Utilizes fumaric acid, L-malic acid, and succinic acid, but not benzoic acid, citric acid, mucic acid, oxalic acid, or L-tartaric acid. Produces acids from L-arabinose, cellobiose, D-fructose, D-galactose, D-glucose, melezitose, L-rhamnose, sucrose, and D-xylose, but not from adonitol, i-erythritol, *myo*-inositol, or D-sorbitol. Tolerates up to 4% (w/v) NaCl. Mesophilic. Contains a major amount of

meso-DAP in the cell wall. Contains neither rhamnose nor 3-*O*-methyl-rhamnose in the whole-cell hydrolyzate.

Source: root of galingale (*Cyperus microiria*), Saitama, Japan.

DNA G+C content (mol%): 70 (reverse HPLC; Tamaoka and Komagata, 1984).

Type strain: I-449, JCM 9960.

Sequence accession no. (16S rRNA gene): AB003933.

5. **Kineosporia succinea** Kudo, Matsushima, Itoh, Sasaki and Suzuki 1998, 1253[VP]

suc.ci′ne.a. L. fem. adj. *succinea* of amber, intended to mean of an amber color.

Substrate mycelia are orange to light brown (especially on yeast extract-starch agar). Produces faint yellow diffusible pigments on oatmeal agar. Decomposes casein, DNA, hypoxanthine, testosterone, and urea, but not adenine, esculin, tyrosine, or xanthine. Utilizes L-arabinose, D-arabitol, cellobiose, dulcitol, D-fructose, D-galactose, D-glucose, D-lactose, maltose, D-mannitol, melezitose, melibiose, L-rhamnose, D-ribose, salicin, starch, sucrose, trehalose, and D-xylose as sole carbon sources, but not adonitol, i-erythritol, methyl α-D-glucoside, raffinose, L-sorbose, or xylitol. Utilizes fumaric acid, L-malic acid, and succinic acid, but not benzoic acid, citric acid, mucic acid, oxalic acid, or L-tartaric acid. Produces acid from L-arabinose, cellobiose, D-fructose, D-galactose, D-glucose, L-rhamnose, sucrose, and D-xylose, but not from adonitol, i-erythritol, *myo*-inositol, melezitose, or D-sorbitol. Tolerates up to 4% (w/v) NaCl. Mesophilic. Contains almost the same amount of LL- and *meso*-DAP or a major amount of LL-DAP in the cell wall. Whole-cell hydrolyzate does not contain rhamnose or 3-*O*-methyl-rhamnose.

Source: fallen leaves, Saitama, Japan.

DNA G+C content (mol%): 70–71 (reverse HPLC; Tamaoka and Komagata, 1984).

Type strain: I-273, JCM 9957.

Sequence accession no. (16S rRNA gene): AB003932.

Genus II. **Kineococcus** Yokota, Tamura, Nishii and Hasegawa 1993, 56[VP]

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Ki.ne.o.coc′cus. Gr. n. *kinesis* motion; N.L. masc. n. *coccus* from Gr. masc. n. *kokkos* a grain, seed; N.L. masc. n. *Kineococcus* a motile coccus.

Cells are spherical, 1.0–1.5 µm in diameter, and occur in pairs, **tetrads, or clusters**. Motile. The **motile cells have tufts of flagella**. Endospores are not formed. Gram-stain-positive. Colonies are circular and rough and may be cream colored to orange. Strictly aerobic. Catalase- and urease-positive. Oxidase-negative. Does not reduce nitrate to nitrite. Acid is produced from glucose and some other sugars. Esculin is hydrolyzed. Starch, gelatin, and casein are not hydrolyzed. The optimum growth temperature is 27°C. The cell wall peptidoglycan contains *meso*-DAP, alanine, and glutamic acid. The major menaquinone is MK-9(H_2). Mycolic acid is not present. The major cellular fatty acid is $C_{15:0}$ anteiso. Diphosphatidylglycerol, phosphatidylglycerol, and unidentified glycolipids are present as polar lipids.

The phylogeny of the genus *Kineococcus* based on 16S rRNA gene sequencing is dubious (Figure 102) given that the topology varies with the algorithm used; in particular, *Kineococcus marinus* is either positioned at the root of the branch leading to the species *Kineococcus radiotolerans* and *Kineococcus aurantiacus*, or else on a distinct branch together with *Quadrisphaera granulorum*. This uncertainty will likely be resolved by further studies based on several genes, which will become possible with the availability of complete genomes and with consideration of more physiological features.

DNA G+C content (mol%): 73.9–74.2.

Type species: **Kineococcus aurantiacus** Yokota, Tamura, Nishii and Hasegawa 1993, 56[VP].

Differentiation of the species of the genus *Kineococcus*

Discriminating features of the three species of the genus *Kineococcus* are listed in Table 69.

TABLE 69. Distinguishing features of the three species of the genus *Kineococcus*[a,b]

Characteristic	*K. aurantiacus*	*K. marinus*	*K. radiotolerans*
Utilization of:			
D-Galactose	−	+	+
D-Lactose	nd	+	−
Maltose	−	+	−
D-Mannose	−	+	+
Raffinose	−	+	−
L-Rhamnose	−	+	−
D-Ribose	−	+	−
Trehalose	−	+	nd
Glycerol	−	+	+
Mannitol	−	+	+
Inositol	−	+	+
Urease activity	+	−	−
Hydrolysis of:			
Esculin	−	+	nd
Gelatin	−	+	nd
Starch	−	+	nd
Temperature range for growth (°C)	9–36	4–37	11–41
pH range for growth	6.0–9.0	5.1–10.1	5.0–9.0
Growth in 7% NaCl	−	+	nd

[a]Data from Lee (2006).

[b]+, Positive; −, negative; nd, not determined

List of species of the genus *Kineococcus*

1. **Kineococcus aurantiacus** Yokota, Tamura, Nishii and Hasegawa 1993, 56[VP]

au.ran.ti.a′cus. N.L. n. *aurantium* generic name of the orange; N.L. masc. adj. *aurantiacus* orange-colored.

Morphology is as described for genus (Yokota et al., 1993). Cells are spherical and 1.0–1.5 µm in diameter and occur in pairs, tetrads, or clusters (Figure 133). Motile. The motile cells have tufts of flagella. Endospores are not formed. Gram-stain-positive. Colonies are circular, rough, and may be cream colored to orange. Strictly aerobic. Catalase- and urease-positive. Oxidase-negative. Does not reduce nitrate to nitrite. Acid is produced from D-glucose, D-xylose, D-fructose, sucrose, and L-arabinose. Acid is not produced from glycerol, D-ribose, D-arabinose, raffinose, maltose, D-galactose, D-mannose, inositol, L-rhamnose, mannitol, or trehalose. Does not hydrolyze esculin, starch, gelatin, or casein. The optimum growth temperature is 28°C. Chemical characteristics, such as amino acid composition of the cell wall peptidoglycan, menaquinone system, mycolic acids, cellular fatty acids, and polar lipids, are the same as given above for the genus.

Source: isolated from soil.

DNA G+C content (mol%): 73.9 (HPLC; Mesbah and Whitman, 1989).

Type strain: RA 333, ATCC 51238, CIP 105426, DSM 7487, NBRC 15268.

Sequence accession no. (16S rRNA gene): AB007420.

2. **Kineococcus marinus** Lee 2006, 1282[VP]

ma.ri′nus. L. masc. adj. *marinus* of the sea, the origin of the sample from which the type strain was isolated.

Aerobic, motile, non-spore-forming, oxidase-negative, catalase-positive, Gram-stain-positive cocci. Cells occur singly, in pairs, or in clusters (Figure 134). Forms capsular polysaccharide-like substances on most solid media. Colonies are circular, smooth, convex, and deep orange in color. Glucose fermentation, H_2S production, and nitrate

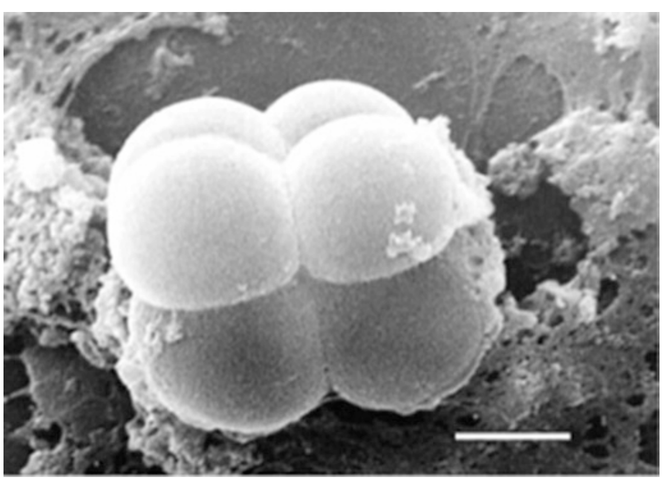

FIGURE 133. Cluster of cells of *Kineococcus aurantiacus* NBRC 15268^T. (From the Digital Atlas of Actinomycetes; photo by T. Tamura, T. Hasegawa and A. Yokota, www.nih.go.jp/saj/DigitalAtlas/; copyright owned by The Society for Actinomycetes, Japan.)

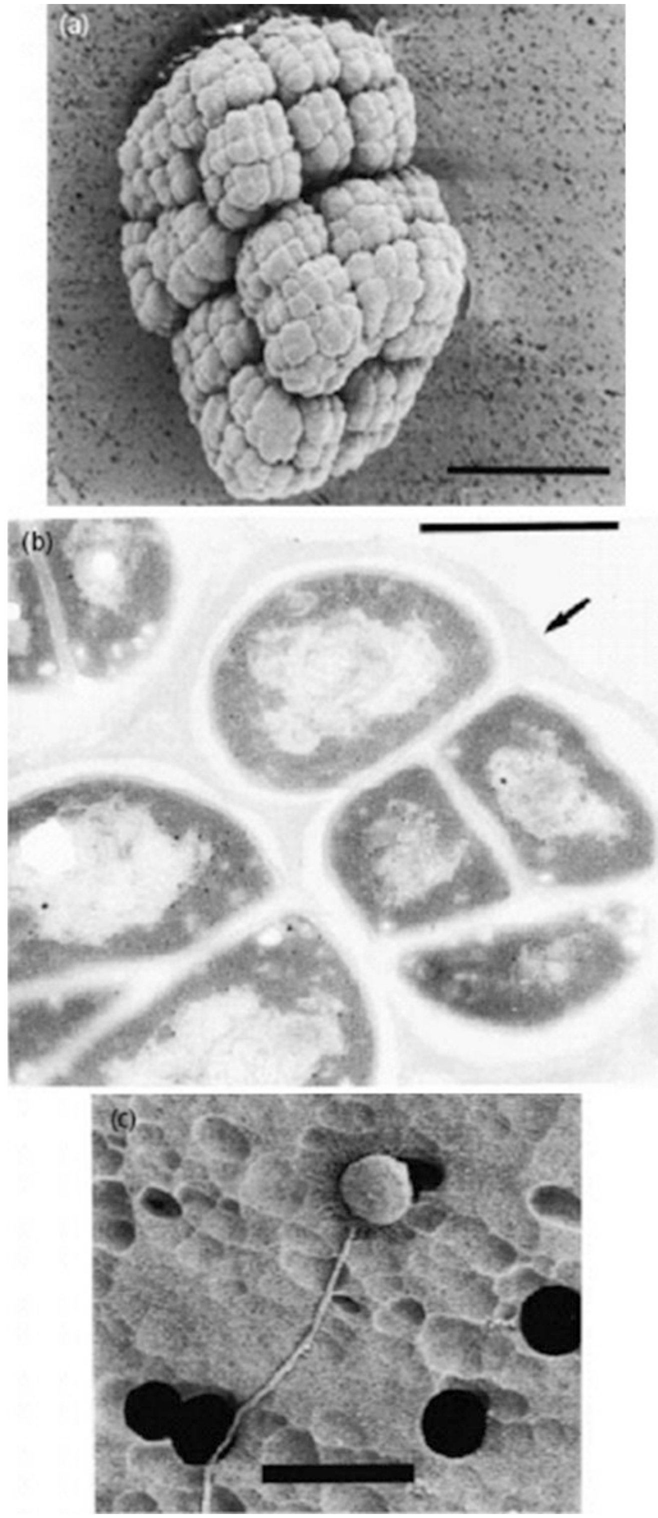

FIGURE 134. (a) Scanning electron micrograph of *Kineococcus radiotolerans* SRS30216^T cultured on PTYG agar (pH 7.0) showing clusters formed by growing cells; bar = 1 µm. (b) Transmission electron micrograph of a thin section of cells of strain SRS30216^T; the *arrow* shows the presence of extracellular matrix; bar = 1 µm. (c) Scanning electron micrograph of a motile cell of strain SRS30216^T exhibiting a single flagellum; bar = 2 µm. (Reprinted with permission from Phillips et al., 2002. Int. J. Syst. Evol. Microbiol. *52*: 933–938.)

reduction are not observed. The temperature range for growth is 4–37°C with optimum growth at 30°C. No growth is observed at or above 40°C. Growth occurs at pH 5.1–10.1, with optimum growth at pH 7.1. β-Galactosidase is present, but urease activity is not detected. DNA, esculin, gelatin, and starch are hydrolyzed, but casein and elastin are not. Salt is required for growth; good growth occurs in 1–4% NaCl, moderate growth in 5–8% NaCl, and poor growth in 9% NaCl. α-Cyclodextrin, β-cyclodextrin, dextrin, glycogen, L-arabinose, D-arabitol, arbutin, cellobiose, D-fructose, L-fucose, D-galactose, D-galacturonic acid, gentiobiose, D-gluconic acid, α-D-glucose, *myo*-inositol, α-D-lactose, lactulose, maltose, D-mannitol, D-mannose, melezitose, methyl β-D-galactoside, 3-methyl D-glucoside, methyl α-D-glucoside, methyl α-D-mannoside, palatinose, D-psicose, raffinose, L-rhamnose, D-ribose, salicin, sedoheptulosan, stachyose, sucrose, D-tagatose, trehalose, turanose, xylitol, D-xylose, acetic acid, α- and β-hydroxybutyric acids, *p*-hydroxyphenylacetic acid, α-ketoglutaric acid, α-ketovaleric acid, lactamide, L-lactic acid, D- and L-malic acid, monomethyl succinate, propionic acid, succinamic acid, L-alaninamide, D-and L-alanine, L-asparagine, glycyl L-glutamic acid, L-serine, glycerol, adenosine, 2′-deoxyadenosine, inosine, AMP, TMP, UMP, D-fructose 6-phosphate, α-D-glucose 1-phosphate, and DL-α-glycerol phosphate are utilized as sole carbon and energy sources for growth. The following substrates are not utilized: inulin, mannan, Tweens 40 and 80, *N*-acetyl-D-glucosamine, *N*-acetyl-β-D-mannosamine, amygdalin, maltotriose, melibiose, methyl α-D-galactoside, methyl β-D-glucoside, D-sorbitol, γ-hydroxybutyric acid, D-lactic acid methyl ester, methyl pyruvate, succinic acid, *N*-acetyl-L-glutamic acid, L-alanyl glycine, L-glutamic acid, L-pyroglutamic acid, putrescine, 2,3-butanediol, thymidine, uridine, and D-glucose 6-phosphate. The diagnostic diamino acid of the peptidoglycan is *meso*-DAP. Whole-cell hydrolyzates contain arabinose and galactose as characteristic sugars. The glycan moiety of the murein structure is acetylated. Mycolic acids are not detected. The predominant menaquinone is MK-9(H_2). Polar lipids contain phosphatidylglycerol and phosphatidylinositol, thus constituting a phospholipid-type PI pattern *sensu* Lechevalier et al. (1981). The major cellular fatty acid is 12-methyltetradecanoic acid ($C_{15:0}$ anteiso).

Source: the type strain was isolated from a sediment sample taken off the coast of Jeju, Republic of Korea.

DNA G+C content (mol%): 76.6 (HPLC).

Type strain: KST3-3, KCCM 42250, NRRL B-24439.

Sequence accession no. (16S rRNA gene): DQ200982.

3. **Kineococcus radiotolerans** Phillips, Wiegel, Berry, Fliermans, Peacock, White and Shimkets 2002, 937[VP]

ra.di.o.to.le′rans. L. n. *radiatio* radiation; N.L. pref. *radio*- pertaining to radiation; L. part. adj. *tolerans* tolerating; N.L. part. adj. *radiotolerans* radiation-tolerating.

Morphology is as described for genus. Cells are cocci, 1.0–1.5 µm in diameter. Cells occur in pairs, tetrads, and large clusters. Colonies are circular, rough, and orange-pigmented. Gram-stain-positive. Cells are motile and produce polar flagella. Catalase-positive. Urease and oxidase tests are negative. A variety of carbon sources is used including glucose, galactose, L-arabinose, sucrose, mannose, xylose, glycerol, mannitol, inositol, and sorbitol, but not raffinose, rhamnose, lactose, citrate, ribose, or maltose. The major fatty acid produced is $C_{15:0}$ anteiso (approx. 90%). Growth occurs at temperatures between 11 and 41°C, between pH 5 and 9, and at NaCl concentrations up to and including 5%. Desiccation-tolerant (100% cell survival after 14 d). Radiation-resistant (>10% cell survival at 3 kGy). An orange pigment, soluble in methanol, with an absorption spectrum with peaks at 444, 471, and 501 nm, is produced.

Source: the type and only strain was isolated from the Savannah River Site in Aiken, South Carolina, USA.

DNA G+C content (mol%): 74.2 (complete genome sequence analysis; http://genome.ornl.gov/microbial/krad/).

Type strain: SRS30216, ATCC BAA-149, DSM 14245.

Sequence accession no.: NZ_AAEF02000006 (complete genome of strain SRS30216[T]).

Genus III. **Quadrisphaera** Maszenan, Tay, Schumann, Jiang and Tay 2005, 1774[VP]

Philippe Normand and David R. Benson

Qua.dri.spha′e.ra. L. pref. numer. adj. *quadr*- four; L. fem. n. *sphaera* a ball, a globe, a sphere; N.L. fem. n. *Quadrisphaera* fourfold balls, coccus in tetrad.

Gram-positive, non-spore-forming cocci, 1.2–3.0 µm in diameter, **occurring in tetrad** arrangements (Figure 135), fitting the morphological description of tetrad-forming organisms described previously in sludge-inhabiting microbes (Cech and Hartman, 1990). MK-8(H_2) is the predominant menaquinone. The phylogenetic position based on 16S rRNA gene sequence analysis (Figure 102) should be in the family *Kineosporiaceae*.

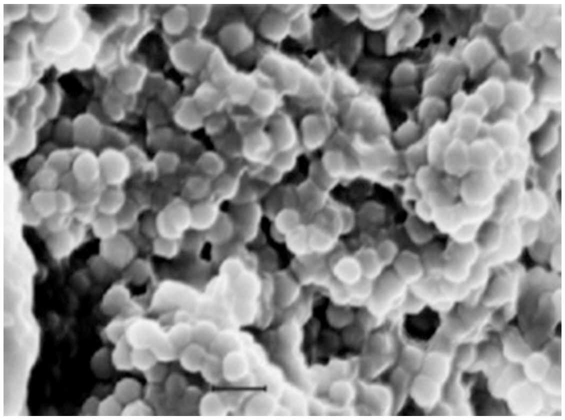

FIGURE 135. Scanning electron micrograph of *Quadrisphaera granulorum* strain AG019[T] showing cocci in tetrad and cluster arrangements. Bar = 3 µm. (Reprinted with permission from Maszenan et al., 2005. Int. J. Syst. Evol. Microbiol. *55*: 1771–1777.)

DNA G+C content (mol%): 75.

Type species: **Quadrisphaera granulorum** Maszenan, Tay, Schumann, Jiang and Tay 2005, 1774[VP].

Further descriptive information

The bacterial genus *Quadrisphaera* (Maszenan et al., 2005) was created to accommodate an isolate obtained from granules suspended in a batch-fed activated sludge reactor. This isolate was given species and genus rank and deemed to be most closely related to members of the family *Frankiaceae*, and later on positioned in the family *Nakamurellaceae* by the NCBI Taxonomy Browser (http://www.ncbi.nlm.nih.gov/Taxonomy/tax.html/) as well as in the TOBA (http://www.taxonomicoutline.org/). The present study however, based on an analysis of 16S rRNA gene sequences, positioned it close to the family *Kineosporiaceae* with a bootstrap value of 99% (Figure 102).

List of species of the genus *Quadrisphaera*

1. **Quadrisphaera granulorum** Maszenan, Tay, Schumann, Jiang and Tay 2005, 1774[VP]

gra.nu.lo′rum. L. gen. pl. n. *granulorum* from or of granules.

In addition to characteristics given in the genus description, also utilizes α-cyclodextrin, α-DL-glycerol phosphate, Tween 40, arbutin, glucose 1-phosphate, Tween 80, glucose 6-phosphate, adonitol, L-arabinose, D-arabitol, glucuronamide, cellobiose, D-psicose, D-mannitol, melezitose, melibiose, L-serine, methyl β-D-glucoside, psicose, D-xylose, methyl pyruvate, pyruvate, 2-aminoethanol, monomethyl succinate, glycerol, L-serine, turanose, and glycerol. It cannot metabolize the following: β-cyclodextrin, dextrin, glycogen, inulin, mannan, *N*-acetyl-D-galactosamine, *N*-acetylglucosamine, *N*-acetylmannosamine, amygdalin, D-arabitol, cellobiose, i-erythritol, D-fructose, L-fucose, D-galactose, D-galacturonic acid, gentiobiose, D-glucuronic acid, α-D-glucose, *myo*-inositol, α-D-lactulose, α-lactose, maltose, D-mannitol, D-mannose, melezitose, methyl α-D-galactoside, methyl β-D-galactoside, 3-methyl glucose, methyl α-D-glucoside, methyl β-D-glucoside, methyl α-D-mannoside, palatinose, raffinose, L-rhamnose, salicin, sedoheptulosan, D-sorbitol, stachyose, sucrose, trehalose, xylitol, acetic acid, α-hydroxybutyric acid, β-hydroxybutyric acid, γ-hydroxybutyric acid, *p*-hydroxyphenylacetic acid, α-ketoglutaric acid, *cis*-aconitic acid, citric acid, formic acid, D-galactonic acid lactone, itaconic acid, malonic acid, quinic acid, D-saccharic acid, sebacic acid, lactamide, D-lactic acid methyl ester, D-malic acid, L-malic acid, propionic acid, succinamic acid, succinic acid, *N*-acetylglutamic acid, bromosuccinic acid, alaninamide, D-alanine, L-alanine, L-alanyl-glycine, L-asparagine, glycyl-L-glutamic acid, L-pyroglutamic acid, putrescine, 2,3-butanediol, glycyl-L-aspartic acid, L-histidine, hydroxyl-L-proline, L-leucine, L-ornithine, L-phenylalanine, L-proline, L-pyroglutamic acid, D-serine, L-threonine, DL-carnitine, γ-aminobutyric acid, adenosine, 2′-deoxyadenosine, inosine, thymidine, uridine, AMP, TMP, UMP, phenylethylamine, or putrescine. The following acid and acid derivatives are utilized: glucuronic acid, α-ketobutyric acid, α-ketovaleric acid, DL-lactic acid, L-aspartic acid, L-glutamic acid, urocanic acid, and pyruvic acid. The following enzyme activities are detected: esterase, esterase lipase, leucine arylamidase, valine arylamidase, naphthol-AS-BI-phosphohydrolase, β-galactosidase, α-glucosidase, and β-glucosidase. The following enzyme activities are not detected: alkaline phosphatase, lipase, cystine arylamidase, trypsin, chymotrypsin, acid phosphatase, α-galactosidase, β-glucuronidase, *N*-acetyl-β-glucosaminidase, α-mannosidase, α-fucosidase, arginine dihydrolase, lysine decarboxylase, ornithine decarboxylase, urease, tryptophan deaminase, and gelatinase. Does not produce H_2S or indole. Voges–Proskauer-negative. Does not produce acetoin. Does not reduce nitrate to nitrite. Catalase-positive, but oxidase-negative (Maszenan et al., 2005).

Source: aerobic granules such as those used in activated sludge reactors.

DNA G+C content (mol%): 75 (reverse HPLC; Schumann et al., 1997).

Type strain: AG019, ATCC BAA-1104, DSM 44889.

Sequence accession no. (16S rRNA gene): AY831385.

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Order X. **Micrococcales** Prévot 1940, 223[AL]

Hans-Jürgen Busse

Mi.cro.coc.ca′les. N.L. fem. n. *Micrococcus* type genus of the order; suff. -*ales* ending to denote an order; N.L. fem. pl. n. *Micrococcales* the *Micrococcus* order.

Representatives of the order are characterized by a pattern of 16S rRNA signatures consisting of nucleotides at positions 127:234 (A–U), 598:642 (U–A), 657:749 (U–A), 953:1228 (G–C), and 1362 (A) as proposed for the suborder *Micrococcineae* (Stackebrandt et al., 1997; Zhi et al., 2009). Quinone systems are composed of menaquinones with 6–14 isoprenoic units in the side chain and one or two subunits may be saturated.

Type genus: **Micrococcus** Cohn 1872, 151[AL] emend. Stackebrandt, Koch, Gvozdiak and Schumann 1995, 691 emend. Wieser, Denner, Kämpfer, Schumann, Tindall, Steiner, Vybiral, Lubitz, Maszenan, Patel, Seviour, Radax and Busse 2002, 635.

Further descriptive information

The order is formed by elevation of the suborder *Micrococcineae* of the class *Actinobacteria* as described by Stackebrandt et al. (1995) in the comprehensive study on Gram-stain-positive bacteria with a high DNA G+C content. The class *Actinobacteria* was proposed on the basis of phylogenetic relatedness and presence of signature nucleotides in the 16S rRNA gene sequences. The order consists of the families *Micrococcaceae*, *Brevibacteriaceae*, *Cellulomonadaceae*, *Dermabacteraceae*, *Dermatophilaceae*, *Intrasporangiaceae*, *Jonesiaceae*, *Microbacteriaceae*, *Promicromonosporaceae* (Stackebrandt et al., 1997), *Bogoriellaceae*, *Dermacoccaceae*, *Rarobacteraceae*, *Sanguibacteraceae* (Stackebrandt and Schumann, 2000), and *Beutenbergiaceae* (Hamada et al., 2009; Zhi et al., 2009). Also the family *Yaniellaceae* (Li et al., 2008), the replacement for the illegitimate name *Yaniaceae* (Li et al., 2005) was considered to be a member of this taxon. However, it was recently shown that the 16S rRNA signature nucleotide patterns of the two representatives, *Yaniella halotolerans* and *Yania flava*, correspond to the pattern defining the family *Micrococcaceae* (except an A in position 196; Zhi et al., 2009). From these observations Yassin et al. (2011) concluded that a separate family for the genus *Yaniella* was not justified and proposed to transfer the genus to the family *Micrococcaceae*. In this volume, the family *Ruaniaceae*, which is composed of the genera *Ruania* (Gu et al., 2007) and *Haloactinobacterium* (Tang et al., 2010), is also assigned to this order. Common to all representatives of the order, as well as other actinobacteria, is the presence of menaquinones as respiratory quinones. The length of the isoprenoid side chains varies (6–14 units). The menaquinones in the majority of taxa within this order (exclusive of the *Microbacteriaceae*) have 7- to 9-unit side chains, but some species exhibit a menaquinone with 10 isoprenoic units and one or two isoprenoid units may be dihydrogenated. Species of the *Microbacteriaceae* are characterized by a predominance of menaquinones with 9–14 unsaturated isoprenoid units in their side chains. The majority of families are characterized by the peptidoglycan type A with the characteristic diamino acids lysine, ornithine, or *meso*-diaminopimelic acid. However, the family *Microbacteriaceae* exhibits peptidoglycan type B with the characteristic diamino acids lysine, ornithine, or diamino butyric acid but never diaminopimelic acid. Most of the fatty acids of representatives of the order are branched acids of the iso- and anteiso-type. Moderate amounts of straight chain fatty acids may be present as well, and *Janibacter* species exhibit a high proportion of unbranched acids (Kämpfer et al., 2006). Diphosphatidylglycerol, phosphatidylglycerol, phosphatidylinositol, and unknown glycolipids are widely distributed among species of the order. Families of this order may be distinguished by a set of signature nucleotides in the 16S rRNA sequence (Table 70).

TABLE 70. Patterns of 16S rRNA signature nucleotides that define families of the order *Micrococcales*[a,b]

Position (s)	*Beutenbergiaceae*	*Bogoriellaceae*	*Brevibacteriaceae*	*Cellulomonadaceae*	*Dermabacteraceae*	*Dermacoccaceae*	*Dermatophilaceae*	*Intrasporangiaceae*	*Jonesiaceae*	*Microbacteriaceae*	*Micrococcaceae*	*Promicromonosporaceae*	*Rarobacteraceae*	*Sanguibacteraceae*	*Ruaniaceae*
120	A	A	A	A	A	A	A	A	A	A	W	A	A	A	A
131:231	C–G	A–G	C–G	C–G	C–G	A–G	Y–K	A–G	A–G	G–R	C–G	A–G	C–G	C–G	A–G
196	G	U	A	U	U	C	A	G	C	U	c	U	A	U	C
342:347	C–G	U–G	C–G	C–G	C–G	C–G	C–G	C–G	C–G	C–G	C–G	C–G	C–G	C–G	C–G
444:490	A–U	A–U	A–U	A–U	A–U	A–U	A–U	a–u	A–U	A–U	A–U	A–U	A–U	A–U	C–U
580:761	C–G	C–A	C–G	C–G	U–A	U–A	U–A	U–A	C–G	C–G	c–g	C–G	C–G	C–G	C–G
602:636	C–G	C–G	C–G	c–g	C–G	C–G	C–G	C–G	C–G	C–G	C–G	G–U	G–U	G–U	S–K
670:736	A–U	A–U	U–A	A–U	A–U	A–U	A–U	A–U	A–U	A–U	A–U	A–U	A–U	A–U	A–U
822:878	G–C	G–C	G–C	G–C	G–C	G–C	G–C	G–C	U–C	G–C	G–C	G–C	G–C	G–C	G–C
823:877	G–C	G–C	G–C	G–C	G–C	G–C	G–C	G–C	A–C	G–C	G–C	G–C	G–C	G–C	G–C
826:874	C–G	C–G	C–G	C–G	C–G	C–G	C–G	C–G	U–G	C–G	C–G	C–G	C–G	C–G	C–G
827	U	U	U	U	U	U	U	U	G	U	U	U	U	U	U
843	U	C	C	U	C	c	U	U	C	C	C	U	U	C	U
950:1231	U–A	U–A	U–A	U–A	U–G	U–A	U–A	U–A	U–A	U–A	U–A	U–A	U–A	U–A	U–A
1047:1210	G–C	G–C	G–U	G–C	G–C	G–C	G–C	G–C	G–C	G–C	G–C	G–C	G–C	G–C	C
1109	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C
1145	G	G	A	G	G	G	G	G	G	R	G	G	G	G	G
1309:1328	G–C	A–U	G–C	G–C	G–U	G–C	G–C	G–C	G–C	G–C	G–C	G–C	G–C	G–C	G–C
1361	G	G	G	G	G	G	G	C	G	G	G	G	G	G	G
1383	U	U	C	Y	C	C	C	C	C	C	C	C	C	C	U

[a] Abbreviations: K = G or T; R = A or G; S = G or C; W = A or U; Y = C or U. Residues in lower-case letters are present in some but not all strains.

[b] Data obtained from Tang et al. (2010), Yassin et al. (2011), and Zhi et al. (2009).

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Family I. **Micrococcaceae** Pribham 1929, 361^{AL} emend. Stackebrandt, Rainey and Ward-Rainey 1997, 479

HANS-JÜRGEN BUSSE

Mi.cro.coc.ca.ce′a.e. N.L. masc. n. *Micrococcus* type genus of the family; suff. -*aceae* ending to denote a family; N.L. fem. pl. n. *Micrococcaceae* the *Micrococcus* family.

Representatives of the family are characterized by a pattern of 16S rRNA signatures consisting of certain nucleotides at positions 293:304 (G–U), 610 (G), 598:640 (U–U), 615:625 (G–C), 839:847 (A–U), 859 (U), 1025:1036 (C–G), 1026:1035 (C–G), 1265:1270 (U–G), and 1278 (U). All species of the family have a type **A** peptidoglycan with the diamino acid **L-lysine**. Respiratory quinones are **menaquinones** predominantly with **7–10 isoprenoid units** in the side chain, which are either completely **unsaturated, dihydrogenated, or a combination of both.** Polar lipid profiles usually contain **phosphatidyglycerol, diphosphatidylglycerol, phosphatidylinositol, and unknown glycolipid(s).** Genera affiliated with this family are *Micrococcus*, *Acaricomes*, *Arthrobacter*, *Citricoccus*, *Kocuria*, *Nesterenkonia*, *Renibacterium*, *Rothia*, *Yaniella*, and *Zhihengliuella*.

Type genus: **Micrococcus** (Cohn 1872) emend. Stackebrandt, Koch, Gvozdiak and Schumann 1995, 691 emend. Wieser Denner, Kämpfer, Schumann, Tindall, Steiner, Vybiral, Lubitz, Maszenan, Patel, Seviour, Radax and Busse 2002, 629.

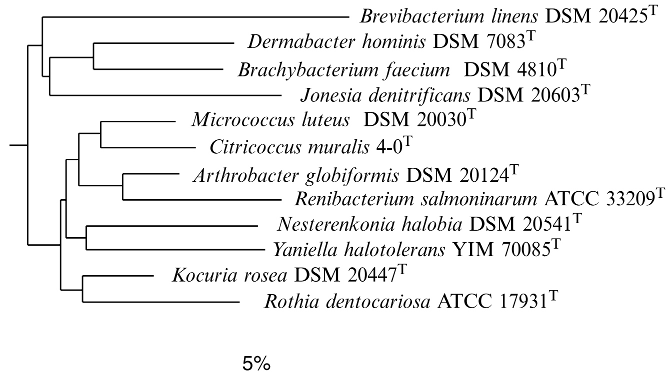

FIGURE 136. Neighbor joining tree (Felsenstein, 1993b) based on 16S rRNA gene sequences of some members of the family *Micrococcaceae* and some related genera, represented by their type species. Bootstrap values (>30%) of 500 resamplings (Felsenstein, 1985) are indicated at nodes. The scale represents 5 inferred nucleotide changes per 100 nucleotides.

Further descriptive information

The family *Micrococcaceae* proposed by Pribham (1929) was later reorganized and emended by Stackebrandt et al. (1997) exclusively based on signature nucleotides in the 16S rRNA gene sequences. At that time, in addition to the type genus *Micrococcus*, the genera *Arthrobacter* (Conn and Dimmick 1947) Koch et al. 1995, *Kocuria*, *Nesterenkonia* Stackebrandt et al. 1995, *Renibacterium* Sanders and Fryer 1980, *Rothia* Georg and Brown 1967, and *Stomatococcus* Bergan and Kocur 1982 were placed into this family. Recently, the single species of the latter genus, *Stomatococcus mucilaginosus*, was reclassified as *Rothia mucilaginosa* Collins et al. 2000. Subsequently, three additional genera were assigned to the family including *Citricoccus* Altenburger et al. 2002, *Acaricomes* Pukall et al. 2006, and *Zhihengliuella* Zhang et al. 2007; in the roadmap to the present volume, the genus *Yaniella* Li, Zhi and Euzéby 2008b has also been incorporated into the family *Micrococcaceae* (Figure 136). Genera of this family share the characteristics of the order *Micrococcales*. The polyamines of the representatives of the family examined thus far, including species of the genera *Micrococcus*, *Kocuria*, *Arthrobacter*, and *Citricoccus* (Altenburger et al., 1997, 2002; Gvozdiak et al., 1998; Hamana, 1994), are mainly spermidine, often with moderate amounts of spermine (exception: *Arthrobacter uratoxydans* exhibiting spermine predominantly).

Genus I. **Micrococcus** Cohn 1872, 151^{AL} emend. Stackebrandt, Koch, Gvozdiak, Schumann 1995, 682 emend. Wieser, Denner, Kämpfer, Schumann, Tindall, Steiner, Vybiral, Lubitz, Maszenan, Patel, Seviour, Radax and Busse 2002, 635

HANS-JÜRGEN BUSSE

Mi.cro.coc′cus. Gr. adj. *mikros* small, little; N.L. masc. n. *coccus* (from Gr. masc. n. *kokkos* grain, seed) coccus; N.L. masc. n. *Micrococcus* small coccus.

Cells are spherical and nonmotile. Endospores are not formed. Gram-stain-positive. Aerobic. **Chemo-organotrophic**, metabolism is strictly respiratory. Catalase- and oxidase-positive. Mesophilic. Non halophilic. The **peptidoglycan contains L-lysine as the diagnostic diamino acid**. The **peptidoglycan type** is either **A2**, with the interpeptide bridge consisting of a stem peptide or **A4α** both with lysine as the diagnostic diamino acid. The **predominant menaquinones** are either **MK-8 and MK-8(H_2)** or **MK-8(H_2)** or **MK-7(H_2) and MK-8(H_2)**. The cytochromes are aa_3, b_{557}, b_{567}, d_{626}; cytochromes c_{550}, c_{551}, b_{563}, b_{564} and b_{567} may be present. Polar lipids are **phosphatidylglycerol, diphosphatidylglycerol, phosphatidylinositol**, an unknown **glycolipid**, and an unknown ninhydrin-negative **phospholipid**. The cellular fatty acids are **iso- and anteiso-branched fatty acids**, with **$C_{15:0}$ anteiso and $C_{15:0}$ iso** predominating. The major aliphatic hydrocarbons (br-Δ-C) are C_{27} to C_{29}. Mycolic acids and teichonic acids are absent; teichuronic acids may be present. Mannosamine-uronic acid may be present as an amino sugar in the cell-wall polysaccharide. D-Arabinose, *p*-arbutin, D-cellobiose, D-galactose, D-melibiose, D-ribose, and salicin are not assimilated. Members of the genus share the *Micrococcaceae*-specific signature nucleotides at positions 293:304, 610, 598, 615:625, 1025:1036, 1026:1035,

1265:1270, and 1278 of the 16S rRNA gene sequence (*Escherichia coli* numbering) and lack the signature nucleotides at positions 640, 839:847, and 859 (Stackebrandt et al., 1997).

DNA G+C content (mol%): 69–76.

Type species: **Micrococcus luteus** (Schroeter) Cohn 1872, 153[AL] emend. Wieser Denner, Kämpfer, Schumann, Tindall, Steiner, Vybiral, Lubitz, Maszenan, Patel, Seviour, Radax and Busse 2002, 635.

Further descriptive information

The genus *Micrococcus* is the type genus of the order *Micrococcales* Prévot 1940 and the family *Micrococcaceae* Pribram 1929. Former members of the genus including *Micrococcus varians*, *Micrococcus kristinae*, *Micrococcus roseus*, *Micrococcus agilis*, *Micrococcus sedentarius*, *Micrococcus nishinomiyanensis*, and *Micrococcus halobius* have been reclassified in other genera based on their distinct phylogenetic positions and differences in the quinone systems, interpeptide bridges of the peptidoglycans, and fatty acids (Koch et al., 1995; Stackebrandt et al., 1995). *Micrococcus varians*, *Micrococcus kristinae*, and *Micrococcus roseus* were reclassified as species of the genus *Kocuria*. *Micrococcus agilis* was transferred to the genus *Arthrobacter*. *Micrococcus sedentarius*, *Micrococcus nishinomiyanensis*, and *Micrococcus halobius* were proposed as species of the genera *Kytococcus*, *Dermacoccus*, and *Nesterenkonia*, respectively.

The genus now consists of six species, *Micrococcus luteus* (the type species of the genus), *Micrococcus lylae*, *Micrococcus antarcticus*, *Micrococcus endophyticus*, *Micrococcus yunnanensis*, and *Micrococcus flavus* (differential characteristics are shown in Table 71). The type strains of the species are separated from each other at 97.8–99.8% 16S rRNA gene sequence similarities. Some representatives of related genera such as *Citricoccus* and *Arthrobacter* show only slightly lower similarity values with the type strains of *Micrococcus* species indicating the limitations of 16S rRNA gene sequence similarities for genus affiliation within this group. Among reliably identified *Micrococcus luteus* strains, the isolate *Micrococcus luteus* strain 118 (accession no. AJ312751; Wieser et al., 2002) exhibits only 98.9% similarity with the type strain of *Micrococcus luteus* DSM 20030 (accession no. AJ536198) in the 16S rRNA gene sequence but 99.1% with the type strain of *Micrococcus yunnanensis*. Hence, 98.9% similarity is not sufficient to indicate species identity, at least for the species *Micrococcus luteus* and *Micrococcus yunnanensis*.

Species of the genus share the coccus shape and the inability to assimilate the carbon sources L-arabinose, cellobiose, D-melibiose, D-ribose, and salicin. The species are characterized by a quinone system with menaquinone MK-8(H_2) [large amounts of MK-8 and MK-7(H_2) may be present as well], a fatty acid profile predominated by $C_{15:0}$ anteiso and $C_{15:0}$ iso (<60%), and a lysine based peptidoglycan type A2 or A4α (Schleifer and Kandler, 1972). *Micrococcus luteus* is so far the only species with strains having different quinone systems, either MK-8 and MK-8(H_2) or MK-8(H_2) and different peptidoglycans, either A2 or A4α (Wieser et al., 2002).

Optimal growth temperature reflects the natural habitat of some *Micrococcus* species. *Micrococcus antarcticus* isolated from Antarctica grows best at 16.8°C; *Micrococcus flavus* isolated from activated sludge grows best at 31°C; and *Micrococcus luteus* and *Micrococcus lylae* both isolated from human and other mammal skin exhibit optimal growth at 37°C. Kloos and Musselwhite (1975) showed that *Micrococcus luteus* is the predominant organism isolated from skin of the head, legs, and arms whereas *Micrococcus lylae* is only occasionally isolated from skin but most frequently during the colder seasons. The association of *Micrococcus luteus* with humans is also indicated by the higher frequency of recovery from air-borne bacteria collected in the "Museo Correr" in Venice, Italy, during visiting hours (Camuffo et al., 1999; Wieser et al., 2002). *Micrococcus luteus* and more rarely *Micrococcus lylae*, can be also isolated from different foodstuffs. During the production of cassava fish among others, *Micrococcus luteus* can be isolated early in the fermentation process (Anihouvi et al., 2007). *Micrococcus luteus* has been isolated in moderate amounts from "androlla" (a Spanish dry-fermented sausage; García Fontán et al., 2007) and detected during ripening of Camembert cheese (Addis et al., 2001) and in goat cheese (Prado et al., 2001). Micrococci are the only Gram-stain-positive bacteria which can be isolated from ice-stored fish, and strains of *Micrococcus luteus* were shown to make up almost 20% of totally recovered strains from this source (Lakshmanan et al., 2002a).

Enrichment and isolation procedures

Micrococcus species do not exhibit special growth requirements. Common media containing yeast extract and peptone and a pH close to 7.0 are suitable for isolation. Specific isolation of *Micrococcus antarcticus* and *Micrococcus yunnanensis* may be done at 4°C. To suppress growth of staphylococci, the medium for isolation of *Micrococcus luteus* should be supplemented with 0.03% (w/v) furazolidone (Baker, 1984) and 10% (w/v) NaCl (Wieser et al., 2002) and incubated at 45°C.

Pathogenicity. There are only a few reports dealing with micrococci related to human infections. However, *Micrococcus luteus* in particular can be considered an opportunistic pathogen. Strains of this species were identified as causative agents of septic shock (Albertson et al., 1978), meningitis (Fosse et al., 1985), septic arthritis (Wharton et al., 1986), endocarditis (Dürst et al., 1991; Glupczynski et al., 1986; Seifert et al., 1995), infections associated with indwelling lines, continuous ambulatory peritoneal dialysis, or a ventriculo-peritoneal shunt (Magee et al., 1990), intracranial suppuration (Selladurai et al., 1993), bacteremia (Peces et al., 1997; von Eiff et al., 1996), chronic cutaneous infections in HIV-positive patients (Smith et al., 1999), and catheter infection (Oudiz et al., 2004).

Another threat to human health is the ability of *Micrococcus luteus* to produce cadaverine in food through the action of lysine decarboxylase (Lakshmanan et al., 2002b). Cadaverine itself has little toxicity but it potentiates the toxicity of histamine in food by inhibiting histamine-metabolizing enzymes such as diamine oxidase and histamine *N*-methyl-transferase (Taylor and Sumer, 1986).

Maintenance procedures

Micrococcus cultures may be lyophilized or stored as a glycerol culture at −80°C by common procedures used for many bacteria. Storage on agar slants at 4°C for several weeks can be recommended.

Differentiation of the genus *Micrococcus* from other genera

Micrococcus species may be distinguished from other genera of the family *Micrococcaceae* and its nearest phylogenetic neighbors, members of the genus *Citricoccus*, by cell-wall peptidoglycan type and menaquinone system (Table 72).

TABLE 71. Characteristics differentiating representatives of the genus *Micrococcus* from other related genera containing coccus-shaped species

Characteristic	*Micrococcus*[a]	*Citricoccus*[b]	*Dermacoccus*[c]	*Kocuria*[d]	*Kytococcus*[e]	*Nesterenkonia*[f]	*Rothia*[g]
Quinone system	MK-8, MK-8(H_2) or MK-8(H_2) or MK-7(H_2), MK-8(H_2)	MK-9(H_2)	MK-8(H_2)	MK-7(H_2) or MK-8(H_2) or MK-7(H_2), MK-8(H_2) or MK-8(H_2), MK-9(H_2), MK-7(H_2) or MK-8(H_2), MK-9(H_2)	MK-8, MK-7 or MK-8, MK-9, MK-10	MK-7 or MK-8 or MK-7, MK-8 or MK-8, MK-7, MK-9 or MK-8, MK-9	MK-7 or MK-7, MK-6(H_2)
Peptidoglycan interpeptide bridge	L-Lys–peptide subunit or L-Lys–D-Asp	Lys–Gly–Glu	L-Lys–L-$Ser_{1–2}$–(L-Ala)–D-Glu	L-Lys–L-$Ala_{3–4}$	L-Lys–Glu_2	L-Lys–Gly–D-Asp or L-Lys–L-Glu or L-Lys–Gly–L-Glu	L-Lys–L-Ala
Major fatty acids (>25%)	$C_{15:0}$ anteiso or $C_{15:0}$ anteiso and $C_{15:0}$ iso	$C_{15:0}$ anteiso or $C_{15:0}$ anteiso and $C_{17:0}$ anteiso	$C_{16:0}$ iso[h]	$C_{15:0}$ anteiso	$C_{17:0}$ anteiso or $C_{17:1}$ anteiso	$C_{16:0}$ iso or $C_{15:0}$ anteiso or $C_{16:0}$, $C_{15:0}$ anteiso or $C_{17:0}$ anteiso, $C_{15:0}$ anteiso	$C_{15:0}$ anteiso
Acid produced from carbohydrates	–	–	–	+	– or weakly +	+[i]	+
DNA G+C content (mol%)	66.4–75.5	63.8–68.0	65.2–69.1	60.0–75.3	68–69	68.0	54.5–57.8

[a]Data from Kocur et al. (1972), Stackebrandt et al. (1995), Wieser et al. (2002), Liu et al. (2000), and Liu et al. (2007).

[b]Data from Altenburger et al. (2002) and Li et al. (2005b).

[c]Data from Stackebrandt et al. (1995) and Pathom-aree et al. (2006b, 2006c).

[d]Data from Stackebrandt et al. (1995), Kovács et al. (1999), Reddy et al., (2003), Kim et al. (2004), Tvrzová et al. (2005a), Li et al. (2006), Mayilraj et al. (2006), and Zhou et al. (2008a).

[e]Data from Stackebrandt et al. (1995) and Becker et al. (2002).

[f]Data from Stackebrandt et al. (1995), Collins et al. (2002b), Li et al. (2005a, 2004b, 2008a), Delgado et al. (2006), and Yoon et al. (2006b).

[g]Data from Stackebrandt et al. (1995), Collins et al. (2000), Fan et al. (2002), Li et al. (2004c), and Chou et al. (2008).

[h]Only *Deferribacter abyssi* was reported to contain $C_{16:0}$ iso predominantly. For other species, such as *Dermacoccus nishinomiyaensis*, *Dermacoccus barathri*, and *Dermacoccus profundi*, the relative amount of major fatty acids was reported not to exceed 20%.

[i]*Nesterenkonia halophila* was negative in acid production from all carbohydrates tested.

TABLE 72. Differential characteristics of the species of the genus *Micrococcus*[a]

Characteristic	*M. luteus*[b]	*M. lylae*[b]	*M. flavus*[c]	*M. antarcticus*[d]	*M. endophyticus*[e]	*M. yunnanensis*[f]
Pigmentation	Yellow	Cream-white	Yellow	Yellow	Yellow	Yellow
Optimal growth temperature (°C)	37	37	31	16.8	28	28
Growth at 4°C[f]	–	–	–	+	–	+
Growth at 45°C	+	nd	–	–	–	+
Major menaquinone(s)	MK-8 and MK-8(H_2) or MK-8(H_2)	MK-8(H_2)	MK-8(H_2), MK-7(H_2)	MK-8, MK-8(H_2)	MK-8(H_2), MK-7(H_2)	MK-8(H_2), MK-7(H_2)
Nitrate reduction[f]	–	–	–	+	+	–
Voges–Proskauer reaction[f]	–	–	–	+	–	–
Hydrolysis of:						
Tween 80	d	+	–	+	–	+
Starch	–	–	+	+	–	–
Casein	+	–	nd	nd	nd	nd
Assimilation of:						
D-Mannose	+	–	–	–	–	nd
D-Trehalose	+	+	+	–	+	nd
Maltose	+	+	–	–	nd	nd
L-Malate	–	–	–	+	nd	nd
Pyruvate	+	+	nd	–	nd	nd
Acetate	d	+	nd	–	nd	nd
Propionate	+	–	nd	–	nd	nd
L-Alanine	d	–	nd	+	nd	nd
Acid production from:[f]						
Adonitol	–	+	–	–	–	–

(continued)

TABLE 72. (continued)

Characteristic	*M. luteus*[b]	*M. lylae*[b]	*M. flavus*[c]	*M. antarcticus*[d]	*M. endophyticus*[e]	*M. yunnanensis*[f]
Amylum	–	+	+	+	–	–
D-Arabinose	+	–	–	–	+	–
L-Arabinose	–	–	–	+	+	–
Arbutin	+	–	–	+	+	–
Cellobiose	–	–	+	+	+	–
Dulcitol	–	+	+	–	–	–
Erythritol	–	–	+	–	–	–
Esculin	–	–	+	+	+	–
Fructose	+	+	–	+	+	–
D-Fucose	–	+	–	–	–	–
Galactose	–	–	–	+	+	–
β-Gentiobiose	–	+	–	+	–	–
Glycogen	–	+	–	+	–	–
Gluconate	–	–	+	–	–	–
Inositol	–	–	–	+	–	–
Inulin	–	+	–	–	–	–
Lactose	–	+	+	+	–	–
D-Lyxose	–	+	–	+	+	–
Mannitol	–	+	–	–	–	–
Melibiose	–	–	–	+	–	+
Melezitose	–	+	–	–	–	–
Methyl α-D-mannoside	–	–	+	–	–	–
Methyl α-D-glucoside	+	–	–	–	+	–
N-Acetylglucosamine	–	–	–	+	+	–
Raffinose	–	–	–	–	+	–
Rhamnose	–	+	–	+	–	–
Ribose	–	–	+	+	+	–
Salicin	+	+	+	+	–	–
Sorbitol	–	+	–	–	+	–
Sorbose	–	+	–	–	–	–
D-Tagatose	–	–	–	+	–	–
Trehalose	+	+	+	–	+	+
D-Xylose	–	–	–	+	–	–

[a]Symbols: +, 90% or more strains positive; –, 90% or more strains negative; d, 11–89% of strains positive; nd, not determined.

[b]Data from Wieser et al. (2002) and Zhao et al. (2009).

[c]Data from Liu et al. (2007) and Zhao et al. (2009).

[d]Data from Liu et al. (2000) and Zhao et al. (2009).

[e]Data from Chen et al. (2009) and Zhao et al. (2009).

[f]Data from Zhao et al. (2009).

List of species of the genus *Micrococcus*

1. **Micrococcus luteus** (Schroeter) Cohn 1872, 153[AL] (synonym: "*Bacteridium luteum*" Schroeter 1872, 126); emend. Wieser et al., 2002, 635

lu′te.us. L. masc. adj. *luteus* golden yellow.

The species shares the properties given in the genus description. Additional characteristics are: cells occur in tetrads and in irregular clusters of tetrads and form smooth, convex colonies with regular edge. Colonies forming cubical packets usually have a granular surface and a matt appearance. Colonies are yellow, yellowish green, or orange pigmented. Some strains form a violet pigment which diffuses into the medium. The optimal growth temperature range is 25–37°C. Growth or weak growth is observed at 45°C, at pH 10.0, and in the presence of 10% NaCl; no growth is observed in the presence of 15% NaCl. Urease-variable. D-Glucose, sucrose, and D-mannose are assimilated. D-Fructose, *N*-acetyl-D-glucosamine, L-rhamnose, gluconate, *cis*-aconitate, *trans*-aconitate, adipate, azelate, itaconate, mesaconate, suberate, β-alanine, L-ornithine, L-tryptophan, L-leucine, 3-hydroxybenzoate, and 4-hydroxybenzoate are not assimilated. *p*-Nitrophenyl (pNP) α-glucopyranoside, and L-alanine *p*-nitroanilide (pNA) are hydrolyzed. pNP D-Galactopyranoside, pNP D-glucuronide, pNP D-glycopyranoside, bis-pNP phosphate, pNP phenylphosphate, pNP phosphorylcholine, 2-deoxythymidine-5′-pNP phosphate, and L-glutamate-3-carboxy pNA are not hydrolyzed. The peptidoglycan variation is either A2 or A4α. The predominant menaquinones are either MK-8 and MK-8(H_2) or MK-8(H_2) alone. MK-7 or MK-7(H_2) and MK-9(H_2) and MK-6(H_2) occur in minor amounts.

Source: mammalian skin.

DNA G+C content (mol%): 70–75.5 (T_m, Bd, HPLC) (Kocur et al., 1972; Wieser et al., 2002); 73 (genome sequence of the type) (Young et al., 2010).

Type strain: ATCC 4698, CCM 169, CCUG 5858, CIP A270, DSM 20030, HAMBI 26, HAMBI 1399, IEGM 391, NBRC3333, JCM 1464, LMG 4050, NCCB 78001, NCTC 2665, NRRL B-287, VKM B-1314.

Sequence accession no. (16S rRNA gene): AJ536198.
Sequence accession no. (complete genome): CP001628 (2.5 MB)
Sequence accession no. (recA gene): AF214783.
Additional remarks: based on substantial chemotaxonomic differences, Wieser et al. (2002) proposed dissection of the species *Micrococcus luteus* into three biovars (see below).

Biovar I. In addition to the properties given in the species description, members of this biovar have the following characteristics. No growth is observed at pH 6.0. Urease-positive. Maltitol, L-aspartate, and propionate are assimilated. D-Maltose, D-trehalose, D-xylose, adonitol, iso-inositol, D-mannitol, D-sorbitol, putrescine, acetate, 4-aminobutyrate, citrate, fumarate, glutarate, DL-3-hydroxybutyrate, DL-lactate, L-malate, oxoglutarate, pyruvate, L-alanine, L-histidine, L-phenylalanine, L-proline, L-serine, and phenylacetate are not assimilated. L-Proline pNA and Tween 20 are hydrolyzed. Casein and Tween 80 are not hydrolyzed. The peptidoglycan type is A2. The major menaquinones are MK-8 and MK-8(H_2). The reference strain for this biovar is the designated type strain for the species, *Micrococcus luteus* DSM 20030.

Biovar II. In addition to the properties given in the species description, members of this biovar have the following characteristics. Colonies are creamy yellow in color, circular, entire, and convex. Growth at pH 6.0 is variable. Urease-variable. D-Maltose, D-trehalose, acetate, propionate, DL-3-hydroxybutyrate, DL-lactate, pyruvate, L-histidine, L-phenylalanine, L-serine, and phenylacetate are assimilated. Oxoglutarate is not assimilated. Assimilation of D-xylose, adonitol, iso-inositol, maltitol, D-mannitol, D-sorbitol, putrescine, 4-aminobutyrate, citrate, fumarate, glutarate, L-malate, L-alanine, L-aspartate, and L-proline is variable. Casein and L-proline pNA are hydrolyzed. Hydrolysis of Tween 20 and Tween 80 is variable. The major menaquinone is MK-8(H_2). The peptidoglycan type is A2.
Source: a medieval wall painting and from indoor air.
DNA G+C content (mol%): 71 (HPLC).
Reference strain: D7, DSM 14234, CCM 4959.
Sequence accession no. (16S rRNA gene): AJ409095

Biovar III. In addition to the properties given in the species description, the single member of this biovar has the following characteristics. Colonies are creamy yellow in color, circular, entire, and convex. No growth is observed at pH 6.0. Urease-positive. D-Maltose, D-trehalose, 4-aminobutyrate, fumarate, DL-3-hydroxybutyrate, DL-lactate, oxoglutarate, pyruvate, L-histidine, and L-proline are assimilated. D-Xylose, adonitol, iso-inositol, maltitol, D-mannitol, D-sorbitol, putrescine, acetate, propionate, citrate, glutarate, L-malate, L-alanine, L-aspartate, L-phenylalanine, L-serine, and phenylacetate are not assimilated. Tween 20 and casein are hydrolyzed. L-Proline pNA and Tween 80 are not hydrolyzed. The main menaquinone is MK-8(H_2). The peptidoglycan variation is A4α. Strain Ballarat was isolated from an activated-sludge plant in Ballarat, Victoria, Australia.
DNA G+C content (mol%): 70 (HPLC).
Reference strain: strain Ballarat, DSM 14235, CCM 4960.
Sequence accession no. (16S rRNA gene): AJ409096.

2. **Micrococcus antarcticus** Liu, Xu, Ma and Zhou 2000, 718[VP]

an.tarc′ti.cus. L. masc. adj. *antarcticus* southern, referring to the Antarctic habitat of the bacterium.

Cells are spherical, have a diameter of 0.5 µm and are nonmotile. Endospores are not formed. Gram-stain-positive, aerobic. Colonies are yellow, mucoid, and fluffy with entire margins. Optimal growth occurs at 15–17°C. Catalase- and oxidase-positive. Positive for indole formation, Voges–Proskauer, methyl red, and nitrate reduction. Starch, Tween 20, Tween 40, and Tween 80 are hydrolyzed. The following carbon sources are utilized: arabitol, fructose, fucose, gluconic acid, mannitol, rhamnose, turanose, xylitol, hydroxybutyric acid, L-lactic acid, L-malic acid, D-malic acid, methyl pyruvate, mono-methyl succinate, succinamic acid, succinic acid, *N*-acetyl L-glutamic acid, alaninamide, D-alanine, L-alanine, L-alanyl-glycine, L-asparagine, L-glutamic acid, glycyl-L-glutamic acid, L-pyroglutamic acid, L-serine, glycerol, adenosine, inosine, thymidine, and uridine. The following biochemical tests are negative: urease, acetoin, and arginine dihydrolase. Gelatin and esculin are not hydrolyzed. Acid production from carbohydrates is negative. There is no utilization of the following carbon sources: dextrin, glycogen, inulin, mannan, amygdalin, L-arabinose, arbutin, cellobiose, D-galactose, D-galacturonic acid, gentiobiose, D-glucose, *myo*-inositol, D-lactose, lactulose, maltose, maltotriose, D-mannose, D-melezitose, D-melibiose, 3-methyl glucose, palatinose, psicose, raffinose, D-ribose, salicin, sorbitol, stachyose, sucrose, D-tagatose, D-trehalose, D-xylose, acetic acid, α-ketoglutaric acid, α-ketovaleric acid, lactamide, propionic acid, pyruvic acid, and 2,3-butanediol. Susceptible to lysozyme, penicillin, tetracycline, erythromycin, novobiocin, streptomycin, methicillin, chloramphenicol, polymyxin, and neomycin. The diagnostic peptidoglycan diamino acid is L-lysine. The predominant menaquinones are MK-8 and MK-8(H_2). The amino sugar in the cell-wall polysaccharide is mannosamine. Major cellular fatty acids are $C_{15:0}$ anteiso and $C_{15:0}$ iso.
Source: Chinese Great-Wall station in Antarctica.
DNA G+C content (mol%): 66.4 (T_m).
Type strain: strain T2, AS 1.2372, JCM 11467.
Sequence accession no. (16S rRNA gene): AJ005932.

3. **Micrococcus endophyticus** Chen, Zhao, Park, Zhang, Xu, Lee, Kim and Li 2009, 1073[VP]

en.do.phy′ti.cus. Gr. pref. *endo-* within; Gr. neut. n. *phyton* plant; L. masc. suff. *-icus* suffix used with the sense of pertaining to; N.L. masc. adj. *endophyticus* within plant, pertaining to the original isolation from plant tissues.

Cells are spherical, Gram-stain-positive, aerobic, non-endospore-forming, coccoid and 0.5–0.7 µm in diameter. Motility is not observed. Colonies on TSA are yellow, slimy, smooth, and circular with entire margins. No pigment is produced. The temperature, pH, and NaCl ranges for growth are 15–37°C, pH 6–9, and 0–10% (w/v). Optimal growth occurs at 28°C and at around pH 7.0–8.0. Catalase-positive and oxidase-positive. Gelatin but not starch and Tween 80 is hydrolyzed. The Voges–Proskauer reaction is negative and the reduction of nitrate is positive. Positive for acid production from *N*-acetylglucosamine, esculin, D-arabinose, L-arabinose, arbutin, cellobiose, fructose, galactose, D-lyxose, methyl α-D-glucoside, raffinose, ribose, sorbitol, trehalose, mannose, maltose, sucrose, D-glucose and 5-ketogluconate, but negative for acid production from adonitol, dulcitol, erythritol, D-fucose, β-gentiobiose, gluconate, glycerol, glycogen, inositol, inulin, lactose, mannitol, melezitose, melibiose, methyl β-D-mannoside, rhamnose, salicin, sorbose, starch,

D-tagatose, L-xylose, D-xylose, methyl β-D-xyloside, amygdalin, D-arabitol, L-arabitol, L-fucose, 2-ketogluconate, and xylitol. The cell-wall peptidoglycan of the type strain contains lysine, glutamic acid, alanine, and glycine. The phospholipids consist of diphosphatidylglycerol, phosphatidylglycerol, phosphatidylinositol, and an unknown ninhydrin-negative phospholipid. The predominant menaquinones are MK-8(H_2) (63.6%) and MK-7(H_2) (21.1%). The major cellular fatty acids are $C_{15:0}$ iso and $C_{15:0}$ anteiso.

Source: surface-sterilized roots of the plant *Aquilaria sinensis*, collected in the tropical rainforest of Yunnan Province in south-west China.

DNA G+C content (mol%): 72.9 (T_m).

Type strain: YIM 56238, DSM 17945, KCTC 19156.

Sequence accession no. (16S rRNA gene): EU005372.

4. **Micrococcus flavus** Liu, Wang, Jiang and Liu 2007, 66[VP]

fla'vus. L. masc. adj. *flavus* yellow, pertaining to the yellow color of the colonies.

Cells are spherical, 0.7–1.0 µm in diameter and nonmotile. Gram-stain-positive, aerobic, and heterotrophic. Colonies are yellow, smooth, and circular with entire margins. Optimal growth occurs at 30.5–31.5°C and pH 6–6.2. Catalase- and oxidase-positive. Starch is hydrolyzed. Negative for the Voges–Proskauer reaction, lipase, reduction of nitrate, and utilization of citric acid. Gelatin is not hydrolyzed. No acid production from carbohydrates. Glycerol, trehalose, and dextrin are used as carbon sources, but D-arabinose, fructose, mannitose, rhamnose, melibiose, xylitol, malic acid, L-glutamic acid, L-lactic acid, nitrilosides, L-arabinose, cellobiose, D-lactose, D-glucose, inositol, maltose, D-mannose, D-melibiose, raffinose, D-ribose, salicin, and sorbitol are not. The predominant menaquinones are MK-8(H_2) and MK-7(H_2). The major cellular fatty acids are $C_{15:0}$ anteiso and $C_{15:0}$ iso. The peptidoglycan is composed of lysine, glutamic acid, alanine, glycine, and aspartic acid suggesting type A4α.

Source: activated sludge in a bioreactor.

DNA G+C content (mol%): 71.4 (T_m).

Type strain: LW4, CGMCC 1.5361, JCM 14000, DSM 19079, CIP 109517.

Sequence accession no. (16S rRNA gene): DQ491453.

5. **Micrococcus lylae** (Kloos, Tornabene and Schleifer 1974) emend. Wieser, Denner, Kämpfer, Schumann, Tindall, Steiner, Vybiral, Lubitz, Maszenan, Patel, Seviour, Radax and Busse 2002; 636[VP]

ly'la.e. N.L. gen. fem. n. *lylae* of Lyla; named for Lyla Kloos, by whom this organism was originally isolated.

The species shares the properties given in the genus description. Additional characteristics are: D-glucose, sucrose, D-fructose, D-maltose, D-trehalose, maltitol, acetate, citrate, fumarate, DL-3-hydroxybutyrate, DL-lactate, pyruvate, L-aspartate, L-histidine, L-leucine, 3-hydroxybenzoate, and 4-hydroxybenzoate are assimilated. L-Mannose, L-xylose, adonitol, iso-inositol, D-mannitol, D-sorbitol, *N*-acetyl-D-glucosamine, L-rhamnose, gluconate, *cis*-aconitate, *trans*-aconitate, adipate, azelate, itaconate, mesaconate, suberate, putrescine, propionate, glutarate, L-malate, oxoglutarate, 4-aminobutyrate, L-ornithine, L-tryptophan, β-alanine, L-alanine, L-phenylalanine, L-proline, L-serine, and phenylacetate are not assimilated. pNP α-Glucopyranoside, L-alanine pNA, L-proline pNA, Tween 20 and Tween 80 are hydrolyzed. pNP D-Galactopyranoside, pNP D-glucuronide, pNP D-glycopyranoside, bis-pNP phosphate, pNP phenylphosphate, pNP phosphorylcholine, 2-deoxythymidine-5′-pNP phosphate, L-glutamate-3-carboxy pNA, and casein are not hydrolyzed. The main menaquinone is MK-8(H_2). The peptidoglycan type is A4α. Major cellular fatty acids are $C_{15:0}$ anteiso and $C_{15:0}$ iso.

Source: mammalian skin.

DNA G+C content (mol%): 68.6 ± 0.8 (T_m) (Kloos et al., 1974).

Type strain: ATCC 27566, CCM 2693, CCUG 33027, CIP 81.70, DSM 20315, IEGM 392, NBRC15355, JCM 11572, LMG 14218, NCTC 11037, VKM B-1815.

Sequence accession no. (16S rRNA gene): X80750.

Sequence accession no. (recA gene): AF214778.

6. **Micrococcus yunnanensis** Zhao, Li, Qin, Zhang, Zhu, Jiang, Xu and Li 2009, 2386[VP]

yun.na.nen'sis. N.L. masc. adj. *yunnanensis* of or pertaining to Yunnan, a province of south-west China.

Cells are Gram-stain-positive, aerobic, non-endospore-forming cocci (0.8–1 µm in diameter). Motility is not observed. Colonies on TSA are yellow, smooth, and circular with entire margins. No pigment is produced. Temperature, pH, and NaCl tolerance ranges for growth are 4–45°C, pH 6–8, and 0–15 % (w/v), respectively. Optimal growth occurs at 28°C and about pH 7.0–8.0. Catalase-positive and oxidase-negative. Gelatin and starch are not hydrolyzed. Negative for methyl red, Voges–Proskauer reaction, urease, and the reduction of nitrate. Acid is produced from melibiose and trehalose but not from adonitol, amylum, D-arabinose, L-arabinose, arbutin, cellobiose, dulcitol, erythritol, esculin, fructose, D-fucose, galactose, β-gentiobiose, glycogen, gluconate, inositol, inulin, lactose, D-lyxose, mannitol, melezitose, methyl α-D-mannoside, methyl α-D-glucoside, *N*-acetylglucosamine, raffinose, rhamnose, ribose, salicin, sorbitol, sorbose, D-tagatose, and D-xylose. The cell-wall peptidoglycan contains lysine, glutamic acid, alanine, glycine, and aspartic acid. The phospholipids consist of diphosphatidylglycerol, phosphatidylglycerol, phosphatidylinositol, and an unknown ninhydrin-negative phospholipid. The predominant menaquinones are MK-8(H_2) (67%) and MK-7(H_2) (26%). Major cellular fatty acids are $C_{15:0}$ anteiso, $C_{16:0}$ iso, and $C_{15:0}$ iso.

Source: surface-sterilized roots of *Polyspora axillaris*, collected in Yunnan Province, south-west China.

DNA G+C content (mol%): 71.7 (HPLC).

Type strain: YIM 65004, CCTCC AA 208060, DSM 21948.

Sequence accession no. (16S rRNA gene): FJ214355.

Genus II. **Acaricomes** Pukall, Schumann, Schütte, Gols and Dicke 2006, 465^{VP}

Hans-Jürgen Busse

A.ca.ri.co′mes. N.L. masc. pl. n. *acari* the mites; L. masc. n. *comes* companion; N.L. masc. n. *Acaricomes* companion of mites.

Gram-stain-positive, aerobic, non-endospore-forming rods. A rod–coccus life cycle is absent. Mesophilic. The pH optimum for growth is pH 6.0–8.0. Catalase-positive, oxidase-negative. The **peptidoglycan type** is **A3α** L-Lys–L-Ala$_3$. The **predominant menaquinone** is dihydrogenated **menaquinone MK-10(H$_2$)**; in addition, methylmenaquinone **MMK-10(H$_2$)** is detectable. The **main fatty acids** determined are **C$_{15:0}$ anteiso and C$_{17:0}$ anteiso**. The major **polar lipids** are **phosphatidylglycerol, diphosphatidylglycerol**, and **phosphatidylinositol**.

DNA G+C content (mol%): 57.7 (HPLC).

Type species: **Acaricomes phytoseiuli** Pukall, Schumann, Schütte, Gols and Dicke 2006, 465^{VP}.

Further descriptive information

Acaricomes phytoseiuli has been isolated from the diseased predator mite *Phytoseiulus persimilis* Athias-Henriot (Pukall et al., 2006). Phylogenetically, the species is placed in the vicinity of the genera *Renibacterium* and *Arthrobacter* sharing 94–95% 16S rRNA gene sequence similarities with certain species including *Renibacterium salmoninarum*, *Arthrobacter globiformis*, *Arthrobacter woluwensis*, and *Arthrobacter russicus*. *Acaricomes phytoseiuli* exhibits peptidoglycan type A3α (L-Lys–L-Ala$_{2–3}$), a fatty acid profile consisting predominantly of anteiso-branched acids (C$_{15:0}$ anteiso and C$_{17:0}$ anteiso), a polar lipid profile with the components phosphatidylinositol, phosphatidylglycerol, and diphosphatidylglycerol and a quinone system with the major compound MK-10(H$_2$). While this peptidoglycan type, fatty acid profile, and polar lipid patterns are common among the family *Micrococcaceae*, the quinone system is rather unusual supporting the placement of this species in a separate genus.

Enrichment and isolation procedures

Acaricomes phytoseiuli may be isolated from its host (the predator mite *Phytoseiulus persimilis*) after surface-sterilization on Luria Bertoni Agar and incubation for 1 week at 25°C under aerobic conditions (Pukall et al., 2006).

Pathogenicity. *Acaricomes phytoseiuli* is assumed to be the causative agent of the "non-responding syndrome" in *Phytoseiulus persimilis* (Bjørnson and Schütte, 2003; Dicke et al., 2000; Schütte et al., 1998) but no information is available suggesting its pathogenic potential for humans.

Maintenance procedures

No information is available concerning maintenance of *Acaricomes phytoseiuli*.

Differentiation of the genus *Acaricomes* from other genera

So far, the genus is only represented by a single strain of a single species. Hence, it is not very clear which of the traits characterizing *Acaricomes phytoseiuli* in fact is sufficiently conserved to be also shared by other species of the genus. However, there is some probability that chemotaxonomic traits such as quinone system, polar lipids, peptidoglycan composition, and fatty acids fulfill the requirement of genus-specificity (Table 105). The quinone system with menaquinone MK-10(H$_2$) and presence of methylmenaquinone MMK-10(H$_2$) is unique among species of related genera such as *Arthrobacter*, *Micrococcus*, *Citricoccus*, and *Renibacterium*. The interpeptide chain of the peptidoglycan consisting of L-Lys–L-Ala$_3$ distinguishes *Acaricomes phytoseiuli* from the majority of related species. Only three *Arthrobacter* species, *Arthrobacter globiformis*, *Arthrobacter ramosus*. and *Arthrobacter methylotrophus* as well as *Kocuria* species exhibit the same peptidoglycan type and most similar types were reported for *Arthrobacter crystallopoietes*, *Rothia* species (Lys–Ala), *Arthrobacter pascens*, *Arthrobacter russicus*, and *Arthrobacter stackebrandtii* (Lys–Ala$_2$) (Borodina et al., 2002b; Chou et al., 2008; Collins et al., 2000; Fan et al., 2002; Kim et al., 2004; Koch et al., 1995; Kovács et al., 1999; Li et al., 2006; Mayilraj et al., 2006; Reddy et al., 2003; Stackebrandt et al., 1995; Tvrzová et al., 2005a; Zhou et al., 2008a).

List of species of the genus *Acaricomes*

1. **Acaricomes phytoseiuli** Pukall, Schumann, Schütte, Gols and Dicke 2006, 465^{VP}

phy.to.sei′u.li. N.L. gen. masc. n. *phytoseiuli* of *Phytoseiulus* the nomenclatural genus name of the host mite.

The characteristics are as described for the genus with the following additional features. Cells are 0.5–0.8 × 1–1.5 μm in size. Colonies on TSA are circular, convex, 1–2 mm in diameter, and colored yellowish. Growth occurs at 15–30°C with an optimum of 25°C. Grows at pH 6.0–9.5. Utilizes dextrin, α-D-glucose, α-D-fructose, maltose, maltotriose, D-mannose, sucrose, turanose, L-glutamic acid, and glucose 1-phosphate. Utilization of Tween 80, palatinose, L-pyroglutamic acid, and glucose 6-phosphate is weak. The following substrates are not utilized: α- and β-cyclodextrin, glycogen, inulin, mannan, Tween 40, *N*-acetyl-D-glucosamine, *N*-acetyl-D-mannosamine, amygdalin, L-arabinose, D-arabitol, arbutin, D-cellobiose, L-fucose, D-galactose, D-galacturonic acid, gentiobiose, D-gluconic acid, *myo*-inositol, α-D-lactose, lactulose, D-mannitol, D-melezitose, D-melibiose, methyl α-D-galactoside, methyl β-D-galactoside, 3-methyl glucose, methyl α-glucoside, methyl β-D-glucoside, methyl α-D-mannoside, D-psicose, D-raffinose, L-rhamnose, D-ribose, salicin, sedoheptulosan, D-sorbitol, stachyose, D-tagatose, D-trehalose, xylitol, D-xylose, acetic acid, α-, β-, and γ-hydroxybutyric acid, *p*-hydroxyphenylacetic acid, α-ketovaleric acid, lactamide, D-lactic acid methyl ester, L-lactic acid, D-malic acid, methylpyruvate, monomethyl succinate, propionic acid, pyruvic acid, succinamic acid, succinic acid, *N*-acetyl-L-glutamic acid, L-alaninamide, D-alanine, L-alanyl glycine, glycyl L-glutamic acid, L-serine, putrescine, 2,3-butanediol, glycerol, adenosine, 2′-deoxyadenosine, inosine, thymidine, uridine, adenosine 5′-monophosphate, thymidine 5′-monophosphate, uridine

5′-monophosphate, fructose 6-phosphate, and DL-α-glycerol phosphate. Cell-wall sugars are galactose and glucose. Cellular fatty acid profile consists mainly of the branched fatty acids $C_{15:0}$ anteiso (53.6%) and $C_{17:0}$ anteiso (31.9%). $C_{16:0}$ iso (6.3%), $C_{16:0}$ (3.6%), and $C_{15:0}$ iso (1.7%). The quinone system is composed of the major compound menaquinone MK-10(H_2) and minor amounts of MK-11(H_2), MK-9(H_2), and MK-8(H_2). Methylmenaquinone is present as well.

Source: the predatory mite *Phytoseiulus persimilis.*

DNA G+C content (mol%): 57.7 (HPLC).

Type strain: CSC, DSM 14247, CCUG 49701, JCM 14300.

Sequence accession no. (16S rRNA gene): AJ812213.

Genus III. **Arthrobacter** Conn and Dimmick 1947, 301[AL] emend. Koch, Schumann and Stackbrandt 1995, 838

Hans-Jürgen Busse, Monika Wieser and Sandra Buczolits

Ar.thro.bac′ter. Gr. n. *arthron* a joint; N.L. masc. n. *bacter* a rod; N.L. masc. n. *Arthrobacter* a jointed rod.

The majority of species exhibit a marked rod–coccus growth cycle when grown in complex media; stationary-phase cultures (generally after 2–7 d) are composed entirely or largely of coccoid cells that are 0.6–1.0 µm in diameter; some species are showing only spherical cells throughout the growth cycle. **Fatty acid**s are predominantly iso- and anteiso-branched with **$C_{15:0}$ anteiso, $C_{15:0}$ iso, $C_{17:0}$ anteiso, and $C_{16:0}$ iso predominating**. Few species exhibit significant amounts of $C_{16:0}$. **Quinone system** is composed of **completely unsaturated or mono-saturated menaquinones with chain lengths of eight to ten isoprenoic units**. The **cell-wall peptidoglycan contains the diagnostic diamino acid lysine** with several variations in the interpeptide bridge conforming to **peptidoglycan type A3α or A4α**.

DNA G+C content (mol%): 55–72.

Type species: **Arthrobacter globiformis** (Conn 1928) Conn and Dimmick 1947, 301[AL].

Further descriptive information

The genus name *Arthrobacter* was proposed by Conn and Dimmick (1947), reviving the old name which had been proposed by Fischer (1895). This name was a *nomen nudum* (naked name) because no species was named, and subsequently the name had been abandoned even by Fischer. Conn and Dimmick (1947) classified three species in the genus *Arthrobacter*, the type species of the genus *Arthrobacter globiforme*, *Arthrobacter helvolum*, and *Arthrobacter tumescens*. Skerman et al. (1980) then listed *Arthrobacter globiforme* as *Arthrobacter globiformis*, and since then this name has been in use. Later, two species of the genus were reclassified in new genera. *Arthrobacter tumescens* first was classified in the genus *Pimelobacter* as *Pimelobacter tumescens* (Suzuki and Komagata, 1983) and later as the type species of the genus *Terrabacter*, *Terrabacter tumescens* (Collins et al., 1989). *Arthrobacter helvolum* was described as the type species of the genus *Pseudoclavibacter* as *Pseudoclavibacter helvolum* (Manaia et al., 2004).

Arthrobacters have been recovered from a huge variety of environments, including soils, sea water, fresh water, human skin, oil, brine, tobacco leaves, air, sewage and activated sludge, mural paintings, clinical specimens, and a cyanobacterial mat. van Waasbergen et al. (2000) even detected *Arthrobacter* strains in terrestrial subsurface sediments at depths between 170 and 220 m.

In the previous edition of *Bergey's Manual of Systematic Bacteriology*, Keddie et al. (1986) subdivided the genus *Arthrobacter* into two groups, the *Arthrobacter globiformis/Arthrobacter citreus* group and the *Arthrobacter nicotianae* group. Common characteristics of the *Arthrobacter globiformis/Arthrobacter citreus* group were the presence of the menaquinone MK-9(H_2) system and peptidoglycan type A3α, with the characteristic diamino acid L-lysine and an interpeptide chain consisting of monocarboxylic L-amino acids, glycine, or both (Schleifer and Kandler, 1972). Characteristics of the *Arthrobacter nicotianae* group were menaquinones with completely unsaturated isoprenoic side chains (MK-8 or MK-9) and peptidoglycan type A4α, with L-lysine and an interpeptide bridge containing a dicarboxylic amino acid. Species assigned to the *Arthrobacter globiformis/Arthrobacter citreus* group were *Arthrobacter globiformis*, *Arthrobacter pascens*, *Arthrobacter oxydans*, *Arthrobacter histidinolovorans*, *Arthrobacter ureafaciens*, *Arthrobacter ramosus*, *Arthrobacter ilicis*, *Arthrobacter aurescens*, *Arthrobacter citreus*, *Arthrobacter crystallopoietes*, and *Arthrobacter atrocyaneus* (Kuhn and Starr, 1960; since reclassified as *Sinomonas atrocyanea* Zhou et al., 2009). Species assigned to the *Arthrobacter nicotianae* group were *Arthrobacter nicotianae*, *Arthrobacter protophormiae*, *Arthrobacter uratoxydans*, and *Arthrobacter sulfureus.* Due to the absence of chemotaxonomic data, several other species were treated as *species incertae sedis*, including *Arthrobacter mysorens* (phylogenetically this species is related to the *Arthrobacter nicotianae* group; Figure 137); *Arthrobacter picolinophilus* [according to Koch et al. (1995) this species is a later heterotypic synonym of *Rhodococcus erythropolis*], *Arthrobacter radiotolerans* [reclassified as *Rubrobacter radiotolerans* (Suzuki et al., 1988)]; and *Arthrobacter siderocapsulatus* [according to Chun et al. (2001), this species is a later heterotypic synonym of *Pseudomonas putida*]. Other species showing *meso*- or LL-diaminopimelic acid as the characteristic diamino acid in the peptidoglycan were listed in two addenda: *Arthrobacter duodecanis* [reclassified as *Tetrasphaera duodecanis* (Ishikawa and Yokota, 2006)]; *Arthrobacter variabilis* [reclassified as *Corynebacterium variabilis* (Collins, 1987) and then renamed *Corynebacterium variabile* (Euzéby, 1998)]; *Arthrobacter viscosus* (the type of this species shares 96.9–99.6% similarity with species of the genus *Rhizobium* and hence reclassification as a species of *Rhizobium* is desirable); *Arthrobacter simplex* [reclassified as *Pimelobacter simplex* (Suzuki and Komagata, 1983)]; and *Arthrobacter tumescens* [reclassified as *Terrabacter tumescens* (Collins et al., 1989)].

Based on the amino acid composition of the peptidoglycan interpeptide chain, another grouping of *Arthrobacter* species was proposed by Komagata and Suzuki (1987) which dissected *Arthrobacter* species into seven groups designated Komagata/Suzuki groups I–VII (Table 73).

At present, the genus *Arthrobacter* consists of 64 recognized species. Based upon the EzTaxon server (Chun et al., 2007), with the exception of *Arthrobacter viscosus*, these *Arthrobacter* species

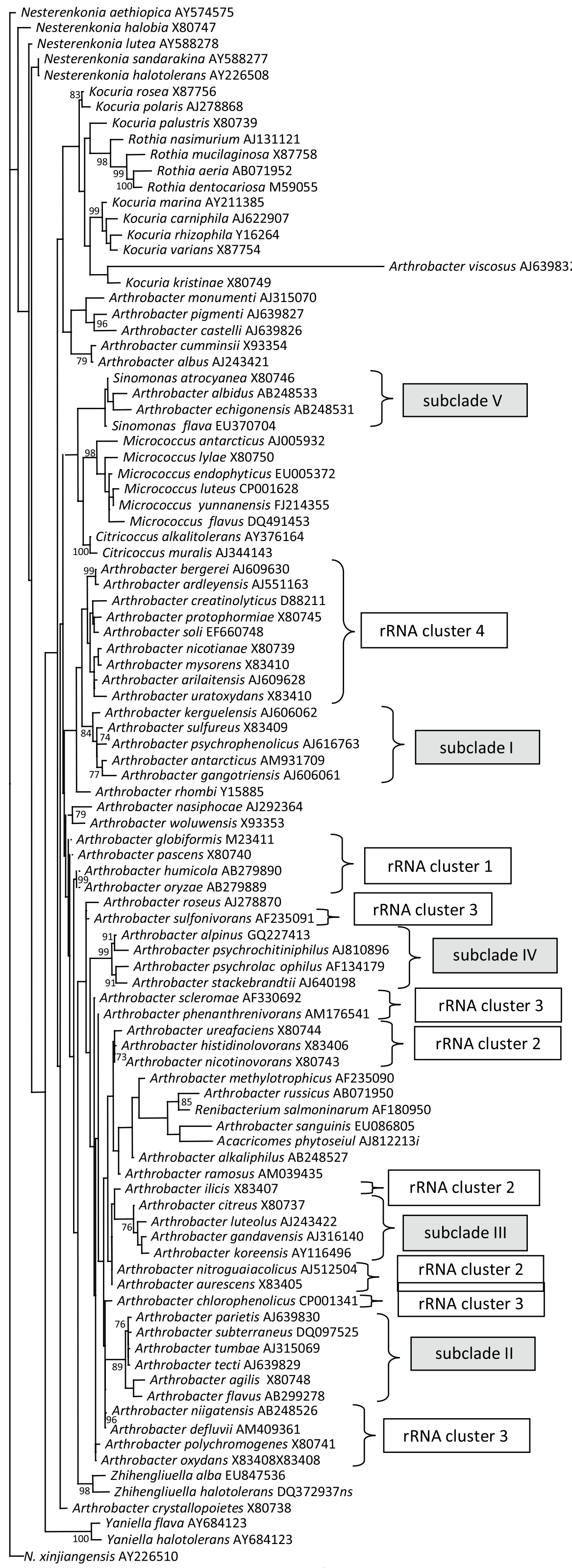

FIGURE 137. Maximum likelihood tree as implemented in the PHYLIP program (Felsenstein, 2009) based on 16S rRNA gene sequences of all recognized *Arthrobacter* species. Sequences were aligned using CLUSTAL_X (Thompson et al., 1997) and manually edited to remove gaps and ambiguous nucleotides in BIOEDIT (Hall, 1999). Bootstrap values of 70% and higher are shown at the nodes. Percentages are based on 100 resamplings. Bar = 10% sequence divergence.

TABLE 73. Classification of *Arthrobacter* species based on the composition of the interpeptide bridge of the peptidoglycan (Komagata and Suzuki, 1987)[a]

Komagata/ Suzuki group	Amino acid composition of the interpeptide bridge	Species
I	Lys–Ser–Thr–Ala	*A. oxydans*, *A. polychromogenes*
II	Lys–Ala–Thr–Ala	*A. aurescens*, *A. histidinolovorans*, *A. ilicis*, *A. nicotinovorans*, *A. ureafaciens*
III	Lys–$Ala_{1–4}$	*A. crystallopoietes*, *A. globiformis*, *A. pascens*, *A. ramosus*
IV	Lys–Ser–$Ala_{2–3}$	*Sinomonas atrocyanea* (formerly *A. atrocyaneus*)
V	Lys–Thr–Ala_2	*A. citreus*
VI	Lys–Ala–Glu	*A. nicotianae*, *A. creatinolyticus*, *A. uratoxydans*, *A. protophormiae*
VII	Lys–Glu	*A. sulfureus*

[a]Abbreviations: Lys, lysine; Ser, serine; Thr, threonine; Ala, alanine; Glu, glutamic acid.

share 16S rRNA gene sequence similarities of 94.4–99.3% with the type strain of the genus, *Arthrobacter globiformis*. Several species of related genera such as *Acaricomes*, *Brachybacterium*, *Citricoccus*, *Kocuria*, *Micrococcus*, *Nesterenkonia*, *Rothia*, *Sinomonas*, and *Zhihengliuella* also possess sequence similarities with *Arthrobacter globiformis* within this range. These observations indicate the heterogeneity of the genus *Arthrobacter*. Hence, identification of novel strains at the genus level only based on 16S rRNA gene sequence similarities are ambiguous if a high degree of similarity is not found with *Arthrobacter globiformis*. Also phylogenetic examinations indicate that *Arthrobacter* is a polyphyletic genus. Several *Micrococcaceae* genera, such as *Micrococcus*, *Citricoccus*, *Sinomonas*, *Acaricomes*, and *Renibacterium*, are included in the clade comprising all *Arthrobacter* species and separate the genus into several subclades (Figure 137). However, the majority of branching points in the phylogenetic tree calculated applying the maximum-likelihood (Figure 137) or neighbor-joining algorithms (not shown) are not supported by significant bootstrap values (>75%), indicating that the phylogenetic relationships among numerous *Arthrobacter* species remain ambiguous at this time.

Species of the *Arthrobacter globiformis*/*Arthrobacter citreus* group (Keddie et al., 1986) are distributed throughout the phylogenetic tree. Although not supported by significant bootstrap values, in many trees *Arthrobacter globiformis* and *Arthrobacter pascens* appear on a common branch, and *Arthrobacter ureafaciens* and *Arthrobacter histidinolovorans* appear on another (Figure 137). The remaining species of the *Arthrobacter globiformis*/*Arthrobacter citreus* group are distributed throughout phylogenetic trees and do not appear to be closely related to each other. For this reason, this group is probably artificial, and it is not used further in this chapter. In contrast, species assigned to the *Arthrobacter nicotianae* group form a distinct clade separate from other *Arthrobacter* species but without significant bootstrap support.

In the tree consisting of all *Arthrobacter* species and representatives of all genera assigned to the family *Micrococcaceae*, five subclades (which may be considered as genus-like clusters) can be identified. These subclades all have at least moderate bootstrap support. Subclade I is composed of *Arthrobacter antarcticus*, *Arthrobacter gangotriensis*, *Arthrobacter kerguelensis*, *Arthrobacter psychrophenolicus*, and *Arthrobacter sulfureus* with 84% bootstrap support. Since this subclade contains *Arthrobacter sulfureus*, the only representative of Komagata/Suzuki group VII, the other four species may also be assigned to this group. In fact, the peptidoglycan types of *Arthrobacter antarcticus*, *Arthrobacter gangotriensis*, *Arthrobacter kerguelensis*, and *Arthrobacter psychrophenolicus* support this assignment. Subclade II comprises six species, *Arthrobacter agilis*, *Arthrobacter flavus*, *Arthrobacter parietis*, *Arthrobacter subterraneus*, *Arthrobacter tecti*, and *Arthrobacter tumbae* with 89% bootstrap support. Subclade III is formed by *Arthrobacter citreus* (originally assigned to the *Arthrobacter globiformis*/*Arthrobacter citreus* group Keddie et al., 1986) or Komagata/Suzuki group V (Komagata and Suzuki, 1987), *Arthrobacter luteolus*, *Arthrobacter gandaviensis*, and *Arthrobacter koreensis* with 76% bootstrap support. A very stable subclade IV with 99% bootstrap support consists of *Arthrobacter alpinus*, *Arthrobacter psychrochitiniphilus*, *Arthrobacter psychrolactophilus*, and *Arthrobacter stackebrandtii*. Subclade V (100% bootstrap support) is composed of the two described *Sinomonas* species *Sinomonas flava* and *Sinomonas atrocyanea* (formerly named *Arthrobacter atrocyaneus* and assigned to *Arthrobacter globiformis*/*Arthrobacter citreus* group and Komagata/Suzuki group IV (Zhou et al., 2009), *Arthrobacter albidus*, and *Arthrobacter echigonensis*. The type species of the genus, *Arthrobacter globiformis*, is not assigned to any of these subclades or any other groups of species whose relatedness is supported by high bootstrap values (>75%), including *Arthrobacter humicola*/*Arthrobacter oryzae*, *Arthrobacter pigmenti*/*Arthrobacter castelli*, *Arthrobacter defluvii*/*Arthrobacter niigatensis*, *Arthrobacter bergerei*/*Arthrobacter ardleyensis*, and *Arthrobacter albus*/*Arthrobacter cumminsii*.

Several groups composed of species sharing high similarities (>97%) in their 16S rRNA gene sequences can also be identified. These rRNA similarity groups are not confirmed upon phylogenetic analyses and are here designated rRNA clusters, which distinguished them from the subclades. rRNA cluster 1 comprises *Arthrobacter globiformis*, *Arthrobacter pascens*, *Arthrobacter humicola*, and *Arthrobacter oryzae*, which share 98.4–99.5% sequence similarity. Two species of this cluster are also classified in the *Arthrobacter globiformis*/*Arthrobacter citreus* group and Komagata/Suzuki group III. Species assigned to rRNA cluster 2 are *Arthrobacter histidinolovorans*, *Arthrobacter nicotinovorans*, *Arthrobacter ureafaciens*, *Arthrobacter ilicis*, *Arthrobacter aurescens*, and *Arthrobacter nitroguajacolicus*; and sequence similarities are in the range 97.6–99.7%. This cluster contains species of the *Arthrobacter globiformis*/*Arthrobacter citreus* group and all species assigned to Komagata/Suzuki group II. rRNA cluster 3 comprises *Arthrobacter chlorophenolicus*, *Arthrobacter sulfonivorans*, *Arthrobacter oxydans*, *Arthrobacter defluvii*, *Arthrobacter polychromogenes*, *Arthrobacter scleromae*, *Arthrobacter niigatensis*, and *Arthrobacter phenanthrenivorans*, with sequence similarities in the range of 97.3–99.7%. The cluster contains one species assigned to the *Arthrobacter globiformis*/*Arthrobacter citreus* group and two species assigned to Komagata/Suzuki group I. rRNA cluster 4 species share 96.7–99.7% sequence similarity. This cluster contains the species *Arthrobacter arilaitensis*, *Arthrobacter ardleyensis*, *Arthrobacter bergerei*, *Arthrobacter creatinolyticus* *Arthrobacter mysorens*, *Arthrobacter nicotianeae*, *Arthrobacter protophormiae*, *Arthrobacter soli*, and *Arthrobacter uratoxydans*. Three of

these species were originally in the *Arthrobacter nicotianae* group and Komagata/Suzuki group VI. Notably, *Arthrobacter bergerei* shares high sequence similarity of 96.6–97.0% with some subclade I species, and subclade I species *Arthrobacter antarcticus*, *Arthrobacter psychrophenolicus*, and *Arthrobacter kerguelensis* also share sequence similarity values in the range 96.7–97.1% with some rRNA cluster 4 species.

In a study on genetic diversity among *Arthrobacter* species collected from terrestrial deep-subsurface sediments, partial *recA* gene sequences were obtained from strains of 14 *Arthrobacter* species (van Waasbergen et al., 2000). Unfortunately, in this study only 360 nucleotides of each strain were analyzed, and hence the deduced phylogeny is of limited statistical significance. However, these phylogenetic analyses demonstrated a close relationship between these groups of *Arthrobacter* species, *Arthrobacter sulfureus/Arthrobacter uratoxydans/Arthrobacter protophormiae/Arthrobacter nicotianae*, *Arthrobacter pascens/Arthrobacter globiformis*, *Arthrobacter aurescens/Arthrobacter nicotinovorans/Arthrobacter histidinolovorans/Arthrobacter ureafaciens*, and *Arthrobacter oxydans/Arthrobacter polychromogenes*, supporting the 16S rRNA based phylogeny. The low sequence similarity of the *Arthrobacter agilis* and *Arthrobacter citreus* genes to those from the other species also agrees with the findings of 16S rRNA studies. Interestingly, two species of *Micrococcus*, *Micrococcus luteus* and *Micrococcus lylae*, were clearly distinguished from all of the *Arthrobacter* species examined. In contrast, the 16S rRNA gene trees do not clearly distinguish these species from *Arthrobacter* (Figure 137). In other studies, the *recA* gene phylogeny also suggests a high degree of relatedness between *Arthrobacter phenanthrenivorans*, *Arthrobacter oxydans*, and *Arthrobacter polychromogenes*, which is similar to the 16S rRNA phylogeny (Kallimanis et al., 2009). Lastly, on the basis of its *recA* sequence, *Arthrobacter chlorophenolicus* does not appear to be closely related to other *Arthrobacter* species.

Chemotaxonomy. Fatty acid profiles of *Arthrobacter* species are composed predominantly of iso- and anteiso-branched fatty acids. The majority of species exhibit fatty acid profiles dominated by $C_{15:0}$ anteiso and possessing usually high amounts of $C_{15:0}$ iso, $C_{16:0}$ iso, and $C_{17:0}$ anteiso. Several species also possess significant amounts of $C_{16:0}$, including *Arthrobacter citreus*, *Arthrobacter nicotianae*, *Arthrobacter cumminsii*, *Arthrobacter niigatensis*, *Arthrobacter oxydans*, and *Arthrobacter polychromogenes*. However, the presence of this fatty acid is not correlated with genetic relatedness. Moreover, *Arthrobacter russicus* possesses an unusual fatty acid profile dominated by $C_{17:0}$ anteiso, but this trait has not been reported in its closest relative *Renibacterium salmonniarum*, which also possesses large amounts of $C_{15:0}$ anteiso (Embley et al., 1983). Therefore, while the fatty acid data may be useful for characterization and identification of *Arthrobacter* species, it does not correlate well with phylogenetic relationships between closely related species.

Arthrobacter species can be distinguished into two major groups based on the presence of monosaturated or completely unsaturated quinones. The first group includes the majority of *Arthrobacter* species, which possess monosaturated menaquinone with nine isoprenoic units in the side chain, MK-9(H_2). This group also includes a smaller number of species with predominantly MK-8(H_2) and corresponds to the polyphyletic *Arthrobacter globiformis/Arthrobacter citreus* group of Keddie et al. (1986). The second group includes several *Arthrobacter* species that exhibit menaquinones with eight, nine, and/or ten completely unsaturated isoprenoic units (MK-8, MK-9, and MK-10) and corresponds to the *Arthrobacter nicotianae* group (Keddie et al., 1986). Since the designation of these groups, all newly proposed *Arthrobacter* species with completely unsaturated menaquinones have proven to be phylogenetically related to the *Arthrobacter nicotianae* group. Species of the *Arthrobacter nicotianae* group (Keddie et al., 1986) whose menaquinone system have been analyzed include *Arthrobacter nicotianae*, *Arthrobacter creatinolyticus*, *Arthrobacter protophormiae*, *Arthrobacter sulfureus*, *Arthrobacter gangotriensis*, *Arthrobacter psychrophenolicus*, *Arthrobacter antarcticus*, and *Arthrobacter kerguelensis*. Unfortunately, the menaquinones of many related species of this group, such as *Arthrobacter arilaitensis*, *Arthrobacter bergerei*, *Arthrobacter mysorens*, *Arthrobacter rhombi*, and *Arthrobacter soli*, have not been examined. However, it would not be surprising if these latter species also possess completely unsaturated quinones.

Several *Arthrobacter* species exhibit quinone systems unusual for the genus. *Arthrobacter koreensis* (subclade III) was reported to have a quinone system with almost equal amounts (43:35) of MK-8(H_2) and MK-9(H_2). Other members of subclade III, *Arthrobacter luteolus* and *Arthrobacter citreus*, were reported to contain a different ratio of MK-8(H_2) and MK-9(H_2), 22:68 and 30:67, respectively (Collins and Kroppenstedt, 1983; Lee et al., 2003), which might reflect their close relatedness. Another quinone system with also almost equal amounts of MK-9(H_2) and MK-8(H_2), a ratio of 56:44, was reported for *Arthrobacter nasiphocae* (Collins et al., 2002a), which was not placed in any of the subclades above. *Arthrobacter albus* and *Arthrobacter cumminsii*, which are phylogenetically quite distant from the type species of the genus (Figure 137), exhibit the major menaquinone MK-8(H_2) (Busse, unpublished results). Species of subclade III, *Arthrobacter parietis*, *Arthrobacter subterraneus*, *Arthrobacter tecti*, and *Arthrobacter tumbae* share a quinone system with largely MK-9(H_2) and significant amounts (>20%) of MK-10(H_2), but this quinone system is also found in the distant relative *Arthrobacter stackebrandtii*. A quinone system most unusual among arthrobacters and many other bacteria was reported for *Arthrobacter scleromae*. It consists of unsaturated and saturated menaquinones of MK-8(H_2) and minor amounts of MK-10 (ratio of 88:12) (Huang et al., 2005b). Likewise, the quinone system of *Arthrobacter phenanthrenivorans* consists of MK-8 and MK-9(H_2) in a ratio 3.6:1 (Kallimanis et al., 2009). Because quinone systems composed of monosaturated and completely unsaturated menaquinones with isoprenoic side chains of different lengths are most unusual among bacteria, re-analyses would be desirable to confirm these two quinone systems.

The peptidoglycans of *Arthrobacter* species contain the diagnostic diamino acid lysine. Applying the scheme of Schleifer and Kandler (1972), differences in the amino acid composition of the interpeptide chains distinguish *Arthrobacter* species into two major groups. The majority of species exhibit peptidoglycan type A3α, which possess monocarboxylic L-amino acids or glycine or both in the interpeptide bridge. *Arthrobacter* species with type A3α peptidoglycan can be distinguished based on the amino acid composition in the interpeptide bridge. *Arthrobacter* species of subclade II and III are characterized by an interpeptide bridge consisting of Lys–Thr–$Ala_{2–3}$. Subclade II species *Arthrobacter agilis*, *Arthrobacter flavus*, *Arthrobacter subterraneus*, *Arthrobacter tecti*, and *Arthrobacter tumbae* possess Lys–Thr–Ala_3,

while *Arthrobacter parietis* possesses Lys–Thr–Ala_2. Subclade III species (*Arthrobacter citreus*, *Arthrobacter gandavensis*, *Arthrobacter koreensis*, and *Arthrobacter luteolus*) also possess Lys–Thr–Ala_2. Other species, *Arthrobacter psychrolactophilus* and *Arthrobacter alpinus* grouped in subclade IV, are also characterized by an interpeptide bridge of Lys–Thr–Ala_3. This interpeptide bridge may also be present in *Arthrobacter psychrochitiniphilus*, whose composition is known to be Lys, Thr, and Ala.

Species of *Arthrobacter* rRNA cluster 2 (*Arthrobacter aurescens*, *Arthrobacter histidinolovorans*, *Arthrobacter ilicis*, *Arthrobacter nicotinovorans*, *Arthrobacter nitroguajacolicus*, and *Arthrobacter ureafaciens*) exhibit an interpeptide bridge of Lys–Ala–Thr–Ala. Another similar interpeptide bridge (Lys–Ser–Thr–Ala) is found in *Arthrobacter chlorophenolicus*, *Arthrobacter sulfonivorans*, *Arthrobacter oxydans*, *Arthrobacter defluvii*, *Arthrobacter polychromogenes*, *Arthrobacter scleromae*, and probably also *Arthrobacter niigatensis* and *Arthrobacter alkaliphilus*. For the latter two species only the predominant amino acids of Lys, Ser, Thr, and Ala were reported (Ding et al., 2009). Except *Arthrobacter alkaliphilus*, which shares highest 16S rRNA gene similarity with *Arthrobacter methylotrophus* (97.4%), these species are grouped in rRNA cluster 3. The peptidoglycan of *Arthrobacter albidus* and *Arthrobacter echigonensis* is composed of the amino acids Lys, Ser, and Ala (Ding et al., 2009), which is consistent with a peptidoglycan interpeptide bridge of Lys–Ser–Ala_x and placement of these two species in subclade V together with the species of the genus *Sinomonas*.

Among arthrobacters, *Arthrobacter nasiphocae* and *Arthrobacter roseus* possess a unique interpeptide bridge composed of Lys–Ala_2–$Gly_{2–3}$–Ala (Gly) and Lys–Gly–Ala_3, respectively, which might reflect their distant phylogenetic relationship to other *Arthrobacter* species. The presence of serine in the interpeptide bridge of *Arthrobacter castelli* (Lys–Ala–Ser–Ala_3) distinguishes this species from its closest relative *Arthrobacter pigmenti*, which possesses Lys–Ala_4 in the interpeptide chain. Nevertheless, the presence of four alanine residues in the interpeptide bridges of both *Arthrobacter castelli* and *Arthrobacter pigmenti* may support the close relatedness of the two species. Other *Arthrobacter* species with interpeptide bridges composed of only alanine in addition to lysine are distributed throughout the genus. These species are *Arthrobacter crystallopoietes* (Lys–Ala), *Arthrobacter pascens* (Lys–Ala_2), *Arthrobacter stackebrandtii* (Lys–Ala_2), *Arthrobacter humicola* (Lys–$Ala_{>2}$), *Arthrobacter oryzae* (Lys–$Ala_{>2}$), *Arthrobacter globiformis* (Lys–Ala_3), *Arthrobacter russicus* (Lys–Ala_2), *Arthrobacter methylotrophus* (Lys–$Ala_{2–4}$), *Arthrobacter monumenti* (Lys–Ala_4), *Arthrobacter pigmenti* (Lys–Ala_4), and *Arthrobacter ramosus* (Lys–Ala_4). *Arthrobacter globiformis*, *Arthrobacter humicola*, *Arthrobacter oryzae*, and *Arthrobacter pascens* are grouped in rRNA cluster 1, and this grouping is supported by the intepeptide composition. In contrast, 16S rRNA analyses do not suggest assignment of the remaining species to rRNA cluster 1. Instead, relatedness to species with different interpeptide bridge compositions is indicated. Hence, this type of interpeptide bridge appears to be less significant for grouping of *Arthrobacter* than other interpeptide bridge structures.

The other major peptidoglycan type found in *Arthrobacter* species is A4α, which contains glutamic acid or glutamic acid and alanine in the interpeptide bridge. It is found in all species in the *Arthrobacter nicotianae* group (species of subclade I and rRNA cluster 4) and in *Arthrobacter rhombi*. Many phylogenetic trees place *Arthrobacter rhombi* at the root of the *Arthrobacter nicotianae* group, but bootstrap support is lacking for assignment to this group (Figure 137). Nevertheless, the type of interpeptide bridge Lys–Ala–Glu supports its affiliation with rRNA cluster 4. Species of subclade I, *Arthrobacter sulfureus*, *Arthrobacter antarcticus*, *Arthrobacter gangotriensis*, *Arthrobacter psychrophenolicus*, and *Arthrobacter kerguelensis*, possess an interpeptide bridge of Lys–Glu. Peptidoglycan type A4α with L-glutamic acid in the interpeptide bridge is also present in *Arthrobacter cumminsii* and *Arthrobacter albus*, which together occupy a phylogenetically distinct branch within the radiation of *Arthrobacter* species (Figure 137). Another species with peptidoglycan type A4α is *Arthrobacter woluwensis*. In this case, the dicarboxylic acid in the interpeptide bridge is D-aspartic acid, which (so far) is a unique trait within the genus *Arthrobacter* but this trait was already reported in *Micrococcus flavus* and a strain of *Micrococcus luteus* (Liu et al., 2007; Wieser et al., 2002).

Cell-wall sugars have been analyzed for approximately half of *Arthrobacter* species, and so far 14 different sugar compositions have been reported. The majority of species possess galactose (Gal) solely or in combination with other sugars, such as glucose (Glc), rhamnose (Rha), mannose (Man), ribose (Rib), and xylose (Xyl). Gal alone is present in the cell walls of *Arthrobacter tumbae*, *Arthrobacter parietis*, and *Arthrobacter citreus*. The cell walls of the type species of the genus, *Arthrobacter globiformis*, and its close relative *Arthrobacter pascens* contain Gal and Glc, and these sugars are also found in *Arthrobacter oryzae*, *Arthrobacter crystallopoietes*, *Arthrobacter histidinolovorans*, *Arthrobacter oxydans*, *Arthrobacter scleromae*, *Arthrobacter nicotianae*, and *Arthrobacter sulfureus*. *Acaricomes phytoseiuli*, which is phylogenetically placed within the radiation of the genus *Arthrobacter*, also possesses these sugars. *Arthrobacter tecti*, *Arthrobacter aurescens*, *Arthrobacter alpinus*, and *Arthrobacter ureafaciens* possess Gal and minor amounts of Man. *Arthrobacter humicola* and *Arthrobacter pigmenti* possess Gal and Rha in their cell walls. The cell wall of *Arthrobacter defluvi* contains Gal, Glc, and Rha. The cell walls of *Arthrobacter subterraneus* and *Arthrobacter flavus* contain Gal, Glc, and Rib. The cell walls of *Arthrobacter ramosus* and *Arthrobacter ilicis* possess Gal, Man, and Rha.The cell walls of *Arthrobacter monumenti* and *Arthrobacter castelli* possess minor amounts of Xyl and Rha in addition to Gal. Four cell-wall sugars, Gal, Glc, Rib, and Rha were detected in *Arthrobacter roseus*, and Gal, Glc, Man, and Rib were found in *Arthrobacter arilaitensis*. Several species lack Gal in the cell wall. Only one cell-wall sugar was detected in *Arthrobacter psychrophenolicus* (Glc) and *Arthrobacter koreensis* and *Arthrobacter luteolus* (Rha). *Arthrobacter agilis* is the sole *Arthrobacter* species which contains glucosamine (GlcN). Three cell-wall sugars, Glc, Man, and Rib, are present in *Arthrobacter bergerei*. Since the cell-wall sugar composition varies among closely related *Arthrobacter* species or certain sugar compositions are found in different subclades/rRNA clusters, apparently this feature is more suitable for characterization of species rather than for identification of closely related groups.

In a study of distribution and composition of teichoic acids, Fiedler and Schäfler (1987) showed that these components are found in the cell walls of A4α peptidoglycan type arthrobacters (*Arthrobacter nicotianae* group *sensu* Keddie et al., 1986), but

not in other representatives of the genus. The teichoic acids of *Arthrobacter nicotianae* are composed of glycerol, glucose, and glucosamine. A qualitatively similar teichoic acid composition is also present in a strain of *Arthrobacter mysorens*, whereas *Arthrobacter protophormiae* and *Arthrobacter sulfureus* also possess galactosamine. It might be assumed that the teichoic acids contribute to the cell-wall sugars, but surprisingly the glucosamine of the teichoic acids of *Arthrobacter nicotianae* and *Arthrobacter sulfureus* was not reported among the cell-wall sugars (Stackebrandt et al., 1983b). Unfortunately, the teichoic acids of only a very limited number of species have been analyzed. Hence, the importance of this tool for classification is unclear.

Information regarding polar lipid composition of arthrobacters is also rather limited. All *Arthrobacter* species which have been examined possess phosphatidylglycerol and diphosphatidylglycerol. In addition, phosphatidylinositol and several glycolipids may be present. In an early study, Walker and Bastl (1967) identified the glycolipids of *Arthrobacter globiformis* strain 616 as dimannosyl-diglyceride, monogalactosyl-diglyceride, and digalactosyl-diglyceride. They also found some evidence for the presence of small amounts of a trimannosyl diglyceride and an even smaller quantity of a tetramannosyl-diglyceride. In a following study, Shaw and Stead (1971) analyzed the lipid composition of the type strains of *Arthrobacter crystallopoietes* and *Arthrobacter pascens* as well as *Arthrobacter globiformis* strain 616. The polar lipid profile of *Arthrobacter crystallopoietes* was composed of phosphatidylglycerol, diphosphatidylglycerol, phosphatidylinositol, dimannosyl-diglyceride, monogalactosyl-diglyceride, digalactosyl-diglyceride, and a fourth glycolipid supposed to be tetramannosyl-diglyceride. No significant differences were reported for *Arthrobacter pascens* and *Arthrobacter globiformis*. These data confirm the close phylogenetic relatedness of *Arthrobacter globiformis* and *Arthrobacter pascens* and may imply relatedness to *Arthrobacter crystallopoietes*, which does not occupy a stable position in phylogenetic trees. The polar lipid profile of *Arthrobacter ilicis* consists of phosphatidylglycerol, diphosphatidylglycerol, phosphatidylinositol, and two unidentified glycolipids G_A and G_B (Collins et al., 1981). The polar lipids of the type strains of *Arthrobacter aurescens*, *Arthrobacter citreus*, *Arthrobacter crystallopoietes*, *Arthrobacter globiformis*, and *Arthrobacter polychromogenes* also comprise phosphatidylglycerol, diphosphatidylglycerol, phosphatidylinositol, and varying numbers of unidentified glycolipids (Collins et al., 1982a). The number of *Arthrobacter globiformis* glycolipids (although not analyzed for chemical structure) was in agreement with the number in previous reports. Collins et al. (1982a) detected only three glycolipids in *Arthrobacter cyrstallopoietes*, but already Shaw and Stead (1971) mentioned the low amounts of a fourth glycolipid in this species. *Arthrobacter aurescens* and *Arthrobacter polychromogenes* showed three unidentified glycolipids, whereas in *Arthrobacter citreus* only two glycolipids were detected.

Collins and Kroppenstedt (1983) investigated the polar lipid profiles of the type strains of *Arthrobacter citreus*, *Arthrobacter nicotianae*, *Arthrobacter protophormiae*, *Arthrobacter sulfureus* (formerly *Brevibacterium sulfureus*) and found polar lipid profiles consisting of phosphatidylglycerol, diphosphatidylglycerol, and unidentified glycolipids, either G_a or G_b. These two glycolipids showed chromatographic behaviors of diglycosyldiacylglycerol, and their staining behavior indicated the presence of galactose and/or mannose residues. Unidentified glycolipid G_a was detected in *Arthrobacter nicotianae*, *Arthrobacter protophormiae*, and *Arthrobacter citreus*, whereas G_b was found in *Arthrobacter sulfureus*. *Arthrobacter citreus* was the only species of this group which possessed phosphatidylinositol. The distribution of G_a and G_b among species of the *Arthrobacter nicotianae* group, here represented by *Arthrobacter nicotianae*, *Arthrobacter protophormiae* and *Arthrobacter sulfureus*, is in accordance with the subdivision of this group based on phylogeny and peptidoglycan type (Lys–Ala–Glu or Lys–Glu). The lipids of *Arthrobacter psychrophenolicus*, placed with *Arthrobacter sulfureus* in subclade I (Figure 137), are phosphatidylglycerol, diphosphatidylglycerol, phosphatidylinositol, and an unidentified glycolipid (Margesin et al., 2004). Unfortunately, no information is provided concerning the chromatographic behavior of this unidentified glycolipid which would allow a comparison to G_b. However, the presence of phosphatidylinositol distinguishes *Arthrobacter psychrophenolicus* from its close relative *Arthrobacter sulfureus*. The unidentified glycolipid G_A of *Arthrobacter ilicis* shows a chromatographic behavior similar to G_a mentioned above, whereas the chromatographic behavior of G_B of *Arthrobacter ilicis* suggests that it is different from G_b. *Arthrobacter russicus* is characterized by the predominant lipids diphosphatidylglycerol and phosphatidylinositol and lesser amounts of phosphatidylglycerol (Li et al., 2004c). In addition to possessing phosphatidylglycerol, diphosphatidylglycerol, and phosphatidylinositol, *Arthrobacter castelli*, *Arthrobacter monumenti* and *Arthrobacter pigmenti* possess one unidentified phospholipid, and one unidentified glycolipid; *Arthrobacter parietis* possesses one unidentified phospholipid and two unidentified glycolipids; and *Arthrobacter tecti* and *Arthrobacter tumbae* possess one unidentified phospholipid (Heyrman et al., 2005). *Arthrobacter flavus*, *Arthrobacter roseus*, and *Arthrobacter phenanthrenivoras* were reported to possess phosphatidylglycerol, diphosphatidylglycerol, and phosphatidylethanolamine (Kallimanis et al., 2009; Reddy et al., 2000; Reddy et al., 2002). Phosphatidylethanolamine is rare among species of *Arthrobacter* and related genera as is the absence of phosphatidylinositol. Re-analysis of the polar lipid profile did not provide any indication of the presence of phosphatidylethanolamine in the type strain of *Arthrobacter roseus*. Like other arthrobacters, the type strain of *Arthrobacter roseus* had a polar lipid profile containing predominantly diphosphatidylglycerol, phosphatidylglycerol, phosphatidylinositol, and an unidentified glycolipid (Busse, unpublished results). Nevertheless, the variability in polar lipid composition suggest that, while this trait is useful for classification of arthrobacters, current data are not complete enough to demonstrate that the distribution of polar lipids is dependent on phylogenetic relatedness.

Information concerning polyamine patterns, which are often useful for classification of bacteria, is limited. Altenburger et al. (2002) analyzed polyamines of 13 *Arthrobacter* species, but the results were not very useful for classification. Spermidine was the most abundant polyamine in most species. While significant differences in the overall polyamine content were detected, the polyamine content was not in accordance with relationships deduced from phylogeny or other chemotaxonomic markers.

Pathogenicity. Although soil is considered the major habitat of arthrobacters, strains have been also isolated from clinical specimens and may have been previously identified as CDC coryneform group B-1 and B-3 (Funke et al., 1997b). Thus, several *Arthrobacter* strains now have been reported to be associated with human diseases.

Arthrobacter cumminsii strains were isolated from urine, a skin infection (Funke et al., 1996), a urinary tract infection, chronic otorrhea, infected amniotic fluid, a vaginal swab, calcaneus osteomyelitis, external otitidis, a deep tissue infection of the upper leg, chronic cervicitis, a blood culture, and a leg wound (Funke et al., 1998). A strain of *Arthrobacter woluwensis* was isolated from blood of an HIV-infected patient hospitalized with fever and chills (Funke et al., 1996) and found to cause a subacute infective endocarditis with involvement of the native mitral valve of an HIV-seronegative injection drug user (Bernasconi et al., 2004) and a catheter-related bacteremia (Shin et al., 2006). Wauters et al. (2000a) reported isolation and characterization of the newly described species *Arthrobacter luteolus* (one strain) and *Arthrobacter albus* (two strains) from an infected surgical wound, and urine and blood from a patient with severe phlebitis, respectively, and detected *Arthrobacter oxydans* (two strains) in blood specimens. The species *Arthrobacter creatinolyticus* was identified in a screening for creatinine-hydrolyzing bacteria from patients with unusually low levels of creatinine in urine samples (Hou et al., 1998), and *Arthrobacter scleromae* was isolated from bloody effusion of a swollen scleroma (Huang et al., 2005b). A bacterial strain associated with a case of Whipple's syndrome in a patient with severe chronic uveitis and systemic inflammatory manifestations was classified within the genus *Arthrobacter* based on partial 16S rRNA gene sequence comparison (Bodaghi et al., 1998). Unnamed *Arthrobacter* strains were recovered from blood cultures or reported to be associated with vaginitis and endophthalmitis (Esteban et al., 1996; Funke et al., 1996). Another unnamed *Arthrobacter* strain was isolated from the blood of a neutropenic patient with acute lymphoblastic leukemia (Hsu et al., 1998).

Among 50 strains of large-colony-forming, whitish-grayish, non-cheese-like-smelling, nonfermentative Gram-stain-positive rods encountered from human clinical specimens, Mages et al. (2008) identified 38 strains of the genus *Arthrobacter*. Fourteen strains isolated from blood, urine, otitis externa, cervix, wound swab, and a tracheal secretion were close relatives of *Arthrobacter cumminsii*. Eleven strains isolated from a blood culture, lung swab after autopsy, eye, vaginal swab, wound swab, or unknown source were closely related to *Arthrobacter oxydans*.

Close relatives of *Arthrobacter aurescens* were recovered from urine and an unknown clinical source. One strain isolated from neck abscess was affiliated with *Arthrobacter oryzae*, two strains related to *Arthrobacter albus* were isolated from blood culture and urine, one strain related to *Arthrobacter protophormiae* was isolated from urine, and some strains with unclear species affiliations were present in specimens from various blood cultures and a hand wound. The novel species *Arthrobacter sanguinis* was also isolated from blood (Mages et al., 2008).

These studies demonstrate that arthrobacters may cause disease in humans and that at least the species *Arthrobacter cumminsii* and *Arthrobacter oxydans* are opportunistic pathogens. Moreover, the pathogenic potential of *Arthrobacter albus*, *Arthrobacter woluwensis*, *Arthrobacter arborescens*, *Arthrobacter oryzae*, *Arthrobacter protophormiae*, and *Arthrobacter sanguinis* should be studied in more detail.

Arthrobacters are generally sensitive to antibiotics. However, only few reports exist on the susceptibility of arthrobacters to antibiotics. Among 24 *Arthrobacter* strains (including the type strains of *Arthrobacter globiformis*, *Sinomonas atrocyanea* [formerly *Arthrobacter atrocyaneus*], *Arthrobacter aurescens*, *Arthrobacter crystallopoietes*, *Arthrobacter cumminsii*, *Arthrobacter histidinolovorans*, *Arthrobacter nicotinovorans*, *Arthrobacter oxydans*, *Arthrobacter pascens*, *Arthrobacter ureafaciens*, *Arthrobacter nicotianeae*, *Arthrobacter protophormiae*, *Arthrobacter uratoxydans*, and *Arthrobacter woluwensis*) tested for susceptibility to 16 antimicrobials (amoxicillin-clavulanic acid, ampicillin, ceftriaxone, cefuroxime, cefalothin, chloramphenicol, ciprofloxacin, clindamycin, erythromycin, gentamicin, imipenem, penicillin G, rifampin, teichoplanin, tetracycline, and vancomycin), only the strain of *Arthrobacter woluwensis* was multidrug resistant. It was only susceptible to teichoplanin, tetracycline, and vancomycin (Funke et al., 1996). The susceptibility patterns of 38 *Arthrobacter* strains was also studied by Mages et al. (2008). Nearly all strains were susceptible to β-lactams, doxycycline, gentamicin, linezolid, rifampin, and vancomycin. Additional strains of *Arthrobacter woluwensis* examined in the latter study were not multidrug resistant, indicating that this trait was not characteristic of the species.

Differentiation of the genus *Arthrobacter* from other genera

Differentiation of the genus *Arthrobacter* from distantly related genera which share phenotypic properties that could lead to misidentifications (e.g. genera of the family *Microbacteriaceae*, *Brevibacterium*, or *Corynebacterium*) can be unambiguously achieved by 16S rRNA gene sequence analyses. Support for phylogenetic relationshhips based on 16S rRNA analyses may be obtained from analyses of the peptidoglycan type, quinone system, and polyamines. Members of the family *Microbacteriaceae* possess exclusively B type peptidoglycan, usually containing ornithine, diaminobutyric acid, or lysine as the diagnostic diamino acid. Completely unsaturated menaquinones with 9–14 isoprenoic units are also useful for differentiation from the majority of *Arthrobacter* species. The polyamine pattern of *Arthrobacter* differentiates it from certain genera, such as *Agrococcus*, *Brevibacterium*, and *Pseudoclavibacter helvolus*, whose polyamine patterns are predominated by spermine, putrescine, or cadaverine (Altenburger et al., 1997; Manaia et al., 2004; Wieser et al., 1999; Zlamala et al., 2002). Differentiation of *Arthrobacter* from other genera of the *Micrococcaceae* is hampered because *Arthrobacter* is not monophyletic and possesses significant phenotypic heterogeneity, especially for chemotaxonomic markers such as the interpeptide chains in the peptidoglycan and quinone systems. Hence, 16S rRNA based phylogeny and sequence similarities do not unambiguously allow identification at the genus level.

The majority of *Arthrobacter* species, except *Arthrobacter cumminsii*, *Arthrobacter albus*, and *Arthrobacter scleromae*, can be distinguished from members of the genus *Micrococcus* by the predominance of MK-7(H_2), MK-8, and/or MK-8(H_2) in the latter genus. Except for *Arthrobacter woluwensis*, which contains L-Lys–D-Asp, no *Arthrobacter* species has a peptidoglycan interpeptide bridge similar to that of *Micrococcus* species, which consists of L-Lys–D-Asp or L-Lys–polymerized peptide subunit (subgroup A2; Schleifer and Kandler, 1972). The only representative of the genus *Acaricomes* contains a MK-10(H_2) quinone system, which has been reported in moderate or low amounts in only a few *Arthrobacter* species.

The MK-9(H_2) quinone system of *Citricoccus* is common in the majority of *Arthrobacter* species, but it distinguishes *Citricoccus* from *Arthrobacter* species with completely unsaturated menaquinones. The peptidoglycan of *Citrobacter* species contains an interpeptide chain composed of Lys–Gly–Glu, which is

not known for *Arthrobacter*, and allows a reliable differentiation between the two genera. *Kocuria* species form a rather stable rRNA gene clade within the *Micrococcaceae*, and phylogenetic analyses can differentiate this genus. The majority of *Arthrobacter* species are also distinguished from *Kocuria* based on the amino acid composition of the interpeptide bridge, which consists of Lys–$Ala_{3–4}$ in *Kocuria*. The same or similar interpeptide bridges are present within *Arthrobacter globiformis* and its close relatives *Arthrobacter pascens*, *Arthrobacter humi*, and *Arthrobacter oryzae* as well as *Arthrobacter crystallopoietes*, *Arthrobacter stackebrandtii*, *Arthrobacter methylotrophicus*, *Arthrobacter russicus*, *Arthrobacter ramosus*, *Arthrobacter monumenti*, and *Arthrobacter pigmenti*. Because of the variability of quinone systems among *Kocuria* species, which includes MK-7(H_2), MK-8(H_2), MK-9(H_2), or combinations of two of these components, this characteristic only distinguishes it from *Arthrobacter* species that possess completely unsaturated menaquinones.

Arthrobacter species with mono-saturated menaquinones can be unambiguously distinguished from *Nesterenkonia* because this genus contains only unsaturated menaquinones (MK-7, MK-8, or a combination of MK-7, MK-8, and MK-9). Differentiation of *Arthrobacter* species with completely unsaturated menaquinones from the majority of *Nesterenkonia* species can be achieved based on the length of the isoprenoic chain. While MK-8 and MK-9 are major components in certain *Arthrobacter* species, they are only reported for *Nesterenkonia flava* and *Nesterenkonia alba* (Luo et al., 2008; Luo et al., 2009). However, these two species have a peptidoglycan containing the interpeptide bridge Lys–Gly–Asp, which is not found in any *Arthrobacter* species. All *Nesteronkonia* species except *Nesteronkonia lacusekhoensis*, which contains a peptidoglycan with Lys–Glu in the interpeptide bridge, can be distinguished from *Arthrobacter* species because the former have an interpeptide bridge of Lys–Gly–Asp or Lys–Gly–Glu.

The peptidoglycan interpeptide bridge consisting of Lys–Gly–Ala (Kusser and Fiedler, 1983) and menaquinone MK-9 of *Renibacterium* distinguishes it from *Arthrobacter*. Also *Rothia* species can be reliably differentiated from *Arthrobacter* because MK-7 is the predominant menaquinone and Lys–Ala is the peptidoglycan interpeptide bridge, the latter only being found in *Arthrobacter crystallopoietes*.

It is harder to differentiate *Arthrobacter* from *Zhihengliuella*. Similar to several *Arthrobacter* species, *Zhihengliuella* possesses a peptidoglycan interpeptide bridge with Lys–Ala–Glu and a quinone system dominated by MK-10 and MK- 9. The presence of the cell-wall sugar tyvelose in *Zhihengliuella* (Tang et al., 2009; Zhang et al., 2007) might be useful for differentiation, but cell-wall sugars have been analyzed only for a limited number of *Arthrobacter* species and the importance of this trait for differentiation has not been substantiated. The problem in differentiation might be related to the proposal of the genus *Zhihengliuella*. This genus was described based on phylogeny of the rRNA gene, but the branching from other taxa had only low bootstrap support (51%), and phenotypic traits distinguishing it from the *Arthrobacter nicotianae* group, which possesses similar or identical quinones and peptidoglycan structure, were provided without considering all the species of the group. For instance, the major fatty acids of *Zhihengliuella* are $C_{15:0}$ anteiso and $C_{15:0}$ iso. For the *Arthrobacter nicotianae* group, only $C_{15:0}$ anteiso was reported as a trait in the latter study. In fact, a number of *Arthrobacter nicotianae* group species contain both $C_{15:0}$ anteiso and $C_{15:0}$ iso as abundant fatty acids, such as *Arthrobacter uratoxydans*, *Arthrobacter protophormiae*, and *Arthrobacter sulfureus* (Funke et al., 1996). Moreover, *Zhihengliuella* species possess high 16S rRNA gene sequence similarities with *Arthrobacter* species from different groups. For instance, the similarities with species of the rRNA cluster 4 are in the range 96.0–97.0%, which is indicative of an affiliation with this rRNA cluster. Hence, the re-evaluation of the taxonomic status of the genus *Zhihengliuella* is desirable. Perhaps, analysis of *recA* or some other housekeeping genes will resolve its taxonomic status more clearly. A possible outcome of these studies may be the transfer of *Arthrobacter* with Lys–Ala–Glu in the interpeptide bridge to the genus *Zhihengliuella*.

Taxonomic comments

As presently defined, the genus *Arthrobacter* comprises species with significant phenotypic variability in their composition of quinones, peptidoglycan, cell-wall sugars and, to a lesser degree, polar lipids. In many cases, groupings based on shared certain chemotaxonomic traits are well supported by comparative 16S rRNA gene sequence analyses, and most intra-group 16S rRNA gene sequence similarities are above 96.0%. These results suggest the genus *Arthrobacter* should be dissected into a number of genera.

Based on phylogeny, 16S rRNA gene sequence similarities, peptidoglycan compositions and/or quinone systems, the following genus-like groups can be defined: "*Arthrobacter globiformis* group" (*Arthrobacter sensu stricto*; corresponding to rRNA cluster 1), "*Arthrobacter aurescens* group" (corresponding to rRNA cluster 2), "*Arthrobacter oxydans* group" (corresponding to rRNA cluster 3), "*Arthrobacter protophormiae* group" (corresponding to rRNA cluster 4), "*Arthrobacter sulfureus* group" (corresponding to subclade I), "*Arthrobacter agilis* group" (corresponding to subclade II), "*Arthrobacter citreus* group" (corresponding to subclade III), "*Arthrobacter psychrolactophilus* group" (corresponding to subclade IV), "*Arthrobacter pigmenti* group", "*Arthrobacter albus/ cumminsii* group", and "*Sinomonas* group" (corresponding to subclade V). Species assigned to the different groups and their major characteristics are listed in Tables 74–84, and their differential characteristics are given in Tables 85–95.

New groups proposed for the genus *Arthrobacter*

"*Arthrobacter globiformis* group" (*Arthrobacter sensu stricto*; rRNA cluster 1). Members of the *Arthrobacter* "*globiformis* group" (Table 74) exhibit 98.4–99.5% 16S rRNA gene similarity, a peptidoglycan type A3α (Lys–Ala_2 or Lys–Ala_3) and menaquinone MK-9(H_2). *Arthrobacter crystallopoietes* is preliminarily assigned to this group because it shares highest 16S rRNA gene sequence similarity (97.6%) with *Arthrobacter globiformis* and possesses a peptidoglycan composed of Lys–Ala. Table 85 lists differential characteristics for this group.

"*Arthrobacter aurescens* group" (rRNA cluster 2). Species of this group (Table 75) share high 16S rRNA similarities (>97.5%), peptidoglycan type A3α (Lys–Ala–Thr–Ala) and the menaquinone MK-9(H_2). Table 86 lists differential characteristics for this group.

"*Arthrobacter oxydans* group" (rRNA cluster 3). Close relatedness of the species of this group is indicated by high 16S rRNA gene similarities (>97%). The peptidoglycan type is A3α

TABLE 74. The "*Arthrobacter globiformis* group" (*Arthrobacter sensu stricto*; rRNA cluster 1) including tentatively assigned species *Arthrobacter crystallopoietes*, *Arthrobacter ramosus*, and *Arthrobacter methylotrophus*)[a,b,c]

	A. globiformis[d]	*A. pascens*[d]	*A. humicola*	*A. oryzae*	*A. crystallopoietes*[d]	*A. ramosus*	*A. methylotrophus*
Peptidoglycan type and interpeptide bridge	A3α, Lys–Ala_3	A3α, Lys–Ala_2	A3α, Lys–$Ala_{>2}$[e]	A3α, Lys–$Ala_{>2}$[e]	A3α, Lys–Ala	A3α, Lys–Ala_4	A3α, Lys–$Ala_{2–4}$
Quinone system	MK-9(H_2)	MK-9(H_2)	MK-9(H_2)	MK-9(H_2)	MK-9(H_2)	MK-9(H_2)	MK-9(H_2), [MK-10(H_2)][f]
Cell-wall sugars	Gal, Glc	Gal, Glc	Gal, Rha	Gal, Glc	Gal, Glc	Gal, Man, Rha	

[a]Abbreviations: Ala, alanine; Asp, aspartic acid; Glu, glutamic acid; Gly, glycine; Lys, lysine; Ser, serine; Thr, threonine; Gal, galactose; Glc, glucose; GlcN, glucosamine; Man, mannose; Rha, rhamnose; Rib, ribose; Xyl, xylose; MK-9(H_2) designates a menaquinone with nine isoprenoic units in the side chain, of which one unit is saturated.

[b]If not indicated otherwise data are taken from the original species description.

[c]98.4–99.5% 16S rRNA gene sequence similarities.

[d]Data from Schleifer and Kandler (1972), Keddie and Cure (1978), Collins and Jones (1981).

[e]Only quantitative amino acid composition of the peptidoglycan was reported (Kageyama et al., 2008). Peptidoglycan type is concluded from the amino acid composition.

[f]Compounds shown in brackets were detected in the range 5–19%.

TABLE 75. The "*Arthrobacter aurescens* group" (rRNA cluster 2)[a,b,c]

	A. aurescens	*A. histidinolovorans*	*A. ilicis*	*A. nicotinovorans*	*A. nitroguajacolicus*	*A. ureafaciens*
Peptidoglycan type and interpeptide bridge[d]	A3α, Lys–Ala–Thr–Ala	A3α, Lys–Ala–Thr–Ala	A3α, Lys–Ala–Thr–Ala	A3α, Lys–Ala–Thr–Ala	A3α, Lys–Ala–Thr–Ala	A3α, Lys–Ala–Thr–Ala
Quinone system[e]	MK-9(H_2)	MK-9(H_2)	MK-9(H_2)	MK-9(H_2)	MK-9(H_2), [MK-8(H_2), MK-10(H_2)][f]	MK-9(H_2)
Cell-wall sugars[g]	Gal, (Man)	Gal, Glc	Gal, Man, Rha		Gal, (Man)	Gal, (Man)

[a]For abbreviations, see footnote to Table 74.

[b]If not indicated otherwise, data are taken from the original species description.

[c]97.6–99.7% 16S rRNA gene sequence similarities.

[d]Data are from Schleifer and Kandler (1972) except *Arthrobacter nicotinovorans* and *Arthrobacter nitroguajaolicus*.

[e]Data are from Collins and Jones (1981) except *Arthrobacter nicotinovorans* and *Arthrobacter nitroguajaolicus*.

[f]Reported to be present in minor amounts.

[g]Sugars listed in parentheses are present only in minor amounts. Data are from Keddie and Cure (1978) except *Arthrobacter nicotinovorans* and *Arthrobacter nitroguajaolicus*.

(Lys–Ser–Thr–Ala). For most species, MK-9(H_2) is the major menaquinone, but MK-8(H_2) predominates in *Arthrobacter scleromae*. In *Arthrobacter phenanthrenivorans* both MK-8 and MK-9(H_2) are abundant (Table 76). Table 87 lists differential characteristics for this group.

"*Arthrobacter protophormiae* group" (rRNA cluster 4). The species of this group (Table 77) possess 16S rRNA gene similarities of 95.5–99.7%. Each species shares at least 96.7% similarity with another species within this group, but most values are higher than 97.5%. Members of this group share the peptidoglycan type A4α (Lys–Ala–Glu) and a quinone system composed of completely unsaturated menaquinones with eight and/or nine isoprenoic units and with MK-8 always predominant. *Arthrobacter rhombi* is assigned to this group based on peptidoglycan type. Table 88 lists differential characteristics for this group.

"*Arthrobacter sulfureus* group" (subclade I). The species in this group (Table 78) share at least 97.2% 16S rRNA gene similarity and form a robust clade supported by high bootstrap values in phylogenetic trees. Members of this group share the peptidoglycan type A4α (Lys–Glu) and a quinone system composed of completely unsaturated menaquinones, with MK-9 predominating but also usually significant amounts of MK-10. Table 89 lists differential characteristics for this group.

"*Arthrobacter agilis* group" (subclade II). Species comprising this group (Table 79) share 97.3–99.6% similarity in the 16S rRNA gene and form a subclade supported by high bootstrap values. The peptidoglycan type is A3α with Lys–Thr–$Ala_{2–3}$. MK-9(H_2) is the predominant menaquinone, but significant amounts of MK-10(H_2) are present in four species of the subclade. Table 90 lists differential characteristics for this group.

"*Arthrobacter citreus* group" (subclade III). Species comprising this group (Table 80) share 16S rRNA gene similarities of 97.6–98.9% and form a subclade supported by moderate bootstrap values. The peptidoglycan type is A3α with Lys–Thr–Ala_2. The quinone system comprises menaquinone MK-9(H_2) or almost equal amounts of MK-8(H_2) and MK-9(H_2). Table 91 lists differential characteristics for this group.

"*Arthrobacter psychrolactophilus* group" (subclade IV). Species comprising this group (Table 81) share >97.0% similarity in the 16S rRNA gene and form a robust clade supported by 99% bootstrap values. The interpeptide bridge of the peptidoglycan is either Lys–Thr–$Ala_{1–3}$ or Lys–Ala_2, and MK-9(H_2) is the predominant menaquinone. Moderate proportions of MK-10(H_2) may be present as well. Species of this group are psychrophilic or psychrotolerant and grow at 0–4°C. It is interesting that in a FASTA sequence comparisons among 28 database entries showing 97.8% or higher similarity with *Arthrobacter psychrochitiniphilus*, 26 sequences were from Antarctica, Spitzbergen, glaciers, or other sources likely to permit low temperature growth (Bai et al., 2006; Cheng and Foght, 2007; Hansen et al., 2007; Reddy et al., 2009; Srinivas et al., 2009). Hence, growth at low temperatures may be another trait of the members of this group. Table 92 lists differential characteristics for this group.

"*Arthrobacter pigmenti* group". The core of this group (Table 82) is represented by *Arthrobacter pigmenti* and *Arthrobacter castelli*, which share 97.9% 16S rRNA gene similarity. *Arthrobacter monumenti* is assigned to this group (96.2% similarity) although it shares higher 16S rRNA gene similarities (but still less than 97.0%) with some other *Arthrobacter* species. However, chemotaxonomic traits of *Arthrobacter monumenti* are in better agreement with those of *Arthrobacter pigmenti* and *Arthrobacter castelli*. *Arthrobacter monumenti* shares with *Arthrobacter pigmenti* and *Arthrobacter castelli* a peptidoglycan with four alanines in the interpeptide chain (Lys–Ala_4 or Lys–Ala–Ser–Ala_3) and the cell-wall sugars Gal and Rha. Notably, the same interpeptide bridge is found in some species of the "*Arthrobacter globiformis* group", including *Arthrobacter ramosus* and *Arthrobacter methylotrophus*. Thus, future studies might combine the "*Arthrobacter globiformis* group" and "*Arthrobacter pigmenti* group" or transfer species between the groups. Table 93 lists differential characteristics for this group.

"*Arthrobacter albus/cumminsii* group". So far, only two species (Table 83) are assigned to this group. They share 99.1% similarity in the 16S rRNA gene and higher sequence similarities with representatives of other genera, such as *Kocuria*, *Citricoccus*, and *Micrococcus*, than with other *Arthrobacter* species. The peptidoglycan type is A4α with Lys–Ala–Glu or Lys–Ser–(Gly)–Glu. The quinone system is MK-8(H_2). Table 94 lists differential characteristics for this group.

"*Sinomonas* group" (subclade V). The two species *Arthrobacter echinogenes* and *Arthrobacter albidus* (Table 84) form a clade with the two species of the genus *Sinomonas* (Zhou et al., 2009). The type strains of the two species share more than 97.0% 16S rRNA gene similarity with at least one of the two species of the genus *Sinomonas* and less than 94.5% similarity with other recognized *Arthrobacter* species. *Arthrobacter albidus* and *Arthrobacter echigonensis* possess Lys, Ser, and Ala in their peptidoglycan, which is consistent with the peptidoglycan type A3α (Lys–Ser–$Ala_{2–3}$) reported for *Sinomonas atrocyanea*. The menaquinone is MK-9(H_2). For these reasons, *Arthrobacter echinogenes* and *Arthrobacter albidus* should be transferred to the genus *Sinomonas*. Table 95 lists differential characteristics for this group.

Species not assigned to the above groups. Due to discrepancies between the 16S rRNA gene sequence and phenotypic data, several species remain ungrouped in this scheme, including *Arthrobacter alkaliphilus*, *Arthrobacter crystallopoietes*, *Arthrobacter methylotrophus*, *Arthrobacter nasiphocae*, *Arthrobacter ramosus*, *Arthrobacter roseus*, *Arthrobacter russicus*, *Arthrobacter sanguinis*, and *Arthrobacter woluwensis*. However, if priority is given to the chemotaxonomic characteristics, several species can be tentatively assigned to certain of the above-mentioned "*Arthrobacter* groups".

Arthrobacter alkaliphilus and *Arthrobacter methylotrophicus* form a separate line of descent within the radiation of the genus *Arthrobacter*. While the high 16S rRNA gene similarity of 97.4% suggest a close relationship, similarities with other species are not much lower, 97.1% to *Arthrobacter nicotinovorans* and 96.9% to *Arthrobacter histidinolovorans*. The peptidoglycan structure of *Arthrobacter alkaliphilus* (Lys–Ser–Thr–Ala) and the quinone system of MK-9(H_2) with significant amounts of MK-10(H_2) distinguishes it from *Arthrobacter methylotrophicus*, which contains Lys–$Ala_{2–4}$ and MK-9(H_2) but no MK-10(H_2). *Arthrobacter nicotinovorans* and *Arthrobacter histidinolovorans* are also distinguishable from *Arthrobacter alkaliphilus* based on peptidoglycan structure and quinone system. The peptidoglycan structure of *Arthrobacter alkaliphilus* is characteristically that of "*Arthrobacter oxydans* group", and hence *Arthrobacter alkaliphilus* is tentatively placed in this group.

Phylogenetic analyses of the rRNA genes of *Arthrobacter crystallopoietes* do not yield a stable assignment to any phylogenetic subclade. It shares 97.6% 16S rRNA gene similarity with the type species of the genus, *Arthrobacter globiformis*, and no other species possesses higher similarity. Sequence similarity values with other species of "*Arthrobacter globiformis* group" (*Arthrobacter sensu stricto*; rRNA cluster 1) are in the range of 96.2–97.1%. The peptidoglycan interpeptide bridge consists of Lys–Ala, which is similar to that of other rRNA cluster 1 species. The cell-wall sugars Gal and Glc are also found in other species of this group. Hence, *Arthrobacter cyrystallopoietes* is tentatively placed in "*Arthrobacter globiformis* group".

Arthrobacter nasiphocea possesses low 16S rRNA gene similarity, 95.5–96.5%, with *Arthrobacter woluwensis*, *Arthrobacter pascens*, *Citricoccus alkalitolerans*, *Arthrobacter oryzae*, *Arthrobacter methylotrophus*, *Arthrobacter nitroguajacolicus*; *Arthrobacter phenanthrenivorans*, *Arthrobacter roseus*, *Arthrobacter humicola*, *Arthrobacter oxydans*, and *Arthrobacter polychromogenes*. Likewise, phylogenetic analyses do not suggest a clear affiliation with any of the *Arthrobacter* groups. The unrelatedness of *Arthrobacter nasiphocea* to other *Arthrobacter* species is also indicated from the peptidoglycan structure (Lys–Ala_2-$Gly_{2–3}$–Ala [Gly]) and quinone system MK-9(H_2) + MK-8(H_2). Hence, *Arthrobacter nasiphocea* might be considered a representative of a novel genus. Table 96 lists differential phenotypic characteristics for this species relative to some other ungrouped species.

In both maximum likelihood and neighbor-joining phylogenetic analyses of the16S rRNA gene, *Arthrobacter methylotrophus* is associated with *Arthrobacter alcaliphilus* but without bootstrap support. The 16S rRNA gene sequences of these two species share 97.4% similarity, but similarities with certain species of "*Arthrobacter globiformis* group", "*Arthrobacter aurescens* group", "*Arthrobacter oxydans* group", and "*Arthrobacter agilis* group" are not much lower, 96.9–97.3%. Hence, 16S rRNA data do not unambiguously indicate an affiliation of *Arthrobacter methylotrophus* to any specific *Arthrobacter* group. It contains the menaquinone MK-9(H_2), which is widely distributed in arthrobacters and not helpful for classification of this species. The peptidoglycan interpeptide bridge is $Ala_{2–4}$ and also similar to those found in

TABLE 76. The "*Arthrobacter oxydans* group" (rRNA cluster 3), including tentatively assigned species *Arthrobacter alkaliphilus*[a,b,c]

	A. chlorophenolicus	*A. defluvii*	*A. niigatensis*	*A. oxydans*[d]	*A. phenanthrenivorans*	*A. polychromogenes*	*A. scleromae*	*A. sulfonivorans*	*A. alkaliphilus*
Peptidoglycan type and interpeptide bridge	A3α, Lys–Ser–Thr–Ala	A3α, Lys–Ser–Thr–Ala	A3α, Lys–Ser–Thr–Ala[e]	A3α, Lys–Ser–Thr–Ala		A3α, Lys–Ser–Thr–Ala[f]	A3α, Lys–Ser–Thr–Ala	A3α, Lys–Ser–Thr–Ala	A3α, Lys–Ser–Thr–Ala[e]
Quinone system[g]	MK-9(H_2), MK-9, [MK-8, MK-11][h]	MK-9(H_2), [MK-8(H_2), MK-7(H_2)][i]	MK-9(H_2), [MK-8(H_2, H_4)]	MK-9(H_2)	MK-8, MK-9(H_2)	MK-9(H_2)[f]	MK-8(H_2), [MK-10]	MK-9(H_2), [MK-10(H_2)]	MK-9(H_2), [MK-10(H_2)]
Cell-wall sugars		Gal, Glc, Rha		Gal, Glc			Gal, Glc		

[a]For abbreviations, see footnote to Table 74.

[b]If not indicated otherwise, data are taken from the original species description.

[c]97.3–99.7% 16S rRNA gene sequence similarities.

[d]Data from Schleifer and Kandler (1972), Keddie and Cure (1978), Collins and Jones (1981).

[e]Only the qualitative amino acid content of the peptidoglycan was reported (Ding et al., 2009). Peptidoglycan type is concluded from the amino acid composition.

[f]Kodama et al. (1992).

[g]Compounds shown in brackets were detected in the range 5–19%.

[h]Ding et al. (2009).

[i]Minor amounts of these two menaquinones were reported.

TABLE 77. The "*Arthrobacter protophormiae group*"(rRNA cluster 4)[a,b,c]

	A. ardleyensis	*A. arilaitensis*	*A. bergerei*	*A. creatinolyticus*	*A. mysorens*	*A. nicotianae*	*A. protophormiae*	*A. rhombi*	*A. soli*	*A. uratoxydans*
Peptidoglycan type and interpeptide bridge	A4α, Lys–Ala–Glu[d]	Lys–Ala–Glu[e]	Lys–Ala–Glu[e]	A4α, Lys–Ala–Glu	A4α, Lys–Ala–Glu[f]	A4α, Lys–Ala–Glu	A4α, Lys–Ala–Glu	A4α, Lys–Ala–Glu		A4α, Lys–Ala–Glu
Quinone system[g]	MK-8, MK-9			MK-8, MK-9		MK-8, MK-9, [MK-7][h]	MK-8, MK-9, [MK-7][h]			MK-8, (MK-7; MK-9)[i]
Cell-wall sugars		Gal, Glc, Man, Rib	Glc, Man, Rib			Gal, Glc				

[a]For abbreviations, see footnote to Table 74.

[b]If not indicated otherwise, data are taken from the original species description.

[c]96.5–99.7% 16S rRNA gene sequence similarities.

[d]Only the quantitative amino acid content of the peptidoglycan was reported (Chen et al., 2005a). Peptidoglycan type is concluded from the amino acid composition.

[e]Only the qualitative amino acid content of the peptidoglycan was reported (Chen et al., 2005a). Peptidoglycan type is concluded from the amino acid composition.

[f]Data are not from the type of the species but from patent strain ATCC 31021 (Stackebrandt et al., 1983b).

[g]Compounds shown in brackets were detected in the range 5–19%.

[h]Data are from Collins and Kroppenstedt (1983).

[i]Quinones in parentheses indicate minor compounds (Bendinger et al., 1992).

TABLE 78. The "*Arthrobacter sulfureus group*" (subclade I)[a,b,c]

	A. antarcticus	*A. gangotriensis*	*A. kerguelensis*	*A. psychrophenolicus*	*A. sulfureus*
Peptidoglycan type and interpeptide bridge	A4α, Lys–Glu	A4α, Lys–Glu	A4α, Lys–Glu	A4α, Lys–Glu	A4α, Lys–Glu
Quinone system[d]	MK-9, MK-8, MK-10[e]	MK-9, MK-10, [MK-8]	MK-9, MK-8, [MK-10]	MK-10 [MK-9]	MK-9 or MK-9, MK-10 [MK-8][f]
Cell-wall sugars	Gal, Glc, Rha			Glc	Gal, Glc

[a]For abbreviations, see footnote to Table 74.

[b]If not indicated otherwise, data are taken from the original species description.

[c]97.2–98.8% 16S rRNA gene sequence similarities.

[d]Compounds shown in brackets were detected in the range 5–19%.

[e]Only major menaquinones reported (Pindi et al., 2010).

[f]Yamada et al. (1976) reported in addition to MK-9 minor amounts of MK-10 for *Arthrobacter sulfureum* AJ 1448 = IAM 1488 (formerly *Brevibacterium sulfureum*). Collins et al. (1979) reported in addition to MK-9 significant amounts of MK-7, MK-8, and MK-10 for the type strain *Brevibacterium sulfureum* c79 (= NCIB 10355). Collins and Kroppenstedt (1983) reported the major menaquinones MK-9 and MK-10 and significant amounts of MK-8 for "*Brevibacterium sulfureum*" NCIB 10355.

TABLE 79. The "*Arthrobacter agilis* group" (subclade II)[a,b,c]

	A. agilis	*A. flavus*	*A. parietis*	*A. subterraneus*	*A. tecti*	*A. tumbae*
Peptidoglycan type and interpeptide bridge	A3α, Lys–Thr–Ala_3	A3α, Lys–Thr–Ala_3	A3α, Lys–Thr–Ala_2	A3α, Lys–Thr–Ala_3	A3α, Lys–Thr–Ala_3	A3α, Lys–Thr–Ala_3
Quinone system[d]	MK-9(H_2), MK-8(H_2)[e,f]	MK-9(H_2)	MK-9(H_2), MK-10(H_2)	MK-9(H_2), MK-10(H_2)	MK-9(H_2), MK-10(H_2), [MK-11(H_2), MK-9]	MK-9(H_2), MK-10(H_2), [MK-11(H_2), MK-7(H_2)]
Cell-wall sugars[g]	GlcN	Gal, Glc, Rib	Gal	Gal, Glc, Rib	Gal, (Man)	Gal

[a]For abbreviations, see footnote to Table 74.

[b]If not indicated otherwise, data are taken from the original species description.

[c]97.3–99.6% 16S rRNA gene sequence similarities.

[d]Compounds shown in brackets were detected in the range 5–19%.

[e]Present in minor amounts.

[f]Recent re-analysis of the quinone system of the type strain of *Arthrobacter agilis* revealed a quinone system composed of 91% MK-9(H_2), 8% MK-8(H_2) and 1% MK-10(H_2) (H.-J. Busse, unpublished results).

[g]Sugars listed in parentheses are present only in minor amounts.

TABLE 80. The "*Arthrobacter citreus* group" (subclade III)[a,bc]

	A. citreus[d]	*A. gandavensis*	*A. koreensis*	*A. luteolus*
Peptidoglycan type and interpeptide bridge	A3α, Lys–Thr–Ala_2	A3α, Lys–Thr–Ala_2	A3α, Lys–Thr–Ala_2	A3α, Lys–Thr–Ala_2
Quinone system[e]	MK-9(H_2), MK-8(H_2)	MK-9(H_2)	MK-8(H_2), MK-9(H_2)	MK-9(H_2), MK-8(H_2)[f]
Cell-wall sugars	Gal[+]		Rha	Rha [e]

[a]For abbreviations, see footnote to Table 74.

[b]If not indicated otherwise, data are taken from the original species description.

[c]97.6–98.9% 16S rRNA gene sequence similarities.

[d]Data from Schleifer and Kandler (1972), Keddie and Cure (1978), and Collins and Kroppenstedt (1983).

[e]Each menaquinone representing >20% of the total quinone content.

[f]Data from Lee et al. (2003).

TABLE 81. The "*Arthrobacter psychrolactophilus* group" (subclade IV)[a,b,c]

	A. psychrolactophilus	*A. stackebrandtii*	*A. psychrochitiniphilus*	*A. alpinus*	*A. russicus*
Peptidoglycan type and interpeptide bridge	A3α, Lys–Thr–Ala_3	A3α, Lys–Ala_2	A3α, Lys–Thr–Ala_{1-2}[d]	A3α, Lys–Thr–Ala_3	A3α, Lys–Ala_2
Quinone system[e]	MK-9(H_2)	MK-9(H_2), [MK-10(H_2)]	MK-9(H_2)	MK-9(H_2)[f]	MK-9(H_2)
Cell-wall sugars				Gal, Rha	

[a]For abbreviations, see footnote to Table 74.

[b]If not indicated otherwise, data are taken from the original species description. including tentatively assigned species *Arthrobacter russicus*.

[c]97.3–98.4% 16S rRNA gene sequence similarities.

[d]Only quantitative amino acid content of the peptidoglycan was reported (Chen et al., 2005a). Peptidoglycan type is concluded from the amino acid composition.

[e]Compounds shown in parentheses were detected in the range 5–19%.

[f]Presence of minor amounts of MK-8(H_2) and MK-9(H_2) were reported as well.

TABLE 82. The "*Arthrobacter pigmenti* group"[a,b,c]

	A. castelli	*A. monumenti*	*A. pigmenti*
Peptidoglycan type and interpeptide bridge	A3α, Lys–Ala–Ser–Ala_3	A3α, Lys–Ala_4	A3α, Lys–Ala_4
Quinone system[d]	MK-9(H_2), [MK-10(H_2)]	MK-9(H_2)	MK-9(H_2), [MK-7(H_2), MK-10(H_2)]
Cell-wall sugars[e]	Gal, (Xyl, Rha)	Gal, (Xyl, Rha)	Gal, Rha

[a]For abbreviations, see footnote to Table 74.

[b]If not indicated otherwise, data are taken from the original species description.

[c]96.2–97.9% 16S rRNA gene sequence similarities.

[d]Compounds shown in brackets were detected in the range 5–19%.

[e]Sugars listed in parentheses are present only in minor amounts.

TABLE 83. The "*Arthrobacter albus/cumminsii* group"[a,b]

	A. albus	*A. cumminsii*
Peptidoglycan type and interpeptide bridge	A4α, Lys–Ala–Glu	A4α, Lys–Ser(Gly)–Glu
Quinone system	MK-8(H_2)	MK-8(H_2)

[a]For abbreviations, see footnote to Table 74.

[b]99.1% 16S rRNA gene sequence similarity.

TABLE 84. The "*Sinomonas* group" (subclade V)[a,b]

	S. atrocyanea	*S. flava*	*A. albidus*	*A. echigonensis*
Peptidoglycan type and interpeptide bridge	A3α, Lys–Ser–Ala_{2-3}	A3α, Lys, Gly, Ala_4[c]	A3α[d], Lys, Ser, Ala	A3α[d], Lys, Ser, Ala
Quinone system[e]	MK-9(H_2), [MK-8(H_2)]	MK-9(H_2), [MK-8(H_2)]	MK-9(H_2), [MK-(10_2)]	MK-9(H_2) [MK-10(H_2)]
Cell-wall sugars	Gal, Glc, Man			

[a]For abbreviations, see footnote to Table 74.

[b]96.0–99.3% 16S rRNA gene sequence similarities.

[c]Only the amino acid content of the peptidoglycan was reported (Zhou et al., 2009). Peptidoglycan type and number of alanines are concluded from the analysis of relative amounts of amino acids in the peptidoglycan.

[d]Only the amino acid content of the peptidoglycan was reported (Ding et al., 2009). Peptidoglycan type is concluded from the amino acids detected in the peptidoglycan.

[e]Compounds shown in brackets were detected in the range 5–19%.

TABLE 85. Characteristics useful for differentiation between species of the "*Arthrobacter globiformis* group" and other species (*Arthrobacter crystallopoietes*, *Arthrobacter methylotrophus*, and *Arthrobacter ramosus*) tentatively assigned to this group[a,b]

Characteristic	*A. globiformis*[b]	*A. humicola*[b]	*A. oryzae*[b]	*A. pascens*[b]	*A. crystallopoietes*	*A. methylotrophus*[c]	*A. ramosus*
Major fatty acids[d]	$C_{15:0}$ anteiso, [$C_{15:0}$ iso], ($C_{16:0}$ iso, $C_{17:0}$ anteiso)	$C_{15:0}$ anteiso, ($C_{17:0}$ anteiso, $C_{16:0}$ iso, $C_{15:0}$ iso)	$C_{15:0}$ anteiso, [$C_{17:0}$ anteiso]	$C_{15:0}$ anteiso, [$C_{17:0}$ anteiso], ($C_{15:0}$ iso)	$C_{15:0}$ anteiso, ($C_{17:0}$ anteiso)	$C_{15:0}$ anteiso, ($C_{15:0}$ iso, $C_{17:0}$ anteiso, $C_{16:0}$ iso)	$C_{15:0}$ anteiso, ($C_{17:0}$ anteiso, $C_{15:0}$ iso)
Motility	−	+	+	−	−[e]	−	+[e]
NaCl range for growth (%, w/v)	0–5	0–3	0–2	0–5	nr	0–2.5	nr
Utilization of L-arabinose	+	−	−	+	nr	nr	nr
Nitrate reductase	−	−	+	−	+[f]	−	nr
Pyrrolidonyl arylamidase	−	−	+	−	nr	nr	nr
Urease	−	−	−	−	+[f]	nr	nr
Enzyme assay (API ZYM):							
Esterase lipase (C8)	−	w	−	−	nr	nr	nr
Acid phosphatase	−	+	+	−	nr	nr	nr
α-Galactosidase	−	+	−	+	nr	nr	−
β-Glucuronidase	−	w	+	−	nr	nr	nr
α-Glucosidase	+	+	+	+	nr	nr	−
α-Mannosidase	+	w	−	+	nr	nr	−

[a]Symbols and abbreviations: +, positive; w, weakly positive; −, negative; nr, not reported. Data are from the original descriptions of the species if not indicated otherwise.

[b]Kageyama et al. (2008).

[c]Borodina et al. (2002b).

[d]Fatty acids in brackets are found in the range between >10–25%; fatty acids in parentheses are present in the range 5–10%. Data from Funke et al. (1996), Borodina et al. (2002b), and Kageyama et al. (2008).

[e]Keddie et al. (1986).

[f]Li et al. (2004c).

TABLE 86. Characteristics useful for differentiation between species of the "*Arthrobacter aurescens* group"[a,b]

Characteristic	*A. aurescens*	*A. histidinolovorans*	*A. ilicis*	*A. nicotinovorans*	*A. nitroguajacolicus*	*A. ureafaciens*
Major fatty acids[c]	$C_{15:0}$ anteiso, ($C_{15:0}$ iso)	$C_{15:0}$ anteiso, [$C_{17:0}$ anteiso], ($C_{16:0}$ iso)	$C_{15:0}$ anteiso, ($C_{16:0}$ iso, $C_{15:0}$ iso, $C_{14:0}$ iso)	$C_{15:0}$ anteiso, [$C_{17:0}$ anteiso]	$C_{15:0}$ anteiso, ($C_{15:0}$ iso, $C_{16:0}$ iso, $C_{17:0}$ anteiso)	$C_{15:0}$ anteiso, $C_{17:0}$ anteiso, ($C_{15:0}$ iso, $C_{16:0}$ iso)
Oxidase	+	nr	−[d]	nr	+	nr
Hydrolysis of:						
Esculin	+	nr	−	nr	+	nr
Starch	+	−	−	v	+	−
Elastase	+	nr	−	nr	+	nr
Pyrrolidonyl arylamidase	−	nr	+	nr	−	nr
Motility	−	−	+	nr	+	−
Utilization of:						
Gluconate	+	nr	−	nr	−	nr
L-Histidine	+	+	+	+	nr	−
L-Leucine	−	−	−	v	nr	−
L-Rhamnose	−	w	w	+	nr	−
Inositol	nr	+	−	+	nr	+
Assimilation of:						
D-Xylose	w	+	+	nr	−	+
Uridine	−	nr	+	nr	−	nr
Arbutin	w	nr	−	nr	w	nr
Sucrose	−	nr	+	nr	+	nr
Propionic acid	−	+	w	+	+	+
Cyclodextrin	+	nr	−	nr	−	nr
D-Melibiose	w	nr	w	nr	−	nr
3-Methylglucose	w	nr	w	nr	−	nr
D-Raffinose	w	nr	w	nr	+	nr
Salicin	−	nr	w	nr	−	nr
Urea formed from creatine	nr	+	−	+	nr	+

[a]Symbols and abbreviations: +, positive; −, negative; w, weakly positive reaction; v, variable. Data are from the original description of the species if not indicated otherwise.

[b]Data from Kotoucková et al. (2004) and Kodama et al. (1992).

[c]Fatty acids in brackets are found in the range beween >10–25%; fatty acids in parentheses are present in the range 5–10%.

[d]Positive in Collins et al. (1981).

TABLE 87. Characteristics useful for differentiation between species of the "*Arthrobacter oxydans* group" and *Arthrobacter alkaliphilus*, tentatively assigned to this group[a]

Characteristic	*A. chlorophenolicus*	*A. defluvii*	*A. niigatensis*	*A. oxydans*	*A. phenanthrenivorans*	*A. polychromogenes*	*A. scleromae*	*A. sulfonivorans*	*A. alkaliphilus*
Major fatty acids[b]	$C_{15:0}$ anteiso, ($C_{15:0}$ iso, $C_{16:0}$ iso, $C_{17:0}$ anteiso)	$C_{15:0}$ anteiso, [$C_{15:0}$ iso, $C_{16:0}$ iso], ($C_{17:0}$ anteiso)	$C_{15:0}$ anteiso, [$C_{16:0}$ iso, $C_{17:0}$ anteiso], ($C_{15:0}$ iso, $C_{16:0}$)	$C_{15:0}$ anteiso, [$C_{17:0}$ anteiso, $C_{16:0}$ iso, $C_{16:0}$], ($C_{15:0}$ iso)[c]	$C_{15:0}$ anteiso, [$C_{16:0}$ iso, $C_{15:0}$ iso, $C_{17:0}$ anteiso], ($C_{16:0}$)	$C_{15:0}$ anteiso, [$C_{17:0}$ anteiso, $C_{16:0}$], ($C_{16:0}$ iso)[c]	$C_{15:0}$ anteiso, [$C_{15:0}$ iso], ($C_{17:0}$ anteiso, $C_{16:0}$ iso, $C_{17:1}$ anteiso ω9*c*)	$C_{15:0}$ anteiso, [$C_{15:0}$ iso], ($C_{16:0}$iso, $C_{17:0}$ anteiso)	$C_{15:0}$ anteiso, $C_{17:0}$ anteiso, ($C_{16:0}$ iso)
Colony color	Pearl gray	Creamy white	Light gray to yellow	Pearl gray to yellow	Cream to yellow	Blue	White	Creamy yellow	Light yellow
Motility	+	–		–	–	–	–	+	–
Nitrate reduction	–	+	+	+		–	(+)	–	–
Growth at/on/in:									
5°C	+	+		–	+	–	–	+	+
37°C	+	+	+	+	+	+	+	–	+
42°C	–	–		–	–	–	–	–	
5% NaCl	+	+		+			+	–	+
10% NaCl	–	–		+			–	–	
Nicotine	+	–		+	+	–	–	–	
Mineral salts medium without biotin	+	+		–	+	–	+	+	
Colony color on nicotine-yeast extract medium	Pearl gray			Blue	Cream to yellow				
Acid production from:									
L-Arabinose	+	–		+		–	–	–	
Galactose	+	–	–	+		+	–	+	
Glucose	+	–	+	+		+	–	+	
Rhamnose	+	–		+		–	–	–	
Inositol	–	+		+		–	–	+	
Mannitol	–	+	(+)	+		–	+	+	
Maltose	+	–	–	+		–	–	–	
Lactose	+	–		+		+	–	+	
Trehalose	+	–		+		+	–	–	
Utilization of:									
Salicin	–	+		–		+	+	+	
Inositol	+	–		–		–	(+)	+	
L-Fucose	–	+		–		–	–	–	
D-Sorbitol	+	–		+		+	+	+	
Hydrolysis of:									
Gelatin	–	–	+	(+)	–	+	–	+	–
Starch	–	(+)		+		(+)	+	–	
2-Naphthyl butyrate[d]	–	+		–		+	–	–	–
2-Naphthyl phosphate (pH 5.4)[d]	–	+		–		–	–	–	+
2-Naphthyl β-D-galactopyranoside[d]	+	–		+		+	–	+	+
6-Bromo-2-naphthyl β-D-glucopyranoside[d]	+	–		–		+	–	–	–

[a]Symbols and abbreviations: +, positive; (+), weakly positive; –, negative. Data are from the original descriptions of the species and from Kim et al. (2008) if not indicated otherwise.

[b]Fatty acids in brackets are found in the range beween >10–25%; fatty acids in parentheses are present in the range 5–10%.

[c]Data from Kodama et al. (1992).

[d]Data from API ZYM tests. The intensity of the color was measured on a scale from 0 to 5 and was interpreted as negative at values of 0 or 1 and positive at values of 2–5 (Mudarris et al., 1994).

TABLE 88. Characteristics useful for differentiation between species of the "*Arthrobacter protophormiae* group"

Characteristic	*A. ardleyensis*[a]	*A. arilaitensis*	*A. bergerei*	*A. creatinolyticus*	*A. mysorens*	*A. nicotianae*	*A. protophormiae*	*A. rhombi*	*A. soli*[b]	*A. uratoxydans*
Fatty acids[c]	$C_{15:0}$ anteiso					$C_{15:0}$ anteiso, [$C_{16:0}$ iso, $C_{17:0}$ anteiso], ($C_{15:0}$ iso, $C_{16:0}$)[d]	$C_{15:0}$ anteiso, $C_{15:0}$ iso, ($C_{16:0}$, $C_{17:0}$ anteiso)[d]		$C_{15:0}$ anteiso, [$C_{17:0}$ anteiso, $C_{16:0}$ iso], ($C_{15:0}$ iso)	$C_{15:0}$ anteiso, $C_{15:0}$ iso, [$C_{17:0}$ iso, $C_{17:0}$ anteiso], ($C_{16:0}$ iso)[d]
Urease		−	−	−	−	−	−	−	−	+
Gelatinase		d	−	+	+	d	+	−	+	+
Galactosidase		+	+	−	−	−	−	+	−	−
Esculin hydrolysis		−	−	−	−	−	−	+	−	−
Nitrate reduction	+	−	−	+	−	+	+	−	+	+
Utilization of:										
5-Aminovalerate		d	d	+	+	d	+	−		+
Malonate		d	d	−	+[e]	d	−	+		−
DL-Glycerate		d	−	−	−	+	−	+		+
(−)-Quinate		−	+	−	+	d	+	+		+
L-Histidine		d	d	−	−[f]	d	+	+		+
D(+)-Glucose	+	d	+	+	+	+	−	+	+	+
D-Xylose	+	d	d	−	+	+	−	−	−	−
D-Ribose	+	d	+	−	+	+	+[g]	+[g]	+	+
L-Arabinose	+	d	+	−	+	+	d	+[g]	−	−
D-Galactose	w	d	d	−	+	d	d	+	+	−
Lactose	−	+	+	−	−	−	−	+	+	−
L-Rhamnose	+	−	d	−	−	−	−	+	−	−
D-Xylitol		−	−	−	−	−	−	−	−	−
Glycerol		+	+	+	+	+	+	+	−	+

[a]Physiological data were generated using the Biolog test.

[b]Physiological data were generated using API 20NE and API 50CH test strips (Roh et al., 2008b).

[c]Fatty acids representing >10–25% of the total are shown in squared brackets, fatty acids representing 5–10% of the total are given in parentheses.

[d]Data published by Funke et al. (1996)

[e]Negative in Stackebrandt et al. (1983b).

[f]Positive in Stackebrandt et al. (1983b).

[g]Negative in Osorio et al. (1999).

other groups, including representatives of the "*Arthrobacter globiformis* group", "*Arthrobacter pigmenti* group", and "*Arthrobacter psychrolactophilus* group". Since 16S rRNA gene sequence similarities with species of the latter two groups are significantly lower, *Arthrobacter methylotrophus* is tentatively assigned to the "*Arthrobacter globiformis* group".

Arthrobacter ramosus exhibits 16S rRNA gene similarities of 97.2–98.2% with species of the "*Arthrobacter aurescens* group", but phylogenetic analyses do not provide support for this or any other affiliation (Figure 137). The peptidoglycan interpeptide bridge of Lys–Ala_4 is also not consistent with an association with the "*Arthrobacter aurescens* group", whose members possess Lys–Ala–Thr–Ala. Sequence similarities with species of "*Arthrobacter globiformis* group" and *Arthrobacter methylotrophicus* are in the range of 96.3–96.8% and 96.3–97.1%, respectively, which also does not support a clear affiliation to these groups. Nevertheless, based on moderate sequence similarities and a similar peptidoglycan interpeptide bridge, *Arthrobacter ramosus* is tentatively placed in the "*Arthrobacter globiformis* group".

In some phylogenetic trees, *Arthrobacter roseus* is placed at the root of the "*Arthrobacter psychrolactophilus* group" but without bootstrap support for assignment to this group. High 16S rRNA gene similarities of 97.0–97.6% identify the "*Arthrobacter oxydans* group" as the next closest relatives. However, the composition of its peptidoglycan interpeptide bridge, Lys–Gly–Ala_3, is unique within *Arthrobacter* and does not provide clear evidence for an assignment to any of the designated *Arthrobacter* groups. Likewise, the menaquinone is MK-9(H_2), which is common within the genus. Hence, this species may be a representative of a novel genus within the *Micrococcaceae*. Table 96 lists differential phenotypic characteristics for this species relative to some other ungrouped species.

Phylogenetic analyses suggest that *Arthrobacter russicus* is closely related to *Renibacterium salmoninarum*, a relationship that is also supported by a high 16S rRNA gene similarity of 97.2%. However, these species differ significantly in G+C content (65.5 and 56.3 mol%, respectively; Li et al., 2004c; Wiens et al., 2008); in the quinone system, and in peptidoglycan structure. *Arthrobacter russicus* has menaquinone MK-9(H_2) and a peptidoglycan with Lys–Ala_2, whereas *Renibacterium salmoninarum* has MK-9 and peptidoglycan with Lys–Ala–Gly (Kusser and Fiedler, 1983; Sanders and Fryer, 1980). These differences may

TABLE 89. Characteristics useful for differentiation between species of the "*Arthrobacter sulfureus* group"

Characteristic	*A. gangotriensis*[a]	*A. kerguelensis*[a]	*A. psychrophenolicus*[b]	*A. sulfureus*[a,b]
Fatty acids[c]	$C_{15:0}$ anteiso, [$C_{15:0}$ iso, $C_{16:0}$ iso,], ($C_{17:0}$ anteiso, $C_{17:0}$ iso, $C_{18:1}$)	$C_{15:0}$ anteiso, $C_{17:0}$ anteiso, ($C_{15:0}$ iso, $C_{17:0}$ iso)	$C_{15:0}$ anteiso, ($C_{15:0}$ iso, $C_{16:0}$ iso, $C_{17:0}$ anteiso)	$C_{15:0}$ anteiso, [$C_{15:0}$ iso], ($C_{16:0}$ iso, $C_{17:0}$ anteiso)[d]
Temperature range for growth (°C)	4–30			
Optimum growth temperature (°C)	22	22	25	25–30
Nitrate reduction			+	−
Esculin hydrolysis	−	+		−
Casein hydrolysis	+	+		−
Skimmed milk hydrolysis at 10°C			+	−
Lysine decarboxylase	−	+		−
Acid production from:				
D-Galactose	+	−		+
D-Mannose	+	−		−
Sucrose	−	−	−	+ or −
D-Xylose	−	+	−	−
Utilization of sole carbon sources:				
D-Cellobiose	+	−		+
D-Mannitol	−	−		+
D-Rhamnose	−	+		+
Trehalose	−	+		+
L-Glutamic acid	−	+		+
L-Histidine	−	+		+
L-Tryptophan	−	−		+
Assimilation of:				
Glucose			−	+
Phenylacetate			+	−
DNA G+C content (mol%)	66	58		64.5–66

[a]Data are from Gupta et al. (2004).

[b]Data are from Margesin et al. (2004).

[c]Fatty acids representing >10–25% of the total are shown in squared brackets, fatty acids representing 5–10% of the total are given in parentheses.

[d]Data are from Collins and Kroppenstedt (1983).

TABLE 90. Characteristics useful for differentiation between species of the "*Arthrobacter agilis* group"[a]

Characteristic	*A. agilis*[b]	*A. flavus*[b]	*A. parietis*[b]	*A. subterraneus*[c]	*A. tecti*[b]	*A. tumbae*[b]
Major fatty acids[d]	$C_{15:0}$ anteiso, [$C_{15:0}$ iso], ($C_{16:1}$ iso)	$C_{15:0}$ anteiso, [$C_{16:0}$ iso, $C_{17:0}$], ($C_{17:0}$ anteiso)	$C_{15:0}$ anteiso, $C_{15:0}$ iso, ($C_{17:0}$ anteiso)	$C_{15:0}$ anteiso, [$C_{15:0}$ iso, $C_{17:1}$ iso, $C_{17:0}$ anteiso], ($C_{16:0}$ iso)	$C_{15:0}$ anteiso, $C_{15:0}$ iso, ($C_{17:0}$ anteiso)	$C_{15:0}$ anteiso, [$C_{15:0}$ iso, $C_{17:0}$ anteiso]
Alkaliphilic		−	−	pH 5.3–10.5	−	+
Growth at 5% NaCl	−	+	+	+	+	+
Oxidase	+	−	−	−	−	−
Nitrate reduction	−	−	+	−	−	v
β-Glucuronidase			−	−	−	+
N-Acetyl-β-glucosaminidase			−	−	+	−
Urease	−	−	v	−	−	v
Gelatin hydrolysis	+	+	+		(+)	(+)
Alkaline phosphatase			−	−	+w	(−)w
Esterase lipase (C_8)			−	+	(+)	(+)w
Lipase (C_{14})	-	+	−	−	−	−
α-Galactosidase			+	−	−	(−)

[a]Symbols and abbreviations: +, all strains positive; (+), ≥80 % positive; v, 21–79% positive; (−), ≤20% positive; −, all strains negative; w, all positive reactions are weak.

[b]Data from Heyrman et al. (2005).

[c]Data from Chang et al. (2007).

[d]Fatty acids in brackets are found in the range beween >10–25%; fatty acids in parentheses are present in the range 5–10%.

TABLE 91. Characteristics useful for differentiation between species of the "*Arthrobacter citreus* group"[a]

Characteristic	*A. citreus*[b]	*A. gandavensis*[b]	*A. koreensis*[c]	*A. luteolus*[b]
Major fatty acids[d]	$C_{15:0}$ anteiso, [$C_{16:0}$], ($C_{15:0}$ iso, $C_{16:0}$ iso, $C_{17:0}$ anteiso)	$C_{15:0}$ anteiso[e], $C_{15:0}$ iso and $C_{17:0}$ anteiso[f]	$C_{15:0}$ anteiso, $C_{15:0}$ iso	$C_{15:0}$ anteiso, [$C_{15:0}$ iso]
β-Galactosidase	+	+	−	−
Urease	−	+	nr	−
Hydrolysis of:				
Esculin	−	+	nr	−
L-Isoleucine-AMC	+	−	nr	+
p-Nitrophenyl phosphate	−	+	nr	−
p-Nitrophenyl β-D-glucoside	−	+	nr	−
Proline-and leucine-*p*-nitroanilide	−	+		−
p-Nitrophenyl phosphate	−	+	nr	−
ONPG and *p*-nitrophenyl α-D-galactoside	−	+	nr	−
Utilization of:				
D-Arabitol	nr	nr	+	−
p-Hydroxyphenylacetic	nr	nr	+	−
Glycerol	nr	nr	−	+
Tween 80	+	nr	+	−
L-Arabinose	+	nr	−	−

[a]Symbols and abbreviations: +, positive; −, negative; AMC, 7-Amino-4- methylcoumarin; ONPG, ortho-nitro-phenyl-β-D-galactopyranoside; nr, not reported.

[b]Data from Storms et al. (2003).

[c]Data from Lee et al. (2003).

[d]Fatty acids in brackets are found in the range beween >10–25%; fatty acids in parentheses are present in the range 5–10%.

[e]Predominant.

[f]Significant.

TABLE 92. Characteristics useful for differentiation between species of the "*Arthrobacter psychrolactophilus* group", including *Arthrobacter russicus* tentatively assigned to the group[a,b]

Characteristic	*A. alpinus*	*A. psychrochitiniphilus*	*A. psychrolactophilus*	*A. stackebrandtii*	*A. russicus*
Major fatty acids[c]	$C_{15:0}$ anteiso, [$C_{16:0}$ iso, $C_{17:0}$ anteiso], ($C_{15:0}$ iso)	$C_{15:0}$ anteiso, [$C_{17:0}$ anteiso]	$C_{15:0}$ anteiso, [$C_{17:0}$ anteiso], ($C_{16:0}$ iso)	$C_{15:0}$ anteiso, [$C_{15:0}$ iso, $C_{16:0}$ iso, $C_{17:0}$ anteiso]	$C_{17:0}$ anteiso, [$C_{15:0}$ anteiso,]
Growth at 30°C	−	−	w[d]	+†	+
Nitrate reduction	−	+	−[d]	+[d]	−
Hydrolysis of urea	+	−	−[d]	+[d]	−
Chitinase	nr	+	−	nr	nr
Gelatinase	nr	−	+	nr	nr
Enzyme assays (API ZYM):					
α-Glucosidase	+	nr	+[d]	+	nr
β-Galactosidase	+	nr	+[d]	w[d]	nr
β-Glucuronidase	+	nr	w[d]	+	nr
Assimilation/utilization of:					
L-Arabinose	+	nr	−[d]	+[d]	+
D-Maltose	+	nr	+[d]	+[d]	−
Lactose	+	+	+†	−[d]	−
Tween 40	w	nr	−[d]	+[d]	+
Tween 80	−	+	−[d]	−[d]	+
Reaction in the Biolog test:					
Raffinose	nr	−	+	nr	−
Cyclodextrin	nr	nr	−	+	−
Tween 40	nr	+	−	+	+
N-Acetyl-D-mannosamine	nr	nr	+	−	−
L-Arabinose	nr	+	−	+	−
L-Fucose	nr	nr	−	+	nr
D-Melezitose	nr	nr	+	−	−
Methyl-D-galactoside	nr	+	−	+	−
Methyl-D-glucoside	nr	nr	+	−	−
Stachyose	nr	nr	−	+	−
L-Asparagine	nr	+	−	+	−
Uridine	nr	+	−	+	v

[a]Symbols and abbreviations: +, positive; w, weakly positive; −, negative; v, variable; nr, not reported.

[b]Data are from the original descriptions of the species if not indicated otherwise.

[c]Fatty acids in brackets are found in the range beween >10–25%; fatty acids in parentheses are present in the range 5–10%.

[d]Data from Zhang et al. (2010).

TABLE 93. Characteristics useful for differentiation between species of the "*Arthrobacter pigmenti* group"[a,b]

Characteristic	*A. castelli*	*A. monumenti*	*A. pigmenti*
Major fatty acids[c]	$C_{15:0}$ anteiso, [$C_{15:0}$ iso, $C_{16:0}$ iso], ($C_{17:0}$ anteiso)	$C_{15:0}$ anteiso, $C_{15:0}$ iso	$C_{15:0}$ iso, $C_{15:0}$ anteiso, ($C_{17:0}$ anteiso)
Alkaliphilic	−	−	+
Nitrate reduction	−	+	v
Urease	+	v	−
Gelatin hydrolysis	−	+	+
Esterase lipase (C_8)	+	v	w
Lipase (C_{14})	w	−	−

[a]Symbols and abbreviations: +, all strains positive; v, variable; −, all strains negative; w, weak reaction

[b]Data from Heyrman et al. (2005).

[c]Fatty acids in brackets are found in the range beween >10–25%; fatty acids in parentheses are present in the range 5–10%.

TABLE 94. Characteristics useful for differentiation between species of the "*Arthrobacter albus/cumminsii* group"[a]

Characteristic	*A. albus*[b]	*A. cumminsii*[c]
Major fatty acids[d]	$C_{15:0}$ anteiso, [$C_{16:0}$ iso, $C_{17:0}$ iso], ($C_{15:0}$ iso)	$C_{15:0}$ anteiso, [$C_{15:0}$ iso, $C_{16:0}$ iso, $C_{16:0}$, $C_{17:0}$ anteiso]
Urease	−	v
DNase activity	−	w
Utilization of:		
Ribose	−	+
Glucose	−	v
Alkaline phosphatase	+	v
Cystine arylamidase	−	v

[a]Symbols and abbreviations: +, positive; w, weakly positive; −, negative; v, variable.

[b]Wauters et al. (2000a).

[c]Funke et al. (1998).

[d]Fatty acids in brackets are found in the range beween >10–25%; fatty acids in parentheses are present in the range 5–10%.

TABLE 95. Characteristics useful for differentiation between species of the "*Sinomonas* group"[a]

Characteristic	*S. atrocyanea*[b]	*S. flava*[b]	*A. albidus*[c]	*A. echigonensis*[c]
Major fatty acids[d]	$C_{15:0}$ anteiso, $C_{17:0}$ anteiso, [$C_{15:0}$ iso,,], ($C_{16:0}$ iso, $C_{17:0}$ iso)	$C_{15:0}$ anteiso, $C_{15:0}$ iso, [$C_{17:0}$ anteiso]	$C_{15:0}$ anteiso, $C_{17:0}$ anteiso, [$C_{15:0}$ iso], ($C_{16:0}$ iso)	$C_{15:0}$ anteiso, [$C_{15:0}$ iso, $C_{17:0}$ anteiso], ($C_{16:0}$ iso)
Optimum pH	6–8	nr	7	7
Optimum temperature (°C)	30–37	nr	25–37	25–37
Reduction of nitrate	+	+	−	−
Pyrrolidonyl arylamidase	nr	nr	+	+
Urease	+	+	+	−
Hydrolysis of gelatin	−	−	−	−
Utilization of:				
Maltose	+	nr	−	(+)
Mannitol	+	+	+	−
Ribose	+	nr	+	+
Sucrose	+	+	(+)	+
Xylose	nr	nr	(+)	−
Esterase (C4)	nr	nr	(+)	+
Cystine arylamidase	nr	nr	+	+
Chymotrypsin	nr	nr	−	−
Galactosidase	nr	nr	−	−
β-Glucosidase	+	+	+	−

[a]Symbols and abbreviations: +, positive; (+), weakly positive; −, negative; nr, not reported.

[b]Data from Zhou et al. (2009).

[c]Data from Ding et al. (2009).

[d]Fatty acids in brackets are found in the range beween >10–25%; values in parentheses are present in the range 5–10%.

be explained by the evolution of *Renibacterium salmoninarum* from an *Arthrobacter*-like ancestor, largely via genome reduction (Wiens et al., 2008), adaption to the salmonid host, and loss of some biosynthetic capabilities. Nevertheless, the absence of significant phenotypic similarities does not support the reclassification of *Arthrobacter russicus* within the genus *Renibacterium*. Compared to *Arthrobacter globiformis*, the 16S rRNA gene of *Arthrobacter russicus* possesses a 12-bp insertion (position 421–432), which is also found in *Renibacterium salmoninarum* and other moderately related *Arthrobacter* species, such as *Arthrobacter psychrolactophilus* (95.5% 16S rRNA gene sequence similarity), *Arthrobacter stackebrandtii* (95.4% 16S rRNA gene sequence similarity), and *Arthrobacter agilis* (94.9% 16S rRNA gene sequence similarity). In other species, such as *Arthrobacter pigmenti*, *Arthrobacter castelli*, *Arthrobacter albus*, and *Arthrobacter cumminsii*, the insertion at this position is a few nucleotides shorter. Whereas peptidoglycan structures clearly distinguish *Arthrobacter russicus* from *Arthrobacter psychrolactophilus* and *Arthrobacter agilis*, no differences in this trait were reported for *Arthrobacter russicus* and *Arthrobacter stackebrandtii*. In sequence comparisons where this insertion is removed from the 16S rRNA gene sequence, *Arthrobacter globiformis* is among the species sharing highest similarities (>96.0%). Since *Arthrobacter russicus* branches in phylogenetic trees which do not include *Renibacterium salmoninarum* at the root of the "*Arthrobacter psychrolactophilus* group" (Ding et al., 2009; Pindi et al., 2010; Tvrzová et al., 2005b; Zhang et al., 2010) and shares with *Arthrobacter stackebrandtii* the peptidoglycan type (Lys–Ala_2) and the quinone system MK-9(H_2), this species is tentatively placed in "*Arthrobacter psychrolactophilus* group".

Arthrobacter sanguinis shares 16S rRNA gene similarities of 94.0–95.2% with *Arthrobacter* species from different *Arthrobacter* groups, such as *Arthrobacter crystallopoietes*, *Arthrobacter cumminsii*, *Arthrobacter globiformis*, *Arthrobacter phenanthrenivorans*, *Arthrobacter pascens*, *Arthrobacter albus*, and *Arthrobacter bergerei* but also with the type strain of *Kocuria rosea*. Phylogenetically, *Arthrobacter sanguinis* forms a separate line of descent with *Acaricomes phytoseiuli*, but this clade is not supported by high bootstrap values, and the two species share only 93.9% 16S rRNA gene similarity. Although it is obvious from these data that *Arthrobacter sanguinis* is not a member of *Arthrobacter sensu stricto* or any of the other *Arthrobacter* groups described herein, it is not placed in a new group because important information is not available, including both the quinone system and peptidoglycan structure. If chemotaxonomic data were available, it might support classification with *Acaricomes phytoseiuli* in the genus *Acaricomes* or placement in a novel genus. Table 96 lists

TABLE 96. Phenotypic traits distinguishing *Arthrobacter* species not assigned to any of the other designated *Arthrobacter* groups

	A. nasiphocea	*A. roseus*	*A. sanguinis*	*A. woluwensis*
Major fatty acids[a]	nr	$C_{14:0}$ iso, $C_{14:0}$, $C_{15:0}$ iso, $C_{15:0}$ anteiso, $C_{15:0}$, $C_{16:0}$ iso, $C_{16:0}$, $C_{16:1}$, $C_{17:0}$ anteiso, $C_{18:0}$, and $C_{18:2}$[b]	$C_{15:0}$ anteiso and $C_{17:0}$ anteiso[c]	$C_{15:0}$ anteiso, [$C_{15:0}$ iso, $C_{16:0}$ iso, $C_{17:0}$ anteiso], ($C_{17:0}$ iso)
Colony color	Grayish-white	Red	Whitish-grayish	Whitish-grayish
Peptidoglycan type	Lys–Ala_2–$Gly_{2–3}$–Ala (Gly) (A3α)	Lys–Gly–Ala_3 (A3α)	nr	L-Lys–D-Asp (A4α)
Quinone system	MK-9(H_2), MK-8(H_2)	MK-9(H_2)	nr	nr
Growth at 37°C	+	−	nr	+
Growth at 5°C	−	+	nr	nr
Hydrolysis of:				
Esculin	−	−	nr	+
Tween 80	nr	nr	nr	nr
Urease	−	−	−	+
Nitrate reduction	−	+	−	−
Utilization of:				
Inositol	nr	−	−	−
Sorbitol	nr	+	+	+
Lactose	nr	−	−	+
Xylitol	nr	nr	−	−
Xylose	nr	+	−	−
Enzyme activity (API ZYM):				
Ester lipase C8	−	nr	+	+
Lipase C14	−	nr	−	+
Cystine arylamidase	−	nr	−	+
Trypsin	−	nr	+	+
β-Glucosidase	−	nr	−	+
α-Fucosidase	−	nr	−	−
α-Galactosidase	−	nr	+	−
β-Galactosidase	−	−	+	+
α-Mannosidase	−	nr	+	+
N-Acetyl-β-glucosaminidase	−	nr	+	+

[a]Fatty acids in brackets are found in the range beween >10–25%; values in parentheses are present in the range 5–10%; nr, not reported.

[b]Only qualitative values were provided by Reddy et al. (2002).

[c]Predominant.

differential phenotypic characteristics for this species relative to some other ungrouped species.

Among species of the genus *Arthrobacter*, *Arthrobacter woluwensis* is the only species with Asp in the peptidoglycan interpeptide bridge, which is designated peptidoglycan type A4α (Schleifer and Kandler, 1972) or A11.31 (http://www.peptidoglycan-types.info). Other *Arthrobacter* species with an A4α type interpeptide bridge possess Glu alone (A11.33), Ala–Glu (A11.35), or Ala–Ser–Glu (A11.38, A11.48, or A11.58). Sequence similarities of 96.7–97.6% in the 16S rRNA gene suggest that this species is a relative of the *Arthrobacter globiformis* group, but there is no bootstrap support for this assignment. Three species of the *Arthrobacter nicotianae* group, two species of *Arthrobacter oxydans* group, and *Arthrobacter methylotrophus*, *Arthrobacter crystallopoietes*, *Arthrobacter roseus*, and *Arthrobacter alkaliphilus* also possess similarity values within this range or slightly lower, 96.4–96.8%. In the absence of a clear assignment to a subclade one rRNA cluster, the unique peptidoglycan of *Arthrobacter woluwensis* suggests that it represents a novel genus, and hence it is not assigned to any of the other *Arthrobacter* groups. Table 96 lists differential phenotypic characteristics for this species relative to some other ungrouped species.

Degradation potential. Numerous arthrobacters have been examined for their potential to degrade xenobiotics and other harmful substances.

Three *Arthrobacter* strains isolated from soil surrounding an outdoor coal storage pile grew with naphthalene vapor as sole source of carbon. However, none of the known genes for naphthalene degradation were detected, such as *nahAc*, *nahAd*, *nahH*, *phnAC*, *xzlE*, or *GST* (Dore et al., 2003). Several phenanthrene-degrading *Arthrobacter* strains have been isolated after long-term exposure to the polycyclic aromatic hydrocarbons (Bodour et al., 2003).

Arthrobacter aurescens TC1 metabolizes 23 different *s*-triazine compounds including atrazine, which it utilizes as a sole source of carbon and nitrogen. Catabolic enzymes involved are TrzN, AtzB, and AtzC (Sajjaphan et al., 2004; Strong et al., 2002). The amidohydrolase TrzN is a zinc-containing enzyme that has a broader specificity than AtzA, the corresponding enzyme of *Pseudomonas* sp. strain ADP and displaces cyano, azido, halide, S-alkyl, and O-alkyl substituents (Shapir et al., 2006; Strong et al., 2002). Further studies suggest that the genes *trzN*, *atzB*, and *atzC* are located on a 380-kb plasmid (Sajjaphan et al., 2004). Another atrazine degrader, *Arthrobacter* AD1 is very closely related to *Arthrobacter ureafaciens* on the basis of 16S rRNA gene similarity. Its *atzA* gene is located on the chromosome (Cai et al., 2003). Three atrazine-degrading strains closely related to *Arthrobacter crystallopoietes* were isolated from French soil (Rousseaux et al., 2001), and the catabolic genes were located on a 117-kb plasmid (Rousseaux et al., 2002).

Degradation of 4-chlorophenol has been reported for several arthrobacters. *Arthrobacter ureafaciens* CPR706 first eliminates the chloro-substituent to form hydroquinone. This strain also degrades other *para*-substituted phenols, including 4-nitro-, 4-bromo-, 4-iodo-, and 4-fluorophenol via the hydroquinone pathway. It does not degrade *ortho*- or *meta*-substituted phenols (Bae et al., 1996). The type strain of *Arthrobacter defluvii* and a second strain of this species were isolated by Kim et al. (2008) after stimulation of a sewage sample with 4-chlorophenol. Another *Arthrobacter* strain, described as *Arthrobacter chlorophenolicus* (Westerberg et al., 2000) was isolated after successively increasing the 4-chlorophenol concentration in a soil suspension from 50 to 350 p.p.m. over a period of 165 d. Two strains of *Arthrobacter nitroguajacolicus* were enriched from forest soil and shown to degrade 4-nitroguaiacol. A third strain of this species produces 4-nitroguaiacol in the course of 4-nitrophenol transformation (Kotoučková et al., 2004).

Arthrobacter sp. strain SU DSM 20407 hydrolytically dehalogenates 4-chlorobenzoate, a breakdown product of polychlorinated biphenyls. The degradation requires three genes organized in an operon on the plasmid pASU1. These genes, designated *fchA*, *fchB*, and *fchC*, encode 4-chlorobenzoate:CoA ligase, 4-chlorobenzoyl-CoA dehalogenase, and 4-hydroxybenzoyl-CoA thioesterase (Schmitz et al., 1992; Zhuang et al., 2003). The same set of genes is also present in *Arthrobacter* sp. strain TM-1, which was originally isolated from sewage sludge by enrichment on 4-chlorobenzoate. *Arthrobacter* sp. strain TM-1 dehalogenates 4-chlorobenzoate, and the product, 4-hydroxybenzoate, is further metabolized via protocatechuate. However, other chlorinated benzoates as well as benzoate itself do not support growth and are presumably not metabolized (Marks et al., 1984). The first enzyme in the pathway, 4-chlorobenzoate:CoA ligase produces 4-chlorobenzoyl CoA and AMP (Zhou et al., 2004). The second enzyme is a trimeric protein, 4-chlorobenzoyl CoA dehalogenase, and has a secondary and three-dimensional structure similar to that of the corresponding enzyme from *Pseudomonas* sp. CBS-3. Dehalogenase activity in *Arthrobacter* sp. strain TM-1 is also inhibited by dissolved oxygen and stimulated by manganese (Zhou et al., 2008b).

Cullington and Walker (1999) isolated a bacterial strain D47 capable of degrading the substituted phenylurea herbicides diuron, isoproturon, chlorotoluron, linuron, monolinuron, and monuron. Turnbull et al. (2001) showed that this strain is closely related to *Arthrobacter oxydans* and *Arthrobacter polychromogenes* and that it hydrolyzes the urea side chain at the carbonyl group. *Arthrobacter* RC100 contains three plasmids, and two of them are involved in mineralization of carbaryl (1-naphthyl *N*-methylcarbamate), which is one of the most commonly used carbamate pesticides for the control of a wide variety of insects. During the mineralization, metabolites that appear early in the suspension are 1-naphthol and salicylic acid. *Arthrobacter* sp. RC100 grows also on salicylic acid, 1-naphthol, gentisic acid, protocatechuic acid, salicylaldehyde, and 2-naphthol (Hayatsu et al., 1999).

The *N*-heteroaromatic quinaldine (2-methylquinoline) is utilized by *Arthrobacter nitroguajacolicus* Rü61a (formerly assigned to the species *Arthrobacter ilicis*) as a source of carbon, nitrogen, and energy. The degradation proceeds via the "anthranilate pathway" (Parschat et al., 2003). The genes for conversion of quinaldine to anthranilate are located on a the conjugative linear catabolic plasmid pAL1, which comprises 112,992 bp (Parschat et al., 2007).

Arthrobacter protophormiae RKJ100 utilizes *o*-nitrobenzoate as sole source of carbon, nitrogen, and energy via an oxygen insensitive pathway that releases ammonia to the culture medium. The catabolic genes are located on a 65-kb plasmid (Chauhan and Jain, 2000). The strain degrades *o*-nitrobenzoate via a reductive pathway leading to the formation of *o*-hydroxylaminobenzoate and anthranilate (Pandey et al., 2003). Also *p*-nitrophenol and 4-nitrocatechol are degraded by *Arthrobacter protophormiae* RKJ100, and the corresponding genes are encoded from the same 65-kb plasmid (Chauhan et al., 2000).

"*Arthrobacter keyseri*" 12B (originally designated *Micrococcus* strain 12B) possesses high 16S rRNA gene similarity to *Arthrobacter ureafaciens*, but the name has never been validly published and hence it does not have standing in nomenclature. The strain converts phthalate to protocatechuate through *cis*-3,4-dihydroxy-3,4-dihydrophthalate and 3,4-dihydroxyphthalate. The genes of the catabolic pathway are plasmid encoded, and sequence analysis reveals genes encoding a transposon resolvase, enzymes required for catabolism of protocatechuate (3,4-dihydroxybenzoate), a putative ATP-binding cassette transporter, a possible phthalate ester hydrolase, a fragment of a norfloxacin resistance-like transporter, and enzymes required for the conversion of phthalate to protocatechuate, respectively (Eaton, 2001).

Arthrobacter sp. strain JBH1 was isolated after enrichment from a nitroglycerin-contaminated site, and it can grow with nitroglycerin as sole source of carbon, nitrogen, and energy. The strain shares 99.5% 16S rRNA gene similarity with *Arthrobacter pascens*, indicating that it is a representative of the genus *Arthrobacter sensu stricto*. It converts nitroglycerin via 1,2-dinitroglycerin to glycerin, which serves as an energy source (Husserl et al., 2010).

Arthrobacter nicotinovorans has the ability to use the tobacco plant alkaloid nicotine as its sole source of carbon and energy. The catabolic genes are located on the 165-kb plasmid pAO1, which also confers the ability to take up nicotine from the medium (Baitsch et al., 2001; Igloi and Brandsch, 2003). Furthermore, the plasmid encodes enzymes essential for biosynthesis of the molybdenum dinucleotide cofactor of nicotine dehydrogenase as well as open reading frames necessary for the uptake and utilization of carbohydrates, sarcosine, and amino acids. pAO1 was successfully transferred by conjugation to a pAO1-negative strain, so genes for conjugation must be present as well.

Enzymes. While a number of enzymes have been identified and characterized in arthrobacters, many are from strains whose identifications are ambiguous. Many of these strains are assigned to the genus *Arthrobacter* on the basis of rod–coccus morphological cycle, presence of lysine in the peptidoglycan, fatty acid profile, and/or 16S rRNA analyses. Since these phenotypic traits are not restricted to arthrobacters and the 16S rRNA gene analysis is sometimes misleading, it is likely that in the future some of the *Arthrobacter* strains listed below will be assigned to other genera.

A strain of *Arthrobacter ilicis* associated with the marine sponge *Spirastrella* sp. produces an extracellular serine type acetylcholinesterase with maximum activity at 45°C and pH 8.0 (Mohapatra and Bapuji, 1998). The enzyme activity is unaffected by Mg^{2+} and Ca^{2+} but inhibited by higher concentrations (25 mM) Co^{2+}, Cu^{2+}, Hg^{2+}, Mn^{2+}, Ni^{2+} and Zn^{2+} as well as EDTA.

Arthrobacter FR-3, isolated from sediment samples of a swamp near the Dow's Lake, Ottawa, Canada, produces a serine-type sulfide oxidase. The free and immobilized sulfide oxidase shows optimal activity at pH 7.5 and 6.0, respectively, and at 35°C. Enzyme activity is not inhibited by 1 mM Ca^{2+}, Mg^{2+}, Na^{+}, and EDTA, but it is completely inhibited by 1 mM Co^{2+} and Zn^{2+} (Mohapatra et al., 2006).

The psychrophilic *Arthrobacter* sp. strain TAD20 produces two cold-adapted, heat labile chitinases, *Ar*ChiA and *Ar*ChiB. The chitinases are active at 4°C but not at 17 or 24°C. They lose 50–60% activity after incubation at 50°C for 30 min. Deduced from the gene sequences, the primary structures of *Ar*ChiA and *Ar*ChiB consist of 846 and 539 amino acids, respectively. Metals are not required for activity, and their activity is unaffected by 10 mM EDTA (Lonhienne et al., 2001).

Three different β-galactosidases have been detected in *Arthrobacter* B7. The sizes of the isozymes are 111 kDa, 71 kDa, and 52 kDa. The 111-kDa isozyme exhibits 22% amino acid sequence similarity with the EbgA protein of *Escherichia coli*, but the optimum temperature is approximately 20°C lower than that of the *Escherichia coli* β-galactosidase. The enzyme requires Mn^{2+} or Mg^{2+} at 1 mM for maximum activity; Ca^{2+} supports 61% activity, whereas Cu^{2+} is completely inhibitory (Trimbur et al., 1994). The amino acid sequence of the 71-kDa isozyme (designated group *lacG*) has little similarity with the enzymes of the *lacZ* family. Highest similarity was found with β-galactosidases from endospore-formers, such as the thermophile *Geobacillus stearothermophilus* (21.8%) and *Bacillus circulans* (17.7%). The enzyme is active over a pH range from 6 to 9 and activity is optimum at pH 6.8. The enzyme activity is reduced after treatment with EDTA but can be restored by addition of 5 mM Mg^{2+}, 10 mM Mn^{2+}, or Ca^{2+}; 10 mM Co^{2+} also increases the activity whereas Ni^{2+} has no effect and Cu^{2+} is inhibitory. The activity is optimal at 45–50°C and is quite stable for at least 70 h at 25, 30, and 35°C (Gutshall et al., 1995). The 52-kDa β-galactosidase of *Arthrobacter* B7 is active as a dimer and hydrolyzes β-1,4 or β-1,3 glycoside linkages. The amino acid sequence is most similar to human and mouse acid β-galactosidase and a corresponding enzyme of *Xanthomonas manihotis* (Gutshall et al., 1997).

The psychrophile *Arthrobacter* strain SB from the Antarctic is a close relative of *Arthrobacter sulfonivorans*. The strain produces a β-galactosidase (BgaS) which exhibits optimal activity at 18°C, 50% activity at 0°C, but is inactivated within 10 min at 37°C. At 4°C, the active enzyme is a homotetramer but dissociates into inactive monomers at 25°C. After dissociation into monomers, the enzyme does not regain activity even when cooled to 4°C. The size of the monomer is approximately 116 kDa. The enzyme is active from pH 6.0 to 9.5, with greatest activity at pH 7.0. Activity is dependent on Mg^{2+}, whereas other mono- or divalent cations have only small stimulatory effects. EDTA inactivates the enzyme at a concentration of 100 mM within 90 min. The nucleotide sequence of the gene that encodes BgaS has highest similarity with those of *lacZ*-like genes of *Arthrobacter* sp. C2-2 and *Arthrobacter psychrolactophilus* (Coker et al., 2003). A cold-adapted β-galactosidase was also characterized from another Antarctic strain, *Arthrobacter* sp. 32c (Hildebrandt et al., 2009). This strain is a close relative of *Arthrobacter oxidans* and *Arthrobacter polychromogenes*. The gene sequence of the enzyme has highest similarity with those of *Arthrobacter* FB24 (77.1%) and *Arthrobacter aurescens* TC1 (71.8%). The enzyme has a temperature optimum at 50°C, with residual activity being 60% at 25°C, and 15% at 0°C. The pH optimum is 6.5, but more than 90% activity remains at pH 8.5. Treatment with 100 mM EDTA for 2 h does not affect the activity. Metal ions such as Na^{+}, K^{+}, Mg^{2+}, Co^{2+}, or Ca^{2+} do not affect activity, whereas Mn^{2+}, Fe^{2+}, and Ni^{2+} strongly and Cu^{2+} or Zn^{2+} completely inhibit the enzyme.

Arthrobacter D10, which was isolated from soil in Pennsylvania, produces an extracellular alkaline phosphatase (de Prada et al., 1996). At pH 9.1, the activity is maximal at 45–50°C. Further examination demonstrated two extracellular alkaline phosphatases, designated D10A and D10B. Alkaline phosphatase

D10B requires calcium for optimal activity, whereas D10A has equal activity with or without calcium. D10A has a maximum pH around 9.5 and is active in the pH range 7.0–11.0. D10B has a narrower pH range, with a maximum at pH 9.0 and less than 10% activity at pH 7.0 and 9.5. D10A loses 60% of activity within 15 min at 55°C, and the less heat-labile D10B loses 20% of activity within 15 min at 55°C.

The soil isolate *Arthrobacter* strain KM possesses activities for several inorganic polyphosphate [poly(P)]- and ATP-dependent kinases including glucokinase, NAD kinase, mannokinase, and fructokinase (Mukai et al., 2003). An enzyme purified from this strain phosphorylates glucose and mannose utilizing either poly(P) or ATP and was designated poly(P)/ATP-glucomannokinase. The enzyme is a monomer with a molecular mass of approximately 30 kDa. The corresponding gene consists of 804 bp. The enzyme has highest activity at pH 7.5 and 45°C, but 50% activity is lost after treatment at 40°C for 5 min. Both enzyme functions, poly(P)- and ATP-dependent gluco- and mannokinase activities require Mg^{2+} or Mn^{2+}. Zn^{2+} and Co^{2+} mediate high activity only for poly(P)-dependent glucomannokinase activity (Mukai et al., 2003).

A pyridoxal-5′-phosphate-dependent D-threonine aldolase has been purified and characterized from *Arthrobacter* sp. DK-38 (Kataoka et al., 1997). The 51-kDa enzyme catalyzes cleavage of D-threonine into glycine and acetaldehyde and the reverse reaction forming D-threonine and D-allothreonine. It is inhibited by chelating reagents such as EDTA, and activity is dependent on divalent cationic salts, including $MnCl_2$, $MgCl_2$, $CoCl_2$, $NiCl_2$, and to a lesser degree $FeSO_4$, whereas $CaCl_2$ has little effect. Other metallic salts including $CuSO_4$, $ZnSO_4$, NaCl, KCl, $AlCl_2$, and $FeCl_2$ have no effect, and Ag_2SO_4, $HgCl_2$, and $CdCl_2$ completely inhibit the enzyme activity. $MnCl_2$ also contributes significantly to thermal stability of the enzyme (Kataoka et al., 1997).

Two extracellular proteinases and an extracellular proline iminopeptidase have been characterized from *Arthrobacter nicotianae* 9458, isolated from the bacterial smear of surface ripened cheeses (Smacchi et al., 1999a, 1999b). The proteinases have molecular masses of approximately 54 kDa (proteinase P1) and 71 kDa (proteinase P2). Both proteinases degrade α_{s1}- and β-casein rapidly at pH 6.5 and 37°C. The optima of the two enzymes are pH 9.5 and 9.0, respectively, but both are still active at pH 6.5. At their optimal pH, the temperature optima are 37°C and 55–60°C, respectively, and both enzymes remain active at 15°C. P1 is irreversibly inactivated after exposure to 70°C for 1 min. The second proteinase, P2, retains 50% of its activity under the same conditions but is inactivated at 85°C after 1 min. EDTA has a strong inhibitory effect on P1 but is only moderately inhibitory to P2. Both enzymes are inhibited by 1 mM Zn^{2+}, Hg^{2+}, Ni^{2+}, Cu^{2+}, or Co^{2+}. At higher concentrations, Fe^{2+} and Mn^{2+} are inhibitory as well. The proline iminopeptidase has a molecular mass of about 54 kDa. Its temperature optimum is 37°C, but activity decreases rapidly above 42°C. Its pH optimum is at 8.0, but 60% of the maximal activity is retained at pH 9.0. It is completely inhibited after treatment for 1 min at 80°C. EDTA is moderately inhibitory. Fe^{2+} and Sn^{2+} completely inactivate the enzyme, and only a little activity is observed in the presence of Zn^{2+}, Hg^{2+}, Ni^{2+}, Cu^{2+}, or Co^{2+}. Mn^{2+} is moderately inhibitory, and Ca^{2+} and Mg^{2+} have little effect.

Genes for degradation of creatinine to glycine and formaldehyde have been identified in *Arthrobacter* sp. TE1826. Together with the repressor gene *soxR*, the *soxA*, *crnA*, and *creA* genes are organized in an operon. The *crnA* gene encodes a 258-amino acid protein, and the *creA* gene encodes a 441-amino acid protein (Nishiya et al., 1998).

A gene encoding a dimethylglycine dehydrogenase (*dmg*) has been identified in *Arthrobacter globiformis* NRRL-B2979, and *soxBDAG* genes encoding the subunits of the heterotetrameric sarcosine oxidase have been identified in *Arthrobacter* spp. 1-IN (Meskys et al., 2001). The *dmg* gene encodes a protein of 830 amino acids whose molecular mass is approximately 90 kDa.

Enrichment and isolation procedures

Due to the high number of species within the genus and their different physiological properties, no general procedure for enrichment and isolation can be recommended. The majority of *Arthrobacter* species are unexacting and grow on standard media containing peptone and yeast extract at or near neutral pH, such as nutrient broth or trypticase soy broth. As shown by Funke et al. (1996), who examined the antibiotic sensitivity of 24 *Arthrobacter* strains, resistance to ciprofloxacin, clindamycin, and gentamicin is widespread among this genus. Hence, supplementation of the enrichment and isolation medium with these antibiotics may be suitable.

Maintenance procedures

Standard procedures for conservation are recommended, such as storage of culture in 25% glycerol at −80°C or lyophilization.

List of species of the genus *Arthrobacter*

1. **Arthrobacter globiformis** (Conn 1928) Conn and Dimmick 1947, 301[AL] (Basonym: *Bacterium globiforme* Conn 1928, 3.)

glo.bi.for′mis. L. n. *globus* ball, globe; L. adj. suff. *-formis -is -e* (from L. n. *forma* figure, shape, appearance) -like, in the shape of; N.L. masc. adj. *globiformis* spherical.

Nonmotile. Colonies on yeast extract-peptone media show no distinctive pigmentation. Either nutritionally nonexacting or require biotin alone. Growth occurs in a suitable mineral salts medium with an ammonium salt or nitrate as sole nitrogen source (with biotin if required) and with glucose as carbon + energy source. The widely used Conn strain ATCC 4336 (NCIB 8602) requires biotin; the type strain does not. Starch is hydrolyzed, nicotine is not utilized. The cell-wall peptidoglycan is of the Lys–Ala_3 type, A3α (Fiedler et al., 1970). The whole-cell sugars are galactose and glucose. The principal isoprenoid quinone is MK-9(H_2) (Collins et al., 1979). The predominant fatty acid is $C_{15:0}$ anteiso (48%), followed by $C_{15:0}$ iso (21%), $C_{17:0}$ anteiso (7%), $C_{16:0}$ iso (7%), $C_{16:0}$ (4%), $C_{14:0}$ iso (2%), and $C_{17:0}$ iso (2%). All other fatty acids are at levels below 1% (Funke et al., 1996).

Source: soil.

DNA G+C content (mol%): 62.0–65.5 (T_m).

Type strain: AS 1.1894, ATCC 8010, BCRC (formerly CCRC) 10598, CCUG 581, CCUG 12157, CCUG 28997, CIP 81.84, DSM 20124, HAMBI 88, HAMBI 1863, IAM 12438, ICPB 3434, NBRC 12137, JCM 1332, LMG 3813, NCIMB 8907, NRIC 0151, NRRL B-2979, VKM Ac-1112.

Sequence accession no. (16S rRNA gene): X80736.

Sequence accession no. (recA): AF214780

Additional remarks: after continuously decreasing oxygen tension in the growth medium, the type strain of *Arthrobacter globiformis* is able to grow in the presence of nitrate, glucose and pyruvate as carbon sources carrying out anaerobic respiratory nitrate reduction with fermentation of carbon sources and also to grow anaerobically in the absence of nitrate, with glucose and pyruvic acid as carbon sources (Eschbach et al., 2003).

2. **Arthrobacter agilis** (Ali-Cohen 1889) Koch, Schumann and Stackebrandt 1995, 837[VP] (*Micrococcus agilis* Ali-Cohen 1889, 36)

a′gi.lis. L. masc. adj. *agilis* agile.

Spheres (0.8–1.2 μm in diameter) occur in pairs and tetrads. Agar colonies are circular, entire, slightly convex, smooth, and matte. Sediment is formed in nutrient broth. No growth occurs on Simmons' citrate medium. Good growth and dark rose-red pigmentation occur on agar slants. The pigment is water insoluble. Motile by means of one to three flagella. Nonmotile strains may occur. Spores are not formed. Gram-stain-positive. The cell-wall peptidoglycan type is type L-Lys–L-Thr–L-Ala (variation A3α). The predominant menaquinone isoprenologue is MK-9(H_2); MK-8(H_2) is a minor component. Contains major amounts of anteiso methyl-branched acids (~65.5% $C_{15:0}$ anteiso) and smaller amounts of iso methyl-branched acids (~13% $C_{15:0}$ iso and ~6% $C_{16:1}$ iso). The polar lipids are phosphatidylglycerol, diphosphatidylglycerol, phosphatidylinositol, and an unknown ninhydrin-negative glycolipid. The amino sugar in the cell-wall polysaccharide is glucosamine. Chemo-organotrophic. Metabolism is respiratory. Aerobic. Acid and gas are not produced from glucose, mannose, lactose, galactose, and glycerol. Catalase-positive. Porphyrin respiratory enzymes are produced. Oxidase-positive. Acetylmethylcarbinol is not produced. β-Galactosidase (*o*-nitrophenyl-β-D-galactopyranoside test) positive. Methyl red negative. Indole and hydrogen sulfide are not produced. Nitrate is not reduced. Gelatin, starch, and esculin are hydrolyzed. Arginine dihydrolase, ornithine and lysine decarboxylases, and phenylalanine deaminase are not produced. Tween 80 may be split. DNase may be produced. Urease, tyrosinase, and phosphatase are not produced. Beta-hemolysis does not occur. Good growth occurs at temperatures between 20 and 30°C. No growth occurs at 37°C. No growth occurs on medium containing 5% NaCl. Susceptible to penicillin, streptomycin, chloramphenicol, tetracycline, erythromycin, novobiocin, ampicillin, carbenicillin, and gentamicin. Resistant to lysozyme. Saprophytic.

Source: water, soil, and human skin.

DNA G+C content (mol%): 67.0–69.0 (T_m).

Type strain: ATCC 966, DSM 20550, CCM 2390, CIP 81.67, NBRC 15319, JCM 2584, LMG 17244, VKM B-1973.

Sequence accession no. (16S rRNA gene): X80748.

Sequence accession no. (recA): AF214779

3. **Arthrobacter albidus** Ding, Hirose and Yokota 2009, 860[VP]

al′bi.dus. L. masc. adj. *albidus* whitish, referring to the color of the colonies.

Cells are nonmotile and non-spore-forming. Gram-stain positive, catalase-positive, oxidase-negative. Shows a rod–coccus growth cycle and produces non-fluorescent pigment. Growth occurs on nutrient broth agar at 10–40°C, optimal temperature for growth is 30°C. Grows in the presence of 3–7% (w/v) NaCl, but no growth occurs in the presence of 7% NaCl. The pH range for growth is 6–10, and the optimum pH is 7. Colonies are round, convex, glossy, with entire margins and are white or light yellow. Using the API CORYNE system, positive reactions are observed for activities of pyrazinamidase, pyrrolidonyl arylamidase, and urease and for the utilization of ribose and mannitol. Negative reactions are obtained for nitrate reduction, hydrolysis of gelatin, and for the utilization of maltose, lactose, and glycogen. Utilization of glucose, xylose, and sucrose is weak. Using the API ZYM system, activity is detected for alkaline phosphatase, esterase lipase C8, leucine arylamidase, valine arylamidase, cystine arylamidase, trypsin, naphthol-AS-BI-phosphohydrolase, β-galactosidase, β-glucuronidase, α-glucosidase, β-glucosidase, and α-mannosidase. No activity is detected for lipase C14, achymotrypsin, α-galactosidase, *N*-acetyl-β-glucosaminidase, or α-fucosidase. Esterase C4 and acid phosphatase activities are weak. The predominant fatty acids are $C_{15:0}$ anteiso, $C_{17:0}$ anteiso, and $C_{15:0}$ iso. The diagnostic diamino acid of the cell-wall peptidoglycan is lysine and the major components are lysine, serine, and alanine. The menaquinone composition is MK-9(H_2) (83%), MK-10(H_2) (14%), and MK-8(H_4) (2%).

Source: a filtration substrate made from volcanic rock from Niigata, Japan.

DNA G+C content (mol%): 70.8 (HPLC).

Type strain: LC13, CCTCC 206018, IAM 15386, JCM 21830.

Sequence accession no. (16S rRNA gene): AB248533.

4. **Arthrobacter albus** Wauters, Charlier, Janssens and Delmée 2000b, 1699[VP] (Effective publication: Wauters, Charlier, Janssens and Delmée 2000a, 2415.)

al′bus. L. masc. adj. *albus* white, because of the white colonies of the organism.

Cells are small coryneform bacteria, nonmotile and without spore formation. Colonies are white and are 1 mm in diameter after 48 h of incubation at 37°C on blood agar. Metabolism is obligately aerobic. Catalase is positive. Urease and esculin are not hydrolyzed, and nitrates are not reduced. Gelatin is slowly and weakly liquefied, and there is no DNase activity. Tyrosine is not hydrolyzed. Simmons' citrate is negative. Pyrrolidonyl peptidase is positive. The organism is resistant to desferrioxamine. No acid is produced from carbohydrates, and there is no utilization of these substrates in the API 50 CH system. Alkaline and acid phosphatase, phosphoamidase, esterase, esterase-lipase, leucine arylamidase, and trypsin are present but not lipase, valine arylamidase, cystine arylamidase, chymotrypsin, α-galactosidase, β-galactosidase, β-glucuronidase, α-glucosidase, β-glucosidase, *N*-acetylglucosaminidase, α-mannosidase, and α-fucosidase. The main cellular fatty acid is $C_{15:0}$ anteiso, and the peptido-

glycan type is L-Lys–L-Ala–L-Glu.

Source: human clinical specimens.

DNA G+C content (mol%): not determined.

Type strain: CF-43, DSM 13068, ATCC BAA-273, CCUG 43812, CIP 106791, JCM 11943.

Sequence accession no. (16S rRNA gene): AJ243421.

5. **Arthrobacter alkaliphilus** Ding, Hirose and Yokota 2009, 859[VP]

al.ka.li′phi.lus. N.L. n. *alkali* (from the Arabic word al-qaliy) the ashes of saltwort; Gr. adj. *philos* loving; N.L. masc. adj. *alkaliphilus* loving alkaline environments.

Cells are nonmotile and non-spore-forming. Gram-stain positive, catalase-positive, and oxidase-negative. Shows a rod–coccus growth cycle and produces non-fluorescent pigment. Growth occurs on nutrient broth agar at 5–40°C, optimal temperature for growth is 30°C. Grows in the presence of 3–7% (w/v) NaCl. The pH range for growth is 6–11 and the optimum pH is 8.5. Colonies are round, convex, glossy, with entire margins and are light yellow. Using the API CORYNE system, positive for pyrazinamidase, pyrrolidonyl arylamidase, and urease activity and for glucose, ribose, maltose, and mannitol utilization. Negative reactions are obtained for nitrate reduction, for hydrolysis of gelatin, and for utilization of glycogen, sucrose, and xylose. Lactose gives a weak reaction. Using the API ZYM system, activity is detected for alkaline phosphatase, acid phosphatase, esterase lipase C8, leucine arylamidase, valine arylamidase, trypsin, naphthol-AS-BI-phosphohydrolase, α-galactosidase, β-galactosidase, β-glucuronidase, α-glucosidase, and α-mannosidase. No activity is detected for esterase C4, lipase C14, cystine arylamidase, β-glucosidase, *N*-acetyl-β-glucosaminidase, or α-fucosidase. The activity of α-chymotrypsin is weak. Predominant fatty acids are $C_{15:0}$ anteiso, $C_{17:0}$ anteiso, and $C_{16:0}$ iso. The diamino acid of the cell-wall peptidoglycan is lysine and the major components are lysine, serine, threonine, and alanine. The menaquinone composition is MK-9(H_2) (77%), MK-10(H_2) (17%), and MK-8(H_4) (3%).

Source: a filtration substrate made from volcanic rock from Niigata, Japan.

DNA G+C content (mol%): 69.0 (HPLC).

Type strain: LC6, CCTCC AB 206013, IAM 15383, JCM 21827.

Sequence accession no. (16S rRNA gene): AB248527.

6. **Arthrobacter alpinus** Zhang, Schumann, Liu, Xin, Zhou, Schinner and Margesin 2010, 2152[VP]

al.pi′nus. L. masc. adj. *alpinus* alpine inhabitant, referring to the isolation of this strain from an alpine environment.

Cells are irregular rods showing a rod–coccus cycle, 0.8–1.0 × 1.3–1.9 µm after 3 d at 20°C on R2A agar plates, often occurring in pairs as typical V-forms. Cells are Gram-stain-positive, aerobic, and nonmotile. Colonies on R2A agar and on nutrient agar are creamy to yellow 176 after 3 d and yellow after 6 d, round, convex, shiny, and with an entire margin; colony diameter is 1–1.5 mm after 3 d at 20°C on R2A agar. Growth occurs in liquid R2A medium and on agar plates at 1–25°C, with fastest growth rates at 20–25°C; growth is absent at 30°C. On R2A agar plates, growth occurs at pH 6–9 and in the presence of 0–5% (w/v) NaCl. Produces catalase but not cytochrome oxidase. Negative for nitrate reduction, indole production, H_2S production, and citrate utilization. Esculin, urea, and starch are hydrolyzed but not casein and Tween 80. Negative for activities of arginine dihydrolase, lysine dihydrolase, ornithine dihydrolase, lipase (C14), *N*-acetyl-β-glucosaminidase, trypsin, α-chymotrypsin, α-fucosidase, and pyrrolidonyl arylamidase. Positive for activities of alkaline phosphatase, acidic phosphatase, esterase (C4), esterase lipase (C8), leucine arylamidase, naphthol-AS-BI-phosphohydrolase, α-glucosidase, β-glucosidase, α-galactosidase, β-galactosidase, β-glucuronidase, and α-mannosidase. Utilizes D-glucose, lactose, L-arabinose, D-maltose, D-mannose, D-mannitol, and *N*-acetylglucosamine but not citrate, capric acid, adipic acid, and phenylacetic acid as sole carbon source; utilization of Tween 40 is weak. Negative for fermentation of glucose, ribose, xylose, mannitol, maltose, lactose, sucrose, glycogen, inositol, sorbitol, rhamnose, melibiose, amygdalin, and L-arabinose. Sensitive to ampicillin, penicillin G, streptomycin, tetracycline, and chloramphenicol (each 30 µg/ml), but resistant to cyclosporin A (100 µg/ml). The predominant cellular fatty acids of the type strain are $C_{15:0}$ anteiso, $C_{16:0}$ iso, and $C_{17:0}$ anteiso. Contains MK-9(H_2) as the major menaquinone and minor amounts of MK-8(H_2) and MK-10(H_2). The cell-wall peptidoglycan is of the type A3α L-Lys–L-Thr–Ala_3. The predominant cell-wall sugars are galactose and rhamnose.

Source (type strain): an alpine soil in Fuschertörl, Hohe Tauern, Austria.

DNA G+C content (mol%): 61.9 (T_m).

Type strain: S6-3; DSM 22274, CGMCC 1.8950.

Sequence accession no. (16S rRNA gene): GQ227413.

7. **Arthrobacter antarcticus** Pindi, Manorama, Begum and Shivaji 2010, 2265[VP]

an.tarc′ti.cus. L. masc. adj. *antarcticus* southern, used to refer to the Antarctic, referring to the place from where the organism was isolated.

Cells have rod–coccus cycle. Rod-shaped cells grown in tryptone soya broth (TSB) at 22°C for 72 h are 1.5–2.2 µm long and 0.2–0.3 µm wide. Cells are Gram-stain-positive, motile, aerobic, and form a yellow colony on TSB agar plates. Growth occurs between 4–25°C, but not at 30°C; good growth is observed at 22–25°C at pH 7. In TSB agar medium, up to 6% NaCl is tolerated. Cells are positive for catalase, phosphatase, citrate (Simons), lysine and ornithine decarboxylase, and nitrate reduction; but negative for indole production, methyl red, Voges–Proskauer's test, H_2S production, gelatinase, lipase, β- galactosidase, and DNase; hydrolyzes urea and starch but not esculin and casein. Produces acid from D- glucose, sucrose, D-fructose, trehalose, rhamnose, D-arabinose, D-galactose, D-xylose, and inositol, but not from erythritol and D-mannose. Utilizes D-glucose, D-galactose, L-rhamnose, L-arabinose, D-raffinose, trehalose, D-melezitose, salicin, sucrose, adonitol, inulin, sodium acetate, glycine, D- alanine, L-glutamic acid, L-proline, L-serine, L-lysine, and L-arginine as sole carbon source but not D-lactose, maltose, D-melibiose, L-sorbose, glycerol, D- cellobiose, mannitol, D-mannose, dulcitol, D-sorbitol, xylitol, erythritol,

L-isoleucine, L-threonine, L-aspartic acid, L-glutamate, L-asparagine, methionine, L-tyrosine, L-phenylalanine, tryptophan, and L-histidine. Resistant to (in μg/ml) norfloxacin (25), colistin (25), and nitrofurantoin (30), but sensitive to (in μg/ml) kanamycin (15), ampicillin (25), tetracycline (10), streptomycin (20), and rifampin (15). Diamino acid in the peptidoglycan is lysine, and alanine is present. The major menaquinones present are MK-8 (37%), MK-9 (61%), and MK-10 (2%). Cells contain phosphatidylethanolamine and diphosphatidylglycerol as major polar lipids. The cell-wall sugars are glucose, galactose, and rhamnose. Mycolic acid not present. Major cellular fatty acids are $C_{14:0}$ (1.30%), $C_{16:0}$ (2.14%), $C_{18:0}$ (2.42%), $C_{15:0}$ iso (11.47%), $C_{15:0}$ anteiso (54.92%), $C_{15:1}$ iso (1.25%), $C_{15:1}$ anteiso (6.38%), $C_{16:0}$ iso (1.79%), $C_{16:0}$ anteiso (1.76%), $C_{17:0}$ anteiso (6.48%), and $C_{19:0}$ iso (1.07%).

Source: Antarctic sea sediments near the Larsemann Hill area.

DNA G+C content (mol%): 68 ± 0.5 (T_m).

Type strain: SPC26, LMG 24542, NCCB 100228.

Sequence accession no. (16S rRNA gene): AM931709.

8. **Arthrobacter ardleyensis** Chen, Xiao, Wang, Zeng and Wang 2005b, 2235[VP] (Effective publication: Chen, Xiao, Wang, Zeng and Wang 2005a, 304.)

ard.ley.en′sis. N.L. masc. adj. *ardleyensis* of or pertaining to Ardley, where the type strain was isolated.

Individual cells show a distinct rod–coccus cycle; some cells are arranged at an angle in V formation, Gram-stain-positive, easily decolorized, rods; motile, aerobic to slightly anaerobic. Catalase is positive, oxidase is negative, and spores or capsules not seen. Colonies in Luria–Bertani medium at 25°C are yellow, circular, convex, smooth, or crumpled in old culture, entire margins, slightly glistening, and semitransparent in young culture. Growth occurs with a suitable carbon source in mineral salts medium; no additional growth factors were required. Grows at 0–30°C; optimal growth temperature is around 25°C. No growth after 30 min at 60°C. Growth is good in 0–3% NaCl and tolerates up to 10% NaCl. Growth is optimal at pH 7.0–8.5. Hydrolyzes Tween 80 and casein but not starch, cellulose, xylan, chitin, and algin. Nitrate is reduced. Methyl red test, Voges–Proskauer test, indole, and H_2S production are negative. NH_3 production is positive. Arginine dihydrolase is produced, but uricase and tryptophan deaminase are negative. Sensitive to (in μg/ml) ampicillin (100) and chloramphenicol (12.5), but resistant to (in μg/ml) kanamycin (50). A Biolog test showed that the following compounds were utilized for respiration: dextrin, glycogen, Tween 40, Tween 80, L-arabinose, D-arabitol, D-cellobiose, D-fructose, D-galactose, α-D-glucose, D-mannose, maltotriose, maltose, DL-α-glycerolphosphatase, D-ribose, L-rhamnose, D-psicose, palatinose, sucrose, D-tagatose, D-trehalose, turanose, D-xylose, acetic acid, α-hydroxybutyric acid, β-hydroxybutyric acid, γ-hydroxybutyric acid, *p*-hydroxyphenyl-acetic acid, α-ketoglutaric acid, α-ketovaleric acid, lactamide, D-lactic acid methyl ester, L-lactic acid, D-malic acid, L-malic acid, pyruvic acid methyl ester, succinic acid mono-methyl ester, propionic acid, pyruvic acid, succinamic acid, *N*-acetyl-L-glutamic acid, L-alaninamide, D-alanine, L-alanine, L-alanyl-glycine, L-asparagine, L-glutamic acid, glycyl-L-glutamic acid, L-pyroglutamic acid, L-serine, putrescine, glycerol, adenosine, and 2′-deoxy adenosine. Negative reactions are observed with α-cyclodextrin, β-cyclodextrin, inulin, mannan, arbutin, amygdalin, *N*-acetyl-β-D-mannosamine, *N*-acetyl-D-glucosamine, L-fucose, D-galacturonic acid, gentiobiose, α-D-lactose, lactulose, D-mannitol, D-melezitese, D-melibiose, β-methyl-D-galactoside, 3-methyl-D-glucose, β-methyl-D-glucoside, salicin, stachyose, uridine, uridine-5′-monophosphate, and α-D-glucose 1-phosphate. The interpeptide bridges of the peptidoglycan consist of alanine and glutamic acid. The peptidoglycan type is A4α. The major menaquinones are MK-8 and MK-9. The cellular fatty acid pattern is dominated by $C_{15:0}$ anteiso.

Source: Antarctic Ardley lake sediment.

DNA G+C content (mol%): 55.2–59.5 (HPLC).

Type strain: An25, CGMCC 1.3685, JCM 12921.

Sequence accession no. (16S rRNA gene; type strain): AJ551163.

Sequence accession no. (16S rRNA gene; other strains): AJ551162, AJ715981.

9. **Arthrobacter arilaitensis** Irlinger, Bimet, Delettre, Lefèvre and Grimont 2005, 459[VP]

a.ri.lai.ten′sis. N.L. masc. adj. *arilaitensis* pertaining to Arilait, arbitrary name formed to honor Arilait-Recherches, a research association that coordinates the collective research programmes of the professional French dairy federations.

The description was based on the study of nine strains. Cells are aerobic, Gram-stain-positive, catalase-positive, oxidase-negative, non-spore-forming, nonmotile, and exhibit a rod–coccus growth cycle. Colonies on BHI agar are yellow, round, smooth, convex, and 2 mm in diameter. Grows between 10°C and 30°C and tolerates up to 10% (w/v) NaCl. Gelatinase, β-galactosidase, pyrazinamidase, pyrrolidonyl arylamidase, phosphatase, and α-glucosidase are produced. Urease and esculin are not hydrolyzed. Nitrate is not reduced. Glucose, ribose, xylose, mannitol, maltose, lactose, sucrose, and glycogen are not fermented. In Biotype 100 strips using biotype 2 medium, most strains (>89%) are able to use the following substrates as sole carbon sources: D-glucose, maltotriose, maltose, α-lactose, D-arabitol, glycerol, 5-keto-D-gluconate, D-gluconate, protocatechuate, 4-hydroxybenzoate, lactate, glycerate, and tyrosine. Other substrates are used less frequently (11–88%): D-galactose, sucrose, D-fructose, D-trehalose, D-mannose, lactulose, L-arabinose, D-ribose, D-xylose, malonate, propionate, 2-oxoglutarate, malate, putrescine, succinate, fumarate, D-glucosamine, 3-hydroxybenzoate, 3-hydroxybutyrate, aspartate, glutamate, proline, alanine, L-histidine, serine, methyl-β-galactopyranoside, D-cellobiose, β-gentiobiose, esculin, D-turanose, D-sorbitol, aconitate, citrate, D-glucuronate, 2-keto-D-gluconate, L-tryptophan, phenylacetate, 4-aminobutyrate, caprylate, and 5-aminovalerate. The following carbon sources are not utilized: sorbose, D-melibiose, D-raffinose, methyl-α-galactopyranoside, methyl-β-glucopyranoside, palatinose, L-rhamnose, fucose, D-melezitose, L-arabitol, xylitol, dulcitol, tagatose, *myo*-inositol, D-mannitol, maltitol, adonitol, lyxose, erythritol, methyl-

α-D-glucopyranoside, methyl-D-glucopyranose, saccharate, mucate, tartrate, tricarballylate, D-galacturonate, *N*-acetyl-D-glucosamine, quinate, gentisate, benzoate, 3-phenylpropionate, *m*-coumarate, trigonelline, betaine, histamine, caprate, glutarate, ethanolamine, tryptamine, and itaconate. The type strain Re117 utilizes the following substrates as sole carbon sources: D-glucose, D-galactose, D-trehalose, sucrose, maltotriose, maltose, lactose, D-cellobiose, ribose, L-arabinose, D-xylose, D-arabitol, glycerol, turanose, 5-keto-D-gluconate, D-gluconate, protocatechuate, 4-hydroxybenzoate, lactate, glycerate, aspartate, glutamate, alanine, serine, and tyrosine.

The cell wall contains lysine, alanine, and glutamic acid. The whole-cell sugars are galactose, glucose, ribose, and mannose.

Source: the surface of Reblochon cheese.

DNA G+C content (mol%): not determined.

Type strain: Re117, CIP 108037, DSM 16368, JCM 13566.

Sequence accession no. (16S rRNA gene; type strain): AJ609628.

Sequence accession no. (16S rRNA gene; other strains): AJ609621, AJ609622, AJ609623, AJ609624, AJ609625, AJ609626, AJ609627, AJ609628.

10. **Arthrobacter aurescens** (*ex* Clark 1951) Phillipps 1953, 241[AL] (*Arthrobacter globiforme* var. aurescens Clark 1951, 180)

au.res′cens. L. v. *auresco* to become golden; L. part. adj. *aurescens* becoming golden.

Colonies of the type strain are reported to show a yellow pigmentation on agar and other media but this strain gives only pale buff colonies on yeast extract-peptone agar when incubated in the dark. When supplied with biotin, growth occurs in suitable mineral salts medium with an ammonium salt or nitrate as nitrogen source and glucose as carbon and energy source (Keddie et al., 1966; Owens and Keddie, 1969). Starch is hydrolyzed, nitrate is not reduced, and there is no growth in 10% NaCl. Nicotine blue is not produced from nicotine. Utilizes L-arginine, L-asparagine, L-histidine, L-arabinose, D-galactose, D-glucose, D-ribose, D-xylose, butanediol, histidinol, 4-aminobutyrate and ρ-hydroxybenzoate but not L-leucine, L-rhamnose, inositol, or malonate. Assimilates adipic acid, citric acid, formic acid, and uric acid but not benzoic acid, glutaric acid, malonic acid, pimelic acid, or propionic acid. Urea is formed from uric acid. Nonmotile (Kodama et al., 1992). Oxidase-positive. Esculin is hydrolyzed. Elastase but not pyrrolidonyl arylamidase is produced. Gluconate is utilized. The following compounds are utilized for respiration: α-cyclodextrin, D-xylose (weak reaction), arbutin (weak reaction), D-melibiose (weak reaction), 3-methylglucose (weak reaction), and D-raffinose (weak reaction). Uridine, sucrose, propionic acid, and salicin are not used for respiration (Kotoučková et al., 2004). The cell-wall peptidoglycan is of the Lys–Ala–Thr–Ala type (A3α) (Fiedler et al., 1970). The whole-cell sugar is galactose (mannose). The major isoprenoid quinone is MK-9(H_2) (Keddie et al., 1986). The predominant fatty acid is $C_{15:0}$ anteiso (~66%), followed by $C_{17:0}$ anteiso (~26%). (Kodama et al., 1992)

Source: soil.

DNA G+C content (mol%): 61.5 (T_m).

Type strain: ATCC 13344, CIP 102364, DSM 20116, HAMBI 1850, NBRC 12136, JCM 1330, LMG 3815, NRRL B-2879, VKM Ac-1105.

Sequence accession no. (16S rRNA gene): X83405.

Sequence accession no. (recA): AF214793.

11. **Arthrobacter bergerei** Irlinger, Bimet, Delettre, Lefèvre and Grimont 2005, 459[VP]

ber.ge′re.i. N.L. gen. masc. n. *bergerei* of Bergère, to honor Jean-Louis Bergère, a French microbiologist.

The description is based on the results of studies of five strains. Cells are aerobic, Gram-stain-positive, catalase-positive, oxidase-negative, non-spore-forming, and nonmotile. They exhibit a rod–coccus growth cycle. Colonies on BHI agar medium are yellow, round, smooth, convex and 2–3 mm in diameter. Grows between 10°C and 30°C and tolerates up to 7.5% (w/v) NaCl. Produce β-galactosidase, pyrazinamidase, pyrrolidonyl arylamidase, and α-glucosidase but not urease, phosphatase, β-glucuronidase, and gelatinase. Esculin is not hydrolyzed. Nitrate is not reduced. D-Glucose, ribose, xylose, mannitol, maltose, lactose, sucrose, and glycogen are not fermented. In Biotype 100 strips using biotype 2 medium, all strains (100%) are able to use the following substrates as sole carbon sources: D-glucose, fructose, D-galactose, sucrose, maltose, lactose, ribose, L-arabinose, D-xylose, D-glycerol, D-gluconate, quinate, protocatechuate, lactate, aspartate, and glutamate. Other substrates are used less frequently (20–80%): lactulose, D-cellobiose, L-rhamnose, D-melezitose, D-mannitol, turanose, D-trehalose, D-mannose, maltotriose, arabitol, methyl-β-galactopyranoside, aconitate, citrate, 2-keto-D-gluconate, L-tryptophan, 4-hydroxybenzoate, 3-hydroxybenzoate, phenylacetate, malate, 5-keto-D-gluconate, betaine, 5-aminovalerate, ethanolamine, malonate, 3-phenylpropionate, coumarate, 4-aminobutyrate, benzoate, putrescine, glucosamine, 3-hydroxybutyrate, histidine, L-alanine, serine, propionate, 2-oxoglutarate, proline, D-alanine, and tyrosine. The following carbon sources are not utilized: sorbose, D-melibiose, D-raffinose, methyl-α-galactopyranoside, β-gentiobiose, methyl-β-glucopyranoside, esculin, palatinose, fucose, L-arabitol, xylitol, dulcitol, tagatose, *myo*-inositol, maltitol, D-sorbitol, adonitol, lyxose, erythritol, methyl-α-D-glucopyranoside, methyl-D-glucopyranose, saccharate, mucate, D-, L-, and *meso*-tartrate, tricarballylate, D-glucuronate, D-galacturonate, *N*-acetyl-D-glucosamine, gentisate, trigonelline, histamine, caprate, caprylate, glutarate, glycerate, tryptamine, and itaconate. The type strain Ca106 utilizes the following substrates as sole carbon sources: D-glucose, fructose, D-galactose, D-mannose, sucrose, lactulose, methyl-β-galactopyranoside, maltotriose, maltose, lactose, D-cellobiose, ribose, L-arabinose, D-xylose, D-arabitol, glycerol, D-gluconate, aconitate, citrate, phenylacetate, quinate, protocatechuate, 3-hydroxybenzoate, benzoate, 4-hydroxybenzoate, putrescine, 4-aminobutyrate, lactate, histidine, glucosamine, aspartate, glutamate, 3-hydroxybutyrate, proline, D-alanine, L-alanine, serine, propionate, and tyrosine. The cell wall contains lysine, alanine, and glutamic acid. The whole-cell sugars are glucose, ribose, and mannose.

Source: the surface of Camembert cheese.

Type strain: Ca106, CIP 108036, DSM 16367, JCM 13567.

Sequence accession no. (16S rRNA gene; type strain): AJ609630.

Sequence accession no. (16S rRNA gene; other strains): AJ609631, AJ609632, AJ609633.

12. **Arthrobacter castelli** Heyrman, Verbeeren, Schumann, Swings and De Vos 2005, 1461[VP]

cas.tel′li. L. gen. n. *castelli* of the castle.

Cells are Gram-stain-positive, short rods and cocci (diameter 0.8–1 μm) occurring in pairs and chains. They are nonmotile and do not form endospores. Colonies on NA after 48 h are small (<1 mm), light yellow, round with entire margins, of low convexity, opaque, and smooth. No growth in an anaerobic chamber on NA. Optimal temperature for growth is 22–37°C. Weak growth at 15°C and no growth at 10 or 45°C. Poor growth on media without NaCl. Catalase-positive and oxidase-negative. By using the API CORYNE system, positive reactions are observed for pyrazinamidase, alkaline phosphatase, β-glucuronidase, β-galactosidase, α-glucosidase, and urease. Negative reactions are obtained for nitrate reduction, pyrrolidonyl arylamidase, *N*-acetyl-β-glucosaminidase, esculin (β-glucosidase), gelatinase, and fermentation of glucose, ribose, xylose, mannitol, maltose, lactose, sucrose, and glycogen. Using the API ZYM system, activity is detected for alkaline phosphatase, acid phosphatase (weak), esterase C4 (weak), esterase lipase C8, lipase C14 (weak), leucine arylamidase, trypsin (weak), phosphoamidase (weak), and α-glucosidase. No activity is detected for valine arylamidase, cystine arylamidase, chymotrypsin, α-galactosidase, β-galactosidase, β-glucuronidase, β-glucosidase, *N*-acetyl-β-glucosaminidase, α-mannosidase, or α-fucosidase. Predominant fatty acids are $C_{15:0}$ anteiso (51%) and $C_{15:0}$ iso (23%). The peptidoglycan type is A3α Lys–Ala–Ser–Ala_3. MK-9(H_2) is the predominant menaquinone (75%), while MK-10(H_2) and MK-9 occur in small amounts. The cell-wall sugars are galactose, xylose, and rhamnose. Polar lipids are phosphatidylinositol, phosphatidylglycerol, diphosphatidylglycerol, one unknown phospholipid, and one unknown glycolipid.

Source: a rosy biofilm overgrowing a mural painting in the Saint-Catherine chapel (castle of Herberstein, Austria).

DNA G+C content (mol%): 68.1 (HPLC).

Type strain: DSM 16402, JCM 21794, LMG 22283.

Sequence accession no. (16S rRNA gene): AJ639826.

13. **Arthrobacter chlorophenolicus** Westerberg, Elväng, Stackebrandt and Jansson 2000, 2090[VP]

chlo.ro.phe.no′li.cus. N.L. n. *chlorophenol* chlorophenol; L. masc. suff. *-icus* suffix used with the sense of pertaining to; N.L. masc. adj. *chlorophenolicus* pertaining to chlorophenols.

Cells are Gram-stain-positive and display a rod–coccus life cycle. The cocci are ~0.75 μm in diameter. The rods are ~0.5–0.7 μm wide and 1–4 μm, depending on the stage in the life cycle and composition of the growth medium. Agar colonies are circular, smooth, and pearl gray in color. Spores are not formed. Growth occurs with a suitable carbon source in mineral salts medium; no additional growth factors are needed. Red to black pigment is formed from several phenolic compounds, only during incubation with shaking from phenols that can be used for growth, and both with and without shaking for phenols that cannot be used for growth. Catalase-positive. Motile. Obligately aerobic. Grows at 3–37°C and optimally between 20 and 30°C. Grows on acetate, ethanol, glycerol, creatine, uric acid, succinate, glucose, tyrosine, ascorbic acid, nicotine, and weakly on creatinine. Niacin and methanol are not utilized. Testing with Biolog showed that the following compounds are oxidized: dextrin, glycogen, D-fructose, D-galactose, D-gluconic acid, α-D-glucose, maltose, maltotriose, D-mannose, D-melezitose, D-melibiose, D-psicose, D-raffinose, D-ribose, stachyose, sucrose, turanose, acetic acid, β-hydroxybutyric acid, ρ-hydroxyphenylacetic acid, α-ketoglutaric acid, D-lactic acid methyl ester, L-malic acid, methyl pyruvate, pyruvic acid, *N*-acetyl-L-glutamic acid, D-alanine, L-alanine, L-alanylglycine, L-asparagine, L-glutamic acid, L-pyroglutamic acid, L-serine, and putrescine. The following substrates were not used for respiration: inulin, α-cyclodextrin, β-cyclodextrin, Tween 40,Tween 80, *N*-acetyl-D-glucosamine, *N*-acetyl-D-mannosamine, amygdalin, L-arabinose, L-arabitol, arbutin, L-fucose, D-galacturonic acid, α-D-lactose, β-methyl-D-galactoside, α-methyl-D-glucoside, β-methyl-D-glucoside, α-methyl-D-mannoside, salicin, sedoheptulosan, D-tagatose, γ-hydroxybutyric acid, α-ketovaleric acid, lactamide, 2,3-butanediol, 2-deoxyadenosine, inosine, thymidine, adenosine-5′-monophosphate, thymidine-5′-monophosphate, uridine-5′-monophosphate, fructose 6-phosphate, glucose 1-phosphate, glucose 6-phosphate, and D-L-α-glycerol phosphate. The cellular fatty acid content is as follows: $C_{15:0}$ anteiso (66%), $C_{16:0}$ iso (10%), $C_{15:0}$ iso (7%), $C_{17:0}$ anteiso (5%), $C_{14:0}$ iso (4%), $C_{16:0}$ (3%), $C_{14:0}$ (2%), $C_{16:1}$ iso h (1%), $C_{16:1}$ ω7*c* (1%), and $C_{17:1}$ anteiso ω9*c* (1%). The cell-wall diamino acid is lysine. The peptidoglycan type is A3α with an L-Lys–L-Ser–L-Thr–L-Ala interpeptide bridge.

Source: soil at Fort Collins, CO, USA.

DNA G+C content (mol%): 65.1 (T_m).

Type strain: A6, ATCC 700700, CIP 107037, DSM 12829, JCM 12360.

Sequence accession no. (16S rRNA gene): AF102267.

Sequence accession no. (complete genome): CP001341.

14. **Arthrobacter citreus** Sacks 1954, 342[AL]

ci′tre.us. L. masc. adj. *citreus* of or pertaining to the citron-tree; intended to mean lemon-yellow, lemon-colored.

The rods are feebly motile. Colonies on yeast extract-peptone media have a yellow, nondiffusible pigment which is insoluble in ether and acetone. Biotin, thiamine, nicotinic acid, tyrosine, methionine, cysteine, and a siderophore such as ferrichrome or mycobactin are required for growth. The siderophores can be replaced by certain synthetic metal chelators. The type strain used about 40 of 180 compounds tested as sole or principal sources of carbon + energy. They included a relatively wide range of carbohydrates, sugar derivatives, and amino acids, together with some fatty acids, dicarboxylic acids, hydroxyacids, oxoacids, non-nitrogenous aromatic compounds, amines, and heterocyclic compounds. Of those tested, no simple alcohols, polyalcohols, or glycols were utilized (Keddie et al.,

1986). Starch is hydrolyzed, nitrate is reduced, and growth occurs in the presence of 10% NaCl. Nicotine blue is not produced from nicotine. Utilizes L-arginine, L-asparagine, L-histidine, L-arabinose, D-galactose, D-glucose, L-rhamnose, D-ribose, D-xylose, 4-aminobutyrate, and malonate but not L-leucine, butanediol, histidinol, inositol, or ρ-hydroxybenzoate. Assimilate citric acid, formic acid, malonic acid, and propionic acid but not adipic acid, benzoic acid, glutaric acid, pimelic acid, or uric acid. Urea is formed from creatinine but not from uric acid. Motile (only the type strain was investigated; Kodama et al., 1992). The type strain is positive for gelatin-, DNase-, Tween 80-, and tyrosine decomposition, β-galactosidase, and α-glucosidase but not for citrate decomposition, pyrrolidonyl peptidase, or *N*-acetylglucosaminidase. Acid is produced from glucose and mannitol (Wauters et al., 2000a). The cell-wall peptidoglycan is of the Lys–Thr–Ala_2 type (A3α) (Fiedler et al., 1970). The only whole-cell sugar is galactose. The principal isoprenoid quinone is MK-9(H_2) (Keddie et al., 1986). The predominant fatty acid is $C_{15:0}$ anteiso (~57%), followed by $C_{16:0}$ (~11%), $C_{17:0}$ anteiso (~8%), $C_{16:0}$ iso (~7%), and $C_{15:0}$ iso (~7%) (Kodama et al., 1992).

Source: chicken feces, but probably a dust or soil contaminant.

DNA G+C content (mol%): 62.9–63.8 (T_m).

Type strain: ATCC 11624, CCUG 23840, CIP 102363, DSM 20133, HAMBI 89, NBRC12957, JCM 1331, LMG 16338, NRRL B-1258, VKM Ac-1106.

Sequence accession no. (16S rRNA gene): X80737.

Sequence accession no. (recA): AF214781.

15. **Arthrobacter creatinolyticus** Hou, Kawamura, Sultana, Shu, Hirose, Goto and Ezaki 1998, 428[VP]

cre.a.ti.no.ly′ti.cus. N.L. n. *creatininum* creatinine; N.L. masc. adj. *lyticus* (from Gr. masc. adj. *lutikos*) able to loosen, able to dissolve; N.L. masc. adj. *creatinolyticus* creatinine-hydrolyzing.

Grows well aerobically at 37°C, and cells are nonmotile, Gram-stain-positive irregular rods with a length of about 1.4–3.6 µm and a width of 0.5–0.8 µm. Cells change into cocci in older cultures and their diameter is 0.5–0.8 µm. Colonies are circular, smooth, and yellow pigmented on brain heart infusion agar. The diameter of the colonies is about 1–1.5 mm after 24 h culture. The cell-wall amino acid components and ratio are Glu:Ala:Lys, 1.9:3.1:1. The diamino acid is lysine. MK-8 and MK-9 are the major menaquinones. Catalase is produced; creatinine, xanthine, and gelatin but not starch are hydrolyzed; and nitrate is reduced; acid is not produced from xylose, ribose, galactose, rhamnose, arabinose, xylitol, or inositol, but is produced from glycerol.

Source: human urine.

DNA G+C content (mol%): 66.0–67.0 (HPLC).

Type strain: CIP 105749, GIFU 12498, JCM 10102.

Sequence accession no. (16S rRNA gene): D88211.

16. **Arthrobacter crystallopoietes** Ensign and Ritterberg 1963, 149[AL]

crys.tal.lo.poi.e′tes. L. n. *crystallus* a crystal, Gr. v. *poieo* to make, form; N.L. masc. adj. *crystallopoietes* crystal forming.

Colonies on yeast extract-peptone media show no distinctive pigmentation. On 2-hydroxypyridine agar, a brilliant green crystalline pigment develops in the colony mass after 3 or 4 d incubation at 30°C. Nutritionally nonexacting: growth occurs in a suitable mineral salts medium with an ammonium salt or nitrate as sole nitrogen source and glucose as carbon + energy source. Starch is not hydrolyzed, nicotine is not utilized. Nonmotile (Keddie et al., 1986). Nitrate is reduced. Urease-positive. Esculin is not hydrolyzed. Inositol but not sorbitol, lactose, xylitol, xylose, glucose, maltose, mannitol, and sucrose is utilized (Li et al., 2004c). The cell-wall peptidoglycan is of the Lys–Ala type. The whole-cell sugars are galactose and glucose. The principal isoprenoid quinone is MK-9(H_2) (Keddie et al., 1986). The predominant fatty acid is $C_{15:0}$ anteiso (75%), followed by $C_{17:0}$ anteiso (7%), $C_{16:0}$ (4%), $C_{16:0}$ iso (4%), and $C_{15:0}$ iso (3%) (Funke et al., 1996).

Source: soil by enrichment in a mineral salt medium containing 2-hydroxypyridine as sole carbon and energy and nitrogen source.

DNA G+C content (mol%): 62.9–63.8 (Bd).

Type strain: ATCC 15481, CIP 102717, DSM 20117, NBRC 14235, JCM 2522, LMG 3819, VKM Ac-1107.

Sequence accession no. (16S rRNA gene): X80738.

17. **Arthrobacter cumminsii** Funke, Hutson, Bernard, Pfyffer, Wauters and Collins 1996, 242[VP] emend. Funke, Pagano-Niederer, Sjödén and Falsen 1998, 1542 (Effective publication: Funke, Hutson, Bernard, Pfyffer, Wauters and Collins, 1996, 2362.)

cum.min′si.i. N.L. gen. masc. n. *cumminsii* of Cummins, to honor Cecil S. Cummins, a prominent American microbiologist and a pioneer of chemotaxonomy.

The cells are coryneform bacteria without irregular branching. No spores are formed. The cells are nonmotile. The organism is obligately aerobic. Colonies are whitish-grayish, smooth, slightly convex, and either of creamy or of sticky consistency and are of less than 2 mm in diameter after 24 h of incubation at 37°C in 5% CO_2 on SBA. Catalase activity is detected, but nitrate reductase activity is not. Urease activity is variable. Esculinase activity is not detected. DNase and gelatinase activities are observed within 10 d. Acid is produced from ribose but not from maltose, sucrose, mannitol, xylose, lactose, or glycogen. Acid production from glucose is variable. The CAMP reaction is negative. The following other enzyme activities are detected: esterase (C4), esterase lipase (C8), leucine arylamidase, acid phosphatase, and phosphoamidase. The activities of pyrazinamidase, pyrrolidonyl arylamidase, alkaline phosphatase, and cystine arylamidase are variable. Chymotrypsin, α-galactosidase, β-galactosidase, β-glucuronidase, α-glucosidase, β-glucosidase, *N*-acetyl-β-glucosaminidase, α-mannosidase, and α-fucosidase are not present. The peptidoglycan type is L-lysine–L-serine–L-glutamic acid. The predominant fatty acid is $C_{15:0}$ anteiso. Significant amounts of $C_{15:0}$ iso, $C_{16:0}$ iso, $C_{16:0}$, and $C_{17:0}$ anteiso are present, depending on the strain. The type strain possesses an A4α, L-lysine–L-serine–L-glutamic acid type murein, but the α-carboxyl group of the D-glutamic acid of the peptide subunit is substituted by a Gly residue.

Source: human clinical specimens.

DNA G+C content (mol%): 60.0–62.0 (HPLC).

Type strain: ATCC 700218, CCUG 36788, CIP 104907, DMMZ 44, DSM 10493, JCM 11675.

Other strains: DMMZ 483, DMMZ 537, CCUG 28802, CCUG 35241, CCUG 36789, CCUG 38876, CCUG 38877, CCUG 38878, CCUG 38880, CCUG 38881, CCUG 38882, CCUG 38883.

Sequence accession no. (16S rRNA gene): X93354.

18. **Arthrobacter defluvii** Kim, Lee, Oh, Kim, Eom and Lee 2008, 1919[VP]

de.flu'vi.i. L. gen. n. *defluvii* of sewage.

Cells are aerobic, Gram-stain-positive, nonmotile, nonsporeforming, and display a rod–coccus life cycle. The cocci are approximately 0.6 µm in diameter. The rods are approximately 0.4–0.66 × 1.0–2.0 µm in size. Catalase-positive but oxidase-negative. Growth occurs at 5–37°C (optimum 25–30°C) and at pH 6–10 (optimum pH 7–8). Growth occurs in the presence of up to 5% NaCl but not with 10% NaCl. Colonies are creamy white, translucent, and circular with entire edges. Indole and H_2S are not produced. Voges–Proskauer reaction is positive. Nitrate is reduced but nitrite is not. Esculin, casein, and starch (weakly) are hydrolyzed, but gelatin and urea are not. Acid is produced from ribose, D-xylose, inositol, mannitol, and esculin, but not from glycerol, erythritol, D-arabinose, L-arabinose, L-xylose, adonitol, methyl-β-D-xylose, galactose, glucose, fructose, mannose, sorbose, rhamnose, dulcitol, sorbitol, methyl-α-D-mannoside, methyl-α-D-glucoside, *N*-acetylglucosamine, amygdalin, arbutin, salicin, cellobiose, maltose, lactose, melibiose, sucrose, trehalose, inulin, melezitose, raffinose, starch, glycogen, xylitol, gentiobiose, turanose, D-lyxose, D-tagatose, D-fucose, L-fucose, D-arabitol, L-arabitol, gluconate, 2-ketogluconate, or 5-ketogluconate. The following compounds are utilized as sole carbon sources: mannitol, rhamnose, D-glucose, salicin, D-ribose, melibiose, L-fucose, sucrose, maltose, mannose, L-arabinose, propionate, malonate, valerate, acetate, citrate, gluconate, DL-lactate, malate, phenylacetate, histidine, L-alanine, 2-ketogluconate, 5-ketogluconate, 3-hydroxybutyrate, glycogen, 3-hydroxybenzoate, 4-hydroxybenzoate, L-proline, and L-serine. The following carbon sources are not utilized: *N*-acetylglucosamine, inositol, D-sorbitol, adipate, itaconate, suberate, and caprate. According to the results from the API ZYM tests, 2-naphthyl butyrate, L-leucyl 2-naphthylamide, 2-naphthyl phosphate (pH 5.4), 6-bromo-2-naphthyl-α-D-galactopyranoside, and 2-naphthyl-α-D-glucopyranoside are hydrolyzed, but 2-naphthyl phosphate (pH 8.5), 2-naphthyl caprylate, 2-naphthyl myristate, L-valyl-2-naphthylamide, L-cystyl-2-naphthylamide, *N*-benzoyl-DL-arginine-2-naphthylamide, *N*-glutaryl-phenylalanine 2-naphthylamide, naphthol-AS-BI-phosphate, 2-naphthyl-β-D-galactopyranoside, naphthol-AS-BI-β-D-glucuronide, 6-bromo-2-naphthyl-β-D-glucopyranoside, 1-naphthyl-*N*-acetyl-β-D-glucosaminide, 6-bromo-2-naphthyl-β-D-mannopyranoside, and 2-naphthyl-α-L-fucopyranoside are not hydrolyzed. The major menaquinone is MK-9(H_2); small amounts of MK-8(H_2) and MK-7(H_2) are also present. Predominant fatty acids are $C_{15:0}$ anteiso (56.4 ±0.4 %), $C_{16:0}$ iso (13.7±0.8 %), and $C_{15:0}$ iso (12.3±2.1 %). Cell-wall peptidoglycan is of A3α type with an L-Lys–L-Ser–L-Thr–L-Ala interpeptide bridge. Cell-wall sugars are galactose, glucose, and rhamnose.

Source: sewage flowing into Geumho River in Daegue, Korea.

DNA G+C content (mol%): 63.5–64.4 (HPLC).

Type strain: 4C1-a, DSM 18782, KCTC 19209.

Sequence accession no. (16S rRNA gene; type strain): AM409361.

Sequence accession no. (16S rRNA gene; another strain): AM409362.

19. **Arthrobacter echigonensis** Ding, Hirose and Yokota 2009, 859[VP]

echi.go.nen'sis. N.L. masc. adj. *echigonensis* of or pertaining to the Echigo region, in Japan.

Cells are nonmotile and non-spore-forming. Gram-stain positive, catalase-positive, oxidase-negative. Cells show a rod–coccus growth cycle and produce non-fluorescent pigment. Growth occurs on nutrient broth agar at 10–40°C, optimal temperature for growth is 30°C. Grows in presence of 3–5% NaCl (w/v) but not in the presence of 7% NaCl. The pH range for growth is 6–10 and the optimum pH is 7. Colonies are round, convex, glossy, with entire margins and are light gray or light yellow. Using the API CORYNE system, positive reactions are observed for activities of pyrazinamidase, pyrrolidonyl arylamidase, and for the utilization of glucose, ribose, and sucrose. Negative reactions are obtained for nitrate reduction, hydrolysis of gelatin, urease activity, and for the utilization of xylose, mannitol, lactose, and glycogen. Utilization of maltose is weak. Using the API ZYM system, activity is detected for alkaline phosphatase, acid phosphatase, esterase C4, esterase lipase C8, leucine arylamidase, valine arylamidase, cystine arylamidase, trypsin, naphthol-AS-BI-phosphohydrolase β-galactosidase, β-glucuronidase, α-glucosidase, and α-mannosidase. No activity detected for lipase C14, α-chymotrypsin, α-galactosidase, β-glucosidase, *N*-acetyl-β-glucosaminidase, or α-fucosidase. The predominant fatty acids are $C_{15:0}$ anteiso, $C_{17:0}$ anteiso, and $C_{15:0}$ iso. The diamino acid of the cell-wall peptidoglycan is lysine and the major components are lysine, serine, and alanine. The menaquinone content is MK-9(H_2) (83%), MK-10(H_2) (6%), and MK-8(H_2) (4%).

Source: a filtration substrate made from volcanic rock from Niigata, Japan.

DNA G+C content (mol%): 71.8 (HPLC).

Type strain: LC10, CCTCC AB 206017, IAM 15385, JCM 21829.

Sequence accession no. (16S rRNA gene): AB248531.

20. **Arthrobacter flavus** Reddy, Aggarwal, Matsumoto and Shivaji 2000, 1559[VP]

fla'vus. L. masc. adj. *flavus* yellow, the color of a pigment that the bacterium produces.

Cells are aerobic, Gram-stain-positive, non-spore-forming, nonmotile, and non-fermentative; they exhibit a rod–coccus growth cycle. Colonies on peptone-yeast extract medium are yellow, round, smooth, convex, and 0.5–2 mm in diameter. The pigment is insoluble in water but soluble in methanol and has a complex absorption spectrum with maxima at 410, 440, and 470 nm. Pigment production is not dependent on growth conditions or media composition. No growth factors

are required. Grows between 5 and 30°C, at pH 6–9, and tolerates up to 11.5% (w/v) NaCl. Optimal growth is observed at 25°C and pH 7. Positive for catalase, lipase, gelatinase, and β-galactosidase but negative for oxidase, urease, phosphatase, indole production, nitrate reduction, and levan formation. Unable to utilize a number of carbon compounds, such as sucrose, rhamnose, cellulose, arabinose, mellibiose, cellobiose, galactose, sucrose, fructose, mannose, trehalose, xylose, mannitol, raffinose, glycerol, ribose, lactose, lactic acid, adonitol, maltose, glucose, glucosamine, sorbitol, melezitol, β-hydroxybutyric acid, dulcitol, glucose, polyethylene glycol, glycine, lysine, sodium citrate, sodium acetate, sodium succinate, cellulose, inulin, *meso*-inositol, glutamic acid, L-alanine, phenylalanine, methionine, glutamine, arginine, serine, potassium hydrogen phosphate, myristic acid, ammonium formate, creatine, methanol, tyrosine, sodium pyruvate, glycogen, erythritol, tryptophan, ethanol, and sodium thioglycolate. Able to utilize sorbitol as the only source of carbon. Unable to oxidize or ferment glucose, galactose, sucrose, thioglycolate, or mannose but able to acidify sucrose and hydrolyze esculin but not starch or cellulose. Sensitive to all antibiotics (in μg) tested: carbenicillin (50), tobramycin (15), chlortetracycline (30), polymyxin B (300), oxytetracycline (30), rifampin (5), nitrofurantoin (300), penicillin (10), bacitracin (10), nitrofurazone (10), gentamicin (10), lincomycin (2), furazolidone (50), colistin (10), furoxone (100), kanamycin (30), nystatin (100), cotrimoxazole (25), chloramphenicol (30), ampicillin (10), tetracycline (30), amoxicillin (100), trimethoprim (5), and erythromycin (15). The cell-wall peptidoglycan type is Lys–Thr–Ala_3 (the A3α variant), and the major menaquinone is MK-9(H_2). The cell-wall sugars are galactose, glucose, and ribose.The cellular fatty acids are $C_{14:0}$, $C_{15:0}$, $C_{15:0}$ anteiso, $C_{16:0}$, $C_{16:0}$ iso, $C_{16:1}$, $C_{17:0}$, $C_{17:0}$ anteiso, $C_{18:0}$ iso, $C_{18:0}$, and $C_{20:0}$. The polar lipids are phosphatidylglycerol, cardiolipin, and phosphatidylethanolamine.

Source: a cyanobacterial mat sample from McMurdo Dry Valley, Antarctica.

DNA G+C content (mol%): 64.0 ±2.0 (T_m).

Type strain: CMS 19Y, JCM 11496, MTCC 3476.

Sequence accession no. (16S rRNA gene): AB299278, AB537168.

21. **Arthrobacter gandavensis** Storms, Devriese, Coopman, Schumann, Vyncke and Gillis 2003, 1882[VP]

gan.da.ven′sis. N.L. masc. adj. *gandavensis* of or belonging to *Gandavum*, the Latin name for Ghent, referring to the place where these strains were first isolated.

Cells are Gram-stain-positive, relatively small coccobacilli with one pointed end. They are catalase-positive. Bright yellow pigment is produced. Growth at 25 and 37°C is about equal; growth is less abundant at 42°C. Obligately aerobic. Cells precipitate partially in BHI broth; unable to grow in 6.5% NaCl. Acid is produced weakly from esculin, D-fructose, and ribose. No acid is produced from adonitol, amygdalin, D- or L-arabinose, D- or L-arabitol, arbutin, cellobiose, dulcitol, erythritol, D- or L-fucose, galactose, β-gentiobiose, gluconate, glycogen, glycerol, inositol, inulin, 2- or 5-ketogluconate, lactose, D-lyxose, maltose, maltotriose, mannitol, melezitose, melibiose, methyl-β-glycoside, methyl-α-D-mannoside, methyl-α-D-glucoside, D-raffinose, sucrose, salicin, sorbitol, L-sorbose, starch, D-tagatose, trehalose, D-turanose, xylitol, or D- or L-xylose. Variable in tests with D-glucose, mannose, and rhamnose. Strains are negative for pyrrolidonyl arylamidase [hydrolysis of L-pyroglutamic acid-7-amino-4-methylcoumarin (AMC)], β-glucuronidase, *N*-acetyl-β-glucosaminidase, enzymic hydrolysis of L-valine-AMC, 4MU-α-D-glucoside, 4-methylumbelliferyl (4MU)-β-D-glucuronide, L-isoleucine-AMC, *p*-nitrophenyl-β-D-cellobioside, and *p*-nitrophenyl-α-D-maltoside. Variable in tests for L-arginine-AMC, L-pyroglutamic acid-AMC, L-tryptophan-AMC, 4MU-*N*-acetyl-β-D-glucosaminide, 4MU-phosphate, 4MU- β-D-glucuronide, and gelatin liquefaction. Cell-wall peptidoglycan is based on the A3α type (L-Lys–L-Thr–L-Ala–L-Ala); major menaquinone is MK-9(H_2). Predominant cellular fatty acid is $C_{15:0}$ anteiso; significant amounts of $C_{15:0}$ iso and $C_{17:0}$ anteiso are also present.

Source: mammary and uterine infections in cattle; its pathogenic role in these processes is uncertain.

DNA G+C content (mol%): 65.0 (HPLC).

Type strain: DSM 15046, JCM 13316, LMG 21285.

Other strains: LMG 21286, LMG 21887.

Sequence accession no. (16S rRNA gene; type strain): AJ316140.

Sequence accession no. (16S rRNA; other strains): AJ491107, AJ491108.

22. **Arthrobacter gangotriensis** Gupta, Reddy, Delille and Shivaji 2004, 2376[VP]

gan.go.tri.en′sis. N.L. masc. adj. *gangotriensis* of or pertaining to the Indian Antarctic station Dakshin Gangotri.

Cells are aerobic, psychrotolerant, Gram-stain-positive, nonmotile, non-spore-forming, and yellow-pigmented: they exhibit a rod–coccus cycle. Grows between 4 and 30°C. The optimum temperature and pH for growth are 22°C and pH 7. Growth occurs in the presence of 6% NaCl. Positive for catalase, oxidase, phosphatase, urease, and gelatinase and negative for methyl red, indole, and Voges–Proskauer tests, β-galactosidase, arginine dihydrolase, lysine decarboxylase, and arginine decarboxylase. Does not hydrolyze esculin, Tween 80, or starch and does not reduce nitrate to nitrite. Acid is produced from D-fructose, D-galactose, and D-mannose but not from D-arabinose, D-glucose, lactose, D-mannitol, D-rhamnose, D-ribose, sucrose, or D-xylose. Can utilize adonitol, D-arabinose, D-cellobiose, dulcitol, D-galactose, inulin, D-fructose, D-glucose, pyruvate, lactose, D-maltose, D-mannose, D-melibiose, D-ribose, sorbitol, sucrose, D-xylose, xylitol, L-arginine, L-asparagine, L-glycine, and L-phenylalanine but not glycerol, D-mannitol, D-rhamnose, trehalose, L-alanine, L-glutamic acid, L-histidine, L-leucine, or tryptophan as sole carbon sources. Resistant to nalidixic acid and nitrofurantoin but sensitive to amikacin, ampicillin, cefoperazone, cefuroxime, ciprofloxacin, co-trimoxazole, erythromycin, chloramphenicol, kanamycin, lincomycin, lomefloxacin, norfloxacin, penicillin, roxithromycin, streptomycin, tetracycline, tobramycin, and vancomycin. The major menaquinones MK-8, MK-9, and MK-10 are present in the ratio 1:4.5:2. The cellular fatty acids at 25°C are $C_{15:0}$ anteiso (61.6%), $C_{17:0}$ anteiso (5.8%), $C_{18:1}$ (9.0%), $C_{17:0}$ iso (5.5%), $C_{16:0}$ iso (4.0%), $C_{15:0}$ iso (3.0%), and $C_{16:1}$ (4.2%).

The yellow pigment is insoluble in water but soluble in methanol and exhibits three absorption maxima, at 494, 528, and 571.5 nm. The cell-wall peptidoglycan type is Lys–Glu (variation A4α).

Source: penguin rookery soil.

DNA G+C content (mol%): 66.0 (method of analysis specified in Shivaji et al., 1989).

Type strain: DSM 15796; JCM 12166.

Sequence accession no. (16S rRNA gene): AJ606061.

23. **Arthrobacter histidinolovorans** Adams 1954, 832[AL]

his.ti.di.no.lo.vo′rans. N.L. n. *histidinolum* histidinol; L. part. adj. *vorans* devouring, consuming; N.L. part. adj. *histidinolovorans* histidinol destroying.

Nonmotile. Colonies on yeast extract-peptone media show no distinctive pigmentation. When supplied with biotin, growth occurs in a suitable mineral salts medium with an ammonium salt as sole nitrogen source and glucose as sole carbon and energy source. Utilizes L-histidinol as major source of carbon and energy and nitrogen when grown in mineral salts medium containing a low concentration of yeast extract (Keddie et al., 1986). There is no hydrolysis of starch, no reduction of nitrate, and no growth in 10% NaCl. Nicotine blue is not produced from nicotine. Utilizes L-arginine, L-asparagine, L-histidine, L-arabinose, D-galactose, D-glucose, L-rhamnose, D-ribose, D-xylose, L-histidinol, inositol, 4-aminobutyrate, and ρ-hydroxybenzoate but not L-leucine, butanediol, or malonate. Assimilates citric acid, formic acid, glutaric acid, propionic acid, and uric acid but not adipic acid, benzoic acid, malonic acid, and pimelic acid. Urea is formed from creatinine and uric acid (Kodama et al., 1992). The cell-wall peptidoglycan is of the Lys–Ala–Thr–Ala type (A3α). The whole-cell sugars are galactose and glucose. The principal isoprenoid quinone is MK-9(H_2) (Keddie et al., 1986). The predominant fatty acid is $C_{15:0}$ anteiso (~64%), followed by $C_{17:0}$ anteiso (~26%) (Kodama et al., 1992).

Source: soil on mineral agar medium containing L-histidinol as carbon and energy and nitrogen source.

DNA G+C content (mol%): 61.3 (Bd).

Type strain: ATCC 11442, CCUG 23888, CIP 106988, DSM 20115, NBRC 15510, JCM 2520, LMG 3822, VKM Ac-1978.

Sequence accession no. (16S rRNA gene): X83406.

Sequence accession no. (recA): AF214788.

24. **Arthrobacter humicola** Kageyama, Morisaki, Ōmura and Takahashi 2008, 56[VP]

hu.mi′co.la. L. masc. n. *humus* soil; L. suff. *-cola* dweller; N.L. masc. or fem. n. *humicola* soil dweller.

Cells have a rod–coccus cycle. Gram-stain-positive, motile by flagella, and aerobic. Colonies are cream colored on YD agar. Growth occurs on YD agar at initial pH 6–10 and at 4–34°C. In YD agar medium, up to 3% NaCl is tolerated. D-Glucose, D-xylose, raffinose, melibiose, D-mannitol, L-rhamnose, L-inositol, and sucrose are assimilated, but L-arabinose and cellulose are not. Esterase (C4), leucine arylamidase, cystine arylamidase, acid phosphatase, naphthol-AS-BI-phosphohydrolase, α-galactosidase, and α-glucosidase are detected by the API ZYM enzyme assay; the assay is negative for alkaline phosphatase, trypsin, chymotrypsin, β-glucuronidase, *N*-acetyl-β-glucosaminidase, and α-fucosidase. Weak reactions are detected for esterase lipase (C8), lipase (C14), valine arylamidase, β-galactosidase, β-glucosidase, and α-mannosidase. Catalase is detected by the API Coryne enzyme assay, but nitrate reductase, pyrrolidonyl arylamidase, and urease are negative. The diagnostic diamino acid of the peptidoglycan is lysine. The acyl type of the peptidoglycan is acetyl. The major menaquinone is MK-9(H_2). The major cellular fatty acids are $C_{15:0}$ anteiso, $C_{17:0}$ anteiso, and $C_{16:0}$ iso. Cell-wall sugars contain galactose and rhamnose.

Source: paddy soil, Japan.

DNA G+C content (mol%): 67.0 (HPLC).

Type strain: KV-653; JCM 15921, NBRC 102056, NRRL B-24479.

Sequence accession no. (16S rRNA gene): AB279890.

25. **Arthrobacter ilicis** Collins, Jones and Kroppenstedt 1981, 384[VP] (Effective publication: Collins, Jones and Kroppenstedt 1981, 321.)

i′li.cis. N.L. n. *Ilex -icis*, a scientific botanical genus name; N.L. gen. n. *ilicis*, of *Ilex*.

Surface colonies on nutrient agar are 0.75–1 mm diameter after 1–2 d, becoming larger (2–4 mm) on extended incubation; convex with entire margin, shiny; yellow pigment produced. Cells show a marked change of form during the growth cycle in complete media. Older cultures are composed entirely of largely of coccoid cells which, on transfer to suitable fresh medium, give rise to irregular rods characteristic of exponential phase cultures. Many cells are arranged at an angle to each other to give V-formations. As growth proceeds, the rods become shorter and are eventually replaced by the coccoid cells characteristic of stationary phase cultures (Cure and Keddie, 1973). Does not required B-vitamins: growth occurs in a mineral salts medium containing an ammonium salt and with glucose as carbon and energy source only when Casamino acids are supplied. Gram-stain-positive, non-acid-fast; endospores are not formed. Motile. Optimum growth temperature 25–30°C; growth at 4 and 10°C but not at 37°C. Slight growth in 5% NaCl and no growth in 10% NaCl. Aerobic, catalase- and cytochrome oxidase-positive. No growth anaerobically. Acids are not formed from glucose and other sugars in soli extract media (Jones, 1975). Weak acid production from glycerol after 5 d (Jones, 1975). Methyl red negative. Casein, chitin, gelatin, hippurate, tyrosine, Tween 20, and xanthine hydrolyzed. Urease, phosphatase, and DNase-positive. Esculin, cellulose, starch, nicotine, Tween 60, and Tween 80 are not hydrolyzed. Indole-negative. Sulfatase-negative. Nitrate is not reduced. Sensitive to erythromycin, tetracycline, and streptomycin. Utilizes L-arginine, L-asparagine, L-histidine, L-arabinose, D-galactose, D-glucose, L-rhamnose, D-ribose, D-xylose, histidinol, inositol, 4-aminobutyrate, and ρ-hydroxybenzoate but not L-leucine, butanediol, or malonate. Assimilate citric acid, formic acid, propionic acid, and uric acid but not adipic acid, benzoic acid, glutaric acid, malonic acid, and pimelic acid. Urea is formed from uric acid but not from creatinine (only type strain investigated, Kodama et al., 1992). Elastase and pyrrolidonyl arylamidase are not produced. Does not utilize

gluconate. Uses uridine, sucrose, D-melibiose (weak reaction), 3-methylglucose (weak reaction), D-raffinose (weak reaction), and salicin (weak reaction) for respiration but not arbutin and α-cyclodextrin (Kotoučková et al., 2004). The cell-wall peptidoglycan is of the Lys–Ala–Thr–Ala type (A3α). The whole-cell sugars are galactose, rhamnose, and mannose. Mycolic acids are not present. The principal isoprenoid quinone is MK-9(H_2). The fatty acid composition is mainly straight-chain, anteiso-, and iso-methyl-branched acids. The major fatty acids are $C_{15:0}$ anteiso and $C_{17:0}$ anteiso. The polar lipids comprise diphosphatidylglycerol, phosphatidylglycerol, phosphatidylinositol, and two glycolipids.

DNA G+C content (mol%): 61.5 (T_m).

Type strain: ATCC 14264, DSM 20138, NCPPB 1228.

Sequence accession no. (16S rRNA gene): X83407.

Additional remarks: Collins et al. (1981) proposed the transfer of *Corynebacterium ilicis* (Mandel et al., 1961) to the genus *Arthrobacter* as *Arthrobacter ilicis.* Several studies however provided evidence that strain ICMP 2608 (= ICPB CI144) and DSM 20138 (= ATCC 14264 = NCPPB 1228) considered to represent the type strain of *Corynbacterium ilicis/Arthrobacter ilicis* are members of different taxa. Based on evidence from a number of publications, it is clear that DSM 20138 = ATCC 14264 = NCPPB 1228 does not show one of the original diagnostic features of *Corynebacterium ilicis,* namely pathogenicity on American holly. In contrast, pathogenicity on American holly is exhibited by ICMP 2608 = ICPB CI14 and hence corresponds with the original description of *Cornyebacterium ilicis.*

After a Request for an Opinion (Young et al., 2004), the Judicial Commission dealt with this problem at its meetings in 2005 at the IUMS Bacteriology and Applied Microbiology Congress in San Francisco. Based on several lines of evidence, the Judicial Commission ruled that the name *Arthrobacter ilicis* (Collins et al., 1982b) is exemplified by DSM 20138 = ATCC 14264 = NCPPB 1228 and that it is not a plant pathogen according to published evidence. The type strain of *Corynebacterium ilicis* (Mandel et al., 1961) is represented by the type strain ICMP 2608 = ICPB CI144 and is reported to be a pathogen of American holly. This strain has also been shown to be a member of the species *Curtobacterium flaccumfaciens,* where it is recognized as the pathotype of *Curtobacterium flaccumfaciens* pv. *ilicis* (Tindall, 2008).

26. **Arthrobacter kerguelensis** Gupta, Reddy, Delille and Shivaji 2004, 2376[VP]

ker.guel.en′sis. N.L. masc. adj. *kerguelensis* of or pertaining to Kerguelen Islands, Antarctica.

Cells are aerobic, psychrotolerant, Gram-stain-positive, nonmotile, non-spore-forming, and undergo a rod–coccus cycle. Colonies are yellow-pigmented. Grows between 4 and 30°C. The optimum temperature and pH for growth are 22°C and pH 7. Growth occurs in the presence of 6% NaCl. Positive for catalase, oxidase, phosphatase, urease, gelatinase, and lysine decarboxylase but negative for methyl red, indole and Voges–Proskauer tests, β-galactosidase, arginine dihydrolase, and arginine decarboxylase. Hydrolyzes esculin but not Tween 80 or starch, and does not reduce nitrate to nitrite. Acid is produced from D-fructose and D-xylose but not from D-arabinose, D-galactose, D-glucose, lactose, D-mannitol, D-mannose, D-rhamnose, D-ribose, or sucrose. Can utilize adonitol, D-arabinose, dulcitol, D-galactose, inulin, D-fructose, D-glucose, L-glutamic acid, L-histidine, pyruvate, lactose, D-maltose, D-mannose, D-melibiose, D-ribose, sorbitol, sucrose, D-xylose, xylitol, L-arginine, L-asparagine, L-glycine, and L-phenylalanine, D-rhamnose, trehalose but not D-cellobiose, glycerol, D-mannitol, L-alanine, L-leucine, or tryptophan as sole carbon sources. Resistant to nalidixic acid, nitrofurantoin, and norfloxacin but sensitive to amikacin, ampicillin, cefoperazone, cefuroxime, ciprofloxacin, co-trimoxazole, erythromycin, chloramphenicol, kanamycin, lincomycin, lomefloxacin, penicillin, roxithromycin, streptomycin, tetracycline, tobramycin, and vancomycin. The major menaquinones MK-8, MK-9, and MK-10 are present in the ratio 4:6:1. The major cellular fatty acids at 25°C are $C_{15:0}$ anteiso (50.0%), $C_{17:0}$ anteiso (25.4%), $C_{15:0}$ iso (6.7%), $C_{17:0}$ iso (5.1%), $C_{16:0}$ iso (4.6%), and $C_{16:1}$ (3.6%).

Source: sea water.

DNA G+C content (mol%): 58.0 (method of analysis specified in Shivaji et al., 1989).

Type strain: KGN15, DSM 15797, JCM 12165.

Sequence accession no. (16S rRNA gene): AJ606062.

27. **Arthrobacter koreensis** Lee, Lee, Pyun and Bae 2003, 1280[VP]

ko.re.en′sis. N.L. masc. adj. *koreensis* of or pertaining to Korea, where the organisms were isolated.

Gram-stain-positive and coryneform. Cells are motile. Colonies on trypticase/soy agar are round, smooth, and yellow. Tests for the reduction of nitrate, the production of catalase, indole, acetoin, pyrazinamidase, alkaline phosphatase, and α-glucosidase, and the assimilation of glucose, mannose, mannitol, maltose, gluconate, and malate are all positive. Oxidizes Tween 40, Tween 80, D-arabitol, D-ribose, xylitol, acetic acid, *p*-hydroxyphenylacetic acid, methyl pyruvate, pyruvic acid, adenosine, 2′-deoxyadenosine, inosine, thymidine, uridine, and thymidine 5′-monophosphate. Cells are alkalitolerant and do not grow at pH 6.0, but they do grow at pH 7.0–12.0, with an optimum at pH 7.0–8.0. Major isoprenoid quinones are menaquinones MK-8(H_2) and MK-9(H_2). Major cellular fatty acids are $C_{15:0}$ anteiso and $C_{15:0}$ iso. Cell-wall peptidoglycan contains Lys–Thr–Ala_2, and the whole-cell sugar is rhamnose.

Source: soil from Daejeon City in Korea.

DNA G+C content (mol%): 63.0 ±2.0 (HPLC).

Type strain: CA15-8, NBRC 16787, JCM 12361, KCTC 9922.

Sequence accession no. (16S rRNA; type strain): AY116496.

Sequence accession no. (16S rRNA; another strain): AY116497.

28. **Arthrobacter luteolus** Wauters, Charlier, Janssens and Delmée 2000b, 1699[VP] (Effective publication: Wauters, Charlier, Janssens and Delmée 2000a, 2414.)

lu.te′o.lus. L. masc. adj. *luteolus* yellowish, because of the yellow-pigmented colonies.

Cells of *Arthrobacter luteolus* are Gram-stain-positive coryneform bacteria. No spores are formed. They are motile by peritrichous flagella. Growth is obligately aerobic. Colonies are slightly yellow, smooth, and ~1.5 mm

in diameter after 24 h of incubation at 37°C on blood agar. Catalase is positive. Nitrate is reduced. No urease is detected. Gelatin and tyrosine are hydrolyzed, but not esculin. Simmons' citrate agar is alkalinized. Not susceptible to desferrioxamine. Tween esterase is negative, but DNase is positive. Pyrrolidonyl peptidase is not detected. Acid is produced oxidatively from glucose, maltose, sucrose, and xylose, but not from mannitol and lactose. Glycerol, ribose, D-xylose, D-glucose, D-fructose, D-mannitol, rhamnose, cellobiose, maltose, sucrose, trehalose, xylitol, L-fucose, and 5-keto-gluconate are utilized. Erythritol, D-arabinose, L-arabinose, L-xylose, adonitol, β-methylxyloside, galactose, L-sorbose, dulcitol, inositol, mannitol, sorbitol, α-methyl-D-mannoside, α-methyl-D-glucoside, *N*-acetylglucosamine, amygdalin, arbutin, esculin, salicin, lactose, melibiose, inulin, melezitose, D-raffinose, starch, glycogen, geniobiose, D-turanose, D-lyxose, D-tagatose, D-fucose, D-arabitol, L-arabitol, and 2-keto-gluconate are not utilized. The following enzymic activities are detected on API ZYM strips: alkaline and acid phosphatase, esterase, esterase-lipase, leucine arylamidase, trypsin, phosphoamidase, and α-glucosidase. Not present are lipase, valine arylamidase, cystine arylamidase, chymotrypsin, α-galactosidase, β-galactosidase, β-glucuronidase, β-glucosidase, *N*-acetylglucosaminidase, α-mannosidase, and α-fucosidase. The major cellular fatty acid is $C_{15:0}$ anteiso, and the peptidoglycan type is A3α L-Lys–L-Thr–L-Ala_2.

Source: an infected surgical wound.

Type strain: CF25; ATCC BAA-272, CCUG 43811, CIP 106789, DSM 13067, JCM 11676.

Sequence accession no. (16S rRNA gene): AJ243422.

29. **Arthrobacter methylotrophus** Borodina, Kelly, Schumann, Rainey, Ward-Rainey and Wood 2002a, 685[VP] (Borodina, Kelly, Schumann, Rainey, Ward-Rainey and Wood 2002b, 180)

me.thy.lo.tro′phus. N.L. n. *methylum* (from French *méthyle*, back-formation from French *méthylène*, coined from Gr. n. *methu* wine and Gr. n. *hulê* wood) the methyl radical; N.L. pref. *methylo-* pertaining to the methyl radical; Gr. n. *trophos* feeder, rearer, one who feeds; N.L. masc. adj. *methylotrophus* feeding on methyl groups.

Cells are spherical or rod-shaped, showing a rod–coccus growth cycle, 0.6 μm in diameter and 1.3 μm in length. Gram-stain-positive, forming clumps and chains in liquid culture, and are nonmotile; spores or capsules not seen; catalase-and oxidase-positive. Grows heterotrophically and aerobically on glucose, fructose, sucrose, galactose, acetate, ethanol, pyruvate, malate, succinate, citrate, serine, alanine, taurine, and yeast extract, and on alkanesulfonates (propane-, butane-, pentane-, and hexane-sulfonate) and diethylsulfone. Grows aerobically on methylated sulfur compounds (dimethylsulfone, dimethylsulfoxide, dimethylsulfide), methanol, methylamine, trimethylamine, and formaldehyde. Methylotrophic growth with dimethylsulfone uses the ribulose monophosphate cycle for formaldehyde assimilation. Methylotrophic growth with methanol uses the serine pathway for C1-assimilation. Does not grow with methanesulfonate or autotrophically on inorganic sulfur compounds. Nitrate is not reduced. Growth on dimethylsulfone occurs optimally at pH 7.2–7.5 and at 25°C. Temperature range for growth is 4–30°C with no growth at 37 or 44°C. Ammonium chloride, nitrate, methylamine, and EDTA are used as nitrogen sources for growth. Growth occurs in the presence of 1.5% (w/v) NaCl, weakly with 2.5% (w/v) NaCl, but not with 5% (w/v) NaCl. On dimethylsulfone-agar medium, they produce creamy-yellow, circular-convex colonies, 1.0–1.3 mm in diameter. MK-9(H_2) is the principal isoprenoid quinone with smaller amounts of MK-10(H_2), MK-8(H_2), MK-9, MK-7(H_2), and MK-11(H_2). The principal cellular fatty acid is $C_{15:0}$ anteiso, with $C_{15:0}$ iso, $C_{16:0}$ iso and $C_{17:0}$ anteiso also present. Peptidoglycan contains lysine as the diagnostic diamino acid, as well as alanine and glutamic acid; provisional peptidoglycan type (L-Lys–L-$Ala_{2–4}$) is an A11.5, A11.6, or A11.7 structure.

Source: soil from the root system of Tagetes minuta.

DNA G+C content (mol%): 61.0 (T_m).

Type strain: TGA, ATCC BAA-111, DSM 14008, JCM 13519.

Sequence accession no. (16S rRNA gene): AF235090.

30. **Arthrobacter monumenti** Heyrman, Verbeeren, Schumann, Swings and De Vos 2005, 1461[VP]

mo.nu.men′ti. L. gen. n. *monumenti* of the monument.

Cells are Gram-stain-positive, short rods and cocci (diameter 0.8–1 μm) occurring in pairs or clusters. They are nonmotile and do not form endospores. Colonies on NA after 48 h are small (<1 mm), light yellow, round with entire margins, of low convexity, opaque and smooth. No growth in an anaerobic chamber on NA. Optimal temperature for growth is 22–30°C. Weak growth at 4°C and 37°C, and no growth at 45°C. Growth on medium with 15% NaCl. Optimum pH for growth is 7–8. Catalase-positive and oxidase-negative. Using the API CORYNE system, positive reactions are observed for nitrate reduction, pyrazinamidase, β-glucuronidase, β-galactosidase, α-glucosidase, esculin (β-glucosidase), and gelatinase. Negative reactions are obtained for *N*-acetyl-β-glucosaminidase and fermentation of ribose, xylose, mannitol, and glycogen. Variable reactions are obtained for pyrrolidonyl arylamidase, urease, and fermentation of glucose, lactose, maltose, and sucrose. In the variable characters listed above for the API CORYNE tests, the type strain is positive for pyrrolidonyl arylamidase but negative for urease and fermentation of glucose, lactose, maltose, and sucrose. In API ZYM, the type strain is positive for β-galactosidase and α-glucosidase (weak) but negative for α-mannosidase. Using the API ZYM system, activity is detected for alkaline phosphatase, esterase C4, esterase lipase C8, leucine arylamidase, trypsin, acid phosphatase (weak), and phosphoamidase (weak). No activity is detected for lipase C14, valine arylamidase, cystine arylamidase, chymotrypsin, α-galactosidase, β-glucuronidase, β-glucosidase, *N*-acetyl-β-glucosaminidase, or α-fucosidase. Variable reactions are obtained for β-galactosidase, α-glucosidase, and α-mannosidase. Predominant fatty acids are $C_{15:0}$ anteiso (57%) and $C_{15:0}$ iso (25%). The peptidoglycan type is A3α Lys–Ala_4. MK-9(H_2) is the predominant menaquinone (87%), while MK-7(H_2), MK-8(H_2), and MK-10(H_2) occur in only small amounts. The cell-wall sugars are galactose, xylose, and rhamnose. Polar lipids of the type strain are

phosphatidylinositol, phosphatidylglycerol, diphosphatidylglycerol, one unknown phospholipid, and one unknown glycolipid.

Source: a biofilm overgrowing a mural painting in the Servilia tomb (Roman necropolis of Carmona, Spain).

DNA G+C content (mol%): 62.2 (HPLC).

Type strain: DSM 16405, JCM 21770, LMG 19502.

Sequence accession no. (16S rRNA gene): AJ315070.

31. **Arthrobacter mysorens** Nand and Rao 1972, 324[AL]

my.so'rens. N.L. masc. adj. *mysorens* of or pertaining to Mysore, India, where the organisms were isolated.

Morphology generally is as for generic description; motility doubtful, flagella not demonstrated. No data available for the peptidoglycan type, cell-wall sugars, or lipids of the type strain.

Growth moderate on nutrient agar; colonies are white and become yellow or lemon-yellow in 3–4 d. Obligately aerobic; weak acid production in glucose, xylose, galactose, glycerol, and cellobiose in 7 d of incubation. Catalase-positive. Growth occurs in Koser's citrate medium; ammonium salts but not nitrate are utilized as sole nitrogen source when provided with glucose as carbon + energy source. No growth on creatine, creatinine, and uric acid agar media; slight growth on nicotine agar. Gelatin and starch hydrolyzed, lecithinase produced. Cellulose not hydrolyzed. Nitrate not reduced to nitrite; urease not produced. Indole, H_2S, and acetylmethylcarbinol not produced. Good growth in 10% NaCl broth; optimum temperature between 20–37°C, no growth at 10°C. Glutamic acid is produced in a mineral salts medium when provided with a suitable carbohydrate; a pink water-soluble pigment is produced in the same medium.

Source: sewage samples in Mysore.

DNA G+C content (mol%): not determined.

Type strain: ATCC 33408, CIP 102716, JCM 11565, LMG 16219, NBRC 103060, NCIB (now NCIMB) 10583.

Sequence accession no. (16S rRNA gene): AJ617482.

Additional remarks: Stackebrandt et al. (1983b) carried out detailed study of a strain named *Arthrobacter mysorens* but unfortunately used ATCC 31021, a patent strain and not the type strain. The authors considered ATCC 31021 to be a member of the *Arthrobacter nicotianae* group of arthrobacters and to be distinct from those species now recognized.

32. **Arthrobacter nasiphocae** Collins, Hoyles, Foster, Falsen and Weiss 2002a, 571[VP]

na.si.pho'ca.e. L. masc. n. *nasus* nose; L. n. *phoca* seal; N.L. gen. n. *nasiphocae* of the nose of a seal.

The cells are Gram-stain-positive, non-spore-forming, irregular-shaped rods; coccoid forms may be observed. Colonies are circular, entire, convex, and approximately 1 mm in diameter after 24 h at 37°C on blood agar. Colonies are grayish-white in color and are nonhemolytic on blood agar. Strictly aerobic and catalase-positive. Growth is produced at 25 and 42°C. Grows in broth containing 5% NaCl but not in 10% NaCl. Acid is not produced from glucose, glycogen, lactose, mannitol, maltose, ribose, sucrose, or D-xylose. Activity is detected for alkaline phosphatase, acid phosphatase, α-glucosidase, esterase C4 (weak), phosphoamidase, pyrazinamidase, pyroglutamic acid arylamidase, and leucine arylamidase. No activity is detected for cystine arylamidase, chymotrypsin, ester lipase C8, α-fucosidase, α-galactosidase, β-galactosidase, β-glucosidase, β-glucuronidase, lipase C14, α-mannosidase, *N*-acetyl-β-glucosaminidase, valine arylamidase, urease, or trypsin. Gelatin and hippurate are hydrolyzed but esculin and starch are not. Nitrate is not reduced. Acetoin is not produced. Cell-wall murein is based on L-lysine variation A3α [type: L-Lys–L-Ala_2–Gly_{2-3}–L-Ala (Gly)]. The major menaquinones are MK-9(H_2) and MK-8(H_2).

Source: the nose of the common seal (*Phoca vitulina*).

DNA G+C content (mol%): 65.0 (HPLC).

Type strain: M597/99/10, CCUG 42953, CIP 107054, JCM 11677.

Sequence accession no. (16S rRNA gene): AJ292364.

33. **Arthrobacter nicotianae** (Giovannozzi-Sermanni 1959) Stackebrandt, Fowler, Fiedler and Seiler 1983b, 481[AL]

ni.co.tí.a'na.e. N.L. n. *Nicotiana* a scientific generic name; N.L. gen. n. *nicotianae* of *Nicotiana*, of the tobacco plant.

Growth of the type strain on agar is bright lemon-yellow. The type strain gives abundant growth on nicotine agar: the medium becomes blue at first and deep wine-red later. Nutritionally nonexacting: growth occurs in a suitable mineral salts medium with an ammonium salt as sole nitrogen source (type strain). Nicotine is utilized by the type strain as a sole or major carbon + energy source. More than 70% of the *Arthrobacter nicotianae* strains assimilate 4-amino-butyrate, 5-amino-valerate, 4-hydroxybenzoate, L-leucine, L-asparagine, D-xylose, D-ribose, L-arabinose, D-galactose, 2,3-butylenglycol, and glycerol; hydrolyze starch and casein; grow in 7% NaCl. The type strain is positive for nitrate reduction, pyrazinamidase, alkaline phosphatase, and pyrrolidonyl arylamidase and assimilates amygdalin, arbutin, cellobiose, D-arbitol, D-xylose, galactose, glycerol, L-arabinose, maltose, ribose, salicin, starch, β-gentiobiose, and glucose. It is negative for β-galactosidase, urease, and cystine arylamidase and cannot assimilate D-mannose, D-turanose, inositol, mannitol, melibiose, rhamnose, sucrose, trehalose, xylitol, *N*-acetylglucosamine, 5-ketogluconate, and phenylacetate (Margesin et al., 2004; Osorio et al., 1999).

The peptide subunit of the peptidoglycan consists of alanine, D-glutamic acid, and lysine. The interpeptide bridge contains L-alanine and D-glutamic acid (Lys–Ala–Glu type, variation A4α; Fiedler et al., 1970). Cell wall (only the type strain is tested) contains galactose and small amounts of glucose. The long-chain fatty acids are primarily of the iso, anteiso, straight chain, and unsaturated acid types, with the anteiso 12-methyl tetradecanoic acid ($C_{15:0}$ anteiso) predominating. The major respiratory isoprenoid quinones are unsaturated menaquinones with 8 and 9 isoprene units. Polar lipids consist of diphosphatidylglycerol (DPG), phosphatidylglycerol (PG), and unidentified glycolipids. The type strain contains diglycosyldiacylglycerol with the chromatographic mobility of dimannosyldiacylglycerol (Collins and Kroppenstedt, 1983).

Source: soil and sewage.

DNA G+C content (mol%): 60.0–66.0 (T_m).

Type strain: AS 1.1895, ATCC 15236, CCM 1648, BCRC (formerly CCRC) 11219, CCUG 23842, CDA 883, CIP 82.107, DSM 20123, HAMBI 1859, IAM 12342, NBRC 14234, IMET 10353, JCM 1333, LMG 16305, NCIMB 9458, NRIC 0153.

Sequence accession no. (16S rRNA gene): X80739.

Additional remarks: in DNA–DNA hybridization studies, Stackebrandt et al. (1983b) found reassociation values of 81–93% between the type strain of *Arthrobacter nicotianae* and the following strains: "*Arthrobacter nucleogenes*" ATCC 21279, *Arthrobacter* sp. NCIB 9863, *Brevibacterium* sp. AJ 1486, and *Corynebacterium liquefaciens* ATCC 14929. Accordingly they considered that all five strains belong to the same species, *Arthrobacter nicotinae.*

After continuously decreasing oxygen tension in the growth medium, the type of *Arthrobacter nicotianae* is able to grow in the presence of nitrate carrying out anaerobic respiratory nitrate reduction (Eschbach et al., 2003).

34. **Arthrobacter nicotinovorans** Kodama, Yamamoto, Amano and Amachi 1992, 237[VP]

ni.co.ti.no.vo′rans N.L. n. *nicotinum* nicotine; L. part. adj. *vorans* devouring, destroying; N.L. part. adj. *nicotinovorans* nicotine devouring.

Aerobic, Gram-stain-positive, not acid-fast. Cells exhibit a marked rod–coccus growth cycle in complex media. The rods are motile by means of a few lateral flagella. Slight growth occurs in the presence of 10% NaCl. Starch is hydrolyzed, and nitrate is not reduced. Nicotine blue is produced from nicotine. Urea is formed from creatine and uric acid. Utilizes L-arabinose, D-galactose, D-glucose, *meso*-inositol, D-ribose, D-xylose, 4-aminobutyrate, L-arginine, L-asparagine, L-histidine, and ρ-hydroxybenzoate but not L-leucine. Citric, formic, malonic, uric, and propionic acids are assimilated but glutaric, adipic, pimelic, and benzoic acids are not. The cell-wall peptidoglycan is of the Lys–Ala–Thr–Ala type and the principal isoprenoid quinone is MK-9(H_2). The fatty acids are mainly straight chain, anteiso- and iso-methyl-branched acids. The major fatty acids are $C_{15:0}$ and $C_{17:0}$ anteiso acids.

DNA G+C content (mol%): 62.4 (HPLC).

Type strain: ATCC 49919, CIP 106990, DSM 420, NBRC 15511, JCM 3874, LMG 16253, VKM Ac-1988.

Sequence accession no. (16S rRNA gene): X80743.

Sequence accession no. (recA): AF214788.

Additional remarks: Arthrobacter oxydans DSM 420 was reclassified as *Arthrobacter nicotinovorans.*

35. **Arthrobacter niigatensis** Ding, Hirose and Yokota 2009, 858[VP]

ni.i.ga.ten′sis. N.L. masc. adj. *niigatensis* of or pertaining to the Niigata region, Japan.

Cells are nonmotile and non-spore-forming. Gram-stain positive, catalase-positive, oxidase-negative, shows a rod–coccus growth cycle, and produces non-fluorescent pigment. Growth occurs on nutrient broth agar at 5–40°C, and optimal temperature for growth is 30°C. Grows in the presence of 3–7% (w/v) NaCl. The pH range for growth is 6–11 and the optimum pH is 7.5. Colonies are round, convex, glossy, with entire margins and are light gray or light yellow. Using the API CORYNE system, a positive reaction is observed for reduction of nitrate, activities of pyrazinamidase, pyrrolidonyl arylamidase, and urease, hydrolysis of gelatin, and for the utilization of glucose, ribose, lactose, and sucrose. Maltose is not utilized and mannitol, xylose, and glycogen are weakly utilized. Using the API ZYM system, activity is detected for alkaline phosphatase, acid phosphatase, leucine arylamidase, trypsin, naphthol-AS-BI-phosphohydrolase, esterase C4, esterase lipase C8, β-galactosidase, β-glucuronidase, α-glucosidase, β-glucosidase, and α-mannosidase. No activity is detected for lipase C14, α-galactosidase, *N*-acetyl-β-glucosaminidase, α-chymotrypsin, or α-fucosidase. Activities of valine arylamidase and cystine arylamidase are weak. The predominant fatty acids are $C_{15:0}$ anteiso, $C_{17:0}$ anteiso, and $C_{16:0}$ iso. The diamino acid of the cell-wall peptidoglycan is lysine and major components are lysine, serine, threonine, and alanine. Menaquinones are MK-9(H_2) (64%), MK-8(H_2) (17%), and MK-8(H_4) (15%).

Source: a filtration substrate made from volcanic rock from Niigata, Japan.

DNA G+C content (mol%): 70.8 (HPLC).

Type strain: LC4, CCTCC AB 206012, IAM 15382, JCM 21826.

Sequence accession no. (16S rRNA gene): AB248526.

36. **Arthrobacter nitroguajacolicus** Kotoučková, Schumann, Durnová, Spröer, Sedláček, Neča, Zdráhal and Němec 2004, 776[VP]

ni.tro.gu.a.ja.co′li.cus. N.L. masc. adj. *nitroguajacolicus* pertaining to the chemical nitroguaiacol, whose name is based on N.L. *guaiacum* from Spanish guayaco, a tropical American tree and the resin derived thereof.

Cells are Gram-stain-positive irregular rods, club-shaped with typical V-forms, motile, and non-acid-fast. They display a rod–coccus life cycle. Cocci are 0.7–1 µm in diameter; rods are 0.6–1.0 µm wide and 1.0–4 µm long. Spores are not formed. Colonies are yellow, circular, convex, and opaque. Growth occurs with a suitable carbon source in mineral salts medium; no additional growth factors are required. Obligately aerobic. Growth at 4–37°C, with optimum at 25–30°C. Growth occurs in the pH range 6.0–8.0 and in the presence of up to 6% (w/v) NaCl. Catalase- and oxidase-positive. Nitrate not reduced. Methyl red test, urease, and hemolysis negative. Hydrolyzes gelatin, starch, casein, esculin, *o*-nitrophenyl-β-D-galactopyranoside, and tyrosine are hydrolyzed. Tween 80, DNA, and lecithin are not hydrolyzed. Elastase is produced. Pyrrolidonyl arylamidase and arginine dihydrolase are not produced. Production of alkaline phosphatase, acid phosphatase, α-galactosidase, and α-fucosidase is variable between strains: the type strain shows positive reactions. Simmons' citrate is utilized, but not gluconate. Acid is not produced from ribose, mannitol, sorbitol, lactose, trehalose, raffinose, sucrose, L-arabinose, D-arabitol, cyclodextrin, glycogen, pullulan, maltose, melibiose, melezitose, methyl-β-D-glucopyranoside, or tagatose. In the Biolog test system, the following compounds are utilized for respiration: dextrin, glycogen, arbutin, D-cellobiose, D-fructose, D-galactose, α-D-glucose, maltose, maltotriose, D-mannitol, D-mannose, palatinose, D-psicose, D-raffinose, D-ribose, D-sorbitol, sucrose, turanose, acetic acid, α-hydroxybutyric

acid, β-hydroxybutyric acid, *p*-hydroxyphenylacetic acid, L-malic acid, methyl pyruvate, propionic acid, pyruvic acid, alaninamide, D-alanine, L-alanine, L-alanyl-glycine, L-asparagine, L-glutamic acid, glycyl-L-glutamic acid, L-pyroglutamic acid, L-serine, putrescine, and glycerol. Utilization of gentiobiose, α-ketoglutaric acid and β-ketoglutaric acid is strain dependent: the type strain is positive. A negative reaction is seen with α-cyclodextrin, β-cyclodextrin, inulin, mannan, Tween 40, Tween 80, *N*-acetyl-D-glucosamine, *N*-acetyl-D-mannosamine, amygdalin, L-arabinose, D-arabitol, L-fucose, D-galacturonic acid, α-D-lactose, lactulose, D-melezitose, D-melibiose, methyl-α-D-galactoside, methyl-β-D-galactoside, 3-methylglucose, methyl-α-D-glucoside, methyl-β-D-glucoside, methyl-α-D-mannoside, L-rhamnose, salicin, sedoheptulosan, D-tagatose, D-trehalose, xylitol, D-xylose, γ-hydroxybutyric acid, lactamide, D-lactic acid methyl ester, D-malic acid, monomethyl succinate, succinamic acid, succinic acid, *N*-acetyl glutamic acid, 2,3-butanediol, adenosine, 2-deoxyadenosine, inosine, thymidine, uridine, adenosine 5′-monophosphate, thymidine 5′-monophosphate, uridine 5′-monophosphate, fructose 6-phosphate, glucose 1-phosphate, glucose 6-phosphate, and DL-α-glycerol phosphate. The cell-wall diamino acid is lysine. The peptidoglycan type is A3α, with an Ala–Thr–Ala interpeptide bridge. The major menaquinone is MK-9(H_2); MK-8(H_2) and MK-10(H_2) occur as minor components. The cellular fatty acid pattern is dominated by $C_{15:0}$ anteiso (65–70%).

Source: forest soil.

DNA G+C content (mol%): 61.9 (HPLC).

Type strain: G2-1, CCM 4924, DSM 15232, JCM 14115.

Other strains: CCM 4925, CCM 7049.

Sequence accession no. (16S rRNA gene): AJ512504.

37. **Arthrobacter oryzae** Kageyama, Morisaki, Ōmura and Takahashi 2008, 55[VP]

o.ry′za.e. L. gen. n. *oryzae* of rice.

Cells have a rod–coccus cycle. Gram-stain-positive, motile by flagella and aerobic. Colonies are cream colored on YD agar. Growth occurs on YD agar at initial pH values between 6 and 11 and at temperatures between 4 and 34°C. In YD agar medium, up to 2% NaCl is tolerated. D-Glucose, raffinose, melibiose, D-mannitol, L-inositol, and sucrose are assimilated, but L-arabinose and cellulose are not. Leucine arylamidase, acid phosphatase, naphthol-AS-BI-phosphohydrolase, β-glucuronidase, and α-glucosidase are detected by the API ZYM enzyme assay; the assay is negative for alkaline phosphatase, esterase lipase (C8), trypsin, chymotrypsin, α-galactosidase, β-galactosidase, β-glucosidase, *N*-acetyl-β-glucosaminidase, α-mannosidase, and α-fucosidase. Weak reactions are detected for esterase (C4), lipase (C14), valine arylamidase, and cystine arylamidase. Nitrate reductase, pyrrolidonyl arylamidase, and catalase are detected by the API Coryne enzyme assay, but urease is negative. The diagnostic diamino acid of the peptidoglycan is lysine. The acyl type of the peptidoglycan is acetyl. The major menaquinone is MK-9(H_2). The major cellular fatty acids are $C_{15:0}$ anteiso, $C_{17:0}$ anteiso, and $C_{15:0}$ iso. Cell-wall sugars contain galactose and glucose.

Source: paddy soil, Japan.

DNA G+C content (mol%): 67.0 (HPLC).

Type strain: KV-651, JCM 15922, NBRC 102055, NRRL B-24478.

Sequence accession no. (16S rRNA gene): AB279889.

38. **Arthrobacter oxydans** Sguros 1954, 21[AL]

o.xy′dans. N.L. part. adj. *oxydans* oxidizing.

Nonmotile (only type strain investigated; Kodama et al., 1992; Li et al., 2004c). Colonies on yeast extract-peptone media are either pearl-gray/white or show a yellow, nondiffusible pigment depending on strain. Growth on nicotine-mineral salts-yeast extract agar is abundant with production of a deep blue, diffusible pigment which turns reddish or yellow-brown in older cultures. When supplied with biotin, growth occurs in a suitable mineral salts medium with an ammonium salt as nitrogen source and glucose as carbon and energy source (Keddie et al., 1986). Starch is hydrolyzed, nitrate is reduced, grows in 10% NaCl. Nicotine blue is produced from nicotine. Esculin is not hydrolyzed. Utilizes L-arginine, L-asparagine, L-histidine, L-leucine, L-arabinose, D-galactose, D-glucose, L-rhamnose, D-ribose, D-xylose, butanediol, histidinol, inositol, malonate, sorbitol, lactose, xylitol, and sucrose but not maltose. Assimilates citric acid, formic acid, propionic acid, and uric acid but not adipic acid, benzoic acid, glutaric acid, malonic acid, or pimelic acid. Urea is formed from creatinine and uric acid. Gelatin and tyrosine are hydrolyzed. Positive for DNase, citrate, β-galactosidase, and α-glucosidase and negative for pyrrolidonyl peptidase and *N*-acetylglucosaminidase. Acid is formed from glucose (Wauters et al., 2000a). Wauters et al. (2000a) described the formation of acid from mannitol, while Li et al. (2004c) showed that the type strain of *Arthrobacter oxydans* does not utilize mannitol. The cell-wall peptidoglycan is of the Lys–Ser–Thr–Ala type (A3α). The whole-cell sugars are galactose and glucose. The principal isoprenoid quinone is MK-9(H_2) (Keddie et al., 1986). The predominant fatty acid is $C_{15:0}$ anteiso (~45%), followed by $C_{17:0}$ anteiso (~17%), $C_{16:0}$ iso (~11%), $C_{16:0}$ (~11%), and $C_{15:0}$ iso (~9%) (only type strain investigated; Kodama et al., 1992).

Source: cured tobacco leaves and associated air. Isolated by enrichment in nicotine-mineral salts-yeast extract medium.

DNA G+C content (mol%): 62.7–64.4 (T_m).

Type strain: AS 1.1925, ATCC 14358, BCRC (formerly CCRC) 11573, CCUG 17757, CIP 107005, DSM 20119, HAMBI 1857, NBRC 12138, IMET 10684, JCM 2521, LMG 3816, NCIMB 9333, NRIC 0154, VKM Ac-1114.

Sequence accession no. (16S rRNA gene): X83408.

Sequence accession no. (recA): AF214789.

39. **Arthrobacter parietis** Heyrman, Verbeeren, Schumann, Swings and De Vos 2005, 1462[VP]

pa.ri′e.tis. L. gen. n. *parietis* of a wall.

Cells are Gram-stain-positive, short rods and cocci (diameter 0.8–1 µm) occurring in pairs or clusters. They are nonmotile and do not form endospores. Colonies on NA after 48 h are 1–2 mm in diameter, yellow-orange, round with entire margins, of low convexity, opaque, and smooth. No growth in an anaerobic chamber on NA. Optimal temperature for growth is 22–30°C. No or only weak growth at 37°C and no growth at 45°C. Good growth after 1 week at 4°C. Growth on medium with 15% NaCl. Growth occurs at pH

6–9, with an optimum of 7–8. Catalase-positive and oxidase-negative. Using the API CORYNE system, positive reactions are observed for nitrate reduction, pyrazinamidase, β-galactosidase, α-glucosidase, esculin (β-glucosidase), gelatinase, and fermentation of glucose. Negative reactions are obtained for alkaline phosphatase, β-glucuronidase, *N*-acetyl-β-glucosaminidase and fermentation of ribose, xylose, mannitol, and glycogen. Variable reactions are obtained for pyrrolidonyl arylamidase, urease, and fermentation of maltose, lactose, and sucrose. Using the API ZYM system, activity is detected for acid phosphatase (weak), esterase C4, leucine arylamidase, trypsin, phosphoamidase (weak), α-galactosidase, β-galactosidase, and α-glucosidase. No activity is detected for alkaline phosphatase, esterase lipase C8, lipase C14, valine arylamidase, chymotrypsin, β-glucuronidase, *N*-acetyl-β-glucosaminidase, and α-fucosidase. Variable results (if positive, weak) were obtained for cystine arylamidase, β-glucosidase, and α-mannosidase. The type strain is negative for the variable characters listed above. Predominant fatty acids are $C_{15:0}$ anteiso (51%) and $C_{15:0}$ iso (29%). The peptidoglycan type is A3α Lys–Thr–Ala_2. MK-9(H_2) (69%) and MK-10(H_2) (20%) are the predominant menaquinones, while MK-8(H_2) and MK-11(H_2) occur in only small amounts. The cell-wall sugar is galactose. Polar lipids of the type strain are phosphatidylinositol, phosphatidylglycerol, diphosphatidylglycerol, one unknown phospholipid, and two unknown glycolipids.

Source: a biofilm overgrowing a mural painting in the Servilia tomb (Roman necropolis of Carmona, Spain).

DNA G+C content (mol%): 63.8 (HPLC).

Type strain: DSM 16404, JCM 14917, LMG 22281.

Sequence accession no. (16S rRNA gene): AJ639830.

40. **Arthrobacter pascens** Lochhead and Burton 1953, 7[AL]

pas′cens. L. part adj. *pascens* nourishing.

Colonies on yeast-peptone medium show no distinctive pigmentation. Nutritionally nonexacting: growth occurs in a suitable mineral salts medium with an ammonium salt or nitrate as sole nitrogen source and glucose as carbon + energy source. Starch is hydrolyzed, nicotine is not utilized. Nonmotile. The cell-wall peptidoglycan is of the Lys–Ala_2 type (A3α) (Fiedler et al., 1970). The whole-cell sugars are galactose and glucose. The principal isoprenoid quinone is MK-9(H_2). The predominant fatty acid is $C_{15:0}$ anteiso (59%), followed by $C_{17:0}$ anteiso (13%), $C_{15:0}$ iso (6%), $C_{16:0}$ iso (3%), $C_{16:0}$ (3%), $C_{16:1}$ ω7*c* (3%), and $C_{18:1}$ ω9*c* (2%) (Funke et al., 1996).

Source: soil.

DNA G+C content (mol%): 63.7 (T_m).

Type strain: ATCC 13346, CCUG 23843, CIP 102362, DSM 20545, HAMBI 1862, NBRC 12139, JCM 11606, LMG 16255, NRRL B-1814, VKM Ac-1116.

Sequence accession no. (16S rRNA gene): X80740.

Sequence accession no. (recA): AF214789.

41. **Arthrobacter phenanthrenivorans** Kallimanis, Kavakiotis, Perisynakis, Spröer, Pukall, Drainas and Koukkou 2009, 278[VP]

phe.nan.thre.ni.vo′rans. N.L. n. *phenanthrenum* phenanthrene; L. v. *vorare* to devour; L. part. adj. *vorans* devouring, digesting; N.L. part. adj. *phenanthrenivorans* digesting phenanthrene.

Cells are aerobic and nonmotile, stain Gram positive, and exhibit a rod–coccus growth cycle. Colonies are cream to yellow in color. Grows at 4–37°C in mineral salts medium with a suitable carbon source; optimum growth occurs between 30 and 37°C. No additional growth factors are required. Catalase- and amylase-positive. Reduces nitrate to nitrite. Negative for oxidase, urease, lipase, and gelatinase. Does not produce H_2S. Acid is not produced from glucose, lactose, or sucrose. Utilizes phenanthrene and anthracene as sole carbon sources. The polar lipid pattern consists of phosphatidylethanolamine, phosphatidylglycerol, and diphosphatidylglycerol. The major menaquinone is MK-8 and the major fatty acids are $C_{15:0}$ anteiso, $C_{16:0}$ iso, $C_{15:0}$ iso, $C_{16:0}$, and $C_{17:0}$ anteiso.

Source: creosote-contaminated soil in Greece.

DNA G+C content (mol%): 65.7 ±0.2 (T_m).

Type strain: Sphe3, DSM 18606, JCM 16027, LMG 23796.

Sequence accession no. (16S rRNA gene): AM176541.

Sequence accession no. (recA): AM931439.

42. **Arthrobacter pigmenti** Heyrman, Verbeeren, Schumann, Swings and De Vos 2005, 1462[VP]

pig.men′ti. L. gen. n. *pigmenti* of pigment or paint.

Cells are Gram-stain-positive, short rods and cocci (diameter 0.8–1 µm) occurring in pairs, chains, or clusters. They are nonmotile and do not form endospores. Colonies on NA after 48 h are small (<1 mm), light yellow, round with entire margins, of low convexity, opaque, and smooth. No growth in an anaerobic chamber on NA. Optimum temperature for growth is 22–30°C. No or only weak growth at 37 or 10°C. No growth at 45 or 4°C. Growth on medium with 15% NaCl; optimal growth at 10% NaCl. Grows at pH 7–10 and optimally at pH 8–9. Catalase-positive and oxidase-negative. Using the API CORYNE system, positive reactions are observed for pyrazinamidase, β-glucuronidase, β-galactosidase, α-glucosidase, and gelatinase. Negative reactions are obtained for pyrrolidonyl arylamidase, *N*-acetyl-β-glucosaminidase, urease, and fermentation of ribose, xylose, mannitol, lactose, and glycogen. Variable reactions are obtained for nitrate reduction, esculin (β-glucosidase) and fermentation of glucose, maltose, and sucrose. Using the API ZYM system, activity is detected for alkaline phosphatase, esterase lipase C8 (weak), leucine arylamidase, trypsin, phosphoamidase (weak), α-galactosidase (weak), and β-glucuronidase. No activity is detected for lipase C14, valine arylamidase, cystine arylamidase, chymotrypsin, α-galactosidase, β-glucosidase, *N*-acetyl-β-glucosaminidase, or α-fucosidase. Variable results (if positive, weak) were obtained for esterase C4, acid phosphatase, and α-mannosidase. Predominant fatty acids are $C_{15:0}$ iso (48%) and $C_{15:0}$ anteiso (40%). The peptidoglycan type is A3α Lys–Ala_4. MK-9(H_2) (66%), MK-10(H_2) (13%), and MK-7(H_2) (14%) are the predominant menaquinones, while MK-9 occurs in only small amounts. The cell-wall sugars are galactose and rhamnose. Polar lipids of the type strain are phosphatidylinositol, phosphatidylglycerol, diphosphatidylglycerol, one unknown phospholipid, and one unknown glycolipid.

Source: a rosy biofilm overgrowing a mural painting in the Saint-Catherine chapel (castle of Herberstein, Austria).

DNA G+C content (mol%): 61.6 (HPLC).

Type strain: DSM 16403, JCM 21771, LMG 22284.

Sequence accession no. (16S rRNA gene): AJ639827.

43. **Arthrobacter polychromogenes** Schippers-Lammertse, Muijsers and Klatser-Oedekerk 1963, 2[AL]

po.ly.chro.mo′ge.nes. Gr. adj. *polu* many; Gr. n. *chroma* color; Gr. v. *gennaio* produce; N.L. part. adj. *polychromogenes* producing many colors.

Cells are nonmotile, long rods and cocci. On nutrient agar, colonies are white, circular, smooth, convex, with an entire edge. Colonies are blue colored on peptone-glucose agar due to large amounts of blue-black crystals in the colonies. Gelatin and starch are hydrolyzed. Negative for indole production, hydrogen sulfide production, acetoin production, and urease. Little or no acid from sugars. Acid from glycerol. Nitrate is reduced to nitrite. Nicotine blue is not produced from nicotine. Utilizes L-arginine, L-asparagine, L-histidine, L-leucine, L-arabinose, D-galactose, D-glucose, L-rhamnose, D-ribose, D-xylose, butanediol, and malonate but not inositol. Assimilates citric acid, formic acid, propionic acid, and uric acid but not adipic acid, benzoic acid, glutaric acid, malonic acid, or pimelic acid (Kodama et al., 1992).

Ammonium or nitrate without biotin is not sufficient as nitrogen source. Sodium citrate is sufficient as sole carbon source, also in the absence of biotin. Excellent growth and much pigment is obtained on a medium of peptone 1%, glycerol 2%, KCl 0.6%, agar 2%, pH 9.0. The bacterium also grows well, though is colorless, when vitamin-free Casamino acids are substituted for peptone in the last mentioned medium; the bacterium does not need vitamins for growth. The color appears in the vitamin-free medium after addition of biotin; biotin is necessary for the formation of the pigments. The temperature optimum for growth and pigment production is about 25°C. Most often growth but no color is observed at 37°C. Growth at 10°C but not at 41°C. A colorless culture, grown at 37°C, soon becomes colored at room temperature. At a pH of about 5, no growth occurs. Optimum pigment formation occurs at the rather high pH value of 9–10. The maximum pH for growth and pigment production is 11 or above. No pigment formation at a pH below 6. The cell-wall peptidoglycan is of the Lys–Ser–Thr–Ala type (A3α) (Fiedler et al., 1970). The whole-cell sugar is galactose. The principal isoprenoid quinone is MK-9(H_2). The predominant fatty acid is $C_{15:0}$ anteiso (~44%), followed by $C_{17:0}$ anteiso (~25%), $C_{16:0}$ iso (~6%), $C_{16:0}$ (~13%), $C_{16:0}$ iso (~6%), and $C_{15:0}$ iso (~3%) (Huang et al., 2005b; Kodama et al., 1992).

Source: air.

DNA G+C content (mol%): 62.9 (T_m; Kodama et al., 1992).

Type strain: ATCC 15216, CIP 106989, DSM 20136, NBRC 15512, JCM 2523, LMG 3821, VKM Ac-1955.

Sequence accession no. (16S rRNA gene): X80741.

44. **Arthrobacter protophormiae** (*ex* Lysenko 1959) Stackebrandt, Fowler, Fiedler and Seiler 1984, 270[VP] (Effective publication: Stackebrandt, Fowler, Fiedler and Seiler 1983b, 482.)

pro.to.phor.mi′a.e. N.L. n. *Protophormia* a genus of dipteran insects, N.L. gen. n. *protophormiae* of *Protophormia*.

In 1-d-old cultures, the cells are slightly club-shaped rods with rounded ends (0.6–0.8 × 0.8–2.0 μm) occurring singly or in irregular groups. Stationary-phase cultures (older than 2 d) are composed almost entirely of spherical cells only. Colonies on nutrient agar show a pale to sulfur yellow, nondiffusible pigment. The peptide subunit of the peptidoglycan consists of alanine, D-glutamic acid, and lysine. The interpeptide bridge contains alanine and glutamic acid (Lys–Ala–Glu type, variation A4α).

Cell wall (only the type strain is tested) contains galactose and small amounts of glucose. The long-chain fatty acids are primarily of the iso, anteiso, straight chain, and unsaturated types, with the anteiso 12-methyl tetradecanoic acid ($C_{15:0}$ anteiso) predominating. The major respiratory isoprenoid quinones are unsaturated menaquinones with 8 and 9 isoprene units (MK-8 and MK-9). Polar lipids consist of diphosphatidylglycerol (DPG), phosphatidylglycerol (PG), and unidentified glycolipids. More than 80% of the 6 strains of *Arthrobacter protophormiae* strains assimilate 5-amino-valerate, 4-hydroxybenzoate, L-asparagine, L-arginine, L-histidine, L-arabinose, D-galactose, and glycerol; resistant to 7% NaCl.

Source: the fly *Protophormia terraenovae* and the soil.

DNA G+C content (mol%): 63.2–65.9 (T_m).

Type strain: ATCC 19271, CIP 106987, DSM 20168, NBRC 12128, JCM 1973, LMG 16324, VKM Ac-2104.

Sequence accession no. (16S rRNA gene): X80745.

Sequence accession no. (recA): AF214790.

45. **Arthrobacter psychrochitiniphilus** Wang, Gai, Chen and Xiao 2009, 2761[VP]

psy.chro.chi.ti.ni′phi.lus. Gr. adj. *psuchros* cold; N.L. n. *chitinum* chitin; Gr. adj. *philos* loving; N.L. masc. adj. *psychrochitiniphilus* a cold, chitin-loving bacterium.

Individual cells show a distinct rod–coccus cycle. Cells are Gram-stain-positive, aerobic, catalase-positive, and oxidase-negative motile rods. Spores or capsules are not seen. Colonies in LB medium at 20°C are yellow, circular, and convex. Growth occurs at 0–25°C, the optimal growth temperature is around 20°C. Grows well at 0–3% NaCl. Optimal growth occurs at pH 6–8. Tween 80, starch, cellulose, lactose, and chitin are hydrolyzed. Gelatin, lecithin, and urea are not hydrolyzed. Nitrate is reduced and NH_3 production is positive. Production of indole and H_2S is negative. Sensitive to ampicillin, chloramphenicol, kanamycin, streptomycin, and tetracycline. Growth occurs on lactose or chitin as the sole carbon source. The cellular fatty acid pattern is dominated by $C_{15:0}$ anteiso. The peptidoglycan type is A3α. The major menaquinone is MK-9(H_2). Biolog tests show that the following compounds are utilized for respiration: dextrin, Tween 40, Tween 80, *N*-acetyl-D-glucosamine, L-arabinose, D-arabitol, cellobiose, D-fructose, D-galactose, D-glucose, α-D-lactose, lactulose, maltose, maltotriose, D-mannitol, D-mannose, D-melezitose, melibiose, methyl-β-D-galactoside, D-psicose, D-ribose, D-sorbitol, sucrose, D-tagatose, trehalose, turanose, xylitol, D-xylose, acetic acid, α-hydroxybutyric acid, α-ketovaleric acid, D-lactic acid methyl ester, L-lactic acid, L-malic acid, pyruvic acid methyl ester, succinic acid monomethyl ester, propionic acid, pyruvic acid, succinamic acid, succinic acid, *N*-acetyl-L-glutamic acid, D-alanine, L-alanyl glycine, L-asparagine, L-glutamic acid, glycyl L-glutamic acid, L-serine, putrescine, 2,3-butanediol, glycerol, adenosine, inosine, thymidine, uridine, adenosine

5′-monophosphate, thymidine 5′-monophosphate, uridine 5′-monophosphate, D-glucose 6-phosphate, and DL-α-glycerol phosphate.

Source: guano of Adelie penguins, Antarctica.

DNA G+C content (mol%): 58.5 (HPLC).

Type strain: GP3, CGMCC 1.6355, JCM 13874.

Sequence accession no. (16S rRNA gene): AJ810896, AB588633.

46. **Arthrobacter psychrolactophilus** Loveland-Curtze, Sheridan, Gutshall and Brechley 2000, 3[VP] (Effective publication: Loveland-Curtze, Sheridan, Gutshall and Brechley 1999, 362.)

psy.chro.lac.to′phi.lus. Gr. adj. *psuchros* cold; L. n. *lac lactis* milk; Gr. masc. adj. *philos* friend, loving; N.L. masc. adj. *psychrolactophilus* a cold, milk (sugar)-loving (bacterium).

Individual cells show a distinct rod–coccus cycle and have a mean cell length of 1.4 μm during exponential growth and 0.5 μm during stationary phase. Gram-stain-positive; easily decolorized; strict aerobe. Contains lysine as the diagnostic amino acid in the peptidoglycan. Non-spore-forming. Nonmotile. No vitamin requirements; grows in mineral salts medium with ammonium chloride as sole source of nitrogen. Colonies on trypticase soy agar without glucose are yellow; degree of pigmentation varies with growth temperature and age of the cells. Able to grow at 0–5°C. Growth range, 0–30°C. Generation time at 10°C in trypticase soy broth with no added carbohydrate is 4.8 h. Does not produce acid with glucose as carbon source. Can utilize lactose, sorbitol, melibiose, cellobiose, glycerol, maltose, raffinose, xylose, galactose, sucrose, and glucose as sole carbon sources. Produces catalase. Produces β-galactosidase, β-glucuronidase, β-glucosidase, β-glucosidase, amylase, and gelatinase. Negative for nitrate reduction, alkaline phosphatase, *N*-acetylglucosaminidase, urease, and DNase. The major cellular fatty acids are anteiso- and isobranched fatty acids. The predominant fatty acid is $C_{15:0}$ anteiso (~73%), followed by $C_{17:0}$ anteiso (~13%), $C_{16:0}$ iso (~8%), $C_{16:0}$ (~2%), and $C_{15:0}$ iso (~1.4%). All other fatty acids are at levels below 1%.

Source: soil.

DNA G+C content (mol%): 60.6 (T_m).

Type strain: B7, ATCC 700733, JCM 12399.

Sequence accession no. (16S rRNA gene): AF134179.

47. **Arthrobacter psychrophenolicus** Margesin, Schumann, Spröer and Gounot 2004, 2070[VP]

psy.chro.phe.no′li.cus. Gr. adj. *psuchros* cold; N.L. masc. adj. *phenolicus* relating to phenol; N.L. masc. adj. *psychrophenolicus* relating to phenol [degradation] at low temperatures.

On nutrient agar, colonies are round, convex, glossy, and have entire margins and a yellow, non-fluorescent pigment. Cells are Gram-stain-positive, aerobic, non-spore-forming, and nonmotile. They exhibit a rod–coccus cycle with irregular rods in the exponential growth phase and predominantly coccoid cells in the stationary growth phase. Positive for phenol degradation, nitrate reduction, and urease, but negative for alkaline phosphatase, pyrrolidonyl arylamidase, and cystine arylamidase. Hydrolyze skimmed milk at 10°C and 25°C. Can utilize mannitol and phenylacetate but not glucose or arabinose as carbon sources. Sensitive to mezlocillin, cefamandole, cefotaxime, tetracycline, amikacin, and imipenem. Good growth and phenol biodegradation occur at 1–25°C (facultative psychrophile). Fully degrades up to 10 mM phenol as the sole carbon source. No growth occurs at pH 5 or 11. The predominant fatty acid (72.1%) is $C_{15:0}$ anteiso, while $C_{17:0}$ anteiso, $C_{16:0}$ iso, $C_{15:0}$ iso, $C_{16:0}$, and $C_{14:0}$ iso are detected in small amounts. The peptidoglycan type is A4α L-Lys–L-Glu. MK-10 is the predominant menaquinone, while MK-9 and MK-11 occur in smaller amounts (ratio of peak areas 72:12:1, respectively). The polar lipids are phosphatidylglycerol, diphosphatidylglycerol, phosphatidylinositol, and an unidentified glycolipid. The only cell-wall sugar is glucose.

Source: an alpine ice cave in Werfen (Salzburg), Austria.

Type strain: AG31, DSM 15454, JCM 13568, LMG 21914.

Sequence accession no. (16S rRNA gene): AJ616763.

48. **Arthrobacter ramosus** Jensen 1960, 131[AL]

ra.mo′sus. L. masc. adj. *ramosus* branched, branching.

Motile by a few lateral flagella. Colonies on yeast extract-peptone media show no distinctive pigmentation. Nutritionally nonexacting: growth occurs in a suitable mineral salts medium with an ammonium salt or nitrate as sole nitrogen source. Starch is not hydrolyzed, and nicotine is not utilized.

The cell-wall peptidoglycan is of the Lys–Ala_4 type (A3α). The whole-cell sugars are galactose, rhamnose, and mannose. The principal isoprenoid quinone is MK-9(H_2) (Keddie et al., 1986) Major fatty acids are $C_{15:0}$ anteiso (63%), $C_{17:0}$ anteiso (8%), and $C_{15:0}$ iso (6%) (Funke et al. 1996).

Source: beech forest soil at depth of 30–35 cm.

DNA G+C content (mol%): 62.2 (T_m).

Type strain: ATCC 13727, CIP 102361, DSM 20546, NBRC12958, JCM 1334, LMG 17309, NRRL B-3159, VKM Ac-1117.

Sequence accession no. (16S rRNA gene): X80742.

49. **Arthrobacter rhombi** Osorio, Barja, Hutson and Collins 1999, 1220[VP]

rhom′bi. L. masc. n. *rhombus* flatfish; L. gen. n. *rhombi* of flatfish.

Cells are Gram-stain-positive, short rods and cocci. They are non-spore-forming and nonmotile. On BHA, smooth convex colonies which are yellowish or whitish in color and with a diameter of approximately 1 mm are formed after 48 h. Growth occurs in 1% (w/v) and 10% (w/v) NaCl and at 4 and 30°C. They are strictly aerobic and catalase- and oxidase-positive. Using the API CORYNE system, positive reactions for β-galactosidase and esculinase are observed. Negative reactions are obtained for nitrate reductase, pyrazinamidase, pyrrolidonyl arylamidase, urease, β-glucuronidase, *N*-acetyl-β-glucosaminidase, and gelatinase. Using the API 50CH system, the following substrates are used as sole carbon sources: glycerol, galactose, D-glucose, D-fructose, D-mannose, mannitol, amygdalin, arbutin, esculin, salicin, cellobiose, maltose, lactose, melibiose, sucrose, trehalose, β-gentiobiose, D-turanose, gluconate, and D-arabitol. Erythritol, D-arabinose, L-arabinose, ribose, D-xylose, adonitol, methyl-β-xyloside, L-sorbose, dulcitol, sorbitol, methyl-α-D-mannoside, methyl-α-D-glucoside, inositol, *N*-acetyl-β-glucosamine, inulin, melezitose, D-raffinose, starch, glycogen, xylitol, D-lyxose, D-tagatose, D-fucose, L-fucose, L-arabitol, 2-ketogluconate, and 5-ketogluconate are not utilized. The

cell-wall murein type is A4α (L-Lys–L-Ala–D-Glu).

Source: organs of Greenland halibut (*Reinhardtius hippoglossoides*) in the deep waters of the Flemish Cap fishing ground (Newfoundland, NW Atlantic).

DNA G+C content (mol%): 61.0 (T_m).

Type strain: F.98.3HR.69, CCUG 38813, JCM 11678.

Other strains of the species: CCUG 38812.

Sequence accession no. (16S rRNA gene; type strain): Y15884.

Sequence accession no. (16S rRNA gene; another strain): Y15885.

50. **Arthrobacter roseus** Reddy, Prakash, Matsumoto, Stackebrandt and Shivaji 2002, 1020[VP]

ro′se.us. L. masc. adj. *roseus* rose-colored.

Rod–coccus growth cycle with irregular rods in the exponential phase and predominantly coccoid cells (~1.0 µm in diameter) in the stationary phase. Gram-stain-positive, nonspore-forming, and nonmotile; colonies on peptone/yeast extract medium are round, smooth, convex, 0.2–1 mm in diameter, and red-pigmented. Pigment is insoluble in water but soluble in methanol and exhibits four absorption maxima, at 437, 467, 494, and 524 nm. Aerobic. Growth occurs at 5–30°C and at pH 6–12, and cells tolerate up to 5.8% NaCl. Optimum growth is observed at 22°C and pH 7. Catalase, phosphatase, gelatinase, and nitrate reduction tests are positive; oxidase, urease, lipase, β-galactosidase, arginine dihydrolase, indole production, methyl red test, Voges–Proskauer test, and levan formation are negative. Esculin and starch are not hydrolyzed. Positive for utilization of mannose, galactose, maltose, fructose, glucose, arabinose, ribose, xylose, rhamnose, raffinose, trehalose, succinic acid, fumaric acid, citric acid, mannitol, sorbitol, adonitol, sucrose, inulin, pyruvate, acetate, alanine, leucine, isoleucine, valine, serine, arginine, aspartic acid, glutamic acid, asparagine, glutamine, proline, and phenylalanine. The following substrates are not utilized as sole carbon sources: dulcitol, inositol, melibiose, lactose, nicotine, cellulose, glycine, threonine, cysteine, methionine, lysine, tyrosine, histidine, and tryptophan. No acid or gas from glucose, arabinose, xylose, rhamnose, fructose, galactose, mannose, lactose, maltose, or sucrose. Sensitive to penicillin, chlortetracycline, chloramphenicol, oxytetracycline, tetracycline, erythromycin, nitrofurantoin, bacitracin, nitrofurazone, leucomycin, rifampin, nystatin, cotrimoxazole, trimethoprim, ampicillin, carbenicillin, gentamicin, amoxicillin, tobramycin, and polymyxin B but resistant to furazolidone, colistin, furoxone, and kanamycin. The peptidoglycan type is Lys–Gly–Ala_3 (variation A3α) and the major menaquinone is MK-9(H_2). Cell-wall sugars are galactose, glucose, ribose, and rhamnose. Cellular fatty acids are $C_{14:0}$ iso, $C_{14:0}$, $C_{15:0}$ iso, $C_{15:0}$ anteiso, $C_{15:0}$, $C_{16:0}$ iso, $C_{16:0}$, $C_{16:1}$, $C_{17:0}$ anteiso, $C_{18:0}$, and $C_{18:2}$. The predominant polar lipids are phosphatidylglycerol, diphosphatidylglycerol, phosphatidylinositol, and an unidentified glycolipid (Busse, unpublished results).

Source: a cyanobacterial mat sample from McMurdo Dry Valleys, Antarctica (77° 32′ 18″ S, 160° 45′ E).

DNA G+C content (mol%): 66.0–69.0 (HPLC).

Type strain: CMS 90r, DSM 14508, JCM 11881, MTCC 3712.

Sequence accession no. (16S rRNA gene): AJ278870.

51. **Arthrobacter russicus** Li, Kawamura, Fujiwara, Naka, Liu, Huang, Kobayashi and Ezaki 2004c, 834[VP]

rus′si.cus. N.L. masc. adj. *russicus* pertaining to Russia (Russian space station).

Grows well under aerobic conditions at 30°C on BHI agar plates, but unable to grow at 37°C. Gram-stain-positive, nonmotile, irregular rods, about 1.3–3.6 µm long and 0.5–0.9 µm wide. Circular, smooth and creamy colonies grow to a diameter of about 1.5 mm after 24 h. Positive for Tween 40, Tween 80, D-fructose, D-mannose, methyl pyruvate, L-alanyl glycine, and putrescine but negative for dextrin, arbutin, D-gluconic acid, maltose, maltotriose, D-melezitose, 3-methyl glucose, methyl-α-D-glucoside, methyl-β-D-glucoside, palatinose, D-ribose, salicin, sucrose, D-trehalose, acetic acid, α-hydroxybutyric acid, β-hydroxybutyric acid, γ-hydroxybutyric acid, α-ketovaleric acid, D-lactic acid methyl ester, L-lactic acid, L-malic acid, propionic acid, succinamic acid, succinic acid, alaninamide, L-asparagine, and 2,3-butanediol, esculin hydrolysis, urease activity, and nitrate reduction. Utilizes glucose but not inositol, sorbitol, lactose, xylitol, xylose, maltose, mannitol, and sucrose. Weakly positive for monomethyl succinate and L-glutamic acid. Variable for turanose, pyruvic acid, uridine, uridine 5′ monophosphate. Cell-wall peptidoglycan is A3α Lys–Ala_2. Predominant isoprenoid quinone is MK-9(H_2). Major cellular fatty acids are $C_{15:0}$ anteiso and $C_{17:0}$ anteiso. Predominant polar lipids are cardiolipin and phosphatidylinositol.

Source: an air sample from the Russian space station Mir.

DNA G+C content (mol%): 65.5 (HPLC).

Type strain: DSM 14555, GTC 863, JCM 11414.

Sequence accession no. (16S rRNA gene): AB071950.

52. **Arthrobacter sanguinis** Mages, Frodl, Bernard and Funke 2008, 2985[VP]

san′gui.nis. L. masc. gen. n. *sanguinis* of blood, indicating that the bacterium was isolated from a blood culture.

The cells are coryneform bacteria without irregular branching, and spores are not formed. The organism is obligately aerobic. The colonies are whitish-grayish, slightly convex, of creamy texture, and up to 2 mm in diameter after 24 h of incubation at 35°C on Columbia SBA plates. Activities of the following enzymes are detected: catalase, acid phosphatase, alkaline phosphatase, esterase (C4), esterase lipase (C8), α-galactosidase, β-galactosidase, gelatinase, *N*-acetyl-β-glucosaminidase, α-glucosidase, leucine arylamidase, α-mannosidase, pyrazinamidase, and trypsin. Activities of α-chymotrypsin, cystine arylamidase, α-fucosidase, β-glucosidase, β-glucuronidase, lipase (C14), nitrate reductase, naphthol-AS-BI-phosphohydrolase, urease, and valine arylamidase are not observed. The bacterium is capable of utilizing *N*-acetylglucosamine, amygdalin, D-arabitol, cellobiose, fructose, galactose, gentiobiose, glucose, glycerol, maltose, mannitol, mannose, melibiose, potassium, gluconate, potassium 2-ketogluconate, raffinose, sucrose, sorbitol, trehalose, and turanose as carbon sources. The type strain did not utilize adonitol, D-arabinose, L-arabinose, L-arabitol, arbutin, dulcitol, erythritol, fucose, methyl-α-D-glucopyranoside, glycogen, inositol, inulin, potassium 5-ketogluconate, lactose, lyxose, methyl-α-D-mannopyranoside, melezitose,

rhamnose, ribose, salicin, sorbose, starch, tagatose, methyl-β-D-xylopyranoside, xylitol, or xylose. Lysine is the diamino acid of the peptidoglycan, and $C_{15:0}$ anteiso and $C_{17:0}$ anteiso are the predominant cellular fatty acids.

Source: a blood culture.

DNA G+C content (mol%): not determined.

Type strain: CCUG 46407, DSM 21259.

Sequence accession no. (16S rRNA gene): EU086805.

53. **Arthrobacter scleromae** Huang, Zhao, He, Wang, Liu, You and Guan 2005a, 1743[VP] (Effective publication: Huang, Zhao, He, Wang, Liu, You and Guan 2005b, 1453.)

scle.ro′ma.e. N.L. gen. n. *scleromae* of scleroma.

Cells are Gram-stain-positive, non-spore-forming, and nonmotile and display a rod–coccus life cycle. They are obligately aerobic, catalase-positive, and ~0.25 to ~0.35 µm in diameter. Colonies on blood agar or nutrient agar are whitish, glistening, convex, smooth surfaced, and circular. The colonies grow to up to 4 to 5 mm in size by 72 h. Growth occurs with a suitable carbon source in mineral salts medium; no additional growth factors are needed. Growth also occurs in the presence of 5% NaCl, at 15–37°C and pH 6–9, but not in 10% NaCl at 5 or 42°C. The organism hydrolyzes casein, DNA, esculin, gelatin, starch, and tyrosine but not lecithin or xanthine. The nitrate reduction test is weakly positive. *N*-Acetylglucosaminidase, β-galactosidase, α-glucosidase, lipase, pyrrolidonyl peptidase, and urease are not produced. Acid is produced from mannitol. It utilizes the following substrates as sole carbon sources: γ-aminobutyrate, L-arginine, L-asparagine, citrate, D-fructose, fumarate, D-galactose, D-gluconate, D-glucose, glycerol, L-histidine, *p*-hydroxybenzoate, 2-oxoglutarate, lactose, DL-malate, maltose, mannose, D-melezitose, phenylacetate, pyruvate, D-raffinose, salicin, sorbitol, succinate, sucrose, trehalose, D-turanose, xylitol, and D-xylose. The following substrates are not utilized: acetamide, adipate, adonitol, L-alanine, ρ-aminobenzoate, D-arabitol, arbutin, azelate, benzoate, 2,3-butanediol, *n*-butyrate, caprylate, D-cellobiose, L-citrulline, L-cysteine, dulcitol, erythritol, formate, glucosamine, D-glucuronate, glutarate, glycogen, glycolate, γ-hydroxybutyrate, inulin, DL-isoleucine, isovalerate, L-leucine, maleate, malonate, DL-methionine, α-methyl-D-glucoside, α-methyl-D-mannoside, nicotine, L-ornithine, oxalate, L-phenylalanine, *o*-phthalate, pimelate, L-rhamnose, D-ribose, sebacate, sorbose, suberate, D-tartrate, L-threonine, L-tryptophan, or DL-valine. Compounds slowly utilized are acetate, L-arabinose, inositol, mannitol, and melibiose. The strain is susceptible to ceftriaxone, chloramphenicol, rifampin, and tetracycline; moderately susceptible to cefazolin, cefotaxime, doxycycline, erythromycin, nitrofurantoin, piperacillin, and vancomycin; and resistant to amikacin, ampicillin, gentamicin, kanamycin, norfloxacin, oxacillin, penicillin G, streptomycin, and tobramycin. The major cellular fatty acid is $C_{15:0}$ anteiso, with significant amounts of $C_{15:0}$ iso, $C_{16:0}$ iso, $C_{17:1}$ anteiso ω9*c*, and $C_{17:0}$ anteiso. The predominant menaquinone is MK-8(H_2). The cell-wall peptidoglycan type is L-Lys–L-Ser–L-Thr–L-Ala (A3α), and the cell-wall sugars are galactose and glucose.

Source: swollen scleromata of a dermatosis patient.

DNA G+C content (mol%): 64.7 (T_m).

Type strain: YH-2001, CGMCC 1.3601, JCM 12642.

Sequence accession no. (16S rRNA gene): AF330692.

54. **Arthrobacter soli** Roh, Sung, Nam, Chang, Kim, Yoon, Jeon, Oh and Bae 2008a, 1993[VP] (Effective publication: Roh, Sung, Nam, Chang, Kim, Yoon, Jeon, Oh and Bae 2008b, 43.)

so′li. L. neut. gen. n. *soli* of soil, the source of the type strain.

The cells are rod-shaped (0.9 × 2.0~3.0 µm), Gram-stain-positive, oxidase-positive, and catalase-negative. Colonies are yellow-pigmented and circular, measuring approximately 1.0–2.0 mm in diameter after 2 d of growth on TSBA at 30°C. The temperature range for growth is 16~40°C, but no growth occurs at 15 and 41°C. No growth occurs in NaCl at concentrations greater than 15%. Optimal pH for growth is 7.0. The strain can reduce nitrate to nitrite. Indole is not produced, and glucose fermentation does not occur. Cells are arginine dihydrolase- and urease-negative. Gelatin hydrolysis occurs, but not esculin and PNPG (*p*-nitrophenyl α-D-glucopyranoside) hydrolysis. Potassium gluconate, malate, trisodium citrate, phenylacetic acid, glycerol, L-arabinose, D-ribose, D-adonitol, methyl-β-D-xyloside, D-galactose, D-glucose, D-fructose, D-mannose, *N*-acetylglucosamine, D-cellobiose, D-maltose, D-lactose, gluconate, 2-ketogluconate, and 5-ketogluconate are assimilated; capric acid, adipic acid, erythritol, D-arabinose, D-xylose, L-xylose, L-sorbose, L-rhamnose, dulcitol, inositol, D-mannitol, D-sorbitol, methyl-α-D-mannoside, methyl-α-D-glucoside, amygdalin, arbutin, esculin, salicin, D-melibiose, sucrose, D-trehalose, inulin, D-melezitose, D-raffinose, starch, glycogen, xylitol, gentiobiose, D-turanose, D-lyxose, D-tagatose, D-fucose, L-fucose, D-arabitol, and L-arabitol are not assimilated. Cells are positive for alkaline phosphatase, esterase (C4), esterase lipase (C8), lipase (C14), leucine arylamidase, valine arylamidase, cystine arylamidase, trypsin, acid phosphatase, naphthol-AS-BI-phosphohydrolase, α-glucosidase, and α-mannosidase. However, cells are negative for α-chymotrypsin, α-galactosidase, β-galactosidase, β-glucuronidase, β-glucosidase, *N*-acetyl-β-glucosaminidase, and α-fucosidase. The predominant fatty acids are $C_{15:0}$ anteiso and $C_{17:0}$ anteiso, $C_{16:0}$ iso, and $C_{15:0}$ iso.

Source: wastewater reservoir sediment collected in Daejeon, Republic of Korea.

DNA G+C content (mol%): 62.0 (HPLC).

Type strain: SYB2, DSM 19449, KCTC 19291.

Sequence accession no. (16S rRNA gene): EF660748.

55. **Arthrobacter stackebrandtii** Tvrzová, Schumann, Spröer, Sedláček, Verbarg, Kroppenstedt and Páčová 2005b, 807[VP]

sta.cke.brand′ti.i. N.L. gen. masc. n. *stackebrandtii* of Stackebrandt, named in honor of Erko Stackebrandt for his pioneering contributions to our insight into the phylogenetic structure of the suborder *Micrococcineae* and of the genus *Arthrobacter* in particular.

Cells are irregular club-shaped rods showing a rod–coccus cycle, 0.6–1 × 1–3 µm, occurring in pairs as typical V-forms. Gram-stain-positive, nonmotile, non-acid-fast, and non-spore-forming. Growth occurs at 4–30°C. Growth occurs at pH 5.7–9.1 and in the presence of 5% NaCl. Obligately aerobic. Positive for catalase, urease, and pyrazinamidase. Negative for oxidase, β-glucuronidase, β-galactosidase,

α-glucosidase, *N*-acetyl-β-glucosaminidase, and pyrrolidonyl arylamidase. Nitrate is reduced to nitrite. Nitrite is not reduced; Tween 80 and esculin are not hydrolyzed. Starch and gelatin are hydrolyzed. Acid production is negative from glucose, ribose, xylose, mannitol, maltose, lactose, sucrose, and glycogen. The following compounds are utilized (Biolog system): α-cyclodextrin, dextrin, Tween 40, L-arabinose, D-arabitol, arbutin, D-cellobiose, D-fructose, L-fucose, D-galactose, α-D-glucose, α-D-lactose, lactulose, maltose, maltotriose, D-mannose, D-melibiose, methyl-α-D-galactoside, methyl-β-D-galactoside, palatinose, D-psicose, D-raffinose, D-ribose, salicin, stachyose, sucrose, D-trehalose, turanose, D-xylose, β-ketovaleric acid, methyl pyruvate, pyruvic acid, L-asparagine, glycerol, adenosine, inosine, thymidine, and uridine. Negative reactions (Biolog) were observed with β-cyclodextrin, glycogen, inulin, mannan, Tween 80, *N*-acetyl-D-glucosamine, *N*-acetyl-D-mannosamine, amygdalin, D-galacturonic acid, gentiobiose, D-gluconic acid, *meso*-inositol, D-melezitose, 3-methyl glucose, methyl-α-D-glucoside, methyl-β-D-glucoside, methyl-α-D-mannoside, L-rhamnose, sedoheptulosan, D-sorbitol, xylitol, β-hydroxybutyric acid, γ-hydroxybutyric acid, *p*-hydroxyphenylacetic acid, α-ketoglutaric acid, lactamide, D-lactic acid methyl ester, D-malic acid, monomethyl succinate, propionic acid, succinamic acid, succinic acid, *N*-acetylglutamic acid, alaninamide, D-alanine, L-alanine, L-alanyl glycine, L-glutamic acid, glycyl L-glutamic acid, L-pyroglutamic acid, putrescine, 2,3-butanediol, adenosine 5′-monophosphate, uridine 5′-monophosphate, fructose 6-phosphate, glucose 1-phosphate, glucose 6-phosphate, and DL-glycerophosphate. The cellular fatty acid contents of $C_{15:0}$ anteiso (48.1%), $C_{17:0}$ anteiso (14.4%), $C_{14:0}$ iso (1.84%), $C_{15:0}$ iso (14.2%), $C_{16:0}$ iso (16.0%), $C_{17:0}$ iso (3.5%), and $C_{16:0}$ (1.4%). The peptidoglycan type is A3α Lys–Ala_2. Strain CCM 2783^T possesses predominantly menaquinone MK-9(H_2) (62%) and MK-10(H_2) (25%) and a small amount of MK-11(H_2) (5%).

Source: poultry litter.

Type strain: CCM 2783, DSM 16005, JCM 14116.

Sequence accession no. (16S rRNA gene): AJ640198.

56. **Arthrobacter subterraneus** Chang, Bae, Nam, Kwon, Park, Shin, Kim, Quan, Rhee, An and Park, 2008, 1993^{VP} (Effective publication: Chang, Bae, Nam, Kwon, Park, Shin, Kim, Quan, Rhee, An and Park 2007, 1878.)

sub.ter.ra′ne.us. L. masc. adj. *subterraneus* under the earth, indicating the source of isolation.

The cells are Gram-stain-positive, short rods, and cocci (diameter 0.8–1 µm) occurring singly, in pairs, or in clusters. They are nonmotile and do not form endospores. Colonies grown on NA after 48 h are small (<1 mm), pale yellow, round with entire margins, of a low convexity, opaque, and smooth. Do not grow in an anaerobic chamber on NA. Optimum temperature for growth is 20–30°C. Weak growth at 37°C, and no growth at 45°C. Growth is apparent at 4°C after 1 week of incubation. Growth occurs on media with 13% NaCl, yet not with 15% NaCl. Grows at pH 5.3–10.5. Catalase-positive and oxidase-negative. Using the API STAPH system, positive reactions are observed for fermentation with D-mannitol, xylitol, D-melibiose, and raffinose, whereas negative reactions are obtained for fermentation with D-glucose, D-fructose, D-mannose, maltose, lactose, D-trehalose, potassium nitrate, β-naphthyl-acid phosphatase, sodium pyruvate, xylose, sucrose, α-methyl-D-glucoside, *N*-acetylglucosamine, arginine, and urea. Using the API ZYM system, activity is detected with esterase (C4), esterase lipase (C8), leucine arylamidase, valine arylamidase, trypsin, acid phosphatase, naphthol-AS-BI-phosphohydrolase, β-galactosidase, and α-glucosidase, whereas no activity is detected with alkaline phosphatase, lipase (14), cystine arylamidase, α-chymotrypsin, α-galactosidase, β-glucuronidase, β-glucosidase, *N*-acetyl-β-glucosaminidase, α-mannosidase, and α-fucosidase. The predominant fatty acids are $C_{15:0}$ anteiso (35%) and $C_{17:1}$ anteiso ω9*c* (15%). The cell-wall peptidoglycan type is A3α, with a Lys–Thr–Ala_3 interpeptide bridge. The major menaquinone is MK-9(H_2). The cell-wall sugars are galactose, glucose, and ribose.

Source: deep subsurface water in Pohang, Gyeongbuk Province, Korea.

Type strain: CH7, DSM 17585, KCTC 9997.

Sequence accession no. (16S rRNA gene): DQ097525.

57. **Arthrobacter sulfonivorans** Borodina, Kelly, Schumann, Rainey, Ward-Rainey and Wood 2002a, 685^{VP} (Effective publication: Borodina, Kelly, Schumann, Rainey, Ward-Rainey and Wood 2002b, 180.)

sul.fo.ni.vo′rans. N.L. n. *sulfonum* sulfone group, L. v. *vorare* to devour, N.L. part. adj. *sulfonivorans* sulfone-devouring.

Cells are spherical, 0.7 µm in diameter. Gram-stain-positive, forming clumps and chains in liquid culture; motile; spores or capsules not seen; catalase- and oxidase-positive. Grows heterotrophically and aerobically on glucose, fructose, sucrose, galactose, acetate, ethanol, pyruvate, malate, succinate, citrate, serine, alanine, taurine, diethylsulfone, and yeast extract. Grows aerobically on methylated sulfur compounds (dimethylsulfone, dimethylsulfoxide, and dimethylsulfide), methanol, methylamine, and dimethylamine. Methylotrophic growth with dimethylsulfone uses the ribulose monophosphate cycle for formaldehyde assimilation. Does not grow with trimethylamine or alkanesulfonates (methane-, propane-, butane-, pentane-, and hexane-sulfonate), or autotrophically on inorganic sulfur compounds. Nitrate is not reduced. Growth on dimethylsulfone occurs optimally at pH 7.3–7.4 and at 20–25°C. Temperature range for growth is 4–30°C with no growth at 37 or 44°C. Ammonium chloride, nitrate, and methylamine are used as nitrogen sources for growth. Growth occurs in the presence of 1.5% or 2.5% (w/v) NaCl, but not with 5% (w/v) NaCl. On dimethylsulfone-agar medium, colonies are creamy-yellow, circular-umbonate, and 0.5–0.7 mm in diameter. The principal isoprenoid quinone is MK-9(H_2), with smaller amounts of MK-10(H_2), MK-8(H_2), MK-9, MK-7(H_2), and MK-11(H_2). The principal cellular fatty acid is $C_{15:0}$ anteiso, with $C_{15:0}$ iso, $C_{16:0}$ iso and $C_{17:0}$ anteiso also present. Peptidoglycan contains lysine as the diagnostic diamino acid, as well as alanine, glutamic acid, threonine, and serine; peptidoglycan type (L-Lys–L-Ser–L-Thr–L-Ala) is A11.23.

Source: soil from the root ball of Allium aflatunense.

DNA G+C content (mol%): 61.0 (T_m).

Type strain: ALL, ATCC BAA-112, DSM 14002, JCM 13520.

Sequence accession no. (16S rRNA gene): AF235091.

58. **Arthrobacter sulfureus** Stackebrandt, Fowler, Fiedler and Seiler 1984, 270[VP] (Effective publication: Stackebrandt, Fowler, Fiedler and Seiler 1983b, 484.)

sul.fu′re.us. L. masc. adj. *sulfureus* of or like sulfur, meaning sulfur-colored.

In young cultures rod-shaped cells, irregular (0.4–0.6 × 1.2 to 1.6 μm), angular or slightly curved, and occasionally club-shaped; snapping divisions are observed. In old cultures, rods become shorter, and in some strains coccoid cells are predominant. Gram-stain-positive. Motile with peritrichous flagella or nonmotile. Colonies large, circular, smooth, entire, raised, glistening, dull, yellow, opaque, butyrous; 2–3 mm in diameter. Slightly turbid in nutrient broth; moderate growth and turbid in glutamate broth; liquefaction in nutrient gelatin stab culture. Nitrate not reduced to nitrite; indole not produced; hydrolysis of starch and casein negative; acetyl-methyl carbinol not produced; catalase-positive. No acid or gas from glycerol, xylose, glucose, sucrose, lactose, dextrin, and starch. Assimilate 4-hydroxybenzoate, L-leucine, L-asparagine, L-arginine, and L-histidine; two of three strains assimilate 5-aminovalerate, malonate, levulinate, D-ribose, D-galactose, D-xylitol, and 2,3-butylenglycol. Cellulose not attacked. The type strain is positive for pyrazinamidase and urease but negative for β-galactosidase. Assimilation of D-arabitol, inositol, maltose, mannitol, xylitol, and 5-ketogluconate, but no assimilation amygdalin, arbutin, cellobiose, D-mannose, D-turanose, D-xylose, glycerol, L-arabinose, melibiose, rhamnose, ribose, salicin, sucrose, trehalose, β-gentiobiose, and *N*-acetylglucosamine (only type strain investigated; Osorio et al., 1999). The peptide subunit consists of alanine, D-glutamic acid, and lysine. The interpeptide bridge consists of glutamic acid (Lys–Ala–Glu type, variation A4α). Cell wall (only one strain tested) contains galactose and traces of mannose. The long-chain fatty acids (two strains tested) are primarily of the iso, anteiso, straight chain, and unsaturated acid types, with the anteiso 12-methyltetradecanoic acid ($C_{15:0}$ anteiso) dominating. The major respiratory quinones are unsaturated menaquinones either with 9 or with equal amounts of 9 and 10 isoprenoic units. Polar lipids consist of diphosphatidylglycerol, phosphatidylglycerol, and of unidentified glycolipids. Optimal temperature and pH for growth are 25–30°C and 7.0–8.0. No growth at pH 5.0.

Source: soil.

DNA G+C content (mol%): 64.5–66.0 (T_m).

Type strain: ATCC 19098, CIP 106986, DSM 20167, NBRC12678, JCM 1338, LMG 16694, NRRL B-14730.

Sequence accession no. (16S rRNA gene): X83409.

Sequence accession no. (recA): AF214787.

59. **Arthrobacter tecti** Heyrman, Verbeeren, Schumann, Swings and De Vos 2005, 1462[VP]

tec′ti. L. gen. n. *tecti* from the ceiling.

Cells are Gram-stain-positive, short rods and cocci (diameter 0.8–1 μm) occurring in pairs or clusters. They are nonmotile and do not form endospores. Colonies on NA after 48 h are small (<1 mm), yellow, round with entire margins, of low convexity, opaque, and smooth. No growth in an anaerobic chamber on NA. Optimum temperature for growth is 22–30°C. Growth on medium with 15% NaCl added. Growth at pH 6–9; optimal growth at pH 8. Catalase-positive and oxidase-negative. Using the API CORYNE system, a positive reaction is observed for *N*-acetyl-β-glucosaminidase. Most strains, including the type strain, test positive for gelatinase but negative for pyrrolidonyl arylamidase and fermentation of ribose and mannitol. Negative reactions are obtained for nitrate reduction, β-glucuronidase, urease, and fermentation of ribose, xylose, maltose, lactose, and glycogen. Variable reactions are obtained for pyrazinamidase, alkaline phosphatase, β-galactosidase, α-glucosidase, esculin (β-glucosidase), and fermentation of glucose and sucrose. Using the API ZYM system, activity is detected for alkaline phosphatase (weak), acid phosphatase (weak), leucine arylamidase, and phosphoamidase (weak). Most strains test positive for esterase C4, esterase lipase C8, trypsin (negative for the type strain), *N*-acetyl-β-glucosaminidase (negative for the type strain), and α-mannosidase (negative for the type strain). No activity is detected for lipase C14, valine arylamidase, cystine arylamidase, α-galactosidase, β-glucuronidase, or α-fucosidase. Variable reactions are obtained for chymotrypsin, β-galactosidase, α-glucosidase, and β-glucosidase. Predominant fatty acids are $C_{15:0}$ anteiso (44%) and $C_{15:0}$ iso (37%). The peptidoglycan type is A3α Lys–Thr–Ala3. MK-9(H_2) (41%), MK-10(H_2) (28%), and MK-11(H_2) (11%) are the predominant menaquinones, while MK-12(H_2), MK-9, MK-10, and MK-11 occur in only small amounts. The cell-wall sugars are galactose and rhamnose. Polar lipids of the type strain are phosphatidylinositol, phosphatidylglycerol, diphosphatidylglycerol, and one unknown phospholipid.

Source: a biofilm overgrowing the ceiling of the main room of the Servilia tomb (Roman Necropolis of Carmona, Spain).

DNA G+C content (mol%): 63.7 (HPLC).

Type strain: DSM 16407, JCM 21772, LMG 22282.

Another strain: LMG 22285.

Sequence accession no. (16S rRNA gene; type strain): AJ639829.

Sequence accession no. (16S rRNA gene; another strain): AJ639828.

60. **Arthrobacter tumbae** Heyrman, Verbeeren, Schumann, Swings and De Vos 2005, 1463[VP]

tum′ba.e. L. gen. n. *tumbae* of a tomb.

Cells are Gram-stain-positive, short rods and cocci (diameter, 0.8–1 μm) occurring singly, in pairs, or in clusters. They are nonmotile and do not form endospores. Colonies on NA after 48 h are small (<1 mm), yellow–orange, round with entire margins, of low convexity, opaque, and smooth. Do not grow in an anaerobic chamber on NA. Optimum temperature for growth is 22–30°C. Weak growth at 37°C, and no growth at 52°C. Growth at 4°C after 1 week of incubation. Growth on medium with 10% NaCl, but not with 15% NaCl. Catalase-positive and oxidase-negative. Alkaliphilic; pH range for growth of 7–10 with an optimum of 8–9. Using the API CORYNE system, positive reactions are observed for pyrazinamidase, β-glucuronidase, β-galactosidase, and α-glucosidase. Most strains, including the type strain, test positive for gelatinase. Negative reactions are obtained for *N*-acetyl-β-glucosaminidase and fermentation of ribose, xylose, mannitol, and glycogen. Variable

reactions are obtained for nitrate reduction, pyrrolidonyl arylamidase, alkaline phosphatase, esculin (β-glucosidase), urease, and fermentation of glucose, maltose, lactose, and sucrose. Using the API ZYM system, activity is detected for esterase C4, leucine arylamidase, and phosphoamidase (weak). Most strains, including the type strain, test positive for esterase lipase C8 and negative for alkaline phosphatase, valine arylamidase, α-galactosidase, and β-galactosidase. No activity is detected for lipase C14, cystine arylamidase, β-glucuronidase, β-glucosidase, *N*-acetyl-β-glucosaminidase, or α-fucosidase. Variable reactions are obtained for trypsin, chymotrypsin, acid phosphatase, α-glucosidase, and α-mannosidase. Predominant fatty acids are $C_{15:0}$ anteiso (57%) and $C_{15:0}$ iso (17%). The peptidoglycan type is A3α Lys–Thr–Ala_3. MK-9(H_2) (54%) and MK-10(H_2) (25%) are the predominant menaquinones, while MK-7(H_2), MK-8(H_2), and MK-11(H_2) occur in only small amounts. The cell-wall sugar is galactose. Polar lipids of the type strain are phosphatidylinositol, phosphatidylglycerol, diphosphatidylglycerol, and one unknown phospholipid.

Source: a biofilm overgrowing a mural painting in the Servilia tomb (Roman Necropolis of Carmona, Spain).

DNA G+C content (mol%): 64.7 (HPLC).

Type strain: DSM 16406, JCM 21773, LMG 19501.

Sequence accession no. (16S rRNA gene): AJ315069.

61. **Arthrobacter uratoxydans** Stackebrandt, Fowler, Fiedler and Seiler 1984, 270[VP] (Effective publication: Stackebrandt, Fowler, Fiedler and Seiler 1983b, 483.)

u.ra.to′xy.dans. N.L. n. *uratum* salt of uric acid; N.L. part. adj. *oxydans* oxidizing; N.L. part. adj. *uratoxydans* uric acid oxidizing.

In young cultures, straight to slightly curved or ellipsoidal rods with metachromic granules to pleomorphic rods which are club-shaped swellings; angular or palisade rods formed by snapping divisions are observed. Gram-stain-positive; 0.8–1.2 × 1.0–5 μm; nonmotile. No capsules; no endospores. Colonies circular, flat, smooth, entire margins, slightly glistening and opaque, 3–4 mm in diameter; creamy white in young cultures to pale yellow in old cultures. Water soluble, pale yellowish brown pigment produced. In nutrient broth, scanty surface growth; no sediments. In gelatin stab cultures, best growth at top and filiform; liquification stratiform. In litmus milk no reaction; slightly alkaline; no coagulation. Nitrate reduced to nitrite. Voges–Proskauer test negative. Indole production negative; hydrogen sulfide production positive; hydrolysis of starch negative; utilization of sodium citrate (Koser's and Christen's medium) positive; utilization of a great variety of organic nitrogen compounds. Uricase strongly positive; hemolysis negative; nonpathogenic. The type strain is positive for pyrazinamidase but negative for β-galactosidase. It can assimilate *N*-acetylglucosamine but not amygdalin, arbutin, cellobiose, D-arabitol, D-mannose, D-turanose, D-xylose, galactose, glycerol, inositol, L-arabinose, maltose, mannitol, melibiose, salicin, sucrose, trehalose, xylitol, β-gentiobiose, or 5-ketogluconate (only type strain investigated; Osorio et al., 1999). Utilizes 5-amino valerate, glyoxylate, L-leucine, L-xylose, and uric acid. Hydrolysis of xanthine and casein; acid is not produced from sugars; cellulose is not decomposed. Aerobic to slightly facultative anaerobic; optimal temperature and pH for growth, 27–35°C and 7–8; thermal death time 10 min at 52°C. The peptide subunit of the peptidoglycan consists alanine, D-glutamic acid, and lysine. The interpeptide bridge contains alanine and glutamic acid (Lys–Ala–Glu type, variation A4α). Major fatty acids are $C_{15:0}$ anteiso (34%), $C_{15:0}$ iso (27%), $C_{17:0}$ anteiso (14%), $C_{17:0}$ iso (11%) and $C_{16:0}$ iso (7%) (Funke et al., 1996).

Source: humus soil.

DNA G+C content (mol%): 61.2–61.5 (T_m).

Type strain: ATCC 21749, CIP 102367, DSM 20647, NBRC 15515, JCM 11944, LMG 16220, VKM Ac-1979.

Sequence accession no. (16S rRNA gene): X83410.

Sequence accession no. (recA): AF214791.

62. **Arthrobacter ureafaciens** (*ex* Krebs and Eggleston 1939) Clark 1955, 112[AL] (*Corynebacterium ureafaciens* Krebs and Eggleston 1939, 310)

u.re.a.fa′ci.ens. N.L. n. *urea* urea; L. v. *facio* to make, produce; N.L. part. adj. *ureafaciens* urea-producing.

Cells are nonmotile. Colonies on yeast extract-peptone media are pale gray, becoming yellow especially when incubated in diffuse daylight at ~20°C. When supplied with biotin, cells grow in suitable mineral salts medium with an ammonium salt as sole nitrogen source and with glucose as carbon and energy source (Keddie et al., 1986). Starch is not hydrolyzed, nitrate is not reduced, and cells do no grow in 10% NaCl. Nicotine blue is not produced from nicotine. Utilizes L-arginine, L-asparagine, L-arabinose, D-galactose, D-glucose, D-ribose, D-xylose, histidinol, inositol, 4-aminobutyrate, and ρ-hydroxybenzoate but not L-histidine, L-leucine, L-rhamnose, butanediol, or malonate. Assimilate citric acid, formic acid, glutaric acid, propionic acid, and uric acid but not adipic acid, benzoic acid, malonic acid, or pimelic acid. Urea is formed from creatinine and uric acid. The cell-wall peptidoglycan is of the Lys–Ala–Thr–Ala type. The whole-cell-wall sugar is galactose (mannose). The principal isoprenoid quinone is MK-9(H_2). The predominant fatty acid is $C_{15:0}$ anteiso (58%), followed by $C_{17:0}$ anteiso (28%), $C_{16:0}$ iso (6%), and $C_{15:0}$ iso (5%) (Kodama et al., 1992).

Source: soil.

DNA G+C content (mol%): 61.7 (T_m).

Type strain: ATCC 7562, CIP 67.3, DSM 20126, NBRC 12140, JCM 1337, LMG 3812, VKM Ac-1121.

Sequence accession no. (16S rRNA gene): X80744.

Sequence accession no. (recA): AF214782.

63. **Arthrobacter woluwensis** Funke, Hutson, Bernard, Pfyffer, Wauters and Collins 1997a, 242[VP] (Effective publication: Funke, Hutson, Bernard, Pfyffer, Wauters and Collins, 1996, 2362.)

wo.lu.wen′sis. N.L. masc. adj. *woluwensis* of or belonging to Woluwe, a town near Brussels, Belgium, where the type strain was isolated from a patient.

The cells are coryneform bacteria which develop into cocci when the cultures become older (72 h). Jointed rods are observed after 1–2 d of incubation. No spores formed. The cells are nonmotile. The organism is obligately aerobic. The colonies are whitish-grayish, smooth, convex, and larger than 2 mm in diameter after 24 h of incubation at 37°C in 5% CO_2 on SBA. The organism is catalase-positive. Nitrate is not reduced. Urea (72 h) and esculin (24 h) are

hydrolyzed. DNase and gelatinase activities are detected within 24 h. Glycerol, galactose, D-glucose, D-fructose, D-mannose, dulcitol, mannitol, sorbitol, *N*-acetylglucosamine, amygdalin, arbutin, salicin, cellobiose, maltose, lactose, melibiose, sucrose, trehalose, melezitose, D-raffinose, β-gentiobiose, D-turanose, D-arabitol, gluconate, 2-keto-gluconate, and 5-keto-gluconate are utilized. Erythritol, D-arabinose, L-arabinose, ribose, D-xylose, L-xylose, adonitol, β-methyl-xyloside, L-sorbose, rhamnose, inositol, α-methyl-D-mannoside, α-methyl-D-glucoside, inulin, starch, glycogen, xylitol, D-lyxose, D-tagatose, D-fucose, L-fucose, and L-arabitol are not utilized. The following enzyme activities are detected: alkaline and acid phosphatase, esterase (C4), esterase lipase (C8), lipase (C14), leucine arylamidase, cystine arylamidase, trypsin, β-galactosidase, α-glucosidase, β-glucosidase, *N*-acetyl-β-glucosaminidase, and α-mannosidase. Chymotrypsin, α-galactosidase, β-glucuronidase, and α-fucosidase are not present. The peptidoglycan type is A4α, L-lysine–D-aspartic acid. Major fatty acids are $C_{15:0}$ anteiso (38%), $C_{15:0}$ iso (22%), $C_{17:0}$ anteiso (15%), $C_{17:0}$ iso (6%), and $C_{16:0}$ iso (11%) (Funke et al., 1996).

Source: cultures of human blood.

DNA G+C content (mol%): 69.0 (HPLC).

Type strain: CUL 1808, ATCC 700220, CCUG 36790, CIP 104908, DSM 10495, JCM 11679.

Sequence accession no. (16S rRNA gene): X93353.

Misclassified species

1. **Arthrobacter viscosus** Gasdorf, Benedict, Cadmus, Anderson and Jackson 1965, 150[AL]

vis.co′sus. L. masc. adj. *viscosus* viscous, sticky; referring to exopolysaccharide production and viscous colonies.

Arthrobacter viscosus was the name given to two polysaccharide-producing bacterial strains. Both strains are considered to be Gram-stain-negative, motile, aerobic rods (ATCC catalog, 1982). The type strain produces from glucose large quantities of a carbohydrate polymer based on galactose, glucose, and mannuronic acid, and contains *meso*-diaminopimelic acid (*meso*-DAP) as cell-wall diamino acid but neither arabinose nor mycolic acids (Keddie and Cure, 1978). The second strain of the species, ATCC 19583, also contains a directly linked peptidiglycan based on *meso*-DAP and lacks arabinose in the cell wall (Schleifer and Kandler, 1972).

Source: Guatemalan soil.

DNA G+C content (mol%): 59.4 (method of analysis specified in Skyring et al., 1971).

Type strain: ATCC 19584; CIP 82.105; DSM 7307; JCM 11566; LMG 16473; NCIB 9729; NRRL B-1973.

Sequence accession no. (16S rRNA gene): AJ639832.

Genus IV. **Citricoccus** Altenburger, Kämpfer, Schumann, Steiner, Lubitz and Busse 2002, 2099[VP]

Hans-Jürgen Busse and Peter Kämpfer

Ci.tri.coc′cus. L. n. *citrus* lemon, citron or citrus, an African tree; N.L. masc. n. *coccus* a sphere; N.L. masc. n. *Citricoccus* lemon-yellow-pigmented coccus.

Cocci, occurring singly, in short chains or in clusters, about 1 µm in diameter; cells are nonmotile, Gram-stain-positive, non-spore-forming, and aerobic. Oxidase-negative and catalase-positive.

The **peptidoglycan consists of the amino acids alanine, glycine, glutamic acid, and lysine** and an **interpeptide chain Lys–Gly–Glu**, peptidoglycan type variation **A4α**. The predominant **menaquinone is MK-9(H_2)**, and moderate amounts of MK-7(H_2) and MK-8(H_2) are present. **Polar lipids are diphosphatidylglycerol, phosphatidylglycerol, phosphatidylinositol, four unknown glycolipids, two unknown phospholipids, and an unknown lipid**. The **major fatty acids are $C_{15:0}$ anteiso, $C_{17:0}$ anteiso, $C_{16:0}$ iso, and $C_{15:0}$ iso**. **Spermidine** is the **predominant polyamine**. This genus is a member of the family *Micrococcaceae*.

DNA G+C content (mol%): 63.8–68.

Type species: **Citricoccus muralis** Altenburger, Kämpfer, Schumann, Steiner, Lubitz and Busse 2002, 2099[VP].

Further descriptive information

With >96% sequence similarity in their 16S rRNA genes, species of the genus *Citricoccus* are closely related to the genus *Micrococcus*, the type genus of the family *Micrococcaceae*. Two species have been described so far: *Citricoccus muralis*, the type species of the genus (Altenburger et al., 2002), and *Citricoccus alkalitolerans* (Li et al., 2005b). These species were isolated from samples of a medieval wall painting in the Church of St. Georgen (Austria) and desert soil in eastern Egypt, respectively. They are phylogenetically closely related, and the sequence similarity of their 16S rRNA genes is 99.6% (Li et al., 2005b). On the basis of their 16S rRNA gene nucleotide sequences in databases, at least four additional strains of the genus may be present in institutional culture collections. These strains are designated *Citricoccus* sp. JM-Le (sequence accession no. AB189330), *Micrococcus* sp. P_wp 0222136 (accession no. AY188937), *Micrococcus* sp. V4.40.20p (accession no. AJ244665), and *Citricoccus* sp. 2216.25.22 (accession no. AB094464) and share >98.5% sequence similarity with *Citricoccus muralis*. They were isolated from a fragment of a stalactite in Jourmon Cave (Gifu Prefecture, Japan), from deep-sea sediment of unknown geographical source, from the Western Mediterranean Sea close to Nice, France (Fritz, 2000), and from subsea floor sediment in the southwestern part of the Sea of Okhotsk (Inagaki et al., 2003). In addition Tiago et al. (2004) found *Citricoccus*-like organisms in nonsaline, alkaline environments.

The two established species are very similar. Their DNA–DNA hybridization is relatively high, 56%. They share major chemotaxonomic characteristics, including the peptidoglycan type A4α (Schleifer and Kandler, 1972), murein type A11.40/56 (DSMZ, 2001), menaquinone MK-9(H_2) as the predominant quinone, a similar polar lipid profile dominated by diphosphatidylglycerol, phosphatidylglycerol, and phosphatidylinositol, anteiso and a similar fatty acid profile comprised almost exclusively of $C_{15.0}$ anteiso and other branched-chain fatty acids. However, significant quantitative differences in the abundance of certain fatty acids

and several physiological traits as well as results from DNA–DNA hybridization confirm their status as separate species (Table 97).

No species of this genus has been reported to be pathogenic.

Enrichment and isolation procedures

No specific isolation medium has been described. Good growth occurs on PYES agar (Altenburger et al., 2002), R2A agar and TS agar (Oxoid), and a very low-nutrient medium, CasMM agar (Altenburger et al., 1996), at 10–28°C. Moderate growth occurs at 4 and 37°C, but no growth is observed at 40°C.

Maintenance procedures

Citricoccus cultures may be lyophilized by common procedures used for many bacteria or stored in 21% glycerol at −80°C.

Differentiation of the genus *Citricoccus* from other genera

Citricoccus may be distinguished from other genera in the family *Micrococcaceae* by their cell-wall peptidoglycan, menaquinone type, colony pigmentation, and G+C content.

TABLE 97. Characteristics distinguishing the two species of the genus *Citricoccus*, *Citricoccus muralis* and *Citricoccus alkalitolerans*[a,b]

Characteristic	*C. muralis*	*C. alkalitolerans*
Growth in the presence of methyl red	+	−
Presence of:		
Lysine decarboxylase	+	−
α-Maltosidase	+	−
Utilization as sole source of carbon:		
Fructose	+	−
Amygdalin	−	+
Glycine	−	+
Arginine	+	−
Fatty acids[a]*:*		
$C_{17:0}$ anteiso	27	4
$C_{15:0}$ iso	6	13
$C_{16:0}$ iso	8	2
DNA G+C content (mol%)	68	64

[a]Symbols: +, positive; −, negative.

[b]Data from Altenburger et al. (2002), and Li et al. (2005b).

[c]Relative amount expressed as percent of total fatty acids.

List of species of the genus *Citricoccus*

1. **Citricoccus muralis** Altenburger, Kämpfer, Schumann, Steiner, Lubitz and Busse 2002, 2099[VP]

mu.ra′lis. L. masc. adj. *muralis* pertaining or belonging to walls.

Morphological, chemotaxonomic and general characteristics are as described for the genus. Colonies reach a maximum size of 3 mm after 2 weeks of incubation. They are yellow pigmented, circular, convex, and opaque. Growth is good at room temperature and 28°C, moderate at 4°C, absent at 37°C, good at NaCl concentrations of up to 7% (w/v), weak at 10% (w/v), and absent at 15% (w/v) and present at initial pH values between 6 and 10. No acid is produced from glucose, sucrose, salicin, inositol, rhamnose, trehalose, cellobiose, and D-mannose. Gluconate, D-glucose, D-maltose, D-mannose, D-sucrose, D-trehalose, acetate, propionate, *cis*-aconitate, fumarate, glutarate, DL-3-hydroxybutyrate, DL-lactate, L-malate, oxoglutarate, pyruvate, L-aspartate, L-leucine, L-phenylalanine, L-proline, L-serine, 3-hydroxybenzoate, 4-hydroxybenzoate, and phenylacetate but not *N*-acetyl-D-galactosamine, *N*-acetyl-D-glucosamine, L-arabinose, *p*-arbutin, D-cellobiose, D-fructose, D-galactose, α-D-melibiose, L-rhamnose, D-ribose, salicin, D-xylose, adonitol, iso-inositol, maltitol, D-mannitol, D-sorbitol, putrescine, *trans*-aconitate, adipate, 4-aminobutyrate, azelate, citrate, itaconate, mesaconate, suberate, L-alanine, β-alanine, L-histidine, L-ornithine, and L-tryptophan serve as sole carbon sources for growth. Esculin, pNP-β-D-glucopyranoside, 2-deoxythymidine-5′-pNP-phosphate, L-alanine-pNA and L-proline-pNA but not pNP-β-D-galactopyranoside, pNP-β-D-glucuronide, pNP-α-D-glucopyranoside, pNP-β-D-xylopyranoside, bis-pNP-phosphate, pNP-phenyl-phosphonate, pNP-phosphoryl-choline, and L-glutamate-γ-3-carboxy-pNA are hydrolyzed. Tween 20 and Tween 80 but not casein and starch are decomposed. Nitrate is not reduced to nitrite. H_2S and indole are not produced. Urease is positive. The peptidoglycan contains of Ala, Gly, Glu, and Lys in a molar ratio of 1.3:0.8:2.0:1.0, and the interpeptide bridge consists of Lys–Gly–Glu (peptidoglycan type A4α, murein type A11.40/56). The type strain contains the menaquinones (molar ratio in parentheses) MK-9(H_2) (64%), MK-7(H_2) (24%), and MK-8(H_2) (14%). The abundant fatty acids are $C_{15:0}$ anteiso (55.6%), $C_{17:0}$ anteiso (27.0%), $C_{16:0}$ iso (8.0), $C_{15:0}$ iso (6.4%), $C_{13:0}$ anteiso (1.2%), $C_{17:0}$ iso (1.1%), and $C_{16:0}$ (0.8%). The polar lipids are diphosphatidylglycerol, phosphatidylglycerol, phosphatidylinositol, four unknown glycolipids, two unknown phospholipids, and a remaining unidentified lipid. Spermidine is the predominant polyamine.

Source: a medieval wall painting in the Church of St Georgen in Styria, Austria.

DNA G+C content (mol%): 68 (HPLC).

Type strain: 4-0, DSM 14442, CCM 4981, CCUG 51768, JCM 12134.

Sequence accession no. (16S rRNA gene): AJ344143.

2. **Citricoccus alkalitolerans** Li, Chen, Zhang, Kim, Park, Lee, Xu and Jang 2005b, 88[VP]

al.ka.li.to′le.rans. N.L. n. *alkali* (from the Arabic articel *al* the; Arabic n. *qaliy* the ashes of saltwort), alkali; L. part. adj. *tolerans* tolerating; N.L. part. adj. *alkalitolerans* referring to the ability of the organism to tolerate alkaline media.

Cells are aerobic, Gram-stain-positive, nonmotile, non-spore-forming cocci, with diameters of 0.5–0.8 µm. Colonies on PYES* medium are light yellow, circular, entire, somewhat convex, opaque, and approximately 1.5 mm in diameter after 24 h of growth at 28°C. Growth occurs between 10 and

*PYES medium: 3 g/l peptone from casein, 3 g/l yeast extract, 2.3 g/l di-sodium succinate, pH 7.2.

37°C and optimally at 28°C. No growth is observed at 4 or 40°C. The optimum growth pH and NaCl (w/v) concentration ranges are 8.0–9.0 and 0–5%, respectively. Catalase-positive and oxidase-negative. Urease- and tyrosinase-negative. Tweens 20 and 80, casein, and starch are not hydrolyzed. H_2S and indole are not produced. Nitrate is not reduced to nitrite. Lipase and α-glucosidase tests are positive. Tests for ornithine and lysine decarboxylase, arginine dihydrolase, β-glucuronidase, α- and β-galactosidase, *N*-acetyl-β-glucosaminidase, and β-glucosidase are negative. Glucose, galactose, sucrose, arabinose, mannose, mannitol, maltose, starch, xylose, ribose, cellobiose, salicin, sorbitol, lactose, dextrin, amygdalin, glycine, and lysine but not fructose, *N*-acetyl-α-glucosamine, L-alanine, β-alanine, L-histidine, L-ornithine, L-tryptophan, and arginine serve as sole carbon sources. The peptidoglycan is of the Lys–Gly–Glu type (A4α). The major quinone is menaquinone MK-9(H_2). The polar lipid profile contains diphosphatidylglycerol, phosphatidylglycerol, phosphatidylinositol, and two unknown glycolipids. The abundant cellular fatty acids are: $C_{15:0}$ anteiso (75%), $C_{15:0}$ iso (13%), $C_{17:0}$ anteiso (4%), $C_{16:0}$ iso (2%), $C_{15:0}$ (2%), $C_{14:0}$ iso (1%), $C_{13:0}$ iso, $C_{17:0}$ iso, and $C_{13:0}$ anteiso. The amounts of $C_{13:0}$, $C_{14:0}$, and $C_{16:0}$ are less than 1%.

Source: an alkaline soil from the eastern desert of Egypt.

DNA G+C content (mol%): 63.8 (T_m).

Type strain: YIM 70010, CCTCC AA 203008, CCUG 51943, DSM 15665, JCM 13012, KCTC 19012.

Sequence accession no. (16S rRNA gene): AY376164.

Genus V. **Kocuria** Stackebrandt, Koch, Gvozdiak and Schumann 1995, 690[VP]

ERKO STACKEBRANDT AND PETER SCHUMANN

Ko.cu′ri.a. N.L. fem. n. Kocuria, named after Miroslav Kocur, a Slovakian microbiologist for his pioneering studies on Gram-stain-positive cocci.

Cells coccoid. Gram-stain-positive. Nonencapsulated. Endospores not formed. Nonmotile. Chemo-organotrophic; metabolism is strictly respiratory. **Aerobic;** strains of one species are slightly facultatively anaerobic. Catalase-positive. Nonhalophilic. Mesophilic. **The peptidoglycan type is A3α (L-Lys–L-Ala$_{3-4}$).** Mycolic acids and teichoic acids are absent. **Menaquinones are hydrogenated; the predominant menaquinones are MK-7(H_2), MK-8, or MK-9(H_2)**, either alone or in combination. Polar lipids include diphosphatidylglycerol and phosphatidylglycerol; phosphatidylinositol is present in one species. **The major fatty acid is $C_{15:0}$ anteiso**.

DNA G+C content (mol%): 66–75.

Type species: **Kocuria rosea** (Flügge 1886) Stackebrandt, Koch, Gvozdiak and Schumann 1995, 690 (*Micrococcus roseus* Flügge 1886 183[AL]).

Further descriptive information

Following a thorough phylogenetic and chemotaxonomic study, the genus *Micrococcus* was dissected (Stackebrandt et al., 1995) leading to the description of *Dermacoccus*, *Kocuria*, *Kytococcus*, and *Nesterenkonia*. The latter three genera are members of the family *Micrococcaceae*, order *Micrococcales*, within the emended order *Actinomycetales* (Buchanan 1917) Stackebrandt et al. 1997. Within this family genera are found which are defined by either spherical cells or cells which undergo a rod–coccus life cycle. The exception is *Renibacterium*, characterized by short rods. This study demonstrated the phylogenetic relevance of chemotaxonomic markers, in that novel combinations of cell-wall structure and profiles of menaquinones, fatty acids, and polar lipids were indicative of a new phylogenetic lineage at the genus rank. The new genus *Kocuria* embraced three former *Micrococcus* species, i.e. *Kocuria rosea* (*Micrococcus roseus* Flügge 1886), *Kocuria varians* (*Micrococcus varians* Migula 1900), and *Kocuria kristinae* (*Micrococcus kristinae* Kloos et al. 1974). Based upon 16S rRNA gene sequence similarities, *Kocuria* species appear to be equidistantly related to members of *Arthrobacter* and *Rothia* but the bootstrap values of the deeply branching lineages within this family are low, thus without statistical significance (Figure 136).

Kocuria species resemble each other in the majority of chemotaxonomic properties and differ from each other mostly in quantitative composition of these markers. The peptidoglycan is characterized by the presence of lysine in position 3 of the peptide subunit. The interpeptide bridge consists of 3 or 4 L-alanine residues, thus defined as the A3α variation (Schleifer and Kandler, 1972). The isoprenoid quinones are hydrogenated menaquinones MK-8(H_2), MK-9(H_2), and MK-7(H_2), occurring either as a single component or in combination with each other. The major fatty acid is $C_{15:0}$ anteiso (>50% of total) but $C_{15:0}$ iso and $C_{16:1}$ represent about 10% each of the total. The base composition of DNA spans a rather broad range (60–75 mol%), which may be due to the different methods used for quantitation. At the interspecies level, a range of 9% and 6% has been determined for *Kocuria rosea* and *Kocuria varians*, respectively, species for which more than the type strain is available for investigation. The extreme values were determined for the recently described type strains of *Kocuria marina* (60 mol%) and *Kocuria himachalensis* (75.3 mol%).

A comparative study on physiological properties which includes all type strains of *Kocuria* is lacking. Therefore only fragmentary information exists on optimal temperature, pH, salt tolerance, and the like for growth. Most *Kocuria* strains grow between 20 and 37°C; *Kocuria aegyptia* can grow at up to 40°C, *Kocuria marina* even up to 43°C. Only the type strains

of *Kocuria polaris* and *Kocuria marina* are able to grow at 5°C. Strains of *Kocuria polaris* and *Kocuria palustris* fail to grow at 37°C. Salt tolerance is variable; if indicated, most strains tolerate 5% NaCl but only one strain tolerates 15% NaCl. The pH range of growth may be broad (5–12 for *Kocuria aegyptia* and 7–12 for *Kocuria polaris*) or narrow, usually slightly alkaliphilic. All species can produce acids from one or more carbohydrates which distinguish them from authentic members of *Micrococcus*. The decrease in medium pH due to acid formation from fermented glucose has only been determined for *Kocuria varians* (pH 4.3–5.9). The degrading capacity differs significantly among species. Most strains do not degrade DNA and starch. Gelatin can be hydrolyzed by some species, though the ability to hydrolyze starch and gelatin is variable in those two species for which more than the type strains have been investigated. Esterase and lipase reactions differ among species; in most cases Tween 80 cannot be utilized. No information is available on metabolic pathways for carbohydrate dissimilation.

DNA–DNA hybridization studies were conducted between several pairs of type strains (Kim et al., 2004; Kotoučková et al., 2004; Kovács et al., 1999; Li et al., 2006; Mayilraj et al., 2006; Rainey et al., 1997; Reddy et al., 2003; Tang and Gillevet, 2003) most of which share lower than 98.8% 16S rRNA similarities. In each case, the corresponding DNA–DNA reassociation values were lower than 60%. Even at a 16 rRNA gene sequence similarity of 99.8% (*Kocuria polaris* versus *Kocuria rosea*), the corresponding DNA reassociation value was as low as 71%. The highest matching values were found for the type strains of *Kocuria erythromyxa* and *Kocuria rosea* (99.9% sequence rRNA gene similarity at 95% DNA similarity) which has led to the union of these species (Rainey et al., 1997). These data clearly indicate a threshold level of 98.8% 16S rRNA gene sequence similarity is essential for membership in *Kocuria*.

Type strains of species originate from a wide range of habitats, including meat (*Kocuria carniphila*, *Kocuria varians*, and *Kocuria kristinae*; Frank et al., 2003), milk (*Kocuria* spp.), mammalian skin (*Kocuria kristinae*, *Kocuria varians*), marine sediment (*Kocuria marina*) and fresh water (*Kocuria rosea*, *Kocuria varians*), cold (*Kocuria himachalensis*), temperate (*Kocuria rosea*) and desert (*Kocuria aegyptia*) soils, cyanobacterial mat (*Kocuria polaris*), and the phyllosphere (*Kocuria palustris*). Many strains, which are affiliated to genus and species, are characterized exclusively on the basis of partial and almost complete 16S rRNA gene sequences. Only these have been deposited in public databases without publication of additional phenotypic circumscriptions. From the information accompanying sequence accession numbers, the origin of isolates support the widespread occurrence of *Kocuria* strains: soil (DQ059671), rhizosphere and phyllosphere (e.g. AF542072, AY838796), aerosolized dust (e.g. AY211105, AY211167, AY278921, AY741253), marine waters (e.g. AY241412, AY269864, AY332098, and AY731367), Mariana Trench deep sediment (Pathom-Aree et al., (2006a) [AY894331]), indoor facilities (e.g. AY030315, AY775563), and cold habitats such as glaciers and the Antarctic environment (AY526638, AY864652, and AY987383). The shielding effect of pigment against UV and the coccoid morphology may contribute to the survival of *Kocuria* strains in dust clouds thousands of kilometers away from their origin (i.e. Saharan dust collected on the US Virgin Islands).

Little information on the occurrence of uncultured *Kocuria* strains has emerged from the analysis of 16S rDNA clone libraries. One clone (AF385532) was found as a new phylotype in lesions of noma cancrum oris, which is a severe gangrenous disease of the oral cavity (Paster et al., 2002). Other single clones were found by Bowman and Nowak (2004) as part of the salmonid gill microbial population, and by McGarvey et al. (2004) in dairy wastewater.

Pathogenicity. Members of the genus *Kocuria* belong to the commensal microbes on human skin, mucous membranes, in the oral cavity and outer ear canal (Frank et al., 2003; Szczerba, 2003b; Szczerba and Krzeminski, 2002) and were detected in indoor environments and in bacterial contaminations of airplanes (Gorny and Dutkiewicz, 2002; McManus and Kelley, 2005). Although caution should be exercised in the differentiation between true infection and contamination, the fact remains that the number of reports implicating members of the family *Micrococcaceae* in clinical cases mainly occurring in immunocompromised patients has increased in recent years (Goodfellow, 1998; Szczerba, 2005). *Kocuria rosea* and *Kocuria kristinae*, e.g. were reported to cause catheter-related bacteremia in patients with severe underlying diseases (Altuntas et al., 2004; Basaglia et al., 2002). An infection by *Kocuria kristinae* associated with acute cholecystitis was diagnosed and successfully treated with levofloxacin (Ma et al., 2005). The following antibiotics are recommended for the treatment of infections caused by *Kocuria* strains or by other representatives of the family *Micrococcaceae*: Amoxicillin with clavulanic acid, doxycycline, cetriaxone, cefuroxime, or amikacin (Szczerba, 2003a). Opportunistic infections are usually serious and difficult to diagnose by routine tests in clinical laboratories. Since the unambiguous identification of strains of the genus *Kocuria* requires a polyphasic strategy, reports on *Kocuria* infections diagnosed using commercial diagnostic test systems are difficult to evaluate. When using only phenotype-based identification systems, phenotypically variable staphylococci may be misidentified as *Kocuria varians* as demonstrated by a polyphasic study (Ben-Ami et al., 2003). Recent molecular sequencing and typing studies aimed at the phylogenetic diversity of environmental samples resulted in the detection of *Kocuria* spp. A combination of 16S rRNA gene amplification and subsequent single strand conformation polymorphisms (SSCP) of the variable region V3 was used to determine the change in microbial populations in goat milk (Callon et al., 2007). *Kocuria* spp. were detected among other bacteria commonly encountered in milk. A combination of community-level physiological profiling and rRNA restriction analysis (ARDRA) on bacterial communities of reed periphyton have also led to the detection of *Kocuria* spp. among the most frequently isolated strains (Borsodi et al., 2007).

Enrichment and isolation procedures

For the isolation of strains from mammalian skin, the procedures of Kloos and Musselwhite (1975) and of Kloos et al. (1976) using P agar (Naylor and Burgi, 1956) are recommended. Although there are no selective procedures to isolate

such strains, spreading colonies of fungi or bacilli may be inhibited by addition of cycloheximide (50 μg/ml; Kloos et al., 1976) or supplementing the isolation media with 7% NaCl (Schleifer et al., 1981), respectively. Repression of staphylococci on skin can be achieved by using furazolidone agar (FTO agar; von Rheinbaben and Hadlock, 1981). Strains of *Kocuria varians* and *Kocuria kristinae* have also been isolated on *Corynebacterium* agar consisting of tryptically digested casein peptone (10.0 g), yeast extract (5.0 g), glucose (5.0 g), NaCl (5.0 g), and agar (15.0 g) per 1000 ml of distilled water (pH 7.2–7.4). *Kocuria* strains may be isolated from soil or sand by the procedure described by Prauser et al. (1997) in which a diluted soil suspension in phosphate buffer is mixed with solubilized organic agar medium with agar content reduced to 0.6% (w/v) at 48°C. This mixture is subsequently poured on organic agar medium containing 1.5% (w/v) agar in Petri dishes and incubated at 28°C for 4–6 d until appearance of colonies. *Kocuria polaris* was isolated from Antarctic Bacterial Medium (ABM) plates containing 0.5% (w/v) peptone, 0.2% (w/v) yeast extract, and 1.5% (w/v) agar (pH 6.9) and incubated at 5°C (Reddy et al., 2003). *Kocuria himachalensis* was obtained on Tryptone Soy Broth (Oxoid CM 129). *Kocuria marina* was isolated on Bacto Marine Broth (Difco 2216). *Kocuria aegyptia* was recovered on a medium containing (g l<MIN>1): glucose, 10.0; peptone, 5.0; yeast extract, 5.0; KH_2PO_4, 1.0; $MgSO_4 \cdot 7H_2O$, 0.2; Na_2CO_3, 10.0, and agar, 15; pH, 10–10.5. Sodium carbonate was sterilized separately and then added to the medium. $NaHCO_3$-Na_2CO_3 buffer was used to adjust the pH (Li et al., 2006).

Cells for taxonomic studies can be cultivated on trypticase soy yeast extract medium consisting of trypticase soy broth (30.0 g), yeast extract (3.0 g), and agar (15.0 g) per 1000 ml of distilled water (pH 7.0–7.2). *Kocuria kristinae* should be cultivated at 37°C, whereas all other representatives of the genus grew optimally at 28–30°C.

Maintenance procedures

Strains have been stored for some weeks as slant cultures at 4°C and as 20% (w/v) glycerol suspensions at −20°C and at −80°C. Long-term preservation methods include freeze drying in skim milk and in liquid nitrogen at −196°C.

Differentiation of the genus *Kocuria* from other genera

Table 98 indicates the characteristics useful to distinguish *Kocuria* from other members of the family *Micrococcaceae*. The isolated phylogenetic position is supported by chemotaxonomic characters which differentiates *Kocuria* from other coccid members of the family. However, this is not achieved by a single feature but by a combination of four chemotaxonomic properties. Cell wall type and variation A3α are also found in members of the phylogenetically neighboring genera *Renibacterium*, *Rothia*, and in *Arthrobacter* group I (*Arthrobacter globiformis* and relatives). Hydrogenated menaquinones of the types MK-8(H_2), MK-9(H_2), and/or MK-7(H_2) are also found in *Arthrobacter* group I, *Citricoccus*, and *Micrococcus*. The range of G+C mol% of DNA for *Kocuria* and members of many other genera in the family is similar, but for *Yania*, *Rothia*, and *Renibacterium* the range is distinctly below 60%. The polar lipids are similar though slight differences exist among the genera. Likewise, the composition of major components of fatty acids do not allow discrimination between genera; qualitative and quantitative assessment of the full range of fatty acid profiles, however, will allow assignment of strains to species.

Taxonomic comments

The species *Kocuria erythromyxa* was proposed by Rainey et al. (1997) as a result of a phylogenetic study on members of *Deinococcus* and *Deinobacter*. The species *Deinococcus erythromyxa* showed 99.9%, 96.6%, and 95.7% 16S rDNA gene sequence similarity to *Kocuria roseus*, *Kocuria varians*, and *Kocuria kristinae* respectively, thus confirming previous chemotaxonomic evidence that *Deinococcus erythromyxa* was more closely related to members of the genera *Arthrobacter* and *Micrococcus* than to *Deinococcus* (Embley et al., 1987). A DNA–DNA reassociation value of 21%, determined for the pair *Deinococcus erythromyxa* (then designated *Sarcina erythromyxa*) and *Kocuria rosea* (then designated *Micrococcus roseus*) (Brooks et al., 1981) was used as evidence to reclassify *Deinococcus erythromyxa* as *Kocuria erythromyxa*. Subsequent pairwise comparison of phenotypic, chemotaxonomic, and genomic properties of the type strains of *Kocuria rosea* and *Kocuria erythromyxa* indicated that these taxa were in fact members of the same species. Above all, this conclusion was based on a DNA–DNA reassociation value of 95%, identical fatty acid patterns, and almost identical physiological reactions against substrates provided by the BIOLOG plate. According to Rule 42 of the International Code of Nomenclature of Bacteria which requires that the oldest legitimate epithet be retained when taxa of equal rank are united, *Kocuria* (*Micrococcus*) *rosea* (Flügge 1886) had priority over *Kocuria* (*Deinococcus*) *erythromyxa* (Brooks and Murray, 1981).

Notably, the name *Kocuria erythromyxa* was proposed for an organism that had a long history of changing genera affiliations. The organism was described as "*Micrococcus erythromyxa*" by Zopf (1891) and as "*Sarcina erythromyxa*" by Chester (1901). Other combinations were "*Bacterium erythromyxa*" (Migula, 1900), "*Bacillus erythromyxa*" (Matzuschita, 1902), and "*Mycobacterium erythromyxa*" (Krasil'nikov, 1941). The species which was not included in the Approved Lists of Bacterial Names (Skerman et al., 1980) received formal status when *Micrococcus roseus* UWO 1045 was described as the type strain of *Deinococcus erythromyxa* (Brooks and Murray, 1981), though as a species *incertae sedis*.

Based upon phylogenetic and chemotaxonomic analyses and supported by a DNA–DNA reassociation value of 87.1% and a G+C content of 72 mol%, it was concluded that *Pelczaria aurantia* ATCC 49321^T (= DSM 12801^T) and *Kocuria rosea* DSM 20447^T are members of the same taxon (Schumann et al., 2000). According to Rule 42 of the Bacteriological Code (Lapage et al., 1992) and on the basis of priority, the two species must be united in the genus *Pelczaria* (Poston, 1994) and in the same species as both species are the type species of the respective genera. As the type strain of *Pelczaria aurantia* did not conform on re-investigation (Schumann et al., 2000) to the description of the type strain of *Pelczaria aurantia* as described by Poston (1993), the Judicial Commission (2005) considered a request of Tindall et al. (2000) to designate the organisms currently deposited as ATCC 49321 and DSM 12801 (i) as members of the species *Kocuria rosea* and (ii) not the species *Pelczaria aurantia*. The genus name

TABLE 98. Differential characteristics of the genus *Kocuria* and phylogenetically related genera[a]

Character	*Kocuria*[b]	*Nesterenkonia*[c]	*Micrococcus*[d]	*Renibacterium*[d]	*Arthrobacter globiformis* group[e]	*Arthrobacternicotianae* group[f]	*Citricoccus*[g]	*Rothia*[h]	*Yania*[i]	*Acaricomes*[j]	*Zhihengliuella*[k]
Morphology	Coccoid	Coccoid	Coccoid	Short rods	Rod–coccus cycle	Rod–coccus cycle	Coccoid	Irregular rods, coccoid forms	Coccoid	Short rods	Short rods
Motility	–	–	–	–	Variable	Variable	–	–	–	nd	–
Peptidoglycan variation	A3α	A4α	A2 or A4α	A3α	A3α	A4α	A4α	A3α	A4α	A3α	A4
Interpeptide bridge of peptidoglycan	Lys–$Ala_{3–4}$	Lys–Gly–Glu, Lys–Glu or Lys–Gly–Asp	Lys–peptide subunit[l] or Lys–Asp	Lys–Ala–Gly[m]	Lys–MCA_{var}	Lys–Glu, Lys–Asp, Lys–Ala–Glu or Lys–Ser–Glu	Lys–Gly–Glu	Lys–Ala_3, Lys–Ala, Lys–Ser, Lys–Gly or Lys–Gly–Ala	Lys–Gly–Glu	Lys–Ala_3	Lys–Ala–Glu
Major menaquinones[n]	MK-7(H_2), MK-8(H_2) MK-9(H_2)	MK-8, MK-9, MK-7	MK-8(H_2) or MK-8 and MK-8(H_2)	MK-9, MK-10	MK-9(H_2), MK-8(H_2), MK-10(H_2)	MK-8, MK-9, MK-10	MK-9(H_2), MK-7(H_2), MK-8(H_2)	MK-7	MK-8, MK-9, MK-7	MK-10(H_2)	MK-9, MK-10
Major fatty acids[l]	$C_{15:0}$ anteiso, $C_{17:0}$ anteiso, $C_{15:0}$ iso	$C_{15:0}$ anteiso, $C_{17:0}$ anteiso, $C_{16:0}$ iso	$C_{15:0}$ anteiso, $C_{15:0}$ iso	$C_{15:0}$ anteiso, $C_{17:0}$ anteiso	$C_{15:0}$ anteiso, $C_{15:0}$ iso, $C_{16:0}$ iso, $C_{17:0}$ anteiso	$C_{15:0}$ anteiso, $C_{17:0}$ anteiso	$C_{15:0}$ anteiso, $C_{17:0}$ anteiso, $C_{15:0}$ iso, $C_{16:0}$ iso	$C_{15:0}$ anteiso, $C_{17:0}$ anteiso, $C_{16:0}$ iso, $C_{16:0}$	$C_{15:0}$ anteiso, $C_{14:0}$ iso, $C_{15:0}$ iso	$C_{15:0}$ anteiso, $C_{17:0}$ anteiso	$C_{15:0}$ anteiso, $C_{15:0}$ iso
Polar lipids[l]	DPG, PG, (PI, PL, GL)	DPG, PG, PI, PL, GL	DPG, PG, PI, PL, GL	DPG, GL	DPG, PG, PI, DMDG, (PE)	DPG, PG, PI, DMDG	DPG, PG, PI, PL, GL	DPG, PG	DPG, PG, PI, PL, GL	PG, DPG, PI	PG, DPG, PI, PL, GL
DNA G+C content (mol%)	60–75	64–72	69–76	52–54	61–69	58–66	63–68	49–60	53–58	58	67

[a]Abbreviations: Ala, alanine; Asp, aspartic acid; Gly, glycine; Glu, glutamic acid; Lys, lysine; Ser, serine; MCA_{var}, monocarboxylic amino acids with variable composition in the interpeptide bridge; abbreviations of menaquinones exemplified by MK-8(H_2), partially saturated menaquinone with one of 8 isoprene units hydrogenated, MK-9, unsaturated menaquinone with 9 isoprene units; DPG, diphosphatidylglycerol; PG, phosphatidylglycerol; PI, phosphatidylinositol; DMDG, dimannosyldiacylglycerol; PL, unidentified phospholipid(s); GL, unidentified glycolipid(s); data in parentheses were reported only for one or few strains of the genus.

[b]Data from Stackebrandt and Schumann (2000b) and Kim et al. (2004).

[c]Data from Stackebrandt and Schumann (2000b), Collins et al. (2002b), and Li et al. (2005a).

[d]Data from Stackebrandt and Schumann (2000b).

[e]Data from Stackebrandt and Schumann (2000b), Reddy et al. (2002), Li et al. (2004a), and Kotoučková et al. (2004).

[f]Data from Stackebrandt and Schumann (2000b), Gupta et al. (2004), and Margesin et al. (2004).

[g]Data from Altenburger et al. (2002) and Li et al. (2005b).

[h]Data from Stackebrandt and Schumann (2000b), Collins et al. (2000), Fan et al. (2002), and Li et al. (2004c).

[i]Data from Li et al. (2004a, 2004c), Li et al. (2005b).

[j]Data from Pukall et al. (2006).

[k]Data from Zhang et al. (2007).

[l]Glycine or glycine amide bound to α-carboxyl group of glutamic acid at position 2 of peptide subunit.

[m]D-Alanine amide bound to α-carboxyl group of glutamic acid at position 2 of peptide subunit.

[n]One, or combinations of more components.

Pelczaria and the species name *Pelczaria aurantia* are placed on the list of *nomina rejicienda*.

Strain ATCC 9341, named "*Sarcina lutea*" by the US Food and Drug Administration, was affiliated to *Micrococcus luteus* by Baird-Parker (1974). Comparison of characteristics between strain ATCC 9341 and the type strain of *Micrococcus luteus* ATCC 4698^T revealed obvious phenotypic differences (Tang and Gillevet, 2003). As strain ATCC 9341 has been used as a quality-control strain in a number of applications, as a standard culture in several official methods, manuals, and the Code of Federal Regulations (for references, see Tang and Gillevet, 2003) it appeared necessary to reinvestigate its taxonomic position. 16S rRNA gene sequence analysis found 99% similarity between strain ATCC 9341 and the type strain of *Kocuria rhizophila* DSM 11926^T (accession no. Y16264). Because of identity in peptidoglycan structure and fatty acid composition, strain ATCC 9341 was affiliated to the species *Kocuria rhizophila*, although DNA–DNA reassociation between ATCC 9341 and the type strain *Kocuria rhizophila* BAA-50^T was 67.6% only and the Riboprint pattern of strain ATCC 9341 did not match that of *Kocuria rhizophila* ATCC BAA-50^T.

Acknowledgements

We thank Rüdiger Pukall and Jennifer Gregor for providing physiological and chemotaxonomic data, respectively, and Jola Swiderski for phylogenetic analyses.

Further reading

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Kocur, M., W.E. Kloos and K.H. Schleifer. 1991. The genus *Micrococcus*. *In* The Prokaryotes: a Handbook on the Biology of Bacteria: Ecophysiology, Isolation, Identification, Applications, 2nd edn, vol. II (edited by Balows, Trüper, Dworkin, Harder and Schleifer). Springer, New York, pp. 1300–1311.

Differentiation of species of the genus Kocuria

Species of the genus *Kocuria* are moderately to closely related among themselves, forming four intrageneric lineages. 16S rRNA gene sequence similarities indicate the presence of two strain clusters and two individual lineages. The branching order is supported by bootstrap values of higher than 70%. A few inconsistencies in some of the original sequences deposited in public databases were corrected and the new accession numbers are indicated in the footnote to Figure 138. Members of the two clusters share higher than 98% similarities among each other, while the four lineages are separated from each other by similarities of 95.5–97.5%. The clustering of strains within each of the two clusters is neither correlated with similarities at the phenotypic level nor with the habitats from which they were isolated (Table 99).

Riboprint analysis by the RiboPrint robot (Qualicon, Dupont) indicates that a unique pattern defines each type strain (Figure 139). As the number of bands is low, the phylogenetic significance is restricted and the resulting dendrogram of ribopatterns bears no similarity with the 16S rRNA gene dendrogram. At the intraspecies level, the patterns may vary significantly and the ribopattern of the type strain does not necessarily characterize additional strains of the species.

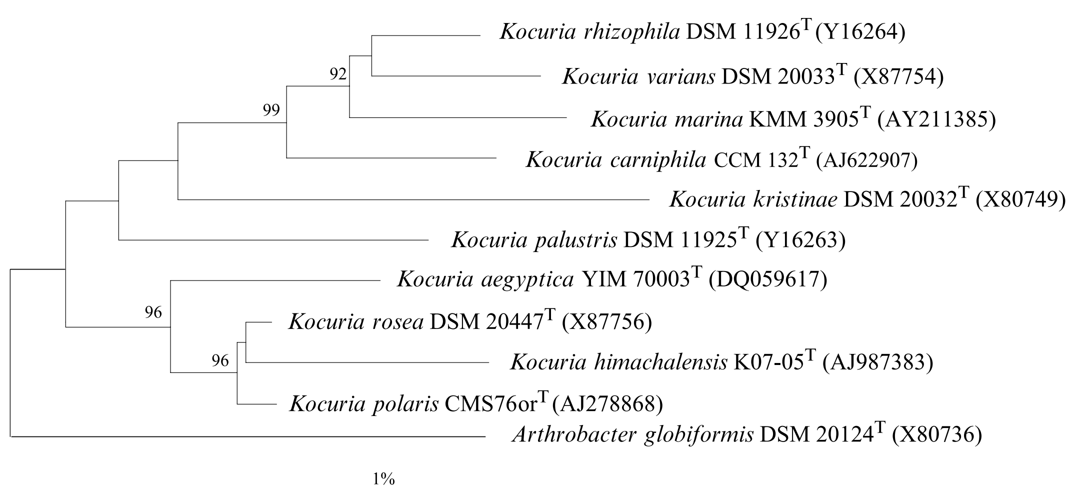

FIGURE 138. Neighbor joining tree (Felsenstein, 1993b) based on 16S rRNA gene sequences of members of the genus *Kocuria*, represented by their type strains. Bootstrap values (>90%) of 500 resamplings (Felsenstein, 1985) are indicated at nodes. The scale represents 1 inferred nucleotide change per 100 nucleotides.

TABLE 99. Characteristics used for differentiation of *Kocuria* spp.[a,b]

Characteristic	*K. rosea*[c]	*K. aegyptia*[d]	*K. carniphila*[e]	*K. himachalensis*[f]	*K. kristinae*[e]	*K. marina*[g]	*K. palustris*[h]	*K. polaris*[i]	*K. rhizophila*[h]	*K. varians*[c]
Oxidase	–	–	–	–	–	–	–	+	–	–
Urease	–	–	–	–	–	+	+	–	–	+
Nitrate reduction	+	–	+	–	–	+	+	+	–	–
H_2S production	–	–	–	–	–	–	(+)	nd	(+)	–
Pigment color	Pink or red	Pink	Yellow	Orange/ pink	Pale cream to pale orange	Orange	Pale yellow	Orange	Yellow	Yellow
Hydrolysis of:										
Gelatin	–	–	–	–	(+)	+	+	–	–	v
Starch	v	–	–	–	–	–	–	–	–	v
Tween 80	–	–	–	–	+	–	+	nd	+	+
Growth at/in:										
5°C	–	nd	–	–	–	+	–	+	–	–
37°C	+	+	+	+	+	+	–	–	+	+
5% NaCl	+	+	+	+	+	+	(+)	–	+	+
10% NaCl	–	–	–	–	+	+	–	–	+	–
15% NaCl	–	–	–	–	–	+	–	–	(+)	–
Utilization as sole carbon source:										
Adonitol	–	nd	nd	–	+	–	+	+	+	+
L-Arabinose	–	–	nd	+	+	–	–	+	+	+
m-Inositol	–	–	+	–	+	–	–	+	–	–
Mannitol	–	+	+	+	+	–	–	–	–	–
Mannose	+	+	+	–	+	+	–	–	+	+
Sorbitol	+	+	+	–	+	–	–	+	–	+
Acid production from:										
L-Arabinose	+	nd	nd	+	–	–	–	+	–	–
Galactose	–	nd	nd	–	–	–	+	(+)	–	–
Glucose	+	–	+	+	+	–	+	+	+	v
Lactose	–	–	+	–	–	–	–	+	+	+
Maltose	–	–	(+)	–	+	–	–	–	–	–
Mannitol	+	–	–	+	–	–	–	+	–	–
Sorbitol	–	–	–	–	+	–	–	+	–	–
Sucrose	–	nd	(+)	–	+	–	+	+	+	–
Xylose	+	nd	–	+	–	–	–	+	–	–
Major menaquinone(s)	MK-8(H_2)	MK-8(H_2) MK-9(H_2)	MK-7 (H_2)	MK-8(H_2)	MK-7(H_2) MK-8(H_2)	nd	MK-7(H_2)	MK-7(H_2) MK-8(H_2)	MK-7(H_2) MK-8(H_2)	MK-7(H_2)
Polar lipids	PG, DPG	PG, DPG	nd	PG, DPG, 2 GLs	PG, DGP, PI, PL, GL	nd	PG, DPG	PG, DPG	PG, DPG	PG, DPG
Whole-cell sugars	Galactosamine	Galactose (glucose)	nd	nd	Glucosamine	nd	nd	Galactose, glucose, ribose	nd	Galactosamine
Major fatty acid(s)	$C_{15:0}$ anteiso	$C_{15:0}$ anteiso, $C_{15:0}$ iso	$C_{15:0}$ anteiso	$C_{15:0}$ anteiso, $C_{15:0}$ anteiso	$C_{15:0}$ anteiso	$C_{15:0}$ anteiso	$C_{15:0}$ anteiso	$C_{15:0}$ anteiso	$C_{17:0}$ anteiso, $C_{15:0}$ anteiso, $C_{15:0}$ iso	$C_{15:0}$ anteiso
DNA G+C content (mol%)	66–75	73	71	75	67	60	70	73	70	66–72

[a]Abbreviations: +, positive reaction; –, negative reaction; v, variable reaction; w, weak reaction; nd, no data available.

[b]Data for the species, except for *Kocuria rosea*, *Kocuria varians*, *Kocuria kristinae*, are based on the type strains only.

[c]Data from Stackebrandt et al. (1995).

[d]Data from Li et al. (2006).

[e]Data from Tvrzová et al. (2005a).

[f]Data from Mayilraj et al. (2006).

[g]Data from Kim et al. (2004).

[h]Data from Kovács et al. (1999).

[i]Data from Reddy et al. (2003).

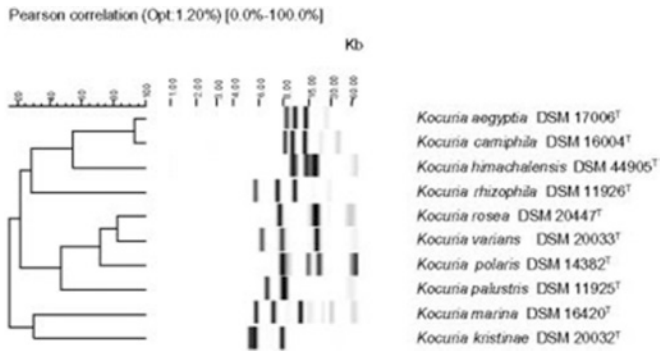

FIGURE 139. Normalized *Eco*RI riboprint profiles (Qualicon, Dupont) of *Kocuria* type strains and a dendrogram of band pattern relatedness as generated by using BioNumerics software (Applied Maths, Kortrijk, Belgium).

List of species of the genus *Kocuria*

1. **Kocuria rosea** (Flügge 1886) Stackebrandt, Koch, Gvozdiak and Schumann 1995, 690 (*Micrococcus roseus* Flügge 1886 183[AL])

ro′se.a. L. fem. adj. *rosea* rose colored, rosy.

In addition to the properties given in the genus description and in Table 99, this species has the following characteristics. Cells are spherical (diameter, 1–1.5 µm) and occur in pairs, tetrads, and clusters. Colonies are circular, slightly convex, smooth (occasionally rough), and pink or red. No water-soluble exopigment is formed. Colony morphology and color become more distinct with age; cell morphology is not culture-, age-, or medium-dependent. Growth is stimulated by cysteine and thiamine or cysteine, thiamine, and pantothenic acid. Growth in nutrient broth is followed by moderate turbidity and a deposit; in some strains a surface ring and pellicle are formed. Grows well in the presence of NaCl concentrations up to 7.5% NaCl. Oxidase test negative (rarely weakly positive); benzidine test positive. Nitrate reduction positive or weakly positive; nitrite reduction negative. Arginine dihydrolase, phenylalanine deaminase, urease, lecithinase, phosphatase, β-galactosidase, and bound human and rabbit coagulase negative. Most strains do not grow on inorganic nitrogen agar and on Simmons' citrate agar. Hydrolysis of esculin and gelatin is negative; hydrolysis of Tween 80 and starch is variable. Production of acetoin is negative or weak; indole and hydrogen sulfide are not produced. Most strains utilize rhamnose, xylose, and glucitol. No acid is formed from mannose, galactose, lactose, and glycerol; acid production from glucose is variable. The optimal growth temperature is in the range 25–37°C. All strains are susceptible to tetracycline, erythromycin, oleandomycin, novobiocin, methicillin, kanamycin, polymyxin, vancomycin, penicillin G, streptomycin, chloramphenicol, and neomycin. Slightly resistant or resistant to lysozyme. Saprophytic. Nonhemolytic. The cytochromes are cytochromes aa_3, c_{550}, c_{557}, b_{564}, and d_{626}.

Source: water and soil.

DNA G+C content (mol%): 66–75 (T_m).

Type strain: ATCC 186, DSM 20447, CCM 679, CCUG 4312, CIP 71.15, IEGM 394, NBRC 3768, JCM 11614, LMG 14224, NCTC 7523, NRRL B-2977, VKM B-1823.

Sequence accession no. (16S rRNA gene): X87756.

2. **Kocuria aegyptia** Li, Zhang, Schumann, Chen, Hozzein, Tian, Xu and Jian 2006, 735[VP]

a.e.gyp′ti.a. L. fem. adj. aegyptia from Egypt, referring to the country of isolation.

In addition to the properties given in the genus description and in Table 99, this species has the following characteristics. Cells are Gram-positive, coccoid, occurring in pairs, tetrads or clusters, and non-motile, and non-endospore-forming. Colonies are pink, circular, opaque, and approximately 2 mm in diameter. The strain cannot grow in ISP 2 medium without salt, but can grow in ISP 2 medium containing 1–5% NaCl, 1–10% KCl, 1–5% $MgCl_2 \cdot 6H_2O$, or 1–5% $CaCl_2$, respectively, with optimum growth occurring at 3%, except for KCl at 5%. Temperature range for growth is 20–40°C, with optimum growth occurring at 28°C. pH range for growth is 5.0–12.0, with optimum growth occurring between 10.0 and 10.5. Oxidase test with

tetramethyl-*p*-phenylenediamine is negative. The strain is negative for urease, *N*-acetyl-glucosaminidase, L-aspartic arylamidase, β-galactosidase, α-galactosidase, α-maltosidase, β-glucuronidase, Tween 20 and Tween 80 esterase, tyrosinase, Methyl red and Voges–Proskauer tests, melanin, indole, and H_2S production, nitrate reduction, gelatin liquefaction, milk peptonization and coagulation, and starch hydrolysis, but positive for catalase-reaction and ornithine decarboxylase, arginine dihydrolase, lysine decarboxylase, lipase, β-glucosidase, and ammonia. Maltose, D-glucose, D-cellobiose, D-trehalose, D-sorbitol, D-fructose, D-mannose, and dextrin can be utilized as the only carbon source. Acid is produced from D-fructose but not from mannose, trehalose, raffinose, ribose, and cellobiose.

Source: A saline alkaline desert soil sample collected from Egypt.

DNA G+C content (mol%): 73 (T_m).

Type strain: CCTCC AA203006, CIP 107966, JCM 14735, YIM 70003, KCTC 19010, DSM 17006.

Sequence accession no. (16S rRNA gene): DQ059617.

3. **Kocuria carniphila** Tvrzová, Schumann, Sedláček, Páčová, Spröer, Verbarg and Kroppenstedt 2005a, 140[VP]

car.ni′phi.la. L. n. *caro* gen. *carnis* meat; N.L. fem. adj. *phila* (from Gr. fem. adj. *philê*) loving; N.L. fem. adj. *carniphila* meat-loving.

In addition to the properties given in the genus description and in Table 99, this species has the following characteristics. Cells are coccoid, 1–1.5 μm in diameter, occurring in pairs and tetrads, Gram-positive, non-motile, non-acid-fast, and non-spore-forming. Colonies are yellow, circular, convex, and opaque. Obligately aerobic. Good growth at 28–37°C. Growth over pH range 7.0–9.1. Very weak growth in the presence of 10% (w/v) NaCl. Catalase-, β-galactosidase- and β-glucuronidase-positive. Negative for oxidase, Voges–Proskauer test, arginine dihydrolase, ornithine decarboxylase, urease, phosphatase, and pyrrolidonyl arylamidase. Nitrate is reduced to nitrite. Nitrite is not reduced. Starch, gelatin, Tween 80, and esculin are not hydrolyzed. Acidification of glucose, fructose, and lactose is positive. Weak acid production from maltose and sucrose. Acid production is negative from mannose, raffinose, trehalose, mannitol, xylitol, melibiose, xylose, turanose, ribose, cellobiose, methyl α-D-glucopyranoside, *N*-acetylglucosamine, and arabinose. The following compounds are utilized: dextrin, Tween 40, Tween 80, *N*-acetyl-D-glucosamine, *N*-acetyl-D-mannosamine, amygdalin, D-fructose, L-fucose, D-galactose, D-galacturonic acid, gentiobiose, D-gluconic acid, α-D-glucose, *myo*-inositol, maltotriose, D-mannitol, D-mannose, D-melibiose, methyl α-D-galactoside, methyl β-D-galactoside, 3-methyl glucose, D-psicose, D-ribose, sucrose, acetic acid, α-hydroxybutyric acid, β-hydroxybutyric acid, γ-hydroxybutyric acid, *p*-hydroxyphenylacetic acid, α-ketoglutaric acid, β-ketovaleric acid, L-lactic acid, mono-methyl succinate, propionic acid, pyruvic acid, succinic acid, *N*-acetyl-glutamic acid, L-glutamic acid, L-pyroglutamic acid, glycerol, thymidine 5′-monophosphate and glucose 6-phosphate. α-Cyclodextrin, β-cyclodextrin, D-arabitol, arbutin, D-galactose, lactulose, D-melezitose, methyl α-D-glucoside, methyl β-D-glucoside, methyl α-D-mannoside, palatinose, D-raffinose, salicin, sedoheptulosan, D-tagatose, xylitol, lactamide, D-lactic acid methyl ester, D-malic acid, L-malic acid, alaninamide, D-alanine, L-alanine, L-alanyl glycine, glycyl-L-glutamic acid, putrescine, inosine, uridine, adenosine 5′-monophosphate, and uridine 5′-monophosphate cannot be utilized.

Source: meat.

DNA G+C content (mol%): 71 (HPLC).

Type strain: CCM 132, DSM 16004, JCM 14118.

Sequence accession no. (16S rRNA gene): AJ622907.

4. **Kocuria himachalensis** Mayilraj, Kroppenstedt, Suresh, Saini and Chakrabarti 2006, 1974[VP]

hi.ma.cha. len′ sis. N.L. fem. adj. himachalensis of or pertaining to the Indian State of Himachal Pradesh, where the type strain was isolated.

In addition to the properties given in the genus description and in Table 99, this species has the following characteristics. Gram-positive cocci, strictly aerobic. Colonies circular, orange/pink colored, and 2 to 5 mm in diameter on TSA agar. No growth occurs at 5°C and 42°C. Growth occurs at 20–30°C (optimal temperature 28°C) and pH up to 10 (optimal pH 7.5). Catalase and oxidase are negative. Does not grow in the presence of 8% NaCl or higher. It is negative for starch hydrolysis, gelatin hydrolysis, indole, methyl red, Voges-Proskauer, and nitrate reduction. Hydrogen sulfide is not produced. Capable of utilizing D-maltose, D-melibiose, L-arabinose, L-alanine, D-cellobiose, D-mannitol, pyruvate, inulin, D-raffinose, and negative for adonitol, *meso*-inositol, lactose as sole carbon sources. Capable of utilizing L-arginine, L-aspartic acid, L-asparagine, L-glutamic acid, L-leucine, and L-phenylalanine as sole nitrogen sources. Acid is produced aerobically from D-glucose, D-mannose, D-mannitol, L-rhamnose, and D-xylose.

Source: soil, one and a half feet below ice glacier, ASL 4200M, Kibber village of Spiti valley, Himachal Pradesh, India.

DNA G+C content (mol%): 75 (T_m).

Type strain: K07-05, JCM 13326, MTCC 7020, DSM 44905.

Sequence accession no. (16S rRNA gene): AJ987383.

5. **Kocuria kristinae** (Kloos, Tornabene and Schleifer 1974). Stackebrandt, Koch, Gvozdiak and Schumann 1995, 690 *Micrococcus kristinae* Kloos, Tornabene and Schleifer 1974, 87[AL]

kris.ti′na.e. N.L. gen. fem. n. *kristinae* of Kristin; named for Kristin Holding, from whom this organism was originally isolated.

In addition to the properties given in the genus description and in Table 99, this species has the following characteristics. Spheres (diameter, 0.7–1.1 μm) occur in tetrads which may form large adherent clusters. Colonies are circular, entire, or crenate, convex, smooth or rough, up to 2 mm in diameter, and pale cream to pale orange; with time the color in the colony center becomes more intense. Colony morphology and color become more distinct with age; cell morphology is not culture age- or medium-dependent.

Does not produce a water-soluble exopigment. Slightly facultatively anaerobic. Grows well on nutrient agar containing up to 10% NaCl; growth is very weak in the presence of 15% NaCl. Oxidase and benzidine test positive. Urease variable; arginine dihydrolase, lecithinase, and phosphatase negative; and β-galactosidase variable. Nitrate reduction is negative in most strains. Nitrite reduction negative. No growth occurs on Simmons' citrate agar, and growth does not occur or is weak on inorganic nitrogen agar. Positive for hydrolysis of esculin but negative for hydrolysis of gelatin, Tween 80, and starch. Produces acetoin. Acid is produced aerobically from glucose, fructose, mannose, sucrose, and glycerol and usually from maltose and glucitol. Does not utilize rhamnose, xylose, ribose, arabinose, raffinose, melibiose, mannitol, ribitol, and galactitol; usually does not utilize galactose and lactose. The amino acid and vitamin requirements are complicated. Usually growth is stimulated by leucine, lysine, valine, and tyrosine. Resistant to lysozyme. Susceptible to tetracycline, erythromycin, novobiocin, methicillin, penicillin, kanamycin, chloramphenicol, neomycin, vancomycin, polymyxin B, and streptomycin. Nonhemolytic. The cytochromes are cytochromes aa_3, C_{548}, C_{557}, b_{561}, and d_{626}.

Source: human skin.

DNA G+C content (mol%): 67 (T_m).

Type strain: DSM 20032, ATCC 27570, CCM 2690, CCUG 33026, CIP 81.69, IEGM 390, NBRC 15354, JCM 7237, LMG 14215, NCTC 11038, NRRL B-14835, VKM B-1811.

Sequence accession no. (16S rRNA gene): X80749.

6. **Kocuria marina** Kim, Nedashkovskaya, Mikhailov, Han, Kim, Rhee and Bae 2004, 1619[VP]

ma.ri'na. L. fem. adj. *marina* of the sea.

In addition to the properties given in the genus description and in Table 99, this species has the following characteristics. Gram-positive, aerobic, non-motile, halotolerant coccoid cells. Positive for catalase, β-galactosidase, and urease, but negative for arginine dihydrolase, lysine and ornithine decarboxylase, oxidase, and alkaline phosphatase. Nitrate is reduced, but hydrogen sulfide is not produced. Production of indole and acetoin (Voges–Proskauer reaction) is negative. Growth occurs in the presence of up to 15% NaCl, although its presence is not required for growth. Grows at 4–43°C. Casein, gelatin, and Tween 40 are hydrolyzed, but agar, alginate, cellulose, DNA, starch, Tween 20, and Tween 80 are not. Glucose, lactose, mannose, and sucrose are utilized as sole carbon sources, but not adonitol, L-arabinose, *meso*-inositol, mannitol, sorbitol, citric acid, or malonic acid. Acid is produced from L-fucose, but not from acetic acid, *N*-acetylglucosamine, adonitol, L-arabinose, cellobiose, dulcitol, fumaric acid, galactose, glucose, glycerol, *meso*-inositol, lactose, malic acid, maltose, mannitol, melibiose, raffinose, rhamnose, sorbitol, sorbose, sucrose, and L-xylose. Growth is inhibited by ampicillin, benzylpenicillin, carbenicillin, gentamicin, lincomycin, neomycin, oleandomycin, streptomycin, and tetracycline, but not by kanamycin or polymyxin B.

Source: marine sediment of the Troitsa Bay of the Gulf of Peter the Great, East Siberian Sea.

DNA G+C content (mol%): 60 (method not reported).

Type strain: KMM 3905, DSM 16420, KCTC 9943, CCUG 51442, JCM 13363.

Sequence accession no. (16S rRNA gene): AY211385.

7. **Kocuria palustris** Kovács, Burghardt, Pradella, Schumann, Stackebrandt and Màrialigeti 1999, 171[VP]

pa.lus'tris. L.fem. adj. *palustris* marshy, swampy.

In addition to the properties given in the genus description and in Table 99, this species has the following characteristics. The cells are spherical (diameter, 1.0–1.5 µm), occurring in pairs, tetrads, and packets, Gram-positive, and non-motile. Endospores not produced. Not acid-fast. The colonies are pale-yellow, 2.0–3.0 mm in diameter, opaque, smooth with irregular edges. Aerobic. Chemoorganotrophic. No growth above 30°C. Good growth between pH 5.7–7.7. Positive for catalase, urease, benzidine, and growth on inorganic nitrogen. Negative for phosphatase, Tween 80 hydrolysis, and esculin hydrolysis. Oxidase, gelatinase, starch hydrolysis, growth on Simmons' citrate, indole, esculin hydrolysis, arginine dihydrolase, and phenylalanine deaminase reaction negative. H_2S reaction weak. Nitrate reduced to nitrite. Acid production from D-glucose, D-fructose, galactose, lactose, and saccharose. No acid production from glycerol, mannitol, sorbitol, ribose, D-xylose, L-arabinose, D-mannose, maltose, β-gentiobiose, D-turanose, arbutin, salicin, trehalose, melezitose, and amidon. Utilization of adonitol, glucuronamide, inosine, Tween 40, and Tween 80. No utilization of cellobiose, turanose, xylitol, methyl-pyruvate, dextrin, glycogen, *N*-acetyl-D-glucosamine, D-trehalose, L-arabinose, D-fructose, L-fucose, *N*-acetyl-D-galactosamine, *m*-inositol, maltose, D-mannitol, D-mannose, D-melibiose, sorbitol, glycerol, L-glutamic acid, and L-alanine not utilized. Growth at 7% NaCl, but no growth at 10% NaCl.

Source: the rhizosphere of *Typha angustifolia* from a floating mat on the Soroksár tributary of the river Danube, Hungary.

DNA G+C content (mol%): 70 (HPLC).

Type strain: TAGA27, DSM 11925, CCUG 52395, CIP 105971, NBRC 16318, JCM 11652.

Sequence accession no. (16S rRNA gene): Y16263.

8. **Kocuria polaris** Reddy, Prakash, Prabahar, Matsumoto, Stackebrandt and Shivaji 2003, 186[VP]

po.la'ris. N.L. fem. adj. *polaris* of or pertaining to a pole.

In addition to the properties given in the genus description and in Table 99, this species has the following characteristics. Cells are coccoid (1.0–1.5 µm in diameter), occur in pairs or tetrads or clusters; non-motile; Gram-positive; aerobic. Colonies on peptone-yeast extract medium were 0.1–2.0 mm in diameter, smooth, round, uniform edged, translucent, extremely mucoid, and orange in color. Psychrotrophic, growing between 5 and 30°C with an optimum at 20°C; cells grow between pH 7 and 12, tolerating up to 2.9% NaCl. Positive for catalase, oxidase, phosphatase, and arginine dihydrolase; negative for urease, lipase, β-galactosidase, hydrolysis of gelatin, esculin, and starch, nitrate to nitrite reduction, indole test, methyl red test, Voges–Proskauer test, and levan formation. Utilizes mannose,

galactose, maltose, fructose, glucose, ribose, xylose, rhamnose, raffinose, trehalose, fumaric acid, dulcitol, inositol, sorbitol, adonitol, sucrose, melibiose, lactose, inulin, pyruvate, acetate, glycine, alanine, leucine, isoleucine, valine, serine, threonine, arginine, and proline; the following substrates were not utilized as the only carbon source: arabinose, succinic acid, citric acid, nicotine, cellulose, cysteine, methionine, lysine, aspartic acid, glutamic acid, asparagine, glutamine, tyrosine, histidine, phenylalanine, and tryptophan. No gas production from glucose, arabinose, xylose, rhamnose, galactose, mannose, lactose, maltose, and sucrose. Sensitive to carbenicillin, tobramycin, chlortetracycline, polymyxin, oxytetracycline, nitrofurantoin, penicillin, bacitracin, nitrofurasone, gentamicin, lincomycin, rifampicin, furazolidone, colistin, furoxone, kanamycin, nystatin, cotrizoxazole, chloramphenicol, ampicillin, tetracycline, amoxicillin, trimethoprim, and erythromycin. Cells contain six water-insoluble pigments which were soluble in methanol; pigment production was not dependent on any specific growth conditions or on the composition of the medium.

Source: a cyanobacterial mat sample from McMurdo Dry Valleys, Antarctica (77°33′3″S, 160°38′E).

DNA G+C content (mol%): 73 (T_m).

Type strain: CMS 76or, DSM 14382, JCM 12076, NBRC 103063, MTCC 3702.

Sequence accession no. (16S rRNA gene): AJ278868.

9. **Kocuria rhizophila** Kovács, Burghardt, Pradella, Schumann, Stackebrandt and Màrialigeti 1999, 172[VP]

rhi.zo′phi.la. Gr. n. *rhiza* a root; Gr. adj. *philos -ê -on* loving; N.L. fem. adj. *rhizophila* root loving.

In addition to the properties given in the genus description and in Table 99, this species has the following characteristics. Cells are spherical, occurring in pairs, tetrads, and packets, 1.0–1.5 µm in diameter, Gram-positive, and non-motile. Endospores not produced. Not acid-fast. The colonies are 1.5–2.5 mm in diameter, opaque, smooth with irregular edges with yellow pigmentation. Aerobic. Chemoorganotrophic. No growth above 40°C. Good growth at pH 5.7–7.7. Catalase, benzidine, and growth on inorganic nitrogen positive. Gelatinase, phosphatase, Tween 80 hydrolysis, growth on Simmons' citrate positive. Oxidase, starch hydrolysis, indole, urease, esculin hydrolysis, arginine dihydrolase, and phenylalanine deaminase reaction negative. H_2S reaction weak. Nitrate not reduced to nitrite. Acid production from D-glucose, D-fructose, D-mannose, and saccharose. No acid production from glycerol, mannitol, sorbitol, ribose, D-xylose, L-arabinose, galactose, lactose, maltose, β-gentiobiose, D-turanose, arbutin, salicin, trehalose, melezitose, and amidon. Utilization of adonitol, L-arabinose, D-fructose, L-fucose, D-glucose, turanose, xylitol, methyl-pyruvate, glucuronamide, dextrin, glycogen, Tween 40, Tween 80, and *N*-acetyl-D-glucosamine. No utilization of cellobiose, D-trehalose, *N*-acetyl-D-galactosamine, *m*-inositol, maltose, D-mannitol, D-melibiose, sorbitol, inosine, glycerol, L-glutamic acid, and L-alanine. Good growth at 10% NaCl.

Source: the rhizosphere of *Typha angustifolia* from a floating mat on the Soroksár tributary of the river Danube, Hungary.

DNA G+C content (mol%): 70 (HPLC).

Type strain: TA68, ATCC BAA-50, CIP 105972, NBRC 16319, JCM 11653, DSM 11926.

Sequence accession no. (16S rRNA gene): Y16264.

10. **Kocuria varians** (Migula 1900) Stackebrandt, Koch, Gvozdiak and Schumann 1995, 690[VP] (*Micrococcus varians* Migula 1900, 135[AL])

va′ri.ans. L. part. adj. *varians* changing, varying.

In addition to the properties given above in the genus description and in Table 99, this species has the following characteristics. Cells are spherical (diameter, 0.9–1.5 µm) and occur in tetrads and irregular clusters of tetrads or rarely in packets or as single cells. Colonies are up to 4 mm in diameter, circular, slightly convex, smooth, and glistening. Some strains may form rough, wrinkled, matt, dry colonies. Colonies are yellow. Does not produce a water-soluble exopigment. Colony morphology and color become more distinct with age; cell morphology is not culture age- or medium-dependent. Slight turbidity and a deposit occur in nutrient broth. Grows well at 22–37°C. The final pH in glucose-containing medium is 4.3–5.9. Growth occurs on nutrient agar containing up to 7.5% NaCl and sometimes on nutrient agar containing 10% NaCl. Growth is stimulated by methionine or cysteine and thiamine. Oxidase test negative; benzidine test positive. Usually positive for urease and nitrate and nitrite reductases; arginine dihydrolase negative. Free and bound coagulase, β-galactosidase, phosphatase, lecithinase, phenylalanine deaminase, and DNase negative. Usually grows on Simmons' citrate agar but not on inorganic nitrogen agar. Negative for hydrolysis of esculin and Tween 80. Hydrolysis of starch and gelatin is variable. Production of acetoin is negative or weak; indole and hydrogen sulfide negative. Acid is produced from glucose, xylose, and fructose, but not from mannose maltose, arabinose, raffinose, galactose, rhamnose, ribitol, lactose, mannitol, glycerol, glucitol, inositol, and salicin. Sucrose utilization is variable. Resistant to lysozyme. Susceptible to kanamycin, neomycin, polymyxin B, and vancomycin; most strains are susceptible to tetracycline, erythromycin, oleandomycin, penicillin G, streptomycin, chloramphenicol, and novobiocin. Very susceptible to methicillin. Nonhemolytic. Saprophytic. The cytochromes are cytochromes aa_3, c_{549}, c_{557}, b_{564}, and d_{626}.

Source: mammalian skin, soil, and water.

DNA G+C content (mol%): 76.6–72 (T_m).

Type strain: CCM 884, ATCC 15306, DSM 20033, CCUG 35392, CIP 81.73, HAMBI 40, HAMBI 1951, IEGM 400, NBRC 15358, JCM 7238, LMG 14231, NCTC 7564, VKM B-1827.

Sequence accession no. (16S rRNA gene): X87754.

Genus VI. **Nesterenkonia** Stackebrandt, Koch, Gvozdiak and Schumann 1995, 689[VP] emend. Collins, Lawson, Labrenz, Tindall, Weiss and Hirsch 2002b emend. Li, Chen, Kim, Zhang, Park, Lee, Xu and Jiang 2005a

Erko Stackebrandt

Ne.ste.ren.ko′ni.a. N.L. fem. n. *Nesterenkonia* honoring Olga Nesterenko, a Ukrainian microbiologist.

Cells may consist of short rods sometimes showing branching, or of cocci. Gram-stain-positive. Non-encapsulated. Endospores are not formed. **Chemo-organotrophic; metabolism is strictly respiratory.** Aerobic. Catalase-positive. Moderately halophilic or halotolerant. Some species are alkaliphilic or alkalitolerant. Mesophilic. **Peptidoglycan type is A4α (L-Lys–Gly–L-Glu, L-Lys–L-Glu or L-Lys–Gly–D-Asp).** Mycolic acids are absent. **Menaquinones with seven, eight, and nine completely unsaturated isoprene units (MK-7, MK-8, and MK-9) predominate.** The phospholipids are diphosphatidylglycerol, phosphatidylglycerol, and phosphatidylinositol. The cellular fatty acids are iso and anteiso-branched fatty acids, with $C_{15:0}$ anteiso and $C_{17:0}$ anteiso predominating. Phylogenetically a member of the family *Micrococcaceae*.

DNA G+C content (mol%): 64–72.

Type species: **Nesterenkonia halobia** Stackebrandt, Koch, Gvozdiak and Schumann 1995, 689[VP]; basonym *Micrococcus halobius* Onishi and Kamekura 1972, 235[AL].

Further descriptive information

The phylogenetic intermixing of *Micrococcus* and *Arthrobacter* species was one of the early results of the 16S rRNA oligonucleotide cataloging approach (Stackebrandt and Woese, 1979). This study confirmed studies on the chemotaxonomic heterogeneity of the genus *Micrococcus* (Faller et al., 1980; Girard, 1971; Jeffries, 1969; Morrison et al., 1971). When almost complete 16S rRNA gene sequences became available (Koch et al., 1994), *Micrococcus lylae* was the only species that phylogenetically grouped closely with the type species *Micrococcus luteus*. Lack of close relationship with other *Micrococcus* species could explain the low DNA–DNA hybridization values found between the pairs *Micrococcus luteus* and *Micrococcus varians* and between *Micrococcus luteus* and *Micrococcus kristinae* (Ogasawara-Fujita and Sakaguchi, 1976; Schleifer et al., 1979). Some *Micrococcus* species had been reclassified before or were subsequently reclassified as members of *Deinococcus* (Brooks et al., 1981), *Stomatococcus* (Bergan and Kocur, 1982), and *Arthrobacter* (Koch et al., 1995), or they formed separate phylogenetic lineages which on the basis of chemotaxonomic properties were assigned to the genera *Nesterenkonia* (for *Micrococcus halobius*), *Kocuria* (for *Micrococcus varians*), *Kytococcus* (for *Micrococcus sedentarius*), and *Dermacoccus* (for *Micrococcus nishinomyaensis*) (Stackebrandt et al., 1995). These four genera had different phylogenetic neighbors, the members of which were not always defined by spherical shape (see Stackebrandt and Woese, 1979, and Figure 140 for neighbors of *Nesterenkonia halobia*). Nesterenkonia was affiliated to the family *Micrococcaceae*, suborder *Micrococcineae* (since elevated to order *Micrococcales* in the taxonomic outline to the present volume), order *Actinomycetales* (Stackebrandt et al., 1997). Recently, more species have been added to the genus *Nesterenkonia*, isolated mostly from hypersaline water and soil samples and halophilic and/or slightly alkaliphilic environments: *Nesterenkonia lacusekhoensis* (Collins et al., 2002b), *Nesterenkonia halotolerans*, and *Nesterenkonia xinjiangensis* (Li et al., 2004b), *Nesterenkonia sandarakina*, and *Nesterenkonia lutea* (Li et al., 2005a), *Nesterenkonia aethiopica* (Delgado et al., 2006) while *Nesterenkonia jeotgali* (Yoon et al., 2006) was isolated from jeotgal, a traditional Korean fermented seafood.

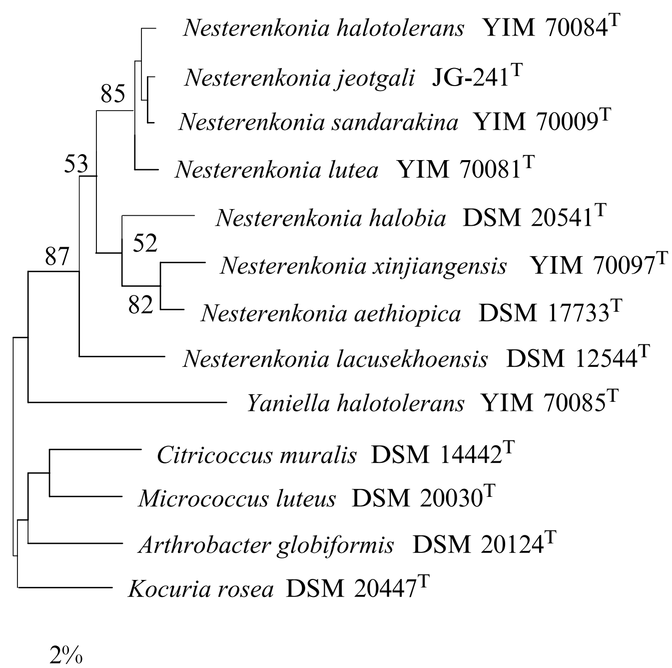

FIGURE 140. Distance matrix tree constructed from 16S rRNA gene sequences (De Soete, 1983) shows the relatedness of the three *Nesterenkonia* species with their nearest neighbors. Bar = 2 inferred nucleotide changes per 100 nucleotides. Numbers on branching points are bootstrap values (1000 resamplings) (Felsenstein, 1985).

Nesterenkonia halobia is the most thoroughly studied species. In all, 150 taxonomic properties of six strains, isolated from ponds of a saltern located in Huelva, Spain (Ventosa et al., 1998) were investigated (Mota et al., 1997). DNA–DNA similarity studies performed among these isolates and the type strain *Nesterenkonia halobia* ATCC 21727[T] revealed 72–100% similarities, confirming the affiliation of the type species. The 16S rRNA gene sequence similarity between *Nesterenkonia halobia* ATCC 21727[T] and one representative of the isolates was 99.1%. Despite the high genomic similarities, differences were found in 9 of the 38 physiological tests also performed on the type strain in the original study of Onishi and Kamegura (1972). The differences were noted in the acid production from some carbohydrates but also in the Voges–Proskauer and Simmons' citrate tests, and hydrolysis of gelatin (Table 100).

Micrographs of *Nesterenkonia halotolerans* and *Nesterenkonia xinjiangensis* (Li et al., 2004b), *Nesterenkonia sandarakina* and *Nesterenkonia lutea* (Li et al., 2005a), and *Nesterenkonia aethiopica* (Delgado et al., 2006) are available as supplementary material in the online publication of these species.

Nesterenkonia spp. have been isolated from various sources and affiliated to described species on the basis of 16S rRNA gene sequences: to *Nesterenkonia lacusekhoensis* for strains from alkaline olive oil extraction waste (Ntougias et al., 2006), to *Nesterenkonia halobia* for strains from decomposing rhizomes of the reed *Phragmites australis* (Borsodi et al., 2005), and a strain from alkaline water of Lake Abijata, Ethiopia (Martins et al., 2001), and to *Nesterenkonia halotolerans* for a strain from an alkaline groundwater environment in Portugal (Tiago et al., 2004).

Enrichment and isolation procedures

Nesterenkonia (*Micrococcus*) *halobia* was isolated from unrefined solar salt of unknown origin obtained from Noda, Japan, in complex medium (Sehgal and Gibbons, 1960) and nutrient broth containing 1 M NaCl. *Nesterenkonia lacusekhoensis* strain IFAM EL-30^T was isolated from a 23 m-deep water sample of Ekho Lake (a hypersaline, meromictic, and heliothermal lake in the ice-free Vestfold Hills, East Antarctica) by means of a Kammerer sampler. The sample was spread directly on plates of medium PYGV (Staley, 1968) agar prepared with Ekho lake water of 10% salinity. Incubation was at 15°C in the dark. *Nesterenkonia sandarakina* YIM 70009^T was isolated from a soil sample collected from the eastern desert of Egypt using a medium containing (g/l): glucose, 10.0; peptone, 5.0; yeast extract, 5.0; $K_2HPO_4 \cdot 3H_2O$, 1.0; $MgSO_4 \cdot 7H_2O$, 0.2; Na_2CO_3, 10.0; agar, 15.0. Furthermore, the sodium carbonate and 15% NaCl (w/v) were separately sterilized before addition to the medium. The pH of the medium was 10.0–10.5; $NaHCO_3/Na_2CO_3$ buffer was used to adjust the pH.

Nesterenkonia halotolerans YIM 70084^T and *Nesterenkonia xinjiangensis* YIM 70097^T were isolated from hypersaline soil samples from Xinjiang Province, Western China, using a modified glycerol/asparagine agar (ISP 5) medium (Shirling and Gottlieb, 1966) supplemented with 15% (w/v) $MgCl_2 \cdot 6H_2O$ and KCl, respectively. The isolation plates were incubated at 28°C for 2 weeks. The pH was adjusted to 7.2 with 1 M NaOH. *Nesterenkonia lutea* YIM 70081^T was isolated from a saline soil sample from China using a modified glycerol/asparagine ISP 5 agar medium, supplemented with 15% (w/v) $MgCl_2 \cdot 6H_2O$. Isolation plates were incubated at 28°C for 2 weeks. *Nesterenkonia jeotgali* JG-241^T was isolated by the usual dilution plating technique on marine agar 2216 (MA; Difco) at 30°C. *Nesterenkonia aethiopica* DSM 17733^T originated from a soiled feather sample collected on the shore of Lake Abjata in Ethiopia; cells were enriched and isolated in complex YP medium (Mota et al., 1997) with shaking at 200 r.p.m. at 37°C.

Maintenance procedures

Nesterenkonia halotolerans YIM 70084^T and *Nesterenkonia xinjiangensis* YIM 70097^T are maintained on medium containing 0.1% (w/v) asparagine, 1% glycerol, 0.1% $K_2HPO_4 \cdot 3H_2O$, 0.5% yeast extract, 10% $MgCl_2 \cdot 6H_2O$ (for strain YIM 70084^T), or 10% KCl (for strain YIM 70097^T). *Nesterenkonia sandarakina* YIM 70009^T and *Nesterenkonia lutea* YIM 70081^T were maintained on modified TSA medium containing 5–10% NaCl (w/v), pH 8.0–9.0, or 5–10% $MgCl_2 \cdot 6H_2O$ (w/v), pH 7.0–8.0 for strains YIM 70009^T and YIM 70081^T, respectively. *Nesterenkonia lacusekhoensis* IFAM EL-30^T is maintained on the modified PYGV medium described above, while *Nesterenkonia halobia* is maintained on modified *Corynebacterium* agar (DSM medium 53 containing 6% NaCl) (Catalogue of strains, DSMZ 2001). All strains can also be stored on medium ISP 5, containing the recommended salt (NaCl or KCl) concentration or in the Microbank system (Biolab Diagnostics, Richmond Hill, ON, Canada). For long-term conservation, freeze drying or storage in the vapor phase of liquid nitrogen is recommended.

Taxonomic comments

Sequence analysis of the 16S rRNA gene of the type strains indicates the presence of a phylogenetic closely related species cluster (*Nesterenkonia sandarakina*, *Nesterenkonia jeotgali*, *Nesterenkonia lutea*, *Nesterenkonia halotolerans*, >99.2% similarity). These species possess D-aspartic acid as a diagnostic amino acid in the interpeptide bridge of peptidoglycan. The other species, characterized by D-glutamic acid in the interpeptide bridge of peptidoglycan are more distantly related (>96.1–<97.1% similarity) (Figure 140). The most obvious chemotaxonomic difference between *Nesterenkonia lacusekhoensis* and other species is the lack of a monocarboxylic acid in the interpeptide bridge (lack of glycine). DNA–DNA hybridization values are available for all type strains though not between *Nesterenkonia aethiopica* and *Nesterenkonia jeotgali*, as these species were described in parallel. *Nesterenkonia sandarakina* YIM 70009^T and *Nesterenkonia lutea* YIM 70081^T shared 45.2% similarity, while *Nesterenkonia halotolerans* YIM 70084^T shared 43.3 and 39.1% similarity, respectively, with these type strains (Li et al., 2005a). Using the microtiter plate method of Ezaki et al. (1989), *Nesterenkonia jeotgali* JG-241^T shared 11–53% DNA relatedness with six type strains of the genus. *Nesterenkonia halotolerans*, *Nesterenkonia sandarakina*, and *Nesterenkonia lutea* had the highest DNA relatedness as well as high 16S rRNA gene similarities to *Nesterenkonia jeotgali* JG-241^T (Figure 140). The spectrophotometric hybridization method (De Ley et al., 1970) revealed that *Nesterenkonia aethiopica* DSM 17733^T had the highest reassociation values with *Nesterenkonia lacusekhoensis* DSM 12544^T (57–64%), *Nesterenkonia halobia* DSM 20541^T (49%), and *Nesterenkonia xinjiangensis* DSM 15475^T (29–36%). These values are in accord with sequence similarities between the type strains (Figure 140).

The phylogenetic affiliation of *Nesterenkonia* to the family *Micrococcaceae* is not obvious from Figure 140, which shows the genus branching more closely to *Yania* (Li et al., 2004a), a member of the family *Yaniaceae* (Li et al., 2005b), now the family *Yaniellaceae* (Li et al., 2008b). While according to Li et al. (2005b) *Yania* branches outside the radiation of a few species representing genera that belong to *Micrococcaceae*, this taxon branches within the radiation of *Micrococcaceae* when all species of all genera of the family are included in a phylogenetic analyses (unpublished). In this tree (Figure 140), *Nesterenkonia* and *Yania* are phylogenetic neighbors, raising doubts about the validity of the family *Yaniellaceae*. With respect to chemotaxonomic properties, *Yania* spp. would be compatible with the diversity of the genera of *Micrococcaceae* (Table 101). Irrespective of selection of strains and the treeing algorithm, bootstrap values for the *Nesterenkonia*/*Yania* lineage are low (Li et al., 2005b; Figure 141) and thus the decision on the higher rank of *Yania* has to await additional taxonomic evidence.

TABLE 100. Differential physiological characteristics of *Nesterenkonia* species[a]

Characteristic	*N. halobia* ATCC 21727[T][b]	*N. lakusekhoensis* DSM 12544[T][c]	*N. halotolerans* YIM 70084[T][d]	*N. xinjiangensis* YIM 70097[T][d]	*N. sandarakina* YIM 70009[T][e]	*N. lutea* YIM 70081[T][e]	*N. aethiopica* DSM 17733[T][f]	*N. jeotgali* JG-241[T][g]
Morphology	Cocci in pairs or tetrads	Short rods, occasionally branching, and cocci	Cocci	Short rods	Cocci	Cocci	Short rods	Cocci
Motility	–	–	+	–	–	+	–	–
Colony pigmentation	Yellow or colorless	Bright yellow	Deep orange–yellow	Light yellow	Orange–yellow	Light yellow to primrose yellow	Yellow	Light yellow
Optimum temperature range for growth (°C)	20–40 (30)	8.5 to >42 (27–33)	4–40 (28)	20–40 (28)	(28)	(28)	25–40 (30–37)	4–36 (25–30)
NaCl tolerance for growth (%)	5.0 to >23	0 to >15	0–25	0–25	1–15	0–20	3–12	0–16
pH tolerance	<6.0–10.0	7.5–9.5	7.0–9.0	7.0–12.0	5.0–12.0	6.5–10.0	7–11	6–9.0
Oxidase activity	+	–	–	–	–	–	+	–
Starch hydrolysis	+	–	–	–	–	–	+	–
H_2S production	–	w	–	–	–	–	–	–
Gelatin liquefaction	–[h]	–	+	+	+	–	+	–
Carbon utilization:								
D-Fructose	nd	+	+	+	+	+	nd	+
D-Mannose	–	+	+	+	+	+	nd	w
D-Trehalose	–	+	–	–	+	–	nd	+
D-Xylose	+	nd	–	+	+	+	–	+
Acid from:								
D-Galactose	+[i]	–	–	–	+[g]	+[g]	–	+
D-Lactose	+[i]	–	–	–	–[g]	+[g]	–	–
D-Mannitol	+[i]	–	–	–	+[g]	+[g]	–	+
D-Xylose	+	–	–	–	+[g]	+[g]	–	+
Trehalose	–	+	–	–	–[g]	+[g]	–	w
Peptidoglycan type	L-Lys–Gly–L-Glu	L-Lys–L-Glu	L-Lys–Gly–Asp	L-Lys–Gly–L-Glu	L-Lys–Gly–D-Asp	L-Lys–Gly–Asp	L-Lys–Gly–L-Glu	L-Lys–Gly–Asp
Polar lipids	DPG, PG, PI, GL	DPG, PG, PC, GL	DPG, PG, PI, GL	DPG, PG, PC	DPG, PG, PI, GL	DPG, PG, PI, GL	nd	DPG, PG, PI, GL
Major Menaquinones	MK-7, MK-8, MK-9	MK-7, MK-8	MK-7, MK-8	MK-7, MK-8, MK-9	MK-7, MK-8	MK-7, MK-8	nd	MK-7, MK-8, MK-9
Major cellular fatty acids (>10 %)	$C_{15:0}$ anteiso, $C_{17:0}$ anteiso	$C_{15:0}$ anteiso, $C_{16:0}$ iso, $C_{15:0}$ iso	$C_{15:0}$ anteiso, $C_{16:0}$ iso, $C_{17:0}$ anteiso	$C_{17:0}$ anteiso, $C_{15:0}$ anteiso	$C_{16:0}$, $C_{15:0}$ anteiso, $C_{17:0}$ anteiso	$C_{15:0}$ anteiso, $C_{16:0}$ iso, $C_{17:0}$ anteiso, $C_{15:1}$ anteiso, $C_{16:1}$ iso	nd	$C_{15:0}$ anteiso, $C_{17:0}$ anteiso
DNA G+C content (mol%)	72[j]	66	64	67	64	65	69	68

[a]Symbols and abbreviations: +, Positive; –, negative; w, weak reaction; for other abbreviations, please refer to footnote in Table 101.

[b]Data from Onishi and Kamekura (1972); Stackebrandt et al. (1995); Mota et al. (1997).

[c]Data from Collins et al. (2002b).

[d]Data from Li et al. (2004b).

[e]Data from Li et al. (2005a).

[f]Data from Delgado et al. (2006).

[g]Data from Yoon et al. (2006).

[h]Positive in Mota et al. (1997).

[i]Negative in Mota et al. (1997).

[j]Range of 70.7–72 mol% for the isolates studies by Mota et al. (1997).

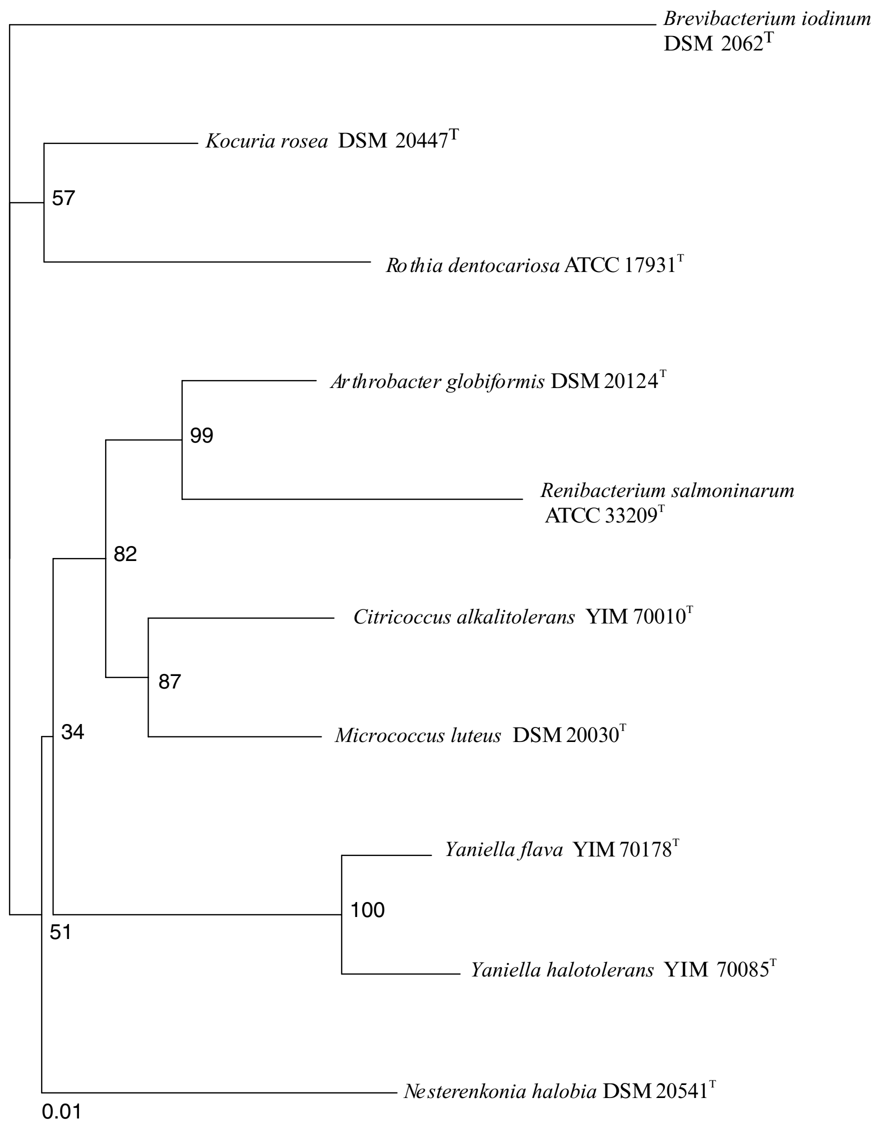

FIGURE 141. 16S rRNA gene sequence neighbor joining tree (Felsenstein, 1993a) of *Yaniella halotolerans* and *Yaniella flava* among representatives of related genera of the order *Micrococcales*. Bootstrap values (>50%) of 1000 resamplings (Felsenstein, 1985) are indicated at nodes. *Brevibacterium iodinum* DSM 2062^T served as root. Bar = 1 nt substitution per 100 nt.

Differentiation of the genus *Nesterenkonia* from other genera

Nesterenkonia, as a member of the family *Micrococcaceae*, differs from other members of the family in morphology, DNA base composition, isoprenoid quinones, and amino acid composition of the peptidoglycan (Table 101). Polar lipid composition distinguishes members of *Nesterenkonia* from *Renibacterium salmoninarum*. The same key properties, especially the DNA G+C content, discriminates *Nesterenkonia* from *Yania*. Affiliation of novel isolates to the genus *Nesterenkonia* should be done on the basis of 16S rRNA gene sequence analysis and from a determination of chemotaxonomic properties, mainly the peptidoglycan type (Table 101). The decision whether to describe novel species should be based on the outcome of studies on the properties listed in Table 100 and Table 101 and, if indicated, by DNA–DNA reassociation studies.

Further reading

Kocur, M. 1986. Genus *Micrococcus*. *In* Bergey's Manual of Systematic Bacteriology, vol. 2 (edited by Sneath, Mair, Sharpe and Holt). Williams & Wilkins, Baltimore, pp. 1004–1008.

Kocur, M., W.E. Kloos and K.H. Schleifer. 1991. The genus *Micrococcus*. *In* The Prokaryotes: a Handbook on the Biology of Bacteria: Ecophysiology, Isolation, Identification, Applications, 2nd edn, vol. II (edited by Balows, Trüper, Dworkin, Harder and Schleifer). Springer, New York, pp. 1300–1311.

Differentiation of the species of the genus *Nesterenkonia*

Differential characters of the species *Nesterenkonia* are presented in Table 100.

TABLE 101. Differentiation of the genus *Nesterenkonia* from phylogenetically neighboring and morphologically similar genera[a]

Characteristic	*Nesterenkonia*[b]	*Yania*[c]	*Kocuria*[d]	*Renibacterium*[e]	*Citricoccus*[f]	*Micrococcus*[g]	*Zhihengliuella*[h]
Morphology	Coccoid	Coccoid	Coccoid	Short rods	Coccoid	Coccoid	Short rods
Peptidoglycan type	L-Lys–Gly–L-Glu, or L-Lys–L-Glu, or L-Lys–Gly–Asp	L-Lys–Gly–L-Glu	L-Lys–$Ala_{3–4}$	L-Lys–Ala–Gly	L-Lys–Gly–Glu	L-Lys–peptide subunit or L-Lys–Asp	L-Lys–L-Ala–L-Glu
Major menaquinones	MK-8, MK-9, MK-7	MK-8, MK-9, MK-7	MK-7(H_2), MK-8(H_2)	MK-9, MK-10	MK-9(H_2), MK-8(H_2), MK-7(H_2)	MK-8(H_2) or MK-8, MK-8(H_2)	MK-9, MK-10
Polar lipids	DPG, PG, PI, PL, GL	DPG, PG, PI, PL, GL	DPG, PG (PI, PL, GL)	DPG, GL	DPG, PG, PI, PL, GL	PG, DPG, PI, GL, PL	PG, DPG, PI, PL, GL
Major fatty acids	$C_{15:0}$ anteiso, $C_{17:0}$ anteiso, $C_{16:0}$ iso	$C_{15:0}$ anteiso, $C_{14:0}$ iso, $C_{15:0}$ iso	$C_{15:0}$ anteiso, $C_{17:0}$ anteiso, $C_{16:0}$ iso, $C_{17:1}$ anteiso	$C_{15:0}$ anteiso, $C_{17:0}$ anteiso	$C_{15:0}$ anteiso, $C_{17:0}$ anteiso, $C_{16:0}$ iso, $C_{16:0}$ iso	$C_{15:0}$ anteiso, $C_{15:0}$ iso	$C_{15:0}$ anteiso, $C_{15:0}$ iso
DNA G+C content (mol%)	64–72	53–58	60–75	52–54	63–68	69–76	66

[a]Abbreviations: Ala, alanine; Asp, aspartic acid; Gly, glycine; Glu, glutamic acid; Lys, lysine; Ser, serine; MCA_{var}, monocarboxylic amino acids with variable composition in the interpeptide bridge; abbreviations of menaquinones exemplified by MK-8(H_2), partially saturated menaquinone with one of 8 isoprene units hydrogenated, MK-9, unsaturated menaquinone with 9 isoprene units; DPG, diphosphatidylglycerol; PG, phosphatidylglycerol; PI, phosphatidylinositol; DMDG, dimannosyldiacylglycerol; PL, unidentified phospholipid(s); GL, unidentified glycolipid(s).

[b]Data from Stackebrandt and Schumann (2000b), Collins et al. (2002b), Li et al. (2005a).

[c]Data from Li et al. (2004a).

[d]Data from Stackebrandt and Schumann (2000b), Kim et al. (2004).

[e]Data from Stackebrandt and Schumann (2000b).

[f]Data from Altenburger et al. (2002), Li et al. (2005b).

[g]Data from Wieser et al. (2002).

[h]Data from Zhang et al. (2007).

List of species of the genus *Nesterenkonia*

1. **Nesterenkonia halobia** (Onishi and Kamekura 1972) Stackebrandt, Koch, Gvozdiak and Schumann 1995, 689[VP] (Basonym: *Micrococcus halobius* Onishi and Kamekura 1972, 235[AL].)

ha.lo′bi.a. Gr. n. *hals*, *halos* salt; Gr. n. *bios* life, N.L. fem. adj. *halobia* living on salt.

Gram-stain-positive. Cells are coccoid (diameter, 0.8–1.5 μm) occur singly, in pairs, and sometimes in tetrads or irregular clusters. Non-encapsulated. Endospores are not formed. The growth temperature range and optimum are 20–40°C and 37°C, respectively. Optimum growth occurs at pH 7.0–7.5, but strains grow well at pH 5–10. Chemo-organotrophic; metabolism is strictly respiratory. Catalase-positive. Moderately halophilic. Mesophilic. Colonies on nutrient agar supplemented with 5% NaCl are circular, smooth, opaque, and nonpigmented. During growth in nutrient broth, the medium is turbid. Optimum growth occurs on media containing 1–2 M NaCl, moderate growth occurs in the presence of 4 M NaCl, and no growth occurs on media lacking NaCl or KCl. Oxidase test positive. Urease, phosphatase, nitrate reductase, and arginine dihydrolase-negative. Most strains grow on Simmons' citrate agar. Starch, gelatin, casein, esculin, and tyrosine are hydrolyzed, while Tween 80 is not hydrolyzed. Acetoin production is positive; indole and hydrogen sulfide production are negative. Low amounts of acid are produced aerobically from glucose, fructose, and D-xylose. No acid is formed from L-arabinose, D-galactose, glycerol, lactose, maltose, mannitol, sucrose, and trehalose. Utilizes D-tagatose, acetic acid, and *p*-hydroxyphenyl acetic acid but not other substrates provided in the Biolog substrate panel. Milk coagulation occurs without acid formation. Resistant to lysozyme; susceptible to ampicillin (10 μg), bacitracin (10 U), cephalothin (30 μg), chloramphenicol (30 μg), erythromycin (15 μg), novobiocin (30 μg), and rifampin (5 μg). Thiamine is required for growth; growth is stimulated by biotin. Nonhemolytic.

Source: unrefined solar salt, Noda, Japan.

DNA G+C content (mol%): 71–72 (T_m).

Type strain: 28-3, ATCC 21727, DSM 20541, CCM 2591, CCUG 38889, CIP 81.68, NBRC15353, JCM 11483, VKM B-1317.

Sequence accession no. (16S rRNA gene): X80747.

2. **Nesterenkonia aethiopica** Delgado, Quillaguamán, Bakhtiar, Mattiasson, Gessesse and Hatti-Kaul 2006, 1232[VP.]

a.e.thio′pi.ca. L. fem. adj. *aethiopica* pertaining to Ethiopia.

Cells are Gram-stain-positive, nonmotile, non-capsule-forming, non-endospore-forming, short rods around 0.7 μm in width and 1.2 μm in length. Catalase- and oxidase-positive and urease-negative. Strictly aerobic and mesophilic: exhibits good growth at 25–40°C, with an optimum between 30 and 37°C; unable to grow at 45°C. Alkaliphilic and moderately halophilic. Optimal growth occurs at initial pH 9 (range pH 7–11) and at 3% (w/v) NaCl (range 3–12%). Carbohydrates utilized as sole carbon sources are D-glucose, D-fructose, and other sugars listed in Table 100. Acid is produced in medium supplied with D-glucose, D-fructose, L-arabinose, and glycerol, but acidification is not detected with D-trehalose, D-galactose, D-lactose, D-mannitol, or D-xylose. Starch, casein, tyrosine, and gelatin are hydrolyzed. Indole is not produced and the Voges–Proskauer test is negative.

Source: a soiled feather sample collected at the shore of Lake Abjata, Ethiopia (7°60′N 38°62′E).

DNA G+C content (mol%): 69.0 (HPLC).

Type strain: DSM 17733, CCUG 48939, JCM 14309.

Sequence accession no. (16S rRNA gene): AY574575.

3. **Nesterenkonia halotolerans** Li, Chen, Zhang, Schumann, Stackebrandt, Xu and Jiang 2004b, 838[VP]

ha.lo.to′le.rans. Gr. n. *hals*, *halos* salt, L. part. adj. *tolerans* tolerating; N.L. part. adj. *halotolerans* salt-tolerating, referring to the ability to tolerate high salt concentrations.

Cells are Gram-stain-positive, non-spore-forming, motile cocci. The colony color on modified ISP 5 medium is deep orange–yellow (the color of the fringe) to light orange–yellow (the color of the center); some colonies resemble concentric rings. Colonies are circular, opaque, and approximately 2.5–3.5 mm in diameter after 24 h at 28°C. The optimum growth temperature is 28°C. The optimum concentration of $MgCl_2 \cdot 6H_2O$ is 10%. The type strain is positive for gelatin liquefaction and urease production and negative for milk peptonization and coagulation, nitrate reduction, growth on cellulose, H_2S and melanin production, and starch hydrolysis. Positive for catalase, milk coagulation, melanin production, growth in cellulose, and lysine decarboxylase, β-glucosidase, β-galactosidase, and α-maltosidase activities. Negative for indole production, decomposition of Tween 20 and Tween 80, casein and starch, and ornithine decarboxylase, arginine dihydrolase, and *N*-acetylglucosaminidase activities. Maltose, glucose, galactose, mannose, fructose, sucrose, maltose, starch, lactose, dextrin, mannitol, and salicin are utilized as sole carbon sources for growth. Rhamnose, acetamide, inositol, adonitol, ribose, arabinose, cellobiose, trehalose, sorbitol, and xylose are not utilized.

Source: a saline soil sample from Xinjiang Province, China.

DNA G+C content (mol%): 64 (T_m).

Type strain: YIM 70084, CCTCC AA001022, DSM , JCM 13018.

Sequence accession no. (16S rRNA gene): AY226508.

4. **Nesterenkonia jeotgali** Yoon, Jung, Kim, Nam and Oh 2006, 2591[VP]

je.ot.ga′li. N.L. gen. n. *jeotgali* of jeotgal, from which the type strain was isolated.

Cells are cocci (diameter 0.5–0.8 μm) on marine agar (MA) at 30°C; some cells are oval in the very early growth phase. Non motile. Colonies are smooth, circular, slightly convex or raised, glistening and 1.0–2.0 mm in diameter after 5 d cultivation at 30°C on MA. Colonies on MA are vivid yellow in color at 30°C, but orange–yellow in color at 25°C. Colonies on solid PYGV medium are light yellow in color at 25 and 30°C. Optimal growth temperature is 25–30°C; growth occurs at 4 and 36°C, but not at 37°C. Optimal pH for growth is 7.5–8.5; growth occurs at pH 6.0, but not at pH 5.5. Optimal growth occurs in the presence of 2–5% (w/v) NaCl; growth occurs in the presence of 0–16% NaCl (w/v). Growth does not occur under anaerobic conditions on MA or on MA supplemented with nitrate. Voges–Proskauer reaction positive. Indole production negative. Tyrosine is hydrolyzed, but esculin, casein, gelatin, hypoxanthine, starch, xanthine, and Tweens 20, 40,

60, and 80 are not hydrolyzed. Nitrate is not reduced. Methyl red reaction is positive. In assays with the API ZYM system, alkaline phosphatase, esterase (C4), esterase lipase (C8), α-glucosidase, and leucine arylamidase are present, but lipase (C14), valine arylamidase, cystine arylamidase, trypsin, α-chymotrypsin, acid phosphatase, naphthol-AS-BI-phosphohydrolase, *N*-acetyl-β-glucosaminidase, α-mannosidase, α-fucosidase, arginine dihydrolase, ornithine decarboxylase, and tryptophan deaminase are absent. D-glucose, D-galactose, L-arabinose, D-cellobiose, D-fructose, maltose, sucrose, acetate, citrate, succinate, L-malate, and pyruvate are utilized. Benzoate, salicin, formate, and L-glutamate are not utilized. Acid is produced from L-arabinose, D-cellobiose, D-glucose, maltose, D-mannose, D-melezitose, D-ribose, and sucrose, but not from D-fructose, melibiose, D-raffinose, L-rhamnose, *myo*-inositol, or D-sorbitol. Susceptible to penicillin G, chloramphenicol, ampicillin, cephalothin, novobiocin, and carbenicillin, but not to polymyxin B, gentamicin, kanamycin, or neomycin.

Source (type strain): jeotgal, a traditional Korean fermented seafood.

DNA G+C content (mol%): 68.0 (HPLC).

Type strain: JG-241, KCTC 19053, JCM 12610.

Sequence accession no. (16S rRNA gene): AY928901.

5. **Nesterenkonia lacusekhoensis** Collins, Lawson, Labrenz, Tindall, Weiss and Hirsch 2002b, 1149[VP]

la.cus.ek.ho.en′sis. N.L. fem. adj. *lacusekhoensis* of or belonging to Ekho Lake, the lake in Antarctica from which the organism was isolated.

Cells consist of short rods with a mean size of 0.8–1.2 μm; sometimes branching is observed. In older cultures, cocci predominate. Cells are nonmotile and do not contain poly β-hydroxybutyrate granules. Colonies show a bright yellow pigmentation. Thiamine HCl is required, and nicotinic acid and biotin stimulate growth. The optimal temperature for growth is 27–33.5°C, and the optimal pH is 8.6. DNA is hydrolyzed, but Tween 80, starch, and alginate are not. Cells form sulfide but not indole. Methyl-red-negative and Voges–Proskauer-negative. Nitrate is not reduced anaerobically. Metabolizes sugars, sugar alcohols and some organic acids.

Source: hypersaline, heliothermal, and meromictic Ekho Lake (23 m) in East Antarctica.

DNA G+C content (mol%): 66.0 (HPLC).

Type strain: IFAM EL-30, DSM 12544, CIP 107030, CCUG 47141, JCM 11953.

Sequence accession no. (16S rRNA gene): AJ290397.

6. **Nesterenkonia lutea** Li, Chen, Kim, Zhang, Park, Lee, Xu and Jiang 2005a, 465[VP]

lu′te.a. L. fem. adj. *lutea* gold–yellow.

Cells are Gram-stain-positive, non-spore-forming, motile cocci with flagella. Colony color on most tested media is light yellow to primrose yellow. Colonies are circular, opaque, somewhat convex and approximately 0.5–1.0 mm in diameter after 24 h at 28°C. Growth occurs at 0–20% (w/v) $MgCl_2 \cdot 6H_2O$ (optimum at 5–10%, w/v) and pH 6.5–10.0 (optimum at 7.0–8.0). Positive for catalase, milk coagulation, melanin production, growth in cellulose, and lysine decarboxylase, β-glucosidase, β-galactosidase, and α-maltosidase activities. Negative for indole production, decomposition of Tween 20 and Tween 80, casein and starch, and ornithine decarboxylase, arginine dihydrolase, and *N*-acetylglucosaminidase activities. Maltose, sucrose, mannitol, salicin, and galactose are utilized as sole carbon sources for growth; rhamnose, acetamide, inositol, adonitol, and sorbitol are not utilized.

Source: a saline soil sample from the Xinjiang Province, China.

DNA G+C content (mol%): 65.0 (T_m).

Type strain: YIM 70081, CCTCC AA 203010, DSM 15666, KCTC 19013, JCM 13019.

Sequence accession no. (16S rRNA gene): AY588278.

7. **Nesterenkonia sandarakina** Li, Chen, Kim, Zhang, Park, Lee, Xu and Jiang 2005a, 464[VP]

san.da.ra′ ki.na. N.L. fem. adj. *sandarakina* (from Gr. fem. adj. *sandarakinê*) of orange color.

Cells are Gram-stain-positive, non-spore-forming cocci. Colony color on most tested media is orange–yellow. Colonies are circular, opaque, and approximately 0.5–1.0 mm in diameter after 24 h at 28°C. Growth occurs at 1–15% (w/v) NaCl (optimum at 5%, w/v) and pH 5.0–12.0 (optimum at 8.0–9.0). Positive for catalase, milk coagulation, melanin production, growth in cellulose, and lysine decarboxylase, β-glucosidase, β-galactosidase, and α-maltosidase activities. Negative for indole production, decomposition of Tween 20 and Tween 80, casein and starch, and ornithine decarboxylase, arginine dihydrolase, and *N*-acetylglucosaminidase activities. Maltose, sucrose, mannitol, salicin, and galactose are utilized as sole carbon sources for growth; rhamnose, acetamide, inositol, adonitol, and sorbitol are not utilized.

Source: a soil sample collected from the eastern desert of Egypt.

DNA G+C content (mol%): 64.0 (T_m).

Type strain: CCTCC AA 203007, DSM 15664, KCTC 19011 JCM 13020, YIM 70009.

Sequence accession no. (16S rRNA gene): AY588277.

8. **Nesterenkonia xinjiangensis** Li, Chen, Zhang, Schumann, Stackebrandt, Xu and Jiang 2004b, 840[VP]

xin.ji.ang.en′sis. N.L. fem. adj. *xinjiangensis* of or pertaining to Xinjiang, the province of western China in which the samples were collected.

Cells are Gram-stain-positive, nonmotile, non-spore-forming, diphtheroid, irregular rods. The colony color on modified ISP 5 medium is light yellow. Colonies are circular, opaque, somewhat convex and approximately 3.5–4.5 mm in diameter after 24 h at 28°C. The optimum growth temperature is 28°C. The optimum concentration of KCl for growth is 10.0%. The type strain is positive for gelatin liquefaction, milk peptonization, and urease production and negative for milk coagulation, nitrate reduction, growth in cellulose, H_2S and melanin production and starch hydrolysis. Almost all tested carbon sources, including glucose, galactose, mannose, fructose, sucrose, maltose, starch, lactose, dextrin, ribose, arabinose, cellobiose, and xylose, are utilized; trehalose and sorbitol are not utilized.

Source: a saline soil sample from Xinjiang Province, China.

DNA G+C content (mol%): 67.0 (T_m).

Type strain: YIM 70097, CCTCC AA001025, DSM 15475, JCM 13021.

Sequence accession no. (16S rRNA gene): AY226510.

Genus VII. **Renibacterium** Sanders and Fryer 1980, 501[VP]

Brian Austin (updated from J.E. Sanders and J.L. Fryer, 1986)

Re.ni.bac.te′ri.um. L. pl. n. *renes* the kidneys; L. neut. n. *bacterium* rod; N.L. neut. n. *Renibacterium* kidney bacterium.

Short rods or **cocco-bacilli**, 0.3–1.0 × 1.0–1.5 µm, often occurring in pairs (= diplococcobacilli) and short chains. **Strongly Gram-stain-positive**. Nonencapsulated. Nonmotile. Endospores are absent.

Aerobic, slow-growing bacterium; temperature for optimum growth 15–18°C; no growth at 37°C. Cysteine required for growth. Growth enhanced by addition of blood, serum (especially fetal calf serum), or charcoal to media. **No acid production from sugars. Catalase-positive. The cell-wall peptidoglycan contains D-alanine**, **D-glutamic acid, glycine, and lysine as the diamino acid.** Cell-wall sugars include galactose, rhamnose, *N*-acetylglucosamine, and *N*-acetylfucosamine. No mycolic acids are present. The major fatty acid is 12-methyltetradecanoic acid ($C_{15:0}$) with 14-methylhexadecanoic acid ($C_{17:0}$ anteiso) also present in significant amounts. The major respiratory quinones are unsaturated menaquinones with nine isoprene units.

DNA G+C content (mol%): 53 (T_m).

Type species: **Renibacterium salmoninarum** Sanders and Fryer 1980, 501[VP].

Further descriptive information

Uniquely stained "bar" forms have been observed *in vivo*, but have never been cultured (Cvitanich, 2004). Cells grown in culture media tend to exhibit consistent, uniform morphology whereas pleomorphic forms may be observed among cells from fish tissues (Fryer and Sanders, 1981).

Enrichment and isolation procedures

Renibacterium may be isolated from diseased fish by streaking diseased kidney tissue onto Mueller–Hinton medium supplemented with 0.1% (w/v) L-cysteine hydrochloride (Wolf and Dunbar, 1959), KDM-2 medium* (Evelyn, 1977), SKDM† (Austin et al., 1983) or charcoal agar‡ (Daly and Stevenson, 1985). Peptone (0.1% w/v)-saline (0.85% w/v) has been recommended as a diluent to remove potentially inhibitory compounds, which may be present in kidney tissue (Evelyn et al., 1981). A "nurse" culture technique based on satellitism or cross feeding enhances recovery of the pathogen, and involves inoculating drops of a dense suspension of a stock culture of *Renibacterium salmoninarum* (= nurse organism) onto the center of isolation media. Then, 25-µl drops of material containing renibacteria are placed around the periphery of the nurse organism (Evelyn et al., 1989). Recovery of *Renibacterium salmoninarum* may be further improved by supplementing the isolation medium with small amounts of spent broth used previously for growing the organism. Here, it is thought that unknown metabolites serve as growth stimulants for the renibacteria (Evelyn et al., 1989, 1990). Inoculated media in Petri dishes should be sealed in containers/bags to prevent loss of moisture, and incubated at 15–18°C. Growth of *Renibacterium* on these media at primary isolation may be slow, and depending on the state of the infection, the size of the inoculum and the nature of the medium may require up to 19 weeks for visible colonies to develop (Benediktsdóttir et al., 1991). Nurse cultures develop more rapidly, i.e. ≥7 d faster than cultures by conventional plating methods. Laboratory cultures may require several weeks of incubation before colonies appear.

On non-selective media, the presence of contaminants may overgrow the organism. The presence of antibiotics in SKDM improves the recovery of *Renibacterium* from diseased fish tissues (Austin et al., 1983; Benediktsdóttir et al., 1991). Also, dropplating reduces problems with contamination (Evelyn et al., 1990).

Shake cultures in KDM-2 broth give uniform growth throughout the medium. However, initial attempts at growth in broth are often characterized by a prolonged lag period; subsequent transfers often reduce this period substantially.

Mueller–Hinton medium supplemented with 0.1% (w/v) L-cysteine hydrochloride has also been used for the growth of *Renibacterium* (Bullock et al., 1974; Wolf and Dunbar, 1959). However, Evelyn (1971) indicated that serum appears to be an essential ingredient for the isolation and continued cultivation of *Renibacterium*. Paterson et al. (1979) added 10% (v/v) fetal calf serum to cysteine supplemented Mueller–Hinton to improve primary isolation. A semi-defined culture medium devoid of serum has been reported for the routine laboratory cultivation of *Renibacterium*, especially for the preparation of biomass for chemical analyses (Embley et al., 1982).

Pathogenicity. Koch's postulates were initially fulfilled by Ordal and Earp (1956) who monitored mortality after infecting chinook salmon by intraperitoneal injection. Murray et al. (1992) succeeded in inducing disease in chinook salmon by immersing them in 10^4–10^6 cells/ml for 15–30 min and by co-habitation of healthy with other experimentally infected fish. The pathogen becomes internalized in non-phagocytic cells (González et al., 1999) and macrophages that produce virulence factors (McIntosh et al., 1997). Within phagocytic cells, the pathogen divides slowly, and survives for >10 d (Gutenberger et al., 1997). An alternative view is that respiratory burst products generated by macrophages kill renibacteria (Campos-Pérez et al., 1997; Hardie et al., 1996).

A hydrophobic, soluble cell surface p57 protein is responsible for agglutination of salmonid leukocytes (Senson and Stevenson, 1999; Wiens et al., 1999), and is encoded by *msa* (major soluble antigen) genes (Coady et al., 2006, 2004; Rhodes et al.,

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*KDM-2 medium contains 1.0% (w/v) peptone, 0.05% (w/v) yeast extract, 0.1% (w/v) L-cysteine hydrochloride, 1.5% (w/v) agar; pH 6.5; sterilized at 121°C for 15 min, cooled to 45°C, then 20% (v/v) sterile fetal calf serum is added.

†SKDM medium contains 1.0% (w/v) tryptone, 0.05% (w/v) yeast extract, 0.005% (w/v) cycloheximide, 1.0% (w/v) agar; pH 6.8; sterilized at 121°C for 15 min, cooled to 50°C, and then sterile fetal calf serum is added to 10% (v/v), and filter-sterilized solutions of L-cysteine hydrochloride (0.1% w/v), D-cycloserine (0.00125% w/v), polymyxin B sulfate (0.0025% w/v), and oxolinic acid (0.00025% w/v).

‡Charcoal agar contains 1.0% (w/v) peptone, 0.05% (w/v) yeast extract, 0.1% (w/v) L-cysteine hydrochloride, 0.1% (w/v) activated charcoal, 1.5% (w/v) agar; pH 6.8; sterilized at 121°C for 15 min (the charcoal may be placed in dialysis tubing prior to sterilization to obtain a clear broth medium).

TABLE 102. Differential characteristics of *Renibacterium* from other representatives of the *Micrococcaceae*[a]

Characteristic	*Arthrobacter*[b]	*Citricoccus*[c]	*Kocuria*[d]	*Micrococcus*[d]	*Nesterenkonia*[d]	*Renibacterium*	*Rothia*[e]	*Yania*[g]
Major menaquinone content	MK-9(H_2) or MK-9 and MK-8 or MK-9 and MK-10	MK-9(H_2)	MK-7(H_2) or MK-7(H_2) and MK-8(H_2) or MK-8(H_2) or MK-8(H_2) and MK-9(H_2)	MK-8 and MK-8(H_2) or MK-8(H_2) or MK-8(H_2) and MK-7(H_2)	MK-7 or MK-7 and MK-8 or MK-8 and MK-9	MK-9, MK-10	MK-7	MK-8 and MK-9 or MK-8
Optimum growth (°C)	25–30	4–28	22–37	25–37	20–40	15–18	35–37	28
Requirement for cysteine	–	–	(+)	–	–	+	–	–
DNA G+C content (mol%)	61–66	63–68	66–75	69–76	70–72	53	49–53	53

[a]Symbols: +, positive for all species; –, negative for all species.

[b]Data from Stackebrandt and Fiedler (1979), Koch et al. (1995).

[c]Data from Altenburger et al. (2002).

[d]Data from Stackebrandt et al. (1995), Li et al. (2004b, 2005a, 2006), Tvrzová et al. (2005a), Mayilraj et al. (2006), Yoon et al. (2006).

[e]Data from Georg and Brown (1967).

[f]Data from Bergan and Kocur (1982).

[g]Data from Li et al. (2004a, 2005c).

2002). A possible role for extracellular products (ECP) in the pathogenicity process is unclear. Some workers have reported the presence of proteases (Sakai et al., 1989) and hemolysins (Grayson et al., 1995a, 2001) in ECPs with the mode of action reflecting inhibition of the respiratory burst but not phagocytic activity in spleen phagocytes (Densmore et al., 1998). Hydrophobicity and auto-aggregation have been linked with virulence (Bruno, 1988). An iron acquisition mechanism has been found (Grayson et al., 1995b). There is evidence that fish respond to infection by the production of stress factors, including plasma cortisol and lactate, and reduced levels of plasma glucose (Mesa et al., 1999). In particular, a 70-kDa stress protein (HSP70) has been recognized in diseased coho salmon (Forsyth et al., 1997).

Maintenance procedures

Renibacterium cultures may be lyophilized by common procedures used for many bacteria. Broth cultures supplemented with 15–20% (v/v) glycerol as cryopreservant may be frozen at −70°C, and maintain viability for one year or longer.

Differentiation of the genus *Renibacterium* from other genera

For routine diagnosis, serological methods, including co-agglutination (Kimura and Yoshimizu, 1981), Western blots (Lovely et al., 1994), the fluorescent antibody test, and the enzyme linked immunosorbent assay (Griffiths et al., 1996, 1991; Jansson et al., 1996; Kozinska and Pekala, 2005; O'Connor and Hoffnagle, 2007; Pascho et al., 1998), have been useful, although cross-reactions have been recorded with coryneforms, a fish pathogenic *Mycobacterium* sp., *Rothia dentocariosa* (Austin and Rayment, 1985), *Pseudomonas* (Yoshimizu et al., 1987), and other unidentified bacteria (Brown et al., 1995; Bullock et al., 1980). The polymerase chain reaction (PCR) amplifying a 320-bp fragment of a 57-kDa protein (p57 antigen; Pascho et al., 1998), nested reverse transcriptase PCR (Cook and Lynch, 1999), quantitiative PCR Powell et al., 2005), real-time PCR (Chase et al., 2006 ; Suzuki and Sakai, 2007), sequencing of the 16S rRNA gene (Konigsson et al., 2005), and terminal restriction fragment length polymorphism (Nilsson and Strom, 2002) are specific and sensitive.

Renibacterium may be distinguished from all other genera in the *Micrococcaceae* (Table 102) (by the requirement for cysteine and preference for serum, low optimum growth temperature, i.e. 15–18°C, and comparatively lengthy time necessary for the development of visible colonies. *Renibacterium* has a distinctive API ZYM profile after incubation for 24 h at 18°C (Austin et al., 1983). Strains are positive for alkaline phosphatase, caprylate esterase, leucine arylamidase, trypsin, acid phosphatase, phosphoamidase, α-glucosidase, and α-mannosidase (+/−), and negative for butyrate esterase, myristate lipase, valine arylamidase, cystine arylamidase, chemotrypsin, α-galactosidase, β-galactosidase, β-glucuronidase, β-glucosidase, *N*-acetyl-β-glucosaminidase, and α-fucosidase.

Taxonomic comments

Prior to the description of *Renibacterium*, the organism was linked with *Brevibacterium* (Smith, 1964), *Corynebacterium* (Sanders et al., 1978), *Lactobacillus* (Vladik et al., 1974), *Listeria* (Bullock et al., 1975) and *Rickettsia* (Snieszko and Griffin, 1955). By 16S rRNA cataloging, *Renibacterium salmoninarum* was considered to comprise a member of the actinomycete subdivision, being related to *Arthrobacter*, *Brevibacterium*, *Cellulomonas*, *Jonesia*, *Micrococcus*, *Promicromonospora*, *Stomatococcus*, and *Terrabacter* (Gutenberger et al., 1991; Stackebrandt et al., 1988). This was supported by Grayson et al. (2000), who linked *Renibacterium* to the high-G+C group of the actinobacteria, notably *Arthrobacter*, on the basis of rRNA gene sequence comparisons (Grayson et al., 2000). The organism has been included in the family *Micrococcaceae* (Stackebrandt and Schumann, 2000a).

A low genetic diversity has been concluded from the results of analysis of 44 enzymes and 40 renibacterial isolates from North America by multilocus enzyme electrophoresis leading to the recognition of 21 electrophoretic types (Starliper, 1996).

List of species of the genus *Renibacterium*

1. **Renibacterium salmoninarum** Sanders and Fryer 1980, 501[VP]

sal.mo.ni.na′rum. N.L. pl. n. *Salmoninae* subfamily of the *Salmonidae*; N.L. gen. pl. n. *salmoninarum* of the *Salmoninae*.

Short rods, cocco-bacilli, 0.3–1.0 × 1.0–1.5 µm, often occurring in pairs (diplococcobacilli) and short chains. May be pleomorphic, and contain intracellular granules. Strongly Gram-stain-positive. Not acid-fast. Nonmotile. Nonencapsulated. Endospores absent. Hemolysis not observed.

On cysteine serum agar, KDM-2 and SKDM media, colonies are circular and convex, white to creamy yellow, and 2 mm in diameter after ~20 d. Old cultures, i.e. ≥12 weeks, may become extremely granular or crystalline in appearance, and a transverse section will reveal the presence of a few Gram-stain-positive rods embedded in a crystalline matrix. Subculturing at this stage often leads to the development of more crystalline "colonies". On Loeffler coagulated serum, a creamy growth with a matt surface is produced, and with Dorset egg medium, growth appears as a raised, smooth shiny yellow layer (Smith, 1964). Creamy yellow growth occurs only at the surface in stationary cultures in cysteine serum broth. Growth on all media is slow, especially at primary isolation, often requiring up to 19 weeks for visible colonies.

Grows very slowly at 5 and 22°C; no growth at 37°C (Smith, 1964). Optimal growth occurs at 15–18°C. Maximum cell yields with laboratory cultures are obtained after 20–30-d incubation at 15–18°C and pH 6.5–7.5 (Fryer and Sanders, 1981).

Aerobic. No acid production from sugars. Does not reduce nitrate. Produces acid and alkaline phosphatase, caprylate esterase, α-glucosidase, leucine arylamidase, and α-mannosidase, but not butyrate esterase, cystine arylamidase, α-fucosidase, α- or β-galactosidase, β-glucosaminidase, β-glucosidase, β-glucuronidase, myristate esterase, or valine arylamidase (Goodfellow et al., 1985). Utilizes 4-umbelliferyl-acetate, 4-umbelliferyl–butyrate, 4-umbelliferyl-heptanoate, 4-umbelliferyl–laurate, 4-umbelliferyl-nonanoate, 4-umbelliferyl–oleate and 4-umbelliferyl–propionate (Goodfellow et al., 1985). All strains isolated require cysteine, and the majority require serum, whole blood, or charcoal for growth. Catalase-positive. Cytochrome-oxidase-negative. Degrades casein, tributyrin, Tween 40, and 60, but not adenine, esculin, arbutin, chitin, chondroitin, DNA, elastin, gelatin, guanine, hyaluronic acid, hypoxanthine, lecithin, RNA, starch, testosterone, Tween 80, tyrosine, or xanthine (Goodfellow et al., 1985).

The cell-wall peptidoglycan contains D-alanine, D-glutamic acid, glycine, and lysine as the diamino acids (Fiedler and Draxl, 1986). The structure contains a glycylalanine interpeptide bridge and the substitution of the α-carboxyl group of D-glutamic acid in position 2 of the peptide subunit with D-alanineamide (Kusser and Fiedler, 1983). Sanders and Fryer (1980) reported the principal cell-wall sugar as glucose, although arabinose, mannose, and rhamnose are also present. However, subsequently, Kusser and Fiedler (1983) demonstrated that the principal cell-wall sugar is galactose, with lesser amounts of *N*-acetylglucosamine, rhamnose, and *N*-acetyl-fucosamine. Methyl-branched fatty acids form over 92% of the total fatty acid component of the cells, with 12-methyltetradecanoic acid (C_{15}) and 14-methylhexadecanoic (C_{17}) as the major components (Embley et al., 1983). The principal respiratory quinones are unsaturated menaquinones with nine isoprene units (Collins, 1982). Straight chain fatty acids generally account for 1% of the total fatty acids, and unsaturated fatty acids are not detected at all. Over 81% of the total fatty acids are composed of the lower melting point anteiso acids, which may contribute to membrane fluidity at low temperatures. All strains contain diphosphatidylglycerol, two major and six or seven minor glycolipids and two unidentified minor phospholipids (Embley et al., 1983). Mycolic acids are absent (Fryer and Sanders, 1981).

Renibacterium salmoninarum is considered to be serologically homogeneous (Bullock et al., 1974; Getchell et al., 1985), although two serogroups have been subsequently described (Bandín et al., 1992).

Renibacterium salmoninarum is an obligate fish pathogen causing a disease known as bacterial kidney disease, Dee disease, and corynebacterial/salmonid kidney disease. The pathogen occurs intracellularly, especially in macrophages (McIntosh et al., 1997) and produces a slowly developing chronic infection characterized by the presence of gray-white enlarged necrotic abscesses primarily in the kidney (Fryer and Sanders, 1981; Wood and Yasutake, 1956). External signs include bilateral exophthalmia, melanosis, and abdominal distension with ascites. There may be blood filled blisters on the flank, and abscesses (Austin and Austin, 2007). Infections develop over a range of water temperatures (4–20.5°C; Sanders et al., 1978). The pathological changes in the fine structure of both the glomerulus and renal tubules of the kidney resemble those observed in mammalian glomerulonephritis and nephritic syndrome (Young and Chapman, 1978). *Renibacterium salmoninarum* is shed from fish feces, and survives in the aquatic environments for short periods of up to 1 week (Balfry et al., 1996).

By disk diffusion, cultures are sensitive to bacitracin, chloramphenicol, cycloserine, erythromycin, terramycin, novobiocin, streptomycin, nitrofurazone, and sulfonamides. *In vivo* tests have indicated that erythromycin gives the best results; however, when this compound is removed from diets the disease quickly reappears (Austin, 1985; Brown et al., 1990; Evelyn et al., 1986; Lee and Evelyn, 1994; Wolf and Dunbar, 1959).

Occurs among populations of mostly farmed and rarely wild salmonid fish in North and South America, Europe, Iceland, and Japan. Has been found mostly in members of the subfamily Salmoninae, i.e. salmon, trout, and char, of the family Salmonidae. There is one report of clinical disease in ayu (*Plecoglossus altivelis*; Nagai and Iida, 2002) and sea lamprey (*Petromyzon marinus*; Eissa et al., 2006).

DNA G+C content (mol%): 53±1.0 (T_m).

Type strain: Lea-1-74, ATCC 33209, CIP 103036, DSM 20767, NBRC15589, JCM 11484, LMG 10846.

Other strains: ATCC 33739 (81-10B-E-BK), NCIMB 1111 (SUND), NCIMB 1112 (CHAT), NCIMB 1113 (CRAW), NCIMB 1114 (1085), NCIMB 1115 (1086) and NCIMB 1116 (TS30).

Sequence accession no. (16S rRNA gene): AF093461 and AF180950.

Genus VIII. **Rothia** Georg and Brown 1967, 68[AL]

Brian Austin (updated from Mary Ann Gerencser and George H. Bowden, 1986)

Roth′i.a. N.L. fem. n. *Rothia* named for Genevieve D. Roth, who performed basic studies with these organisms.

Coccoid, spherical, **dipththeroid, filamentous** mycelial cells, usually up to 1.0 µm in diameter. Irregular swellings and clubbed ends up to 5.0 µm in diameter may be present. Growth may be exclusively coccoid, diphtheroid, or filamentous or a mixture of these forms. **Gram-stain-positive**. Nonmotile. Endospores are absent. Non acid-fast. Mature colonies (4–7 d) 2–6 mm in diameter, usually off-white/cream, smooth or rough, usually soft in texture but may be dry and crumbly or mucoid. **Optimum temperature for growth is 30–37°C.** Usually catalase-positive**. Chemo-organotrophic.** Ferments carbohydrates. **Major product of glucose fermentation is lactic acid. Does not produce propionic acid. The cell-wall peptidoglycan type is A3α, and contains alanine, glutamic acid, and lysine**, but not **diaminopimelic acid (DAP).** Contains **MK-7** as the major isoprenoid quinone. Cell-wall sugars include **fructose**, **galactose**, and **glucose**, but not **6-deoxy-talose** or **arabinose.**

DNA G+C content (mol%): 54–60 (T_m).

Type species: **Rothia dentocariosa** (Onishi 1949) Georg and Brown 1967, 68[AL].

Further descriptive information

Growth may consist entirely of coccoid, diphtheroidal, filamentous, or mycelial forms, but mixtures of these cell types are more common. Filamentous forms are seen in cultures grown on solid media whereas coccoid forms are more common in broth cultures. Mycelial forms are composed of branched filaments, which fragment into bacillary or coccoid forms (Daneshvar et al., 2004). Cells in 2- to 3-d-old broth cultures may be entirely coccoid, but it is difficult to maintain a culture in the coccoid form. Occasional diplo-coccoid forms occur (Daneshvar et al., 2004).

Lai and Listgarten (1980) described the cell wall as a moderately electron-dense layer, 19–32 nm thick. Multiple cleavage planes parallel to each other and to the cell surface gave the impression of a multilayered cell wall. Markedly thickened segments of cell-wall material were seen also. No periplasmic space was observed. When grown on blood agar, the contour of the wall was rough. Neither long nor short surface fuzz or other structures were present outside the cell wall. Mesosomes or intracellular membrane intrusions and electron-dense-cytoplasmic inclusions were present. Nucleoids were seen in cells grown in broth, but not on agar. No variation in the wall structure at the ultrastructural level was found in the various morphological types.

Young, i.e. 18–24 h, colonies grown anaerobically are microscopic and usually highly filamentous, frequently resembling the "spider" colonies of *Actinomyces*. Young colonies grown aerobically are larger, i.e. ≥1 mm in diameter, smooth or granular, and may or may not have a fringed border.

Older colonies, i.e. 4–7 d old, may be smooth and convex, or have rough, highly convoluted (folded) but glistening surfaces. The texture may be creamy, dry, crumbly, or mucoid.

Rothia grows well on brain heart infusion agar (BHIA), tryptone soya agar (TSA) and on nutrient agar. The best growth occurs aerobically at 30–37°C, but some growth occurs anaerobically in an atmosphere of N_2:H_2:CO_2 (80:10:10). Little or no growth is obtained on media with inorganic nitrogen, such as Czapek. Citrate does not serve as the sole source of carbon for energy and growth.

Rothia has a fermentative metabolism, producing mainly lactic and acetic acid from glucose. The lactate dehydrogenase (LDH) produced by *Rothia* is activated by fructose 1,6-diphosphate, and inhibited by adenosine 5′-triphosphate, and thus resembles the LDH of *Actinomyces viscosus* (Eisenberg et al., 1976).

Enrichment and isolation procedures

The culture media recommended for primary isolation and maintenance are BHIA and TSA with incubation aerobically at 37°C for 2–7 d. *Rothia amarae* and *Rothia terrae* grow well in Luria–Bertani broth. Selective media have been described for the isolation of *Rothia* (Beighton, 1976; Beighton and Colman, 1976; Ritz, 1966).

Pathogenicity. Rothias, notably *Rothia dentocariosa* and *Rothia mucilaginosa*, are commonly found in the human oral cavity and the pharynx, respectively (Collins et al., 2000; Schaal, 1992; Yang et al., 2007). Although regarded as having low virulence to humans particularly children (Yang et al., 2007), *Rothia dentocariosa* is associated with dental plaque and periodontal disease (Binder et al., 1997; Yang et al., 2007) and opportunistic diseases, i.e. endocarditis, endophthalmitis, peritonitis, septicemias, and other serious infections including those of immunocompromised and intensive care patients (Bibashi et al., 1999; Binder et al., 1997; Boudewijns et al., 2003; Ergin et al., 2000; Ferraz et al., 1998; Kong et al., 1998; Lee et al., 2008; Martínez-Martínez, 1998; Minato and Abiko, 1984; Nguyen et al., 2000; Pape et al., 1979; Pers et al., 1987; Salamon and Prag, 2002; Schafer et al., 1979; Schiff and Kaplan, 1987; Vaccher et al., 2007). There is evidence that *Rothia dentocariosa* occurs in infections which may lead to complications or be associated with other medical conditions, for example, pneumonia in an immunocompromised patient with lung cancer (Wallet et al., 1997), vertebral osteomyelitis complicating endocarditis (Llopis and Carratala, 2000), endocarditis in a child with congenital heart disease (Braden et al., 1999), endocarditis in patients with periodontal disease and endocarditis, multiple brain abscesses and severe heart failure in another patient with periodontal disease (Binder et al., 1997), endocarditis in association with multiple cranial hemorrhages (Ricaurte et al., 2001), and renal disease (Habib et al., 1999). In some cases, oral surgery is associated with infection, for example, *Rothia dentocariosa* was recovered from a patient with endocarditis complicated by multiple cerebellar hemorrhages (Sadhu et al., 2005). Also, *Rothia dentocariosa* has been recovered from a child with endophthalmitis (Partner et al., 2006) and a child having a corneal ulcer and the oropharynx was thought to be the source of infection (Morley and Tuft, 2006). In the latter case, the organism in saliva may have been transferred via fingers (Morley and Tuft, 2006).

Other pediatric infections involving *Rothia dentocariosa* include severe acute tonsillitis characterized by a thick white membrane covering the tonsils, leukocytosis, and elevated C-protein level (Ohashi et al., 2005), septicemia in the absence of endocarditis in a neonatal infant with meconium aspiration syndrome (Shin et al., 2004), and sepsis in a renal transplant patient (Wiesmayr et al., 2006). *Rothia dentocariosa* isolates forming charcoal-black pigmented colonies have been recovered from the female genitourinary tract (Daneshvar et al., 2004).

Rothia mucilaginosa has been reported as an opportunistic cause of meningitis leading to death in seriously immunocompromised pediatric patients with malignant tumors or undergoing hematopoietic stem cell transplantation (Lee et al., 2008). Also, the organism has been associated with bacteremia in patients with Shwachman-Diamond syndrome and cancer/leukemia (Fanourgiakis et al., 2003; Paci et al., 2000; Vaccher et al., 2007), with lower respiratory tract infections including pneumonia and recurrent lung abscesses (Korsholm et al., 2007) and AIDS (Cunniffe et al., 1994). It has been associated with meningitis/central nervous system infections (Goldman et al., 1998; Skogen et al., 2001) in immunocompromised patients with cancer and immunocompromised recipients of hematopoietic stem cell transplants (Goldman et al., 1998; Lee et al., 2008).

Abscess formation has been documented in experimental studies in mice (Miksza-Zylkiewicz and Linda, 1980; Roth and Flanagan, 1969; Scharfen, 1975).

Maintenance procedures

Cultures on BHIA or TSA slopes remain viable for 3–4 months at room temperature. For long-term storage, suspensions should be lyophilized.

Procedures for testing special characters

Microcolonies of *Rothia* are best demonstrated by dropping a sterile coverslip over a portion of the inoculum on a BHIA plate with incubation aerobically at 35°C for 18–24 h. Microcolonies will be filamentous under the coverslip, and smooth outside the coverslip (Roth and Thurn, 1962).

In general, methods described for testing biochemical characteristics of *Actinomyces* spp. are suitable for *Rothia* except that aerobic incubation is important. The catalase test is best performed by flooding growth on slopes with 3.0% H_2O_2, and observing for effervescence (a positive test). Anaerobic cultures need to be exposed to air for 15–30 min before testing.

A satisfactory fermentation base medium is 3 g of meat extract, 10 g of peptone, 5 g of NaCl, 10 ml of Andrade's indicator, and 1000 ml of distilled water. The oxidative-fermentative test (Hugh and Leifson, 1953) is useful for differentiating *Rothia* from *Nocardia*, which oxidizes or does not utilize glucose in this medium. Degradation of gelatin may be detected and evaluated in gelatin agar (BHIA supplemented with 4 g of gelatin/l). After 7 d of incubation, the plates are flooded with saturated ammonium sulfate, and observed for the development of clear zones around the bacterial growth. For chemotaxonomic analysis, *Rothia* is best grown in bulk in tryptone soya broth or nutrient broth on a shaker.

Differentiation of the genus *Rothia* from other genera

Rothia may be differentiated from all other genera in the family *Micrococcaceae* by the G+C content of the DNA and the menaquinone content (see Table 100 and Table 102). The distinguishing traits between *Rothia* spp. have been included in Table 103.

Taxonomic comments

The genus *Rothia* was created to accommodate organisms originally regarded as *Nocardia* (Davis and Freer, 1960; Roth, 1957; Roth and Thurn, 1962) and assigned to the family *Actinomycetaceae* (Roth, 1957; Schaal, 1992). These organisms are normal inhabitants of the mouth and throat. On the basis of molecular studies, the genus was reassigned to the family *Micrococcaceae* (Stackebrandt et al., 1997). *Stomatococcus mucilaginosus* (Bergan and Kocur, 1982) was reclassified as *Rothia mucilaginosa* after 16S rRNA gene sequencing and whole-cell protein analysis (Collins et al., 2000). *Rothia nasimurium* was described to accommodate isolates from the nose of a mouse (Collins et al., 2000); *Rothia aeria* was found in the air from the Russian space station, Mir (Li et al., 2004c). *Rothia amarae* was recovered from sludge (Fan et al., 2002) and *Rothia terrae* was isolated from soil (Chou et al., 2008). *Rothia* resembles *Actinomyces* morphologically but grows better aerobically. Only one study (Schofield and Schaal, 1981) reported stimulation of growth by CO_2.

TABLE 103. Differential characteristics of *Rothia* spp.[a]

Characteristic	1. *R. dentocariosa*	2. *R. amarae*	3. *R. aeria*	4. *R. mucilaginosa*	5. *R. nasimurium*	6. *R. terrae*
Production of:						
Alkaline phosphatase	–	–	–	+	–	+
Catalase	+	+	+	–	+	+
β-Glucosidase	+	–	+	+	–	+
Trypsin	–	–	?	–	+	–
Acid production from:						
Ribose	+	+	–	–	–	–

[a]Based on Chou et al. (2008).

List of species of the genus *Rothia*

1. **Rothia dentocariosa** (Onishi 1949) Georg and Brown 1967, 68[AL] (*Actinomyces dentocariosa* Onishi 1949, 282)

den.to.car.i.o′sa. L. n. *dens, dentis* tooth; L. adj. *cariosus -a -um* decayed or decaying; N.L. fem. adj. *dentocariosa* tooth-decaying.

Produces off white/cream colonies of 2–6 mm in diameter after 4–7 d. Cells (usually 1.0 μm in diameter) are nonmotile, Gram-stain-positive, coccoid, diphtheroid, filamentous, or mycelial. In addition, isolates form charcoal-black pigmented colonies (Daneshvar et al., 2004).

Aerobic to microaerophilic. No or very poor growth anaerobically. CO_2 is not required for growth. Possesses a fermentative metabolism. Optimum growth at 35–37°C. Catalase variable. Produces acetic and lactic acid, trace amounts of pyruvic and succinic acids, but no butyric, caproic, isovaleric, or propionic acid from glucose fermentation. Reduces nitrate and nitrite. The results of indole production are contradictory. The Voges–Proskauer reaction is positive. Produces acid from fructose, glucose, maltose, salicin, sucrose, and trehalose but not adonitol, amygdalin, cellobiose, dulcitol, erythritol, glycogen, inositol, inulin, mannitol, α-methyl-D-mannoside, melibiose, raffinose, sorbitol, starch, or xylose. Produces leucine aminopeptidase, trypsin, and α-glucosidase, but not alkaline phosphatase, chemotrypsin, α- and β-galactosidase, β-glucoronidase, lipase (C_{14}), α-mannosidase, phosphoamidase, or β-xylosidase. Degrades esculin and usually gelatin, but not albumin, casein, starch, or urea. No hemolysis on human and sheep blood, but variable hemolysis on rabbit blood. Grows in 2% (w/v) sodium chloride, 5% and 10% (w/v) bile, 0.01% (w/v) potassium tellurite, 0.005% (w/v) sodium azide, 0.01% (w/v) sodium selenite, and 0.2% (w/v) sodium taurocholate but not 6% (w/v) sodium chloride and 0.05% (w/v) crystal violet (Holmberg and Hallander, 1973; Kilian, 1978; Miksza-Zylkiewicz, 1980; Schofield and Schaal, 1981; Slack and Gerenscer, 1975).

Characterized by the presence of iso- even-numbered fatty acids and only anteiso- odd-numbered fatty acids. Specifically, $C_{15:0}$ anteiso, C_{16}; $C_{16:0}$, and $C_{17:0}$ anteiso fatty acids are present with only trace amounts of other fatty acids. The peptidoglycan is of the A3α type with D-alanine and L-lysine in position 4 and 3, respectively and L-alanine in the interpeptide bridge (Von Graevenitz, 2004). The polar lipids are mono- and di-phosphatidylglycerol and the major isoprenoid quinone is MK-7. The cell wall contains fructose, galactose, glucose, and ribose (Embley et al., 1984; Hammond, 1970a; Von Graevenitz, 1999).

Heterogeneity has been recognized within the species on the basis of phenotype and serotyping (Lesher et al., 1974), pyrolysis mass spectrometry (Sutcliffe et al., 1997), and 16S rRNA gene sequencing (Kronvall et al., 1998), with the resulting delineation of two phenotypically indistinguishable genomovars that do not correspond to biotypes (Kronvall et al., 1998); the second of these genomovars was elevated to a new species, *Rothia aeria*.

Using antiserum to killed whole cells of *Rothia* for serotyping by immunofluorescence, *Rothia* was separated into at least three serovars. However, some isolates failed to react with any of the available antisera (Lesher et al., 1974). This suggests the existence of additional serovars.

Hammond (1970a) isolated a soluble polysaccharide antigen from the cell wall with a major antigenic determinant containing fructose, glucose, galactose, and ribose. This antigen was found in all strains of *Rothia* tested, and may account for the cross-reactions seen between serovars.

The G+C content of the DNA reported by Hammond (1970b) was questioned by Gustafson et al. (1985), who determined the value for the type strain ATCC 17931 as 53.7–54.7 ±0.43 mol%, with a slightly different result for ATCC 19426. The compromise suggested by Gustafson et al. (1985) was a value for the species of 54–57 mol%.

DNA G+C content (mol%): 54–57 (T_m).

Type strain: ATCC 17931, CCUG 35437, CIP 81.83, DSM 43762, DSM 46363, NBRC 12531, JCM 3067, NCTC 10917, NRRL B-8017.

Sequence accession no. (16S rRNA gene): M59055.

2. **Rothia aeria** Li, Kawamura, Fujiwara, Naka, Liu, Huang, Kobayashi and Ezaki 2004c, 833[VP]

a.e′ri.a. L. fem. adj. *aeria* of the air, referring to the isolation of the type strain from the air in the Russian Mir space station.

Young colonies are creamy white and smooth and become rough, dry, folded, convex, and adherent to the agar surface with age. Cells are Gram-stain-positive coccoid, coccobacillary, or filamentous. Growth occurs on BHIA at 30°C. Catalase-positive. Does not produce alkaline phosphatase. Utilizes arbutin, dextrin, D-fructose, α-hydroxybutyric acid, L-lactic acid, maltose, maltotriose, D-mannose, D-melezitose, 3-methyl glucose, glycerol, methyl-α-D-glucoside, α-D-glucose, methyl-β-D-glucoside, palatinose, D-psicose, salicin, sucrose, D-trehalose, and turanose but not acetic acid, *N*-acetyl D-glucosamine, *N*-acetyl L-glutamic acid, *N*-acetyl D-mannosamine, adenosine, adenosine 5′-monophosphate, alaninamide, L- or D-alanine, L-alanyl glycine, amygdalin, L-arabinose, D-arabitol, L-asparagine, 2,3-butanediol, cellobiose, α- and β-cyclodextrin, 2′-deoxyadenosine, L-fructose, fructose 6-phosphate, D-galactose, D-galacturonic acid, gentiobiose, D-gluconic acid, glucose 1-phosphate, glucose 6-phosphate, L-glutamic acid, DL-α-glycerol phosphate, glycogen, glycyl L-glutamic acid, β-hydroxybutyric acid, γ-hydroxybutyric acid, *p*-hydroxyphenyl acetic acid, *m*-inositol, inosine, inulin, α-ketoglutaric acid, α-ketovaleric acid, D-lactic acid methyl ester, lactamide, α-D-lactose, lactulose, D- and L-malic acid, D-mannitol, D-melibiose, methyl α-D-galactoside, methyl-β-D-galactoside, methyl-α-D-mannoside, methyl pyruvate, mannan, monomethyl succinate, propionic acid, L-pyroglutamic acid, pyruvic acid, putrescine, D-raffinose, L-rhamnose, D-ribose, sedoheptulosan, L-serine, D-sorbitol, stachyose, succinamic acid, succinic acid, D-tagatose, thymidine, thymidine 5′-monophosphate, Tween 40 or 80, uridine, uridine 5′-monophosphate, xylitol, or D-xylose (Li et al., 2004c).

The cell-wall peptidoglycan is the A3α type. The predominant isoprenoid quinone is MK-7. The major cellular fatty acids are $C_{15:0}$ anteiso, $C_{16:0}$ iso, and $C_{17:0}$ anteiso. The major polar lipids are phosphatidylglycerol and cardiolipin (Li et al., 2004c).

The species includes cultures formerly recognized as *Rothia dentocariosa* genomovar II.

The 16S rDNA sequence similarities with *Rothia dentocariosa*, *Rothia mucilaginosa*, *Rothia amarae*, and *Rothia nasimurium* were 98.0, 96.4, 96.0, and 95.4%, respectively.

Source (type strain): air in the Russian space laboratory, Mir.
DNA G+C content (mol%): 57 (T_m).
Type strain: A1-17B, DSM 14556, JCM 11412, GTC 867.
Sequence accession no. (16S rRNA gene): AB071952.

3. **Rothia amarae** Fan, Jin, Tong, Li, Pasciak, Gamian, Liu and Huang 2002, 2259[VP]

a.ma′ra.e. Gr. n. *amara* trench, conduit, channel; here a sewage duct; N.L. gen. n. *amarae* of a sewage duct.

Produces cream, mucoid, smooth, convex colonies, which roughen with age. Cells are nonmotile, Gram-stain-positive, and ovoid to spherical (0.6–0.9 μm in diameter) and occur either singly, in pairs, tetrads, or packets. Facultatively anaerobic; grows well aerobically, but only poorly anaerobically. Grows optimally at 30–37°C, and not at all at 15 and 45°C. Catalase-positive. Reduces nitrate to nitrite. Produces acid from glucose, glycerol, maltose, mannose, ribose, salicin, sucrose, and trehalose but not lactose, mannitol, raffinose, or sorbitol. Produces ester lipase C8 and valine arylamidase both weakly, but not phosphatase, trypsin, and urease. Degrades esculin and gelatin (Fan et al., 2002). The cell-wall peptidoglycan type is A3α. Phosphatidylglycerol and diphosphatidylglycerol are the main phospholipids and phosphatidylinositol is a minor component. The major isoprenoid quinones are MK-6(H_2) and MK-7. The major cellular fatty acid is $C_{15:0}$ anteiso (Fan et al., 2002). The 16S rDNA sequence similarities with *Rothia dentocariosa*, *Rothia mucilaginosa*, and *Rothia nasimurium* were 96.0, 96.7, and 97.3%, respectively.

Source: sludge (regarded as deodorized dung and sewage) in a foul water sewer in China.
DNA G+C content (mol%): 54.5 (T_m).
Type strain: J18, JCM 11375, AS 4.1721, CCUG 47294.
Sequence accession no. (16S rRNA gene): AY043359.

4. **Rothia mucilaginosa** (Bergan and Kocur 1982) Collins, Hutson, Båverud and Falsen 2000, 1250[VP] (*Stomatococcus mucilaginosus* Bergan and Kocur 1982, 374)

mu.ci.la.gi.no′sa. L. fem. adj. *mucilaginosa* slimy.

Colonies (1–1.5 mm in diameter) are mucoid, transparent or whitish, adherent, convex, non-hemolytic with entire edges on blood agar. Cells are Gram-stain-positive, encapsulated, nonmotile, facultatively anaerobic, non-sporeforming cocci (0.9–1.3 μm in diameter) and are arranged mostly in clusters and occasionally in pairs and tetrads. Growth occurs optimally at 30–37°C but not at all on nutrient agar supplemented with 5% (w/v) NaCl. Catalase is weakly positive or negative. Acid is produced from fructose, glucose, glycerol, maltose, mannose, sucrose, salicin and trehalose, but not from adonitol, lactose, mannitol, raffinose, sorbitol, or xylose. Simmons' citrate is not utilized. The Voges–Proskauer reaction is positive. Nitrates are reduced to nitrite. Esculin and gelatin are degraded, but not DNA, starch, Tween 80, tyrosine, or urea. Arginine, H_2S, indole, coagulase, lysine and ornithine decarboxylase, oxidase, and phosphatase are not produced (Bergan and Kocur, 1982; Stackebrandt et al., 1983a). The dominant fatty acids are C_{15} and C_{16}. The cell wall contains peptidoglycan of the L-Lys–L-Ser$_2$–D-Glu type. Mannose containing lipoglycan is present rather than lipoteichoic acid (Sutcliffe and Old, 1995). The major glycolipid is dimannosyl acylmonoglyceride (Pasciak et al., 2004).

Source: oral cavity, pharynx, and upper respiratory tract of humans.
DNA G+C content (mol%): 56–60.4 (T_m).
Type strain: CCUG 20962, ATCC 25296, NCTC 10663, CCM 2417, CIP 71.14, DSM 20746, NBRC 15673, JCM 10910.
Sequence accession no. (16S rRNA gene): X87758.

5. **Rothia nasimurium** Collins, Hutson, Båverud and Falsen 2000, 1250[VP]

na.si.mu′ri.um. L. n. *nasus* nose; L. gen. pl. n. *murium* of mice; N.L. pl. gen. n. *nasimurium* of the nose of mice.

Growth occurs aerobically and in 5% CO_2 on Columbia blood agar base supplemented with 5% (v/v) defibrinated horse blood at 37°C. Colonies are non-pigmented and cells are facultatively anaerobic, Gram-stain-positive, nonmotile, and ovoid. Catalase-positive. Weakly α-hemolytic, and degrades esculin but not sodium hippurate. Reduces nitrate. The Voges–Proskauer reaction is negative. Produces acid from glucose, lactose, maltose, methyl-β-D-glucopyranoside, sucrose, and trehalose, but not from L-arabinose, D-arabitol, cyclodextrin, glycogen, mannitol, melibiose, melezitose, *N*-acetylglucosamine, pullulan, raffinose, ribose, sorbitol, tagatose, or D-xylose. Produces alanine-phenylalanine-proline arylamidase, cystine arylamidase, ester lipase C8, β-galacturonidase, α-glucosidase, leucine arylamidase, valine arylamidase, pyrazinamidase, and trypsin, but not alkaline phosphatase, arginine dihydrolase, esterase C4, α-fucosidase, α-galactosidase, β-glucosidase, β-glucuronidase, lipase C14, α- and β-mannosidase, or urease (Collins et al., 2000). The main cellular fatty acids are iso- and anteiso-methyl branched types with $C_{15:0}$ predominating. The 16S rRNA gene sequence revealed 96.6% similarity to both *Rothia dentocariosa* and *Rothia mucilaginosa*.

Source: the nose of a healthy mouse.
DNA G+C content (mol%): 56 (T_m).
Type strain: CCUG 35957, CIP 106912, JCM 10909.
Sequence accession no. (16S rRNA gene): AJ131121.

6. **Rothia terrae** Chou, Chou, Lin, Lin, Wei, Arun, Young and Chen 2008, 87[VP]

ter′ra.e. L. gen. n. *terrae* of the earth, i.e. the organism was isolated from soil.

Colonies are white/cream colored. Cells are aerobic, spherical to ovoid (0.5–1.5 × 0.5–1.0 μm) and occur singly, in pairs, or tetrads. Fermentative, with poor growth occurring in anaerobic conditions. Growth occurs at 15–40°C, optimally at 35°C, in 0–7% (w/v) NaCl (optimally in 0–0.5% [w/v] NaCl) and at pH 7.0–8.0. Poly-β-hydroxybutyrate granules do not accumulate intracellularly. Catalase-positive. The Voges–Proskauer reaction is positive. Nitrate is reduced. H_2S is not produced. Esculin and gelatin are hydrolyzed, but not casein, starch, Tween 20, 40, 60, or 80, or urea. Produces alkaline phosphatase, esterase C4, β-galactosidase, α- and β-glucosidase, leucine arylamidase, lipase C8, naphthol-AS-B1-phosphohydrolase, and pyrazinamidase but not *N*-acetyl-β-glucoaminidase, acid phosphatase, α-chemotrypsin, α-fucosidase, α-galactosidase, β-glucuronidase, cysteine arylamidase, lipase C14, α-mannosidase, pyrrolidonyl arylamidase, trypsin, or valine arylamidase. Acid is produced from fructose, glucose, glycerol, lactose, maltose, mannose, sucrose, and trehalose but not adonitol, glycogen, mannitol, ribose, salicin, or xylose. Glycerol, 3-methylglucose, D-psicose, L-lactic acid, and methylpyruvate are

oxidized, but 2,3-butanediol, DL-α-glycerol phosphate, α-hydroxybutyric acid, malic acid, methyl-α-D-glucoside, methyl-β-D-glucoside, monomethyl succinate, pyruvic acid, salicin, and succinic acid are not. Dextrin, D-fructose, α-D-glucose, glycerol, glycogen, maltose, D-mannose, 3-methyl-D-glucose, L-lactic acid, D-lactic acid methyl ester, D-psicose, pyruvic acid methyl acid, L-serine, sucrose, D-trehalose, and turanose are utilized in the Biolog GP2 system, but not acetic acid, *N*-acetyl-D-glucosamine, *N*-acetyl-L-glutamic acid, *N*-acetyl-β-D-mannosamine, adenosine, adenosine-5′-monophosphate, L-alaninamide, D- and L-alanine, L-alanylglycine, amygdalin, L-arabinose, D-arabitol, arbutin, L-asparagine, 2,3- butanediol, D-cellobiose, α- or β-cyclodextrin, 2′-deoxyadenosine, D-fructose 6-phosphate, L-fucose, D-galactose, D-galacturonic acid, gentiobiose, α-D-glucose 1-phosphate, α-D-glucose 6-phosphate, L-glutamic acid, DL-α-glycerol phosphate, glycyl-L-glutamic acid, α-, γ-and β-hydroxybutyric acid, *p*-hydroxyphenylacetic acid, inosine, *myo*-inositol, inulin, α-ketoglutaric acid, α-ketovaleric acid, lactamide, α-D-lactose, lactulose, L- and D-malic acid, maltotriose, mannan, D-mannitol, D-melezitose, D-melibiose, α-and β-methyl-D-galactoside, α- and β-methyl-D-glucoside, α-methyl-D-mannoside, palatinose, propionic acid, L-pyroglutamic acid, putrescine, pyruvic acid, D-raffinose, L-rhamnose, D-ribose, salicin, sedoheptulosan, D-sorbitol, stachyose, succinamic acid, succinic acid, succinic acid monomethyl ester, D-tagatose, thymidine, thymidine-5′-monophosphate, Tween 40 and 80, uridine, uridine-5′-monophosphate, xylitol, or D-xylose. Resistant to gentamicin, kanamycin, and nalidixic acid, but not to ampicillin, chloramphenicol, erythromycin, novobiocin, penicillin G, rifampin, streptomycin, or tetracycline (Chou et al., 2008). The peptidoglycan type is A3α and contains alanine, glutamic acid, and lysine. Phosphatidylglycerol and diphosphatidylglycerol are the main phospholipids and some unknown glyco- and phospholipids are minor components. The predominant fatty acids are $C_{15:0}$ anteiso, $C_{17:0}$ anteiso, and $C_{16:0}$. The major isoprenoid quinone is MK-7.

Source: soil in Taiwan.

DNA G+C content (mol%): 56.1 ± 0.5 (T_m).

Type strain: L-143, BCRC 17588, LMG 23708.

Sequence accession no. (16S rRNA gene): DQ822568.

Genus IX. **Yaniella** (Li, Chen, Xu, Zhang, Schumann, Tang, Xu and Jiang 2004a) Li, Zhi and Euzéby 2008b, 526[VP] (Illegitimate homotypic synonym: *Yania* Li, Chen, Xu, Zhang, Schumann, Tang, Xu and Jiang 2004a, 529)

Peter Schumann

Ya.ni.el′la. N.L. fem. dim. n. *Yaniella* named after Sun-Chu Yan (1912–1994), a Chinese microbiologist who devoted his life to the study of actinomycete taxonomy and antibiotics.

Cells are nonmotile, aerobic, Gram-stain-positive, nonsporeforming, coccoid or oval, about 0.4–0.8 µm in diameter, and occur singly or in clusters. Moderately **halophilic or halotolerant**. **Oxidase negative** and **catalase positive**. Cell-wall peptidoglycan **type** is **A4α**, **L-Lys←Gly←L-Glu**. Whole-cell sugars are xylose, mannose, and galactose. Phospholipids are diphosphatidylglycerol, phosphatidylglycerol, one unknown phospholipid, and one unknown glycolipid. Predominant **menaquinones** are **MK-8 and MK-9 or MK-8**. Major cellular fatty acids are $C_{15:0}$ anteiso and $C_{15:0}$ iso or $C_{15:0}$ anteiso. The description is based on those of the species *Yaniella halotolerans* (Li et al., 2004a) and *Yaniella flava* (Li et al., 2005c).

DNA G+C content (mol%): 53–58.

Type species: **Yaniella halotolerans** (Li, Chen, Xu, Zhang, Schumann, Tang, Xu and Jiang 2004a) Li, Zhi and Euzéby 2008b, 526[VP] (*Yania halotolerans* Li, Chen, Xu, Zhang, Schumann, Tang, Xu and Jiang 2004a, 530).

Further descriptive information

The genus *Yaniella* was established to harbor two isolates from geographically distant saline habitats in China which showed less than 94.3% 16S rRNA gene sequence similarity to their phylogenetic neighbors (Li et al., 2004a, 2005c). Due to the low 16S rRNA gene sequence similarity to other members of the suborder *Micrococcineae*, the family *Yaniellaceae* with *Yaniella* as type genus was proposed by Li et al. (2005c). A phylogenetic study of type species of all genera of the suborder *Micrococcineae* (subsequently expanded and elevated to order *Micrococcales* in the taxonomic outline to the present volume) revealed that the genus *Nesterenkonia* in the family *Micrococcaceae* was the closest phylogenetic neighbor of the genus *Yaniella* (Figure 140, *Nesterenkonia* chapter, above). The type strains of *Yaniella halotolerans* and *Yaniella flava* show 98.4% 16S rRNA gene sequence similarity at a bootstrap value of 100%. Their DNA–DNA similarity is only 35.4%, and both type strains can be differentiated by a set of species-specific phenotypic properties (Li et al., 2005c; Table 104). The cells of both type strains are Gram-stain-positive nonmotile cocci. The peptidoglycan type is A4α, L-Lys←Gly←L-Glu (A11.56 according to http://www.peptidoglycan-types.info). The menaquinones MK-8 (83%), MK-7 (12%), and MK-9 (1.5%) occur in *Yaniella halotolerans* while *Yaniella flava* contains the menaquinones MK-8, MK-9, and MK-7 in the ratio 64:29:1. The cellular fatty acid profile of *Yaniella halotolerans* consists of $C_{15:0}$ iso (44.3%), $C_{15:0}$ anteiso (35.6%), $C_{17:0}$ anteiso (9.7%), $C_{15:0}$ (0.3%), $C_{16:0}$ (0.9%), $C_{14:0}$ iso (1.5%), $C_{16:0}$ iso (3.3%), $C_{17:0}$ iso (2.8%), $C_{19:0}$ anteiso (0.4%), $C_{16:1}$ ω7*c* (0.5%), and $C_{15:1}$ iso (0.7%), while that of *Yaniella flava* contained $C_{15:0}$ anteiso (58.2%), $C_{14:0}$ iso (12.5%), $C_{15:1}$ iso (9.3%), $C_{16:0}$ iso (8.0%), $C_{17:0}$ anteiso (5.8%), $C_{13:0}$ iso (2.1%), $C_{17:0}$ iso (1.9%), $C_{16:0}$ (0.6%), $C_{15:1}$ anteiso (0.5%), $C_{13:0}$ anteiso (0.4%), $C_{16:1}$ ω7*c* (0.3%), $C_{18:1}$ ω9*c* (0.3%), and $C_{14:0}$ (0.1%). The polar lipid pattern of *Yaniella halotolerans* contained traces of phosphatidylinositol in addition to the pattern consisting of diphosphatidylglycerol, phosphatidylglycerol, one unknown phospholipid, and one unknown glycolipid shared by both species. The colonies of strains YIM 70085[T] and YIM 70178[T] on ISP5 agar supplemented with 10% KCl (w/v) are light yellow, circular, lubricous, and opaque. Both type strains grow optimally at pH 7.0–8.0 and at salt concentrations of 10–15% KCl, NaCl, or $MgCl_2{\cdot}6H_2O$ under aerobic conditions.

Enrichment and isolation procedures

Yaniella halotolerans YIM 70085[T] was isolated by using the dilution plating method from a saline soil sample from Xinjiang

TABLE 104. Characteristics differentiating *Yaniella halotolerans* YIM 70085[T] and *Yaniella flava* YIM 70178[T,a,b]

Characteristic	*Y. halotolerans* strain YIM 70085[T]	*Y. flava* strain YIM 70085[T]
Optimal concentration of KCl for growth (% w/v)	10	10–15
Range of salt concentrations for growth (% w/v):		
KCl	0–20	0.5–30
$MgCl_2 \cdot 6H_2O$	0–15	0.5–30
NaCl	0–25	0.5–25
pH Range for growth	6.5–8.5	6.0–9.0
Utilization of sucrose	+	−
Enzyme activities:		
N-Acetyl-glucosaminidase	−	+
β-Galactosidase	−	+
β-Glucosidase	+	−
α-Maltosidase	+	−
Urease	+	−
Major menaquinones (>25%)	MK-8	MK-8 and MK-9
Polar lipids	DPG, PI, PG, PL and GL	DPG, PG, PL and GL
Major fatty acids (>10%)	$C_{15:0}$ iso, $C_{15:0}$ anteiso	$C_{15:0}$ anteiso
DNA G+C content (mol%)	53.5	57.9

[a]Symbols: +, Positive; −, negative; DPG, diphosphatidylglycerol; PG, phosphatidylglycerol; PI, phosphatidylinositol; PL, unidentified phospholipid; GL, unidentified glycolipid.

[b]Data from Li et al. (2005c).

TABLE 105. Differential chemotaxonomic characteristics of the genus *Zhihengliuella* and related genera of the family *Micrococcaceae*[a]

Characteristic	*Zhihengliuella*[b]	*Micrococcus*[c]	*Arthrobacter globiformis* group[d]	*Arthrobacter nicotianae* group[e]	*Arthrobacter albus/cumminsii* group[f]	*Citricoccus*[g]	*Acaricomes*[h]
Interpeptide bridge	L-Ala–L-Glu	Peptide subunit or D-Asp	MCA_{var} or D-Asp	Ala–Glu or Glu	L-Ala–L-Glu or L-Ser–L-Glu	Gly–Glu	Ala_3
Cell-wall sugars[i]	Tyv, (Glc, Man)	Gal or Man	Gal, (Glc, Rha, Man, Xyl) or GlcN	Glc, (Gal)			Gal, Glc
Predominant menaquinone(s)	MK-9, MK-10	MK-8, MK-8(H_2) or MK-8(H_2) or MK-7(H_2), MK-8(H_2)	MK-8(H_2), 9(H_2) or MK-9(H_2) or MK-8(H_2)	MK-8, MK-9, MK-10 or MK-8, MK-9 or MK-9, MK-10 or MK-9, MK-10, MK-11	MK-8(H_2)	MK-9(H_2)	MK-10(H_2) MMK-10(H_2)
Polar lipids	DPG, PI, PG, PL, GL(s)	DPG, PI, PG, PL, GL	DPG, PG, PI, DMDG	DPG, PG, PI, DMDG		DPG, PI, PG, PL, GL	DPG, PG, PI
Major fatty acid(s)[i]	$C_{15:0}$ anteiso, ($C_{15:0}$ iso, $C_{17:0}$ anteiso)	$C_{15:0}$ anteiso, $C_{15:0}$ iso	$C_{15:0}$ anteiso, ($C_{15:0}$ iso, $C_{16:0}$ iso, $C_{17:0}$ anteiso)	$C_{15:0}$ anteiso, ($C_{15:0}$ iso, $C_{16:0}$ iso, $C_{16:0}$, $C_{17:0}$ anteiso)	$C_{15:0}$ anteiso, ($C_{16:0}$ iso, $C_{16:0}$, $C_{17:0}$ anteiso)	$C_{15:0}$ anteiso	$C_{15:0}$ anteiso, $C_{17:0}$ anteiso
DNA G+C content (mol%)	66.5–70.3	66–76	59–70	55–66	60–62	63–68	57.7

[a]Abbreviations: L-Ala, L-alanine; L-Glu, L-glutamic acid; D-Asp, D-aspartic acid; MCAvar, MCA_{var}, Variable monocarboxylic amino acid; L-Ser, L-serine; Gly, glycine; Ara, arabinose; Fru, fructose; Gal, galactose; Glc, glucose; GlcN, glucosamine; Man, mannose; Rha, rhamnose; Rib, ribose, Tyv, tyvelose; Xyl, xylose; MMK, methylmenaquinone; DMDG, dimannosyldiacylglycerol; DPG, diphosphatidylglycerol; GL, unidentified glycolipid; PG, phosphatidylglycerol; PI, phosphatidylinositol; PL, unidentified phospholipid.

[b]Data from Zhang et al. (2007) and Tang et al. (2009).

[c]Data from Stackebrandt and Schumann (2000a), Liu et al. (2000), and Wieser et al. (2002).

[d]*Arthrobacter globiformis* group is defined here as containing predominantly dihydrogenated menaquinones and a peptidoglycan with monocarboxyl amino acids only in the interpeptide bridge (peptidoglycan type A3α) or with a D-aspartate. The definition of this group is not in line with that given by Keddie et al., (1986) for the *Arthrobacter globiformis/citreus* group. Data from Keddie et al. (1986), Kodama et al. (1992), Koch et al. (1995), Stackebrandt et al. (1995), Funke et al. (1996), and Heyrman et al. (2005).

[e]*Arthrobacter nicotianae* group is defined here as containing only quinones with unsaturated isoprenoid units in the side chain and a peptidoglycan with a dicarboxyl amino acid (glutamic acid) in the interpeptide bridge (peptidoglycan type A4α). The definition of this group is not in line with that given by Keddie et al., (1986) for *Arthrobacter nicotianae* group. Data from Collins and Kroppenstedt (1983), Keddie et al. (1986), Koch et al. (1995), Stackebrandt et al. (1995), Funke et al. (1996), Gupta et al. (2004), Margesin et al. (2004), and Chen et al. (2005a).

[f]*Arthrobacter cumminsii/albus* group is here defined as containing dihydrogenated menaquinone with eight isoprenoid units in the side chain [MK-8(H_2)] and a peptidoglycan with a dicarboxyl amino acid (glutamic acid) in the interpeptide bridge (A4α). Data from Funke et al. (1996), Wauters et al. (2000a), and Busse, unpublished results.

[g]Altenburger et al. (2002) and Li et al. (2005b).

[h]Pukall et al. (2006).

[i]Parentheses indicate variation among species or strains.

Province, west China. The medium used for selective isolation was glycerol/asparagine agar (ISP 5 medium: 1.0 g L-asparagine, 10.0 g glycerol, 1.0 g K_2HPO_4, 1.0 ml trace salts solution, and 20.0 g agar per 1000 ml distilled water; Shirling and Gottlieb, 1966) that was supplemented with 15% KCl (w/v). *Yaniella flava* YIM 70178^T was isolated by dilution plating on SGA agar (7.5 g Casamino acids, 10.0 g yeast extract, 20.0 g $MgSO_4 \cdot 7H_2O$, 3.0 g sodium citrate, 2.0 g KCl, 1.0 ml trace salts solution, and 15.0 g agar per 1000 ml distilled water, pH 7.5–7.6; Al-Tai and Ruan, 1994) supplemented with 20% KCl (w/v) from a saline soil sample originating from Qinghai Province in north-west China. The trace salts solution for both strains consisted of 0.1g $FeSO_4 \cdot 7\ H_2O$, 0.1g $MnCl_2 \cdot 4\ H_2O$, and 0.1g $ZnSO_4 \cdot 7\ H_2O$ per 1000 ml distilled water, pH 7.0–7.4. Both strains were obtained from cultures incubated at 28°C for 2 weeks. Biomass for chemical and molecular systematic studies was obtained by cultivation in shaken flasks (about 150 rpm) of modified ISP5 broth (KCl 10% w/v, pH 7.0) at 28°C for 1 week.

Maintenance procedures

Strains of the genus *Yaniella* were maintained on ISP5 agar slants containing 10% KCl (w/v) at 4°C and in glycerol suspensions (20% v/v) at −20°C. Long-term preservation methods include freeze drying in skim milk and maintenance in liquid nitrogen at −196°C.

Differentiation of the genus *Yaniella* from other genera

Table 98 of the chapter on the genus *Kocuria* shows the differential phenotypic characteristics of the genus *Yaniella* and the phylogenetically related genera of the family *Micrococcaceae*. The genus *Yaniella* shares the peptidoglycan variation A4α Lys←Gly←Glu with the genera *Citricoccus* (Altenburger et al., 2002; Li et al., 2005b) and *Nesterenkonia* (Collins et al., 2002b; Li et al., 2005a; Stackebrandt et al., 1995), but differs from both genera in a remarkably lower range of DNA G+C values and from the genus *Citricoccus* additionally in the occurrence of completely unsaturated menaquinones.

Taxonomic comments

Recently, the genus name *Yania* was proved to be illegitimate because of the precedence of the genus name *Yania* in zoological descriptions and was replaced by the name *Yaniella* (Li et al., 2008b).

List of species of the genus *Yaniella*

1. **Yaniella halotolerans** (Li, Chen, Xu, Zhang, Schumann, Tang, Xu and Jiang 2004a) Li, Zhi and Euzéby 2008b, 526^{VP} (Illegitimate homotypic synonym: *Yania halotolerans* Li, Chen, Xu, Zhang, Schumann, Tang, Xu and Jiang 2004a, 530)

ha.lo.to′le.rans. Gr. n. *hals halos* salt; L. pres. part. *tolerans* tolerating; N.L. part. adj. *halotolerans* salt-tolerating, referring to the organism's ability to tolerate high salt concentrations.

In addition to the properties given in the genus description, this species has the following characteristics. Colonies reach maximum size (2 mm in diameter) after 1 week incubation at 28°C. They are yellow, circular, lubricious, and opaque. Almost all carbon sources tested are utilized, including glucose, galactose, arabinose, starch, cellobiose, lactose, mannose, mannitol, fructose, sucrose, maltose, and xylose, but acid is produced only from glucose, maltose, sucrose, and fructose. Positive for milk peptonization and urease, but negative for milk coagulation, nitrate reduction, gelatin liquefaction, growth on cellulose, and production of H_2S and melanin. Starch and Tweens 20, 40, and 80 are not hydrolyzed. Cells do not form indole; methyl red and Voges–Proskauer tests are negative. Temperature range for growth is 10–40°C, with an optimum temperature of 28–30°C. Growth pH is optimal at 7.0–8.0. Concentration ranges of NaCl, KCl, and $MgCl_2 \cdot 6H_2O$ for growth are 0–25, 0–20 and 0–15%, respectively. Menaquinones are MK-8, MK-7, and MK-9. Major cellular fatty acids are $C_{15:0}$ iso and $C_{15:0}$ anteiso.

Source: saline soil that was collected in Xinjiang Province, west China.

DNA G+C content (mol%): 53.5 (T_m).

Type strain: YIM 70085, CCTCC AA 001023, DSM 15476, JCM 13527.

Sequence accession no. (16S rRNA gene): AY228479.

2. **Yaniella flava** (Li, Schumann, Zhang, Xu, Chen, Xu, Stackebrandt and Jiang 2005c), Li, Zhi and Euzéby 2008b, 526^{VP} (Illegitimate homotypic synonym: *Yania flava* Li, Schumann, Zhang, Xu, Chen, Xu, Stackebrandt and Jiang 2005c, 1937)

fla′va. L. fem. adj. *flava* golden yellow, referring to the color of the colonies.

In addition to the properties given in the genus description, this species has the following characteristics. Colonies are light yellow, circular, lubricous, and opaque. Acid is produced from glucose, maltose, and fructose. It is negative for milk peptonization, milk coagulation, urease, and nitrate reduction, gelatin liquefaction, growth in cellulose, H_2S and melanin production. Some other physiological and biochemical characteristics are listed in Table 104. The cell-wall peptidoglycan type is A4α, L-Lys←Gly←L-Glu. The polar lipids contain diphosphatidylglycerol, phosphatidylglycerol, an unknown phospholipid, and an unknown glycolipid. The predominant menaquinones are MK-8 and MK-9. The major cellular fatty acid is $C_{15:0}$ anteiso.

Source: a saline soil collected from Qinghai Province, north-west China.

DNA G+C content (mol%): 57.9 (HPLC).

Type strain: YIM 70178, DSM 16377, JCM 13595, KCTC 19047.

Sequence accession no. (16S rRNA gene): AY684123.

Genus X. **Zhihengliuella** Zhang, Schumann, Yu, Liu, Zhang, Xu, Stackebrandt, Jiang and Li 2007, 1018[VP] emend. Tang, Wang, Chen, Lou, Cao, Xu, and Li 2009, 2029

Hans-Jürgen Busse

Zhi.heng.li.u.el′la. N.L. fem. dim. n. *Zhihengliuella* named after Zhi-Heng Liu (1940–), a Chinese microbiologist who devotes himself to the study of actinomycete taxonomy.

Gram-stain-positive, mesophilic and aerobic. Cells are **non-motile, non-spore-forming, short rods** (0.6–1.0 × 1.5–2.0 µm). Catalase-positive and oxidase-negative. The **peptidoglycan type is A4**, L-lys–L-ala–L-glu. The predominant **menaquinones** are **MK-9 and MK-10;** MK-8 occurs in smaller amounts. The **major fatty acids** are **$C_{15:0}$ anteiso and $C_{15:0}$ iso, or $C_{15:0}$ anteiso and $C_{17:0}$ anteiso**. The major **polar lipids** are **phosphatidylglycerol, diphosphatidylglycerol, and phosphatidylinositol**. **Diagnostic cell-wall sugars** are **tyvelose and mannose**.

DNA G+C content (mol%): 66.5–70.3 (HPLC).

Type species: **Zhihengliuella halotolerans** Zhang, Schumann, Yu, Liu, Zhang, Xu, Stackebrandt, Jiang and Li 2007, 1018[VP].

Further descriptive information

The genus *Zhihengliuella* consists of two species, *Zhihengliuella halotolerans* and *Zhihengliuella alba* which are sharing 97.5% 16S rRNA gene sequence similarity; differential characteristics are given in Table 106. Phylogenetically, the genus *Zhihengliuella* is placed in the family *Micrococcaceae* but the position within the family is not stable as indicated by different positions in different phylogenetic trees and low bootstrap support (Tang et al., 2009; Zhang et al., 2007). The type species of the genus *Zhihengliuella halotolerans* shares highest 16S rRNA gene sequence similarities with the type strains of certain *Micrococcus* and *Arthrobacter* species including *Arthrobacter oryzae* (96.4%), *Arthrobacter pascens* (96.2%), *Arthrobacter woluwensis* (96.1%), *Arthrobacter kerguelensis*, *Micrococcus antarcticus*, *Micrococcus luteus* (all 96.0%), *Arthrobacter psychrophenolicus*, *Arthrobacter arilaitensis*, *Arthrobacter mysorens* (all 95.9%), *Micrococcus lylae*, and *Arthrobacter globiformis* (95.7%). These data do not suggest close affiliation with either *Micrococcus* or *Arthrobacter*. Since no sequences of unnamed strains are deposited in gene banks with strains that share more than 97% 16S rRNA gene similarities, availability of other strains of this genus in institutional collections is not indicated. The fatty acid profile consists predominantly (>90%) of iso- and anteiso-branched acids which is similar to other representatives of the family *Micrococcaceae*. The uniqueness of *Zhihengliuella* species is indicated by the sugar tyvelose found with glucose in the cell wall. This characteristic has not been reported for any related taxa. The diagnostic diamino acid lysine supports affiliation of *Zhihengliuella halotolerans* with the *Micrococcaceae*, whereas the interpeptide bridge L-Ala–L-Glu is only reported as a characteristic of certain species of the *Arthrobacter nicotianae* group (*sensu* Keddie et al., 1986), including *Arthrobacter*

TABLE 106. Characteristics distinguishing between *Zhihengliuella halotolerans* and *Zhihengliuella alba*

Characteristic	*Z. halotolerans*	*Z. alba*
Pigmentation	Pale yellow[a]	White
NaCl range for growth (%, w/v)	0–25[a]	0–15
Optimal NaCl concentration (%, w/v)	10[a]	5
pH range for growth	6–10[a]	5–9
Optimum pH range	8–9[a]	6–8
Hydrolysis of:		
Starch	+	–
Tween 20	+	–
Utilization of (Biolog GP2):		
L-Arabinose, β-cyclodextrin, gentiobiose, maltotriose, melibiose, methyl β-D-galactoside, methyl β-D-glucoside, methyl α-D-mannoside, salicin, sedoheptulosan, D-sorbitol, stachyose, turanose, acetic acid, α-hydroxybutyric acid, β-hydroxybutyric acid, γ-hydroxybutyric acid, α-D-ketoglutaric acid, D-malic acid, L-malic acid, pyruvic acid methyl ester, pyruvic acid, succinamic acid, D-alanine, L-alanyl glycine L-glutamic acid, 2,3-butanediol, inosine, uridine, thymidine-5′-monophosphate, uridine-5′-monophosphate, D-fructose 6-phosphate, α-D-glucose 1-phosphate, D-glucose 6-phosphate, α-DL-glycerol phosphate	+	–
L-Asparagine, L-serine, 2′-deoxyadenosine, *N*-acetyl-D-galactosamine, cellobiose, α-D-lactose	–	+
Enzyme activities (API ZYM):		
Alkaline phosphatase, lipase (C14)	+	–
Leucine arylamidase, valine arylamidase, cystine arylamidase, trypsin, α-chymotrypsin, acid phosphatase, naphthol-AS-BI-phosphohydrolase, β-galactosidase, β-glucosidase	–	+
Acid production from (API 50CHB):		
L-Arabinose, glycerol, D-adonitol, D-sorbitol, methyl β-D-xylopyranoside, inositol, arbutin, esculin, salicin, raffinose, turanose, glycogen, xylitol, gentiobiose, D-arabitol, potassium 5-ketogluconate	+	–
L-Xylose, D-glucose, sucrose	–	+
DNA G+C content (mol%)	66.5[a]	70.3

[a]Data for *Zhihengliuella halotolerans* are from Zhang et al. (2007). All other data are from Tang et al. (2009).

gangotriensis, *Arthrobacter kerguelensis*, *Arthrobacter psychrophenolicus*, and *Arthrobacter sulfureus*. The quinone system consisting predominantly of completely unsaturated menaquinones MK-9, MK-10, and small amounts of MK-8 is a characteristic similarly found in certain species of the *Arthrobacter nicotianae* group and in *Renibacterium*. Both the quinone system and interpeptide bridge might suggest a closer affiliation of *Zhihengliuella* with the *Arthrobacter nicotianae* group but this proposal is so far not supported by 16S rRNA based phylogeny.

Pathogenicity. No pathogenic potential was reported for any of the *Zhihengliuella* species, and neither the source of isolation (saline soil) nor NaCl preference (5–10%) suggest any pathogenic potential for humans.

Maintenance procedures

Cultures of *Zhihengliuella halotolerans* may be stored on marine agar (Difco 2216) slants at 4°C and as 20% (w/v) glycerol suspensions at −20°C. Cultures of *Zhihengliuella alba* may be stored on ISP 5 agar* slants containing 5% NaCl at 4°C and as 20% (w/v) glycerol suspensions at −20°C.

Differentiation of the genus *Zhihengliuella* from other genera

Zhihengliuella species may be differentiated chemotaxonomically from related genera based on the quinone system, peptidoglycan composition, cell-wall sugars, and with some restrictions, also fatty acid profile.

List of species of the genus *Zhihengliuella*

1. **Zhihengliuella halotolerans** Zhang, Schumann, Yu, Liu, Zhang, Xu, Stackebrandt, Jiang and Li 2007, 1018[VP]

ha.lo.to′le.rans. Gr. n. *hals halos* salt; L. part. adj. *tolerans* tolerating; N.L. part. adj. *halotolerans* salt-tolerating, referring to the organism's ability to tolerate high salt concentrations.

The characteristics are as described for the genus with the following additional features. Pale-yellow colonies are circular, opaque, and approximately 1.0 mm in diameter after 24 h at 28°C. Optimum growth occurs at pH 8.0–9.0 and at 28–30°C with 10% NaCl. Negative for ornithine decarboxylase, urease, *N*-acetylglucosaminidase, β-galactosidase, α-glucosidase, gelatin liquefaction, methyl red test, Voges–Proskauer test, and nitrate reduction, but positive for arginine dihydrolase, lysine decarboxylase, lipase, β-glucosidase, α-galactosidase, ammonia production, starch, hydrolysis of Tween 20 and 80, and milk peptonization. Malonate is utilized and acid is produced from maltose, glucose, sucrose, L-arabinose, and trehalose. The cell wall contains glucose and tyvelose. Polar lipids include an unknown phospholipid and an unknown glycolipid. The menaquinones are MK-9, MK-10, and MK-8 (ratio, 5:2:1). The cellular fatty acid profile contains $C_{15:0}$ anteiso (63.9%), $C_{15:0}$ iso (15.0%), $C_{17:0}$ anteiso (8.5%), $C_{16:0}$ iso (7.1%), $C_{16:0}$ (2.5%), $C_{17:0}$ iso (1.0%), $C_{14:0}$ iso (0.8%), $C_{14:0}$ (0.8%), $C_{15:0}$ (0.2%), and $C_{15:1}$ anteiso (0.2%).

Source: saline soil sample collected from Qinghai province, north-west China.

DNA G+C content (mol%): 66.5 (HPLC).

Type strain: YIM 70185, DSM 17364, JCM 15629, KCTC 19085.

Sequence accession no. (16S rRNA gene): DQ372937.

2. **Zhihengliuella alba** Tang, Wang, Chen, Lou, Cao, Xu and Li 2009, 2029[VP]

al′ba. L. fem. adj. *alba* white, referring to the white color of colonies.

Cells are aerobic, Gram-stain-positive, short rods, 0.5–0.9 × 1.3–1.8 µm in size, non-motile, and without flagella. Colonies are circular, smooth, and white on modified ISP 5 medium.* Growth occurs in 0–15% (w/v) NaCl, with good growth at 5% (w/v) NaCl. Growth occurs at 4–45°C and pH 5.0–9.0, with optimum growth at 28–37°C and pH 6.0–8.0. Catalase-positive and oxidase-negative. Negative for milk peptonization, methyl red, and Voges–Proskauer tests, H_2S production, nitrate reduction, and hydrolysis of starch, urea, cellulose, casein, chitin and Tween 20. Positive for hydrolysis of esculin, gelatin, Tween 40, and Tween 80. Enzyme activities (API ZYM) are observed for esterase (C4), esterase lipase (C8), leucine arylamidase, valine arylamidase, cystine arylamidase, trypsin, α-chymotrypsin, acid phosphatase, naphthol-AS-BI-phosphohydrolase, β-galactosidase, α-glucosidase and β-glucosidase. Negative for alkaline phosphatase, lipase (C14), α-galactosidase, β-glucuronidase, *N*-acetyl-β-glucosaminidase, α-mannosidase, and α-fucosidase. Acid is produced (API 50CHB) from erythritol, D-ribose, DL-xylose, D-glucose, D-fructose, L-sorbose, L-rhamnose, D-mannitol, amygdalin, maltose, sucrose and starch, but not from glycerol, L-arabinose, D-arabinose, D-adonitol, methyl β-D-xylopyranoside, D-galactose, D-mannose, dulcitol, inositol, D-sorbitol, methyl α-D-mannopyranoside, methyl α-D-glucopyranoside, *N*-acetylglucosamine, turanose, arbutin, aesculin, cellobiose, salicin, D-lactose, melibiose, trehalose, inulin, melezitose, raffinose, glycogen, xylitol, gentiobiose, D-lyxose, D-tagatose, DL-fucose, DL-arabitol, potassium gluconate, potassium 2-ketogluconate, and potassium 5-ketogluconate. The following substrates are oxidized in the Biolog GP2 test: dextrin, mannan, Tweens 40 and 80, *N*-acetyl-D-galactosamine, D-arabitol, cellobiose, D-fructose, D-galactose, α-D-glucose, *myo*-inositol, α-D-lactose, maltose, D-mannitol, D-mannose, methyl α-D-glucoside, palatinose, D-psicose, raffinose, L-rhamnose, D-ribose, sucrose, D-tagatose, trehalose, xylitol, D-xylose, α-ketovaleric acid, succinic acid monomethyl ester, propionic acid, pyruvic acid, L-asparagine, L-serine, glycerol, 2′-deoxyadenosine, and thymidine. The following substrates are not oxidized: α-cyclodextrin, β-cyclodextrin, starch, inulin, *N*-acetyl-D-glucosamine, amygdalin, L-arabinose, arbutin, L-fucose, D-galacturonic acid, gentiobiose, D-gluconic acid, lactulose, melezitose, melibiose, methyl α-D-galactoside, methyl

*ISP 5 medium comprising (per 1 distilled water, final pH 7.5): 1 g L-asparagine, 10 g glycerol, 5 g yeast extract, 1 g K_2HPO_4, 5 g KNO_3, 100 g NaCl, and 15 g agar. NaCl is sterilized separately before being added to the medium.

β-D-galactoside, 3-methyl-D-glucose, methyl β-D-glucoside, methyl α-D-mannoside, salicin, sedoheptulosan, D-sorbitol, stachyose, turanose, acetic acid, α-hydroxybutyric acid, β-hydroxybutyric acid, γ-hydroxybutyric acid, ρ-hydroxyphenylacetic acid, α-ketoglutaric acid, lactamide, D-lactic acid methyl ester, L-lactic acid, D-malic acid, L-malic acid, pyruvic acid methyl ester, pyruvic acid, succinamic acid, succinic acid, *N*-acetyl-L-glutamic acid, L-alaninamide, DL-alanine, L-alanyl glycine, L-glutamic acid, glycyl L-glutamic acid, L-pyroglutamic acid, putrescine, 2,3-butanediol, adenosine, inosine, uridine, adenosine-5′-monophosphate, thymidine-5′-monophosphate, uridine-5′-monophosphate, D-fructose 6-phosphate, α-D-glucose 1-phosphate, D-glucose 6-phosphate, and DL-α-glycerol phosphate. Peptidoglycan type is A4α, L-Lys–L-Ala–L-Glu. Major cell sugars contain tyvelose and mannose; minor amounts of glucose, rhamnose and galactose are also detected. Predominant menaquinones are MK-10 (64.0%) and MK-9 (32.7%); a minor amount of MK-8 (3.3%) is also detected. Polar lipids are diphosphatidylglycerol, phosphatidylglycerol, phosphatidylinositol, an unknown phospholipid, and two unknown glycolipids. Major cellular fatty acids are $C_{15:0}$ anteiso and $C_{17:0}$ anteiso; minor amounts of $C_{14:0}$, $C_{14:0}$ iso, $C_{15:0}$ iso, $C_{15:1}$ anteiso, $C_{16:0}$, $C_{16:0}$, $C_{17:0}$ iso, and $C_{18:0}$ are also detected.

Source: saline soil sample collected from Ganjiahu Suosuo Forest National Nature Reserve in Xinjiang province, northwest China.

DNA G+C content (mol%): 70.3 (HPLC).

Type strain: YIM 90734, KCTC 19375, DSM 21143.

Sequence accession no. (16S rRNA gene): EU847536.

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Family II. **Beutenbergiaceae** Zhi, Li and Stackebrandt 2009, 597[VP] emend. Hamada, Iino, Tamura, Iwami, Harayama and Suzuki 2009, 2813[VP] emend. Ue, Matsuo, Kasai and Yokota 2011, 125[VP]

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Beu′ten.ber.gi.a.ce′a.e. N.L. fem. n. *Beutenbergia* type genus of the family; suff. *-aceae* ending to denote a family; N.L. fem. pl. n. *Beutenbergiaceae* the family of *Beutenbergia*.

Cells are irregular rods and cocci which occur singly, in pairs, or in small clusters. A rod–coccus growth cycle may be expressed. Gram-stain-positive. Endospores are not observed. **Nonmotile. Aerobic and facultatively anaerobic**. Colonies are circular, convex, and vary in color from white to yellow. Members of the family grow well on complex organic media and utilize a broad spectrum of carbon sources. Optimum growth between 28 and 37°C. Oxidase-positive or negative, **catalase-positive**. The characteristic diamino acids in the peptidoglycan are L-lysine or L-ornithine which correspond to the **peptidoglycan types A4α or A4β**, respectively. The major menaquinone is **MK-8(H_4)**. The pattern of polar lipids typically consists of diphosphatidylglycerol and (with the exception of *Serinibacter*) several unidentified phospholipids, and possibly phosphatidylglycerol, phosphatidylinositol, and phosphatidylethanolamine. The cellular fatty acid profiles are dominated by **iso- and anteiso-branched-chain acids and minor amounts of saturated straight-chain acids**. The family currently accommodates the genera *Beutenbergia*, *Salana*, *Serinibacter*, and *Miniimonas*. The pattern of 16S rRNA gene sequence signatures consists of nucleotides at positions 144:178 (C–G), 280 (U), 293:304 (G–U), 668:738 (A–U), 1003:1038 (G–U), 1027:1034 (U–A), and 1414:1486 (U–A). Members of the family were isolated from cave soil, river sediments, sea sand, and the intestinal tract of a fish.

DNA G+C content (mol%): 71–75.

Type genus: **Beutenbergia** Groth, Schumann, Schuetze, Augsten, Kramer and Stackebrandt 1999b, 1738[VP].

Taxonomic comments

The genus *Beutenbergia* has been established by Groth et al. in 1999 for two strains isolated from cave soil. Comparison of 16S rRNA gene sequences showed that the two strains shared a 100% sequence similarity between each other and that they were phylogenetically members of the order *Micrococcales*. However, the low sequence similarity values to members of other families together with the fact that *Beutenbergia cavernae* shared only 9–59% of signature nucleotides defining families within the *Micrococcales* indicated that these strains could not be affiliated to any described genus or family of this order. With the descriptions of the genera *Salana* (von Wintzingerode et al., 2001) and *Serinibacter* (Hamada et al., 2009), it became obvious that the relationship of these two genera is closer to *Beutenbergia* than to all previously described genera of the *Actinobacteria*. These findings supported the proposal of the new family *Beutenbergiaceae* (Zhi et al., 2009) containing the genera *Beutenbergia*, *Georgenia*, and *Salana*. Hamada et al. (2009) emended the family descriptions of the *Beutenbergiaceae* and the *Bogoriellaceae*. Hamada et al. (2009) defined the family *Bogoriellaceae* on the basis of the pattern of 16S rRNA gene sequence signatures, i.e. nucleotides at positions 144:178 (U–G), 293:304 (G–U), 479 (U), 602:636 (C–G), 668:738 (A–U), 1003:1038 (G–C), 1027:1034 (C–G), and 1409:1491 (C–G) and to include the genera *Bogoriella* (Groth et al., 1997a) and *Georgenia* (Altenburger et al., 2002; Li et al., 2007). The family *Beutenbergiaceae* comprised the genera *Beutenbergia*, *Salana*, and *Serinibacter* and was defined mainly on the basis of the pattern of 16S rRNA gene sequence signatures, i.e. nucleotides at positions 144:178 (C–G), 280 (U), 293:304 (G–U), 668:738 (A–U), 1003:1038 (G–U), 1027:1034 (U–A), and 1414:1486 (U–A) (Hamada et al., 2009). Recently the genus *Miniimonas* (Ue et al., 2011) was described containing the signature nucleotides of the family *Beutenbergiaceae*, and the family description was emended by Ue et al. (2011) to change the nucleotide pair at position 131:231 to A–G, not C–G per Zhi et al. (2009). Differential characteristics of the genera *Beutenbergia*, *Salana*, *Serinibacter*, and *Miniimonas* are indicated in Table 107. Patterns of selected 16S rRNA gene signature nucleotides are given in Table 108. The 16S rRNA gene sequence-based phylogenetic tree showing the positions of the members of the family *Beutenbergiaceae* is given in Figure 142.

TABLE 107. Differentiation of the genera *Beutenbergia*, *Miniimonas*, *Salana*, and *Serinibacter*[a,b]

Characteristic	*Beutenbergia*	*Miniimonas*	*Salana*	*Serinibacter*
Cell morphology	Irregular rods and cocci, rod–coccus cycle	Rods–coccoid	Short rods and cocci, club- like forms, rod–coccus cycle	Irregular, short rods
Relationship to oxygen	Aerobic to microaerobic	Aerobic and anaerobic	Aerobic and anaerobic	Aerobic and anaerobic
Oxidase	–	–	w	–
Cell-wall diamino acid	L-Lys	L-Orn	L-Orn	L-Lys
Peptidoglycan type	A4α	A4β	A4β	A4α
Predominant cellular fatty acids	$C_{15:0}$ iso, $C_{15:0}$ anteiso	$C_{15:0}$ anteiso, $C_{16:0}$	$C_{15:0}$ anteiso, $C_{14:0}$	$C_{15:0}$ anteiso, $C_{16:0}$
Polar lipids	DPG, PI, 3 PL	PG, DPG, PI, 1 PL	DPG, PG, PLs	PE, PG
DNA G+C content (mol%)	71	74	75	71

[a]Symbols: –, negative; w, weakly positive. Abbreviations: DPG, diphosphatidylglycerol; PG, phosphatidylglycerol; PE, phosphatidylethanolamine; PI, phosphatidylinositol; PL, unidentified phospholipid.

[b]Data from Groth et al. (1999b), von Wintzingerode et al. (2001), Hamada et al. (2009), and Ue et al. (2011).

TABLE 108. Patterns of selected 16S rRNA gene signature nucleotides of the genera of the family *Beutenbergiaceae*[a]

Position	*Beutenbergia*	*Miniimonas*	*Salana*	*Serinibacter*
140:223	G–U	G–C	G–U	G–U
589:650	U–A	U–A	U–A	C–G
610	A	U	U	U
612:628	G–C	C–G	C–G	C–G
616:624	G–C	G–C	G–U	G–C
839:847	U–A	C–A	C–A	C–A
863	A	A	U	U

[a]According to Ue et al. (2011).

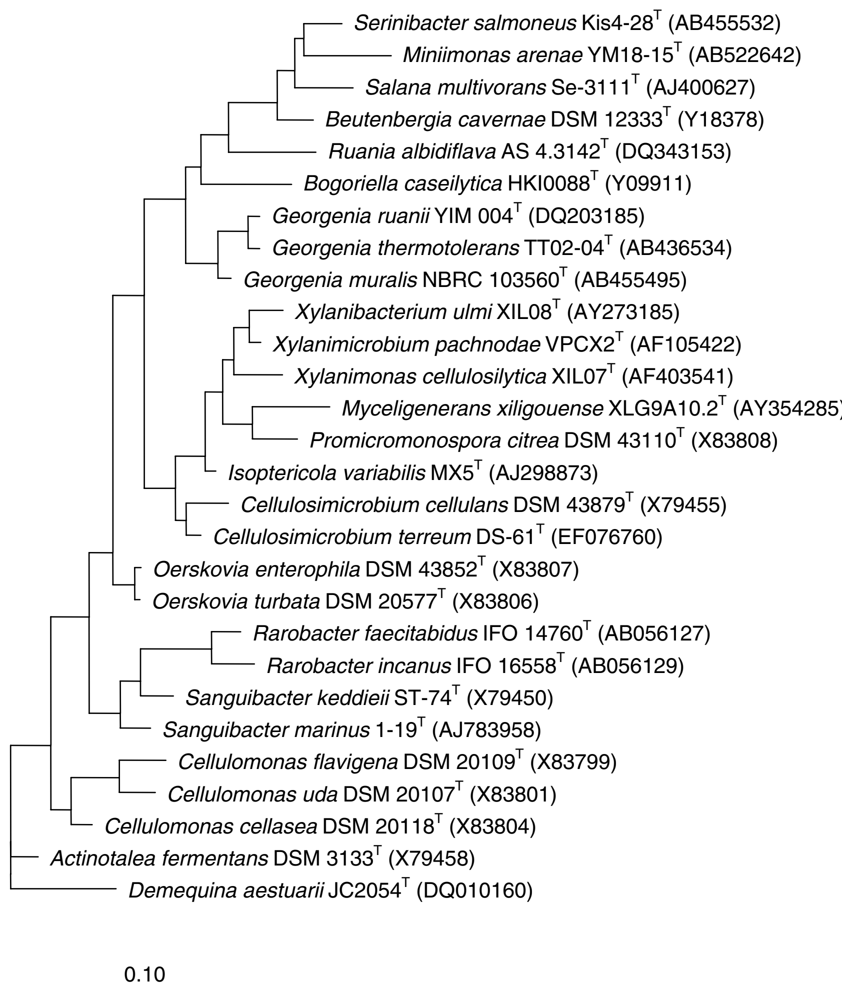

FIGURE 142. Phylogenetic analysis based on 16S rRNA gene sequences available from EMBL (accession numbers in parentheses). The phylogenetic tree was constructed using the ARB software package. Tree building was performed using the maximum-likelihood method with fastDNAml without filters. Bar = 0.10 nucleotide substitutions per nucleotide position.

Genus I. **Beutenbergia** Groth, Schumann, Schuetze, Augsten, Kramer and Stackebrandt 1999b, 1738[VP]

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Beu.ten.ber′gi.a. N.L. fem. n. *Beutenbergia* referring to Beutenberg, the geographical location of the institute in which the soil sample was studied.

Cells are irregular rods (0.5–0.8 × 1.0–3.1 μm) **and cocci** (0.7–1.0 μm). They occur singly, in pairs, short chains, or clusters and exhibit a **rod–coccus growth cycle (Figure 143 and Figure 144)**. Gram-stain-positive, not acid-fast. **Endospores are not formed. Nonmotile**. **Aerobic to microaerobic.** Colonies grown on rich (R) agar are smooth, circular, convex, opaque with entire margins and cream to bright yellow in color. Good growth on complex organic media at 28°C. NaCl in the culture medium may be tolerated up to 4%. **Oxidase-negative, catalase-positive**. The **peptidoglycan type is A4α** based on L-lysine. The **acyl type is acetyl**. Whole cell sugars are **glucose, mannose, and galactose**. The predominant menaquinone is **MK-8(H_4)**; MK-8(H_2), MK-8, and MK-9(H_4) occur in minor amounts. The polar lipids consist of **phosphatidylinositol, diphosphatidylglycerol, and unknown phospholipids. The cellular fatty acid profile** is dominated by the occurrence of **iso- and anteiso-branched-chain acids.** Mycolic acids are absent. Based on 16S rRNA gene sequence similarities, strains of *Beutenbergia* are closely related both to genera of the families *Beutenbergiaceae* (*Salana*, *Serinibacter* and *Miniimonas*) and *Bogoriellacea* (*Georgenia* and *Bogoriella*) and to the genera *Cellulosimicrobium* (*Promicromonosporaceae*), *Sanguibacter* (*Sanguibacteraceae*), and *Intrasporangium* (*Intrasporangiaceae*), and the genus *Ruania*. The 16S rRNA gene signature nucleotides that differentiate the genus from the other genera of the *Beutenbergiaceae* are listed in Table 108.

DNA G+C content (mol%): 71 (HPLC).

Type species: **Beutenbergia cavernae** Groth, Schumann, Schuetze, Augsten, Kramer and Stackebrandt 1999b, 1738[VP].

Further descriptive information

Currently the genus is represented by only one species *Beutenbergia cavernae* which includes two strains of the same origin. 16S rRNA gene sequence comparisons showed that the two strains share a 100% sequence similarity between one another and form a clade in the 16S rRNA gene tree of the suborder *Micrococcineae* (elevated to order *Micrococcales* in the taxonomic outline to the present volume) (Stackebrandt et al., 1997) with *Salana multivorans* (von Wintzingerode et al., 2001), *Serinibacter salmoneus* (Hamada et al., 2009) and *Miniimonas arenae* (Ue et al., 2011; Figure 142, chapter on *Beutenbergiaceae*). *Beutenbergia cavernae* shares a 16S rRNA gene sequence similarity of 97.1% with *Miniimonas arenae*, 96.5% with *Serinibacter salmoneus*, and 95.9% with *Salana multivorans*, and 94.6–95.3% with two species of *Georgenia* (Altenburger et al., 2002; Li et al., 2007), 94.0% with *Ruania albidiflava* (Gu et al., 2007), and 93.9% with *Bogoriella caseilytica* (Groth et al., 1997a). Interestingly, a high value of 96.0% 16S rRNA gene sequence similarity is also found

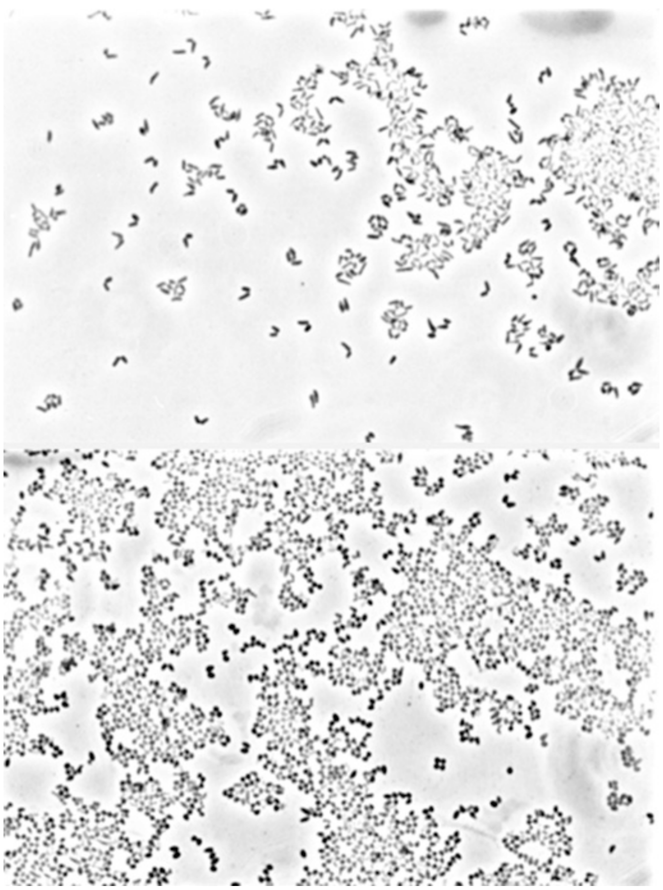

FIGURE 143. Micrographs of cells from *Beutenbergia cavernae* DSM 12333[T] grown at 28°C in liquid R medium for (top) 24 h or (bottom) 96 h. Bars = 10 μm.

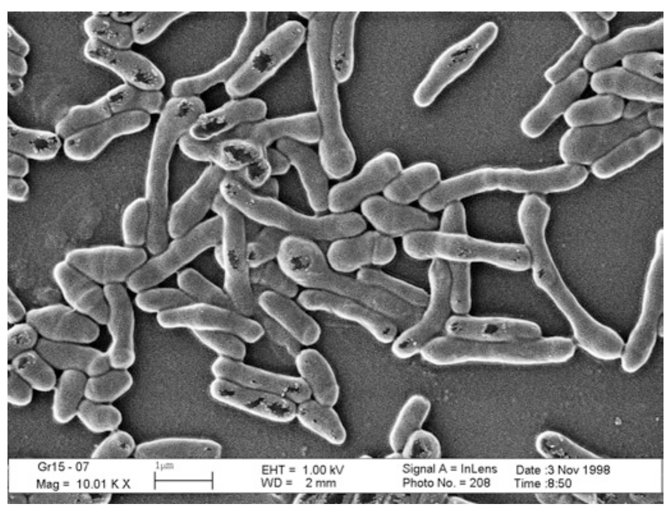

FIGURE 144. Scanning electron micrograph of cells from a 72-h-old culture of *Beutenbergia cavernae* DSM 12333[T] grown at 28°C on R agar. Bar = 1 μm.

between *Beutenbergia cavernae* and *Cellulosimicrobium cellulans* (Schumann et al., 2001). Furthermore *Beutenbergia cavernae* shares a relatively high level of 16S rRNA gene sequence similarity with *Sanguibacter keddieii* (94.1%; Fernández-Garayzábal et al., 1995) or *Intrasporangium calvum* (94.0%; Kalakoutskii et al., 1967). The 16S rRNA gene sequence of *Beutenbergia cavernae* possesses almost all of the signature nucleotides which are described to define members of the order *Micrococcales*. Only the A–A pair in position 722:723 is replaced by a G–G pair, which is also present in the strains of *Georgenia muralis* (Altenburger et al., 2002). The 16S rRNA gene signature nucleotides that define the genus *Beutenbergia* are listed in Table 108. The peptidoglycan of *Beutenbergia cavernae* contains Ala, Glu, and Lys in a molar ratio of 2.1:2.2:1.0. The interpeptide bridge consists of L-Lys–L-Glu which is in accordance with the peptidoglycan type A11.54 (DSMZ Catalogue of Strains, 1998, or http://www.dsmz.de), a variation of the peptidoglycan type A4α (Schleifer and Kandler, 1972).

Enrichment and isolation procedures

Beutenbergia cavernae grows readily in complex liquid or on solidified media, i.e. Bacto nutrient agar,* rich (R) medium† (Yamada and Komagata, 1972), Bacto tryptic soy broth,‡ and peptone-yeast extract-brain heart infusion medium** (PY-BHI, Yokota et al., 1993). The two strains of *Beutenbergia cavernae* were isolated from a cave on casein mineral medium†† (Altenburger et al., 1996) and on PY-BHI agar using a standard dilution plate procedure.

Maintenance procedures

Cultures of *Beutenbergia cavernae* can be maintained by serial transfers on the solidified complex media listed above. Growth on agar slants in screw-capped tubes can be kept at 4°C for about 2–4 weeks. Long-term preservation of liquid cultures supplemented with 5% dimethylsulfoxide is recommended in the vapor phase of liquid nitrogen (−140°C).

Differentiation of the genus *Beutenbergia* from other genera

Beutenbergia can be distinguished from the other genera of the family *Beutenbergiaceae* by differences in the patterns of 16S rRNA gene signature nucleotides (Table 108), 16S rRNA gene sequence similarities ranging from 95.9–97.1%, and by a broad spectrum of phenotypic characteristics (Table 107). As all the members of the family *Beutenbergiaceae* are currently represented by only one species, the phylogenetic and phenotypic differences between the genera will become more obvious as further strains of the family become available. The closest phylogenetic neighbor of *Beutenbergia* within the *Beutenbergiaceae* is the genus *Miniimonas* which can be distinguished from *Beutenbergia* by the presence of L-ornithine as cell-wall diaminoacid (peptidoglycan type A4β), the composition of the polar lipids and a DNA G+C content of 74 mol%. The genus *Serinibacter* shares with *Beutenbergia* the peptidoglycan type A4α, but is characterized by the presence of serine and the absence of L-alanine in the peptidoglycan and by differences in the pattern of polar lipids. The genus *Salana* can be distinguished from *Beutenbergia* by the peptidoglycan type A4β. Further differentiating characteristics between these two genera are quantitative differences in the contents of diagnostic cellular fatty acids, the absence of phosphatidylinositol in *Salana*, and differences in numerous physiological properties (Groth et al., 1999b; von Wintzingerode et al., 2001). *Beutenbergia cavernae* shares with the closely related *Georgenia muralis* the peptidoglycan type A11.54 with the interpeptide bridge L-Lys–L-Glu (Altenburger et al., 2002), which is not present in *Georgenia ruanii* (Li et al., 2007). In the one currently available strain of this species, a further peptidoglycan type (A11.35) with the interpeptide bridge L-Lys–L-Ala–L-Glu is found. Differences in the patterns of polar lipids, the fatty acid profiles, and a broad spectrum of physiological markers equally contribute to distinguish *Beutenbergia* from *Georgenia*. The rare peptidoglycan type A11.54, present in *Beutenbergia cavernae*, *Serinibacter salmoneus*, and *Georgenia muralis*, corresponds to that found in *Arthrobacter sulfureus* (Stackebrandt et al., 1983), a member of the family *Micrococcaceae* (Stackebrandt et al., 1997). Although the strains of *Beutenbergia cavernae* and *Arthrobacter sulfureus* are morphologically similar, they can be readily distinguished from one another by their low level of phylogenetic relationship and differences in the DNA G+C content, menaquinone type, pattern of polar lipids, and fatty acid profiles. The composition of the interpeptide bridge also serves to distinguish *Beutenbergia* from *Bogoriella* and *Ruania*. *Bogoriella* has an interpeptide bridge consisting of L-Lys–L-Ala–L-Ala–L-Glu, and that of *Ruania* consists of L-Lys–Gly–L-Glu–L-Glu. Further differences in phenotypic characteristics underline the phylogenetic distinctiveness between *Beutenbergia* and these two genera (Groth et al., 1997a; Gu et al., 2007). The genus *Demetria* (Groth et al., 1997b), which is also characterized by the presence of the peptidoglycan type A4α in combination with the menaquinone type MK-8(H_4), shares with *Beutenbergia* a 16S rRNA gene sequence similarity of only 91.4%. Numerous phenotypic characteristics including the composition of the interpeptide bridge of the peptidoglycan, pattern of polar lipids, fatty acid profile, and morphological and physiological properties clearly separate *Demetria* from *Beutenbergia*. The phylogenetically close genera *Cellulosimicrobium*, *Sanguibacter*, and *Intrasporangium* show the following phenotypic characteristics that distinguish them from *Beutenbergia*. Young cultures of *Cellulosimicrobium cellulans* form a primary mycelium that fragments later into irregular curved or club shaped rods. The peptidoglycan type is A4α with a D-Ser–D-Asp interpeptide bridge, and the major menaquinone is MK-9(H_4). Cells of *Sanguibacter* are typically facultative anaerobic, motile short rods. The peptidoglycan type A4α is based on L-Lys–L-Ser–D-Glu.

*Bacto nutrient agar consists of (g/l): Bacto beef extract, 3.0; Bacto peptone, 5.0, and Bacto agar, 15.0 (all from Difco); pH 6.8.

†Rich (R) medium consists of (g/l): Bacto peptone (Difco), 10.0; yeast extract (Difco), 5.0; Casamino acids (Difco), 5.0; beef extract (Difco), 2.0; malt extract (Difco), 5.0; glycerol, 2.0; $MgSO_4 \cdot 7H_2O$, 1.0; Tween 80, 0.05, and agar, 20.0; pH 7.2.

‡Bacto tryptic soy broth consists of (g/l): Bacto tryptone, 17.0; Bacto soytone, 3.0; glucose, 2.5; NaCl, 5.0; K_2HPO_4, 2.5 (all from Difco); pH 7.3.

**Peptone-yeast extract-brain heart infusion medium consists of (g/l): peptone, 10.0; yeast extract, 2.0; Bacto brain heart infusion (Difco), 2.0; NaCl, 2.0, and glucose, 2.0; pH 7.0.

††Casein mineral medium consists of (g/l): K_2HPO_4, 0.6; $Na_2HPO_4 \cdot 2H_2O$, 0.5; $MgSO_4 \cdot 7H_2O$, 0.05; $MgCl_2 \cdot 7H_2O$, 0.1; KNO_3, 0.2; $FeCl_3 \cdot 6H_2O$, 0.01; casein, 0.8, and yeast extract 0.4; pH 7.0.

$MK\text{-}9(H_4)$ is the predominant menaquinone. *Intrasporangium* is characterized by the formation of a branching mycelium, a peptidoglycan based on LL-A_2pm-Gly_3 (type A3γ), MK-8 as major menaquinone, and a DNA G+C content of 68 mol%.

Taxonomic comments

The genus *Beutenbergia* was established by Groth et al. (1999) for two strains isolated from cave soil. Comparison of 16S rRNA gene sequences showed that the two strains were phylogenetically members of the suborder *Micrococcineae*. However, the low sequence similarity values to members of other families together with the fact that *Beutenbergia cavernae* shared only 9–59% of the signature nucleotides that phylogenetically define families within the *Micrococcineae* indicated that these strains could not be affiliated to any described genus or family of this suborder (elevated to order *Micrococcales* in the taxonomic outline to the present volume). With the descriptions of the genera *Salana* (von Wintzingerode et al., 2001) and *Georgenia* (Altenburger et al., 2002), it became obvious that these two genera are more closely related to *Beutenbergia* than to previously described genera of the actinobacteria. These findings supported an affiliation of the genera *Beutenbergia*, *Salana*, and *Georgenia* to the tentative family "*Beutenbergiaceae*" (Garrity et al., 2007). However, when the 16S rRNA gene sequence of *Ruania* (Gu et al., 2007) was added to the database of the actinobacteria, it was expected from the correspondence in the 16S rRNA gene signature nucleotides that the genera *Beutenbergia*, *Bogoriella*, *Georgenia*, *Ruania*, and *Salana* could be affiliated to a common family. However, the update of the structure and definition (based on 16S rRNA gene sequence) of the higher ranks of the class *Actinobacteria* supported the proposal of the new family *Beutenbergiaceae* (Zhi et al., 2009) comprising the genera *Beutenbergia*, *Georgenia*, and *Salana*. Hamada et al. (2009) emended the descriptions of the families *Beutenbergiaceae* and *Bogoriellaceae*. Based on their phylogenetic studies, the family *Beutenbergiaceae* accommodates the genera *Beutenbergia*, *Salana*, and *Serinibacter*, and the genus *Georgenia* is placed into the family *Bogoriellaceae*. Ue et al. (2011) confirmed the results of Hamada et al. (2009) and added the genus *Miniimonas* to the family *Beutenbergiaceae*.

List of species of the genus *Beutenbergia*

1. **Beutenbergia cavernae** Groth, Schumann, Schuetze, Augsten, Kramer and Stackebrandt 1999b, 1738[VP]

ca.ver′na.e. L. n. *caverna* cave; L. gen. n. *cavernae* of a cave, referring to the habitat of the organism.

The description is the same as given for the genus. In addition, the organism displays the following phenotypic characteristics. Colonies grown on rich (R) agar vary in size from 0.7 to 1.8 mm. Young cultures are composed almost entirely of irregular rods arranged in palisades, clusters or pairs at an angle to give V-forms. In stationary phase cultures, coccoid cells dominate. Optimum temperature for growth is 28°C. Growth is reduced at 37°C and does not occur at 42°C and in the presence of 6% NaCl. Acids are produced from L-arabinose, D-cellobiose, dextrin, D-fructose, D-galactose, D-glucose, glycerol, inulin, maltose, D-mannose, D-raffinose, L-rhamnose, D-ribose, salicin, starch, sucrose, trehalose, and D-xylose. No acid production from D-glucitol, lactose, and D-mannitol. Acetate, aconitate, benzoate, citrate, formate, malate, succinate, and DL-tartrate are not utilized. Nitrate is reduced to nitrite, H_2S is produced, but not indole. Methyl red and Voges–Proskauer reactions are negative. Casein, esculin, gelatin, and potato starch are decomposed; but not adenine, hippurate, hypoxanthine, xanthine, Tween 80, tyrosine, and urea. Reactions of the API ZYM enzyme assay (bioMerieux) are positive for alkaline phosphatase, esterase (C 4), esterase lipase (C 8), leucine arylamidase, cystine arylamidase, acid phosphatase, naphthol-AS-BI-phosphohydrolase, α-galactosidase, β-galactosidase, α-glucosidase, *N*-acetyl-β-glucosaminidase, α-mannosidase, and α-fucosidase, but negative for lipase (C 14), valine arylamidase, trypsin, chymotrypsin, β-glucuronidase, and β-glucosidase. Cells are susceptible to the following antibiotics (μg/disc): ampicillin (10), chloramphenicol (30), erythromycin (15), neomycin (30), oxytetracycline (30), penicillin G (2 IU/disc), polymyxin B (weakly, 300 IU/disc), rifampin (2), but not susceptible to ciprofloxacin (5), gentamicin (10), kanamycin (30), lincomycin (2), nitrofuran (300), oxacillin (5), streptomycin (10), and sulfonamide (300). The predominant cellular fatty acids are $C_{15:0}$ iso, $C_{15:0}$ anteiso, and $C_{16:0}$.

DNA G+C content (mol%): 71 (HPLC).

Type strain: HKI 0122, DSM 12333, ATCC BAA-8, CCUG 43141, CIP 106362, IFO (now NBRC) 16432, JCM 11478.

Sequence accession no. (16S rRNA gene): Y18378.

Genus II. **Miniimonas** Ue, Matsuo, Kasai and Yokota 2011, 125[VP]

Peter Kämpfer

Mi.ni.i.mo′nas. L.adj. *minius* cinnabar-red, vermilion; L. fem. n. *monas* a unit, monad; N.L. fem. n. *Miniimonas* vermilion monad, referring to the cell mass color.

Cells are irregular rods 0.6 × 3.1 μm) **and cocci** (1.0–1.7 μm). Gram-stain-positive, not acid-fast. **Endospores are not formed. Nonmotile**. **Facultatively** an**aerobic.** Good growth on complex organic media at 28–30°C. NaCl in the culture medium may be tolerated up to 5%. **Oxidase-negative, catalase-positive**. The **peptidoglycan type is A4β** based on L-ornithine. The **acyl type is acetyl**. Whole cell sugars are **glucose, xylose, and ribose**. The predominant menaquinone is **$MK\text{-}8(H_4)$**. The

polar lipids consist of **phosphatidylglycerol, diphosphatidylglycerol, phosphatidylinositol, and one unknown phospholipid. The cellular fatty acid profile** is dominated by the occurrence of **iso- and anteiso-branched-chain acids.** Mycolic acids are absent. Based on 16S rRNA gene sequence similarities, the genus is closely related the other genera of the family *Beutenbergiaceae* (96.3–97.3%).

DNA G+C content (mol%): 74.2 (HPLC).

Type species: **Miniimonas arenae** Ue, Matsuo, Kasai and Yokota 2011, 125[VP].

Further descriptive information

Currently the genus is represented by the only species *Miniimonas arenae* which accommodates only one strain. Comparison of 16S rRNA gene sequences showed that the type strain of *Miniimonas arenae* shares similarity with the type strains of *Beutenbergia cavernae* (96.3%), *Serinibacter salmoneus* (95.1%), and *Salana multivorans* (96.1%). The peptidoglycan of *Miniimonas arenae* contains Ala, Ser, Glu, and Orn in a molar ratio of 0.4:0.5:1.0:0.4. The interpeptide bridge consists of L-Orn–L-Glu which is in accordance with the peptidoglycan type A4β (Schleifer and Kandler, 1972).

Enrichment and isolation procedures

Miniimonas arenae grows readily in complex liquid or on solidified media, i.e. Bacto nutrient agar (Difco, g/l: Bacto beef extract, 3.0; Bacto peptone, 5.0, and Bacto agar, 15.0; pH 6.8), tryptone soy agar (Oxoid), H medium (Ue et al., 2011), and IL8 medium [(g/l): lactose 10 g, polypeptone 15 g, yeast extract 15 g, NaCl 10 g, and agar 15 g] at 28–30°C. The type strain of *Miniimonas arenae* was isolated from sea sand collected from Teguma fishing harbor in Nagasaki Perfecture (Japan) on H medium (Ue et al., 2011).

Maintenance procedures

Cultures of *Miniimonas arenae* can be maintained by serial transfers on the solidified complex media listed above. Growth on agar slants in screw-capped tubes can be kept at 4°C for about 2–4 weeks. Long-term preservation of liquid cultures supplemented with 5% dimethylsulfoxide is recommended in the vapor phase of liquid nitrogen (–140°C).

Differentiation of the genus *Miniimonas* from other genera

Miniimonas can be distinguished from the other genera of the family *Beutenbergiaceae* by differences in the family specific signature nucleotides. The pattern of 16S rRNA signatures consists of nucleotides at positions 140:223 (G–C), 589:128 650 (U–A), 610 (U), 612:628 (C–G), 616:624 (G–C), 839:847 (C–A) and 863 (A) (Ue et al., 2011), which is different from the other genera of the family *Beutenbergiaceae*. The 16S rRNA gene sequence similarity to the type strains of the genera *Beutenbergia*, *Salana*, and *Serinibacter* is 95.1–96.3%, and some phenotypic characteristics (Table 107) are characteristic for the genus. As all the members of the family *Beutenbergiaceae* are currently represented by only one or two species (Groth et al., 1999b; Hamada et al., 2009; von Wintzingerode et al., 2001), the phylogenetic and phenotypic differences between the genera will become more obvious when other strains of the family become available. The closest phylogenetic neighbor of *Miniimonas* within the *Beutenbergiaceae* is the genus *Serinibacter* (Figure 142, chapter *Beutenbergiaceae*).

List of species of the genus *Miniimonas*

1. **Miniimonas arenae** Ue, Matsuo, Kasai and Yokota 2011, 125[VP]

a.re′na.e. L.gen. n. *arenae* of sand, isolated from sea sand, referring to the source of isolation of the organism.

The description is the same as given for the genus. In addition the organism displays the following phenotypic characteristics. Cells sizes in rods vary from 0.6–3.7 µm in length. The diameters of the cocci are 1.0–1.7 µm. Growth occurs at 25–30°C and a wide pH range (5–11) and optimum (7–7.5). Cells grow in absence of NaCl but tolerate up to 5% NaCl (w/v). When grown aerobically for 7 d on LB agar or IL8 medium, a vermilion cell mass is formed. The organism does not form spores. Cells form circular and smooth colonies of 1–4 mm diameter. Nitrate is reduced to nitrite. H_2S is not produced. Hydrolysis of starch is negative. Based on API ZYM tests, cells are positive for esterase lipase (C8), leucine arylamidase, naphthol AS-BI-phosphohydrolase, α-galactosidase, β-galactosidase, α-glucosidase, and β-glucosidase but negative for alkaline phosphatase, lipase (C14), valine arylamidase, cystine arylamidase, trypsin, chymotrypsin, β-glucuronidase, *N*-acetyl-β-glucosaminidase, α-mannosidase, and α-fucosidase. Cells are weakly positive for esterase (C4) and acid phosphatase. Utilizes the following substrates a sole carbon sources: L-arabinose, D-mannose, D-xylose, D-glucose, D-fructose, L-rhamnose, D-maltose monohydrate, glucose, but cannot utilize D-mannitol. Anaerobic formation of acid from API 20A is positive for glucose, sucrose, maltose, D-xylose, L-arabinose, D-mannose, D-raffinose, L-rhamnose, and D-trehalose, weakly positive for D-cellobiose and negative for D-mannitol, lactose, salicin, glycerol, D-melezitose, and D-sorbitol. Based on API 20A tests, cells are anaerobically positive for the hydrolysis of esculin but negative for the hydrolysis of gelatin, the production of indole, arginine dihydrolase, and urease. The peptidoglycan type, polar lipids, fatty acid pattern, and major menaquinone are as described for the genus. The predominant cellular fatty acids (for the type strain in %) are $C_{15:0}$ anteiso (46.7) and $C_{16:0}$ (23.2), and minor fatty acids are $C_{17:0}$ anteiso (9.4), $C_{14:0}$ (5.4), $C_{16:0}$ iso (3.8), $C_{15:0}$ iso (3.4 %), $C_{14:0}$ iso (2.8), $C_{15:0}$ (2.3), and $C_{17:0}$ iso (1.5).

Source: sea sand from Teguma fishing harbor in Nagasaki Prefecture (Japan).

DNA G+C content (mol%): 74.2 (HPLC).

Type strain: YM18-15, NBRC 106267, KCTC 19750, MBIC 08348.

Sequence accession no. (16S rRNA gene): AB522642.

Genus III. **Salana** von Wintzingerode, Göbel, Siddiqui, Rösick, Schumann, Frühling, Rohde, Pukall and Stackebrandt 2001, 1659[VP]

Erko Stackebrandt

Sa.la′na. N.L. fem. n. *Salana* referring to the German river Saale, the source of the bioreactor culture.

Irregular rods and cocci, sometimes club-like forms (Figure 145). Gram-stain-positive. Facultatively anaerobic bacteria. **Anaerobically reduces selenate to elemental selenium.** A variety of organic electron donors may be utilized. The peptidoglycan is of the A4β type with an L-Orn ← L-Glu interpeptide bridge and an L-Ser residue at position 1 of the peptide subunit. The polar lipids are phosphatidylglycerol, diphosphatidylglycerol, and several unidentified phospholipids in minor amounts. The fatty acid pattern is of the iso and anteiso branched and straight chain saturated type. The major menaquinone is MK-8(H_4).

DNA G+C content (mol%): 75.

Type species: **Salana multivorans** von Wintzingerode, Göbel, Siddiqui, Rösick, Schumann, Frühling, Rohde, Pukall and Stackebrandt 2001, 1659[VP].

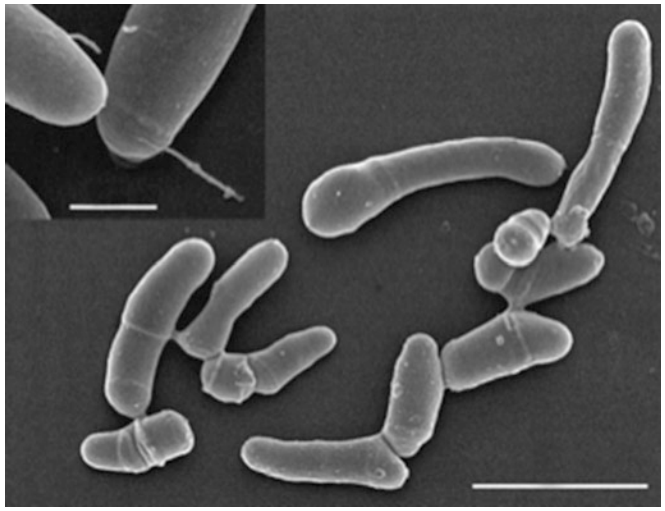

FIGURE 145. Scanning electron micrograph of cells of *Salana multivorans* Se-3111[T]. (main) Rod-shaped, coccoid and club-like cell morphotypes of strain Se-3111[T] (bar = 2 μm). (inset) Stalk-like elements located at the cell pole (bar = 0.2 μm).

Further descriptive information

The type strain DSM 13521[T] and two additional strains were characterized and found identical in all tests performed. Only the type strain was maintained in public collections. Strains were able to grow under aerobic and anaerobic conditions. Acidification of growth medium occurred in the presence of a large range of sugars and carbohydrates and the acidification by *Salana multivorans* was more pronounced than that by its phylogenetic neighbor, *Beutenbergia cavernae* (Groth et al., 1999b). API ZYM reactions were as follows: positive for alkaline phosphatase, esterase (C_4), esterase lipase (C_8), leucine arylamidase, cystine arylamidase, phosphatase acid, naphthol-AS-BI-phosphohydrolase, α-galactosidase, β-glucosidase, β-galactosidase, α-glucosidase, *N*-acetyl-β-glucosaminidase, α-mannosidase, valine arylamidase; negative for trypsin, chymotrypsin, α-fucosidase, and β-glucuronidase. Lipase (C_{14}) weak. Acid was formed under aerobic conditions (according to API 50CH) from glycerol, D-arabinose, L-arabinose, ribose, D-xylose, methyl β-D-xyloside, methyl α-D-mannoside, amygdalin, arbutin, salicin, cellobiose, lactose, inulin, melezitose, starch, glycogen, xylitol, gentobiose, galactose, D-glucose, D-fructose, D-mannose, maltose, melibiose, sucrose, trehalose, D-raffinose, D-turanose, and D-lyxose. Negative acid formation from erythritol, L-xylose, adonitol, L-sorbose, rhamnose, dulcitol, inositol, mannitol, sorbitol, α-methyl-D-glucoside, *N*-acetylglucosamine, D-tagatose, D-fucose, L-fucose, D-arabitol, L-arabitol, gluconate, 2-keto-gluconate, and 5-keto-gluconate. Anaerobically acid was produced from glucose, mannitol, lactose, sucrose, maltose, xylose, salicin, arabinose, glycerol, cellobiose, mannose, melezitose, raffinose, sorbitol, rhamnose, and trehalose. All strains formed insoluble, elemental selenium when grown anaerobically in selenate containing medium (3.9×10^{-6}–2.8×10^{-5} mol/l). Cells formed slightly red colored colonies (diameter, 0.3–0.6 mm) when grown under anaerobic conditions for 14 d on selenate containing medium. When grown aerobically for 6 d on LB or Columbia blood agar, strains form yellow or white-gray colonies of 1.0 or 2.0 mm diameter. Stalk-like elements of up to 300 nm were visible at the poles of some cells (Figure 145, inset). The peptidoglycan contained Ala, Ser, Orn, and Glu in a molar ratio of 0.6:0.9:1.0:1.5. Detailed analysis (von Wintzingerode et al., 2001) indicated that *Salana multivorans* strains were characterized by novel peptidoglycan structure with an L-Orn ← L-Glu interpeptide bridge and an L-Ser residue at position 1 of the peptide subunit.

Enrichment and isolation procedures

Using culture conditions favoring enrichment of anaerobic, selenate respiring organisms, three strains (DSM 13521[T], Se-13111, and Se-1311) were isolated (von Wintzingerode et al., 1999). Only the type strain has been maintained in public collections. Samples were taken from a fluidized bed reactor (FBR) inoculated with an anaerobic, trichlorobenzene-dechlorinating consortium enriched from sediment of the river Saale near Jena, Germany (Selent, 1999). Within the FBR the dechlorinating consortium was immobilized on polyurethane foam cubes. For enrichment of anaerobic, selenate-reducing bacteria, foam cubes were removed from the bioreactor by a sterile forceps and transferred to reduced RAMM-medium (Shelton and Tiedje, 1984). After 7 d of incubation at 30°C under anaerobic conditions (GasPak anaerobic jars, Anaerogen), enrichment cultures were set up by transferring one foam cube each to Erlenmeyer flasks containing 50 ml of Se-medium (Macy et al., 1989): 2.2 g/l NaCl, 0.3 g/l KCl, 0.3 g/l NH_4Cl, 0.2 g/l KH_2PO_4, 0.15 g/l $CaCl_2{\cdot}2\,H_2O$, 0.4 g/l $MgCl_2{\cdot}6\,H_2O$, 0.6 g/l $NaHCO_3$, 3.78 g/l Na_2SeO_4, 3.78 g/l potassium acetate, 10 ml/l trace metal solution SL8, 10 ml/l vitamin solution, 800 μl/l methanol. After 5 d of anaerobic incubation in anaerobic jars (CO_2, N_2, and H_2 atmosphere), a red precipitate was visible and served as an inoculum for subcultures in modified Se-medium (1–4 g/l yeast extract, 1.2 g/l NaCl, 0.3 g/l Na_2SO_4, and 2.02 g/l KNO_3).

Pure cultures were obtained by repeated streaking on agar plates containing the above medium.

Maintenance procedures

After isolation cells were stored at −70°C in liquid growth medium supplemented with 30–50% (v/v) glycerol. Long-term preservation in liquid nitrogen.

Differentiation of the genus *Salana* from other genera

16S rRNA gene sequence analysis reveals the moderate relationship of *Salana multivorans* and *Beutenbergia cavernae* (95% similarity), suborder *Micrococcineae* (elevated to order *Micrococcales* in the present volume), class *Actinobacteria* (Stackebrandt et al., 1997), while relationships with the other members of the order were slightly lower (90% and 93%). The distance between branching points of the *Salana/Beutenbergia* lineage is statistically insignificant in distance matrix (De Soete, 1983; Figure 146) and maximum-likelihood (Felsenstein, 1993; not shown) 16S rDNA gene dendrograms. In the present volume, *Salana* is classified in the family *Beutenbergiaceae*, along with the genera *Beutenbergia*, *Serinibacter*, and *Miniimonas*. Physiological differences between *Salana multivorans* and *Beutenbergia cavernae* are shown in Table 109. Few morphological and chemotaxonomic characteristics differentiate *Salana multivorans* DSM 13521[T] from species of *Beutenbergia*, *Ruania*, *Georgenia*, *Bogoriella*, *Ornithinococcus*, and *Ornithinomicrobium*. These include mainly the composition, type, and variant of the peptidoglycan (Table 110). *Salana multivorans* and *Beutenbergia cavernae* also differ in the amino acid at position 1 of the peptide side chain (L-Ser and L-Ala, respectively). The latter two taxa also contain ornithine (A4β) in their murein and isoprenoid quinones of the MK-8(H_4) type.

TABLE 109. Differences in physiological characteristics of *Salana multivorans* and *Beutenbergia cavernae*[a,b]

Reaction	*Salana multivorans*	*Beutenbergia cavernae*
API 20A, acidification of:		
Glucose	+	v
Mannitol	+	−
Lactose	+	−
Sucrose	+	v
Maltose	+	v
Xylose	+	v
Salicin	+	v
Arabinose	+	v
Glycerol	+	−
Cellobiose	+	−
Mannose	+	v
Melezitose	+	−
Raffinose	+	−
Sorbitol	+	−
Rhamnose	+	−
Trehalose	+	−
API ZYM:		
Lipase (C14)	v	−
Valine arylamidase	+	−
Trypsin	v	−
β-Glucosidase	+	−
α-Fucosidase	−	+
API 50 CH, aerobic utilization with acid formation of:	v	−
Glycerol		
D-Arabinose	v	+
Ribose	v	+
D-Xylose	+	+
β-Methyl-D-xyloside	v	−
α-Methyl-D-mannoside	+	−
Amygdalin	v	−
Arbutin	+	−
Maltose	+	+
Lactose	+	−
Melezitose	+	−
Glycogen	+	−
Xylitol	+	−
Gentiobiose	+	−

[a]Abbreviations: +, positive; −, negative; v, variable; w, weak.

[b]Both type strains are negative for acidification of gelatin and esculin.

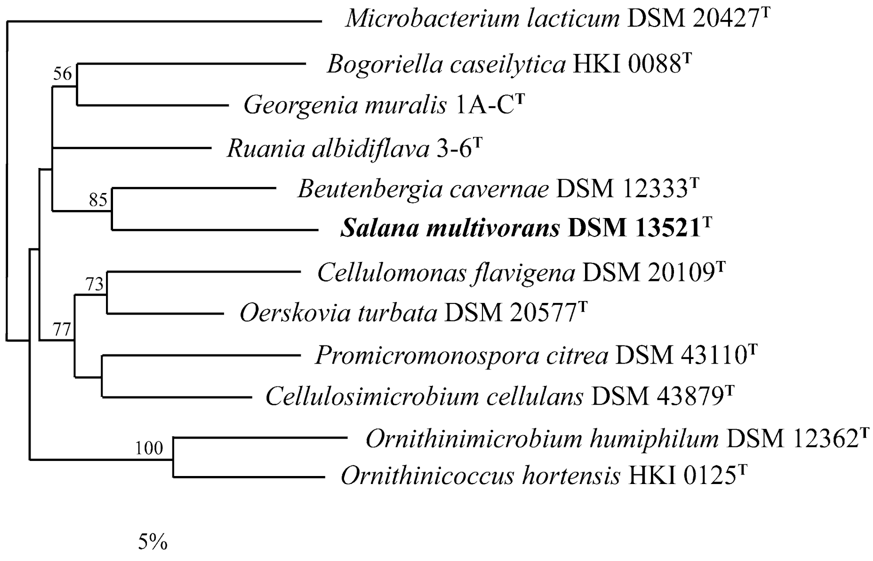

FIGURE 146. 16S rDNA based phylogenetic dendrogram (De Soete, 1983) constructed from a distance matrix shows the phylogenetic position of strain *Salana multivorans* Se-3111^{T} and some members of the order *Micrococcales*. Numbers at relevant branching point refer to bootstrap values (500 resamplings; Felsenstein, 1985). Scale bar represents 5% sequence divergence.

TABLE 110. Differential morphological and chemotaxonomic characteristics of *Salana multivorans*, phylogenetic neighbors, and two actinobacterial taxa with murein containing ornithine (A4β) and MK-8(H_4)[a]

Characteristics	*Salana*	*Beutenbergia*[b]	*Ruania*[c]	*Georgenia*[c]	*Bogoriella*[c]	*Ornithinococcus*[d]	*Ornithinomicrobium*[e]
Cell morphology	Rods, cocci, club-like	Rods/cocci cycle	Coccoid	Rod, cocci	Irregular rods, cocci	cocci	Irregular rods, cocci
DNA G+C content (mol%)	75	71	70	70	70	72	70
Peptidoglycan[f]	L-Orn–L-Glu	L-Lys–L-Glu	L-Lys–Gly–L-Glu–L-Glu	L-Lys–L-Glu	L-Lys–L-Ala$_2$-L-Glu	L-Orn–Gly$_{(1–2)}$-D-Glu	L-Orn–L-Ala–Gly–D-Asp
Murein type	A4β	A4α	A4α	A4α	A4α	A4β	A4β
Polar lipids	PG, DPG, PLs	PI, DPG, 3 PLs	PG, DPG, GL	PG, DPG, PIM, PL, GL	PI, PG, DPG, PL	PI, PG, DPG, Pser, PLs	PI, PG, DPG, PLs, GLs

[a]Abbreviations: L-Ala, L-alanine; D-Asp, D-aspartic acid; L-Glu, L-glutamic acid; L-Lys, L-lysine; L-Ser, L-serine; L-Orn, L-ornithine; DPG, diphosphatidylglycerol; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; PI, phosphatidylinositol; Pser, phosphatidylserine, PL(s), unknown phospholipid(s); GL(s), unknown glycolipid(s).

[b]Data from Groth et al. (1999b).

[c]Data from Gu et al. (2007).

[d]Data from Groth et al. (1999a).

[e]Data from Groth et al. (2001).

[f]Amino acid at pos. 1/Interpeptide bridge.

List of species of the genus *Salana*

1. **Salana multivorans** von Wintzingerode, Göbel, Siddiqui, Rösick, Schumann, Frühling, Rohde, Pukall and Stackebrandt 2001, 1659[VP]

mul.ti.vo′rans. L. adj. *multus* many, numerous; L. v. *vorare* to devour, swallow; N.L. part. adj. *multivorans* devouring many, referring to the utilization of numerous kinds of substrates.

Rod-shaped to circular, sometimes club-like cells (0.4–0.7 μm in width, 1.2–3.1 μm in length). Electron microscopy showed the presence of polar stalk-like elements of up to 300 nm in length in some cells. Gram-stain-positive, nonmotile. Growth with formation of acid occurs under both aerobic and anaerobic conditions (API 20A and API 50CH substrate panels). Gelatin and esculin not hydrolyzed. Indole not formed, urease-negative. Catalase and oxidase reactions weak. Under anaerobic conditions, cells reduce selenate to elemental selenium. Amino-functional lipids and glycolipids are not found. The cellular fatty acid profile contains: 23.0%

tetradecanoic acid, 1.0% pentadecanoic acid, 17.0% hexadecanoic acid, 2.9% 12-methyl tridecanoic acid, 2.3% 14-methyl pentadecanoic acid, 50.4% 12-methyl-tetradecanoic acid, and 1.5% 14-methyl hexadecanoic acid. The major menaquinone is MK-8(H_4). MK-6(H_4), MK-7(H_4), and MK-8(H_2) occur as minor components. Some metabolic properties are described in *Further descriptive information*, above, and in Table 109.

Source: an anaerobic, dechlorinating bioreactor culture enriched from sediment of the German river Saale.

DNA G+C content (mol%): 75 (HPLC).

Type strain: Se-3111, DSM 13521, NRRL B-24118, JCM 13524.

Sequence (EMBL) accession no. (16S rRNA gene): AJ400627.

Genus IV. **Serinibacter** Hamada, Iino, Tamura, Iwami, Harayama and Suzuki 2009, 2812^{VP}

Peter Kämpfer

Se.ri.ni.bac′ter. N.L. n. *serinum* serine; N.L. masc n. *bacter* rod; N.L. masc. n. *Serinibacter* a rod with serine in the cell wall.

Cells are irregular rods (0.5 × 1.0–2.0 µm). Gram-stain-positive, not acid-fast. **Endospores are not formed. Nonmotile. Aerobic to anaerobic.** Good growth on complex organic media at 28–30°C. NaCl in the culture medium may be tolerated up to 10%. **Oxidase-negative, catalase-positive**. The **peptidoglycan type is A4α** with an L-Ser residue at position 1 of the peptide subunit. The **acyl type is acetyl**. The major cell wall sugar is **galactose**. The predominant menaquinone is **MK-8(H_4)**. The major polar lipids consist of **phosphatidylethanolamine and phosphatidylglycerol. The cellular fatty acid profile** is dominated by the occurrence of **iso- and anteiso-branched-chain acids.** Mycolic acids are absent. Based on 16S rRNA gene sequence similarities, the genus is closely related to other genera of the family *Beutenbergiaceae* (95.1–96.3%).

DNA G+C content (mol%): 70.7 (HPLC).

Type species: **Serinibacter salmoneus** Hamada, Iino, Tamura, Iwami, Harayama and Suzuki. 2009, 2812^{VP}.

Further descriptive information

Currently the genus is represented by the only species *Serinibacter salmoneus* which accommodates only one strain. Comparison of 16S rRNA gene sequences showed that the type strain of *Serinibacter salmoneus* strains shares similarity with the type strains of *Beutenbergia cavernae* (96.3%), *Miniimonas arenae* (95.1%), and *Salana multivorans* (96.1%). Patterns of selected 16S rRNA nucleotide signatures are given in Table 108. The peptidoglycan of *Serinibacter salmoneus* contains Ala, Glu, Lys, and Ser in a molar ratio of 1.0:2.1:0.9:1.0. The enantiomeric analysis of the peptidoglycan amino acids revealed the presence of D-Ala, D-Glu, L-Glu, L-Ser, and L-Lys; however, L-Ala was absent. These data indicated that the cell-wall peptidoglycan of strain Kis4-28^T was of the A4α type (Schleifer and Kandler, 1972), with lysine as the diagnostic cell-wall diamino acid, an interpeptide bridge of the peptidoglycan comprising L-Lys–L-Glu, and an L-Ser residue at position 1 of the peptide subunit (Hamada et al., 2009). The major cellular fatty acids of the type strain of *Serinibacter salmoneus* were $C_{15:0}$ anteiso (45.7), $C_{16:0}$ (24.4%), $C_{16:0}$ iso (11.5%), $C_{17:0}$ anteiso (10.5%), and minor fatty acids including $C_{14:0}$ (4.1%) and $C_{14:0}$ iso (3.8%). Beside galactose, which was the major cell-wall sugar, mannose, ribose, and xylose were detected as minor components (Hamada et al., 2009).

Enrichment and isolation procedures

Serinibacter salmoneus grows readily in complex liquid or on solidified media, i.e. Bacto nutrient agar (Difco, g/l: Bacto beef extract, 3.0; Bacto peptone, 5.0 and Bacto agar, 15.0; pH 6.8) and tryptone soy agar (Oxoid). The type strain of *Serinibacter salmoneus* was isolated from Japanese sillago (*Sillago japonica*), a fish collected from Kyonan beach on the coast of Tokyo Bay, Japan (Hamada et al., 2009). The sillago was dissected, and an intestinal tract sample of approximately 1 g was used for the isolation of bacteria. The procedure employed for bacterial isolation was as described by Iino et al. (2007). NBRC medium 802 containing 1.0% Polypeptone (Wako), 0.2% yeast extract (Difco), 0.1% $MgSO_4 \cdot 7H_2O$, and 1.5% agar (if required) was used for general laboratory cultivation (Hamada et al., 2009).

Maintenance procedures

Cultures of *Miniimonas arenae* can be maintained by serial transfers on the solidified complex media listed above. Growth on agar slants in screw-capped tubes can be kept at 4°C for about 2–4 weeks. Long-term preservation of liquid cultures supplemented with 5% dimethylsulfoxide is recommended in the vapor phase of liquid nitrogen (−140°C).

Differentiation of the genus *Serinibacter* from other genera

Serinibacter can be distinguished from the other genera of the family *Beutenbergiaceae* by differences in the family specific signature nucleotides (Table 108). A detailed phylogenetic analysis performed on the basis of the 16S rRNA gene sequence revealed that *Serinibacter salmoneus* formed a separate lineage with the type strains of *Salana multivorans*, *Beutenbergia cavernae*, and *Miniimonas arenae* (Figure 142). The amino acid composition of the peptidoglycan of *Serinibacter* was different from that of the genera *Salana*, *Beutenbergia*, and *Miniimonas*. The peptidoglycan of the isolate was of the A4α type, with L-Lys as the diagnostic cell-wall diamino acid. In contrast, the peptidoglycan of the genus *Salana* is reported to be of the A4β type, with L-ornithine as the diagnostic diamino acid (von Wintzingerode et al., 2001). The peptidoglycan of the genus *Miniimonas* is also reported to be of the A4β type but contained L-ornithine as the diagnostic diamino acid (Ue et al., 2011). In addition, the polar lipids are significantly different. Although the peptidoglycan type in species of the genus *Beutenbergia* is also A4α (Groth et al., 1999b), *Serinibacter* is different from this genus in that it contained L-Ser at position 1 of the peptide subunit. In addition, *Serinibacter* possesses phosphatidylethanolamine as a polar lipid. These data indicated that the chemotaxonomic characteristics were distinct from those of its phylogenetic neighbors (Table 107).

List of species of the genus *Serinibacter*

1. **Serinibacter salmoneus** Hamada, Iino, Tamura, Iwami, Harayama and Suzuki 2009, 2813[VP]

sal.mo′ne.us. N.L. masc. adj. *salmoneus* salmon-colored, yellowish pink, because the cells grown in liquid culture are yellowish pink.

Cells are 0.4–0.5 × 1.0–2.0 µm and nonmotile. Colonies are circular, smooth and yellowish orange, but cells grown in liquid culture are yellowish pink. Catalase-positive, oxidase-negative. The Voges–Proskauer test is positive, while the methyl red test is negative. Growth occurs at NaCl concentrations of 0–10% (w/v) but not at 15%, and the optimal NaCl concentration range for growth is 0–5%. The temperature range for growth is 10–37°C, and the optimal temperature is 28°C. The pH range for growth is 6.0–9.0, and the optimal pH is 7.0. Acid is produced from *N*-acetylglucosamine, cellobiose, D-fructose, D-galactose, gentiobiose, D-glucose, glycogen, lactose, maltose, D-mannose, methyl-α-D-glucopyranoside, methyl-β-D-xylopyranoside, starch, sucrose, trehalose, and D-xylose. Esterase lipase (C8), leucine arylamidase, acid phosphatase, α-galactosidase, β-galactosidase, α-glucosidase, pyrazinamidase, and pyrrolidonyl arylamidase are present, whereas alkaline phosphatase, lipase (C4), valine arylamidase, cystine arylamidase, trypsin, chymotrypsin, β-glucuronidase, *N*-acetyl-β-glucosaminidase, α-fucosidase, arginine dihydrolase, lysine- and ornithine decarboxylases, and tryptophan- and phenylalanine deaminases are absent. H_2S and indole are not produced. Esculin is hydrolyzed, while gelatin, urea, and DNA are not hydrolyzed. Nitrate is not reduced. The interpeptide bridge of the cell-wall peptidoglycan is L-Lys–L-Glu. The major cellular fatty acids are $C_{15:0}$ anteiso and $C_{16:0}$, followed by $C_{16:0}$ iso and $C_{17:0}$ anteiso.

Source: intestinal tract of sillago fish (*Sillago japonica*) collected from Kyonan beach on the coast of Tokyo Bay, Japan.

DNA G+C content (mol%): 70.7 (HPLC).

Type strain: Kis4-28, NBRC 104924, DSM 21801.

Sequence accession no. (16S rRNA gene): AB455532.

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Family III. **Bogoriellaceae** Stackebrandt and Schumann 2000, 1283^{VP} emend. Zhi, Li and Stackebrandt 2009, 597^{VP} emend. Hamada, Iino, Tamura, Iwami, Harayama and Suzuki 2009, 2813

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Bo.go.ri.el.la.ce′a.e. N.L. fem. n. *Bogoriella* type genus of the family; -*aceae* ending to denote a family; N.L. fem. pl. n. *Bogoriellaceae* the family of *Bogoriella*.

Cell morphology comprises **irregular rods, cocci** and occasionally filaments. Cells occur singly, in pairs, or in small clusters. A rod–coccus growth cycle may be expressed. Gram-stain-positive. **Nonsporeforming**. **Nonmotile** or **motile. Aerobic to microaerobic or facultatively anaerobic**. Colonies are circular, convex, and vary in their color from white to yellow. Members of the family grow well on complex organic media. Growth temperature 10–45°C. Tolerance to alkaline pH values and to NaCl in the culture medium is variable. Some of the strains are alkaliphilic and may tolerate up to 10% NaCl. **Oxidase-positive or -negative**; **catalase-positive**. The characteristic **diamino acid in the peptidoglycan is L-lysine** which corresponds to the **peptidoglycan type A4α**. The major menaquinone is **MK-8(H_4)**. The polar lipids typically comprise **diphosphatidylglycerol and phosphatidylglycerol; phosphatidylinositol or phosphatidylinositol mannoside**, and unidentified phospholipids or glycolipids may additionally be present. The cellular fatty acid profiles are dominated by **iso- and anteiso-branched-chain acids** and minor amounts of saturated straight-chain acids. Mycolic acids are absent. The family is a member of the order *Micrococcales* and currently accommodates the genera *Bogoriella* and *Georgenia*. The common 16S rRNA gene signature nucleotides of the members of the family are: nucleotides at positions 144:178 (U–G), 293:304 (G–U), 479 (U), 602:636 (C–G), 668:738 (A–U), 1003:1038 (G–C), 1027:1034 (C–G) and 1409:1491 (C–G).

DNA G+C content (mol%): 70–73.

Type genus: **Bogoriella** Groth, Schumann, Rainey, Martin, Schuetze and Augsten 1997a, 793.

Further descriptive information

The genus *Georgenia* (Altenburger et al., 2002; Li et al., 2007) is represented by three species, while the genus *Bogoriella* (Groth et al., 1997a) accommodates only one species. The members of the *Bogoriellaceae* are morphologically similar and comprise cells exhibiting coccus or rod–coccus morphologies. However, a broad spectrum of physiological properties together with the chemotaxonomic characteristics listed in Table 111 serve to distinguish the genera from one another. The phylogenetic positions of the members of the family *Bogoriellaceae* are shown in the 16S rRNA gene sequence based phylogenetic tree (Figure 142). Differentiating signature nucleotides are given in Table 112. Strains of the *Bogoriellaceae* originate from differ-

TABLE 111. Phenotypic characteristics differentiating the genera of the family *Bogoriellaceae*[a,b]

Characteristic	*Bogoriella*[c]	*Georgenia*[d]
Cell morphology	Irregular rods, coccoid cells, filaments	Short rods and cocci, rod–coccus cycle
Relationship to oxygen	Aerobic to microaerobic	Aerobic and facultatively anaerobic
Oxidase	–	+
Cell-wall diamino acid	L-Lys	L-Lys
Interpeptide bridge	L-Lys–L-Ala–L-Ala–L-Glu	L-Lys–L-Glu or L-Lys–L-Ala–L-Glu
Predominant cellular fatty acids	$C_{15:0}$ anteiso, $C_{17:0}$ anteiso	$C_{15:0}$ anteiso, $C_{15:0}$ iso, $C_{14:0}$ iso
Polar lipids	PG, DPG, PI, 1 PL	DPG PG; PI or PIM, PL, GL
DNA G+C content (mol%)	70	70–73

[a]Symbols: +, positive; –, negative.

[b]Abbreviations: DPG, diphosphatidylglycerol; GL, unidentified glycolipid; PG, phosphatidylglycerol; PI, phosphatidylinositol; PIM, phosphatidylinositol mannoside; PL, unidentified phospholipid.

[c]Data from Groth et al. (1997a).

[d]Data from Altenburger et al. (2002); Li et al. (2007), and Hamada et al. (2009a).

TABLE 112. 16S rRNA gene signature nucleotides that differentiate the members of the family *Bogoriellaceae*[a]

Position	*Bogoriella*	*Georgenia*
66:99	A–U	A–U
140:223	G–U	G–U
144:178	U–G	U–G
589:650	C–G	U–A
602:636	C–G	C–G
610	A	A
612:628	C–G	G–C
616:624	G–C	G–C
615:625	G–C	A–U
839:847	C–G	C–G
863	U	U
1133:1141	A–U	G–C
1134:1140	C–G	G–C
1254:1283	G–C	G–C
1263:1272	A–U	G–U
1414:1486	C–G	C–G

[a]Gu et al. (2007).

ent habitats: soda soil (*Bogoriella*), soil from a forest (*Georgenia ruanii*, *Georgenia thermotolerans*; Li et al., 2007; Hamada et. al., 2009a) and from a medieval wall painting in case of *Georgenia muralis* (Altenburger et al., 2002).

Taxonomic comments

The family *Bogoriellaceae* was established by Stackebrandt and Schumann (2000) for the monospecific genus *Bogoriella*. At that time it had become evident that the phylogenetic lineage of *Bogoriella caseilytica* branches among other lineages within the suborder *Micrococcineae* described as individual families. The subsequent descriptions of the genera *Salana* (von Wintzingerode et al., 2001), *Georgenia* (Altenburger et al., 2002) and *Ruania* (Gu et al., 2007) indicated a close phylogenetic relationship between these three genera and the genera *Beutenbergia* (Groth et al., 1999) and *Bogoriella*, based on the levels of 16S rRNA gene sequence similarities and the correspondence in the patterns of 16S rRNA gene sequence signature nucleotides. But the update of the patterns of 16S rRNA gene sequence signature nucleotides revealed that the genera *Beutenbergia*, *Georgenia* and *Salana* were members of the new family *Beutenbergiaceae*, and the genus *Bogoriella* remained the only member of the family *Bogoriellaceae* (Zhi et al., 2009). With the subsequent emendation of the descriptions of the families *Beutenbergiacea* and *Bogoriellaceae* by Hamada et al. (2009b), the genus *Georgenia* was grouped into the family *Bogoriellaceae*. The family *Beutenbergiaceae* was defined mainly on the basis of the following 16S rRNA gene sequence signature pattern consisting of nucleotides at positions 144:178 (C–G), 280 (U), 293:304 (G–U), 668:738 (A–U), 1003:1038 (G–U), 1027:1034 (U–A) and 1414:1486 (U–A), and comprised the genera *Beutenbergia*, *Salana* and *Serinibacter* (Hamada et al., 2009b). Recently the genus *Miniimonas* (Ue et al., 2011) was included into this family as its pattern of signature nucleotides was in accordance with that described for the *Beutenbergiaceae*. The genus *Ruania* could not be affiliated to one of the above mentioned related families due to the differences in the 16S rRNA gene sequence signature nucleotides (Hamada et al., 2009b).

Genus I. **Bogoriella** Groth, Schumann, Rainey, Martin, Schuetze and Augsten 1997a, 793[VP]

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Bo.go.ri.el′la. N.L. dim. fem. n. *Bogoriella* named after Lake Bogoria, Kenya.

Cells are irregularly shaped rods or cocci (0.5–0.8 × 1.0–2.5 µm). **Filaments**, up to 10 µm, may appear. Cells occur singly, in pairs, or in small clusters. Gram-stain-positive. Not acid fast. **Endospores are not formed**. **Nonmotile**. **Aerobic to microaerobic**. Colonies are circular, smooth, slightly convex, glistening, and pale to intense yellow. Growth temperature 28–37°C. **Alkaliphilic.** The optimum initial pH for growth in liquid medium A ranges from 8.8–10.1. NaCl in the alkaline medium A is well tolerated up to 8%. **Oxidase-negative; catalase-positive**. The **peptidoglycan type is A4α** with **L-lysine** as the diagnostic diamino acid and an **L-Lys–L-Ala–L-Ala–L-Glu interpeptide bridge.** The **acyl type is acetyl**. **Galactose** is the only whole-cell sugar. The predominant respiratory quinone is **MK-8(H_4).** The phospholipids are **phosphaditylglycerol, diphosphaditylglycerol, phosphaditylinositol, and an unknown phospholipid.** The cellular fatty acid profile is dominated by the occurrence of **anteiso-branched-chain acids**; iso-branched-chain acids occur in minor amounts Mycolic acids are absent. Based on 16S rRNA gene sequence comparison, *Bogoriella* is most closely related to the genera *Georgenia* and *Beutenbergia* (Figure 142). The 16S rRNA gene signature nucleotides that differentiate the genus from the other genus of the *Bogoriellaceae* are listed in Table 11.

DNA G+C content (mol%): 70.

Type species: **Bogoriella caseilytica** Groth, Schumann, Rainey, Martin, Schuetze and Augsten 1997a, 793[VP].

Further descriptive information

Currently the genus is represented by the only species, *Bogoriella caseilytica*, and accommodates a single strain. 16S rRNA gene sequence comparison shows that this strain forms a common branch in the 16S rRNA gene tree of the suborder *Micrococcineae* (Stackebrandt et al., 1997) with the three species of *Georgenia* (Altenburger et al., 2002; Li et al., 2007; Hamada et al., 2009a) within the cluster of the family *Bogoriellaceae* (Stackebrandt and Schumann, 2000) (Figure 142, chapter on *Beutenbergiaceae*, above). *Bogoriella caseilytica* shares 94.7–95.0% 16S rRNA gene sequence similarity with the type strains of *Georgenia muralis* (Altenburger et al., 2002) and *Georgenia ruanii* (Li et al., 2007). The sequence similarities to other related genera are 93.9% (*Beutenbergia*), 93.2% (*Ruania*), and 92.9% (*Salana*), respectively.

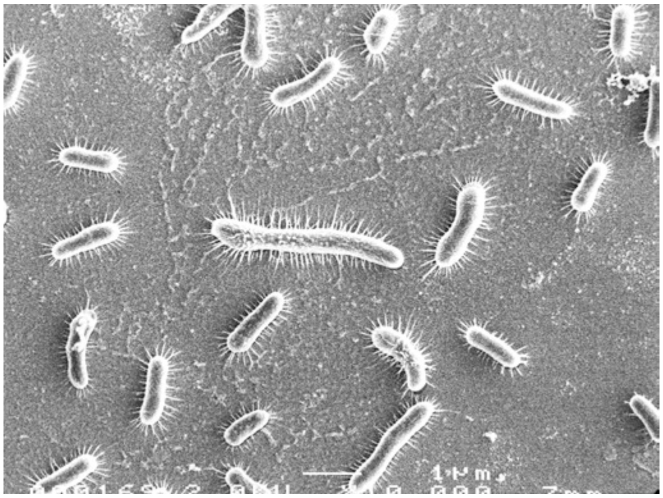

FIGURE 147. Scanning electron micrograph of cells from a 48-h-old culture of *Bogoriella caseilytica* DSM 11294^{T} grown at 28°C on solid medium A. Bar = 1 μm.

Interestingly, a comparable level of 16S rRNA gene sequence similarity (93.7% or 92.8%) is also found between *Bogoriella* and members of the genera *Cellulosimicrobium* (Schumann et al., 2001) or *Promicromonospora* (Krasil'nikov et al., 1961). Both genera are members of the family *Promicromonosporaceae* (Stackebrandt et al., 1997). Scanning electron micrographs reveal that the cells of *Bogoriella caseilytica* possess spiky structures which are distributed over the whole surface of each cell (Figure 147). The peptidoglycan is characterized by the presence of lysine, alanine, and glutamic acid at a molar ratio of 1.0:4.3:2.1 and represents a new variation of the peptidoglycan type A4α (Schleifer and Kandler, 1972) with an L-Lys–L-Ala–L-Ala–L-Glu interpeptide bridge. *Bogoriella caseilytica* grows well on alkaline complex media containing peptone and casein as nitrogen sources and utilizes a broad spectrum of carbon sources.

Enrichment and isolation procedures

Bogoriella caseilytica can be enriched using solidified or liquid medium A (Duckworth et al., 1996) which contains (g/l): glucose, 10.0; peptone (Difco), 5.0; yeast extract (Difco), 5.0; KH_2PO_4, 1.0; $MgSO_4 \cdot 7H_2O$, 0.2; NaCl, 40.0; Na_2CO_3; 10.0, and agar, 20.0; pH 9.6, and incubation temperatures 28–37°C. It is important for the preparation of medium A that NaCl and Na_2CO_3 have to be sterilized separately. They have to be added to the remaining compounds at 60°C before the agar medium is poured into the Petri dishes. A cultivation of the strain is also possible for a limited number of transfers on complex organic media like Bacto nutrient agar (Difco) at a neutral pH value. However, under these conditions growth is reduced. *Bogoriella caseilytica* was isolated from soda soil (pH 10.0) near Lake Bogoria in the Kenyan–Tanzanian Rift Valley using a standard dilution plate procedure and medium A.

Maintenance procedures

Cultures of *Bogoriella caseilytica* are maintained for laboratory cultivation by serial transfers on solidified or in liquid medium A. Growth on agar slants in screw-capped tubes can be kept for about 2–4 weeks at 4°C. Long-term preservation of liquid cultures supplemented with 5% dimethylsulfoxide is recommended in the vapor phase of liquid nitrogen (−140°C).

Differentiation of the genus *Bogoriella* from other genera

The genus *Bogoriella* can be clearly differentiated from the genus *Georgenia* of the family *Bogoriellaceae* by unique morphological, physiological, and chemotaxonomic characteristics; among them are the unusual spiky structures on the cell surfaces which have so far not been reported before in coryneform actinobacteria. *Bogoriella* shares with strains of the genera *Georgenia* (Altenburger et al., 2002; Li et al., 2007; Hamada et al., 2009a), *Beutenbergia* (Groth et al., 1999), *Serinibacter* (Hamada et al., 2009b), *Ruania* (Gu et al., 2007), and *Demetria* (Groth et al., 1997b) the combination of the peptidoglycan type A4α (Schleifer and Kandler, 1972) and MK-8(H_4) as predominating menaquinone. However, while *Bogoriella* has an L-Lys←L-Ala←L-Ala←L-Glu interpeptide bridge, in *Georgenia* two different structures may occur, e.g. L-Lys←L-Glu or L-Lys←L-Ala←L-Glu, respectively. *Beutenbergia* and *Serinibacter* share with *Georgenia muralis* (Altenburger et al., 2002) the interpeptide bridge of L-Lys←L-Glu, while *Ruania* has an interpeptide bridge consisting of L-Lys←Gly←L-Glu←L-Glu which represents a novel murein type (Gu et al., 2007). *Demetria* (family *Dermacoccaceae*, Stackebrandt and Schumann, 2000; Zhi et al., 2009) is characterized by an L-Lys←Ser←D-Asp interpeptide bridge and can be additionally distinguished from *Bogoriella* by its remote phylogenetic relationship (90.4% 16S rRNA gene sequence similarity) and a broad spectrum of physiological characteristics. The genera *Cellulosimicrobium* and *Promicromonospora* can be also readily distinguished from *Bogoriella* by the formation of hyphae, the structure of the peptidoglycan, the presence of MK-9(H_4) as major menaquinone and numerous further phenotypic properties (Schumann et al., 2001; Krasil'nikov et al., 1961).

Taxonomic comments

The genus *Bogoriella* has been established in 1997 to accommodate an unusual actinobacterial strain from an alkaline habitat. At that time, the low degree of 16S rRNA gene similarity with other members of the order *Actinomycetales* (less than 95%) did not justify an affiliation to a higher taxonomic rank for a single strain. However, in 2000, the family *Bogoriellaceae* was established by Stackebrandt and Schumann to accommodate the monospecific genus *Bogoriella* as it became evident that the phylogenetic lineage of *Bogoriella caseilytica* branches among other lineages within the suborder *Micrococcineae* (since elevated to order *Micrococcales* in the taxonomic outline to the present volume) described as individual families. The descriptions of the genera *Beutenbergia*, *Georgenia*, *Ruania* and *Salana* revealed a close phylogenetic relationship of these genera with *Bogoriella*. However, with the update of the structure and 16S rRNA gene sequence based definition of higher ranks of the class *Actinobacteria* it was obvious that the genera *Beutenbergia*, *Georgenia* and *Salana* should be grouped into the new family *Beutenbergiaceae* (Zhi et al., 2009). *Bogoriella* continued to be the only genus in the family *Bogoriellaceae* until the emendation of the descriptions of the families *Beutenbergiaceae* and *Bogoriellaceae* by Hamada et al. (2009b). According to Hamada et al. (2009b) the family *Bogoriellaceae* accommodates the genera *Bogoriella* and *Georgenia* while the family *Beutenbergiaceae* contains the genera *Beutenbergia*, *Salana*, *Serinibacter* and the genus *Miniimonas* which was recently included in this family (Ue et al., 2011).

List of species of the genus *Bogoriella*

1. **Bogoriella caseilytica** Groth, Schumann, Rainey, Martin, Schuetze and Augsten 1997a, 793[VP]

ca. sei.ly′ti.ca. L. n. *caseus* cheese; N.L. adj. *lyticus -a -um* (from Gr. adj. *lutikos -ê -on*), able to loosen, able to dissolve; N.L. fem. adj. *caseilytica* loosening or dissolving casein.

Description is the same as for the genus. In addition, the strain shows the following phenotypic characteristics. The following organic acids are not used as sole carbon sources: acetate, aconitate, benzoate, citrate, formate, malate, succinate, and DL-tartrate. Acids are produced from L-arabinose, D-cellobiose, dextrin, D-fructose (weak), D-galactose, D-glucose, D-glucitol (weak), glycerol, inulin, lactose (weak), maltose (weak), D-mannitol (weak), D-mannose, D-raffinose (weak), L-rhamnose, D-ribose, salicin, sucrose (weak), potato starch, trehalose, and D-xylose. Starch, casein, esculin, and gelatin are hydrolyzed. Adenine, hippurate, hypoxanthine, Tween 80, tyrosine, urea, and xanthine are not decomposed. Furthermore, the following substrates provided in the GP microplates (Biolog) are used as sole carbon sources: amygdalin, L-arabinose, D-arabitol, arbutin, cellobiose, α- and β-cyclodextrin, D-fructose, L-fucose, D-galactose, gentiobiose, α-D-glucose, glycerol, glycogen, α-hydroxybutyric acid, α-D-lactose, lactulose, maltose, maltotriose, D-mannitol, D-mannose, D-melezitose, D-melibiose, methyl-β-D-galactoside, 3-methyl-glucose, methyl-β-D-glucoside, methyl-α-D-mannoside, palatinose, D-raffinose, D-ribose, salicin, D-sorbitol, stachyose (weak), sucrose, D-tagatose, D-trehalose, turanose, D-xylose, methyl pyruvate, and pyruvic acid. Nitrate is not reduced to nitrite. Hydrogen sulfide is produced (weak); indole is not produced. Methyl red and Voges–Proskauer reactions are negative. 48-h-old cultures of *Bogoriella caseilytica* display positive enzymic reactions in the API ZYM enzyme assay (bioMerieux) for acid phosphatase, alkaline phosphatase, esterase (C4), esterase-lipase (C8), leucine arylamidase, β-galactosidase, α-glucosidase, β-glucosidase, *N*-acetyl-β-glucosaminidase, and α-mannosidase. Chymotrypsin, cystine arylamidase, α-fucosidase, α-galactosidase, β-glucuronidase, lipase (C14), naphthol-AS-BI-phosphohydrolase, trypsin, and valine arylamidase are negative. Cells are susceptible to the following antibiotics (μg/disc): ampicillin (10) chloramphenicol (30), ciprofloxacin (5), erythromycin (15), gentamicin (10), kanamycin (30), lincomycin (2), neomycin (30), oxacillin (5), oxytetracycline (30), penicillin G (2 IU/disc), polymyxin B (2 IU/disc), rifampin (2), and streptomycin (10). The cellular fatty acid profile is characterized by the predominance of $C_{15:0}$ anteiso and minor amounts of $C_{17:0}$ anteiso, $C_{15:1}$ iso, and $C_{16:0}$ iso.

DNA G+C content (mol%): 70 (HPLC).

Type strain: HKI 0088, ATCC 700413, CIP 105404, DSM 11294, JCM 11479.

Sequence accession no. (16S rRNA gene): Y09911.

Genus II. **Georgenia** Altenburger, Kämpfer, Schumann, Vybiral, Lubitz and Busse 2002, 880[VP] emend. Li, Xu, Schumann, Zhang, Pukall, Xu, Stackebrandt and Jiang 2007, 1426

Hans-Jürgen Busse

Ge.or.gen′i.a. N.L. fem. n. *Georgenia* referring to the village St Georgen in Styria (Austria), where strains 1A-C[T] and 3A-1 were isolated.

Cells may exhibit a rod–coccus growth cycle. Rods and cocci occur singly or in small clusters. Cocci are 1 μm in diameter; rods are 1–2 ×0.5–1 μm. Gram-stain-positive, non-spore-forming, motile and nonmotile. Growth occurs under both aerobic and anaerobic conditions. Oxidase and catalase positive. Peptidoglycan type is A4α with L-Lys ← L-Glu or L-Lys–L-Ala–L-Glu as the interpeptide bridge (murein type A11.54 or A11.35). Quinone system is menaquinone MK-8(H_4). Polar lipids are diphosphatidylglycerol, phosphatidylglycerol, phosphatidylinositol mannoside or phosphatidylinositol together with unidentified phospholipids, and one unknown glycolipid. The predominant fatty acid is $C_{15:0}$ anteiso; significant amounts of $C_{15:1}$ anteiso, $C_{15:0}$ iso and $C_{14:0}$ iso are present. Polyamine content is low. Major polyamines are spermidine and spermine. Phylogenetically, this genus is a member of the family *Bogoriellaceae*, order *Micrococcales*, class *Actinobacteria*.

DNA G+C (mol%): 70–73.

Type species: **Georgenia muralis** Altenburger, Kämpfer, Schumann, Vybiral, Lubitz and Busse 2002, 880[VP].

Further descriptive information

The genus *Georgenia* was described for two Gram-stain-positive, yellow-pigmented strains which had been isolated from a medieval wall painting in St Georgen, Styria, Austria (Altenburger et al., 2002). Similarity values in the 16S rRNA gene sequences indicate a moderate relatedness to representatives of the genera *Cellulomonas*, *Cellulosibacterium*, *Beutenbergia*, *Bogoriella*, and *Oerskovia*. The two strains of *Georgenia muralis* exhibit a quinone system with the major compound MK-8(H_4). The peptidoglycan type is A4α with an interpeptide bridge L-Lys ← L-Glu. In the polar lipid profile, diphosphatidylglycerol is predominating. Moderate to minor amounts of phosphatidylinositol-mannosides, two unknown phospholipids, an unknown glycolipid, and phosphatidylglycerol are present as well. The yellow pigmentation is based on at least five different compounds. The fatty acid profile is predominated by the presence of methyl-branched fatty acids of the iso- and anteiso-type with the major compound $C_{15:0}$ anteiso. Differential characteristics for the *Georgenia* versus some related genera are shown in Table 113.

Based on high 16S rRNA gene sequences similarities two suspected, but so far not described, members of the genus are apparently present in institutional collections; these include *Georgenia* sp. (99.6% similarity; accession no AB094466) isolated from sub-seafloor sediment of the sea of Okhotsk (Inagaki et al., 2003) and *Georgenia* sp. T04-04 (97.0% similarity; accession no: AY880044) cultivated from within oral squamous cell carcinoma tissue removed from a 96-year-old female patient (Hooper et al., 2006).

TABLE 113. Characteristics differentiating *Georgenia* from related taxa[a]

Characteristic	*Georgenia*[b]	*Beutenbergia*[c]	*Bogoriella*[d]	*Cellulomonas*[e]	*Cellulosimicrobium*[f]	*Demetria*[g]	*Salana*[h]
Cell morphology	Rod–coccus	Rod–coccus	Irregular rods, coccoid	Branched rods	Rod-shaped	Coccoid, short rods	Rod-shaped-circular or club-like
Major menaquinone	MK-8(H_4)	MK-8(H_4)	MK-8(H_4)	MK-9(H_4)	MK-9(H_4)	MK-8(H_4)	MK-8(H_4)
Peptidoglycan type	A4α	A4α	A4α	A4β	A4α	A4α	A4β
Amino acid in position 1	L-Ala	L-Ala	L-Ala	nr	nr	L-Ala	L-Ser
Interpeptide bridge	L-Lys ← L-Glu, L-Lys ← L-Ala ← L-Glu	L-Lys ← L-Glu	L-Lys ← L-Ala ← L-Ala ← L-Glu	L-Orn ← D-Asp or L-Orn ← D-Glu	L-Lys ← D-Ser ← D-Asp	L-Lys ← L-Ser ← D-Asp	L-Orn ← L-Glu
DNA G+C content (mol%)	70–73	71	70	72–76	74–75	66	75

[a]Abbreviations: L-Ala, L-alanine; D-Asp, D-aspartic acid; L-Glu, L-glutamic acid; L-Lys, L-lysine; L-Ser, L-serine; L-Orn, L-ornithine; nr, not reported.

[b]Altenburger et al. (2002) and Li et al. (2007).

[c]Groth et al. (1999).

[d]Groth et al. (1997a).

[e]Stackebrandt and Prauser (1991) and Funke et al. (1995).

[f]Schumann et al. (2001), and Brown et al. (2006).

[g]Groth et al. (1997b).

[h]von Wintzingerode et al. (2001).

Enrichment and isolation procedures

For isolation, temperatures of approximately 28°C, neutral pH, and media with rather low nutrient content such as R2A* or ISP 2† agar are preferred by strains of this genus.

Pathogenicity

Nothing is known about pathogenicity of *Georgenia muralis*, *Georgenia ruanii*, or *Georgenia* sp., but due to its source of isolation, *Georgenia* sp. T04-04 should be carefully examined for its potential to act as an opportunistic pathogen.

List of species of the genus *Georgenia*

1. **Georgenia muralis** Altenburger, Kämpfer, Schumann, Vybiral, Lubitz and Busse 2002, 880[VP]

mu.ra′lis. L. adj. *muralis*, *-le* pertaining or belonging to walls.

Morphological, chemotaxonomic, and general characteristics are as described for the genus. Colonies reach a maximum diameter of 2 mm. They are yellow pigmented, transparent, circular, and convex. Good growth occurs at NaCl concentrations up to 5% (w/v), with weak growth at 7% (w/v) NaCl. Growth is observed at pH 6–9.

N-Acetyl- D-glucosamine, L-arabinose, *p*-arbutin, D-cellobiose, D-fructose, D-galactose, gluconate, D-glucose, D-maltose, α-D-melibiose, sucrose, salicin, D-trehalose, D-xylose, and D-mannitol are assimilated. L-Rhamnose, D-ribose, adonitol, *i*-inositol, maltitol, D-sorbitol, putrescine, acetate, propionate, *cis*-aconitate, *trans*-aconitate, adipate, 4-aminobutyrate, azelate, citrate, fumarate, glutarate, DL-3-hydroxybutyrate, itaconate, DL-lactate, L-malate, mesaconate, oxoglutarate, pyruvate, suberate, L-alanine, β-alanine, L-aspartate, L-histidine, L-leucine, L-ornithine, L-phenylalanine, L-proline, L-serine, L-tryptophan,3-hydroxybenzoate, 4-hydroxybenzoate, and phenylacetate are not assimilated. Assimilation of D-mannose is variable. *p*-Nitrophenyl (pNP) β-D-galactopyranoside, pNP β-D-glucuronide, pNP α-D-glucopyranoside, pNP β-D-glucopyranoside, bis-pNP phosphate, pNP phenylphosphonate, L-alanine *p*-nitroanilide (pNA), and L-proline pNA are hydrolyzed. pNP Phosphorylcholine, 2-deoxythymidine-5′-pNP phosphate, and L-glutamate γ-3-carboxy-pNA are not hydrolyzed. Esculin, cellulose azur, and potato starch are hydrolyzed. α-Cellulose, casein, and Tween 80 are not decomposed. Nitrate is reduced to nitrite. Negative for H_2S production, indole production, and urease activity. Good growth occurs at 28°C and 37°C, weak growth at 4°C, and no growth at 44°C. An unidentified compound is present in the fatty acid profile in significant amounts.

Source: medieval wall painting in the church of St Georgen in Styria, Austria.

DNA G+C content (mol%): 70 (HPLC).

Type strain: 1A-C, CCM 4963, DSM 14418, JCM 12241, NBRC 103560.

Sequence accession no. (16S rRNA gene): AB455495, X94155.

2. **Georgenia ruanii** Li, Xu, Schumann, Zhang, Pukall, Xu, Stackebrandt and Jiang 2007, 1426[VP]

ru.a′ni.i. N.L. gen. n. *ruanii* of Ruan, named in honor of Ji-Sheng Ruan (1926–), a Chinese microbiologist who devotes himself to the study of actinomycete taxonomy.

Displays the following properties in addition to those described above for the genus. Colonies are 1.1–1.2 mm in diameter, circular, entire, slightly convex, opaque, and pale white–yellow to straw-colored on ISP 2 agar. Cells are short-rod-shaped, 0.5–0.8 × 1.4–2.0 µm in size, and motile (by means of a single flagellum at each end of the cell). Chemoorganotrophic, with respiratory-type metabolism. Endospores and polyhydroxyalkanoate are not formed. Does not grow in the presence of sodium chloride at concentrations above 5%. Temperature range for growth is 10–37°C, with optimum growth at 28–30°C. pH Range for growth is 6.5–10.0, with optimum growth at pH 7.0. Tests for the hydrolysis of gelatin, for melanin production, H_2S production and indole production, for resistance to KCN, for milk coagulation and for peptonization are negative. Nitrate is not reduced to nitrite. Dextrin, rhamnose, fructose, glucose, arabinose, sorbose, lactose, galactose, sucrose, dulcitol, inositol, maltose, mannose, cellobiose, turanose, mannitol, melibiose, melezitose, raffinose, ribose, salicin, xylitol, adonitol, arabitol, galacturonate, and sorbitol are utilized as sole carbon and energy sources. Acid is produced from fructose, galactose, mannose, mannitol, xylose, ribose, lactose, glucose, and sucrose. Starch and esculin are hydrolyzed. Acetamide, xanthine, hypoxanthine, and Tweens 20 and 80 are degraded weakly; urea is not degraded. Lipase, lysine decarboxylase, β-glucosidase, *N*-acetyl- β-glucosaminidase, β-galactosidase, α-maltosidase, and L-aspartic arylamidase tests are positive, while arginine dihydrolase, ornithine decarboxylase, α-galactosidase, and β-glucuronidase tests are negative. Resistant to oleandomycin and nalidixic acid, but sensitive to penicillin G, vancomycin, polymyxin B, erythromycin, terramycin, aureomycin, tetracycline, streptomycin sulfate, novobiocin, gentamicin, and chloramphenicol. The peptidoglycan type is A4 (L-Lys–L-Ala–L-Glu). The cell-wall sugars are galactose and rhamnose. The polar lipids are phosphatidylinositol, phosphatidylglycerol, diphosphatidylglycerol, and an unknown phospholipid. The menaquinones are MK-8(H_4), MK-7(H_4), and MK-9(H_4) (90:2:1). The cellular fatty acid profile consists mainly of $C_{15:0}$ anteiso (78.3%) and $C_{15:0}$ iso (14.9%), with other minor components, including $C_{17:0}$ anteiso (1.6%), $C_{14:0}$ (1.0%), $C_{15:1}$ anteiso (1.0%), $C_{16:0}$ (1.0%), $C_{13:0}$ anteiso (0.7%), $C_{16:0}$ iso (0.6%), $C_{17:0}$ iso (0.5%), $C_{14:0}$ iso (0.5%), and $C_{13:0}$ iso (0.1%).

DNA G+C content (mol%): 72.9 (HPLC).

Type strain: YIM 004, CCTCC AB 204065, DSM 17458, JCM 15130, KCTC 19029, NBRC 103883.

Sequence accession no. (16S rRNA gene): DQ203185.

3. **Georgenia thermotolerans** Hamada, Tamura, Ishida and Suzuki 2009a, 1878[VP]

ther.mo.to′le.rans. Gr. n. *therme* heat; L. pres. part. *tolerans* tolerating; N.L. part. adj. *thermotolerans* able to tolerate high temperatures.

*R2A: 0.5 g/l yeast extract, 0.5 g/l casein hydrolysate, 0.5 g/l, glucose, 0.5 g/l starch, 0.3 g/l K_2HPO_4, 0.024 g/l $MgSO_4$, 0.3 g/l Na_2-pyruvate, 15 g/l agar, pH 7.2 ± 0.2.

†ISP 2 agar: 4 g/l yeast extract (Difco), 10 g /l malt extract (Difco), 4 g/l dextrose (Difco), 50 g/l NaCl, 20g/l agar, pH 7.4 ± 0.2.

Displays the following properties in addition to those described above for the genus. Colonies are 1–2 mm in diameter, circular, opaque, smooth and whitish–yellow. Cells are irregular, rod-shaped, 0.5–0.7 × 1.0–2.0 µm in size, and motile. Good growth occurs under both aerobic and anaerobic conditions and in the presence of sodium chloride at concentrations of 0–5%. Moderate growth occurs at 7% NaCl and no growth at 10% NaCl. Temperature range for growth is 10–45°C, optimum growth at 28°C. pH range for growth is 6.0–9.0, optimum growth at pH 7.0. Acid is produced from D-ribose, D-xylose, D-glucose, methyl α-D-glucopyranoside, amygdalin, arbutin, salicin, D-cellobiose, maltose and sucrose. No acid production occurs from L-arabinose, L-xylose, D-mannose, D-mannitol, melibiose, trehalose, D-raffinose, starch or glycogen. Esterase (C4), esterase lipase (C8), leucine aminopeptidase, phosphohydrolase, β-glucuronidase, β-glucosidase, α-glucosidase, pyrazinamidase and pyrrolidonyl arylamidase are present; alkaline phosphatase, lipase (C14), valine aminopeptidase, cysteine aminopeptidase, trypsin, chymotrypsin, α-galactosidase, β-galactosidase, *N*-acetyl-β-glucosaminidase, α-mannosidase, α-fucosidase, arginine dihydrolase, lysine decarboxylase, ornithine decarboxylase and tryptophan deaminase are absent. H_2S and indole are not produced. Aesculin, gelatin and urea are not hydrolyzed. Nitrate is reduced to nitrite. The peptidoglycan is of the A4α type with aspartic acid. The cell-wall sugar is galactose. Major polar lipids are diphosphatidylglycerol, phosphatidylglycerol and phosphatidylinositol mannosides. The predominant fatty acids are: $C_{15:0}$ anteiso (89.3%), $C_{14:0}$ iso (6%) and $C_{15:0}$ iso (4.7%).

Type strain was isolated from forest soil in Iriomote Island, Japan.

DNA G+C content (mol%): 73.0 (HPLC).

Type strain: TT02-04, NBRC 104148, DSM 21501.

Sequence accession no. (16S rRNA gene): AB436534.

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Family IV. **Brevibacteriaceae** Breed 1953b, 13^AL emend. Zhi, Li and Stackebrandt 2009, 597^VP

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Brev.i.bac.te.ri.a.ce′a.e. N.L. neut. *Brevibacterium* type genus of the family; suff *-aceae* ending to denote a family; N.L. fem. pl. n *Brevibacteriaceae* the *Brevibacterium* family.

The family contains the genus *Brevibacterium*. A distinct pattern of 16S rRNA signatures shown by members of the family, namely **120(A), 131:231 (C–G), 196 (A), 342:347 (C–G), 444:490 (A–U), 580:761 (C–G), 602:636 (C–G), 670:736 (U–A), 822:878 (G–C), 823:877 (G–C), 826:874 (C–G), 827 (U), 843 (C), 950:1231 (U–A), 1047:1210 (G–U), 1109 (C), 1145 (A), 1309:1328 (G–C), 1361 (G), 1383 (C)**, distinguishes it from all other families classified in the order *Micrococcales*. Additional properties of the family are found in the genus description.

DNA G+C content (mol%): 55–70.

Type genus: **Brevibacterium** Breed 1953b, 13^AL emend. Collins, Jones, Keddie and Sneath 1980, 6.

Further descriptive information

The suborder *Micrococcineae* (Breed 1953b) emend. Zhi et al. 2009 has been elevated into the order *Micrococcales* (see section on this order, above). The genus *Brevibacterium* forms a distinct clade in the 16S rRNA *Micrococcales* tree (see section on the order *Micrococcales*) and can be distinguished from other families in the order by using chemotaxonomic properties, notably on the basis of menaquinone composition and polar lipid and polyamine patterns (Stackebrandt and Schumann, 2000).

Genus I. **Brevibacterium** Breed 1953a, 13^AL emend. Collins, Jones, Keddie and Sneath 1980, 6

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Bre.v.i.bac.te′ri.um. L. adj. *brevis* short; L. neut. n. *bacterium* a small rod; N.L. neut. n. *Brevibacterium* a short rodlet.

Aerobic, **catalase-positive, non-acid-fast, asporogenous actinomycetes which have a rod–coccus cell cycle when grown on complex media**. Usually nonmotile, but some species show motility. **Both rod and coccoid forms are Gram-stain-positive, but some strains and older cultures decolorize readily**. Cells are variable in length but generally are 0.6–1.0 µm in diameter; those from older cultures (3–7 d) are composed mainly or entirely of coccoid cells or coccobacilli. On transfer to a suitable fresh medium, these forms grow out to give the irregular, slender rods characteristic of exponential phase cultures. **Many cells are arranged at an angle to give V-forms**. **Primary branching may occur but not a true mycelium. Respiratory mode of metabolism.** Optimum growth temperature is 20–37°C depending on the species and strain. Grows well on peptone-yeast extract agar at neutral pH. Little or no acid is produced from glucose or other carbohydrates in a peptone medium. Proteinases produced. **The cell-wall peptidoglycan contains *meso*-diaminopimelic acid as the diamino acid and is of the A1γ type. Contains dihydrogenated menaquinones with eight isoprene units as the predominant isoprenologue and major proportions of branched cellular fatty acids, notably $C_{15:0}$ anteiso and $C_{17:0}$ anteiso**. Mycolic acids are absent.

Isolated from clinical material, dairy products, poultry, and marine and terrestrial habitats.

DNA G+C content (mol%): 55–70.

Type species: **Brevibacterium linens** (Wolff 1910) Breed 1953a, 13^AL emend. Collins, Jones, Keddie and Sneath 1980, 7 ("*Bacterium linens*" Wolff 1910).

Further descriptive information

Phylogeny. The genus *Brevibacterium* forms a distinct line of descent within the high G+C containing actinomycetes (Cai and Collins, 1994; Stackebrandt et al., 1997; Zhi et al., 2009). The 24 species assigned to the genus form a monophyletic group in the 16S rRNA gene tree (Figure 148) and share sequence similarities which range from 92.5% between *Brevibacterium aurantiacum* and *Brevibacterium paucivorans*, to 99.8% between *Brevibacterium sanguinis* and *Brevibacterium celere*. DNA–DNA relatedness experiments have not been carried out between the type strains of the two latter species which were described around the same time in 2004.

Cell morphology. Brevibacteria grow on a range of media though morphology and staining reactions are best seen on EYGA medium (Cure and Keddie, 1973). Strains tend to show a distinct rod–coccus cycle when grown on complex media (Collins, 2006; Collins et al., 1980). Both young (6–24 h) and older (3–7 d) cultures should be examined as cell shape (Figure 149) and staining properties change during the growth cycle (Crombach, 1974; Cure and Keddie, 1973; Mulder and Antheumisse, 1963). Coccoid cells are mainly found in older cultures and when transferred to fresh media give rise to slender irregular rods. Coccoid and rod-shaped cells are Gram-stain-positive but generally decolorize with age (Jones and Keddie, 1986). Members of the genus are typically nonmotile, though motility has been detected in *Brevibacterium album* (Tang et al., 2008), *Brevibacterium iodinum* (Colwell et al., 1969), and *Brevibacterium samyangense* (Lee, 2006).

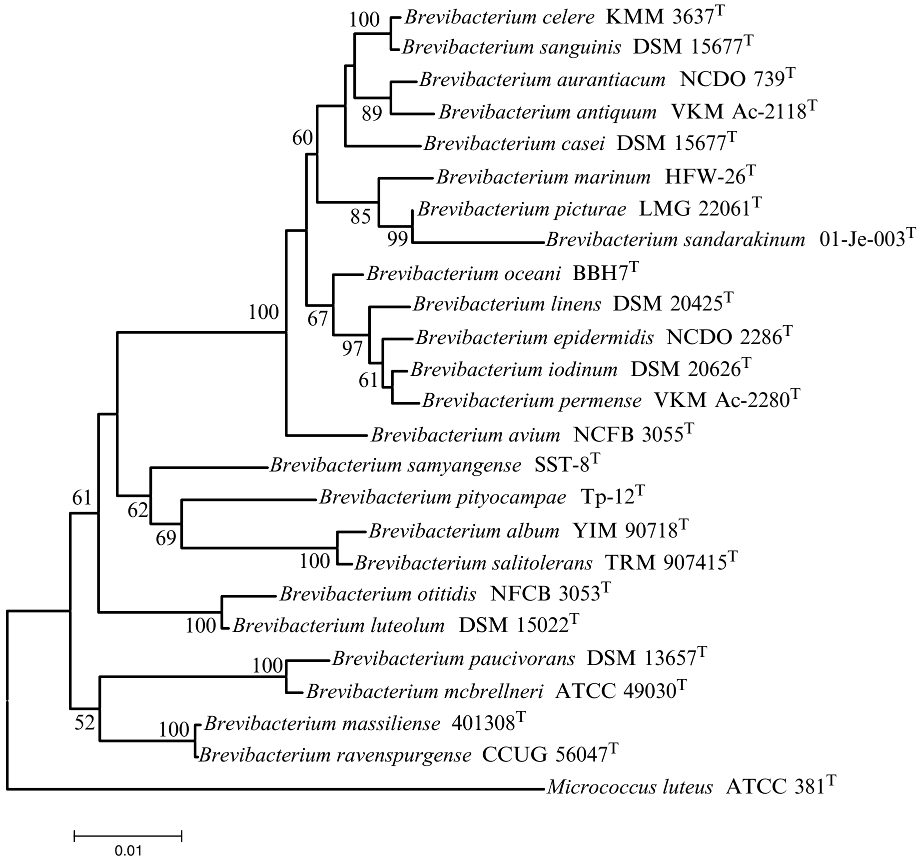

FIGURE 148. Neighbor-joining tree based on 16S rRNA gene sequences showing the phylogenetic relationships of *Brevibacterium* species. Bootstrap values are shown at branching points. *Micrococcus luteus* ATCC 381^T was used as outgroup. Bar = 1 substitution per 100 nucleotides.

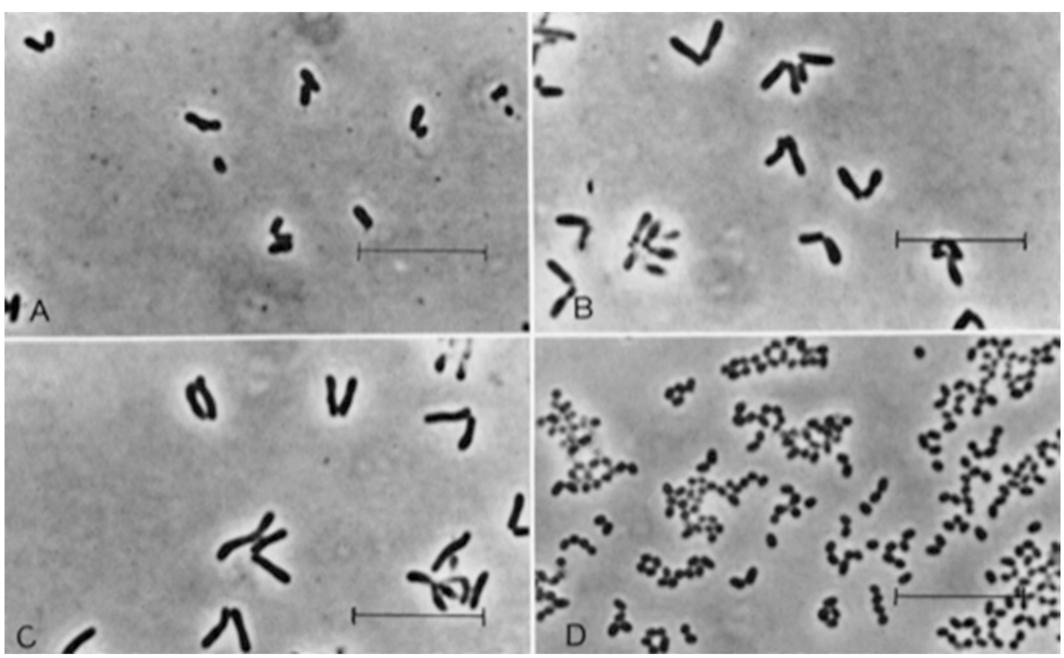

FIGURE 149. *Brevibacterium linens* (ATCC 9175) when grown on medium EYGA at 25°C; inoculum coccoid cells as in (D). (A) After 6 h, showing outgrowth of rods from coccoid cells; (B) after 12 h; (C) after 24 h; and (D), after 3 d. Bars = 10 µm. (Reproduced with permission from Keddie and Jones, 1981. The genus *Brochothrix* (formerly *Microbacterium thermosphactum*, McLean and Sulzbacher). *In* The Prokaryotes: a Handbook on Habitats, Isolation, and Identification of Bacteria (edited by Starr, Stolp, Trüper, Balows and Schlegel). Springer, New York, pp. 1866–1869.)

Chemotaxonomy. The cell-wall peptidoglycan contains *meso*-diaminopimelic acid (Collins et al., 1983a; Fiedler et al., 1981; Kämpfer et al., 2010; Keddie and Cure, 1977, 1978; Pascual and Collins, 1999; Tang et al., 2008; Yamada and Komagata, 1972b) and is of the A1γ type (Schleifer and Kandler, 1972). A unique feature of *Brevibacterium paucivorans* is the presence of glycine and glutamic acid as non-peptidoglycan components of the cell wall (Wauters et al., 2001). Galactose is present in the cell wall of some species such as *Brevibacterium album* (Tang et al., 2008), *Brevibacterium avium* (Pascual and Collins, 1999), *Brevibacterium casei*, and *Brevibacterium epidermidis* (Sharpe et al., 1977, 1978). Teichoic acids were initially detected in the cell walls of *Brevibacterium casei* (Fiedler and Bude, 1989), *Brevibacterium epidermidis* (Fiedler and Bude, 1989), *Brevibacterium iodinum* (Anderton and Wilkinson, 1980) and *Brevibacterium linens* (Fiedler et al., 1981) but have been found in additional *Brevibacterium* species (Gavrish et al., 2005).

Brevibacterium species have similar fatty acid patterns. All contain major amounts of anteiso- and iso-methyl branched acids together with small amounts of straight-chain saturated acids. The major components are 12-methyltetradecanoic ($C_{15:0}$ anteiso) and 14-methylhexadecanoic ($C_{17:0}$ anteiso) acids (Bousfield et al., 1983; Bowie et al., 1972; Collins et al., 1980, 1983a). These fatty acids usually account for over 75% of the total fatty acid composition (Bhadra et al., 2008; Funke and Carlotti, 1994; Gruner et al., 1993; Ivanova et al., 2004; Kati et al., 2010) though not in species such as *Brevibacterium mcbrellneri* (McBride et al., 1993), *Brevibacterium otitidis* (Wauters et al., 2003), and *Brevibacterium paucivorans* (Wauters et al., 2001). In contrast, mycolic acids are not found in brevibacteria (Goodfellow et al., 1976; Heyrman et al., 2004; Keddie and Cure, 1977; Lee, 2006).

Menaquinones are the sole respiratory quinones present in the cell membranes of brevibacteria. The major component is usually dihydrogenated menaquinone with eight isoprene units [MK-8(H_2)] (Collins et al., 1980, 1983a; Heyrman et al., 2004; Kämpfer et al., 2010; Lee, 2006, 2008), though smaller amounts of other components such as MK-8, MK-7(H_2), and MK-9(H_2) may be present (Collins et al., 1979, 1980, 1983a). In contrast, *Brevibacterium casei* and *Brevibacterium linens* contain comparable amounts of MK-7(H_2) and MK-8(H_2) (Collins et al., 1983a).

Diphosphatidylglycerol (DPG) and phosphatidylglycerol (PG) usually feature as major components in brevibacterial polar lipid patterns (Bhadra et al., 2008; Collins et al., 1983a; Kämpfer et al., 2010). Collins et al. (1980) reported both of these lipids and a glycolipid, dimannosyldiacylglycerol, in strains of *Brevibacterium iodinum* and *Brevibacterium linens*. Some species also contain phosphatidylinositol (PI) (Collins et al., 1980, 1983a; Heyrman et al., 2004; Lee, 2008), though the presence of this component has been shown to vary with cultural conditions (Jones and Keddie, 1986). In addition to DPG and PG, an unidentified glycolipid has been detected in *Brevibacterium picturae* (Heyrman et al., 2004) and *Brevibacterium sandarakinum* (Kämpfer et al., 2010). Cadavarine and putrescine are characteristic components of brevibacterial polyamine patterns (Altenburgera et al., 1996; Kämpfer et al., 2010).

Colony morphology. Opaque, white or pale orange, convex colonies (0.5–1.0 mm in diameter) with entire edges and smooth, shiny surfaces are formed within 2 d on nutrient agar. Colonies become larger (2.0–4.0 mm in diameter) after 4–7 d (Jones and Keddie, 1986). Opaque gray-white, convex colonies with smooth, shiny surfaces up to 2 mm in diameter are formed on blood agar after 24 h; they later increase in size and turn slightly greenish or yellowish in color (Funke et al., 1997). Collins et al. (1983a) described colonies of *Brevibacterium casei* as gray-white in color, but in an earlier study of the same strains, Sharpe et al. (1976) recorded the slow production of a brown, water-soluble pigment when the organisms were cultured on milk agar.

Brevibacterium linens forms yellow to deep orange-yellow colonies when grown on a range of media, with pigment production in most strains being light-dependent (Crombach, 1974; Mulder et al., 1966). Jones et al. (1973) suggested that the pigment of *Brevibacterium linens* was a carotenoid which could be recognized by distinctive color reactions occurring when pigmented growth was treated with either glacial acetic acid or solutions of strong bases. The pigments of *Brevibacterium linens* were subsequently shown to consist of the aromatic carotenoids 3,3′-dihydroxyisorenieratene, isorenieratene, and 3-hydroxyisorenieratene (Kohl et al., 1983).

The characteristic metallic purple to blue color of *Brevibacterium iodinum* is due to the production of purple, extracellular crystals of the phenazine derivative iodinin on and in the colonies and adjacent medium (Collins et al., 1980; Colwell et al., 1969; Davis, 1939; Sneath, 1960). The synthesis of iodinin is enhanced on certain media such as Blood Agar Base (Difco) plates incubated at 30°C for 2 d, but is not affected by light (Sneath, 1960).

Nutritional and growth conditions. Brevibacteria grow well on routine laboratory media such as blood, nutrient, tryptone soy, yeast extract–malt extract, and yeast extract–peptone agars. They tolerate and are sometimes stimulated by the addition of NaCl to the medium (Crombach, 1974; Heyrman et al., 2004; Kati et al., 2010; Lee, 2006, 2008; Mulder et al., 1966; Sharpe et al., 1977). They grow well at neutral pH and at 30°C, though not all strains grow optimally at this temperature. *Brevibacterium linens*, for instance, grows best between 20–25°C (Keddie and Jones., 1981), *Brevibacterium avium* at 37°C (Pascual and Collins, 1999), *Brevibacterium casei* and *Brevibacterium epidermidis* between 30 and 37°C (Pitcher and Noble, 1978; Sharpe et al., 1976, 1977), and *Brevibacterium iodinum* at 28°C (Jones, 1975).

Metabolism. Brevibacteria are aerobic, catalase-positive, chemoorganotrophic actinomycetes with an oxidative type of metabolism. Most strains are proteolytic, metabolizing casein, gelatin, and milk (Bousfield, 1972; Colwell et al., 1969; Crombach, 1974; Keddie and Jones., 1981; Wauters et al., 2003). In general, acid is not produced from glucose or other carbohydrates (Bousfield, 1972; Collins et al., 1983a; Jones, 1975; Kati et al., 2010), though a range of compounds is used as sole carbon sources (Crombach, 1974; Ivanova et al., 2004; Kämpfer et al., 2010; Tang et al., 2008; Yamada and Komagata, 1972a). Several species including *Brevibacterium casei*, *Brevibacterium epidermidis*, *Brevibacterium linens*, and *Brevibacterium paucivorans* produce methanethiol from L-methionine (Pitcher and Noble, 1978; Sharpe et al., 1976, 1977, 1978; Wauters et al., 2003).

Genetics. Little is known about brevibacterial genetics, though plasmids designated pBL100 and pBL33 have been isolated from *Brevibacterium linens* strains (Kato et al., 1989; Sandoval et al., 1985) and purified (Holtz et al., 1992).

Antibiotic sensitivity. Few studies have been directed toward establishing the antibiotic sensitivity patterns of *Brevibacterium* species (Bhadra et al., 2008; Colwell et al., 1969; Ivanova et al., 2004; Jones, 1975; McBride et al., 1993; Sneath, 1960), though this is likely to change given the clinical significance of species such as *Brevibacterium casei* (Brazzola et al., 2000; Funke et al., 1997). Members of this taxon tend to be resistant to β-lactams, ciprofloxacin, clindamycin, and erythromycin and sensitive to gentamicin, rifampin, and tetracycline (Funke et al., 1996). Pitcher and Noble (1978) noted that nearly 90% of methanethiol-producing skin brevibacteria isolated from the feet of patients infected with *Trichophyton* species were resistant to penicillin. The antibiotic sensitivity profiles of individual *Brevibacterium* species are cited in the species descriptions.

Bacteriocins and antimicrobials. Several bacteriocins and antimicrobials are produced by *Brevibacterium linens* (Kato et al., 1984, 1991). These include Linocin A which shows inhibition to some other members of this species but not to other *Brevibacterium* species or to *Corynebacterium*, *Listeria*, and *Micrococcus* species (Valdes-Stauber and Scherer, 1994). Further, Linenscin OC2 from another *Brevibacterium linens* strain inhibits food borne pathogens including *Listeria monocytogenes* and *Staphylococcus aureus*. In addition, *Brevibacterium linens* strains isolated from brine used to salt red-smear cheeses produce an antimicrobial agent active against *Listeria* species (Martin et al., 1995).

Clinical significance. Members of the genus *Brevibacterium*, notably *Brevibacterium casei*, have quite recently been associated with human infections (Collins, 2006; Funke et al., 1997; McCaughey and Damani, 1991; Pitcher and Malnick, 1984). They have been implicated as agents of continuous ambulatory peritoneal dialysis (Antoniou et al., 1997; Gruner et al., 1993; Hummel et al., 1996; Wauters et al., 2000), corneal ulcers (Ghosheh et al., 2007), endocarditis (Dass et al., 2002), osteomyelitis (Neumeister et al., 1993), peritonitis (Cannon et al., 2005), septicemia (Carlotti et al., 1993; Kaukoranta-Tolvanen et al., 1995; Lina et al., 1994; Lipsky et al., 1982; Reinert et al., 1995; Ulrich et al., 2006), and sepsis in AIDS patients (Brazzola et al., 2000; Janda et al., 2003). In addition, *Brevibacterium luteolum* has been isolated from an ear discharge (Wauters et al., 2003), *Brevibacterium mcbrellneri* from infected genital hair of patients with white piedra in association with *Trichosporon beigelli* (McBride et al., 1993), *Brevibacterium massiliense* from an ankle discharge (Roux and Raoult, 2009), *Brevibacterium otitidis* from an ear infection (Pascual et al., 1996a), and *Brevibacterium paucivorans* from clinical samples (Wauters et al., 2001).

Ecology. Brevibacteria have been isolated from numerous habitats, notably ones with a high salt concentration. Most species have been derived either from dairy milk products, human skin, or clinical material. The usual habitat of *Brevibacterium linens* is the exterior of surface-ripened cheeses such as Limburger (Brennan et al., 2002), but it also occurs on cheeses such as Brick, Camembert, and Roquefort (El-Erian, 1969; Mulder et al., 1966). This organism is considered to be responsible for the surface color of red-smear cheeses and also, in part, by its proteolytic activity, to the ripening of such cheeses (Brennan et al., 2002). It is for this latter reason that *Brevibacterium linens* is often inoculated onto the surface of smear cheeses during the early stages of ripening, either as a commercial preparation or as the so-called "old-young" smearing method whereby young cheeses are washed with a brine suspension of microorganisms from the surface of mature cheese.

Strains now classified as *Brevibacterium casei* (Collins et al., 1983a) have been isolated from cheese curd, milk, and cheddar cheese, though the primary habitat of this organism is considered to be the human skin (Sharpe et al., 1976). *Brevibacterium epidermidis* is also thought to be part of the resident flora of human skin (Collins et al., 1983a; Pitcher and Noble, 1978). Other *Brevibacterium* species isolated from human material include *Brevibacterium luteolum* (corrig. Wauters et al., 2003), *Brevibacterium mcbrellneri* (McBride et al., 1993), *Brevibacterium otitidis* (Pascual et al., 1996a), and *Brevibacterium paucivorans* (Wauters et al., 2004b).

Brevibacteria have been recovered from marine habitats, as seen by the isolation of *Brevibacterium celere* from the degraded thallus of the brown alga *Fucus evanescens* (Ivanova et al., 2004), *Brevibacterium marinum* from sea water (Lee, 2008), *Brevibacterium oceani* from deep-sea sediment, and *Brevibacterium samyangense* from beach sand (Lee, 2006). Additional species have been isolated from more unusual sources such as *Brevibacterium album* from saline soil (Tang et al., 2008), *Brevibacterium salitolerans* from a hypersaline lake sediment sample (Guan et al., 2010), *Brevibacterium picturae* from a damaged mural painting (Heyrman et al., 2004), *Brevibacterium pityocampae* from healthy larvae of the moth, *Thaumelopoea pityocampae*, a pest of *Pinus* species in Mediterranean countries, including Turkey (Kati et al., 2010), and *Brevibacterium sandarakinum* from an indoor wall colonized by fungi (Kämpfer et al., 2010).

Enrichment and isolation procedures

Brevibacteria can be isolated on nutrient media based on glucose, peptone, and yeast extract, as exemplified by trypone soy agar supplemented with 4% (w/v) NaCl (TSAS; [Oxoid] 17 g; peptone, 3 g; NaCl, 9 g; K_2HPO_4, 2.5 g; glucose, 2.5 g; agar, 12 g; pH is adjusted to 7.0. The medium is sterilized at 121°C for 15 min). Isolation of the organisms is usually achieved by the use of such media supplemented with cheese, milk, and an elevated concentration of NaCl or sea water, as appropriate, depending on the environmental sample. Inoculated plates should be incubated at 20–25°C for 3–7 d, though temperatures of 30–37°C are better for the isolation of skin brevibacteria. It is important when isolating *Brevibacterium linens* that plates are exposed to light during some period of active growth to enhance pigment formation (Crombach, 1974; Mulder et al., 1966). A convenient practice is to incubate plates at 25°C until small colonies are evident and then to expose plates to light on the laboratory bench for the rest of the incubation period (Jones and Keddie, 1986).

Since isolation media are non-selective for brevibacteria, it is necessary to randomly select pigmented and nonpigmented colony types to establish whether cells have a coryneform morphology. When identification is limited to a consideration of isolates showing a rod–coccus growth cycle care must be taken to distinguish brevibacteria from organisms such as arthrobacters and rhodococci. Tests for this purpose are available (see section on differentiating the genus *Brevibacterium* from other genera).

Isolation from cheese. Samples of cheese or cheese curd are homogenized in 2% (w/v) trisodium citrate supplemented with NaCl (El-Erian, 1969). This medium is based on Tryptone

Soy Broth (Oxoid) though similar products are available from other sources such as BBL and Difco. Colonies growing on the isolation plates are examined as described above.

Sharpe et al. (1976) isolated brevibacteria from various dairy products by direct plating onto a medium containing 30% (w/v) skim milk and 2% (w/v) agar. All isolates were tested for the production of methanethiol from L-methionine (see *Procedures for testing for special characteristics*, below) and then examined for a coryneform morphology.

A medium which has been reported to be particularly suitable for the isolation of *Brevibacterium linens* from cheese is that of Albert et al. (1944). On this medium, which contains ripened cheese, *Brevibacterium linens* is reputed to give good pigment production in 5–7 d at 21°C or at room temperature, especially when plates are incubated in an oxygen-enriched atmosphere (Keddie and Jones., 1981).

Isolation from skin. Swabs moistened in physiological saline are rubbed firmly over the appropriate skin area and then streaked onto TSAS agar or Blood Agar Base no. 2 (Difco). After incubation at 30°C for 5–7 d colonies are randomly selected and examined for coryneform morphology. Further identification tests are then carried out.

Isolation from other sources. *Brevibacterium marinum* and *Brevibacterium samyangense* were isolated from sea water and sediment samples, respectively, using starch casein agar (Küster and Williams, 1964) prepared using 60% natural sea water (Lee, 2006, 2008). Similarly, *Brevibacterium oceani* was isolated by plating out a sediment sample suspended in 2% (w/v) NaCl onto yeast extract–peptone plates (Bhadra et al., 2008) and *Brevibacterium album* was isolated by inoculating a saline soil suspension onto glycerol-asparagine agar (Tang et al., 2008). Similarly, *Brevibacterium picturae* strains were isolated on R2A agar (Difco) supplemented with 1% (w/v) NaCl and on tryptic soy agar supplemented with 10% (w/v) NaCl, *Brevibacterium pityocampae* by inoculating suspensions of the contents of surface-sterilized insect larvae onto nutrient agar (Kati et al., 2010), and *Brevibacterium sandarakinum* by enriching a sample taken from a wall colonized by fungi in a 10 ml 0.9 (w/v) NaCl solution containing 0.01% (w/v) Tween 80 and plating dilutions onto M79 agar (Kämpfer et al., 2010).

Maintenance procedures

Brevibacteria may be preserved for some months by stab inoculation in nutrient agar (Oxoid) or similar media in screw-capped containers. After overnight incubation with the caps loose at 25 or 30°C, the caps should be screwed tightly to prevent evaporation. The containers may be stored at room temperature (~20°C), or better at ~4°C in the dark. Cultures may be preserved for longer periods (at least 7 years) by freezing in glass beads at −70°C (Feltham et al., 1978) or by lyophilization.

Procedures for testing for special characters

Presence of rod–coccus cycle. This may be demonstrated by using the methods described by Cure and Keddie (1973).

Cell-wall composition. Satisfactory results may be obtained with one of the methods described by Bousfield et al. (1985).

Detection of mycolic acids. This may be achieved by whole-organism methanolysis as described by Minnikin et al. (1984).

Polar lipid patterns. These may be determined by two dimensional TLC analysis of free lipid extracts (Collins et al., 1983a).

Test for the production of methanethiol. Sharpe et al. (1978) recommended the DTNB method of Laakso (1976) because it is more rapid and, in their experience, much more sensitive than the Conway diffusion method (Sharpe et al., 1978).

Organisms grown on nutrient agar slopes are suspended (to give an E_{580} of 10) in 0.05 M Tris/HCl buffer at pH 8.0; 1 ml samples are then incubated in rubber-stoppered test tubes with 12.5 mM L-methionine and 0.25 mM 5.5-dithiols (2-nitrobenzoic acid) (DTNB) in a total volume of 5 ml for 2 h at 30°C. Methanethiol production is indicated by the development of a yellow color in the test incubations. Positive tests are discernable by eye, but weak methanethiol producers may be confirmed colorimetrically at E_{420} after centrifugation of the bacterial growth. Controls (a) without cell suspensions and (b) without L-methionine should be incubated.

In addition, Pitcher and Malnick (1984) described a rapid method for the detection of methanethiol.

Differentiation of the genus *Brevibacterium* from other genera

16S rRNA gene sequencing is currently the most effective way of distinguishing brevibacteria from other actinomycetes, notably from those which show a rod–coccus growth cycle and contain *meso*-A_2pm in the wall peptidoglycan. Morphologically, members of the genus can be confused with *Corynebacterium*, *Dietzia*, *Gordonia*, *Rhodococcus*, and *Williamsia* strains but can be readily distinguished from the latter as they have fatty acids rich in iso- and anteiso-methyl branched components but lack mycolic acids and phosphatidylinositol dimannosides (Collins et al., 1980, 1983a; Goodfellow and Maldonado, 2006). Similarly, brevibacteria can be distinguished from arthrobacters and cellulomonads as they contain *meso*-A_2pm and not lysine or ornithine in the wall peptidoglycan (Collins, 2006). Bousfield et al. (1983) suggested that computer-assisted interpretation of fatty acid data combined with morphological examination may be sufficient to identify strains as members of the genera *Brevibacterium*, *Arthrobacter*, *Cellulomonas*, *Corynebacterium*, *Kurthia*, or *Oerskovia*.

Taxonomic comments

The genus *Brevibacterium* was proposed by Breed (1953a) to accommodate several Gram-stain-positive, asporogenous, nonbranching rod-shaped bacteria which had previously been classified in the genus *Bacterium*; *Brevibacterium linens* was designated the type species. The genus was recognized in the 7th edition of the *Manual* where it was classified with the genus *Kurthia* in the family *Brevibacteriaceae*. At that time, the genus *Brevibacterium* contained 23 species which were described as typically short, unbranched rods that were usually nonmotile; no mention was made of a coryneform morphology.

It was subsequently shown that *Brevibacterium linens* had a coryneform morphology and that it exhibited a rod–coccus growth cycle similar to that seen in *Arthrobacter globiformis* (Mulder and Antheumisse, 1963; Schefferle, 1966). In some earlier numerical taxonomic studies, it was even proposed that *Brevibacterium linens* should be transferred to the genus *Arthrobacter* as *Arthrobacter linens* (Bousfield, 1972; da Silva and Holt, 1965;

Davis and Newton, 1969). However, Fiedler et al. (1970) showed not only that the peptidoglycan in *Brevibacterium linens* was quite different from that in *Arthrobacter globiformis* and closely related species, but also that the 28 species named *Brevibacterium* which they examined were heterogeneous in peptidoglycan type. It was mainly for these reasons that the genus *Brevibacterium* was listed as *incertae sedis* in the 8th edition of the *Manual* (Rogosa and Keddie, 1974).

The heterogeneity of the genus was underpinned by chemotaxonomic (Keddie and Cure, 1978; Minnikin et al., 1978; Schleifer and Kandler, 1972) and numerical taxonomic (Jones, 1978; Seiler et al., 1977) studies and *Brevibacterium linens* was seen as a distinct taxon which could form the basis of a redefined genus (Jones, 1975; Keddie and Cure, 1977; Sharpe et al., 1978; Yamada and Komagata, 1972a). These and further studies led Collins et al. (1980) to redefine the genus *Brevibacterium* and at the same time to reclassify the organism previously designated "*Chromobacterium iodinum*" as *Brevibacterium iodinum*. Later, two groups of methanethiol-producing coryneform bacteria, one isolated from cheese and milk and the other from human skin, were classified as *Brevibacterium casei* and *Brevibacterium epidermidis* by Collins et al. (1983a). The methanethiol-producing organisms from cheese and milk were first isolated and well described by Sharpe et al. (1978, 1976, 1977) and those from skin by Pitcher and Noble (1978) and Sharpe et al. (1978). All of these investigators emphasized the similarity between these isolates and *Brevibacterium linens*, though at that time the genus *Brevibacterium* was *incertae sedis*.

The four *Brevibacterium* species mentioned above were recognized in the last edition of *Bergey's Manual of Systematic Bacteriology* by Jones and Keddie (1986) who also listed additional species that required further study so that they might be reclassified with confidence. In the meantime, new species have been assigned to the genus *Brevibacterium*, which now encompasses 24 validly published species forming a monophyletic branch in the 16S rRNA gene tree (Figure 148). In contrast, species previously associated with the genus have been transferred to other genera, as exemplified by the reclassification of *Brevibacterium ammoniagenes* Cooke and Keith 1927 and *Brevibacterium stationis* (ZoBell and Upham 1944) Breed 1953a as *Corynebacterium ammoniagenes* comb. nov. Collins 1987 and *Corynebacterium stationis* comb. nov. Bernard et al. 2010.

Differentiation of the species of the genus *Brevibacterium*

Brevibacterium species can be distinguished using a combination of phenotypic tests (Table 114) but not always with confidence, as a common set of tests are not yet available for the delineation of all species. *Brevibacterium linens* is most easily recognized by its ability to produce yellow to deep orange colonies (Jones et al., 1973; Mulder et al., 1966), though it needs to be remembered that DNA relatedness studies show that this taxon encompasses at least two species (Fiedler et al., 1981). The color reaction of *Brevibacterium linens* illicited by various chemicals has been used to distinguish members of this taxon (Grecz and Dack, 1961), but this test is not exclusively selective (Jones et al., 1973). The production of distinct purple extracellular crystals of iodinin by *Brevibacterium iodinum* is diagnostic for this organism (Clemo and Daglish, 1950; Davis, 1939; Sneath, 1960). In practice, closely related *Brevibacterium* species are distinguished using a combination of 16S rRNA gene sequence and phenotypic data (Kämpfer et al., 2010; Kati et al., 2010). Preliminary evidence suggests that *Brevibacterium* species can be separated on the basis of their MALDI-TOF mass spectra (Roux and Raoult, 2009).

TABLE 114. Characteristics differentiating species of the genus *Brevibacterium*[a]

Characteristic	*B. linens*	*B. album*	*B. antiquum*	*B. aurantiacum*	*B. avium*	*B. casei*	*B. celere*	*B. epidermidis*	*B. iodinum*	*B. luteolum*	*B. massiliense*	*B. marinum*	*B. mcbrellneri*	*B. oceani*	*B. otitidis*	*B. paucivorans*	*B. permense*	*B. picturae*	*B. pityocampae*	*B. ravenspurgense*	*B. salitolerans*	*B. samyangense*	*B. sandarakinum*	*B. sanguinis*
Colony color[b]	yo	wh	wo	o	gw	gw	wy	wy	g	y	wb	y	wb	po	wy	g	o	wh	y	gw	wy	bry	lr	gw
Colony texture[c]	s	s	nd	nd	s	s	s	s	s	s	s	s	d	st	sc	s–st	nd	nd	nd	st	s	nd	nd	st–s
Growth at:																								
10°C	−	−	+	+	−	−	−	−	−	−	−	+	−	+	−	−	+	−	−	nd	−	+	+	nd
37°C	w	+	−	−	+	+	+	+	+	+	+	−	+	−	+	+	+	v	+	+	+	+	−	+
42°C	−	+	−	−	nd	nd	nd	−	+	nd	+	−	nd	−	nd	nd	nd	−	+	nd	+	+	−	nd
NaCl tolerance (%, w/v)	10	5	18	15	nd	15	15	15	12	10	10	10	nd	12	nd	nd	18	15	10	nd	18	9	10	10
Caseinase	+	nd	+	+	+	+	−	−	+	+	nd	+	+	+	+	−	+	nd	nd	nd	nd	−	nd	+
Lipase	−	−	nd	nd	−	nd	nd	nd	−	+	w	−	nd	−	−	−	nd	−	nd	+	nd	−	nd	nd
Oxidase	w	−	+	+	+	−	+	−	+	nd	−	−	nd	−	nd	nd	+	+	−	nd	−	−	nd	−
Pyrazinamidase	+	+	+	+	nd	+	−	+	+	+	w	−	−	+	+	−	+	+	nd	+	+	+	nd	+
α-Glucosidase	−	−	−	−	−	v	+	−	−	−	−	+	−	−	−	−	−	−	nd	−	−	+	nd	+
Nitrate reduction	+	−	−	+	+	v	−	v	+	−	−	+	−	−	−	−	−	v	+	−	−	−	nd	v
Acid from phenylacetate	+	nd	nd	nd	nd	+	nd	nd	+	nd	nd	nd	+	+	−	−	nd	nd	nd	nd	nd	nd	nd	+
Utilization of:																								
D-Arabinose	−	nd	nd	−	−	+	−	+	−	−	−	nd	−	−	−	−	−	nd	nd	−	−	nd	nd	+
Gluconate	−	nd	+	−	−	+	−	+	−	−	nd	nd	−	+	−	−	+	nd	nd	−	nd	nd	nd	+
Mannitol	−	+	+	+	+	−	−	+	−	−	−	+	−	−	−	−	+	nd	−	−	−	+	−	−

[a] Symbols: +, positive; −, negative; w, weak; v, variable; nd, not determined.

[b] bry; bright yellow; g, grayish; gw, grayish-white; light red; o, orange; po, pale-orange; y, wb, whitish-beige; wh, white, wo, white to orange; wy, whitish-yellow; yellow; yo, yellow to orange.

[c] s, smooth; st, sticky; d, dry; sc, smooth-creamy.

List of species of the genus *Brevibacterium*

1. **Brevibacterium linens** (Wolff 1910) Breed 1953a, 13[AL] emend. Collins, Jones, Keddie and Sneath, 1980, 7 ("*Bacterium linens*" Wolff 1910)

li′nens. L. part. adj. *linens* spreading over, smearing.

Aerobic, Gram-stain-positive, nonmotile actinomycetes which have a coryneform morphology and show a rod–coccus growth cycle when grown on complex media. Surface colonies on nutrient agar are small (0.1–0.2 mm in diameter) after 1–2 d, becoming larger (2.5 mm) on extended incubation. Mature colonies are shiny, convex, and have entire margins. On peptone–yeast extract media, an orange to orange-red pigment is produced. Pigment production by most strains, including the type strain, is light dependent. Pigmented growth gives characteristic color reactions with various acids and bases. Optimum growth temperature is 20–25°C; growth is generally poor or absent at 5°C and 37°C. Grows in the presence of 10% (w/v) NaCl. Acid is not produced from glucose or other sugars in peptone media. Gelatin is liquefied, hippurate hydrolyzed, and extracellular DNase produced. Weakly oxidase-positive. Methanethiol is produced from L-methionine. Additional phenotypic characteristics are shown in Table 114. The major fatty acids are $C_{17:0}$ anteiso and $C_{15:0}$ anteiso.

Common on surface-ripened smear cheeses.

Source: harzer cheese.

DNA G+C content (mol%): 60–64 (T_m).

Type strain: ATCC 9172, CIP 101125, DSM 20425, HAMBI 2038, JCM 1327, NBRC 12142, NRRL B-4210, VKM Ac-2112.

Sequence accession no. (16S rRNA gene): X77451.

Additional comments: Brevibacterium linens has been shown to be heterogeneous on the basis of nutritional properties (Mulder et al., 1966), electrophoretic protein patterns (Foissy, 1974) and DNA–DNA relatedness studies (Fiedler et al., 1981). Fiedler and his colleagues showed that strains designated *Brevibacterium linens* in public and private culture collections fell into at least two distinct species.

2. **Brevibacterium album** Tang, Wang, Schumann, Stackebrandt, Lou, Jiang, Xu and Li 2008, 576[VP]

al′bum. L. neut. adj. *album* white.

Aerobic, Gram-stain-positive, motile actinomycete which forms rods (1.8 × 4.0–5.0 μm). Colonies are white, smooth, circular, opaque, and 1–2 mm in diameter after incubation for 2 d on gelatin-asparagine agar (ISP medium 5) supplemented with 5% (w/v) NaCl. Growth occurs at 28–45°C with an optimum of 37°C; pH range 6–8 with an optimum of 7.5. Grows in the presence of NaCl, 0–10% (w/v) with an optimum of 0–5%.

The following carbon sources are used for growth in Biolog GP2 microplates: D-alanine, β-cyclodextrin, *N*-acetyl-D-glucosamine, fructose 6-phosphate, L-fucose, D-gluconic acid, *myo*-inositol, lactulose, D-mannitol, L-mannose, methyl β-D-galactoside, methyl α-D-glucoside, D-malic acid, palatinose, putrescine, salicin, succinic acid, trehalose, turanose, L-alanyl glycine, Tween 80, and uridine 5′-monophosphate.

Positive for alkaline phosphatase, gelatin hydrolysis, pyrazinamidase, pyrrolidonyl arylamidase, and acid production from D-ribose (API CORYNE tests). In addition, it is positive for acid and alkaline phosphatase, α-chemotrypsin, cystine arylamidase, esterase (C4), esterase lipase (C8), leucine arylamidase, naphthol-AS-BI-phosphohydrolase, trypsin, and valine arylamidase (API ZYM tests). Additional phenotypic characteristics are shown in Table 114.

Cell wall contains galactose. Major fatty acids are anteiso $C_{15:0}$, $C_{17:0}$ anteiso, $C_{15:0}$ iso, and $C_{16:0}$ iso. The predominant isoprenologue is MK-8(H_2); also contains MK-6, MK-6(H_2), MK-7(H_2), MK-8, and MK-9(H_2). The major phospholipids are diphosphatidylgylcerol and phosphatidylglycerol.

Source: a saline soil collected in northwest China.

DNA G+C content (mol%): 70.0 (HPLC).

Type strain: YIM 90718, CCTCC AB 20612, DSM 18261, JCM 15617, KCTC 19173.

Sequence accession no. (16S rRNA gene): EF158852.

3. **Brevibacterium antiquum** Gavrish, Krauzova, Potekhina, Karasev, Plotnikova, Altyntseva, Korosteleva and Evtushenko 2005, 1[VP] (Effective publication: Gavrish, Krauzova, Potekhina, Karasev, Plotnikova, Altyntseva, Korosteleva and Evtushenko 2004, 181.)

an.ti′qu.um. L.neut. adj. *antiquum* ancient, old.

Aerobic, Gram-stain-positive, nonmotile actinomycete which is composed mainly of coccoid cells in old cultures. Colonies are white to orange, circular, and slightly convex. Orange pigment is produced in the light. Grows at 7°C, but not at 37°C; optimal growth is between 24–26°C. Grows in the presence of 18% (w/v) NaCl.

Gelatin is hydrolyzed, but esculin and starch are not. Oxidase-positive. The ability to hydrolyze urea and to produce H_2S is strain-specific. L-Arabinose (slowly), D-fructose, D-galactose (slowly), D-glucose, glycerol, D-mannose, and D-xylose are used as carbon sources in a mineral medium supplemented with 0.05% (w/v) yeast extract, but D-melibiose, melezitose, raffinose and sorbitol are not. Acid is produced from fructose, D-galactose, D-glucose, mannose, and sorbitol, but not from inositol, inulin, D-mannitol, melezitose, raffinose, sorbose, rhamnose, or trehalose. Pyrrolidonyl arylamidase-positive, but negative for β-galactosidase, β-glucuronidase, and *N*-acetyl-β-glucosaminidase (API Coryne tests). Methyl red and Voges–Proskauer tests are negative. Additional phenotypic characteristics are shown in Table 114. The major fatty acids are $C_{15:0}$ anteiso and $C_{17:0}$ anteiso, and predominant menaquinone is MK-8(H_2). Cell-wall teichoic acids contain glucose, glycerol, and mannitol.

Source: 1.8–3-million-year-old ancient permafrost sediments located on the Kolyma Lowland, East Siberia, Russia.

DNA G+C content (mol%): 60.1–64.3 (T_m).

Type strain: JCM 13317, LMG 22206, UCM Ac-411, VKM Ac-2118.

Sequence accession no. (16S rRNA gene): AY243344.

Additional comments: the specific epithet *antiquum* is a "Latin adjective in the neuter gender" not a "modern noun adjective" (*sic*) as cited in the publication by Gavrish et al. (2005). The culture collection accession number LMG 22206 was provided on request for validation (List Editor, 2005).

4. **Brevibacterium aurantiacum** Gavrish, Krauzova, Potekhina, Karasev, Plotnikova, Altyntseva, Korosteleva and Evtushenko 2005, 1[VP] (Effective publication: Gavrish, Krauzova, Potekhina, Karasev, Plotnikova, Altyntseva, Korosteleva and Evtushenko 2004, 181.)

au.ran.ti′a.cum. N.L. neut. adj. *aurantiacum* orange-colored.

Aerobic, Gram-stain-positive, nonmotile actinomycetes which are mainly present as coccoid cells in older cultures. Colonies are orange, circular, slightly convex, and glistening. Grows at 7°C and optimally between 24–26°C. Grows in the presence of 15% (w/v) NaCl.

H_2S is produced. Does not hydrolyze esculin, starch, or urea. Production of acid from fructose and gelatin hydrolysis are strain-specific. Cellobiose, fructose, D-galactose, D-glucose, glycerol, D-mannose, and D-xylose are used as carbon sources in a mineral medium supplemented with 0.05% (w/v) yeast extract, but adonitol, L- and D-arabinose, D-arabitol, dulcitol, *meso*-erythritol, L-fucose, inositol, lactose, D-melibiose, raffinose, salicin, sorbitol, and trehalose are not. Acid is produced from galactose, D-glucose, mannose, and salicin, but not from inositol, inulin, D-mannitol, melezitose, raffinose, sorbose, sorbitol, or trehalose. Alkaline phosphatase-positive, but negative for pyrrolidonyl arylamidase, β-glucuronidase, β-galactosidase, and *N*-acetyl-β-glucosaminidase (API Coryne system). Additional phenotypic characteristics are shown in Table 114.

Major fatty acids are $C_{15:0}$ anteiso and $C_{17:0}$ anteiso, and the predominant menaquinone MK-8(H_2). Contains glycerol teichoic acids.

Source: cheese.

DNA G+C content (mol%): 60.1–64.3 (T_m).

Type strain: ATCC 9175, JCM 2590, LMG 22546, NCIMB 8546), VKM Ac-2111.

Sequence accession no. (16S rRNA gene): X76566.

Additional comments: the culture collection accession number LMG 22546 was provided on request for validation (List Editor, 2005).

5. **Brevibacterium avium** Pascual and Collins 1999, 1529[VP]

a′vi.um. L. n. *avis* a bird; L. gen. pl. n. *avium* of birds.

Aerobic, Gram-stain-positive, non-acid-fast, nonmotile actinomycetes which have a coryneform morphology. Colonies are convex, with entire margins and are round, smooth, grayish-white and butyrous. Grows well at 22 and 30°C and optimally at 37°C.

Tyrosine is decomposed and extracellular DNase produced. Gelatin and xanthine are degraded, but not esculin, starch or urea. L-Arabinose, D-arabitol, D-fructose, galactose, D-glucose, glycerol, D-mannose, and mannitol are used as sole carbon sources, but not adonitol, amygdalin, L-arabitol, arbutin, cellobiose, dulcitol, erythritol, D-fucose, L-fucose, β-gentiobiose, 2-ketogluconate, 5-ketogluconate, *N*-acetylglucosamine, methyl-α-D-glucoside, glycogen, inositol, inulin, lactose, D-lyxose, maltose, methyl α-D-mannoside, melezitose, melibiose, D-raffinose, rhamnose, ribose, salicin, sorbitol, L-sorbose, starch, sucrose, D-tagatose, trehalose, D-turanose, xylitol, D-xylose, or methyl β-xyloside. Does not produce acid from glucose, maltose, mannitol, sucrose, or xylose. Positive for alkaline and acid phosphatase, esterase (C4), ester lipase (C8), leucine arylamidase, and phosphoamidase, but negative for α-chymotrypsin, α-fucosidase, β-fucosidase, α-galactosidase, β-glucuronidase, α-mannosidase, *N*-acetyl-β-glucosaminidase, trypsin, and valine arylamidase (API ZYM tests). Additional phenotypic characteristics are shown in Table 114.

Galactose, but not arabinose, is present in the cell wall.

Source: bumble-foot-like manifestations of poultry.

DNA G+C content (mol%): not determined.

Type strain: CCUG 43455, CIP 106532, JCM 11680, NCIMB 703055.

Sequence accession no. (16S rRNA gene): Y17962.

6. **Brevibacterium casei** Collins, Farrow, Goodfellow and Minnikin 1983b, 896[VP] (Effective publication: Collins, Farrow, Goodfellow and Minnikin 1983a, 393.)

ca′se.i. L. n. *caseus* cheese; L. gen. n. *casei* of cheese.

Aerobic, Gram-stain-positive, nonmotile actinomycetes which have a coryneform morphology. Gray-white colonies are produced on nutrient agar. A brown, water soluble pigment is formed on milk agar. Grows well at 40°C, and optimally between 30–37°C. Survives heating in nutrient broth (Oxoid) at 60°C for 30 min. Tolerant to 15% (w/v) NaCl.

Gelatin is hydrolyzed and extracellular DNase produced. Reduction of nitrate is variable. Weak acid production from glucose has been reported using the method of Hugh and Leifson (1953). Glucose, sucrose, acetate, and lactate are used as sole carbon sources. Additional phenotypic characteristics are shown in Table 114.

DNA–DNA relatedness values between the type strain and representatives of related species are as follows: *Brevibacterium epidermidis* (22–23%), *Brevibacterium iodinum* (14% with type strain), and *Brevibacterium linens* (19% with type strain).

Source: cheddar cheese, cheese curd, and raw milk.

DNA G+C content (mol%): 66–67 (T_m).

Type strain: ATCC 35515, CCM 4100, BCRC 12214, CCUG 29001, CIP 102111, DSM 20657, IMET 10997, JCM 2594, KCTC 3082, KCTC 3085, NBRC 14812, NCIMB 702048, VKM Ac-2114.

Sequence accession no. (16S rRNA gene): AJ251418, X76564.

Additional comments: Brevibacterium casei Collins et al., (1983a) was previously known as CDC coryneform groups B-1 and B-3. The name *Brevibacterium casei* (effective publication: Collins et al. 1983a), type strain CMD1 = NCIMB 702048 (formerly NCDO 2048) appears on validation lists 12 and 13. The date of valid publication of the name is the first citation, namely the one in validation list 12 (Euzéby and Kudo, 2001).

7. **Brevibacterium celere** Ivanova, Christen, Alexeeva, Zhukova, Gorshkova, Lysenko, Mikhailov and Nicolau 2004, 2110[VP]

ce′le.re. L. neut. adj. *celere* rapid, indicating the rapid growth on nutrient media.

Aerobic, Gram-stain-positive, non-acid-fast, nonmotile actinomycetes which have a coryneform morphology. Colonies are circular, convex with entire margins, whitish yellow with a butyrous consistency. Growth occurs between 12–42°C, and from pH 5–10 with an optimum at 8.5–9.0. Tolerant to 15% (w/v) NaCl.

Alginate, gelatin, and laminarin are degraded but starch is not. Negative for urease production. The following car-

bon sources are used for growth in Biolog GP2 microplates: acetic acid, *cis*-aconitic acid, alaninamide, γ-aminobutyric acid, bromosuccinic acid, D-and L-alamine, D-and L-carnitine, D-gluconic acid, α-D-glucose, D- and L-lactic acid, D-trehalose, dextrin, formic acid, glycerol, glycyl L-glutamic acid, α-hydroxybutyric acid, β-hydroxybutyric acid, γ-hydroxybutyric acid, *p*-hydroxyphenylacetic acid, hydroxyproline, α-ketobutyric acid, L-alanyl glycine, L-asparagine, L-aspartic acid, L-fucose, L-glutamic acid, L-histidine, L-leucine, L-ornithine, L-phenylalanine, L-proline, L-pyroglutamic acid, L-serine, maltose, methylpyruvate, monomethyl succinate, *N*-acetyl-D-galactosamine, *N*-acetyl-D-glucosamine, phenylethylamine, propionic acid, putrescine, quinic acid, sebacic acid, succinic acid, sucrose, turanose, Tween 40, Tween 80, and urocanic acid. Does not form acid from galactose, glucose, lactose, maltose, sorbose, or xylose.

Susceptible to (μg/ml): carbenicillin (10) and oleandomycin (30), but resistant to: ampicillin (10), benzylpenicillin (10), gentamicin (10), kanamycin (30), lincomycin (15), neomycin (30), streptomycin (30), and polymyxin B (25). Additional phenotypic characteristics are shown in Table 114.

Major fatty acids are $C_{15:0}$ anteiso and $C_{17:0}$ anteiso.

Source: the brown alga *Fucus evanescens*.

DNA G+C content (mol%): 61.4 (T_m).

Type strain: ATCC BAA-809, DSM 15453, JCM 13521, KMM 3637.

Sequence accession no. (16S rRNA gene): AY228463.

8. **Brevibacterium epidermidis** Collins, Farrow, Goodfellow and Minnikin 1983b, 896[VP] (Effective publication: Collins, Farrow, Goodfellow and Minnikin 1983a, 393.)

e.pi.der′mi.dis. Gr. n. *epidermida* the outer skin; N.L. gen. n. *epidermidis* of the epidermis.

Aerobic, Gram-stain-positive, nonmotile actinomycetes which have a coryneform morphology. Gray-white colonies are formed on nutrient agar. Grows optimally between 30–37°C; some strains grow at 40°C. Grows in presence of 15% (w/v) NaCl.

Gelatin is hydrolyzed and extracellular DNase is produced. Nitrate reduction is strain-dependent. Acetate and lactate are used as sole carbon sources. Acid is not produced from glucose in peptone media. Additional phenotypic characteristics are shown in Table 114.

DNA–DNA relatedness values between the type strain and representatives of related species are as follows: *Brevibacterium casei* (31–44%), *Brevibacterium iodinum* (33% with type strain), and *Brevibacterium linens* (14% with type strain).

Source: human skin.

DNA G+C content (mol%): 63.5 (T_m).

Type strain: ATCC 35514, BCRC 2215, CCM 4098, CCUG 30540, CIP 102110, DSM 20660, IMET 10998, JCM 2593, KCTC 3090, NBRC 14811, NCIMB 702286, NCTC 12997, VKM Ac-2108.

Sequence accession no. (16S rRNA gene): X76565.

Additional comments: the species name *Brevibacterium epidermidis* [effective publication: Collins et al. 1983a; type strain: strain D731 = NCIMB 702286 formerly NCDO 2286] appears on Validation Lists 12 and 13. The date of the valid publication of this name is the first citation, namely the one cited in Validation List 12 (Euzéby and Kudo, 2001).

9. **Brevibacterium iodinum** (*ex* Davis 1939) Collins, Jones, Keddie and Sneath 1981, 216[VP] (Effective publication Collins, Jones, Keddie and Sneath 1980, 7.)

i.o.di′num. N.L. neut. n. *iodinum* iodine.

Aerobic, nonmotile actinomycetes which have a rod–coccus growth cycle. Cells frequently appear Gram-stain-negative. In very young cultures (approx. 8 h), cells frequently stain one-half Gram-positive, the other half Gram-negative. Colonies on nutrient agar are whitish-gray and smooth, convex with entire margin, and shiny. Blue to purple extracellular crystals of iodinin are produced on a variety of media. Iodinin production is not influenced by light. Grows between 20–37°C, optimally between 25–30°C, but does not grow below 12°C.

Catalase-positive. Gelatin is hydrolyzed. DNase is produced. Hippurate is not hydrolyzed. H_2S is produced from cysteine but not from sodium thiosulfate. Does not produce acid from glucose or other sugars in peptone based media. Citrate is utilized. Additional phenotypic characteristics are shown in Table 114.

Major fatty acids are $C_{15:0}$ anteiso and $C_{17:0}$ anteiso.

Source: milk.

DNA G+C content (mol%): 60–63 (T_m).

Type strain: ATCC 49514, BCRC 12216, DSM 20626, IMET 10995, JCM 2591, KCTC 3083, NBRC 15230, NCIMB 700613, NCTC 12955, NRRL B-1717, VKM Ac-2106.

Sequence accession no. (16S rRNA gene): X76567, X83813.

10. **Brevibacterium luteolum** corrig. Wauters, Avesani, Laffineur, Charlier, Janssens, Van Bosterhaut and Delmée 2003, 1324[VP]

lu.te′o.lum. L. neut. adj. *luteolum* yellowish, because colonies exhibit a yellow pigment.

Aerobic, Gram-stain-positive rods (2–3 μm long) which show a diphtheroid morphology, but not a rod–coccus growth cycle. Colonies are smooth, yellowish, and approximately 1–2 mm in diameter after incubation for 2 d at 37°C. Grows optimally between 30 and 37°C; some strains grow at 20°C. Grows in the presence of 10% (w/v) NaCl.

Gelatin is degraded, but esculin, tyrosine, and xanthine are not. Ethylene glycol is acidified. γ-Aminobutyric acid is utilized and alkalinized on Simmon's agar base. Phenylacetate is acidified and methanethiol produced from L-methionine. Pyrrolidonyl peptidase-positive. *N*-Acetyl-β-D-glucosaminidase is positive when the nitrophenyl compound is used as substrate (Rosco). Positive for lipase esterase (C8), leucine arylamidase, and phosphoamidase, and acid and alkaline phosphatase variable (negative for the type strain) (API ZYM tests). Additional phenotypic characteristics are shown in Table 114.

Main fatty acids are $C_{15:0}$ anteiso and $C_{17:0}$ anteiso. Isolated from clinical and environmental samples.

Source: peritoneal fluid of a patient undergoing dialysis.

DNA G+C content (mol%): 68.8 (T_m).

Type strain: CF87, CCUG 46604, DSM 15022, JCM 12367.

Sequence accession no. (16S rRNA gene): AJ488509.

Additional comments: Wauters et al. (2003) proposed the specific epithet *lutescens*. However, *lutescens* (L. part. adj. from L. v. *lutesco*) means becoming muddy, not yellowish as cited in the paper by Wauters et al. Consequently, this

specific epithet was corrected on notification with the author's agreement (Euzéby, 2004).

11. **Brevibacterium marinum** Lee 2008, 503[VP]

ma.ri'num. L. neut. adj. *marinum* of the sea, marine.

Aerobic, Gram-stain-positive, nonmotile actinomycete which forms long rods (1.5–2.4 × 0.6–0.9 μm) at the exponential growth phase; as the cultures age these elements fragment into short rods (0.5–0.6 × 0.7–1.0 μm) which occasionally occur singly but more often, in pairs or in chains. V-forms are observed. Colonies are translucent, convex, smooth, and circular with entire margins. Pigment production is variable depending on the culture conditions. In the dark colonies are white; in the light, they are bright yellow. Grows from 10–30°C, optimally at 30°C; does not grow at 4 or 37°C. Growth occurs in the pH range 5–12. Tolerant to 10% (w/v) NaCl.

Hypoxanthine is degraded, but not DNA, gelatin, starch, cellulose, tyrosine, or xanthine. Positive for nitrate reduction, β-galactosidase, and urease, but negative for alkaline phosphatase, β-glucosidase, β-glucuronidase, *N*-acetyl-β-glucosaminidase, and pyrrolidonyl arylmidase (API Coryne tests). Esterase (C4) and esterase lipase (C8) are weakly positive, but acid phosphatase, alkaline phosphatase, α-chemotrypsin, cysteine arylamidase, galactosidase, β-glucosidase, leucine arylamidase, lipase (C14), α-mannosidase, naphthol-AS-BI-phosphohydrolase, trypsin, and valine arylamidase are negative (API ZYM tests). Does not produce acid from D-glucose, D-lactose, maltose, D-mannitol, D-ribose, sucrose, or D-xylose. Additional phenotypic characteristics are shown in Table 114. The major fatty acids are $C_{15:0}$ anteiso, $C_{17:0}$ anteiso, and $C_{18:0}$. The predominant menaquinone is MK-8(H_2), and the major polar lipids are diphosphatidylglycerol, phosphatidylglycerol, and phosphatidyl methyl inositol; an unknown phospholipid is present as a minor component.

Source: seawater from Hwasun Beach in Jeju, Republic of Korea.

DNA G+C content (mol%): 71.4 (HPLC).

Type strain: HFW-26, DSM 18964, JBRI 2001, JCM 15618, KCTC 19221.

Sequence accession no. (16S rRNA gene): AM421807.

12. **Brevibacterium massiliense** Roux and Raoult 2009, 1962[VP]

mas.si.li.en'se. L. neut. adj. *massiliense* of Massilia, the old Roman name for Marseille, where the type strain was isolated.

Aerobic, Gram-stain-positive, nonmotile actinomycetes which form short, irregular straight rods (0.4–1.4 × 0.3–0.5 μm). Colonies are beige, shiny, smooth, circular, and 0.2 mm in diameter after growth for 24 h in 5% CO_2 on sheep blood agar. Grows between 25–45°C, optimally between 30–37°C. Good growth in the presence of 1–10% (w/v) NaCl.

Does not hydrolyze gelatin. Positive for esterase (C4) and leucine arylamidase, but negative for alkaline phosphatase, α-fucosidase, β-glucosidase, β-glucuronidase, α-mannosidase, *N*-acetyl-β-glucosaminidase, and naphthol-AS-BI-phosphohydrolase (API ZYM tests). Positive for alanine arylamidase, arginine arylamidase, glycine arylamidase, leucine arylamidase, leucyl-glycine arylamidase, phenylalanine arylamidase, proline arylamidase, serine arylamidase, and tyrosine arylamidase (API 32A tests).

The following sole carbon sources are used for growth in Biolog GP2 microplates: acetic acid, 2,3-butanediol, D-alanine, glycyl L-glutamic acid, α-hydroxybutyric acid, β-hydroxybutyric acid, γ-hydroxybutyric acid, α-ketovaleric acid, L-alaninamide, L-alanine, L-alanyl glycine, L-asparagine, L-glutamic acid, L-lactic acid, L-serine, *N*-acetyl-L-glutamic acid, propionic acid, pyruvic acid, pyruvic acid methyl ester, succinic acid monomethyl ester, Tween 40, and Tween 80. Additional phenotypic characteristics are shown in Table 114. The major fatty acids are $C_{15:0}$ anteiso, $C_{17:0}$ anteiso, $C_{15:0}$ iso, and $C_{16:0}$ iso.

Source: a human ankle discharge sample.

DNA G+C content (mol%): not determined.

Type strain: 5401308, CCUG 53855, CIP 109422, CSUR P26.

Sequence accession no. (16S rRNA gene): EU868814.

13. **Brevibacterium mcbrellneri** McBride, Ellner, Black, Clarridge and Wolf 1994, 852[VP] (Effective publication: McBride, Ellner, Black, Clarridge and Wolf 1993, 260.)

mc.brell'ne.ri. N.L. masc. gen. n. *mcbrellneri* of McBrEllner, named for two of the authors (M.E. McBride and K.M. Ellner) of the publication describing its isolation and characteristics.

Aerobic, Gram-stain-positive, nonmotile actinomycetes which show a rod–coccus growth cycle. Colonies are whitish-beige, dry, and friable. Grows at 37°C, but not at 20°C. Tolerates 6.5% (w/v) NaCl but not higher concentrations of salt.

DNA and gelatin are hydrolyzed. Xanthine degradation is variable. Pyrrolidone peptidase is negative. Produces methanethiol from L-methionine. Ribose is used as a sole carbon source, but mannose is not. Acid is produced from 2,3-butylene glycol.

Sensitive (μg/ml) to ampicillin (10), cephalothin, methoprim sulfate (24), penicillin (2), and vancomycin (15), but resistant to erythromycin (15), oxacillin (10), and tetracycline (30). Additional phenotypic characteristics are shown in Table 114. The major fatty acids are $C_{15:0}$ antieso and $C_{17:0}$ anteiso. Cell walls contain galactose, glucose, and xylose.

Source: infected genital hair of patients with white piedra.

DNA G+C content (mol%): 63.05 (T_m).

Type strain: E2cr, ATCC 49030, CCUG 37313, DSM 9583, JCM 11682.

Sequence accession no. (16S rRNA gene): X93594.

14. **Brevibacterium oceani** Bhadra, Raghukumar, Pindi and Shivaji 2008, 59[VP]

o.ce.a'ni. L. gen n. *oceani* of the ocean.

Aerobic, Gram-stain-positive, nonmotile, rod-shaped (2.0–3.0 × 1.0–1.2 μm) actinomycetes. Sticky, pale orange colonies (~1.5–2.0 mm in diameter) with entire margins are formed at 28°C on nutrient agar. Growth occurs between 10–35°C, but not at 5 or 37°C. pH for growth is between 5.2–9.5 with an optimum of 6.8.

L-Arginine, L-asparagine, L-lysine, malonate, L-proline, L-serine, and L-tyrosine are used as carbon sources, but adon-

itol, L-cystine, erythritol, D-glucuronate, and 2-oxoglutarate are not. Acid is produced from phenylacetate, but not from adonitol, D- and L-arabinose, D-cellobiose, dulcitol, fructose, D-galactose, glucose, inositol, lactose, maltose, D-mannitol, mannose, D-melibiose, D-raffinose, rhamnose, D-ribose, sucrose, trehalose, or D-xylose. Positive for lysine decarboxylase and ornithine decarboxylase, but negative for citrate utilization, esculin hydrolysis, β-galactosidase, H_2S production, indole production, methyl red and Voges–Proskauer tests, oxidase, and phenylalanine deamination.

Resistant to (μg/ml): chloramphenicol (25), nalidixic acid (15), and polymixin B (15), but sensitive to ampicillin (25), kanamycin (30), roxithromycin (30), streptomycin (25), and tetracycline (25). Additional phenotypic characteristics are shown in Table 114. The major fatty acids are $C_{15:0}$ anteiso and $C_{17:0}$ anteiso. The predominant menaquinone is MK-8(H_2), and the major polar lipids are diphosphatidylglycerol and phosphatidylglycerol.

Source: a 50–70 cm section of a deep-sea sediment core of 4.6 m length obtained from the Chagos Trench, Indian Ocean, at a water depth of 5904 m.

DNA G+C content (mol%): 59.8–60.2 (T_m).

Type strain: BBH7, IAM 15353, JCM 21798, LMG 23457.

Sequence accession no. (16S rRNA gene): AM158906.

15. **Brevibacterium otitidis** Pascual, Collins, Funke and Pitcher 1996b, 1189[VP] (Effective publication: Pascual, Collins, Funke and Pitcher 1996a, 121.)

o.ti′ti.dis. Gr. n. *ous, otos* ear; N.L. suff. *-itis, idis* suffix used in names of inflammations; N.L. gen. n. *otitidis* of inflammation of the ear.

Aerobic, Gram-stain-positive, nonmotile actinomycetes which have a coryneform morphology. Colonies are whitish-yellowish and friable with a dry texture. Growth is observed at 37°C but not at 20°C.

Gelatin and xanthine are degraded. Positive for production of pyrrolidone peptidase. Additional phenotypic characteristics are shown in Table 114.

Source: a human ear infection.

DNA G+C content (mol%): not determined.

Type strain: A37/73, ATCC 700348, BBH7, CCUG 37257, CIP 10544, DSM 10718, JCM 11683, NCIMB 703053.

Sequence accession no. (16S rRNA gene): X93593.

16. **Brevibacterium paucivorans** Wauters, Charlier and Janssens Delmée 2001, 1706[VP]

pau.ci.vo′rans. L. adj. *paucus* little; L. v. *vorare* to eat; N.L. part. adj. *paucivorans* eating little, because few substrates are assimilated.

Aerobic, Gram-stain-positive, nonmotile actinomycetes which form short, plump rods with a coryneform morphology. Colonies are grayish, glistening and smooth or sticky and approximately 2 mm in diameter after incubation for 2 d at 37°C on blood agar. Grows at 37°C, but not 20°C.

Carbohydrates are neither acidified nor assimilated. Gelatin is liquefied weakly and slowly. Does not hydrolyze esculin, or degrade tyrosine or xanthine. Ethylene glycol is acidified but 2,3-butylene glycol is not. Methanethiol is produced from L-methionine. Urea is hydrolyzed. Pyrrolidonyl peptidase is negative. Positive for leucine arylamidase and phosphoamidase; weakly positive for esterase lipase (C8), but negative for acid phosphatase, alkaline phosphatase, α-chymotrypsin, cystine arylamidase, esterase (C4), α-fucosidase, α-and β-glucosidase, α-mannosidase, *N*-acetyl-β-glucosaminidase, and valine arylamidase; some strains are positive for trypsin (API ZYM tests). Additional phenotypic characteristics are shown in Table 114.

The cell wall contains an excess of glutamic acid and glycine moieties that are not bound to the peptidoglycan. The cellular fatty acids are of the branched type with $C_{15:0}$ anteiso and $C_{17:0}$ anteiso as major components. Isolated from clinical material.

Source: blood.

DNA G+C content (mol%): 55.8 (T_m).

Type strain: CF62, CCUG 45688, DSM 13657, JCM 11567, LMG 19814.

Sequence accession no. (16S rRNA gene): AJ251463.

17. **Brevibacterium permense** Gavrish, Krauzova, Potekhina, Karasev, Plotnikova, Altyntseva, Korosteleva and Evtushenko 2005, 1[VP] (Effective publication: Gavrish, Krauzova, Potekhina, Karasev, Plotnikova, Altyntseva, Korosteleva and Evtushenko 2004, 182.)

per.men′se. N.L. neut. adj. *permense* pertaining to Perm, a region of Russia.

Aerobic, Gram-stain-positive, nonmotile actinomycetes which are mainly present as coccoid cells in older cultures. Colonies are white to orange, circular, slightly convex, and glistening. Orange pigment is produced in the light. Grows at 37°C; optimal temperature for growth is 24°C. Grows in the presence of 18% (w/v) NaCl.

Gelatin is hydrolyzed but esculin and urea are not. H_2S is produced. The methyl red and Voges–Proskauer tests are negative. Does not degrade starch. L-Arabinose, cellobiose, fructose, D-galactose, D-glucose, gluconate, glycerol, D-mannose, raffinose, salicin, trehalose, and D-xylose are used as carbon sources when prepared in a mineral medium supplemented with 0.05% (w/v) yeast extract, but D-arabinose, D-arabitol, adonitol, dulcitol, *meso*-erythritol, L-fucose, inositol, lactose, D-melibiose, and sorbitol are not. Acid is produced from D-galactose, D-glucose, fructose, mannose, salicin, and sorbitol, but not from inositol, inulin, D-mannitol, melezitose, raffinose, rhamnose, sorbose, or trehalose. Positive for alkaline phosphatase, but negative for pyrrolidonyl arylmidase, β-glucuronidase, β-galactosidase, and *N*-acetyl-β-glucosaminidase (API Coryne tests). Additional phenotypic characteristics are shown in Table 114. The major menaquinone is MK-8(H_2). Cell-wall teichoic acid contains glycerol, mannose, and mannitol.

Source: salt-contaminated soil samples collected in the Perm region of Russia.

DNA G+C content (mol%): 60.1–64.3 (T_m).

Type strain: JCM 13318, LMG 22207, UMC Ac-413, VKM Ac-2280.

Sequence accession no. (16S rRNA gene): AY243343.

Additional comments: the culture collection number was provided on request for validation (List Editor, 2005).

18. **Brevibacterium picturae** Heyrman, Verbeeren, Schumann, Devos, Swings and De Vos 2004, 1540[VP]

pic.tu′ra.e. L. gen. n. *picturae* of a painting.

Aerobic, Gram-stain-positive, nonmotile actinomycetes which do not form a clear rod–coccus cycle on nutrient agar. Single coccoid cells (0.8 × 1.0 μm) are formed after growth for 6 and 16 h, single coccoid to oval cells after 24 h; after 72 h growth, cells are oval or short rods which occur singly or in pairs (1.0–1.5 × 4.0 μm). Colonies on nutrient agar are white, low-convex, slightly transparent to opaque, and circular with entire margins. Growth is variable at 37°C, optimal between 20–30°C, but does not grow at 10°C. The pH range for growth is 6–9, with an optimum at pH 7.0. Tolerant to 10% (w/v) NaCl.

Positive for alkaline phosphatase, urease, and gelatin hydrolysis, and negative for pyrrolidonyl arylamidase, β-glucuronidase, esculin, and fermentation of ribose, xylose, mannitol, and glycogen; variable for nitrate reduction, β-galactosidase, and acid production from glucose, lactose, maltose, and sucrose (API Coryne tests).

Weakly positive for esterase (C4), esterase lipase (C8), and naphthol-AS-BI-phosphohydrolase; variable for acid phosphatase and leucine arylamidase, and negative for α-chymotrypsin, cystine arylamidase, α-fucosidase, α-galactosidase, β-glucosidase, α-mannosidase, trypsin, and valine arylamidase (API ZYM tests). Additional phenotypic characteristics are shown in Table 114. The major fatty acids are $C_{15:0}$ anteiso and $C_{17:0}$ anteiso. The predominant menaquinone is MK-8(H_2), and the major polar lipids are diphosphatidylglycerol, phosphatidylglycerol, phosphatidylinositol, an unknown glycolipid, and an unknown phospholipid.

Source: a mural painting discolored by microbial growth.

DNA G+C content (mol%): 63.3 (HPLC).

Type strain: DSM 16132, JCM 13319, LMG 22061.

Sequence accession no. (16S rRNA gene): AJ620364.

19. **Brevibacterium pityocampae** Katı, İnce, Demir and Demirbağ 2010, 315[VP]

pi.ty.o.cam′pae. L. fem. n. *pityocampa* a pine grub; L. fem. gen. n. *pityocampae* of a pine grub, referring to the isolation of the type strain from larvae of *Thaumetopoea pityocampa*.

Aerobic, Gram-stain-positive, nonmotile actinomycete which forms rods (1.0 × 0.5 μm). Colonies are yellow, circular, and convex with entire margins. Grows at 25–40°C, from pH 6.0–9.0, and optimally at pH 8.0. Grows in 0–10%, but not 15% (w/v) NaCl.

Arginine dihydrolase, β-galactosidase, lysine decarboxylase, ornithine decarboxylase, and tryptophan deaminase are not formed. Does not produce acetoin, hydrogen sulfide, or indole; gelatin is not degraded and citrate is not utilized. Does not produce acid from amygdalin, L-arabinose, D-glucose, inositol, D-mannitol, melibiose, L-rhamnose, D-sorbitol, or sucrose. Additional phenotypic characteristics are shown in Table 114. The major fatty acids are $C_{15:0}$ anteiso and $C_{17:0}$ anteiso.

Source: healthy larvae of *Thaumetopoea pityocampa* from the Middle Black Sea region of Turkey.

DNA G+C content (mol%): 69.8 (HPLC).

Type strain: Tp12, DSM 21720, NCCB 100255.

Sequence accession no. (16S rRNA gene): EU484189.

20. **Brevibacterium ravenspurgense** Mages, Frodl, Bernard and Funke 2009, 1[VP] (Effective publication: Mages, Frodl, Bernard and Funke 2008, 2985.)

ra.vens.pur.gen′se. N.L. adj. *ravenspurgense* pertaining to Ravenspurgum, Latin name of the town of Ravensburg, Germany, where the type strain of this species was isolated.

Aerobic, Gram-stain-positive, nonmotile actinomycetes which show a coryneform morphology. Colonies are whitish-grayish, slightly convex, have a sticky consistency, and are up to 2 mm in diameter after incubation for 24 h at 35°C on Columbia SBA plates.

Positive for esterase (C4), esterase lipase (C8), leucine arylamidase, and naphthol-AS-BI-phosphohydrolase, but negative for acid phosphatase, α-chymotrypsin, α-fucosidase, α-galactosidase, β-galactosidase, gelatinase, *N*-acetyl-β-glucosaminidase, α-glucosidase, β-glucosidase, β-glucuronidase, lipase (C14), α-mannosidase, nitrate reductase, pyrrolidonyl arylamidase, urease, and valine arylamidase; variable for alkaline phosphatase and trypsin (API ZYM tests). Does not use carbohydrates in the system described by Funke and Carlotti (1994). Additional phenotypic characteristics are shown in Table 114. The major fatty acids are $C_{15:0}$ anteiso and $C_{17:0}$.

Source: the town of Ravensburg in Germany.

DNA G+C content (mol%): not determined.

Type strain: CCUG 56047, DSM 21258.

Sequence accession no. (16S rRNA gene): EU086793.

21. **Brevibacterium salitolerans** Guan, Zhau, Xiao, Liu, Xia, Zhang and Zhang 2010, 2993[VP]

sa.li.to.le′rans. L. n. *sal salis* salt; L. part. adj. *tolerans* tolerating; N.L. part. adj. *salitolerans* salt-tolerating, originating from a saline habitat.

Aerobic, Gram-stain-positive, nonmotile actinomycete which forms rods (1.3–1.8 × 0.6–1.0 μm) but does not show a rod–coccus growth cycle. Colonies on glycerol-asparagine agar (ISP 5 medium) supplemented with 2% (w/v) NaCl, 1% (w/v) KCl, and 0.5% (w/v) $MgCl_2$ are 2.0–3.5 mm in diameter, smooth, circular, and whitish-yellow after 5 d. Colonial pigmentation is always whitish-yellow in both the dark and light. Grows from 15–50°C, optimally between 28 and 40°C, but does not grow at 10°C or 52°C. Grows from pH 5.0–9.0, optimally at pH 7.0–7.5. Tolerant up to 18% (w/v) NaCl, but grows optimally in the presence of 3.8% (w/v) NaCl.

Degrades gelatin, but not cellulose, starch, or Tweens 20, 40, or 80. Positive for alkaline phosphatase, gelatin hydrolysis, β-glucosidase, pyrazinamidase, pyrrolidonyl arylamidase, and acid production from D-ribose (API CORYNE tests). Does not coagulate or peptonize milk or produce H_2S. Methyl red and Voges–Proskauer tests are negative.

The following sole carbon sources are used for growth in Biolog GP2 microplates: acetic acid, *N*-acetyl-L-glutamic acid, adenosine, L-alaninamide, D-alanine, L-alanine, L-alanyl glycine, L-asparagine, 2,3-butanediol, α-cyclodextrin, 2′-deoxy adenosine, dextrin, L-fucose, D-gluconic acid, L-glutamic acid, glycogen, glycyl L-glutamic acid, α-, β- and γ-hydroxybutyric acid, inosine, α-ketovaleric acid, lactamide, L-lactic acid, D-lactic acid methyl ester, D-malic acid, L-malic acid, methyl pyruvate, monomethyl succinate, propionic

acid, pyruvic acid, L-serine, succinamic acid, succinic acid, thymidine, Tween 40, and Tween 80. Additional phenotypic characteristics are shown in Table 114.

The DNA–DNA relatedness value between the type strain and the corresponding strain of *Brevibacterium album* is 41.3%. The major fatty acids are $C_{17:0}$ anteiso and $C_{15:0}$ anteiso. The predominant menaquinone is MK-8(H_2), but smaller amounts of MK-6(H_2), MK-7, MK-7(H_2), and MK-8 are also present. The major polar lipids are diphosphatidylglycerol and phosphatidylglycerol.

Source: a hypersaline sediment sample collected from Lop Nur salt lake in Xinjiang Province, north-west China.

DNA G+C content (mol%): 69 (HPLC).

Type strain: TRM 415, CCTCC AB 208328, JCM 15900, KCTC 19616.

Sequence accession no. (16S rRNA gene): GU117109.

22. **Brevibacterium samyangense** Lee 2006, 1891[VP]

sam.yang.en′se. N.L. neut. adj. *samyangense* of Samyang Beach, Jeju, Republic of Korea, from where the type strain was isolated.

Aerobic, Gram-stain-positive, motile actinomycetes which show an apparent rod–coccus growth cycle on yeast extract-sea water agar; single or paired coccoid cells are seen after 6 h together with rare short rods; after 12–16 h most cells are irregular, slender rods, arranged in V-forms, while coccoid cells occur singly, in pairs, or in chains after 24–72 h. Colonies are opaque, convex, circular, and bright yellow in color with an entire edge. Grows from 10–45°C, but not at 4 or 55°C, from pH 6.1–10.1, and in the presence of 15% (w/v) NaCl.

Degrades D- and L-tyrosine, but not xanthine. The following sole carbon sources are used for growth in Biolog GP2 microplates: acetic acid, *N*-acetyl-L-glutamic acid, *N*-acetyl-D-glucosamine, *N*-acetyl-β-mannosamine, adenosine, adenosine 5′-monophosphate, L-alaninamide, D- and L-alanine, L-alanylglycine, amygdalin, L-arabinose, arabitol, L-asparagine, D-cellobiose, β-cyclodextrin, 2-deoxyadenosine, dextrin, D-fructose, L-fucose, D-galactose, D-galacturonic acid, α-D-glucose, D-glucose 6-phosphate, α-D-glucoside 1-phosphate, L-glutamic acid, DL-α-glycerol phosphate, glycogen, glycyl L-glutamic acid, *p*-hydroxyphenylacetic acid, α-, β-, and γ-hydroxybutyric acids, inosine, *myo*-inositol, inulin, α-ketoglutaric acid, lactamide, L-lactic acid, D-lactic acid methyl ester, α-D-lactose, lactulose, L-malic acid, maltose, maltotriose, mannan, D-mannose, D-melezitose, D-melibiose, methyl ethyl α-D-mannoside, methyl α-D-galactoside, 3-methyl D-glucoside, methyl β-D-glucoside, methyl pyruvate, monomethyl succinate, palatinose, propionic acid, D-psicose, putrescine, L-pyroglutamic acid, D-raffinose, L-rhamnose, D-ribose, salicin, sedoheptulosan, L-serine, D-sorbitol, stachyose, succinamic acid, succinic acid, sucrose, D-tagatose, thymidine, thymidine 5′-monophosphate, D-trehalose, turanose, Tweens 40 and 80, uridine, uridine 5′-monophosphate, xylitol, and D-xylose.

Positive for alkaline phosphatase, β-galactosidase, and pyrrolidonyl arylamidase, but negative for *N*-acetyl-β-glucosaminidase, gelatin hydrolysis, β-glucosidase, β-glucuronidase, urease, and fermentation of glycogen, D-lactose, D-mannitol, and D-ribose (API Coryne tests). Positive for esterase (C4), esterase lipase (C8), leucine arylamidase, naphthol-AS-BI-phosphohydrolase, and trypsin, and weakly positive for cystine arylamidase and valine arylamidase (API ZYM tests). Additional phenotypic characteristics are shown in Table 114. The major fatty acids are $C_{17:0}$ anteiso, $C_{15:0}$ anteiso, and $C_{15:0}$ iso. The predominant menaquinone is MK8(H_2). The polar lipid pattern contains phosphatidylglycerol as a major component but lacks diphosphatidylglycerol and phosphatidylinositol.

Source: sand sediment of Samyang Beach, Jeju Island, Republic of Korea.

DNA G+C content (mol%): 70.7 (T_m).

Type strain: SST-8, JCM 14546, KCCM 42316, NRRL B-41420.

Sequence accession no. (16S rRNA gene): DQ344485.

23. **Brevibacterium sandarakinum** Kämpfer, Schäfer, Lodders and Busse 2010, 911[VP]

san.da.ra.ki′num. N.L. neut. adj. *sandarakinum* (from Gr. neut. adj. *sandarakinos -e -on*) of light-red color.

Aerobic, Gram-stain-positive, nonmotile actinomycete which does not form a clear rod–coccus cycle on nutrient agar. After 12 h growth, cells are coccoid (0.8–1.2 µm) and occur singly; after 24 h cells are coccoid to oval. Good growth occurs after 3 d of incubation on nutrient agar at 25–30°C. Growth occurs between 4°C (weak) and 36°C (but not above this temperature), from pH 5.5–12.5 (optimally from pH 7.5–9.5), and in the presence of 1–10% NaCl.

Acetate (weak), *cis*-aconitate, citrate, fumarate (weak), D-galactose, D-glucose, glutarate, histidine, DL-3-hydroxybutyrate, D-mannose, 2-oxoglutarate, propionate, pyruvate, ribose, and D-sorbitol are used as sole carbon sources, but *N*-acetyl-D-galactosamine, *N*-acetyl-D-glucosamine, *trans*-aconitate, D-adonitol, 4-aminobutyrate, L-arabinose, arbutin, azelate, cellobiose, D-fructose, *myo*-inositol, itaconate, D-maltitol, maltose, D-mannitol, melibiose, mesaconate, 2-oxoglutarate, putrescine, L-rhamnose, salicin, sucrose, trehalose, and D-xylose are not. Additional phenotypic characteristics are shown in Table 114.

Major fatty acids are anteiso-branched fatty acids. Menaquinones include MK-8(H_2), MK-7(H_2), and minor amounts of MK-9(H_2). The polar lipid profile includes diphosphatidylglycerol, phosphatidylglycerol, an unidentified aminophospholipid, an unidentified glycolipid, an unidentified polar lipid, and minor amounts of three unidentified phospholipids. Cadaverine and putrescine are major components in the polyamine profile.

Source: a sample from the wall of a house colonized with molds in Jena, Germany.

DNA G+C content: not determined.

Type strain: 01-Je-003, CCM 7649, DSM 22082.

Sequence accession no. (16S rRNA gene): FN293377.

Additional comments: the type strain of *Brevibacterium sandarakinum* showed relatively low DNA–DNA relatedness to *Brevibacterium marinum* (31.0%, reciprocal 35.7%) and *Brevibacterium picturae* (36.3%, reciprocal 37.9%) (Kämpfer et al., 2010).

24. **Brevibacterium sanguinis** Wauters, Haase, Avesani, Charlier, Janssens, Van Broeck and Delmée 2004a, 1425[VP] (Effective

publication: Wauters, Haase, Avesani, Charlier, Janssens, Van Broeck and Delmée 2004b, 2831.)

san′gui.nis. L. gen. n. *sanguinis* of blood, because most strains were isolated from blood.

Aerobic, Gram-stain-positive, nonmotile actinomycete which shows a coryneform morphology. Colonies are grayish white, opaque, somewhat sticky, and reach a diameter of 2 mm after 48 h at 37°C on blood agar.

Gelatin, tyrosine, and xanthine are degraded. D-Arabinose, L-fucose, gluconate, glucose, glycerol, maltose, *myo*-inositol, *N*-acetylglucosamine, sucrose, trehalose, and turanose are used as carbon sources (API 50CH tests and ID 32 GN strips). Does not produce acid from glucose or other carbohydrates. Positive for alkaline phosphatase, esterase (C4), esterase lipase (C8), leucine arylamidase, acid phosphatase, and phosphoamidase (API ZYM tests). Does not hydrolyze esculin; methanethiol is produced from L-methionine. Quinate and γ-aminobutyrate are assimilated and alkalinized on Simmons' mineral base. Tyramine is assimilated and acidified on Simmons' mineral base by most strains.

Highly sensitive to thallium acetate. The major fatty acids are $C_{15:0}$ anteiso and $C_{17:0}$ anteiso.

DNA G+C content (mol%): 69.9 (T_m).

Type strain: CF63, CCUG 47857, DSM 15677, JCM 13386.

Sequence accession no. (16S rRNA gene): AJ564859.

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ERKO STACKEBRANDT AND PETER SCHUMANN

Cel.lu.lo.mo.na.da.ce′a.e. N.L. fem. n. *Cellulomonas* type genus of the family; suff. *-aceae* ending to denote a family; N.L. fem. pl. n. *Cellulomonadaceae* the *Cellulomonas* family.

Members exhibit a **large variety of morphological forms which often change during growth and under the influence of defined external factors** such as the kind of culture (liquid, chemostat, agar), degree of aeration, and the kind of C- and N-sources. Slender irregular rods of varying length, which may show primary branching, short filaments, and coccoid forms, may occur in late exponential phase cultures; vegetative mycelia with the oldest parts of the mycelium fragmenting into sections of different size and more or less irregular shape may occur. Fragmentation may continue to yield rod-like cells of coryneform appearance and arrangement; aerial mycelium is not formed. **Motile by means of one or several polar or subpolar flagella or by means of peritrichous flagella; some species are nonmotile**. Gram-stain-positive, but many of the older cells stain Gram-negative. **Aerobic to facultatively anaerobic**, producing acid from a variety of carbohydrates. Strains for which information is available form lactic acid and/or acetic acid as the main acidic intermediary products of glucose dissimilation. Glucose is degraded via the Embden–Meyerhof–Parnas pathway and the hexose-monophosphate shunt.

Mycolic acids and diagnostic whole-cell sugars are absent. Cross-linkage of the peptidoglycan is by the A type; **diagnostic amino acids are either ornithine or lysine**. **Interpeptide bridges contain either aspartic acid or glutamic acid**; **L-Thr in combination with a dicarboxylic amino acid occurs in one genus. 12-Methyltetradecanoic ($C_{15:0}$ anteiso) is the prominent fatty acid; $C_{16:0}$, $C_{15:0}$ iso, $C_{14:0}$, and $C_{17:0}$ anteiso may also occur. Menaquinone MK-9(H_4) is the predominant isoprenoid quinone.** Teichoic acids are absent.

The pattern of 16S rRNA gene signatures consists of nucleotides at positions 120 (A), 131:231 (C–G), 196 (U), 342:347 (C–G), 444:490 (A–U), 580:761 (U–A), 602:636 (c–g), 670:736 (A–U), 822:878 (G–C), 823:877 (G–C), 826:874 (C–G), 827 (U), 843 (U), 950:1231 (U–A), 1047:1210 (G–C), 1109 (C), 1145 (A), 1309:1328 (G–C), 1361 (G), and 1383 (y) (Zhi et al., 2009). Usually found in soil, compost, and cellulose enriched environments such as decaying plant materials, bark, wood, sugar fields, rumen, and activated sludge; occasionally isolated from various clinical specimens.

DNA G+C content (mol%): 68–76.

Type genus: **Cellulomonas** Bergey, Harrison, Breed, Hammer and Huntoon 1923, 154 emend. mut. char. Clark 1953, 50.

Further descriptive information

Phylogenetically a member of the order *Micrococcales*. The family contains the type genus *Cellulomonas* Bergey et al. 1923 emend. Clark 1953 emend. Stackebrandt et al. 1982 and the genus *Oerskovia* Prauser et al. 1970 emend. Lechevalier 1972.

The original description of the family *Cellulomonadaceae* Stackebrandt and Prauser 1991b encompassed the genera *Cellulomonas*, *Oerskovia*, *Promicromonospora*, and *Jonesia*. The rational for including these morphologically and chemotaxonomically heterogeneous genera into the same genus was their placement within the same individual line of descent defined by 16S rRNA gene sequence analysis. In the following years, the number of newly described genera of the order *Actinomycetales* was significantly increased, leading to a refinement of the phylogenetic structure of the family (Rainey et al., 1995). The proposal for a new hierarchic structure of the *Actinobacteria* classis nov. Stackebrandt et al. 1997 led to the exclusion of the genera *Promicromonospora* and *Jonesia*, for which the families *Promicromonosporaceae* Rainey, Ward-Rainey and Stackebrandt and *Jonesiaceae* Stackebrandt, Rainey and Ward-Rainey, respectively, were described. As, at the time of this proposal, the type species of the genus *Rarobacter* Yamamoto et al. 1988 was phylogenetically placed adjacent to *Cellulomonas* and *Oerskovia*, this genus was included in the family *Cellulomonadaceae*. A few years later, with more descriptions released, *Rarobacter* changed its position to an individual branch outside the confines of the family *Cellulomonadaceae*. The genus was therefore excluded from this family and transferred to a new family *Rarobacteraceae* Stackebrandt and Schumann 2000b leaving the family *Cellulomonadaceae* to contain only *Cellulomonas* and *Oerskovia*. For the signatures listed in the family description, 16S rRNA gene sequence information of recent descriptions of *Cellulomonas* (An et al., 2005; Brown et al., 2005a; Jones et al., 2005; Kang et al., 2007; Rivas et al., 2004) and *Oerskovia* species (Stackebrandt et al., 2002a) was taken into account. *Cellulomonas fermentans* has been reclassified as *Actinotalea fermentans* gen. nov. (Yi et al., 2007) which is now placed outside the realm of *Cellulomonadaceae* due to the occurrence of MK-10(H_4) and differences in five signature nucleotides. As the set of 16S rRNA signature nucleotides may change with each newly described species added to the family, we refrain from emending the family description on the basis of these signatures alone.

Genus I. **Cellulomonas** Bergey, Harrison, Breed, Hammer and Huntoon 1923, 154 emend. mut. char. Clark 1952, 50[AL]

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Cel.lu.lo.mo′nas. N.L. n. *cellulosum* cellulose; L. fem. n. *monas* a unit, monad; N.L. fem. n. *Cellulomonas* cellulose monad.

In young cultures, slender, irregular rods ~0.3–0.7 µm × ~1.0–4.0 µm or more which may be straight, angular, or slightly curved; some of the rods are arranged at an angle to each other giving V formations. Occasional cells may show primary branching. A mycelium is produced by one species. Cultures a week or more old usually contain mainly short rods, but a small proportion of the cells may be coccoid. Nonsporeforming. Gram-stain-positive, but the cells are very readily decolorized. Not acid fast. Motile by one (usually polar or subpolar) or a few lateral flagella, or nonmotile. **The cell-wall peptidoglycan contains ornithine; dicarboxylic acids are either glutamic acid or aspartic acid. The major isoprenoid quinones are tetrahydrogenated quinones with nine isoprene units [MK-9(H_4)].** Aerobic; most strains also capable of anaerobic growth as stab cultures. Optimum temperature ~30°C. Growth moderate on peptone-yeast extract media at neutral pH giving opaque, usually convex, white, yellowish, or yellow colonies. **Chemoorganotrophic: metabolism primarily respiratory, but also fermentative; most strains produce acid from glucose both aerobically and anaerobically**. Most strains are cellulolytic and reduce nitrate. Phylogenetically a member of the order *Micrococcales*.

DNA G+C content (mol%): 68.5–76.

Type species: **Cellulomonas flavigena** (Kellerman and McBeth 1912) Bergey, Harrison, Breed, Hammer and Huntoon 1923, 165 (*Bacillus flavigena* Kellerman and McBeth 1912, 488).

Further descriptive information

Young cultures consist of slender, irregular rods ranging between ~0.4–0.8 µm in diameter. The rods vary considerably in length and may appear as short filaments in late exponential phase cultures (24 h) when examined by the methods described by Cure and Keddie (1973). They may also show primary branching. As growth proceeds, the rods become shorter and V formations become more obvious. Cultures a week or more old are usually composed mainly of short rods, but a proportion of the cells may be coccoid. When placed on fresh solid medium, growth of coccoid cells occurs by elongation from one or sometimes two parts of the cell to give rods which appear club-shaped or jointed. However, they do not show the marked rod–coccus cycle characteristic of *Arthrobacter* and *Brevibacterium*. Only *Cellulomonas humilata* forms a mycelium with true branching that fragments into diphtheroid and coccoid elements (Collins and Pascual, 2000).

Moderate growth occurs in air at 30°C on meat extract, peptone agar, or yeast-extract, peptone-based agar media at near neutral pH (Keddie and Jones, 1981). Colonies on such media are opaque, usually convex, ~1–3 min in diameter, and usually yellow, but sometimes white. *Cellulomonas humilata* forms branched, filamentous microcolonies (Gledhill and Casida, 1969). Some strains grow at 10°C, some at 5°C, and the maximum temperatures are in the range 36–43°C (cited in Stackebrandt and Keddie, 1986). If tested, strains do not survive heating at 63°C for 30 min (Keddie et al., 1966). All strains grow best aerobically and most give markedly reduced growth under anaerobic conditions; a few are aerobic or give equivocal results (Keddie, 1974; Keddie and Cure, 1977). *Cellulomonas humilata* is microaerophilic (Gledhill and Casida, 1969). The main products of glucose dissimilation in resting cell suspensions of several type strains are acetic acid, L-lactic acid, formic acid, succinic acid, ethanol, and CO_2. The major acid produced from glucose in growing cultures is acetic acid; most strains additionally produce L-lactic acid (Stackebrandt and Kandler, 1979, 1980b). Lactic acid is the main end product of sugar dissimilation by *Cellulomonas humilata* (Gledhill and Casida, 1969); end products were not determined for strains described in the past years. The major route for glucose dissimilation is the Embden–Meyerhof–Parnas pathway; a small amount of glucose is metabolized via the hexose monophosphate pathway (Stackebrandt and Kandler, 1974, 1980b).

Biotin and thiamine are the only exogenous organic growth factors required by the type strains of *Cellulomonas flavigena*, *Cellulomonas biazotea*, *Cellulomonas cellasea*, *Cellulomonas gelida*, and *Cellulomonas uda* (Keddie et al., 1966); when provided with these vitamins, growth occurs in suitable mineral media with glucose as carbon and energy source and an ammonium salt (or nitrate for most strains) as nitrogen source (Owens and Keddie, 1969).

Little information is available on the habitats of *Cellulomonas* species; most of the original cultures were isolated from soil (Bergey et al., 1923), and this has generally been considered to be their major habitat. Decayed wood, cellulose-containing material, compost, and municipal waste were other sources of cellulomonads. Strains of *Cellulomonas denverensis* and *Cellulomonas hominis*, rare human pathogens, were isolated from blood, cerebrospinal fluid, and some other specimen (Brown et al., 2005a; Funke et al., 1995).

A wide range of carbohydrate-binding modules, carbohydrate esterases, and glycoside hydrolases have been studied in detail at the molecular level. Several enzymes have been identified to be involved in the hydrolysis of xylane (xylanases), cellulose (endo-1,4-glucanases, β-glucosidase, cellobiohydrolases), chitin (chitinase), and mannan (β-mannanase). Most of these studies were carried out on *Cellulomonas flavigena* and *Cellulomonas fimi*. The molecular data on these enzymes were compiled by Stackebrandt et al. (2002b). The literature contains more examples of novel cellulomonad enzymes, but evaluation of their properties is confined to function and application. As long as comparative studies of these enzymes on a wider selection of *Cellulomonas* strains is missing, the significance of this data for classification is restricted.

Pathogenicity. A polyphasic study of two bacterial strains isolated from cerebrospinal fluids of a male and a female patient, respectively, revealed that the isolates belong to the genus *Cellulomonas* and represent a new species, *Cellulomonas hominis* (Funke et al., 1995). Although no patients' records were available for the evaluation of the clinical significance of the

isolates, this was the first report on members of the genus *Cellulomonas* that were isolated from clinical specimens. Because of lacking evidence for its pathogenicity, *Cellulomonas hominis* has been affiliated to risk group 1 (Merkblatt B 006 der Berufsgenossenschaft der Chemischen Industrie, July 2005, Germany). Three additional clinical isolates from cerebrospinal fluid (strain W7335), pilonidal cyst (strain W7336), and a lip wound (strain W7387) have been identified as *Cellulomonas hominis* mainly on the basis of DNA–DNA hybridization (Brown et al., 2005a). Two isolates from a patient with endocarditis from Denver (W6929^T from blood and W6124 from a homograft valve) and a blood isolate (strain W6117) from a patient from Ohio differed in their ability to ferment sorbitol and had DNA–DNA similarity values sufficiently different from *Cellulomonas hominis* to be classified in a new species, *Cellulomonas denverensis* (Brown et al., 2005a). *Cellulomonas hominis* strains DMMZ CE40^T and DMMZ CE39 were susceptible to tetracycline, rifampin, and vancomycin (Funke et al., 1995). Rifampin and vancomycin were also suitable antimicrobial agents for *Cellulomonas denverensis* (Brown et al., 2005a). Additionally, clarithromycin, clindamycin, imipenem, and minocycline were considered active against *Cellulomonas hominis* and *Cellulomonas denverensis* strains (Brown et al., 2005a). Two isolates from blood cultures from patients at the Gaziantep University Hospital (southeast Turkey) were identified as members of the genus *Cellulomonas* that were susceptible to teicoplanin, rifampin, vancomycin, gentamicin, and ampicillin-sulbactam and were reported by Balci et al. (2002). Although it is difficult to differentiate between true infection and contamination and to identify *Cellulomonas* strains unambiguously by phenotype-based commercial diagnostic test systems, the role of members of the genus *Cellulomonas* as potential pathogens in clinical cases, especially in immunocompromised patients, must be taken into consideration.

The environmental type strain of *Cellulomonas bogoriensis* (Jones et al., 2005) is susceptible to a wide range of antibiotics; resistance and susceptibility data are also available for *Cellulomonas terrae* (An et al., 2005).

Enrichment and isolation procedures

Cultures may be enriched in a mineral-based medium containing a low (0.05–0.1%) concentration of yeast extract to provide the necessary organic growth factors with filter paper as cellulose source. This is followed by plating on a similar solid medium but containing cellulose in dispersed form. Cellulolytic bacteria produce colonies surrounded by zones of clearing. Direct plating on cellulose agar, or R2A medium without previous enrichment, may also be used. The methods are not selective for *Cellulomonas* and isolates must be screened for those with a coryneform morphology.

Suspensions or macerates of the material being examined (soil, compost, etc.) may be streaked directly onto the surface of cellulose agar (Stewart and Leatherwood, 1976) of the following composition (g/100 ml of distilled water): $NaNO_3$, 0.1; K_2HPO_4, 0.1; KCl, 0.05; $MgSO_4$, 0.05; yeast extract, Difco, 0.05; agar, 1.7; ball-milled filter paper, 0.1; glucose 0.1; pH 7.0. To prepare the ball-milled filter paper, a 3% (w/v) aqueous suspension of filter paper (Whatman no. 1) is ball-milled for 3 d. Other suitable sources of dispersed cellulose may be used, e.g. microcrystalline cellulose (Avicel, FNIL) at a concentration of 0.1% (w/v) (Kaufmann et al., 1976). In other similar versions of the medium, the glucose is omitted. The plates are incubated at 30°C for 5–7 d; colonies showing zones of clearing are replated on the same medium until pure cultures are obtained. Yellow to yellowish isolates which show a coryneform morphology in combination with cellulolytic activity are presumptive members of the genus *Cellulomonas*.

Cellulomonas enrichments may be prepared by a method similar to that described by Han and Srinivasan (1968). A liquid version of the cellulose agar described above is used but with the glucose omitted and with strips of filter paper replacing the finely divided cellulose. Other mineral bases may be used, e.g. that of Han and Srinivasan (1968), Owens and Keddie (1969), Bagnara et al., (1985), and Malekzadeh et al. (1993). Xylanic strains may be isolated on XED medium (xylan, 0.7%; yeast extract, 0.3%; agar, 2.5%) (Rivas et al., 2004). Strains from clinical specimens were cultured on Columbia agar or Trypticase soy agar (Becton Dickinson) with 5% sheep blood at 37°C in a 5% CO_2 atmosphere. Alkaliphilic cellulomonas may be enriched at 37°C on an alkaline casein medium containing the following (g/l): glucose (10), Difco peptone (5), Difco yeast extract (5), K_2HPO_4 (1), $MgSO_4 \cdot 7H_2O$ (0.2), NaCl (40), Na_2CO_3 (10), casein (20), and agar (20) (Jones et al., 2005). Cultivation is achieved in glucose alkaline medium consisting of two parts. Solution A contained the following dissolved in 800 ml distilled water and sterilized: glucose (10 g), Difco peptone (5 g), Difco yeast extract (5 g), K_2HPO_4 (1 g), and $MgSO_4 \cdot 7H_2O$ (0.2 g). Solution B contained 40 g NaCl and 10 g Na_2CO_3 dissolved in 200 ml distilled water and sterilized. The two solutions were then mixed. Solid medium was prepared by adding agar (2%, w/v) to solution A before sterilization (Duckworth et al., 1996).

Maintenance procedures

For short-term preservation (a few weeks), stab cultures (by loop) in TSX semisolid medium (Keddie et al., 1966) should remain viable for 3 months or more at room temperature (20°C). For long-term preservation (several years), cultures may be preserved by lyophilization.

Taxonomic comments

Most species of *Cellulomonas* which have been included in taxonomic studies have been represented only by single strains. It is therefore difficult to know whether or not the few phenetic differences reported to occur between species (see Table 115) are species-specific or only strain-specific. As all species described around 1913 resembled each other phenetically, only *Cellulomonas flavigena* was recognized in the 8th edition of the *Manual*, while *Cellulomonas biazotea*, *Cellulomonas cellasea*, *Cellulomonas gelida*, and *Cellulomonas uda* were considered to be subjective synonyms and *Cellulomonas fimi* a possible subjective synonym of *Cellulomonas flavigena* (Keddie, 1974). However, the reduction to a single species was questioned by Braden and Thayer (1976) on the basis of serological relatedness using purified cell wall preparations as antigens. DNA–DNA reassociation studies on representatives of *Cellulomonas* revealed the presence of seven genetically well-defined species (Stackebrandt and Kandler, 1979); "*Cellulomonas subalbus*" NCIB 8075

TABLE 115. Characteristics differentiating species of the genus *Cellulomonas*[a]

Characteristic	*C. flavigena*[b,c]	*C. biazotea* 704[b,c]	*C. bogoriensis*[d]	*C. cellasea*[b,c]	*C. composti*[e]	*C. denverensis*[f]	*C. fimi*[b,c,f]
Morphology	Straight or curved rods	Straight or curved rods	Straight and rod-shaped	Straight or curved rods	Rods	Short rods	Straight or curved rods
Mycelium	–	–	–	–	–	–	–
Motility	+[b,c]	+[b]	+	–[b]	–	+	+[b]
Catalase	+	+	+	+	–	+	+
Nitrate reduction	+	+	–	+	+	+	+
Urease	–	–	–	–	–	–	–
Growth on[j]*:*							
Acetate	+	+	nd	+	nd	nd	–
Dextrin	+	–	nd	–	nd	nd	w
Gluconate	+	–	–	–	–	nd	–
Lactose	–	+	–	–	w	nd	+
Lactate	–	+	–	+	nd	nd	+
Mannitol	–	–	–	+	–	nd	–
Mannose	nd	+	+	+	+	nd	+
Raffinose	–	+	–	–		–	–
Rhamnose	–	+	w	–	–	nd	+
Ribose	+	–	–	–	w	nd	–
Xylose	+	+	+	+	+	nd	+
Hydrolysis of:							
Casein	nd	nd	+	nd	nd	–	nd
Cellulose	+	+	+	+	+	nd	+
DNase	–	–	+	–	+	nd	–
Esculin	+	+	+	+	+	+	+
Gelatin	+	+	+	–	w	–	+
Starch	+	nd	+	nd	+	nd	nd
Peptidoglycan composition[k]	L-Orn←Asp	L-Orn←D-Glu	L-Orn←D-Asp	L-Orn←D-Glu	L-Orn←D-Glu	nd	L-Orn←D-Glu
Predominant cellular fatty acids[l]	$C_{15:0}$ anteiso	$C_{15:0}$ anteiso, $C_{15:0}$ iso, $C_{16:0}$	$C_{15:0}$ anteiso, $C_{16:0}$	$C_{15:0}$ anteiso, $C_{16:0}$, $C_{17:0}$ anteiso	$C_{15:0}$ anteiso, $C_{16:0}$, $C_{14:0}$, $C_{18:0}$,	$C_{15:0}$ iso, $C_{15:0}$ anteiso, $C_{17:0}$ anteiso	$C_{16:0}$, $C_{17:0}$ anteiso
Cell-wall sugars[m]	$GlcNH_2$, Rha, Man, Rib	$GlcNH_2$, Rha, Gal, 6dTal	nd	Rha, Man, 6dTal	Man, Glu	Man, Rha, Rib	$GlcNH_2$,Rha, Fuc, Glc
DNA G+C content (mol%)	72.7–74.8	71.5–75.6	71.5	75	73.7	68.5	71.0–72.0

[a]Symbols and abbreviations: +, positive; –, negative; w, weakly positive; nd, not determined.

[b]Stackebrandt and Kandler (1979); Stackebrandt et al. (1982); Stackebrandt and Prauser (1991b).

[c]Funke et al. (1995).

[d]Jones et al. (2005).

[e]Kang et al. (2007).

[f]Brown et al. (2005a).

[g]Rivas et al. (2004).

[h]Elberson et al. (2000).

[i]An et al. (2005).

[j]For *Cellulomonas humilata*, recorded under "acid from" in Table 15.49 by Schaal (1986). All strains grow on glucose, maltose and sucrose.

[k]Asp, aspartic acid; Glu, glutamic acid; Orn, ornithine.

[l]A number before a colon indicates the number of carbons; the number after the colon is the number of double bonds; iso, indicates a methyl branch at the iso position; anteiso, indicates a methyl branch at the anteiso position.

[m]6dTal, 6-Deoxytalose; Fuc, fucose; Gal, galactose, Glc, glucose; Man, mannose; Rha, rhamnose.

C. gelida[b,c]	*C. hominis*[c,f]	*C. humilata*[g]	*C. iranensis*[h]	*C. persica*[h]	*C. terrae*[i]	*C. uda*[b,c]	*C. xylanilytica*[g]
Straight or curved rods	Regular short rods	Filaments, fragmenting into diphtheroid or coccoid forms	Straight or curved rods	Straight or curved rods	Straight rods	Straight or curved rods	Curved rods or coccoid
	−	+	−	−	−	−	−
+a	+	−	+	+	−	−	−
+	+	−	nd	nd	−	+	+
−	+	−	+	+	+	+	+
−	−	−	+	+	−	−	−
+	nd	−	+	+	−	+	−
−	+	+	+	+	nd	+	nd
−	+	+	−	−	−	−	−
d	+	+	−	−	+	+	+
−	nd	−	−	−	−	−	−
nd	−	+	nd	nd	−	−	−
nd	nd	+	+	+	+	nd	+
−	+	W1	−	−	−	−	nd
−	+	+	nd	nd	(+)	−	+
−	−	W1	−	−	−	−	−
+	+	w	nd	nd	+	+	+
nd	nd	+	nd	nd	−	nd	−
+	−	w	+	+	+	+	+
+	+	nd	+	+	+	+	nd
nd	+	+	nd	nd	+	+	
+	+	w	W	w	nd	+	w
+	nd	+	+	+	+	+	+
L-Orn←D-Glu	L-Orn←D-Glu	L-Orn←D-Glu	L-Orn←D-Asp	L-Orn←D-Asp	L-Orn←D-Glu	L-Orn←D-Glu	L-Orn←D-Glu
$C_{15:0}$ anteiso, $C_{15:0}$	$C_{15:0}$ anteiso, $C_{16:0}$, $C_{17:0}$ anteiso	$C_{15:0}$ anteiso, $C_{17:0}$ anteiso, $C_{16:0}$	nd	nd	$C_{15:0}$ anteiso, $C_{15:0}$ iso, $C_{16:0}$	nd	$C_{15:0}$ anteiso, $C_{15:0}$ iso, $C_{18:0}$
GlcNH$_2$, Glc	Man, Fuc, Rham	Rha, Fuc, Glc	GlcNH$_2$, Rha, (Man)	GlcNH$_2$, Rha, (Man)	Rha, Gal, Glc	GlcNH$_2$, Man	Rha, Man, Fuc
72.4–74.4	76	73	nd	nd	73.9	72	73

was found to be identical with *Cellulomonas gelida* ATCC 488[T] on the basis of genetic and phenotypic evidence. The Approved Lists of Bacterial Names (Skerman et al., 1980) contains six species: *Cellulomonas flavigena*, *Cellulomonas biazotea*, *Cellulomonas gelida*, *Cellulomonas uda*, *Cellulomonas fimi*, and *Cellulomonas cellasea.* A seventh species, *Cellulomonas cartae*, was described later, and *Nocardia cellulans*, *Oerskovia xanthineolytica*, *Brevibacterium fermentans*, and *Brevibacterium lyticum* were considered to be subjective synonyms of *Cellulomonas cartae* (Stackebrandt and Kandler, 1980a). As the specific epithet *cellulans* (Metcalfe and Brown, 1957) antedates the name *cartae* (Stackebrandt and Kandler, 1980a), the latter epithet was illegitimate and the legitimate name for this species is *Cellulomonas cellulans* (Stackebrandt and Keddie, 1986). Differences in biochemical and morphological characters (Keddie and Jones, 1981) and phylogenetic analyses (Stackebrandt et al., 1980; Stackebrandt et al., 1982) including newly described and undescribed species indicate that *Cellulomonas cellulans* groups more distantly from the core of *Cellulomonas* than observed previously (Rainey et al., 1995). *Cellulomonas cellulans* clusters next to members of the family *Promicromonosporaceae* and differs in chemotaxonomic characteristics from established genera resulting in the reclassification of this species as *Cellulosimicrobium cellulans* (Schumann et al., 2001).

The species *Cellulomonas turbata* was created by the union of *Cellulomonas* and *Oerskovia* (Stackebrandt et al., 1982). However, even at the time of unification, it was evident that *Cellulomonas turbata* differed from all other *Cellulomonas* species in its susceptibility against *Oerskovia* phages (Prauser, 1986), in the amino acid composition of peptidoglycan, and in differences in the formation of mycelia. The emended genus *Oerskovia* with *Oerskovia turbata* as the type species was reestablished by Stackebrandt et al. (2002a). Depending upon the number and phylogenetic origin of reference sequences, *Oerkovia* species may branch as a clade of their own within the radiation of *Cellulomonas* (e.g. Kang et al., 2007). This indicates that distinctness in morphological and chemotaxonomic traits does not always correlate with phylogenetic separation. A similar case is *Actinotalea* (*Cellulomonas*) *fermentans* that tends to occupy different positions within *Cellulomonas* (e.g. An et al., 2005).

Phylogeny. *Cellulomonas* strains share >95% 16S rRNA gene sequence similarities with strains of *Oerskovia.* Independent of the number and selection of outgroup sequences from the order *Micrococcales*, *Oerskovia* strains branch within the radiation of *Cellulomonas* type strains. It is worth noting that the two *Cellulomonas* type strains from clinical specimens, representing *Cellulomonas hominis* and *Cellulomonas denverensis*, cluster specifically with oerskovias, some of which are of clinical relevance as well. The chemotaxonomic clustering of *Cellulomonas* strains according to the dicarboxylic amino acid of the peptidoglycan interpeptide bridge (glutamic versus aspartic acid) is not reflected by the phylogenetic position of the strains. Asp-containing strains are found in three lineages; specific relationships are found between *Cellulomonas flavigena* and *Cellulomonas persica* and between *Cellulomonas fermentans* and *Cellulomonas bogoriensis.* The mostly low bootstrap values separating the Asp-containing strains, make possible a different branching when more strains will be included into the genus. Similarly, the position of the *Oerskovia* lineage is of low statistical significance, and this position should not be considered settled. A few signature base pairs define members of the two highly related genera: At positions 185:192, 602:636, 612:628, and 1120:1153 cellulomonads exhibit the composition R–Y, C–G, Y–G, and C–G, while oerskovias have the composition C–G, G–U, U–A, and U–A, respectively. Recently the species *Cellulomonas fermentans* has been reclassified as *Actinotalea fermentans* (Yi et al., 2007). The decision was based on the change of phylogenetic position of *Cellulomonas fermentan* due to the addition of the 16S rRNA gene sequence of a new member of *Micrococcales*, described as *Demequina aestuarii* (Yi et al., 2007).

Some of the close 16S rRNA gene sequence relationships are supported by moderate DNA–DNA similarity values among the type strains, e.g. *Cellulomonas biazotea* and *Cellulomonas fimi* (50% homology) and *Cellulomonas gelida* and *Cellulomonas uda* (45% similarity; Stackebrandt and Kandler, 1979; 66%, Elberson et al., 2000). A similar range has also been determined for *Cellulomonas flavigena* and *Cellulomonas uda* (range 41–56%; Elberson et al., 2000) and for *Cellulomonas flavigenea*, *Cellulomonas iranensis*, and *Cellulomonas persica* (39–62%), and *Cellulomonas persica* and *Cellulomonas gelida* (48%; Elberson et al., 2000). *Cellulomonas gelida* is genotypically and phenotypically indistinguishable from the strain named "*Cellulomonas subalbus*" (NCIB 8075) (Stackebrandt and Kandler, 1979), thus supporting the earlier proposal by Clark (1953) to reduce "*Cellulomonas* subalbus" to synonymy.

Low values have been determined for *Cellulomonas cellasea* (<35%) and other *Cellulomonas* strains, between *Cellulomonas denverensis* and *Cellulomonas hominis* (24–33%; Brown et al., 2005a) and between *Cellulomonas xylanilytica* XIL11[T] and *Cellulomonas humilata* ATCC 25174[T] (37%; An et al., 2005) (Figure 150).

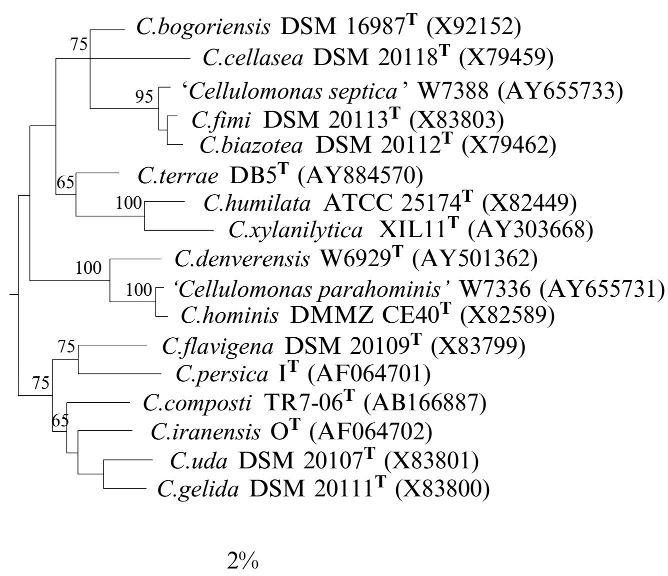

FIGURE 150. 16S rRNA gene sequence dendrogram (De Soete, 1983) of members of the genus *Cellulomonas*, represented by their type strains and two non-validly described species. Bootstrap values (>60%) of 1000 resamplings (Felsenstein, 1989) are indicated at nodes. Members of the genus *Cellulosimicrobium* define the root. The scale represents 2% inferred nucleotide changes.

List of species of the genus *Cellulomonas*

1. **Cellulomonas flavigena** (Kellerman and McBeth 1912) Bergey, Harrison, Breed, Hammer and Huntoon 1923, 165[AL] (*Bacillus flavigena* Kellerman and McBeth 1912, 488)

fla.vi.ge′na. L. adj. *flavus* yellow; L. suff. *genus -a -um* (from L. v. *gigno* to produce, give birth to, beget) producing; N.L. fem. adj. *flavigena* yellow-producing.

Cell morphology as given for the genus. Colonies irregular, 0.9–3.1 mm in diameter, lemon yellow, and opaque, flat, edge fimbriate. Physiological, biochemical, and chemotaxonomic characteristics are given in Table 115 and in the generic description. In addition, cells grow on L-arabinose, D-galactose, cellobiose, and trehalose. The major fatty acid is $C_{15:0}$ anteiso with smaller amounts of $C_{15:0}$ and traces of other iso-, anteiso- and straight chain saturated acids.

DNA G+C content (mol%): 72.7–74.8 (T_m)

Type strain: ATCC 482, CCUG 12162, CCUG 28996, CFBP 4223, CIP 82.10, DSM 20109, NBRC 3754, JCM 1489, LMG 16263, NCAIM B.01383, VKM Ac-1137.

Sequence accession no. (16S rRNA gene): X83799.

2. **Cellulomonas biazotea** (Kellerman, McBeth, Scales and Smith 1913) Bergey, Harrison, Breed, Hammer and Huntoon 1923, 158[AL] (*Bacillus biazoteus* Kellerman, McBeth, Scales and Smith 1913, 506)

bi.a.zo′te.a. L. prefix *bis*, *bi* two; N.L. n. *azotum* (from Fr. n. *azote*) nitrogen; L. fem. suff. *-ea* suffix used with various meanings, but signifying in general made of or belonging to; N.L. fem. adj. *biazotea* two nitrogen sources utilized (i.e. organic and inorganic).

Cell morphology as given for the genus. Colonies circular, slightly irregular, 0.6–2.8 mm in diameter, canary yellow, and opaque, low convex, edge entire or erose. Physiological, biochemical, and chemotaxonomic characters are given in Table 115 and in the generic description. In addition, cells grow on L-arabinose, D-galactose, cellobiose, and trehalose. The major cell-wall sugar is rhamnose while galactose, mannose, and 6-desoxytalose are minor components. The major fatty acid is $C_{15:0}$ anteiso with smaller amounts of $C_{15:0}$ iso.

DNA G+C content (mol%): 71.5–75.6 (T_m).

Type strain: ATCC 486, CFBP 4269, CIP 82.11, DSM 20112, NBRC 12680, JCM 1340, LMG 16695, NCAIM B.01385, VKM Ac-1410.

Sequence accession no. (16S rRNA gene): X79462, X83802.

3. **Cellulomonas bogoriensis** Jones, Grant, Duckworth, Schumann, Weiss and Stackebrandt 2005, 1713[VP]

bo.go.ri.en′sis. N.L. fem. adj. *bogoriensis* pertaining to Lake Bogoria, Kenya.

Cell morphology as given for the genus. On alkaline GAM agar, colonies are opaque, glistening, pale yellow, circular, and convex or domed, the margins are entire, and they are about 2 mm in diameter after 2–3 d at 37°C. Colonies are viscous or slimy and tend to clump when scraped with a loop. On neutral tryptone soy agar (Oxoid), growth is less vigorous, colonies are translucent and yellow and generally <1 mm in diameter. The temperature range for growth is 20–37°C, with an optimum around 30–37°C. No growth occurs at 15 or 45°C. Alkaliphilic and slightly halotolerant. Growth occurs at pH values of 6.0–10.5, with an optimum around pH 9–10. No growth occurs at pH 11 or pH 5.5. Growth below pH 7 is less vigorous and less abundant. Growth occurs in medium containing 0–8.0% (w/v) NaCl. Chemoorganotrophic. Growth occurs on complex substrates such as yeast extract and peptone. Facultatively anaerobic. Physiological, biochemical, and chemotaxonomic characters are given in Table 115 and in the generic description. In addition, acid is produced aerobically and anaerobically (API 50 CH) from the following: L-arabinose, D-xylose, D-glucose, D-fructose, D-mannose, cellobiose, maltose, sucrose, trehalose, gentiobiose, D-turanose, D-lyxose, rhamnose (weak), and 5-ketogluconate (weak). Utilizes amygdalin, arbutin, and salicin. Unable to utilize ribose, lactose, galactose, melibiose, D-raffinose, glycogen, glycerol, erythritol, inositol, mannitol, sorbitol, xylitol, arabitol, gluconate, or lactate. Hydrolyzes carboxymethylcellulose and amorphous cellulose. The following enzymes are produced (API ZYM, API Coryne): C4-esterase, C8-esterase, leucine arylamidase, α-chymotrypsin, α-glucosidase, β-glucosidase, and pyrazinamidase. Major fatty acids are $C_{15:0}$ anteiso and $C_{16:0}$; $C_{15:0}$ iso, $C_{16:0}$ iso, $C_{14:0}$, $C_{15:1}$anteiso, $C_{14:0}$ iso, and $C_{17:0}$ anteiso occur in amounts <6%. Phosphatidylglycerol is the only identified phospholipid; three unknown phospholipids occur as well.

Source: the littoral zone of Lake Bogoria, Kenya, at Acacia Camp.

DNA G+C content (mol%): 71.5.

Type strain: 69B4, CIP 108683, DSM 16987.

Sequence accession no. (16S rRNA gene): AJ863164, X92152.

4. **Cellulomonas cellasea** (Kellerman, McBeth, Scales and Smith 1913) Bergey, Harrison, Breed, Hammer and Huntoon 1923, 158[AL] (*Bacillus cellaseus* Kellerman, McBeth, Scales and Smith 1913, 508)

cel.la′se.a. N.L. n. cellulosum cellulose; L. fem. suff. *-ea* suffix used with various meanings, but signifying in general made of or belonging to; N.L. fem. adj. *cellasea* pertaining to cellulose.

Cell morphology as given for the genus. Colonies circular, becoming irregular, 0.6–3.4 mm diameter, lemon yellow, opaque, edge entire or slightly erose. Physiological, biochemical, and chemotaxonomic characters are given in Table 115 and in the generic description. In addition, cells grow on L-arabinose, D-galactose, cellobiose, and trehalose. The major cell-wall sugar is rhamnose; mannose and 6-desoxytalose occur in smaller amounts.

DNA G+C content (mol%): 75.0 (T_m).

Type strain: ATCC 487, CFBP 4260, CIP 102222, DSM 20118, NBRC 3753, JCM 9967, LMG 16323, NCCB 29015, VKM Ac-1136.

Sequence accession no. (16S rRNA gene): X79459, X83804.

5. **Cellulomonas composti** Kang, Im, Jung, Kim, Goodfellow, Kim, Yang, An and Lee 2007, 1259

com.pos′ti. N.L. n. *compostum* compost; N.L. gen. n. *composti* of compost.

Cell morphology as given for the genus. Nonmotile. Colonies on R2A agar plates are circular, smooth, yellow

circles within 3 d at 25°C. Optimal growth occurs at 30°C and pH 6.8–7.5. Aerobic or facultatively anaerobic. Utilizes D-glucose, maltose, and glycogen as sole carbon sources. Catalase negative. Acid is produced aerobically and anaerobically from L-arabinose, D-xylose, D-galactose, D-glucose, D-fructose, D-mannose, D-cellobiose, D-maltose, sucrose, D-trehalose, starch, glycogen, and gentiobiose. Acid is produced weakly under anaerobic conditions from D-ribose, D-lactose, inulin, and D-lyxose. Hydrolyzes gelatin weakly. Positive for nitrate reduction. β-Glucosidase, amylase, cellulose, and DNase are produced. Negative for catalase and urease. Physiological, biochemical, and chemotaxonomic characters are given in Table 115 and in the generic description. Cell-wall sugars are mannose and glucose.

Source: compost from a cattle farm near Daejeon, Republic of Korea.

DNA G+C content (mol%): 73.7 (HPLC).

Type strain: TR7-06, JCM 14898, KCTC 19030, NBRC 100758.

Sequence accession no. (16S rRNA gene): AB166887.

6. **Cellulomonas denverensis** Brown, Frazier, Morey, Steigerwalt, Pellegrini, Daneshvar, Hollis and McNeil 2005b, 1395[VP] (Effective publication Brown, Frazier, Morey, Steigerwalt, Pellegrini, Daneshvar, Hollis and McNeil 2005a, 1736.)

den.ver.en′sis, N.L. fem. adj. *denverensis* of or pertaining to Denver, Colorado, USA, the city of origin of the type strain.

Cell morphology as given for the genus. Cells are short (1 μm), thin, Gram-stain-positive, nonsporeforming rods that are motile by polar and lateral flagella. Colonies are circular, smooth, and convex, and are pale yellow after about 3 d. Cells are fermentative. Growth occurs at 35 and 45°C but not at 25°C. Catalase positive. Physiological, biochemical, and chemotaxonomic characters are given in Table 115 and in the generic description. Acid is produced from cellobiose, D-galactose, D-glucose, lactose, maltose, mannose, L-rhamnose, salicin, D-sorbitol, sucrose, D-trehalose, and D-xylose, and sometimes from L-arabinose (2/3), glycerol (2/3), and melibiose (1/3). No acid is produced from adonitol, dulcitol, *i*-erythritol, *i*-*myo*-inositol, D-mannitol, melezitose, or raffinose. The diagnostic whole-cell sugars are mannose, ribose, and rhamnose.

Source: blood and other specimens of human origin.

DNA G+C content (mol%): 68.5.

Type strain: W6929, ATCC BAA-788, CCUG 18431, DSM 15764, JCM 14733.

Sequence accession no. (16S rRNA gene): AY501362.

7. **Cellulomonas fimi** (McBeth and Scales 1913) Bergey, Harrison, Breed, Hammer and Huntoon 1923, 166[AL] (*Bacterium fimi* McBeth and Scales 1913, 30)

fi′mi. L. n. *fimus* dung; L. gen. n. *fimi* of dung.

Cell morphology as given for the genus. Colonies circular, 0.4–1.8 mm in diameter, cream or yellowish, opaque, glistening, edge entire. Physiological, biochemical, and chemotaxonomic characteristics are given in Table 115 and in the generic description. The major cell-wall sugar is rhamnose; fucose and glucose are minor components.

DNA G+C content (mol%): 71.3–72.0 (T_m).

Type strain: ATCC 484, CCUG 24087, CFBP 4254, CIP 102114, DSM 20113, HAMBI 93, NBRC 15513, JCM 1341, LMG 16345, NCAIM B.01386, NCCB 29016, NCTC 7547, VKM Ac-1411.

Sequence accession no. (16S rRNA gene): X79460, X83803.

8. **Cellulomonas gelida** (Kellerman, McBeth, Scales and Smith 1913) Bergey, Harrison, Breed, Hammer and Huntoon 1923, 162[AL] (*Bacillus gelidus* Kellerman, McBeth, Scales and Smith 1913, 510)

ge′li.da. L. fem. adj. *gelida* very cold.

Cell morphology as given for the genus. Colonies circular, 0.8–2 mm in diameter, cream or yellowish, opaque, convex domed, edge entire or slightly erose. Physiological, biochemical, and chemotaxonomic characteristics are given in Table 115 and in the generic description. In addition, cells grow on L-arabinose, D-galactose, cellobiose, and trehalose. The major fatty acid is $C_{15:0}$ anteiso with smaller amounts of $C_{15:0}$ and traces of other iso-, anteiso- and straight chain saturated acids. [These studies were carried out on "*C. subalbus*" C222 (NCIB 8075) which has been regarded as a strain of *Cellulomonas gelida*.]

DNA G+C content (mol%): 72.4–74.4 (T_m)

Type strain: ATCC 488, CFBP 4257, CIP 102221, DSM 20111, NBRC 3748, JCM 1490, LMG 16697.

Sequence accession no. (16S rRNA gene): X83800.

9. **Cellulomonas hominis** Funke, Ramos and Collins 1996, 362[VP] (Effective publication: Funke, Ramos and Collins 1995, 2096.)

ho′mi.nis L. gen. n. *hominis* of man, indicating that the two isolates included in the present description are derived from humans and not from the environment.

Cell morphology as given for the genus. The cells are short (1 μm), thin rods without irregular branching. Nonsporeforming. The cells are motile. The colonies are circular, smooth, convex, and whitish initially but with yellowish pigmentation in about 3 d. The colony size is about 1 mm in diameter after 24 h of incubation at 37°C in 5% CO_2. Physiological, biochemical and chemotaxonomic characteristics are given in Table 115 and in the generic description. In addition, acid is produced from L-arabinose, cellobiose, dextrin, D-fructose, galactose, β-gentiobiose, gluconate, glucose, glycerol, lactose, maltose, mannose, α-methyl-D-mannoside, rhamnose, salicin, sucrose, xylose, and β-methylxyloside. No acid is produced from adonitol, dulcitol, erythritol, inositol, 2-ketogluconate, or mannitol. The following enzyme activities are detected: esterase (C4), esterase lipase (C8), leucine arylamidase, α-glucosidase, and α-mannosidase. Valine arylamidase, chymotrypsin, β-glucuronidase, and α-fucosidase are not present.

DNA G+C content (mol%): 73–76 (HPLC).

Type strain: ATCC 51964, CCUG 34783, CIP 104574, DMMZ CE40, DSM 9581, NBRC 16055, JCM 12133, LMG 17240.

Sequence accession no. (16S rRNA gene): not available.

10. **Cellulomonas humilata** (corrig. Gledhill and Casida 1969) Collins and Pascual 2000, 662[VP] (*Actinomyces humiferus* Gledhill and Casida 1969, 119)

hu.mi.la′ta. L. masc. n. *humus* soil; L. part. adj. *latus -a, -um* borne; N.L. fem. adj. *humilata* soil-borne.

Cells are predominantly filamentous and branched and often have swollen ends. They stain Gram-positive and are non-acid-fast. After prolonged incubation, they usually fragment into diphtheroid or coccoid elements of varied size and shape. In liquid media, growth is granular or flocculent, forming a white sediment without turbidity. Mature colonies are small, opaque, smooth, entire, and convex with a dark central region. Rough colony variants occur occasionally. Pigmentation is not evident. Microaerophilic to aerobic; there is poor or no growth in anaerobic conditions. Growth is not stimulated by increased CO_2 tension. Oxidase negative.

The optimum temperature for growth is approximately 30°C; poor or no growth is observed at 37°C. Does not grow on media lacking organic nitrogen. In addition, little if any growth is obtained in certain chemically defined media or those that contain simple peptones. Cells are sensitive to lysis by lysozyme. Fermentation of sugars produces lactic acid as a major end product and no gas formation. Physiological, biochemical, and chemotaxonomic characteristics are given in Table 115 and in the generic description. In addition, casein, esculin, and starch are hydrolyzed, whereas xanthine, tyrosin, and urea are not. Gelatin is weakly decomposed but not liquefied. Litmus milk is acidified and reduced. Nitrates are not reduced to nitrites. Production of indole from tryptophan and of ammonia from peptone and arginine are negative. Methyl-red test is positive, whereas Voges–Proskauer reaction is negative. Hydrogen sulfide is produced. No growth is obtained in the presence of 4% NaCl. Pyruvate, fumarate, 2-oxoglutarate, and gluconate are utilized. Acid is produced from cellobiose, dextrin, D-fructose, D-glucose, D-mannose, D-raffinose, D-xylose, galactose, L-arabinose, maltose, mannitol, melezitose, melibiose, rhamnose, salicin, starch, sucrose, turanose, and β-gentiobiose, whereas acid is not produced from adonitol, dulcitol, inositol, inulin, ribose, or sorbitol. Acid production from glycerol, lactose, and trehalose is variable. Xylane is hydrolyzed. Cell-wall peptidoglycan is of the L-Orn←D-Glu type. Rhamnose is the predominant cell-wall sugar, but glucose and fucose may be present in trace amounts. Thrives in organically rich soil, from which the organism may be recovered in large numbers. The type strain produces acid from glycerol, lactose and trehalose.

Comment: Lysine reported by Collins and Pascual (2000) could not be confirmed as diamino acid of peptidoglycan and the type L-Orn←D-Glu was detected as cited in Stackebrandt et al. (2002a).

Source: organically rich soils.

DNA G+C content (mol%): 73 (Bd).

Type strain: ATCC 25174, CCUG 35596, CIP 104614, JCM 11945, NRRL B-3752.

Sequence accession no. (16S rRNA gene): not available.

11. **Cellulomonas iranensis** Elberson, Malekzadeh, Yazdi, Kameranpour, Noori-Daloii, Matte, Shahamat, Colwell and Sowers 2000, 996[VP]

i.ra.nen′sis. N.L. fem. adj. *iranensis* of or belonging to Iran, where the organism was isolated.

Cell morphology as given for the genus. Irregular, straight, or slightly curved rods 0–4 × 2–54–0 µm, which occasionally exhibit typical coryneform branching. Cells are motile, with 1–4 peritrichous flagella. Gram-stain-positive, but readily decolorized. Colonies are light yellow, approximately 1 mm in diameter, circular, convex, and glossy. Optimal growth temperature is 30°C. Optimal pH for growth is 7.2–7.4. Physiological, biochemical, and chemotaxonomic characteristics are given in Table 115 and in the generic description. In addition, produces acid from dextrin. D-ribose and raffinose are not utilized. Starch and gelatin (weakly) are hydrolyzed. Nitrate is reduced to nitrite, DNase produced. Urea hydrolyzed as nitrogen source in cellulose fermentation. Peptidoglycan is of the L-Orn←D-Asp type and contains the sugars rhamnose, glucosamine, and minor amounts of mannose.

Source: forest humus soil located under walnut, fig, hornbeam, mimosa and box trees in Ramsar Forest, 1.6 km south of Ramsar, Iran, along the border of the Caspian Sea.

DNA G+C content (mol%): not available.

Type strain: O, ATCC 700643, DSM 14785, JCM 12410, NBRC 16734, NBRC 101100.

Sequence accession no. (16S rRNA gene): AF064702.

12. **Cellulomonas persica** Elberson, Malekzadeh, Yazdi, Kameranpour, Noori-Daloii, Matte, Shahamat, Colwell and Sowers 2000, 995[VP]

per′si.ca. L. fem. adj. *persica* Persian, from Persia, classical name of Iran, where the organism was isolated.

Cell morphology as given for the genus. Irregular, straight or slightly curved rods 0–6 × 2–04–0 mm, which occasionally exhibit typical coryneform branching. Cells are motile, with 1–4 peritrichous flagella. Gram-stain-positive, but readily decolorized. Colonies are light yellow, approximately 1 mm in diameter, circular, convex, and glossy. Optimal growth temperature is 30°C. Optimal pH for growth is 7.2–7.4. Physiological, biochemical, and chemotaxonomic characteristics are given in Table 115 and in the generic description. In addition, acid production from dextrin. Does not utilize D-ribose or raffinose. Starch and gelatin are hydrolyzed, the latter weakly. Nitrate is reduced to nitrite, DNase produced. Urea hydrolyzed as nitrogen source in cellulose fermentation. Peptidoglycan is of the L-Orn←D-Asp type and contains the sugars rhamnose, glucosamine, and minor amounts of mannose.

Source: forest humus soil located under walnut, fig, hornbeam, mimosa, and box trees in Ramsar Forest, 1–6 km south of Ramsar, Iran, along the border of the Caspian Sea.

DNA G+C content (mol%): not available.

Type strain: I, ATCC 700642, DSM 14784, JCM 12411, NBRC 16733, NBRC 101101.

Sequence accession no. (16S rRNA gene): AF064701.

13. **Cellulomonas terrae** An, Im, Yang, Kang, Kim, Jin, Kim and Lee 2005, 1708[VP]

ter′rae. L. gen. n. *terrae* of the earth.

Cell morphology as given for the genus. Grows well under aerobic or facultatively anaerobic conditions at 30°C on R2A agar. Cells are Gram-stain-positive, straight rods,

and produce creamy yellow, circular, smooth colonies. No growth at 4 or 45°C or at 4% NaCl. Physiological, biochemical, and chemotaxonomic characteristics are given in Table 115 and in the generic description. In addition, negative for oxidase activity and production of indole and acylamidase, and positive for β-galactosidase, β-glucosidase, and hydrolysis of xylan. Can also use the following as sole carbon sources: arabinose, fructose, galactose, gentiobiose, glycogen, inositol, 5-ketogluconate, mannose, D-melibiose, *N*-acetylglucosamine, rhamnose, starch, and xylane. No growth with the following: acetate, adipate, L-alanine, caprate, citrate, L-fucose, 3-hydroxybenzoate, 3-hydroxybutyrate, 4-hydroxybutyrate, histidine, itaconate, 2-ketogluconate, malate, malonate, propionate, L-proline, phenylacetate, salicin, L-serine, D-sorbitol, suberate, and valerate. Can produce acid from L-arabinose, cellobiose, fructose, galactose, gentiobiose, glycerol, glycogen, glucose, D-lyxose, maltose, mannose, melezitose, melibiose, *N*-acetylglucosamine, raffinose, sucrose, trehalose, D-turanose, and D-xylose. The predominant isoprenoid quinones are MK-9(H_4) and MK-8(H_4). The most abundant cellular fatty acids (>10%) are $C_{15:0}$ anteiso, $C_{15:0}$ iso, and $C_{16:0}$. The cell-wall sugars are galactose, glucose, and rhamnose.

Source: soil near Yusong in Daejeon City, Republic of Korea.

DNA G+C content (mol%): 73.9 (HPLC).

Type strain: DB5, JCM 14899, KCTC 19081, NBRC 100819.

Sequence accession no. (16S rRNA gene): AY884570.

14. **Cellulomonas uda** (Kellerman, McBeth, Scales and Smith 1913) Bergey, Harrison, Breed, Hammer and Huntoon 1923, 166[AL] (*Bacterium udum* Kellerman, McBeth, Scales and Smith 1913, 514)

u′da. L. fem. adj. *uda* moist, wet.

Cell morphology as given for the genus. Colonies punctiform or circular, 0.2–1.8 mm in diameter, off-white, opaque, smooth and shiny surface, edge entire. Physiological, biochemical, and chemotaxonomic characteristics are given in Table 115 and in the generic description. In addition, cells grow on L-arabinose, D-galactose, cellobiose, and trehalose.

DNA G+C content (mol%): 72 (T_m).

Type strain: ATCC 491, CFBP 4256, CIP 102089, DSM 20107, NBRC 3747, JCM 1492, LMG 16327, NCAIM B.01382, VKM Ac-1140.

Sequence accession no. (16S rRNA gene): X83801.

15. **Cellulomonas xylanilytica** Rivas, Trujillo, Mateos, Martínez-Molina and Velázquez 2004, 535[VP]

xy.la.ni.ly′ti.ca. N.L. n. *xylanum* xylan a vegetal polymer, N.L. adj. *lyticus -a -um* (from Gr. adj. *lutikos -ê -on*) able to loosen, able to dissolve; N.L. fem. adj. *xylanilytica* xylan-dissolving.

Cell morphology as given for the genus. Coccoid or rod-shaped cells (2.4 × 1.4 µm). Colonies on YED medium are smooth, yellow, and usually 1–3 mm in diameter within 7 d at 28°C. Optimal growth temperature is 30°C. Optimal growth pH is 7. Oxidase positive. Physiological, biochemical, and chemotaxonomic characteristics are given in Table 115 and in the generic description. Aerobic or facultatively anaerobic and chemoorganotrophic. In addition, utilizes arabinose, cellulose, gentiobiose, mannose, *N*-acetylglucosamine, starch, and xylan as sole carbon sources. By contrast, no growth occurs in acetate, adipate, caprate, citrate, malate, mannitol, phenylacetate, or ribose. Acid is produced from amygdalin, arbutin, cellobiose, D-fructose, galactose, glucose, glycerol, glycogen, inulin, lactose, D-lyxose, maltose, D-mannose, melezitose, melibiose, D-raffinose, rhamnose, salicin, L-sorbose, sucrose, trehalose, xylitol, D-xylose, methyl α-D-glucoside, methyl α-D-mannoside, *N*-acetylglucosamine, and β-gentiobiose. Amylases, cellulases, β-galactosidase, and xylanases are actively produced. Esculin, casein, and gelatin are hydrolyzed. Nitrate is reduced to nitrite. Arginine dehydrolase, indole, tryptophan deaminase, and urease are not produced. Major fatty acids are $C_{15:0}$ anteiso, $C_{16:0}$, and $C_{18:0}$. Major isoprenoid quinones are MK-9(H_4) and MK-8(H_4). Peptidoglycan is of the L-Orn←D-Glu type (A4β). Cell-wall sugars are rhamnose, mannose, and fucose.

Comment: L-Glu as peptidoglycan interpeptide bridge was erroneously stated in the species description of Rivas et al. (2004).

Source: the bark of a decaying elm tree.

DNA G+C content (mol%): 73 (T_m).

Type strain: XIL11, CECT 5729, JCM 14281, LMG 21723, NBRC 101102.

Sequence accession no. (16S rRNA gene): AY303668.

Genus II. *Incertae sedis* **Actinotalea** Yi, Schumann and Chun 2007, 155[VP]

Hans-Jürgen Busse

Ac.ti.no.ta′le.a. Gr. n. *aktis -inos* ray; L. fem. n. *talea* a slender staff, rod, stick; N.L. fem. n. *Actinotalea* ray stick.

Gram-stain-positive, coryneform rods exhibiting polymorphism. Nonmotile. **Facultatively anaerobic**. **Diagnostic diamino acid** of the peptidoglycan is L-ornithine and the **interpeptide bridge is D-aspartate**. **Major fatty acids** are **$C_{14:0}$, $C_{15:0}$ anteiso, and $C_{16:0}$**. The **major isoprenoid quinone is MK-10(H_4)**.

DNA G+C content (mol%): 76.

Type species: **Actinotalea fermentans** (Bagnara, Toci, Gaudin and Belaich 1985) Yi, Schumann and Chun 2007, 155[VP] (*Cellulomonas fermentans* Bagnara, Toci, Gaudin and Belaich 1985, 506).

Further descriptive information

Cellulomonas fermentans was reclassified in a novel genus as *Actinotalea fermentans* based mainly on 16S rRNA gene phylogeny and the predominance of the quinone system menaquinone MK-10(H_4) (Yi et al., 2007) which is rare among bacteria.

Both phylogeny and quinone system distinguish the genus from *Cellulomonas* which exhibits menaquinone MK-9(H_4) as the major compound. Although *Actinotalea fermentans* was placed phylogenetically quite distant from *Cellulomonas* species, 16S rRNA gene sequence similarities (94.5–96.1%) with species of this genus suggest at least a closer relatedness than with other genera. This relatedness is supported by similar fatty acid profiles which consist mainly of branched acids and a significant proportion of the unbranched acid $C_{16:0}$ and by the peptidoglycan of *Actinotalea fermentans* consisting of the diagnostic diamino acid L-ornithine and an interpeptide bridge with D-aspartic acid which is also exhibited by some *Cellulomonas* species (Jones et al., 2005). On the other hand, adding *Actinotalea fermentans* to the family *Cellulomonadaceae* would increase the phenotypic heterogeneity within the family whose representatives share the characteristic menaquinone MK-9(H_4).

Five additional *Actinotalea* strains, as indicated by 16S rRNA gene sequence similarities higher than 96.8%, might be accessible from institutional culture collections: *Actinotalea* species GF, accession no. EU016408 (99.5%), isolated from a benzene-degrading, iron-reducing laboratory enrichment culture (Kunapuli et al., 2007); *Cellulomonas* species ANA-WS2, accession no. EU303275 (97.9%), isolated from a flood field; *Actinotalea fermentans* SAFR-046, AY167843, (97.2%), isolated from a spacecraft assembly facility; actinobacterium PB90-5, accession no. AJ229241 (97.0%), isolated from anoxic bulk soil of a flooded rice microcosm; and actinobacterium SOC A20(63), accession no. DQ628959 (96.9%), isolated from a subglacial bacterial community (Cheng and Foght, 2007).

Enrichment and isolation procedures

The type strain was isolated from an enrichment culture from a municipal dumping ground sample in basal medium* supplemented with 8% barley straw and, after autoclaving, with (per liter) 10 ml of sterile cysteine-hydrochloride solution (5.0%) and 10 ml of sterile sodium bicarbonate solution (8.0%); it was incubated under anaerobic conditions for five weeks. Samples from the enrichment were used to inoculate cellulose agar roll tubes [basal medium supplemented with 4.0% (w/v) ball-milled MN300 cellulose and 2.0% agar] and incubated according to the Hungate roll-tube technique (Hungate, 1969). After 2 weeks of incubation, colonies developing cleared zones of cellulose digestion were detected.

Pathogenicity. No reports exist which indicate a pathogenic potential of this organism.

Maintenance procedures

For maintenance, basal medium supplemented with 0.5% MN300 cellulose in 10 ml volumes under an O_2-free argon atmosphere is recommended (Bagnara et al., 1985).

Differentiation of the genus *Actinotalea* from other genera

The most useful trait for differentiation of the genus from other taxa is the quinone system consisting of the major menaquinone MK-10(H_4). This characteristic unambiguously differentiates the genus from the next closely related genera as indicated by 16S rRNA gene sequence similarities, including *Cellulomonas* and *Oerskovia* which both exhibit the major quinone menaquinone MK-9(H_4) (An et al., 2005; Brown et al., 2005a; Collins and Pascual, 2000; Elberson et al., 2000; Jones et al., 2005; Kang et al., 2007; Rivas et al., 2004; Stackebrandt et al., 2002a, 2002b). So far, MK-10(H_4) has only been reported to be the major quinone in certain species of the genus *Arcanobacterium* (Lehnen et al., 2006). Differentiation between *Actinotalea* and *Arcanobacterium* can be easily achieved by analysis of the fatty acid profile. *Actinotalea* exhibits a fatty acid profile in which branched acids predominate, whereas in *Arcanobacterium*, unbranched acids predominate.

List of species of the genus *Actinotalea*

1. **Actinotalea fermentans** (Bagnara, Toci, Gaudin and Belaich 1985) Yi, Schumann and Chun 2007, 155[VP] (*Cellulomonas fermentans* Bagnara, Toci, Gaudin and Belaich 1985, 506)

fer.men′tans. L. part. adj. *fermentans* fermenting.

The species exhibits the characteristics listed in the genus description. Size of the cells is 0.2–0.5 x 0.6–1.7 µm. Gram-behavior after staining is negative, but ultrastructure of the murein is Gram-positive. Colonies are white with an undulate margin, 2–3 mm in diameter. On cellulose agar, digestion extending from the periphery of the colony is observed. Optimum pH is 7.4, and growth is substantially reduced at pH lower than 7.0 or higher 8.0. Optimum growth is observed at 30–37°C, but no growth at 50°C. Nitrate is reduced, gelatin is liquefied, and NH_4 is produced from peptone. Acetyl methyl carbinol and indol are not produced. Catalase and urease are negative. Arabinose, cellulose, fructose, galactose, glucose, glycogen, lactose, maltose, mannose, mannitol, raffinose, starch, sucrose, trehalose, xylose, and xylan are used as a single source of carbon and energy, but asparagine, arbutin, glycerol, inulin, melibiose, melezitose, pectin, rhamnose, ribose, pyruvate, salicin, sorbitol, and urea are not. Does not grow under aerobic conditions with butanol, butyrate, citrate, ethanol, gluconate, lactate, propanol, propionate, or succinate, but it grows slowly with acetate. Growth is only slightly supported in the presence of yeast extract (0.05%) or Casamino acids (0.2%). Growth factors other than biotin and thiamin together are required and can be provided by 0.05% yeast extract. Products of fermentation are acetic acid, formic acid, ethanol, occasionally L-(+)-lactic acid and, in small amounts, CO_2 and succinic acid. Cell-wall sugars are glucose, rhamnose, and ribose.

Source: a methanogenic enrichment culture under anaerobic conditions from a municipal dumping ground, Montaubert (Essonne, France).

DNA G+C content (mol%): 75.8 (T_m).

Type strain: M, ATCC 43279, CFBP 4259, CIP 103003, DSM 3133, JCM 9966, LMG 16154, NBRC 15517.

Sequence accession no. (16S rRNA gene): X79458, X83805.

*Basal medium composition: 2.21g/l K_2HPO_4, 1.50 g/l KH_2PO_4, 1.30 g/l $(NH_4)SO_4$, 0.10 g/l $MgCl_2 \cdot 6H_2O$, 0.02 g/l $CaCl_2 \cdot 2H_2O$, 0.25 ml $FeSO_4$ solution [0.5% (w/v)], 5g/l yeast extract, pH 7.4.

Genus III. *Incertae sedis* Demequina Yi, Schumann and Chun 2007, 154[VP]

Hans-Jürgen Busse

De.me.qui'na. N.L. fem. n. *Demequina* arbitrary name derived from demethylmenaquinone, an unusual quinone found in this organism.

Gram-stain-positive, strictly aerobic, and slightly halophilic. Oxidase negative, catalase positive and acid-fast negative. Non-spore-forming. Cells are nonmotile rods with round ends. The only isoprenoid quinone is **demethylmenaquinone DMK-9(H_4)**. The peptidoglycan contains the **diagnostic diamino acid L-ornithine** and alanine, glycine, serine, D-glutamate, and aspartate. **Cross-linkage** is of **A-type.** D-Glutamate represents the N-terminus of the interpeptide bridge. Predominant **cellular fatty acids** are of the **anteiso-branched and straight-chain types**. **Major polar lipids** are **phosphatidylinositol, diphosphatidylglycerol**, and an **unknown phospholipid**. Phylogenetically, the genus is affiliated to the order *Micrococcales*.

DNA G+C content (mol%): 67.

Type species: **Demequina aestuarii** Yi, Schumann and Chun 2007, 154[VP].

Further descriptive information

The genus *Demequina* consist so far only of a single species, *Demequina aestuarii*, and its description is based on a single strain (Yi et al., 2007). The strain was isolated from a tidal flat sediment sample from Ganghwa Island, South Korea. Phylogenetically, the species was shown to branch close to the origin of the *Intrasporangiaceae* clade. However, this branching is not supported by significant bootstrap values. The uncertainty of the phylogenetic placement is also supported by similar 16S rRNA gene sequence similarities (94.0–94.2%) with the type species of *Actinotalea* and representatives of the *Sanguibacteraceae*, *Cellumonadaceae*, and *Intrasporangiaceae*. *Demequina aestuarii* exhibits a fatty acid profile in which methyl-branched acids and significant amounts of $C_{16:0}$ predominate. Similar fatty acids have been only reported for *Actinotalea fermentans* and *Cellulomonas* species. The presence of the diagnostic diamino acid L-ornithine in the cell wall clearly distinguishes *Demequina aestuarii* from *Sanguibacteracea* which were reported to contain L-lysine (Fernández-Garayzábal et al., 1995; Huang et al., 2005; Pascual et al., 1996). The other two related families are heterogeneous concerning this trait. Taxa within *Cellulomonadaceae* are characterized either by the presence of L-ornithine or L-lysine (An et al., 2005; Brown et al., 2005a; Collins and Pascual, 2000; Elberson et al., 2000; Jones et al., 2005; Kang et al., 2007; Rivas et al., 2004; Stackebrandt et al., 2002a, 2002b), and *Intrasporangiaceae* (Groth et al., 1999, 2001; Hanada et al., 2002) have been reported to be comprised of taxa which exhibit the presence of L-ornithine or isomers of diaminopimelic acid as the diagnostic diamino acid in the peptidoglycan. The quinone system consisting of the major compound demethylmenaquinone DMK-9(H_4) has not been reported for any species of the *Intrasporangiaceae*, *Sanguibacteraceae*, or *Cellulomonadaceae*, nor for any other taxon of the *Actinobacteria*. Hence, placement of *Demequina aestuarii* in a certain family is not supported by chemotaxonomic characteristics and would create more phenotypic heterogeneity in each family. However, it would be highly desirable if other potential members of the genus (see below) would be examined to determine whether they share this trait.

In addition to the single described species of the genus *Demequina*, members of the genus might be available from institutional culture collections, as can be concluded from 16S rRNA gene sequence entries in gene banks which are seen to share more than 95.0% 16S rRNA gene sequence similarity with the type strain of *Demequina aestuarii*: *Cellulomonas* species KAR82, accession no. EF451710 (97.0%), isolated from Arctic permafrost soil from Spitsbergen, Northern Norway (Hansen et al., 2007); *Cellulomonas* species NP4, accession no. EU196315 (96.0%), isolated from cold saline (7.5% salt) sulfidic spring, Canadian High Arctic; *Sanguibacter* species NH20, accession no. EU109730 (95.9%), isolated from tidal paddy soil; actinobacterium A11, accession no. AB302329 (95.6%), isolated from Indonesian seawater (Harwati et al., 2007); and *Cellulomonas* species F11, accession no. EU697083 (95.2%), isolated from mangrove sediments in Fujian, Fugong, south of the Jiulong River estuary (China). Three additional gene bank entries of bacterial strains held at the IFO culture collection were found with high sequence similarities to *Demequina aestuarii*. Recently, strains from this collection were transferred to NITE Biological Resource Center (NBRC) keeping the IFO numbers. However, it is not clear whether these strains are yet available from NBRC because they could not be found in the catalog of this collection. These strains are: *Cellulomonas* species IFO 16240, accession no. AB023361 (96.7%); *Cellulomonas* species IFO 16241, accession no. AB023362 (96.5%); and *Cellulomonas* species IFO 16246, accession no. AB023367 (96.3%).

Enrichment and isolation procedures

Demequina aestuarii can be isolated from sediment by standard dilution plating on marine agar (Difco 2216).

Pathogenicity. No reports concerning pathogenic potential of *Demequina aestuarii* can be found in literature.

Maintenance procedures

Demequina aestuarii can be maintained as a glycerol suspension (20%, w/v) at −80°C.

Differentiation of the genus *Demequina* from other genera

The most striking characteristic of *Demequina aestuarii* is the quinone system consisting demethylmenaquinone DMK-9(H_4) as its only component. No other actinobacterial taxon has been reported to share this quinone system, hence it can be considered as a diagnostic trait of the species and perhaps for the genus as well.

List of species of the genus *Demequina*

1. **Demequina aestuarii** Yi, Schumann and Chun 2007, 154[VP]

ae.stu.a′ri.i. L. gen. n. *aestuarii* of a tidal flat, isolated from tidal flat sediment.

In addition to the characteristics listed in the genus description the species exhibits the following properties: size of cells is 0.25–0.35 x 0.60–1.20 µm. Growth occurs at pH 6–11 (optimum, pH 7), in 0–12% (w/v) NaCl (optimum, 2–4 %), and at 5–35°C (optimum, 33.5°C). Minimum doubling time is 6.4 h. Extended incubation (up to 15 d) is required at 5°C. Growth occurs on marine agar (Difco 2216), ISP medium No. 3 (Difco) supplemented with sea salts, ISP medium No. 4 (Difco) supplemented with sea salts, and PYGV (Staley, 1968) supplemented with sea salts, but not on Oatmeal agar supplemented with sea salts. Colonies on marine agar at 30°C are yellow, translucent, and circular, with entire margins, approximately 0.2 mm in diameter after 2 d, with a maximum diameter of 3–4 mm after 7 d. Negative for arginine dihydrolase and urease activities. Positive for β-galactosidase activity. Nitrate is not reduced to nitrite. Does not produce H_2S. Acid is not produced from glucose, nor indole from tryptophan. Decomposes carboxymethylcellulose, DNA, and esculin, but not adenine, alginate, casein, chitin, gelatin, hypoxanthine, starch, Tween 80, tyrosine, or xanthine. On the basis of the API ZYM system, the organism is positive for leucine arylamidase, β-galactosidase, α-glucosidase, and β-glucosidase; weakly positive for naphthol-AS-BI-phosphohydrolase; negative for alkaline phosphatase, esterase (C4), esterase lipase (C8), lipase (C14), valine arylamidase, cystine arylamidase, trypsin, chymotrypsin, acid phosphatase, α-galactosidase, β-glucuronidase, *N*-acetyl-β-glucosaminidase, mannosidase, and fucosidase. Utilizes D-cellobiose, D-fructose, D-galactose, D-glucose, D-mannitol, D-mannose, D-salicin, D-trehalose, D-xylose, glycerol, L-arabinose, and sucrose as a sole carbon source. Utilization of lactose is weak. Does not utilize acetamide, acetate, benzoate, citrate, D-raffinose, D-ribose, D-sorbitol, ethanol, glycine, inositol, inulin, 2-propanol, L-arginine, L-ascorbate, L-asparagine, L-lysine, L-ornithine, L-rhamnose, *N*-acetylglucosamine, polyethylene glycol, salicylate, succinate, tartrate, or thiamine. Major fatty acids are $C_{15:0}$ anteiso, $C_{17:0}$ anteiso, $C_{16:0}$, and $C_{15:1}$ anteiso (relative amounts 10–50%); $C_{16:0}$ iso, $C_{15:0}$, and $C_{15:0}$ iso are found in moderate amounts (3–10%)

Source: a tidal flat sediment sample from Ganghwa Island, South Korea.

DNA G+C content (mol%): 67 (HPLC).

Type strain: JC2054, IMSNU 14027, JCM 12123, KCTC 9919.

Sequence accession no. (16S rRNA gene): DQ010160.

Genus IV. **Oerskovia** Prauser, Lechevalier and Lechevalier 1970, 534[AL] emend. Stackebrandt, Breymann, Steiner, Prauser, Weiss and Schumann 2002a, 1108

Erko Stackebrandt and Peter Schumann

O.er.sko′vi.a. N.L. fem. n. *Oerskovia* in honor of J. Ørskov who first described this organism.

Extensively branching vegetative hyphae (~0.5 µm in diameter) growing on the surface of and penetrating into the agar, **which break up into motile or nonmotile rod-like elements**. Growth appears bacteroid in smears. No aerial mycelium. Motile or nonmotile. Gram-stain-positive; catalase-positive; oxidase-positive. **Aerobic to facultatively anaerobic**; strict aerobic strains may occur. Major fatty acids are $C_{15:0}$ anteiso, and $C_{16:0}$; $C_{15:0}$ iso, $C_{14:0}$, and $C_{17:0}$ anteiso occur in smaller amounts. **Major isoprenoid quinone is MK-9(H_4). Diamino acid of peptidoglycan is L-lysine; the interpeptide bridge consists of either L-Thr←D-Asp or L-Thr←D-Glu**. Muramic acid residues are acetylated. Major polar lipids are phosphatidylglycerol, diphosphatidylglycerol, and phosphatidylinositol. Widely distributed in soil.

DNA G+C content (mol%): 70.5–75.0.

Type species: **Oerskovia turbata** (Erikson 1954) Prauser, Lechevalier and Lechevalier 1970, 534[AL] emend. Lechevalier 1972, 263 emend. Stackebrandt, Breymann, Steiner, Prauser, Weiss and Schumann 2002a, 1109 (*Nocardia turbata* Erikson 1954).

Further descriptive information

Erikson (1954) described "Oerskov's motile nocardia, strain 27", which was first reported by Ørskov (1938), as *Nocardia turbata*. The rational for the reclassification of this species as the type species of the new genus *Oerskovia*, *Oerskovia turbata* (Prauser et al., 1970), was based on evidence that members of this species were phenetically different from strains of *Nocardia* of the *asteroides–farcinica* type (Jones and Bradley, 1964; Prauser, 1967; Sukapure et al., 1970). This species differs significantly from authentic *Nocardia* strains by exhibiting lysine as the diagnostic diamino acid of the peptidoglycan rather than *meso*-diaminopimelic acid as found in nocardiae. Many strains of the species are motile by flagellation of fragmented hyphal parts (Sukapure et al., 1970) (named motile "spores" by Higgins et al., 1967). The genus was emended by Lechevalier (1972) based on the capability of the species to grow anaerobically on trypticase-soy agar (one of 15 media tested), to metabolize glucose oxidatively and fermentatively, and because it is catalase negative when grown under anaerobic conditions.

Oerskoviae were randomly and rarely isolated from various types of soils from different geographical regions, composts, decaying plant materials, and occasionally from various clinical specimens (Reller et al., 1975; Sottnek et al., 1977; Cruickshank et al., 1979; see below). *Oerskovia enterophila* constitutes the major part of the actinomycete microflora of the intestines and feces of litter-inhabiting millipedes, e.g. *Chromatoiulus projectus* (Dzingov et al., 1982; Jáger et al., 1983; Szabó et al., 1986). This species also occurs in the feces of the cave-inhabiting blind isopode, *Mesoniscus graniger* (Bodnar et al., 1989). A large homogeneous

population of facultatively anaerobic *Oerskovia*-type nocardioforms was also found in the gut contents of adult specimens of the common earthworm *Lumbricus polyphemus* (Ravasz et al., 1987; Szabó et al., 1986). None of these oerskoviae could be isolated from the surrounding feeding habitats of the animals, nor did they survive in the natural soil and litter habitat (Márialigeti et al., 1985). On the other hand, 13 out of 311 culturable cellulolytic bacterial isolates from soil of an agricultural encatchment were closely related (99.6% 16S rDNA gene sequence similarity) to *Oerskovia* (*Promicromonospora*) *enterophila* (Ulrich and Wirth, 1999).

Pathogenicity. *Oerskovia* strains have been implicated as agents of disease since the first report of Reller et al. (1975). Isolates from blood, heart tissues and heart valves have been identified as *Oerskovia turbata* strains while *Oerskovia xanthineolytica* strains have been isolated from blood, cerebrospinal fluid, sputum, urine, and wounds (Sottnek et al., 1977). *Oerskovia* strains have been reported to cause cholecystitis (Thomas et al., 2007), endocarditis (Reller et al., 1975; Urbina et al., 2003), endophthalmitis (Hussain et al., 1987), keratitis (Shah et al., 1996), meningitis (Kailath et al., 1988), peritonitis (Lujan-Zilbermann et al., 1999; Rihs et al., 1990), pyonephritis (Cruickshank et al., 1979), and different types of bacteremia (LeProwse et al., 1989; Niamut et al., 2003). Successful antibiotic therapy has been achieved with vancomycin in several cases of foreign-body-associated infections involving catheters or prosthetic joints (Harrington et al., 1996; Rihs et al., 1990). The unambiguous identification of *Oerskovia* strains requires a polyphasic strategy, hence reports on *Oerskovia* infections diagnosed only by using commercial, phenotype-based diagnostic test systems are difficult to evaluate. Several isolates from clinical specimens identified as *Oerskovia turbata* were reclassified as *Cellulosimicrobium funkei* (Brown et al., 2006).

Taxonomic comments

The discovery that the genera *Cellulomonas* and *Oerskovia* were highly related phylogenetically led to the latter becoming a synonym of the former (Stackebrandt et al., 1982) and the consequential reclassification of *Oerskovia turbata* as *Cellulomonas turbata*. However, the distinctiveness of *Cellulomonas turbata* from other *Cellulomonas* species was evident even at the time of unification, as authentic cellulomonads were not susceptible to *Oerskovia* phages (Prauser, 1986) and showed differences in peptidoglycan composition and the ability to form mycelia. The emphasis at the time of unification was placed on the phylogenetic position of strains rather than on the presence of distinctly different chemotaxonomic properties (Stackebrandt and Prauser, 1991b). As more species of *Cellulomonas* were described, members of the genus *Oerskovia* were pushed towards the periphery of the *Cellulomonas* phylogenetic tree. The application of different tree-making algorithms to the 16S rRNA gene sequence dataset composed of members of the suborder *Micrococcineae* (since elevated to order *Micrococcales* in the roadmap to the present volume) showed that *Cellulomonas* [*Oerskovia*] *turbata* and its relatives branched close to the root of the *Cellulomonas* tree in most analyses, though the branching was never supported by bootstrap values that would have signaled statistically significant differences. Nevertheless, the phylogenetic position of *Cellulomonas* [*Oerskovia*] *turbata* and the notion that there was a high correlation between phylogenetic position and chemotaxonomic uniqueness among actinobacterial genera (Stackebrandt and Schumann, 2000a) led to the re-establishment of the genus *Oerskovia* (Stackebrandt et al., 2002a). These workers also transferred *Promicromonospora enterophila* to the genus *Oerskovia* as *Oerskovia enterophila* on the basis of phylogenetic (99.6% 16S rRNA gene sequence similarity with *Oerskovia turbata*), chemotaxonomic, and physiological data (Jáger et al., 1983) as well as on phage susceptibility studies (Prauser, 1986). Two new *Oerkovia* species, *Oerkovia jenensis* and *Oerkovia paurometabola*, were also described in this study to accommodate strains which, until then, had been recognized as either *Cellulomonas turbata* or *Promicromonospora enterophila*.

The description of additional *Cellulomonas* species led to the genus *Oerskovia* being placed back into the radiation of *Cellulomonas* species, though low bootstrap values indicate the tentativeness of this relationship (Figure 151). The high phylogenetic relatedness found between the four *Oerskovia* species is reflected by moderately high DNA–DNA reassociation values which range 55–75% similarity. *Oerskovia paurometabola* and *Oerskovia jenensis* are particularly close neighbors, as strains of these species share 64–75% DNA–DNA similarity (Stackebrandt et al., 2002a).

Oerskovia xanthineolytica (Lechevalier, 1972) has also had a checkered taxonomic history. When the type species *Oerskovia turbata* was transferred to the genus *Cellulomonas* as *Cellulomonas turbata*, *Oerskovia xanthineolytica*, the second species in the genus *Oerskovia*, was not described as the type species of the genus, hence this species stayed without genus affiliation. Stackebrandt and Kandler (1980a) proposed *Oerskovia xanthineolytica*, *Nocardia cellulans*, and some other taxa as subjective synonyms of *Cellulomonas cartae* which was described for the then non-validly published species "*Cellulomonas cartalyticum*". However, *Nocardia cellulans* Metcalf and Brown (1957) had priority over *Cellulomonas cartae*, hence this assemblage of strains was reclassified as *Cellulomonas cellulans* (Stackebrandt and Keddie, 1986). Besides *Cellulomonas cartae* and *Oerskovia xanthineolytica*, other taxa included in this species were "*Arthrobacter luteus*" (Kaneko et al., 1969), *Brevibacterium fermentans* (Chatelain and Second, 1966), *Brevibacterium lyticum* (Takayama et al., 1960), and "*Corynebacterium manihot*" (Collard, 1963). These organisms were considered to belong to the same species as they shared the peptidoglycan type L-Lys←D-Ser←D-Asp (Seidl et al., 1980; for additional references, see below), were susceptible to *Oerskovia* phages (particularly to those active against *Oerskovia xanthineolytica*; Prauser, 1984, 1986), and were found to be similar based on numerical taxonomic data (Jones, 1975; Seiler, 1983; Seiler et al., 1977). In addition, high DNA–DNA relatedness values were obtained for *Brevibacterium fermentans*, *Cellulomonas cellulans*, "*Corynebacterium manihot*", and *Oerskovia xanthineolytica* (60–68%; Stackebrandt et al., 1980; Prauser, 1986), while *Cellulomonas cartae* and *Nocardia cellulans* were found to be closely related based on 16S rRNA cataloging data (Stackebrandt et al., 1980). *Cellulomonas cellulans* (including the synonym *Oerskovia xanthineolytica*) has recently been reclassified as *Cellulosimicrobium cellulans* (Schumann et al., 2001).

Isolation and enrichment

Oerskoviae, like many other soil bacteria, grow on a wide range of media. The main problem in their isolation is the exclusion

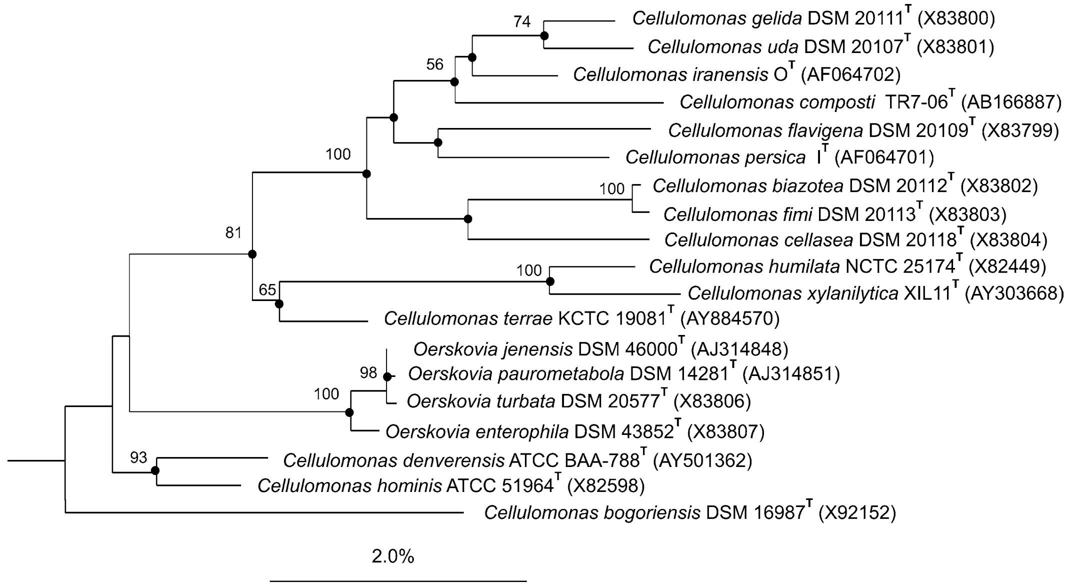

FIGURE 151. Neighbor-joining tree based on almost complete 16S rRNA gene sequences showing relationships between type strains of *Oerskovia* and *Cellulomonas*. Dots at branching points indicate that the same topologies were recovered by maximum likelihood analysis (Felsenstein, 1981). Numbers at branching points are per cent bootstrap values (1000 resamplings) (only values of 60% and above are shown). Members of the order *Micrococcales* were used to root the tree.

of contaminants that can cover large areas of isolation plates, e.g. swarming bacilli, pseudomonads, and hyphal fungi, as well as the suppression of streptomycetes, which may be confused at first sight with young stages on the isolation plates and which may antagonize oerskoviae. Procedures and media recommended for the isolation of *Promicromonospora* strains (Stackebrandt and Prauser, 1991b) can be used to isolate oerskoviae. Tapwater agar (1.5% crude agar in tap water; Lechevalier and Lechevalier, 1986) has been used to isolate oerskoviae.

Maintenance procedures

Serial transfers on nutrient agar at 4-week intervals followed by maintenance at 4°C are recommended for maintaining oerskoviae. Storage of cells as 20% (w/v) glycerol suspensions at −20°C and at −80°C is a suitable preservation method. Long-term preservation methods include freeze drying in skim milk and maintenance in liquid nitrogen at −196°C.

Differentiation of *Oerskovia* from related genera

The most reliable way of placing an unidentified strain into the genus *Oerskovia* is to determine its 16S rRNA gene sequence and peptidoglycan type. In addition, *Oerskovia* strains are characterized by MK-9(H_4) as the predominant menaquinone, by a phospholipid pattern consisting of phosphatidylglycerol, diphosphatidylglycerol, phosphatidylinositol, and three unidentified phospholipids, and by an A4α peptidoglycan type in which the amino acid moiety at position 3 of the peptide side chain consists of lysine and the interpeptide bridge is composed of threonine and either D-aspartic acid (L-Lys←L-Thr←D-Asp) or D-glutamic acid (L-Lys←L-Thr←D-Glu) (Seidl et al., 1980). In contrast, strains of *Cellulomonas* possess L-ornithine at position 3 of the side chain and a single dicarboxylic amino acid (either D-aspartic acid or D-glutamic acid) defines the interpeptide bridge. The main fatty acids of oerskoviae are similar to those of phylogenetically close taxa.

A set of phages has been proposed as specific to the genus *Oerskovia* (Prauser, 1984, 1986; Prauser and Falta, 1968; Stackebrandt and Prauser, 1991b). The reclassification of *Oerskovia xanthineolytica* first as *Cellulomonas cellulans* and then as *Cellulosimicrobium cellulans* means that phages O5 (specific for DSM 49112) and O13 (for DSM 49139) have to be removed from the *Oerskovia* phage set. Although studies on the phage susceptibility of members of the family *Cellulomonadaceae* have not been performed recently, it seems reasonable to restrict the set of *Oerskovia*-specific phages to O6 (DSM 49111) (host *Oerskovia turbata* DSM 20577^T), O2 (DSM 49109) (host *Oerskovia turbata* DSM 43878), and O3 (DSM 49138) (host *Oerskovia jenensis* DSM 46000^T). Phages have not been described for the other *Oerskovia* species.

Differentiation of the species of the genus *Oerskovia*

Oerskovia enterophila DSM 43852^T utilizes a broad range of Biolog compounds (76 out of 95 substrates metabolized), whereas *Oerskovia paurometabola* DSM 14281^T mainly gives negative results towards the Biolog substrate panel (28 out of 95 positive). *Oerskovia jenensis* and *Oerskovia turbata* are metabolically similar (Stackebrandt et al., 2002a). Phenotypic properties that can be used to differentiate between *Oerskovia* species are shown in Table 116. *Oerskovia turbata* possesses the peptidoglycan type L-Lys←L-Thr←D-Asp, while the other members of the genus are defined by the L-Lys←L-Thr←D-Glu peptidoglycan type. *Oerskovia* strains fall into species specific clusters based on RiboPrint patterns generated by using the restriction enzyme *Pst*I (Figure 152; Stackebrandt et al., 2002a).

TABLE 116. Characteristics differentiating phenotypic properties of *Oerskovia* species as determined by using API Coryne kits[a,b]

Characteristic	*O. turbata*	*O. jenensis*	*O. enterophila*	*O. paurometabola*
β-Galactosidase	+	w	+	−
Gelatin hydrolysis	+	−	−	−
Glycogen fermentation	v	+	+	+
Pyrazinamidase	−	−	+	−
Urease	−	v	+	+

[a]Symbols: +, >85% positive; -, 0–15% positive; w, weak reaction; v, variable.

[b]All strains were positive in the following reactions: *N*-acetyl-β-glucosidase, catalase, oxidase, α- and β-glucosidase, nitrate reduction, pyrazimidase, and in fermenting glucose, maltose, ribose saccharose, and xylose. They were negative for β-glucuronidase, pyrrolidonyl arylamidase, and in fermenting lactose and mannitol. All strains utilized the following Biolog GP substrates: *N*-acetylglucosamine, adenosine, adenosine-5′-monophosphate, β-cyclodextrin, 2-deoxyadenosine, dextrin, D-gluconic acid, α-D-glucose, glycogen, glycerol, inosine, maltose, maltotriose, mannose, methyl-pyruvate, ribose, salicin, sucrose, thymidine, trehalose, turanose, Tween 40, uridine, and D-xylose but not for: L-alanine, L-D-alanine, alaninamide, L-alanyl-glycine, D-arabitol, glucose 1-phosphate, glucose 6-phosphate, inulin, *p*-hydroxyphenyl acetic acid, α-ketoglutaric acid, and melezitose.

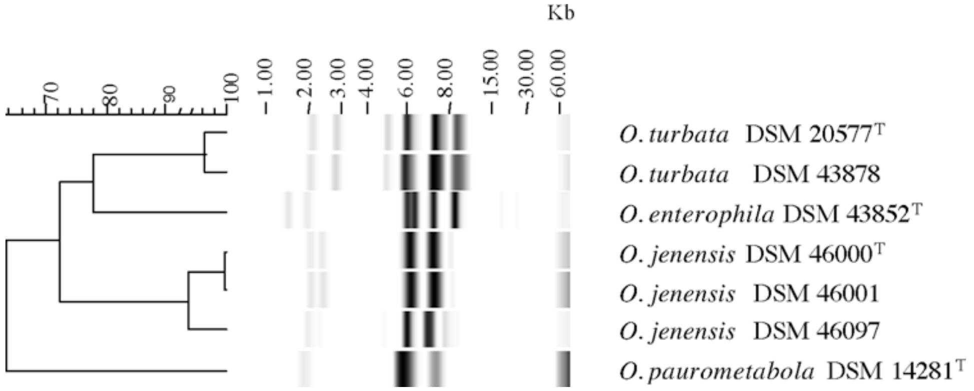

FIGURE 152. RiboPrint pattern of strains of the genus *Oerskovia* generated with *Pst*I. The dendrogram (Pearson correlation) was generated using BioNumerics software (Applied Maths, Kortrijk, Belgium).

List of species of the genus *Oerskovia*

1. **Oerskovia turbata** (Erikson 1954) Prauser, Lechevalier and Lechevalier 1970, 534[AL] emend. Lechevalier 1972, 263; emend. Stackebrandt, Breymann, Steiner, Prauser, Weiss and Schumann 2002a, 1109 (*Nocardia turbata* Erikson 1954)

tur.ba′ta L. fem. adj. *turbata* agitated.

Extensively branching vegetative hyphae fragment into motile rod-like elements which are usually monotrichous when small and peritrichous when long (~0.4–1.1 µm). Colonies on CASO (Difco) are lemon yellow and 0.2–0.4 mm in diameter at 28°C after 2 d. Nitrite is produced from nitrate. Casein, gelatin, and starch are hydrolyzed. Catalase positive. Not acid fast. The type strain is aerobic to facultatively anaerobic. Mesophilic. Isolated from soil. Acid is produced from arabinose, cellobiose, dextrin, fructose, galactose, glucose, glycerol, glycogen, α-methyl-D-glucose, β-methyl-D-xyloside, lactose, maltose, mannose, salicin, sucrose, trehalose, and xylose but not from adonitol or inositol. Acetate, lactate, and pyruvate are used as sole carbon sources, but benzoate, succinate, tartrate, and tyrosine are not. The following tests are variable (percent positive strains): degradation of hypoxanthine (37%), xanthine (37%), use of citrate (37%), and propionate (88%) as sole carbon sources and acid production from mannitol (12%), melibiose (37%), raffinose (25%), rhamnose (12%), sorbitol (25%), and sorbose (12%). In addition to the physiological reactions shown in Table 116 the following Biolog substrates are utilized: α-acetyl mannosamine, amygdalin, arbutin, cellobiose, D-fructose, D-galactose, gentobiose, D-lactic acid methylester, L-lactic acid, α-D-lactose, mannan, D-melibiose, α-methyl-D-galactoside, and sodium, acetic. Peptidoglycan type is L-Lys←L-Thr←D-Asp. $C_{15:0}$ anteiso is the major fatty acid (>40%); $C_{15:0}$ iso, $C_{16:0}$, $C_{14:0}$, $C_{16:0}$ iso, and $C_{17:0}$ anteiso occur in smaller amounts (<5–20%).

Source: soil.

DNA G+C content (mol%): 71–75 (T_m).

Type strain: 891, Ørskov strain 27, ATCC 25835, CFBP 4266, CIP 100331, DSM 20577, NBRC 15015, JCM 3160, LMG 4072, NCIMB 10587, NCTC 11973, NRRL B-8019, VKM Ac-1024.

Sequence accession no. (16S rRNA gene): X79454, X83806.

2. **Oerskovia enterophila** (Jáger, Márialigeti, Hauck and Barabás 1983) Stackebrandt, Breymann, Steiner, Prauser, Weiss and Schumann 2002a, 1110[VP] (*Promicromonospora enterophila* Jáger, Márialigeti, Hauck and Barabás 1983, 530)

en.ter.o′phi.la. Gr. n. *enteron* intestine; Gr. adj. *philos, ê, on* loving; N.L. fem. adj. *enterophila* gut-loving.

Gram-stain-positive, non-acid-fast, facultatively anaerobic organism with branching vegetative hyphae. Aerial mycelium absent. Substrate hyphae fragment into nonmotile bacillary and coccoid or sporelike elements. In general, colonies are glistening, soft, pasty, and cream yellow in color. On CASO agar (DIFCO) they are 0.3–0.5 mm in diameter at 28°C after 2 d. On starch-yeast extract media rough, wrinkled, dull colony variants may occur. Does not form water-soluble diffusible pigments or melanin pigments. Growth occurs at pH 3.0, in 5% NaCl, and at 32°C. Cellulose is not degraded. Indole and Voges–Proskauer tests are negative. Does not degrade hypoxanthine, ribonucleic acid, tyrosine, Tween 80, or xanthine. Reaction for β-glucosidase (esculin) is positive in the API "Coryne" substrate panel but negative according to Jáger et al. (1983). Glucose is metabolized both oxidatively and fermentatively. Dextrin, lactose, maltose, mannose, sucrose, salicin, trehalose, and xylose used as sole carbon sources, but adonitol, dulcitol, inositol, inulin, mannitol, melibiose, melezitose, raffinose, rhamnose, sorbitol, and sorbose are not. In addition to the physiological reactions shown in Table 116, the following Biolog substrates are utilized: α-acetyl mannosamine, amygdalin, arbutin, L-asparagine, lactic acid, cellobiose, D-fructose, fructose-6-phosphate, DL-fucose, D-galactose, D-galacturonic acid, gentobiose, α-hydroxybutyric acid, γ- hydroxybutyric acid, lactamide, *meso*-inositol, D-lactic acid methylester, α-D-lactose, D-malic acid, mannan, D-melibiose, α-methyl-D-galactoside, sedoheptulosan, sodium acetate, and stachyose. Susceptible to cephalosporine (10 μg ml), pristinamycin (10 μg ml), spiramycin (30 μg ml), and vancomycin (50 μg ml), but resistant to nalidixic acid (30 μg ml), nitrofurantoin (300 μg ml), and nystatin (100 IU). The peptidoglycan is of the A4α type, variation L-Lys←L-Thr←D-Glu. $C_{15:0}$ anteiso is the major fatty acid (~51%); $C_{15:0}$ iso, $C_{16:0}$, $C_{14:0}$, $C_{16:0}$ iso, and $C_{17:0}$ anteiso occur in smaller amounts (<4–17%). The cell-wall sugars are fucose, mannose, and rhamnose.

Source: fresh fecal pellets of *Chromatoiulus projectus* Verh. (Diplopoda, Myriapoda).

DNA G+C content (mol%): 71 (T_m).

Type strain: DFA-19, ATCC 35307, DSM 43852, HMGB B1078, NBRC 14295, JCM 7350, NRRL B-16223.

Sequence accession no. (16S rRNA gene): X83807.

3. **Oerskovia jenensis** Stackebrandt, Breymann, Steiner, Prauser, Weiss and Schumann 2002a, 1110[VP]

je.nen′sis N.L. fem. adj. *jenensis* pertaining to Jena, Germany.

Gram-stain-positive, non-acid-fast, facultatively anaerobic organism with branching vegetative hyphae. Aerial mycelium absent. Substrate hyphae fragment into motile bacillary and coccoid or sporelike elements. On CASO agar (DIFCO) colonies are cream-yellow, 0.2–0.4 or 0.5–0.7 mm in diameter at 28°C after 2 d. Does not produce water-soluble diffusible pigments or melanin. Glucose is metabolized both oxidatively and facultative fermentatively. In addition to the physiological reactions indicated in Table 116 the following Biolog substrates are utilized: α-acetyl mannosamine, amygdalin, arbutin, cellobiose, D-fructose, D-galactose, gentobiose, D-lactic acid methylester, L-lactic acid, mannan, D-melibiose, α-methyl-D-galactoside, and sodium acetate. The peptidoglycan is of the A4 α type, variation L-Lys←L-Thr←D-Glu. The major fatty acid is $C_{15:0}$ anteiso (54%); $C_{16:0}$, $C_{17:0}$ anteiso, $C_{15:0}$ iso, and $C_{14:0}$ (8.0–15%) occur in smaller amounts.

Source: spruce-forest soil and brookled-bed.

DNA G+C content (mol%): not determined.

Type strain: CCUG 47142, CIP 100330, DSM 46000, JCM 9969.

Sequence accession no. (16S rRNA gene): AJ314848.

4. **Oerskovia paurometabola** Stackebrandt, Breymann, Steiner, Prauser, Weiss and Schumann 2002a, 1110[VP]

pau.ro.me.ta′bo.la. Gr. adj. *pauros* little; Gr. adj. *metabolos* changeable; N.L. fem. adj. *paurometabola* producing little change.

Gram-stain-positive, non-acid-fast, facultatively anaerobic organism with branching vegetative hyphae. Aerial mycelium absent. Substrate hyphae fragment into motile bacillary and coccoid or sporelike elements. On CASO agar (DIFCO) colonies are cream-yellow, 0.5–0.6 mm in diameter at 28°C after 2 d. Does not form water-soluble diffusible pigments or melanin pigments. Glucose is metabolized both oxidatively and facultative fermentatively. Additional physiological reactions are indicated in Table 116. The peptidoglycan is of the A4 α type, variation L-Lys←L-Thr←D-Glu. Major fatty acid is $C_{15:0}$ anteiso (58%); $C_{16:0}$, $C_{17:0}$ anteiso, $C_{15:0}$ iso, and $C_{14:0}$ (8.0–18%) occur in smaller amounts.

Source: soil of a leafy wood, in the Carpathian Mountains.

DNA G+C content (mol%): not determined.

Type strain: CCUG 47105, DSM 14281, JCM 11755, LMG 20385.

Sequence accession no. (16S rRNA gene): AJ314851.

Genus V. **Tropheryma** La Scola, Fenollar, Fournier, Altwegg, Mallet and Raoult 2001, 1478[VP]

Matthias Maiwald and David A. Relman

Tro.phe.ry′ma. Gr. n. *trophê* nourishment, food; Gr. neut. n. *eruma* fence, a defence against, barrier; N.L. fem. (*sic*) n. *Tropheryma* barrier to nourishment, so named because it causes malabsorption.

Straight, regular, small rods, generally 0.2–0.25 × 1.0–2.5 μm. Forms chains or cords in co-culture with human fibroblasts (La Scola et al., 2001; Maiwald et al., 2003b). Stains inconsistently Gram-stain-positive in infected human tissues (Dobbins, 1987) and Gram-stain-negative in culture (La Scola et al., 2001). Ziehl–Neelsen stain (acid-fastness) is negative. Periodic acid-Schiff (PAS) stain is positive (red color). **Does not grow in standard microbiological media**; growth in laboratory animals has not been described. Grows in tissue culture medium in co-culture with human fibroblasts (La Scola et al., 2001), as well as axenically in tissue culture medium supplemented with extra amino acids (Renesto et al., 2003). Intact cells in culture are

observed in intra- and extracellular locations. Grows at **37°C** in a **5% carbon dioxide** atmosphere; anaerobic or completely aerobic growth unknown. **Nonmotile**. Extremely **slow growth**; estimated bacterial generation time in culture between 28 and 96 h. When viewed by electron microscopy in infected human tissues, bacterial cells are surrounded by an outer membrane with symmetrical leaflets (Silva et al., 1985). Due to a lack of measurable biochemical characteristics, DNA sequence analysis is the most suitable technique for identification; commonly used genetic loci are 16S rRNA gene and 16S–23S rRNA intergenic spacer (accession no. X99636). *Tropheryma whipplei*, the etiologic agent of Whipple's disease, is the only species in the genus. The only naturally infected hosts are humans.

DNA G+C content (mol%): 46 (genome sequence).

Type species: **Tropheryma whipplei** La Scola, Fenollar, Fournier, Altwegg and Raoult 2001, 1478[VP].

Further descriptive information

Phylogeny. Phylogenetic information from the Whipple's disease bacterium was first obtained via broad-range 16S rDNA PCR and sequencing directly from infected human tissue, in the absence of any cultivated strain (Relman et al., 1992; Wilson et al., 1991). A phylogenetic placement within the actinomycetes (phylum *Actinobacteria*, class *Actinobacteria*) was established, and the organism was provisionally named "*Tropheryma whippelii*" (Relman et al., 1992). A more detailed phylogenetic analysis (Maiwald et al., 1996) established an intermediate position between the family *Cellulomonadaceae* and a cluster composed of actinomycetes with group B peptidoglycan (Figure 153). The highest 16S rDNA sequence similarities were found to the species *Leifsonia aquatica* (previously "*Corynebacterium aquaticum*," with group B peptidoglycan) and *Cellulomonas cellasea* (with group A peptidoglycan), both at 91.6%. However, with this level of similarity to its closest known relatives, the relationships of *Tropheryma whipplei* to any other known bacteria are quite distant. The placement of *Tropheryma whipplei* within the actinomycetes (phylum *Actinobacteria*) was confirmed on the basis of 23S rRNA, 5S rRNA, DNA-dependent RNA polymerase (*rpoB*), 65 kDa heat-shock protein (*groEL2*), elongation factor Tu (*tuf*), ATPase beta subunit (*atpD*), and RNase P RNA (*rnpb*) gene sequences (Maiwald et al., 2000, 2003a).

Cell morphology, cell-wall composition, and fine structure. Multiple electron-microscopic studies, summarized by Dobbins (1987), showed a uniform appearance of bacteria in tissues of patients with Whipple's disease, before the availability of cultures. The bacteria are small, rod-shaped, measuring approximately 0.2–0.25 × 1.0–2.5 µm. In human tissue, intact bacteria are found in intra- and extracellular locations; intracellular forms are also seen in various stages of degradation. Dividing bacterial shapes appear to be more commonly located extracellularly (Silva et al., 1985). In co-culture with human fibroblasts, the bacteria are arranged in chains (Figure 154) or cords (Maiwald et al., 2003b); the cords are also described as "rope-like structures" (La Scola et al., 2001). The bacteria are positively stained by PAS reagent, by acridine orange, by YO-PRO nucleic acid stain, and by specific fluorescently labeled human and mouse antibodies (La Scola et al., 2001; Maiwald et al., 2003b). They can also be visualized by bacterial fluorescent *in situ* hybridization using specific nucleic acid probes (Fredricks and Relman, 2001; Maiwald et al., 2003b). When examined by electron microscopy in human tissues, the bacteria have a distinctive shape and size and a "trilaminar" appearance of the cell envelope (Dobbins, 1987; Silva et al., 1985). The cell envelope consists – from inside to outside – of (i) a cytoplasmic membrane, (ii) a ~20 nm-thick cell wall with an inner polysaccharide and an outer peptidoglycan layer, and (iii) a symmetric outer membrane (Silva et al., 1985). The outer membrane is unlike other bacterial outer membranes and it has been suspected to be of human host origin (Silva et al., 1985). The polysaccharide (glycopeptide) layer confers reactivity with the PAS stain, which is used in the histopathological diagnosis of Whipple's disease. A corresponding gene cluster for the biosynthesis and export of extracellular polysaccharide has been found in the *Tropheryma whipplei* genome (Bentley et al., 2003).

Culture-based characteristics and growth conditions. *Tropheryma whipplei* can be propagated in cell culture with human fibroblasts (Raoult et al., 2000) and axenically in cell culture medium supplemented with extra amino acids (Renesto et al., 2003). It does not grow on any standard microbiological media. In fact, after the initial discovery by electron microscopy in 1961 that bacteria were associated with the pathogenesis of Whipple's disease, many culture attempts were undertaken and all failed or yielded contaminants (Dobbins, 1987). For decades, it was regarded as one of the classic uncultivated bacteria involved in human disease, along with *Treponema pallidum* and *Mycobacterium leprae*. The first *bona fide* culture isolate of *Tropheryma whipplei* was obtained from an infected aortic heart valve of a patient with endocarditis and is now the type strain, designated Twist-Marseille (CNCM I-2202) (Raoult et al., 2000).

Metabolism and metabolic pathways. Due to the difficulties with cultivation, biochemical tests are not currently used for the identification and characterization of *Tropheryma whipplei*. Information on metabolism has become available from the organism's genome sequence (Bentley et al., 2003; Raoult et al., 2003; Renesto et al., 2003). *Tropheryma whipplei* appears to be missing genes for the biosynthesis of some amino acids, as well as for some aspects of carbohydrate and energy metabolism. Genes for the biosynthesis of arginine, tryptophan, and histidine are completely absent, as are genes for the NADH dehydrogenase complexes and the tricarboxylic acid cycle. This renders *Tropheryma whipplei* an organism that is dependent on the supply of preformed nutrients from external sources, such as the human host.

Genetics and genomics. The genome sequences of two *Tropheryma whipplei* strains have been determined and analyzed (Bentley et al., 2003; Raoult et al., 2003). One of the strains, TW08/27, was obtained from cerebrospinal fluid (Maiwald et al., 2003b), and the other strain, the type strain Twist-Marseille, was obtained from a heart valve (Raoult et al., 2000). The sequence accession numbers are BX072543 and AE014184, respectively. Both genomes are markedly reduced in size and content, measuring slightly smaller than 1 Mbp (925,938 and 927,303 bp, respectively). By comparison, the known genomes of other actinomycetes range from about 2.1–10.1 Mbp (http://www.ncbi.nlm.nih.gov/). The genomic G+C content is 46% which is quite low considering that actinomycetes are generally high G+C-organisms. These features, plus the metabolic deficiencies, suggest that *Tropheryma whipplei* has undergone reductive genome evolution and occupies a host-dependent ecological niche.

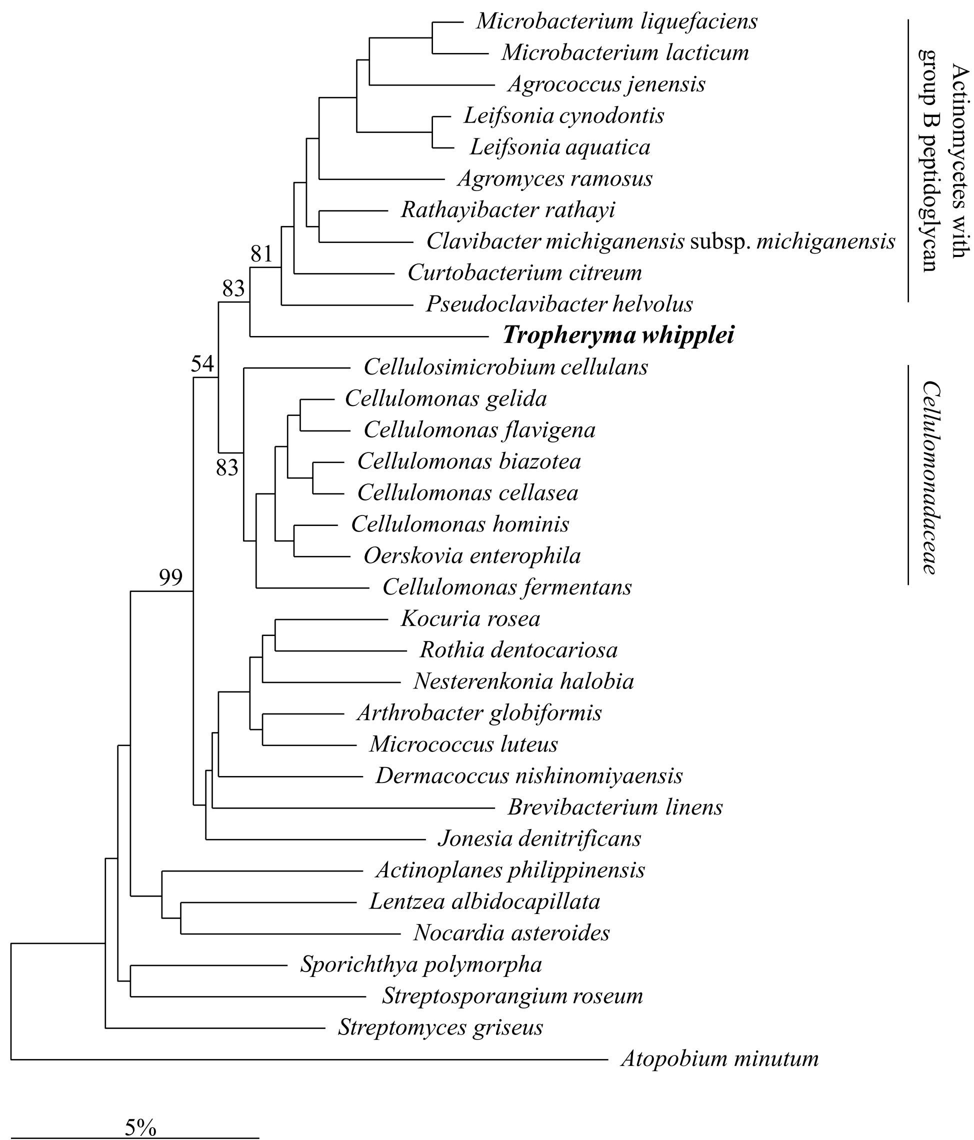

FIGURE 153. Phylogenetic tree displaying the relationships of *Tropheryma whipplei* to other actinomycetes. The scale bar represents five inferred nucleotide substitutions per 100 nucleotides. Numbers at branching points indicate bootstrap values. Modified and reprinted from Maiwald et al. (1996) with permission of the publisher. Strain designations (when available) and sequence accession numbers (in parentheses) are: *Actinoplanes philippinensis* DSM 43019^T (X93187), *Agrococcus jenensis* DSM 9580^T (X92492), *Agromyces ramosus* DSM 43045^T (X77447), *Arthrobacter globiformis* DSM 20124^T (M23411), *Atopobium minutum* ATCC 33267^T (M59059), *Brevibacterium linens* DSM 20425^T (X77452), *Cellulomonas biazotea* DSM 20112^T (X83802), *Cellulomonas cellasea* DSM 20118^T (X83804), *Cellulomonas fermentans* DSM 3133^T (X83805), *Cellulomonas flavigena* DSM 20109^T (X83799), *Cellulomonas gelida* DSM 20111^T (X83800), *Cellulomonas hominis* DSM 9581^T (X82598), *Cellulosimicrobium cellulans* DSM 43879^T (X83809), *Clavibacter michiganensis* subsp. *michiganensis* DSM 46364^T (X77435), *Curtobacterium citreum* DSM 20528^T (X77436), *Dermacoccus nishinomiyaensis* DSM 20448^T (X87757), *Jonesia denitrificans* DSM 20603^T (X83811), *Kocuria rosea* DSM 20447^T (X87756), *Leifsonia aquatica* DSM 20146^T (X77450), *Leifsonia cynodontis* (M60935), *Lentzea albidocapillata* DSM 44073^T (X84321), *Microbacterium lacticum* DSM 20427^T (X77441), *Microbacterium liquefaciens* DSM 20638^T (X77444), *Micrococcus luteus* (M38242), *Nesterenkonia halobia* DSM 20541^T (X80747), *Nocardia asteroides* DSM 43757^T (X80606), *Oerskovia enterophila* DSM 43852^T (X83807), *Pseudoclavibacter helvolus* DSM 20419^T (X77440), *Rathayibacter rathayi* DSM 7485^T (X77439), *Rothia dentocariosa* ATCC 17931^T (M59055), *Sporichthya polymorpha* DSM 46113^T (X72377), *Streptomyces griseus* (M76388), *Streptosporangium roseum* DSM 43021^T (X89947), and *Tropheryma whipplei* (X99636).

Despite the loss of genes for metabolic functions, the organism has a relative abundance of genes encoding cell surface molecules. About 15% of the organism's proteins are predicted to be located at the cell surface, and a significant proportion of these have no homologue in sequence databases. A new family of surface proteins was identified and termed WiSP (for *Tropheryma whipplei* surface proteins). Interestingly, the two genome-sequenced strains (Bentley et al., 2003; Raoult et al., 2003) are

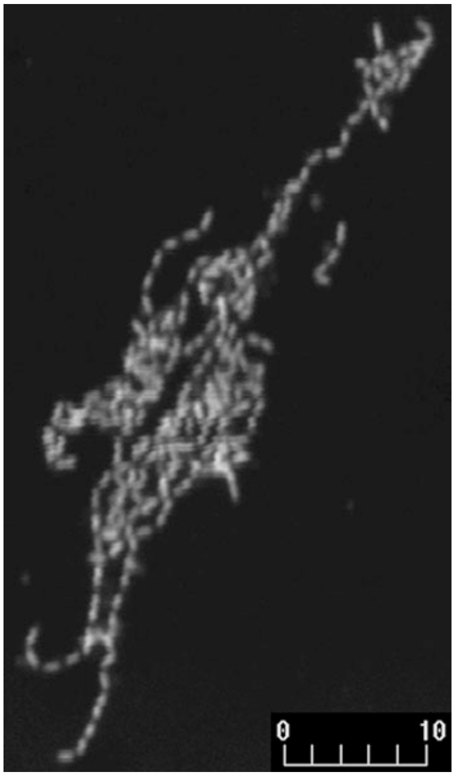

FIGURE 154. Photomicrograph of supernatant from a culture of *Tropheryma whipplei*. Bacteria were stained with YO-PRO-1 nucleic acid dye. Scale bar represents micrometers. Reprinted from Maiwald et al. (2003b) with permission from University of Chicago Press. Copyright 2003 by the Infectious Diseases Society of America.

distinguished by a large inversion of about 57% of the genome located between two WiSP genes. There are several apparent mechanisms by which *Tropheryma whipplei* creates genetic variability. It possesses several stretches of variable-number tandem repeat (VNTR) sequences and two unusual, large regions of non-coding repetitive DNA termed repeat clusters (RC) 1 and 2. VNTR sequences are known sources of antigenic and phase variation. In addition, one WiSP gene sequence that is embedded in a repeat cluster shows unusual, localized sequence hypervariation and is predicted to cause amino acid substitutions or frameshift mutations. Both the abundance and variability of surface molecules suggest that the organism possesses mechanisms to escape the host immune response.

Comparative genomic hybridization with a DNA microarray derived from the type strain Twist-Marseille using the DNA of 15 other isolated strains revealed remarkably little variation in gene content between different strains, with the percentage of conserved genes ranging between 97.8% and 99.9% (La et al., 2007). WiSP protein genes were significantly overrepresented among the divergent genes. The single strain with the lowest degree of conservation had a single deletion of 17 adjacent genes (19,052 bp) located between two WiSP genes. These findings indicate that the genome of *Tropheryma whipplei* is conserved and lacks exogenous genetic exchange to a similar degree as that of other small-genome, host-dependent organisms, that genomic variation between *Tropheryma whipplei* strains is derived mostly from WiSP gene differences, and that WiSP genes can act as "hot spots" for genomic rearrangements.

Strain typing. Differences between individual *Tropheryma whipplei* strains were first recognized in the 16S–23S rRNA intergenic spacer sequence (Hinrikson et al., 1999; Maiwald et al., 2000). At the present time, seven spacer types have been described and designated types 1–7 (Geissdörfer et al., 2001; Hinrikson et al., 1999; Maiwald et al., 2000). Most types only differ by a few nucleotides along the 293–294 bp spacer sequence. Types 1 and 2 are most common; the ratio is about 1:2 (type 1:type 2) in patients from Europe and North America (Maiwald et al., 2000). The other types are rare. The same spacer type is generally found in different sample types and at different time points from the same patient, which suggests systemic dissemination of individual *Tropheryma whipplei* strains in affected patients.

Differences at the nucleotide level are also found in a 23S rRNA insertion sequence that is common to the class *Actinobacteria* (the *Tropheryma whipplei* insertion sequence is 80 bp; those of other actinomycetes, 86–116 bp) and in the *groEL2* heat-shock protein gene sequence (Hinrikson et al., 2000; Morgenegg et al., 2000). The possibility of typing by VNTR sequence analysis was explored in one study (Maiwald et al., 2003a). Samples from 11 patients were examined at three VNTR loci and for their 16S–23S rRNA spacer type. A distinct typing pattern was found for each individual *Tropheryma whipplei* strain, however, given the unstable nature of VNTRs, it remains unclear if the patterns remain stable during the course of the disease or during culture passages. A more comprehensive typing method was developed with microarray-based comparative genomic analysis (La et al., 2007). Of 16 analyzed strains, all were individually distinguishable based on their hybridization patterns. However, the approach, as published, depended on the availability of cultivated strains as opposed to the suitability of the other methods for fixed and unfixed tissues and body fluids, as well as culture material.

Antigenic structure. Antibodies in patients' sera and antibodies raised in rabbits or mice against cultivated *Tropheryma whipplei* can be used to detect bacterial material in histological sections and in tissue culture cells (Baisden et al., 2002; La Scola et al., 2001; Raoult et al., 2000). Cultivated bacteria have also been tested for their usefulness as a source of antigen in a serological assay with patients' sera (Raoult et al., 2000). However, sensitivity and specificity were limited: elevated IgM titers were detected in 7 of 9 sera from patients and 3 of 40 sera from controls and elevated IgG titers in 9 of 9 patient sera and 29 of 40 controls.

Immunoblotting of whole-cell extract from a cultivated *Tropheryma whipplei* strain with mouse polyclonal antiserum showed strongly reactive bands at 213, 110, and 85 kDa (La Scola et al., 2001). A mouse monoclonal antibody to an 84 kDa immunodominant antigen of *Tropheryma whipplei* was raised and found to be specific when tested with control bacteria (Liang et al., 2002). However, in both studies, the nature of the antigens was not further examined.

Genome sequencing has revealed the WiSP family of proteins as unique to *Tropheryma whipplei* (Bentley et al., 2003; Raoult et al., 2003). Members of the WiSP family differ considerably in size (103–2308 aa), but are characterized by several common features such as common N-terminal or C-terminal domains or beta-strand motifs. They are likely to be important major antigens, but no data are available concerning antigenicity of WiSPs and the human immune response against these proteins.

A proteomic approach has been applied in an effort to characterize antigens for use in serological tests (Kowalczewska et al.,

2006). Proteins of cultivated *Tropheryma whipplei* organisms were separated with two-dimensional gel electrophoresis, immunoblotted with patient and control antisera, and analyzed by mass spectrometry. Twenty-three candidate antigens were identified, of which six were recognized significantly more frequently by patient than control antisera, and 17 recognized only by some patient antisera. However, no single antigen had sufficient sensitivity and specificity to be used alone in a serological test, so it was proposed to use a combination. Only one of the proteins was predicted to be located at the cell membrane; most were predicted to be cytoplasmic proteins.

Antibiotic sensitivity. Antibiotic therapy for *Tropheryma whipplei* infection is primarily based on empirical observations (Dobbins, 1987). The disease responds well initially to tetracyclines, but since tetracyclines do not penetrate the blood-brain barrier well, patients tend to suffer from relapses affecting the central nervous system. Currently, treatment of choice is an initial two-week intravenous regimen with either penicillin plus streptomycin or with a third-generation cephalosporin followed by oral therapy with trimethoprim-sulfamethoxazole for 1 year or more (Maiwald et al., 2010).

Laboratory data on antibiotic susceptibilities have become available from work with cultivated *Tropheryma whipplei* strains. Growth in the presence of antibiotics was assessed with real-time PCR. In co-culture with fibroblasts, the bacterium appears sensitive to doxycycline, macrolides, penicillins, rifampin, teicoplanin, and trimethoprim-sulfamethoxazole, variably sensitive to imipenem, and only moderately sensitive or resistant to cephalosporins, fluoroquinolones, and vancomycin (Boulos et al., 2004). In axenic medium, the results were very similar to those in cell culture, except that *Tropheryma whipplei* was sensitive to ceftriaxone and vancomycin (Boulos et al., 2005). Interestingly, the target gene for trimethoprim action, dihydrofolate reductase, is absent from the *Tropheryma whipplei* genome, so that the activity of the trimethoprim-sulfamethoxazole combination is presumably based solely on its sulfamethoxazole component.

Pathogenicity. *Tropheryma whipplei* is the agent of Whipple's disease, a chronic systemic illness of humans affecting the intestinal tract as well as multiple other organs. Intestinal manifestations most commonly include a malabsorption syndrome with weight loss, diarrhea, and abdominal pain. The onset is protracted, usually over 1–2 years, and is often preceded by several years of episodes of arthralgias or arthritis. The intestinal lamina propria as well as intestinal and mesenteric lymph nodes are filled with lipid deposits and with macrophages containing PAS-positive bacterial inclusions. Among the various other manifestations of Whipple's disease are central nervous system disease with dementia and movement disorders, joint manifestations (arthralgias and arthritis), and "blood culture-negative" endocarditis (Maiwald et al., 2010; Marth and Raoult, 2003).

The virulence mechanisms of *Tropheryma whipplei* are largely unknown; in particular, it is unclear how the bacterium enters the human body and human tissues, how it establishes infection, and how it avoids immunological clearance. It is thought that host immunological factors play a role in the establishment of disease. Clearly defined immunological defects have not been identified, but patients with Whipple's disease have quantitative abnormalities in certain immunological functional parameters. For example, peripheral blood monocytes of Whipple's disease patients, when challenged with various stimuli, show quantitative imbalances in cytokine secretion when compared to controls (Marth et al., 2002).

Genes with a presumed function in pathogenesis are scarce in the *Tropheryma whipplei* genome. Two genes that are predicted to encode surface proteins are similar to a gene (*tadA*) required for pilus-mediated tight adherence of the pathogen *Actinobacillus actinomycetemcomitans*; gene homologues are also widely distributed among other bacteria. In addition, the abundance of predicted surface molecules, many of which are novel and variable, suggests that *Tropheryma whipplei* has mechanisms to escape immunologic recognition and clearance.

Ecology and epidemiology. The questions of natural habitat and mode of acquisition of *Tropheryma whipplei* are unresolved. There is a moderate overrepresentation of outdoor workers (farmers, carpenters) among patients with Whipple's disease (Dobbins, 1987). In addition, *Tropheryma whipplei* sequences have been detected by PCR in sewage samples (Maiwald et al., 1998). This might be suggestive of an environmental habitat. On the other hand, *Tropheryma whipplei* DNA has been detected in saliva and in intestinal specimens of healthy people (Ehrbar et al., 1999; Street et al., 1999), suggesting that *Tropheryma whipplei* may be a human commensal that becomes pathogenic when the control by host immunity lapses.

There is also debate about whether *Tropheryma whipplei* replicates as an intracellular or extracellular organism. While macrophages with PAS-positive intracellular inclusions are the histopathologic hallmark of Whipple's disease, intact bacteria undergoing binary division are consistently seen in extracellular locations by electron microscopy (Silva et al., 1985). In addition, the rRNA of *Tropheryma whipplei* (presumably from metabolically active bacteria) can be detected in extracellular locations (directly underneath the intestinal epithelial basement membrane) by fluorescent *in situ* hybridization (Fredricks and Relman, 2001). *Tropheryma whipplei* has been found to replicate inside monocytes and macrophages, in a manner dependent on the presence of the cytokine interleukin-16 (Desnues et al., 2005). Given that *Tropheryma whipplei* replicates in axenic medium supplemented with extra amino acids (Renesto et al., 2003), it is quite conceivable that it replicates directly underneath the intestinal basement membrane in the lamina propria, supplied by a rich influx of nutrients from the intestinal lumen. However, such requirements might also be met if it replicates in an environment in association with a polymicrobial community.

Enrichment and isolation procedures

Tropheryma whipplei has been isolated from infected human heart valves, ocular vitreous fluid, duodenal biopsy specimens, peripheral blood, and cerebrospinal fluid (Fenollar et al., 2003; Maiwald et al., 2003b; Raoult et al., 2000, 2001). However, so far, this has only been achieved in specialized research laboratories. Culture-based approaches are not suitable for routine diagnostic purposes in clinical microbiology laboratories. Cell lines that have supported cultivation are HEL and MRC-5 primary human embryonic lung fibroblasts (CCL-34 and CCL-171, respectively; ATCC, Manassas, VA, USA) (La Scola et al., 2001) and primary human foreskin fibroblasts (Maiwald et al., 2003b). Minimal essential medium (MEM) supplemented with

fetal calf serum and L-glutamine can be used to cultivate and maintain fibroblast cell monolayers and to propagate *Tropheryma whipplei* in association with these cells (La Scola et al., 2001). One culture passage may require about 2–4 weeks. For primary isolation from clinical specimens, shell vials (as for viral cultures) are helpful (La Scola et al., 2001). Pre-incubation with antibiotics (amphotericin B, colistin, and cephalothin or ciprofloxacin) is useful for isolation from contaminated duodenal tissue (Fenollar et al., 2003; Raoult et al., 2001). Growth of *Tropheryma whipplei* in culture can be assessed by qualitative or quantitative PCR for *Tropheryma whipplei* nucleic acids, by staining with PAS reagent or YO-PRO fluorescent nucleic acid stain (Figure 154), by fluorescent *in situ* hybridization, and by immunofluorescent staining using specific antisera (La Scola et al., 2001; Maiwald et al., 2003b; Raoult et al., 2000). Bacteria are found both in the fibroblast cell monolayers and in the culture supernatant. Medium suitable for the propagation of *Tropheryma whipplei* in cell-free conditions can be made from tissue culture medium by supplementation with 10% fetal calf serum, 1% L-glutamine, and 1% human non-essential amino acids (Renesto et al., 2003). So far, this recipe has supported the growth of several strains after primary cell-based isolation and allowed the primary isolation of one strain from a heart valve sample (Renesto et al., 2003).

Differentiation of the genus *Tropheryma* from other genera

Standard microbiological tests are generally unsuitable for identification and differentiation of the genus *Tropheryma* from other genera. In human specimens, the detection of PAS-positive inclusions in macrophages by histology or cytology, followed by confirmation of typical bacterial morphology by electron microscopy, is considered proof of *Tropheryma whipplei* infection (Maiwald et al., 2010). Although some other genera (e.g. *Mycobacterium*, *Rhodococcus*) may stain PAS-positive, no known organisms associated with PAS-positive inclusions have the same small, regular, rod-shaped appearance with a symmetric outer membrane when viewed by electron microscopy (Silva et al., 1985). The most appropriate method for bacterial identification, however, is PCR followed by sequencing. Suitable targets are the 16S rRNA gene or 16S–23S rRNA intergenic spacer because comparative sequence information is readily available. Primers for the 16S rRNA gene include *Tropheryma whipplei*-specific tw1013f (5′-AGAGATACGCCCCCCGCAA) and tw1243r (5′-ATTCGCTCCACCTTGCGA), which are modified versions (without extra restriction enzyme recognition sites) of the primers pW3FE and pW2RB published by Relman et al. (1992) and yield a 266 bp amplification product. Alternatively, any bacterial broad-range primers spanning a longer 16S rDNA segment may be used (Maiwald, 2011). Primers spanning the 16S–23S intergenic spacer are *Tropheryma whipplei*-specific tws3f (5′-CCGGTGACTTAACCTTTTTGGAGA) and tws4r (5′-TCCCGAGGCTTATCGCAGATTG); they yield a 490 bp amplification product (Hinrikson et al., 1999). Another suitable target is a repetitive sequence element found on the *Tropheryma whipplei* genome (Fenollar et al., 2004). The primers are 53.3F (5′-AGAGAGATGGGGTGCAGGAC) and 53.3R (5′-AGCCTTTGCCAGACAGACAC) and the product size is 164 bp. It should be emphasized that visualization of amplification products on gels is not sufficient for identification. With any of the PCR assays, sequencing is necessary.

Taxonomic comments

Relman et al. (1992) proposed the name "*Tropheryma whippelii*" for the bacterium, after molecular identification directly from human tissue using bacterial broad-range PCR, sequencing, and phylogenetic analysis. The name contains the Greek words for "nourishment" and "barrier" to describe the malabsorption syndrome that is commonly associated with Whipple's disease and honors George H. Whipple, the American pathologist who first described the disease (Whipple, 1907). The bacterium was then formally described after a cultivated isolate became available (La Scola et al., 2001; Raoult et al., 2000). In the formal description, latinization of the species name resulted in a change to *whipplei* (La Scola et al., 2001).

Tropheryma whipplei is not closely related to any other bacterium on the basis of its 16S rRNA gene sequence or its genome contents. It is a member of the phylum *Actinobacteria*. The order and family designations of the genus *Tropheryma* have not been formally established. The National Center for Biotechnology Information (NCBI) website (http://www.ncbi.nlm.nih.gov/), in its Taxonomy Browser, lists class *Actinobacteria*, subclass *Actinobacteridae*, order *Actinomycetales*, suborder *Micrococcineae*, family *Cellulomonadaceae*, genus *Tropheryma*, although this is not based on any formal publication. According to a thorough description of the class *Actinobacteria* (Stackebrandt et al., 1997), the families most closely related to *Tropheryma whipplei* are *Cellulomonadaceae* (with group A peptidoglycan) and *Microbacteriaceae* (with group B peptidoglycan), both in the suborder *Micrococcinae* (since elevated to order *Micrococcales* in the roadmap to the present volume). However, the relationships of *Tropheryma whipplei* to both families are quite distant, with more than 8% 16S rRNA gene sequence divergence (Maiwald et al., 1996).

Miscellaneous. Unlike many other bacterial diseases, anatomic and surgical pathology are most commonly involved in the laboratory diagnosis of Whipple's disease (using histopathology, cytology, and electron microscopy). Clinical microbiology has become increasingly involved since the early 1990s, with the availability of PCR tests. There are four main diagnostic laboratory methods for Whipple's disease: (i) histology from paraffin-embedded tissue specimens (commonly intestinal biopsies) using the PAS stain, (ii) cytology from body fluids using the PAS stain, (iii) electron microscopy looking for typical bacteria in tissues or body fluids, and (iv) diagnostic PCR for DNA of *Tropheryma whipplei*. Most PCR assays are based on the 16S rRNA gene sequence of *Tropheryma whipplei* as originally published by Relman et al. (1992); some are based on alternative sequences, such as a repetitive sequence element found in the *Tropheryma whipplei* genome (Fenollar et al., 2004). While PCR testing has become important as a second-line confirmatory test for histopathology on intestinal biopsy specimens, it can also be used as a first-line test on extra-intestinal specimens such as cerebrospinal fluid, joint fluid, and ocular vitreous fluid (Maiwald et al., 2010).

Further reading

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List of species of the genus *Tropheryma*

1. **Tropheryma whipplei** La Scola, Fenollar, Fournier, Altwegg, Mallet and Raoult 2001, 1478[VP]

whip′ple.i. N.L. gen. masc. n. *whipplei* of Whipple, after George H. Whipple, the pathologist who described the disease that became known as Whipple's disease.

Since *Tropheryma whipplei* is the only species of the genus, the identification criteria given in the section on the genus *Tropheryma* apply equally to the species. Since the organism does not grow on standard microbiological media and is nonreactive in conventional biochemical tests, identification is best achieved using PCR and nucleic acid sequence analysis. *Tropheryma whipplei* is commonly found in the intestinal mucosa of patients with Whipple's disease and visualized by histopathology. Cultivated isolates have been obtained from heart valves, peripheral blood, ocular vitreous fluid, intestinal biopsies, and cerebrospinal fluid. The type strain Twist-Marseille (CNCM I-2202) was isolated from the aortic valve of a patient with endocarditis (La Scola et al., 2001; Raoult et al., 2000) and represents the first successful culture of the organism after several decades of unsuccessful attempts by various researchers.

DNA G+C content (mol%): 46 (genome sequence).

Type strain: Twist-Marseille, CNCM I-2202.

Sequence accession no. (16S rRNA gene): AF251035.

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Family VI. **Dermabacteraceae** Stackebrandt, Rainey and Ward-Rainey 1997, 485^{VP} emend. Zhi, Li and Stackebrandt 2009, 597

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Der.ma.bac.te.ra.ce′a.e. N.L. masc. n. *Dermabacter* type genus of the family; suff. *-aceae* ending to denote a family; N.L. fem. pl. n. *Dermabacteraceae* the *Dermabacter* family.

Gram-stain-positive, non-acid-fast, nonsporeforming, nonmotile actinomycetes which contain ***meso*-diaminopimelic acid as the cell wall diamino acid and two residues of dicarboxylic acids in the interpeptide bridge (A4γ peptidoglycan), straight-chain saturated, iso-, and anteiso-branched fatty acids, unsaturated menaquinones with 7–9 isoprene units, but not mycolic acids. The pattern of rRNA gene signatures consists of nucleotides at positions 120 (A), 131:231 (G–C), 196 (U), 342:347 (C–G), 444:490 (A–U), 580:761 (U–A), 602:636 (C–G), 670:736 (A–U), 822:878 (G–C), 823:877 (G–C), 826:874 (C–G), 827 (U), 843 (C), 950:1231 (U–G), 1047:1210 (G–C), 1109 (C), 1145 (G), 1309:1328 (G–U), 1361 (G) and 1383 (C)**.

Source: a wide range of natural habitats and clinical material.

DNA G+C content (mol%): 62–72 (T_m).

Type genus: **Dermabacter** Jones and Collins 1989, 93^{VP} (Effective publication: Jones and Collins 1988, 54).

Further descriptive information

The family contains the genus *Dermabacter* Jones and Collins 1988 and the genus *Brachybacterium* Collins et al. 1988 (Figure 155). These taxa form a distinct phyletic line in the 16S rRNA *Micrococcineae* (elevated to order *Micrococcales* in the present volume) gene tree (Cai and Collins, 1994; Schubert et al., 1996). The family can be distinguished from other families classified in the order *Micrococcales* by a set of 16S rRNA signature nucleotides (Zhi et al., 2009) and by a combination of chemotaxonomic markers (see sections on other families classified in the order *Micrococcales*).

Acknowledgements

Thanks are due to Ingrid Goth, Peter Schumann, and Peter Kämpfer for helping improve the original draft of this chapter and to the latter for providing Figure 155.

Genus I. **Dermabacter** Jones and Collins 1989, 93^{VP} (Effective publication: Jones and Collins 1988, 54.)

Michael Goodfellow

Der.ma.bac′ter. Gr. n. *derma* skin; N.L. masc. n. *bacter* masc. equivalent of the Gr. neut. dim. n. *baktron* small rodlet; N.L. masc. n. *Dermabacter* small rod living on skin.

Facultatively anaerobic, catalase-positive, oxidase-negative actinomycetes which ferment a range of sugars and **decarboxylate lysine and ornithine. Cell wall peptidoglycan contains *meso*-diaminopimelic acid, alanine, and glutamic acid. Long-chain fatty acids are predominantly of the iso-, anteiso-methyl branched type. The major polar lipids are diphosphatidylglycerol, phosphatidylglycerol, and a diglucosyldiacylglycerol with some phospholipids. Unsaturated menaquinones with nine isoprene units predominate, though corresponding components with seven and eight isoprene units occur in substantial amounts**. The phylogenetic position of *Dermabacter*, as determined by 16S rRNA gene sequence analysis, is in the family *Dermabacteraceae*.

Source: skin of healthy adults.

DNA G+C content (mol%): 62.0–63.0 (T_m).

Type species: **Dermabacter hominis** Jones and Collins 1989, 93^{VP} (Effective publication: corrig. Jones and Collins 1988, 54.).

Further descriptive information

The genus *Dermabacter* forms a separate line of descent within the order *Micrococcales* and is most closely related to the genus *Brachybacterium* (see Figure 155) (Buczolits et al., 2003; Collins et al., 1988; Heyrman et al., 2002). These taxa, together with the genera *Demetria*, *Dermacoccus*, and *Kytococcus*, form the family *Dermabacteraceae*. The genus, which is monospecific, was proposed to accommodate four strains of coryneform bacteria isolated from the skin of healthy adults (Jones and Collins, 1988). Sometime later it was discovered that strains dating back to the 1970s with phenotypic properties similar to *Dermabacter hominis* had been referred to the Laboratory Center for Disease Control in Canada and to the Communicable Disease Center in the USA (Bernard et al., 1994; Funke et al., 1997). These strains, isolated primarily from blood cultures and less commonly from other body sites of patients in Belgium, Canada, Sweden, and the USA, were provisionally designated CDC coryneform groups 3 (xylose fermenter) and 5 (xylose nonfermenter). Subsequently, 15 Swiss clinical isolates belonging to these taxa were classified as *Dermabacter hominis* based on chemotaxonomic and 16S rRNA gene sequence data (Funke et al., 1994). Similar results were reported by Gruner et al. (1994) who also found that 22 out of 30 strains of CDC groups 3 and 5 could be designated *Dermabacter hominis* based on DNA–DNA relatedness data; the remaining strains were considered to belong to other *Dermabacter* species even though they could not be separated from *Dermabacter hominis* using fatty acid and phenotypic data.

Dermabacter strains form small, convex, grayish-white colonies which have a distinct pungent odor (Bernard et al., 1994). They ferment a broad range of sugars (Funke et al., 1994; Jones and Collins, 1988), produce lysine and ornithine decarboxylases (Bernard et al., 1994; Funke et al., 1994) and acetate and lactate as major products of glucose metabolism (Funke et al., 1994). In general, they are susceptible to cephalosporins, glycopeptides, teichoplanin, and vancomycin, whereas the activities of ciprofloxacin, contrimoxazole, erythromycin, gentamicin, pipemidic acid, sulfamethoxazole, and tetracycline

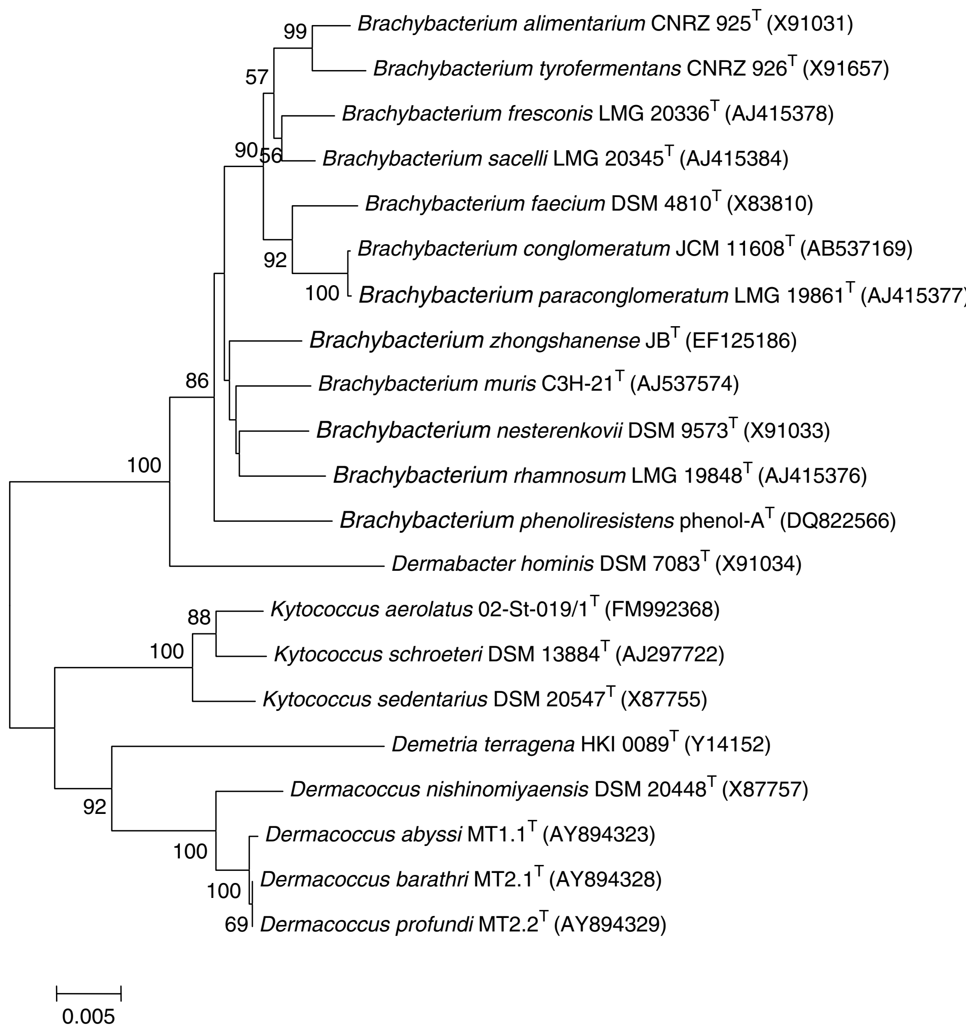

FIGURE 155. Phylogenetic analysis based on 16S rRNA gene sequences available from the European Molecular Biology Laboratory data library (accession numbers are given in brackets). Multiple alignment, distance calculations (distance options according to the Kimura-2 model), and clustering with the neighbor-joining method were performed by using the software package MEGA (Molecular Evolutionary Genetics Analysis) version 4 (Tamura et al., 2007). Bootstrap values based on 1000 replications are listed as percentages at the branching points. Bar = 0.005 nucleotide substitutions per nucleotide position.

are limited, as recorded by their Mic_{80} values (Funke et al., 1996; Troxler et al., 2001).

The peptidoglycan of dermabacters contains *meso*-diaminopimelic acid, alanine and glutamic acid; this is consistent with the directly cross-linked murein type A1γ (Jones and Collins, 1988). They contain major proportions of anteiso- and iso-methyl branched fatty acids, but lack mycolic acids (Bernard et al., 1994; Funke et al., 1994; Jones and Collins, 1988). This fatty acid profile is sufficiently distinctive to separate members of the genus from related taxa using the Microbial Identification (MIDI) system (Bernard et al., 1994). The predominant component in the menaquinone profile is MK-7, though substantial amounts of MK-7 and MK-8 are present (Jones and Collins, 1988). These workers found that the polar lipid patterns of dermabacters contained major amounts of diphosphatidylglycerol, phosphatidylglycerol, several unknown phospholipids, and a single glycolipid which had the chromatographic mobility of diglycosyldiacylglycerol.

Initially, *Dermabacter hominis* strains were considered to be nonpathogenic, though their isolation from blood cultures and other bodily sites of patients such as abscesses, eyes, skin lesions, and wounds, implied clinical significance (Funke et al., 1994, 1997; Gruner et al., 1994). The organism is now considered to be an opportunistic human pathogen which can cause abscesses (Bavbek et al., 1998), bacteremia (Gomez-Garces et al., 2001), chronic osteomyelitis (Van Bosterhaut et al., 2002), and peritoneal-dialysis associated peritonitis (Radtke et al., 2001). In addition, *Dermabacter*-like strains cause chronic inflammation of the integument, septicemia, and, notably, cheilitis in lizards (Koplos et al., 2000; Pasmans et al., 2004, 2008). Cheilitis appears to be highly contagious with morbidity and mortality rates of 100% in the desert agama (*Agama impalearis*) (Pasmans et al., 2004).

Enrichment and isolation procedures

Dermabacter strains can be isolated from clinical material including blood by plating onto standard media such as Columbia agar supplemented with sheep blood and incubating aerobically at 37°C with 5% CO_2. They also grow on Columbia chocolate and MacConkey agars.

Maintenance procedures

Dermabacter strains may be lyophilized or preserved as glycerol cultures at −80°C using standard procedures.

Differentiation of the genus *Dermabacter* from other genera

16S rRNA gene sequencing provides an effective way of distinguishing *Dermabacter* strains from related taxa. They can be separated from related taxa using a combination of chemical markers, quantitative analysis of fatty acid profiles (Bernard et al., 1994), and from their nearest phylogenetic neighbor, the genus *Brachybacterium*, on the basis of colony morphology, major fatty acids, G+C content, and menaquinone and cell wall peptidoglycan types (see Table 117, below).

TABLE 117. Chemotaxonomic traits suitable for phenotypical differentiation between *Brachybacterium* and its next phylogenetic relative *Dermabacter*

Characteristic	*Brachybacterium*[a]	*Dermabacter*[b]
Major fatty acid	$C_{15:0}$ anteiso or $C_{15:0}$ anteiso and $C_{17:0}$ anteiso	$C_{17:0}$ anteiso
Quinone system	MK-7 or MK-7 and MK-8	MK-9 or MK-9 and MK-8
Number of glycolipids in the polar lipid profile	2–5	1
DNA G+C content (mol%)	68–73	62
Peptidoglycan type	A4γ[c] (interpeptide bridge consisting of D-Asp-D-Glu or D-Glu–D-Glu)	A1γ[c] (directly cross-linked)

[a]Data from Collins et al. (1988), Gvozdyak et al. (1992), Takeuchi et al. (1995), Schubert et al. (1996), Heyrman et al. (2002), Buczolits et al. (2003), Chou et al. (2007), and Zhang et al. (2007).

[b]Data from Jones and Collins (1988).

[c]For explanation, see Schleifer and Kandler (1972).

List of species of the genus *Dermabacter*

1. **Dermabacter hominis** Jones and Collins 1989, 93[VP] (Effective publication: corrig. Jones and Collins 1988, 54)

ho′mi.nis. L. n. *homo* man; L. gen. n. *hominis* of man, signifying the isolation of strains from human skin.

Gram-stain-positive, nonmotile organism which forms short rods and coccoid elements. Grows at 37°C, but not at 10°C; some strains grow at 45°C. Grows in the presence of 5% (w/v) NaCl.

Esculin is hydrolyzed, but hippurate and urea are not. Gelatin is liquefied and lecithinase is produced. Does not reduce nitrate. Negative for CAMP, indole, and Voges–Proskauer tests and for the production of arginine dehydrolase and tryptophan deaminase. Positive for alkaline phosphatase, esterase (C4), β-galactosidase, α-and β-glucosidases, α-mannosidase, and *N*-acetyl-β-glucosaminidase, but negative for acid phosphatase, L-chymotrypsin, cystine arylamidase, L-fucosidase, β-glucuronisidase, lipase (C14), and valine arylamidase (API ZYM tests). DNA and starch are degraded, but cellulose, tyrosine, and xanthine are not. Acid is produced from *N*-acetylglucosamine, amygdalin, cellobiose, fructose, galactose, β-gentiobiose, glucose, lactose, maltose, mannose, melezitose, melibiose, raffinose, ribose, sucrose, trehalose, and turanose, but not from adonitol, D- or L-arabinose, D- or L-arabitol, dulcitol, erythritol, D- or L-fucose, glycerol, glycogen, 2- and 5-ceto-gluconate, gluconate, inositol, inulin, mannitol, α-methyl-D-mannoside, β-methyl-D-xyloside, rhamnose, sorbitol, tagalose, xylose, or xylitol. Does not use citrate as a sole carbon source.

Cellular fatty acids contain major proportions of 14-methylhexadecanoic ($C_{17:0}$ anteiso) and 12-methyltetradecanoic ($C_{15:0}$ anteiso) acids.

Additional chemotaxonomic and phenotypic data are cited in the genus description and in the text.

Source: human skin, blood cultures, and other body parts of humans, and from blood cultures and integuments of lizards.

DNA G+C content (mol%) 61–63 (T_m).

Type strain: S69, ATCC 49369, CCUG 32998, CIP 105144, DSM 7083, JCM 7448, NCIMB 13131.

Sequence accession no. (16S rRNA gene): X76728, X91034.

Additional comments: Dermabacter hominis was incorrectly cited as *Dermabacter hominus* in Validation List no. 28 (Euzéby and Kudo, 2001).

Genus II. **Brachybacterium** Collins, Brown and Jones 1988, 46[VP]

Sandra Buczolits and Hans Jürgen Busse

Bra.chy.bac.te′ri.um. Gr. adj. *brachys* short; L. neut. dim. n. *bacterium* rod; N.L. neut. n. *Brachybacterium* a small rodlet.

Cells vary in shape from coccoid forms in the stationary phase, which occur in large agglomerations, to club-shaped rods in the exponential phase, which occur predominantly in pairs or agglomerations. Cells are nonmotile, nonsporeforming, and stain Gram-positive. Surface colonies are small, white to yellow, circular, convex, and smooth.

Essentially aerobic, but may show growth under microaerophilic conditions. The temperature range for growth is 4–42°C, with optimal growth at 25–35°C. Can **grow at pH 6.0–9.0** and in the **presence of up to 15% NaCl**. **Catalase-positive and oxidase-negative.**

Acid is produced from D-glucose, D-galactose, and L-arabinose. Esculin is hydrolyzed. Tyrosine, chitin, and cellulose are not hydrolyzed. Voges–Proskauer-negative. Nonhemolytic.

Cell wall peptidoglycan contains ***meso*-diaminopimelic acid, alanine, and glutamic acid; aspartic acid may be present**. Mycolic acids are absent. **The principal menaquinone is MK-7**. The **fatty acid profile** consists of the predominant compound **$C_{15:0}$ anteiso with significant amounts $C_{16:0}$ iso and $C_{17:0}$ anteiso**. The **polar lipid profile** is composed of **diphosphatidylglycerol, phosphatidylglycerol, and two unknown glycolipids**. **Spermine and spermidine** are the **predominant components of the polyamine pattern**.

DNA G+C content (mol%): 68–73.

Type species: **Brachybacterium faecium** Collins, Brown and Jones 1988, 46[VP].

Further descriptive information

The genus *Brachybacterium* encompasses a separate line of descent within the *Micrococcales*, with *Dermabacter hominis* as the next phylogenetic relative (Buczolits et al., 2003; Heyrman et al., 2002). The species share more than 96% 16S rRNA gene sequence similarities and less than 95% with *Dermabacter hominis*. Genus and first species, *Brachybacterium faecium*, were described by Collins et al. (1988) based on the unique characteristics of three strains which had been isolated from poultry deep litter (Schefferle, 1966). In the following, one of these strains was described to represent a novel species, *Brachybacterium paraconglomeratum*, and the second strain was assigned to the reclassified species *Brachybacterium conglomeratum* (formerly "*Micrococcus conglomeratus*") which had been isolated from oil brine. The same authors (Takeuchi et al., 1995) also described another novel species of the genus, *Brachybacterium rhamnosum*, which had been isolated from corn steep liquor. Other species of the genus such as *Brachybacterium nesterenkovii*, *Brachybacterium alimentarium*, *Brachybacterium tyrofermentans*, *Brachybacterium fresconis*, *Brachybacterium sacelli*, *Brachybacterium muris*, *Brachybacterium phenoliresistens*, and *Brachybacterium zhongshanense* were recovered from cheese, from the surface of French Gruyère and Beaufort cheeses, from a medieval wall painting of the chapel of castle Herberstein (Austria), from the liver of a laboratory mouse, oil-contaminated sand (Pintunt County, Taiwan), and from river sediments (Zhongshan City, China), respectively (Buczolits et al., 2003; Chou et al., 2007; Gvozdyak et al., 1992; Heyrman et al., 2002; Schubert et al., 1996; Zhang et al., 2007). Strains of the genus were also detected in numerous sites: a surgical ward (Xu et al., 2004), Antarctic sea ice brine (Junge et al., 1998), melt water from accretion ice (Lake Vostok, the largest subglacial lake in Antarctica) at a depth of approximately 3.5 km (Christner et al., 2001), a flowerpot-using fed-batch reactors for composting of household biowaste (Hiraishi et al., 2003), Wijdefjord, Spitzbergen, capable of hydrolyzing amylopectin, pullulan, and skim milk (Groudieva et al., 2004), and Mediterranean sediments (Suess et al., 2004). Based on deposited 16S rRNA gene sequences, brachybacteria were also detected in sewage sludge compost (strain SSCS6, accession no. AB210986), in a commercial nitrifying inoculum (strain R-23117, accession no. AJ786820; strain R-23108, accession no. AJ786818), in sea sediment (strain BBH3, accession no. DQ358873), at the west coast of India (*Brachybacterium* sp. DV1, accession no. DQ190429), in a space craft assembly facility (SAFR 049, accession no. AY167842), as a jellyfish-degrading bacterium (TN-2003; accession no. AB101583), and from undesignated sources (*Brachybacterium* sp. LB25, accession no. AB257583; strain B-116, accession no. DQ399745; *Brachybacterium* sp. B-4021, accession no. DQ347542; *Brachybacterium* sp. B-4051, accession no. DQ347546).

All species exhibit a quinone system consisting of the predominant compound menaquinone MK-7; moderate amounts of MK-8 may be present as well. Fatty acid profiles are predominated by the presence of iso- and anteiso methyl-branched acids. Common to the species of the genus is the presence of diphosphatidylglycerol, phosphatidylglycerol, and some unknown phospho- and glycolipids in their polar lipid profiles, but the presence of other unknown polar lipids appears useful for discrimination between species. The peptidogylcan type is A4γ, but variations were identified in the composition of the interpeptide bridge. The muramic residues can be either *N*-acetylated or *N*-glycolated (Buczolits et al., 2003; Collins et al., 1988; Gvozdyak et al., 1992; Heyrman et al., 2002; Schubert et al., 1996; Takeuchi et al., 1995). The presence of teichoic acids with the unusual compound erythritol as the polyol and diaminoglucuronic acid as a substituent in *Brachybacterium alimentarium* and *Brachybacterium tyrofermentans* was reported by Schubert et al. (1996, 1993).

Cystathionine lyase activity, assumed to play an important role in the development of the flavor of certain cheeses, has been reported for two *Brachybacterium* strains (Curtin et al., 2001). Certain strains of *Brachybacterium conglomeratum* and *Brachybacterium tyrofermentans* are suspected to be able to carry out the proteolytic degradation of infectious prion protein PRP[SC] (Müller-Hellwig et al., 2006).

Enrichment and isolation procedures

No specific isolation medium has been described so far. Good growth occurs on PYES* agar, TSA (trypticase soy agar), and

*0.3% peptone, 0.3% yeast extract, 0.23% Na_2-succinate, 1.5% agar, pH 7.2.

R2A agar. Addition of 5–7.5% NaCl (w/v) supports growth of most *Brachybacterium* strains, but *Brachybacterium nesterenkovii* strains grow best in the presence of 0–0.5% NaCl (w/v), and *Brachybacterium phenoliresistens* grows best in the presence of 2–3% NaCl (w/v). Temperature range for growth of *Brachybacterium* strains is 4–42°C, but 28°C may be suitable to grow all strains.

Pathogenicity. No reports on pathogenicity of *Brachybacterium* species have been published so far. However, one case has been reported showing that *Brachybacterium paraconglomeratum* has been isolated from a hospital environment (Xu et al., 2004).

Maintenance procedures

Brachybacterium cultures may be lyophilized or stored as glycerol cultures at −80°C by common procedures used for many bacteria.

Differentiation of genus *Brachybacterium* from other genera

Brachybacterium may be distinguished from other genera in the family *Micrococcaceae* and its next phylogenetic neighbor, *Dermabacter hominis*, by cell-wall peptidoglycan, menaquinone type, colony pigmentation, major fatty acid, and DNA G+C content (Table 117).

List of species of the genus *Brachybacterium*

1. **Brachybacterium faecium** Collins, Brown and Jones 1988, 46[VP]

fae′ci.um. L. n. *faex* feces, dregs; L. gen. pl. n. *faecium* of dregs, of feces.

Surface colonies are small, gray, white or pale lemon, low convex, and smooth. Cells vary in shape from coccoid forms in the stationary phase to rods (0.5–0.75 × 1.5–2.5 μm) in the exponential phase. Cells are Gram-stain-positive, non-acid-fast, and nonmotile. Aerobic (but may show weak anaerobic growth). Optimum temperature 25–30°C. No growth in the presence of thallous acetate (0.01%) or sodium azide (0.01%). Grows well in the presence of 15% NaCl. Does not grow at pH 5.0 and pH 10.0. Catalase is produced; oxidase is not produced. Acid is produced from L-arabinose, D-galactose, D-glucose, and maltose. Acid is not produced from adonitol, dulcitol, inositol, salicin, and sorbose. Some strains produce acid from cellobiose and sucrose. Catalase-positive. Negative for desoxyribonuclease, oxidase, phosphatase, and sulfatase. Esculin and starch are hydrolyzed. Cellulose, chitin, gelatin, tyrosine, and xanthine are not hydrolyzed. Some strains degrade arginine, hippurate, urea, uric acid, and casein. Some strains reduce nitrate to nitrite. H_2S is not produced. Voges–Proskauer-negative.

Cell-wall peptidoglycan is based on *meso*-diaminopimelic acid. Galactose and glucose are the wall sugars. Mycolic acids are not present. The principal menaquinone is MK-7. The long-chain fatty acids are primarily of the straight-chain saturated, iso-and anteiso-methyl branched types, with 12-methyltetradecanoic ($C_{15:0}$ anteiso) and 14-methylhexadecanoic ($C_{17:0}$ anteiso) acids predominating. The major polar lipids consist of diphosphatidylglycerol, phosphatidylglycerol, several phospholipids, and several glycolipids and unknown lipids.

Characteristics differentiating the species of *Brachybacterium* are listed in Table 118.

Source: poultry deep litter.

DNA G+C content (mol%): 68–71.5 (T_m).

Type strain: Schefferle 6-10, ATCC 43885, CCUG 32293, CIP 103333, DSM 4810, IEGM 403, NBRC 14762, JCM 11609, NCIMB 9860, VKM Ac-1423.

Sequence accession no. (16S rRNA gene): X83810, X91032.

2. **Brachybacterium alimentarium** Schubert, Ludwig, Springer, Kroppenstedt, Accolas and Fiedler 1996, 86[VP]

a.li.men.ta′ri.um. L. neut. adj. *alimentarium* pertaining to food.

Surface colonies are medium sized, vivid lemon colored, low convex, and smooth. Cells vary in shape from coccoid forms in the stationary phase, which occur in small clusters of 4–8 cells, to club-shaped rods in the exponential phase, which occur in pairs. Facultatively anaerobic. The optimum temperature is 30°C, and the optimum pH is 7.3. Grows well in the presence of 15% NaCl. Grows weakly at pH 5.0, but not at pH 10.0. Catalase and urease are produced; oxidase is not produced. Starch, esculin, casein, gelatin, and Tween 20 are hydrolyzed. Tween 80 and tributyrin are not hydrolyzed. Nitrate is reduced to nitrite. Acid is produced from D-arabinose, L-arabinose, D-galactose, D-glucose, D-mannose, melibiose, raffinose, L-rhamnose, D-ribose, and D-xylose but not from D-fructose, lactose, maltose, sucrose, and D-sorbitol.

The cell-wall peptidoglycan is based on *meso*-diaminopimelic acid with a D-Asp–D-Glu interpeptide bridge (variation A4γ). Two teichoic acids are present; one contains erythritol and *N*,*N*9-diacetyl-2,3-diamino-2,3-dideoxyglucuronic acid, and the other contains *N*-acetylglucosamine and galactose as sugar constituents. The principal menaquinone is MK-7; MK-8 is present in substantial amounts. The long-chain fatty acids are primarily straight-chain, saturated, iso- and anteiso-methyl, branched fatty acids, with 12-methyltetradecanoic ($C_{15:0}$ anteiso) acid predominating. The major polar lipids consist of diphosphatidylglycerol, phosphatidylglycerol, a phospholipid, glycolipids, and unknown lipids.

Source: the surfaces of Beaufort and Gruyère cheeses.

DNA G+C content (mol%): 73.0 (T_m).

Type strain: ATCC 700067, CCUG 50349, CIP 104811, CNRZ 925, DSM 10672, NBRC 16118, JCM 11607, LMG 19551.

Sequence accession no. (16S rRNA gene): X91031.

3. **Brachybacterium conglomeratum** (*ex* Migula 1900) Takeuchi, Fang and Yokota 1995, 166[VP] ("*Micrococcus conglomerates*" Migula 1900)

con.glo.me.ra′tum. L. part. neut. adj. *conglomeratum* rolled together.

TABLE 118. Characteristics differentiating species of the genus *Brachybacterium*[a]

Characteristic	*B. faecium*[b]	*B. alimentarium*[b]	*B. conglomeratum*[b,c]	*B. fresconis*[b]	*B. muris*[b]	*B. nesterenkovii*[b]	*B. paraconglomeratum*[c]	*B. phenoliresistens*[d]	*B. rhamnosum*[b]	*B. sacelli*[b]	*B. tyrofermentans*[b]	*B. zhongshanense*[e]
Urease production	−	+	+	+	−	−	+	−	+	−	−	+
H_2S production	−	−	d	−	−	+	+	−	+	−	−	nd
Indole formation	(+)	−	(+)	−	−	−	+	−	−	−	−	−
Nitrate reduction	+	+	+	+	−	+	+	−	+	+	+	+
Growth at:												
4°C	+	+	−	−	−	−	nd	+	+	−	+	−
37°C	+	−	+	(+)	+	+	nd	+	+	(+)	−	+
42°C	(+)	−	(+)	−	(+)	−	nd	nd	−	−	−	(+)
pH 5.0	−	(+)	d	+	−	(+)	−	+	+	+	−	+
pH 10.0	−	−	+	+	+	−	nd	+	−	−	−	+
at 15% NaCl	+	+	+	+	−	−	(+)	−	−	+	(+)	−
Hydrolysis of:												
Casein	−	+	−	−	−	−	nd	(+)	+	−	−	nd
Starch	+	+	+	−	+	−	+	+	+	−	−	−
Tween 20	−	+	−	−	+	+	−	+	−	−	−	−
Tween 80	−	−	d	−	(+)	−	−	−	−	−	−	−
Acid production from:												
D-Arabinose	(+)	(+)	−	−	+	−	−	+	−	−	−	nd
D-Fructose	−	−	+	+	+	−	+	+	+	+	−	+
DL-Lactose	−	−	d	−	+	+	(+)	(+)	−	−	−	nd
Maltose	(+)	−	d	−	+	(+)	+	+	+	−	−	+
D-Mannose	−	(+)	d	+	(+)	−	+	+	+	(+)	+	+
Melibiose	+	+	+	+	−	−	nd	−	+	−	+	nd
Raffinose	−	+	−	+	−	(+)	−	+	+	−	(+)	nd
L-Rhamnose	−	+	−	(+)	+	−	−	+	−	(+)	(+)	+
D-Ribose	(+)	(+)	d	−	+	(+)	−	−	(+)	−	(+)	nd
D-Sorbitol	−	−	−	−	−	−	−	−	+	−	−	+
Sucrose	−	−	−	−	+	+	+	+	+	−	−	+
D-Xylose	−	(+)	d	(+)	−	+	−	+	(+)	−	−	nd
Peptidoglycan with:												
Aspartic acid	−	+	−	−	+	−	−	+	−	−	+	nd
Glycine	+	+	+	+	+	−	+	+	+	+	+	nd
Distinguishing polar lipid(s):												
Unknown glycolipids	3	2	3	3	3	2	3	4	3	3	3	nd
Unknown phospholipids	4	1	3	2	2	3	nd	1	3	2	2	nd
Unknown phosphoglycolipid	1	−	1	−	−	−	nd	−	1	−	−	nd
Unknown aminophosphoglycolipids	−	−	2	−	−	−	2	−	−	−	−	nd
Quinone system MK-7 supplemented with:												
MK-8 (>25%)	−	+	nd	+	−	−	nd	nd	−	+	−	nd
MK-7(H_2) present	−	−	nd	+	−	−	nd	nd	−	−	−	nd

[a]Symbols: +, >89% positive; (+), weakly positive; d, 11–89% positive; −, >89% negative; nd, not determined.

[b]Buczolits et al. (2003).

[c]Takeuchi et al. (1995).

[d]Chou et al. (2007).

[e]Zhang et al. (2007).

Cells are Gram-stain-positive, nonmotile, and nonspore-forming. In the stationary growth phase, cells are coccoid; however, when a culture is transferred to fresh medium, the cells become oval or rod-shaped. Colonies are circular, entire, low convex, smooth, opaque, and whitish yellow or pale brown. Aerobic or facultatively anaerobic. Catalase and urease are produced; oxidase is not produced. Grows well in the presence of 15% NaCl. Some strains grow at pH 5.0 and pH 10.0. Starch and esculin are hydrolyzed, but Tween 20 and Tween 40 are not hydrolyzed. Some strains hydrolyze Tween 60 and Tween 80. Nitrate is reduced to nitrite. Acid is produced from L-arabinose, D-galactose, and

D-glucose. Acid is not produced from trehalose, mannitol, sorbitol, and xylitol.

The cell-wall peptidoglycan is based on *meso*-diaminopimelic acid. The cell wall sugars are galactose and glucose. The cell wall muramic acid occurs in the *N*-glycolyl form. Mycolic acids are absent. The principal menaquinone is MK-7. The long-chain fatty acids are primarily straight-chain, saturated, iso and anteiso methyl-branched fatty acids; $C_{15:0}$ anteiso and $C_{17:0}$ anteiso are the predominant fatty acids. The major polar lipids consist of diphosphatidylglycerol, phosphatidylglycerol, several glycolipids, phospholipids, unidentified aminophosphoglycolipids, and unknown lipids.

Source: an oil brine.

DNA G+C content (mol%): 70.6 (HPLC).

Type strain: AJ 1015, Komagata 5-2, CCM 2589, CCUG 50160, CCUG 50354, CIP 104400, DSM 10241, NBRC 15472, JCM 11608, VKM Ac-1975.

Sequence accession no. (16S rRNA gene): AB537169.

4. **Brachybacterium fresconis** Heyrman, Balcaen, De Vos, Schumann and Swings 2002, 1644[VP]

fres.co′nis. N.L. gen. n. *fresconis* pertaining or belonging to fresco(es).

Cells vary in shape from coccoid forms (single or in agglomerates) in the stationary phase, to short rods in the exponential phase. Cells are nonmotile, nonsporeforming, and stain Gram-positive. Colonies on marine agar after 24 h are small (<1 mm), cream-colored, smooth, glistening, circular in outline, and low convex. No growth in an anaerobic chamber on marine agar. The temperature range for growth is 5–40°C, with optimal growth between 20 and 30°C. Grows well in the presence of 15% NaCl. Grows at pH 5.0 and pH 10.0. Catalase and urease are produced; oxidase is not produced. Esculin and gelatin are hydrolyzed, starch, casein, Tween 20, and Tween 80 are not hydrolyzed. Nitrate is reduced to nitrite. Indole is not produced. Arginine dihydrolase-negative, and β-galactosidase-positive. Assimilation of arabinose, glucose, maltose, and mannose is positive; caprate, citrate, and malate are not assimilated. The cell-wall peptidoglycan contains *meso*-diaminopimelic acid and is of the type A4γ (*m*-Dpm ← D-Glu ← D-Glu) with glycine bound to the *a*-carboxyl group of D-glutamic acid at position 2 of the peptide subunit. Muramic acid residues are glycolylated. Mycolic acids are absent. The principal menaquinone is MK-7; MK-8 is present in substantial amounts, and MK-7(H_2) in minor amounts. The main fatty acid is $C_{15:0}$ anteiso. The major polar lipids consist of diphosphatidylglycerol, phosphatidylglycerol, phospholipids, glycolipids, and unknown lipids.

Source: a medieval wall painting.

DNA G+C content (mol%): 70.4 (HPLC).

Type strain: DSM 14564, JCM 12138, LMG 20336.

Sequence accession no. (16S rRNA gene): AJ415378.

5. **Brachybacterium muris** Buczolits, Schumann, Weidler, Radax and Busse 2003, 1959[VP]

mu′ris. L. gen. n. *muris* of the mouse; the type strain was isolated from a mouse.

Cells are small, coccoid to ovoid, 1.2–1.5 µm in diameter. Cells occur singly, in pairs, or in agglomerates. Gram-stain-positive and nonsporeforming. Motility is not observed. Cells grow best on sheep blood agar plates and Brucella broth supplemented with 8% fetal bovine serum. Colonies on PYES agar are translucent, have light-yellow pigmentation, and are circular, convex, and smooth. Best growth is observed under microaerobic conditions; moderate growth is observed under aerobic conditions, but no growth occurs anaerobically. Colony diameters on sheep blood agar are 3–5 mm after 4 days incubation at 37°C under microaerobic conditions. The temperature range for growth is 15–42°C, with optimal growth between 25 and 37°C; no growth at 4°C. No growth is observed at pH 5.0, but the strain grows at pH 10.0. Does not grow in the presence of 15% NaCl.

Catalase is produced; oxidase and urease are not produced. Esculin, starch, Tween 20, and Tween 80 are hydrolyzed; casein is not hydrolyzed. Nitrate is not reduced to nitrite. Indole is not produced. Acid is produced from L-arabinose, D-arabinose, D-fructose, D-galactose, D-glucose, lactose, maltose, D-mannose, L-rhamnose, D-ribose, and sucrose, but not from melibiose, raffinose, D-xylose, and D-sorbitol.

The diagnostic cell-wall diamino acid is *meso*-diaminopimelic acid, and the peptidoglycan type is A31.3 (variation A4γ) containing the amino acids *meso*-diaminopimelic acid, alanine, glycine, aspartic acid, and glutamic acid. Muramic acid residues are *N*-acetylated and not *N*-glycolated. Mycolic acids are absent. The principal menaquinone is MK-7; MK-8 and MK-6 are present in minor amounts. The fatty acid profile consists of the predominant compound $C_{15:0}$ anteiso with significant amounts of $C_{14:0}$ iso, $C_{15:0}$ iso, $C_{16:0}$ iso, $C_{17:0}$ anteiso, and $C_{19:0}$. The polar lipid profile is composed of diphosphatidylglycerol, phosphatidylglycerol, three unknown glycolipids, two unknown phospholipids, and six unidentified polar lipids. Spermine and spermidine are the predominant components of the polyamine pattern.

Source: the liver of a laboratory mouse strain.

DNA G+C content (mol%): not available.

Type strain: C3H-21, CCM 7047, CCUG 50352, DSM 15460, JCM 14117.

Sequence accession no. (16S rRNA gene): AJ537574.

6. **Brachybacterium nesterenkovii** Gvozdyak, Nogina and Schumann 1992, 77[VP]

nes.te.ren.ko′vi.i. N.L. gen. masc. n. *nesterenkovii* of Nesterenko, named for O.A. Nesterenko, a Ukrainian microbiologist who has made a valuable contribution to the systematics of coryneform and nocardioform bacteria.

In the stationary growth phase, cells are coccoid (diameter 0.5–1 µm). During the exponential growth phase the cells are coccoid, oval, angular, and short rods that vary in size from strain to strain (up to 2 µm long). V-shaped cells are observed.

Colonies are matte and convex with regular edges; their surfaces are usually smooth but may be rough. The colony diameter on beef extract agar is up to 1.5 mm (rarely, 3 mm). The color of the colonies is lemon yellow on beef extract

agar and milk agar and pale grayish on glucose-asparagine agar. Facultatively anaerobic. Gram-stain-positive, non-acid-fast, nonmotile, and nonsporeforming. Grows well at 18–37°C. Grows on medium containing 20 μg of furazolidone per ml, and forms acid from glycerol in the presence of erythromycin. Does not grow on Simmons citrate agar. Grows weakly at pH 5.0, but not at pH 10.0. Catalase is produced; oxidase and urease are not produced. Acid is produced aerobically from L-arabinose, D-galactose, D-glucose, maltose, D-ribose, sucrose, and glycerol. D-Arabinose, erythrose, melezitose, rhamnose, L-sorbose, trehalose, adonitol, D-arabitol, L-arabitol, dulcitol, inositol, mannitol, and sorbitol are not attacked. Variable results are observed for acid formation from D-mannose, D-melibiose, D-xylose, salicin, and starch. Utilizes tartrate, oxaloacetate, and maleate. Some strains poorly utilize acetate, lactate, pyruvate, and succinate. Negative for phenylalanine deaminase and for lysine and ornithine decarboxylase. Ammonia production from arginine is detected 21 days after inoculation. Esculin is hydrolyzed. Tyrosine, gelatin, xanthine, hypoxanthine, Tween 40, Tween 60, and Tween 80 are not hydrolyzed. Tween 20 is hydrolyzed from some strains. Negative for lecithinase, hydrogen sulfide, and acetoin.

Susceptible to ampicillin, oxacillin, methicillin, carbenicillin, benzylpenicillin, streptomycin, neomycin, kanamycin, erythromycin, oleandomycin, rifampin, lincomycin, ristomycin, tetracycline, and chloramphenicol. Not susceptible to polymyxin.

The cell-wall peptidoglycan is based on *meso*-diaminopimelic acid with alanine and glutamic acid but without glycine. There are significant amounts of glucose, galactose, and rhamnose in the cells. Mycolic acids are absent. The principal menaquinone is MK-7. The main fatty acids are $C_{15:0}$ anteiso and $C_{17:0}$ anteiso. The major polar lipids consist of diphosphatidylglycerol, phosphatidylglycerol, several phospholipids, and several glycolipids, and unknown lipids.

Source: milk and milk products.

DNA G+C content (mol%): 70.0 (T_m).

Type strain: 35, CIP 104813, DSM 9573, NBRC 15384, IMV Ac-752, JCM 11648, LMG 19549.

Sequence accession no. (16S rRNA gene): X91033.

7. **Brachybacterium paraconglomeratum** Takeuchi, Fang and Yokota 1995, 167[VP]

pa.ra.con.glo.me.ra′tum. Gr. prep. *para* alongside of, resembling; L. neut. part. adj. *conglomeratum* specific epithet of *Brachybacterium conglomeratum*; N.L. neut. part. adj. *paraconglomeratum* resembling *Brachybacterium conglomeratum*.

Cells are Gram-stain-positive, nonmotile, and nonsporeforming. In the stationary growth phase, cells are coccoid; however, when a culture is transferred to fresh medium, the cells become oval or rod-shaped. Colonies are circular, entire, low convex, smooth, and opaque; the colony color is pale brown. Aerobic or facultatively anaerobic. Catalase and urease are produced; oxidase is not produced. Grows weakly in the presence of 15% NaCl. Does not grow at pH 5.0. Starch and esculin are hydrolyzed, but Tween 20, Tween 40, Tween 60, and Tween 80 are not hydrolyzed. H_2S and indole are produced. Nitrate is reduced to nitrite. Acid is produced from L-arabinose, cellobiose, D-fructose, D-galactose, D-glucose, lactose, maltose, D-mannose, melezitose, salicin, sucrose, adonitol, and inositol. Acid is not produced from D-arabinose, raffinose, L-rhamnose, D-ribose, trehalose, D-xylose, and sorbitol.

The cell-wall peptidoglycan is based on *meso*-diaminopimelic acid. The cell wall sugars are galactose and glucose. The cell wall muramic acid occurs in the *N*-glycolyl form. Mycolic acids are absent. The principal menaquinone is MK-7. The long-chain fatty acids are primarily straight-chain, saturated, iso and anteiso methyl-branched fatty acids; $C_{15:0}$ anteiso and $C_{17:0}$ anteiso are the predominant fatty acids. The major polar lipids are diphosphatidylglycerol, phosphatidylglycerol, unidentified glycolipids, and unidentified aminophosphoglycolipids.

Source: poultry deep litter.

DNA G+C content (mol%): 68.6 (HPLC).

Type strain: ATCC 51843, CIP 103334, DSM 46341, IEGM 404, NBRC 15224, JCM 11649, LMG 19861, NCIMB 9861, VKM Ac-1425.

Sequence accession no. (16S rRNA gene): AJ415377.

8. **Brachybacterium phenoliresistens** Chou, Lin, Lin, Sheu, Wei, Arun, Young and Chen 2007, 2677[VP]

phe.no.li.re.sis′tens. N.L. neut. n. *phenol* phenol; L. part. adj. *resistens* resisting; N.L. part. adj. *phenoliresistens* phenol resisting, referring to the organism's ability to resist phenol.

Cells are small, coccoid to ovoid shaped, 0.8–1.3 μm in diameter. Cells occur singly, in pairs or as agglomerates. Facultatively anaerobic. Gram-stain-positive (Gram staining and KOH test), nonmotile, and nonsporeforming. Colonies on marine 2216 agar are pale yellow, circular, and convex in shape with entire edges. Colonies are approximately 0.9–2.1 mm in diameter on marine 2216 agar after 48 h incubation at 28°C. No accumulation of poly-β-hydroxybutyrate granules is observed. Flexirubin-type pigments are present. Optimum growth occurs aerobically, while only moderate anaerobic growth is observed. Good growth occurs at temperatures of 4–40°C, 0.5–7.0% NaCl, and pH 5.0–10.0. Optimum growth occurs at 28–35°C, 2–3% NaCl, and pH 7.0. Acid is produced from arabinose, fructose, mannose, raffinose, rhamnose, sucrose, xylose, cellobiose, galactose, glucose, lactose, and trehalose, but not from ribose or sorbitol. Positive for catalase, DNase, and hydrolysis of starch and Tween 20. Weakly positive for hydrolysis of casein and Tween 40. Negative for oxidase, nitrate reduction, nitrite reduction, lipase (corn oil), and hydrolysis of Tweens 60 and 80. In the API Coryne system, positive for hydrolysis of pyrazinamidase, pyrrolidonyl arylamidase, alkaline phosphatase, β-glucuronidase, β-galactosidase, α-glucosidase, *N*-acetyl-β-glucosaminidase, and esculin; acid production from D-glucose, D-xylose, D-mannitol, maltose, D-lactose, and sucrose. Negative for nitrate reduction, urease, gelatin hydrolysis, and acid production from D-ribose and glycogen. With the API 20E system, positive for ONPG test, acetoin production, and acid production from glucose, mannitol, inositol, rhamnose, sucrose, amygdalin, and arabinose, but negative for nitrate reduction, arginine dihydrolase, lysine decarboxylase, orni-

thine decarboxylase, citrate utilization, H_2S production, urease, tryptophan deaminase, indole production, gelatinase, and acid production from sorbitol and melibiose. Positive enzyme reactions (API ZYM) for alkaline phosphatase, C4 esterase, C8 lipase, leucine arylamidase, α-galactosidase, β-galactosidase, α-glucosidase, β-glucosidase, *N*-acetyl-β-glucosaminidase, α-mannosidase, and α-fucosidase; weak positive reactions for C14 lipase, valine arylamidase, cystine arylamidase, acid phosphatase, naphthol-AS-BI-phosphohydrolase, and β-glucuronidase, but negative reactions for trypsin and α-chymotrypsin. The following carbon substrates (Biolog GP2) are oxidized: β-cyclodextrin, dextrin, glycogen, Tween 40, *N*-acetyl-D-glucosamine, L-arabinose, D-arabitol, arbutin, D-cellobiose, D-fructose, D-galactose, gentiobiose, α-D-glucose, maltose, maltotriose, D-mannitol, D-mannose, D-melezitose, methyl β-D-glucoside, palatinose, D-psicose, D-raffinose, salicin, D-sorbitol, stachyose, sucrose, trehalose, turanose, xylitol, pyruvic acid methyl ester, glycerol, adenosine, and 2′-deoxyadenosine. Unable to oxidize α-cyclodextrin, inulin, mannan, Tween 80, *N*-acetyl-β-D-mannosamine, amygdalin, L-fucose, D-galacturonic acid, D-gluconic acid, *myo*-inositol, α-D-lactose, lactulose, D-melibiose, methyl α-D-galactoside, methyl β-D-galactoside, 3-methyl D-glucose, methyl α-D-glucoside, methyl α-D-mannoside, L-rhamnose, D-ribose, sedoheptulosan, D-tagatose, D-xylose, acetic acid, α-hydroxybutyric acid, β-hydroxybutyric acid, γ-hydroxybutyric acid, p-hydroxyphenylacetic acid, α-ketoglutaric acid, α-ketovaleric acid, lactamide, D-lactic acid methyl ester, L-lactic acid, D-malic acid, L-malic acid, succinic acid monomethyl ester, propionic acid, pyruvic acid, succinamic acid, succinic acid, *N*-acetyl-L-glutamic acid, L-alaninamide, D-alanine, L-alanine, L-alanyl glycine, L-asparagine, L-glutamic acid, glycyl L-glutamic acid, L-pyroglutamic acid, L-serine, putrescine, 2,3-butanediol, inosine, thymidine, uridine, adenosine, uridine 5′-monophosphate, D-fructose 6-phosphate, α-D-glucose 1-phosphate, D-glucose 6-phosphate, or DL-α-glycerol phosphate. Resistant to ampicillin, gentamicin, nalidixic acid, and tetracycline, but sensitive to chloramphenicol, erythromycin, kanamycin, novobiocin, penicillin G, rifampin, and streptomycin.

The diagnostic cell-wall diamino acid is *meso*-diaminopimelic acid, and the peptidoglycan type is variation A4γ containing amino acids *meso*-diaminopimelic acid, alanine, glycine, aspartic acid, and glutamic acid. The polar lipid profile consists of diphosphatidylglycerol, phosphatidylglycerol, three unknown glycolipids, and one unknown phospholipid. The predominant fatty acid is $C_{15:0}$ anteiso with significant amounts of $C_{16:0}$ iso, $C_{14:0}$ iso, and $C_{17:0}$ anteiso. The major menaquinone is MK-7.

Source: oil-contaminated coastal sand, Pingtung County, Taiwan.

DNA G+C content (mol%): 70.8 (HPLC).

Type strain: phenol-A, BCRC 17589, JCM 15157, LMG 23707.

Sequence accession no. (16S rRNA gene): DQ822566.

9. **Brachybacterium rhamnosum** Takeuchi, Fang and Yokota 1995, 167[VP]

rham.nos′um. L. n. *rhamnosum* rhamnose, referring to the abundance of rhamnose in the cell wall.

Cells are Gram-stain-positive, nonmotile, and nonsporeforming. In the stationary growth phase, cells are coccoid; however, when a culture is transferred to fresh medium, the cells become oval or rod-shaped. Colonies are circular, entire, low convex, smooth, opaque, and whitish yellow. Aerobic or facultatively anaerobic. Catalase and urease are produced; oxidase is not produced. Does not grow in the presence of 15% NaCl. Grows at pH 5.0, but not at pH 10.0. Starch, esculin, and casein are hydrolyzed, but Tween 20, Tween 40, Tween 60, and Tween 80 are not hydrolyzed. H_2S is produced. Nitrate is reduced to nitrite. Acid is produced from L-arabinose, cellobiose, D-fructose, D-galactose, D-glucose, maltose, D-mannose, melibiose, melezitose, raffinose, D-ribose, salicin, sucrose, trehalose, D-xylose, adonitol, inositol, and sorbitol. Acid is not produced from D-arabinose, lactose, and L-rhamnose.

The cell-wall peptidoglycan is based on *meso*-diaminopimelic acid. The cell wall sugars are galactose, glucose, and rhamnose. The cell wall muramic acid occurs in the *N*-glycolyl form. Mycolic acids are absent. The principal menaquinone is MK-7. The long-chain fatty acids are primarily straight-chain, saturated, iso and anteiso methyl-branched fatty acids; $C_{15:0}$ anteiso and $C_{17:0}$ anteiso are the predominant fatty acids. The major polar lipids consist of diphosphatidylglycerol, phosphatidylglycerol, several phospholipids, and several glycolipids and unknown lipids.

Source: commercially available corn steep liquor.

DNA G+C content (mol%): 71.5 (HPLC).

Type strain: H-6S, ATCC 51844, CIP 104398, DSM 10240, NBRC 15203, JCM 11650, LMG 19848, VKM Ac-1976.

Sequence accession no. (16S rRNA gene): AJ415376.

10. **Brachybacterium sacelli** Heyrman, Balcaen, De Vos, Schumann and Swings 2002, 1644[VP]

sa.cel′li. L. gen. n. *sacelli* of the chapel.

Cells vary in shape from coccoid forms (single or in agglomerates) in the stationary phase, to short rods in the exponential phase. Cells are nonmotile, nonsporeforming, and stain Gram-positive. Colonies on marine agar after 24 h are small (<1 mm), cream-colored, smooth, glistening, circular in outline, and low convex. No growth in an anaerobic chamber on marine agar. The temperature range for growth is 5–40°C, with optimal growth between 20 and 30°C. Grows well in the presence of 15% NaCl. Grows at pH 5.0, but not at pH 10.0. Catalase is produced, oxidase and urease are not produced. Esculin is hydrolyzed, starch, casein, Tween 20, Tween 80, and gelatin are not hydrolyzed. Nitrate is reduced to nitrite. Indole is not produced. Arginine dihydrolase-negative, and β-galactosidase-positive. Strains assimilate gluconate, maltose, mannose, mannitol, and *N*-acetylglucosamine, but not adipate, arabinose, caprate, citrate, glucose, and malate.

The cell-wall peptidoglycan contains *meso*-diaminopimelic acid and is of the type A4γ (*m*-Dpm ← D-Glu ← D-Glu) with glycine bound to the *a*-carboxyl group of D-glutamic acid at position 2 of the peptide subunit. Muramic acid residues are glycolylated. Mycolic acids are absent. The principal menaquinone is MK-7 in combination with either MK-8 in lower but still considerable amounts, or in approximately equal amounts. MK-7(H_2) or MK-9 are present in minor

amounts in some strains. The main fatty acid is $C_{15:0}$ anteiso. The major polar lipids consist of diphosphatidylglycerol, phosphatidylglycerol, phospholipids, glycolipids, and unknown lipids.

Source: a medieval wall painting.

DNA G+C content (mol%): 70.3 (HPLC).

Type strain: CCUG 50347, DSM 14566, JCM 12139, LMG 20345.

Sequence accession no. (16S rRNA gene): AJ415384.

11. **Brachybacterium tyrofermentans** Schubert, Ludwig, Springer, Kroppenstedt, Accolas and Fiedler 1996, 86[VP]

ty.ro.fer.men′tans. Gr. n. *tyros* cheese; L. part. adj. *fermentans* leavening; N.L. part. adj. *tyrofermentans* fermenting cheese.

Surface colonies are medium sized, pale flaxen, low convex, and smooth. Cells vary in shape from coccoid forms in the stationary phase, which occur in large agglomerations, to club-shaped rods in the exponential phase, which occur predominantly in pairs or agglomerations of four cells. Facultative anaerobic. The optimum temperature is 30°C, and the optimum pH is 7.3. Grows weakly in the presence of 15% NaCl. Does not grow at pH 5.0 and pH 10.0. Catalase is produced; oxidase and urease are not produced. Esculin and gelatin are hydrolyzed. Starch, casein, Tween 20, Tween 80, and tributyrin are not hydrolyzed. Tyrosine and xanthine are not utilized. Nitrate and nitrite are reduced. Acid is produced from L-arabinose, D-galactose, D-glucose, D-mannose, melibiose, raffinose, L-rhamnose, and D-ribose, but not from D-arabinose, D-fructose, lactose, maltose, sucrose, D-xylose, and D-sorbitol.

The cell-wall peptidoglycan is based on *meso*-diaminopimelic acid with a D-Asp–D-Glu interpeptide bridge (variation A4γ). One teichoic acid structure is present; this structure contains erythritol, *N*-acetylglucosamine, and *N*,*N*9-diacetyl-2,3-diamino-2,3-dideoxyglucuronic acid. The principal menaquinone is MK-7. The long-chain fatty acids are primarily straight-chain, saturated, iso- and anteiso-methyl, branched fatty acids, with 12-methyltetradecanoic ($C_{15:0}$ anteiso) acid predominating. The major polar lipids consist of diphosphatidylglycerol, phosphatidylglycerol, phospholipids, glycolipids, and unknown lipids.

Source: the surfaces of Beaufort and Gruyère cheeses.

DNA G+C content (mol%): 73.0 (T_m).

Type strain: ATCC 700068, CCUG 50353, CIP 104812, CNRZ 926, DSM 10673, NBRC 16119, JCM 11610, LMG 19552.

Sequence accession no. (16S rRNA gene): X91657.

12. **Brachybacterium zhongshanense** Zhang, Zeng, Cai, Deng, Luo and Sun 2007, 2523[VP]

zhong.shan.en′se. N.L. neut. adj. *zhongshanense* pertaining to Zhongshan, a city in China, from where the type strain was isolated.

Cells vary in shape from coccoid forms (irregular) in the stationary phase to short rods in the exponential phase. Cells are nonmotile, Gram-stain-positive, and non-sporeforming. Colonies on LB plates are cream-colored, smooth, glistening, and grow well in an anaerobic chamber on LB plates. Temperature range for good growth is 25–40°C; no growth occurs at 4 or 45°C. Grows in 0–10% (w/v) NaCl. Grows well at pH 5.0–8.0 and weakly at pH 9.0–11.0; no growth is observed at pH 12.0–14.0. Catalase and urease are produced. Oxidase is not produced. Esculin and gelatin are hydrolyzed, but starch is not. Nitrate is reduced to nitrite. Indole is not produced. Positive for arginine dihydrolase and denitrification. Acid is produced from glucose. Utilizes the following carbon sources: α-cyclodextrin, β-cyclodextrin, dextrin, glycogen, inulin, amygdalin, L-arabinose, arbutin, D-cellobiose, D-fructose, D-galactose, D-galacturonic acid, gentiobiose, α-D-glucose, α-D-lactose, lactulose, maltose, maltotriose, D-mannose, D-melezitose, D-melibiose, methyl α-D-galactoside, methyl β-D-galactoside, methyl β-D-glucoside, palatinose, D-psicose, D-raffinose, stachyose, sucrose, D-tagatose, trehalose, turanose, xylitol, D-xylose, acetic acid, L-lactic acid, pyruvic acid methyl ester, pyruvic acid, glycerol, adenosine, inosine, and thymidine. Predominant fatty acids are $C_{15:0}$ anteiso and $C_{17:0}$ anteiso.

Source: sediments along the Qijiang River, Zhongshan City, China.

DNA G+C content (mol%): 71.2 (T_m).

Type strain: JB, CGMCC 1.6508, DSM 18832, JCM 15471, LMG 23926.

Sequence accession no. (16S rRNA gene): EF125186.

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Family VII. **Dermacoccaceae** Stackebrandt and Schumann 2000, 1285VP emend. Zhi, Li and Stackebrandt 2009, 597

Erko Stackebrandt

Der.ma.coc.ca.ce′a.e. N.L. masc. n. *Dermacoccus* type genus of the family; suff. *-aceae* ending to denote a family; N.L. fem. pl. n. *Dermacoccaceae* the *Dermacoccus* family.

Gram-stain-positive cocci to short rods; nonmotile; non-acid-fast; nonencapsulated; nonsporeforming. Chemoorganotrophic; aerobic or microaerophilic with formation of acids from some carbohydrates. Catalase positive. Mesophilic and, when tested, nonhalophilic. **The peptidoglycan type is either L-Lys–L-Ser$_{1-2}$–D-Glu or L-Lys–L-Ser$_2$(L-Ala)–D-Glu, or L-Lys–Glu$_2$, or Lys–L-Ser–D-Asp, variation A4α.** When tested, muramic acid is acetylated; mycolic acids and teichoic acids are absent. **The menaquinones are either hydrogenated or completely unsaturated. Main fatty acids are anteiso-methyl and iso-methyl-branched; straight-chain saturated and monounsaturated fatty acid may occur.** Polar lipids are diphosphatidylglycerol, phosphatidylglycerol, and phosphatidylinositol; phosphatidyl-ethanolamine and two unknown phospholipids occur in one genus.

The pattern of 16S rRNA gene signatures consists of nucleotides at positions 120 (A), 131:231 (A–G), 196 (C), 342:347 (C–G), 444:490 (A–U), 580:761 (U–A), 602:636 (C–G), 670:736 (A–U), 822:878 (G–C), 823:877 (G–C), 826:874 (C–G), 827 (U), 843 (c), 950:1231 (U–A), 1047:1210 (G–C), 1109 (C), 1145 (G), 1309:1328 (G–C), 1361 (G), and 1383 (C) (Zhi et al., 2009).

DNA G+C content (mol%): 66–71.

Type genus: **Dermacoccus** Stackebrandt, Koch, Gvozdiak and Schumann 1995, 689VP.

Taxonomic comments

According to the taxonomic outline for this volume, the family *Dermacoccaceae* Stackebrandt and Schumann 2000 is a member of the order *Micrococcales* (elevated from suborder *Micrococcineae* of Stackebrandt et al., 1997), originally established on the basis of 16S rRNA gene sequence signatures. In the first attempt to use such signatures for an intra-class taxonomic structure of the *Actinobacteria* (Stackebrandt et al., 1997), the genera *Dermacoccus* and *Kytococcus* were placed within the family *Dermatophilaceae*. However, with the description of the genus *Demetria* and several other new genera of the *Micrococcineae*, the necessity to describe novel families within the suborder became obvious. Based upon a revised set of signature nucleotides, the genera *Dermacoccus* and *Kytococcus* were removed from *Dermatophilaceae* and formed, together with *Demetria*, the family *Dermacoccaceae* (Stackebrandt and Schumann, 2000).

The high degree of sequence similarity shared between type strains of genera of *Micrococcineae* made the topologies of phylogenetic trees prone to the number and selection of organisms of the suborder and outside reference organisms. In certain dendrograms *Serinicoccus marinus*, a member of *Intrasporangiaceae*, clustered with all three members of *Dermacoccaceae* (ARB, Ludwig et al., 2004; Mayilraj et al., 2006) while, in another dendrogram, *Serinicoccus marinus* branched outside the radiation of *Dermacoccaceae* [with *Ornithinobacterium humiphilum* (Yi et al., 2004)]. However, most of the recently published studies on novel taxa of *Micrococcineae* (now *Micrococcales*) did not include both members of *Dermacoccaceae* and *Seriniccocus marinus*. In the absence of members of *Dermacoccaceae*, *Seriniccocus marinus* did often cluster with *Ornithinimicrobium* species (Montero-Barrientos et al., 2005; Jung et al., 2006; Lee, 2006; Liu et al., 2008; Groth and Kämpfer, 2012, this volume), but not exclusively (Lee and Lee, 2007).

As shown by Groth and Kämpfer (2012, this volume) and Schumann (2012, this volume) in the descriptions of *Intrasporangiaceae* and *Serinicoccus*, respectively, the presence of ornithine as diagnostic amino acid in the peptidoglycan links *Serinibacter marinus* to certain members of *Intrasporangiaceae*; also, members of *Dermacoccaceae* do not match the revised set of signature nucleotides of *Intrasporangiaceae* (Groth and Kämpfer, 2012) and *Serinicoccus marinus* fails to match the set provided for *Dermacoccaceae* (Zhi et al., 2009).

The main characteristics differentiating members of *Dermacoccaceae* and phylogenetically related genera are depicted by Schumann (2012, this volume) in the description of *Serinicoccus*, while Groth and Kämpfer (2012, this volume) provide an overview of the morphological and chemotaxonomic heterogeneity of the family *Intrasporangiaceae*.

Genus I. **Dermacoccus** Stackebrandt, Koch, Gvozdiak and Schumann 1995, 689VP

Erko Stackebrandt

Der.ma.coc′cus. N.L. n. *derma* skin: Gr. masc. n. *kokkos* a grain, berry: N.L. masc. n. *Dermacoccus* coccus living on skin.

Cells are coccoid. Gram-stain-positive. Nonencapsulated. Nonsporeforming. **Chemoorganotrophic; aerobic; rarely weak growth occurs under microaerophilic conditions.** Catalase positive. Nonhalophilic. Mesophilic. **The peptidoglycan type is type L-Lys–L-Ser$_{1-2}$–D-Glu or L-Lys–L-Ser$_2$(L-Ala)–D-Glu, variation A4α.** Muramic acid is acetylated. Mycolic acids and teichoic acids are absent. Galactosamine is the major constituent of cell wall polysaccharide. **The menaquinones are hydrogenated; the predominant menaquinone is MK-8(H$_2$).** The cytochromes are cytochromes aa_3, c_{549}, c_{555}, b_{559}, b_{564}, and d_{626} (one type strain analyzed).

The polar lipids include diphosphatidylglycerol, phosphatidylglycerol, and phosphatidylinositol. **The major fatty acids are $C_{15:0}$ iso, $C_{16:0}$ iso, $C_{17:0}$ iso, and $C_{17:0}$ ante.** If present, the long-chain aliphatic hydrocarbons are C22 and C23 hydrocarbons, as well as minor amounts of C25, C26, and C27 hydrocarbons.

DNA G+C content (mol%): 65.2–71.1.

Type species: **Dermacoccus nishinomiyaensis** (Oda 1935) Stackebrandt, Koch, Gvozdiak and Schumann 1995, 689[VP] emend. Kocur, Schleifer and Kloos 1975, 291 (*Micrococcus nishinomiyaensis* Oda 1935, 1202).

Further descriptive information

Originally described by Oda (1935) on the basis of a single strain, the species *Micrococcus nishinomiyaensis* was emended by Kocur et al. (1975) on the basis of the characterization of 31 strains isolated from human skin (Kloos et al., 1974). Subsequent chemotaxonomic (Faller et al., 1980) and 16S rRNA gene sequence analysis (Koch et al., 1994) data have shown the taxonomic separateness of *Micrococcus nishinomiyaensis* from authentic *Micrococcus* species, leading to its reclassification as *Dermacoccus nishinomiyaensis* (Stackebrandt et al., 1995). This genus, together with *Kytococcus* (Stackebrandt et al., 1995) and *Demetria* (Groth et al., 1997a) were enclosed in the family *Dermacoccaceae* on the basis of phylogenetic relatedness and the presence of a unique set of nucleotides of the 16S rRNA gene sequences (Stackebrandt and Schumann, 2000).

The strains affiliated to the species differ in chemotaxonomic and physiological properties which, however, may be within the range of intra-species diversity (Kocur et al., 1975). Only two of the 31 strains investigated show a profile identical to that of the type strain Oda no. 59^T in the 24 properties tested. None of the strains produce acid from glucose anaerobically, but about one third of the strains are able to produce acid aerobically. The interpeptide bridge of peptidoglycan shows some variations. While the majority of strains show an L-Ser–L-Ser_2–D-Glu composition, two strains have an L-Ser–D-Glu variation and, in five strains, a part of the second serine residue is replaced by L-alanine [L-Ser–L-Ser (L-Ala)–D-Glu]. With respect to the fatty acid composition of *Dermacoccus nishinomiyaensis*, the literature indicates conflicting results: while Stackebrandt et al. (1995) indicates the presence of a significant amount of unsaturated branched-chain acids, notably $C_{17:0}$ iso (11–33% of total), this component is neither detected in *Dermacoccus nishinomiyaensis* nor in the type strains of *Dermacoccus abyssi* (Pathom-aree et al., 2006a) *Dermacoccus barathi*, and *Dermacoccus profundi* (Pathom-aree et al., 2006b). These authors discuss the possibility that growth conditions may influence the composition of fatty acids in dermacocci and related organisms.

While most strains have been isolated from the skin of humans, one report indicates the presence of *Dermacoccus* species (5.5% of 369 strains isolated) in salt used in the production of Spanish dry-cured ham (Cordero and Zumalacarregui, 2000). As these findings conflict with the lack of growth of *Dermacoccus nishinomiyaensis* strains in 7.5% NaCl (Kocur et al., 1975), it can be assumed that either the Spanish isolates belong to a new taxon or these organisms are transient but not actively growing on the surface of salted ham.

The production of monensin has been reported for three *Dermacoccus nishinomiyaensis* strains (Pospisil et al., 1998).

Phylogenetic analysis indicates a very high similarity between the type strains of *Dermacoccus profundi* MT2.2^T and *Dermacoccus barathri* MT2.1^T which share 99.9% 16S rRNA gene similarity and differ in a single nucleotide only. Almost as highly related is *Dermacoccus abyssi* MT1-1^T (99.8%), while *Dermacoccus nishinomiyaensis* DSM 20448^T is slightly more distantly related (98.5–98.6%) (Pathom-aree et al., 2006a, 2006b). Despite the high gene sequence similarities, all strains are genomically well separated, as DNA–DNA reassociation experiments among the four type strains of the genus (Pathom-aree et al., 2006a, 2006b), using the microplate method of Ezaki et al. (1989), shows less than 25% DNA–DNA reassociation similarities.

Dermacoccus abysii MT1-1^T grows well at 40 Mpa and can be considered a piezotolerant actinobacterium. At this pressure, strains of *Dermacoccus nishinomiyaensis* DSM 20448^T show a decrease in cell numbers (Pathom-aree et al., 2006a); information on the other two type strains is not available (Pathom-aree et al., 2006b).

Enrichment and isolation procedures

Samples from human skin were obtained by sterile cotton swabs moistened with a sterile detergent containing 0.1% Triton X-100 (Packard) in 0.075 M phosphate buffer, pH 7.9. Each swab was used to inoculate the surface of an agar plate (10 cm diameter) which was incubated at 34°C for 4 d (Kloos et al., 1974). The isolation medium was non-selective, containing gram per liter: peptone (Difco), 10; yeast extract (Difco), 5; sodium chloride, 5; glucose, 1; agar (Difco), 15.

Strains of the other three species were isolated from a sediment sample from the Mariana Trench (Challenger deep) at a depth of 10,898 m using sterilized mud samplers and the remotely operated submersible *Kaiko* (Kato et al., 1997). The sample (2 ml) was transported to the UK in an insulated container at 4°C and then stored at −20°C. The strains were isolated from a suspension of the sediment sample used to inoculate a raffinose-histidine agar plate (Vickers et al., 1984) supplemented with cycloheximide and nystatin. The isolates were maintained on glucose-yeast extract agar plates (Gordon and Mihm, 1962) at room temperature and as glycerol suspensions (20%, v/v) at −20°C.

Maintenance procedures

Serial transfers on nutrient agar at 4-week intervals followed by maintenance at 4°C are recommended. Storage of cells as 20% (w/v) glycerol suspensions at −20°C and at −80°C is a suitable preservation method. Long-term preservation methods include freeze-drying in skim milk and maintenance in liquid nitrogen at −196°C.

Further reading

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Schleifer, K.H., W.E. Kloos and M. Kocur. 1981. The genus *Micrococcus*. *In* The Prokaryotes: a Handbook on Habitats, Isolation, and Identification of Bacteria (edited by Starr, Stolp, Trüper, Balows and Schlegel). Springer, New York, pp. 1539–1547.

Differentiation of the genus *Dermacoccus* from other genera

By comparative 16S rRNA gene sequence analysis, the genus *Dermacoccus* is about equidistantly related to the genera of the family *Dermacoccaceae*, i.e. *Kytococcus* and *Demetria*. Including the sequences of the type species of all genera of *Micrococcineae* (now *Micrococcales*) into a neighbor-joining analysis (not shown), *Serinicoccus* appears to be the nearest genus outside the family. This contrasts the study by Yi et al. (2004) in which the type species *Serinicoccus marinus* branched adjacent to *Ornithinomicrobium*. As the same publication, however, also finds *Kytococcus sedentarius* to branch outside the family *Dermacoccaceae*, the inclusion of only a small number of reference sequences may cause an erroneous branching pattern. Depending on the selection of reference organisms and treeing algorithm used, the topology of phylogenetic trees may differ significantly. None of the deeper branchings are of statistical significance, and in no case will a species from a neighboring genus be so close to *Dermacoccus* species that it may be regarded a member of *Dermacoccus*. As seen in Table 119 of the description of *Dermacoccaceae*, *Dermacoccus*, *Kytococcus*, and *Demetria* contain L-lysine as diagnostic amino acid, while L-ornithine is present in *Ornithinococcus*, *Serinicoccus*, and *Ornithinomicrobium*. The latter two genera and *Demetria* possess MK-8(H_4) as major menaquinone; *Dermacoccus* possesses MK-8(H_2), while *Kytococcus* has fully saturated menaquinones. Sufficient differences occur in the combination of phylogenetic position, morphological traits, and chemotaxonomic markers to unambiguously affiliate new isolates to the genus *Dermacoccus*.

TABLE 119. Phenotypic properties differentiating the type strains of *Dermacoccus* species[a,b]

Characteristic	*D. nishinomiyaensis* DSM 20448^T	*D. abyssi* DSM 17573^T	*D. barathri* MT2.1^T	*D. profundi* MT2.2^T
H_2S production	−	−	−	+
Urea hydrolysis	−	−	+	+
Degradation of:				
Arbutin	−	+	−	−
DNA	+	+	+	−
Gelatin	+	−	−	−
Starch	+	+	−	−
Tween 80	−	+	−	−
API ZYM:				
α-Fucosidase	+	+	+	−
β-Glucosidase	+	+	+	−
Lipase (C14)	+	+	+	−
Trypsin	+	−	+	+
Growth at/on:				
10°C	−	+	+	+
10% NaCl	−	+	+	+
12.5% NaCl	−	−	−	+

[a]Symbols: +, >85% positive; −, 0–15% positive.

[b]Data from Pathom-aree et al. (2006a, 2006b).

List of species of the genus *Dermacoccus*

1. **Dermacoccus nishinomiyaensis** (Oda 1935) Stackebrandt, Koch, Gvozdiak and Schumann 1995, 689^{VP} emend. Kocur, Schleifer and Kloos 1975, 291 (*Micrococcus nishinomiyaensis* Oda 1935, 1202)

ni.shi.no.mi.ya.en′sis. N.L. masc. adj. *nishinomiyaensis* pertaining to Nishinomiya, a city in Japan.

In addition to the properties given in the genus description, this species has the following properties. Percentages in parentheses indicate the percent of the 32 strains studied which are positive for the character cited (Kocur et al., 1975). Cells are spherical (diameter, 0.9–1.6 mm) and occur in pairs, tetrads, or irregular clusters of tetrads. Colonies are up to 2 mm in diameter, circular, entire, slightly convex, smooth with glistening (rarely matt) surfaces, and bright orange. Colony morphology and color become more distinct with age; cell morphology is not culture age or medium dependent. Some strains produce a water-soluble orange exopigment. Slight turbidity and a deposit occur during growth in nutrient broth.

The final pH in glucose broth is 5.4–6.9. Growth is weak or absent on inorganic nitrogen agar and growth does not occur on Simmons' citrate agar (85%); benzidine test is positive. Negative for free or bound coagulase, phosphatase, lecithinase, arginine dihydrolase, ornithine and lysine decarboxylases, phenylalanine deaminase, β-galactosidase, and DNase. Most strains produce urease (80%). Gelatin is hydrolyzed (100%), but esculin is not hydrolyzed. Hydrolysis of starch (35%) and Tween 80 is variable (60%). Acetoin, indole, and hydrogen sulfide are not produced. Does not utilize sucrose, lactose, galactose, rhamnose, xylose, glycerol, and mannitol. Most strains do not utilize fructose and mannose. Utilization of glucose and galactose is variable. Reduction of nitrate to nitrite is variable (67%); nitrite reduction negative. Methyl red test is negative. Cysteine or methionine and niacin are required. Growth is stimulated by tryptophan, valine, aspartic acid, glutamic acid, proline, and lysine. Susceptible to novobiocin and neomycin. Most strains are susceptible to erythromycin, penicillin, streptomycin, and chloramphenicol; resistant to furazolidone, resistant or slightly resistant to lysozyme, and weakly resistant to methicillin. Grows well in the presence of up to 5% NaCl; no growth occurs in the presence of 7% NaCl. Grows well at 25–37°C. Saprophytic. Nonhemolytic.

Source: type strain isolated from water using for brewing sake. Other strains isolated from skin of humans and other sources.

DNA G+C content (mol%): 67.8 (T_m).

Type strain: Oda no. 59, ATCC 29093, CCM 2140, CCUG 33028, CIP 81.71, DSM 20448, IEGM 393, NBRC 15356, JCM 11613, LMG 14222, NCTC 11039, VKM B-1818.

Sequence accession no. (16S rRNA gene): X87757.

2. **Dermacoccus abyssi** Pathom-aree, Nogi, Sutcliffe, Ward, Horikoshi, Bull and Goodfellow 2006a, 1235[VP]

a.bys′si. L. gen. n. *abyssi* of an abyss.

Non-acid–alcohol-fast, nonmotile actinomycete. Forms coccoid cells (diameter 0.8–1.5 μm) that occur in irregular clusters. Cream to pale yellow, circular, entire, convex, smooth, glistening colonies are formed on glucose-yeast extract agar after 5 d at 28°C. Grows well on tryptic soy agar but poorly on inorganic nitrogen agar. Growth occurs between 10 and 37°C, with optimum growth around 28°C. In addition to the properties given in the genus description, this species has the following properties. Casein, cellulose, hypoxanthine, starch, and uric acid are degraded. Growth occurs in the presence of 7.5% NaCl. Piezotolerant grows well in glucose-yeast extract broth at 40 MPa. Additional phenotypic properties are shown in Table 119.

Source: sediment collected from the Challenger Deep of the Mariana Trench at a depth of 10,898 m.

DNA G+C content (mol%): 65.2 (HPLC).

Type strain: MT1.1, DSM 17573, JCM 14339, NCIMB 14084.

Sequence accession no. (16S rRNA gene): AY894323.

3. **Dermacoccus barathri** Pathom-aree, Nogi, Ward, Horikoshi, Bull and Goodfellow 2006b, 2306[VP]

ba.ra′thri. L. neut. n. *barathrum* a deep pit, an abyss; L. gen. n. *barathri* of an abyss.

Aerobic, Gram-stain-positive, non-acid–alcohol-fast, nonmotile actinomycete that forms coccoid cells (diameter 0.8–1.5 μm) that occur in irregular clusters. Light yellow, circular, entire, convex, smooth, glistening colonies are formed on glucose-yeast extract agar after 5 d at 28°C. Grows well on tryptic soy agar, but poorly on inorganic nitrogen agar. Growth occurs between 10 and 37°C, with an optimum around 28°C. In addition to the properties given in the genus description, hypoxanthine, Tween 20, Tween 40, and uric acid are degraded. Acid is not formed from (+)-D-arabinose, (+)-D-arabitol, (−)-L-arabitol, (+)-D-cellobiose, dextran, *meso*-erythritol, (+)-D-fructose, (+)-D-galactose, (+)-D-glucose, glycerol, glycogen, *myo*-inositol, inulin, (+)-D-maltose, (+)-D-mannitol, (+)-D-mannose, (+)-D-melezitose, (+)-D-melibiose, (+)-D-raffinose, (−)-L-rhamnose, (+)-D-salicin, (+)-D-sucrose, (+)-D-trehalose, (+)-D-sorbitol, (+)-L-sorbose, xylitol, or (+)-D-xylose. Additional phenotypic properties are shown in Table 119.

Source: sediment collected from the Challenger Deep of the Mariana Trench at a depth of 10,898 m.

DNA G+C content (mol%): 66.8 (HPLC).

Type strain: MT2.1, DSM 17574, JCM 14588, NCIMB 14081.

Sequence accession no. (16S rRNA gene): AY894328.

4. **Dermacoccus profundi** Pathom-aree, Nogi, Ward, Horikoshi, Bull and Goodfellow 2006b, 2306[VP]

pro.fun′di. L. neut. n. *profundum* depth, abyss; L. gen. n. *profundi* of an abyss.

Aerobic, Gram-stain-positive, non-acid–alcohol-fast, nonmotile actinomycete that forms coccoid cells (diameter 0.8–1.5 μm) that occur in irregular clusters. Brilliant yellow, irregular, entire, pulvinate, rough colonies with matted surfaces are formed on glucose-yeast extract agar after 5 days at 28°C. Grows well on tryptic soy agar but poorly on inorganic nitrogen agar. Growth occurs between 10 and 37°C, with an optimum around 28°C. In addition to the properties given in the genus description, hypoxanthine, Tween 20, Tween 40, and uric acid are degraded. Acid is not formed from (+)-D-arabinose, (+)-D-arabitol, (−)-L-arabitol, (+)-D-cellobiose, dextran, *meso*-erythritol, (+)-D-fructose, (+)-D-galactose, (+)-D-glucose, glycerol, glycogen, *myo*-inositol, inulin, (+)-D-maltose, (+)-D-mannitol, (+)-D-mannose, (+)-D-melezitose, (+)-D-melibiose, (+)-D-raffinose, (−)-L-rhamnose, (+)-D-salicin, (+)-D-sucrose, (+)-D-trehalose, (+)-D-sorbitol, (+)-L-sorbose, xylitol, or (+)-D-xylose. Additional phenotypic properties are shown in Table 119.

Source: sediment collected from the Challenger Deep of the Mariana Trench at a depth of 10,898 m.

DNA G+C content (mol%): 69.1 (HPLC).

Type strain: MT2.2, DSM 17575, JCM 14589, NCIMB 14126.

Sequence accession no. (16S rRNA gene): AY894329.

Genus II. **Demetria** Groth, Schumann, Rainey, Martin, Schuetze and Augsten 1997a, 1132[VP]

Ingrid Groth and Peter Kämpfer

De.me′tri.a. Gr. n. *Demeter* Greek god of agriculture and wives; L. fem. suff. *-ia* suff. denoting belonging to; N.L. fem. *Demetria* a bacterium being responsible for fertility.

Cells are irregularly **coccoid to rod-shaped**, 0.8–1.2 × 0.8–3.0 μm. They occur singly, in pairs, short chains, or in small irregular clusters. Gram-stain-positive. Not acid fast. Nonsporeforming. **Nonmotile. Aerobic to microaerobic**. Colonies are circular, smooth, convex, white to pale yellow, and 1–3 mm in diameter. Optimum temperature for growth is 28°C; no growth occurs at 37°C. **Oxidase negative, catalase positive**. The **peptidoglycan type is A4α** with L-lysine as the characteristic diamino acid. The **acyl type is acetyl**. **Galactose** is the only whole-cell sugar. The only **respiratory quinone** is **MK-8(H_4).** The **polar lipids** are composed of **phosphaditylinositol, phophaditylglycerol, diphophaditylglycerol, phosphatidylethanolamine, and two unknown phospholipids**. The cellular fatty acid profile is complex with large amounts of saturated and monounsaturated straight-chain acids and smaller amounts of iso- and anteiso-branched-chain acids. Mycolic acids are absent. Based on 16S rRNA gene sequence comparison, the genus *Demetria* is most closely related to members of the genera *Dermacoccus* and *Kytococcus*.

DNA G+C content (mol%): 66.

Type species: **Demetria terragena** Groth, Schumann, Rainey, Martin, Schuetze and Augsten 1997a, 1132[VP].

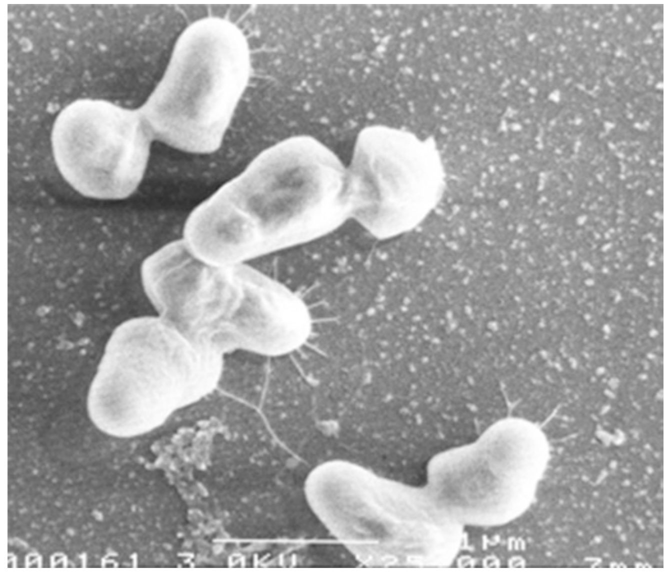

FIGURE 156. Scanning electron micrograph of cells from a 48-h-old culture of *Demetria terragena* DSM 12295[T] grown at 28°C on R agar. Bar = 1 μm.

Further descriptive information

At present the genus is represented by the type species *Demetria terragena* and accommodates a single strain. 16S rRNA gene sequence comparison shows that this strain forms a distinct lineage within the order *Micrococcales* (formerly suborder *Micrococcineae* of Stackebrandt et al., 1997) adjacent to those of members of the genera *Dermacoccus* (Stackebrandt et al., 1995) and *Kytococcus* (Stackebrandt et al., 1995). The 16S rRNA gene sequence similarities between *Demetria* and the species of the most closely related genus *Dermacoccus* are 96.6–96.7%, and those to the slightly less closely related species of the genus *Kytococcus* 95.4–95.8%. The genera of the family *Dermabacteraceae*, *Dermabacter* and *Brachybacterium* share with *Demetria* 16S rRNA gene sequence similarities of 94.3% and 93.9–95.1%, respectively.

Cells of *Demetria terragena* are typically characterized by filopodia at their bottom sides (Figure 156) which are different from the spiky structures reported for *Bogoriella caseilytica* (Groth et al., 1997b). The peptidoglycan of *Demetria* contains Lys, Ala, Asp, Glu, and Ser in a molar ratio of 0.8:1.8:1.2:1.0:0.8. The interpeptide bridge consists of L-Lys–L-Ser–D-Asp which is in accordance with variation A11.36 of the peptidoglycan type A4α (DSMZ Catalogue of Strains, 1998, or http://www.dsmz.de/species/murein.htm). The cellular fatty acid profile is characterized by the predominance of $C_{18:1}$, $C_{18:0}$, $C_{17:0}$ and smaller amounts of $C_{17:0}$ anteiso, $C_{16:0}$, $C_{16:0}$ iso, and $C_{17:0}$ iso. *Demetria terragena* grows well on complex organic media containing peptone, yeast extract, and meat extract as nitrogen sources and utilizes a broad spectrum of different carbon sources.

Enrichment and isolation procedures

Demetria terragena grows readily in complex liquid or on solidified media, i.e. Bacto nutrient agar* (Difco), rich (R) medium† (Yamada and Komagata, 1972), Bacto tryptic soy broth‡ (Difco), and peptone-yeast extract brain heart infusion medium§ (PY-BHI, Yokota et al., 1993). *Demetria terragena* was isolated from a sample of frozen compost soil on nutrient agar** using a standard dilution plate procedure.

Maintenance procedures

Cultures of *Demetria terragena* can be maintained by serial transfers on complex agar media. Growth on agar slants in screw-capped tubes can be kept for about 2–4 weeks at 4°C. Long-term preservation of liquid cultures supplemented with 5% dimethylsulfoxide is recommended in the vapor phase of liquid nitrogen (−140°C).

*Bacto nutrient agar consists of (g/l): Bacto beef extract, 3.0; Bacto peptone, 5.0, and Bacto agar, 15.0 (all from Difco); pH 6.8.

†Rich (R) medium consists of (g/l): Bacto peptone (Difco), 10.0; yeast extract (Difco), 5.0; Casamino acids (Difco), 5.0; beef extract (Difco), 2.0; malt extract (Difco), 5.0; glycerol, 2.0; $MgSO_4 \cdot 7H_2O$, 1.0; Tween 80, 0.05, and agar, 20.0; pH 7.2.

‡Bacto tryptic soy broth consists of (g/l): Bacto tryptone, 17.0; Bacto soytone, 3.0; glucose, 2.5; NaCl, 5.0, and K_2HPO_4, 2.5 (all from Difco); pH 7.3.

§Peptone-yeast extract-brain heart infusion medium consists of (g/l): peptone, 10.0; yeast extract, 2.0; Bacto brain heart infusion (Difco), 2.0; NaCl, 2.0, and glucose, 2.0; pH 7.0.

**Nutrient agar consists of (g/l): peptone, 20.0; NaCl, 5.0, and agar, 12.0; pH 7.2.

TABLE 120. Morphological and chemotaxonomic characteristics that differentiate the genus *Demetria* from the closest relatives[a]

Characteristic	*Demetria*[b]	*Dermacoccus*[c]	*Kytococcus*[d]
Morphology	Coccoid, short rods	Coccoid	Coccoid
Peptidoglycan type	L-Lys–L-Ser–D-Asp	L-Lys–L-Ser_{1-2}–D-Glu or L-Lys–L-Ser_{1-2}–L-Ala–D-Glu	L-Lys–Glu_2
Major menaquinone(s)	MK-8(H_4)	MK-8(H_2)	MK-7, MK-8, MK-9, MK-10
Predominant cellular fatty acids	$C_{18:1}$, $C_{18:0}$, $C_{17:0}$, $C_{17:0}$ anteiso	$C_{15:0}$ iso, $C_{16:0}$ iso $C_{17:0}$ iso, $C_{17:0}$ anteiso, $C_{17:1}$ iso	$C_{15:0}$ iso, $C_{17:0}$ iso, $C_{17:0}$ anteiso, $C_{17:1}$ ω9*c* iso, $C_{15:0}$, $C_{17:0}$, $C_{17:1}$ ω8*c*
Polar lipids	PI, PE, PG, DPG, PL	PI, PG, DPG	PI, PG, DPG, PSer, PIM
DNA G+C content (mol%)	66	66–71	68–69

[a]Abbreviations: DPG, diphosphatidylglycerol; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; PI, phosphatidylinositol; PIM, phosphatidylinositol mannoside, PL, unidentified phospholipid; PSer, phosphatidylserine.

[b]Data taken from Groth et al. (1997a).

[c]Data taken from Kocur (1986), Stackebrandt et al. (1995), and Pathom-aree et al.(2006a, 2006b).

[d]Data taken from Kocur (1986), Stackebrandt et al. (1995), Becker et al. (2002), and Kämpfer et al. (2009).

Differentiation of the genus *Demetria* from other genera

The distinct phylogenetic position of *Demetria terragena* within the cluster of the genera *Dermacoccus* and *Kytococcus* is shown in the 16S rRNA sequence based tree of the family *Dermabacteraceae* (Figure 155, above) Furthermore, *Demetria* can readily be distinguished from these two genera by the presence of the rare structure of the peptidoglycan (murein type A11.36) in combination with the major menaquinone MK-8(H_4), and by the morphological and chemotaxonomic characteristics listed in Table 120. It should be mentioned in this connection that a similar combination of peptidoglycan type A4α (Schleifer and Kandler, 1972) and the predominating menaquinone MK-8(H_4) has been found in *Beutenbergia cavernae* (Groth et al., 1999), in *Bogoriella caseilytica* (Groth et al., 1997b) and *Georgenia muralis* (Altenburger et al., 2002), which are members of the families *Beutenbergiaceae* and *Bogoriellaceae*, respectively (Hamada et al., 2009). However, these organisms can clearly be differentiated from *Demetria terragena* and from each other both by their separate lineages in the phylogenetic tree of the order *Micrococcales* and by a broad spectrum of phenotypic characteristics (Altenburger et al., 2002; Groth et al., 1997b, 1999; Stackebrandt and Schumann, 2000). On the basis of the chemotaxonomic properties *Demetria* can also readily be distinguished from the genera *Dermabacter* (Jones and Collins, 1988) and *Brachybacterium* (Collins et al., 1988). The monospecific genus *Dermabacter*, with the type species *Dermabacter hominis*, is characterized by a directly cross-linked peptidoglycan based on *meso*-diaminopimelic acid, type A1γ (Schleifer and Kandler, 1972), major menaquinones consisting of MK-9, together with MK-8 and MK-7 in substantial amounts, polar lipids consisting of PG, DPG, PLs and GL, and $C_{17:0}$ anteiso, $C_{15:0}$ anteiso, and $C_{16:0}$ iso as the predominant fatty acids (Jones and Collins, 1988). Strains of the genus *Brachybacterium* are characterized by the peptidoglycan type A4γ based on *meso*-diaminopimelic acid, a muramic acid that occurs mainly in the *N*-glycolated form, MK-7 as the principal menaquinone, polar lipids consisting of PG, DPG and GL, and a fatty acid profile which is dominated by $C_{15:0}$ anteiso and $C_{17:0}$ anteiso (Collins et al., 1988; Gvozdyak et al., 1992; Schubert et al., 1996; Takeuchi et al., 1995).

Taxonomic comments

The genus *Demetria* was established in 1997 for a single strain isolated from compost soil which could be clearly differentiated from all previously described actinobacteria on the basis of genotypic and phenotypic characteristics. At that time, the phylogenetic tree based on 16S rRNA gene sequences showed that *Demetria terragena* clustered together with the genera *Dermacoccus*, *Kytococcus*, and *Dermatophilus* (Van Saceghem, 1915). These three genera represented the family *Dermatophilaceae* at the time of the description of *Demetria* (Stackebrandt et al., 1997). The affiliation of *Demetria terragena* to the *Dermatophilaceae* was supported both by high 16S rRNA gene similarities to these genera and the presence of all signature nucleotides defined for this family (Stackebrandt et al., 1997). Later it became obvious from new 16S rRNA gene sequences added to the database of the actinomycetes that the type genus *Dermatophilus* clustered separately from the genera *Dermacoccus*, *Kytococcus*, and *Demetria*. Based on this finding, Stackebrandt and Schumann (2000) split the family *Dermatophilaceae* into the family *Dermatophilaceae* and the new family *Dermacoccaceae*. The family *Dermatophilaceae* comprised the sole genus *Dermatophilus* and the family *Dermacoccaceae* was represented by the genera *Dermacoccus*, *Kytococcus*, and *Demetria*. Recent phylogenetic studies revealed that *Kineosphaera* (Liu et al., 2002) is a member of the family *Dermatophilaceae*, and that *Dermacoccus*, *Kytococcus*, and *Demetria* should be included in the family *Dermabacteraceae*.

Acknowledgements

B. Schütze is acknowledged for critical reading of this article.

List of species of the genus *Demetria*

1. **Demetria terragena** Groth, Schumann, Rainey, Martin, Schuetze and Augsten 1997a, 1132[VP]

ter.ra.ge′na. L. n. *terra* soil; L. suff. *-genus*, *-a*, *-um* suffix denoting an origin; N.L. fem. adj. *terragena* coming from soil.

Description is the same as for the genus. In addition, the strain shows the following phenotypic characteristics: acids are produced from D-fructose, D-galactose, D-glucose, glycerol, D-mannitol, D-mannose, and D-ribose. No acid production from L-arabinose, D-cellobiose, dextrin, D-glucitol, inulin, lactose, maltose, D-raffinose, L-rhamnose, salicin, sucrose, potato starch, trehalose, and D-xylose. Acetate, aconitate, citrate, malate, and succinate are utilized as sole carbon sources; benzoate, formate, and DL-tartrate are not utilized. Nitrate is not reduced to nitrite. Hydrogen sulfide is produced; indole is not produced. Methyl red and Voges–Proskauer reactions are negative. Casein, potato starch, tyrosine, xanthine, and Tween 80 are decomposed; adenine, hypoxanthine, esculin, gelatin, hippurate, and urea are not decomposed. NaCl in combination with rich (R) medium is well tolerated up to 8%. 72h-old cultures display positive enzymic reactions in the API ZYM assay (bioMerieux) for alkaline phosphatase, chymotrypsin, esterase-lipase (C8), leucine arylamidase, acid phosphatase, naphthol-AS-BI-phosphohydrolase, and α-glucosidase. Weakly positive reactions are recorded for esterase (C4), lipase (C14), valine arylamidase, trypsin, and α-mannosidase. Negative results are obtained for cystine arylamidase, α-galactosidase, β-galactosidase, β-glucuronidase, β-glucosidase, *N*-acetyl-β-glucosamidase, and α-fucosidase. Cells are susceptible to the following antibiotics: ampicillin (10 μg/disc), chloramphenicol (30 μg/disc), ciprofloxacin (5 μg/disc), erythromycin (15 μg/disc), gentamicin (10 μg/disc), kanamycin (30 μg/disc), lincomycin (2 μg/disc), neomycin (30 μg/disc), nitrofuran (300 μg/disc), oxacillin (5 μg/disc), oxytetracycline (30 μg/disc), penicillin G (2 IU/disc), polymyxin B (300 IU/disc), rifampin (2 μg/disc), and streptomycin (10 μg/disc).

DNA G+C content (mol%): 66 (HPLC).

Type strain: 2002-046, HKI 0089, CIP 105501, DSM 11295, NBRC 16164, JCM 11480.

Sequence accession no. (16S rRNA gene): Y14152.

Genus III. **Kytococcus** Stackebrandt, Koch, Gvozdiak and Schumann 1995, 687[VP]

Erko Stackebrandt

Ky.to.coc′cus. Gr. neut. n. *kytos* skin (*sic*); Gr. masc. n. *kokkos* a grain; N.L. masc. n. *Kytococcus* a coccus from skin.

Cells are coccoid. Gram-stain-positive. Nonencapsulated. Nonsporeforming. Nonmotile. **Chemoorganotrophic; metabolism is strictly respiratory**. Aerobic. Catalase positive. Nonhalophilic. Mesophilic. **The peptidoglycan type is type L-Lys–Glu$_2$**, variation A4a. Mycolic acids and teichoic acids are absent. **Menaquinones with 7, 8, 9, and 10 completely unsaturated isoprene units (MK-7, MK-8, MK-9, and MK-10) predominate**. **The major cellular fatty acids are $C_{17:1}$ iso, $C_{17:0}$ anteiso, and $C_{17:0}$ iso**. In addition, significant amounts of $C_{15:0}$ iso, or $C_{17:0}$ and $C_{15:0}$ may be present. The cytochromes of the type species are cytochromes aa_3, c_{626}, c_{550}, b_{557}, b_{561}, and b_{564}. The type species contains the polar lipids diphosphatidylglycerol, phosphatidylglycerol, and phosphatidylinositol. The major aliphatic hydrocarbons of the type species are C_{30}–C_{33} hydrocarbons. Phylogenetically a member of the family *Dermacoccaceae*, order *Actinomycetales*.

DNA G+C content (mol%): 68–69.

Type species: **Kytococcus sedentarius** (ZoBell and Upham 1944) Stackebrandt, Koch, Gvozdiak and Schumann, 1995, 687[VP] (*Micrococcus sedentarius* ZoBell and Upham 1944; Kloos, Tornabene and Schleifer 1974, 84).

Further descriptive information

Micrococcus sedentarius was described by ZoBell and Upham (1944) based on a single strain designated 541 (ATCC 14392; CCM 314) that was isolated from a submerged slide in sea water. Isolation of micrococci from the skin of healthy people in the USA resulted in the characterization of several strains that matched the original description of *Micrococcus sedentarius*. Consequently, Kloos et al. (1974) validly published the name *Micrococcus sedentarius*, describing strain 541[T] (ATCC 14392[T]; CCM 314[T]) as the type strain. Of the properties investigated for the 20 strains including the type strain, some were variable. The type strain did not produce an exopigment and was weakly nitrate reduction positive (the majority of strains were negative), but was similar to the majority of strains in growth on 10% NaCl agar, oxidase negative reaction, and lack of acid formation from glucose and lactose.

Comparative analysis of the 16S rRNA gene sequences of *Micrococcus* species led to the dissection of the genus, leaving only *Micrococcus luteus* and *Micrococcus lylae* as authentic members of the genus (Stackebrandt et al., 1995). Though superficially resembling *Micrococcus* species, the phylogenetic distance of the other species was so large and the chemotaxonomic differences so profound that it was justified to reclassify *Micrococcus kristinae*, *Micrococcus varians*, and *Micrococcus roseus* as members of *Kocuria*, *Micrococcus nishinomiyaensis* as a member of *Dermacoccus*, and *Micrococcus sedentarius* as a member of *Kytococcus*, i.e. *Kytococcus sedentarius*.

While the strains investigated in the early study by Kloos et al. (1974) were obtained from apparently healthy people, there is a report on a possible involvement of a *Kytococcus sedentarius* strain in the pathogenesis of hemorrhagic pneumonia. This organism, identified by morphology and 16S rRNA gene sequences, was isolated from blood cultures and was recovered post-mortem from lung and spleen tissues (Levenga et al., 2004). Other cases

describe the presence of *Micrococcus sedentarius* in endocarditis (Old and McNeill, 1979) and in shunt infection (Leport et al., 1989). The association of *Kytococcus sedentatius* with "pitted keratolysis," a condition characterized by malodor and erosion of the soles of the feet, has been discussed by Nordstrom et al. (1987). Isolated strains from the foot were proteolytic and were able to degrade callus *in vitro* (Holland et al., 1992). Two keratin-degrading enzymes (serine proteases), capable of degrading human callus, have been identified (Longshaw et al., 2002). The pathogenic potential of members of *Kytococcus* has recently been strengthened by the description of *Kytococcus schroeteri* for strains isolated from patients with endocarditis (Becker et al., 2002; Becker et al., 2003) and bacteremic pneumonia (Mohammedi et al., 2005).

The type strains of *Kytococcus sedentarius* and *Kytococcus schroeteri* are 97.9% similar in their 16S rRNA genes sequences. Moderately high values are found when compared with several members of the family *Dermacoccaceae* (*Dermacoccus*: 94.3–96.6% and *Demetria*: 92.4–93.2%) and neighboring genera, e.g. members of *Dermatophilaceae* (*Dermatophilus* and *Kineosphaera*: 91.5–92.5%); *Serinicoccus*, 93.0%; *Ornithinomicrobium*, 93.7–94.0%; and *Intrasporangium*, 94.0–94.7%. The DNA–DNA reassociation values for the type strains of *Kytococcus* species is 45.5%.

Enrichment and isolation procedures

For the isolation of human and mammalian strains, the PY medium described by Kloos and Musselwhite (1975) is recommended: peptone (Difco), 10 g; yeast extract (Difco), 5 g; NaCl, 5 g; glucose, 1 g; agar (Difco), 15 g, distilled water, 1000 ml, pH 7.2–7.4. Cycloheximide (50 µg/ml) or 7% NaCl can be added to inhibit growth of fungi and spreading of bacilli, respectively. Strains of *Kytococcus schroeteri* were isolated by the Bactec 9249 system (Becton Dickinson).

Maintenance procedures

Kytococcus sedentarius is maintained on *Corynebacterium* agar (g/l: casein peptone tryptic digest, 10; yeast extract, 5; glucose, 5; NaCl, 5; agar, 15; pH 7.2–7.4) at 30°C, while *Kytococcus schroeteri* is maintained on Columbia agar (Becton Dickinson) at 37°C, supplemented with 5% sheep blood, or on nutrient broth (Oxoid). Medium-term maintenance is provided 4°C with serial transfers at 4-week intervals. Cells can be stored as 20% (w/v) glycerol suspensions at −20°C and at −80°C Long-term preservation methods include freeze-drying in skim milk and maintenance in liquid nitrogen at −196°C.

TABLE 121. Characteristics differentiating the species of the genus *Kytococcus*[a]

Characteristics	*K. sedentarius*	*K. schroeteri*
Pigmentation of colonies	Cream white/deep buttercup yellow	Muddy yellow
α-D-glucosidase	+	−
Hydrolysis of Tween 80	−	+
Menaquinone composition	MK-8, MK-9, MK-10	MK-7, MK-8, MK-9
Major fatty acids	$C_{17:0}$ anteiso, $C_{15:0}$, $C_{17:0}$, $C_{10:0}$ iso and $C_{17:1}$ iso	$C_{17:1}$ iso, $C_{17:0}$ iso, $C_{15:0}$ iso and $C_{17:0}$ anteiso

[a]Symbols: +, >85% positive; −, 0–15% positive.

Differentiation of the genus *Kytococcus* from other genera

The salient differentiating properties of members of *Dermacoccaceae* and phylogenetically related genera are indicated in the description of *Dermacoccaceae*. *Kytococcus* shares with *Dermacoccus* and *Demetria* an L-Lys residue at position 3 of the peptidoglycan side chain but differs from these organisms in the composition of the interpeptide bridge. A major difference is the presence of fully saturated menaquinones in *Kytococcus* species, while other members of the family possess MK-8(H_2 or H_4) compounds. *Kytococcus* and *Dermacoccus* species share similar polar lipids, but both genera differ in fatty acid composition among themselves and that of *Demetria* species. Genera that show a moderate relatedness with *Kytococcus* differ significantly in peptidoglycan type, menaquinone composition, and the other properties indicated in Table 121 of the family description.

Differentiation of the species of the genus *Kytococcus*

Characteristics that differentiate the two *Kytococcus* species are listed in Table 121 (from Becker et al., 2002).

List of species of the genus *Kytococcus*

1. **Kytococcus sedentarius** (ZoBell and Upham 1944) Stackebrandt, Koch, Gvozdiak and Schumann, 1995, 687[VP] (*Micrococcus sedentarius* ZoBell and Upham 1944; Kloos, Tornabene and Schleifer 1974, 84)

se.den.ta´ri.us. L. masc. adj. *sedentarius* of or belonging to sitting, sitting, sedentary.

In addition to the properties given above in the genus description, the following properties are characteristic for this species. Cells are spherical (diameter, 0.8–1.1 mm) and occur predominantly in tetrads or in tetrads in cubical packets. In smears, cells are often surrounded by a slimy Gram-stain-negative layer. Colonies are up to 3.5 mm in diameter, circular, entire, convex to pulvinate, and usually smooth and develop rather slowly. The colonies are cream white or deep buttercup yellow. Colony morphology and color become more distinct with age; cell morphology is not culture age or medium dependent. Some strains produce a brownish exopigment. Grows well in the presence of NaCl concentrations up to 10%. Growth does not occur on inorganic nitrogen agar and Simmons' citrate agar. Positive in the benzidine test. Most strains are negative in the oxidase test. Arginine dihydrolase positive. Negative for urease, lecithinase, β-galactosidase, and phosphatase. A few strains can reduce nitrate to nitrite; nitrite reduction does not occur. Hydrolyzes gelatin, but does not hydrolyze esculin, starch, and Tween 80. Acetoin is not produced. Metabolically inert for acid production from carbohydrates. No acid is produced from glucose, galactose, rhamnose, xylose, mannose, maltose, sucrose, ribose, raffinose, melibiose, arabinose,

galactose, ribitol, glucitol, galactitol, mannitol, and glycerol. Most strains do not utilize fructose and lactose. Methionine is required. Most strains require tyrosine, arginine, valine, lysine, leucine, and pantothenic acid. The optimum growth temperature range is 28–36°C. Nonhemolytic. Menaquinones are MK-8, MK-9 and MK-10. The major cellular fatty acids are $C_{17:0}$ anteiso, $C_{15:0}$, $C_{17:0}$, $C_{10:0}$ iso, and $C_{17:1}$ iso. Minor components are $C_{15:1}$, $C_{16:0}$, $C_{16:1}$, and $C_{15:0}$ iso. All strains are resistant to methicillin and penicillin G and susceptible to streptomycin, novobiocin, tetracycline, neomycin, vancomycin, and polymyxin B. Most strains are susceptible to erythromycin, kanamycin, and chloramphenicol.

DNA G+C content (mol%): 68–69 (T_m).

Type strain: 541, ATCC 14392, CCM 314, CCUG 33030, CIP 81.72, DSM 20547, NBRC 15357, JCM 11482, LMG 14228, NCTC 11040, VKM B-1316.

Sequence accession no. (16S rRNA gene): X87755.

2. **Kytococcus schroeteri** Becker, Schumann, Wüllenweber, Schulte, Weil, Stackebrandt, Peters and von Eiff 2002, 1613[VP]

schro.e′te.ri. NL. gen. masc. n. *schroeteri* of Schroeter, honoring Joseph Schroeter, a German microbiologist.

In addition to the properties given in the genus description, the species has the following properties. Cells are spherical (diameter 1.1–1.5 μm) and occur predominantly in pairs or tetrads. Colonies are up to 2–5 mm in diameter (after 48 h incubation), circular, entire, convex, and smooth and develop rather slowly. Colonies are muddy yellow. Grows well in the presence of NaCl concentrations up to 12%. No growth above 15% NaCl. Growth does not occur in Simmons' citrate agar. Oxidase negative. Positive for alkaline phosphatase, arginine dihydrolase, and pyrazinamidase. Negative for lecithinase, β-galactosidase, and urease. Nitrate is not reduced. Hydrolyzes gelatin and Tween 80, but does not hydrolyze esculin or starch. Acetoin is not produced. Metabolically inert for acid production from carbohydrates. No acid is produced from L-arabinose, D-galactose, D-glucose, maltose, D-mannose, D-melibiose, raffinose, L-rhamnose, D-ribose, sucrose, D-xylose, adonitol, glycerol, or D-mannitol. The optimum growth temperature is 37°C. Nonhemolytic. Resistant to penicillin G, oxacillin, and erythromycin, and susceptible to ampicillin, chloramphenicol, ciprofloxacin, gentamicin, tetracycline, vancomycin, and teicoplanin. Menaquinones with eight and seven completely unsaturated isoprene units (MK-8, MK-7) predominate. The major cellular fatty acids are $C_{17:1}$ iso, $C_{17:0}$ iso, $C_{15:0}$ iso, and $C_{17:0}$ anteiso. Minor components are $C_{16:0}$ iso, $C_{15:0}$ anteiso, and $C_{17.1}$ anteiso.

Source: blood from a patient with endocarditis.

DNA G+C content (mol%): not available.

Type strain: Muenster 2000, CCM 4918, DSM 13884, JCM 12136.

Sequence accession no. (16S rRNA gene): AJ297722.

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Family VIII. **Dermatophilaceae** (Austwick 1958) emend. Gordon 1964, 521[AL] emend. Stackebrandt, Rainey and Ward-Rainey 1997, 484 emend. Stackebrandt and Schumann 2000, 1283 emend. Zhi, Li and Stackebrandt 2009, 597

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Der.ma.to.phi.la.ce′a.e. N.L. masc. n. *Dermatophilus* type genus of the family; suff. *-aceae* ending to denote family; N.L. pl. fem. n. *Dermatophilaceae* the *Dermatophilus* family.

The family embraces two genera which differ from each other in morphology. In *Dermatophilus*, mycelia consist of narrow tapering filaments which divide transversely and in at least two longitudinal planes to form zoospores, forming germ tubes that elongate into filaments. In *Kineosphaera*, motile cocci grow singly, in pairs, or in packets. Aerial mycelium is absent, but in *Dermatophilus* sometimes inducible by 10% CO_2. Gram-stain-positive. Not acid-fast. Chemoorganotrophic; facultatively anaerobic or strictly aerobic. Peptidoglycan type is the directly linked *meso*-diaminopimelic acid (*meso*-A_2pm). Major menaquinone is MK-8(H_4). Polar lipids are diphosphatidylglycerol, phosphatidylglycerol, and phosphatidylinositol; in addition, *Kineosphaera* contains phosphatidylethanolamine and phosphatidylcholine. Major fatty acids of *Dermatophilus* strains are $C_{16:0}$, $C_{15:0}$, and $C_{14:0}$; $C_{16:0}$, $C_{17:1}$ $C_{18:1}$, and $C_{17:0}$ were reported for *Kineosphaera* species.

The family is a member of the order *Micrococcales*. The 16S rRNA gene signature nucleotides that define members of *Dermatophilaceae* are at positions 120 (A), 131:231 (Y–K), 196 (A), 342:347 (C–G), 444:490 (A–U), 580:761 (U–A), 602:636 (C–G), 670:736 (A–U), 822:878 (G–C), 823:877 (G–C), 826:874 (C–G), 827 (U), 843 (U), 950:1231 (U–A), 1047:1210 (G–C), 1109 (C), 1145 (G), 1309:1328 (G–C), 1361 (G), and 1383 (C) (Zhi et al., 2009).

Includes pathogenic organisms causing skin lesions in mammals including humans.

DNA G+C content (mol%): 57 ± 59 (T_m; *Dermatophilus*) – 71 ± 3 (HPLC; *Kineosphaera*).

Type genus: **Dermatophilus** Van Saceghem 1915, 357 emend. mut. char. Gordon 1964, 521.

Further descriptive information

Type strains of the two genera included in *Dermatophilaceae* show a moderate relatedness of 95% 16S rRNA gene sequence similarity. Members of *Dermatophilus* and *Kineosphaera* form clusters of cuboid cells or coccoid strains but differ in morphology and, except for the composition of peptidoglycan, in chemotaxonomic properties, including a broad range of DNA G+C contents. The rationale to include them into the same family is the phylogenetic position of *Kineosphaera limosa* Lpha5[T], branching adjacent to the radiation of the two type species of the genus *Dermatophilus* (Stackebrandt, 2003). Diagnostic morphological and chemotaxonomic characteristics are listed in Table 122.

Taxonomic comments

Dermatophilus congolensis was described (Van Saceghem, 1915) as the causative organism of an economically important acute, subacute, or chronic exudative and proliferative skin disease which is prevalent, but not exclusively found, in the tropics and subtropics, affecting more than 30 animal species and humans (Zaria, 1993). The disease is referred to as either streptotrichosis, pitted keratolysis, strawberry foot rot, lumpy wool, rain scald, or dermatophilosis, depending on the host infected and clinical signs. *Kineosphaera limosa* (Liu et al., 2002), isolated from a sequential batch reactor running under alternating anaerobic and aerobic conditions, has not been reported to be pathogenic.

The family *Dermatophilaceae* was first described to include the two actinomycete genera *Dermatophilus* and *Geodermatophilus* (Austwick, 1958). In the absence of phylogenetic and chemotaxonomic data, the classification was based on a single but very unique morphological feature, i.e. division in both transverse and longitudinal planes leading to the formation of packets or clusters of cuboid cells or cocci. The two species *Dermatophilus congolensis* and *Geodermatophilus obscurus* differ, however, in growth characteristics and developmental stages (Luedemann, 1968), chemical properties (Lechevalier, 1981), and phylogenetic position among the actinomycetes (Stackebrandt et al., 1983). While *Geodermatophilus obscurus* clusters with species of *Frankia* and *Blastococcus* (Ahrens and Moll, 1970) constituting an individual subline of descent (Hahn et al., 1989; Normand, 2006; Stackebrandt et al., 1983), *Dermatophilus congolensis* was found to group by itself within a broad taxon cluster that was later classified as the suborder *Micrococcineae* (Stackebrandt et al., 1997), and which has been elevated to order *Micrococcales*

TABLE 122. Morphological, metabolic, and chemotaxonomic characteristics of genera of the family *Dermatophilaceae*[a,b]

Characteristic	*Dermatophilus*	*Kineosphaera*
Morphology	Branching filaments which divide by transverse and longitudinal septa to form zoospores which form germ tubes, elongate into filaments	Gram-stain-positive motile cocci which grow in pairs and packets
Metabolism	Facultatively anaerobic; acid from glucose	Obligately aerobic; acid not produced
Polar lipids	PG, DPG, PI	PG, DPG, PI, PE, PC
Predominant cellular fatty acids	$C_{16:0}$, $C_{15:0}$, $C_{14:0}$	$C_{16:0}$, $C_{17:1}$, $C_{18:1}$, $C_{17:0}$
DNA G+C content (mol%)	57–59	71.3

[a]Abbreviations: A_2pm, diaminopimilic acid; $C_{14:0}$, tetradecanoic acid; $C_{15:0}$, pentadecanoic acid; $C_{16:0}$, hexadecanoic acid; $C_{16:0}$ iso, 14-methylpentadecanoic acid; $C_{17:0}$, heptadecanoic acid; $C_{17:0}$ iso, 15-methylhexadecanoic acid; $C_{17:0}$ anteiso, 14-methylhexadecanoic acid; $C_{17:1}$, heptadecenoic acid; $C_{17:1}$ iso, 15-methylhexadecenoic acid; $C_{18:0}$, octadecanoic acid; $C_{18:1}$, octadecenoic acid. PI, phosphatidylinositol; PG, phosphatidylglycerol; PC, phosphatidylcholine; DPG, diphosphatidylglycerol; PE, phosphatidylethanolamine; PL, unidentified phospholipid.

[b]Data from Liu et al. (2002) and Stackebrandt and Schumann (2000).

in the taxonomic roadmap to the present volume. The family *Dermatophilaceae* was emended twice, first on the basis of 16S rRNA gene signature nucleotides (Stackebrandt et al., 1997) and shortly after by a modified set of signature nucleotides (Stackebrandt and Schumann, 2000). The public database of gene sequences lists 16S rRNA gene sequences of two invalidly named taxa, i.e. "*Tonsillophilus suis*" (accession no. AB096084) (Murakami et al., 1991) and "*Dermatophilus crocodyli*" (AF226615), both branching adjacent to *Dermatophilus* type strains (Figure 157). The unresolved branching point of *Kineosphaera limosa* is discussed in the chapter on *Kineosphaera*, below. Irrespective of the treeing algorithm used [distance matrix of DeSoete (1983), neighbor-joining (Felsenstein, 1993), or maximum-likelihood (Olsen et al., 1994)] the nearest neighbors of *Dermatophilaceae* within the order *Micrococcales* are members of *Dermacoccaceae*, *Brevibacteraceae*, *Jonesiaceae*, and *Dermabacteraceae*, but neither the intra- nor the inter-family branching of the *Dermatophilaceae* lineages is supported by bootstrap values of >70%.

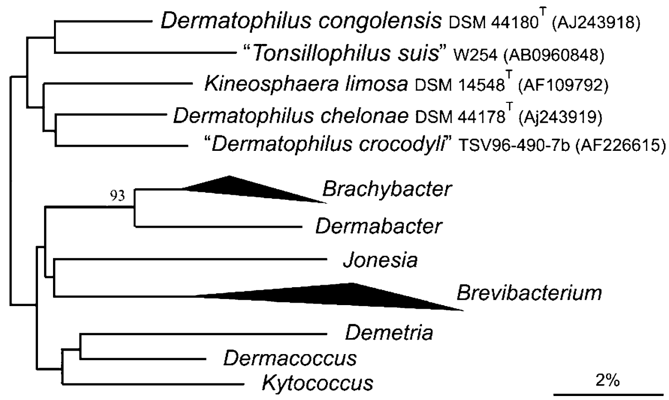

FIGURE 157. Maximum-likelihood tree of 16S rRNA gene sequences showing the nearest neighbors of members of *Dermatophilaceae*. The bootstrap value was calculated for 500 replicate trees. The bar corresponds to a 2% difference in nucleotide sequences.

Genus I. **Dermatophilus** Van Saceghem 1915, 357 emend. mut. char. Gordon 1964, 521[AL]

ERKO STACKEBRANDT

Der.ma.to.phi′lus. Gr. n. *derma -atos* skin; Gr. adj. *philos* loving; N.L. masc. n. *Dermatophilus* skin loving.

Aerial mycelium develops in atmospheres containing added CO_2. **Substrate mycelium consists of long tapering filaments, branching laterally at right angles; septa formed in transverse and in horizontal and vertical longitudinal planes giving rise to up to eight parallel rows of coccoid cells (spores), each of which becomes motile by a tuft of flagella.** Gram-stain-positive. Not acid-fast. Catalase-positive. **Aerobic and facultatively anaerobic**. Nonfermentative, but acid is produced from certain carbohydrates. Growth reported only on complex media; minimum nutritional requirements unknown. Temperature optimum ~37°C. Chondroitinase activity against chondroitin 4-sulfate positive. No hyaluronidase or elastase activity. As determined for the type species, **peptidoglycan contains *meso*-diaminopimelic acid** (*meso*-A_2pm); madurose is present in whole-cell hydrolysates; **polar lipids include phosphatidylglycerol, diphosphatidylglycerol, and phosphatidylinositol; major fatty acids are straight chain unsaturated $C_{16:0}$, $C_{15:0}$, and $C_{14:0}$.** Chemoorganotrophic. **Parasitic on mammals, especially domestic herbivores.** Pathology is usually limited to exudative dermatitis which may, however, be severe and life-threatening; on rare occasions causes subcutaneous abscesses and lymph node granulomas. Also found in skin lesions and abscesses in chelonids.

DNA G+C content (mol%): 57–59 (T_m).

Type species: **Dermatophilus congolensis** Van Saceghem 1915, 357 emend. mut. char. Gordon 1964, 521.

Further descriptive information

The natural habitat of the type species, *Dermatophilus congolensis*, is the diseased tissue of infected animals, where it is found in or on the integument where it causes a chronic or acute exudative dermatitis. Apparently, this bacterium does not grow saprophytically in nature. It has been isolated mainly from mammals, e.g. cattle, sheep, goats, and equines, and, less-commonly, from other sources including camels, giraffes, gazelles, rodents, monkeys, a polar bear, seals, pigs, and humans, but also from lizards (Gordon, 1976; Zaria, 1993). From a medical point of view, *Dermatophilus congolensis* is unique in that its natural growth cycle is restricted to the living layer of the epidermis. A few human isolates are known, most probably as a result of direct contact with infected animals (Kaplan, 1976). One strain, isolated from a wrist infection of an adult male, is on deposit as ATCC 14639. The second species, *Dermatophilus chelonae* has so far only been isolated from non-mammalian hosts, i.e. tortoise and turtles (Masters et al., 1995).

Attempts to find *Dermatophilus* species in soil have been unsuccessful and Masters et al. (1995) failed to isolate *Dermatophilus chelonae* from the surrounding environment of the infected cheloids, i.e. the Perth Zoo, Perth, Western Australia. As pointed out by Roberts (1967), this may be due to the inability of the organism to survive outside the host environment, especially under dry conditions.

Isolation and enrichment

Dermatophilus congolensis is readily isolated from cutaneous crusts or newly formed scabs by chopping or mincing material from the base of the scab in a small volume of sterile nutrient broth or distilled water and then plating on blood agar (5–10% defibrinated sheep blood in a blood agar base). As this material might be heavily contaminated with other bacteria, isolation requires a good streaking technique on beef infusion-horse blood agar plates which are incubated aerobically at 37°C. A modified procedure uses Haalstra's method (1965), growing the isolates on defibrinated 10% human blood agar plates containing 1.000 units of polymyxin B sulfate/ml of medium. To obtain a high yield of good-sized colonies, the plates are incubated at 37°C for about 2 d in an atmosphere of 10% CO_2 or in

a candle jar followed by a day in air. Isolation of *Dermatophilus congolensis* from old scabs is difficult because of reduced viability of aged cells or contamination of wetted scabs with other bacteria. Inoculation of the suspended scab material on the scarified skin of guinea pig or sheep and the use of the resulting scabs for unambiguous identification has been recommended by Roberts (1981). While the natural habitat of *Dermatophilus congolensis* is the diseased tissue of infected animals, it has been isolated mainly from cattle, sheep, goats, and equines, and, less- commonly, from other sources, including camels, giraffes, gazelles, rodents, monkeys, a polar bear, seals, pigs, humans, and even from the non-mammalian host sources, lizards and turtles. A compilation of recent literature is compiled by Stackebrandt (2006). A strain of *Dermatophilus chelonae* has recently been isolated from a king cobra (Wellehan et al., 2004).

Strains of *Dermatophilus chelonae* were isolated on 9% bovine blood agar (in Oxoid Columbia agar base no. 2; Masters et al., 1995). Growth is observed after 2–3 d of incubation at 37°C in the presence of 10% CO_2 or at 27°C in the ambient atmosphere. Growth also occurs in tryptose-phosphate medium supplemented with 10% ovine serum after 3 d at 37°C when the broth is inoculated with a dense suspension of zoospores.

Zoospores survive under unfavorable conditions and can resist drying. They can withstand heating at 100°C when dried, and they survive in dry scabs at temperatures of 28–31°C for up to 42 months.

Maintenance procedures

Good growth of *Dermatophilus* strains is achieved in brain heart infusion agar (Difco 0418) or trypticase soy broth agar supplemented with 5% blood. Well-grown cultures in Parafilm-sealed Erlenmeyer flasks may be kept at 4°C for several weeks and frozen at −20°C for several months. For lyophilization, good recovery rates are obtained when fresh materials from colonies on blood agar are suspended in nutrient broth. Vanbreuseghem et al. (1976) recommended maintaining a bacterial suspension in 2 ml of a mixture of equal volumes of ox serum, a 10% solution of sucrose in distilled water, and a 5% solution of neopeptone (Difco) in distilled water. Viable isolates have been maintained in this state for more than 10 years. Subcultures are made by mixing the lyophilized cells with a solution consisting of 2% glucose and 1% neopeptone in tap water. Freeze-dried cultures are stored at 8°C. Strains can also be maintained in N_2 vapor.

Differentiation of the genus *Dermatophilus* from other genera

Despite striking morphological similarities in certain stages of their life cycles, members of *Dermatophilus* and *Geodermatophilus* differ in their ecological niches, their chemotaxonomic features, colonial pigmentation, location and number of flagella, DNA base composition, mode of branching, and pathogenicity. Main chemotaxonomic differences between *Dermatophilus* and phylogenetically related and morphologically similar species are indicated in Table 123 of the description of *Dermatophilaceae.*

Differentiation of the species of the genus *Dermatophilus*

The salient differentiating features are listed in Table 123. In addition *Dermatophilus chelonae* differs from *Dermatophilus congolensis* in a number of cultural characteristics including faster growth at 27°C than at 37°C and production of a distinctive odor. The DNA restriction enzyme digestion and protein electrophoresis patterns are distinct (Masters et al., 1995). The electrophoretic mobilities of enzymes differ from the mobilities observed with *Dermatophilus congolensis* strains. Biochemical differences between *Dermatophilus chelonae* and *Dermatophilus congolensis* include the ability of the chelonid isolates to reduce nitrate to nitrite and the fact that the chelonid isolates exhibit collagenase activity *in vitro*. Several studies identify *Dermatophilus congolensis* strains by molecular methods including random amplified polymorphic DNA (RAPD) and oligonucleotide probing (Garcia-Sanchez et al., 2004a, 2004b; Larrasa et al., 2002) as well as restriction length polymorphism (Razafindraibe et al., 2006) and pulsed-field gel electrophoresis of PCR products (Larrasa et al., 2004).

The type strains of the two *Dermatophilus* species share a moderate 16S rRNA gene sequence similarity of 93.9%. As a higher value of 95.4% similarity has been determined for the type strain of *Kineophaera limicola* DSM 14548^T and *Dermatophilus chelonae* DSM 44178^T than for strain DSM 14548^T and *Dermatophilus congolensis* DSM 44180^T (93.7%), the genus *Kineosphaera* appear to be phylogenetically placed within the radiation of *Dermatophilus* species (Stackebrandt, 2003).

TABLE 123. Diagnostic properties of the species of the genus *Dermatophilus*[a,b]

Characteristic	*Dermatophilus congolensis*	*Dermatophilus chelonae*
Colony morphology	On blood agar colonies rough, often becoming viscous, white to gray at first, becoming orange to yellow. On brain-heart-infusion medium, strains have a gray-yellow tint, a wrinkled and shiny surface, and either a rough or smooth consistency, depending on the number of filaments and cocci. Convex colonies with crateriform center may occur.	On bovine blood agar colonies initially dry and adherent, gray-white. After transfer to room temperature, growth continues, colonies are adherent, white, raised, and umbonate or annilliform, becoming sticky after several days.
Motility of zoospores	High	Low
Hemolysis	Hemolytic on media containing sheep blood but not horse blood	Beta-hemolytic on media containing ovine blood; on horse blood nonhemolytic at 37°C, and weakly beta-hemolytic at 27°C. Forms two hemolysis zones around colonies on blood agar at 37°C in the presence of 10% CO_2.
Urease	+	v
Antibody $2F_4$ reaction	+	−

[a]Symbols: +, >85% positive; −, 0–15% positive; v, variable.

[b]Masters et al. (1995).

List of species of the genus *Dermatophilus*

1. **Dermatophilus congolensis** Van Saceghem 1915, 357 emend. mut. char. Gordon 1964, 521[AL]

con.go.len′sis. N.L. masc. adj. *congolensis* pertaining to the Congo (named for the Belgian Congo).

The description has been taken from Gordon (1989). Hyphae, 0.5–1.5 µm in diameter, develop from germ tubes. After several transverse and longitudinal divisions, hyphae may be up to 5 µm in diameter, with branches at right angles tapering to nonseptate apices. Become converted entirely into eight-ranked packets of isodiametric segments encased in a gelatinous sheath (Figure 158). Each segment is released as a motile spore bearing a tuft of five or more flagella. The spores subsequently lose motility and germinate. On blood agar, colonies are rough, often becoming viscous; they are adherent through invasion of substrate by hyphae; often white to gray at first, usually becoming orange to yellow; hemolytic on media containing sheep but not horse blood. Good growth on Loeffler's medium, light yellow; medium liquefied by most strains. No growth on Sabouraud glucose agar, Czapek agar, or tomato paste-oatmeal agar. Broth is clear with a flocculent or ropy sediment, sometimes with a surface ring of growth. Acid from glucose and fructose; transient acid (within 48 h) from galactose; often late production from maltose. Acid not produced from lactose, sucrose, xylose, dulcitol, mannitol, sorbitol, or salicin. Starch hydrolyzed. Gelatin and casein hydrolyzed. Tyrosine and xanthine not hydrolyzed. Indole not formed; methyl red and Voges–Proskauer tests negative. Nitrates not reduced. Aerobic, facultatively anaerobic. In an atmosphere of 10% carbon dioxide at 37°C, growth is accelerated and aerial hyphae are formed, while septation and spore formation are delayed. Susceptible to a wide range of antibacterial antibiotics including penicillin, streptomycin, chloramphenicol, erythromycin, and the tetracyclines. Resistant to the antifungal agents griseofulvin, nystatin, and tolnaftate.

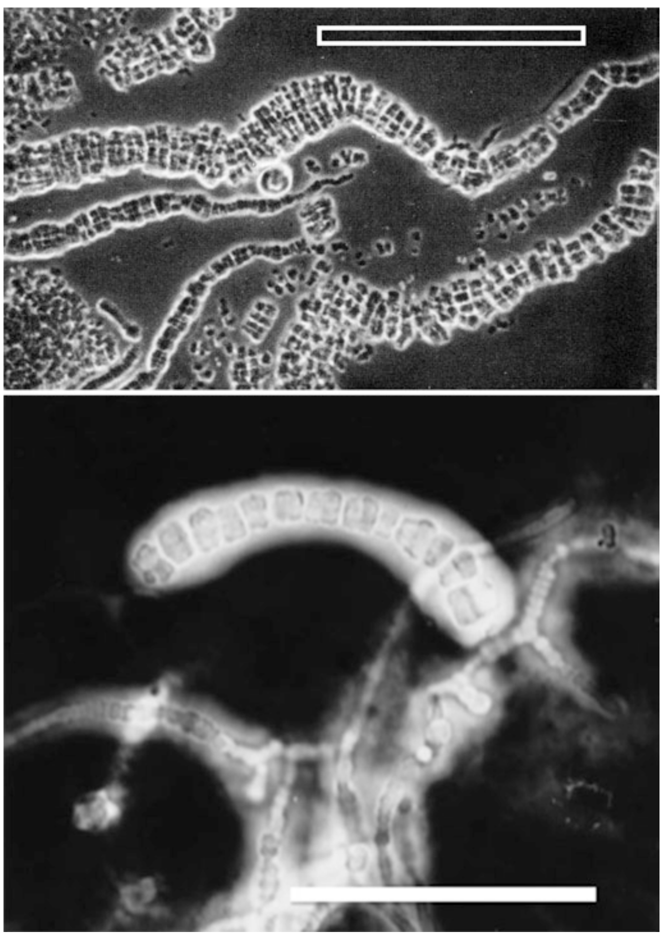

FIGURE 158. Morphology of *Dermatophilus congolensis.* (top) Wet mount of broth culture, showing fully segmented hyphae with cubical packets of coccoid spores. Scale bar = 20 µm. (Reprinted with permission from Gordon, 1964. J. Bacteriol. *88*: 509–522.) (bottom) Thick capsulate around mature filaments of mammalian strain W15. Scale bar = 30 µm. (Reprinted with permission from Masters et al., 1995. Int. J. Syst. Bacteriol. *45*: 50–56.)

Etiological agent of dermatophilosis, an exudative, often severe, dermatitis affecting large numbers of cattle, sheep, goats, and domesticated equines and causing important economic problems in many parts of the world. Occasional infections have been reported in many additional mammalian species, including humans.

DNA G+C content (mol%): not available.

Type strain: ATCC 14637, CCUG 47448, DSM 44180, JCM 8106, NCTC 13039, NRRL B-2350.

Sequence accession no. (16S rRNA gene): AJ243918.

2. **Dermatophilus chelonae** Masters, Ellis, Carson, Sutherland and Gregory 1995, 55[VP]

chel.lo′na.e. L. gen. n. *chelonae* of a turtle or tortoise, the source of the first isolates.

The description has been taken from Master et al. (1995). Coccoid zoospores that are 0.7 × 0.8 µm–1.2 × 1.6 µm. Produce germ tubes (diameter, ~1 µm) which elongate into filaments, eventually producing branching mycelia. Initially, septa form transversely, and there is a slight bulging of the filament on one side of the septum. Longitudinal septa are produced later, creating mature filaments with transverse and longitudinal divisions at regular intervals; the filaments are up to five cuboidal segments (~4 µm) in diameter and are surrounded by a thin capsule (thickness, 0.13–1.3 µm) (Figure 159). The segments separate, becoming zoospores with 0–6 flagella; aflagellate zoospores are the most common zoospores. All forms (zoospores, germ tubes, filaments, and segments) are Gram-stain-positive. Negative for fluorescent-antibody staining with monoclonal antibody 2F. Colonies on bovine blood agar after 3 d of growth are initially dry and adherent; the colonies are small, very slightly raised, and gray-white and have two hemolysis zones (beta and alpha) at 37°C; they are white, raised, and larger and have a beta-hemolysis zone at 27°C. After transfer to room temperature, growth continues, and colonies are adherent, white, raised, and umbonate or annelliform; the colonies become sticky after several days and caseous after prolonged storage. On ovine blood agar, colonies are beta-hemolytic; on equine blood agar, colonies are nonhemolytic at 37°C and weakly beta-hemolytic at 27°C. Good growth occurs on 1% tryptone-1.75% casein

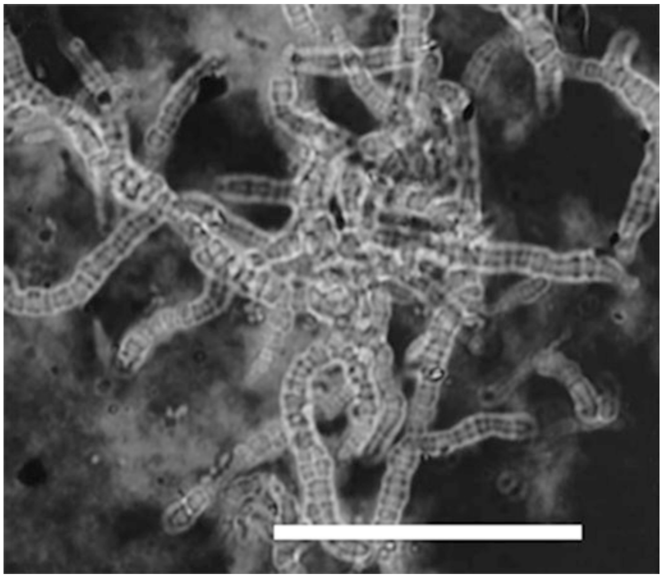

FIGURE 159. Morphology of *Dermatophilus chelonae.* Thin capsule (0.5 µm thick) around cells of strain W16[T]. Scale bar = 30 µm. (Reprinted with permission from Masters et al., 1995. Int. J. Syst. Bacteriol. *45*: 50–56.)

hydrolysate-0.5% NaCl agar, continuing for several weeks at room temperature. Cultures in tryptosephosphate-10% ovine serum broth produce a flocculent deposit and a clear supernatant, and no growth is suspended or occurs at the surface. Cultures produce a distinctive putrid odor. Acid is produced from glucose and is often produced from fructose and galactose (variable) as well as maltose. Acid is not produced from lactose, sucrose, xylose, dulcitol, mannitol, sorbitol, salicin, or trehalose. Gelatin and casein are hydrolyzed. Urease is not always produced. Weak reduction of nitrate to nitrite occurs. Indole is not formed; methyl red and Voges–Proskauer tests are negative. Chondroitinase activity occurs with chondroitin 4-sulfate but not with chondroitin 6-sulfate. Collagenase activity occurs with *in vitro*-assembled collagen.

Resistant to polymyxin B (1000 IU/ml), streptomycin (10 µg), and neomycin (30 µg). Susceptible to penicillin G (10 U), tetracycline (30 µg), chloramphenicol (30 µg), and sulfafurazole (100 µg).

Isolated from chelonids with skin lesions or abscesses at the Perth Zoo, in Perth, Western Australia. Low levels of infectivity on sheep, rabbits, and guinea pigs.

DNA G+C content (mol%): not available.

Type strain: W16, ATCC 51576, CCUG 47447, CIP 104541, DSM 44178, JCM 9706.

Sequence accession no. (16S rRNA gene): AJ243919.

Genus II. **Kineosphaera** Liu, Hanada, Marsh, Kamagata and Nakamura 2002, 1847[VP]

Erko Stackebrandt

Ki.ne.o.spha.e′ra. Gr. n. *kinesis* motion; L. fem. n. *sphaera* sphere, N.L. fem. n. *Kineosphaera* a motile sphere.

Cells are strictly aerobic, nonsporeforming, Gram-stain-positive, motile cocci (1–2 µm in diameter) which grow in pairs and packets. Catalase negative and oxidase positive. Cells do not use nitrite as an electron acceptor. The cell wall contains *meso*-A2pm as its characteristic diamino acid. The major menaquinone is MK-8(H4). The predominant cellular fatty acids are $C_{16:0}$; $C_{17:1}$, $C_{18:1}$, $C_{17:0}$, and $C_{16:1}$ occurs in smaller amounts. Phospholipids are phosphatidylglycerol, phosphatidylinositol, diphosphatidylglycerol, phosphatidylethanolamine, and phosphatidylcholine. The genus is a member of the family *Dermatophilaceae*, order *Micrococcales*, class *Actinobacteria*.

DNA G+Ccontent (mol%): 71.

Type species: **Kineosphaera limosa** Liu, Hanada, Marsh, Kamagata and Nakamura 2002, 1848[VP].

Further descriptive information

The spherical cells of strain Lpha5[T] have a rough surface (Figure 160A) and contain intracellular granules (Figure 160B) consisting of 3-hydroxybutyrate and 3-hydroxyvalerate as main components (Liu et al., 2002). Cells appear to divide by binary fission. The morphology resembles that of *Dermatophilus* strains grown in liquid growth media in which division is not confined so that cuboid packets of cells are formed.

Enrichment and isolation procedures

Kineosphaera limosa Lpha5[T] (DSM 14548[T]) and its closest relative, strain Lpha7 were isolated only once from an activated sludge reactor enriched with 2% phosphorous (Liu, 1995;

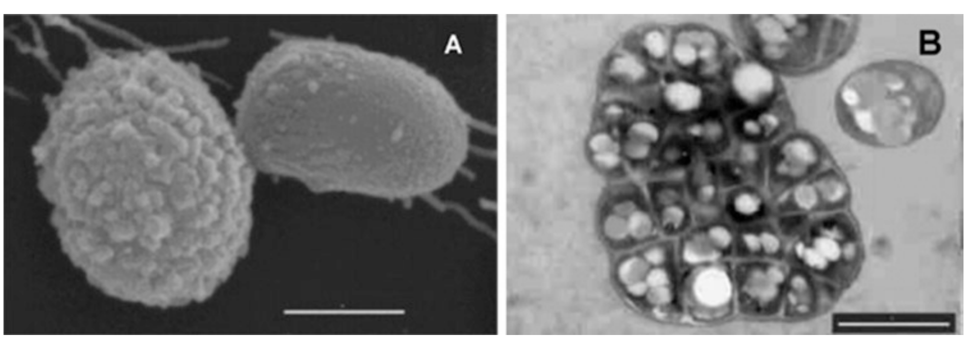

FIGURE 160. Morphology of *Kineosphaera limosa* strain Lpha5[T]. (A) Scanning electron micrograph. (B) Transmission electron micrograph, showing intracellular PHA granules. Bar = 1.0 µm. (Reprinted with permission from Liu et al., 2002. Int. J. Syst. Evol. Microbiol. *52*: 1845–1849.)

Liu et al., 2000, 2002). *Kineosphaera limosa* was isolated on medium GM1 (Liu et al., 1997) at 30°C at pH 7.0. On this medium strains require 1–2 weeks for colonies to appear. In liquid GM1 Medium, the doubling time is 1.7 d. Medium GM1 contains per liter at pH 7.0: glucose, 0.5 g; Na-acetate·$3H_2O$, 2 g; peptone, 0.5 g; yeast extract, 0.5 g; KH_2PO_4, 0.44 g; $MgSO_4$·$7H_2O$, 0.5 g; $(NH_4)_2SO_4$, 0.5 g; vitamin solution, 10 ml; sludge extract (autoclaved and filtered), 100 ml; and agar, 16 g.

Maintenance procedures

Kineosphaera limosa is maintained aerobically on DSM medium 776 (Nakamura et al., 1995) containing in grams per liter: glucose, 0.5 g; peptone, 0.5 g; yeast extract, 0.5 g; Na-glutamate, 0.5 g; KH_2PO_4, 0.5 g; $(NH_4)_2SO_4$, 0.1 g; $MgSO_4$·$7H_2O$, 0.1 g; and at pH 7.0, at 30°C. Cells also grow well on Bacto (Becton & Dickinson).

Differentiation of the genus *Kineosphaera* from other genera

Kineosphaera limosa Lpha5^T differs morphologically from dermatophili in the lack of formation of motile spores, a germ tube that extends at its growing tip to elongate into filaments, and a mycelium. Differences also occur in DNA base composition and in the composition of fatty acids and polar lipids (Table 122), while members of both genera are similar in peptidoglycan type and menaquinones; they share the same set of 16S rRNA gene signature nucleotides defined for members of the genus *Dermatophilus* (Stackebrandt, 2003; Stackebrandt and Schumann, 2000).

Taxonomic comments

The type strain of *Kineosphaera limosa* Lpha5^T was described for a coccoid organism from activated sludge that showed 94% 16S rRNA gene sequence similarity to *Dermatophilus congolensis* DSM44180^T. However, as only the 16S rRNA gene sequence of *Dermatophilus congolensis* ATCC 14637^T but not that of the second *Dermatophilus* species, *Dermatophilus chelonae* DSM 44178^T, was included in the phylogenetic analysis, the impression was given that the type strain Lpha5^T branched outside the radiation of the genus *Dermatophilus*. When sequences of the two type strains of *Dermatophilus*, *Kineosphaera limosa* Lpha5^T, and other *Dermatophilus* species were included in the phylogenetic analysis, it was obvious that *Kineosphaera limosa* branched within the radiation of the genus *Dermatophilus* (Stackebrandt, 2003).

The involvement of *Kineosphaera* species in the removal of phosphate is unknown. No information is available about pathogenicity of these organisms.

List of species of the genus *Kineosphaera*

1. **Kineosphaera limosa** Liu, Hanada, Marsh, Kamagata and Nakamura 2002, 1848^{VP}

li.mo′sa. L. fem. adj. *limosa* muddy, pertaining to sludge, the natural habitat of the species.

Colonies are light-yellow, irregular, and umbonate (2–3 µm in diameter) with undulating margins when the strain is grown on GM1 medium. Neisser-stain-negative and polyhydroxybutyrate-stain-positive. The optimum growth temperature is 30°C (temperature growth range 10–35°C), and the optimum growth pH is 7.0 (pH growth range is pH 6.0–10.2). Grows in the presence of up to 3.0% (w/v) NaCl. Utilizes dextrin, D-arabitol, fructose, α-D-glucose, maltose, 3-*O*-methylglucose, psicose, trehalose, turanose, xylitol, adenosine, Tween 40, Tween 80, *N*-acetyl-D-mannosamine, D-mannose, methyl-β-D-glucoside, palatinose, salicin, D-sorbitol, sucrose, D-trehalose, β-hydroxybutyric acid, methylpyruvate, propionic acid, succinamic acid, succinic acid, and glycerol. Cells accumulate PHA. The major cellular fatty acids are $C_{16:0}$, $C_{17:1}$, $C_{18:1}$, $C_{17:0}$, and $C_{16:1}$; $C_{15:0}$ and $C_{14:0}$ occur in smaller amounts.

Source: activated sludge.

DNA G+C content (mol%): 71.3 (HPLC).

Type strain: Lpha5, DSM 14548, JCM 11399, NBRC 100340.

Sequence accession no. (16S rRNA gene): AF109792.

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Family IX. **Intrasporangiaceae** Rainey, Ward-Rainey and Stackebrandt *in* Stackebrandt, Rainey and Ward-Rainey 1997, 485^{VP} emend. Stackebrandt and Schumann 2000, 1284 emend. Zhi, Li and Stackebrandt 2009, 597

Peter Kämpfer and Ingrid Groth

In.tra.spo.ran.gi.a.ce′a.e. N.L. neut. n. *Intrasporangium* type genus of the family; suff. *-aceae* ending to denote a family; N.L. fem. pl. n. *Intrasporangiaceae* the family of *Intrasporangium*.

Cell morphology comprises **branching mycelium and filaments**, but **typically irregular short rods and cocci**. In some genera, a rod–coccus growth cycle occurs (*Knoellia*, *Terrabacter*). Gram-stain-positive. **Nonsporeforming. Generally nonmotile** with the **exception of some strains of *Terrabacter*. Aerobic and facultatively anaerobic**. Colonies are smooth, circular, and vary from white to yellow. Growth usually between 20 and 37°C, but may also occur at 40 or 42°C. **Oxidase-negative and in a few genera variable; catalase-positive**. The characteristic diamino acids in the peptidoglycan are LL- and *meso*-diaminopimelic acid (LL-A_2pm, *meso*-A_2pm,) and L-ornithine which is consistent with the **peptidoglycan types A1γ, A3γ, and A4β**, respectively. The major menaquinone is typically **MK-8(H_4)**, but **MK-8** may occur as main component, too. Common phospholipids are **phosphatidylglycerol**, **diphosphatidylglycerol**, and **phosphatidylinositol;** phosphatidylethanolamine and unidentified phospho- and glycolipids may additionally be present in some genera. The **cellular fatty acid profiles are complex** with **major amounts of iso- and anteiso-branched-chain acids together with saturated and monounsaturated straight-chain acids. Mycolic acids are absent**.

The family is a member of the order *Micrococcales* and currently accommodates 16 genera, among them *Intrasporangium* (Kalakoutskii et al., 1967), *Arsenicicoccus* (Collins et al., 2004), *Humihabitans* (Kageyama et al., 2007a), *Janibacter* (Martin et al., 1997), *Knoellia* (Groth et al., 2002), *Kribbia* (Jung et al., 2006), *Lapillicoccus* (Lee and Lee, 2007), *Marihabitans* (Kageyama et al., 2008a), *Ornithinicoccus* (Groth et al., 1999), *Ornithinimicrobium* (Groth et al., 2001), *Oryzihumus* (Kageyama et al., 2005), *Phycicoccus* (Lee, 2006), *Serinicoccus* (Yi et al., 2004), *Terrabacter* (Collins et al., 1989), *Terracoccus* (Prauser et al., 1997), and *Tetrasphaera* (Maszenan et al., 2000). The 16S rRNA gene signature nucleotides that define all genera of the family are: position 30:553 (C–G), 660:745 (G–C), 838:848 (C–G), and 859 (C). The signatures that differentiate the genera from one another are listed in Table 124.

DNA G+C content (mol%): 68–74.

Type genus: **Intrasporangium** Kalakoutskii, Kirillova and Krassil'nikov 1967, 79[AL].

Further descriptive information

The genera of the family *Intrasporangiaceae* are rather heterogeneous concerning their phenotypic characteristics. This is obvious from the diagnostic morphological and

TABLE 124. 16S rRNA gene signature nucleotides that differentiate the members of the family *Intrasporangiaceae* and related genera

Position	*Intrasporangium*	*Arsenicicoccus*	*Humihabi-tans*	*Janibacter*	*Knoellia*	*Kribbia*	*Lapillicoccus*	*Marihabitans*	*Ornithinicoccus*	*Ornithinimicrobium*	*Oryzihumus*	*Phycicoccus*	*Serinicoccus*	*Terrabacter*	*Terracoccus*	*Tetrasphaera*	*Actinotalea*	*Demequina*	*Sanguibacter*
69:99	G–N	A–U	G–U	G–U	G–G	A–U	G–U	A–U	G–U	A–U	G–U	G–U	G–U	G–U	G–U	G–U	G–U	G–U	G–U
140:223	G–C	C–G	G–C	G–C/ G–U[a]	G–C/ G–U[b]	G–U	G–C	G–U	U–A	C–G	C–G	G–C	C–G	G–C	G–C	G–C	G–C	G–C	G–C
157:164	N–C	G–C	G–C	U–G	U–G	U–C	G–C	U–G	G–C	G–C	G–C	G–C	U–G	G–C	G–C	G–C/ U–A[c]	G–C	G–C	G–C
258:268	A–U	A–U	A–U	A–U	A–U	A–U	A–U	A–U	G–C	G–C	A–U	A–U	G–C	A–U	A–U	A–U	A–U	A–U	A–U
630	C	C	C	C	C	C	C	C	C	C/U[d]	C	C	U	C	C	C	C	C	C
658:748	G–U	G–A	G–U	G–U	G–U	G–U	G–A	G–U	A–A	G–U	G–A	G–U/ G–A[e]	G–G	G–U	G–U	G–U/ A–U[f]/ G–C[g]	G–A	G–U	G–U
659:746	U–A	U–A	U–A	U–A	U–A	U–A	U–A	U–A	U–A	U–A	U–A	U–A	U–A	U–A	U–A	U–A	C–G	U–A	U–A
694	G	A	G	A	A	A	A	A	G	G	A	A	G	G	G	A	G	G	G
839:847	U–A	U–A	U–A	U–A	U–A	C–G	U–A	U–A	U–A	C–G	U–A	U–A	C–G	U–A	U–A	U–A/ C–G[h]	U–A	U–A	U–A
1003:1037	G–C	G–U	G–C	G–U	G–U	G–C	G–U	G–C	G–U	G–U	G–U	G–C	G–C	G–C	G–C	G–U/ G–A[i]/ G–C[j]	A–G	T—	G–C
1007:1022	C–G	C–G	C–G	C–G	C–G	C–G	C–G	C–G	C–G	C–G	C–G	G–U/ G–A[k]	A–U	C–G	C–G	C–G/ G–C[l]	C–G	U–A	C–G
1133:1141	A–U	G–C	A–U	A–U	A–U/ G–C[m]	G–C	G–C	A–U	A–U	A–U	A–U	A–U/ G–C[n]	A–U	A–U	A–U	G–C	G–C	A–U	A–U
1134:1140	C–G	C–G	C–G	C–G	C–G	G–C	G–C	C–G	C–G	C–G	C–G	C–G	C–G	C–G	U–G	C–G/ G–C[o]	G–C	C–G	C–G

[a] *Janibacter limosus, Janibacter terrae, Janibacter corallicola.*

[b] *Knoellia subterranea.*

[c] *Tetrasphaera australiensis, Tetrasphaera elongata, Tetrasphaera jenkinsii, Tetrasphaera vanveenii, Tetrasphaera veronensis.*

[d] *Ornithinimicrobium kibberense.*

[e] *Phycicoccus jejuensis.*

[f] *Tetrasphaera australiensis, Tetrasphaera vanveenii.*

[g] *Tetrasphaera veronensis, Tetrasphaera duodecadis.*

[h] *Tetrasphaera vanveenii.*

[i] *Tetrasphaera australiensis.*

[j] *Tetrasphaera veronensis.*

[k] *Phycicoccus dokdonensis.*

[l] *Tetrasphaera veronensis.*

[m] *Knoellia aerolata.*

[n] *Phycicoccus jejuensis.*

[o] *Tetrasphaera japonica.*

TABLE 125. Morphological and chemotaxonomic characteristics of the genera of the *Intrasporangiaceae* and related genera[a,b]

Characteristic	*Intrasporangium*	*Arsenicicoccus*	*Humihabitans*	*Janibacter*	*Knoellia*	*Kribbia*	*Lapillicoccus*	*Marihabitans*	*Ornithinicoccus*
Cell morphology	Branching mycelium	Cocci	Branching mycelium	Short rods, cocci	Irregular rods and cocci, (rod–coccus growth cycle)	Irregular short rods, cocci	Cocci	Irregular short rods	Cocci
Cell-wall diamino acid	LL-A_2pm-glycine$_3$	LL-A_2pm-glycine$_1$	LL-A_2pm	*meso*-A_2*pm*	*meso*-A_2pm	*meso*-A_2*pm*	LL-A_2pm	*meso*-A_2pm	L-Orn
Major menaquinone	MK-8	MK-8(H_4)	MK-8(H_4)	MK-8(H_4)	MK-8(H_4)	MK-8(H_4)	MK-8(H_4)	MK-8(H_4)	MK-8(H_4)
Predominant cellular fatty acids	$C_{15:0}$ iso, $C_{15:0}$ anteiso, $C_{16:0}$ iso	$C_{16:1}$ ω7*c*, $C_{15:0}$ iso, $C_{18:1}$ ω9*c*, $C_{15:0}$ anteiso	$C_{15:0}$ iso, $C_{16:0}$ iso, $C_{14:0}$ iso	$C_{16:0}$ iso, $C_{17:1}$ ω8*c*, $C_{17:0}$ $C_{18:1}$ ω9*c*	$C_{15:0}$ iso, $C_{16:0}$ iso, $C_{17:0}$ iso, $C_{17:1}$ iso, $C_{17:0}$ anteiso $C_{17:1}$ ω8*c*	$C_{18:0}$ 10methyl, $C_{16:0}$ iso, $C_{18:1}$ ω9*c*, $C_{16:0}$, $C_{18:0}$	$C_{16:0}$ iso, $C_{17:1}$ ω8*c*, $C_{15:0}$ iso	$C_{17:0}$ iso, $C_{17:1}$ ω8*c*, $C_{18:1}$ ω9*c*, $C_{15:0}$ iso	$C_{15:0}$ iso, $C_{15:0}$ anteiso
Polar lipids	PI, PIM, PG, DPG	nd	nd	DPG, PG, PI	PE, PI, DPG, PG, PL	nd	DPG, PI	nd	PI, PG, DPG, PSer, PLs
DNA G+C content (mol%)	68	72	70	69–73	68–73	69–70	74	70	72

[a]Abbreviations: nd, not determined; DMK, demethylmenaquinone; DPG, diphosphatidylglycerol; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; PI, phosphatidylinositol; PIM, phosphatidylinositol mannosides; PSer, Phosphatidylserine; APL, unidentified aminophospholipid; GL, unidentified glycolipid; PL, unidentified phospholipid.

[b]Data taken from: Kalakoutskii et al. (1967), Schumann et al. (1997) (*Intrasporangium*); Collins et al. (2004) (*Arsenicicoccus*); Kageyama et al. (2007a) (*Humihabitans*); Lee and Lee (2007) (*Lapillicoccus*); Collins et al. (1989), Montero-Barrientos et al. (2005), Weon et al. (2007b) (*Terrabacter*); Prauser et al. (1997) (*Terracoccus*); Martin et al. (1997), Yoon et al. (2004), Kämpfer et al. (2006) (*Janibacter*); Groth et al. (2002) and Weon et al. (2007a) (*Knoellia*); Jung et al. (2006) (*Kribbia*); Kageyama et al. (2008a) (*Marihabitans*); Kageyama et al. (2005) (*Oryzihumus*); Lee (2006), Yoon et al. (2008) (*Phycicoccus*); Maszenan et al. (2000), Ishikawa and Yokota (2006), McKenzie et al. (2006) (*Tetrasphaera*); Yi et al. (2007) (*Actinotalea*); Yi et al. (2007) (*Demequina*); Groth et al. (1999) (*Ornithinicoccus*); Groth et al. (2001), Liu et al. (2008) (*Ornithinimicrobium*); Yi et al. (2004) (*Serinicoccus*); Fernández-Garayzábal et al. (1995a), Huang et al. (2005) (*Sanguibacter*).

Ornithinimicrobium	*Oryzihumus*	*Phycicoccus*	*Sernicoccus*	*Terrabacter*	*Terracoccus*	*Tetrasphaera*	*Actinotalea*	*Demequina*	*Sanguibacter*
Rods, cocci	Irregular rods	Cocci	Cocci	Rods, cocci, (rod–coccus growth cycle)	Cocci in packets	Cocci, irregular short rods, filaments	Irregular rods	Rods	Irregular motile rods
L-Orn	*meso*-A_2pm	*meso*-A_2pm	L-Orn, Ser	LL-A_2pm-glycine$_3$	LL-A_2pm-glycine$_3$	*meso*-A_2pm	L-Orn	L-Orn	L-Lys
MK-8(H_4)	MK-8(H_4)	MK-8(H_4)	MK-8(H_4)	MK-8(H_4)	MK-8(H_4)	MK-8(H_4), MK-8	MK-10(H_4)	DMK-9(H_4)	MK-9(H_4)
$C_{16:0}$ iso, $C_{15:0}$ iso, $C_{15:0}$ anteiso, $C_{17:1}$ ω9*c*, $C_{17:0}$ iso	$C_{16:0}$ iso, $C_{15:0}$ iso, $C_{14:0}$ iso, $C_{15:0}$ anteiso	$C_{17:1}$ ω8*c*, $C_{16:0}$ iso, $C_{15:0}$ iso, $C_{15:0}$, $C_{17:0}$	$C_{15:0}$ iso, $C_{16:0}$ iso, $C_{17:1}$ iso ω9*c*, $C_{15:0}$ anteiso, $C_{17:0}$ anteiso	$C_{15:0}$ iso, $C_{14:0}$ iso, $C_{16:0}$ iso, $C_{17:0}$ iso, $C_{15:0}$ anteiso	$C_{15:0}$ iso, $C_{15:0}$ anteiso, $C_{16:0}$	$C_{16:0}$ iso, $C_{15:0}$ iso, $C_{17:0}$ anteiso	$C_{14:0}$, $C_{15:0}$ anteiso, $C_{16:0}$	$C_{15:0}$ anteiso, $C_{17:0}$ anteiso, $C_{16:0}$,$C_{15:1}$ anteiso	$C_{15:0}$ anteiso, $C_{15:1}$ anteiso, $C_{16:0}$, $C_{14:0}$, $C_{18:0}$
PI, PG, DPG, PLs, GLs	nd	PE, PI, PG, DPG, PL	PG, DPG, PI, GL	DPG, PI, PE, PL	PE, PI, PG, DPG	DPG, PG, PI, PE, APL, PL	nd	PI, DPG, PL	nd
70–71	72–73	71–74	72	71–73	73	68–73	76	67	69–73

chemotaxonomic characteristics listed in Table 125. According to the isomer and the kind of the diagnostic diamino acid in the peptidoglycan, the members of the *Intrasporangiaceae* can be divided into three groups. The first group contains the genera having LL-A_2pm in the peptidoglycan, namely *Intrasporangium* (Kalakoutskii et al., 1967), *Arsenicicoccus* (Collins et al., 2004), *Humihabitans* (Kageyama et al., 2007a), *Lapillicoccus* (Lee and Lee, 2007), *Terrabacter* (Collins et al., 1989), and *Terracoccus* (Prauser et al., 1997). The second group accommodates the genera possessing *meso*-A_2pm, including *Janibacter* (Martin et al., 1997), *Knoellia* (Groth et al., 2002), *Kribbia* (Jung et al., 2006), *Marihabitans* (Kageyama et al., 2008a), *Oryzihumus* (Kageyama et al., 2005), *Phycicoccus* (Lee, 2006), and *Tetrasphaera* (Maszenan et al., 2000). The genera falling into the third group have L-ornithine in the peptidoglycan; these are *Ornithinicoccus* (Groth et al., 1999), *Ornithinimicrobium* (Groth et al., 2001), and *Serinicoccus* (Yi et al., 2004). Furthermore, a group of related strains comprises the L-lysine-containing genus *Sanguibacter* (Fernández-Garayzábal et al., 1995a, 1995b), now classified in the family *Sanguibacteraceae*, and the two genera *Actinotalea* (Yi et al., 2007) and *Demequina* (Yi et al., 2007), both with L-ornithine in the cell wall, and which are classified in this volume within the *Cellulomonadaceae*.

The genera *Intrasporangium, Terracoccus, Terrabacter*, and *Janibacter* share putrescine as the major polyamine (Busse and Schumann, 1999). The type genus *Intrasporangium* shares with its most closely related genus, *Humihabitans*, the occurrence of a branched vegetative mycelium with the tendency to fragmentation, but differs from this genus by possessing MK-8 as major menaquinone. Interestingly, MK-8 as main component of the menaquinones is also found in the pleomorphic species *Tetrasphaera vanveenii* (McKenzíe et al., 2006) which shows filamentous growth when grown on glucose sulfide (GS) medium under laboratory conditions (Williams and Unz., 1985). The filaments are similar to those observed in the biomass samples obtained from activated sludge plants. In contrast to *Intrasporangium*, minor amounts of MK-8(H_2) and MK-8(H_4) are additionally present in *Tetrasphaera vanveenii*, and the formation of a branched mycelium is not observed. The occurrence of spherical or lemon-shaped vesicles in cultures of *Intrasporangium calvum* caused controversial discussions about their function. While Kalakoutskii et al. (1967) believed that the vesicles are comparable to the sporangia of *Actinoplanes* and other sporangia-forming actinomycetes, Lechevalier and Lechevalier (1969) did not find evidence of any stage of sporangiospore formation in *Intrasporangium calvum* vesicles by electron microscopic studies. Furthermore, Lechevalier and Lechevalier observed similar swellings in different actinomycetes isolated from soil and grown on media which were nutritionally unsatisfactory and in older cultures such as those of *Intrasporangium calvum*. Based on these studies, the vesicles should not be considered as morphological structures of taxonomic importance (Lechevalier and Lechevalier, 1969; Prauser et al., 1997).

The majority of strains belonging to the family were isolated from activated sludge or soils from different geographic regions including agricultural fields, soil from caves, permafrost soil, and marine sediments. Some of the strains affiliated to the genera *Arsenicicoccus, Kribbia, Ornithinimicrobium*, and *Tetrasphaera* are involved in the degradation of different kinds of toxic chemical compounds in waste water treatment plants or in contaminated soils. Some of the strains belonging to the genus *Janibacter* are associated with insects, hard corals, or plants. The phylogenetic positions of the current members of the family *Intrasporangiaceae* are shown in the 16S rRNA gene sequence based phylogenetic tree (Figure 161).

Taxonomic comments

The family *Intrasporangiaceae* was established by Rainey et al. 1997 (in Stackebrandt et al., 1997) to accommodate the genera *Intrasporangium, Sanguibacter*, and *Terrabacter*. Because of their genomic relatedness and the correspondence in the signature nucleotides, the genera *Janibacter* and *Terracoccus* were later included in this family (Stackebrandt and Schumann, 2000). With the addition of these taxa into the *Intrasporangiaceae*, it became evident that *Sanguibacter* formed a separate line of descent within the suborder *Micrococcineae* (elevated to order *Micrococcales* in the present volume) and had to be excluded from the *Intrasporangiaceae*. Consequently the 16S rRNA gene signature nucleotides specific for the emended family *Intrasporangiaceae* had to be redefined and *Sanguibacter* was proposed as the type genus of a novel family (Stackebrandt and Schumann, 2000). The genus *Ornithinicoccus* was at that time considered not included into the *Intrasporangiaceae* as the 16S rRNA gene signatures showed significant deviations from this set. However, with the addition of further actinobacterial 16S rRNA gene sequences to the databases, it has become obvious that *Ornithinicoccus, Ornithinimicrobium, Knoellia, Tetrasphaera, Arsenicicoccus, Serinicoccus*, and *Oryzihumus* should also be affiliated to the *Intrasporangiaceae* together with "*Candidatus* Nostocoida limicola" Blackall et al. 2000 (Garrity et al., 2007). Based on the later finding that the isolates of "*Candidatus* Nostocoida limicola" are members of the genus *Tetrasphaera* and represent three novel species of this genus (McKenzíe et al., 2006), the term "*Candidatus* Nostocoida limicola" lost its standing in nomenclature for these organisms and consequently should no longer be used.

From the phylogenetic data, it is very likely that the recently described genera *Kribbia, Phycicoccus, Lapillicoccus, Humihabitans*, and *Marihabitans* belong to the family *Intrasporangiaceae*.

It should be noted that the genera *Lapillicoccus* and *Phycicoccus* show a close relationship to members of the genus *Tetrasphaera* (Figure 161). A tree on the basis of the maximum-likelihood algorithm revealed the separate position of these genera. The genus *Lapillicoccus* is chemotaxonomically clearly different from *Tetrasphaera*, in contrast to *Phycicoccus* (Table 125). The genus *Tetrasphaera* is heterogeneous and should perhaps be divided in future studies on the basis of more strains. The genera *Demequina* and *Actinotalea* are grouped more closely to *Sanguibacter* and *Cellulomonas*, which also show a closer relationship to *Rarobacter, Cellulosimicrobium*, and *Promicromonospora*.

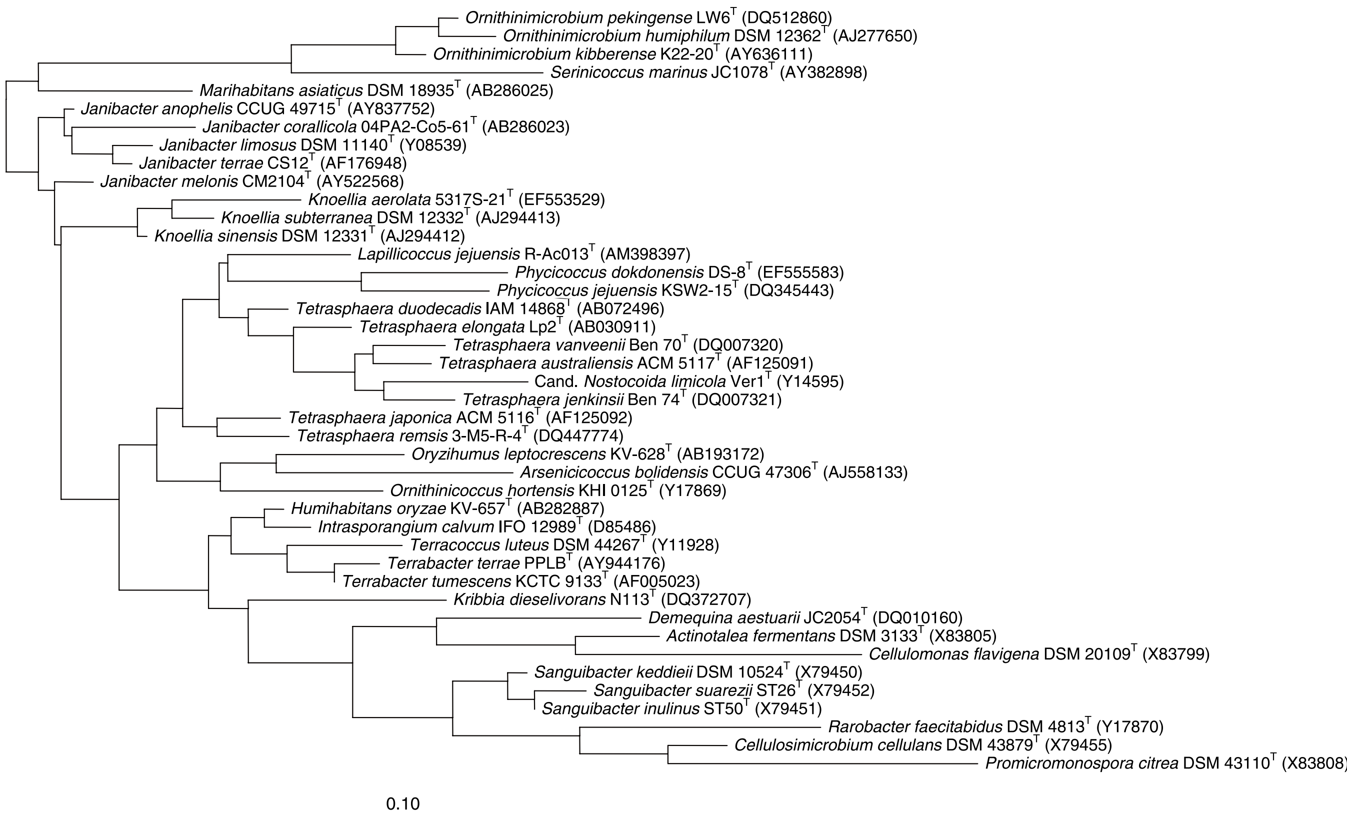

FIGURE 161. Phylogenetic tree showing the relationship of members of the family *Intrasporangiaceae* on the basis of 16S rRNA gene sequences available from the European Molecular Biology Laboratory databank (accession numbers are given in brackets). The tree was calculated with the maximum-likelihood method, using the software environment ARB (Ludwig et al., 2004). A filter defining positions which share identical residues in at least 50% of all included sequences from the family *Intrasporangiaceae*, was used for reconstructing the tree. Bar = 0.10 substitutions per site.

Genus I. **Intrasporangium** Kalakoutskii, Kirillova and Krassilnikov 1967, 79[AL]

INGRID GROTH

In.tra.spo.ran′gi.um. L. prep. *intra* within; Gr. n. *spora* a seed; Gr. n. *angeion* a vessel; N.L. neut. n. *Intrasporangium* a name coined to emphasize the possibility of intercalary formation of sporangia in mycelial filaments.

Branching mycelium, 0.4–1.2 µm in diameter, **with a tendency to break into fragments of various size and shape**. **No formation of aerial mycelium**. Gram-stain-positive, not acid-fast. Spherical and lemon-shaped vesicles (5–15 µm in diameter) are formed intercalary and/or at the hyphal apices depending on the age of the cultures. **Nonmotile**. **Aerobic**. Colonies are compact, 1–5 mm, circular, glistening and cream-whitish. Good growth on complex organic media containing peptone and meat extract and at temperatures of 28–37°C. No growth at 45°C and on the majority of mineral synthetic media routinely employed for actinomycetes. The **peptidoglycan type is A3γ** based on LL-A_2pm as the characteristic diamino acid. The predominant menaquinone is **MK-8**. The polar lipids consist of **phosphatidylinositol, phosphatidylinositol mannoside, phosphatidylglycerol, and diphosphatidylglycerol.** The cellular fatty acid profile is dominated by **iso- and anteiso-branched-chain acids** with major amounts of $C_{15:0}$ iso, $C_{15:0}$ anteiso, and $C_{16:0}$ iso. Mycolic acids are absent. Based on 16S rRNA gene sequence comparison, *Intrasporangium* is most closely related to the genera *Humihabitans* and *Terracoccus*. Further close relatives are the genera, *Terrabacter*, *Janibacter*, *Knoellia*, and *Lapillicoccus*. The 16S rRNA gene signature nucleotides that differentiate the genus from the other genera of the family *Intrasporangiaceae* are listed in Table 124.

DNA G+C content (mol%): 68.

Type species: **Intrasporangium calvum** Kalakoutskii, Kirillova and Krassilnikov 1967, 79[AL].

Further descriptive information

The genus is represented by the sole species *Intrasporangium calvum* (Kalakoutskii et al., 1967) and accommodates a single strain. A second strain is mentioned by Sukapure et al. (1970). However, this strain has not been deposited in any type culture collection, and a detailed description is missing. The closest phylogenetic neighbors of *Intrasporangium calvum* are *Terracoccus luteus* (Prauser et al., 1997) and *Humihabitans oryzae* (Kageyama

et al., 2007a). Both genera are currently represented by only a single strain, too. *Intrasporangium* shares with *Terracoccus* and *Humihabitans* 16S rRNA gene sequence similarities of 97.7% and 97.6%, respectively. Slightly less closely related are the genera *Terrabacter* (Collins et al., 1989), *Janibacter* (Martin et al., 1997), *Knoellia* (Groth et al., 2002)and *Lapillicoccus* (Lee and Lee, 2007) showing sequence similarities of 96.5–97.4%; 95.6–97.0%; 94.9–96.4% and 96.2%, respectively. The chemotaxonomic data referring to the composition of the interpeptide bridge, the pattern of the polar lipids, and the profile of cellular fatty acids for *Intrasporangium calvum* were obtained by Schumann et al. (1997) as a result of a comprehensive study with type strains of LL-A_2pm-containing taxa. The authors supplemented the data of the structure of the interpeptide bridge reported by Kalakoutskii et al. (1989) in the previous edition of this *Manual*. The interpeptide bridge of *Intrasporangium calvum* was found to consist of three glycine residues and a fourth glycine residue bound to the α-carboxyl group of the D-glutamic acid in position 2 of the peptide subunit (Prauser et al., 1997). The previously given data related to the fatty acid profile and the pattern of phospholipids could not be confirmed by Schumann et al. (1997) and had to be replaced. The characteristic vesicles occurring in old cultures of *Intrasporangium calvum* are not comparable to true sporangia, as was shown by electron microscopic studies (Lechevalier and Lechevalier, 1969). Prauser et al. (1997) confirmed that *Intrasporangium calvum* was mistakenly thought to form sporangia.

Enrichment and isolation procedures

Intrasporangium calvum grows only moderately and rather slowly even on peptone containing media. Subculturing of the organism on yeast peptone agar (g/l tap water: baker's yeast, 5.0; peptone, 5.0; glucose, 10.0 and agar, 15; pH 7.0) and in different variations of meat-extract-peptone broth or agar (g/l: peptone, 5.0; beef extract, 5.0; NaCl, 5.0 and agar 15.0–20.0, pH 7.2–7.4) supplemented with either 0.5% glycerol, 0.1% yeast extract, 0.5% glucose, 5% starch, 0.001% Na-thioglycollate, or 10% blood serum did not improve growth. Growth was sparse on oatmeal agar, rice agar, yeast glucose agar, and wheat meal agar. No growth was obtained on malt agar supplemented with 1% $CaCO_3$, potato agar, Czapek agar, glutamic acid glucose agar, glucose asparagine agar, soil extract- or water agar. Growth was also not enhanced on complex media upon enrichment of the atmosphere by H_2 and/or CO_2. The type strain was isolated under nonselective conditions on plates of meat-peptone agar exposed to the atmosphere of a school dining room (Kalakoutskii et al., 1967).

Maintenance procedures

Cultures of *Intrasporangium calvum* can be maintained by serial transfers on the above-mentioned complex media containing peptone and meat-extract. For long-term preservation, routine procedures of lyophilization or storage in the vapor phase of liquid nitrogen (−140°C) by adding 5% dimethylsulfoxide to the cultures can be applied.

Differentiation of the genus *Intrasporangium* from other genera

In the 16S rRNA gene tree of the *Intrasporangiaceae*, the genus *Intrasporangium* forms a common branch with the genus *Humihabitans* adjacent to the genera *Terracoccus* and *Terrabacter*. The phenotypic differences between *Intrasporangium* and the very closely related *Humihabitans* are mainly based on the menaquinone type. In contrast to *Humihabitans* and all other genera of the family *Intrasporangiaceae*, *Intrasporangium* has MK-8 as major menaquinone. However, one species of the genus *Tetrasphaera* (*Tetrasphaera vanveenii*) also possesses MK-8 as main menaquinone (McKenzíe et al., 2006). As both *Intrasporangium* and *Humihabitans* are represented by a single strain, the phylogenetic and phenotypic variability within the genus cannot currently be assessed. *Intrasporangium* shares with *Terracoccus* and *Terrabacter* the peptidoglycan type A3γ (Schleifer and Kandler, 1972), but it can be readily distinguished from these two genera by the menaquinone type, differences in the composition of fatty acids and polar lipids, and differences in morphological properties. The susceptibility of *Intrasporangium calvum* to genus-specific phages (Prauser and Falta, 1968; Wellington and Williams, 1981) and the results of extensive numerical taxonomic studies (Williams et al., 1983) additionally underline the differences from *Terracoccus*, *Terrabacter*, and equally from all other aerobic mycelium-forming actinomycetes. The slightly less closely related genera *Janibacter* and *Knoellia* can be readily distinguished from *Intrasporangium* by the presence of *meso*-A_2pm in their peptidoglycan, menaquinones of the MK-8(H_4) type, and morphological and further chemotaxonomic characteristics listed in Table 125.

Taxonomic comments

The given description is currently based on a single species with a single strain and therefore cannot reflect the phylogenetic depth and the phenotypic variability of the entire taxon. Ecological aspects cannot be considered as the real habitat of *Intrasporangium* is unknown. Comparative studies based on further isolates would also be useful to clarify the function of the intercalar vesicles (Figure 162), which should not be termed sporangia.

Acknowledgements

L.V. Kalakoutskii wrote the section on *Intrasporangium* in the previous edition of this *Manual* (1986 and 1989). Most of the data referring to morphological, physiological, and cultural characteristics have been retained from that version. I am grateful to H. Prauser for his helpful discussions and to B. Schütze for critical reading of this article.

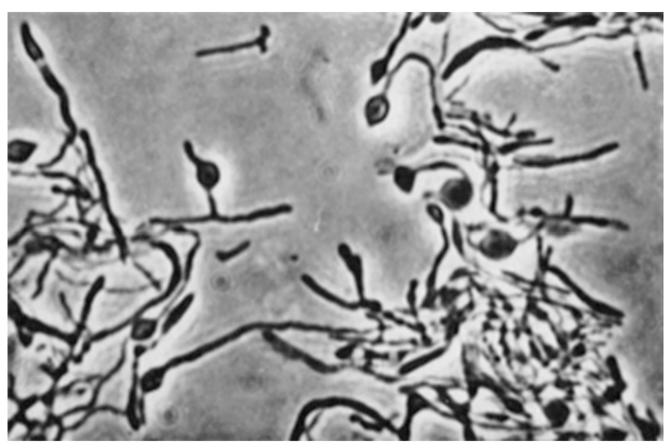

FIGURE 162. 6-d-old slide culture of *Intrasporangium calvum* on meat-peptone agar (4000×). Phase contrast. Branching mycelium and intramycelial vesicles in both terminal and intercalary positions (Reproduced with permission from Kalakoutskii et al., 1967. J. Gen. Microbiol. *48*: 79–85.).

List of species of the genus *Intrasporangium*

1. **Intrasporangium calvum** Kalakoutskii, Kirillova and Krassilnikov 1967, 79[AL]

cal′vum. L. neut. adj. *calvum* bald, referring to the absence of aerial mycelium.

The description is the same as for the genus. Macroscopically visible colonies appear on peptone containing media after 3–5 d of incubation at 28°C. Colonies are viscous, round, glistening, and whitish, becoming creamy on aging. The vesicles begin to appear following 5–6 d of cultivation on solid media, but are rare under conditions of submerged cultivation. Growth in liquid cultures usually occurs in a sedimented form. Vesicles are abundant in older cultures (about 12 d). The temperature range for growth is 10–42°C. Nitrate is reduced to nitrite. According to Williams et al. (1983) the organism is able to utilize L-arginine, L-cysteine, L-methionine, L-phenylalanine, and L-serine as sole nitrogen source (0.1%, w/v) in a basal medium containing glucose and mineral salts. On ISP medium 9 the following compounds are able to support growth if added as sole carbon sources (1.0%, w/v): D-glucose, cellobiose, D-fructose, mannitol, L-rhamnose, salicin, trehalose, D-xylose, and sodium pyruvate (0.1%, w/v). Degradation of esculin, arbutin, casein, gelatin, elastin, Tween 80, and DNA was noted on modified Bennett's agar and Bacto DNase test agar (Difco). On modified Bennett's agar, growth was possible in the presence of any of the following compounds: phenylethanol (0.1%, w/v), potassium tellurite (0.01%, w/v), thallous acetate (0.001%, w/v), cephaloridine (100 μg/ml), gentamicin (100 μg/ml), neomycin (50 μg/ml), or tobramycin (50 μg/ml).

DNA G+C content (mol%): 68 (T_m).

Type strain: ATCC 23552, DSM 43043, JCM 3097, KIP-7, NBRC 12989, NRRL B-3866, VKM Ac-701.

Sequence accession no. (16S rRNA gene): AJ566282, D85486.

Genus II. **Arsenicicoccus** Collins, Routh, Saraswathy, Lawson, Schumann, Welinder-Olsson and Falsen 2004, 607[VP]

Paul A. Lawson

Ar.se.ni.ci.coc′cus. L. n. *arsenicum* arsenic; N.L. masc. n. *coccus* berry (from Gr. masc. n. *kokkos* grain, seed), N.L. masc. n. *Arsenicicoccus* arsenic coccus, because the type species was recovered from an arsenic enrichment).

Cells are cocci that occur in clusters. Gram-stain-positive and nonsporeforming. **Facultatively anaerobic and catalase-positive.** Acid is formed from glucose and some other carbohydrates. Nitrate is reduced. Voges–Proskauer-negative. **The major long-chain cellular fatty acids are a complex mixture of straight-chain saturated, monounsaturated, iso- and anteiso-methyl-branched acids.** Hydroxy fatty acids are not present. **The major respiratory quinone is MK-8(H_4). Cell-wall murein is based on LL-diaminopimelic acid (type: LL-Dpm-glycine$_1$).** Isolated from arsenic enrichment from sediment containing mine waste (Adak, Boliden region, Sweden).

DNA G+C content (mol%): 72.2.

Type species: **Arsenicicoccus bolidensis** Collins, Routh, Saraswathy, Lawson, Schumann, Welinder-Olsson and Falsen 2004, 608[VP].

Further descriptive information

The genus contains only one species, *Arsenicicoccus bolidensis*, and therefore the characteristics provided below refer to this species. Using commercially available API kits, acid is formed from fructose, glucose, glycogen, mannitol, mannose, sucrose, and D-xylose. Depending on the test kit, acid may or may not be produced from cellobiose, lactose, maltose, and ribose. Using the API ZYM system, alkaline phosphatase, esterase C4, ester lipase C8, α- and β-galactosidase, and α- and β-glucosidase are positive; acid phosphatase and phosphoamidase are either weakly positive or negative. Chymotrypsin, cystine arlyamidase, α-fucosidase, β-glucuronidase, α-mannosidase, lipase C14, leucine arylamidase, *N*-acetyl-β-glucosaminidase, trypsin, and valine arylamidase are not detected. Using the API Coryne test kit, alkaline phosphatase, β-galactosidase, α-glucosidase, pyrazinamidase, and pyroydonyl arylamidase are detected, but not β-glucuronidase or *N*-acetyl-β-glucosaminidase. Esculin and gelatin are hydrolyzed, but urea is not. Chemotaxonomic characteristics are as given in the genus description. Possess As(V) reduction mechanisms that are coupled to respiration or impart resistance to As toxicity.

Isolation procedures

Arsenicicoccus bolidensis was isolated from sediment from a man-made lake near the town of Adak (Boliden region, Sweden) containing mine waste with high concentrations of As, Cu, and Zn present. The abandoned mine trailings are underlain by peat bogs and are mixed with glacial till (Routh and Saraswathy, 2005). Several 30–45 cm long undisturbed sediment cores were collected across the lake using a gravity corer, transported to the laboratory within 48 h, and immediately sliced for various geochemical and microbiological assays. During slicing, the outer surface of the cores was pared with a sterile knife and samples stored in zip lock plastic bags. Sediments were added to a basal salts medium (BSM) for enrichment. BSM contained the following constituents (in g/l): NH_4Cl, 0.25; K_2HPO_4, 0.14; KCl, 0.5; $CaCl_2·2H_2O$, 0.15; NaCl, 1.0: $MgCl_2·6H_2O$, 0.62; and 1 ml of trace elements solution containing $MnCl_2·4H_2O$, 0.1 mg; $FeCl_2·4H_2O$, 1.5 mg; $Co(NO_3)_2·6H_2O$, 0.12 mg; $ZnCl_2$, 0.07 mg; $CuCl_2·2H_2O$, 0.015 mg; H_3BO_3, 0.06 mg; $Na_2MoO_4·2H_2O$, 0.025 mg; $Ni(NO_3)_2·6H_2O$, 0.025 mg; and *para*-aminobenzoic acid, 0.05 mg. Lactate (0.089 mM) was used as the sole carbon source in the medium. The medium was later spiked with 0.435 mM As to facilitate the isolation of As resistant strains. The As enriched cultures were diluted 10-fold, and 0.1 ml aliquots of the extract were spread onto Tryptic Soy Agar (TSA) plates spiked with

0.435 mM As. After 72 h of incubation at 22°C, colonies were selected and replated on the same medium until pure cultures were obtained.

Maintenance procedures

Strains grow well on Soy Yeast Extract medium or Columbia agar base supplemented with 5% defibrinated sheep blood at 22–25°C. For long-term preservation, strains can be maintained in a medium containing 15–20% glycerol at −70°C or lyophilized.

Taxonomic comments

The genus *Arsenicicoccus* was proposed in 2004 to accommodate a phylogenetically distinct Gram-stain-positive, catalase-positive, facultatively anaerobic, nonsporeforming coccus-shaped organism originating from a manmade lake sediment (Collins et al., 2004). Phylogenetic analysis using 16S rRNA gene sequences shows that the genus is phylogenetically related to the order *Actinomycetales* of the class *Actinobacteria*. The taxon is monospecific and belongs to the family *Intrasporangiaceae* within the suborder *Micrococcineae* (Stackebrandt et al., 1997), which has been elevated to order *Micrococcales* in the taxonomic roadmap to the present volume. The family *Intrasporangiaceae* is a phenotypically heterogeneous taxon which contains actinomycetes that can be divided into three groups on the basis of the diagnostic diamino acid in the cell-wall peptidoglycan. The genera *Intrasporangium* (Kalakoutskii et al., 1967), *Terrabacter* (Collins et al., 1989), *Terracoccus* (Prauser et al., 1997), *Arsenicicoccus* (Collins et al., 2004), *Kribbia* (Jung et al., 2006), and *Lapillicoccus* (Lee and Lee, 2007) contain LL-diaminopimelic acid; the genera *Janibacter* (Martin et al., 1997), *Tetrasphaera* (Maszenan et al., 2000), *Knoellia* (Groth et al., 2002), *Oryzihumus* (Kageyama et al., 2005), and *Phycicoccus* (Lee, 2006) *meso*-diaminopimelic acid, and the genera *Ornithinicoccus* (Groth et al., 1999), *Ornithinimicrobium* (Groth et al., 2001), and *Serinicoccus* (Yi et al., 2004) contain L-ornithine.

Arsenicicoccus bolidensis forms a distinct lineage within this family of organisms, but does not display a particularly close affinity with any of the aforementioned taxa. Figure 163 depicts a phylogenetic reconstruction using the neighbor-joining method. *Arsenicicoccus bolidensis* clusters with *Oryzihumus leptocrescens*, *Ornithinicoccus hortensis*, and *Lapillicoccus jejuensis*. However, this grouping is not supported by significant bootstrap values nor is this cluster preserved when maximum-parsimony and maximum-likelihood tree-making algorithms are employed (data not shown).

Differentiation of the genus *Arsenicicoccus* from other genera

In addition to differentiation by 16S rRNA gene sequence analysis, *Arsenicicoccus bolidensis* can be readily distinguished from its closest phylogenetic relatives using a combination of biochemical, chemotaxonomic and morphological criteria (see Table 126).

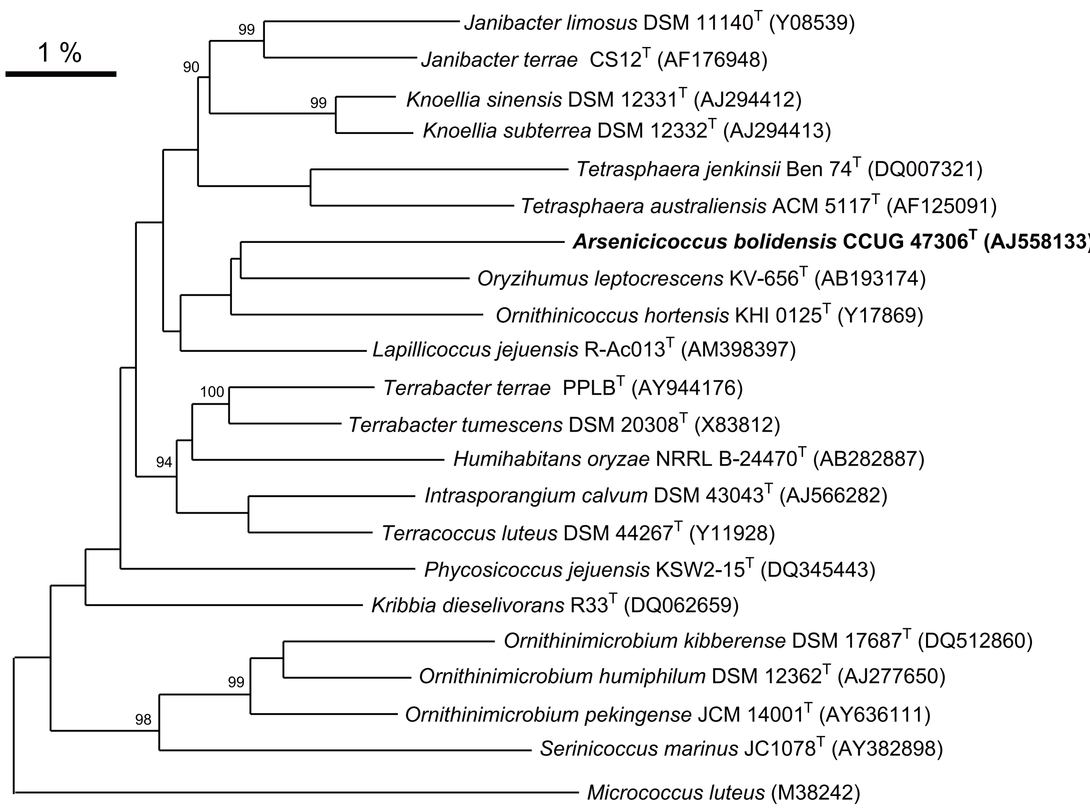

FIGURE 163. Unrooted neighbor-joining tree depicting the estimated phylogenetic relationships of members of the genus *Arsenicicoccus* and other members of the family *Intrasporangiaceae*; *Micrococcus luteus* was used as the outgroup. The numbers on the branches refer to bootstrap values determined from 1000 replications. Only values above 90% are shown. Bar = 1% sequence divergence.

TABLE 126. Characteristics distinguishing the genus *Arsenicicoccus* and genera in the family *Intrasporangiaceae*

Characteristic	*Arseniciococcus*[a]	*Intrasporangium*[b]	*Janibacter*[c]	*Knoellia*[d]	*Kribbia*[e]	*Lapillicoccus*[f]	*Ornithinicoccus*[g]	*Ornithinimicrobium*[h]	*Oryzihumus*[i]	*Phycicoccus*[j]	*Serinicoccus*[k]	*Terrabacter*[l]	*Terracoccus*[m]	*Tetrasphaera*[n]
Cell morphology	Cocci	Hyphae	Coccoid to rod-shaped	Irregular rods and cocci	Irregular short rods or cocci	Cocci	Cocci	Irregular rods and cocci	Irregular rods	Cocci	Cocci	Irregular rods	Cocci	Cocci or short rods
Cell-wall diamino acid	LL-A$_2$pm	LL-A$_2$pm	*meso*-A$_2$pm	*meso*-A$_2$pm	*meso*-A$_2$pm	LL-DAP	L-Orn	L-Orn	*meso*-A$_2$pm	*meso*-DAP	L-Orn	LL-A$_2$pm	LL-A$_2$pm	*meso*-A$_2$pm
Major menaquinone	MK-8(H$_4$)	MK-8	MK-8(H$_4$)	MK-8(H$_4$)	MK-8(H$_4$)	MK-8(H$_4$)	MK-8(H$_4$)	MK-8(H$_4$)	MK-8(H$_4$)	MK-8(H$_4$)	MK-8(H$_4$)	MK-8(H$_4$)	MK-8(H$_4$)	MK-8(H$_4$)
Major fatty acid(s)	$C_{16:1}$ ω7*c*, $C_{15:0}$ iso, $C_{18:1}$ ω9*c*, $C_{15:0}$ anteiso	$C_{15:0}$ iso, $C_{16:0}$ iso, $C_{15:0}$ anteiso	$C_{16:0}$ iso, $C_{17:0}$, $C_{17:1}$ ω8*c*, $C_{18:1}$ ω9*c*	$C_{15:0}$ iso, $C_{16:0}$ iso, $C_{17:0}$ iso, $C_{17:0}$ anteiso	$C_{16:0}$, $C_{16:0}$ iso, $C_{18:1}$ ω9*c*, $C_{18:0}$, $C_{18:0}$ 10 methyl	$C_{15:0}$ iso, $C_{16:0}$ iso, $C_{17:1}$ ω8*c*	$C_{15:0}$ iso, $C_{15:0}$ anteiso	$C_{15:0}$ iso, $C_{15:0}$ anteiso, $C_{16:0}$ iso	$C_{14:0}$ iso, $C_{15:0}$ iso, $C_{16:0}$ iso	$C_{15:0}$, $C_{15:0}$ iso, $C_{16:0}$ iso, $C_{17:0}$, $C_{17:1}$ ω8*c*	$C_{15:0}$ iso, $C_{16:0}$ iso, $C_{17:1}$ iso ω9*c*	$C_{15:0}$ iso, $C_{15:0}$ anteiso, or $C_{14:0}$ iso, $C_{15:0}$, $C_{16:0}$ iso[p]	$C_{15:0}$ anteiso, $C_{15:0}$ iso, $C_{16:0}$,	$C_{15:0}$ anteiso, $C_{15:0}$ iso[q], or $C_{16:0}$ iso, $C_{17:0}$ anteiso, $C_{15:0}$ iso[r], or $C_{16:0}$ iso, $C_{17:0}$ anteiso[s]
DNA G+C content (mol%)	72.2	68.2	69–73	68–69	69–70	74.1	72	70	72–73	74	72	71–73	73	68–71

[a] *Arseniciococcus* (data from Collins et al., 2004).

[b] *Intrasporangium* (Kalakoutskii et al., 1967).

[c] *Janibacter* (Imamura et al., 2000; Kämpfer et al., 2006; Martin et al., 1997; Yoon et al., 2000, 2004).

[d] *Knoellia* (Groth et al., 2002).

[e] *Kribbia* (Jung et al., 2006).

[f] *Lapillicoccus* (Lee and Lee, 2007).

[g] *Ornithinicoccus* (Groth et al., 1999).

[h] *Ornithinimicrobium* (Groth et al., 2001).

[i] *Oryzihumus* (Kageyama et al., 2005).

[j] *Phycicoccus* (Lee, 2006).

[k] *Serinicoccus* (Yi et al., 2004).

[l] *Terrabacter* (Collins et al., 1989; Montero-Barrientos et al., 2005).

[m] *Terracoccus* (Prauser et al., 1997).

[n] *Tetrasphaera* (Hanada et al., 2002; Maszenan et al., 2000).

[o] *Tetrabacter terrae.*

[p] *Tetrabacter tumescens.*

[q] *Tetrasphaera elongate.*

[r] *Tetrasphaera australiensis.*

[s] *Tetrasphaera japonica.*

List of species of the genus *Arsenicicoccus*

1. **Arsenicicoccus bolidensis** Collins, Routh, Saraswathy, Lawson, Schumann, Welinder-Olsson and Falsen 2004, 608[VP]

bo.li.den′sis. N.L. masc. adj. *bolidensis* pertaining to the Boliden region in Vasterbotten district of northern Sweden, where the type strain was isolated.

Characteristics are as given for the genus with the following information. Depending on the test kit, acid may or may not be produced from cellobiose, lactose, maltose, and ribose. Using the API ZYM system, alkaline phosphatase, esterase C4, ester lipase C8, α- and β-galactosidase, α- and β-glucosidase are positive; acid phosphatase and phosphoamidase are either weakly positive or negative. Chymotrypsin, cystine arlyamidase, α-fucosidase, β-glucuronidase, α-mannosidase, lipase C14, leucine arylamidase, *N*-acetyl-β-glucosaminidase, trypsin, and valine arylamidase are not detected. Using the API Coryne test kit, alkaline phosphatase, β-galactosidase, α-glucosidase, pyrazinamidase, and pyroydonyl arylamidase are detected but β-glucuronidase and *N*-acetyl-β-glucosaminidase are not. Esculin and gelatin are hydrolyzed, but urea is not. Chemotaxonomic characteristics are as given in the genus description. Possess As(V) reduction mechanisms that are coupled to respiration or impart resistance to As toxicity. Other characteristics are as given for the genus.

Source: arsenic enrichment from sediment containing mine waste (Boliden, Sweden).

DNA G+C content (mol%): 72.2 (HPLC).

Type strain: CCUG 47306, DSM 15745, IAM 15342, JCM 13385.

Sequence accession no. (16S rRNA gene): AJ558133.

Genus III. **Humihabitans** Kageyama, Takahashi and Ōmura 2007a, 2165[VP]

The Editorial Board

Hu.mi.ha′bi.tans. L. masc. n. *humus* soil; L. masc. n. *habitans* an inhabitant; N.L. masc. n. *Humihabitans* an inhabitant of soil.

Aerobes that form **branching hyphae** and fragmentary vegetative mycelium. Cell length is 1.0–2.2 µm with a mean diameter of 0.5 µm. **Stain Gram-positive. Catalase-positive.** The **major menaquinone is MK-8(H_4)**. The cell-wall peptidoglycan is type A3c and contains the diagnostic diamino acid, **LL-diaminopimelic acid**. Mycolic acids are absent. Phylogenetic analysis of the 16S rRNA gene sequence positions this genus within the family *Intrasporangiaceae*. Its closest relative is *Intrasporangium calvum*, from which it can be distinguished by the presence of the menquinone MK-8(H_4). The type strain was isolated from soil samples of a rice paddy field in Saitama Prefecture, Japan, using agar medium containing oxidant scavengers.

DNA G+C content (mol%): 70.

Type species: **Humihabitans oryzae** Kageyama, Takahashi and Ōmura 2007a, 2165[VP].

Enrichment and isolation procedures

Humihabitans type strain KV-657 was isolated from soil samples of rice paddies when grown on nutrient agar medium supplemented with superoxide dismutase. This technique was found to increase the number of colonies that appeared when isolating organisms from soil samples (Takahashi et al., 2003). Morphological and phenotypic studies were conducted on cells grown in GPM agar medium (1% D-glucose, 0.5% peptone, 0.5% meat extract, 0.3% NaCl and 1.2% agar; pH 7.0) and 1/5-strength GPM medium, respectively. Cells were isolated for studies after 2 d growth at 27°C.

Differentiation of the genus *Humihabitans* from other genera

Humihabitans can be distinguished from other members of the *Intrasporangiaceae* by phenotypic and chemotaxonomic properties. The major cellular fatty acids of isolates of this family are a complex mixture of straight-chain saturated, monounsaturated, iso- and anteiso-methyl-branched chains. *Humihabitans* contains a mixture of all these fatty acids, as well as the presence of the major menaquinone, MK-8(H_4), both characteristics of which distinguish it from the genera *Intrasporangium*. *Humihabitans* form branching and fragmentary hyphae, distinguishing the isolate from other genera including *Arsenicicoccus*, *Terrabacter*, and *Terracoccus*. Isolates of the genus *Arsenicicoccus* have a similar complex mixture of major fatty acids but differ from *Humihabitans* in cell morphology.

On the basis of 16S rRNA gene sequence, *Humihabitans* is related to *Intrasporangium calvum* with a similarity of 97.6%. The DNA–DNA hybridization with the same species is a relatively high 33–34%.

List of species of the genus *Humihabitans*

1. **Humihabitans oryzae** Kageyama, Takahashi and Ōmura 2007a, 2165[VP]

o.ry′za.e. L. gen. n. *oryzae* of rice, pertaining to the isolation of the type stain from rice paddy soil.

Cells form branching hyphae or fragmentary vegetative mycelium and range in length between 1.0 and 2.2 µm, with a mean diameter of 0.5 µm. Colonies are pale yellow in color. Growth occurs at pH 5–11 and at 8°C and 40°C. In 1/5-strength nutrient agar medium, NaCl is tolerated up to 3%. Utilizes D-fructose, D-glucose, maltose, D-mannitol, D-mannose, sucrose, trehalose and D-xylose but not L-arabinose, D-galactose, raffinose and L-rhamnose. Enzymic

activities present include esterase (C4), esterase lipase (C8), valine arylamidase, cystine arylamidase, trypsin, chymotrypsin, acid phospatase, α-galactosidase, β-galactosidase and α-glucosidase. Weak reactions for lipase (C14), leucine arylamidase, naphthol-AS-BI-phosphohydrolase and β-glucosidase were detected. The cellular fatty acid components include $C_{14:0}$ iso (7.3%), $C_{15:0}$ iso (39.6%), $C_{15:0}$ anteiso (5.8%), $C_{15:0}$ (4.8%), $C_{16:0}$ iso (13.3%), $C_{17:0}$ iso (2.0%), $C_{17:0}$ anteiso (2.0%), $C_{17:0}$ ω8*c* (6.4%) and $C_{17:0}$ (5.8%).

DNA G+C content (mol%): 70 (HPLC).

Type strain: KV-657.

Sequence accession no. (16S rRNA gene): AB282887.

Genus IV. **Janibacter** Martin, Schumann, Rainey, Schuetze and Groth 1997, 533[VP]

Ingrid Groth

Ja.ni.bac′ter L. n. *Janus* a god in Roman mythology, who is said to have had two faces; N.L. masc. n. *bacter* a rod; N.L. masc. n. *Janibacter* referring to the changing morphology of the microorganisms.

Cells are typically **cocci**; in young cultures **irregular short rods or rod-like cells** may occur. Cells occur singly, in pairs, short chains, or irregular clumps. Gram-stain-positive. Not acid-fast. **Nonsporeforming. Nonmotile. Aerobic**. Colonies are smooth, circular, convex, and vary in color from white to yellow. Good growth on complex organic media. Optimal temperatures for growth 23–35°C. NaCl in the culture medium may be tolerated up to 10%. **Oxidase variable**; **catalase-positive**. The **peptidoglycan type** is **A1γ**, *meso*-A_2pm directly cross-linked, which is in accordance with type A31. The **acyl type is acetyl**. **Whole-cell sugars** so far analyzed are **glucose, mannose, and ribose**. The **predominant menaquinone is MK-8(H_4)**. The **polar lipids** consist of **phosphatidylglycerol, diphosphatidylglycerol, and phosphatidylinositol**. The **cellular fatty acid profiles are complex with major amounts of iso-branched-chain acids together with straight-chain saturated and monounsaturated acids** ($C_{16:0}$ iso, $C_{17:0}$, $C_{17:1}$ ω8*c*, $C_{18:1}$ ω9*c*). **Mycolic acids are absent**. 16S rRNA gene sequence analysis reveals that members of *Janibacter* are closely related to strains of *Knoellia*, *Tetrasphaera*, *Terrabacter*, *Intrasporangium*, *Lapillicoccus*, and *Terracoccus* with which they share 16S rRNA gene sequence similarities of 95.0–98.2%, 94.1–97.1%, 95.2–97.0%, 95.6–97.0%, 96.1–96.4%, and 95.0–96.4%, respectively. The 16S rRNA gene signature nucleotides that differentiate the genus from the other genera of the family *Intrasporangiaceae* are listed in Table 124, chapter *Intrasporangiaceae*.

DNA G+C content (mol%): 69–73.

Type species: **Janibacter limosus** Martin, Schumann, Rainey, Schuetze and Groth 1997, 533[VP].

Further descriptive information

The genus *Janibacter* currently accommodates six species, *Janibacter limosus* (Martin et al., 1997), *Janibacter anophelis* (Kämpfer et al., 2006), *Janibacter corallicola* (Kageyama et al., 2007b, 2007c), *Janibacter hoylei* (Shivaji et al., 2009), *Janibacter melonis* (Yoon et al., 2004), and *Janibacter terrae* (Yoon et al., 2000; emend. Lang et al., 2003). While strains of *Janibacter limosus* and *Janibacter terrae* were isolated from wastewater contaminated soils *Janibacter anophelis*, *Janibacter corallicola*, and *Janibacter melonis* were associated with insects, a hard coral, or plants, respectively. *Janibacter hoylei* was isolated from air.

The 16S rRNA gene sequence similarity values between the type strains of the *Janibacter* species range from 97.0–98.9%. The low levels of DNA–DNA relatedness obtained in DNA–DNA hybridization studies with different combinations of the type strains clearly separate these strains at the species level from one another (Kämpfer et al., 2006; Lang et al., 2003; Shivaji et al., 2009; Yoon et al., 2000, 2004). The same is true for the cellular fatty acid profiles which are useful in the differentiation of *Janibacter* species.

Cell morphology of *Janibacter* species comprises cocci together with short rods or rod-like cells. While young cultures either consist of cocci or short rods (Figure 164), stationary cells are always coccoid. A marked rod–coccus growth cycle is not reported. Neither substrate nor primary mycelium is formed. Growth occurs up to a temperature of 38°C, but 40°C may be also tolerated by some strains depending on the composition of the culture medium (Lang et al., 2003; Shivaji et al., 2009; Yoon et al., 2004). NaCl in the culture medium is tolerated up to a concentration of 7% and, in some cases, up to 10%. No growth occurs under anaerobic conditions.

The type strains of *Janibacter limosus*, *Janibacter hoylei*, *Janibacter melonis*, and *Janibacter terrae* are able to reduce nitrate to nitrite (data for *Janibacter anophelis* and *Janibacter corallicola* are not reported).The type strains of *Janibacter limosus*, *Janibacter anophelis*, *Janibacter corallicola*, *Janibacter melonis* and *Janibacter terrae* utilize D-glucose, D-maltose, D-mannose, and D-trehalose, in some cases at least in weak or delayed reactions (Kageyama et al., 2007b; Kämpfer et al., 2006). *Janibacter hoylei* does not utilize these carbon sources. The following physiological properties given for *Janibacter limosus* DSM 11140[T] in the original description (Martin et al., 1997) should be considered to be variable: decomposition of gelatin, starch, and Tween 80; utilization of acetate, benzoate, and citrate. Yoon et al. (2000) and Lang et al. (2003) obtained conflicting results when they used this strain as a reference in these tests. Acid production from various carbon sources was not observed in all currently described *Janibacter* species, both when tests based on the method of Hugh and Leifson (1953) or Kämpfer et al. (1991) were applied (Kämpfer et al., 2006; Martin et al., 1997; Shivaji et al., 2009; Yoon et al., 2000, 2004) and when commercially available test systems like the API 50CH galleries (bioMérieux) were used (Lang et al., 2003).

Susceptibility to antibiotics is only reported for two strains of *Janibacter limosus* (Martin et al., 1997). Some of the strains belonging to *Janibacter terrae* were found to be able to degrade environmental pollutants like dibenzofuran (Engesser et al., 1989; Strubel et al., 1989) or trichloroethylene (Imamura

TABLE 127. Characteristics differentiating the species of the genus *Janibacter*[a,b]

Characteristic	*J. limosus* DSM 11140^T	*J. anophelis* CCUG 49715 ^{T}	*J. corallicola* DSM 18906^T	*J. hoylei* DSM 21601^T	*J. melonis* DSM 16063^T	*J. terrae* DSM 13876^T
Cell morphology	Cocci, rods	Cocci, rod-like cells	Cocci	Cocci	Cocci	Cocci, rods
Oxidase	–	+	nd	+	–	– or w
Growth at 37°C	–	+	+	+	+	+
Utilization of sole carbon sources[c]:						
Acetate	–	+	nd	nd	+	+
N-Acetyl-D-glucosamine	d	–	nd	nd	w	d
cis-Aconitate	–	–	nd	nd	–	+
Adipate	–	–	nd	nd	w	+
4-Aminobutyrate	–	w	nd	nd	+	–
L-Azelate	–	–	nd	nd	–	w
D-Fructose	+	–	+	nd	–	+
D-Gluconate	+	+	nd	nd	–	+
i-Inositol	w	+	nd	nd	–	+
2-Oxoglutarate	–	–	nd	nd	+	+
L-Proline	w	w	nd	nd	+	+
Putrescine	+	–	nd	–	–	+
L-Suberate	w	–	nd	nd	w	+
Sucrose	+	+	–	–	w	+

[a]Symbols: +, positive; w, weakly positive; –, negative; d, delayed reaction; nd, not determined.

[b]Data taken from: Martin et al. (1997) (*Janibacter limosus*); Kämpfer et al. (2006) (*Janibacter anophelis*); Kageyama et al. (2007b) (*Janibacter corallicola*); Shivaji et al. (2009) (*Janibacter hoylei*); Yoon et al. (2004) (*Janibacter melonis*); and Lang et al. (2003) (*Janibacter terrae*).

[c]Data for *Janibacter limosus, Janibacter anopheles, Janibacter melonis* and *Janibacter terrae* from Kämpfer et al. (2006).

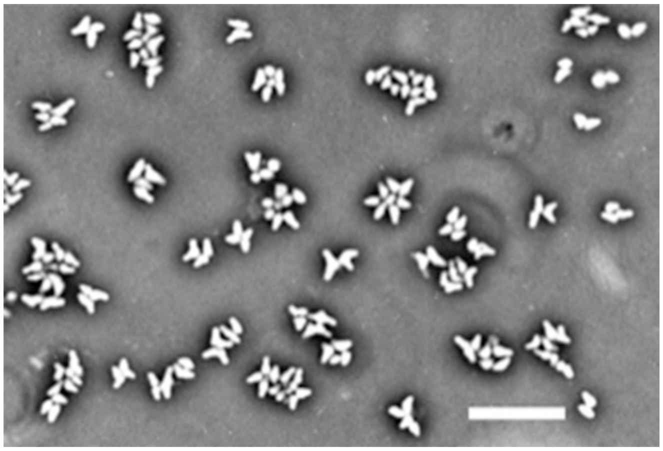

FIGURE 164. Cells of *Janibacter limosus* DSM 11140[T] grown on an agar slide for 12 h at 28°C. Bar = 10 μm. Photo scanned from negative.

et al., 2000). The two strains of *Janibacter melonis* are endophytic and are considered to cause abnormal spoilage in oriental melons (Yoon et al., 2004). The recently described type strain of *Janibacter anophelis* was isolated from the midgut of *Anopheles arabiensis* which may indicate an endosymbiontic life cycle (Kämpfer et al., 2006). The two strains of *Janibacter corallicola* (Kageyama et al., 2007b) derived from the hard coral *Acropora gemmifera* collected in the Angauru Coral Garden in Palau, a marine habitat. The type strain of *Janibacter hoylei* was isolated from an air sample collected at an altitude of 40–41.4 km.

Enrichment and isolation procedures

Strains of *Janibacter* grow readily in liquid or on solidified complex media, i.e. Bacto nutrient agar (Difco, g/l: Bacto beef extract, 3.0; Bacto peptone, 5.0; and Bacto agar, 15.0; pH 6.8), rich (R) medium (Yamada and Komagata, 1972) which is composed of (g/l): Bacto peptone (Difco), 10.0; yeast extract (Difco), 5.0; Casamino acids (Difco), 5.0; beef extract (Difco), 2.0; malt extract (Difco), 5.0; glycerol, 2.0; $MgSO_4 \cdot 7H_2O$, 1.0; Tween 80, 0.05; and agar, 20.0; pH 7.2, Bacto tryptic soy broth (Difco, g/l: Bacto tryptone, 17.0; Bacto soytone, 3.0; glucose, 2.5; NaCl, 5.0; and K_2HPO_4, 2.5; pH 7.3), and peptone-yeast extract-brain heart infusion medium (Yokota et al., 1993) containing (g/l): peptone, 10.0; yeast extract, 2.0; Bacto brain heart infusion (Difco), 2.0; NaCl, 2.0; and glucose, 2.0; pH 7.0, and GPM agar (Kageyama et al., 2007b) containing (g/l): glucose, 10.0; peptone, 5.0; meat extract, 5.0; NaCl, 3.0; and agar, 12.0; pH 7.0. The strains of *Janibacter limosus* were isolated from their special habitat on plate count agar (Difco, g/l: Bacto tryptone, 5.0; Bacto yeast extract, 2.5; Bacto glucose, 1.0; and Bacto agar, 15.0; pH 7.0) and those of *Janibacter melonis* on Bacto nutrient agar using standard dilution plate procedures. *Janibacter anophelis* was isolated by streaking the midgut suspension of the mosquito on agar plates containing LB agar (g/l: tryptone, 10; yeast extract, 5.0; NaCl, 10.0; glucose, 1.0; pH 7.0; and agar supplemented with $CaCl_2$ to a final concentration of 2.5 mM before pouring) (Lindh et al., 2005). In case of some strains of *Janibacter terrae*, special enrichment methods were applied using either dibenzofuran as sole source of carbon and energy (Lang et al., 2003) or incubating the isolation plates in a trichloroethylene enriched atmosphere (Imamura et al., 2000). The two strains of *Janibacter corallicola* were isolated from homogenized corals, suspended and diluted in sterile seawater, on MA + Ca medium which consists of Difco Marine Broth 2216 (Becton Dickinson), 3.74 g/l; filtrated seawater, 750 ml; distilled water with 1% $CaCO_3$, 250 ml; and agar, 15 g/l (Kageyama et al., 2007b). The type strain of *Janibacter hoylei* was isolated from an air sample collected at an altitude of 40–41.4 km using a balloon with a cryosampler. Under sterile laboratory conditions the air sample from the cryotube was sequentially filtered through a 0.45 μm filter and a 0.22 μm filter using a milipore filtration unit. The filters were cut into pieces and placed on agar plates containing nutrient agar, LB agar and minimal salt agar. Growth of strain PVAS[T] (*Janibacter hoylei*) was observed after 24 d of incubation at 30°C as a single colony on a minimal salt agar plate, which consisted of (per 100 ml) 20 ml of a 3.39% (w/v) sodium dihydrogen phosphate solution, 20 ml 1.5% (w/v) potassium phosphate, 20 ml 0.25% (w/v) NaCl, 20 ml 0.5% (w/v) ammonium chloride and 18 ml Millipore water. The final agar concentration was 2%. The medium was supplemented with 2 ml sterile 20% glucose and 0.2 ml sterile 0.1 M $MgSO_4$ solution; pH 7 (Shivaji et al., 2009).

Maintenance procedures

Cultures of *Janibacter* strains can be maintained by serial transfers on the complex organic media mentioned above. For long-term preservation, storage of liquid cultures supplemented with 5% dimethylsulfoxide is recommended in the vapor phase of liquid nitrogen (−140°C).

Differentiation of the genus *Janibacter* from other genera

The genus *Janibacter* shares with the genera *Knoellia* (Groth et al., 2002), *Kribbia* (Jung et al., 2006), *Marihabitans* (Kageyama et al., 2008a), *Oryzihumus* (Kageyama et al., 2005), *Phycicoccus* (Lee, 2006), and *Tetrasphaera* (Maszenan et al., 2000) the peptidoglycan type A1γ (Schleifer and Kandler, 1972), based on *meso*-A_2pm, and the menaquinone type MK-8(H_4). However, *Janibacter* can be distinguished from these genera by the compositions of cellular fatty acids and phospholipids together with differences in morphological, cultural, and physiological characteristics. Among the *Actinobacteria*, the common occurrence of the peptidoglycan type A1γ and the menaquinone type MK-8(H_4) is also reported for some other genera which do not possess mycolic acids. These are the genera *Nakamurella* (*ex* Yoshimi et al. 1996) Tao et al. 2004, *Dermatophilus* Van Saceghem 1915, *Kineosphaera* Liu et al. 2002, and *Pseudonocardia* (Henssen 1957) emend. Warwick et al. 1994 emend. Reichert et al. 1998 emend. Huang et al. 2002. While *Nakamurella* and *Pseudonocardia* are the type genera of their own families; *Nakamurellaceae* (*ex* Yoshimi et al. 1996) Tao et al. 2004 emend. Zhi et al. 2009 and *Pseudonocardiaceae* (Embley et al. 1988, 1989) emend. Stackebrandt et al. 1997 emend. Zhi et al. 2009, respectively, the genera *Dermatophilus* and *Kineosphaera* are members of the *Dermatophilaceae* (Austwick 1958) emend. Stackebrandt et al. 1997 emend. Stackebrandt and Schumann 2000 emend. Zhi et al. 2009. The phylogenetic separateness of these genera from *Janibacter* is supported by a broad spectrum of different phenotypic characteristics. The above-mentioned close phylogenetic neighbors *Terrabacter*, *Intrasporangium*, *Lapillicoccus*, and *Terracoccus* have LL-A_2pm as the diagnostic diamino acid in their peptidoglycan

and can additionally be distinguished from *Janibacter* by morphological and further chemotaxonomic characteristics listed in Table 125 (chapter *Intrasporangiaceae*).

Taxonomic comments

An emended description of the species *Janibacter terrae* (Yoon et al., 2000) was given by Lang et al. (2003) due to the inclusion of ten dibenzofuran-degrading strains and *Janibacter brevis* as its later heterotypic synonym. Both species *Janibacter terrae* and *Janibacter brevis* were independently described by two research groups (Yoon et al., 2000; Imamura et al., 2000). Lang et al. (2003) carried out DNA–DNA hybridization with the type strains of *Janibacter brevis* DSM 13953^T and *Janibacter terrae* DSM 13876^T. As the DNA–DNA similarity between these strains was about 80% and the 16S rRNA gene sequence similarity was found to be 99.9%, it became obvious that *Janibacter brevis* and *Janibacter terrae* should be considered as synonyms of one species. According to Rule 24b of the Bacteriological Code the name *Janibacter terrae* has priority (Lang et al., 2003; Lapage et al., 1992).

List of species of the genus *Janibacter*

1. **Janibacter limosus** Martin, Schumann, Rainey, Schuetze and Groth 1997, 533^{VP}

li.mo′sus. L. masc. adj. *limosus* muddy, pertaining to sludge, the natural habitat of the species.

See Table 127 and the generic description. Cells are coccoid (0.3–1.2 μm) or short rods (0.4 × 1.4 μm). Optimum growth temperature is 28°C. Tolerates up to 10% NaCl in the culture medium. Utilizes formate and malate. Hippurate, succinate, and DL-tartrate are not utilized. Casein and tyrosine are hydrolyzed; adenine, esculin, hypoxanthine, urea, and xanthine are not hydrolyzed. H_2S is produced; indole and acetoin are not produced. The strains are susceptible to the following antibiotics (μg/disc): chloramphenicol (30), ciprofloxacin (5), erythromycin (15), gentamicin (10), kanamycin (30), lincomycin (2), neomycin (30), nitrofuran (300), oxytetracycline (30), and polymyxin B (300 IU/disc); but are not susceptible to penicillin G (2 IU/disc), rifampin (2), and sulfonamide (300). The predominant cellular fatty acids are $C_{17:1}$ ω8*c*, $C_{17:0}$, $C_{18:1}$ ω9*c*, and $C_{16:0}$ iso.

DNA G+C content (mol%): 70 (HPLC).

Type strain: HKI 83, ATCC 700321, CCUG 37980, CIP 105276, DSM 11140, NBRC 16128, JCM 10980.

Sequence accession no. (16S rRNA gene): Y08539.

2. **Janibacter anophelis** Kämpfer, Terenius, Lindh and Faye 2006, 391^{VP}

a.no.phe′lis. N.L. gen. n. *anophelis* of a mosquito of the genus *Anopheles*; the strain was isolated from the midgut of *Anopheles arabiensis.*

See Table 127 and the generic description. Optimum growth temperature in Luria–Bertani (LB) broth is 35°C with a generation time of 44 min. Utilizes propionate, fumarate, glutarate, 3-hydroybutyrate, DL-lactate, L-malate, pyruvate, L-alanine, L-aspartate, L-histidine, L-leucine, and L-serine as sole carbon sources; the following carbon sources are not utilized: *N*-acetyl-D-galactosamine, L-arabinose, *p*-arbutin, D-cellobiose, D-galactose, melibiose, L-rhamnose, D-ribose, salicin, D-xylose, adonitol, maltitol, D-mannitol, sorbitol, *trans*-aconitate, citrate, itaconate, mesaconate, β-alanine, L-ornithine, L-phenylalanine, L-tryptophan, DL-3-hydroxybenzoate, DL-4-hydroxybenzoate, and L-phenylacetate. The predominant cellular fatty acids are $C_{16:0}$ iso, $C_{17:0}$, and $C_{17:1}$ ω8*c*.

DNA G+C content (mol%): not available.

Type strain: H2.16B, CCUG 49715, CIP 108728, JCM 21813.

Sequence accession no. (16S rRNA gene): AY837752.

3. **Janibacter corallicola** Kageyama, Takahashi, Yasumoto-Hirose, Kasai, Shizuri and Ōmura 2007c, 2449^{VP} (Effective publication: Kageyama, Takahashi, Yasumoto-Hirose, Kasai, Shizuri and Ōmura 2007b, 187.)

co.ral.li′co.la. L. n. *corallum* coral; L. suff. *-cola* inhabitant, dweller, N.L. n. *corallicola* coral-dweller.

See Table 127 and the generic description. Cells are cocci and vary in their diameter from 0.6–1.1 μm. Colonies are pale yellow. Growth occurs in a range of pH 5–9 and a temperature range of 16–38°C, optimum growth at pH 6–8 and temperatures 23–36°C. NaCl is tolerated up to 7% in the culture medium. D-Galactose and raffinose are used as sole carbon sources, but L-arabinose, D-mannitol, and xylose are not. Using the API ZYM system, activity is detected for esterase (C4), esterase lipase (C8), α-glucosidase, leucine arylamidase, and naphthol-AS-BI-phosphohydrolase. Weak reactions are displayed for lipase (C14), valine arylamidase, acid phosphatase, and β-glucosidase. Activities for cystine arylamidase and trypsin are variable. No activity is detected for alkaline phosphatase, chymotrypsin, α-galactosidase, β-galactosidase, β-glucuronidase, *N*-acetyl-β-glucosamidase, α-mannosidase, and α-fucosidase. The predominant cellular fatty acids are $C_{16:0}$ iso, $C_{17:0}$, $C_{17:1}$ ω8*c*, and $C_{18:1}$ ω9*c*.

DNA G+C content (mol%): 70–71 (HPLC).

Type strain: 04PA2-Co5-61, MBIC 08265, DSM 18906, JCM 15571.

Sequence accession no. (16S rRNA gene): AB286023.

4. **Janibacter hoylei** Shivaji, Chaturvedi, Begum, Pindi, Manorama, Ananth Padmanaban, Shouche, Pawar, Vaishampayan, Dutt, Datta, Manchanda, Rao, Bhargava and Narlikar 2009, 2981^{VP}

hoy′le.i. N.L. gen. masc. n. *hoylei* of Hoyle, named after Sir Fred Hoyle, the famous English astronomer.

See Table 127 and the generic description. Cells are cocci, 0.4–0.7 in diameter. Colonies on LB agar are creamish in color, entire, circular and 2 mm in diameter. Good growth on nutrient agar at 25–30°C. Grows in LB broth at 20–40°C and at pH 5–10, optimum growth at 30°C and pH 9. Grows in the presence of 5% (w/v) NaCl in LB broth, weak growth in LB broth containing 10% (w/v) NaCl. Oxidase-positive. H_2S and indole are not produced. Voges–Proskauer reaction is negative. Casein is hydrolyzed, Tween 80 and esculin are not hydrolyzed. D-malate, sodium benzoate, sodium citrate, sodium formate, sodium succinate, D-gluconate, inositol,

putrescine, sucrose and D-galactose are not utilized, but monomethyl succinate is utilized. The predominant cellular fatty acids are: $C_{16:0}$ iso, $C_{17:0}$ 10 methyl, $C_{17:1}$ ω9*c* and $C_{18:1}$ ω9*c*.

DNA G+C content (mol%): 72.8 (HPLC)

Type strain: PVAS-1, MTCC 8307, DSM 21601, CCUG 56714.

Sequence accession no. (16S rRNA gene): DQ317608

5. **Janibacter melonis** Yoon, Lee, Yeo and Choi 2004, 1979[VP]

me.lo′nis. L. gen. n. *melonis* of melon (*Cucumis melo*), referring to the fruit from which the organisms were isolated.

See Table 127 and the generic description. Cells are cocci (0.8–1.0 µm). Colonies on rich (R) medium are cream-colored and 1.5–3.0 mm in diameter. Optimum growth temperature is 30°C. Growth occurs at 10°C, but not at 4°C. In liquid brain heart infusion medium, 40°C is tolerated for growth; in rich (R) medium, no growth at 40°C. Optimal pH value for growth is around pH 7.0; no growth at pH 5.0. Utilizes citrate and formate; benzoate and succinate are not utilized. Tween 20, 40, 60, 80, esculin, casein, starch, and tyrosine are hydrolyzed. Hypoxanthine, xanthine, gelatin and urea are not hydrolyzed. H_2S, acetoin, and indole are not produced. The predominant cellular fatty acids are $C_{16:0}$ iso, $C_{17:1}$ ω8*c*, $C_{18:1}$ ω9*c*, or $C_{17:0}$.

DNA G+C content (mol%): 73 (HPLC).

Type strain: CM2104, DSM 16063, JCM 12321, KCTC 9987.

Sequence accession no. (16S rRNA gene): AY522568.

6. **Janibacter terrae** Yoon, Lee, Kang, Kho, Kang and Park 2000, 1826[VP] emend. Lang, Kroppenstedt, Swiderski, Schumann, Ludwig, Schmid and Weiss 2003, 2003.

ter′ra.e. L. gen. n. *terrae* of the earth.

See Table 127 and the generic description. Optimum growth temperature is in the range of 25–30°C. Maximum growth temperature in liquid rich (R) medium is 40°C. Growth occurs in liquid brain heart infusion medium in the presence of 10% NaCl. Casein, gelatin, and starch are hydrolyzed. Tween 80 and esculin are not hydrolyzed. H_2S may be produced from cysteine. DL-Malate and turanose are utilized, but cellobiose, *m*- or *p*-hydroxybenzoate, citrate, succinate, DL-tartrate, ethanolamine, histamine, sarcosine, spermine, β alanine, L-arginine, L-leucine, L-ornithine, and L-valine are not utilized. Negative results are also obtained for production of acetoin, indol, arginine dihydrolase, urease, and β-galactosidase. The predominant cellular fatty acids are $C_{16:0}$ iso, $C_{17:1}$ ω8*c*, and $C_{18:1}$ ω9*c*.

DNA G+C content (mol%): 69–73 (HPLC).

Type strain: CS12, ATCC BAA-130, CCUG 45369, CIP 107018, DSM 13876, JCM 10705, KCCM 80001

Sequence accession no. (16S rRNA gene): AF176948.

Genus V. **Knoellia** Groth, Schumann, Schuetze, Augsten and Stackebrandt 2002, 81[VP]

INGRID GROTH

Kno.el′li.a. N.L. fem. n. *Knoellia* named after Hans Knöll (1913–1978), a German pioneer in antibiotic research.

Cells are irregular **rods** (1.5–6.0 × 0.4–1.2 µm) and **cocci** (0.6–1.5 µm) (Figure 165). They occur singly, in pairs, short chains, or clusters. A marked **rod–coccus growth cycle** may be expressed (Figure 166). Gram-stain-positive, **not acid-fast**, **nonsporeforming**. **Nonmotile**. **Aerobic to microaerobic**. Colonies are smooth, circular, convex, opaque with entire margins, and white to cream in color. Good growth occurs on complex organic media between pH 5–9 and at temperatures of 28–35°C; no growth at 42°C. NaCl in the culture medium is tolerated up to 2 or 4%. **Oxidase-negative; catalase-positive**. The **peptidoglycan** is of the **A1γ type** based on *meso*-A_2pm, direct cross-linkage, which is in accordance with type A31. **The acyl type is acetyl**. No diagnostic whole cell sugars are found. The major menaquinone is **MK-8(H_4);** MK-7(H_4) and MK-6(H_4) may occur in traces. The **polar lipids** are mainly composed of **phosphatidylethanolamine, phosphatidylinositol, diphosphatidylglycerol;** phosphatidylglycerol may occur in minor amounts. The cellular **fatty acid profile** is characterized by the predominance of **iso-branched-chain acids** ($C_{15:0}$ iso, $C_{16:0}$ iso, $C_{17:0}$ iso, $C_{17:1}$ iso). Anteiso-branched ($C_{17:0}$ anteiso) and straight-chain saturated or monounsaturated acids may also occur in higher amounts ($C_{17:0}$, $C_{17:1}$ ω8*c*). **Mycolic acids are absent**. Based on 16S rRNA gene sequence comparison strains of *Knoellia* are most closely related to members of the genera *Janibacter*, *Lapillicoccus*, and *Tetrasphaera*. The 16S rRNA gene signature nucleotides that differentiate the genus from the other genera of the family *Intrasporangiaceae* are listed in Table 124, chapter *Intrasporangiaceae*.

DNA G+C content (mol%): 68–73.

Type species: **Knoellia sinensis** Groth, Schumann, Schuetze, Augsten and Stackebrandt 2002, 82[VP].

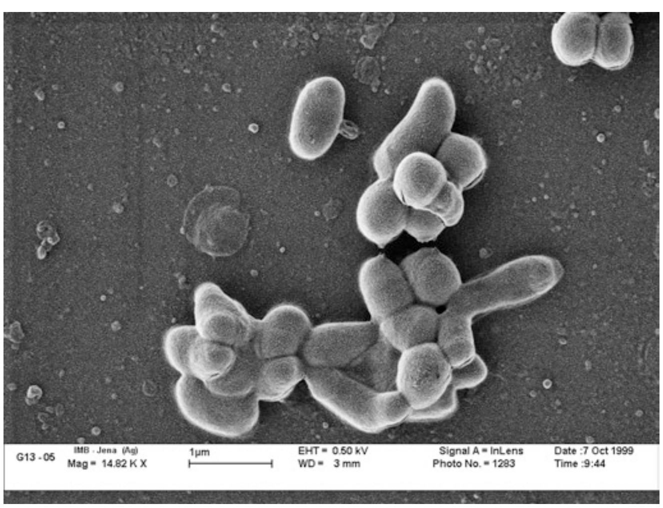

FIGURE 165. Scanning electron micrograph of cells from a 3-d-old culture of *Knoellia sinensis* DSM 12331[T] grown on rich (R) agar at 28°C. Bar = 1 µm.

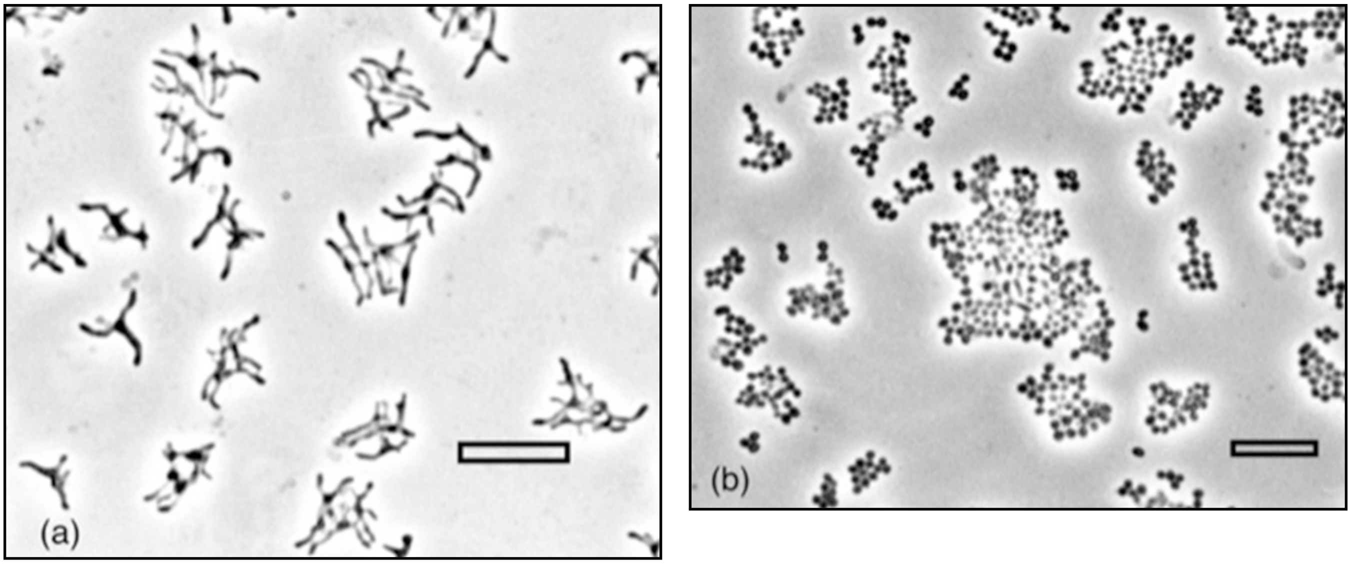

FIGURE 166. Micrographs of *Knoellia sinensis* DSM 12331[T] grown (a) on rich (R) agar at 28°C for 24 h and (b) in liquid rich (R) medium for 72 h. Bars = 10 μm.

Further descriptive information

In the 16S rRNA gene sequence tree of the *Intrasporangiaceae* (Figure 161), the strains of *Knoellia* form a common cluster with the species of *Janibacter* (Martin et al., 1997) and *Tetrasphaera* (Maszenan et al., 2000) with which they share 95.0–98.2% and 94.9–97.8% sequence similarities, respectively. The monospecific genus *Lapillicoccus* (Lee and Lee, 2007) forms a separate lineage adjacent to the *Janibacter-Knoellia-Tetrasphaera* cluster and is similar closely related (96.4%). Furthermore, the type strains of *Knoellia sinensis* and *Knoellia subterranea* possess a deviation from the 16S rRNA gene signature of members of the suborder *Micrococcineae* (elevated in this volume to order *Micrococcales*) that is shared with members of the genus *Tetrasphaera* (nt 952:1229, T–A base pair instead of C–G; Maszenan et al., 2000). The genus *Knoellia* currently accommodates three species, *Knoellia sinensis* (Groth et al., 2002), *Knoellia aerolata* (Weon et al., 2007a), and *Knoellia subterranea* (Groth et al., 2002). Each species is represented by a single strain. While *Knoellia sinensis* and *Knoellia subterranea* were isolated from soil samples collected at different sites of a cave, *Knoellia aerolata* was obtained from an air sample collected in Korea. The type strain of *Knoellia sinensis* shares 16S rRNA gene sequence similarities of 97.8 and 98.8%, respectively, with the type strains of *Knoellia aerolata* and *Knoellia subterranea*. Low levels of DNA–DNA relatedness (29.2, 37.0, and 41.0%) and numerous differences in physiological characteristics (Table 128) between the three organisms clearly indicate that they represent separate species.

The type strains of *Knoellia sinensis* and *Knoellia subterranea* are morphologically very similar. Their colonies grown on rich (R) medium (Yamada and Komagata, 1972) vary in size from 0.4–1.7 mm. The coccoid cells of the stationary growth phase grow out with several germ tubes (up to 6) to irregular rods after transfer to fresh medium. As the cocci remain in their positions within the chains or clusters during the outgrowth, the impression of a rudimentary mycelium is given. The occurrence of a comparable rod–coccus growth cycle is not reported in the description of *Knoellia aerolata* (Weon et al., 2007a).

The type strains of the three species share the following physiological properties. They are positive for reduction of nitrate, hydrolysis of esculin, starch and Tween 80, but negative for hydrolysis of hypoxanthine and xanthine and for the production of indole. By using the API 20NE and API ID 32GN test strips (bioMérieux), the three strains show positive reactions for the utilization of sodium acetate, D-glucose, maltose, sucrose, glycogen, salicin, valeric acid, 3-hydroxy-butyric acid, propionic acid, and L-proline as sole carbon sources, while fermentation of glucose, production of arginine dihydrolase, and utilization of L-arabinose, capric acid, trisodium citrate, phenylacetic acid, L-rhamnose, itaconic acid, suberic acid, sodium malonate, potassium 5-ketogluconate, 3-hydroxybenzoic acid, D-melibiose, L-fucose, potassium 2-ketogluconate, and 4-hydroxybenzoic acid are negative (Weon et al., 2007a). By using the API ZYM system (bioMérieux), positive reactions are obtained for the production of alkaline phosphatase, esterase (C4), esterase lipase (C8), leucine arylamidase, α-glucosidase, and β-glucosidase, but negative results for the production of trypsin, chymotrypsin, α-galactosidase, *N*-acetyl-β-glucosamidase, α-mannosidase, and α-fucosidase. The production of β-galactosidase seems to be variable. Weon et al. (2007a) obtained positive results for the type strains of the three species using API 20NE test strips, while Groth et al. (2002) obtained negative results for *Knoellia sinensis* and *Knoellia subterranea* by using the API ZYM assay. The type strains of *Knoellia sinensis* and *Knoellia subterranea* produce H_2S, but not acetoin; they hydrolyze hippurate (weakly), but not adenine. They produce acid from D-cellobiose, D-fructose, D-galactose, D-glucose, maltose, D-mannose, and D-ribose, but not from L-arabinose, D-glucitol, glycerol, inulin, lactose, D-mannitol, D-raffinose, L-rhamnose, salicin, or D-xylose. They utilize succinate as sole carbon source, but not aconitate, benzoate, citrate, and DL-tartrate. Both strains are susceptible to the following

TABLE 128. Characteristics differentiating species of the genus *Knoellia*[a]

Characteristic	*K. sinensis* DSM 12331^T	*K. aerolata* DSM 18566^T	*K. subterranea* DSM 12332^T
Growth at 37°C[b,c]	−	−	+
Decomposition of[b,c]:			
Casein	−	−	+
Gelatin	−[d]	+	+
Tyrosine	+	−	+
Urea	−	−	w[d]
Susceptibility to ampicillin (10 µg/disc)[b]	+	nd	−
Acid production from:[b]			
Dextrin	−	nd	+
Starch	−	nd	+
Sucrose	+	nd	−
Trehalose	−	nd	w
Utilization of:			
Formate[b]	−	nd	w
API 20NE /API ID 32GN test strips:[c]			
N-Acetylglucosamine	−	+	+
Adipic acid	+	−	−
L-Alanine	−	+	+
L-Histidine	+	−	+
Inositol	−	+	−
Lactic acid	+	−	+
Malic acid	−	−	+
D-Mannitol	−	+	+
D-Mannose	−	+	+
Potassium gluconate	−	+	−
D-Ribose	−	−	+
L-Serine	+	−	+
D-Sorbitol	−	+	+
BIOLOG test results:[b]			
Adenosine	−	nd	+
D-Alanine	−	nd	+
L-Alanyl glycine	−	nd	+
Glucose 1-phosphate	+	nd	−
Glucose 6-phosphate	+	nd	−
γ-Hydroxybutyric acid	+	nd	−
p-Hydroxyphenyl acetic acid	+	nd	−
D-Lactic acid methyl ester	+	nd	−
D-Mannitol	−	nd	+
Succinamic acid	−	nd	+
Xylitol	−	nd	+

[a]Symbols: +, positive; w, weakly positive; −, negative; nd, not determined.

[b]Data from Groth et al. (2002).

[c]Data from Weon et al. (2007a).

[d]Conflicting results reported by Weon et al. (2007a).

antibiotics (µg/disc): chloramphenicol (30), ciprofloxacin (5), erythromycin (15), gentamicin (10), kanamycin (30), lincomycin (2), neomycin (30), nitrofuran (300), oxytetracycline (30), penicillin G (2 IU/disc), polymyxin B (300 IU/disc), rifampin (2), streptomycin (10), and sulfonamide (300).

Enrichment and isolation procedures

The strains of *Knoellia sinensis* and *Knoellia subterranea* grow readily in liquid or on solidified complex media, i.e. Bacto nutrient agar (Difco, g/l: Bacto beef extract, 3.0; Bacto peptone, 5.0; and Bacto agar, 15.0; pH 6.8), rich (R) medium (Yamada and Komagata, 1972) which is composed of (g/l): Bacto peptone (Difco), 10.0; yeast extract (Difco), 5.0; Casamino acids (Difco), 5.0; beef extract (Difco), 2.0; malt extract (Difco), 5.0; glycerol, 2.0; $MgSO_4 \cdot 7H_2O$, 1.0; Tween 80, 0.05; and agar, 20.0; pH 7.2, Bacto tryptic soy broth (Difco, g/l: Bacto tryptone, 17.0; Bacto soytone, 3.0; glucose, 2.5; NaCl, 5.0; and K_2HPO_4, 2.5; pH 7.3,) and peptone-yeast extract-brain heart infusion medium (Yokota et al., 1993) containing (g/l): peptone, 10.0; yeast extract, 2.0; Bacto brain heart infusion (Difco), 2.0; NaCl, 2.0; and glucose, 2.0; pH 7.0. The type strain of *Knoellia sinensis* was isolated on casein mineral medium (Altenburger et al., 1996) containing (g/l): K_2HPO_4, 0.6; $Na_2HPO_4 \cdot 2H_2O$, 0.5; $MgSO_4 \cdot 7H_2O$, 0.05; $MgCl_2 \cdot 7H_2O$, 0.1; KNO_3, 0.2; $FeCl_3 \cdot 6H_2O$, 0.01; casein, 0.8 and yeast extract 0.4; pH 7.0, and that of *Knoellia subterranea* on peptone-yeast extract-brain heart infusion medium using standard dilution plate procedures. The type strain of *Knoellia aerolata* was isolated on R2A agar (Difco, g/l: Bacto yeast

extract, 0.5; Bacto proteose peptone no. 3, 0.5; Bacto Casamino acids, 0.5; glucose, 0.5; soluble starch, 0.5; sodium pyruvate 0.3; potassium phosphate dibasic, 0.3; magnesium sulfate, 0.05; and Bacto agar, 15.0; pH 7.2) by using an air sampler. For subculturing of *Knoellia aerolata*, R2A agar is recommended, but the strain grows also well on Bacto nutrient agar and Bacto tryptic soy agar.

Maintenance procedures

Cultures of *Knoellia* strains can be maintained by serial transfers on the complex organic media mentioned above. For long-term preservation, storage of liquid cultures supplemented with 5% dimethylsulfoxide is recommended in the vapor phase of liquid nitrogen (−140°C).

Differentiation of the genus *Knoellia* from other genera

The genus *Knoellia* has in common with the genera *Janibacter* (Martin et al., 1997), *Kribbia* (Jung et al., 2006), *Marihabitans* (Kageyama et al., 2008a), *Oryzihumus* (Kageyama et al., 2005), *Phycicoccus* (Lee, 2006), and *Tetrasphaera* (Maszenan et al., 2000) the presence of *meso*-A_2pm, in the cell-wall peptidoglycan and MK-8(H_4) as main menaquinone, while the other members of the *Intrasporangiaceae* possess either LL-A_2pm, or L-ornithine, as diagnostic diamino acids. However, the genus *Knoellia* can be readily distinguished from the phylogenetically closest genus *Janibacter* by differences in the cellular fatty acid profiles and in the composition of polar lipids (Table 125, chapter *Intrasporangiaceae*). Furthermore, a rod–coccus growth cycle similar to that of *Knoellia* is not observed in *Janibacter* species. Physiological characteristics like acid production from selected carbon sources and tolerance to NaCl additionally contribute to differentiate these two genera from one another. The slightly less closely related genus *Tetrasphaera* can be distinguished from *Knoellia* by the occurrence of *meso*- or 3-hydroxy-A_2pm in the peptidoglycan and by differences in the fatty acid profiles. Morphological characteristics which referred to the coccoid cells in tetrads of the originally described *Tetrasphaera* species, *Tetrasphaera japonica* and *Tetrasphaera australiensis* (Maszenan et al., 2000), can generally no longer be used to distinguish these two genera from one another. With the description of further *Tetrasphaera* species, the variability in cell morphology within this genus has become obvious (Hanada et al., 2002; Ishikawa and Yokota, 2006; McKenzíe et al., 2006). The remaining genera of the *meso*-A_2pm containing group, *Kribbia*, *Marihabitans*, *Oryzihumus*, and *Phycicoccus* can also be well separated from *Knoellia* by their phylogenetic distinctiveness (16S rRNA gene sequence similarities below 96% and differences in the 16S rRNA gene signature nucleotides) together with the phenotypic characteristics listed in Table 125 (chapter *Intrasporangiaceae*). Interestingly, the genus *Lapillicoccus* (Lee and Lee, 2007), which shares with *Knoellia* a 16S rRNA gene sequence similarity of 96.4%, a value similar to that of *Janibacter* species, possesses LL-A_2pm as diagnostic diamino acid in the peptidoglycan. This characteristic, together with the missing phosphatidylethanolamine in the polar lipid pattern and the typical coccoid cell morphology, clearly distinguish *Lapillicoccus* from *Knoellia*. As the genera *Kribbia*, *Lapillicoccus*, *Marihabitans*, and *Oryzihumus*, are represented by only one species, the variability of the phenotypic markers within the genus cannot be reflected by the current descriptions. The availability of further strains of these genera will make the differentiation between the genera more efficient.

List of species of the genus *Knoellia*

1. **Knoellia sinensis** Groth, Schumann, Schütze, Augsten and Stackebrandt 2002, 82[VP]

si.nen′sis. N.L. fem. adj. *sinensis* of China, the origin of the type strain.

See Table 128 and the generic description. Rod-shaped cells vary in their dimensions from 1.7–4.5 × 0.4–0.9 µm and the cocci from 0.6–0.9 µm. By using the API ZYM system, activity is detected for valine arylamidase and cystine arylamidase. No activity is detected for β-glucuronidase. Weak reactions are displayed for lipase (C14), naphthol-AS-BI-phosphohydrolase, and acid phosphatase. The predominant cellular fatty acids are $C_{17:1}$ iso, $C_{15:0}$ iso, $C_{16:0}$ iso, $C_{17:0}$ iso, and $C_{17:0}$ anteiso.

DNA G+C content (mol%): 68 (HPLC).

Type strain: HKI 0119, CCUG 47139, CIP 106775, DSM 12331, JCM 11536, NBRC 100350.

Sequence accession no. (16S rRNA gene): AJ294412.

2. **Knoellia aerolata** Weon, Kim, Schumann, Kroppenstedt, Noh, Park and Kwon 2007a, 2863[VP]

a.e.ro.la′ta. Gr. n. *aer* air; L. part. adj. *latus*, *-a*, *-um* carried; N.L. fem. part. adj. *aerolata* airborne.

See Table 128 and the generic description. Rod-shaped cells vary in their dimensions from 1.0 × 1.5–2.0 µm and the cocci from 1.0–1.5 µm. Optimal growth at 30°C, pH 6.0–7.0 and up to1% NaCl. Chitin from crab shells, pectin, carboxymethylcellulose, and DNA are not hydrolyzed. By using the API ZYM system, activity is detected for β-glucuronidase. No activity is detected for cystine arylamidase, lipase (C14), naphthol-AS-BI-phospho-hydrolase, valine arylamidase, and acid phosphatase. The predominant cellular fatty acids are: $C_{16:0}$ iso, $C_{17:1}$ ω8*c*, and $C_{15:0}$ iso.

DNA G+C content (mol%): 73 (HPLC).

Type strain: 5317S-21, DSM 18566, KACC 20583.

Sequence accession no. (16S rRNA gene): EF553529.

3. **Knoellia subterranea** Groth, Schumann, Schütze, Augsten and Stackebrandt 2002, 82[VP]

sub.ter.ra′ne.a. L. fem. adj. *subterranea* under the earth, referring to the place of isolation.

See Table 128 and the generic description. Rod-shaped cells vary in their dimensions from 1.9–6.0 × 0.5–1.2 µm and the cocci from 0.8–1.4 µm. By using the API ZYM system, activity is detected for valine arylamidase, cystine arylamidase, and naphthol-AS-BI-phosphohydrolase. No activity is detected for β-glucuronidase. Weak reactions are displayed for lipase (C14) and acid phosphatase. The predominant cellular fatty acids are: $C_{15:0}$ iso, $C_{17:0}$ iso, $C_{17:0}$ anteiso, and $C_{16:0}$ iso.

DNA G+C content (mol%): 69 (HPLC).

Type strain: HKI 0120, CCUG 48178, CIP 106776, DSM 12332, JCM 11537, NBRC 100351.

Sequence accession no. (16S rRNA gene): AJ294413.

Genus VI. **Kribbia** Jung, Kim, Song, Lee, Oh and Yoon 2006, 2430[VP]

Jung Hoon Yoon

Krib.bi′a. N.L. fem. n. *Kribbia* arbitrary name formed from the acronym of the Korea Research Institute of Bioscience and Biotechnology, KRIBB, where taxonomic studies of this taxon were performed.

Cells are irregular short rods or cocci, 0.4–0.6 µ × 1.0–1.5 µm. Gram-stain-positive. **Nonmotile**. **Facultatively anaerobic**. Catalase-positive. Oxidase-negative. Nitrate is reduced to nitrite. **The cell-wall peptidoglycan contains *meso*-diaminopimelic acid**. **The predominant menaquinone is MK-8(H_4)**. The cellular fatty acid profile consists of straight-chain, branched, unsaturated, and 10-methyl fatty acids.

DNA G+C content (mol%): 69–70.

Type species: **Kribbia dieselivorans** Jung, Kim, Song, Lee, Oh and Yoon 2006, 2430[VP].

Further descriptive information

Kribbia dieselivorans is the sole species of this genus. *Kribbia dieselivorans* was isolated from an enrichment culture with diesel oil-degradation activity. Two strains of *Kribbia dieselivorans* were isolated by plating the enrichment culture on 10× diluted nutrient agar and R2A agar, respectively. *Kribbia dieselivorans* was found to have the ability to degrade diesel oil. *Kribbia dieselivorans* grows well on a wide range of media including nutrient agar, trypticase soy agar, and R2A agar. Characteristics are given in detail in the species description or are shown in Table 129. Phylogenetically, the genus *Kribbia* is a member of the family *Intrasporangiaceae* (Jung et al., 2006) within the order *Micrococcales*, class *Actinobacteria*. In the phylogenetic trees constructed using the neighbor-joining, maximum-likelihood, and maximum-parsimony algorithms, the genus *Kribbia* forms an independent lineage within the clade comprising members of the family *Intrasporangiaceae*. The 16S rRNA gene sequence similarity values between the genus *Kribbia* and phylogenetically related genera (for type strains of species) are as follows: *Arsenicicoccus* (95.5%), *Humibacillus* (95.6%), *Humihabitans* (95.8%), *Intrasporangium* (95.6%), *Janibacter* (95.4–96.1%), *Knoellia* (94.9–95.0%), *Lapillicoccus* (95.3%), *Ornithinicoccus* (94.8%), *Ornithinimicrobium* (94.3–94.8%), *Oryzihumus* (94.2%), *Phycicoccus* (93.6–94.2%), *Sanguibacter* (93.2–94.0%), *Serinicoccus* (94.2%), *Terrabacter* (95.7–96.0%), *Terracoccus* (95.6%), and *Tetrasphaera* (93.8–94.5%).

Enrichment and isolation procedures

The genus *Kribbia* was isolated from oil-contaminated tidal flat sediment collected from Kwangyang in Korea. The sediment samples (each 0.5 mg) were inoculated in 100 ml of Bushnell-Haas broth (Difco) that contained 2% (w/v) diesel oil as a single carbon source. These media were incubated at 30°C on a horizontal shaker at 150 rpm. The enrichment culture **with diesel oil-degradation activity** was diluted serially, spread on 10× diluted nutrient agar and R2A agar, and **incubated at 30°C**. No selective media or enrichment procedures have yet been described which are specific for organisms of this genus. The genus *Kribbia* may grow on a wide range of media. If similar samples are available, isolation of the genus *Kribbia* may be achieved by the oil-enrichment and the dilution plating methods using a wide range of media.

Maintenance procedures

For short-term preservation, serial transfer from agar slants of appropriate media is recommended. Agar slants can be kept at 4°C for at least 2 months. For long-term preservation, lyophilization and storage in liquid nitrogen or in frozen glycerol suspensions are suitable. For lyophilization, the cell mass is suspended in an appropriate fluid such as 20% (w/v) skim milk. For storage in liquid nitrogen, cell mass is inoculated into cryo-tubes containing an appropriate fluid such as 20% (w/v) glycerol. Glycerol suspension is prepared by making suspension with cell mass in aqueous glycerol in appropriate vial or tube. The vial or tube is stored at −20°C or −70°C.

Differentiation of the genus *Kribbia* from other genera

The genus *Kribbia* and most genera belonging to the family *Intrasporangiaceae* have MK-8(H_4) as the predominant menaquinone, but the genus *Intrasporangium* has MK-8 as the predominant menaquinone (Jung et al., 2006; Kageyama et al., 2007a, 2008b; Lee and Lee, 2007). The genus *Kribbia* is distinguishable from members of the family *Intrasporangiaceae* by differences in the diamino acid type in position 3 of the peptidoglycan and the fatty acid profile (Table 129). The genus *Kribbia* contains *meso*-diaminopimelic acid as the diamino acid in the position 3 of the peptidoglycan, whereas the genera *Arsenicicoccus*, *Humihabitans*, *Humibacillus*, *Intrasporangium*, *Lapillicoccus*, *Terrabacter*, and *Terracoccus* and the genera *Ornithinicoccus*, *Ornithinimicrobium*, and *Serinicoccus* contain LL-diaminopimelic acid and L-ornithine, respectively, as the diamino acid in the

TABLE 129. Characteristics differentiating the genus *Kribbia* from the other meso-A_2pm-containing genera of the family *Intrasporangiaceae*[a]

Characteristics[b]	*Kribbia*	*Janibacter*	*Knoellia*	*Oryzihumus*	*Phycicoccus*
Cell morphology	Irregular short rods or cocci	Coccoid to rod-shaped	Irregular rods and cocci	Irregular rods	Cocci
Major fatty acid (>10% of total fatty acids)	$C_{18:0}$ 10 methyl, $C_{16:0}$ iso, $C_{18:1}$ ω9*c*, $C_{16:0}$, $C_{18:0}$	$C_{16:0}$ iso, $C_{17:1}$ ω8*c*, $C_{18:1}$ ω9*c*, $C_{17:0}$	$C_{15:0}$ iso, $C_{16:0}$ iso	$C_{16:0}$ iso, $C_{15:0}$ iso, $C_{14:0}$ iso	$C_{15:0}$ iso, $C_{16:0}$ iso
DNA G+C content (mol%)	69–70	69–73	68–73	72–73	71–74

[a]Abbreviation: *meso*-A_2pm, *meso*-diaminopimelic acid.

[b]Data taken from Martin et al. (1997), Imamura et al. (2000), Yoon et al. (2000, 2004, 2008), Groth et al. (2002), Kageyama et al. (2005), Jung et al. (2006), Kämpfer et al. (2006), Lee (2006), and Weon et al. (2007a).

position 3 of the peptidoglycan (Jung et al., 2006; Kageyama et al., 2008b, 2007a; Lee and Lee, 2007). The fatty acid profiles, particularly major fatty acids, distinguish the genus *Kribbia* from *meso*-diaminopimelic acid-containing genera of the family *Intrasporangiaceae* (Table 129). The genus *Kribbia* is also distinguishable from members of the genus *Tetrasphaera* by differences in the predominant menaquinone type and/or the fatty acid profile (McKenzíe et al., 2006).

List of species of the genus *Kribbia*

1. **Kribbia dieselivorans** Jung, Kim, Song, Lee, Oh and Yoon 2006, 2430[VP]

die.se.li.vo′rans. N.L. n. *dieselum* diesel; L. v. *vorare* to devour; N.L. part. adj. *dieselivorans* diesel oil-devouring.

Cells are irregular short rods or cocci, 0.4–0.6 μm × 1.0–1.5 μm. Gram-stain-positive. Nonmotile. Facultatively anaerobic. Catalase-positive. Oxidase-negative. Urease-negative. Growth occurs between 8 and 42°C with an optimum temperature of 30°C. Optimal pH for growth is 6.5–7.5. Anaerobic growth occurs on TSA and on TSA with nitrate. Casein, gelatin, esculin, Tween 20, Tween 40, Tween 60, and Tween 80 are hydrolyzed. Starch, hypoxanthine, xanthine, and tyrosine are not hydrolyzed. Arginine dihydrolase, lysine decarboxylase, and ornithine decarboxylase are absent. H_2S and indole are not produced. Nitrate is reduced to nitrite. Acetate, benzoate, D-cellobiose, and pyruvate are utilized as sole carbon and energy sources. D-Glucose, D-trehalose, sucrose, and maltose are weakly utilized. D-Fructose, D-galactose, D-mannose, D-xylose, L-arabinose, citrate, formate, salicin, succinate, L-glutamate, and L-malate are not utilized as sole carbon and energy sources. Acid is produced from D-glucose, D-cellobiose, sucrose, maltose, and D-trehalose. Acid is not produced from D-sorbitol, *myo*-inositol, D-ribose, D-fructose, D-mannitol, melibiose, L-arabinose, D-melezitose, D-galactose, L-rhamnose, lactose, and D-raffinose. Susceptible to streptomycin, penicillin G, chloramphenicol, ampicillin, cephalothin, gentamicin, novobiocin, tetracycline, lincomycin, oleandomycin, neomycin, and carbenicillin, but not to polymyxin B and kanamycin. In assays with the API ZYM system (bioMérieux), esterase (C4), esterase lipase (C8), leucine arylamidase, naphthol-AS-BI-phosphohydrolase, β-galactosidase, α-glucosidase, and β-glucosidase are present, but alkaline phosphatase, lipase (C14), valine arylamidase, cystine arylamidase, trypsin, α-chymotrypsine, acid phosphatase, α-galactosidase, β-glucuronidase, *N*-acetyl-β-glucosaminidase, α-mannosidase, and α-fucosidase are absent. The major fatty acids (> 10% of total fatty acids) are $C_{18:0}$ 10 methyl, $C_{16:0}$ iso, $C_{18:1}$ ω9*c*, $C_{16:0}$, and $C_{18:0}$.

Source: a tidal flat sediment.

DNA G+C content (mol%): 69.6–69.9 (HPLC).

Type strain: N113, JCM 13585, KCTC 19143.

Sequence accession no. (16S rRNA gene): DQ372707.

Genus VII. **Lapillicoccus** Lee and Lee 2007, 2796[VP]

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La.pil.li.coc′cus. L. masc. n. *lapillus* a little stone; N.L. masc. n. *coccus* (from Gr. masc. n. *kokkos* grain, seed) coccus; N.L. masc. n. *Lapillicoccus* a coccus attached to a little stone, referring to the isolation source.

Nonsporeforming cocci (0.2–0.3 μm) that occur singly or in pairs. **Gram-stain-positive.** Nonmotile. **Aerobic**. Temperature range for growth 10–37°C, pH range 4.1–11.1, NaCl tolerance 0–2%. Cell wall contains LL-diaminopimelic acid as the diagnostic diamino acid. The acyl type of the muramic acid is acetylated. Mycolic acids are not present. MK-8(H_4) is the major menaquinone. Polar lipid profile comprises diphosphatidylglycerol and phosphatidylinositol.

DNA G+C content (mol%): 74.1.

Type species: **Lapillicoccus jejuensis** Lee and Lee 2007, 2796[VP].

Further descriptive information

Major fatty acids are $C_{16:0}$ iso (21.2%), $C_{17:1}$ ω8*c* (15.5%) and $C_{15:0}$ iso (13.7%).

Phylogenetic analysis of the 16S rRNA gene positions the genus within family *Intrasporangiaceae*. The closest phylogenetic neighbor is *Tetrasphaera japonica* (97.1% sequence similarity) (Maszenan et al., 2000). Environmental clones with high 16S rRNA gene sequence similarity have been detected in siliceous sedimentary rock (99.1%, accession no. AB179506, unpublished) and Mn-oxidizing microbiota associated with Mn nodules in rice field soils (98.8%, accession no. AB354142, unpublished).

Enrichment and isolation procedures

Strain R-Ac013[T] was isolated from a small stone collected from an agricultural field in Jeju, Republic of Korea. A piece of the stone was crushed into a powder (using a pestle) and suspended in 10 ml sterilized, distilled water. Serial diluents of the sample are transferred onto starch-casein agar (Kuester and Williams, 1964), and the plates are incubated at 30°C for 14 d. Colony morphology and pigmentation is visible after 7 d incubation on trypticase soy agar (TSA; Difco) at 28°C.

Maintenance procedures

Stock cultures are maintained as a 20% glycerol suspension at −20 and −80°C.

Differentiation of the genus *Lapillicoccus* from closely related genera

Lapillicoccus contains the diamino acid LL-DAP, while other phylogenetically related genera (*Tetrasphaera*, *Janibacter*, *Knoellia*,

Oryzihumus, and *Fodinibacter*) contain *meso*-DAP. *Intrasporangium* contains LL-DAP, but hyphal growth and possession of MK-8 as the major menaquinone differentiates it from *Lapillicoccus*. *Terrabacter* has long rod-shaped cell morphology and a rod–coccus life cycle, while *Lapillicoccus* has coccoid shape. The presence of phosphatidylethanolamine as the diagnostic phospholipid and absence of $C_{17:1}$ ω8*c* as a major fatty acid further distinguishes both *Terrabacter* and *Terracoccus*.

List of species of the genus *Lapillicoccus*

1. **Lapillicoccus jejuensis** Lee and Lee 2007, 2797[VP]

je.ju.en′sis. N.L. masc. adj. *jejuensis* of Jeju, Republic of Korea, referring to the site from which the type strain was isolated.

Colonies are circular, flat, undulate and bright yellow in color. Growth occurs at mesophilic temperature and pH 4.1–11.1, with optima at 30°C and pH 7.1. β-Galactosidase is present, but urease and arginine dihydrolase are not. Nitrate is not reduced to nitrite. Esculin is degraded, but indole production, glucose fermentation and gelatin hydrolysis does not occur. API ZYM detects leucine arylamidase, acid phosphatase, aglucosidase, *N*-acetyl-β-glucosaminidase, esterase lipase (C8) and α-chymotrypsin, but not alkaline phosphatase, esterase (C4), lipase (C14), valine arylamidase, cystine arylamidase, trypsin, naphthol-AS-BI-phosphohydrolase, α-galactosidase, β-glucosidase, β-glucuronidase, α-mannosidase and α-fucosidase. D-Arabinose, D-mannitol and malate are assimilated. Growth is not affected by D-glucose, D-mannose, *N*-acetyl-D-glucosamine, maltose, gluconate, caprate, adipate, citrate and phenylacetate. Positive for protease, cellulase and amylase activity. DNA is hydrolyzed. Hypoxanthine, tyrosine and xanthine are degraded.

For all other characteristics, refer to the genus description.

DNA G+C content (mol%): 74.1 (HPLC).

Type strain: R-Ac013, KCTC R-Ac013, DSM 18607.

Sequence accession no. (16S rRNA gene): AM398397.

Genus VIII. **Ornithinicoccus** Groth, Schumann, Martin, Schuetze, Augsten, Kramer and Stackebrandt 1999, 1722[VP]

Ingrid Groth

Or.ni.thi.ni.coc′cus. N.L. n. *ornithinum* ornithine (an amino acid named after the Gr. n. *ornithos*, bird); Gr. masc. n. *kokkos* a grain; N.L. masc. n. *Ornithinicoccus* a coccus with ornithine.

Cells are irregular **cocci** (0.8–1.3 μm) that occur singly, in pairs, short chains, and clusters (Figure 167). Gram-stain-positive, not acid-fast, nonsporeforming. **Nonmotile. Aerobic to microaerobic**. Colonies are smooth, circular, opaque with entire margins and white to cream in color when grown on rich (R) medium. Additionally, colonies may appear that have a depressed center which is surrounded by a uniform or radial structured wall and a wrinkled marginal zone. Colonies vary in size from 0.6–4.3 mm. Good growth occurs on complex organic media at temperatures 28–37°C; no growth at 42°C. NaCl in the culture medium is well tolerated up to 4%. **Oxidase-negative; catalase-positive**. The **peptidoglycan type is A4β** with an L-Orn–$Gly_{(1,2)}$–D-Glu interpeptide bridge which is in accordance with variation A21.13. **The acyl type is acetyl**. No diagnostic whole cell sugars are found. The major menaquinone is **MK-8(H_4);** MK-8(H_2) is present in minor amounts. The polar lipids are composed of **phosphatidylinositol, phosphatidylglycerol, diphosphatidylglycerol, phosphatidylserine**, and two unknown phospholipids. The cellular fatty acid profile is distinctly dominated by the occurrence of **iso- and anteiso-branched-chain acids**. Mycolic acids are absent. Based on 16S rRNA gene sequence comparison, the genus *Ornithinicoccus* is most closely related to members of the genera *Humihabitans* and *Oryzihumus*. The 16S rRNA gene signature nucleotides that differentiate the genus from the other genera of the family *Intrasporangiaceae* are listed in Table 124.

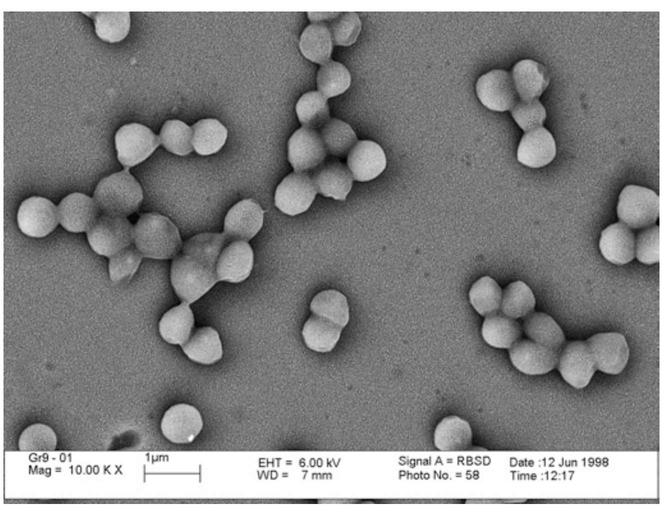

FIGURE 167. Scanning electron micrograph of cells from a 24-h-old culture of *Ornithinicoccus hortensis* DSM 12335[T] grown on rich (R) agar for 24 h.

DNA G+C content (mol%): 72.

Type species: **Ornithinicoccus hortensis** Groth, Schumann, Martin, Schuetze, Augsten, Kramer and Stackebrandt 1999, 1723[VP].

Further descriptive information

The genus *Ornithinicoccus* currently accommodates the sole species *Ornithinicoccus hortensis* (Groth et al., 1999). *Ornithinicoccus* forms a common branch with the monospecific genus

Oryzihumus (Kageyama et al., 2005) in the 16S rRNA gene tree of the *Intrasporangiaceae*, but the branching point is not supported by a high bootstrap value (Figure 161). The 16S rRNA gene sequence similarity between *Ornithinicoccus hortensis* and the strains of *Oryzihumus* is 95.6%. A slightly higher sequence similarity (95.8%) is found between *Ornithinicoccus* and the equally monospecific genus *Humihabitans* (Kageyama et al., 2007a). Furthermore, a comparable level of 16S rRNA gene sequence similarity (95.0–95.8%) is also present between *Ornithinicoccus hortensis* and *Intrasporangium calvum* (95.4%) (Kalakoutskii et al., 1967), two of the three species of *Ornithinimicrobium* (*Ornithinimicrobium kibberense*, 95.2%; *Ornithinimicrobium pekingense*, 95.1% (Liu et al., 2008; Mayilraj et al., 2006), two of the three species of *Knoellia* (*Knoellia sinensis*, 95.5%; *Knoellia subterranea*, 95.0%) (Groth et al., 2002), three of the five species of *Janibacter* (Martin et al., 1997) (*Janibacter anophelis*, 95.6%; *Janibacter melonis*, 95.4%; *Janibacter terrae*, 95.1%) (Kämpfer et al., 2006; Yoon et al., 2000, 2004), three of the eight species of *Tetrasphaera* (*Tetrasphaera remsis*, 95.8%; *Tetrasphaera japonica*, 95.6%; *Tetrasphaera duodecadis*, 95.4%) (Ishikawa and Yokota, 2006; Maszenan et al., 2000; Osman et al., 2007), and *Terrabacter tumescens* (95.0%) (Collins et al., 1989). Two strains of *Ornithinicoccus hortensis* (HKI 0125, DSM 12335) share a 16S rRNA gene sequence similarity of 100% between one another and exhibit a high similarity in all tested phenotypic characteristics. Both strains reduce nitrate to nitrite and produce H_2S. Indole is not produced. Aconitate, formate, malate, and succinate are utilized as sole carbon sources. While sodium malate is readily utilized, disodium succinate, sodium formate, and sodium aconitate are utilized after a prolonged incubation time (about 7 d). Benzoate and DL-tartrate are not utilized. The utilization of acetate and citrate is variable. Acid production from carbohydrates is generally weakly expressed: i.e. D-glucose, maltose, D-mannitol, D-glucitol, trehalose, starch, dextrin, sucrose. No acid production from L-arabinose, lactose, D-cellobiose, D-galactose, inulin, D-raffinose, L-rhamnose, D-ribose, salicin, and D-xylose. Variable results are obtained from D-fructose, glycerol, and D-mannose. Casein, gelatin, hippurate, hypoxanthine, xanthine, and Tween 80 are hydrolyzed; esculin and tyrosine are not hydrolyzed. On esculin-iron agar both strains produce a brown pigment after prolonged incubation (above 7 d). This pigment is different from the black staining of the agar in the case of positive esculin hydrolysis. It stains the colonies and penetrates into the agar. Methyl red and Voges–Proskauer reactions are negative. Urease activity is variable. Cells of *Ornithinicoccus hortensis* are susceptible to the antibiotics (µg/disc): ampicillin (10), chloramphenicol (30), ciprofloxacin (5), erythromycin (15), gentamicin (10), kanamycin (30), neomycin (30), nitrofuran (300), oxytetracycline (30), penicillin G (2 IU/disc), polymyxin B (300 IU/disc), rifampin (2), and streptomycin (10). Susceptibility to oxacillin (5) is unclear; no susceptibility to sulfonamid.

Enrichment and isolation procedures

Strains of *Ornithinicoccus hortensis* grow readily in liquid or on solidified complex organic media, i.e. rich (R) medium (Yamada and Komagata, 1972) which is composed of (g/l): Bacto peptone (Difco), 10.0; yeast extract (Difco), 5.0; Casamino acids (Difco), 5.0; beef extract (Difco), 2.0; malt extract (Difco), 5.0; glycerol, 2.0; $MgSO_4 \cdot 7H_2O$, 1.0; Tween 80, 0.05; and agar, 20.0; pH 7.2, peptone-yeast extract-brain heart infusion medium (Yokota et al., 1993) containing (g/l): peptone, 10.0; yeast extract, 2.0; Bacto brain heart infusion (Difco), 2.0; NaCl, 2.0; and D-glucose, 2.0; pH 7.0, Bacto tryptic soy broth (Difco, g/l: Bacto tryptone, 17.0; Bacto soytone, 3.0; glucose, 2.5; NaCl, 5.0 and K_2HPO_4, 2.5; pH 7.3), and Bacto nutrient agar (Difco, g/l: Bacto beef extract, 3.0; Bacto peptone, 5.0; and Bacto agar, 15.0; pH 6.8). Both strains of *Ornithinicoccus hortensis* were isolated from a soil sample (garden soil) on peptone-yeast extract-brain heart infusion agar by using a standard dilution plate procedure.

Maintenance procedures

Cultures of *Ornithinicoccus* strains can be maintained by serial transfers on the complex organic media mentioned above. For long-term preservation, storage of liquid cultures supplemented with 5% dimethylsulfoxide is recommended in the vapor phase of liquid nitrogen (−140°C).

Differentiation of the genus *Ornithinicoccus* from other genera

Ornithinicoccus can be clearly distinguished from the closest phylogenetic neighbor *Humihabitans* (Kageyama et al., 2007a) which forms branching hyphae and has a peptidoglycan containing LL-A_2pm, Ala, Gly and Glu (type A3γ, Schleifer and Kandler, 1972). Similarly, morphological characteristics and the peptidoglycan type differentiate *Ornithinicoccus* from *Oryzihumus* (Kageyama et al., 2005). The strains of *Oryzihumus* are irregular slender rods and have *meso*-A_2pm together with Ala and Glu in their peptidoglycan (type A1γ; Schleifer and Kandler, 1972). *Ornithinimicrobium* (Groth et al., 2001) and *Ornithinicoccus* sharing 16S rRNA gene sequence similarities of 94.9–95.2% have in common the peptidoglycan type A4β (Schleifer and Kandler, 1972). However, *Ornithinimicrobium* has an interpeptide bridge that consists of L-Orn–L-Ala–Gly–D-Asp, while *Ornithinicoccus* is characterized by an L-Orn–$Gly_{(1,2)}$-D-Glu interpeptide bridge. The more distantly related genus *Serinicoccus* (Yi et al., 2004), which exhibits a 16S rRNA gene sequence similarity to *Ornithinicoccus* of only 92.8%, is also characterized by the peptidoglycan type A4β. However, the structure of the peptidoglycan has not yet been elucidated in all details. It seems that *Serinicoccus* represents a novel variation of the peptidoglycan type A4β containing ornithine and serine. The genera of the *Intrasporangiaceae* with a typical coccus morphology, e.g. *Arsenicicoccus* (Collins et al., 2004), *Lapillicoccus* (Lee and Lee, 2007), *Terracoccus* (Prauser et al., 1997), and *Phycicoccus* (Lee, 2006) share with *Ornithinicoccus* 16S rRNA gene sequence similarities below 95.0%. A low 16S rRNA gene sequence similarity (92.4–93.0%) is also present for the rod-shaped genera *Demequina* and *Actinotalea* (Yi et al., 2007) which share with *Ornithinicoccus* ornithine as the diagnostic diamino acid of the peptidoglycan. Both genera can be distinguished from *Ornithinicoccus* in that their interpeptide bridges consist of D-Glu and D-Asp, respectively. The degree of relationship between *Ornithinicoccus* and the other genera of the *Intrasporangiaceae* cannot currently be definitely determined as the closely related genera are represented by a low number of strains. The available phylogenetic data of genera with more than one or two species, *Janibacter* (Martin et al., 1997), *Knoellia* (Groth et al., 2002), *Tetrasphaera* (Maszenan et al., 2000), and *Terrabacter* (Collins et al., 1989), show that some species within the same genus may exhibit a similar level of relationship as the above-mentioned closest phylogenetic neighbors. These

genera can easily be differentiated from *Ornithinicoccus* by morphological and chemotaxonomic characteristics, especially by their peptidoglycan types (Table 125).

Taxonomic comments

The genus *Ornithinicoccus* was published in 1999. At that time, the family *Intrasporangiaceae* contained the genera *Intrasporangium* (Kalakoutskii et al., 1967), *Sanguibacter* (Fernández-Garayzábal et al., 1995a, 1995b), and *Terrabacter* (Collins et al., 1989; Stackebrandt et al., 1997). As in the 16S rRNA gene sequence of the type strain of *Ornithinicoccus hortensis*, only 12 of the 17 signature nucleotides were found that were described for the genera *Intrasporangium*, *Sanguibacter*, and *Terrabacter*, a family affiliation of *Ornithinicoccus* was postponed at that time until further strains would be available for analyses. The later exclusion of the genus *Sanguibacter* from the *Intrasporangiaceae* and the inclusion of *Terracoccus* and *Janibacter* into the emended family did not affect the affiliation of *Ornithinicoccus* to a higher taxonomic rank (Stackebrandt and Schumann, 2000). However, the descriptions of further novel actinobacteria required again a revision of the family *Intrasporangiaceae*. Based on new insights obtained from further 16S rRNA gene sequences, *Ornithinicoccus* was included in the *Intrasporangiaceae*, too (Garrity et al., 2007) Zhi et al. (2009) emended the description of the family *Intrasporangiaceae* and confirmed the affiliation of *Ornithinicoccus* to this family.

List of species of the genus *Ornithinicoccus*

1. **Ornithinicoccus hortensis** Groth, Schumann, Martin, Schuetze, Augsten, Kramer and Stackebrandt 1999, 1723[VP]

hor.ten′sis. L. masc. adj. *hortensis* of or belonging to a garden, referring to the place where the organism was isolated.

See generic description. By using the API ZYM system (bioMérieux), activity is detected for alkaline phosphatase, esterase (C 4), esterase lipase (C 8), lipase (C 14), leucine arylamidase, valine arylamidase, cystine arylamidase, chymotrypsin, phosphatase acid, naphthol-AS-BI-phosphohydrolase, and α-glucosidase. Negative results are obtained for trypsin, α-galactosidase, β-galactosidase, β-glucuronidase, β-glucosidase, *N*-acetyl-β-glucosamidase, α-mannosidase, and α-fucosidase. The predominant cellular fatty acids are $C_{15:0}$ iso and $C_{15:0}$ anteiso. Minor amounts of $C_{15:0}$ and $C_{17:1}$ iso also are found.

DNA G+C content (mol%): 72 (HPLC).

Type strain: HKI 0125, ATCC BAA-9, CCUG 43142, CIP 106364, DSM 12335, NBRC 16434, JCM 11538.

Sequence accession no. (16S rRNA gene): Y17869.

Genus IX. **Ornithinimicrobium** Groth, Schumann, Weiss, Schuetze, Augsten and Stackebrandt 2001, 85[VP]

Ingrid Groth

Or′ni.thi.ni.mic.ro.bi.um. N.L. n. *ornithinum* ornithine (an amino acid named after the Gr. n. *ornithos*, bird); N.L. neut. n. *microbium* (from Gr. adj. *mikros* small and Gr. n. *bios* life) a microbe; N.L. neut. n. *Ornithinimicrobium* a microbe with ornithine.

Cells are irregular **short rods** (1.0–3.2 × 0.5–1.0 µm) and/or **cocci** (0.7–1.4 µm) that occur singly, in pairs, short chains, or clusters (Figure 168). Gram-stain-positive, not acid-fast, **nonsporeforming. Nonmotile. Aerobic to microaerobic**. Colonies are smooth, circular, opaque, convex with entire margins, and cream to yellow in color. Good growth on complex organic media between 25–38°C and pH 6–8. NaCl in the culture medium is tolerated up to 6–7%. **Oxidase-negative; catalase-positive**. The **peptidoglycan type is A4β** with L-Orn–L-Ala–Gly–D-Asp as interpeptide bridge which is in accordance with variation A21.14. **The acyl type is acetyl**. **Whole cell sugars** are glucose, **arabinose, rhamnose, and xylose**. The major menaquinone is **MK-8(H_4);** MK-8(H_2), MK-8 and MK-6 may occur in minor amounts. The **polar lipids** are composed of **phosphatidylinositol, phosphatidylglycerol, diphosphatidylglycerol**, and unidentified phospho- and glycolipids. The cellular **fatty acid profile** is distinctly dominated by the occurrence of **iso-branched-chain acids;** anteiso-branched acids may also occur in a higher amount. Mycolic acids are absent. Based on 16S rRNA gene sequence comparison, strains of *Ornithinimicrobium* are most closely related to members of the genera *Serinicoccus* and *Ornithinicoccus*; slightly less closely related are the genera *Arsenicicoccus*, *Humihabitans*, *Oryzihumus*, and *Kribbia*. The 16S rRNA gene signature nucleotides that differentiate the genus from the other genera of the family *Intrasporangiaceae* are listed in Table 124.

DNA G+C content (mol%): 69–71.

Type species: **Ornithinimicrobium humiphilum** Groth, Schumann, Weiss, Schuetze, Augsten and Stackebrandt 2001, 85[VP].

Further descriptive information

Ornithinimicrobium shares with members of the most closely related genera *Serinicoccus* (Yi et al., 2004) and *Ornithinicoccus* (Groth et al., 1999) 16S rRNA gene sequence similarities of 95.0–95.8% and 94.9–95.2%, respectively. The sequence similarities between *Ornithinimicrobium* and *Arsenicicoccus* (Collins et al., 2004), *Humihabitans* (Kageyama et al., 2007a), *Oryzihumus* (Kageyama et al., 2005), and *Kribbia* (Jung et al., 2006) are 94.8–95.0%, 94.2–94.6%, 94.2–94.6%, and 93.8–94.3%, respectively. The genus *Ornithinimicrobium* currently accommodates three species, *Ornithinimicrobium humiphilum* (Groth et al., 2001), *Ornithinimicrobium kibberense* (Mayilraj et al., 2006), and *Ornithinimicrobium pekingense* (Liu et al., 2008). Each species is represented by a

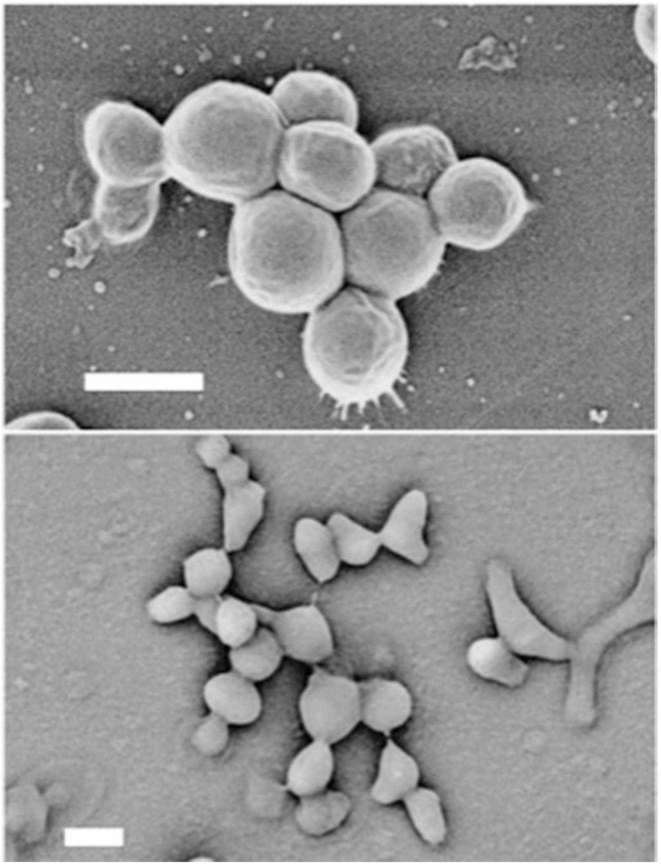

FIGURE 168. Scanning electron micrographs of cells from a 3-d-old culture of *Ornithinimicrobium humiphilum* DSM 12362^T grown on rich (R) agar at 28°C. Bars = 1 μm.

single strain. The 16S rRNA gene sequence similarities between the type strain of *Ornithinimicrobium humiphilum* and those of *Ornithinimicrobium kibberense*, and *Ornithinimicrobium pekingense* are 98.0 and 98.7%, respectively. DNA–DNA relatedness values of 64.5 and 31.5%, respectively, together with pronounced differences in phenotypic characteristics separate the three strains at the species level (Liu et al., 2008; Mayilraj et al., 2006).The type strains of *Ornithinimicrobium humiphilum*, *Ornithinimicrobium kibberense*, and *Ornithinimicrobium pekingense* have in common the following physiological characteristics: they are positive for hydrolysis of starch, but negative for Voges–Proskauer reaction and acid production from lactose, D-mannose, D-melibiose, L-rhamnose, and salicin. Some of the physiological properties reported for *Ornithinimicrobium humiphilum* seem to be variable depending on the applied cultural conditions. While Groth et al. (2001) obtained positive results for acid production from L-arabinose, D-cellobiose, D-glucose, maltose, trehalose (weakly positive), and xylose (weakly positive) using the oxidation-fermentation (O-F) medium described by Hugh and Leifson (1953), Mayilraj et al. (2006) obtained negative results for these characteristics by using the methods of Smith et al. (1952). The same is true for the reduction of nitrate to nitrite and the production of H_2S.

The phenotypic characteristics that differentiate the three species of *Ornithinimicrobium* are listed in Table 130.

Enrichment and isolation procedures

Strains of *Ornithinimicrobium* grow readily in liquid or on solidified complex organic media, i.e. rich (R) medium (Yamada and Komagata, 1972) which is composed of (g/l): Bacto peptone (Difco), 10.0; yeast extract (Difco), 5.0; Casamino acids (Difco), 5.0; beef extract (Difco), 2.0; malt extract (Difco), 5.0; glycerol, 2.0; $MgSO_4 \cdot 7H_2O$, 1.0; Tween 80, 0.05; and agar, 20.0; pH 7.2, peptone-yeast extract-brain heart infusion medium (Yokota et al., 1993) containing (g/l): peptone, 10.0; yeast extract, 2.0; Bacto brain heart infusion (Difco), 2.0; NaCl, 2.0 and D-glucose, 2.0; pH 7.0, and Bacto tryptic soy broth (Difco, g/l: Bacto tryptone, 17.0; Bacto soytone, 3.0; glucose, 2.5; NaCl, 5.0; and K_2HPO_4, 2.5; pH 7.3). The type strain of *Ornithinimicrobium humiphilum* was isolated from garden soil (Germany) on peptone-yeast extract-brain heart infusion medium and that of *Ornithinimicrobium kibberense* from soil, 45 cm below an ice glacier, 4200 m above sea level, in Kibber village of the Spiti valley, Himachal Pradesh (India), on tryptic soy agar using standard dilution plate procedures. The type strain of *Ornithinimicrobium pekingense* was isolated from activated sludge of a waste water treatment bioreactor. The waste water contained various nitroaromatic compounds (nitrobenzene, nitrophenol, 2, 4-dinitrophenol) and aniline. The reactor had been operated for one year. The sludge sample was suspended in sterile water and a portion of the suspension was directly spread on LB agar plates (g/l: tryptone, 10; yeast extract, 5.0; NaCl, 10.0; glucose, 1.0; and agar supplemented with $CaCl_2$ to a final concentration of 2.5 × 10 <MIN>3 M before pouring).

Maintenance procedures

Cultures of *Ornithinimicrobium* strains can be maintained by serial transfers on the complex organic media mentioned above. Stock cultures can be maintained at −80°C by adding 20% glycerol. For long-term preservation, storage of liquid cultures supplemented with 5% dimethylsulfoxide is recommended in the vapor phase of liquid nitrogen (−140°C).

Differentiation of the genus *Ornithinimicrobium* from other genera

The genus *Ornithinimicrobium* has in common with members of the closely related genera *Serinicoccus* (Yi et al., 2004) and *Ornithinicoccus* (Groth et al., 1999) and with the distantly related genera *Actinotalea* and *Demequina* (Yi et al., 2007) L-ornithine as the diagnostic diamino acid in the peptidoglycan. However, there are pronounced differences in the structure of the interpeptide bridges between these genera. The interpeptide bridge of *Ornithinimicrobium* consists of L-Orn–L-Ala–Gly–D-Asp, while that of *Ornithinicoccus* consists of L-Orn–$Gly_{(1,2)}$–D-Glu. The structure of the peptidoglycan of *Serinicoccus* has not yet been elucidated in all details. It seems that *Serinicoccus* represents a novel variation of the peptidoglycan type A4β (Schleifer and Kandler, 1972) containing ornithine and serine (Yi et al., 2004). *Actinotalea* and *Demequina* are characterized by the presence of an L-Orn–D-Asp or an L-Orn–D-Glu interpeptide bridge, respectively, (Yi et al., 2007) and share with *Ornithinimicrobium* 16S rRNA gene

TABLE 130. Differential characteristics of the species of the genus *Ornithinimicrobium*[a]

Characteristic	*O. humiphilum*[b] DSM 12362^T	*O. kibberense*[c] JCM 12763^T	*O. pekingense*[d] JCM 14001^T
Cell morphology	Irregular rods and cocci	Cocci	Irregular short rods
Growth at 42°C	+	–	–
Growth at pH 9	–[c]	+	+
Growth at 7% NaCl	–	+	+
Hydrolysis of casein	+	v	–
Hydrolysis of Tween 80	–	–	+
Acid production from:			
L-Arabinose	+[e]	–[f]	–
D-Cellobiose	+[e]	–	–
D-Fructose	–	+	–
D-Galactose	+	+	–
D-Glucose	+[e]	–	–
Maltose	+[e]	+	–
D-Raffinose	–	+	–
D-Ribose	+	nd	–
Sucrose	w	+	–
Utilization as sole carbon source:			
Acetate	–	+	+
L-Alanine[g]	–	+	nd
D-Arabitol[g]	–	+	+
Arbutin[g]	–	+	nd
Benzoate	–	+	+
D-Cellobiose[g]	–	+	+
Citrate	–	+	+
D-Fructose[g]	–	+	+
D-Galactose[g]	–	+	+
Gentiobiose[g]	–	+	nd
D-Gluconic acid[g]	–	+	nd
Glycogen[g]	–	+	nd
β-Hydroxybutyric acid[g]	–	+	nd
α-Ketovaleric acid[g]	+	–	nd
Mannan[g]	–	+	nd
Palatinose[g]	–	+	nd
Propionic acid[g]	+	–	nd
D-Psicose[g]	–	+	nd
D-Raffinose[g]	–	+	w
Salicin[g]	–	+	nd
Stachyose[g]	–	+	nd
Succinic acid monomethyl ester[g]	+	–	nd
Turanose[g]	–	+	nd

[a]Symbols: +, positive; w, weakly positive; –, negative; v, variable; nd, not determined.

[b]Data from Groth et al. (2001).

[c]Data from Mayilraj et al. (2006).

[d]Data from Liu et al. (2008).

[e,f]Conflicting results reported by Mayilraj et al. (2006) or Liu et al. (2008), respectively.

[g]Data for *Ornithinimicrobium humiphilum* and *Ornithinimicrobium kibberense* from Mayilraj et al. (2006) using Biolog GP2 MicroPlates.

sequence similarities below 93% (91.6–92.0% and 90.8–91.2%, respectively). In addition to these differences in the structure of the peptidoglycan, further phenotypic characteristics contribute to distinguish all these genera from *Ornithinimicrobium* (Table 125). The less closely related genera *Arsenicicoccus*, *Humihabitans*, *Oryzihumus*, and *Kribbia* can also be readily differentiated from *Ornithinimicrobium* by phenotypic properties. Apart from other differentiating characteristics, *Arsenicicoccus* and *Humihabitans* possess LL-A_2pm as the diagnostic diamino acid in the peptidoglycan while *Oryzihumus* and *Kribbia* possess *meso*-A_2pm. Furthermore, the cells of *Arsenicicoccus* are typical cocci, and *Humihabitans* forms branched hyphae that undergo fragmentation.

Taxonomic comments

The genus *Ornithinimicrobium* could not be affiliated to any family of the suborder *Micrococcineae* at the time of the description in 2001. The highest 16S rRNA gene sequence similarity

values were found with *Ornithinicoccus* (Groth et al., 1999) and members of the family *Intrasporangiaceae* (Rainey et al., 1997 in Stackebrandt et al., 1997). According to Stackebrandt and Schumann (2000) the family contained the genera *Intrasporangium* (Kalakoutskii et al., 1967), *Terrabacter* (Collins et al., 1989; Stackebrandt et al., 1997), *Terracoccus* (Prauser et al., 1997) and *Janibacter* (Martin et al., 1997). Due to the lack of five of 17 family specific 16S rRNA gene signature nucleotides described for the *Intrasporangiaceae*, *Ornithinimicrobium* was not included in this family. However, the descriptions of further novel actinobacteria required again a revision of the family *Intrasporangiaceae*. Based on new insights obtained from further 16S rRNA gene sequences and signature nucleotides *Ornithinimicrobium* and *Ornithinicoccus* were now included in the *Intrasporangiaceae*, too (Garrity et al., 2007). Zhi et al. (2009) emended the description of the family *Intrasporangiaceae* and confirmed the affiliation of *Ornithinimicrobium* to this family.

List of species of the genus *Ornithinimicrobium*

1. **Ornithinimicrobium humiphilum** Groth, Schumann, Weiss, Schuetze, Augsten and Stackebrandt 2001, 85[VP]

hu.mi′phi.lum. L. n. *humus* soil; N.L. neut. adj. *philum* (from Gr. neut. adj. *philon*) friend, loving; N.L. neut. adj. *humiphilum* loving soil.

See Table 130 and the generic description. Subsequent to the isolation and later in few cultures, in addition to the typical rods and cocci both filamentous cells with swollen club shaped ends or a tendency to primary branching and single spherical cysts were observed. Colonies are circular, convex, glistening, and smooth with an entire margin (0.3–1.2 mm). Color varies within the yellow shade from whitish to bright yellow. Two colony types may be observed on rich (R) medium differing in size, color, and growth rate. While colonies of the pale yellow, opaque type grow rapidly after their transfer on a fresh medium, the growth of the smaller transparent yellow colonies is delayed and somewhat reduced. Optimum temperature for growth is 37–42°C; no growth at 50°C. Acids are produced from starch and, in a low amount, from dextrin. No acid production from D-glucitol, glycerol, and D-mannitol. Formate is used as sole carbon source. Utilization of malate and succinate is delayed and weakly expressed. Aconitate and DL-tartrate are not utilized. Gelatin, hypoxanthine, and xanthine are hydrolyzed; adenine, esculin, hippurate, tyrosine, and urea are not hydrolyzed. Indole is not produced. Cells are susceptible to (μg/disc): ampicillin (10), chloramphenicol (30), ciprofloxacin (5), erythromycin (15), gentamicin (10), kanamycin (30), lincomycin (2), neomycin (30), nitrofuran (300), oxytetracycline (30), penicillin G (2 IU/disc), polymyxin B (300 IU/disc), rifampin (2), and streptomycin (10); no susceptibility to oxacillin (5) and sulfonamide (300). By using the API ZYM system (bioMérieux), activity is detected for alkaline phosphatase, esterase (C 4), esterase lipase (C 8), leucine arylamidase, valine arylamidase, cystine arylamidase, acid phosphatase, naphthol-AS-BI-phosphohydrolase, and α-glucosidase. No activity is detected for lipase (C 14), α-galactosidase, β-galactosidase, β-glucuronidase, β-glucosidase, *N*-acetyl-β-glucosamidase, α-mannosidase, and α-fucosidase. A weak reaction is observed for trypsin and chymotrypsin. The predominant cellular fatty acids are $C_{16:0}$ iso, $C_{15:0}$ iso, and $C_{15:0}$ anteiso.

DNA G+C content (mol%): 70 (HPLC).

Type strain: HKI 0124, CIP 106634, DSM 12362, JCM 11540.

Sequence accession no. (16S rRNA gene): AJ277650.

2. **Ornithinimicrobium kibberense** Mayilraj, Saha, Suresh and Saini 2006, 1660[VP]

kib.be.ren′se. N. L. neut. adj. *kibberense* pertaining to Kibber, a village in the Indian Himalayas, where the type strain was isolated.

See Table 130 and the generic description. Growth temperature ranges from 20–37°C; optimum temperature for growth is 28°C; no growth at 4 and 42°C. Colonies on TSA are buff-yellow and 1–3 mm in diameter. Acids are produced from trehalose. No acid production from inulin, D-mannitol, and D-xylose. Nitrate reductase is present. H_2S and indole are not produced. Cells are susceptible to (μg/disc): colistin (10), lincomycin (2), and polymyxin B (300). For detailed results of the utilization of carbon sources using the Biolog GP2 MicroPlate test, see Mayilraj et al. (2006).

The predominant cellular fatty acids are $C_{17:1}$ ω9*c*, $C_{17:0}$ iso, $C_{15:0}$ iso, and $C_{16:0}$ iso.

DNA G+C content (mol%): 71 (T_m).

Type strain: K22-20, DSM 17687, JCM 12763, MTCC 6545.

Sequence accession no. (16S rRNA gene): AY636111.

3. **Ornithinimicrobium pekingense** Liu, Wang, Jiang and Liu 2008, 118[VP]

pe.king.en′se. N.L.neut. adj. *pekingense* of Peking, the former name of Beijing city, the origin of the type strain.

See Table 130 and the generic description. Cells are short rods and cocci. The rods vary in dimensions from 1.0–1.6 × 0.5–0.8 μm. Colonies grown on TSA are light yellow and 0.2–1.0 mm in diameter. Optimal growth occurs between 33–37°C and pH 7.8–8.2. Nitrate reductase is present. Gelatin is hydrolyzed. Acid production is negative from melezitose, sorbitol, and xylose. The predominant cellular fatty acids are $C_{15:0}$ iso and $C_{17:1}$ ω9*c*.

DNA G+C content (mol%): 69 (T_m).

Type strain: LW6, CGMCC 1.5362, JCM 14001.

Sequence accession no. (16S rRNA gene): DQ512860.

Genus X. **Oryzihumus** Kageyama, Takahashi, Seki, Tomoda and Ōmura 2005, 2557$^{\mathrm{VP}}$

Akiko Kageyama and Yōko Takahashi

Ory.zi.hu′mus. L. fem. n. *oryza* rice; L. masc. n. *humus* soil; N.L. masc. n. *Oryzihumus* rice soil.

Gram-stain-positive, catalase-positive, aerobic, nonmotile irregular rods. The peptidoglycan is of the A type of direct cross-linkage and contains *meso*-diaminopimelic acid, alanine, and glutamic acid. The acyl type of the glycan chain of peptidoglycan is acetyl. Mycolic acids are absent. The major menaquinone is **MK-8(H$_4$)**. The fatty acid profile is dominated by **$C_{16:0}$ iso and $C_{15:0}$ iso**. Phylogenetically, this genus is a member of the family *Intrasporangiaceae*, order *Micrococcales*.

DNA G+C content (mol%): 72–73.

Type species: **Oryzihumus leptocrescens** Kageyama, Takahashi, Seki, Tomoda and Ōmura 2005, 2558$^{\mathrm{VP}}$.

Further descriptive information

The family *Intrasporangiaceae* was proposed by Rainey et al. (in Stackebrandt et al., 1997) and emended by Stackebrandt and Schuman (2000). The genera belonging to this family can be divided into three groups on the basis of the diagnostic diamino acid types in the cell-wall peptidoglycan: LL-diaminopimelic acid (DAP), *meso*- DAP acid, or L-ornithine. Genera belonging to the *meso*- DAP group are *Janibacter* (Martin et al., 1997), *Knoellia* (Groth et al., 2002), *Kribbia* (Jung et al., 2006), *Oryzihumus* (Kageyama et al., 2005), *Phycicoccus* (Lee, 2006), and *Tetrasphaera* (Maszenan et al., 2000).

16S rRNA gene sequence data show that the genus *Oryzihumus* is closely related to the genus *Ornithinicoccus* of the family *Intrasporangiaceae* (Figure 169). Almost complete 16S rRNA gene sequences have been determined for *Oryzihumus* strains. The sequence similarity values for three *Oryzihumus leptocrescens* strains were above 99.9%, whereas the sequence similarity values between these three strains and *Ornithinicoccus hortensis* were below 95.6%.

Enrichment and isolation procedures

Strains KV-628^T, KV-641, and KV-656 were isolated from soil samples collected from a paddy field in Saitama prefecture, Japan. Soil samples (2 g) were suspended in 18 ml sterile water and then mixed. Soil particles were allowed to sediment, the liquid phase was diluted by 10^5 times and 100 µl was spread onto the surface of four kinds of GPM agar (1.0% glucose, 0.5% peptone, 0.5% meat extract, 0.3% NaCl, and 1.2% agar, pH 7.0) plated (Takahashi et al., 2003), as follows: (1) unsupplemented, (2) supplemented with SOD (300 units/plate), (3) supplemented with catalase (2100 units/plate) or supplemented with SOD plus catalase. Each plate was cultured at 27°C. Biomass for biochemical and chemotaxonomic characteristics was prepared by culture in Todd–Hewitt broth (Difco™) or Nutrient broth (Difco™) at 27°C for 3–4 d.

Maintenance procedures

Oryzihumus strains can be maintained for several weeks on nutrient agar plates. Long-term storage can be achieved at −80°C in nutrient broth or by lyophilization in the presence of stabilizers such as skim milk.

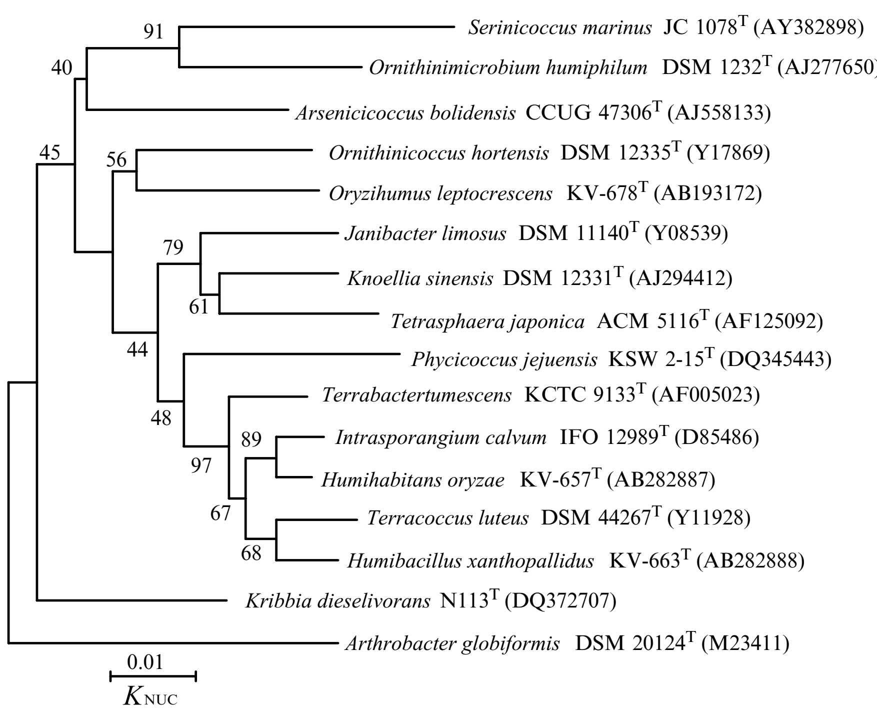

FIGURE 169. Phylogenetic tree derived from 16S rRNA gene sequences of the members of the family *Intrasporangiaceae* and constructed using the neighbor-joining method and K_{nuc} values. Numbers at branching points are bootstrap values (1000 resamplings). The tree was unrooted and *Arthrobacter globiformis* was used as an outgroup.

TABLE 131. Characteristics differentiating the genus *Oryzihumus* and *meso*-diaminopimelic acid-containing members of the family *Intrasporangiaceae*[a]

Characteristic	*Oryzihumus*	*Janibacter*	*Knoellia*	*Kribbia*	*Phycicoccus*	*Tetrasphaera*
Cell morphology	Irregular rods	Coccoid to rod-shaped	Irregular rods and cocci	Irregular short rods or cocci	Cocci	Cocci or short rods
Major fatty acid (~10% of total fatty acid)	$C_{16:0}$ iso, $C_{15:0}$ iso, $C_{14:0}$ iso	$C_{16:0}$ iso, $C_{17:1}$ ω8*c*, $C_{18:1}$ ω9*c*, $C_{17:0}$	$C_{15:0}$ iso, $C_{17:0}$ iso, $C_{16:0}$ iso, $C_{17:0}$ anteiso	$C_{18:0}$ 10 methyl, $C_{16:0}$ iso, $C_{18:1}$ ω9*c*, $C_{16:0}$, $C_{18:0}$	$C_{17:1}$ ω8*c*, $C_{16:0}$ iso, $C_{15:0}$ iso	($C_{15:0}$ anteiso, $C_{15:0}$ iso)[b] or ($C_{16:0}$ iso, $C_{17:0}$ anteiso, $C_{15:0}$ iso)[c] or ($C_{16:0\ iso}$, $C_{17:0}$ anteiso)[d]
Polar lipids[e]	nd	DPG, PG, PI	DPG, PE, PI PG, PL	nd	DPG, PE, PI	PE[f], PL[f]
DNA G+C content (mol%)	72–73	69–73	68–69	69–70	74	68–71

[a]Data for reference genera were taken from Jung et al. (2006) (*Kribbia*); Martin et al. (1997), Lang et al. (2003), Yoon et al. (2004) (*Janibacter*); Groth et al. (2002) (*Knoellia*); Kageyama et al. (2005) (*Oryzihumus*); Lee (2006) (*Phycicoccus*); Maszenan et al. (2000) and Hanada et al. (2002) (*Tetrasphaera*).

[b]Data for *Tetrasphaera elongate*.

[c]Data for *Tetrasphaera australiensis*.

[d]Data for *Tetrasphaera japonica*.

[e]DPG, disphosphatidylglycerol; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; PI, phosphatidylinositol; PL, unknown phospholipid(s).

[f]Component detected in only some representatives of the genus.

Differentiation of the genus *Oryzihumus* from other genera

Characteristics that can be used to distinguish the genus *Oryzihumus* from other genera classified in *meso*-DAP acid group of family *Intrasporangiaceae* are shown in Table 131, these properties include cell morphology, major fatty acid, and polar lipids. The genus *Oryzihums* can be differentiated from other genera belong to the family *Intrasporangiaceae* on the basis of cell morphology and the pattern of phospholipids.

List of species of the genus *Oryzihumus*

1. **Oryzihumus leptocrescens** Kageyama, Takahashi, Seki, Tomoda and Ōmura 2005, 2558[VP]

lep.to.cres′cens. Gr.adj. *leptos* thin, fine, delicate, slender; L. part. adj. *crescens* growing; N.L. part. adj. *leptocrescens* slender growing.

Cells are irregular rods with diameters 0.4–0.9 × 0.9–1.9 µm. Colonies are pale yellow. Aerobic to microaerophilic. Growth occurs at pH 4–9; temperature range for growth is 15–37°C. In 1/5 Nutrient agar medium, NaCl is tolerated up to 5%. D-Glucose, maltose, sucrose, and trehalose are assimilated, but L-rhamnose and D-xylose are not. Susceptible to erythromycin (15 µg/ml), rifampin (30 µg/ml), novobiocin (30 µg/ml), streptomycin (10 µg/ml), and chloramphenicol (30 µg/ml). Not susceptible to aztreonam (100 µg/ml). Esterase (C4), esterase lipase (C8), leucine arylamidase, valine arylamidase, acid phosphatase, naphthol-AS-BI-phosphohydrolase, β-galactosidase, α-glucosidase, and β-glucosidase are detected by APIZYM enzyme assay; alkaline phosphatase, cystine arylamidase, trypsin, chymotrypsin, β-glucosidase, *N*-acetyl-β-glucosaminase, α-mannosidase, and α-fucosidase are negative. Weak reaction for lipase (C14) is detected. Variable reactions for α-galactosidase are detected.

Source: paddy soil.

DNA G+C content (mol%): 72–73 (HPLC).

Type strain: KV-628, JCM 12835, NBRC 100762, NRRL B-24347.

Sequence accession no. (16S rRNA gene): AB193172.

Genus XI. **Phycicoccus** Lee 2006, 2371[VP] emend. Zhang, Liu and Liu 2011, 74

Soon Dong Lee

Phy.ci.coc′cus. L. n. *phycos -i* from Gr. n. *phukos* seaweed; N.L. masc. n. *coccus* from Gr. n. *kokkos* a grain or berry; N.L. masc. n. *Phycicoccus* coccus from seaweed.

Cells are **spherical** (0.3–1.0 µm in diameter) or rod-shaped (0.5–0.9 × 1.1–2.0 µm). **Aerobic**. Gram-stain-positive. **Nonspore-forming. Nonmotile. Catalase-positive**. Colonies are circular, smooth, convex and white, cream, or yellow in color. Optimum growth temperature is 28–30°C. **Chemoorganotrophic.** Several carbohydrates are assimilated. Cell wall contains *N*-acetylated murein and *meso*-diaminopimelic acid (*meso*-DAP). The predominant menaquinone is tetrahydrogenated with eight

isoprene units [MK-8(H_4)]. The fatty acids are mainly of *iso*-methyl-branched, unsaturated and saturated types with substantial amount of *anteiso*-methyl-branched and 10-methyl fatty acids. Mycolic acids are not present. The phospholipids include diphosphatidylglycerol and phosphatidylinositol. The presence of phosphatidylethanolamine or phosphatidylglycerol is variable depending upon strains. Phylogenetically the genus belongs to the family *Intrasporangiaceae*.

DNA G+C content (mol%): 70.5–74.0.

Type species: **Phycicoccus jejuensis** Lee 2006, 2371[VP].

Further descriptive information

The cells of *Phycicoccus* are strictly aerobic, Gram-stain-positive cocci (0.3–1.0 µm in diameter) that occur singly, in pairs, in chains, or in clusters or rod-shaped (0.5–0.9 × 1.1–2.0 µm). All of the cells are nonmotile. Colonies are circular, smooth, convex and white, cream, or yellow in color.

No growth occurs on any medium under anaerobic conditions. The temperature range for growth is 5–37°C. Optimum growth occurs at pH 6.0–8.0. All strains give positive reactions in the following tests: catalase activity; hydrolysis of casein and Tween 80; assimilation of D-glucose, D-mannose, D-mannitol, *N*-acetyl-D-glucosamine, D-maltose, gluconate, and malate (API 20NE); esterase lipase (C8), leucine arylamidase, acid phosphatase, β-galactosidase, β-glucosidase, and naphthol-AS-BI-phosphohydrolase activities (API ZYM); and tolerance to 5% NaCl. They give negative reactions for indole production, urease and α-fucosidase activities, and assimilation of caprate and citrate.

The diagnostic diamino acid in the cell-wall peptidoglycan is *meso*-diaminopimelic acid, and the peptidoglycan is A1γ type. For those strains that have been examined, the glycan moiety of their cell walls is acetylated, indicating that the muramic acid occurs in the usual *N*-acetyl form. Weon et al. (2008) examined whole cell sugars in the type strain of *Phycicoccus aerophilus* and found glucose and ribose as diagnostic cell-wall sugars. The major menaquinone is the same in all species, tetrahydromenaquinone with eight isoprene units [MK-8(H_4)]. The polar lipid composition is variable. In the original description of the genus (Lee, 2006), the polar lipid profile comprised phosphatidylethanolamine (PE) as a characteristic phospholipid together with diphosphatidylglycerol and phosphatidylinositol. This profile is also found in *Phycicoccus aerophilus* (Weon et al., 2008). However, PE was not detected in *Phycicoccus dokdonensis* (Yoon et al., 2008) and *Phycicoccus bigeumensis* (Dastager et al., 2008). Instead, they contain phosphatidylglycerol in their polar lipid profiles. In addition, unknown glycolipids were reported in *Phycicoccus cremeus* (Zhang et al., 2011). All stains of *Phycicoccus* exhibit similar fatty acid profiles. The fatty acids consist mainly of *iso*-methyl-branched, unsaturated, and saturated components with substantial amounts of *anteiso*-methyl-branched and 10-methyl fatty acids. Small amounts of 3-hydroxyl fatty acid are present in some strains. The predominant fatty acids (>10% of a total) commonly detected in all strains examined are $C_{15:0}$ iso and $C_{16:0}$ iso. The other fatty acids present in substantial amounts are $C_{17:1}$ ω8*c*, $C_{17:0}$, $C_{15:0}$, $C_{15:0}$ anteiso, $C_{17:0}$ 10-methyl, and $C_{14:0}$ iso. Mycolic acids are not present. The DNA base ratios reported by members of the genus are 70.5–74.0 mol% G+C, as measured by HPLC or thermal renaturation.

Strains of *Phycicoccus* have been isolated from a variety of sources including dried seaweed, air, and soils (see list of species).

Enrichment and isolation procedures

Species have been isolated by plating. Nutrient agar (Difco), R2A (Difco) agar with or without 10-folded dilution, or chemically synthetic medium V2M (Zhang et al., 2010) have been used for air or soil samples with incubation times of 5–7 d. For isolation of *Phycicoccus jejuensis*, a piece of dried seaweed was directly applied onto plates of WAT agar, containing 0.05% $MgSO_4 \cdot 7H_2O$, 0.05% $CaCl_2 \cdot 2H_2O$, 1.5% agar, plus 60% (v/v) sterilized natural seawater (Lee, 2006).

Maintenance procedures

Phycicoccus strains can be preserved for several months in 20% glycerol suspension with or without the addition of 60% (v/v) natural seawater (−20°C). Lyophilization is recommended for longer preservation of several years.

Differentiation of the genus *Phycicoccus* from other genera

The genus *Phycicoccus* may be differentiated from genera which contain *meso*-DAP as cell-wall diamino acid and MK-8(H_4) as major menaquinone in the family *Intrasporangiaceae* by the characteristics given in Table 132.

Taxonomic comments

The genus *Phycicoccus* was established by Lee (2006) with the description of *Phycicoccus jejuensis* as the sole and type species. In the original description, the comparative analysis of 16S rRNA gene sequences indicated that *Phycicoccus* possesses about 96% sequence similarity to strains of "*Candidatus* Nostocoida limicola" which have been described as a filamentous bacterium isolated from activated sludge (Blackall et al., 2000) of the family *Intrasporangiaceae*. Because of the practical difficulty in obtaining biomass for polyphasic characterization, the description of this filamentous bacterium has not been not validated. *Phycicoccus* is readily differentiated from strains of "*Candidatus* Nostocoida limicola," based on the comparison of limited features, namely 16S rRNA gene sequence similarity and morphological characteristics (Blackall et al., 2000). At the same time when the *Phycicoccus* was proposed, strains of "*Candidatus* Nostocoida limicola," were assigned to the genus *Tetrasphaera* (McKenzíe et al., 2006). Among the *Intrasporangiaceae*, *Terrabacter* possesses high 16S rRNA gene sequence similarities to *Phycicoccus* but also LL-DAP instead of *meso*-DAP as the diagnostic cell-wall diamino acid and it lacks the unsaturated fatty acids common in *Phycicoccus* (Martin et al., 1997; Montero-Barrientos et al., 2005).

The genus *Phycicoccus* as presently defined exhibits a close chemotaxonomic relationship to bacteria of the family *Intrasporangiaceae* which contain *meso*-DAP as principal cell-wall diamino acid and MK-8(H_4) as major menaquinone. These include the genera *Janibacter*, *Knoellia*, *Kribbia*, *Oryzihumus*, and *Tetrasphaera*.

Differentiation and characteristics of the species of the genus *Phycicoccus*

Species of *Phycicoccus* can be differentiated using the characteristics described in Table 133.

TABLE 132. Characteristics differentiating *Phycicoccus* and genera which contain *meso*-DAP as cell-wall diamino acid and MK-8(H_4) as major menaquinone in the family *Intrasporangiaceae*[a]

Characteristic	*Phycicoccus*	*Janibacter*	*Knoellia*	*Kribbia*	*Oryzihumus*	*Tetrasphaera*
Cell morphology	Cocci or rods	Cocci or short rods	Cocci or irregular rods	Irregular rods or cocci	Irregular rods	Cocci or irregular rods
Polar lipids[b,c]	DPG, (PE), (PG), (PI), (PL), (GL)	DPG, PG, PI	DPG, PE, PI, PG, PL	nd	nd	DPG, PG, PI,, (PE), (APL), (PL)
Acid types[d]	S, A, I, U	S, I, U	S, I, A	S, I, U, T	S, I, A	S, I, A, (U)
DNA G+C content (mol%)	71–74	70	68–69	69–70	72–73	68–71
Origin of isolation	Dried seaweed, air, soil	Sludge, sewage waste	Soil of cave	Tidal flat sediment	Paddy soil	Sludge, sewage waste

[a]Data from Martin et al. (1997), Maszenan et al. (2000), Groth et al. (2002), Hanada et al. (2002), Kageyama et al. (2005), Jung et al. (2006), Lee (2006), McKenzíe et al. (2006), Dastager et al. (2008), Weon et al. (2008), Yoon et al. (2008), and Zhang et al. (2011).

[b]DPG, diphosphatidylglycerol; PE, phosphatidylethanolamine; PG, Phosphatidylglycerol; PI, phosphatidylinositol; PL, unknown phospholipid(s); APL, unknown amino phospholipid; GL, unknown glycolipids; nd, not determined.

[c]( ), variable depending on species.

[d]A, anteiso-methyl-branched; I, iso-methyl-branched; S, straight-chain, saturated; U, monounsaturated; T, tuberculostearic acid ($C_{18:0}$ 10-methyl); ( ), sometimes present.

TABLE 133. Characteristics differentiating species of the genus *Phycicoccus*[a,b]

Characteristic	*P. jejuensis*	*P. aerophilis*	*P. bigeumensis*	*P. cremeus*	*P. dokdonensis*
Cell morphology	Cocci	Rods	Cocci	Rods	Cocci
Cell size (μm)	1	0.0–0.9 × 1.1–1.5	0.4–0.5	0.5–0.8 × 1.5–2.0	0.3–0.7
Growth at 4–5°C	+	+	−	−	−
Growth at pH 10.0	+	−	+	+	−
Growth in 7% NaCl	+	+	−	+	−
Oxidase	−	−	−	−	+
Nitrate reduction	+	−	+	+	−
Hydrolysis of:					
Esculin	+	+	−	+	+
DNA	+	−	−	−	−
Gelatin	+	+	−	+	+
Starch	+	+	+	−	+
Assimilation of (API 20NE):					
Adipate	(+)	−	(+)	−	−
L-Arabinose	+	−	+	−	−
Phenylacetate	+	−	−	−	−
Enzyme activities (API ZYM):					
Alkaline phosphatase	+	−	+	(+)	+
α-Chymotrypsin	+	+	−	−	−
Cystine arylamidase	+	−	−	−	+
Esterase (C4)	+	−	+	−	+
α-Galactosidase	+	−	−	+	+
α-Glucosidase	+	+	−	+	+
β-Glucuronidase	−	−	+	−	−
Lipase (C14)	+	−	−	−	−
α-Mannosidase	−	−	+	+	(+)
Trypsin	+	−	−	+	−
Valine arylamidase	+	−	−	+	+
Polar lipids[c]	DPG, PE, PI	DPG, PE, PI	DPG, PG	DPG, PI, GL	DPG, PG, PI, PL
DNA G+C content (mol%)	74	70.5	73.4	72	70.7

[a]Symbols: +, positive; −, negative; (+), weakly positive.

[b]Based on data from Lee (2006); Dastager et al. (2008); Weon et al. (2008); Yoon et al. (2008); and Zhang et al. (2011).

[c]DPG, diphosphatidylglycerol; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; PI, phosphatidylinositol; PL, unknown phospholipid(s); GL, unknown glycolipids.

List of species of the genus *Phycicoccus*

1. **Phycicoccus jejuensis** Lee 2006, 2371[VP]

je.ju.en′sis. N.L. masc. adj. *jejuensis* of Jeju, Republic of Korea, referring the site from which the type strain was isolated.

Cells are nonmotile cocci that occur singly, in pairs, or in clusters. Colonies are circular, smooth, translucent, and moderate yellow in color. Other characteristics are given in Table 133 and in *Further descriptive information*. The temperature range for growth is 4–37°C, with an optimum at 30°C. The pH for growth is pH 5.1–10.1, with an optimum at pH 7.1. Growth occurs up to 7% (w/v) NaCl. Hydrolyzes elastin. Negative for *N*-acetyl-β-glucosaminidase activity and glucose fermentation. Utilizes a variety of carbohydrates as sole carbon and energy sources. The major cellular fatty acids (>10% of total) are $C_{17:1}$ ω8*c*, $C_{16:0}$ iso, $C_{15:0}$ iso, $C_{15:0}$, and $C_{17:0}$. The polar lipids include phosphatidylethanolamine, phosphatidylinositol and diphosphatidylglycerol.

Source: dried seaweed.

DNA G+C content (mol%): 74 (HPLC).

Type strain: KSW2-15, JCM 15616, KCCM 42315, NRRL B-24460.

Sequence accession no. (16S rRNA gene): DQ345443.

2. **Phycicoccus aerophilus** Weon, Yoo, Kim, Schumann, Kroppenstedt, Hong and Kwon 2008, 2391[VP]

ae.ro.phi′lus. Gr. n. *aer aeros* air; N.L. adj. *philus -a -um* (from Gr. adj. *philos -ê -on*) friend, loving; N.L. masc. adj. *aerophilus* air-loving.

Cells are nonmotile, short rods (0.8–0.9 × 1.1–1.5 µm). Colonies on R2A medium are white, round, and convex with clear margins. Other characteristics are given in Table 133 and in *Further descriptive information*. The temperature range for growth is 5–37°C, with an optimum at 30°C. The pH for growth is pH 5.0–9.0, with an optimum at pH 6.0–7.0. Growth occurs at up to 7% (w/v) NaCl. Hydrolyzes tyrosine and xanthine, but not chitin, carboxymethyl-cellulose, hypoxanthine, or pectin. Positive for β-galactosidase, but negative for glucose fermentation and arginine dihydrolase. Utilizes sucrose, sodium acetate, L-alanine (weak), glycogen, salicin, melibiose, L-fucose, propionic acid, valeric acid, and 3-hydroxybutyric acid as sole carbon and energy sources. The major fatty acids (>10% of total) are $C_{17:1}$ ω8c, $C_{15:0}$ iso, $C_{16:0}$ iso, and $C_{17:0}$. The polar lipids include phosphatidylethanolamine, phosphatidylinositol, and diphosphatidylglycerol.

Source: air.

DNA G+C content (mol%): 70.5 (HPLC).

Type strain: 5516T-20, DSM 18548, KACC 20658.

Sequence accession no. (16S rRNA gene): EF493847.

3. **Phycicoccus bigeumensis** Dastager, Lee, Ju, Park and Kim 2008, 2427[VP]

bi.geu.men′sis. N.L. masc. adj. *bigeumensis* pertaining to Bigeum Island, Republic of Korea, the source of soil sample from where the type strain was isolated.

Cells are cocci with a warty appearance and occur singly or in short chains. Colonies are circular, small, smooth, and yellow in color. Other characteristics are given in Table 133 and in *Further descriptive information*. The temperature range for growth is 20–37°C, with an optimum at 28°C. The pH range for growth is 7.0–12.0, with an optimum at pH 7.4 ± 0.1. Growth occurs up to 5% (w/v) NaCl. Negative for *N*-acetyl-β-glucosaminidase activity. Utilizes L-arabinose, cellobiose, D-fructose, D-galactose, D-glucose, *myo*-inositol, D-lactose, D-mannose, D-mannitol, melibiose, raffinose, L-rhamnose, and trehalose as sole carbon and energy sources. Acid is not produced from any of the carbon sources. The major fatty acids (>10% of total) are $C_{15:0}$ iso, $C_{16:0}$ iso, $C_{17:0}$, and $C_{15:0}$ anteiso. The polar lipids include diphosphatidylglycerol and phosphatidylglycerol.

Source: soil.

DNA G+C content (mol%): 73.4 (HPLC).

Type strain: MSL-03, DSM 19264, KCTC 19266.

Sequence accession no. (16S rRNA gene): EF466128.

4. **Phycicoccus cremeus** Zhang, Liu and Liu 2011, 74[VP]

cre′me.us. N.L. masc. adj. *cremeus* cream-white.

Cells are nonmotile rods (0.5–0.8 × 1.5–2.0 µm). Colonies on R2A agar (after 2 d at 30°C) are 0.3–0.8 mm in diameter, circular, entire, smooth, circular, and cream in color. Other characters are given in Table 133 and in *Further descriptive information*. The temperature range for growth is 14–35°C, with an optimum at 29°C. The pH for growth is pH 4.1–10.0, with an optimum at pH 7.0–8.0. Growth occurs up to 7% (w/v) NaCl. Growth occurs on diluted TSB agar (1/10 and 1/20 strength of TSB) and on R2A agar. Does not hydrolyze Tween 20. Negative for arginine dihydrolase and *N*-acetyl-β-glucosaminidase activities, Voges–Proskauer reaction, and glucose fermentation. The major fatty acids (>10% of a total) are $C_{15:0}$ iso, $C_{16:0}$ iso, $C_{17:1}$ ω8*c*, and $C_{17:0}$ 10-methyl. The polar lipids include diphosphatidylglycerol, phosphatidylinositol, and three unknown glycolipids.

Source: forest soil.

DNA G+C content (mol%): 72.0 (T_m).

Type strain: V2M29, CGMCC 1.6963, NBRC 104261.

Sequence accession no. (16S rRNA gene): FJ529696.

5. **Phycicoccus dokdonensis** Yoon, Lee, Kang and Oh 2008, 599[VP]

dok.do.nen′sis. N.L. masc. adj. *dokdonensis* of Dokdo, Republic of Korea, the source of the soil sample from which the type strain was isolated.

Cells are nonmotile cocci (0.3–0.7 µm in diameter). Colonies on Trypticase soy agar after 7 d of incubation at 30°C are 0.6–1.2 mm in diameter, circular, smooth, glistening, convex, and grayish yellow in color. Other characteristics are given in Table 133 and in *Further descriptive information*. The temperature range for growth is 10–36°C, with an optimum at 30°C. The pH range for growth is pH 5.0–8.5, with an optimum at pH 6.5–7.5. Growth occurs at up to 5% (w/v) NaCl. Hydrolyzes hypoxanthine and Tweens 20, 40, 60 but not xanthine and L-tyrosine. Negative for arginine dihydrolase, lysine decarboxylase, ornithine decarboxylase, and tryptophan deaminase. H_2S is not produced. Utilizes esculin, melezitose, starch, and D-lyxose as sole carbon and energy sources. The major fatty acids (>10% of total) are $C_{15:0}$ iso and $C_{16:0}$ iso. The polar lipids contain phosphatidylinositol, phosphatidylglycerol, diphosphatidylglycerol, and two unidentified phospholipids.

Source: soil.

DNA G+C content (mol%): 70.7 (HPLC).

Type strain: DS-8, CCUG 54521, KCTC 19248.

Sequence accession no. (16S rRNA gene): EF555583.

Genus XII. **Serinicoccus** Yi, Schumann, Sohn and Chun 2004, 1587[VP]

Peter Schumann

Se.ri.ni.coc′cus. N.L. neut. n. *serinum* serine; N.L. masc. n. *coccus* (from Gr. masc. n. *kokkos*) a grain, seed; N.L. masc. n. *Serinicoccus* a coccus with serine in the cell wall.

Cells are nonmotile cocci. Gram-stain-positive, strictly aerobic, moderately halophilic. Oxidase-negative, catalase-positive, not acid-fast. Nonsporeforming. The **peptidoglycan** type is of the **A type** of cross-linkage and **contains ornithine, alanine, lysine, serine, and glutamic acid**. The acyl type of the glycan chain of peptidoglycan is acetyl. The major menaquinone is **MK-8(H_4)**. The major **cellular fatty acids** are of the **iso- and anteiso-methyl-branched type**. The polar lipids are **phosphatidylglycerol, diphosphatidylglycerol, phosphatidylinositol**, and one unknown glycolipid. Phylogenetically, this genus is affiliated to the family *Intrasporangiaceae*.

DNA G+C content (mol%): 72.

Type species: **Serinicoccus marinus** Yi, Schumann, Sohn and Chun 2004, 1587[VP].

Further descriptive information

The genus *Serinicoccus* is a member of the family *Intrasporangiaceae* belonging to the order *Micrococcales*, class *Actinobacteria* according to the taxonomic outline for the present volume. The genus *Serinicoccus* clusters in neighbor-joining trees based on 16S rRNA gene sequences together with the genera *Ornithinimicrobium*, *Kribbia*, and *Arsenicicoccus* (Jung et al., 2006; Lee, 2006) but also with *Demetria terragena* and *Dermacoccus nishinomiyaensis* (Mayilraj et al., 2006; Yi et al., 2004). A search by using the EzTaxon server (Chun et al., 2007) revealed the following pair-wise similarity values for close relatives: *Ornithinimicrobium kibberense* DSM 17687[T] (96.3%), *Kribbia dieselivorans* N113[T] (95.5%), *Dermacoccus nishinomiyaensis* DSM 20448[T] (95.2%), *Kytococcus sedentarius* DSM 20547[T] (94.7%), *Arthrobacter cumminsii* DSM 10493[T] (94.3%), *Demetria terragena* DSM 11295[T] (93.9%), *Arthrobacter albus* DSM 13068[T] (94.0), *Janibacter limosus* DSM 11140[T] (93.7%), and *Arseniciccoccus bolidensis* CCUG 47306[T] (93.7%). Except for the two *Arthrobacter* species which represent a distant lineage of their genus (Pukall et al., 2006), the closest phylogenetic neighbors belong to the families *Dermacoccaceae* and *Intrasporangiaceae*. The comparison of the available 16S rRNA gene sequences of *Serinicoccus marinus* JC1078[T], *Ornithinimicrobium humiphilum* DSM 12362[T], and *Demetria terragena* DSM 11295[T] is hampered by the fact that the first ~50 nucleotides of sequence AY382898 (JC1078[T], 1421 nucleotides in total) and the last ~140 nucleotides of sequence AJ277650 (DSM 12362[T], 1306 nucleotides in total) are missing when compared to the sequence of *Demetria terragena* DSM 11295[T] (1457 nucleotides in total). The revised set of signature nucleotides for the family *Intrasporangiaceae* (Family *Intrasporangiaceae*, this volume), the phylogenetic association, and agreement in chemotaxonomic characteristics with members of this family support the affiliation of the genus *Serinicoccus* to the family *Intrasporangiaceae* as it has been proposed recently (Garrity et al., 2007; Montero-Barrientos et al., 2005).

The cells of the type strain of *Serinicoccus marinus* JC1078[T] are nonmotile cocci and share the presence of the diamino acid L-ornithine in the peptidoglycan with the closest phylogenetic neighbor *Ornithinimicrobium humiphilum* (Groth et al., 2001). The peptidoglycan of strain JC1078[T] contains the amino acids ornithine (Orn), alanine (Ala), glycine (Gly), serine (Ser), and glutamic acid (Glu). Ser represents the *N*-terminus of the interpeptide bridge. The peptide L-Ala→D-Glu typical of peptidoglycans cross-linked according to the A-type was detected in the partial hydrolysate of the peptidoglycan. The muramic acid residues of the glycan chain are acetylated. However, a detailed peptidoglycan structure could not be concluded from the analytical data.

Colonies of strain JC1078[T] on Marine agar 2216 (MA; Difco) are yellow, circular, convex, entire, glistening, opaque, and butyraceous. They are approximately 1 mm in diameter after 3 d and 3–4 mm after incubation for 5 d at 30°C. The type strain grows optimally at 35°C in the presence of 2–3% NaCl at pH 8. Growth is slow without NaCl and concentrations up to 14% NaCl were tolerated. An extended incubation time is required at low temperatures (e.g. 20 d at 5°C). Sparse growth was observed at pH 6 and 11. Strain JC1078[T] is strictly aerobic.

Serinicoccus marinus JC1078[T] originates from a surface seawater sample of the East Sea, Korea.

Isolation and maintenance procedures

Strain JC0178[T] was isolated from a surface seawater sample collected from the East Sea (Korea) by the standard dilution plating technique using MA and incubation at 30°C. The isolate was routinely cultured on MA with serial transfers at 4-week intervals. The strain was maintained as agar cultures at 4°C and as 20% (w/v) glycerol suspension at −80°C. Long-term preservation is possible by freeze-drying in skim milk or maintenance in liquid nitrogen.

Differentiation of the genus *Serinicoccus* from other genera

The genus *Serinicoccus* shares with the genus *Ornithinimicrobium* the diagnostic diamino acid L-Orn but can be differentiated by this criterion from members of the families *Intrasporangiaceae* and *Dermacoccaceae* (Table 134) as well as from *Arthrobacter albus* (Wauters et al., 2000) and *Arthrobacter cumminsii* (Funke et al., 1996) which show either the peptidoglycan variation A4α based on the diamino acid L-lysine or peptidoglycans based on diaminopimelic acid isomers like *Kribbia* and *Arsenicicoccus*. The menaquinone MK-8(H_4) occurs in the genera *Serinicoccus*, *Ornithinimicrobium*, *Kribbia*, *Arsenicicoccus*, and *Demetria* whereas MK-8(H_2) or completely unsaturated menaquinones were characteristic of *Dermacoccus* or *Kytococcus*, respectively.

The genus *Serinicoccus* differs from the phylogenetically closest genus *Ornithinimicrobium* in displaying Ser but lacking aspartic acid in the peptidoglycan and in its invariably coccoid morphology. Additional differentiating characteristics of *Serinicoccus* are its capability to decompose Tween 80 and tyrosine but negative

TABLE 134. Characteristics differentiating the genus *Serinicoccus* and phylogenetically related genera[a]

Characteristic	*Serinicoccus*[b]	*Arsenicicoccus*[c]	*Demetria*[d]	*Dermacoccus*[e]	*Kribbia*[f]	*Kytococcus*[e]	*Ornithinimicrobium*[g]
Morphology	Cocci	Cocci	Irregular cocci to short rods	Cocci	Irregular short rods or cocci	Cocci	Cocci
Peptidoglycan variation	nd	A3γ	A4α	A4α	nd	A4α	A4β
Interpeptide bridge of peptidoglycan	nd (L-Orn as diamino acid)	LL-Dpm–Gly	L-Lys–L-Ser–D-Asp	L-Lys–L-Ser$_{1-2}$–D-Glu or L-Lys–L-Ser$_{1-2}$–L-Ala–D-Glu	nd (*meso*-Dpm as diamino acid)	L-Lys–D-Glu$_2$	L-Orn–L-Ala–Gly–D-Asp
Major menaquinones	MK-8(H$_4$)	MK-8(H$_4$)	MK-8(H$_4$)	MK-8(H$_2$)	MK-8(H$_4$)	MK-8, MK-9, MK-10	MK-8(H$_4$)
Major fatty acids	$C_{15:0}$ iso, $C_{16:0}$ iso, $C_{17:1}$ iso, $C_{17:0}$ iso, $C_{15:0}$ anteiso	$C_{16:1}$ ω7*c*, $C_{15:0}$ iso, $C_{18:1}$	$C_{18:1}$, $C_{18:0}$, $C_{17:0}$, $C_{17:0}$ anteiso	$C_{17:0}$ iso, $C_{17:0}$ anteiso, $C_{17:1}$ iso	$C_{18:0}$ 10-methyl, $C_{16:0}$ iso, $C_{18:1}$, $C_{16:0}$, $C_{18:0}$	$C_{17:0}$ anteiso, $C_{17:0}$, $C_{17:1}$ iso	$C_{15:0}$ iso, $C_{16:0}$ iso
Polar lipids	PG, DPG, PI, GL	nd	DPG, PG, PI, PE, PL	DPG, PG, PI	nd	DPG, PG, PI	DPG, PG, PI, PL, GL
DNA G+C content (mol%)	72	72	66	66–71	69–70	68–69	70

[a]Abbreviations: Ala, alanine; Asp, aspartic acid; Dpm, diaminopimelic acid; Glu, glutamic acid; Gly, glycine; Lys, lysine; Orn, ornithine; Ser, serine; peptidoglycan variations according to Schleifer and Kandler (1972); MK-8(H$_4$), partially saturated menaquinone with two of eight isoprene units hydrogenated; MK-8, unsaturated menaquinone with eight isoprene units; DPG, diphosphatidylglycerol; PG, phosphatidylglycerol; PE, phosphatidylethanolamine; PI, phosphatidylinositol; PL, unidentified phospholipid(s); GL, unidentified glycolipid(s); nd, no data available.

[b]Data from Yi et al. (2004).

[c]Data from Collins et al. (2004).

[d]Data from Groth et al. (1997).

[e]Data from Stackebrandt et al. (1995).

[f]Data from Jung et al. (2006).

[g]Data from Groth et al. (2001).

reactions for nitrate reduction and decomposition of xanthine and hypoxanthine as well as the absence of alkaline and acid phosphatases, cystine arylamidase, and naphthol-AS-BI phosphohydrolase as revealed by the API ZYM test. *Serinicoccus marinus* can tolerate a NaCl concentration of 12% (Yi et al., 2004) and differs in this feature from *Ornithinimicrobium humiphilum* (Groth et al., 2001) and *Ornithinimicrobium kibberense* (Mayilraj et al., 2006). In contrast to *Ornithinimicrobium humiphilum* DSM 12362[T], *Serinicoccus marinus* is unable to grow at temperatures above 35°C (Groth et al., 2001; Yi et al., 2004).

List of species of the genus *Serinicoccus*

1. **Serinicoccus marinus** Yi, Schumann, Sohn and Chun 2004, 1587[VP]

ma.ri′nus. L. masc. adj. *marinus* of or belonging to the sea, marine.

In addition to the characteristics that define the genus, it has the characteristics described below. Growth occurs at NaCl concentrations of 0–14% (w/v), the optimum being 2–3%. The pH range for growth is pH 6–11, the optimum being pH 8. The temperature range for growth is 10–35°C, with the optimum being 35°C. Extended incubation (up to 20 d) is required at 5°C. Cells are cocci with diameters of 0.5–0.9 μm. Colonies on MA are yellow, circular, convex, entire, glistening, opaque, and butyraceous. Colonies are approximately 1 mm in diameter after 3 d (on MA at 30°C) and reach the maximum diameter of 3–4 mm after 5 d. Nitrate is reduced to nitrite. Does not show arginine dihydrolase activity. β-Galactosidase activity is weakly present. Does not produce H_2S on TSI agar, acid from glucose in API 20NE kit, or indole from tryptophan. Casein, DNA, esculin, gelatin, starch, Tween 80, and tyrosine are decomposed; adenine, alginate, cellulose, chitin, hypoxanthine, urea, and xanthine are not. Tested by the API ZYM enzyme assay, strain JC1078[T] is positive for esterase (C4), esterase lipase (C8), leucine arylamidase, valine arylamidase, and α-glucosidase; variable for trypsin and β-glucosidase; negative for alkaline phosphatase, lipase (C14), cystine arylamidase, α-chymotrypsin, acid phosphatase, naphthol-AS-BI-phosphohydrolase, α-galactosidase, β-galactosidase, β-glucuronidase, *N*-acetyl-β-glucosaminidase,

α-mannosidase, and α-fucosidase. Utilizes acetate, D-cellobiose, D-fructose, D-galactose, D-glucose, D-mannitol, D-mannose, D-sorbitol, D-trehalose, glycerol, and sucrose as a sole carbon source. Does not utilize acetamide, benzoate, citrate, D-ribose, ethanol, glycine, inulin, 2-propanol, lactose, L-arginine, L-ascorbate, L-lysine, L-rhamnose, *N*-acetylglucosamine, polyethylene glycol, salicylate, or tartrate. The major fatty acids are $C_{15:0}$ iso, $C_{16:0}$ iso, $C_{17:1}$ ω9*c* iso, $C_{17:0}$ iso, $C_{15:0}$ anteiso and $C_{17:0}$ anteiso.

Source: a seawater sample from the East Sea, Korea.

DNA G+C content (mol%): 72 (HPLC).

Type strain: JC1078, DSM 15273, JCM 13024, KCTC 9980, IMSNU 14026.

Sequence accession no. (16S rRNA gene): AY382898.

Genus XIII. **Terrabacter** Collins, Dorsch and Stackebrandt 1989, 4^VP

Erko Stackebrandt

Ter.ra.bac′ter. L. n. *terra* earth; N.L. masc. n. *bacter* masc. equivalent of Gr. neut. n. *baktron* a rod; N.L. masc. n. *Terrabacter* earth (soil) rod.

A rod–coccus growth cycle occurs during growth in complex media. Irregular rods are observed in exponential phase cultures; stationary phase cultures are composed mainly of coccoid cells. Both rods and coccoid forms are Gram-stain-positive. Cells are non-acid-fast and nonsporeforming. Rods nonmotile or motile. Growth at 10°C and 35°C. Optimum temperature for growth approximately 25–30°C. Growth in 5% NaCl. Catalase-positive. Oxidase-negative. **Obligatory aerobic**. Acid is not produced from glucose and other sugars in peptone-based media. Cellulose is not hydrolyzed. Nitrate is reduced to nitrite by two species. **The cell-wall peptidoglycan contains LL-2,6-diaminopimelic acid (variation A3γ) as the diamino acid; the interpeptide bridge consists of three glycine residues. Mycolic acids are absent. Major long-chain cellular fatty acid is $C_{15:0}$ iso; $C_{14:0}$ iso, $C_{15:0}$ anteiso, and $C_{16:0}$ iso are present in lesser amounts; $C_{17:0}$ anteiso and $C_{16:1}$ iso may be present. The polar lipids comprise diphosphatidylglycerol, phosphatidylethanolamine, and phosphatidylinositol. The major isoprenoid quinones are tetrahydrogenated menaquinones with eight isoprene units [MK-8(H_4)].**

DNA G+C content (mol%): 69.8 to 73.4.

Type species: **Terrabacter tumescens** (Jensen 1934) Collins, Dorsch and Stackebrandt 1989, 6 (*Corynebacterium tumescens* Jensen 1934, 45; *Arthrobacter tumescens* Conn and Dimmick 1947, 302; *Pimelobacter tumescens* Suzuki and Komagata 1983, 70).

Further descriptive information

The genus *Terrabacter* belongs to the family *Intrasporangiaceae* (Stackebrandt et al., 1997), defined by phylogenetic position and the presence of a common set of 16S rRNA gene signature nucleotides. Originally described as *Arthrobacter tumescens* (Jensen) the species was reclassified as *Pimelobacter tumescens* (Suzuki and Komagata, 1983). Differences in some chemotaxonomic properties of members of this genus (Collins et al., 1983; O'Donnell et al., 1982) pointed towards the taxonomic heterogeneity of the genus, which was dissolved by Collins et al. (1989) on the basis of the phylogenetic analysis of reverse transcribed 16S rRNA gene sequences. While *Pimelobacter jensenii* was reclassified as *Nocardioides jensenii*, *Pimelobacter tumescens* formed the nucleus of the new genus *Terrabacter*. The phylogenetically closest neighbors of *Terrabacter* species are *Terracoccus luteus* (97.6% 16S rRNA gene sequence similarity) and *Intrasporangium calvum* (97.3%). This intrafamily substructure is in accord with the composition of the peptidoglycan of its members (see the chapter on *Intrasporangiaceae*, above, and Table 125 for a description of characteristics that differentiate between the genera of the *Intrasporangiaceae*).

The type strain PPLB^T of the species *Terrabacter terrae*, (Montero-Barrientos et al., 2005) differs from *Terrabacter tumescens* DSM 20308^T in morphology, fatty acid composition, and some phenotypic properties (Table 135). The two type strains share 98.8% 16S rRNA gene sequence similarity and a low DNA–DNA reassociation value of 36.6%. The third species *Terrabacter aerolatus* 5516J-36^T (Weon et al., 2007b) differs from *Terrabacter terrae* CECT 3379^T and *Terrabacter tumescens* DSM 20308^T with respect to fatty acid composition and morphological, cultural, and phenotypic properties (Table 135). Phylogenetically, the three type strains are closely related (16S rRNA gene sequence similarities 98.7–98.9%). DNA–DNA reassociation values of less than 52% determined among the three *Terrabacter* type strains support their genomic separateness (Weon et al., 2007b).

According to API 20NE and API ZYM strips, the type strains of the three *Terrabacter* species are positive for esculin hydrolysis, gelatin hydrolysis, and β-galactosidase, esterase (C4), esterase lipase (C8), leucine arylamidase, acid phosphatase, naphthol-AS-BI-phosphohydrolase, α-galactosidase, and α-glucosidase activities. All strains are negative for indole production, glucose fermentation, and arginine dihydrolase, urease, alkaline phosphatase, cystine arylamidase, trypsin, α-chymotrypsin, β-glucuronidase, *N*-acetyl-β-glucosaminidase, and α-fucosidase activities. According to API 20NE and API ID32GN strips, all strains assimilate D-glucose, D-mannose, *N*-acetylglucosamine, maltose, potassium gluconate, malic acid, inositol, sucrose, sodium malonate, sodium acetate, L-alanine, glycogen, propionic acid, valeric acid, L-histidine, and 3-hydroxybutyric acid. None of the strains assimilate capric acid, trisodium citrate, phenylacetic acid, L-rhamnose, itaconic acid, suberic acid, potassium 5-ketogluconate, salicin, L-fucose, potassium 2-ketogluconate, or 4-hydroxybenzoic acid. The following reactions differ from the description of *Terrabacter terrae* listed as negative by Montero-Barrientos et al. (2005): mannose, *N*-acetylglucosamine, gluconate, esterase, *β*-galactosidase, and -glucosidase; these reactions are indicated in the species description below as in the original description.

Terrabacter species have frequently been isolated from unpolluted but also from polluted soil, e.g. from biphenyl-adapted soils (Wagner-Döbler et al., 1998). Organisms affiliated to the genus *Terrabacter* have been demonstrated to metabolize a variety of polycyclic aromatic hydrocarbons. Several catabolites were determined and genes characterized involved in

TABLE 135. Characteristics differentiating *Terrabacter tumescens*, *Terrabacter aerolatus* (Weon et al., 2007b), and *Terrabacter terrae* (Montero-Barrientos et al., 2005)[a]

Characteristic	*T. tumescens* DSM 20308^T	*T. aerolatus* 5516J-36^T	*T. terrae* strain PPLB^T
Cell shape	Rod–coccus cycle	Rods or cocci	Long rods
Colony color	White/gray	White	Yellow
Growth on 7% NaCl	–	–	+
Nitrate reduction	+	+	–
Urease	–	–	+[b]
Hydrolysis of DNA	+	–	–
Assimilation of:			
Adipic acid	w	–	w
L-Arabinose	+	–	+
α-D-Glucosamine	+	nd	–
3-Hydroxybenzoic acid	+	–	–
Lactic acid	–	–	+
D-Mannitol	+	+	–
D-Melibiose	+	+	–
L-Proline	+	–	–
D-Ribose	–	–	+
L-Serine	–	–	+
D-Sorbitol	+	+	–
Enzyme activities:			
β-Glucosidase	–	w	–
Lipase (C14)	–	+	–
α-Mannosidase	–	w	–
Valine arylamidase	–	+	–

[a]Symbols: +, Positive; –, negative; nd, not determined; w, weak.

[b]Tested negative by Weon et al. (2007b).

the degradation of biphenyl (Aislabie et al., 1999), dibenzofuran (Iida et al., 2002; Kasuga et al., 2001), fluorene analogs (Habe et al., 2004), and chlorinated dioxin (Habe et al., 2002). Whether these strains represent novel species will have to await their thorough characterization.

Enrichment and isolation procedures

Terrabacter tumescens can be isolated by standard methods on "poor" medium to obtain arthrobacters from soil: (g/liter) $Ca(H_2PO_4)_2$, 0.25; K_2HPO_4, 1.0; $MgSO_4·7H_20$ 0.25; $(NH_4)_2SO_4$, 0.25; Casein, 1.0; yeast extract, 0.7; glucose, 1.0; agar, 10.0; pH 6.9–7.0. Other media have been listed by Keddie and Jones (1981).

Terrabacter terrae strain PPLB^T was isolated from a soil sample mixed with Iberian pig hair by dilution plating on feather agar (g/liter: NH_4Cl, 0.5; NaCl, 0.5; K_2HPO_4, 0.3; KH_2PO_4, 0.4; $MgCl_2·6H_2O$, 0.1; yeast extract, 0.1; hammer-milled chicken feathers, 10; agar, 20) at 32°C (Kim et al., 2001; Williams et al., 1990). Colonies showed transparent "haloes" produced by the hydrolysis of keratin from chicken feathers.

Terrabacter aerolatus strain 5516J-36^T was isolated from an air sample from the Jeju region of Korea, by collecting air using an MAS-100 air sampler (Merck; single-stage multiple-hole impactor) which contained Petri dishes with R2A agar (BBL) amended with 200 mg cycloheximide ml21 (Sigma). Plates were incubated at 30°C and strain 5516J-36^T was recovered after 5 d.

Maintenance procedures

All strains grow on R2A medium (pH 7.0; Difco) at 30°C. Serial transfers on nutrient agar at 4-week intervals followed by maintenance at 4°C are recommended. Storage of cells as 20% (w/v) glycerol suspensions at −20°C and at −80°C is a suitable preservation method. Long-term preservation methods include freeze drying in skim milk and maintenance in liquid nitrogen at −196°C.

Differentiation of the genus *Terrabacter* from other genera

Properties in which the genus *Terrabacter* differ from phylogenetically neighboring taxa are indicated in Table 135 of the chapter on the genus *Terracoccus.*

List of species of the genus *Terrabacter*

1. **Terrabacter tumescens** (Jensen 1934) Collins, Dorsch and Stackebrandt 1989, 6^{VP} (*Corynebacterium tumescens* Jensen 1934, 45; *Arthrobacter tumescens* Conn and Dimmick 1947, 302; *Pimelobacter tumescens* Suzuki and Komagata 1983, 70)

   tu.mes´cens. L. part. adj. *tumescens* swelling up.

   A rod–coccus cycle occurs during growth on complex media. Cells from older cultures are coccoid (diameter, 0.5–8 µm). After transfer to fresh complex media, long, irregular rods (0.6–1.2 × 2.0–6.0 µm) are formed. The long rods show primary branching. Aerial mycelium is not

formed. Generally nonmotile (occasionally motile strains occur). Colonies are glossy, entire, smooth, and gray or white. Growth at 10 and 35°C; may or may not grow at 37°C. Optimum temperature for growth approximately 25–30°C. Most strains grow in 5% NaCl. Obligatory aerobic. Acid is not formed from glucose and other sugars in peptone-based media. Thiamine is the only growth factor required (when thiamine is provided, the organism utilizes an ammonium salt or nitrate as the sole nitrogen source in mineral salts medium with glucose as the carbon-plus-energy source). A wide range of organic compounds is utilized as sole or principal carbon-plus-energy sources for growth including D-alanine, crotonate, inositol, α-D-glucosamine, and raffinose; D-glucuronate, L-ornithine, D-phenylalanine, and uric acid are not utilized. Hippurate, and tyrosine are hydrolyzed. Starch may or may not be hydrolyzed. Some strains hydrolyze Tween 80. Sulfatase-negative. Some strains produce H_2S. Other reactions are listed in Table 135 and above. The major polyamine is putrescine, while spermidine and spermin occur in smaller amounts. There are major amounts of $C_{14:0}$ iso, $C_{15:0}$ iso, and $C_{16:0}$ iso whole cell fatty acids. In addition to polar lipids described for the genus, unidentified phospholipids may occur.

In most respects the description of the type strain corresponds to that of the species. The type strain is nonmotile. Starch and Tween 80 are hydrolyzed. H_2S is not produced.

Source: soil.

DNA G+C content (mol%): 69.8–72.4 (T_m).

Type strain: ATCC 6947, CCUG 23928, CIP 102515, DSM 20308, IAM 12345, NBRC 12960, JCM 1365, LMG 3818, NCIMB 8914, NRRL B-4012, VKM Ac-1120.

Sequence accession no. (16S rRNA gene): X83812, X83815.

2. **Terrabacter aerolatus** Weon, Schumann, Kroppenstedt, Kim, Song, Kwon, Go and Stackebrandt 2007b, 2108[VP]

a.e.ro.la′tus. Gr. n. *aer*, *aeros* air; L. part. adj. *latus* carried; N.L. masc. part. adj. *aerolatus* airborne.

Cells are strictly aerobic, Gram-stain-positive, motile rods or cocci (0.9–1.06 × 1.0–3.5 mm). Catalase-positive and oxidase-negative. Colonies are white, convex, and round. Optimal temperature for growth is 30°C. Growth occurs in the range 5–35°C. Optimal pH for growth is 7.0–8.0; growth occurs in the range pH 4–9. Growth occurs in the presence of 0–5% (w/v) NaCl. Casein, starch, tyrosine, and Tween 80 are hydrolyzed, but chitin, CMcellulose, pectin, hypoxanthine, and xanthine are not. Other reactions are listed in Table 135 and above. Whole-cell sugars are glucose, ribose, rhamnose, xylose, and galactose (in decreasing order of abundance). The major fatty acids (>5% of total fatty acids) are $C_{15:0}$iso (49.0%), $C_{16:0}$iso (14.5%), $C_{14:0}$iso (7.9%), $C_{17:0}$iso (5.8%), and $C_{15:0}$ anteiso (5.2%). In addition to polar lipids described for the genus, an unidentified phosphoglycolipid is present.

Source: from an air sample in Jeju, Korea.

DNA G+C content (mol%): 71.7 (HPLC).

Type strain: 5516J-36, DSM 18562, KACC 20556.

Sequence accession no. (16S rRNA gene): EF212039.

3. **Terrabacter terrae** Montero-Barrientos, Rivas, Velázquez, Monte and Roig 2005, 2293[VP]

ter′ra.e. L. gen. n. *terrae* of the earth.

Gram-stain-positive cells are long irregular rods 2.0 × 6.0 μm in length. Aerial mycelium is not formed. Nonmotile, nonsporeforming, and aerobic. Colonies are glossy and yellow on feather and nutrient agar. Oxidase-negative and catalase-positive. Chemoorganotrophic. Metabolism is respiratory; acid is not produced from glucose or other carbohydrates. A wide range of organic compounds are utilized for growth including mannitol, citrate, and chicken feathers. Does not assimilate arabinose, mannose, *N*-acetylglucosamine, maltose, gluconate, caprate, adipate, malate, or phenylacetate. Lipase, keratinase, and valine aminopeptidase are produced. Esterase, leucine aminopeptidase, cystine aminopeptidase, trypsin, chymotrypsin, acid phosphatase, β-galactosidase, α-glucosidase, β-glucosidase, and α-mannosidase are not produced. Negative for the Voges–Proskauer reaction and H_2S production. Negative for decomposition of esculin and Tween 80. Hydrolyzes urea, arginine, starch, and casein. Other reactions are listed in Table 135 and above. In addition to polar lipids described for the genus an unidentified phosphoglycolipid is present.

Source: soil mixed with Iberian pig hair.

DNA G+C content (mol%): 71 (T_m)

Type strain: PPLB, CECT 3379, JCM 14283, LMG 22921.

Sequence accession no. (16S rRNA gene): AY944176.

Genus XIV. **Terracoccus** Prauser, Schumann, Rainey, Kroppenstedt and Stackebrandt 1997, 1222[VP]

Peter Schumann

Ter.ra.coc′cus. L. fem. n. *terra* soil; Gr. masc. n. *kokkos* berry; N.L. masc. n. *Terracoccus* coccus isolated from soil.

Spherical to slightly ellipsoidal cells existing singly, in pairs, in tetrads, in irregular small clusters, or in more or less regular sarcinoid packets which may form large irregular clusters. Gram-stain-positive. Not acid-fast. Nonmotile. Nonsporeforming. Strictly aerobic. Chemoorganotrophic. Oxidase-negative. Catalase-positive. The **LL-diaminopimelic acid**-containing **peptidoglycan** of group **A3γ** has **three glycine residues as an interpeptide bridge** and **a fourth glycine residue bound to the α-carboxyl group of D-glutamic acid** in position 2 of the peptide subunit. The major **menaquinone** is **MK-8(H_4)**. The **fatty acid profile** represents the **iso, anteiso type** with predominately $C_{15:0}$ iso followed by $C_{15:0}$ anteiso. Minor straight-chain fatty acids occur. The **polar lipids** are **phosphatidylethanolamine, phosphatidylinositol**, **phosphatidylglycerol, and diphosphatidylglycerol**. Mycolic acids are absent. Phylogenetically, this genus is affiliated to the family *Intrasporangiaceae*, order *Micrococcales.*

DNA G+C content (mol%): 73.

Type species: **Terracoccus luteus** Prauser, Schumann, Rainey, Kroppenstedt and Stackebrandt 1997, 1223^{VP}.

Further descriptive information

The genus *Terracoccus* belongs to the family *Intrasporangiaceae* (Stackebrandt et al., 1997) as revealed by the occurrence of signature nucleotides defining this family (Stackebrandt and Schumann, 2000) except those at base pair positions 1134:1140, at which strain IMET 7848^T has an A–U rather than a C–G pair. At signature nucleotide positions which are variable for members of the family *Intrasporangiaceae*, *Terracoccus luteus* has a U–A pair (586:55), C (223), and a G–C pair (615:625). Initially, the family *Intrasporangiaceae* (Stackebrandt et al., 1997) was established to harbor only the type genus *Intrasporangium* (Kalakoutskii et al., 1967), the genus *Terrabacter* (Collins et al., 1989), and the peripherally related genus *Sanguibacter* (Fernández-Garayzábal et al., 1995a). The genus *Terracoccus* was described as the fourth member of the family *Intrasporangiaceae* (Prauser et al., 1997). As a consequence of the rapid taxonomic developments within the phylogenetic radiation of the genus *Terracoccus*, 15 genera are now included in the family *Intrasporangiaceae*. Therefore, the set of family-specific 16S rRNA gene signature nucleotides has been modified, and the family *Intrasporangiaceae* has been redefined recently under inclusion of phenotypic characteristics (see the family *Intrasporangiaceae*, above). A search by using the EzTaxon server (Chun et al., 2007) revealed the following pair-wise 16S rRNA gene similarity values for the closest relatives of *Terracoccus* that share the membership in the family *Intrasporangiaceae*: *Terrabacter tumescens* DSM 20308^T (98.1%) (Collins et al., 1989), *Intrasporangium calvum* DSM 43043^T (97.7%) (Kalakoutskii et al., 1967), *Terrabacter aerolatus* 5516J-36^T (97.57%) (Weon et al., 2007b), *Terrabacter terrae* LMG 22921^T (97.5%) (Montero-Barrientos et al., 2005), and *Humihabitans oryzae* KV-657^T (96.8%) (Kageyama et al., 2007a). The phylogenetic clustering of these genera (see Figure 161) is in agreement with the detection of a peptidoglycan structure which is unique among bacteria for all strains of the genera *Terracoccus* (Prauser et al., 1997), *Terrabacter* (Montero-Barrientos et al., 2005; Schleifer and Kandler, 1972; Weon et al., 2007b), and *Intrasporangium* (Schumann et al., 1997). The diamino acid at position 3 of the peptide subunit is LL-diaminopimelic acid (LL-A_2pm), the interpeptide bridge consists of three glycine residues, and a fourth glycine residue is bound to the α-carboxyl group of D-glutamic acid at position 2 of peptide subunit (variation A41.2 of peptidoglycan type A3γ according to http://www.peptidoglycan-types.info). Unfortunately, no information on this significant feature is available for the phylogenetic relative *Humihabitans oryzae* (Kageyama et al., 2007a).

The cells of *Terracoccus luteus* are invariably coccoid. Colonies were yellow with slight tendency to orange and appeared after 24–48 h under optimal conditions. The type strain of *Terracoccus luteus* DSM 44267^T gives rise to two stable colony forms on all applied agar media: (1) rough, irregularly raised and lobed (DSM 44274), and (2) smooth, convex, and circular (DSM 44275). Cells of the two colony forms cannot be differentiated by using 16S rRNA gene sequence comparison, DNA–DNA hybridization, analysis of cellular fatty acids, and RiboPrinting (P. Schumann, unpublished data), suggesting that *Terracoccus luteus* DSM 44267^T can develop rough as well as smooth colonies (Prauser et al., 1997).

Strain DSM 44267^T grows well on organic media such as R medium (Yamada and Komagata, 1972), medium 79m (Schumann et al., 1997), and tryptone soya broth (Oxoid CM129) medium, on agar media as well as in liquid shaken cultures. The addition of vitamins did not influence the growth. Growth on these media was optimal at 28°C and pH 6.0–7.2 but was sparse at pH 4.5 and 9.5. Growth was less rapid at 20°C, very poor at 15 and 37°C, and no growth occurred at 10 and at 45°C.

Cells grew only at the surface of stab cultures, and no growth was detectable on agar slant cultures covered with paraffin oil, indicating that *Terracoccus luteus* DSM 44267^T is strictly aerobic.

The type strain was not susceptible to any of eleven phages active against LL-A_2pm-containing actinomycetes according to the study of Prauser et al. (1997).

Strain DSM 44267^T originated from soil sampled from a dug pond. Information provided with sequences deposited in EMBL point also to the occurrence of *Terracoccus* strains in the phyllosphere (AB242812) and rhizosphere (AY143777). *Terracoccus luteus* is considered nonpathogenic.

Enrichment and isolation procedures

Strain DSM 44267^T was isolated from soil at the water-soil interface of the bank of a duck pond by the procedure described by Prauser et al. (1997). A diluted soil suspension in phosphate buffer was mixed with solubilized agar medium 79m modified by reduction of the agar content to 0.6% (w/v) at 48°C. This mixture was subsequently poured on agar medium 79m containing 1.5% (w/v) agar in Petri dishes and incubated at 28°C for 4–6 d until colonies became visible for isolation under a binocular microscope of low magnification.

Maintenance procedures

Serial transfers at 4-week intervals followed by maintenance at 4°C and storage of cells as 20% (w/v) glycerol suspensions at −20°C and at −80°C were suitable provisions for preservation. Long-term preservation methods include freeze drying in skim milk and maintenance in liquid nitrogen at −196°C.

Procedures for testing special characters

Since the peptidoglycan type A3γ, variation A41.2, is an extraordinary feature of members of the genera *Terracoccus*, *Terrabacter*, and *Intrasporangium*, its elucidation is described in the following.

The peptidoglycan was isolated and purified by disintegration of cells by shaking with glass beads and subsequent trypsine digestion as described by Schleifer and Kandler (1972) and Schleifer (1985). The peptidoglycan structure was determined by analyzing two hydrolysates of purified cell walls according to Schleifer (1985). The first hydrolysis was carried out with 4N HCl at 100°C for 16 h and resulted in amino acids. The second hydrolysate obtained under milder conditions (4 N HCl, 100°C, 45 min), in addition, contained dipeptides. The amino acids and peptides were separated and identified by two-dimensional ascending TLC on cellulose plates with the solvent systems of Schleifer and Kandler (1972). The isomer of A_2pm was determined by ascending chromatography on cellulose plates and comparison with authentic standards according to Rhuland et al. (1955). The structural variation A41.2 of peptidoglycan type A3γ was concluded from the presence of the amino acids LL-A_2pm, alanine (Ala), glycine (Gly), and glutamic acid (Glu)

as well as of the characteristic peptides L-Ala→D-Glu, Gly→D-Glu, LL-A_2pm→D-Ala, Gly→LL-A_2pm, and Gly→Gly. The structure was confirmed by the molar ratio of the amino acids of approximately 1:1:4:2 for LL-A_2pm:Glu:Gly:Ala, determined by gas chromatography as described by MacKenzie (1987).

Differentiation of the genus *Terracoccus* from other genera

The genus *Terracoccus* is differentiated from all members of the family *Intrasporangiaceae* (except its closest phylogenetic neighbors *Intrasporangium* and *Terrabacter*) by its peptidoglycan type A3γ, variation A41.2 (Table 125, in the family *Intrasporangiaceae*, above). *Arsenicicoccus bolidensis* is only distantly related to *Terracoccus luteus* (94.9%) and its peptidoglycan based on LL-A_2pm contains only a single glycine residue in the interpeptide bridge (Collins et al., 2004). *Terracoccus* differs in morphology from representatives of its most closely related genera *Intrasporangium*, *Terrabacter*, and *Humihabitans*. *Terracoccus* and *Terrabacter* contain menaquinone MK-8(H_4), which is found in all representatives of the family *Intrasporangiaceae* except for the genus *Intrasporangium* (MK-8) (Table 125). The DNA G+C value of *Terracoccus luteus* exceeds that of *Intrasporangium calvum* by 5 mol%. *Terracoccus* and *Terrabacter* show phosphatidylethanolamine as characteristic phospholipid which is absent from *Intrasporangium*. *Terracoccus luteus* differs from *Terrabacter* in containing phosphatidylglycerol and is not susceptible to the *Terrabacter tumescens* phages TB1 (DSM 49116) and Tb2 (DSM 49158). *Terracoccus luteus* contains $C_{15:0}$ iso, $C_{15:0}$ anteiso, and $C_{16:0}$ as major components of the cellular fatty acid profile, differing significantly from that of its closest relatives *Terrabacter tumescens* and *Intrasporangium calvum* by lower amounts of $C_{16:0}$ iso and higher amounts of $C_{15:0}$ anteiso (Prauser et al., 1997; Schumann et al., 1997).

Strain DSM 44267^T shows DNA–DNA similarity values of only 38.8% and 36.5% to the type strains of *Intrasporangium calvum* and *Terrabacter tumescens*, respectively (Prauser et al., 1997). These low values suggest that the level at which execution of DNA–DNA hybridization for organisms of the peptidoglycan type A3γ, variation A41.2, is indicated lies above a 16S rRNA gene sequence similarity threshold of 98% (see also: Stackebrandt and Ebers, 2006).

Further reading

Stackebrandt, E. and P. Schumann. 2006. Introduction to the taxonomy of actinobacteria. *In* The Prokaryotes: a Handbook on the Biology of Bacteria, 3rd edn, vol. 3, *Archaea, Bacteria, Firmicutes,* Actinomycetes (edited by Dworkin, Falkow, Rosenberg, Schleifer and Stackebrandt). Springer, New York, pp. 297–321.

List of species of the genus *Terracoccus*

1. **Terracoccus luteus** Prauser, Schumann, Rainey, Kroppenstedt and Stackebrandt 1997, 1223^{VP}

lu′te.us. L. masc. adj. *luteus* yellow.

In addition to the properties given in the genus description and in Table 125, this species has the following characteristics. The cells are 0.7–1.3 µm in diameter. The colonies, 0.05–2.0 mm in diameter, may be rough or smooth. Rough colonies are lobed and irregularly raised; smooth colonies are circular and convex. The cells of rough colonies are mostly arranged in more or less regular sarcinoid packets which may form large irregular clusters. The cells of smooth colonies occur singly, in pairs, in tetrads, or in small irregular clusters. Urease and H_2S are produced. Nitrate is not reduced to nitrite. Formation of acids from sugars and alcohols is not observed. Only a few of the tested compounds are utilized for growth, i.e. D-arabitol, gluconate, *N*-acetyl-D-glucosamine, *myo*-inositol, L-proline, succinate, sucrose, and D-turanose. Starch, casein, gelatin, esculin, and DNA are hydrolyzed; Tween 80 is not hydrolyzed. Sodium acetate, formate, aconitate, benzoate, citrate, hippurate, disodium succinate, and potassium tartrate are not utilized. Xanthine, hypoxanthine, adenine, and DL-tyrosine are not decomposed.

Source: soil at the water-soil interface of a duck pond on the island of Hiddensee, Germany.

DNA G+C content (mol%): 73 (HPLC).

Type strain: ATCC 700812, CCUG 39186, CIP 105511, DSM 44267, NBRC 16165, IMET 7848, JCM 10981.

Sequence accession no. (16S rRNA gene): Y11928.

Genus XV. **Tetrasphaera** Maszenan, Seviour, Patel, Schumann, Burghardt, Tokiwa and Stratton 2000, 601^{VP} emend. Ishikawa and Yokata 2006, 1371

Robert J. Seviour and Abdul M. Maszenan

Te.tra.spha.e′ra. Gr.n. *tetra* four; L. fem. n. *sphaera* sphere; N.L. fem. n. *Tetrasphaera* four spherical bacterial cells.

Cells show a highly variable morphology with some growing as highly irregularly septate filaments, cocci in tetrads, often clustered or in pairs, as single cells, or irregular elongated clusters of rods, or showing an ability to change from rods to cocci, from filamentous forms to single often swollen cocci, or to and from V or T shaped arrangements. Gram-stain-positive, but can stain Gram variably. Member of the family *Intrasporangiacaeae*, order ***Micrococcales***. Cells are nonmotile, and do not form endospores. Aerobic and chemoheterotrophic, often growing very slowly on a limited range of substrates. All strains may store polyphosphate

granules; poly-β-hydroxyalkanoates are only detected in some strains. Growth occurs at 10–35°C, but not in all strains, and at pH 6.0–9.0. Catalase-positive, and usually oxidase-positive, but indole-negative. Where tested, all strains can grow on acetate and glucose, and, in most cases, on propionate. **Cell-wall peptidoglycan contains *meso*-diaminopimelic acid, characterizing the A1γ murein, or 3-hydroxy-*meso*-diaminopimelic acid (A4γ) *for Tetrasphaera duodecadis* (formerly known as *Arthrobacter duodecadis*)**. **Menaquinone composition varies, but, in most strains, the major menaquinone is MK-8**(H_4). Polar lipid composition of isolates varies too, but diphosphatidylglycerol, phosphatidylinositol, and phosphatidylglycerol are generally present. The major fatty acids in all isolates except *Tetrasphaera elongata* Lp2^T are the branched fatty acids ($C_{16:0}$ iso and $C_{17:0}$ anteiso), although 10-methyl branched fatty acids may be present.

Isolated from activated sludge and arable soil.

DNA G+C content (mol%): 68–73.

Type species: **Tetrasphaera japonica** Maszenan, Seviour, Patel, Schumann, Burghardt, Tokiwa and Stratton 2000, 601^{VP}.

Further descriptive information

It is now clear that members of the genus *Tetrasphaera* are very diverse morphologically, and not all grow as cocci in tetrads. Sequence analyses of 16S rRNA and *rpoc1* genes from several isolates of the filamentous bacterium "*Candidatus* Nostocoida limicola" (Blackall et al., 2000) obtained from activated sludge plants suggested that these were probably members of the genus *Tetrasphaera* (Figure 170) despite showing marked differences in their cell morphologies from those originally described as typifying this genus (Maszenan et al., 2000). This view was substantiated after analyses of their peptidoglycan, fatty acid compositions, polar lipid and menaquinone compositions (Table 136), DNA–DNA hybridization values, and 16S–23S intergenic spacer region fingerprints. Thus, the "*Candidatus* Nostocoida

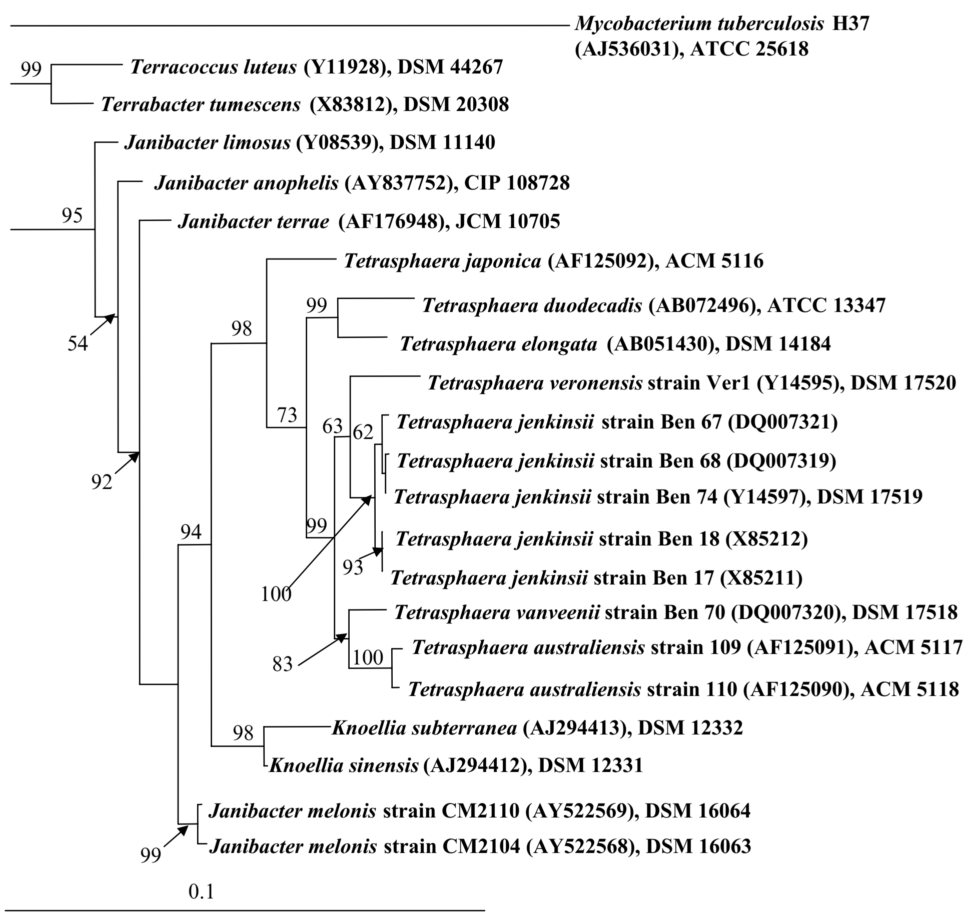

FIGURE 170. Phylogenetic analysis of 16S rRNA gene sequences of currently described members of the genus *Tetrasphaera* species, and their nearest relatives based on almost complete sequences, using maximum-likelihood. Bootstrap values based on 1000 replicates. Scale bar = 0.1 nucleotide substitutions per site.

TABLE 136. Characteristics of species within the genera *Tetrasphaera*, *Knoellia*, and *Janibacter*[a,b]

Characteristic	*T. japonica*	*T. australiensis* strain Ben 109	*T. australiensis* strain Ben 110	*T. duodecadis*	*T. elongata* strain LP2	*T. jenkinsii* strain Ben 17/74	*T. vanveenii* strain Ben 70	*T. veronensis* strain Ver 1	*J. anonphelis*	*J. limosus*	*J. melonis*	*J. terrae*	*K. sinensis*	*K. subterranean*
Polar lipids	DPG, PG, PI, PL, APL	DPG, PG, PI, PL	DPG, PG, PI, PE	nr	DPG, PG PI, PE, APL	PI, PG, APL	PG, DPG, PI, APL	DPG, PI, PG, APL	nr	DPG, PG, PI	PG, DPG, PI	DPG, PG, PI	PE, PI, DPG.	PE, PI DPG
Menaquinones	$MK-8(H_4)$ $MK-7(H_4)$ $MK-6(H_4)$	$MK-8(H_4)$ $MK-8(H_2)$ MK-8	$MK-8(H_4)$ $MK-6(H_4)$ $MK-8(H_2)$	$MK-8(H_4)$	$MK-8(H_4)$	$MK-8(H_4)$ $MK-6(H_4)$ $MK-8(H_2)$ MK-8	MK-8 $MK-8(H_2)$ $MK-8(H_4)$	nd	nr	MK-$8(H_4)$	MK-$8(H_4)$	$MK-8(H_4)$	$MK-8(H_4)$ $MK-7(H_4)$ $MK-6(H_4)$	$MK-8(H_4)$ $MK-7(H_4)$ $MK-6(H_4)$
Cell-wall type	A1γ	A1γ	A1γ	A4γ	A1γ	A1γ	A1γ	A1γ	A1γ	A1γ	A1γ	A1γ	A1γ	A1γ
Fatty acids	$C_{16:0}$ iso, $C_{17:0}$ anteiso	$C_{16:0}$ iso, $C_{17:0}$ anteiso, $C_{15:0}$ iso	$C_{16:0}$ iso, $C_{17:0}$ anteiso	$C_{17:0}$ 10 methyl, $C_{16:0}$ iso, $C_{15:0}$ iso	$C_{15:0}$ anteiso, $C_{15:0}$ iso, $C_{14:0}$ iso, $C_{16:0}$ iso	$C_{17:0}$ anteiso, $C_{16:0}$ iso, $C_{15:0}$ iso, $C_{16:1}$ iso	$C_{16:0}$ iso, $C_{15:0}$ iso	$C_{16:0}$ iso, $C_{16:1}$ iso, $C_{15:0}$ iso	$C_{16:0}$ iso, $C_{17:0}$, $C_{17:1}$ ω8*c*	$C_{16:0}$ iso, $C_{17:1}$ ω8*c*, $C_{18:1}$ ω9*c*	$C_{17:1}$ ω8*c*, $C_{16:0}$ iso, $C_{17:0}$, $C_{18:1}$ ω9*c*	$C_{16:0}$ iso, $C_{17:1}$ ω8*c*, $C_{18:1}$ ω9*c*	$C_{17:1}$ iso, $C_{15:0}$ iso, $C_{16:0}$ iso, $C_{17:0}$ iso, $C_{17:0}$ anteiso *c*	$C_{15:0}$ iso, $C_{17:0}$ iso, $C_{17:0}$ anteiso, $C_{16:0}$ iso

[a]Symbols: APL, unknown aminophospholipid; DPG, diphosphatidylglycerol; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; PI, phosphatidylinositol; PL, unknown phospholipid; nr not reported; nd not detected.

[b]Data for *Tetrasphaera duodecadis* from Ishikawa and Yokata (2006); for *Tetrasphaera australiensis* and *Tetrasphaera japonica* from Maszenan et al. (2000); for *Tetrasphaera veronensis*, *Tetrasphaera jenkinsii*, and *Tetrasphaera vanveenii* from McKenzíe et al. (2006); for *Knoellia sinensis* and *Knoellia subterranea* from Groth et al. (2002); for *Janibacter limosus, Janibacter melonis, Janibacter terrae,* and *Janibacter anophelis* from Kämpfer et al. (2006).

limicola" isolates currently in culture have been allocated to three new *Tetrasphaera* species (McKenzíe et al., 2006).

Several fluorescently labeled 16S rRNA targeted FISH probes have been designed and applied for the *in situ* identification of members of the filament morphotype in wastewater treatment plants, but not for each of the individual species (Liu and Seviour, 2001). This filament was originally isolated and named by van Veen (1973), and has been held responsible in several countries for episodes of bulking and possibly foaming in activated sludge systems. In pure culture, the *Tetrasphaera* and "*Candidatus* Nostocoida limicola" isolates described by Maszenan et al. (2000), Blackall et al. (2000), and Hanada et al. (2002) all accumulated polyphosphate granules. Later, Liu et al. (2001) showed that *Tetrasphaera*-related organisms identified by FISH and filamentous bacteria morphologically closely resembling "*Candidatus* Nostocoida limicola" also contained polyphosphate granules in a wastewater treatment plant designed to remove phosphorus (P), and thus were thought to behave as polyphosphate-accumulating organisms (PAO) there (Eschenhagen et al., 2003). All characterized isolates of *Tetrasphaera* contain polyphosphate (Hanada et al., 2002; McKenzíe et al., 2006) except *Tetrasphaera duodecadis* (Ishikawa and Yokota, 2006), which was isolated from arable soil (Lochhead, 1958). *Tetrasphaera elongata* (Hanada et al., 2002) was isolated from a laboratory scale plant designed to remove polyphosphate. Kong et al. (2005) confirmed that *Tetrasphaera*-related organisms were important PAO in activated sludge, but with a phenotype different from that expected of a PAO (Seviour et al., 2003), since these cells identified by FISH probing could not accumulate acetate anaerobically.

Furthermore, and agreeing with the pure culture data of Maszenan et al. (2000) and *in situ* observations of Liu et al. (2001), these strains did not accumulate polyhydroxyalkanoates, although all the characterized "*Candidatus* Nostocoida limicola" isolates do (Blackall et al., 2000; McKenzíe et al., 2006). Instead, the PAO *Tetrasphaera* species appeared to preferentially assimilate amino acids under anaerobic conditions (Kong et al., 2005). Kong et al. (2005) could not culture their *Tetrasphaera* related PAO, but, on the basis of the 16S rRNA gene sequences retrieved in their clone library study, they appear to differ from the *Tetrasphaera* species so far characterized in pure culture. Those *Tetrasphaera* described by Kong et al. (2005) did not fluoresce with the FISH probe of Kong et al. (2002) designed against the *Tetrasphaera* strains described by Maszenan et al. (2000). This suggests further phylogenetic diversity exists among members of this genus. Ishikawa and Yokata (2006) later reclassified *Arthrobacter duodecadis* Lochhead (1958) as another species, *Tetrasphaera duodecadis*, using both genotypic and chemotaxonomic characters.

In pure culture, cells of members of this genus demonstrate considerable morphological variations which are often medium dependent. This is especially true of the "*Candidatus* Nostocoida limicola" members (Blackall et al., 2000; McKenzíe et al., 2006). Unusual filamentous growth is formed on GS medium (Williams and Unz, 1985), where swollen, flattened, and irregularly septate cells are present. On R2A medium (Reasoner and Geldreich, 1985), the same strain grows as swollen and irregular cocci with occasional budding, often in short chains or clusters (Figure 171). *Tetrasphaera duodecadis* can switch its morphology between rods and coccoid forms (Ishikawa and Yokota, 2006).

Enrichment and isolation procedures

Strain T1-X7 was first isolated by Kataoka et al. (1996), after a prolonged enrichment of activated sludge under starvation conditions at very high biomass levels in a 0.1% peptone/0.1% glucose medium, and repeatedly fed batch culture (fill and draw conditions) with very low feed rate of the carbon source. After 6 months, the enriched cultures were streaked out onto a cell extract/agar medium. Colonies that grew were isolated and cultured. The strain T1-X7 obtained in this way grew as cocci in pairs and tetrads, and on the basis of a few phenotypic characters, was tentatively identified as a *Micrococcus*. It was shown subsequently to be *Tetrasphaera japonica*^{T} (Maszenan et al., 2000). Strain Lp2, *Tetrasphaera elongata*^{T} was isolated from the community of an anaerobic:aerobic sequencing batch reactor designed to remove PAO microbiologically, onto GM1 medium (Hanada et al., 2002). *Tetrasphaera duodecadis* (previously known as *Arthrobacter duodecadis*) was isolated from soil on medium containing vitamin B_{12} as an essential growth factor (Lochhead, 1958).

All other cultured strains including Ben 17, Ben18, Ben 67, Ben 68, and Ben 74^{T} (members of *Tetrasphaera jenkinsii*; McKenzíe et al., 2006), Ben 70^{T} (*Tetrasphaera vanveenii*; McKenzíe et al., 2006), Ver 1^{T} (*Tetrasphaera veronensis*; McKenzíe et al. (2006), and both Ben109^{T} and Ben 110 (both *Tetrasphaera australiensis*; Maszenan et al., 2000), were obtained after micromanipulation (Skerman, 1968) from activated sludge biomass onto either GS or R2A (Reasoner and Geldreich, 1985) medium, where they grew, but very slowly (Blackall et al., 2000; Maszenan et al., 2000; McKenzíe et al., 2006). Once in culture, these isolates could then grow on a range of other media, but always very slowly.

Differentiation of the genus *Tetrasphaera* from other genera

Members of the genus *Tetrasphaera* are most closely phylogenetically related to those of the genera *Janibacter* (Martin et al., 1997) and *Knoellia* (Groth et al., 2002) (Table 137). They share many chemotaxonomic characters with them, including cell-wall type (A1γ), although *Tetrasphaera duodecadis* (Ishikawa and Yokota, 2006) is distinctive in possessing an A4γ peptidoglycan. Groth et al. (2002) suggested that the type strain of *Tetrasphaera*, *Tetrasphaera japonica* T1-X7^{T}, differed from the known *Knoellia* species in possessing phosphatidylethanolamine. While *Tetrasphaera australiensis* (strain BEN110) and *Tetrasphaera elongata* strain Lp2^{T} contain this phospholipid (Hanada et al., 2002; Maszenan et al., 2000), neither *Tetrasphaera jenkinsii*, *Tetrasphaera vanveenii*, or *Tetrasphaera veronensis* (McKenzíe et al., 2006) do so. It is not clear whether it is present in *Tetrasphaera duodecadis* but has not been detected in any *Janibacter* species.

Fatty acid profiles can help distinguish *Tetrasphaera* species from *Knoellia* species and *Janibacter* species, as shown in Table 136. For example, although $C_{17:0}$ iso is a major fatty acid in *Knoellia* species (Groth et al., 2002), it is often absent or only a minor component in *Tetrasphaera* species (Hanada et al., 2002; Ishikawa and Yokota, 2006; Maszenan et al., 2000; McKenzíe et al., 2006), which in most cases produce $C_{16:0}$ iso as their major fatty acid. *Tetrasphaera elongata* Lp2^{T} has a distinctive profile, with $C_{15:0}$ anteiso as its major fatty acid (Hanada et al., 2002), as does *Tetrasphaera jenkinsii* (McKenzíe et al., 2006) with $C_{17:0}$ anteiso. With *Janibacter* species, $C_{17:1}$ ω8*c* and $C_{18:1}$ ω9*c* are major fatty

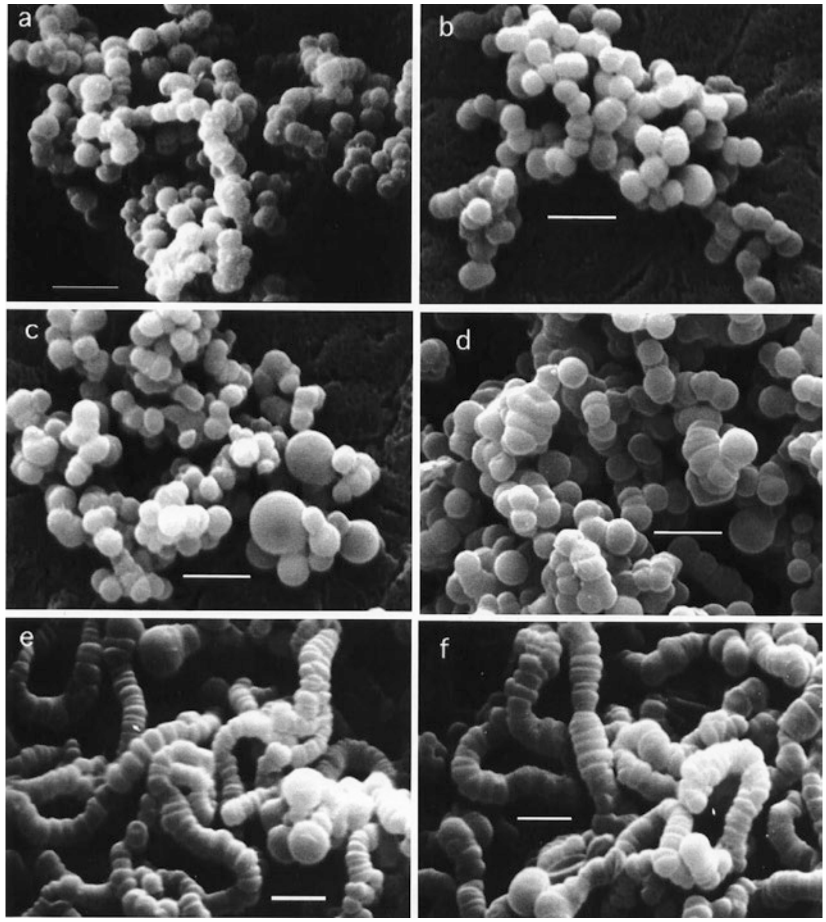

FIGURE 171. SEM of *Tetrasphaera jenkinsii* (Ben 17, Ben 67, Ben 68, and Ben74) and *Tetrasphaera veronensis* (Ver1^{T}) strains showing medium-dependent morphological variations. (a and b) Ben 17 and Ben 70, respectively, grown on R2A, where both appear as regular cocci in clusters and short chains. In Ben 70 (c) and Ben 74 (d), these cocci can appear inflated and, in some cases, appear to bud. (e and f) Ben 67 and Ben 68, respectively, showing typical features of all these strains when grown on GS agar, i.e. irregular flattened discoid cells in chains with occasional terminal swollen coccoid cells (McKenzíe et al., 2006).

acids, making their profiles quite different from those detected in *Tetrasphaera* species (Kämpfer et al., 2006). However, their fatty acid profiles vary with growth conditions (Ishikawa and Yokota, 2006; Kämpfer et al., 2006; Martin et al., 1997; McKenzíe et al., 2006; Yoon et al., 2004; Yoon et al., 2000), hence their taxonomic value should be interpreted cautiously.

Taxonomic comments

Individual members of the genus *Tetrasphaera* are morphologically very variable and differ in many phenotypic characteristics. 16S rRNA gene sequence analyses, while clearly placing the genus *Tetrasphaera* in the family *Intrasporangiaceae* in the order *Micrococcales*, also suggest that seven species of *Tetrasphaera* can currently be recognized as falling within the radiation of this genus (Figure 170). Thus, the 16S rRNA gene sequences of strains Ben 17, Ben 18, Ben 67, Ben 68, and Ben 74 (members of the genus *Tetrasphaera jenkinsii*) are more than 99.5% similar to each other, while those of Ben 109 and Ben 110 (*Tetrasphaera australiensis*) are 99.7% similar (Maszenan et al., 2000; McKenzíe et al., 2006). On the other hand, strains T1-X7 (*Tetrasphaera japonica*), Lp2 (*Tetrasphaera elongata*), Ver1 (*Tetrasphaera veronensis*), Ben 70 (*Tetrasphaera vanveenii*), and *Tetrasphaera duodecadis* are less than 98.5% similar to each other and to all of the other *Tetrasphaera* strains. Available DNA–DNA hybridization and certain chemotaxonomic data (Table 136) support this speciation (Hanada et al., 2002; Ishikawa and Yokota, 2006; Maszenan et al., 2000; McKenzíe et al., 2006).

Differentiation of the species of the genus *Tetrasphaera*

The individual *Tetrasphaera* species are separated mainly on the basis of their 16S rRNA gene sequences, chemotaxonomic attributes, and available DNA–DNA hybridization values, since, where examined, intraspecies differences are common in many of the biochemical characters used in their descriptions. This variation might reflect their very slow growth rates and overall low levels of metabolic activity. Furthermore, some species contain only a single isolate. The chemotaxonomic features which help differentiate the seven species of *Tetrasphaera* from each other and from members of their most closely related genera *Janibacter* and *Knoellia* are given in Table 136.

TABLE 137. Characteristics of *Tetrasphaera* and the genera *Janibacter* and *Knoellia*[a]

Characteristics	*Tetrasphaera*	*Janibacter*	*Knoellia*
Morphology	Varies with growth conditions; rods or cocci, sometimes swollen in pairs, tetrads, or irregular aggregates, showing V or T arrangements, and in some, flattened irregular discoid cells arranged as filaments	Coccoid to rod-shaped cells appearing in short chains or aggregates	Coccoid cells formed in stationary phase but reverted to irregular rod-shaped cells in fresh medium, appearing mycelial like, with outgrowths
Motility	Nonmotile	Nonmotile	Nonmotile
Endospores	Absent	Absent	Absent
Habitat	Activated sludge or soil	Sewage sludge, melon, mosquito gut, soil	Soil in a cave
DNA G+C content (mol%)	68–73	70–73	68–69
Cell-wall type	A1γ or A4γ	A1γ	A1γ
Catalase	+	+	+
Oxidase	+/−	−, weak for *J. terrae*	−
Nitrate reduction to nitrite	+/− (+ for *T. elongata*)	+ *J. terrae*	+
Polyphosphate granules	+	−	−
Signature nucleotides:			
952/1229	T–A	C–G	na
1133/1141	G–C	A–T	na

[a]Symbols: +, positive; −, negative; na, not available.

List of species of the genus *Tetrasphaera*

1. **Tetrasphaera japonica** Maszenan, Seviour, Patel, Schumann, Burghardt, Tokiwa and Stratton 2000, 601[VP]

ja.po′ni.ca. N.L. fem. adj. *japonica* pertaining to Japan, the source of the type strain.

Grows very slowly as cocci in clusters of tetrads. Weakly catalase-positive and oxidase-positive. Cell-wall type is A1γ. The major respiratory menaquinone is MK-8(H_4), although MK-7(H_4) and MK-6(H_4) are also produced, and it contains an unknown amino phospholipid. Its major fatty acids are $C_{16:0}$ iso and $C_{17:0}$ anteiso, and it produces tuberculostearic acid. It utilizes pyruvate, but failure to detect utilization of other substrates may be an artifact, reflecting its very low level of metabolic activity.

DNA G+C content (mol%): 71 (T_m).

Type strain: T1-X7, ACM 5116, DSM 13192, JCM 21381, NBRC 103088.

Sequence accession no. (16S rRNA gene): AF125092.

2. **Tetrasphaera australiensis** Maszenan, Seviour, Patel, Schumann, Burghardt, Tokiwa and Stratton 2000, 601[VP]

aus.tra.li.en′sis. N.L. fem. adj. *australiensis* pertaining to Australia, the source of the type strain.

Grows very slowly as cocci in clusters of tetrads. Cell-wall type is A1γ. The major menaquinone is MK-8(H_4), and MK-8(H_2) is present in smaller amounts. One strain, Ben 110[T], contains large amounts of MK-6(H_4), while Ben 109[T] has trace amounts of MK-8. Can utilize adenosine-5′-monophosphate, 3-methylglucose, sucrose, D-trehalose, Tween 40, Tween 80, and D-xylose. Major fatty acids are $C_{16:0}$ iso and $C_{17:0}$ anteiso.

DNA G+C content: 68–70 (T_m).

Type strain: Ben 109, ACM 5117, DSM 12890, JCM 21391, NBRC 103087.

Sequence accession no. (16S rRNA gene): 125091.

3. **Tetrasphaera duodecadis** (Lochhead 1958) Ishikawa and Yokata 2006, 1371 (*Arthrobacter duodecadis* Lochhead 1958, 170)

du.o.de′ca.dis. L. n. *duodecas -adis* the number twelve; L. gen n. *duodecadis* of twelve, referring to the requirement of the organism for vitamin B_{12}.

Cells are Gram-stain-variable and can change morphology from rods to coccoid cells. Require vitamin B_{12} and thiamine for growth. Grows at 10°C but not at 37°C. Negative for aminopeptidase, H_2S, acetoin, and indole production, but positive for catalase and nitrate reduction, β-galactosidase production, and for the oxidative and fermentative (O/F) test. Assimilates glucose, fructose, mannitol, and sucrose. Acetamide, esculin, and gelatin are hydrolyzed, but arginine or urea are not. Cell-wall peptidoglycan is type A4γ, where 3-hydroxy *meso*-diaminopimelic acid is present. The menaquinone is MK-8(H_4). Major fatty acids are $C_{17:0}$ 10 methyl, $C_{15:0}$ iso, and $C_{16:0}$ iso, Tuberculostearic acid and $C_{16:0}$ 10 methyl are also found.

DNA G+C content(mol%): 73 (HPLC).

Type strain: ATCC 13347, CIP 102696, IAM 14868, JCM 11564, LMG 17308, NBRC 12959, NCIMB 9222, VKM Ac-1108.

Sequence accession no. (16S rRNA gene): AB072496.

4. **Tetrasphaera elongata** Hanada, Liu, Shintani, Kamagata and Nakamura 2002, 886[VP]

e.lon′ga.ta. L. fem. part. adj. *elongata* elongated, pertaining to the formation of elongated clumps.

Grows as oval to short rods, sometimes forming elongated linear clumps. Can reduce nitrate to nitrite but not to dinitrogen. Acetate, alanine, arginine, cellobiose, glucose, lactose, maltose, mannose, sucrose, melibiose, trehalose, xylose, dulcitol, inositol, mannitol, and pyruvate are oxidized

aerobically; starch and glycogen are hydrolyzed. Cell-wall type is A1γ. The major menaquinone is MK-8(H_4), and major fatty acids are $C_{15:0}$ anteiso, $C_{15:0}$ iso, $C_{14:0}$ iso, and $C_{16:0.}$

DNA G+C content (mol%): 70 (T_m).

Type strain: Lp2, DSM 14184, JCM 11141, NBRC 103079.

Sequence accession no. (16S rRNA gene): AB030911.

5. **Tetrasphaera jenkinsii** McKenzie, Seviour, Schumann, Maszenan, Liu, Webb, Monis, Saint, Steiner and Seviour 2006, 2288[VP]

jen.kin′si. i. N.L. gen. masc. n. *jenkinsii* of Jenkins, referring to David Jenkins, a contemporary American environmental engineer who has made a considerable contribution to our understanding of the filamentous bacteria causing bulking and foaming in activated sludge processes.

The phenotypic features of this species are those given by Blackall et al. (2000) for "*Candidatus* Nostocoida limicola" strains Ben 17, Ben 18, Ben 67, and Ben 68, supplemented by results for Ben 74[T], as follows: morphology varies from long irregular filaments on GS medium to swollen clustered cells on R2A medium. Cells are often Gram-stain-variable. Nonfermentative. No anaerobic growth or with nitrite as sole electron acceptor, but can reduce nitrate to nitrite. Utilizes acetate, fructose, glucose, glycerol, lactose, pyruvate, propionate, mannose, and Tween 80, but not citrate, ethanol, lactate, oleate, or succinate, as sole carbon sources, and nitrate, nitrite, peptone and urea as N sources. Catalase- and oxidase-positive. All strains stained positively for polyphosphate and on some media for poly-β-hydroxyalkanoates. Cell-wall type is A1γ. Major menaquinone is MK-8(H_4), and MK-6(H_4), MK-8, and MK-8(H_2) occur in minor amounts. The polar lipid pattern contains phosphatidylinositol, phosphatidylglycerol, and an unidentified aminophospholipid. Major cellular fatty acids are $C_{17:0}$ anteiso, $C_{16:0}$ iso, $C_{16:1}$ iso, and $C_{15:0}$ iso.

DNA G+C content (mol%): not available.

Type strain: Ben 74, DSM 17519, JCM 15590, NCIMB 14128.

Sequence accession no. (16S rRNA gene): DQ007321.

6. **Tetrasphaera vanveenii** McKenzie, Seviour, Schumann, Maszenan, Liu, Webb, Monis, Saint, Steiner and Seviour 2006, 2288[VP]

van.vee′ni.i. N.L. gen. masc. n. *vanveenii* of van Veen, referring to a contemporary Dutch microbiologist, W.L. van Veen, who originally isolated this filamentous bacterium from activated sludge.

The morphological and phenotypic attributes of this species are the same as those described for *Tetrasphaera jenkinsii.* Cells stain positively for both poly-β hydroxy alkanoates and polyphosphate. Cell-wall type is A1γ. The major menaquinone is MK-8, with MK-8(H_2) and MK-8(H_4) produced in smaller amounts. The polar lipid pattern consists of phosphatidylinositol, phosphatidylglycerol, diphosphatidylglycerol, and an unidentified aminophospholipid. Major cellular fatty acids are $C_{16:0}$ iso and $C_{15:0}$ iso.

DNA G+C content (mol%): not available.

Type strain: Ben 70, DSM 17518, JCM 15591, NCIMB 14127.

Sequence accession no. (16S rRNA gene): DQ007320.

7. **Tetrasphaera veronensis** McKenzie, Seviour, Schumann, Maszenan, Liu, Webb, Monis, Saint, Steiner and Seviour 2006, 2288[VP]

ve.ro.nen′sis. L. fem. adj. *veronensis* of Verona, Italy, where the isolates originated.

The morphological and phenotypic features of this species are the same as those described for *Tetrasphaera jenkinsii*[T], except that sucrose can be used as a carbon source. Cell-wall type is A1γ. Isoprenoid quinones could not be detected in the type strain. The polar lipid pattern contains phosphatidylinositol, phosphatidylglycerol, diphosphatidylglycerol, and an unidentified phospholipid. Major cellular fatty acids are $C_{16:0}$ iso, $C_{16:1}$ iso, and $C_{15:0}$ iso.

DNA G+C content (mol%): not available.

Type strain: Ver 1, DSM 17520, JCM 15592, NCIMB 14129.

Sequence accession no. (16S rRNA gene): Y14595.

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Family X. **Jonesiaceae** Stackebrandt, Rainey and Ward-Rainey 1997, 485[VP] emend. Zhi, Li and Stackebrandt 2009, 598

Erko Stackebrandt

Jone.si.a′ce.ae. N.L. fem. n. *Jonesia* type genus of the family; *-aceae* ending to denote a family; N.L. fem. pl. n. *Jonesiaceae* the *Jonesia* family.

Gram-stain-positive, branched rods. Mycelium absent. Chemotaxonomic and metabolic properties as for the genus *Jonesia*.

The pattern of 16S rRNA gene signatures consists of nucleotides at positions 120 (A), 131:231 (A–G), 196 (C), 342:347 (C–G), 444:490 (A–U), 580:761 (C–G), 602:636 (C–G), 670:736 (A–U), 822:878 (U–C), 823:877 (A–C), 826:874 (U–G), 827 (G), 843 (C), 950:1231 (U–A), 1047:1210 (G–C), 1109 (C), 1145 (G), 1309:1328 (G–C), 1361 (G), and 1383 (C) (Zhi et al., 2009). The set of signature nucleotides is defined on the basis of the two type strains, representing the family. It may need to be changed when more genera and species are added to *Jonesiaceae*.

DNA G+C content (mol%): 56–58.

Type genus: **Jonesia** Rocourt, Wehmeyer and Stackebrandt 1987, 268[VP].

Genus I. **Jonesia** Rocourt, Wehmeyer and Stackebrandt 1987, 268[VP]

Erko Stackebrandt

Jones′i.a. L. fem. n. *Jonesia* of Jones, honoring Dorothy Jones, a British microbiologist

Nonsporeforming rods (0.3 × 0.5 µm) showing branched Y- and club-like forms. Irregular, branching rods may occur. Gram-stain-positive, but many cells, especially in older cultures, are readily decolorized. Filamentous and coccoid cells may also occur in older cultures. Colonies on nutrient agar are 0.5–1.5 mm in diameter (24–48 h), convex, smooth, grayish, and translucent to opaque, becoming yellowish in 10–20 d. Catalase-positive; oxidase-negative. Cell wall contains peptidoglycan of the L-Lys–L-Ser–D-Glu type (A4α) and teichoic acid of the poly(ribitol phosphate) type (ribitol:galactose-amine:galactose, 1:0.2:0.1) (Only *Jonesia denitrificans* was investigated.) No lipoteichoic acid. Predominant fatty acids are $C_{15:0}$ ante and $C_{16:0}$; $C_{16:0}$ iso may occur. Polar lipids are phosphatidylglycerol and phosphatidylinositol; diphosphatidylglycerol, unknown phosphoglycolipids and small amounts of unidentified phospholipids may occur. No mycolic acids. Major isoprenoid type is MK-9. Members of the genus are phylogenetically remotely related to the genera *Dermabacter* and *Brachybacterium*.

DNA G+C content (mol%): 56–58.

Type species: **Jonesia denitrificans** (Prévot 1961) Rocourt, Wehmeyer and Stackebrandt 1987, 269[VP] (*Listeria denitrificans* Prévot 1961, 512.)

Further descriptive information

Jonesia (*Listeria*) *denitrificans* is serologically distinct from *Listeria* species and from *Erysipelothrix* (Welshimer and Meredith, 1971; Wilkinson and Jones, 1975). It is pathogenic to rats and mice when injected intraperitoneally but does not cause conjunctivitis when instilled into the eyes of rabbits and guinea pigs.

Enrichment and isolation procedures

Jonesia (*Listeria*) *denitrificans* was isolated from cooked ox blood (Sohier et al., 1948), but the natural habitat of the organism is not known. It grows well on tryptic soy broth agar (TSBA, Difco, containing 1.5% Difco agar).

Jonesia qinghaiensis (Schumann et al., 2004) was isolated from a mud sample of a soda lake (~pH 9.0) in the west of China. Isolation was done at 28°C by dilution plating on Bacto marine broth agar (MBA), pH 7.2.

Maintenance procedures

Strains may be preserved for some months by stab inoculation in nutrient agar (Tryptose Agar or other similar media) in screw-capped vials. These should be stored at room or, preferably, refrigerator temperature. Strains may be preserved long-term by lyophilization or in liquid nitrogen.

Differentiation from closely related taxa

The genus *Jonesia* is not closely related to any other genus of the suborder *Micrococccineae* (Rainey et al., 1995; Stackebrandt et al., 1997; Figure 172), which has been elevated to order *Micrococcales* in the present volume. Some diagnostic morphological and chemotaxonomic properties of *Jonesia denitrificans* and other taxa to which this species appears related today and has appeared to be related in the past are indicated in Table 138. The closest relatives are members of the family *Dermabacteraceae* Stackebrandt et al. 1997 embracing *Dermabacter* and *Brachybacterium* and, more distantly related, *Brevibacteriaceae* Breed 1953 emend. Stackebrandt et al. 1997, containing *Brevibacterium. Jonesia* species differ from those of *Dermabacter* and *Brachybacterium* in peptidoglycan composition, but they share fully unsaturated menaquinones and similar composition in fatty acids and polar lipids; these, however, are also frequently represented in other members of *Micrococcales*. A loose relationship between *Jonesia* (*Listeria*) *denitrificans* and *Brachybacterium faecium* was observed earlier in a numerical phenetic study (Jones, 1975).

Taxonomic comments

Originally placed into the genus *Listeria*, evidence accumulated on the basis of results from morphological, biochemical, serological, chemical, and nucleic acid studies that *Listeria denitrificans* is not a member of the genus *Listeria* (Chatelain and Second, 1976; Collins et al., 1983; Fiedler et al., 1984; Fiedler and Seger, 1983; Jones, 1975; Stuart and Welshime, 1973, 1974;

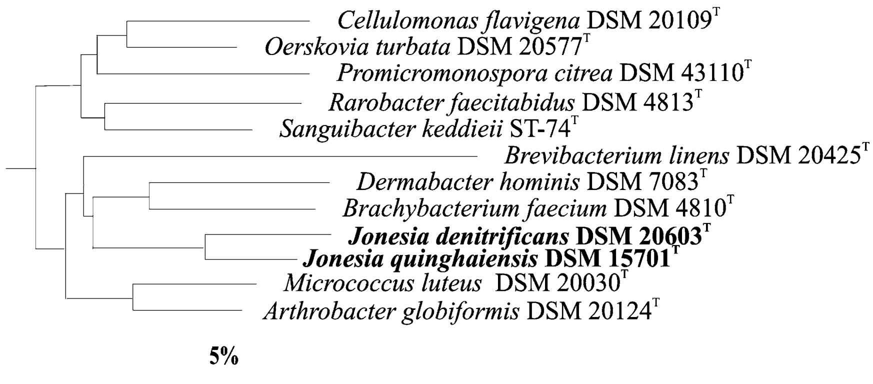

FIGURE 172. Neighbor-joining dendrogram of nearly complete 16S rRNA gene sequences displaying the phylogenetic relationship between *Jonesia* type strains and some representatives of members of the order *Micrococcales*. Numbers at nodes refer to bootstrap values (1000 resamplings). Scale bar = 5% nucleotide substitutions.

Welshimer and Meredith, 1971; Wilkinson and Jones, 1975; Wilkinson and Jones, 1977). In the pre-16S rRNA gene sequencing era, *Listeria denitrificans* was found more closely related to coryneform bacteria than to other members of the genus *Listeria*. Relatedness to *Oerskovia*, *Renibacterium*, and *Arthrobacter* was discussed but excluded on the basis of individual chemotaxonomic properties (see Seeliger and Jones, 1986). 16S rRNA oligonucleotide analysis then separated *Listeria denitrificans* CIP 55134^T from the other members of *Listeria*, leading to the description of *Jonesia denitrificans* (Rocourt et al., 1987), which was placed among the Gram-positive bacteria with a high DNA G+C mol%.

Major differences in the DNA G+C value (mol%), peptidoglycan structure, lipid pattern, isoprenoid quinone and the 16S rRNA oligonucleotides supported the taxonomic placement of *Jonesia denitrificans* within the class *Actinobacteria*. This conclusion was challenged on the basis of results of a comparative study of intracellular proteins (Finn and Rowe, 1996) of *Listeria* species and *Jonesia* (*Listeria*) *denitrificans*. However, the recent description of a second *Jonesia* species (Schumann et al., 2004) fully supports the membership of *Jonesia* within the class *Actinobacteria* (Stackebrandt et al., 1997) and outside the realm of authentic *Listeria* and related taxa.

The higher order relationship of the genus *Jonesia* has seen some changes over the past decade. Based on a rather restricted dataset of 16S rRNA gene sequences and emphasizing chemotaxonomic data, especially the peptidoglycan type, the genera *Cellulomonas*, *Oerskovia*, *Promicromonospora*, and *Jonesia* were included in a new family *Cellulomonadaceae* (Stackebrandt and Prauser, 1991). The distinct position of *Jonesia* within the new family was clearly stated as it was the only species that differed in the possession of teichoic acids, menaquinone type (MK-9), and a significantly lower base composition of DNA (56–58 mol% G+C) (properties also found in *Jonesia quinghaiensis*) from other members of the family [MK-9(H_4) and 70–76 mol% G+C, respectively]. A few years later, the sequence database of members of the order *Micrococcales* (Stackebrandt et al., 1997; Zhi et al., 2009) increased considerably, resulting in a phylogenetic picture that differs significantly from that in 1991. While *Cellulomonas* and *Oerskovia* are still seen as phylogenetic neighbors, the type species of *Promicromonospora* branches separately from, though closely to, the *Cellulomonas/ Oerskovia* lineage, but this branching is not supported by high statistical significance. The phylogenetic separation of *Jonesia* from these organisms is supported by chemotaxonomic evidence, and the new data finds *Jonesia* to be only remotely related to any member of the *Micrococcales*. *Brachybacterium*, *Dermabacter*, and *Brevibacterium* are the genera grouping most closely to *Jonesia*, but the low branching points are not statistically significant. As a consequence, *Jonesia* was proposed to be excluded from the family *Cellulomonadaceae* (Rainey et al., 1995); a formal description of the family *Jonesiaceae* Stackebrandt, Rainey and Ward-Rainey, (1997) followed a few years later (Stackebrandt et al., 1997).

Differentiation of the species of the genus *Jonesia*

The type strains of the two *Jonesia* species share 96.6% 16S rRNA gene sequence similarity. The nearest neighbors of the order *Micrococcales* are less than 93.5% related. According to API 50 CHE (*indicates a positive reaction also in the Biolog GP2 substrate panel) the type strains of both species utilize: glycerol, L-arabinose*, D-xylose*, galactose, D-glucose*, D-fructose*, D-mannose*, arbutin*, esculin, salicin*, cellobiose, maltose*, lactose, sucrose*, trehalose, starch, glycogen, β-gentiobiose, D-turanose, and 5-ceto-gluconate. None of them utilized (*negative reaction also in the Biolog GP2 substrate panel): erythritol, D-arabinose, ribose, L-xylose, adonitol, β-methyl-xyloside, L-sorbose, rhamnose*, dulcitol, inositol*, mannitol*, sorbitol, inulin, melezitose*, D-raffinose*, xylitol, D-tagatose, D-fucose, L-fucose*, D-arabitol*, L-arabitol, 2-keto-gluconate, α-methyl-D-mannoside*, α-methyl-D-glucoside*, and *N*-acetyl-D-glucosamine*. According to the Biolog GP2 substrate panel, the following substrates were utilized in addition: dextrin, glycerol, maltotriose, D-psicose, D-ribose, adenosine, and 2′-deoxyadenosin. The other substrates of the Biolog GP2 substrate panel were not used.

Differentiating properties are indicated in Table 139.

TABLE 138. Diagnostic properties in which *Jonesia* differ from some other genera of the order *Micrococcales*[a,b]

Characteristic	*Jonesia*	*Beutenbergia*	*Brachybacterium*	*Brevibacterium*	*Cellulomonas*	*Cellulosimicrobium*	*Dermabacter*	*Promicromonospora*
Branched rods	v	–	–	+	+	–	–	–
Branching mycelium	–	–	–	–	–	+	–	+
Fragmentation of mycelium in older cultures	–	–	–	–	–	+	–	+
Aerial mycelium	–	–	–	–	–	–	–	+
Motility or motile elements	v	–	–	–	+	–	–	–
Peptidoglycan diamino acid	L-Lys	L-Lys	*meso*-A_2pm	*meso*-A_2pm	L-Orn	L-Lys	*meso*-A_2pm	L-Lys
Interpeptide bridge	Ser–Glu	L-Glu	D-Glu_2 or D-Asp-D-Glu	None	D-Asp or D-Glu	D-Ser–D-Asp	L-$Ser_{1–2}$ or L-$Ser_{1–2}$–L-Ala–D-Glu	L-Ala–D-Glu
Polar lipids[c]	PI, PGL, DPG, PL, GL, or PG, DPG, PI, PL	PI, DPG, PL	PG, DPG, PL, GL	PI, PG, DPG, PL, GL	PI, PGL	nd	PG, DPG, PL, GL	PG, DPG, PL, GL
Fatty acid pattern	$C_{15:0}$ ante, $C_{16:0}$ iso, $C_{16:0}$	$C_{15:0}$ iso, $C_{15:0}$ ante	$C_{15:0}$ ante, $C_{17:0}$ ante	$C_{15:0}$ ante, $C_{17:0}$ ante	$C_{15:0}$ ante, $C_{16:0}$, $C_{18:0}$	$C_{15:0}$ ante, $C_{16:0}$ iso, $C_{16:0}$	$C_{15:0}$ ante, $C_{16:0}$ iso, $C_{17:0}$ ante	$C_{15:0}$ ante, $C_{16:0}$ iso, $C_{16:0}$, $C_{17:0}$ ante
Menaquinone type	MK-9	MK-8(H_4),	MK-7, MK-8	MK-8(H_2), MK-7(H_2)	MK-9(H_4), MK-8(H_4),	MK-9(H_4), MK-9(H_2), MK-8(H_4), MK-7(H_4)	MK-9, MK-8, MK-7	MK-9(H_4)
DNA G+C content (mol%)	56–58	71	68–72	56–69	71–76	72.9–76.5	62	70–75

[a]Symbols: +, >85% positive; –, 0–15% positive; v, variable; nd, not determined.

[b]Data from Bakalidou et al. (2002), Collins et al. (1983), Minnikin et al. (1979), Schumann et al. (2001), Schumann et al. (2004), Schumann et al. (2001) and Kalakoutskii et al. (1989), DSMZ Catalogue of Strains (2001), and Schumann, unpublished.

[c]DPG, Diphosphatidylglycerol; PG, phosphatidylglycerol; PI, phosphatidylinositol; PL, unidentified phospholipid; GL, unidentified glycolipid.

TABLE 139. Physiological characteristics of *Jonesia denitrificans* DSM 20603[T] and *Jonesia quinghaiensis* DSM 15701[T] determined using the Biolog GP2 MicroPlate system[a]

Characteristic	*J. denitrificans* DSM 20603[T]	*J. quinghaiensis* DSM 15701[T]
Acetic acid	+	−
N-Acetyl L-glutamic acid	w	−
Amygdalin	−	+
2,3-Butanediol	w	−
D-Cellobiose	+	−
D-Galactose	+	−
D-Gluconic acid	−	+
Inosine	+	−
Mannan	w	−
β-Methyl-D-galactoside	w	−
3-Methyl-glucose	+	−
Methyl pyruvate	−	+
Palatinose	w	−
Propionic acid	w	−
Pyruvic acid	w	−
D-Sorbitol	+	−
D-Tagatose	w	−
Thymidine	+	−
Turanose	+	−
Uridine	+	−

[a]Symbols: +, positive; −, negative; w, weakly positive.

List of species of the genus *Jonesia*

1. **Jonesia denitrificans** (Prévot 1961) Rocourt, Wehmeyer and Stackebrandt 1987, 269[VP]

de.ni.tri′fi.cans. N.L. a inf. *denitrificare* to denitrify; N.L. part. adj. *denitrificans* denitrifying.

In addition to properties given in the genus description, the species is characterized as follows: irregular rods, 0.3–0.5 µm in diameter and 2–3 µm in length. Filamentous forms may develop. Gram-stain-positive, but many cells, especially in older cultures, fail to retain the Gram stain completely. Coccoid forms which develop in older cultures always stain Gram-positive. The coccoid forms give rise to rod forms on transfer to a fresh medium. Non-acid-fast. Nonsporeforming. Motile by peritrichous flagella at both 25°C and 37°C. Facultatively anaerobic; organoheterotrophic. Colonies on nutrient agar are 0.5–1.5 mm in diameter (24–48 h), convex, smooth, edge entire, grayish, and translucent to opaque. Rough forms produce rough type colonies with a depressed center. As cultures become older (10–21 d) a yellowish pigmentation may develop. Optimum growth temperature ~30°C. Temperature limits of growth 10–40°C. Cells do not survive heating at 60°C for 30 min. Grow in 5% but not 10% (w/v) NaCl. Grow in presence of 4.5% (w/v) potassium thiocyanate, 0.01% (w/v) potassium tellurite, 0.01% (w/v) 2,3,5-triphenyltetrazolium chloride, and 0.02% (w/v) thallous acetate. Do not grow in the presence of 0.01% (w/v) sodium azide. Catalase-positive. Oxidase-negative. Acid but no gas produced from glucose, cellobiose, fructose, mannose, galactose, maltose, lactose, melibiose, sucrose, trehalose, glycerol, L-arabinose, D-xylose, amygdalin, arbutin, glycogen, starch, dextrin, β-gentiobiose, D-turanose, D-lyxose, salicin, and esculin. Weak acid production from melezitose. No acid produced from L-fucose, D-arabinose, sorbose, D-tagatose, ribose, L-xylose, adonitol, methyl β-xyloside, rhamnose, erythritol, inositol, mannitol, dulcitol, sorbitol, α-methyl-D-glucoside, α-methyl-D-mannoside, *N*-acetylglucosamine, inulin, D-raffinose, xylitol, D-fucose, D-arabitol, L-arabitol, gluconate, 2-keto-gluconate, and 5-keto-gluconate. Amygdalin, melibiose D-lyxose, and gluconate not utilized (API50CH). Inosine, D-cellobiose, D-galactose, 3-methyl-glucose, D-sorbitol, turanose, acetic acid, thymidine, and uridine are utilized (Biolog GP2).

Methyl red-positive. Voges–Proskauer-negative. Exogenous citrate not utilized. Growth requirements not determined. Sensitive, *in vitro*, by agar diffusion method to penicillin, streptomycin, chloramphenicol, aureomycin, terramycin, erythromycin, tetracycline, bacitracin, novobiocin, oleandomycin, kanamycin, vancomycin, colomycin, polymyxin B, and nitrofurantoin. Resistant to sulfonamide, neomycin, and nalidixic acid. β-Hemolysis negative; CAMP test against *Staphylococcus aureus* and *Rhodococcus equi* are negative.

Extracellular enzymes hydrolyze DNA, RNA, cellulose, and starch but not gelatin, chitin, casein, lecithin (egg yolk), xanthine, tyrosine, Tween 20, or Tween 80; slight hydrolysis of Tweens 40 and 60 takes place after 7 d. Phosphatase, sulfatase, and urease are not produced. Esculin is hydrolyzed. Sodium hippurate is not hydrolyzed or only weakly hydrolyzed (H_2SO_4 method) after 10 d. Acid produced in litmus

milk. Nitrates are reduced to nitrites. H_2S-negative. Indole-negative. Galactosamine is a diagnostic whole-cell sugar. Mycolic acids are not present.

Source: cooked ox blood.

DNA G+C content (mol%): 56–58 (T_m).

Type strain: ATCC 14870, CCUG 15532, CIP 55.134, DSM 20603, NBRC 15587, JCM 11481, = NCTC 10816.

Sequence accession no. (16S rRNA gene): X78420, X83811.

2. **Jonesia quinghaiensis** Schumann, Cui, Stackebrandt, Kroppenstedt, Xu and Jiang 2004, 2183[VP]

quing.hai.en′sis. N.L. fem. adj. *quinghaiensis* pertaining to Qinghai, Western province of China, where the type strain was isolated.

Gram-stain-positive; non-acid-fast. On tryptic soy broth agar, rhizoidal colonies are 4 mm in diameter after 7 d incubation at 28°C. On marine broth agar, medium light-yellowish colonies (about 0.5 mm in diameter) with rhizoid appearance (diameter 4–5 mm). Cells are rod shaped (0.5 x 1.5–2 μm) and nonmotile. Optimal growth temperature is 20–30°C; optimal pH is 7–9; optimal salt concentration is 2.0–7.5% (w/v) NaCl. Amygdalin, melibiose, D-lyxose, and gluconate utilized (API 50CH). Amygdalin, D-gluconic acid, and methyl pyruvate utilized (Biolog GP2). The polar lipids consist of phosphatidylglycerol, diphosphatidylglycerol, phosphatidylinositol, and two unknown phospholipids. The major cellular fatty acids are $C_{15:0}$ anteiso, $C_{16:0}$ iso, and $C_{16:0}$.

Source: mud of a soda lake in Qinghai, a Western province of China.

DNA G+C content (mol%): 57.3 (HPLC).

Type strain: QH3A7, CGMCC 1.3459, DSM 15701, JCM 13009.

Sequence accession no. (16S rRNA gene): AJ626896.

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Family XI. **Microbacteriaceae** Park, Suzuki, Yim, Lee, Kim, Yoon, Kim, Kho, Goodfellow and Komagata 1995, 418^VP (Effective publication: Park, Suzuki, Yim, Lee, Kim, Yoon, Kim, Kho, Goodfellow and Komagata 1993, 312.) emend. Rainey, Ward-Rainey and Stackebrandt 1997, 485

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Mi.cro.bac.te.ri.a.ce′a.e. N.L. neut. n. *Microbacterium* type genus of the family; suff. *-aceae* denoting a family; N.L. fem. pl. n. *Microbacteriaceae* the *Microbacterium* family.

Predominantly slender irregular rods of varying shape and length, often **showing V forms. Irregular spherical or ovoid cells** to short rods or **branched fragmenting vegetative mycelium** are typical of some taxa. Aerial hyphae are observed on very rare occasions. Nonmotile or motile. Nonsporeforming**. Gram-stain-positive** in terms of cell-wall architecture, but **the Gram reaction frequently appears to be negative or variable.** Non-acid-fast. **Colonies are mostly colored due to carotenoid pigments.**

Chemo-organotrophic; metabolism is primarily respiratory with oxygen as the terminal electron acceptor. **Predominantly aerobic;** microaerophilic or facultatively anaerobic bacteria occur. **Mostly catalase-positive;** some species display negative reaction for catalase. Strains usually grow on standard laboratory media based on yeast extract and peptone. Various carbon compounds are commonly used as sole carbon and energy sources. Many species require vitamins and amino acids for growth. Some species are nutritionally very exacting. Mostly **mesophiles** and **neutrophiles;** psychrophylic, psychrotolerant, acidophilic, and alkaliphilic species occur in some genera.

Cell-wall peptidoglycan is of the B group; contains the diamino acids lysine, ornithine, 2.4-diaminobutyric acid (Dab), or their combinations. The only respiratory quinones detected are **menaquinones** with 7–14 **unsaturated** isoprene units in the side chain. Fatty acids are predominantly **saturated anteiso- and iso-methyl branched**; ω-cyclohexylundecanoic acid and 1,1-dimethyl alkanes occur in some taxa in significant amounts. At a lower growth temperature, 12-methyl-tetradecenoic ($C_{15:1}$ anteiso) acid can be produced. **Mycolic acids are absent**. **Polar lipid patterns** include mostly **diphosphatidylglycerol, phosphatidylglycerol, and a variety of glycolipids**; other components may be detected in some genera.

An insertion between helices 54 and 55 of the 23S rRNA gene typical of actinobacteria has been determined for some species.

Natural habitats are various terrestrial and aquatic environments. Some species are associated with plants, animals, algae, fungi, as well as clinical specimens. Several species and subspecies include plant pathogens, some of which are under regulatory control in different countries.

DNA G+C content (mol%): 60–76.

Type genus: **Microbacterium** Orla-Jensen 1919, 179^AL emend. Takeuchi and Hatano 1998b, 744^VP.

Further descriptive information

The family *Microbacteriaceae* (Park et al., 1993; Stackebrandt et al., 1997) is the member of the order *Micrococcales* (formerly suborder *Micrococcineae*), class *Actinobacteria* (Stackebrandt et al., 1997), and currently embraces the following genera: *Microbacterium*, *Agreia*, *Agrococcus*, *Agromyces*, *Clavibacter*, *Cryobacterium*, *Curtobacterium*, *Frigoribacterium*, *Frondhabitans**, *Gulosibacter*, *Humibacter*, *Labedella*, *Leifsonia*, *Leucobacter*, *Microcella*, *Microterricola*, *Mycetocola*, *Okibacterium*, *Phycicola*, *Plantibacter*, *Pseudoclavibacter*, *Rathayibacter*, *Rhodoglobus*, *Salinibacterium*, *Subtercola*, *Yonghaparkia*, and *Zimmermannella*† (a homotypic synonym of *Pseudoclavibacter*).

Bacteria of the family *Microbacteriaceae* form a coherent 16S rRNA-based clade and are clearly distinguished from all other families of the order *Actinomycetales* mainly by their unusual B group cell-wall peptidoglycan (Schleifer and Kandler, 1972) and unsaturated respiratory menaquinones (Collins and Jones, 1981) with isoprene units ranging from 7–8 (*Frondicola*) to 13–14 (representatives of *Microbacterium* and *Microcella*) (Table 140). Group B peptidoglycan is characterized by a cross-linkage between the α-carboxyl group of D-glutamic acid in position 2 of the peptide subunit and the C-terminal D-alanine of an adjacent subunit. In contrast, other actinobacteria are known to have A type peptidoglycans, in which cross-linkages are formed between the ω-amino group of the diamino acid in position 3 of the peptide subunit to the carboxyl group of the C-terminal D-alanine in position 4 of the adjacent subunit (Schleifer and Kandler, 1972). A diamino acid such as 2,4-diaminobutyric acid (Dab), lysine, or ornithine in L- or/and D-configuration is always present in the interpeptide bridge of B-type peptidoglycan, since two carboxyl groups must be cross-linked (Schleifer and Seidl, 1985). An amino acid (Dab, homoserine, or lysine) in L-configuration occupies position 3 of the peptide subunit in the structural types so far determined (DSMZ, 2001; Schleifer and Kandler, 1972).

Cells of the majority of species are slender irregular rods (~0.2–0.7 µm) which vary considerably in length and may be arranged in V-formation. The morphological features are prominent for some members of the family. Irregular spherical or ovoid cells to short rods (0.7–1.0 × 1.1–1.7 µm) are characteristic of most *Agrococcus* species; very small rods (~0.2–0.4 µm × ~0.5–1.1 µm) have been reported, for instance, for representatives of *Microbacterium*, *Frondicola*, *Leucobacter*, *Pseudoclavibacter*, and *Zimmermannella*. A fragmenting vegetative mycelium (commonly penetrating into agar media) is characteristic of many *Agromyces* species. A budding-like mode of cell division has been observed in *Phycicola gilvus*. Ultramicro-sized cells (width, 0.15–0.25 µm; volume, <0.1 μm^3) of selenoid shape, occasionally forming rings (when dissociation does not occur after septum formation), were reported for freshwater aerobic isolates affili-

*The name *Frondihabitans* Greene et al. (2009) was proposed to replace the illegitimate name *Frondicola* Zhang et al. (2007c) (Greene et al., 2009).

†According to the Bacteriological Code, the genus name *Zimmermannella* is a later (junior) homotypic synonym of the genus name *Pseudoclavibacter* Manaia et al. (2004) (see the *Taxonomic comments* section and the chapter on the genus *Pseudoclavibacter* for more information).

TABLE 140. Characteristics differentiating the genera of the family *Microbacteriaceae*[a,b]

Characteristic	*Microbacterium*	*Agreia*	*Agrococcus*	*Agromyces*	*Clavibacter*	*Cryobacterium*	*Curtobacterium*	*Frigoribacterium*	*Frondihabitans (Frondicola)*	*Gulosibacter*	*Humibacter*	*Labedella*
Colony color[e]	Y, W, O, Cr	O, R, Y	Y, O, W, Cr	Y, W, O, PG	Y, O, P, W, BL	P, Y	Y, O, P, I	Y	W	W	W	Y
Morphology[f]	Irregular rods	Irregular rods	Spherical cells to short rods	Fragmenting hyphae to irregular rods	Irregular rods	Irregular rods	Irregular rods	Irregular rods	Irregular rods	Irregular rods	Irregular rods	Irregular rods
Motility	D−	D+	−	−	−	D−	D+	+	−	−	+	−
Relation to growth temperature[g]	M	M	M	M	M	Ps (9–22°C), M	M	Ps (4–10°C), M	M	M (35–37°C)	M	M
Diamino acid and characteristic amino acids in peptidoglycan[h]	L-Lys/D-Orn; (Hsr, Hyg, Hyo)	L-Dab, D-Orn; Hyg	L-Dab; Asp (Thr)[i]	L-Dab	L-Dab, D-Dab	L-Dab	D-Orn; Hsr, (Hug)	D-Lys; Hsr	D-Orn; Hsr	D-Orn[j]	Dab, Orn	Orn
N-Glycolil residues	+	−	−	−	−	−	−	−	−		−	−
Predominant menaquinones[k]	Various, 10 to 14[l]	10 or 10, 11	11, 12 or 10, 11	12 or 12, 11 or 12, 13 or 11, 12 or 13,12	9 or 9, 10	10 or 11, or 11, 12	9	9	8, 7	9	11, 12	10, 11
Predominant (>10%) and characteristic fatty acids and DMA[m]	$C_{15:0}$ anteiso, $C_{17:0}$ anteiso, ($C_{16:0}$ iso, $C_{15:0}$ iso)	$C_{15:0}$ anteiso, $C_{16:0}$ iso, $C_{17:0}$ anteiso, [$C_{15:0}$ anteiso DMA]	$C_{15:0}$ anteiso, ($C_{17:0}$ anteiso, $C_{15:0}$ iso, $C_{16:0}$ iso)	$C_{15:0}$ anteiso, $C_{17:0}$ anteiso, $C_{16:0}$ iso	$C_{15:0}$ anteiso, $C_{17:0}$ anteiso, $C_{16:0}$ iso	$C_{15:0}$ anteiso, $C_{15:1}$ anteiso[n], $C_{15:0}$ iso, $C_{17:0}$ anteiso	$C_{15:0}$ anteiso, $C_{17:0}$ anteiso, $C_{16:0}$ iso / $C_{17:0}$ Ch, $C_{17:0}$ anteiso ($C_{15:0}$ anteiso)	$C_{15:0}$ anteiso, $C_{16:0}$ iso; $C_{16:0}$, ($C_{15:1}$ anteiso[n]), $C_{15:0}$ anteiso DMA	$C_{18:1}$ ω7*c*, [$C_{17:1}$ ω9*c*, $C_{14:0}$ 2-OH, $C_{14:0}$ iso 3-OH][o]	$C_{15:0}$ anteiso, $C_{16:0}$ iso, $C_{17:0}$ anteiso	$C_{17:0}$ anteiso, $C_{16:0}$ iso $C_{17:0}$ Ch	$C_{15:0}$ anteiso, $C_{16:0}$ iso, $C_{17:0}$ anteiso, [$C_{18:0}$ CH_3]
Polar lipids[p]	DPG, PG, (PI, PGM, GL)	DPG, PG, GL	PG, DPG, GL, PL, (UL)	DPG, PG, GL, (PL)	DPG, PG, GL	DPG, PG, GL	DPG, PG, GL	PG, DPG, GL	DPG, PG, Gl, PL	DPG, PG, GL		PG, DPG
DNA G+C content (mol%)	64–72	65–67	74–75/65[s]	69–73/65.3[s]	70–76	65–67/70[s]	68–74	71	71	65	68	68

[a]Data compiled from the original descriptions of the genera listed; the species composing these genera and publications are cited in respective generic chapters in this volume.

[b]Symbols, +, present; −, absent; D, different between species (character for the type species is indicated).

[c]Data for the *Leifsonia sensu stricto* species (*Leifsonia aquatica*, *Leifsonia naganoensis*, *Leifsonia poae*, *Leifsonia shinshuensis*, and *Leifsonia xyli*). See chapters on *Leifsonia* and *Rhodoglobus* in this volume for information concerned with the remaining *Leifsonia* species.

[d]The genus name *Zimmermannella* Lin et al. (2004) is a later (junior) homotypic synonym of the genus name *Pseudoclavibacter* Manaia et al. (2004); the data presented are as given in the original description of the genus *Zimmermannella* which includes, along with *Zimmermannella helvola*, the species *Zimmermannella alba*, *Zimmermannella bifida*, and *Zimmermannella faecalis* (see the text and the chapter Genus *Pseudoclavibacter* in this volume for more information).

[e]Abbreviations: BL, blue to black (bipyridyl pigment, indigiodine Starr, 1958; Kuhn et al., 1965); Cr, cream; O, orange; P, pink; PG, pink gray to pink white; PB, pale brown; R, reddish to red; RO, rose-orange; W, white; Y, yellow. The pigmentation intensity and tints may vary depending on the growth conditions and age of culture.

[f]Rods usually become shorter with the age of culture and a proportion of cells may be coccoid in many genera (see the generic chapters for details). Aerial hyphae were reported for *Agromyces aurantiacus* on glycerol-asparagine agar (Shirling and Gottlieb, 1966); young cells of *Subtercola frigoramans* are frequently swollen at the pole or in the middle.

[g]Abbreviations: M, mesophilic to psychrotolerant organisms with temperature optima for growth ~25–30°C unless indicated; Ps, psychrophilic or psychrotolerant organisms with lower temperature optima (in parentheses).

[h]Abbreviations: Dab, 2,4-diaminobutyric acid; Orn, ornithine; Lys, lysine; Asp, aspartic acid; Thr, threonine; Gab, γ-aminobutyric acid; Hyg, 3-*threo*-hydroxy glutamic acid; Hyo, 3-*threo*-hydroxy ornithine. In addition, alanine, glutamic acid, and glycine are usually present in all organisms. L- and D-, enantiomeric isomers of diamino acids (where available at least for the type species). Oblique strokes separate diamino acids reported for different species within a genus. Amino acids given in parentheses occur in some species of a genus.

Leifsonia[c]	*Leucobacter*	*Microcella*	*Microterricola*	*Mycetocola*	*Okibacterium*	*Phycicola*	*Plantibacter*	*Pseudoclavibacter*	*Rathayibacter*	*Rhodoglobus*	*Salinibacterium*	*Subtercola*	*Yonghaparkia*	*Zimmermannella*[d]
Y, W, PB	W, PB, Y	Y	Y	Y	Y	Y	Y	Y	Y, RO	R	Y	Y	Y	W, Y
Irregular rods, filaments	Irregular rods	Irregular rods	Irregular rods	Irregular rods	Irregular rods	Short rods to small coccoid and bud-like forms	Irregular rods	Irregular rods	Irregular rods	Irregular rods with bulbous protuberances	Irregular rods	Irregular rods	Irregular rods	Irregular rods
D+	–	–	+	–	–	+	–	–	–		–	–	–	–
M	M	M (35°C)	M	M	M	M	M	M	M	Ps (18°C)	M	Ps (15–17°C)	M	M
D-Dab, L-Dab/Dab	L-Dab/Dab; (Gab, Thr)	Lys/D-Orn; (Hsr)	Dab	Lys	Lys; Hsr	Dab	L-Dab	L-Dab, D-Dab	L-Dab	Orn	Orn, Lys	Dab; Hug	Dab	L-Dab, D-Dab/L-Dab; (Hsr)
–	–		–	–	+	–	–	–			–			–
11 or 11, 10 or 11, 12	11 or 11, 10	12, 13 or 13, 14	12, 11	10	10, 11	11	10 or 10, 9	9	10	12, 11	11, 10	9, 10	12	9 or 8, 9 or 10
$C_{15:0}$ anteiso, $C_{17:0}$ anteiso, $C_{16:0}$ iso	$C_{15:0}$ anteiso, $C_{17:0}$ anteiso, $C_{16:0}$ iso, ($C_{16:0}$)	$C_{16:0}$ iso, $C_{15:0}$ anteiso, $C_{14:0}$ iso, $C_{15:0}$ iso	$C_{15:0}$ anteiso, $C_{17:0}$ anteiso	$C_{15:0}$ anteiso, $C_{17:0}$ anteiso	$C_{15:0}$ anteiso, $C_{16:0}$ iso, $C_{17:0}$ anteiso	$C_{15:0}$ anteiso, $C_{17:0}$ anteiso	$C_{15:0}$ anteiso, $C_{16:0}$ iso, $C_{17:0}$ anteiso	$C_{15:0}$ anteiso, $C_{16:0}$ iso, $C_{17:0}$ anteiso	$C_{15:0}$ anteiso, $C_{16:0}$ iso, $C_{17:0}$ anteiso	$C_{15:0}$ anteiso, $C_{17:0}$ anteiso, $C_{16:0}$ iso	$C_{15:0}$ anteiso, $C_{16:0}$ iso, $C_{14:0}$ iso	$C_{15:0}$ anteiso, ($C_{16:0}$ iso; $C_{14:0}$ iso); $C_{16:0}$ iso DMA, $C_{15:0}$ anteiso DMA	$C_{15:0}$ anteiso, $C_{16:0}$ iso, $C_{17:0}$ anteiso	$C_{15:0}$ anteiso, $C_{17:0}$ anteiso, $C_{16:0}$ iso, $C_{16:0}$
PG, DPG, GL	PG, DPG, GL, (AP, PL)				DPG, PG, GL	DPG, PG, PI, PC	PG, DPG, GL	DPG, PG, GL	PG, DPG, GL, (PL)		PG, DPG[q]	PG, DPG, PL, GL	DPG, PG, PL, GL	DPG, PG, GL[r]
68–71	66–69	67–71	70	64–65	67	70	68–70	67	60–69	62	61	64–68	71–72	62–68

[i]Both aspartic acid and threonine are absent in the cell-wall peptidoglycan of *Agrococcus casei*.

[j]No data are available on the peptidoglycan amino acid composition for *Gulosibacter*.

[k]Unsaturated menaquinones, ~20% and more (were available). Numerals indicate the numbers of isoprene units in the side chain.

[l]The majority of *Microbacterium* species contain MK-11 and MK-12 or MK-12 and MK-13 as the predominant menaquinones.

[m]Abbreviations: Ch, ω-cyclohexylundecanoic acid; DMA, 1,1-dimethoxy-alkanes. Brackets indicate that a compound reaches more than 10% only in some species as reported; minor (less than 10%) but characteristic components if available are given in square brackets. Oblique stroke separate different fatty acid patterns reported for different species within a genus. *Note*: Relative amounts of the predominant fatty acids may vary depending on growth conditions and growth phase.

[n]Along with *Cryobacterium* species and *Frigoribacterium* species, the monounsaturated 12-methyl-tetradecenoic acid ($C_{15:1}$ anteiso) may be present in organisms of other genera grown at lower temperatures.

[o]The retention time of the methyl esters of $C_{18:1}$ ω7*c* and $C_{14:0}$ 2-OH fatty acids is almost identical to that of cyclohexyl-$C_{17:0}$ and $C_{15:0}$ DMA, respectively, in the standard MIDI system (Kämpfer et al., 2000; Qiu et al., 2007). The following fatty acids have recently been reported by Greene et al. (2009) as characteristic components: $C_{16:0}$, $C_{14:0}$ 2-OH, $C_{14:0}$ iso 3-OH, $C_{17:1}$ ω9*c* and $C_{18:1}$ ω7*c* (no quantitative data are available).

[p]Abbreviations: DPG, diphosphatidylglycerol; PG, phosphatidylglycerol; PI, phosphatydylinositol; AP, aminophospholipid; PC, phosphatidylcholine; PGM, phosphatidylglycerol mannoside; PL, unknown phospholipid(s); GL, glycolipid(s). Trace amounts of polar lipids may present additionally in representatives of some genera (see respective genera chapters in this volume).

[q]No data on studying the possible presence of glycolipids.

[r]Data for *Pseudoclavibacter helvolus* (*Zimmermannella helvolum*) (see the text and the chapter Genus *Pseudoclavibacter* in this volume for more information).

[s]Oblique strokes separate the lower values reported for *Agrococcus casei* and *Agromyces neolithicus* and higher value for *Cryobacterium mesophilum*.

ated to the family (Hahn et al., 2003). Cells of a copiotrophic isolate, strain 12–8 (>99% 16S rRNA gene sequence similarity with *Yonghaparkia alkaliphila*) are divided by multiple longitudinal and transverse septa resulting in formation of very small cells of different size and shape (Iizuka et al., 1998).

Bacteria of the family *Microbacteriaceae* are predominantly heterotrophic and obligate aerobes, but some show microaerophilic growth, e.g. representatives of *Agromyces* (Jurado et al., 2005a, 2005b, 2005c) and *Microbacterium* (Buczolits et al., 2008b), or require microaerobic conditions for growth (*Agromyces ramosus*). Some *Microbacterium* species were reported to give weak anaerobic growth and produce acids under anaerobic conditions (for references, see Collins and Keddie, 1986). Representatives of the family, identified as members of the genera *Microbacterium* and *Leifsonia*, were shown to grow chemolithotrophically in a medium containing thiosulfate and exhibit growth coupled with thiosulfate oxidation (Anandham et al., 2008). A strain identified as *Microbacterium* was reported to reduce toxic Cr(VI) under anaerobic conditions at the expense of acetate as the electron donor (Pattanapipitpaisal et al., 2001). Some strains from alkaline environments, that exhibited close phylogenetic relatedness to *Yonghaparkia alkaliphila*, *Microcella alkaliphila* or *Microbacterium* species, are dependent on elevated levels of CO_2 for growth (Ueda et al., 2008).

Most bacteria of the family are pigmented on complex media in shades of yellow, orange, pink, and red due to production of carotenoid pigments (Saperstein and Starr, 1954; Saperstein et al., 1954; Starr and Saperstein, 1953; Trutko et al., 2005). The color intensity and shade may vary depending on the species, growth medium, culture age, exposure to light, and incubation temperature. Qualitative and quantitative changes in the carotenoid pigments were observed, for instance, for *Curtobacterium flaccumfaciens* (pv. *poinsettiae*), when the level of the required growth factor, thiamine, is altered in the basal medium. The cells grown under conditions of low thiamine concentration were red-pigmented, with two major pigments produced: spirilloxanthin and lycoxanthin. Under conditions of high thiamine concentrations, the cells were orange-yellow and three major pigments were produced: lycoxanthin, cryptoxanthin, and a small amount of spirilloxanthin (Starr and Saperstein, 1953). In some bacteria tested so far (e.g. representatives of the genera *Agrococcus*, *Agromyces*, *Leifsonia*, *Microbacterium*, and *Leucobacter*), the pigments are light-induced (Groth et al., 1996; Muir and Tan, 2007; Trutko et al., 2005; Zlamala et al., 2002a). Most strains of *Clavibacter michiganensis* subsp. *insidiosus* contain the dark blue-gray bipyridyl pigment indigiodine (Kuhn et al., 1965; Starr, 1958). Similar dark blue-gray extracellular pigment, along with a rose carotenoid-like pigment, is also produced by some *Clavibacter* strains isolated from desert ecosystems (Dobrovolskaya et al., 1999).

The DNA base ratios range from 60 mol% as determined for *Rathaibacter toxicus* by HPLC (Sasaki et al., 1998) to 76.1 mol% (T_m) reported for *Clavibacter michiganensis* subsp. *insidiosus* (Döpfer et al., 1982). Somewhat higher values were reported in early works for other members of the family *Microbacteriaceae*.

Members of the family *Microbacteriaceae* are widely distributed in nature and have been isolated from or detected by molecular methods in diverse terrestrial, marine, and freshwater environments (for references and details, see respective generic chapters in this volume). Cells of some representatives of the family were reported to survive rather extreme conditions and were found, for instance, in more than 500,000-year-old glacial ice (Christner, 2002), in a deep Greenland glacier ice core at least 120,000 years old (Miteva et al., 2004a), in 1.8–3 million years old permafrost sediments (Kochkina et al., 2001; Vishnivetskaya et al., 2006), and in desert ecosystems including the Atacama Desert which represents one of the driest places on Earth (Osman et al., 2008b). Some *Microbacterium* species (along with *Arthrobacter* species) were shown to be highly resistant to UV-radiation (Osman et al., 2008b; Wu and Liu, 2007), desiccation, and a simulated Martian atmosphere (Osman et al., 2008b). These actinobacteria were suggested for consideration as prime candidates for possible contamination of Mars (Osman et al., 2008b). Representatives of the family were also reported to resist and reduce heavy metal contaminations due to specific mechanisms (Idris et al., 2004; Mokashi and Paknikar, 2002; Morais et al., 2004; Pattanapipitpaisal et al., 2001; Sarangi and Krishnan, 2008; Sheng et al., 2008; Zhu et al., 2008).

Microbacteriaceae can be associated with different eukaryotic organisms (Evtushenko and Takeuchi, 2006; Mendes et al., 2007; Muir and Tan, 2007; Nishiwaki et al., 2007; Somvanshi et al., 2007; Wang et al., 2007; Wichels et al., 2006; Xin et al., 2008; Zachow et al., 2008), and are being cited among opportunistic human pathogens and clinical isolates (mostly *Microbacterium* species and also representatives of *Leifsonia*, *Leucobacter*, *Okibacterium*, *Pseudoclavibacter*, and some others) (Adderson et al., 2008b; Bosshard et al., 2003; Dempsey et al., 2007; Funke et al., 1997; Gneiding et al., 2008; Mages et al., 2008; Siala et al., 2008). There are also plant pathogens among *Microbacteriacea* which belong to several species and subspecies of the genera *Clavibacter*, *Curtobacterium*, *Leifsonia*, and *Rathayibacter* (Young et al., 1996, 2004a).

Besides the microorganisms belonging to the genera reviewed by the following chapters, great diversity of not yet precisely identified members of the family *Microbacteriaceae* has been discovered in various ecosystems. As it might be supposed from the analysis of 16S rRNA gene sequences available in public databases, many of these bacteria represent separate lines of descent and form the nuclei of novel genera to be described in the future.

Taxonomic comments

The first isolation of bacteria of the family *Microbacteriaceae* was reported about a century ago (Hutchinson, 1917; Smith, 1910, 1913; Spieckermann and Kotthoff, 1914). Those included the plant-pathogenic organisms currently belonging to *Clavibacter michiganensis* subsp. *michiganensis*, *Rathayibacter rathayi*, *Clavibacter michiganensis* subsp. *sepedonicus*, and *Rathayibacter tritici*. The type species of the type genus of the family, *Microbacterium lacticum*, isolated from dairy products, was described shortly afterwards (Orla-Jensen, 1919).

The chemical analyses of cell walls and lipids played the most important role in development of the classification system of the family in the "pre-molecular" era (for details and references see Collins and Bradbury, 1986). Early phylogenetic studies, e.g. oligonucleotide cataloguing (Stackebrandt et al., 1980), DNA/rRNA similarities (Döpfer et al., 1982), 5S rRNA gene sequence clustering (Park et al., 1987), and the levels of sequence similarity of an insertion within the 23S rRNA between helices 54 and 55 (Roller et al., 1992) confirmed the relatedness of bacteria

possessing the group B type peptidoglycan and menaquinones with unsaturated isoprene units in the side chain. Collins and Bradbury (1986) suggested that the target bacteria possessing a group B type peptidoglycan merit a family status (the name *Microbacteriaceae* was provisionally proposed).

Later on, Park et al. (1993) concluded from the results of 5S rRNA gene sequence comparisons of representatives of the target bacterial group and representatives of other actinomycete genera that the sequence similarity and associated chemotaxonomic characteristics provide compelling grounds for classifying the bacteria with a group B type peptidoglycan in a single family. The authors established the family *Microbacteriaceae* comprising the type genus *Microbacterium* and the genera *Aureobacterium*, *Agromyces*, *Curtobacterium*, and *Clavibacter* (the family name was validated in 1995). In 1997, Stackebrandt and co-authors suggested a hierarchic classification scheme of actinobacteria, in which delineation of higher taxa was based on the 16S rRNA gene sequence-based phylogenetic clustering and distribution of signature nucleotides. Along with other taxonomic proposals, the authors emended the description of the family *Microbacteriaceae*, adding the genera *Agrococcus* and *Rathayibacter*. The following characteristic patterns of 16S rRNA gene signature nucleotides were proposed for the family *Microbacteriaceae*: nucleotides at positions 45:396 (U–A), 144:178 (C–G), 258:268 (A–U), 497 (A), 615:625 (A–U), 694 (G), 771:808 (G–C), 839:847 (G–U), 1256 (G), 1310:1327 (A–U), and 1414:1486 (U–A) (Stackebrandt et al., 1997).

The current classification system of the family *Microbacteriaceae* and affiliated genera (Figure 173) is an example of using a taxonomic strategy based on the polyphasic approach (Colwell, 1970; Stackebrandt, 2006; Vandamme et al., 1996) which assumes a certain level of consensus while interpreting genotypic and phenotypic data in establishing of taxa and delineating their boundaries. Delineation of genera within this family is primarily based on the 16S rRNA-based phylogenetic clustering (as a rule the genus taxon is considered to be monophyletic) and chemotaxonomic characteristics, such as cell-wall diamino acids and other diagnostic amino acids, the cell-wall peptidoglycan composition, the type of acyl groups on the muramyl residues of the peptidoglycans determined in a glycolate test (detection of *N*-glycolyl residues) (Uchida and Aida, 1977), the number of isoprene units of the major menaquinone(s), the predominant or specific components of fatty acid profiles including various 1,1-dimethyl alkanes (Kämpfer et al., 2000; Schumann et al., 2003) and the DNA G+C content (Table 140) and some morphological features mentioned above. Physiological, biochemical and some other chemotaxonomic characteristics including the cell-wall sugar composition and quantitative fatty acid profiles (determined from cells grown under similar conditions and standardized analysis) are presently considered to be the phenotypic traits of secondary importance for differentiation of genera within this family but are commonly used for species delineation.

The 16S rRNA gene sequence similarity between representatives of different genera of the family *Microbacteriaceae* ranges from ~91 to 97–98%. It is noteworthy that in other contexts (and with other bacteria) the 97–98% cut-off value is often assumed to delineate species (rather than genus) category. In some cases, the establishment of novel genera phylogenetically very close to previously described genera is based on the suggested priority of chemotaxonomic features over the high 16S rRNA gene sequence similarity. One example is the genus *Yonghaparkia* (Yoon et al., 2006b) exhibiting 2,4-diaminobutyric acid in the peptidoglycan and more than 98% 16S rRNA gene sequence similarity to the type species of the genus *Microcella* (Tiago et al., 2005b) which contains a lysine-based peptidoglycan. Several closely related genera and species which are phylogenetically interspersed among such genera (Figure 173) have been described almost simultaneously by different authors (without their being aware of the similarity of their research subjects) from different taxonomic perspectives. Those include (i) the genera *Rhodoglobus* (Sheridan et al., 2003), *Salinibacterium* (Han et al., 2003), and the species *Leifsonia aurea* and *Leifsonia rubra* (Reddy et al., 2003b); (ii) *Pseudoclavibacter*, *Gulosibacter* (Manaia et al., 2004) and *Zimmermannella* with four species (Lin et al., 2004); (iii) *Microterricola* (Matsumoto et al., 2008), *Phycicola* (Lee et al., 2008), and *Leifsonia pindariensis* (Reddy et al., 2008).

It is worth noting in this context that the descriptions of type species of the genera *Zimmermannella* and *Pseudoclavibacter* are based on the same strain ("*Brevibacterium helvolum*" ATCC 13715 = DSM 20419 = JCM 9491= NBRC 15775) designated the type strain. The genus name *Pseudoclavibacter* Manaia et al. (2004) that was validly published somewhat earlier is therefore a senior homotypic synonym of the genus name *Zimmermannella* Lin et al. (2004) according to Rule 24b of the *Bacteriological Code* (1990 Revision) (Lapage et al., 1992). Strict application of the Rules of the Bacteriological Code also indicates that the genus name *Zimmermannella* Lin et al. 2004 is illegitimate [Rule 51b(1)] and the species included (*Zimmermannella alba*, *Zimmermannella bifida*, and *Zimmermannella faecalis*) cannot be maintained in the genus *Zimmermannella* in accordance with Rule 37a(1) (Euzéby, 2009). Moreover, the genera *Pseudoclavibacter* and *Zimmermannella* were reported to differ in their circumscriptions, where *Zimmermannella* with its four species has broader boundaries in terms of the essential diagnostic characteristics, as follows from the formal descriptions of these genera (Lin et al., 2004; Manaia et al., 2004). The species *Zimmermannella alba*, *Zimmermannella bifida*, and *Zimmermannella faecalis* are distinguished from each other and from *Pseudoclavibacter helvolus* at least in their peptidoglycan structures and/or predominant menaquinones (both characteristics usually delineate genera within the family *Microbacteriaceae*). The above data, along with the similarity values of the 16S rRNA gene sequences of these species that are of the same level as recorded for representatives of separate genera of the family, suggest that *Zimmermannella alba*, *Zimmermannella bifida*, and *Zimmermannella faecalis* can be classified into at least two different genera.

In some of the aforementioned examples, different interpretations of the data on 16S rRNA gene sequence comparison, along with different chemotaxonomic characteristics recorded for the phylogenetically very close organisms lead authors to the discrepant taxonomic conclusions (prominent examples are the pairs *Rhodoglobus vestalii*–*Leifsonia rubra* and *Microterricola viridarii*–*Leifsonia pindariensis*). It is thus apparent that the taxonomic status of such genera and species is in need of further revision. It also seems quite likely that the taxonomic status of *Agrococcus casei* (Bora et al., 2007), which branches outside the cluster of all remaining *Agrococcus* species and differs from these at least by its cell-wall peptidoglycan composition and a

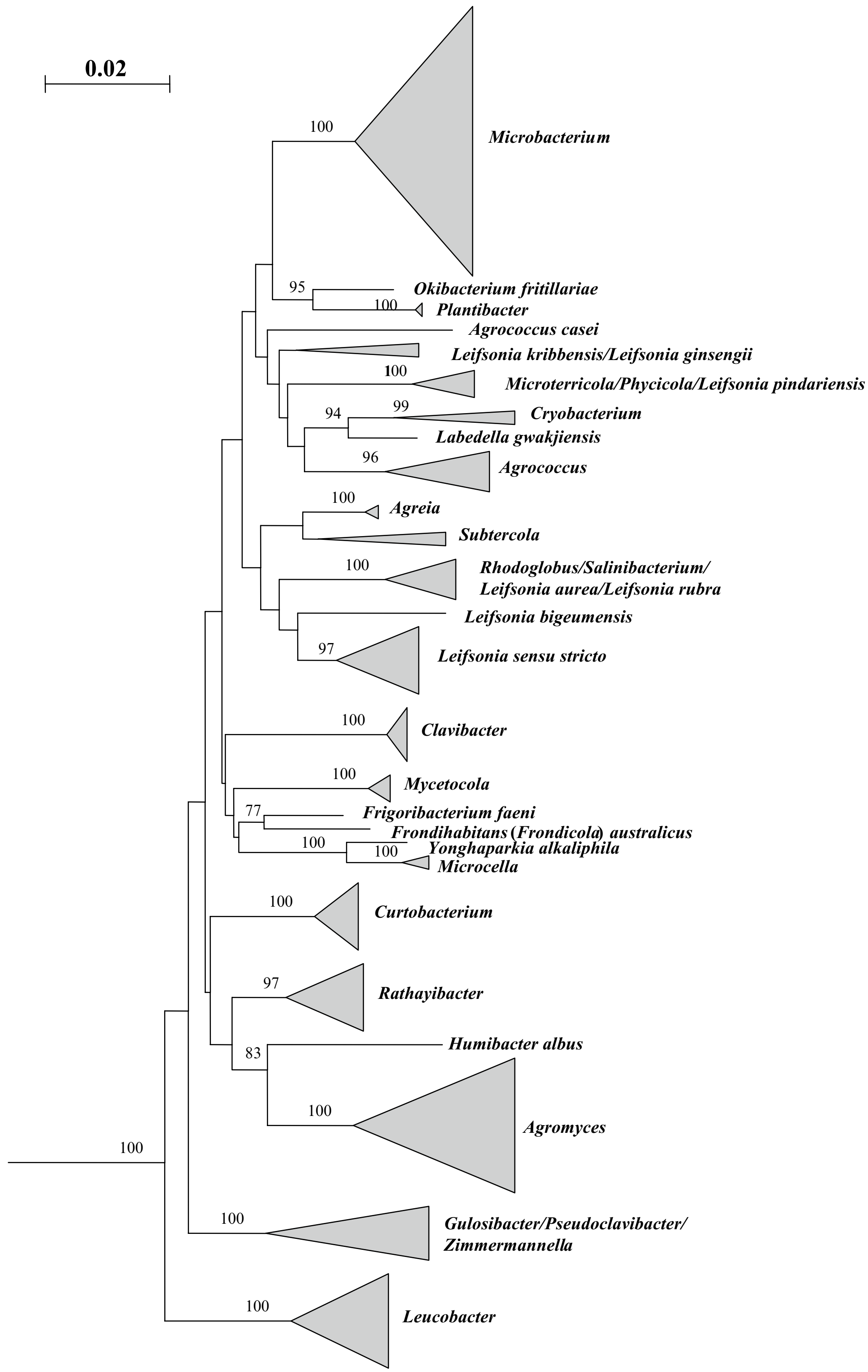

FIGURE 173. Phylogenetic dendogram based on 16S rRNA comparison of the type strains of the type species of the family *Microbacteriaceae*. Bar = 0.02 inferred nucleotide substitution per nucleotide. Values at nodes indicate bootstrap values for 500 replicates.

lower G+C content of DNA (65 mol%), requires re-evaluation. Similarly, the generic assignment of some recently described *Leifsonia* species, such as *Leifsonia ginsengi* (Qiu et al., 2007), *Leifsonia kribbensis* (Dastager et al., 2009), and *Leifsonia bigeumensis* (Dastager et al., 2008c), which do not form a robust 16S rRNA-based phylogenetic cluster with the *Leifsonia sensu stricto* species and tend to differ from them in the phenotypic characteristics available, is in need of re-evaluation (see the chapter Genus *Leifsonia* for more information).

On the other hand, the distantly related species within the type genus *Microbacterium* (which so far comprises the largest number of species within the family) share relatively low 16S rRNA gene sequence similarities (~94–95%), which are equal to or lower than values reported for representatives of different genera of this family. The genus *Microbacterium* also looks heterogeneous in terms of chemotaxonomic markers (the species are characterized at least by different structural types of the cell-wall peptidoglycans based on different diamino acids and different quinone systems). At the same time, members of this genus form a well-defined 16S rRNA-based clade (Figure 173) and the species with different structural types of peptidoglycan are phylogenetically intermixed (Takeuchi and Hatano, 1998b).

Recently, an attempt has been made to clarify the phylogeny and taxonomic structure of the genus *Microbacterium* and the family *Microbacteriaceae* by analyzing the partial housekeeping genes encoding DNA gyrase subunit B (*gyrB*), RNA-polymerase subunit B (*rpoB*), recombinase A (*recA*), and polyphosphate kinase (*ppk*) (Richert et al., 2007; Stackebrandt et al., 2007). It was shown that the phylogenetic groupings of the type strains of species within the genus *Microbacterium* differed with each protein-coding gene sequenced and from the 16S rRNA based phylogeny as well. Only the *ppk* gene tree and a concatenated tree based on the sequences of 16S rRNA and all four housekeeping genes, placed *Microbacterium* strains with lysine-based peptidoglycan as a coherent cluster within the radiation of *Microbacterium* species having ornithine in the peptidoglycan (Richert et al., 2007). The extended multilocus sequence analysis (Stackebrandt et al., 2007) performed with the type strains of 32 species of 13 genera of the family *Microbacteriaceae* demonstrated that members of most genera with more than one strain formed phylogenetically coherent clusters based on nucleic acid sequences and protein sequences of housekeeping genes. However, the topology of phylogenetic trees differed from one another and from the 16S rRNA-based tree. Furthermore, problems identified related to drawing conclusions from individual gene sequences for bacterial systematics and lack of conserved PCR primer targets in the genes applicable to the full set of type strains under investigation have also been illustrated. The result of this study led the authors to the conclusion that the 16S rRNA-based phylogenetic analysis will continue to serve as "gold standard" for decisions about taxonomic ranks from species to family categories until a substantially higher degree of genomic information is available (Stackebrandt et al., 2007).

In recent years, the study of newly isolated organisms has highlighted some problems in the current taxonomy of the family associated with the shortage of chemotaxonomic markers and other phenotypic characteristics routinely examined in bacteria that are useful for delineating phylogenetically distant bacterial groups or single strains (supposedly belonging to new genera). This particularly concerns the organisms whose generic assignment cannot be unambiguously interpreted in terms of 16S rRNA phylogeny and probably other genes used for phylogenetic analysis. Meanwhile, more detailed analysis of the cell-wall composition, e.g. determination of stereoisomers of Dab of the cell-wall peptidoglycan (Sasaki et al., 1998), or identification of some "unknown" or minor lipid components including polar lipids (Buczolits et al., 2008b; Kämpfer et al., 2000; Lee et al., 2008; Lee, 2007; Schumann et al., 2003) uncover additional chemotaxonomic markers useful in differentiating genera and species of this family. One may note that some other cell molecules and polymers reported for several representatives of the family appear to be useful for taxonomic purposes in the group concerned, e.g. the polyamine pattern (Altenburger et al., 1997), the heme composition and the associated cytochrome and quinole oxidases of respiratory chains (Trutko et al., 2003), the cell wall anionic polymers (Naumova et al., 2001; Ortiz-Martinez et al., 2004; Shashkov et al., 1993, 1995), and probably some biochemical characteristics. Further, employment of fourier-transform infrared (FT-IR) spectroscopy, which reflects common physical-chemical profiles of bacterial cells (Oberreuter et al., 2002), was shown to be helpful in differentiating members of related genera within this family (Behrendt et al., 2002). In addition, some studies, as mentioned before, revealed unusual cell morphology in some representatives of the family. Such findings, along with clearly distinguishable morphological features of some so-called "irregular rods", as well as different reactions in Gram-staining and KOH tests (Gregersen, 1978) and sensitivity to lysozyme (indicating differences in the cell envelope chemistry), point to potential usefulness of the detailed study of cell morphology, life cycles, and the fine cell architecture for discovering and documenting additional phenotypic characteristics associated with phylogenetic distinctness of organisms.

Comparative genomics has recently greatly facilitated the evaluation of the current taxonomic system and contributed substantially to exploration of the limits of the currently recognized species and genus categories in some bacterial groups (Konstantinidis and Tiedje, 2007). The prospects for harmonizing genomic and phenotypic information at the level of family (at least with some *Proteobacteria*) are not fully satisfying. So far, genome sequences of three representatives of the family *Microbacteriaceae*, including the plant pathogens *Leifsonia xyli* subsp. *xyli* (Monteiro-Vitorello et al., 2004), *Clavibacter michiganensis* subsp. *michiganensis* (Gartemann et al., 2008), and *Clavibacter michiganensis* subsp. *sepedonicus* (Bentley et al., 2008), are available. Showing the genome characteristics typical of actinobacteria as a whole, these species/subspecies have also many similar genes and proteins indicative of their lifestyle and recent niche adaption. The published data on comparative genomics and proteomics of some actinobacteria, which emphasize also some aspects relevant to the possible future development in taxonomy of these organisms, have been discussed in a review (Ventura et al., 2007).

Acknowledgements

This work was supported by the program MCB of the Russian Academy of Sciences. The author is grateful to Dr Vladimir N. Akimov for valuable discussions on the phylogenetic structure of the family *Microbacteriaceae* and construction of phylogenetic tree.

Further comments

After this chapter was completed, three novel genera of the family *Microbacteriaceae* were added to the family: *Klugiella* (Cook et al., 2008), *Glaciibacter* (Katayama et al., 2009), and *Schumannella* (An et al., 2008, 2009).

Genus I. **Microbacterium** Orla-Jensen 1919, 179[AL] emend. Takeuchi and Hatano 1998b, 744[VP]

Ken-ichiro Suzuki and Moriyuki Hamada

Mic.ro.bac.te′ri.um. Gr. adj. *mikros* small; L. neut. n. *bacterium* a small rod; N.L. neut. n. *Microbacterium* a small rodlet.

Cells of young cultures (from 12 to 24 h) are small, slender, irregular rods, generally 0.4–0.6 µm in diameter by 1.0–2.0 µm in length. Some species form cells up to 6.0 µm in length. Some of the rods are arranged at angles to each other to give a "V" formation. Primary branching may occur but is not common in mycelia formation. Cells of old cultures (from 3 to 7 d) are shorter and coccoid cells are often formed. However, the rod–coccus cycle does not occur. Cells stain Gram-positive and are not acid-fast. Endospores are not formed. Nonmotile or motile. Colonies on solid media are yellowish white, yellow to orange in color. **Generally strictly aerobic and catalase-positive. Chemo-organotrophic.** Acid is produced from glucose and some carbohydrates, but only slowly or weakly on peptone. Some organic acids are assimilated.

The cell-wall peptidoglycan **contains L-lysine or D-ornithine** with glycine, alanine, and D-glutamic acid, which is often partially substituted by hydroxylglutamic acid. D-Ornithine-containing species and some L-lysine-containing species contain homoserine. **Muramic acid of the peptidoglycan occurs in the *N*-glycolyl form.** Cell-wall sugars are principally galactose and rhamnose. The presence of glucose, mannose, fucose, 6-deoxytalose, and/or xylose is variable, depending on the species. Major menaquinones are unsaturated **MK-11, MK-12, MK-13, and/or MK-14.** MK-10 is found as a minor component in some species. The **polar lipids consist of diphosphatidylglycerol, phosphatidylglycerol, and one or some glycolipids.** Cellular fatty acids are predominantly $C_{15:0}$ anteiso, $C_{17:0}$ anteiso, and $C_{16:0}$ iso. Signature nucleotides in the 16S rRNA gene sequences are at positions: 69:99 (G/A–T), 129 (C/T), 232 (G), 279 (T), 443:491 (C/T–G), 770:809 (G–C/G), 780 (A), 830:856 (T–A), and 929:1386 (A–T).

DNA G+C content (mol%): 63–75 (HPLC, T_m).

Type species: **Microbacterium lacticum** Orla-Jensen 1919, 179[AL].

Further descriptive information

The genus *Microbacterium*, represented by *Microbacterium lacticum*, was established for small, rod-shaped bacteria isolated from dairy products and equipment (Orla-Jensen, 1919). *Microbacterium lacticum* was characterized by its thermotolerance, surviving at 63°C for 30 min in skim milk or 72°C for 15 min (Collins and Keddie, 1986). Since the establishment of the genus *Microbacterium* by Orla-Jensen, the first reclassification of the genus *Microbacterium* was done by Collins et al. (1983c), who defined the genus for species containing peptidoglycan with lysine. At the same time, Collins et al. (1983a) proposed the genus *Aureobacterium* for those species containing ornithine in the peptidoglycan, with *Aureobacterium liquefaciens* as the type species. While these proposals were in accordance with the peptidoglycan diamino acids, phylogenetic relationships based on 16S rRNA gene sequences of the two genera were intermixed (Rainey et al., 1994; Takeuchi and Yokota, 1994). Considering these phylogenetic relationships, Takeuchi and Hatano (1998b) proposed the union of the two genera into the emended genus *Microbacterium*. Thus, the variation of the diamino acids is regarded as a species and not a genus characteristic.

Cells of species of the genus *Microbacterium* are short rods, generally as small as those of members of other genera of the family *Microbacteriaceae*. Although cells of older cultures become shorter, no pleomorphic cell cycle is observed.

Principally strictly aerobic. Although *Microbacterium gubbeenense* and *Microbacterium ulmi* are described as facultatively anaerobic, good growth is shown in aerobic culture.

Chemotaxonomy. Chemotaxonomic profiles are important for characterization and identification of species of the genus *Microbacterium*. While polar lipids and cellular fatty acids are similar for most species of the genus, the structure of the cell-wall peptidoglycan and menaquinone composition vary, as shown in Table 141.

Cell-wall peptidoglycan. Like other members of the family *Microbacteriaceae*, the genus *Microbacterium* possesses B group peptidoglycan (Collins et al., 1983a, 1983c; Schleifer and Kandler, 1972). The peptidoglycan contains either lysine or ornithine as the characteristic diamino acid in the interpeptide bridge. In addition, variation is found in the amino acid at position 3 of the peptide side-chain. So far, five variations are known (Figure 174). Note that the classification of species based upon the presence of either ornithine or lysine in the cell-wall is no longer used (Rainey et al., 1994; Takeuchi and Yokota, 1994).

Muramic acid of the glycan moiety of the peptidoglycan is glycolated, as also found in the genera *Mycobacterium* and *Nocardia* (Uchida and Aida, 1979). The glycolyl type of the cell-wall peptidoglycan is one of the useful differential characteristics that distinguish the genus *Microbacterium* from other members of the family *Microbacteriaceae*, except for the genus *Okibacterium* (Evtushenko et al., 2002). It is determined by the glycolate test developed by Uchida and Aida (1979). Early studies of Uchida and Aida (1977) analyzed the molar ratio of glycolyl residues in the unit cell-wall to determine the ratio of substitution by glycolyl muramic acid. *N*-Glycolyl muramic acid is formed by hydroxylation of the *N*-acetyl muramic acid. This activity is also responsible for the hydroxylation of glutamic acid of the peptide subunit. Similarly, some species of the family *Micromonosporaceae* have both glycolyl muramic acid and a hydroxylated diaminopimelic acid in their peptidoglycan.

Cell-wall sugars. The cell-wall sugars of members of the genus *Microbacterium* have been well characterized by the studies of Yokota et al. (1993a, 1993b) and Takeuchi and Hatano (1998a). Most of the species contain galactose and rhamnose. The presence of glucose, mannose, fucose, 6-deoxytalose, and/or xylose varies depending on the species. The first observation of 6-deoxytalose was reported by Imai et al. (1984) in *Microbacterium arborescens*. 6-Deoxytalose has a different configuration of

the hydroxyl base at the C4 position of rhamnose, which is a common component of the cell-walls of *Microbacterium*.

Isoprenoid quinones. Species of the genus *Microbacterium* contain menaquinone exclusively as the respiratory quinone. The isoprenoid side-chains are unsaturated and range from 9 to 14 (Collins et al., 1979, 1980; Collins and Jones, 1981; Yamada et al., 1976). The majority of species contain MK-12 as a major component, together with MK-11 or MK-13 as a second major compound (Table 141). Some species have MK-11 as the major component, with MK-10 as the second major compound, and other species have MK-13 and MK-14 as major components. Often, small amounts of menaquinones with one more or less isoprenoid units in the side-chain may be detected. However, as demonstrated by Buczolits et al. (2008a) for *Microbacterium paraoxydans*, the relative amounts of the different menaquinones in a single species are rather constant. Thus, quantitative analysis by HPLC is desirable for determination of menaquinone profile.

Polar lipids. Diphosphatidylglycerol, phosphatidylglycerol, and a glycolipid are generally found as major components of the polar lipids studied so far. In addition, other unidentified glycolipids, phosphoglycolipids, phospholipids, and lipids not containing a sugar moiety, a phosphate, or an amino group may also be present.

Cellular fatty acids. The cellular fatty acid profiles of species of the genus *Microbacterium* are rather similar. Branched fatty acids, either iso or anteiso, are abundant, and the proportion of straight-chain acids is small. The major cellular fatty acids

TABLE 141. Phenotypic characteristics of the genus *Microbacterium* species[a]

Characteristic	*M. lacticum*	*M. aerolatum*	*M. agarici*	*M. aoyamense*	*M. aquimaris*	*M. arabinogalactanolyticum*	*M. arborescens*	*M. aurantiacum*	*M. aurum*
Colony pigmentation	GW/Y	Y	Y	PY	Y	YW	O	O	Y
Motility	−	−	nd	−	−	−	+	−	−
Growth at 37°C	−	+	nd	−	+	nd	+	+	nd
Optimum temperature	30	nd	25–30	nd	25–30	28	28–37	28	30
pH range for growth	nd	nd	nd	5.0–11.0	6-10	nd	nd	nd	nd
Growth in the presence of:									
5% NaCl	nd	+	nd	+	+	nd	nd	+	nd
7% NaCl	nd	nd	nd	−	+	nd	nd	nd	nd
Catalase	+	+	+	+	+	nd	nd	nd	nd
Oxidase	nd	−	+	nd	+	nd	nd	nd	nd
Hydrolysis of:									
Esculin	nd	nd	+	nd	+	nd	nd	nd	+
Gelatin	−	−	nd	nd	−	+	+	−	−
Starch	+	nd	nd	nd	−	+	nd	+	+
Tween 80	−	nd	nd	nd	nd	+	−	+	−
H_2S production	−	nd	nd	nd	−	+	+	+	+
Peptidoglycan diamino acid	Lys	Orn	Orn	Orn	Orn	Orn	Lys	Orn	Lys
Cell-wall sugars	Gal, Man, Rha	nd	nd	Gal, Rha, Xyl	Gal, Glc	Gal	6dT, Gal, Man	Fuc, Gal, Rha	Fuc, Gal, Glc
Major menaquinones	MK-11, 12	MK-12, 13	MK-11, 12	MK-13	MK-11	MK-12, 13	MK-11, 12	MK-12	MK-11, 12
Major fatty acids[b]	ai-C15, ai-C17	ai-C15, ai-C17, i-C16	ai-C15, ai-C17, i-C16	ai-C15, ai-C17, i-C16	ai-C15, ai-C17, i-C16	ai-C15, ai-C17	ai-C15, ai-C17, i-C16	ai-C15, ai-C17, i-C15, i-C16	ai-C15, ai-C17, i-C16
Polar lipids	DPG, PG, PGL, PL	DPG, PG, GL	DPG, PG, GL	nd	DPG, PG, GL	DPG, PG, PGL, GL	DPG, PG, GL	nd	DPG, PG, GL
DNA G+C content (mol%)	70	69.3–69.7	nd	69	69.3–69.6	69.3	71	70.1–70.3	69.2

(continued)

TABLE 141. (continued)

Characteristic	*M. awajiense*	*M. azadirachtae*	*M. barkeri*	*M. binotii*	*M. chocolatum*	*M. deminutum*	*M. dextranolyticum*	*M. esteraromaticum*	*M. flavescens*
Colony pigmentation	LY	LY	Y	Y	O	PY	Y	Y	Y
Motility	–	+	+	–	–	–	–	+	–
Growth at 37°C	+	+	nd	+	+	–	–	nd	–
Optimum temperature	nd	28	30	nd	28	nd	30	28	25
pH range for growth	6.0–11.0	5.0–10.0	nd	nd	nd	6.0–9.0	nd	nd	nd
Growth in the presence of:									
5% NaCl	+	+	nd	+	–	–	nd	–	nd
7% NaCl	–	–	nd	–	–	–	nd	–	nd
Catalase	+	+	+	+	nd	+	nd	nd	+
Oxidase	nd	–	nd	–	nd	nd	nd	nd	+
Hydrolysis of:									
Esculin	nd	+	+	nd	nd	nd	+	nd	nd
Gelatin	nd	–	nd	–	–	nd	–	–	+
Starch	nd	–	+	+	+	nd	–	+	+
Tween 80	nd	nd	–	nd	+	nd	+	+	+
H_2S production	nd	–	nd	–	+	nd	+	+	+
Peptidoglycan diamino acid	Orn	Orn	Orn	Orn	Orn	Orn	Lys	Orn	Orn
Cell-wall sugars	nd	nd	Gal, Glc, Rha	Gal, Man, Rha	Gal, Man, Rha, Xyl	Fuc, Gal, Glc, Rha, Xyl	6dT, Gal, Glc, Rha	Gal and Glc	Gal, Glc, Rha
Major menaquinones	MK-12, 13, 14	MK-12, 13	MK-11, 12	MK-10, 11, 12	MK-12	MK-13	MK-11, 12	MK-12, 13	MK-13, 14
Major fatty acids[b]	ai-C15, ai-C17, i-C16, i-C15	ai-C15, ai-C17, i-C16	ai-C15, ai-C17	ai-C15, ai-C17, i-C16	ai-C17, ai-C15, i-C16	ai-C17, ai-C15	ai-C15, i-C16	ai-C15, ai-C17	ai-C15, ai-C17, i-C16
Polar lipids	nd	nd	DPG, PG, GL	DPG, PG, PL, GL	nd	nd	DPG, PG, PGL, GL	DPG, PG, GL	DPG, PG, GL
DNA G+C content (mol%)	70	69.5	68.7	70–72	69.5	69	68.3	68.8	66.9

(continued)

TABLE 141. (continued)

Characteristic	*M. flavum*	*M. fluvii*	*M. foliorum*	*M. ginsengisoli*	*M. ginsengiterrae*	*M. gubbeenense*	*M. halophilum*	*M. halotolerans*	*M. hatanonis*
Colony pigmentation	Y	PY	Y	Y	PY	C	YW	W	LY
Motility	–	–	+	–	–	nd	–	–	–
Growth at 37°C	+	–	+	–	+	–	+	–	+
Optimum temperature	nd	nd	25	nd	30	nd	28	28–30	30
pH range for growth	5.0–11.0	6.0–11.0	nd	5.0–9.0	5.0–9.0	nd	nd	nd	6.0–9.0
Growth in the presence of:									
5% NaCl	+	–	nd	–	+	+	+	+	–
7% NaCl	+	–	nd	–	+	+	nd	+	–
Catalase	nd	+	+	+	+	+	nd	+	+
Oxidase	nd	nd	–	+	+	–	nd	–	–
Hydrolysis of:									
Esculin	nd	nd	+	+	nd	nd	nd	nd	+
Gelatin	nd	nd	+	–	nd	–	+	–	–
Starch	nd	nd	nd	–	nd	–	+	–	nd
Tween 80	nd	nd	–	nd	nd	–	+	–	nd
H_2S production	nd	nd	–	nd	nd	nd	nd	–	nd
Peptidoglycan diamino acid	Lys	Orn	Orn	Orn	Orn	Lys	Orn	Orn	Orn
Cell-wall sugars	nd	nd	Gal, Man, Rha	Rha	Gal, Rib, Xyl	Gal, Rha	Gal, Glc, Man	Gal, Glu, Rib, Xyl	Gal, Rha
Major menaquinones	MK-11, 12	MK-11, 12	MK-10, 11, 12	MK-12	MK-12, 13	MK-11, 12	MK-11, 12, 13	MK-10, 11	MK-11, 12
Major fatty acids[b]	ai-C15, ai-C17, i-C16	ai-C15, ai-C17, i-C16	ai-C15, ai-C17, i-C16	ai-C17, ai-C15, i-C16	ai-C15, ai-C17, i-C16	ai-C15, ai-C17, i-C16	ai-C15, i-C16, i-C15	ai-C15, ai-C17, i-C16	ai-C15, ai-C17, i-C16
Polar lipids	nd	nd	nd	nd	nd	DPG, PI, PGM	nd	nd	nd
DNA G+C content (mol%)	70	70	67	69.4	64.5	69–75	67.2	63.8	69.0

(continued)

TABLE 141. (continued)

Characteristic	*M. hominis*	*M. humi*	*M. hydrocarbonoxydans*	*M. imperiale*	*M. indicum*	*M. insulae*	*M. invictum*	*M. keratanolyticum*	*M. ketosireducens*
Colony pigmentation	YW	Y	Y	O	PY	Y	W/Y	Y	Y
Motility	−	nd	+	+	nd	−	+	+	−
Growth at 37°C	+	nd	+	+	−	+	+	nd	−
Optimum temperature	28	25–30	nd	25–30	22	30	nd	28	28
pH range for growth	nd	nd	nd	nd	5.0–9.0	5.5–8.0	6.0–9.5	nd	nd
Growth in the presence of:									
5% NaCl	+	nd	nd	nd	+	+	+	nd	nd
7% NaCl	nd	nd	nd	nd	−	−	−	nd	−
Catalase	nd	+	+	+	−	−	+	nd	nd
Oxidase	nd	+	−	−	−	+	−	nd	nd
Hydrolysis of:									
Esculin	nd	+	nd	nd	+	+	+	nd	nd
Gelatin	−	nd	nd	−	nd	+	+	+	+
Starch	−	nd	nd	−	nd	+	+	−	+
Tween 80	+	nd	nd	−	nd	+	nd	−	+
H_2S production	+	nd	−	nd	nd	−	−	+	nd
Peptidoglycan diamino acid	Lys	Orn	Orn	Lys	Orn	Orn	Lys	Orn	Orn
Cell-wall sugars	6dT, Gal, Man, Rha	nd	nd	Gal, Man, Rha	Gal, Glu, Rha (whole cell)	Gal, Rha, Rib	nd	Gal	nd
Major menaquinones	MK-11, 12	MK-10, 11	MK-11, 12	MK-11, 12	MK-11, 12	MK-12, 13, 14	MK-11, 12	MK-12, 13	MK-13
Major fatty acids[b]	ai-C15, ai-C17, i-C16	ai-C15, ai-C17, i-C16	nd	ai-C15, ai-C17	i-C16, ai-C15, ai-C17	ai-C15, ai-C17, i-C17, i-C16	ai-C15, ai-C17	ai-C15, ai-C17	ai-C15, ai-C17, i-C16, i-C15
Polar lipids	nd	DPG, PG, GL	nd	DPG, PG, GL	PG, PI	DPG, PG, PL, GL	nd	DPG, PG, GL	nd
DNA G+C content (mol%)	71.2	nd	nd	71.0–75.4	65.5	69.9	70.1	66.5	69.7–69.8

(continued)

TABLE 141. (continued)

Characteristic	*M. kitamiense*	*M. koreense*	*M. kribbense*	*M. lacus*	*M. laevaniformans*	*M. lindanitolerans*	*M. liquefaciens*	*M. luteolum*	*M. luticocti*
Colony pigmentation	O	LY	PY	PY	Y	Y	BY	Y	W
Motility	–	–	–	–	–	nd	–	–	+
Growth at 37°C	+	+	+	–	nd	+	+	–	+
Optimum temperature	nd	30	28	nd	30	28–37	30	28	36
pH range for growth	6.0–9.0	6.0–8.0	7.0–11.0	6.0–11.0	nd	6.0–11.0	nd	nd	5.5–9.7
Growth in the presence of:									
5% NaCl	+	+	–	–	nd	–	+	–	+
7% NaCl	+	+	–	–	nd	–	+	–	+
Catalase	+	+	–	nd	+	+	+	nd	–
Oxidase	–	–	nd	nd	nd	–	nd	nd	–
Hydrolysis of:									
Esculin	nd	+	+	nd	nd	+	+	nd	+
Gelatin	+	–	–	nd	+	–	+	–	+
Starch	+	nd	+	nd	+	+	–	–	+
Tween 80	–	nd	+	nd	–	nd	–	–	nd
H_2S production	–	+	nd	nd	+	–	+	+	nd
Peptidoglycan diamino acid	Orn	Lys	Lys	Orn	Lys	Orn	Orn	Orn	Orn
Cell-wall sugars	nd	Gal, Xyl	Gal, Glc, Rib, Xyl	nd	Gal, Man, Rha, Xyl	Gal, Rib	Rha	Gal, Glc, Rha	Man
Major menaquinones	MK-11	MK-11, 12	MK-11, 12	MK-12, 13	MK-11, 12	MK-11, 12	MK-11, 12	MK-12	MK-12
Major fatty acids[b]	ai-C15, ai-C17, i-C16	ai-C15, ai-C17, i-C16	C18:1ω7c, ai-C15, i-C16	ai-C15, ai-C17, i-C16	ai-C15, ai-C17	ai-C15, ai-C17, i-C16, i-C15	ai-C15, ai-C17	ai-C15, ai-C17	ai-C17, ai-C15
Polar lipids	nd	nd	DPG, PG	nd	DPG, PG, GL	DPG, PG, PL	DPG, PG, GL	DPG, PG, GL	nd
DNA G+C content (mol%)	69.2	68	71.0	69	70.0–73.7	65.3	68.2–72.5	70.6	72

(continued)

TABLE 141. (continued)

Characteristic	*M. marinilacus*	*M. maritypicum*	*M. natoriense*	*M. oleivorans*	*M. oxydans*	*M. paludicola*	*M. paraoxydans*	*M. phyllosphaerae*	*M. profundi*
Colony pigmentation	Y	LY	Y	O	Y	Y	BY	Y	Y
Motility	nd	+	−	−	+	−	+	+	nd
Growth at 37°C	+	+	+	+	+	+	+	+	−
Optimum temperature	nd	28	30	nd	30	25–30	25–37	25	28
pH range for growth	6.0–11.0	nd	5.0–9.0	nd	nd	6.0–9.0	nd	nd	6.0–9.5
Growth in the presence of:									
5% NaCl	+	+	+	nd	+	+	+	−	+
7% NaCl	+	nd	−	nd	+	nd	+	−	+
Catalase	+	nd	+	+	+	+	+	+	+
Oxidase	nd	nd	−	−	−	+	−	−	−
Hydrolysis of:									
Esculin	nd	nd	nd	+	+	+	+	+	+
Gelatin	nd	+	nd	nd	+	−	+	+	+
Starch	nd	−	nd	nd	nd	+	nd	v	−
Tween 80	nd	+	nd	nd	nd	nd	nd	v	−
H_2S production	nd	−	nd	−	+	nd	−	−	−
Peptidoglycan diamino acid	Orn	Orn	Orn	Lys	Orn	Orn	Orn	Orn	Orn
Cell-wall sugars	nd	Gal	nd	nd	Gal, Gul, Man, Rha	Gal, Glu, Man, Rha, Fuc	nd	Gal, Rha	nd
Major menaquinones	MK-11, 12	MK-12	MK-9, 10, 11, 12	MK-11, 12	MK-11, 12	MK-11, 12	MK-11, 12	MK-11, 12	MK-12, 13
Major fatty acids[b]	ai-C15, ai-C17, i-C16	ai-C15, i-C16	ai-C15, i-C15, ai-C17	nd	ai-C15, ai-C17, i-C16	ai-C15, ai-C17, i-C16	ai-C15, ai-C17, i-C16	ai-C15, i-C16, i-C15, ai-C17	ai-C15, i-C15, i-C16, ai-C17
Polar lipids	nd	nd	nd	nd	DPG, PG, GL	DPG, PG, PL, GL	nd	nd	nd
DNA G+C content (mol%)	71.6	71.6	69.1	nd	70–71	66.5	69.9	64	66.8

(continued)

TABLE 141. (continued)

Characteristic	*M. pseudoresistens*	*M. pumilum*	*M. pygmaeum*	*M. radiodurans*	*M. resistens*	*M. saperdae*	*M. schleiferi*	*M. sediminicola*	*M. soli*
Colony pigmentation	Y	PY	PY	W	Y	Y	Y	PY	PY
Motility	nd	–	–	+	–	+	–	nd	–
Growth at 37°C	nd	–	–	–	nd	–		+	+
Optimum temperature	25–30	nd	nd	nd	nd	30	28	nd	30
pH range for growth	nd	7.0–10.0	5.0–9.0	6.0–10.0	nd	nd	nd	6.0–11.0	nd
Growth in the presence of:									
5% NaCl	nd	–	+	+	nd	nd	nd	+	nd
7% NaCl	nd	–	–	–	nd	–	nd	+	nd
Catalase	+	+	+	+	+	+	nd	+	+
Oxidase	+	nd	nd	–	nd	nd	nd	nd	–
Hydrolysis of:									
Esculin	+	nd	nd	nd	+	+	nd	nd	nd
Gelatin	nd	nd	nd	–	nd	–	–	nd	nd
Starch	nd	nd	nd	nd	–	+	–	nd	nd
Tween 80	nd	nd	nd	nd	nd	+	v	nd	nd
H_2S production	nd	nd	nd	–	nd	+	–	nd	–
Peptidoglycan diamino acid	Orn	Orn	Orn	Orn	Orn	Orn	Orn	Orn	Orn
Cell-wall sugars	nd	Gal, Rha	nd	Rha	nd	Gal, Glc	6dT, Gal, Man	nd	Gal
Major menaquinones	MK-13	MK-12, 13	MK-11, 12, 13	MK-10, 11, 12	MK-12, 13	MK-11, 12	MK-11, 12	MK-10, 11	MK-11, 12, 13
Major fatty acids[b]	ai-C15, ai-C17, i-C16	ai-C15, i-C16, ai-C17	ai-C17, ai-C15	ai-C15, i-C16, i-C15	ai-C17, ai-C15, i-C16	ai-C15, ai-C17, i-C16	ai-C15, ai-C17	ai-C15, ai-C17, i-C16	ai-C15, i-C15, i-C16, ai-C17
Polar lipids	DPG, PG,GL	nd	nd	DPG, PG, PL, GL	nd	DPG, PG, GL	DPG, PG, GL	nd	nd
DNA G+C content (mol%)	nd	71	68	67.7	64	69.1	66.9	67.8	68.9–73.5

(continued)

TABLE 141. (continued)

Characteristic	*M. terrae*	*M. terregens*	*M. terricola*	*M. testaceum*	*M. thalassium*	*M. trichothecenolyticum*	*M. ulmi*	*M. xylanilyticum*
Colony pigmentation	Y	YB	Y	O	Y/YW	Y	W	Y
Motility	−	−	−	+	−	−	−	nd
Growth at 37°C	nd	−	−	nd	−	nd	+	+
Optimum temperature	28	20–26	nd	30	28	28	nd	25–30
pH range for growth	nd	nd	6.0–11.0	nd	nd	nd	5.0–8.0	6.0–9.0
Growth in the presence of:								
5% NaCl	nd	nd	−	nd	+	nd	nd	−
7% NaCl	nd	nd	−	nd	nd	nd	nd	−
Catalase	nd	+	+	+	nd	nd	−	+
Oxidase	nd	−	nd	nd	nd	nd	−	+
Hydrolysis of:								
Esculin	nd	nd	nd	nd	nd	nd	+	+
Gelatin	+	−	nd	+	+	−	+	nd
Starch	+	−	nd	−	+	+	nd	+
Tween 80	+	−	nd	+	+	+	nd	nd
H_2S production	+	−	nd	nd	−	+	nd	−
Peptidoglycan diamino acid	Orn	Orn	Orn	Orn	Orn	Orn	Orn	Orn
Cell-wall sugars	Gal, Glc, Rha	6dT, Gal, Rha	nd	Rha, Gal	Gal, Glc, (Man, Rha)	Gal, Glc	Fuc, Gal, Rha, Xyl	Gal, Glu
Major menaquinones	MK-13, 14	MK-12, 13	MK-12, 13	MK-11	MK-11, 12	MK-13	MK-12, 13	MK-12
Major fatty acids[b]	ai-C15, ai-C17	ai-C15, ai-C17	ai-C15, ai-C17, i-C16	ai-C15, ai-C17, i-C16	ai-C15, i-C16, i-C15, ai-C17	ai-C15, ai-C17	ai-C15, ai-C17, i-C16	ai-C15, i-C16, ai-C17
Polar lipids	DPG, PG, PGL	DPG, PG, GL	nd	DPG, PG, GL	nd	DPG, PG, GL	nd	DPG, PG, PL, GL
DNA G+C content (mol%)	70.7	68.6	70	67.7	69.1–69.7	69.0	69	69.7

[a]Symbols and abbreviations: +, positive; −, negative; v, variable; nd, no data available; BY, bright yellow; C, cream; GW, gray-white; LY, light yellow, O, orange; PY, pale yellow; W, white; Y, yellow; YB, yellow-brown; YW, yellow-white; Lys, lysine; Orn, ornithine; 6dT, 6-deoxytalose; Fuc, fucose; Gal, galactose; Glc, glucose; Man, mannose; Rha, rhamnose; Rib, ribose; Xyl, xylose; ai, anteiso-branched; i, iso-branched; DPG, diphosphatidylglycerol; PG, phosphatidylglycerol; PGL, phosphoglycolipid; PGM, phosphatidylglycerolmannoside; PI, phosphatidylinositol; PL, unknown phospholipid; GL, unknown glycolipid.
[b]In decreasing order of abundance.

are usually $C_{15:0}$ anteiso, followed by $C_{17:0}$ anteiso, $C_{16:0}$ iso, and $C_{15:0}$ iso.

Phylogeny. The family *Microbacteriaceae* was proposed by Park et al. (1993) based on the phylogenetic relationship derived from the 5S rRNA sequences and confirmed by subsequent analyses of the 16S rRNA genes (Rainey et al., 1994; Takeuchi and Yokota, 1994). Although the genus *Microbacterium* accommodates a large number of species, it forms a single cluster in the phylogenetic tree of the family (Figure 175). This observation is in agreement with studies of Takeuchi and Hatano (1998b), who showed that species containing lysine in the peptidoglycan belonging to the genus *Microbacterium sensu* Collins et al. (1983c) and those containing ornithine, formerly classified in the genus *Aureobacterium* (Collins et al., 1983a), could not be separated from each other on the basis of 16S rRNA gene phylogeny. Thus, they proposed the union of the two genera. The 16S rRNA gene sequence similarity values between species in the genus *Microbacterium* emended by Takeuchi and Hatano (1998b) range from 92.2 to 100%. For comparison, the similarity values of *Microbacterium lacticum*, the type species of the genus, to the other type species of the genera of the family *Microbacteriaceae* range from 89.6 to 96.4% among the type strains.

The phylogeny of species with different types of peptidoglycan was studied extensively using housekeeping genes, such as DNA gyrase subunit B (*gyrB*), RNA-polymerase subunit B (*rpoB*), recombinase A (*recA*), and polyphosphate kinase (*ppk*) (Richert et al., 2007). Analysis of the *ppk* genes and the concatenate tree, combining sequences of all five genes, demonstrated a coherent cluster for the species which contain lysine in the peptidoglycan within the radiation of the current genus *Microbacterium*, including former *Aureobacterium* species and *Microbacterium* species with ornithine in the peptidoglycan (Figure 175).

Biochemical and physiological characteristics. Species of the genus *Microbacterium* are generally aerobic, catalase-positive, and grow at 25–30°C. Oxidase and other activities, such as hydrolysis of starch, gelatin, and Tweens, are variable. Acid is produced from various carbohydrates. However, the production of acid is generally slow or weak. Organic acids and other carbon compounds are assimilated as sole carbon sources, indicated by turbidity in liquid media or by alkalinization of solid media due to consumption of organic acids. These characteristics are dependent on the species. The properties are summarized in Table 141.

Some species of the genus *Microbacterium*, such as *Microbacterium flavescens* and *Microbacterium terregens*, require special nutritional supplements for good growth (Lochhead, 1958). Terregens factor, biotin, thiamine, pantothenic acid, and L-methionine may be required for growth. The terregens factor is replaceable by other siderophores, such as coprogen and ferrichrome (Komagata and Suzuki, 1986b; Lochhead, 1958).

Enrichment and isolation procedures

Species of the genus *Microbacterium* have been isolated from various sources. The type strain of the type species, *Microbacterium lacticum*, was isolated from dairy products and is thermotolerant (Orla-Jensen, 1919). Many species have been isolated from soil, sediment, or activated sludge, as well as from freshwater and seawater. Some have been isolated from animals. *Microbacterium flavum* and *Microbacterium marinilacus* were isolated from a marine ascidian and hydroid, respectively (Kageyama et al., 2007c, 2007d). *Microbacterium imperiale* and *Microbacterium saperdae* were isolated from the moth *Eacles imperialis* and dead *Saperda caracharias* insects, respectively (Lysenko, 1959; Steinhaus, 1941). Some species were isolated from clinical specimens. *Microbacterium binotii* and *Microbacterium paraoxydans* were isolated from human blood (Clermont et al., 2009; Laffineur et al., 2003a), *Microbacterium hominis* was from a lung aspirate (Funke et al., 1995), *Microbacterium oxydans* was from hospital material (Chatelain and Second, 1966), and *Microbacterium resistens* was from a corneal ulcer (Funke et al., 1998).

While most species were isolated without special selective media, medium containing superoxide dismutase and catalase to remove oxygen radicals (Takahashi et al., 2003) was used to isolate *Microbacterium aoyamense*, *Microbacterium deminutum*, *Microbacterium pumilum*, and *Microbacterium terricola* (Kageyama et al., 2006, 2007a)

Microbacterium hydrocarbonoxydans and *Microbacterium oleivorans* were isolated by enrichment culture with 1–5% crude oil in the medium. Some *Microbacterium* strains were isolated through some special screening procedures; D-arabinofuranosidase production by *Microbacterium arabinogalactanolyticum* (Kotani et al., 1972), keratansulfate endo-β-galactosidase production by *Microbacterium keratanolyticum* (Nakazawa et al., 1975; Nakazawa and Suzuki, 1975), reduction of 2,5-diketo-D-gluconic acid to 2-keto-gluconic acid by *Microbacterium ketosireducens* (Takeuchi and Hatano, 1998a), production of *N*-acyl-D-amino acid amidohydrolase by *Microbacterium natoriense* (Liu et al., 2005), degradation of trichothecene mycotoxin by *Microbacterium trichothecenolyticum* (Nakayama et al., 1980), and production of exopolysaccharide by *Microbacterium kitamiense* (Matsuyama et al., 1999).

Maintenance procedures

Most of the species grow well on nutrient agar or trypticase soy agar (TSA). Supplementation with yeast extract generally enhances growth. Cultures of most species stored at 4–8°C will remain viable for 1 or 2 months, depending upon the strain and medium. For long-term preservation, freezing at −80°C or lower or in the vapor phase of liquid nitrogen and freeze-drying are recommended. For freezing, cells are suspended in liquid medium containing 5–20% glycerol. For freeze-drying, cells are suspended in 10% skimmed milk containing 1% monosodium glutamate. Attention should be paid to repeated serial transfers to fresh medium that may lead to weak growth.

Differentiation of the genus *Microbacterium* from other genera

The species of the genus *Microbacterium* can be differentiated from those in other genera of the order *Micrococcales* by chemotaxonomic features, as well as by phylogenetic analysis. Amino acid composition of the peptidoglycan suggesting the B group structure (Schleifer and Kandler, 1972) and menaquinones with

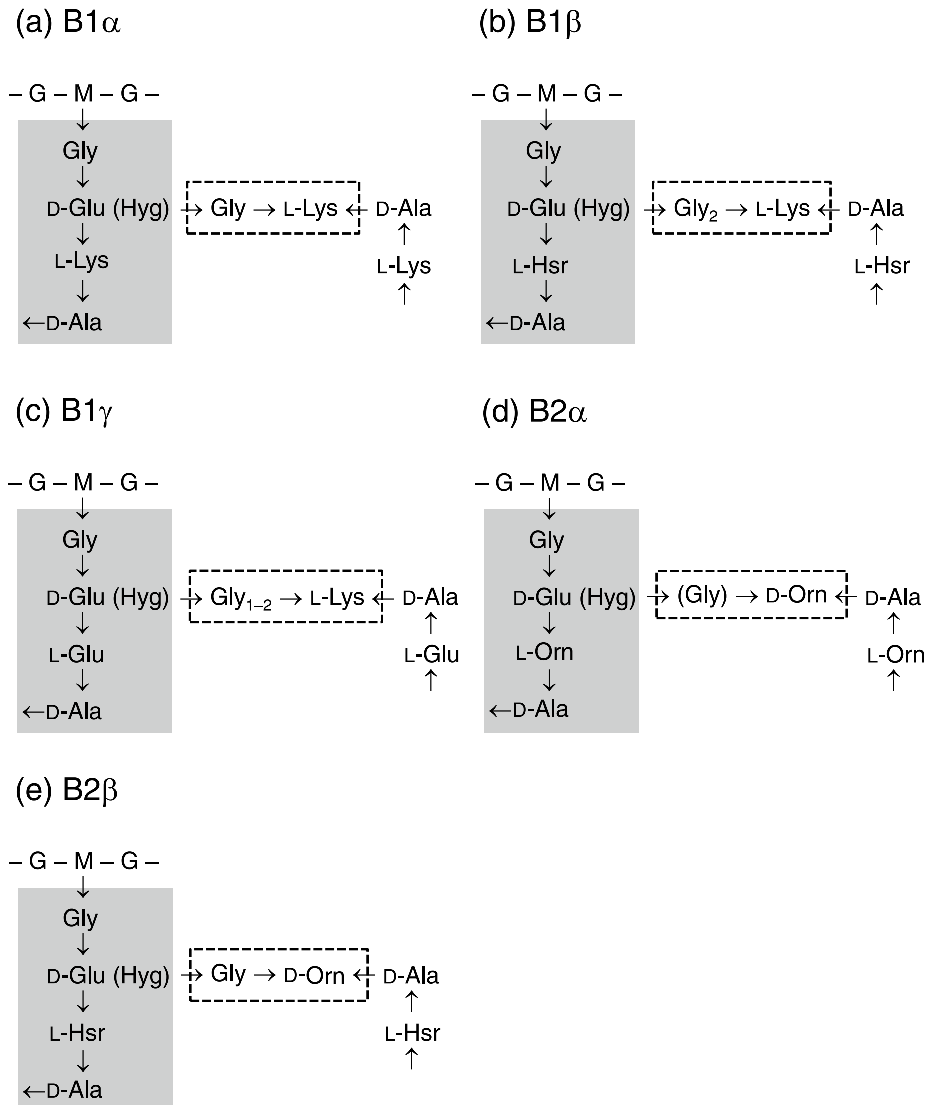

FIGURE 174. Varieties of peptidoglycan structure found in the members of the genus *Microbacterium*. M, *N*-acetyl or glycolyl muramic acid; G, *N*-acetylglucosamine; Ala, alanine; Glu, glutamic acid; Gly, glycine; Hsr, homoserine; Hyg, hydroxyglutamic acid; Lys, lysine; Orn, ornithine. The shaded box and the box with broken line indicate the peptide subunit and interpeptide bridge, respectively. In addition to these units, the amino acids outside of the boxes are those of the neighboring side-chains.

long unsaturated isoprenoid side-chains, i.e. MK-11 to MK-13 as the major components (Collins and Jones, 1981; Schumann et al., 2009), are indicative of the genus. Differentiation of the genus from other genera of the family *Microbacteriaceae* can be also observed by 16S rRNA gene analysis including phylogenetic grouping and chemotaxonomically. The members of the genus *Microbacterium* form a distinct cluster within the family in the phylogenetic tree. Signature nucleotides in the 16S rRNA gene sequences have been proposed by Takeuchi and Hatano (1998b) as follows: positions 69:99 (G/A–T), 129 (C/T), 232 (G), 279 (T), 443:491 (C/T–G), 770:809 (G–C/G), 780 (A), 830:856 (T–A), and 929:1386 (A–T).

While the amino acid composition of the peptidoglycan and the menaquinone profile are useful tools, the glycolation of the cell-wall, as determined by the glycolate test (Uchida et al., 1999), is especially informative. Among the family *Microbacteriaceae*, this trait is only shared by members of the genus *Okibacterium* (Evtushenko et al., 2002), which can be distinguished by phylogenetic analyses. The ornithine-containing *Microbacterium* species can also be further distinguished from *Curtobacterium* by the

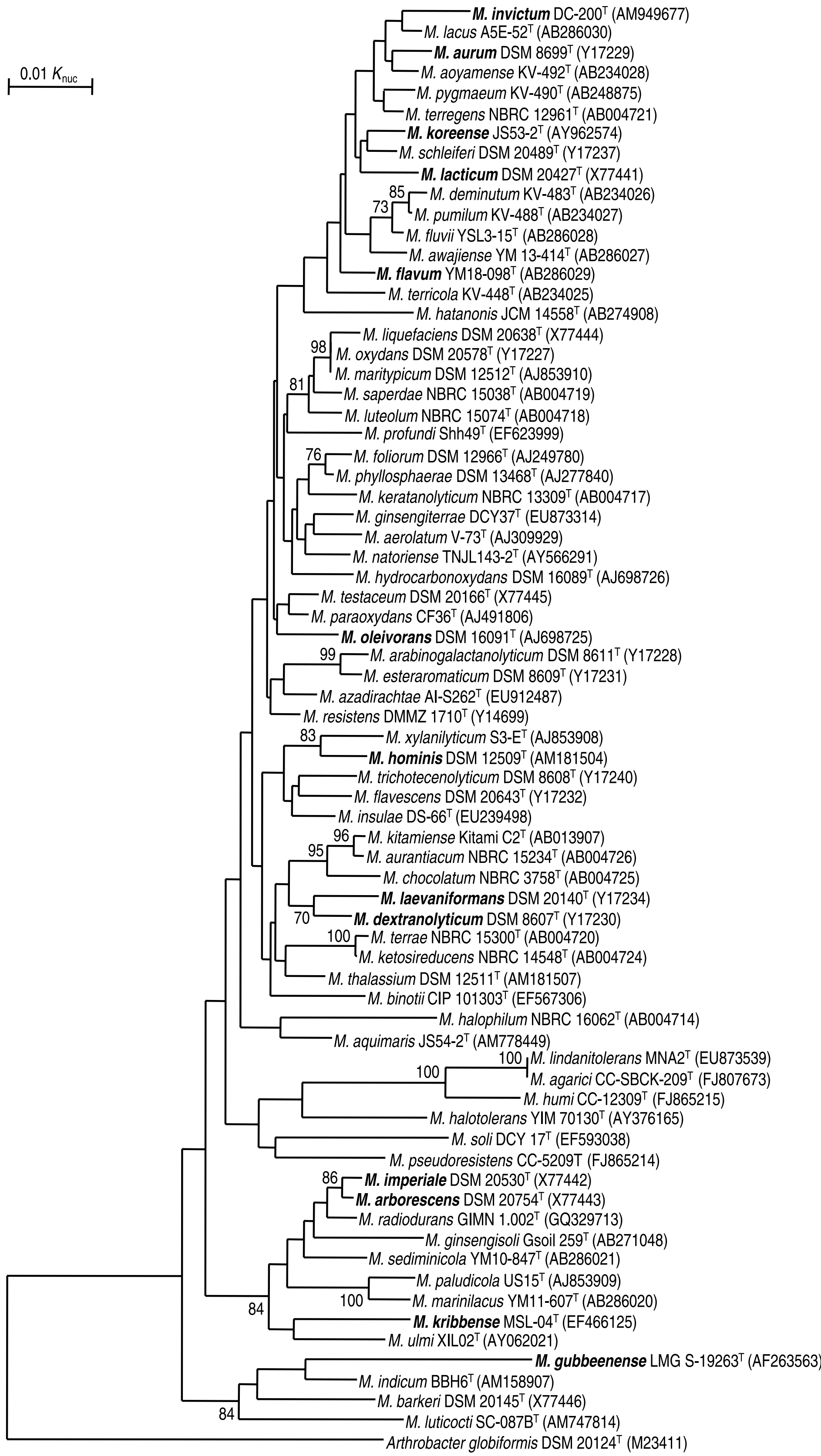

FIGURE 175. Phylogenetic tree derived from 16S rRNA gene sequences of the members of the genus *Microbacterium* constructed with the neighbor-joining method. Bootstrap values (>70%) based on 1000 replicates are shown at branch nodes. The species with names in bold contain lysine in the cell-wall peptidoglycan. Bar = 0.01 K_{nuc} substitutions per nucleotide position.

presence of 1 mole of glycine in the interpeptide bridge and the length of the menaquinone side-chain. Confirmation of the taxonomic placement by polyphasic characterization combining several methods is also recommended.

Taxonomic comments

Orla-Jensen described the genus *Microbacterium* in 1919 with three species, *Microbacterium lacticum*, *Microbacterium liquefeciens*, and "*Microbacterium flavum*," for strains isolated from dairy products or associated equipment. Later, Orla-Jensen (1921) recognized the heterogeneity of the genus *Microbacterium*. Schleifer (1970) revealed that each of the three species had different peptidoglycan structures and only *Microbacterium lacticum* appeared in the Approved Lists of Bacterial Names (Skerman et al., 1980). Improved chemotaxonomy enhanced the reclassification of the Gram-stain-positive, aerobic, irregular rods, namely coryneform-shaped bacteria. "*Microbacterium flavum*" Orla-Jensen (1919) was reclassified in the genus *Corynebacterium* as *Corynebacterium flavescens* (Barksdale et al., 1979). This species is different from the recognized species *Microbacterium flavum* proposed by Kageyama et al. (2007a). Strains with group B peptidoglycan were also found in the coryneform group of bacteria, i.e. *Corynebacterium laevaniformans* and *Brevibacterium imperiale*, which possess type B1α or B1β peptidoglycan (Schleifer and Kandler, 1972).

Collins et al. (1983c) reclassified those bacteria containing lysine in the peptidoglycan in the genus *Microbacterium* with *Microbacterium lacticum*, the type species of the genus. In addition, Collins et al. (1983a) proposed a new genus *Aureobacterium*, with *Aureobacterium liquefaciens* (formerly "*Microbacterium liquefaciens*" Orla-Jensen 1919) as the type species, for species possessing group B peptidoglycan with D-ornithine (Schleifer, 1970). The genus *Aureobacterium* also accommodated the coryneform group of bacteria classified in the genera *Arthrobacter* (Lochhead, 1958), *Corynebacterium* (Dias et al., 1962), and *Curtobacterium* (Yamada and Komagata, 1972a), containing the same peptidoglycan (namely B2β; Schleifer and Kandler, 1972). These genera were well characterized and novel species were added to both genera (Imai et al., 1984; Yokota et al., 1993a, 1993b). Later, Rainey et al. (1994) and Takeuchi and Yokota (1994) revealed that the genera were not phylogenetically distinct. Consequently, Takeuchi and Hatano (1998b) proposed the union of the two genera. At that time, the genera *Microbacterium* and *Aureobacterium* contained 6 and 13 species, respectively. Since the union of the genera, the number of species assigned to the emended genus *Microbacterium* rapidly increased to 71 species.

Phylogenetic trees including all these 71 species also indicate that the species formerly assigned to the two genera are not distinguishable in 16S rRNA gene trees. Because the majority of species share sequence similarities higher than 97%, 16S rRNA gene phylogenies may not be informative. Analyses of house-keeping genes, which may be more reliable molecular chronometers, were able to distinguish species possessing lysine in their peptidoglycan from those with ornithine (Richert et al., 2007). The question then arises as to whether the 16S rRNA gene is a suitable marker for the phylogenetic relationships within the genus *Microbacterium*. Until this question has been answered, the chemotaxonomic varieties in the genus *Microbacterium* must be regarded as species characteristics.

Differentiation of species of the genus *Microbacterium*

Species differentiation is finally concluded by phylogenetic analysis based on 16S rRNA gene sequences and DNA–DNA relatedness analysis. The novelty of species can be reliably determined by DNA–DNA hybridization if the values are less than 70%. When the 16S rRNA gene similarity is 98.0% or greater, DNA–DNA hybridization should be performed for the proposal of a novel species (Schumann et al., 2009). Since many of the species of the genus *Microbacterium* are represented by a single strain, little is known concerning intraspecies variability. Hence, biochemical and physiological characteristics are not reliable for identification of species or differentiation between species. Nevertheless, these traits should be considered for the differentiation and characterization of *Microbacterium* species.

List of species of the genus *Microbacterium*

1. **Microbacterium lacticum** Orla-Jensen 1919, 179[AL]

   lac′ti.cum. L. masc. n. *lac*, *lactis* milk; L. neut. -*icum* suffix used with the sense of pertaining to; N.L. neut. adj. *lacticum* pertaining to milk, lactic.

   The following characteristics are based mainly on Orla-Jensen (1919), Yamada and Komagata (1972b), Schleifer and Kandler (1972), Collins et al. (1983c), and Yokota et al. (1993b).

   Cells are small, slender, nonmotile rods arranged at angles; V forms are observed but no branching. In older cultures, rods become shorter but no rod–coccus cycle is seen. Colonies are 1–3 mm in diameter, circular, convex with entire margins, opaque, and moist. Color of colonies varies from gray-white to yellow. Aerobic. Catalase-positive. Optimum temperature for growth is 30°C. No growth occurs at 37°C. Starch is hydrolyzed. Gelatin, and Tweens 20, 40, 60 and 80 are not hydrolyzed. Urease-negative. Nitrate is reduced. H_2S is not produced. Arginine is not decarboxylated. Acid is produced from glucose, fructose, mannose, galactose, lactose, maltose, cellobiose, dextrin, starch, mannitol, *N*-acetylglucosamine, arbutin, esculin, and salicin, but not from L-arabinose, xylose, rhamnose, ribose, sorbose, sucrose, trehalose, raffinose, inulin, glycerol, erythritol, adonitol, dulcitol, sorbitol, inositol, or methyl α-glucoside. The following organic acids are assimilated: acetate, pyruvate, L-lactate, D-lactate, malate, succinate, fumarate, citrate, and butyrate. The following organic acids are not assimilated: 2-oxoglutarate, formate, propionate, oxalate, malonate, glutarate, adipate, pimelate, glycolate, glyoxylate, gluconante, hippurate, and uriate.

   Cell-wall peptidoglycan contains L-lysine as the diamino acid, conforming to type B1α (Figure 174). Cell-wall acyl

type is glycolyl. Cell-wall sugars are rhamnose, mannose, and galactose. Major menaquinones are MK-11 and MK-12. The polar lipids are diphosphatidylglycerol, phosphatidylglycerol, and dimannosyldiacylglycerol. In addition, an unknown monoglycosyldiacylglycerol and a phosphoglycolipid are also present. Cellular fatty acids are predominantly $C_{15:0}$ anteiso and $C_{17:0}$ anteiso.

Source: usually found in milk, dairy products and on dairy equipment.

DNA G+C content (mol%): 69–74.9 (T_m), 70 (HPLC; type strain).

Type strain: ATCC 8180, CCUG 28998, CIP 69.3, DSM 20427, HAMBI 2287, JCM 1379, NBRC 14135, VKM Ac-1145.

Sequence accession no. (16S rRNA gene): X77441.

2. **Microbacterium aerolatum** Zlamala, Schumann, Kämpfer, Valens, Rosselló-Mora, Lubitz and Busse 2002b, 1233[VP]

a.e.ro.la′tum. Gr. n. *aer* air; L. neut. part. adj. *latum* carried; N.L. neut. part. adj. *aerolatum* airborne.

Cells are nonmotile, small rods from 0.5 to 0.7 µm wide by 1.5 to 1.9 µm long, occurring singly or in irregular clusters. Colonies are translucent, yellow-pigmented, circular, slightly convex, opaque, moist, and 2–7 mm in diameter on TSA.

Aerobic. Good growth is observed at 4 and 37°C. Growth occurs in the presence of 6.5% (w/v) NaCl. Catalase-positive and oxidase-negative. Nitrate is reduced to nitrite. β-Galactosidase is positive. Casein and gelatin are not hydrolyzed.

Acid is produced from D-glucose, L-arabinose, D-sucrose, D-galactose, D-mannose, D-maltose, L-rhamnose, D-mannitol, and D-xylose but not from D-trehalose, D-lactose, or glycerol. The following carbon compounds are utilized: L-arabinose, *N*-acetyl-D-glucosamine, cellobiose, D-fructose, D-galactose, gluconate, D-glucose, D-mannose, maltose, L-rhamnose, D-ribose, maltitol, sucrose, trehalose, D-xylose, and DL-lactate.

The cell-wall peptidoglycan is B2β type, [HSr]–D-Glu(Hyg)–Gly–D-Orn (Figure 174), containing ornithine as characteristic diamino acid and glycine and homoserine at positions 1 and 3 of the peptide side-chain, respectively. The polar lipid profile is composed of diphosphatidylglycerol, phosphatidylglycerol, and two unknown glycolipids. The major menaquinones are MK-12 and MK-13, with a minor amount of MK-11. Cellular fatty acids contain predominantly $C_{15:0}$ anteiso, $C_{17:0}$ anteiso, and $C_{16:0}$ iso.

Source: air in the chapel "Virgilkapelle" in Vienna, Austria.

DNA G+C content (mol%): 69.3–69.7 (HPLC).

Type strain: V-73, CCM 4955, DSM 14217, JCM 12137, NBRC 103071.

Sequence accession no. (16S rRNA gene): AJ309929.

3. **Microbacterium agarici** Young, Busse, Langer, Chu, Schumann, Arun, Shen, Rekha and Kämpfer 2010, 857[VP]

a.ga.ri′ci. N.L. gen. n. *agarici* of *Agaricus*, the generic name of the mushroom *Agaricus blazei* from where the type strain was isolated.

Cells are short rods, approximately 1–1.2 µm in width and 2–3 µm in length. Aerobic. Catalase and oxidase are positive. Good growth occurs on nutrient agar and R2A agar (Oxoid) at 25–30°C and yellow colonies appear in 3 d.

Acid is produced from D-mannitol and weakly produced from adonitol but not from L-arabinose, cellobiose, glucose, lactose, D-mannose, sucrose, D-xylose D-arabitol, dulcitol, erythritol, maltose, melibiose, methyl D-glucoside, raffinose, rhamnose, salicin, sorbitol, or trehalose. Positive for hydrolysis of esculin (weak reaction), pNP-α-D-glucopyranoside, pNP-β-D-glucopyranoside, L-alanine-pNA oNP-β-D-galactopyranoside, pNP-β-D-xylopyranoside, and L-proline-pNA (oNP, *o*-nitrophenyl; pNP, *p*-nitrophenyl; pNA, *p*-nitroanilide), but negative for pNP-β-D-glucuronide, bis-pNP-phosphate, pNP-phenyl-phosphate, pNP-phosphoryl-choline, 2-deoxythymidine-5′-pNP-phosphate, and L-glutamate-γ-3-carboxy-pNA. Cellobiose, D-fructose, D-glucose, DL-lactate, D-mannose, maltose, D-mannitol, sucrose, trehalose, L-arabinose, D-galactose, L-rhamnose, D-ribose, salicin, D-xylose, adonitol, maltitol, D-sorbitol, putrescine, L-malate, L-proline, and L-aspartate are assimilated, but *N*-acetyl-D-glucosamine, *N*-acetyl-D-galactosamine, *p*-arbutin, gluconate, α-melibiose, *i*-inositol, acetate, propionate, *cis*-aconitate, citrate, fumarate, oxoglutarate, pyruvate, *trans*-aconitate, adipate, 4-aminobutyrate, azelate, glutarate, DL-3-hydroxybutyrate, itaconate, L-leucine, mesaconate, phenylacetate, L-phenylalanine, suberate, L-tryptophan, L-alanine, β-alanine, L-histidine, L-ornithine, L-serine, 3-hydroxybenzoate, and 4-hydroxybenzoate are not assimilated.

The cell-wall peptidoglycan is B2α with an interpeptide bridge of D-Glu–Gly–D-Orn (Figure 174). The predominant quinones are MK-11 and MK-12, with MK-10 as a minor component. The polar lipid profile is composed of diphosphatidylglycerol, phosphatidylglycerol, and one unknown glycolipid. Minor amounts of two phospholipids and another glycolipid are also found. Major fatty acids are $C_{15:0}$ anteiso, $C_{16:0}$ iso, and $C_{17:0}$ anteiso. Predominant polyamines are spermidine and spermine.

Source: the type strain was isolated from the stalk of the edible mushroom *Agaricus blazei* cultivated in the laboratory.

DNA G+C content (mol%): Not known.

Type strain: CC-SBCK-209, CCM 7686, DSM 21798.

Sequence accession no. (16S rRNA gene): FJ807673.

4. **Microbacterium aoyamense** Kageyama, Takahashi and Ōmura 2006, 2115[VP]

a.o.ya.men′se. N.L. neut. adj. *aoyamense* of or belonging to Aoyama, Tokyo, Japan, where the type strain was isolated.

Cells are nonmotile, irregular rods, 0.3–0.5 × 0.4–0.8 µm in size. Colony color is pale yellow. Aerobic and catalase-positive. Growth occurs at pH 5–11 and 14–34°C. Tolerates up to 5% NaCl on 5× diluted nutrient agar medium. D-Glucose, D-galactose, maltose, mannitol, D-mannose, raffinose, L-rhamnose, sucrose, and trehalose are assimilated, but arabinose, fructose, and xylose are not. Esterase (C4), esterase lipase (C8), lipase (C14), leucine arylamidase, acid phosphatase, naphthol-AS-BI-phosphohydrolase, β-galactosidase, α-glucosidase, β-glucosidase, and *N*-acetyl-β-glucosamidase are detected with the API ZYM enzyme assay; alkaline phosphatase, cystine arylamidase, trypsin, chymotrypsin, α-galactosidase, β-glucuronidase, α-mannosidase, and α-fucosidase are negative; weak reaction for valine arylamidase.

The peptidoglycan contains ornithine as the diagnostic amino acid. Cell-wall acyl type is glycolyl. The menaquinone profile is predominantly MK-13, with small amounts of MK-14 and MK-12. Major cellular fatty acids are $C_{15:0}$ anteiso, $C_{17:0}$ anteiso, and $C_{16:0}$ iso. Cell-wall sugars are rhamnose, galactose, and xylose.

The type strain was isolated by adding superoxide dismutase and catalase to the medium to scavenge oxygen radicals.

Source: the type strain was isolated from soil from Aoyama Cemetery, Tokyo, Japan.

DNA G+C content (mol%): 69 (HPLC; type strain).

Type strain: KV-492, NBRC 101280, NRRL B-24451.

Sequence accession no. (16S rRNA gene): AB234028.

5. **Microbacterium aquimaris** Kim, Lee, Oh and Lee 2008a, 1619[VP]

a.qui.ma′ris. L. n. *aqua* water; L. n. *mare -is* sea; N.L. gen. n. *aquimaris* of seawater.

Cells are nonmotile, short rods, 0.6–0.8 × 1.0–1.5 µm in size. Yellowish, translucent, and circular colonies with entire edges (1–2 mm in diameter) are formed in 3 d culture on TSA at 30°C. Aerobic. Catalase and oxidase are positive. Growth occurs at 15–37°C, pH 6–10, and in up to 10% (w/v) NaCl. Indole and H_2S are not produced. Nitrate is not reduced to nitrite. Esculin is hydrolyzed but starch, gelatin, and urea are not. β-Galactosidase is produced but arginine dihydrolase, ornithine dehydroxylase, and lysine decarboxylase are not. Voges–Proskauer test is positive but methyl red test is negative.

In the API 50 CH gallery, acid is produced from esculin, arbutin, cellobiose, glucose, maltose, mannitol, mannose, and salicin and weakly from fructose, galactose, gluconate, glycerol, 5-ketogluconate, methyl β-D-xylose, ribose, and turanose, but not from *N*-acetylglucosamine, adonitol, amygdalin, D- and L-arabinose, D- and L-arabitol, dulcitol, erythritol, D- and L-fucose, gentiobiose, glycogen, inositol, inulin, 2-ketogluconate, lactose, D-lyxose, melezitose, melibiose, methyl α-D-glucoside, methyl α-D-mannoside, raffinose, sorbitol, sorbose, starch, D-tagatose, trehalose, xylitol, or L-xylose. 5-Ketogluconate is utilized as a sole carbon source, but acetate, *N*-acetylglucosamine, adipate, L-alanine, L-arabinose, caprate, citrate, L-fucose, gluconate, D-glucose, glycogen, histidine, 3-hydroxybenzoate, 4-hydroxybenzoate, 3-hydroxybutyrate, inositol, itaconate, 2-ketogluconate, DL-lactate, malate, malonate, maltose, mannitol, mannose, melibiose, phenylacetate, L-proline, propionate, rhamnose, D-ribose, salicin, L-serine, D-sorbitol, suberate, sucrose, and valerate are not.

The peptidoglycan contains ornithine and conforms to type B2β: [L-Hsr]–D-Glu(Hyg)–Gly–D-Orn (Figure 174). The cell-wall acyl type is glycolyl. The cell-wall sugars are glucose and galactose. The menaquinone is composed of predominantly MK-11, with MK-12 and MK-10. Major cellular fatty acids are $C_{15:0}$ anteiso, $C_{17:0}$ anteiso, and $C_{16:0}$ iso. The polar lipids consist of diphosphatidylglycerol, phosphatidylglycerol, and an unknown glycolipid.

Source: the type strain was isolated from seawater at Jeju, Korea.

DNA G+C content (mol%): 69.3–69.9 (HPLC), 69.3 (type strain).

Type strain: JS54-2, DSM 19713, JCM 15625, KCTC 19124.

Sequence accession no. (16S rRNA gene): AM778449.

6. **Microbacterium arabinogalactanolyticum** (Yokota, Takeuchi, Sakane and Weiss 1993a) Takeuchi and Hatano 1998b, 745[VP] (*Aureobacterium arabinogalactanolyticum* Yokota, Takeuchi, Sakane and Weiss 1993a, 560)

a.ra.bi.no.ga.lac.ta.no.ly′ti.cum. N.L. n. *arabinogalactanum* arabinogalactan, polysaccharide produced by the bacterium *Mycobacterium tuberculosis*; N.L. neut. adj. *lyticum* (from Gr. neut. adj. *lutikon*) able to loosen, able to dissolve; N.L. neut. adj. *arabinogalactanolyticum* arabinogalactan dissolving.

The following characteristics are based mainly on the data of Yokota et al. (1993a).

Cells are nonmotile rods arranged at angles; V forms are observed but no branching. Colonies are 1–3 mm in diameter, circular, low convex with entire margins, opaque, and moist. No pigment is produced. Aerobic. Optimum temperature for growth is 28°C. Growth occurs in the presence of 2% NaCl. Oxidative. Gelatin, starch, and Tweens 60 and 80 are hydrolyzed but Tweens 20 and 40 are not. Nitrate is not reduced. Voges–Proskauer test is negative. Positive for methyl red test, urease, and arginine decarboxylase. Positive for production of H_2S. Acetate, malate, succinate, oxalate, fumarate, DL-lactate, propionate, and hippurate are assimilated. Formate and citrate are assimilated weakly.

Cell-wall peptidoglycan contains ornithine, homoserine, glutamic acid (and hydroxyglutamic acid), glycine, and alanine, conforming to type B2β (Figure 174). A small amount of lysine is also detected. Cell-wall acyl type is glycolyl. Cell-wall sugar is galactose. Major menaquinones are MK-12 and MK-13. Major polar lipids are diphosphatidylglycerol, phosphatidylglycerol, an unidentified glycolipid, and an unidentified phosphoglycolipid. Cellular fatty acids are predominantly $C_{15:0}$ anteiso and $C_{17:0}$ anteiso.

Type strain produces α-D-arabinofuranosidase (Kotani et al., 1972; Misaki et al., 1977).

Source: the type strain was isolated from soil (Kotani et al., 1972).

DNA G+C content (mol%): 69.3 (HPLC; type strain).

Type strain: ATCC 51926, CIP 103814, DSM 8611, JCM 9171, LMG 16469, NBRC 14344, VKM Ac-1943.

Sequence accession no. (16S rRNA gene): Y17228.

7. **Microbacterium arborescens** (*ex* Frankland and Frankland 1889) Imai, Takeuchi and Banno 1985, 535[VP] (Effective publication: Imai, Takeuchi and Banno 1984, 283.) ["*Flavobacterium arborescens*" (Frankland and Frankland 1889) Bergey, Harrison, Breed, Hammer and Huntoon 1923]

ar.bo.res′cens. L. part. adj. *arborescens* becoming a tree.

Motile rods, 0.5 by 2.5 µm (Breed et al., 1957). Growth on nutrient agar is poor; addition of 0.5% brain heart infusion (BHI) gives good growth. Color of colonies is orange. Aerobic. Good growth is shown at 28–37°C. Gelatin is hydrolyzed but Tweens 20, 40, and 80 are not. Hydrolysis of Tween 60 is weak. Production of H_2S is positive. Nitrate is not reduced. Voges–Proskauer test is negative. Arginine dihydrolase-negative. Acid is produced from D-xylose, sucrose, L-arabinose, and trehalose (weak reaction), but not from raffinose or inulin (Imai et al., 1984; Yokota et al., 1993b). Acetate and fumarate are assimilated but lactate, malate, and propionate are not (Yokota et al., 1993b).

Cell-wall peptidoglycan contains lysine as the diamino acid. The molar ratio of amino acids, alanine, glycine, lysine, glutamic acid with 3-hydroxyglutamic acid, and homoserine indicates that the peptidoglycan structure is type B1β (Figure 174). Cell-wall acyl type is glycolyl. Cell-wall sugars are mannose, galactose, and 6-deoxytalose. Major menaquinones are MK-11 and MK-12. Major polar lipids are diphosphatidylglycerol, phosphatidylglycerol, and unidentified glycolipids (Yokota et al., 1993b). Cellular fatty acids are predominantly $C_{15:0}$ anteiso, $C_{16:0}$ iso, and $C_{17:0}$ anteiso (Yokota et al., 1993b).

Source: isolated from river and lake water (Breed et al., 1957).

DNA G+C content (mol%): 71 (type strain; Weeks, 1974), 71.0 (HPLC; Yokota et al., 1993b).

Type strain: ATCC 4358, CIP 55.81, DSM 20754, HAMBI 1892, JCM 5884, LMG 4009, NBRC 3750, VKM Ac-1944.

Sequence accession no. (16S rRNA gene): X77443.

8. **Microbacterium aurantiacum** Takeuchi and Hatano 1998a, 978[VP]

au.ran.ti′a.cum. N.L. n. *aurantium* specific name of the orange; L. neut. suff. *-acum* adjectival suffix used with the sense of belonging to; N.L. neut. adj. *aurantiacum* orange-colored.

Cells are nonmotile, irregular, short rods, 0.2–0.5 × 0.6–2.0 µm in size, arranged at angles; V forms but no branching or rod–coccus cycle are observed. Colonies are 2–4 mm in diameter, orange-pigmented, circular, low convex with entire margins, opaque, and moist. Growth occurs at 37°C (optimal at 28°C). Grows in 5% NaCl but weak growth is observed in 6.5% NaCl. Starch, and Tweens 20, 40, 60, and 80 are hydrolyzed but gelatin is not. H_2S is produced. Methyl red and Voges–Proskauer tests are negative. Arginine dihydrolase is not produced. Acid is produced from mannose and sucrose; acid production is variable from glucose and xylose and negative from L-arabinose, galactose, inulin, melezitose, raffinose, rhamnose, ribose, and trehalose. The following compounds are assimilated: maltose, mannose, mannitol, fumarate, gluconate, and propionate. Assimilation of arabinose, lactate, and malate is variable. *N*-Acetylglucosamine, acetate, adipate, caprate, citrate, and phenylacetate are not assimilated.

Cell-wall peptidoglycan contains ornithine, homoserine, glutamic acid (and hydroxyglutamic acid), glycine, and alanine corresponding to peptidoglycan type B2β (Figure 174). Cell-wall sugars are rhamnose, galactose, and fucose. Major menaquinone is MK-12. Major cellular fatty acids are $C_{15:0}$ anteiso, $C_{17:0}$ anteiso, $C_{15:0}$ iso, and $C_{16:0}$ iso.

Source: the type strain was isolated from sewage.

DNA G+C content (mol%): 70.1–70.3 (HPLC).

Type strain: ATCC 49090, CIP 105730, DSM 12506, JCM 9177, NCFB 2288, NBRC 15234, VKM Ac-2075.

Sequence accession no. (16S rRNA gene): AB004726.

9. **Microbacterium aurum** Yokota, Takeuchi and Weiss 1993b, 552[VP]

au′rum. L. n. *aurum* gold.

Cells are nonmotile, small, slender rods arranged at angles; V forms are observed but no branching. In older cultures, rods become shorter but no rod–coccus cycle is shown. Colonies are 1–2 mm in diameter, golden yellow-pigmented, circular, low convex with entire margins, opaque, and moist. Aerobic. Optimum temperature for growth is approximately 30°C. Esculin, starch, and Tween 60 are hydrolyzed but gelatin and Tweens 20, 40 and 80 are not. Urease-negative. H_2S is produced. Voges–Proskauer test is negative. Arginine is not decarboxylated. Acid is produced weakly from glucose, mannose, fructose, maltose, cellobiose, mannitol, galactose, L-rhamnose, sucrose, trehalose, raffinose, starch, and inulin but not from D-xylose, L-arabinose, D-arabinose, glycerol, arbutin, esculin, salicin, ethanol, butanol, melibiose, melezitose, ribose, L-sorbose, xylitol, erythritol, adonitol, dulcitol, sorbitol, inositol, methanol, or methyl α-D-glucoside. Acetate, 2-oxoglutarate, and succinate are assimilated but pyruvate, lactate, malate, maleate, formate, butyrate, oxalate, pimelate, glycolate, glyoxylate, gluconate, propionate, and hippurate are not.

Cell-wall peptidoglycan contains lysine as the diamino acid and conforms to type B1β (Figure 174). Cell-wall acyl type is glycolyl. Cell-wall sugars are fucose, galactose, and glucose. Major menaquinones are MK-11 and MK-12. The polar lipids are diphosphatidylglycerol, phosphatidylglycerol, and dimannosyldiacylglycerol. Cellular fatty acids are predominantly $C_{15:0}$ anteiso, $C_{17:0}$ anteiso, and $C_{16:0}$ iso.

Source: the type strain was isolated from corn steep liquor.

DNA G+C content (mol%): 69.2 (HPLC; type strain).

Type strain: H-5, ATCC 51345, CIP 103994, DSM 8600, JCM 9179, NBRC 15204, VKM Ac-1950.

Sequence accession no. (16S rRNA gene): Y17229.

10. **Microbacterium awajiense** Kageyama, Matsuo, Kasai, Shizuri, Ōmura and Takahashi 2008b, 2471[VP] (Effective publication: Kageyama, Matsuo, Kasai, Shizuri, Ōmura and Takahashi, 2008a, 4.)

awa.ji.en′se. N.L. neut. adj. *awajiense* of or pertaining to Awaji Island, Hyogo, Japan, where the strain was isolated.

Cells are irregular rods, 0.5–0.8 × 0.7–1.2 µm in size. Nonmotile. Colony color is light yellow. Catalase-positive, aerobic. Grows at pH 6–11 and 13–38°C. Grows in up to 5% NaCl. D-Galactose, D-glucose, maltose, D-mannitol, D-mannose, raffinose, L-rhamnose, trehalose, and xylose are assimilated. Sucrose is not assimilated. Alkaline phosphatase, esterase (C4), esterase, lipase (C8), lipase (C14), leucine arylamidase, valine arylamidase, cystine arylamidase, chymotrypsin, acid phosphatase, naphthol-AS-BI-phosphohydrolase, α-glucosidase, β-glucosidase, *N*-acetyl-β-glucosaminidase, and α-fucosidase are detected by the API ZYM enzyme assay; trypsin, α-galactosidase, β-glucuronidase, and α-mannosidase are negative. β-Galactosidase reaction is weak.

The peptidoglycan contains ornithine as the diagnostic amino acid. Cell-wall acyl type is glycolyl. The major menaquinones are MK-12, MK-13, and MK-14. Predominant cellular fatty acids are $C_{15:0}$ anteiso and $C_{17:0}$ anteiso, followed by $C_{16:0}$ iso and $C_{15:0}$ iso.

Source: the type strain was isolated from sediment collected from the shore of Yura, Awaji Island, Japan.

DNA G+C content (mol%): 70 (HPLC) for type strain.

Type strain: YM13-414, DSM 18907, MBIC08276, NBRC 103565.

Sequence accession no. (16S rRNA gene): AB286027.

11. **Microbacterium azadirachtae** Madhaiyan, Poonguzhali, Lee, Lee, Saravanan and Santhanakrishnan 2010, 1690[VP]

a.za.di.rach′ta.e. N.L. n. *Azadirachta* a botanical genus name; N.L. gen. n. *azadirachtae* of *Azadirachta* isolated from *Azadirachta indica*.

Cells are motile, short rods, approximately 0.3–0.4 µm wide and 0.6–0.7 µm long, occurring singly or in pairs. Colonies are 1–2 mm in diameter, smooth, circular, and light-yellow after 48 h at 28°C on R2A agar. Growth is observed at 0–5.5% NaCl, pH 5.0–10.0, and 10–37°C, with optimal growth at 0–1% NaCl, pH 7.0–8.0, and 28°C.

Strictly aerobic. Catalase-positive and oxidase-negative. Nitrate is not reduced to nitrite. Esculin, casein, and tributyrin are hydrolyzed. Starch, gelatin, and urea are not hydrolyzed. No growth on MacConkey agar. Tolerates up to 1 mM $NiCl_2$ and 0.5 mM $CdCl_2$. The type strain tolerates bacitracin, cephalosporin, nalidixic acid, and polymyxin B (all at 500 µg/ml). Acid is produced from glucose and rhamnose. The following carbon substrates are oxidized (Biolog GP2 MicroPlates): β-cyclodextrin, dextrin, Tween 40, Tween 80, *N*-acetyl-D-galactosamine, *N*-acetyl-β-D-mannosamine, amygdalin, L-arabinose, D-arabitol, arbutin, cellobiose, D-fructose, L-fucose, D-galactose, gentiobiose, D-gluconic acid, α-D-glucose, *myo*-inositol, maltose, maltotriose, D-mannitol, D-mannose, melezitose, methyl β-D-glucoside, palatinose, D-psicose, L-rhamnose, salicin, D-sorbitol, sucrose, D-tagatose, trehalose, turanose, acetic acid, β-hydroxybutyric acid, γ-hydroxybutyric acid, *p*-hydroxyphenylacetic acid, α-ketobutyric acid, D-lactic acid methyl ester, L-lactic acid, D-malic acid, L-malic acid, pyruvic acid methyl ester, pyruvic acid, succinamic acid, succinic acid, *N*-acetyl-L-glutamic acid, L-alaninamide, D-alanine, L-alanine, L-alanyl glycine, L-asparagine, L-glutamic acid, glycyl L-glutamic acid, L-serine, putrescine, glycerol, adenosine, 2′-deoxyadenosine, inosine, thymidine, uridine, and adenosine-5′-monophosphate. Negative for assimilation of α-cyclodextrin, glycogen, inulin, mannan, D-galacturonic acid, lactose, lactulose, melibiose, methyl α-D-galactoside, methyl β-D-galactoside, 3-methyl-D-glucose, methyl α-D-glucoside, methyl α-D-mannoside, raffinose, D-ribose, sedoheptulosan, stachyose, xylitol, D-xylose, α-hydroxybutyric acid, α-ketovaleric acid, lactamide, succinic acid monomethyl ester, propionic acid, L-pyroglutamic acid, 2,3-butanediol, thymidine-5′-monophosphate, uridine-5′-monophosphate, D-fructose 6-phosphate, α-D-glucose 1-phosphate, D-glucose 6-phosphate, and DL-α-glycerol phosphate.

The cell-wall peptidoglycan is of type B2β containing ornithine (Figure 174). Cell-wall acyl type is glycolyl. The predominant quinones are MK-12 and MK-13. Major fatty acids are $C_{15:0}$ anteiso, $C_{17:0}$ anteiso, and $C_{16:0}$ iso.

The type strain shows plant-growth-promoting traits including indole-3-acetic acid production, phosphate-solubilization, 1-aminocyclopropane-1-carboxylate deaminase activity, and sulfur oxidation in plate assays, but is negative for siderophore production (Madhaiyan et al., 2010).

Source: the type strain was isolated from the rhizoplane of neem seedlings at Tamilnadu Agricultural University (TNAU), Coimbatore, Tamilnadu, India.

DNA G+C content (mol%): 69.5 (HPLC; type strain).

Type strain: AI-S262, JCM 15681, KCTC 19668, LMG 24772.

Sequence accession no. (16S rRNA gene): EU912487.

12. **Microbacterium barkeri** (Collins, Jones, Keddie, Kroppenstedt and Schleifer 1983a) Takeuchi and Hatano 1998b, 745[VP] (*Aureobacterium barkeri* Collins, Jones, Keddie, Kroppenstedt and Schleifer 1983a, 249; "*Corynebacterium barkeri*" Dias, Bilimoria and Bhat 1962, 66)

bar′ker.i. N.L. gen. masc. n. *barkeri* of Barker; named for H.A. Barker, an American biochemist.

The following characteristics are based mainly on Schleifer and Kandler (1972), Collins et al. (1983a), Suzuki and Komagata (1983), and Yokota et al. (1993a).

Cells are motile, small, slender rods, arranged at angles; V forms are observed in young cultures but no branching. In older cultures, rods become shorter but no rod–coccus cycle is seen. Good growth is observed on nutrient agar. Colonies are 1–3 mm in diameter, low convex, circular with entire margins, shiny, and moist. Colonies are yellow in color. Obligately aerobic. Catalase-positive. Optimum temperature for growth is 30°C. Esculin, starch, hippurate, and Tweens 20, 40 and 60 are hydrolyzed. Tween 80 is not hydrolyzed. Urease-negative. Arginine dihydrolase is positive. Nitrate is reduced. H_2S is produced. Voges–Proskauer test is negative but methyl red test is positive. Tellurite (0.05%) is reduced. Acid is produced slowly and weakly from glucose oxidatively. Acetate, malate, succinate, and fumarate are assimilated. Propionate and hippurate are assimilated weakly. Formate, citrate, DL-lactate, and oxalate are not assimilated.

Cell-wall peptidoglycan contains D-ornithine as the diamino acid and conforms to type B2β: [L-Hsr]–D-Glu–Gly–D-Orn (Figure 174). Cell-wall acyl type is glycolyl. Cell-wall sugars are rhamnose, galactose, and glucose. Major menaquinones are MK-11 and MK-12. The polar lipids consist of diphosphatidylglycerol, phosphatidylglycerol, and a diglycosyldiacylglycerol. Cellular fatty acids are predominantly $C_{15:0}$ anteiso and $C_{17:0}$ anteiso.

Source: the type strain was isolated from raw domestic sewage.

DNA G+C content (mol%): 68.7 (HPLC; type strain).

Type strain: ATCC 15954, CCM 1928, CCUG 33090, BCRC 11642, CIP 102692, DSM 20145, HAMBI 1894, IAM 12585, IMET 10688, JCM 1343, LMG 16341, NBRC 15036, NCIMB 9658, VKM Ac-1020.

Sequence accession no. (16S rRNA gene): X77446.

13. **Microbacterium binotii** Clermont, Diard, Bouchier, Vivier, Bimet, Motreff, Welker, Kallow and Bizet 2009, 1020[VP]

bi.no′ti.i. N.L. gen. masc. n. *binotii* of Binot, named in honor of Jean Binot (1867–1909), who created the Collection de l'Institut Pasteur.

Aerobic, nonmotile, short rods. Colonies are yellow, circular, and 1–2 mm in diameter after 48 h culture on

trypto-casein-soy agar at 30°C. Catalase-positive and oxidase-negative. Growth occurs at 15–37°C and in 0–6% NaCl. Nitrate is not reduced to nitrite. Starch is decomposed but gelatin and casein are not. Voges–Proskauer and indole production tests are negative. Positive for esterase, acid phosphatase, α- and β-galactosidases, *N*-acetyl-β-glucosaminidase, and α- and β-glucosidases. Negative for ornithine decarboxylase, arginine dihydrolase, lysine decarboxylase, urease, trypsin, β-glucuronidase, α-mannosidase, and α-fucosidase. Acid is produced from glycerol, L-arabinose, D-xylose, galactose, D-glucose, D-fructose, D-mannose, mannitol, arbutin, esculin, salicin, cellobiose, maltose, lactose, sucrose, trehalose, starch, β-gentiobiose, turanose, and gluconate.

The diamino acid of peptidoglycan is ornithine associated with alanine, glycine, homoserine, glutamic acid, and hydroxyglutamic acid, and conforms to type B2β. The cell-wall sugars are galactose, mannose, and rhamnose. The predominant menaquinones are MK-10, MK-11, and MK-12. Main polar lipids are diphosphatidylglycerol, phosphatidylglycerol, an unknown glycolipid, and an unknown phospholipid. Major cellular fatty acid is $C_{17:0}$ anteiso; $C_{15:0}$ anteiso and $C_{16:0}$ iso are also found.

Source: the type strain was isolated from human blood.

DNA G+C content (mol%): 70–72 (HPLC), 71.7 (type strain).

Type strain: CIP 101303, DSM 19164, JCM 16365.

Sequence accession no. (16S rRNA gene): EF567306.

Further comments: CIP 102116 is a reference strain.

14. **Microbacterium chocolatum** Takeuchi and Hatano 1998a, 979[VP]

cho.co.la′tum. N.L. neut. adj. *chocolatum* chocolate-colored, derived from the Mexican *chocolatl*, chocolate.

Cells are nonmotile, irregular, short rods, 0.2–0.5 × 0.6–2.0 µm in size, arranged at angles; V forms but no branching or rod–coccus cycle are observed. Colonies are 2–4 mm in diameter, orange or dull orange in color, circular, low convex with entire margins, opaque, and moist. Growth occurs at 37°C (optimal at 28°C). Weak growth occurs in 2% NaCl but not in 5% NaCl. Starch, and Tweens 20, 40, 60, and 80 are hydrolyzed but gelatin is not. H_2S is produced. Methyl red and Voges–Proskauer tests are negative. Arginine dihydrolase is not produced. Acid is produced from mannose, sucrose, and xylose but not from L-arabinose, galactose, glucose, inulin, melezitose, raffinose, rhamnose, ribose, or trehalose. Maltose and gluconate are assimilated; mannose and mannitol are weakly assimilated; and arabinose, *N*-acetylglucosamine, acetate, adipate, caprate, citrate, fumarate, lactate, malate, phenylacetate, and propionate are not assimilated.

Cell-wall peptidoglycan contains ornithine, homoserine, glutamic acid (and hydroxyglutamic acid), glycine, and alanine, corresponding to peptidoglycan type B2β (Figure 174). Cell-wall sugars are rhamnose and galactose, with small proportion of mannose and xylose. Major menaquinone is MK-12. Cellular fatty acids are composed of $C_{17:0}$ anteiso, $C_{15:0}$ anteiso, and $C_{16:0}$ iso.

Source: the type strain was isolated from culture contamination.

DNA G+C content (mol%): 69.5 (HPLC).

Type strain: BUCSAV 207, CIP 105729, DSM 12507, JCM 12412, NCIMB 8181, NBRC 3758, VKM Ac-2078.

Sequence accession no. (16S rRNA gene): AB004725.

Further comments: The type strain was originally isolated by M.H. Knutsen and named "*Chromobacterium chocolatum*" Knutsen (1944) (Clise, 1948), although the name does not appear in the Approved Lists of Bacterial Names.

15. **Microbacterium deminutum** Kageyama, Takahashi and Ōmura 2006, 2115[VP]

de.mi.nu′tum. L. part. neut. adj. *deminutum* diminutive.

Cells are nonmotile, irregular rods, 0.3–0.7 × 0.5–0.9 µm. Colony color is pale yellow. Aerobic and catalase-positive. Growth occurs at pH 6–9 and 17–31°C. Tolerates up to 4% NaCl on 5× diluted nutrient agar medium. D-Glucose, D-galactose, maltose, D-mannose, L-rhamnose, and sucrose are assimilated, but arabinose, fructose, mannitol, raffinose, trehalose, and xylose are not. Esterase (C4), esterase lipase (C8), leucine arylamidase, valine arylamidase, cystine arylamidase, trypsin, chymotrypsin, acid phosphatase, naphthol-AS-BI-phosphohydrolase, α-galactosidase, β-galactosidase, β-glucuronidase, α-glucosidase, β-glucosidase, and *N*-acetyl-β-glucosamidase are detected with the API ZYM enzyme assay; α-fucosidase is not detected. Reactions for alkaline phosphatase, lipase (C14), and α-mannosidase are weak.

The peptidoglycan contains ornithine as the diagnostic amino acid. Cell-wall acyl type is glycolyl. Cell-wall sugars are rhamnose, fucose, galactose, glucose, and xylose. The quinone system contains predominantly menaquinone MK-13, with moderate amounts of MK-12 and MK-14. Major cellular fatty acids are $C_{17:0}$ anteiso and $C_{15:0}$ anteiso.

The type strain was isolated by adding superoxide dismutase and catalase to the culture medium to scavenge oxygen radicals.

Source: the type strain was isolated from soil from Aoyama Cemetery, Tokyo, Japan.

DNA G+C content (mol%): 69 (HPLC; type strain).

Type strain: KV-483, NBRC 101278, NRRL B-24453.

Sequence accession no. (16S rRNA gene): AB234026.

16. **Microbacterium dextranolyticum** Yokota, Takeuchi and Weiss 1993b, 551[VP]

dex.tra.no.ly′ti.cum. N.L. n. *dextranum* dextran, polysaccharide produced by bacteria; N.L. neut. adj. *lyticum* (from Gr. neut. adj. *lutikon*) able to loosen, able to dissolve; N.L. neut. adj. *dextranolyticum* dextran dissolving.

Cells are nonmotile, small, slender rods arranged at angles; V forms are observed but no branching. In older cultures, rods become shorter but no rod–coccus cycle is seen. Colonies are 2–4 mm in diameter, yellow-pigmented, circular, low convex with entire margins, opaque, and moist. Aerobic. Optimum temperature for growth is 30°C. Esculin, and Tweens 20, 40, 60, and 80 are hydrolyzed but gelatin and starch are not. Urease-negative. H_2S production and Voges–Proskauer test are positive. Arginine is not decarboxylated. Acid is produced weakly from glucose, mannose, fructose, maltose, cellobiose, mannitol, xylose, L-arabinose, galactose, sucrose, lactose, trehalose, raffinose, inulin, arbutin, esculin, ethanol, butanol, melibiose, and melezitose but not from ribose, D-arabinose, L-sorbose, xylitol, erythritol,

adonitol, dulcitol, sorbitol, inositol, methanol, methyl α-D-glucoside, L-rhamnose, starch, glycerol, or salicin. Pyruvate, fumarate, and succinate are assimilated but acetate, lactate, malate, 2-oxoglutarate, maleate, formate, butyrate, oxalate, pimelate, glycolate, glyoxylate, gluconate, propionate, and hippurate are not.

Cell-wall peptidoglycan contains L-lysine as the diamino acid, conforming to type B1α (Figure 174). Cell-wall acyl type is glycolyl. Cell-wall sugars are rhamnose, 6-deoxytalose, galactose, and glucose. Major menaquinones are MK-11 and MK-12. The polar lipids are diphosphatidylglycerol, phosphatidylglycerol, and dimannosyldiacylglycerol. In addition, an unknown monoglycosyldiacylglycerol and phosphoglycolipid are also present. Cellular fatty acids are predominantly $C_{15:0}$ anteiso and $C_{16:0}$ iso.

The type strain is known to produce a dextran-α-1,2-debranching enzyme (Kobayashi et al., 1978; Mitsuishi et al., 1979).

Source: the type strain was isolated from soil.

DNA G+C content (mol%): 68.3 (HPLC; type strain).

Type strain: M-73, ATCC 51344, CCUG 38517, CIP 103993, DSM 8607, JCM 9180, NBRC 14592, NRRL B-23242, VKM Ac-1940.

Sequence accession no. (16S rRNA gene): AB007417.

17. **Microbacterium esteraromaticum** (Omelianski 1923) Takeuchi and Hatano 1998b, 745[VP] ("*Bacterium esteraromaticum*" Omelianski 1923; *Flavobacterium esteraromaticum* Bergey, Harrison, Breed, Hammer and Huntoon 1930, 150; *Aureobacterium esteraromaticum* Yokota, Takeuchi, Sakane and Weiss 1993a, 560)

es.ter.a.ro.ma′ti.cum. N.L. n. *ester* ester; L. neut. adj. *aromaticum* aromatic, fragrant; N.L. neut. adj. *esteraromaticum* smelling sweet due to esters.

The following characteristics are based mainly on the data of Yokota et al. (1993a).

Cells are rods arranged at angles; V forms are observed but no branching. Motile with lateral flagella. Colonies are 1–3 mm in diameter, yellow-pigmented, circular, low convex with entire margins, opaque, and moist. Aerobic. Optimum temperature for growth is 28°C. No growth occurs in the presence of 2% NaCl. Oxidative. Starch, and Tweens 40, 60, and 80 are hydrolyzed but hydrolysis of gelatin and Tween 20 is variable depending on strain. Nitrate is not reduced. Methyl red and Voges–Proskauer tests are negative. Urease-negative. Positive for production of H_2S. Arginine decarboxylase-negative. Acetate, malate, succinate, oxalate (weak reaction), fumarate, DL-lactate, propionate, and hippurate are assimilated but formate and citrate are not.

Cell-wall peptidoglycan contains ornithine, homoserine, glutamic acid (and hydroxyglutamic acid), glycine, and alanine, conforming to type B2β (Figure 174). Cell-wall acyl type is glycolyl. Cell-wall sugars are galactose and glucose. Major menaquinones are MK-12 and MK-13. The polar lipids are diphosphatidylglycerol and phosphatidylglycerol. An unidentified glycolipid is also present. Cellular fatty acids are predominantly $C_{15:0}$ anteiso and $C_{17:0}$ anteiso.

Source: the type strain was isolated from soil (Omelianski, 1923).

DNA G+C content (mol%): 69 (T_m) (Weeks, 1974), 68.8 (HPLC; type strain).

Type strain: ATCC 8091, CIP 103916, DSM 8609, JCM 9172, LMG 4020, NBRC 3751, VKM Ac-1942.

Sequence accession no. (16S rRNA gene): Y17231.

18. **Microbacterium flavescens** (Lochhead 1958) Takeuchi and Hatano 1998b, 745[VP] (*Arthrobacter flavescens* Lochhead 1958, 170; *Aureobacterium flavescens* Collins, Jones, Keddie, Kroppenstedt and Schleifer 1983a, 247)

fla.ves′cens. L. v. *flavesco* to become golden yellow; L. part. adj. *flavescens* becoming yellow.

The following characteristics are based mainly on Lochhead (1958), Collins et al. (1983a), and Yokota et al. (1993a).

Small, irregular, nonmotile rods. Cells in young cultures are 0.4–0.6 × 1.0–6.0 µm in size, arranged at angles; V forms are observed but no branching. In older cultures, coccoid forms, 0.5 µm in diameter, are observed but no rod–coccus cycle is seen. Nutritionally exacting; terregens factor, biotin, and thiamine are required for growth. Colonies are 0.2–0.5 mm in diameter, low convex, and circular with entire edge. A yellow non-diffusible pigment is produced. Obligately aerobic. Catalase-positive. Oxidase is weakly positive. Optimum temperature for growth is 25°C. Growth occurs at 10°C but not at 37°C. Soil extract is required for growth. Gelatin, starch, and DNA are hydrolyzed. Tweens 20, 40, 60, and 80 are hydrolyzed. Urease-negative. Arginine is not decarboxylated. Nitrate is reduced. H_2S is produced. The terregens factor may be replaced by other siderophores such as coprogen and ferrichrome. When supplied with terregens factor, vitamins, and glucose as carbon plus energy source, ammonium salts or nitrates are utilized as nitrogen source. Acid is produced slowly from D-glucose, galactose, and few other sugars. Acetate is assimilated. Succinate, fumarate, DL-lactate, propionate, and hippurate are assimilated weakly. Formate, citrate, malate, and oxalate are not assimilated.

Cell-wall peptidoglycan contains D-ornithine as the diamino acid and L-homoserine, conforming to type B2β (Figure 174). Cell-wall acyl type is glycolyl. Cell-wall sugars are rhamnose, galactose, and glucose. Major menaquinones are MK-13 and MK-14. The polar lipids consist of diphosphatidylglycerol, phosphatidylglycerol, and diglycosyldiacylglycerol. Cellular fatty acids are predominantly $C_{15:0}$ anteiso, $C_{17:0}$ anteiso, and $C_{16:0}$ iso.

Source: the type strain was isolated from soil.

DNA G+C content (mol%): 66.9 (HPLC; type strain).

Type strain: ATCC 13348, CCUG 29000, CIP 102401, DSM 20643, HAMBI 1909, JCM 11568, JCM 3877, LMG 3028, NCIMB 9221, NBRC 15039, VKM Ac-1415.

Sequence accession no. (16S rRNA gene): Y17232.

19. **Microbacterium flavum** Kageyama, Takahashi, Matsuo, Adachi, Kasai, Shizuri and Ōmura 2008c, 529[VP] (Effective publication: Kageyama, Takahashi, Matsuo, Adachi, Kasai, Shizuri and Ōmura 2007a, 57.)

fla′vum. L. neut. adj. *flavum* yellow.

Cells are nonmotile, irregular rods, 0.5–0.8 × 0.7–1.1 µm. Colony color is lemon yellow. Aerobic. Growth occurs at pH

5–11 and 5–37°C. Tolerates up to 7% NaCl on 5× diluted nutrient agar medium. D-Glucose, fructose, D-galactose, maltose, D-mannose, raffinose, L-rhamnose, sucrose (strong reaction), and trehalose are assimilated, but arabinose, mannitol, and xylose are not. Esterase (C4), esterase lipase (C8), leucine arylamidase, valine arylamidase, cystine arylamidase, trypsin, acid phosphatase, naphthol-AS-BI-phosphohydrolase, β-galactosidase, α-glucosidase, *N*-acetyl-β-glucosaminidase are detected by the API ZYM enzyme assay; α-galactosidase, β-glucuronidase, α-mannosidase, and α-fucosidase are negative. Reactions for alkaline phosphatase, lipase (C14), chymotrypsin, and β-glucosidase are weak.

The cell-wall peptidoglycan contains lysine as the diagnostic amino acid. Cell-wall acyl type is glycolyl. The major menaquinones are MK-11 and MK-12. Predominant cellular fatty acids are $C_{15:0}$ anteiso, $C_{17:0}$ anteiso, and $C_{16:0}$ iso.

Source: the type strain was isolated from the ascidian *Didemnum moseleyi* collected from near the Nomazaki Peninsula, Nagasaki, Japan.

DNA G+C content (mol%): 70 (HPLC; type strain).

Type strain: YM18-098, DSM 18909, JCM 15574, MBIC08278, NBRC 103923.

Sequence accession no. (16S rRNA gene): AB286029.

20. **Microbacterium fluvii** Kageyama, Matsuo, Kasai, Shizuri, Ōmura and Takahashi 2008b, 2471[VP] (Effective publication: Kageyama, Matsuo, Kasai, Shizuri, Ōmura and Takahashi, 2008a, 4.)

flu'vi.i. L. gen. n. *fluvii* of a river.

Cells are irregular rods, 0.4–0.6 × 0.6–1.2 µm. Nonmotile. Colony color is pale yellow. Catalase-positive, aerobic. Grows at pH 6–11 and 16–36°C. Grows in up to 3% NaCl. D-Galactose, D-glucose, maltose, D-mannitol, D-mannose, and L-rhamnose are assimilated. Raffinose, sucrose, and xylose are not assimilated. Esterase (C4), esterase lipase (C8), lipase (C14), leucine arylamidase, valine arylamidase, cystine arylamidase, trypsin, chymotrypsin, acid phosphatase, naphthol-AS-BI-phosphohydrolase, β-glucosidase, and *N*-acetyl-β-glucosaminidase are detected by the API ZYM enzyme assay; α-galactosidase, β-glucuronidase, α-glucosidase, α-mannosidase, and α-fucosidase are negative. Weak reaction for alkaline phosphatase and β-galactosidase.

The peptidoglycan contains ornithine as the diagnostic amino acid. Cell-wall acyl type is glycolyl. The major menaquinones are MK-11 and MK-12. Predominant cellular fatty acids are $C_{15:0}$ anteiso, $C_{17:0}$ anteiso, and $C_{16:0}$ iso.

Source: the type strain was isolated from driftwood collected at the estuary of Maera River in Iriomote Island, Japan.

DNA G+C content (mol%): 70 (HPLC; type strain).

Type strain: YSL3-15, DSM 18908, MBIC08277, NBRC 105003.

Sequence accession no. (16S rRNA gene): AB286028.

21. **Microbacterium foliorum** Behrendt, Ulrich and Schumann 2001, 1273[VP]

fo.li.o'rum. L. pl. gen. neut. n. *foliorum* of the leaves.

Cells are irregularly shaped rods. In older cultures, rods are shorter, but a marked rod–coccus cycle does not occur. Motile by means of a single polar or lateral flagellum. Colonies are yellow, shiny, slightly convex, and round with entire margins. Strictly aerobic. Optimum growth temperature is approximately 25°C. Growth occurs in the presence of 2% (w/v) NaCl. Catalase-positive and oxidase-negative. Nitrate is not reduced to nitrite. β-Galactosidase is positive. Esculin, DNA, and gelatin are hydrolyzed. Urease, arginine dihydrolase, Voges–Proskauer test, H_2S production, and indole production are negative. Starch is weakly utilized. Casein and Tween 80 are not hydrolyzed. Hydrolysis of Tween 60 is strain-dependent. Acid is produced from amygdalin, L-arabinose, arbutin, cellobiose, D-fructose, galactose, glycerol, maltose, mannitol, D-mannose, salicin, sucrose, trehalose, and D-xylose but not from adonitol, D-arabitol, dulcitol, D-fucose, erythritol, gluconate, 2-ketogluconate, 5-ketogluconate, inositol, L-sorbose, D-tagatose, xylitol, or L-xylose. Acid production from D-arabinose, methyl α-D-glucoside, L-fucose, β-gentiobiose, glycogen, inulin, lactose, methyl α-D-mannoside, melibiose, melezitose, raffinose, rhamnose, ribose, sorbitol, starch, and methyl β-xyloside is variable between strains. Negative for the fermentative and oxidative production of acid from glucose by the method of Hugh and Leifson (1953) but positive for oxidative acid production by using API 20NE and API50CH.

The cell-wall peptidoglycan is type B2β containing ornithine, [L-Hsr]–D-Glu–Gly–D-Orn. The cell-wall acyl type is glycolyl. The cell-wall sugars are galactose, rhamnose, and mannose. The major menaquinones are MK-12, MK-11, and MK-10. Cellular fatty acids contain $C_{15:0}$ anteiso, $C_{17:0}$ anteiso, and $C_{16:0}$ iso.

Source: strains are isolated from the phyllosphere of grasses and from the litter layer after mulching of the sward. The type strain was isolated from phyllosphere of grasses.

DNA G+C content (mol%): 67 (HPLC; type strain).

Type strain: P 333/02, CIP 107137, DSM 12966, JCM 11569, LMG 19580, NBRC 103072.

Sequence accession no. (16S rRNA gene): AJ249780.

22. **Microbacterium ginsengisoli** Park, Kim, Kim, Im, Yi, Kim, Soung and Yang 2008, 430[VP]

gin.seng.i.so'li. N.L. n. *ginsengum* ginseng; L. n. *solum* soil; N.L. gen. n. *ginsengisoli* of soil of a ginseng field, the source of type strain.

Cells are short rods, 0.3–0.4 × 0.6–0.8 µm in size. Nonmotile. Colonies are yellow, smooth, circular, convex, and transparent after 2 d cultivation on R2A agar. Aerobic. Catalase and oxidase are positive. Growth occurs at 18–30°C, at pH 5–9, and in up to 4% (w/v) NaCl. Nitrate is not reduced to nitrite. Esculin and DNA are hydrolyzed but starch, xylan, gelatin, chitin, cellulose, and skim milk are not. Urease and arginine dihydrolase are absent. In the API 50CH tests, acid is produced from D-fructose, cellobiose, D-mannose, L-rhamnose, sucrose, trehalose, turanose, D-xylose, glycerol, and mannitol. Acid is not produced from gluconate, 2-ketogluconate, 5-ketogluconate, D- and L-arabinose, D- and L-fucose, galactose, gentiobiose, lactose, D-lyxose, melezitose, melibiose, raffinose, ribose, sorbose, D-tagatose, L-xylose, methyl α-D-glucoside, methyl α-D-mannoside, methyl β-D-xyloside, adonitol, D- and L-arabitol, dulcitol, erythritol, inositol, sorbitol, xylitol, *N*-acetylglucosamine, starch, amygdalin,

arbutin, inulin, salicin, or glycogen. The carbon sources utilized in the API 20NE and API 32GN systems are D-glucose, maltose, D-mannose, rhamnose, sucrose, inositol, mannitol, L-alanine, L-proline, L-serine, 3-hydroxybutyrate, acetate, gluconate, malate, propionate, and valerate. Negative for utilization of the following substrates: L-arabinose, L-fucose, melibiose, D-ribose, D-sorbitol, L-histidine, *N*-acetylglucosamine, salicin, glycogen, 2-ketogluconate, 5-ketogluconate, 3-hydroxybenzoate, 4-hydroxybenzoate, adipate, caprate, citrate, itaconate, D- and L-lactate, malonate, phenylacetate, and suberate. In API ZYM assays, esterase (C4), esterase lipase (C8), leucine arylamidase, valine arylamidase, trypsin, acid phosphatase, β-galactosidase, α-glucosidase, β-glucosidase, and *N*-acetyl-β-glucosaminidase activities are present, but alkaline phosphatase, lipase (C14), cystine arylamidase, α-chymotrypsin, naphthol-AS-BI-phosphohydrolase, α-galactosidase, β-glucuronidase, α-mannosidase, and α-fucosidase are absent.

The peptidoglycan contains ornithine. The cell-wall sugar is rhamnose. The predominant menaquinone is MK-12, followed by MK-11. Major cellular fatty acids are $C_{17:0}$ anteiso, $C_{15:0}$ anteiso, and $C_{16:0}$ iso.

Source: the type strain was isolated from soil from a ginseng field in Pocheon in the Republic of Korea.

DNA G+C content (mol%): 69.4 (HPLC; type strain).

Type strain: Gsoil 259, DSM 18659, JCM 15304, KCTC 19189.

Sequence accession no. (16S rRNA gene): AB271048.

23. **Microbacterium ginsengiterrae** Kim, Kim, Bui, Kim, Srinivasan and Yang 2010, 2811[VP]

gin.seng.i.ter′ra.e. N.L. n. *ginsengum* ginseng; L. n. *terra* soil; N.L. gen. n. *ginsengiterrae* of soil of a ginseng field, the source of the type strain.

Cells are nonmotile and rod-shaped, 0.5–0.7 µm in diameter and 1.5–2.0 µm in length. Colonies are circular with regular edges, yellow, and translucent with a diameter of 2–3 mm after 2 d incubation on R2A agar. Strictly aerobic. Grows at 15–45°C and pH 5–9, with optimal growth at 30°C and pH 7.0. Slightly halophilic. Grows poorly in the absence of NaCl and at concentrations greater than 8% NaCl. Catalase, oxidase, and DNase are positive. In the API ZYM kit, α-glucosidase, β-glucosidase, esterase (C4), esterase lipase (C8), naphthol-AS-BI-phosphohydrolase, and α-mannosidase activities are positive, whereas alkaline phosphatase, lipase (C14), leucine arylamidase, valine arylamidase, cystine arylamidase, trypsin, α-chymotrypsin, acid phosphatase, α-galactosidase, β-galactosidase, β-glucuronidase, *N*-acetyl-β-glucosaminidase, and α-fucosidase activities are negative. In the API 20NE kit, arginine dihydrolase and urease reactions are positive, but the following reactions are negative: reduction of nitrate to nitrite, reduction of nitrate to nitrogen, indole production, protease, β-galactosidase, glucose acidification, and assimilation of L-arabinose, D-mannitol, *N*-acetylglucosamine, maltose, potassium gluconate, capric acid, adipic acid, malic acid, trisodium citrate, and phenylacetic acid. In the API 32GN kit, the following substrates are assimilated: D-ribose, L-rhamnose, sucrose, 3-hydroxybenzoic acid, and 4-hydroxybenzoic acid. The following substrates are not assimilated: glucose, inositol, itaconic acid, suberic acid, sodium malonate, sodium acetate, L-alanine, glycogen, L-serine, salicin, melibiose, L-fucose, D-sorbitol, propionic acid, capric acid, valeric acid, L-histidine, 3-hydroxybutyric acid, and L-proline.

The cell-wall peptidoglycan is of type B2β: (L-Hsr)–D-Glu–Gly–D-Orn. Major whole-cell sugars are ribose, xylose, and galactose. The predominant quinones are MK-12 and MK-13. The polar lipids contain one unknown glycolipid and aminolipids, in addition to diphosphatidylglycerol and phosphatidylglycerol. Major fatty acids are $C_{15:0}$ anteiso, $C_{17:0}$ anteiso, and $C_{16:0}$ iso.

Source: the type strain was isolated from soil of a ginseng field in the Republic of Korea.

DNA G+C content (mol%): 64.5 (HPLC; type strain).

Type strain: DCY37, JCM 15516, KCTC 19526.

Sequence accession no. (16S rRNA gene): EU873314.

24. **Microbacterium gubbeenense** Brennan, Brown, Goodfellow, Ward, Beresford, Vancanneyt, Cogan and Fox 2001, 1974[VP]

gub.be.en.en′se. N.L. neut. adj. *gubbeenense* of or belonging to Gubbeen House, County Cork, Ireland, where the cheese containing the organism is produced.

Cells are small, slender rods, 0.5 to 2.0 µm in length, showing typical coryneform morphology with cells arranged at angles to V-shapes. No primary branching or rod–coccus cycle are observed. Colonies on peptone-yeast extract agar are irregular, cream, matt, slightly raised, and 1–5 mm in diameter.

Facultatively anaerobic. Catalase-positive and oxidase-negative. Growth occurs at 30°C but not at 37°C. Tolerates up to 12% (w/v) NaCl. Nitrate is reduced to nitrite. Starch, gelatin, urea, and Tweens 20, 40, 60, and 80 are not hydrolyzed. H_2S production, methyl red test, and Voges–Proskauer test are negative. Indole is not produced. β-Galactosidase is produced, but arginine dihydrolase is not. Acid is produced from arabinose, mannose, glucose, galactose, trehalose, and lactose and not from rhamnose, melezitose, xylose, inulin, raffinose, or ribose. Gluconate, mannose, malate, citrate, propionate, and fumarate are assimilated but adipate, caprate, phenylacetate, acetate, and lactate are not. The organism produces alkaline phosphatase, esterase (2-naphthyl butyrate as substrate), esterase lipase (2-naphthyl caprylate as substrate), leucine arylamidase (L-leucine 2-naphthylamide as substrate), acid phosphatase, naphthol-AS-BI-phosphohydrolase, α-galactosidase, β-galactosidase, α-glucosidase, β-glucosidase, and *N*-acetylglucosaminidase. The organism does not produce lipase (2-naphthyl myristate as substrate), valine arylamidase, cystine arylamidase, trypsin, β-glucuronidase, chymotrypsin, α-mannosidase, α-fucosidase, or arginine dihydrolase.

The peptidoglycan contains lysine, conforming to type B1. The cell-wall acyl type is glycolyl. The cell-wall sugars are rhamnose and galactose. The menaquinone is composed of MK-11 and MK-12. The presence of phosphatidylglycerol mannosides in the polar lipids of this species, in addition to diphosphatidylglycerol and phosphatidylinositol, has been reported (Brennan et al., 2001). Major cellular fatty acids are $C_{15:0}$ anteiso, $C_{17:0}$ anteiso, and $C_{16:0}$ iso.

Source: the type strain was isolated from surface of Gubbeen, Irish farmhouse smear-ripened cheese.

DNA G+C content (mol%): 69–75 (HPLC).

Type strain: DPC 5286, JCM 12075, LMG S-19263, NBRC 103073, NCIMB 30129.

Sequence accession no. (16S rRNA gene): AF263563.

Further comments: phosphatidylglycerol mannosides are reported in the polar lipid profile of this species in addition to diphosphatidylglycerol and phosphatidylinositol. There are no other species of the genus *Microbacterium* that have a similar polar lipid profile. If the identification of these lipids is correct and not originating from a contaminant, this is the distinctive characteristic of this species.

25. **Microbacterium halophilum** Takeuchi and Hatano 1998a, 980[VP]

ha.lo′phi.lum. Gr. n. *hals halos* salt; N.L. neut. adj. *philum* (from Gr. neut. adj. *philon*), friend, loving; N.L. neut. adj. *halophilum* salt-loving.

Cells are nonmotile, irregular short rods, 0.2–0.5 × 0.6–2.0 μm in size, arranged at angles; V forms but no branching or rod–coccus cycle are observed. Colonies are 2–4 mm in diameter, yellowish white in color, circular, low convex with entire margins, opaque, and moist. Growth occurs at 37°C (optimal at 28°C). Good growth is observed in 2 to 6.5% NaCl. Starch, and Tweens 40, 60, and 80 are hydrolyzed and gelatin and Tween 20 are weakly hydrolyzed. Methyl red test is positive. Voges–Proskauer test, production of H_2S, and arginine dihydrolase are negative. Acid is produced from glucose, mannose, and rhamnose but not from L-arabinose, galactose, inulin, melezitose, raffinose, ribose, sucrose, trehalose, or xylose. Maltose, mannose, mannitol, and gluconate are assimilated whereas arabinose, *N*-acetylglucosamine, acetate, adipate, caprate, citrate, fumarate, lactate, malate, phenylacetate, and propionate are not.

Cell-wall peptidoglycan contains ornithine, homoserine, glutamic acid (and hydroxyglutamic acid), glycine, and alanine, corresponding to peptidoglycan type B2β (Schleifer and Kandler, 1972). Cell-wall sugars are mannose, galactose, and glucose. Major menaquinones are MK-11, MK-12, and MK-13. Cellular fatty acid profile is composed of almost equal amounts of $C_{15:0}$ anteiso, $C_{16:0}$ iso, and $C_{15:0}$ iso, followed by $C_{17:0}$ anteiso and $C_{17:0}$ iso.

Source: the type strain was isolated from mangrove rhizosphere soil.

DNA G+C content (mol%): 67.2 (HPLC; type strain).

Type strain: No. 76, DSM 12508, JCM 12077, NBRC 16062, VKM Ac-2080.

Sequence accession no. (16S rRNA gene): AB004714.

26. **Microbacterium halotolerans** Li, Chen, Kim, Park, Tang, Lee, Xu and Jiang 2005a, 69[VP]

ha.lo.to′le.rans. Gr. n. *hals halos* salt; L. part. adj. *tolerans* tolerating; N.L. part. adj. *halotolerans* salt-tolerating, referring to the ability of the organism to tolerate high salt concentrations.

Cells are nonmotile, short rods. Colonies are white, circular, convex with entire margins, shiny, moist, and 1–2 mm in diameter after 2–3 d incubation. Grows optimally at 28–30°C and pH 7.0–8.0. Tolerant to salts; grows in 0–15% NaCl, 0–20% KCl, and 0–30% $MgCl_2 \cdot H_2O$, with optimal growth at 5% NaCl, 5–10% KCl, and 10% $MgCl_2 \cdot H_2O$. Catalase-positive and oxidase-negative. Tweens 20 and 80, gelatin, casein, and starch are not decomposed. Voges–Proskauer test, H_2S production, and indole production are negative. Nitrate is not reduced to nitrite. Acid is produced from glucose, mannitol, mannose, ribose, fructose, maltose, and xylose. Uses the following compounds as sole carbon and energy sources: glucose, mannitol, mannose, ribose, fructose, sucrose, maltose, galactose, arabinose, starch, cellobiose, lactose, and xylose. Amygdalin, salicin, dextrin, galacturonate, 5-ketogluconate, L-arabitol, trehalose, rhamnose, inositol, sorbitol, malonate, and adonitol cannot be assimilated. Positive for lipase and β-galactosidase. Negative for ornithine decarboxylase, arginine dihydrolase, lysine decarboxylase, urease, α-galactosidase, *N*-acetyl-β-glucosaminidase, β-glucuronidase, and L-aspartic arylamidase. In the original description, the species is described as being both positive and negative for α-maltosidase and β-glucosidase.

Cell-wall peptidoglycan is type B2β: [Gly]–L-Hsr–D-Glu(Hyg)–Gly–D-Orn. The acyl type is glycolyl. Cell-wall sugars are ribose, galactose, glucose, and trace amounts of xylose. Predominant menaquinones are MK-10 and MK-11. Major cellular fatty acids are $C_{15:0}$ anteiso, $C_{17:0}$ anteiso, and $C_{16:0}$ iso.

Source: the type strain was isolated from saline soil collected from Qinghai Province in the west China.

DNA G+C content (mol%): 63.8 (T_m; type strain) [66.5 mol% is reported by Li et al. (2005a)].

Type strain: YIM 70130, CIP 108071, JCM 13013, KCTC 19017.

Sequence accession no. (16S rRNA gene): AY376165.

27. **Microbacterium hatanonis** Bakir, Kudo and Benno 2008, 656[VP]

ha.ta.no′nis. N.L. gen. masc. n. *hatanonis* of Hatano, in honor of Dr Kazunori Hatano, for his contribution to the understanding of the genus *Microbacterium*.

Cells are nonmotile rods, approximately 0.8 μm wide and 1.7 μm long. Colonies are light yellow, circular, convex with entire margins, moist, and shiny. Aerobic. Catalase-positive and oxidase-negative. Growth occurs at 10–37°C and pH 6.0–9.0, with optimal growth at 30°C and pH 7.0. No growth occurs at 2% NaCl; optimum growth is seen in the absence of NaCl. Nitrate is not reduced. Arginine dihydrolase, urease, gelatin hydrolysis, indole production, and D-glucose fermentation are negative. Esculin hydrolysis and β-galactosidase are positive. Arabinose, D-Glucose, D-mannose, mannitol, maltose, and potassium gluconate are assimilated. Malate is assimilated weakly. *N*-Acetylglucosamine, capric acid, adipic acid, trisodium citrate, and phenylacetic acid are not utilized. In the API ZYM test system, positive for the production of esterase, esterase lipase, leucine arylamidase, valine arylamidase, cystine arylamidase, trypsin, acid phosphatase, naphthol-AS-BI-phosphohydrolase, α-galactosidase, β-glucuronidase, α-glucosidase, β-glucosidase, and α-fucosidase; negative for alkaline phosphatase, lipase, α-chymotrypsin, and α-mannosidase.

The cell-wall peptidoglycan contains ornithine as the diagnostic diamino acid. Cell-wall sugars are rhamnose and galactose. The major menaquinones are MK-12 and MK-11,

with moderate amounts of MK-10. Predominant cellular fatty acids are $C_{15:0}$ anteiso, $C_{17:0}$ anteiso, and $C_{16:0}$ iso.

Source: the type strain was isolated from a contaminant of hairspray.

DNA G+C content (mol%): 69.0 (HPLC; type strain).

Type strain: FCC-01, DSM 19179, JCM 14558.

Sequence accession no. (16S rRNA gene): AB274908.

28. **Microbacterium hominis** Takeuchi and Hatano 1998a, 980[VP]

ho′mi.nis. L. masc. n. *homo -inis* man; L. gen. masc. n. *hominis* of man.

Cells are nonmotile, irregular, short rods, 0.2–0.5 × 0.6–2.0 µm, arranged at angles; V forms but no branching or rod–coccus cycle are observed. Colonies are 2–4 mm in diameter, yellowish white in color, circular, low convex with entire margins, opaque, and moist. Growth occurs at 37°C (optimal growth at 28°C). Good growth is observed in the presence of 2% NaCl but only weak growth is observed at 5 and 6.5% NaCl. H_2S is formed. Methyl red and Voges–Proskauer tests are positive. Tweens 20, 40, 60, and 80 are hydrolyzed but starch and gelatin are not. Acid is produced from L-arabinose, galactose, glucose, mannose, melezitose, sucrose, and trehalose but not from inulin, raffinose, rhamnose, ribose, or xylose. Arabinose, maltose, mannose, mannitol, *N*-acetylglucosamine, acetate, citrate, fumarate, gluconate, lactate, malate, and propionate are assimilated but not adipate, caprate, or phenylacetate.

Cell-wall peptidoglycan contains lysine, glutamic acid (and hydroxyglutamic acid), glycine, and alanine. Cell-wall sugars are rhamnose, 6-deoxy-L-talose, galactose, and mannose. Major menaquinones are MK-11 and MK-12. Cellular fatty acids are composed of $C_{15:0}$ anteiso, $C_{17:0}$ anteiso, and $C_{16:0}$ iso.

Source: the type strain was isolated from lung aspirate.

DNA G+C content (mol%): 71.2 (HPLC; type strain).

Type strain: CIP 105731, DSM 12509, JCM 12413, LCDC 84-209, NBRC 15708, VKM Ac-2081.

Sequence accession no. (16S rRNA gene): AM181504.

29. **Microbacterium humi** Young, Busse, Langer, Chu, Schumann, Arun, Shen, Rekha and Kämpfer 2010, 859[VP]

hu′mi. L. gen. n. *humi* of earth, soil, of soil, the source of the type strain.

Cells are short rods, about 1–1.2 µm in width and 2–3 µm in length. Aerobic. Catalase and oxidase are positive. Good growth occurs on nutrient agar and R2A agar (Oxoid) at 25–30°C and yellow colonies appear in 3 d.

Acid is produced from D-mannitol and sucrose. Acid is produced weakly from glucose and lactose but not from adonitol, L-arabinose, cellobiose, D-mannose, D-xylose, D-arabitol, dulcitol, erythritol, maltose, melibiose, methyl D-glucoside, raffinose, rhamnose, salicin, sorbitol, or trehalose. Esculin, pNP-α-D-glucopyranoside, pNP-β-D-glucopyranoside, oNP-β-D-galactopyranoside, pNP-β-D-glucuronide, pNP-β-D-xylopyranoside, bis-pNP-phosphate, pNP-phenylphosphate, and L-alanine-pNA are hydrolyzed but pNP-phosphoryl-choline, 2-deoxythymidine-5′-pNP-phosphate, L-glutamate-γ-3-carboxy-pNA, and L-proline-pNA are not hydrolyzed. Cellobiose, D-fructose, D-glucose, DL-lactate, D-mannose, maltose, D-mannitol, L-proline, sucrose, trehalose, *N*-acetyl-D-glucosamine, L-arabinose, D-galactose, α-melibiose, L-rhamnose, D-ribose, D-xylose, maltitol, D-sorbitol, fumarate, L-malate, oxoglutarate, pyruvate, and L-aspartate are assimilated but *N*-acetyl-D-galactosamine, *p*-arbutin, gluconate, salicin, adonitol, i-inositol, putrescine, acetate, propionate, *cis*-aconitate, citrate, *trans*-aconitate, adipate, 4-aminobutyrate, azelate, glutarate, DL-3-hydroxybutyrate, itaconate, L-leucine, mesaconate, phenylacetate, L-phenylalanine, suberate, L-tryptophan, L-alanine, β-alanine, L-histidine, L-ornithine, L-serine, 3-hydroxybenzoate, and 4-hydroxybenzoate are not assimilated.

The cell-wall peptidoglycan is of type B2α with an interpeptide bridge D-Glu–Gly–D-Orn. The predominant quinones are MK-11 and MK-10, with MK-12 as a minor component. The polar lipid profile is composed of diphosphatidylglycerol, phosphatidylglycerol, and one unknown glycolipid. Major fatty acids are $C_{15:0}$ anteiso, $C_{16:0}$ iso, and $C_{17:0}$ anteiso. Predominant polyamines are spermidine and spermine.

Source: isolated from the stalk of the edible mushroom *Agaricus blazei* cultivated in the laboratory.

DNA G+C content (mol%): not known.

Type strain: CC-12309, CCM 7687, DSM 21799.

Sequence accession no. (16S rRNA gene): FJ865215.

30. **Microbacterium hydrocarbonoxydans** Schippers, Bosecker, Spröer and Schumann 2005, 657[VP]

hy.dro.car.bon.ox′y.dans. N.L. n. *hydrocarbonum* hydrocarbon; N.L. part. adj. *oxydans* oxidizing; N.L. part. adj. *hydrocarbonoxydans* oxidizing hydrocarbons.

Cells are motile, irregular rods, 0.3–1.5 µm in size. Colonies are circular, smooth, translucent, and yellow-pigmented with a maximum diameter of 5 mm after 2 weeks of cultivation. Obligately aerobic. Growth occurs at 30–37°C and in 2–4% NaCl. Catalase-positive and oxidase-negative. Arginine is not hydrolyzed. Urease-negative. H_2S is not produced and the Voges–Proskauer reaction is negative. Acid is produced from rhamnose, sucrose, and xylose but not from D-glucose, adonitol, inositol, or sorbitol. The following compounds are utilized as a sole carbon sources: *N*-acetyl-D-glucosamine, L-arabinose, cellobiose, D-fructose, D-galactose, gluconate, D-glucose, glycogen, maltose, D-mannose, L-rhamnose, D-ribose, sucrose, salicin, trehalose, L-xylose, D-mannitol, acetate, propionate, citrate, fumarate, DL-lactate, L-malate, pyruvate, L-alanine, L-aspartate, L-histidine, L-hydroxyproline, L-proline, L-serine, putrescine, phenylacetate, and *N*-acetyl-D-galactosamine. α-D-Galacturonate, α-D-melibiose, adonitol, i-inositol, sorbitol, *trans*-aconitate, adipate, DL-3-hydroxybutyrate, suberate, 3-hydroxybenzoate, 4-hydroxybenzoate, and L-ornithine are not utilized. The following are hydrolyzed: pNP-*N*-acetyl-β-D-galactosaminide, pNP-*N*-acetyl-β-D-glucosaminide, pNP-α-L-arabinopyranoside, pNP-β-D-cellopyranoside, pNP-β-D-galactopyranoside, pNP-α-D-glucopyranoside, pNP-β-D-glucopyranoside, pNP-β-D-lactoside, pNP-α-D-mannoside, pNP-α-D-maltoside, pNP-β-D-xyloside, bis-pNP-phosphate, benzolphosphonacid-pNP ester, pNP-phosphoryl-choline, 2-deoxythymidine-5′-pNP-phosphate, L-alanine-pNA, glycine-pNA, L-leucine-pNA, L-lysine-pNA, and L-proline-pNA.

The following are not hydrolyzed: pNP-β-D-glucuronide, γ-L-glutamate-pNA, L-glutamate-γ-3-carboxy-pNA, and L-valine-pNA. Crude oil is used as a substrate for growth.

The cell-wall peptidoglycan contains L-ornithine as the diagnostic diamino acid, conforming to type B2β: [L-HSr]–D-Glu(Hyg)–Gly–D-Orn. The predominant menaquinones are MK-11 and MK-12.

The type strain was isolated by enrichment with medium containing 1–5% crude oil.

Source: the type strain was isolated from oil-contaminated soil in Germany.

Type strain: BNP48, DSM 16089, JCM 14340, NBRC 103074, NCIMB 14002.

Sequence accession no. (16S rRNA gene): AJ698726.

31. **Microbacterium imperiale** (Steinhaus 1941) Collins, Jones and Kroppenstedt 1983b, 672[VP] (Effective publication: Collins, Jones and Kroppenstedt 1983c, 75.) ("*Bacterium imperiale*" Steinhaus 1941, 777; *Brevibacterium imperiale* Breed 1953, 13)

im.pe.ri.a′le. L. neut. adj. *imperiale* imperial, from the specific epithet of name of insect host (*Eacles imperialis* Dru).

The following characteristics are based mainly on Steinhaus (1941), Schleifer and Kandler (1972), Yamada and Komagata (1972b), Jones (1975), Collins et al. (1983c), and Yokota et al. (1993b).

Cells are small, slender rods, arranged at angles; V forms are observed but no branching. In older culture, rods become shorter but no rod–coccus cycle is seen. Very weak motility is observed. A few cells may be motile by means of one (Steinhaus, 1941), two, or three (Jones, 1975) flagella. Colonies are 1–4 mm in diameter, circular, low-convex with entire margins, opaque, and moist. Mucoid growth occurs on solid media containing glucose or lactose. Reddish orange pigment is produced. Aerobic and weak anaerobic growth is shown (Jones, 1975). Catalase-positive. Oxidase-negative. Optimum temperature for growth is 25–30°C. Growth occurs at 10°C and 37°C, but not at 45°C. Does not survive heating at 60°C for 30 min. Hydrolysis of starch and gelatin is negative or weakly positive. Tweens 40 and 60 are weakly hydrolyzed, whereas Tweens 20 and 80 are not hydrolyzed. Urease-negative. Arginine is not decarboxylated. Acid is produced from L-arabinose, glucose, fructose, mannose, galactose, sucrose, trehalose, cellobiose, and esculin, but not from rhamnose, sorbose, maltose, starch, inulin, glycerol, erythritol, adonitol, mannitol, dulcitol, sorbitol, inositol, arbutin, or methyl α-glucoside. Slow, weak acid production from lactose, xylose, salicin, raffinose, and dextrin is observed. The following organic acids are assimilated: acetate, pyruvate, L-lactate, D-lactate, malate, succinate, fumarate, 2-oxoglutarate, citrate, and propionate. The following organic acids are not assimilated: formate, butyrate, oxalate, malonate, glutarate, adipate, pimelate, glycolate, glyoxylate, gluconante, hippurate, and uriate.

Cell-wall peptidoglycan contains L-lysine as the diamino acid, conforming to type B1β (Figure 174). Cell-wall acyl type is glycolyl. Cell-wall sugars are rhamnose, mannose, and galactose. Major menaquinones are MK-12 and MK-11. The polar lipids consist of diphosphatidylglycerol, phosphatidylglycerol, and dimannosyldiacylglycerol. Cellular fatty acids are predominantly $C_{15:0}$ anteiso and $C_{17:0}$ anteiso.

Source: the type strain was isolated from alimentary tract of the imperial moth *Eacles imperialis*.

DNA G+C content (mol%): 71–75.4 (T_m) (Yokota et al., 1993b).

Type strain: ATCC 8365, CIP 82.108, DSM 20530, HAMBI 117, HAMBI 1891, JCM 1378, NCIMB 9888, NBRC 12610, VKM Ac-1447.

Sequence accession no. (16S rRNA gene): X77442.

32. **Microbacterium indicum** Shivaji, Bhadra, Rao, Chaturvedi, Pindi and Raghukumar 2007, 1821[VP]

in.di′cum. L. neut. adj. *indicum* pertaining to India.

Cells are rod-shaped. Colonies are circular and pale yellow with an entire margin on yeast extract-peptone agar after 2 d incubation at 22°C. Growth occurs between 8°C and 30°C but not at 5°C or 37°C. Growth occurs between pH 5 and 9. Tolerates up to 6% (w/v) NaCl at pH 6.8. Catalase- and oxidase-negative. Negative for Voges–Proskauer reaction, indole production, and methyl red test. Ornithine decarboxylase, lysine decarboxylase, phenylalanine deaminase, and urease are also negative. Esculin is hydrolyzed. Nitrate is reduced. Acid is produced from D-glucose, fructose, L-arabinose, D-arabinose, D-xylose, cellobiose, maltose, trehalose, melezitose, and sucrose but not from D-galactose, L-rhamnose, mannose, or sorbitol. The following compounds are used as sole carbon sources: D-glucose, D-fructose, D-galactose, L-arabinose, cellobiose, lactose, maltose, sucrose, acetate, L-serine, L-arginine, L-lysine, D-xylose, L-xylose, raffinose, D-ribose, melezitose, inositol, mannitol, malonate, and L-asparagine but not L-rhamnose, mannose, D-glucuronate, citrate, or L-alanine. Sensitive to amikacin (30 μg), cefuroxime (30 μg), rifampin (25 μg), bacitracin (10 μg), roxithromycin (30 μg), tobramycin (15 μg), gentamicin (30 μg), erythromycin (15 μg), ciprofloxacin (30 μg), chloramphenicol (30 μg), penicillin (10 μg), and kanamycin (30 μg), but resistant to ampicillin (25 μg) and nalidixic acid (30 μg).

The cell-wall peptidoglycan contains D-ornithine as the diagnostic amino acid and has possible structure B2β (Figure 174). Whole-cell sugars are rhamnose, galactose, and glucose. The menaquinones are MK-11 and MK-12. Predominant cellular fatty acids are $C_{16:0}$ iso, $C_{15:0}$ anteiso, $C_{16:0}$, and $C_{17:0}$ anteiso. In addition to phosphatidylglycerol, the presence of phosphatidylinositol was reported by Shivaji et al. (2007) in the polar lipid profile of this species. These authors also reported the same result in *Microbacterium barkeri*, which had not been reported previously. However, the presence of phosphatidylinositol is an uncommon trait among species of this genus.

Source: the type strain was isolated from deep-sea sediment of the Chagos Trench at a depth of 5904 m.

DNA G+C content (mol%): 65.5 (T_m; type strain).

Type strain: BBH6, IAM 15355, JCM 21800, LMG 23459.

Sequence accession no. (16S rRNA gene): AM158907.

33. **Microbacterium insulae** Yoon, Schumann, Kang, Lee, Lee and Oh 2009, 1741[VP]

in.su′la.e. L. fem. gen. n. *insulae* of an island, where the type strain was isolated.

Cells are nonmotile rods to cocci (0.2–0.5 × 0.5–1.8 µm). Colonies are yellow, circular, convex, smooth, glistening, and 1.5–2.0 mm in diameter after 7 d culture at 30°C on TSA. Grows at 20–38°C, at pH 5.5–8.0, and in up to 5% (w/v) NaCl; optimal growth is at 30°C, at pH 6.5 and 7.0, and in 0.5–1.0% NaCl. Anaerobic growth does not occur on TSA or on TSA supplemented with nitrate. Catalase-negative and oxidase-positive. Negative reaction for H_2S and indole production and hydrolysis of urea, casein, hypoxanthine, tyrosine, and xanthine. Positive for hydrolysis of gelatin, esculin, starch, and Tweens 20, 40, 60, and 80. Utilizes glycerol, L-arabinose, D-xylose, galactose, glucose, fructose, mannose, mannitol, rhamnose, amygdalin, esculin, salicin, cellobiose, maltose, lactose, sucrose, trehalose, melezitose, starch, gentiobiose, turanose, methyl α-D-glucoside, and gluconate; utilization of glycogen is weak; negative for utilization of D-arabinose, D-ribose, methyl β-D-xyloside, inositol, *N*-acetylglucosamine, arbutin, melibiose, raffinose, D-lyxose, L-fucose, 2-ketogluconate, 5-ketogluconate, citrate, malate, phenylacetate, erythritol, L-xylose, adonitol, sorbose, dulcitol, sorbitol, methyl α-D-mannoside, inulin, xylitol, D-tagatose, D-fucose, D-arabitol, L-arabitol, caprate, and adipate. Negative for arginine dihydrolase, lysine decarboxylase, ornithine decarboxylase, and tryptophan deaminase activities.

In the API ZYM test, positive for leucine arylamidase, β-glucosidase, α-glucosidase, and *N*-acetyl-β-glucosaminidase, weakly positive for esterase (C4) and esterase lipase (C8), but negative for acid phosphatase, alkaline phosphatase, lipase (C14), valine arylamidase, cystine arylamidase, trypsin, α-chymotrypsin, naphthol-AS-BI-phosphohydrolase, α-galactosidase, β-galactosidase, α-mannosidase, β-glucuronidase, and α-fucosidase. Susceptible to ampicillin, carbenicillin, cephalothin, chloramphenicol, lincomycin, novobiocin, oleandomycin, penicillin G, streptomycin, and tetracycline but not to gentamicin, kanamycin, neomycin, or polymyxin B.

Cell-wall peptidoglycan is type B2β: [L-HSr]–D-Glu(Hyg)–Gly–D-Orn. The acyl type is glycolyl. Cell-wall sugars are galactose, ribose, and rhamnose. Predominant menaquinone is MK-13; MK-12 and MK-14 are present in moderate amounts. Major polar lipids are diphosphatidylglycerol, phosphatidylglycerol, an unidentified phospholipid, and an unidentified glycolipid. Major fatty acids are $C_{15:0}$ anteiso, $C_{17:0}$ anteiso, $C_{17:0}$ iso, and $C_{16:0}$ iso.

Source: the type strain was isolated from soil of Dokdo, Korea.

DNA G+C content (mol%): 69.9 (T_m; type strain).

Type strain: DS-66, CCUG 54523, KCTC 19247.

Sequence accession no. (16S rRNA gene): EU239498.

34. **Microbacterium invictum** Vaz-Moreira, Lopes, Faria, Spröer, Schumann, Nunes and Manaia 2009, 2039[VP]

in.vic′tum. L. neut. adj. *invictum* invincible, powerful, that survives composting, also an ancient and popular designation attributed to Oporto city.

Cells are motile, short rods (0.5–0.6 µm long by 0.7–0.8 µm). Colonies are white and convex in 2-d-old cultures, turning yellowish in 4–6 d. Grows at 10–37°C, at pH 6.0–9.5, and in up to 5% NaCl. Catalase-positive and oxidase-negative. Nitrate is reduced to nitrite. Citrate is not used and H_2S and indole are not produced. Anaerobic growth is observed in the presence of nitrate. Voges–Proskauer test is positive. Gelatin, esculin, starch (weakly), and ε-poly-caprolactone (weakly) are hydrolyzed. Acid is produced from *N*-acetylglucosamine, amygdalin, L-arabinose, arbutin, cellobiose, D-fructose, D-glucose, D-mannitol, D-mannose, L-rhamnose, and salicin, with weak reactions observed for D-galactose, D-sorbitol, and D-xylose, but not from D-adonitol, D-arabinose, D- and L-arabitol, dulcitol, erythritol, D- and L-fucose, gentiobiose, gluconate, 2- and 5-ketogluconate, glycerol, glycogen, inositol, inulin, lactose, D-lyxose, maltose, melezitose, melibiose, raffinose, D-ribose, L-sorbose, starch, sucrose, D-tagatose, trehalose, turanose, xylitol, L-xylose, methyl α-D-glucopyranoside, methyl α-D-mannopyranoside, or methyl β-D-xylopyranoside. The following compounds are oxidized (API 20E) and assimilated: L-arabinose, D-glucose, D-mannitol, L-rhamnose, and D-sorbitol. In addition, assimilates *N*-acetylglucosamine, cellobiose, D-fructose, gentiobiose, potassium gluconate, potassium 2-ketogluconate, maltose, D-mannose, turanose, xylitol (weakly), and D-xylose, but not phenylacetate, D-adonitol, adipate, esculin, amygdalin, D-arabinose, D- and L-arabitol, caprate, citrate, dulcitol, erythritol, D- and L-fucose, D-galactose, potassium 5-ketogluconate, glycerol, glycogen, inositol, inulin, lactose, D-lyxose, malate, melezitose, melibiose, raffinose, D-ribose, salicin, L-sorbose, starch, sucrose, D-tagatose, trehalose, L-xylose, methyl α-D-glucopyranoside, methyl α-D-mannopyranoside, or methyl β-D-xylopyranoside. Glucose fermentation is weak and slow. Alkaline phosphatase, esterase (C4), esterase lipase (C8), leucine arylamidase, valine arylamidase, acid phosphatase, naphthol-AS-BI-phosphohydrolase, β-galactosidase, β-glucuronidase, α- and β-glucosidase, and *N*-acetyl β-glucosamidase are produced, but not arginine dihydrolase, lysine and ornithine decarboxylases, urease, tryptophan deaminase, lipase (C14), cystine arylamidase, trypsin, α-chymotrypsin, α-galactosidase, α-mannosidase, or α-fucosidase. Growth occurs in the presence of (µg/l) sulfamethoxazole/trimethoprim (23.75/1.25), meropenem (10), and ceftazidime (30).

Cell-wall peptidoglycan type is B1α containing lysine, glycine, alanine, and glutamic acid but not homoserine or hydroxyglutamic acid. The acyl type is glycolyl. Predominant menaquinones are MK-11 and MK-12 (approx. 40% each). Major fatty acids are $C_{15:0}$ anteiso and $C_{17:0}$ anteiso.

Source: the type strain was isolated from homemade compost.

DNA G+C content (mol%): 70.1 ± 0.3 (HPLC; type strain).

Type strain: DC-200, DSM 19600, LMG 24557.

Sequence accession no. (16S rRNA gene): AM949677.

35. **Microbacterium keratanolyticum** (Yokota, Takeuchi, Sakane and Weiss 1993a) Takeuchi and Hatano 1998b, 745[VP] (*Aureobacterium keratanolyticum* Yokota, Takeuchi, Sakane and Weiss 1993a, 561)

ker.a.ta.no.ly′ti.cum. N.L. n. *keratanum* keratan, sulfur-containing polysaccharide produced by mammals; N.L. neut. adj. *lyticum* (from Gr. neut. adj. *lutikon*) able to loosen, able to dissolve; N.L. neut. adj. *keratanolyticum* keratan dissolving.

The following characteristics are based mainly on the data of Yokota et al. (1993a).

Cells are motile rods arranged at angles; V forms are observed. Colonies are 1–3 mm in diameter, circular, low convex with entire margins, opaque, and moist. Yellow pigment is produced. Aerobic. Optimum temperature for growth is 28°C. Growth occurs in presence of 2% NaCl. Oxidative. Esculin and gelatin are hydrolyzed. Starch, and Tweens 20, 40, 60, and 80 are not hydrolyzed. Nitrate is reduced. Negative for the methyl red test and urease. Arginine decarboxylase-positive. Produces H_2S. Acetate, DL-lactate, malate, succinate, oxalate, fumarate, propionate, and hippurate are assimilated. Citrate is assimilated weakly but formate is not.

Cell-wall peptidoglycan contains ornithine, glutamic acid (and hydroxyglutamic acid), glycine, and alanine but not homoserine, conforming to peptidoglycan type B2α (Figure 174). A small amount of lysine is also detected. Cell-wall acyl type is glycolyl. Cell-wall sugar is galactose. Major menaquinones are MK-12 and MK-13. The polar lipids are diphosphatidylglycerol and phosphatidylglycerol. An unidentified glycolipid is also present. Cellular fatty acids are predominantly $C_{15:0}$ anteiso and $C_{17:0}$ anteiso.

Type strain is reported to produce keratansulfate endo-β-galactosidase (EC 3.2.1.103).

Source: the type strain was isolated from soil (Nakazawa et al., 1975; Nakazawa and Suzuki, 1975).

DNA G+C content (mol%): 66.5 (HPLC; type strain).

Type strain: ATCC 35057, CIP 103815, DSM 8606, JCM 9173, LMG 16470, NBRC 13309, VKM Ac-1958.

Sequence accession no. (16S rRNA gene): AB004717.

36. **Microbacterium ketosireducens** Takeuchi and Hatano 1998a, 980[VP]

ke.to.si.re.du′cens. N.L. n. *ketosum* ketose; L. part. adj. *reducens* reducing (deoxidizing); N.L. part. adj. *ketosireducens* ketose-deoxidizing.

Cells are nonmotile, irregular short rods of 0.2–0.5 × 0.6–2.0 µm in size, arranged at angles; V forms but no branching or rod–coccus cycle are observed. Colonies are 2–4 mm in diameter, yellow in color, circular, low convex with entire margins, opaque, and moist. Optimal growth occurs at 28°C; no growth at 37°C. Good growth occurs in 2% NaCl but not at 6.5%. H_2S is formed and methyl red test is positive. Voges–Proskauer test is negative. Nitrate is reduced to nitrite. Arginine dihydrolase is negative. Starch, gelatin, and Tweens 20, 40, 60, and 80 are hydrolyzed. Acid is produced from L-arabinose, glucose, mannose, sucrose, and xylose; variable from galactose and inulin; and negative from melezitose, raffinose, rhamnose, ribose, and trehalose. Arabinose, maltose, mannose, mannitol, and gluconate are assimilated; acetate, fumarate, and lactate are weakly assimilated; citrate assimilation is variable; and *N*-acetylglucosamine, adipate, caprate, malate, phenylacetate, and propionate are not assimilated.

Cell-wall peptidoglycan contains ornithine, homoserine, glutamic acid (and hydroxyglutamic acid), glycine, and alanine, corresponding to peptidoglycan type B2β (Figure 174). Major menaquinone is MK-13. Cellular fatty acids are mainly composed of $C_{15:0}$ anteiso, $C_{17:0}$ anteiso, $C_{16:0}$ iso, and $C_{15:0}$ iso.

Strains of this species have the ability to reduce 2,5-diketo-D-gluconic acid (2,7-anhydro-β-arabino-2,5-hepto-diulo-pyranose) to 2-keto-gulonic acid (2,7-anhydro-β-D-ido-heptulopyranose).

Source: the type strain was isolated from soil.

DNA G+C content (mol%): 69.7–69.8 (HPLC; type strain).

Type strain: CIP 105732, DSM 12510, JCM 12078, NBRC 14548, VKM Ac-2082.

Sequence accession no. (16S rRNA gene): AB004724.

37. **Microbacterium kitamiense** Matsuyama, Kawasaki, Yumoto and Shida 1999, 1356[VP]

kita.mi.en′se. N.L. neut. adj. *kitamiense* of or belonging to Kitami, a city in Hokkaido, Japan, where the organism was isolated.

Cells are nonmotile, irregular rods, 0.5–0.8 × 1.0–2.0 µm in size. Colonies grown on TSA for 5 d at 25°C are round, capitate, smooth, orange in color, glistening, and about 1.5 to 3.0 mm in diameter. Strictly aerobic. Catalase-positive and oxidase-negative. Growth occurs between 15 and 37°C and between pH 6.0 and 9.0. Tolerates up to 7% NaCl. Nitrate reduction is variable depending on strain. Indole is not formed and production of H_2S is negative. Positive for hydrolysis of gelatin and starch. Hydrolysis of casein, urea, DNA, and Tweens 20, 40, 60, and 80 is negative. Tyrosine is decomposed. Acid is produced from glucose, fructose, galactose, mannose, maltose, arabinose, sucrose, xylose, trehalose, glycerol, mannitol, and raffinose but not from inositol, sorbose, lactose, ribose, salicin, sorbitol, melibiose, or adonitol. Utilization of citrate, succinate, fumarate, and propionate is negative.

The peptidoglycan contains D-ornithine as the diagnostic diamino acid. Cell-wall acyl type is glycolyl. The predominant menaquinone is MK-11, followed by MK-12. Major cellular fatty acids are $C_{15:0}$ anteiso, $C_{17:0}$ anteiso, and $C_{16:0}$ iso.

The strains of *Microbacterium kitamiense* were isolated in the course of screening of producers of exopolysaccharide. The strains produce soluble and insoluble polysaccharides (yields of 0.5 to 5.0 g/l) containing glucose, galactose, mannose, and rhamnose.

Source: the type strain was isolated from wastewater of a sugar-beet factory in Kitami City, Hokkaido, Japan.

DNA G+C content (mol%): 69.2 (HPLC; type strain).

Type strain: Kitami C2, CIP 106320, DSM 13237, JCM 10270, NBRC 16414.

Sequence accession no. (16S rRNA gene): AB013907.

38. **Microbacterium koreense** Lee, Lee and Park 2006, 426[VP]

ko.re.en′se. N.L. neut. adj. *koreense* of or pertaining to Korea, where the type strain was isolated.

Cells are nonmotile, short rods, approximately 0.7 µm in diameter and 3.0 to 4.0 µm in length. Cells of old cultures become shorter or spherical. Colonies are circular, convex with entire margins, moist, shiny, and light yellow in color. Aerobic. Catalase-positive. Oxidase-negative. Growth occurs at 20–37°C (optimum at 30°C), pH 6.0–8.0 (optimum at pH 7.0). Grows at 5% NaCl but not at 10% (w/v) or higher. Optimum growth is observed without addition of NaCl to the medium. H_2S is not produced and indole is not formed. Esculin is hydrolyzed but gelatin is not. Nitrate is not reduced to nitrite. Arginine dihydrolase, urease, and β-galactosidase

are negative. Acid is produced from glucose, fructose, mannose, rhamnose, mannitol, *N*-acetylglucosamine, cellobiose, maltose, sucrose, and starch and produced weakly from D-xylose, galactoside, trehalose, glycogen, and D-turanose. Acid is not produced from glycerol, erythritol, D-arabinose, L-arabinose, ribose, L-xylose, adonitol, methyl β-D-xyloside, sorbose, dulcitol, inositol, sorbitol, methyl α-D-mannoside, methyl α-D-glucoside, amygdalin, arbutin, salicin, lactose, melibiose, inulin, melezitose, raffinose, xylitol, gentiobiose, D-lyxose, D-tagatose, D-fucose, L-fucose, D-arabitol, L-arabitol, gluconate, 2-ketogluconate, or 5-ketogluconate.

The diamino acid of peptidoglycan is lysine. The cell-wall sugars are galactose and xylose. The predominant menaquinones are MK-11 and MK-12. Major cellular fatty acids are $C_{15:0}$ anteiso, $C_{17:0}$ anteiso, and $C_{16:0}$ iso. The polar lipids consist of diphosphatidylglycerol and phosphatidylglycerol.

Source: the type strain was isolated from seawater in the South Sea of Korea.

DNA G+C content (mol%): 68 (HPLC; type strain).

Type strain: JS53-2, CCUG 50754, CIP 108696, JCM 15305, KCTC 19074.

Sequence accession no. (16S rRNA gene): AY962574.

39. **Microbacterium kribbense** Dastager, Lee, Ju, Park and Kim 2008b, 2539[VP]

krib.ben′se. N.L. neut. adj. *kribbense* of or belonging to KRIBB, arbitrary adjective formed from the acronym of the Korea Research Institute of Bioscience and Biotechnology, KRIBB, where the taxonomic studies on the type strain were performed.

Cells are irregular rods, 0.4–0.6 × 0.5–0.8 µm in size. Nonmotile. Colonies are pale to lemon yellow. Aerobic but catalase-negative. Growth occurs at 20–37°C (optimum at 28°C), at pH 7–11 (optimum at pH 7.2–7.4), and in up to 3% (w/v) NaCl. Nitrate is reduced to nitrite. Esculin, starch, and Tween 80 are hydrolyzed but cellulose, gelatin, and urea are not. Arabinose, galactose, glucose, maltose, mannitol, mannose, melibiose, raffinose, rhamnose, sucrose, and trehalose are assimilated but ribose, fructose, and xylose are not. Esterase (C4), esterase lipase (C8), leucine arylamidase, acid phosphatase, naphthol-AS-BI-phosphohydrolase, α-galactosidase, β-glucuronidase, α-glucosidase, β-glucosidase and *N*-acetyl-β-glucosaminidase are detected with the API ZYM enzyme assay; alkaline phosphatase, lipase (C14), valine arylamidase, cystine arylamidase, trypsin, α-chymotrypsin, β-galactosidase, α-mannosidase, and α-fucosidase are negative.

The peptidoglycan contains lysine. The cell-wall acyl type is glycolyl. The cell-wall sugars detected are ribose, galactose, glucose, and xylose. The major menaquinones are MK-11 and MK-12. Main polar lipids are diphosphatidylglycerol and phosphatidylglycerol. Cellular fatty acids contain a large proportion of $C_{18:1}$ ω7*c* in addition to $C_{15:0}$ anteiso and $C_{16:0}$ iso.

Source: the type strain was isolated from soil collected from Bigeum Island, Korea.

DNA G+C content (mol%): 71.0 (T_m; type strain).

Type strain: MSL-04, DSM 19265, KCTC 19269.

Sequence accession no. (16S rRNA gene): EF466125.

Further comments: although the diamino acid of the species is lysine in the description, in the text of the same reference, peptidoglycan is type B2β containing ornithine, alanine, glycine, homoserine, and hydroxyglutamic acid (Dastager et al., 2008b).

There are no other species of the genus *Microbacterium* that have a large proportion of $C_{18:1}$ ω7*c* in the cellular fatty acids. If the identification of this fatty acid is correct and not originating from a contaminant, this is the distinctive characteristic of this species.

40. **Microbacterium lacus** Kageyama, Takahashi, Matsuo, Adachi, Kasai, Shizuri and Ōmura 2008c, 529[VP] (Effective publication: Kageyama, Takahashi, Matsuo, Adachi, Kasai, Shizuri and Ōmura 2007a, 57.)

la′cus. L. gen. n. *lacus* of a lake.

Cells are nonmotile, irregular rods, 0.6–0.7 × 0.9–1.5 µm. Colony color is pale yellow. Aerobic. Growth occurs at pH 6–11 and 13–33°C. Tolerates up to 2% NaCl on 5× diluted nutrient agar medium. D-Glucose, D-galactose, maltose, and sucrose are assimilated, but arabinose, fructose, mannitol, D-mannose, raffinose, L-rhamnose, trehalose, and xylose are not. Esterase (C4), esterase lipase (C8), lipase (C14), leucine arylamidase, valine arylamidase, cystine arylamidase, trypsin, chymotrypsin, acid phosphatase, naphthol-AS-BI-phosphohydrolase, α-galactosidase, α-glucosidase, and *N*-acetyl-β-glucosaminidase are detected by the API ZYM enzyme assay; β-galactosidase, β-glucuronidase, β-glucosidase, α-mannosidase, and α-fucosidase are negative. Reaction for alkaline phosphatase is weak.

The cell-wall peptidoglycan contains ornithine as the diagnostic amino acid. Cell-wall acyl type is glycolyl. The major menaquinones are MK-12 and MK-13. Predominant cellular fatty acids are $C_{17:0}$ anteiso, $C_{15:0}$ anteiso, and $C_{16:0}$ iso.

Source: the type strain was isolated from sediments collected at the shore of Lake Shinjiko, Shimane, Japan (salinity of the sample and the depth of the site were approximately 0.5% and 2 m, respectively).

DNA G+C content (mol%): 69 (HPLC; type strain).

Type strain: A5E-52, DSM 18910, JCM 15575, MBIC08279, NBRC 103566.

Sequence accession no. (16S rRNA gene): AB286030.

41. **Microbacterium laevaniformans** (*ex* Dias and Bhat 1962) Collins, Jones and Kroppenstedt 1983b, 673[VP] (Effective publication: Collins, Jones and Kroppenstedt 1983c, 75.) ("*Corynebacterium laevaniformans*" Dias and Bhat 1962, 68.)

la.e.van.i.for′mans. N.L. n *laevanum* laevan, levan polysaccharide; L. part. adj. *formans* forming; N.L. part. adj. *laevaniformans* levan forming.

The following characteristics are based mainly on Dias and Bhat (1962, 1964), Schleifer and Kandler (1972), Collins et al. (1983c), Yokota et al. (1993b), and Takeuchi and Hatano (1998a).

Cells are nonmotile, small, slender rods, arranged at angles; V forms are observed but no branching. In older cultures, rods become shorter but no rod–coccus cycle is seen. Colonies are 1–3 mm in diameter, circular, low convex with entire margins, opaque, and moist. Produces a

yellow pigment. Aerobic. Optimum temperature for growth is 30°C. Survives heating at 60°C. Catalase-positive. H_2S is formed. Methyl red and Voges–Proskauer tests are positive. Nitrate is not reduced. Starch and gelatin are hydrolyzed. Tweens 20, 40, 60, and 80 are not hydrolyzed. Urease-negative. Arginine is decarboxylated. Acid is produced from glucose, galactose, fructose, mannose, lactose, maltose, cellobiose, dextrin, sorbitol, mannitol, glycerol, and glycogen. Acid production is variable from rhamnose, raffinose, sucrose, and trehalose (Takeuchi and Hatano, 1998a). Acid is not produced from *N*-acetylglucosamine, xylose, arabinose, inulin, inositol, salicin, arbutin sorbose, dulcitol, ribose, melibiose, or melezitose. Maltose, mannose, mannitol, gluconate, acetate, lactate, malate, fumarate, and propionate are assimilated; citrate assimilation is variable but phenylacetate, caprate, and adipate are not assimilated. Biotin, thiamine, and pantothenic acid are required for growth.

Cell-wall peptidoglycan contains L-lysine as the diamino acid, conforming to type B1α (Figure 174). Cell-wall acyl type is glycolyl. Cell-wall sugars are rhamnose, mannose, galactose, and xylose. Major menaquinones are MK-11 and MK-12. The polar lipids consist of diphosphatidylglycerol, phosphatidylglycerol, and dimannosyldiacylglycerol. In addition, an unknown monoglycosyldiacylglycerol is sometimes present. Cellular fatty acids are predominantly $C_{15:0}$ anteiso and $C_{17:0}$ anteiso.

Source: the type strain was isolated from activated sludge.

DNA G+C content (mol%): 70–73.7 (T_m).

Type strain: ATCC 15953, CIP 100934, DSM 20140, JCM 9181, NCIMB 9659, NBRC 14471, VKM Ac-1138.

Sequence accession no. (16S rRNA gene): Y17234.

42. **Microbacterium lindanitolerans** Lal, Gupta, Schumann and Lal 2010, 2637[VP]

lin.da.ni.to′le.rans. N.L. n. *lindanum* lindane; L. part. adj. *tolerans* tolerating; N.L. part. adj. *lindanitolerans* lindane-tolerating.

Cells are rod-shaped and not flagellated. Colonies are smooth, circular, 1.0–1.5 mm in diameter, and yellow after incubation on LB agar at 28°C for 3 d. Growth occurs at 25–37°C, pH 6–11, with optimal growth at 28–37°C and pH 7–8. Optimal growth is shown in the presence of 0–2% NaCl. No growth in 5% NaCl. Catalase-positive and oxidase-negative. Nitrate is reduced to nitrite. Hydrolysis of esculin and starch is positive. Xanthine and hypoxanthine are hydrolyzed after 3 weeks. Urease, DNase, gelatin hydrolysis, H_2S production, and Voges–Proskauer reaction are negative.

Acid production is observed from L-arabinose, D-glucose, raffinose, sucrose, and D-mannitol, but not from adonitol, lactose, *myo*-inositol, D-ribose, D-sorbitol, or D-xylose. As sole carbon sources, cellobiose, D-fructose, D-galactose, D-glucose, lactose, D-mannose, raffinose, sucrose, D-sorbitol, and trehalose are utilized, but adonitol, L-arabinose, citrate, D-mannitol, *myo*-inositol, D-ribose, and D-xylose are not. Hexachlorocyclohexane (CHC, lindane) is not degraded.

The peptidoglycan is of the B type of cross-linkage containing ornithine as the diagnostic diamino acid and glycolyl residues, but not homoserine or hydroxyglutamic acid. The whole-cell sugars include ribose and galactose. The menaquinones are MK-12, MK-11, MK-13, and MK-10, in an approximate molar ratio of 45:40:13:3. The polar lipids comprise diphosphatidylglycerol, phosphatidylglycerol, and two unknown polar lipids. The major cellular fatty acids are $C_{15:0}$ anteiso, $C_{17:0}$ anteiso, $C_{16:0}$ iso, and $C_{15:0}$ iso.

Source: the type strain was isolated from soil contaminated with hexachlorocyclohexane (HCH, a herbicide called lindane) in North India.

DNA G+C content (mol%): 65.3 (HPLC; type strain).

Type strain: MNA2, CCM 7585, DSM 22422.

Sequence accession no. (16S rRNA gene): EU873539.

43. **Microbacterium liquefaciens** (Collins, Jones, Keddie, Kroppenstedt and Schleifer 1983a) Takeuchi and Hatano 1998b, 745[VP] ("*Microbacterium liquefaciens*" Orla-Jensen (1919); *Aureobacterium liquefaciens* Collins, Jones, Keddie, Kroppenstedt and Schleifer 1983a, 246)

li.que.fa′ciens. L. part. adj. *liquefaciens* dissolving.

The following characteristics are based mainly on Orla-Jensen (1919), Schleifer and Kandler (1972), Collins et al. (1983a), Yokota et al. (1993a), and Buczolits et al. (2008a).

Aureobacterium liquefaciens is the type species of the genus *Aureobacterium.*

Cells are nonmotile, small, slender rods arranged at angles; V forms are observed in young cultures but no branching. In older cultures, rods become shorter but no rod–coccus cycle is seen. Colonies are 1–3 mm in diameter, convex, circular with entire margins, opaque, and glistening. A bright yellow pigment is produced. Obligately aerobic. Catalase-positive. Optimum temperature for growth is 30°C. Growth occurs at pH 10, and at 4 and 37°C but not at 42°C. Weak growth is observed in 7% (w/v) NaCl. H_2S is produced. Arginine, gelatin, esculin, and casein are hydrolyzed. Starch, hippurate, and Tweens 20, 40, 60, and 80 are not hydrolyzed. Nitrate is reduced. Voges–Proskauer test is negative. Methyl red test, urease, and arginine dihydrolase are positive. Tellurite (0.05%) is not reduced. B vitamins are required. Acid is produced slowly from D-glucose, galactose, and few other sugars. Acetate, malate, succinate, fumarate, propionate, and hippurate are assimilated. Oxalate and DL-lactate are weakly assimilated. Formate and citrate are not assimilated.

Cell-wall peptidoglycan contains D-ornithine as the diamino acid, conforming to type B2β: [L-Hsr]–D-Glu–Gly–D-Orn. Cell-wall acyl type is glycolyl. Cell-wall sugar is rhamnose. Major menaquinones are MK-11 and MK-12. Moderate amounts of MK-10 or MK-13 may occur. The polar lipids consist of diphosphatidylglycerol, phosphatidylglycerol, and diglycosyldiacylglycerol. Additionally, an unidentified glycolipid and three unidentified polar lipids may occur. Cellular fatty acids are predominantly $C_{15:0}$ anteiso and $C_{17:0}$ anteiso.

Source: milk, cheese, dairy products, and dairy equipment.

DNA G+C content (mol%): 68.6 (HPLC; type strain).

Type strain: ATCC 43647, BCRC 14859, CCUG 33091, CIP 102402, DSM 20638, HAMBI 1895, JCM 3879, LMG 16342, NBRC 15037, NCIMB 11509, VKM Ac-1018.

Sequence accession no. (16S rRNA gene): X77444.

44. **Microbacterium luteolum** (Yokota, Takeuchi, Sakane and Weiss 1993a) Takeuchi and Hatano 1998b, 745[VP] (*Aureobacterium luteolum* Yokota, Takeuchi, Sakane and Weiss 1993a, 562)

lu.te.o′lum. L. neut. adj. *luteolum* yellowish.

The following characteristics are based mainly on the data of Yokota et al. (1993a) and Buczolits et al. (2008a).

Cells are nonmotile rods arranged at angles; V forms are observed. Colonies are 1–3 mm in diameter, circular, low convex with entire margins, opaque, and moist. Yellow pigment is produced. Aerobic. Optimum temperature for growth is 28°C. Weak growth occurs at 4°C but no growth is seen at 37°C. No growth occurs in the presence of 2% NaCl. Oxidative. Gelatin, starch, and Tweens 20, 40, 60, and 80 are not hydrolyzed. Nitrate is reduced. Methyl red test and urease are positive. Arginine decarboxylase-negative. Produces H_2S. Acetate, DL-lactate, malate, succinate, fumarate, propionate, and hippurate are assimilated. Formate and citrate are not assimilated. Oxalate is assimilated weakly.

Cell-wall peptidoglycan contains ornithine, homoserine, glutamic acid with hydroxyglutamic acid, glycine, and alanine, corresponding to type B2β (Figure 174). A small amount of lysine is also detected. Cell-wall acyl type is glycolyl. Cell-wall sugars are rhamnose, galactose, and glucose. Major menaquinone is MK-12. Menaquinones MK-11 and MK-13 are found in moderate amounts. The polar lipids are diphosphatidylglycerol and phosphatidylglycerol. One or two unidentified glycolipids and three unidentified polar lipids are also present. Cellular fatty acids are predominantly $C_{15:0}$ anteiso and $C_{17:0}$ anteiso.

Source: the type strain was isolated from soil.

DNA G+C content (mol%): 70.6 (HPLC; type strain).

Type strain: ATCC 51474, CIP 102086, DSM 20143, JCM 9174, LMG 16207, NBRC 15074.

Sequence accession no. (16S rRNA gene): AB004718.

45. **Microbacterium luticocti** Vaz-Moreira, Lopes, Falsen, Schumann, Nunes and Manaia 2008a, 1703[VP]

lu.ti.coc′ti. L. neut. n. *lutum* mud, sludge; L. part. adj. *coctus -a -um* digested; N.L. gen. n. *luticocti* of digested sludge.

Cells are short rods, 0.4–0.6 × 1.3–1.9 µm in size. Motile. Colonies are white, opaque, and circular (1–2 mm diameter) on BHI agar. Aerobic but catalase and oxidase are negative. Growth occurs between 27 and 45°C (optimally 36°C), between pH 5.5 and 9.7 (optimally pH 8), and in NaCl up to 10% (optimally 1–3%). Nitrate is reduced to nitrite. Starch, gelatin, and esculin are hydrolyzed. Urease is produced. Acid is produced from D-arabitol, D-fructose, D-mannitol, L-rhamnose, sucrose, and trehalose. Acid is produced weakly from cellobiose, L-fucose, D-galactose, maltose, D-mannose, potassium 5-ketogluconate, and turanose. The carbon sources assimilated are *N*-acetylglucosamine, amygdalin, D-arabinose, cellobiose, D-fructose, L-fucose, D-galactose, gentiobiose, D-lactose, maltose, D-mannitol, D-mannose, potassium gluconate, L-rhamnose, D-ribose, salicin, and trehalose. Negative for Tweenase, arginine dihydrolase, lysine and ornithine decarboxylases, citrate utilization, and indole and acetoin production. In the original description, the species is described as being both positive and negative for β-galactosidase.

Growth on glucose as a single carbon source is poor. The following compounds are not assimilated: L-arabinose, caprate, adipate, malate, citrate, phenylacetate, glycerol, erythritol, D-xylose, L-xylose, D-adonitol, methyl β-D-xylopyranoside, L-sorbose, dulcitol, inositol, D-sorbitol, methyl α-D-mannopyranoside, methyl α-D-glucopyranoside, arbutin, melibiose, sucrose, inulin, melezitose, raffinose, starch, glycogen, xylitol, turanose, D-lyxose, D-tagatose, D-fucose, D-arabitol, L-arabitol, potassium 2-ketogluconate, or potassium 5-ketogluconate. Does not produce acid from glycerol, erythritol, D-arabinose, L-arabinose, D-ribose, D-xylose, L-xylose, D-adonitol, methyl β-D-xylopyranoside, D-glucose, L-sorbose, dulcitol, inositol, D-sorbitol, methyl α-D-mannopyranoside, methyl α-D-glucopyranoside, *N*-acetylglucosamine, amygdalin, arbutin, salicin, D-lactose, melibiose, inulin, melezitose, raffinose, starch, glycogen, xylitol, gentiobiose, D-lyxose, D-tagatose, D-fucose, L-arabitol, potassium gluconate, or potassium 2-ketogluconate. Cannot ferment or oxidize (API 20E) D-glucose, D-mannitol, inositol, D-sorbitol, sucrose, melibiose, amygdalin, or L-arabinose. Growth occurs in the presence of ciprofloxacin (5 µg), meropenem (10 µg), ceftazidime (30 µg), colistin sulfate (50 µg), and sulfamethoxazole (25 µg). Unable to grow in the presence of amoxycillin (25 µg), gentamicin (10 µg), tetracycline (30 µg), SXT (sulfamethoxazole/trimethoprim, 23.5/1.25 µg), cephalothin (30 µg), streptomycin (10 µg), or ticarcillin (75 µg).

The peptidoglycan is type B2β, containing ornithine, homoserine, glycine, alanine, and glutamic acid (and hydroxyglutamic acid). Cell-wall acyl type is glycolyl. The cell-wall sugar is mannose, with a trace amount of xylose. The major menaquinone is MK-12, with MK-11 as a moderate component. Predominant cellular fatty acids are $C_{17:0}$ anteiso and $C_{15:0}$ anteiso, with moderate amounts of $C_{15:0}$ iso and $C_{16:0}$ iso.

Source: the type strain was isolated from municipal sewage sludge compost.

DNA G+C content (mol%): 72±0.3 (HPLC; type strain).

Type strain: SC-087B, CCUG 54537, DSM 19459, JCM 15576.

Sequence accession no. (16S rRNA gene): AM747814.

46. **Microbacterium marinilacus** Kageyama, Takahashi, Matsuo, Kasai, Shizuri and Ōmura 2007b, 2358[VP]

mari.ni.la′cus. L. adj. *marinus* marine; L. n. *lacus -us* lake; N.L. gen. n. *marinilacus* of a marine lake.

Rod-shaped cells are variable in size, 0.4–0.6 × 0.9–1.4 µm. Colony color is lemon yellow. Catalase-positive, aerobic. Grows between pH 6 and 11, and at 12°C and 38°C. Grows in the presence of up to 8% NaCl. L-Arabinose, D-fructose, D-glucose, maltose, D-mannitol, D-mannose, L-rhamnose, trehalose, and D-xylose are assimilated. D-Galactose, raffinose, and sucrose are not assimilated. Esterase (C4), esterase lipase (C8), leucine arylamidase, valine arylamidase, cystine arylamidase, trypsin, naphthol-AS-BI-phosphohydrolase, α-glucosidase, β-glucosidase, *N*-acetyl-β-glucosaminidase, and α-fucosidase are detected by the API ZYM enzyme assay; alkaline phosphatase, α-galactosidase, β-galactosidase, β-glucuronidase, and α-mannosidase are negative. Reactions for lipase (C14), chymotrypsin, acid phosphatase, and trypsin are weak.

The peptidoglycan contains ornithine as the diagnostic amino acid. Cell-wall acyl type is glycolyl. The major menaquinones are MK-11 and MK-12. Major cellular fatty acids are $C_{15:0}$ anteiso, $C_{17:0}$ anteiso, and $C_{16:0}$ iso.

Source: the type strain was isolated from an unidentified hydroid collected from the Sano Marine Lake, Republic of Palau.

DNA G+C content (mol%): 71.6 (HPLC; type strain).

Type strain: YM11-607, DSM 18904, MBIC07778, NBRC 104188.

Sequence accession no. (16S rRNA gene): AB286020.

47. **Microbacterium maritypicum** corrig. (ZoBell and Upham 1944) Takeuchi and Hatano 1998a, 981[VP] (*Flavobacterium marinotypicum* ZoBell and Upham 1944, 268)

ma.ri.ty′pi.cum. L. n. *mare* the sea; L. neut. adj. *typicum* typical; N.L. neut. adj. *maritypicum* typical of the sea.

Cells are irregular short rods of 0.2–0.5 × 0.6–2.0 µm in size, arranged at angles; V forms but no branching or rod–coccus cycle are observed. Motile with peritrichous flagella. Colonies are 2–4 mm in diameter, light yellow in color, circular, low convex with entire margins, opaque, and moist. Growth occurs at 28°C (optimal) and 37°C. Good growth occurs in 2% and 5% NaCl, but growth is weak in 6.5% NaCl. H_2S formation, methyl red test, and Voges–Proskauer test are negative. Arginine dihydrolase is not produced. Gelatin, and Tweens 20, 40, 60, and 80 are hydrolyzed but starch is not. Acid is produced from glucose and mannose and weakly from sucrose but not from L-arabinose, galactose, inulin, melezitose, raffinose, rhamnose, ribose, trehalose, or xylose. Maltose, mannose, mannitol, *N*-acetylglucosamine, citrate, gluconate, malate, and phenylacetate are assimilated but arabinose, acetate, adipate, caprate, fumarate, lactate, and propionate are not.

Cell-wall peptidoglycan contains ornithine, homoserine, glutamic acid (plus hydroxyglutamic acid), glycine, and alanine, corresponding to peptidoglycan type B2β (Figure 174). Cell-wall sugar is galactose. Major menaquinone is MK-12. Predominant cellular fatty acids are $C_{15:0}$ anteiso and $C_{16:0}$ iso; $C_{15:0}$ iso and $C_{17:0}$ anteiso are present in small amounts.

Source: the type strain was isolated from seawater and marine mud.

DNA G+C content (mol%): 71.6 (HPLC; type strain).

Type strain: ATCC 19260, CIP 105733, DSM 12512, JCM 11570, LMG 8374, NCIMB 559, NBRC 15779, VKM Ac-2079.

Sequence accession no. (16S rRNA gene): AJ853910.

Further comments: The specific epithet *marinotypicum* (*sic*) was corrected by Takeuchi and Hatano (1998a).

48. **Microbacterium natoriense** Liu, Nakayama, Hemmi, Asano, Tsuruoka, Shimomura, Nishijima and Nishino 2005, 664[VP]

na.to.ri.en′se. N.L. neut. adj. *natoriense* of or belonging to Natori, Miyagi, Japan, where the type strain was isolated.

Cells are nonmotile rods, 0.5–0.6 × 1.5 µm in size. Colonies are yellow in color. Obligately aerobic. Growth occurs at 10 to 40°C (optimum at 30°C) and pH 5 to 9 (optimum at pH 5–7). Grows in 6% (w/v) NaCl. Catalase-positive and oxidase-negative. Pyradinamidase, alkaline phosphatase, β-galactosidase, α-glucosidase, *N*-acetyl-β-glucosaminidase, β-glucosidase, and gelatinase are positive. Casein hydrolysis, nitrate reduction, pyrrolidonyl arylamidase, β-glucuronidase, and urease are negative.

Acid is produced from glycerol, L-arabinose, D-xylose, D-galactose, D-glucose, D-fructose, D-mannose, methyl α-D-glucopyranoside, *N*-acetyl-D-glucosamine, amygdalin, arbutin, esculin, salicin, cellobiose, maltose, melibiose, sucrose, trehalose, inulin, melezitose, raffinose, starch, glycogen, and D-turanose. Acid production is not observed from erythritol, D-arabinose, D-ribose, L-xylose, D-adonitol, methyl β-D-xylopyranoside, L-sorbose, L-rhamnose, dulcitol, inositol, D-mannitol, D-sorbitol, lactose, xylitol, gentiobiose, D-tagatose, D-fucose, D-arabitol, L-arabitol, potassium gluconate, potassium 2-ketogluconate, or potassium 5-ketogluconate.

The cell-wall peptidoglycan contains ornithine as the diagnostic amino acid. The menaquinones are MK-9, MK-10, MK-11, and MK-12. Cellular fatty acids are composed of $C_{15:0}$ anteiso, $C_{15:0}$ iso, and $C_{17:0}$ anteiso.

The type strain was isolated by using a selective medium containing 0.1% (w/v) *N*-acetyl-D-phenylalanine to screen for the activity of *N*-acyl-D-amino acid amidohydrolase (D-aminoacylase).

Source: the type strain was isolated from soil obtained at Natori, Miyagi, Japan.

DNA G+C content (mol%): 69.1 (HPLC; type strain).

Type strain: TNJL143-2, ATCC BAA-1032, JCM 12611.

Sequence accession no. (16S rRNA gene): AY566291.

49. **Microbacterium oleivorans** Schippers, Bosecker, Spröer and Schumann 2005, 657[VP]

o.le.i.vo′rans. L. n. *oleum* oil; L. part. adj. *vorans* devouring; N.L. part. adj. *oleivorans* oil-devouring, capable of utilizing oil (hydrocarbons).

Cells are nonmotile, irregular rods, 0.3–1.1 µm in size. Colonies are circular, smooth, translucent, and orange-pigmented with a maximum diameter of 3 mm after 2 weeks of cultivation. Obligately aerobic. Growth occurs at 30–37°C and in 2–4% (w/v) NaCl. Catalase-positive and oxidase-negative. Arginine is not hydrolyzed. Urease-negative. H_2S is not produced and the Voges–Proskauer test is negative. Acid is produced from sucrose and xylose but not from D-glucose, rhamnose, adonitol, inositol, or sorbitol. The following compounds are utilized as sole sources of carbon: L-arabinose, cellobiose, D-fructose, D-galactose, gluconate, D-glucose, maltose, D-mannose, α-D-melibiose, L-rhamnose, D-ribose, sucrose, salicin, trehalose, L-xylose, D-mannitol, sorbitol, fumarate, DL-lactate, L-malate, pyruvate, L-aspartate, L-histidine, putrescine, and 4-hydroxybenzoate. The following are not utilized: *N*-acetyl-D-glucosamine, α-D-galacturonate, glycogen, adonitol, i-inositol, acetate, propionate, *trans*-aconitate, adipate, citrate, DL-3-hydroxybutyrate, suberate, L-alanine, L-hydroxyproline, L-proline, L-serine, 3-hydroxybenzoate, phenylacetate, *N*-acetyl-D-galactosamine, and L-ornithine. Crude oil is used as a substrate for growth. The following are hydrolyzed: esculin, pNP-*N*-acetyl-β-D-galactosaminide, pNP-*N*-acetyl-β-D-glucosaminide, pNP-α-L-arabinopyranoside, pNP-β-D-cellobioside, pNP-β-D-galactopyranoside, pNP-α-D-glucopyranoside, pNP-β-D-glucopyranoside, pNP-α-D-mannoside, pNP-α-D-maltoside,

pNP-β-D-xyloside, bis-pNP-phosphate, benzolphosphonacid-pNP ester, L-alanine-pNA, glycine-pNA, L-leucine-pNA, L-lysine-pNA, and L-proline-pNA. The following are not hydrolyzed: pNP-β-D-glucuronide, pNP-β-D-lactoside, pNP-phosphoryl-choline, 2-deoxythymidine-5′-pNP-phosphate, γ-L-glutamate-pNA, L-glutamate-γ-3-carboxy-pNA, and L-valine-pNA.

The cell-wall peptidoglycan contains L-lysine as the diagnostic diamino acid, conforming to type B1γ: [L-Glu]–D-Glu(Hyg)–Gly_{1-2}–L-Lys. The menaquinones are predominantly MK-11 and MK-12.

The type strain was isolated by enrichment with medium containing 1–5% crude oil.

Source: the type strain was isolated from oil storage cavern 126 near Etzel, Germany.

Type strain: BAS69, DSM 16091, JCM 14341, NBRC 103075, NCIMB 14003.

Sequence accession no. (16S rRNA gene): AJ698725.

50. **Microbacterium oxydans** (Chatelain and Second 1966) Schumann, Rainey, Burghardt, Stackebrandt and Weiss 1999, 176[VP] (*Brevibacterium oxydans* Chatelain and Second 1966, 642)

o′xy.dans. N.L. part. adj. *oxydans* oxidizing.

The following description is based mainly on Schumann et al. (1999) and Buczolits et al. (2008a).

Cells are motile rods, 1–2 µm in length, occurring singly or in random groups. Colonies cultured on nutrient agar at 30°C for 24 h are very small, round with entire edges, and produce a yellow color within 2–3 d. Grows at 20 and 37°C, with optimum growth at 30°C. Growth is also observed at 4°C, pH 10, and in the presence of 7% (w/v) NaCl. Aerobic. Catalase-positive and oxidase-negative. Nitrate is not reduced to nitrite. Gelatin is liquefied and most strains peptonize milk. Esculin is hydrolyzed. β-Galactosidase is produced. H_2S is produced. Methyl red test is negative. Acetylmethylcarbinol is not produced. Lysine decarboxylase is not produced. Urease-negative. Cellulose is not attacked. Acid is produced from glucose, fructose, galactose, mannose, sucrose, maltose, mannitol, glycerol, salicin, and dextrin but not from inositol. Acid production from xylose, arabinose, rhamnose, lactose, and sorbitol is variable between strains. The type strain utilizes caprylate, citrate, 4-hydroxybenzoate, D-gluconate, and D-ribose as carbon sources.

The cell-wall peptidoglycan contains D-ornithine as the diagnostic amino acid and is possibly type B2β, [L-Hsr]-D-Glu–Gly D-Orn. Cell-wall acyl type is glycolyl. Cell-wall sugars are rhamnose, mannose, glucose, and galactose. The menaquinones are mainly MK-11 and MK-12, with MK-9 and MK-10 as minor components. Polar lipids detected are diphosphatidylglycerol and phosphatidylglycerol, as well as one or two unknown glycolipids. In addition, three unidentified polar lipids are also found (Buczolits et al., 2008a). Predominant cellular fatty acids are $C_{15:0}$ anteiso, $C_{17:0}$ anteiso, and $C_{16:0}$ iso.

Source: contaminated hospital material, habitat unknown.

DNA G+C content (mol%): 70–71 (HPLC; type strain).

Type strain: CIP 66.12, DSM 20578, JCM 12414, NBRC 15586, NCIMB 9944, VKM Ac-2116.

Sequence accession no. (16S rRNA gene): Y17227.

51. **Microbacterium paludicola** Park, Kim, Jin and Lee 2006, 536[VP]

pa.lu.di′co.la. L. n. *palus -udis* swamp; L. suff. *-cola* from L. masc. or fem. n. *incola* inhabitant; N.L. masc. or fem. n. *paludicola* inhabitant of a swamp.

Cells are nonmotile, short rods, 0.8–1.0 × 1.0–2.5 µm in size. Colonies are lemon yellow, translucent, and circular with entire edges. Aerobic. Growth occurs at 15–37°C (optimum at 25–30°C), pH 6–9 (optimum at pH 6–8), and in up to 6.5% (w/v) NaCl. Catalase- and oxidase-positive. Nitrate is not reduced to nitrite. Xylan, starch, and esculin are hydrolyzed but casein, cellulose, and gelatin are not. Arginine dihydrolase and urease are produced, but ornithine and lysine decarboxylases are not produced. Methyl red and Voges–Proskauer tests are negative. Acid is produced from glycerol, L-arabinose, D-xylose, galactose, glucose, fructose, mannose, rhamnose, mannitol, methyl α-D-mannoside, esculin, salicin, cellobiose, maltose, sucrose, trehalose, melezitose, starch, glycogen, and D-turanose, but not from erythritol, D-arabinose, ribose, L-xylose, adonitol, methyl β-D-xylose, sorbose, dulcitol, inositol, sorbitol, methyl α-D-glucoside, *N*-acetylglucosamine, amygdalin, arbutin, lactose, melibiose, inulin, raffinose, xylitol, gentiobiose, D-lyxose, D-tagatose, D-fucose, L-fucose, D-arabitol, L-arabitol, gluconate, 2-ketogluconate, or 5-ketogluconate. The following compounds are utilized as sole carbon sources: mannose, gluconate, adipate, mannitol, D-glucose, salicin, L-arabinose, propionate, valerate, 2-ketogluconate, rhamnose, D-sucrose, maltose, DL-lactate, 5-ketogluconate, and glycogen. The following carbon sources are not utilized: malate, phenylacetate, melibiose, L-fucose, D-sorbitol, caprate, citrate, histidine, 3-hydroxybutyrate, 4-hydroxybenzoate, L-proline, *N*-acetylglucosamine, D-ribose, inositol, itaconate, suberate, malonate, acetate, L-alanine, glycogen, 3-hydroxybenzoate, and L-serine. According to the results from the API ZYM test, 2-naphthyl butyrate, 2-naphthyl caprylate, 2-naphthyl myristate, L-leucyl 2-naphthylamide, L-valyl 2-naphthylamide, 2-naphthyl phosphate (pH 5.4), naphthol-AS-BI-phosphate, 2-naphthyl α-D-glucopyranoside, and 6-bromo-2-naphthyl α-D-mannopyranoside are hydrolyzed, but 2-naphthyl phosphate (pH 8.5), L-cystyl 2-naphthylamide, *N*-benzoyl-DL-arginine 2-naphthylamide, *N*-glutaryl-phenylalanine 2-naphthylamide, 6-bromo-2-naphthyl α-D-galactopyranoside, 2-naphthyl β-D-galactopyranoside, naphthol-AS-BI-β-D-glucuronide, 6-bromo-2-naphthyl β-D-glucopyranoside, 1-naphthyl *N*-acetyl-β-D-glucosaminide, and 2-naphthyl α-L-fucopyranoside are not hydrolyzed.

The peptidoglycan is type B2β: [L-Hsr]–D-Glu–Gly–D-Orn. The cell-wall acyl type is glycolyl. The cell-wall sugars detected are glucose, galactose, and mannose, with trace amounts of rhamnose and fucose. The menaquinones are predominantly MK-11 and MK-12, with small amounts of MK-13. Main polar lipids are diphosphatidylglycerol, phosphatidylglycerol, and unknown polar lipids including glycolipids and phospholipids. Cellular fatty acids contain a large proportion of $C_{15:0}$ anteiso, followed by $C_{17:0}$ anteiso and $C_{16:0}$ iso.

The type strain was isolated by the enrichment culture for xylan degradation.

Source: the type strain was isolated from swamp forest soil in Ulsan, Korea.

DNA G+C content (mol%): 66.5 (HPLC; type strain).

Type strain: US15, DSM 16915, JCM 14308, KCTC 19080.

Sequence accession no. (16S rRNA gene): AJ853909.

52. **Microbacterium paraoxydans** Laffineur, Avesani, Cornu, Charlier, Janssens, Wauters and Delmée 2003b, 936[VP] (Effective publication: Laffineur, Avesani, Cornu, Charlier, Janssens, Wauters and Delmée 2003a, 2244.) emend. Buczolits, Schumann, Valens, Rosselló-Mora and Busse 2009, 1559 (Effective publication: Buczolits, Schumann, Valens, Rosselló-Mora and Busse 2008a, 249.)

pa.ra.o′xy.dans. Gr. prep. *para* beside, alongside of, near, like; N.L. part. adj. *oxydans* a bacterial specific epithet; N.L. part. adj. *paraoxydans* like (*Microbacterium*) *oxydans*, because the organism resembles to *Microbacterium oxydans*.

Coryneform rods. Motile by peritrichous flagella. Growth occurs under aerobic and microaerobic conditions, but not anaerobically. Growth occurs between 20 to 40°C on blood agar and at 15–42°C but not at 4°C on PYES agar (0.3% peptone from casein, 0.3% yeast extract, 0.1% succinic acid, pH 7.2). Optimum growth is observed between 25 and 37°C. Growth is observed at pH 10 and in the presence of 7% (w/v) NaCl. Colonies are bright yellow in color, smooth, sometimes sticky, and reach a diameter of 2 mm after 48 h of incubation at 37°C. On PYES agar after 48 h incubation at 28°C, colonies are 4–6 mm in diameter. Catalase-positive and oxidase-negative. Negative for urease, H_2S production, and nitrate reduction. Acid is produced from glucose, sucrose, maltose, galactose, fructose, mannose, D-ribose, and mannitol but not from salicin, D-arabinose, L-arabinose, DL-lactose, or raffinose. Acid production from melibiose, L-rhamnose, and D-xylose is variable. The following compounds are assimilated (API 50CH test): glycerol, D-arabinose, ribose, glucose, galactose, fructose, mannose, rhamnose, mannitol, methyl α-D-glucoside, *N*-acetylglucosamine, esculin, salicin, cellobiose, maltose, lactose, sucrose, trehalose, melezitose, gentiobiose, D-turanose, L-fucose, and gluconate. Assimilation is variable for sorbose, sorbitol, amygdalin, xylitol, and D-fucose.

The peptidoglycan is type B2β containing the amino acids D-ornithine, glycine, alanine, glutamic acid, hydroxyglutamic acid, and homoserine. Muramic acid residues are glycolylated. Major menaquinones are MK-12 and MK-11; MK-13 and MK-10 are present in minor amounts. The polar lipid profile is composed of diphosphatidylglycerol, phosphatidylglycerol, two unidentified glycolipids, one unknown phospholipid, and four unidentified polar lipids. Main cellular fatty acids are $C_{15:0}$ anteiso, $C_{17:0}$ anteiso, and $C_{16:0}$ iso. Phylogenetically, closely related to *Microbacterium luteolum*, *Microbacterium oxydans*, and *Microbacterium saperdae* based on 16S rRNA gene sequences.

Source: the type strain was isolated from human blood (of a patient of bacteremia).

DNA G+C content (mol%): 69.9 (HPLC; type strain).

Type strain: CF36, CCUG 46601, DSM 15019, JCM 12372, NBRC 103076.

Sequence accession no. (16S rRNA gene): AJ491806, AJ581908.

Further comments: C57-33 (=DSM 15461) is a reference strain.

53. **Microbacterium phyllosphaerae** Behrendt, Ulrich and Schumann 2001, 1273[VP]

phyl.lo.sphae′rae. Gr. n. *phullon* leaf; L. fem. n. *sphaera* ball, sphere; N.L. fem. n. *phyllosphaera*, phyllosphere; N.L. gen. fem. n. *phyllosphaerae* of the phyllosphere.

Cells are irregularly shaped rods. In older cultures, rods are shorter, but a marked rod–coccus cycle does not occur. Motile by means of a single polar or lateral flagellum. Colonies are yellow, shiny, slightly convex, and round with entire margins. Strictly aerobic. Optimum growth temperature is approximately 25°C. Growth occurs in the presence of 2% (w/v) NaCl. Catalase-positive and oxidase-negative. Nitrate is not reduced to nitrite. β-Galactosidase is positive. Esculin, DNA, and gelatin are hydrolyzed. Urease, arginine dihydrolase, Voges–Proskauer test, H_2S production, and indole production are negative. Hydrolysis of starch, casein, and Tween 80 is variable between strains. Acid is produced from amygdalin, arbutin, cellobiose, D-fructose, galactose, β-gentiobiose, glycerol, maltose, mannitol, D-mannose, rhamnose, salicin, sucrose, and trehalose and but not from adonitol, D-arabitol, dulcitol, D-fucose, erythritol, gluconate, 2-ketogluconate, 5-ketogluconate, L-sorbose, D-tagatose, or L-xylose. Acid production from L-arabinose, D-arabinose, methyl α-D-glucoside, L-fucose, glycogen, inositol, inulin, lactose, methyl α-D-mannoside, melibiose, melezitose, raffinose, ribose, sorbitol, starch, xylitol, D-xylose, and methyl β-xyloside is variable between strains. Oxidative production of acid from glucose is observed by using API 20NE. Assimilation of citrate is negative.

The cell-wall peptidoglycan is type B2β: [L-Hsr]–D-Glu–Gly–D-Orn. The cell-wall acyl type is glycolyl. The cell-wall sugars are galactose and rhamnose. The major menaquinones are MK-12, MK-11, and MK-10. Cellular fatty acids contain $C_{15:0}$ anteiso, $C_{16:0}$ iso, $C_{15:0}$ iso, and $C_{17:0}$ anteiso.

Source: strains are isolated from the phyllosphere of grasses and from the litter layer after mulching of the sward. The type strain was isolated from phyllosphere of grasses.

DNA G+C content (mol%): 64 (HPLC; type strain).

Type strain: P 369/06, CIP 107138, DSM 13468, JCM 11571, LMG 19581, NBRC 103077.

Sequence accession no. (16S rRNA gene): AJ277840.

54. **Microbacterium profundi** Wu, Wu, Wang, Wang, Yang, Oren and Xu 2008, 2932[VP]

pro.fun′di. L. gen. n. *profundi* of the depths of the sea, of the deep-sea.

Cells are short rods, 0.4–0.6 × 1.0–2.0 µm in size, occurring singly or in pairs. Colonies are 1–2 mm in diameter, smooth, circular, elevated, and yellow in color after 48 h at 28°C. Aerobic. Growth occurs at 4–35°C (optimum at 28°C) and pH 6–9.5 (optimum at pH 7–8). Grows in 0–7.5% (w/v) NaCl, but optimum growth is observed in 1% NaCl or less. Catalase-positive and oxidase-negative. Nitrate is not reduced to nitrite. Esculin, DNA, and gelatin are hydrolyzed but casein, starch, Tweens 20 and 80, and tyrosine are not. Urease, arginine dihydrolase, gluconate oxidation, β-galactosidase, and lecithinase are negative. Acid is produced (API 50CH) from D- and L-arabinose, arbutin, cellobiose, erythritol, esculin, ferric citrate, D-galactose,

D-glucose, D- fructose, L-fucose, glycerol, D-lactose, maltose, D-mannitol, D-mannose, methyl β-D-glucoside, L-rhamnose, D-ribose, salicin, and D-xylose. Negative for the production of acid from sucrose and trehalose, but positive when tested according to the method of Leifson (1963). The substrates utilized as sole carbon source are acetate, L-arabinose, L-arginine, L-aspartate, cellobiose, D-fructose, fumarate, D-galactose, D-glucose, glycerol, isoleucine, lactate, lactose, lysine, malate, maltose, D-mannitol, D-mannose, propionate, pyruvate, raffinose, rhamnose, ribose, salicin, L-serine, succinate, sucrose, trehalose, L-valine, and xylose but not *N*-acetylglucosamine, adipic acid, L-alanine, capric acid, citrate, L-cysteine, ethanol, formate, glycine, L-histidine, *myo*-inositol, malonate, L-methionine, potassium gluconate, phenylacetic acid, sorbitol, sorbose, or trisodium citrate. Susceptible to ampicillin (10 µg), bacitracin (0.04 IU), carbenicillin (100 µg), cefalexin (30 µg), cefoxitin (30 µg), ceftriaxone (30 µg), chloramphenicol (30 µg), erythromycin (15 µg), gentamicin (10 µg), minocycline (30 µg), neomycin (30 µg), novobiocin (30 µg), penicillin (10 µg), polymyxin B (300 IU), rifampin (5 µg), streptomycin (10 µg), and tetracycline (30 µg), but not to kanamycin (30 µg), nitrofurantoin (300 µg), nystatin (100 µg), or tobramycin (10 µg).

The purified peptidoglycan contains ornithine, alanine, and glycine, plus two unidentified amino acids. The menaquinones are MK-12 and MK-13. Cellular fatty acids contain $C_{15:0}$ anteiso, $C_{15:0}$ iso, $C_{16:0}$ iso, and $C_{17:0}$ anteiso.

Source: the type strain was isolated from deep-sea sediment collected from a polymetallic nodule region in the East Pacific Ocean.

DNA G+C content (mol%): 66.8 (T_m; type strain).

Type strain: Shh49, CGMCC 1.6777, JCM 14840.

Sequence accession no. (16S rRNA gene): EF623999.

55. **Microbacterium pseudoresistens** Young, Busse, Langer, Chu, Schumann, Arun, Shen, Rekha and Kämpfer 2010, 859[VP]

pseu.do.re.sis′tens. Gr. adj. *pseudês* false; L. part adj. *resistens* resisting and also a bacterial specific epithet; N.L. part. adj. *pseudoresistens* the false *Microbacterium resistens*.

Cells are short rods, about 1–1.2 µm in width and 2–3 µm in length. Aerobic. Catalase and oxidase are positive. Good growth occurs on nutrient agar and R2A agar (Oxoid) at 25–30°C and yellow colonies appear in 3 d.

Does not produce acid from D-arabitol, adonitol, dulcitol, erythritol, D-mannitol, L-arabinose, D-xylose, glucose, cellobiose, lactose, maltose, D-mannose, sucrose, melibiose, methyl D-glucoside, raffinose, rhamnose, salicin, sorbitol, or trehalose. Esculin, pNP-α-D-glucopyranoside, pNP-β-D-glucopyranoside, pNP-phenyl-phosphate, L-alanine-pNA, and L-proline-pNA are hydrolyzed but oNP-β-D-galactopyranoside, pNP-β-D-glucuronide, pNP-β-D-xylopyranoside, bis-pNP-phosphate, pNP-phosphoryl-choline, 2-deoxythymidine-5′-pNP-phosphate, and L-glutamate-γ-3-carboxy-pNA are not hydrolyzed. Cellobiose, D-fructose, D-glucose, DL-lactate, D-mannose, maltose, D-mannitol, L-proline, sucrose, trehalose, *N*-acetyl-D-glucosamine, D-ribose, salicin, and fumarate (weak reaction) are assimilated but L-arabinose, *p*-arbutin, D-galactose, gluconate, α-melibiose, L-rhamnose, D-xylose, adonitol, *i*-inositol, maltitol, D-sorbitol, putrescine, *N*-acetyl-D-galactosamine, acetate, propionate, *cis*-aconitate, citrate, L-malate, oxoglutarate, pyruvate, *trans*-aconitate, adipate, 4-aminobutyrate, azelate, glutarate, DL-3-hydroxybutyrate, itaconate, L-leucine, mesaconate, phenylacetate, L-phenylalanine, suberate, L-tryptophan, L-alanine, β-alanine, L-aspartate, L-histidine, L-ornithine, L-serine, 3-hydroxybenzoate, and 4-hydroxybenzoate are not assimilated.

The cell-wall peptidoglycan is of type B2α with an interpeptide bridge D-Glu–D-Orn. The predominant quinone is MK-13, with MK-12 and MK-14 as minor components. The polar lipid profile is composed of diphosphatidylglycerol, phosphatidylglycerol, and one unknown glycolipid. Trace amounts of three unknown lipids are also found. Major fatty acids are $C_{15:0}$ anteiso, $C_{16:0}$ iso, and $C_{17:0}$ anteiso. Predominant polyamines are spermidine and spermine.

Source: isolated from the stalk of the edible mushroom *Agaricus blazei* cultivated in the laboratory.

DNA G+C content (mol%): Not known.

Type strain: CC-5209, CC-005209, CCM 7688, DSM 22185.

Sequence accession no. (16S rRNA gene): FJ865214.

56. **Microbacterium pumilum** Kageyama, Takahashi and Ōmura 2006, 2115[VP]

pu′mi.lum. L. neut. adj. *pumilum* dwarfish, diminutive, little.

Cells are nonmotile, irregular rods, 0.2–0.6 × 0.4–1.2 µm. Colony color is pale yellow. Aerobic and catalase-positive. Growth occurs at pH 7–10 and 17–32°C. Tolerates up to 2% NaCl on 5× diluted nutrient agar medium. D-Glucose, arabinose, D-galactose, maltose, D-mannose, and sucrose are assimilated, but fructose, mannitol, raffinose, L-rhamnose, trehalose, and xylose are not. Alkaline phosphatase, esterase lipase (C8), leucine arylamidase, valine arylamidase, cystine arylamidase, trypsin, chymotrypsin, acid phosphatase, naphthol-AS-BI-phosphohydrolase, α-galactosidase, β-galactosidase, β-glucuronidase, α-glucosidase, β-glucosidase, *N*-acetyl-β-glucosamidase, and α-fucosidase are detected with the API ZYM enzyme assay; α-mannosidase is not detected. Reactions for esterase (C4) and lipase (C14) are weak.

The peptidoglycan contains ornithine as the diagnostic amino acid. Cell-wall acyl type is glycolyl. Cell-wall sugars are rhamnose and galactose. The predominant menaquinones are MK-13 and MK-12, with minor amounts of MK-14. Major cellular fatty acids are $C_{15:0}$ anteiso, $C_{16:0}$ iso, and $C_{17:0}$ anteiso.

The type strain was isolated by cultivation with superoxide dismutase and catalase in the isolation medium to scavenge oxygen radicals.

Source: the type strain was isolated from soil from Aoyama Cemetery, Tokyo, Japan.

DNA G+C content (mol%): 71 (HPLC; type strain).

Type strain: KV-488, JCM 14902, NBRC 101279, NRRL B-24452.

Sequence accession no. (16S rRNA gene): AB234027.

57. **Microbacterium pygmaeum** Kageyama, Matsuo, Kasai, Shizuri, Ōmura and Takahashi 2008b, 2471[VP] (Effective

publication: Kageyama, Matsuo, Kasai, Shizuri, Ōmura and Takahashi 2008a, 5.)

pyg.ma′e.um. L. neut. adj. *pygmaeum* dwarf.

Cells are nonmotile, irregular rods, 0.3–0.4 × 0.5–0.9 µm. Colony color is pale yellow. Catalase-positive, aerobic. Grows between pH 5 and 9, and 17°C and 31°C. Grows in up to 5% NaCl. Galactose, glucose, maltose, mannitol, mannose, raffinose, sucrose, and trehalose are assimilated. L-Rhamnose and xylose are not assimilated. Esterase (C4), esterase lipase (C8), leucine arylamidase, acid phosphatase, naphthol-AS-BI-phosphohydrolase, α-glucosidase, β-glucosidase, and *N*-acetyl-β-glucosaminidase are detected by the API ZYM enzyme assay; phosphatase alkaline, lipase (C4), cystine arylamidase, trypsin, chymotrypsin, α-galactosidase, α-galactosidase, β-glucuronidase, α-mannosidase, and α-fucosidase are negative. Weak reaction for valine arylamidase.

The peptidoglycan contains ornithine as the diagnostic amino acid. Cell-wall acyl type is glycolyl. The major menaquinones are MK-11, MK-12, and MK-13. The major cellular fatty acids are $C_{17:0}$ anteiso and $C_{15:0}$ anteiso.

Source: the type strain was isolated from soil collected from Aoyama Cemetery, Japan.

DNA G+C content (mol%): 68 (HPLC; type strain).

Type strain: KV-490, JCM 15925, NBRC 101800, NRRL B-24469.

Sequence accession no. (16S rRNA gene): AB248875.

58. **Microbacterium radiodurans** Zhang, Zhu, Yuan, Yao, Tang, Lin, Yang, Li and Chen 2010, 2668[VP]

ra.di.o.du′rans. L. n. *radius* a beam or ray; N.L. pref. *radio-* pertaining to radiation; L. part. adj. *durans* enduring; N.L. part. adj. *radiodurans* resisting radiation.

Cells are weakly motile, short rods, about 0.4–0.6 µm wide and 0.5–0.8 µm long. Colonies grown on nutrient agar at 28°C for 2 d are smooth, nearly circular, convex, transparent, and white. Growth occurs at 18–30°C, at pH 6.0–10.0, and with <7% (w/v) NaCl. Catalase is weakly positive and oxidase is negative. Strictly aerobic and heterotrophic. Nitrate reduction is negative. Gelatin is not hydrolyzed. With the Biolog GP2 MicroPlate system, β-cyclodextrin, cellobiose, gentiobiose, α-D-glucose, D-xylose, D-fructose, maltose, D-mannose, salicin, D-sorbitol, inositol, maltotriose, D-mannitol, trehalose, 3-methyl D-glucose, α-keto-glutaric acid, valerate, succinic acid monomethyl ester, pyruvic acid methyl ester, glycyl L-glutamic acid, uridine, uridine 5′-monophosphate, putrescine, and α-D-glucose 1-phosphate are oxidized but the following compounds are not utilized: L-arabinose, sucrose, raffinose, L-rhamnose, D-tagatose, xylitol, α-cyclodextrin, dextrin, glycogen, D-galacturonic acid, sedoheptulosan, methyl α-D-glucoside, *N*-acetylglucosamine, acetate, adipate, L-lactate, D-malate, L-malate, propionic acid, citrate, 3-hydroxybutyrate, phenylacetate, Tweens 40 and 80, L-alanine, *N*-acetyl-L-glutamic acid, L-serine, L-proline, L-alaninamide, L-pyroglutamic acid, L-glutamic acid, lactamide, D-lactic acid methyl ester, and adenosine 2′-deoxyadenosine.

The diagnostic diamino acid of the cell-wall peptidoglycan is ornithine. The cell-wall acyl type is glycolyl. The cell-wall sugar is rhamnose. The major menaquinones are MK-11, MK-12, and MK-10. The major polar lipids are diphosphatidylglycerol, phosphatidylglycerol, an unidentified phospholipid, and an unidentified glycolipid. The major cellular fatty acids are $C_{15:0}$ anteiso, $C_{16:0}$ iso, and $C_{15:0}$ iso.

The type strain shows high resistance to UV radiation (254 nm, 200 mW/cm^2, 5 min) compared to *Microbacterium arborescens*, *Microbacterium imperiale*, and *Escherichia coli* K-12 (Zhang et al., 2010).

Source: the type strain was isolated from the upper sand layer of the Gobi desert, Xinjiang, China.

DNA G+C content (mol%): 67.7 (HPLC; type strain).

Type strain: GIMN 1.002, CCTCC M208212, NRRL B-24799.

Sequence accession no. (16S rRNA gene): GQ329713.

59. **Microbacterium resistens** (Funke, Lawson, Nolte, Weiss and Collins 1998) Behrendt, Ulrich and Schumann 2001, 1275[VP] (*Aureobacterium resistens* Funke, Lawson, Nolte, Weiss and Collins 1998, 91)

re.sis′tens. L. pres. part. *resistens* being resistant, referring to the vancomycin resistance which is very unusual for coryneform bacteria.

The following characteristics are taken from Funke et al. (1998) and Behrendt et al. (2001).

Cells are nonmotile, small, slim rods, 1–2 µm in length. Colonies are up to 1.5 mm in diameter after 24 h incubation, glistening, convex, of creamy consistency, and exhibit yellow pigment. Grows in 2% NaCl. Catalase-positive. DNase activity is present. Arginine dihydrolase and Voges–Proskauer reactions are negative. Nitrate is not reduced. Esculin, tyrosine, casein, and DNA are hydrolyzed but not urea or starch. Acid is produced from glucose, maltose, and sucrose but not from mannitol or xylose. The following carbon compounds are utilized: glycerol, ribose, galactose, D-fructose, D-mannose, rhamnose, *N*-acetylglucosamine, amygdalin, salicin, cellobiose, lactose, trehalose, xylitol, D-turanose, D-lyxose, gluconate, 2-keto-gluconate, and 5-keto-gluconate. The following are not utilized: erythritol, L-arabinose, adonitol, methyl β-xyloside, L-sorbose, dulcitol, sorbitol, methyl α-D-mannoside, methyl α-D-glucoside, melibiose, inulin, melezitose, raffinose, starch, glycogen, D-fucose, D-arabitol, and L-arabitol. Activities of pyrazinamidase, esterase (C4), esterase lipase (C8), leucine arylamidase, cystine arylamidase, acid phosphatase, phosphoamidase, β-galactosidase, α-glucosidase, β-glucosidase, *N*-acetyl-glucosaminidase, α-mannosidase, and α-fucosidase are present whereas pyrrolidonyl arylamidase, chymotrypsin, α-galactosidase, and β-glucuronidase activities are not detected.

Cell-wall peptidoglycan contains ornithine in both D and L forms as diamino acid and is possibly type B2α: [L-Orn]–D-Glu–D-Orn. Cell-wall acyl type is glycolyl. Major menaquinones are MK-12 and MK-13. Major cellular fatty acids are $C_{17:0}$ anteiso, $C_{15:0}$ anteiso, and $C_{16:0}$ iso.

Strains of this species are resistant to vancomycin but susceptible to teicoplanin.

Source: human clinical specimens. The type strain was isolated from a corneal ulcer of a 14-year-old female wearing contact lenses

DNA G+C content (mol%): 64 (HPLC; type strain).

Type strain: CCUG 38312, DMMZ 1710, DSM 11986, JCM 11686, NBRC 103078.

Sequence accession no. (16S rRNA gene): Y14699.

60. **Microbacterium saperdae** (Lysenko 1959) Takeuchi and Hatano 1998b, 745[VP] (*Brevibacterium saperdae* Lysenko 1959, 41; *Curtobacterium saperdae* Yamada and Komagata 1972a, 425; *Aureobacterium saperdae* Collins, Jones, Keddie, Kroppenstedt and Schleifer 1983a, 248)

sa.per′da.e. N.L. gen. nov. *saperdae* of *Saperda* (scientific name for a genus of insects), isolated from *Saperda caracharias*.

The following characteristics are based mainly on Yamada and Komagata (1972b), Schleifer and Kandler (1972), Collins et al. (1980, 1983a), Suzuki and Komagata (1983), Yokota et al. (1993a), and Buczolits et al. (2008a).

Cells are motile, small, slender rods arranged at angles; V forms are observed in young cultures but no branching. In older cultures, rods become shorter but no rod–coccus cycle is seen. Good growth on nutrient agar. Colonies are 1–3 mm in diameter, convex, circular with entire margins, opaque, glistening, and yellow in color. Obligately aerobic. Catalase-positive. Optimum temperature for growth is 30°C. Growth is also observed at 4°C but not at 37°C, pH 10, or in the presence of 7% (w/v) NaCl. H_2S is produced. Arginine, esculin, starch, and Tweens 20, 40, 60 and 80 are hydrolyzed. Gelatin and hippurate are not hydrolyzed. Urease-positive. Arginine dicarboxylase is positive. Nitrate is not reduced. Tellurite (0.05%) is not reduced. Acid is produced weakly from L-arabinose, xylose, rhamnose, glucose, fructose, mannose, galactose, sucrose, maltose, cellobiose, dextrin, mannitol, arbutin, esculin, and salicin, but not from sorbose, lactose, trehalose, raffinose, starch, inulin, glycerol, erythritol, adonitol, dulcitol, sorbitol, inositol, or methyl α-glucoside.

The following organic acids are assimilated: acetate, pyruvate, L-lactate, D-lactate, malate, succinate, fumarate, 2-oxoglutarate, propionate, butyrate, glycolate, glyoxylate and hippurate. The following organic acids are not assimilated: citrate, malonate, glutarate, adipate, pimelate, gluconate, and uriate.

Cell-wall peptidoglycan contains D-ornithine as the diamino acid, conforming to type B2β: [Hsr]–D-Glu–Gly–D-Orn (Figure 174). Cell-wall acyl type is glycolyl. Cell-wall sugars are galactose and glucose. Major menaquinones are MK-11 and MK-12. The polar lipids consist of diphosphatidylglycerol, phosphatidylglycerol, and diglycosyldiacylglycerol. In addition, an unidentified glycolipid and three unidentified polar lipids may found (Buczolits et al., 2008a). Cellular fatty acids are predominantly $C_{15:0}$ anteiso, $C_{17:0}$ anteiso, and $C_{16:0}$ iso.

Source: the type strain was isolated from body cavity of dead insects (*Saperda caracharias*).

DNA G+C content (mol%): 69.1 (HPLC; type strain).

Type strain: ATCC 19272, BCRC 12119, CCEB 366, CCUG 33092, CIP 104420, DSM 20169, HAMBI 1890, IAM 12547, IMET 11076, JCM 1352, LMG 16343, NBRC 15038, NRRL B-14833, VKM Ac-1414.

Sequence accession no. (16S rRNA gene): AB004719.

61. **Microbacterium schleiferi** (Yokota, Takeuchi, Sakane and Weiss 1993a) Takeuchi and Hatano 1998b, 745[VP] (*Aureobacterium schleiferi* Yokota, Takeuchi, Sakane and Weiss 1993a, 563)

schlei′fer.i. N.L. gen. masc. n. *schleiferi* of Schleifer, referring to K.H. Schleifer, a German microbiologist who contributed to the elucidation of the primary structure of peptidoglycan and to taxonomic studies of the strains belonging to this species.

The following characteristics are based mainly on the data of Yokota et al. (1993a).

Cells are nonmotile rods arranged at angles; V forms are observed. Colonies are 1–3 mm in diameter, circular, low convex with entire margins, opaque, and moist. Yellow pigment is produced. Aerobic. Optimum temperature for growth is 28°C. Growth occurs in the presence of 2% NaCl. Oxidative. Gelatin and starch are not hydrolyzed. Tween 40 is hydrolyzed but hydrolysis of Tweens 20, 60, and 80 is strain-dependent. Nitrate is not reduced. Voges–Proskauer test is positive. Methyl red test, urease, and production of H_2S are negative. Formate, acetate, citrate, malate, succinate, oxalate, fumarate, DL-lactate, propionate, and hippurate are not assimilated.

Cell-wall peptidoglycan contains ornithine with hydroxylornithine, homoserine, glutamic acid with hydroxyglutamic acid, glycine, and alanine, corresponding to peptidoglycan type B2β (Figure 174). A small amount of lysine is also detected. Cell-wall acyl type is glycolyl. Cell-wall sugars are 6-deoxytalose, mannose, and galactose. Major menaquinones are MK-11 and MK-12; MK-10 is also found in moderate amounts. The polar lipids are diphosphatidylglycerol and phosphatidylglycerol; an unidentified glycolipid is also present. Cellular fatty acids are predominantly $C_{15:0}$ anteiso and $C_{17:0}$ anteiso.

Source: the type strain was isolated from soil.

DNA G+C content (mol%): 66.9 (HPLC; type strain).

Type strain: ATCC 51473, CIP 102087, DSM 20489, JCM 9175, LMG 16153, NBRC 15075, VKM Ac-1946.

Sequence accession no. (16S rRNA gene): Y17237.

62. **Microbacterium sediminicola** Kageyama, Takahashi, Matsuo, Kasai, Shizuri and Ōmura 2007b, 2357[VP]

se.di.mi.ni′co.la. L. n. *sedimen -inis* sediment; L. suff. *-cola* inhabitant dweller; N.L. n. *sediminicola* sediment-dweller.

Rod-shaped cells, variable in size, 0.4–0.7 × 0.8–1.5 µm. Colony color is pale yellow. Catalase-positive, aerobic. Grows between pH 6 and 11 and between 19°C and 35°C. Grows in the presence of up to 7% NaCl. L-Arabinose, D-galactose, D-glucose, maltose, D-mannitol, D-mannose, L-rhamnose, trehalose, and D-xylose are assimilated. D-Fructose, raffinose, and sucrose are not assimilated. Esterase (C4), esterase lipase (C8), leucine arylamidase, valine arylamidase, cystine arylamidase, acid phosphatase, α-galactosidase, β-galactosidase, α-glucosidase, β-glucosidase, and *N*-acetyl-β-glucosaminidase are detected by the API ZYM enzyme assay; alkaline phosphatase, chymotrypsin, naphthol-AS-BI-phosphohydrolase, β-glucuronidase, α-mannosidase, and α-fucosidase are negative. Reactions for lipase (C14) and trypsin are weak.

The peptidoglycan contains ornithine as the diagnostic diamino acid. Cell-wall acyl type is glycolyl. The major menaquinones are MK-10 and MK-11. Predominant cellular fatty acids are $C_{15:0}$ anteiso, $C_{17:0}$ anteiso, and $C_{16:0}$ iso.

Source: the type strain was isolated from sediment collected from the mouth of the Samambula River, Fiji.

DNA G+C content (mol%): 67.8 (HPLC; type strain).

Type strain: YM10-847, DSM 18905, JCM 15577, MBIC 08264.

Sequence accession no. (16S rRNA gene): AB286021.

63. **Microbacterium soli** Srinivasan, Kim, Sathiyaraj, Kim, Jung, In and Yang 2010, 482[VP]

so′li. L. gen. n. *soli* of soil, the source of the first strains.

Cells are nonmotile and rod-shaped, approximately 0.2–0.5 µm wide and 0.5–0.9 µm long after growth on R2A agar (Difco) at 30°C for 5 d. Colonies are circular and pale yellow on R2A agar after 5 d of incubation at 30°C. Growth occurs at 25–42°C, with optimal growth at 30°C on R2A agar. Aerobic. Catalase-positive and oxidase-negative. Nitrate is not reduced to nitrite or nitrogen gas. Positive for production of acid phosphatase, cysteine arylamidase, α-galactosidase, α-glucosidase, β-galactosidase, β-glucosidase (esculin hydrolysis), leucine arylamidase, α-mannosidase, and valine arylamidase, but negative for production of arginine dihydrolase, α-fucosidase, β-glucuronidase, lipase (C14), protease (gelatin hydrolysis), and urease. Acid is produced from cellobiose, esculin, and trehalose but not from D-adonitol, D- or L-arabitol, dulcitol, erythritol, D- or L-fucose, glycerol, glycogen, inositol, inulin, 2-ketogluconate, D-lyxose, methyl α-D-mannoside, raffinose, L-rhamnose, sorbitol, L-sorbose, starch, D-tagatose, L-xylose, or xylitol. *N*-Acetyl-D-glucosamine, L-arabinose, gluconate, glucose, maltose, mannitol, mannose, phenylacetate, L-proline, salicin, and sucrose are assimilated. Acetate, adipate, L-alanine, caprate, citrate, L-fucose, glycogen, L-histidine, 3-hydroxybenzoate, 3-hydroxy-DL-butyrate, inositol, itaconate, 2-ketogluconate, 5-ketogluconate, DL-lactate, L-malate, malonate, melibiose, propionate, L-rhamnose, D-ribose, L-serine, sorbitol, suberate, and n-valerate are not assimilated.

The possible cell-wall peptidoglycan structure is of type B2β, (L-Hsr)–D-Glu–Gly–D-Orn with glycolyl resides. Cell-wall sugar is galactose, with traces of an unknown sugar. The predominant quinones are MK-12, MK-11, and MK-13. The major polar lipids are diphosphatidylglycerol, phosphatidylglycerol, an unidentified phospholipid, and an unidentified glycolipid. The major cellular fatty acids are $C_{15:0}$ anteiso, $C_{15:0}$ iso, $C_{16:0}$ iso, and $C_{17:0}$ anteiso.

Four strains were also isolated from the same source and classified in this species based on the results of DNA–DNA hybridization experiments (Srinivasan et al., 2010).

Source: soil of a ginseng field in Daejeon, Republic of Korea.

DNA G+C content (mol%): 68.9–73.5 (HPLC), 70.2 (type strain).

Type strain: DCY 17, JCM 17024, KCTC 19237, LMG 24010.

Sequence accession no. (16S rRNA gene): EF593038.

Further comments: although the results of peptidoglycan analysis give Orn, Gly, Ala and Asp, the description shown in the text, B2α with [L-Orn]–D-Glu–D-Orn is more likely.

64. **Microbacterium terrae** (Yokota, Takeuchi, Sakane and Weiss 1993a) Takeuchi and Hatano 1998b, 745[VP] (*Aureobacterium terrae* Yokota, Takeuchi, Sakane and Weiss 1993a, 562[VP])

ter′ra.e. L. n. *terra* soil; L. gen. n. *terrae* of soil.

The following characteristics are based mainly on the data of Yokota et al. (1993a).

Cells are nonmotile rods arranged at angles; V forms are observed. Colonies are 1–3 mm in diameter, circular, low convex with entire margins, opaque, and moist. Yellow pigment is produced. Aerobic. Optimum temperature for growth is 28°C. Growth occurs in presence of 2% NaCl. Oxidative. Gelatin, starch, and Tweens 40, 60, and 80 are hydrolyzed. Hydrolysis of Tween 20 is variable. Nitrate is reduced. Voges–Proskauer and methyl red tests are negative. Urease-positive. Arginine decarboxylase-negative. Produces H_2S. Acetate, fumarate, and DL-lactate are assimilated. Malate, succinate, and hippurate are assimilated weakly. Oxalate, formate, and citrate are not assimilated. Propionate assimilation is variable.

Cell-wall peptidoglycan contains ornithine, homoserine, glutamic acid with hydroxyglutamic acid, glycine, and alanine, corresponding to type B2β (Figure 174). A small amount of lysine is also detected. Cell-wall acyl type is glycolyl. Cell-wall sugars are rhamnose, galactose, and glucose. Major menaquinones are MK-13 and MK-14. The polar lipids are diphosphatidylglycerol and phosphatidylglycerol. Unidentified phosphoglycolipids are also present. Cellular fatty acids are predominantly $C_{15:0}$ anteiso and $C_{17:0}$ anteiso.

Source: the type strain was isolated from soil.

DNA G+C content (mol%): 70.7 (HPLC; type strain).

Type strain: ATCC 51476, CIP 103816, DSM 8610, JCM 9176, LMG 16471, NBRC 15300, VKM Ac-1945.

Sequence accession no. (16S rRNA gene): AB004720.

65. **Microbacterium terregens** (Lochhead and Burton 1953) Takeuchi and Hatano 1998b, 745[VP] (*Arthrobacter terregens* Lochhead and Burton 1953, 254; *Aureobacterium terregens* Collins, Jones, Keddie, Kroppenstedt and Schleifer 1983a, 247)

ter.re′gens. L. n. *terra* soil; L. part. adj. *egens* requiring; N.L. part. adj. *terregens* soil-requiring.

The following characteristics are based mainly on Lochhead and Burton (1953), Schleifer and Kandler (1972), Collins et al. (1983a), Suzuki and Komagata (1983), and Yokota et al. (1993a).

Cells are nonmotile, small, irregular rods, 0.6–0.8 × 1.0–5.0 µm in size, arranged at angles; V forms are observed in young cultures but no branching. In older cultures, shorter coccoid forms are observed but no rod–coccus cycle is seen. Yellowish brown colonies are 1–2 mm in diameter, low convex, and circular with entire edges on soil extract agar. A yellow non-diffusible pigment is produced. Obligately aerobic. Catalase-positive. Oxidase-negative. Optimum temperature for growth is 20 to 26°C. Growth occurs at 10°C but not at 37°C. Soil extract is required for growth. Starch, casein, gelatin, cellulose, and Tweens 20, 40, 60, and 80 are not hydrolyzed. Urease-negative. Arginine dihydrolase-negative. Nitrate is reduced to nitrite. H_2S is not produced. Voges–Proskauer test is negative but methyl red test is positive. Terregens factor, biotin, thiamine, pantothenic acid, and methionine are required for growth. Good growth is

not observed without supplementation with a source of terregens factor or similar factors and, preferably, yeast extract. The terregens factor is replaceable by other siderophores such as coprogen and ferrichrome. Acetate, malate, succinate, fumarate, and propionate are assimilated. Hippurate is assimilated weakly. Formate, citrate, oxalate, and DL-lactate are not assimilated.

Cell-wall peptidoglycan contains D-ornithine as the diamino acid, conforming to type B2β: [Hsr]–D-Glu–Gly–D-Orn. Cell-wall acyl type is glycolyl. Cell-wall sugars are rhamnose, 6-deoxytalose, and galactose. Major menaquinones are MK-12 and MK-13, with a minor amount of MK-14. The polar lipids consist of diphosphatidylglycerol, phosphatidylglycerol, and diglycosyldiacylglycerol. Cellular fatty acids are predominantly $C_{17:0}$ anteiso, and $C_{15:0}$ anteiso.

Source: the type strain was isolated from soil.

DNA G+C content (mol%): 68.6 (HPLC; type strain).

Type strain: ATCC 13345, CCUG 23845, CIP 103038, DSM 20449, JCM 1342, LMG 17311, NBRC 12961, NCIMB 8909, NRRL B-1824, VKM Ac-2083.

Sequence accession no. (16S rRNA gene): AB004721.

66. **Microbacterium terricola** corrig. Kageyama, Takahashi and Ōmura 2007d, 1372[VP] (Effective publication: Kageyama, Takahashi and Ōmura 2007c, 3.)

te.rri.co′la. L. n. *terricola* dweller in soil.

Irregular rods, 0.2–0.5 × 0.3–0.7 µm in size. Nonmotile. Colony color is yellow. Catalase-positive. Grows between pH 6 and 11, and at 10 and 35°C but not at 37°C. Grows in up to 4% NaCl. Assimilates glucose, galactose, maltose, mannose, raffinose, sucrose, and trehalose. In the API ZYM enzyme assay, alkaline phosphatase, esterase (C4), esterase lipase (C8), leucine, arylamidase, valine arylamidase, cystine arylamidase, trypsin, acid phosphatase, β-galactosidase, α-glucosidase, β-glucosidase, and *N*-acetyl-β-glucosaminidase are positive whereas chymotrypsin, β-glucuronidase, α-mannosidase, and α-fucosidase are negative. Weak reaction for lipase (C14) and variable reactions for naphthol-AS-BI-phosphohydrolase and α-galactosidase are observed.

The peptidoglycan contains ornithine, alanine, glycine, homoserine, and glutamic acid (partially substituted by hydroxyglutamic acid). Cell-wall acyl type is glycolyl. The major menaquinones are MK-12 and MK-13. Predominant cellular fatty acid is $C_{15:0}$ anteiso, followed by $C_{17:0}$ anteiso and $C_{16:0}$ iso.

The strains of this species were isolated by selective medium supplemented by superoxide dismutase and catalase (Takahashi et al., 2003).

Source: the type strain was isolated from soil collected from Aoyama Cemetery, Tokyo, Japan.

DNA G+C content (mol%): 70 (HPLC; type strain).

Type strain: KV-448, NBRC 101801, NRRL B-24468.

Sequence accession no. (16S rRNA gene): AB234025.

Further comments: the specific epithet *Microbacterium terricolae* (*sic*) has been corrected on validation according to Rule 61.

67. **Microbacterium testaceum** (Komagata and Iizuka, 1964) Takeuchi and Hatano 1998b, 745[VP] (*Brevibacterium testaceum* Komagata and Iizuka 1964, 497; *Curtobacterium testaceum* Yamada and Komagata 1972a, 425; *Aureobacterium testaceum* Collins, Jones, Keddie, Kroppenstedt and Schleifer 1983a, 249)

tes.ta′ce.um. L. neut. adj. *testaceum* brick-colored.

The following characteristics are based mainly on Yamada and Komagata (1972b, 1972a), Collins et al. (1980, 1983a), Suzuki and Komagata (1983), Yokota et al. (1993a), and Zlamala et al. (2002b).

Cells are motile, small, slender rods arranged at angles; V forms are observed in young cultures but no branching. In older cultures, rods become shorter but no rod–coccus cycle is seen. Good growth on nutrient agar. Colonies are 1–3 mm in diameter, low convex, circular with entire margins, shiny, and moist. Colonies are orange in color. Obligately aerobic. Catalase-positive. Optimum temperature for growth is 30°C. Gelatin, casein, arginine, and Tweens 20, 40, 60, and 80 are hydrolyzed. Starch is not hydrolyzed. Urease-positive. Arginine decarboxylation is strain-dependent. Nitrate is reduced. Tellurite (0.05%) is reduced. Acid is produced from xylose, glucose, fructose, mannose, sucrose, maltose, trehalose, cellobiose, raffinose, starch, and inulin, but not from L-arabinose, rhamnose, galactose, sorbose, lactose, dextrin, glycerol, erythritol, adonitol, mannitol, dulcitol, sorbitol, inositol, arbutin, esculin, salicin, or methyl α-glucoside. The following organic acids are assimilated: acetate, pyruvate, L-lactate, D-lactate, malate, succinate, fumarate, 2-oxoglutarate, propionate, butyrate, glycolate, gluconate, and hippurate. The following organic acids are not assimilated: malonate, glutarate, adipate, pimelate, glyoxylate, and uriate.

Cell-wall peptidoglycan contains D-ornithine as the diamino acid, conforming to type B2β (Figure 174). Cell-wall acyl type is glycolyl. Cell-wall sugars are rhamnose and galactose. In addition, some strains contain xylose. Major menaquinone is MK-11, with small amounts of MK-10 and MK-12. The polar lipids consist of diphosphatidylglycerol, phosphatidylglycerol, and diglycosyldiacylglycerol. Cellular fatty acids are predominantly $C_{17:0}$ anteiso, $C_{15:0}$ anteiso, and $C_{16:0}$ iso.

Source: the type strain was isolated from rice paddy.

DNA G+C content (mol%): 67.7 (HPLC; type strain).

Type strain: ATCC 15829, BCRC 12120, CCM 2299, CCUG 23849, CIP 104324, DSM 20166, IAM 1561, IMET 10361, JCM 1353, LMG 16344, NBRC 12675, VKM Ac-1019.

Sequence accession no. (16S rRNA gene): X77445.

68. **Microbacterium thalassium** Takeuchi and Hatano 1998a, 981[VP]

tha.las′si.um. N.L. neut. adj. *thalassium* based on Gr. adj. *thalassios* pertaining to the sea.

Cells are nonmotile, irregular, short rods, 0.2–0.5 × 0.6–2.0 µm in size, arranged at angles; V forms but no branching or rod–coccus cycle are observed. Colonies are 2–4 mm in diameter, yellow or yellowish white in color, circular, low convex with entire margins, opaque, and moist. Growth occurs at 28°C (optimal) and but not at 37°C. Good growth occurs in the presence of 2% and 5% NaCl but only weakly in 6.5% NaCl. H_2S and arginine dihydrolase are not formed and Voges–Proskauer test is negative; methyl red

test is positive. Starch, and Tweens 40, 60, and 80 are hydrolyzed. Gelatin and Tween 20 are hydrolyzed weakly. Acid is produced from glucose, mannose, and xylose but is not produced from L-arabinose, galactose, inulin, melezitose, raffinose, rhamnose, ribose, sucrose, or trehalose. Maltose, mannose, mannitol, and gluconate are assimilated but arabinose, *N*-acetylglucosamine, acetate, adipate, caprate, citrate, fumarate, lactate, malate, phenylacetate, and propionate are not.

Cell-wall peptidoglycan contains ornithine, homoserine, glutamic acid (hydroxyglutamic acid), glycine, and alanine, corresponding to peptidoglycan type B2β (Schleifer and Kandler, 1972). Cell-wall sugars of the type strain are galactose and glucose, whereas those of another strain are rhamnose, mannose, galactose, and glucose. Major menaquinones are MK-11 and MK-12. Predominant cellular fatty acids are $C_{15:0}$ anteiso, $C_{16:0}$ iso, $C_{15:0}$ iso, and $C_{17:0}$ anteiso.

Source: the type strain was isolated from soil from mangrove rhizosphere.

DNA G+C content (mol%): 69.1–69.7 (HPLC; type strain).

Type strain: CIP 105728, DSM 12511, JCM 12079, NBRC 16060, VKM Ac-2084.

Sequence accession no. (16S rRNA gene): AM181507.

69. **Microbacterium trichothecenolyticum** (Yokota, Takeuchi, Sakane and Weiss 1993a) Takeuchi and Hatano 1998b, 745[VP] (*Aureobacterium trichothecenolyticum* Yokota, Takeuchi, Sakane and Weiss 1993a, 562[VP])

tri.cho.the.ce.no.ly′ti.cum. N.L. n. *trichothecenum* trichothecene, a mycotoxin produced by the fungus *Trichothecium roseum*; N.L. neut. adj. *lyticum* (from Gr. neut. adj. *lutikon*) able to loosen, able to dissolve; N.L. neut. adj. *trichothecenolyticum* trichothecene decomposing.

The following characteristics are based mainly on the data of Yokota et al. (1993a).

Cells are nonmotile rods arranged at angles; V forms are observed. Colonies are 1–3 mm in diameter, circular, low convex with entire margins, opaque, and moist. Yellow pigment is produced. Aerobic. Optimum temperature for growth is 28°C. Weak growth occurs in the presence of 2% NaCl. Oxidative. Starch, and Tweens 20, 40, 60, and 80 are hydrolyzed. Gelatin is not hydrolyzed. Nitrate is reduced. Voges–Proskauer and methyl red tests are negative. Urease-positive and arginine decarboxylase-negative. Produces H_2S. Acetate, malate, succinate, and fumarate are assimilated. DL-Lactate, propionate, and hippurate are assimilated weakly. Formate, citrate, and oxalate are not assimilated.

Cell-wall peptidoglycan contains ornithine, homoserine, glutamic acid plus hydroxyglutamic acid, glycine, and alanine, corresponding to peptidoglycan type B2β (Figure 174). A small amount of lysine is also detected. Cell-wall acyl type is glycolyl. Cell-wall sugars are galactose and glucose. Major menaquinone is MK-13; MK-12 and MK-14 are present in moderate amounts. The polar lipids are diphosphatidylglycerol and phosphatidylglycerol. An unidentified glycolipid is also present. Cellular fatty acids are predominantly $C_{15:0}$ anteiso and $C_{17:0}$ anteiso.

The type strain is reported to decompose trichothecene mycotoxin (Nakayama et al., 1980).

Source: the type strain was isolated from soil.

DNA G+C content (mol%): 69.0 (HPLC; type strain).

Type strain: ATCC 51475, CIP 103817, DSM 8608, JCM 1358, LMG 16696, NBRC 15077, VKM Ac-1948.

Sequence accession no. (16S rRNA gene): Y17240.

70. **Microbacterium ulmi** Rivas, Trujillo, Sánchez, Mateos, Martínez-Molina and Velázquez 2004b, 516[VP]

ul′mi. L. gen. fem. n. *ulmi* of the elm tree.

Cells are nonmotile rods with coryneform morphology. Colonies are convex, smooth, opaque, white, and 1–3 mm in diameter within 7 d of cultivation at 28°C. Aerobic or facultatively anaerobic. Growth occurs between 15 and 37°C and between pH 5 and 8. Catalase- and oxidase-negative. Arginine dihydrolase, ornithine decarboxylase, lysine decarboxylase, tryptophan deaminase, and urease are not produced. Esculin, casein, and gelatin are hydrolyzed. Nitrate is not reduced. D-Arabinose, carboxymethylcellulose, cellobiose, D-fructose, gentiobiose, maltose, mannitol, D-mannose, phenylacetate, starch, and xylan are utilized as sole carbon sources. Does not utilize L-arabinose, malate, *N*-acetyl-D-galactosamine, or citrate as sole carbon sources.

The cell-wall peptidoglycan contains D-ornithine as the diagnostic amino acid and possible structure is type B2β (Figure 174). Cell-wall sugars are galactose, fucose, xylose, and rhamnose. The menaquinones are predominantly MK-12 and MK-13, with minor amounts of MK-11 and MK-14, and trace amounts of MK-10. Predominant cellular fatty acids are $C_{15:0}$ anteiso, $C_{17:0}$ anteiso, and $C_{16:0}$ iso.

The type strain was isolated by enrichment in the medium containing 0.7% xylan. In addition to xylanolytic activity, this strain possesses phosphate-solubilizing activity (Rivas et al., 2004b).

Source: the type strain was isolated from sawdust of a decayed tree of *Ulmus nigra*.

DNA G+C content (mol%): 69 (T_m; type strain).

Type strain: XIL02, CECT 5976, JCM 14282, LMG 20991.

Sequence accession no. (16S rRNA gene): AY062021.

71. **Microbacterium xylanilyticum** Kim, Park, Park, Kim and Lee 2005, 2077[VP]

xy.la.ni.ly′ti.cum. N.L. n. *xylanum* xylan, a plant polymer; N.L. neut. adj. *lyticum* (from Gr. neut. adj. *lutikon*) able to loose, able to dissolve; N.L. neut. adj. *xylanilyticum* xylan-dissolving.

Cells are nonmotile rods showing primary branching. Colonies are yellowish, rough, slightly convex, and circular with irregular margins. Aerobic. Growth occurs at 15–37°C (optimum at 25–30°C), at pH 6–9 (optimum at pH 6–8), and in up to 4% (w/v) NaCl. Catalase- and oxidase-positive. Indole and H_2S are not produced. The methyl red test is negative, but the Voges–Proskauer test is positive. Nitrate is reduced to nitrite but nitrite is not reduced. Esculin, starch, and xylan are hydrolyzed but casein, cellulose, and urea are not. Acid is produced from glycerol, D-arabinose, L-arabinose, ribose, D-xylose, galactose, glucose, fructose, mannose, mannitol, methyl α-D-glucoside, amygdalin, esculin, salicin, cellobiose, maltose, lactose, melibiose, sucrose, trehalose, melezitose, raffinose, starch, glycogen, and D-turanose but not from erythritol, L-xylose, adonitol, methyl β-D-xyloside,

sorbose, rhamnose, dulcitol, inositol, sorbitol, methyl α-D-mannoside, *N*-acetylglucosamine, arbutin, inulin, xylitol, gentiobiose, D-tagatose, D-fucose, L-fucose, D-arabitol, L-arabitol, gluconate, 2-ketogluconate, or 5-ketogluconate. The compounds utilized as sole carbon sources are mannitol, *N*-acetylglucosamine, D-glucose, D-ribose, D-melibiose, D-sucrose, maltose, L-arabinose, DL-lactate, histidine, glycogen, and L-proline but not rhamnose, salicin, inositol, L-fucose, D-sorbitol, itaconate, propionate, suberate, caprate, malonate, valerate, acetate, citrate, malate, L-alanine, 2-ketogluconate, 5-ketogluconate, 3-hydroxybutyrate, 4-hydroxybenzoate, 3-hydroxybenzoate, or L-serine. In the API ZYM assay, L-leucyl 2-naphthylamide, 2-naphthyl phosphate (pH 5.4), 6-bromo-2-naphthyl α-D-galactopyranoside, 2-naphthyl α-D-glucopyranoside, and 2-naphthyl α-L-fucopyranoside are hydrolyzed, but 2-naphthyl phosphate (pH 8.5), 2-naphthyl butyrate, 2-naphthyl caprylate, 2-naphthyl myristate, L-valyl 2-naphthylamide, L-cystyl 2-naphthylamide, *N*-benzoyl-DL-arginine 2-naphthylamide, *N*-glutaryl-phenylalanine 2-naphthylamide, naphthol-AS-BI-phosphate, 2-naphthyl β-D-galactopyranoside, naphthol-AS-BI-β-D-glucuronide, 6-bromo-2-naphthyl β-D-glucopyranoside, 1-naphthyl *N*-acetyl-β-D-glucosaminide, and 6-bromo-2-naphthyl α-D-mannopyranoside are not hydrolyzed.

The cell-wall peptidoglycan is type B2β: [L-Hsr]–D-Glu–Gly–D-Orn. The cell-wall acyl type is glycolyl. The cell-wall sugars are glucose and galactose. The major menaquinone is MK-12; MK-11 and MK-13 are present in moderate amounts. Main polar lipids are diphosphatidylglycerol, phosphatidylglycerol, and unknown polar lipids including glycolipids and phospholipids. Cellular fatty acids contain a large proportion of $C_{15:0}$ anteiso, followed by $C_{16:0}$ iso and $C_{17:0}$ anteiso.

The type strain was isolated by enrichment culture for xylan degradation; cultivation was carried out on R2A agar supplemented with 2.5% (w/w) insoluble chromogenic xylan.

Source: the type strain was isolated from biofilm of a membrane bioreactor for wastewater treatment.

DNA G+C content (mol%): 69.7 (HPLC; type strain).

Type strain: S3-E, DSM 16914, JCM 13591, KCTC 19079.

Sequence accession no. (16S rRNA gene): AJ853908.

Genus II. **Agreia** Evtushenko, Dorofeeva, Dobrovolskaya, Streshinskaya, Subbotin and Tiedje 2001, 2077[VP]

Lyudmila I. Evtushenko

Ag.re′i.a. N.L. fem. n. *Agreia* named in honor of Nina S. Agre, a Russian microbiologist.

Irregular, slender rods (mean 0.4–0.6 × 1.5–2.5 µm). Some cells are arranged at an angle to each other to give V-formations. **Nonsporeforming**. **Gram-stain-positive** in terms of cell-wall composition, but the cells are **readily decolorized**. Non-acid-fast. **Chemo-organotrophs**, having a respiratory type of metabolism. **Obligately aerobic; catalase-positive**. Acid is produced from some carbohydrates oxidatively. Nutritionally fastidious. Growth is moderate to good on standard laboratory media based on peptone, yeast extract, and glucose at near neutral pH. Colonies are typically yellow or yellow-orange and may become red-orange or brown-orange with age. Mostly mesophilic with a growth optimum at ~24–26°C.

The cell-wall peptidoglycan **contains L-diaminobutyric acid and D-ornithine** as diamino acids; **glutamic acid is 3-hydroxylated to a high degree.** Glycan moiety of the peptidoglycan is acetylated. Menaquinones are the sole respiratory quinones; the major component is an unsaturated **menaquinone with 10 isoprene units (MK-10), followed by MK-11 and/or MK-9.** Predominant fatty acids are $C_{15:0}$ anteiso and $C_{17:0}$ anteiso; **1,1-dimethoxy-anteiso-pentadecane ($C_{15:0}$ anteiso DMA) is present.** Mycolic acids are lacking. The principal polar lipids detected are phosphatidylglycerol, diphosphatidylglycerol, and a characteristic glycolipid. Spermine is the single major compound in the polyamine pattern. Commonly associated with plants and related sources.

DNA G+C content (mol%): 65–67.

Type species: **Agreia bicolorata** Evtushenko, Dorofeeva, Dobrovolskaya, Streshinskaya, Subbotin and Tiedje 2001, 2077[VP].

Further descriptive information

The genus *Agreia* belongs to the family *Microbacteriaceae*, the order *Micrococcales*. Strains of the genus show the highest 16S rRNA gene sequence similarities (up to ~97%) with *Okibacterium* and *Subtercola*, and form a clade with *Subtercola* species. (Figure 173). The close relatedness of *Agreia* and *Subtercola* is also supported by results of housekeeping gene analyses (Stackebrandt et al., 2007). The type strains of two recognized *Agreia* species, *Agreia bicolorata* and *Agreia pratensis*, exhibit 99.6% 16S rRNA gene sequence similarity and 47.8% DNA–DNA relatedness (Schumann et al., 2003).

Visible growth is observed after 2–3 d on *Corynebacterium* medium CB (DSMZ medium 53), and modified *Corynebacterium* medium MCB (Casamino acids, 3 g; peptone, 5 g; beef extract, 2 g; yeast extract, 5 g; glucose, 5 g; NaCl, 5 g; agar, 15 g; tap water, 1 liter; pH 7.0) at 24–26°C. Colonies are round, glistening, opaque, and sometimes fluid on certain media due to production of abundant polysaccharide slime (*Agreia bicolorata*). The colony color is usually yellow to orange; *Agreia bicolorata* may become red-orange to brown-orange with age (5–7 d) on some media (e.g. MCB-agar). The determined pigment is of the carotenoid nature (Trutko et al., 2005). The inhibition of pigmentation by fosmidomycin at the concentration lower than those inhibitory for growth suggests the nonmevalonate pathway of pigment biosynthesis (Kuzuyama, 2002; Lichtenthaler, 2000; Trutko et al., 2005). Various carbon sources are used for growth and energy. None of the strains

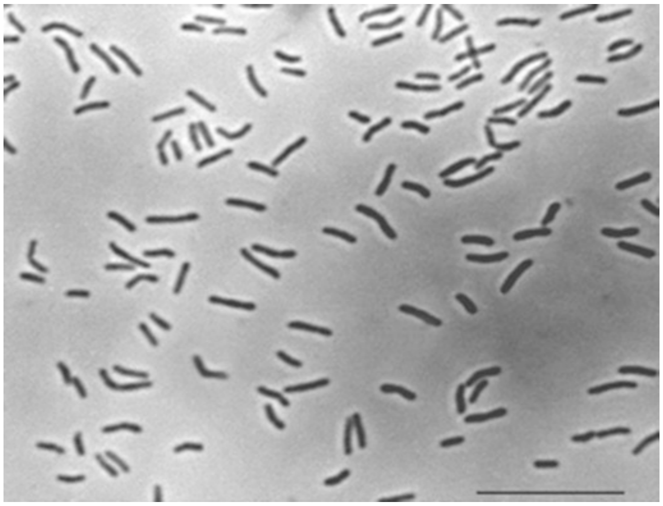

FIGURE 176. Cellular morphology of *Agreia bicolorata*, 2-d-old slide culture on CB agar. Phase-contrast microscopy. Bar = 10 µm. (Courtesy of N.E. Suzina.)

examined so far decompose starch, casein, gelatin, DNA, cellulose, hypoxanthine, and xanthine.

Cells are slender irregular rods under light microscopy, 0.4–0.6 µm in diameter × 1.5–2.5 µm or occasionally even longer, sometimes slightly curved. Some of the rods are arranged at an angle to each other giving V-formations (Figure 176). Branching forms occasionally occur, and longer cells might be composed of divided but not separated cells due to capsular material like that in *Rathayibacter* species. In older cultures, cells are usually shorter; they are single or may be arranged in pairs or short chains. Some cells show a weak but distinct motility, however, no flagella were observed. The electron microscopic study revealed the cell-wall structure typical of Gram-stain-positive bacteria, but the test with Gram-staining is indistinct or negative; KOH test also shows a reaction typical of Gram-stain-negative bacteria.

The unusual cell-wall peptidoglycan of group B-type contains two diamino acids, diaminobutyric acid (L-Dab) and D-ornithine (D-Orn), in equimolar amounts, along with alanine, glycine, glutamate, and 3-hydroxylated glutamate (Behrendt et al., 2002; Evtushenko et al., 2001; Schumann et al., 2003). The molar ratios of glutamate and 3-hydroxyglutamate vary in different strains or experiments, but 3-hydroxyglutamate always significantly predominates. The L-Dab supposedly occupies position 3 of the peptide subunit and D-Orn is present in the bridge (Schumann et al., 2003). Muramic acid residues of the peptidoglycans of both *Agreia pratensis* and *Agreia bicolorata* are acetylated (Schumann et al., 2003), as determined according to Uchida et al. (1999). Cell-wall sugars determined in three strains of *Agreia bicolorata* are rhamnose, mannose, and fucose.

Oxidase reaction applying tetramethyl-*p*-phenylenediamine varies between species or strains. Three terminal oxidases, the bb_3- and aa_3- type cytochrome oxidases and nonheme cyanide-resistant oxidase, were revealed in the respiratory chain of *Agreia bicolorata* in varying proportions depending on the strain, the growth medium, and growth phase at which biomass was harvested, and the oxygen concentration. Under oxygen deficiency, the *bd*-oxidase is additionally produced (Trutko et al., 2003). The major respiratory menaquinone is MK-10, with lower amounts of MK-11 and/or MK-9. The predominant fatty acids are $C_{15:0}$ anteiso (30–49%), $C_{17:0}$ anteiso (14–23%), and $C_{16:0}$ iso (17–41%) as determined for cells grown in different media. In addition, 1,1-dimethoxy-anteiso-pentadecane ($C_{15:0}$ anteiso-DMA) is present constituting more than 4% of the fatty compounds (Schumann et al., 2003). Principal polar lipids, as recently determined for *Agreia bicolorata*, include phosphatidylglycerol, diphosphatidylglycerol, and a characteristic glycolipid co-migrating with the glycolipid G_2 as designated by Collins and Jones (1980) and with the same staining characteristics. In addition, a few unknown α-naphthol-positive glycolipids in trace amounts were revealed. It is worth noting that the glycolipid G_2 was also found, along with other glycolipids, in representatives of several genera of the family *Microbacteriaceae* including *Agromyces*, *Clavibacter*, *Leifsonia*, *Rathayibacter* (Collins and Jones, 1980), and *Frigoribacterium* (Kämpfer et al., 2000). *Agreia bicolorata* exhibits a polyamine pattern with the single major compound spermine, which is quite an unusual feature (H.-J. Busse, unpublished results). Similar polyamine patterns have so far only been detected in representatives of the genera *Agrococcus* and *Plantibacter* species (Altenburger et al., 1997; Schumann et al., 2009; Wieser et al., 1999; Zlamala et al., 2002a).

Strains of *Agreia bicolorata* are sensitive to the following antibiotics (10 µg/ml): ampicillin, doxycycline, gentamicin, penicillin, rifampin, and tetracycline; they are resistant to neomycin and streptomycin. A representative of the genus, strain "*Subtercola pratensis*" beet 1-3-7 (AJ969075), was reported to possess antifungal activity against a plant pathogen, *Pythium ultimum* (Zachow et al., 2008).

Members of the genus *Agreia* are primarily associated with plants and plant-derived materials; some may be endophytes. Strains of *Agreia pratensis* were isolated from grass phyllosphere. *Agreia bicolorata* and some related strains were recovered from leaf galls induced by the plant parasitic gall-forming nematode *Heteroanguina graminophila* (the subfamily *Anguininae*) on the nematode plant host, *Calamagrostis neglecta*. A single *Agreia bicolorata* strain was also found in the stem gall induced by *Anguina agropyri* on *Elymus repens* sampled at the same site. The bacteria might be nematode-vectored like *Rathayibacter* species (Bird, 1981; Bradbury, 1986; Riley et al., 2001) or represent a secondary bacterial infection of galls, descending from phyllosphere or healthy plant tissue. Two yellow-pigmented bacteria, strains VKM Ac-2052 and VKM Ac-1783, which were isolated from plant galls induced by the nematode *Heteroanguina graminophila* on *Calamagrostis neglecta* and *Anguina agropiry* on *Elymus repens*, respectively (Evtushenko et al., 1994; Evtushenko and Takeuchi, 2006), most likely represent two novel species of the genus *Agreia*, as might be supposed from the 16S rRNA gene sequence comparison and phenotypic characteristics. A few strains belonging to the genus *Agreia* were isolated from internal parts of sugar beet body (Zachow et al., 2008), soil (Davis et al., 2005), permafrost (Vishnivetskaya et al., 2006), and some other environments. Information concerning these and several other environmental isolates is limited, but some might also prove to be representatives of novel species of the genus. No bacteria pathogenic for warm-blooded animals and humans or associated with clinical specimens have been identified within this genus up to now.

Enrichment and isolation procedures

Bacteria of the genus *Agreia* can be isolated on MCB agar, PYGV agar (Staley, 1968), R2A agar (Difco), and other media based on glucose, peptone, and yeast extract commonly used for isolation of plant-associated actinobacteria. Various approaches and techniques, e.g. described by Dunleavy (1989), Behrendt et al. (1997), Zinniel et al. (2002), might be applied. For isolation of bacteria from plant galls, the following procedure may be used. The galls are detached from a plant with a sterile scalpel, treated by a detergent or/and surface sterilized, rinsed by sterile water, and pre-incubated in water or in tenfold-diluted nutrient broth (e.g. MCB broth) for 1–2 h. Then the galls are cut into pieces, transferred to 1–2 ml of 0.85% solution of NaCl (w/v), ground with a pestle, and the suspensions are plated onto appropriate media. The following isolation medium was used for isolation of *Agreia bicolorata*: 2 g peptone, 1 g glucose, 1 g yeast extract, 1 g casein peptone, 10 ml glycerol, 5 g chalk, 100 ml wort, 15 g agar, 900 ml distilled water, pH 7.2–7.4.

Maintenance procedures

Agreia strains can be maintained on CB agar or R2A agar for up to 2 months at 10°C. For long-term preservation, strains can be lyophilized by standard procedures. The three strains of *Agreia bicolorata* lyophilized in skim milk as a cryoprotectant have been safely maintained for more than 15 years since the isolation. Various agar media mentioned in the above section can be used for subculturing.

Differentiation of the genus *Agreia* from other genera

Phenotypic characteristics that delineate *Agreia* from other genera of the family *Microbacteriaceae* are listed in Table 140. The unique composition of peptidoglycan containing L-Dab and D-Orn as the diamino acids along with a high proportion of 3-hydroxylated glutamate, is the most striking chemotaxonomic marker of *Agreia*. The detection of DMA among the fatty acids is another feature delineating *Agreia* from other genera including phylogenetically neighboring *Subtercola*. In particular, both *Agreia bicolorata* and *Agreia pratensis* grown at 25°C were reported to have exclusively $C_{15:0}$ anteiso DMA (up to 4.2% of the total fatty acids), whereas the *Subtercola* species contain a higher content of this compound (10–11%) and, in addition, $C_{16:0}$ iso DMA (11.9–13.3%) and $C_{17:0}$ anteiso DMA (2.9–4.0%) (Männistö et al., 2000; Schumann et al., 2003).

Differentiation of the species of the genus *Agreia*

In contrast to the permanently yellow-pigmented *Agreia pratensis*, colonies of *Agreia bicolorata* are yellow-orange pigmented, often becoming red-orange to brown-orange with age (e.g. on MCB medium), and usually produce abundant polysaccharide slime. The cells of *Agreia bicolorata* are oxidase-positive, while *Agreia pratensis* shows negative oxidase reaction applying tetramethyl-*p*-phenylenediamine. The major menaquinone of *Agreia bicolorata* is MK-10 with MK-9 as a minor component and traces of MK-11 and MK-8, whereas the quinone system of *Agreia pratensis* was reported to consist of MK-10 (51%), MK-11 (21%), and MK-9 (13%). In addition, *Agreia bicolorata* displays higher tolerance to fosmidomycin (0.5 mg/ml), and pigment formation is not suppressed by 0.3 mg/ml of the antibiotic, whereas *Agreia pratensis* loses the pigmentation at a substantially lower (0.05 mg/ml) fosmidomycin concentration (Trutko et al., 2005). DNA–DNA hybridization tests or analyses of other adequate genomic characteristics, e.g. the comparison of housekeeping gene sequences (*gyrB*, *rpoB*, *recA*, and *ppk*) available for *Agreia bicolorata* (Stackebrandt et al., 2007) is expedient to support affiliation of an isolate with *Agreia bicolorata* or *Agreia pratensis*, or obtain evidence for its possible assignment to a new species.

Acknowledgements

This work was supported by the program MCB of the Russian Academy of Sciences. The author is grateful to Dr Hans-Jürgen Busse for sharing unpublished information on the polyamine composition of *Agreia*.

List of species of the genus *Agreia*

1. **Agreia bicolorata** Evtushenko, Dorofeeva, Dobrovolskaya, Streshinskaya, Subbotin and Tiedje 2001, 2077[VP]

bi.co.lo.ra′ta. L. adv. num. *bis* twice; L. part. adj. *coloratus -a -um* coloured; N.L. fem. part. adj. *bicolorata* two-coloured.

The characteristics are as described for the genus with the following additional information. Young cells are irregular rods (0.4–0.5 × 1.5–2.5 µm). Some cells display a weak but distinct motility, though flagella are not observed. Liquid cultures usually become viscous with age (4–7 d) in CB medium. Oxidase and catalase-positive. L-Arabinose, D-glucose, D-galactose, cellobiose, fructose, inositol (weak), inulin, lactose, maltose, mannose, mannitol, melibiose, raffnose, L-rhamnose, ribose, salicin, sorbitol, trehalose, turanose, or D-xylose are used as a carbon source for growth and energy on basal medium (Shirling and Gottlieb, 1966) supplemented with 0.1% (w/v) of yeast extract and 0.1% (w/v) of casitone. Adonitol, dextran, lyxose, *meso*-erythritol, or sorbose are not used for growth on the same basal medium. Alkaline reaction is observed with citrate, fumarate, gluconate (weak), 2-oxoglutarate, and malate, but negative with oxalate or tartrate applying bromocresol purple as a pH indicator (for the method, see Zgurskaya et al., 1993). Weak growth is observed only with a few amino acids (DL-leucine, DL-methionine, or DL-proline) used as a nitrogen source. H_2S is produced from peptone. Tween 40, Tween 80, and esculin are hydrolyzed, but starch, hypoxanthine, and xanthine are not. Sensitive to 6% NaCl. Rhamnose is the major cell-wall sugar; mannose, and fucose are present in minor amounts (molar ratio ~7:1:0.5 for the type strain).

Source: the leaf gall induced by the nematode *Heteroanguina graminophila* on *Calamagrostis neglecta*, Moscow region, Russia.

DNA G+C content (mol%): 67 (T_m).

Type strain: DL-4, DSM 14575, JCM 12206, NBRC 103053, VKM Ac-1804.

Sequence accession no. (16S rRNA gene): AF159363, AM410672.

2. **Agreia pratensis** (Behrendt et al., 2002) Schumann, Behrendt, Ulrich and Suzuki 2003, 2043[VP] (*Subtercola pratensis* Behrendt, Ulrich, Schumann, Naumann and Suzuki 2002, 1452)

pra.ten′sis. L. fem. adj. *pratensis* found in meadows/grassland.

The characteristics are as described for the genus with the following additional information based on the data of Behrendt et al. (2002), Schumann et al. (2003), and own recent morphological observations.

Young cells are irregularly shaped rods (0.4–0.5 × 1.5–2.3 µm). Colonies are yellow, shiny, slightly convex, and circular, with entire margins. Strictly aerobic. Catalase-positive and oxidase-negative. The optimum growth temperature is about 25°C; growth occurs at 4°C (slow) and at −2°C (very slow). In the Biolog test systems, L-arabinose, D-fructose, D-galactose, D-glucose, inulin, D-maltose, D-mannitol, D-mannose, raffinose, sucrose, D-trehalose, ribose, and D-xylose are oxidized. Weak reactions are observed with melibiose and are strain-dependent with inositol (type strain negative) and L-rhamnose (type strain positive). Reaction is negative with adonitol, lactose, D-melezitose, D-sorbitol, and L-sorbose. Esculin, Tween 60, and Tween 80 are hydrolyzed. None of the tested strains hydrolyses starch, casein, gelatin, DNA, or cellulose. Voges–Proskauer test and formation of levan from sucrose are negative.

Source: the phyllosphere of grasses.

DNA G+C content (mol%): 65 (HPLC).

Type strain: P 229/10, DSM 14246, JCM 12145, LMG 21000, NBRC 103054, VKM Ac-2510.

Sequence accession no. (16S rRNA gene): AJ310412.

Genus III. **Agrococcus** Groth, Schumann, Weiss, Martin and Rainey 1996, 239[VP]

Peter Kämpfer and Hans-Jürgen Busse

Ag.ro.coc′cus. Gr. n. *agros* field or soil; N.L. masc. n. *coccus* (from Gr. masc. n. *kokkos* grain, seed) coccus; N.L. masc. n. *Agrococcus* a coccus from soil.

Irregular, spherical, ovoid or short rods, 0.7–1.0 µm in diameter and 1.1–1.7 µm in length. Cells occur singly and in pairs, short flexible chains, or small irregular clusters. Nonsporeforming. **Gram-stain-positive** and **nonmotile. Aerobic** or **microaerobic**. Colonies are circular, convex, and smooth; the pigmentation varies between white, cream, yellow, and orange depending on culture conditions. Colony size ranges from 2–7 mm. Catalase-positive; oxidase-negative.

The diagnostic **cell-wall diamino acid** is **diaminobutyric** acid and the **acyl type** is **acetyl**. The major respiratory quinones are **menaquinone MK-10, or MK-10 and MK-11, or MK-11 and MK-12**. The predominant **fatty acids** are **iso-** and **anteiso-branched acids** (>96%), with the major compounds being $C_{15:0}$ anteiso, $C_{17:0}$ anteiso, $C_{16:0}$ iso, and $C_{15:0}$ iso. **Spermidine** is the only abundant **polyamine** (Altenburger et al., 1997; Wieser et al., 1999; Zlamala et al., 2002a).

Phylogenetically, this genus is a member of the family *Microbacteriaceae*.

DNA G+C content (mol%): 65–75.

Type species: **Agrococcus jenensis** Groth, Schumann, Weiss, Martin and Rainey 1996, 239[VP].

Further descriptive information

The genus *Agrococcus* and its type species *Agrococcus jenensis* were described to include two strains containing diaminobutyric acid (DAB) in their cell wall; they were isolated during a study of actinomycetes that produce bioactive compounds (Groth et al., 1996). Six additional species were subsequently described (Table 142). *Agrococcus citreus* was isolated from a medieval wall painting in the chapel of castle Herberstein in Styria, Austria (Wieser et al., 1999). *Agrococcus baldri* was collected from the air within the "Virgilkapelle" underneath the "Stephansplatz" in Vienna, Austria (Zlamala et al., 2002a). *Agrococcus lahaulensis* was isolated from a soil sample from Lahaul-Spiti Valley, Himalayas, India (Mayilraj et al., 2006). *Agrococcus casei* was isolated from smear-ripened cheeses (Bora et al., 2007). *Agrococcus jenjuensis* (AM396260) was isolated from dried seaweed found at Samyang Beach, Jeju, Republic of Korea (Lee, 2008). *Agrococcus versicolor* was isolated from the phyllosphere of potato plants in Dahnsdorf, Germany (Behrendt et al., 2008a). Phylogenetically, the genus is a member of the family *Microbacteriaceae* (Groth et al., 1996). The seven species share more than 95% 16S rRNA gene sequence similarity and several characteristic which support their affiliation in a single genus. Their peptidoglycan contains the diamino butyric acid (DAP). The peptidoglycans of all species except *Agrococcus casei* also contain the amino acids alanine, glycine, aspartate, and glutamate. The peptidoglycans of *Agrococcus jenensis*, *Agrococcus versicolor*, *Agrococcus jejuensis*, and *Agrococcus lahaulensis* also contain threonine. *Agrococcus casei* exhibits the most unique peptidoglycan lacking both of the amino acids aspartate and threonine (Bora et al., 2007). Most species contain a quinone system consisting of the predominant quinones MK-11 and MK-12. *Agrococcus lahaulensis* also contains large amounts of MK-10 (Bora et al., 2007); the quinone system of *Agrococcus versicolor* comprises the major compounds MK-10 and MK-11 and that of *Agrococcus jejuensis* MK-10 and significant amounts of MK-9. The major fatty acids are $C_{15:0}$ anteiso, $C_{17:0}$ anteiso, $C_{16:0}$ iso, and $C_{15:0}$ iso. In addition to some species-specific compounds, the polar lipids are diphosphatidylglycerol, phosphatidylglycerol, and an unknown glycolipid (Bora et al., 2007; Mayilraj et al., 2006; Wieser et al., 1999; Zlamala et al., 2002a). The predominant polyamine is spermine, although so far only *Agrococcus jenensis* (Altenburger

TABLE 142. Characteristics differentiating the genus *Agrococcus* from other actinobacterial cocci

Characteristic	*Agrococcus*[a]	*Arthrobacter agilis*[b]	*Arthrobacter sulfonivorans*[c]	*Citricoccus*[d]	*Demetria*[e]	*Dermacoccus*[f]	*Kineosphaera*[g]	*Kocuria*[h]	*Kytococcus*[i]	*Micrococcus*[j]	*Nesterenkonia*[k]
Peptidoglycan type	B	A	A	A	A	A	A	A	A	A	A
Diamino acid	Diaminobutyric acid	L-Lysine	L-Lysine	L-Lysine	L-Lysine	L-Lysine	*meso*-Diaminopimelic acid	L-Lysine	L-Lysine	L-Lysine	L-Lysine
Quinone system	MK-10 or MK-10, MK-11 or MK-11, MK-12[l]	MK-9(H_2)	MK-9(H_2)	MK-9(H_2)	MK-8(H_4)	MK-8(H_2) [MK-7(H_2), MK-9(H_2)]	MK-8(H_4)	MK-7(H_2) and/or MK-8(H_2)	MK-8 [MK-9, MK-10] or MK-8, MK-7	MK-8, MK-8(H_2) or MK-8(H_2)	MK-7 or MK-8 or MK-7, MK-8 or MK-8, MK-7, MK-9 or MK-8, MK-9
Major fatty acids[l]	**$C_{15:0}$anteiso**, $C_{17:0}$ anteiso, $C_{16:0}$ iso, $C_{15:0}$ iso	**$C_{15:0}$anteiso**, $C_{15:0}$ iso, $C_{17:0}$ anteiso, $C_{16:0}$	**$C_{15:0}$anteiso**, $C_{15:0}$ iso, $C_{17:0}$ anteiso, $C_{16:0}$ iso	**$C_{15:0}$anteiso**, $C_{17:0}$ anteiso, $C_{16:0}$ anteiso, $C_{15:0}$ iso	$C_{18:1}$, $C_{18:0}$, $C_{17:0}$, $C_{17:0}$ anteiso, $C_{16:0}$, $C_{16:0}$ iso	$C_{17:1}$ iso, $C_{17:0}$ iso, $C_{17:0}$ anteiso, $C_{15:0}$ iso, $C_{16:1}$, $C_{16:0}$ iso,	$C_{16:0}$, $C_{17:1}$, $C_{18:1}$, $C_{17:0}$	**$C_{15:0}$anteiso**, ($C_{16:0}$ iso, $C_{15:0}$ iso, $C_{17:0}$ anteiso, $C_{16:0}$, $C_{16:1}$)	$C_{17:1}$iso, $C_{17:0}$ anteiso, $C_{17:0}$ iso, ($C_{17:0}$, $C_{15:0}$ iso, $C_{15:0}$)	**$C_{15:0}$anteiso, $C_{15:0}$iso**, $C_{17:0}$ anteiso, ($C_{16:0}$ iso)	($C_{15:0}$ anteiso, $C_{17:0}$ anteiso, $C_{15:0}$ iso, $C_{15:1}$ anteiso, $C_{16:0}$ iso)
Pigmentation	White, cream, yellow, orange, dark pink or red	Pink	Cream yellow	Yellow	White to pale yellow	Bright orange or yellow	Light yellow	Yellow or orange	Cream-white, or yellow	Colorless or yellow	Colorless or bright yellow or primrose yellow
Characteristic polyamine	Spermine	Spermidine	Unknown	Spermidine	Unknown	Spermidine, spermine	Unknown	Spermidine	Spermidine, spermine	Spermidine	Spermidine

[a]Groth et al. (1996); Wieser et al. (1999); Zlamala et al. (2002a); Mayilraj et al. (2006); and Bora et al. (2007)

[b]Koch et al. (1995); Hamana (1994); Gvozdiak et al. (1998); Kämpfer, unpublished results.

[c]Borodina et al. (2002).

[j]Stackebrandt et al. (1995); Hamana (1994); Gvozdiak et al. (1998); and Wieser et al. (2002).

[h]Stackebrandt et al. (1995); Hamana (1994); Gvozdiak et al. (1998), Kovács et al. (1999); Reddy et al. (2003a); Kim et al. (2004); and Tvrzová et al. (2005).

[k]Stackebrandt et al. (1995); Hamana (1994); Gvozdiak et al. (1998); Collins et al. (2002); Li et al. (2004b, 2005b), Delgado et al. (2006); and Yoon et al. (2006a).

[f]Stackebrandt et al. (1995) ; Hamana (1994); Gvozdiak et al. (1998); and Pathom-aree et al. (2006a, 2006b).

[i]Stackebrandt et al. (1995); Hamana (1994); Gvozdiak et al. (1998); and Becker et al. (2002).

[d]Altenburger et al. (2002) and Li et al. (2005c).

[g]Liu et al. (2002).

[e]Groth et al. (1997).

[l]Sum of relative amounts >80%; compounds shown in bold face are usually present in relative amounts >40%.

et al., 1997), *Agrococcus citreus* (Wieser et al., 1999), and *Agrococcus baldri* (Zlamala et al., 2002a) have been analyzed for this trait. This polyamine pattern is unique within the family *Microbacteriaceae* (Altenburger et al., 1997) and may be a specific marker of the genus *Agrococcus*. Analyses of *Agrococcus jenensis*, *Agrococcus citreus*, and *Agrococcus baldri* using Fourier-transform infra-red (FT-IR) revealed a homogeneous cluster for the five strains of *Agrococcus baldri*, whereas two strains of *Agrococcus jensensis* and the type strain of *Agrococcus citreus* were intermingled (Zlamala et al., 2002a). Thus, FT-IR is of limited suitability to identify *Agrococcus* at the species level. On the other hand, MALDI-TOF analyses have shown that strains of *Agrococcus versicolor* can be reliably identified and distinguished from related species such as *Agrococcus casei*, *Agrococcus baldri*, *Agrococcus citreus*, *Agrococcus lahaulensis*, and *Agrococcus jenensis* (Behrendt et al., 2008a).

Based upon 16S rRNA gene sequences deposited in databases, the following strains reveal higher than 96.0% sequence similarity to one of the type strains of *Agrococcus*. These strains are designated "*Corynebacterium* cf. *aquaticum*" V4.B0.26 (accession no. AJ244681; Fritz, 2000), *Agrococcus* sp. QSSC2–2 (AF170759), *Agrococcus* sp. Bt 15 (AJ971867), bacterium PH2 (AY345361), *Agrococcus* sp. Tibet-IT72 (DQ177486), *Microbacteriaceae* bacterium 4_C7/16_17 (EF540491), *Agrococcus* sp. 8_1Kb (EF540451), *Agrococcus* sp. G2DM-57 (DQ416789), *Agrococcus* sp. DNG5 (FJ423764), *Agrococcus* sp. m8–15 (DQ923165), *Agrococcus* sp. Everest-gws-11 (EU584505), *Agrococcus* sp. ZXM075 (FJ436727), *Agrococcus* sp. MSCB-4 (EF103201), *Corynebacteriaceae* bacterium NR121 (DQ520815), *Agrococcus jenenensis* B16 (EU169180), *Agrococcus jenensis* strain 1RN-3D2 (EU379252), *Agrococcus jenenensis* XJU-1 (EF672044), *Agrococcus jenensis* zf-IIRht16 (DQ223675) *Agrococcus* sp. RCML-30 (FJ005071), and *Agrococcus* sp. Ens24 (DQ339623). These strains were isolated from sea water of the Mediterranean Sea near to Nice, from an Antarctic quartz stone sublithic community, the gut of a bumble bee, Loihi submarine volcano, permafrost at Qinghai-Tibet Plateau, semi-coke, north eastern region of Estonia, a chromium polluted landfill site near a contaminated stream in Gorwa Industrial Estate, Baroda, Gujarat, India (Desai et al., 2009), forest soil, an aquatic system in the valley of Cuatro Cienegas, Coahuila, Mexico, glacial meltwater, Mount Everest, during massive green algae blooms in coastal water of Qingdao, China, snow in Western China, soil sample from north Delhi, India, the rhizosphere soil of *Bashania fangiana*, the Wolong Nature Reserve, Sichuan Province, China, commercial airline cabin air (Osman et al., 2008a), necrotic trunk, China, ice cores, East Rongbuk Glacier, Mt. Qomolangma (Zhang et al., 2006), Xinjiang Province, China, and from alpine subnival plants, respectively. *Microbacteriaceae* bacterium 4_C7/16_17 shares 100% 16S rRNA gene similarity with the type strain of *Agrococcus versicolor* suggesting a relatedness at the species level. *Agrococcus* sp. Tibet-IT72, *Agrococcus jenenensis* B16, and "*Corynebacterium* cf. *aquaticum*" share 99.9%, 99.7%, and 99.6%, respectively, 16S rRNA gene sequence similarity with the type strain of *Agrococcus jenensis* and, hence, they can be considered authentic members of this species. *Agrococcus* sp. MSCB-4, *Agrococcus jenensis* strain 1RN-3D2, and *Agrococcus* sp. Everest-gws-11 most likely are representatives of *Agrococcus citreus* with which they share 99.9–100% 16S rRNA gene similarity. In addition, Tiago et al. (2004) found an *Agrococcus*-like organism, designated *Agrococcus jenensis* AC72 (AJ717350), in nonsaline alkaline groundwater samples in Cabeco de Vide, Portugal. 16S rRNA gene sequence similarity (98.0%) with the type strain of *Agrococcus jenensis* suggests that this strain was misidentified. Also, *Agrococcus jenensis* zf-IIRht16 must be considered to be misidentified because its 16S rRNA gene sequence similarity with the type strain is even lower. Other potential members of the genus of unknown sources are the catechol-degrading *Microbacterium* sp. Atl-19 (EF028128), *Agrococcus* sp. CGMCC 1.3033 (EU660308), and *Agrococcus* sp. CGMCC 1.3033 (EU660307). Recently, an *Agrococcus* strain was also detected in the air above the Atacama Desert in Chile applying a culture-independent approach (Fred Rainey, pers. communication). These results emphasize the very wide distribution of this genus.

Strains of at least two species, *Agrococcus jenensis* and *Agrococcus baldri*, show photochromogenic pigmentation. Strains usually grow well on complex media at 28°C supplemented with 0–4% NaCl (w/v). Their source of isolation (sandstone, wall painting, air, quartz stone) may indicate that at least some members of the genus *Agrococcus* have developed mechanisms to withstand desiccation, but so far this assumption has not been investigated. No specific isolation medium has been described. Characteristics useful for differentiation of species are listed in Table 143.

Pathogenicity. The pathogenic potential of species of this genus has not been reported.

Maintenance procedures

Agrococcus cultures may be lyophilized by procedures commonly used for many aerobic heterotrophic bacteria of the class *Actinobacteria*.

Differentiation of the genus *Agrococcus* from other genera

The genus *Agrococcus* can be distinguished from other genera in the family *Microcbacteriaceae* and other cocci of the order *Micrococcales* by the presence of diamino butyric acid in the cell-wall peptidoglycan, the menaquinone type, and the predominance of the polyamine spermine. Differential features are specified in Table 142. Note added in proof: *Agrococcus* sp. DNG5 (FJ423764) has since been described as *Agrococcus terreus*.

TABLE 143. Characteristics differentiating the species of the genus *Agrococcus*[a,b]

Characteristic	*A. versicolor*	*A. baldri*	*A. casei*	*A. citreus*	*A. jejuensis*	*A. jenensis*	*A. lahaulensis*
Chemotaxonomic:							
Aspartate in the cell wall	+	+	−	+	+	+	+
Threonine in the cell wall	+	−	−	−	+	+	+
Major menaquinones	MK-10, MK-11	MK-11, MK-12	MK-11, MK-12	MK-11, MK-12	MK-9, MK-10	MK-11, MK-12	MK-10, MK-11, MK-12
Utilization of:							
N-Acetyl-D-galactosamine, 4-aminobutyrate,	nd	+	nd	−	nd	−	nd
L-Arabinose, D-xylose	nd	−(+)[c]	nd	+	nd	−(+)[c]	+
D-Fructose	nd	+	nd	+	nd	−(+)[c]	+
D-Glucose, D-mannose, D-maltose	nd	+	nd	+	+	−	nd
L-Ribose, L-rhamnose, L-proline	nd	+	nd	+	nd	−	nd
D-Gluconate	nd	+	nd	+	−	−	nd
D-Cellobiose, D-trehalose,	nd	+(−)[c]	nd	+(−)[c]	nd	−	−
Hydrolysis of:							
pNP[d] -β-D-glucopyranoside	nd	−	nd	+	nd	+	nd
Decomposition of:							
Esculin	+	−(+)[e]	−	+	+	+	−
Tributyrin		+	−	+		+	nd
Tween 20	nd	−	−	+(−)[e]		+	+
Tween 80	nd	− (+)[e]	+	−(+)[e]	−	−	nd
Acid production from:							
L-Arabinose	nd	−	nd	+	−	+(−)[c]	−
Cellobiose	nd	+	−	3		+	−
Fructose	nd	−	−	+	+	+	+
Glucose	nd	−(+)[e]	+	−(+)[e]	+	−	nd
Maltose	nd	v	−	−(+)[e]	+	−	−
Mannitol	nd	+	−	+	−	+	−
Mannose	nd	−	+	−	+	−	nd
Sucrose	nd	+	−	+(−)[e]	+	+(−)[e]	−
Trehalose	nd	−(+)[e]	−	−(+)[e]	+	−	+
Oxidation of carbon sources (Biolog GN microplates):[f]							
N-Acetyl-D-glucosamine, sucrose	+	−	−	−	nd	−	−
L-Alaninaminde	+	−	−	−	nd	+	−
L-Arabinose, D-fructose, D-xylose	+	+	−	+	nd	+	+
2′-Deoxyadenosine	−	−	+	−	nd	+	+
Dextrin, maltotriose	+	−	−	−	nd	−	+
D-Fructose 6-phosphate	−	−	−	+	nd	−	−
D-Gluconic acid, D-mannose	+	+	−	+	nd	+	−
α-D-Glucose	+	+	−	−	nd	+	−
Glycerol, adenosine	+	+	+	−	nd	−	+
α-Ketovaleric acid, propionic acid	w	−	w	−	nd	−	−
D-Psicose	+	+	+	+	nd	+	−
L-Rhamnose, inosine, D-fructose 6-phosphate	−	−	−	−	nd	−	+
D-Sorbitol	−	+	w	−	nd	−	−
D-Tagatose	w	+	−	−	nd	−	−
Thymidine	−	+	+	−	nd	−	+
D-Trehalose	+	−	w	−	nd	−	−
Turanose	+	−	−	+	nd	−	−
Tweens 40 and 80, methyl pyruvate, *N*-acetyl-L-glutamic acid	−	−	+	−	nd	−	−

[a]Symbols: +, positive; −, negative; v, variable; w, weakly positive; nd, not determined.

[b]Zlamala et al. (2002a); Mayilraj et al. (2006); and Bora et al. (2007).

[d]pNP, *p*-nitrophenyl.

[c]Values in brackets indicate differing results reported by Mayilraj et al. (2006).

[e]Values in brackets indicate differing results reported by Bora et al. (2007).

[f]Only type strains were examined (Behrendt et al., 2008a).

List of species of the genus *Agrococcus*

1. **Agrococcus jenensis** Groth, Schumann, Weiss, Martin and Rainey 1996, 239[VP]

je.nen′sis. N.L. masc. adj. *jenensis* of or belonging to the Thuringian town Jena, where the organism was isolated.

Cells are irregular, spherical or ovoid to short rods, 0.7–1.0 × 0.7–1.7 µm. A few cells may be up to 2.6 µm long. Cells occur singly, in pairs, in short flexible chains of about 3–6 cells, or small irregular clusters. Colonies are circular, slightly convex, and smooth. Colony diameter ranges from 2–4 mm. The color of the colonies varies depending on culture conditions and illumination from whitish to yellow or intense orange. Nitrate is not reduced to nitrite; hydrogen sulfide is produced. Starch and esculin are hydrolyzed. Hydrolysis of casein is variable or negative, depending on the method applied (Zlamala et al., 2002a). Acids are produced from fructose, mannitol, L-rhamnose, and D-glucitol. Acid production from sucrose, glycerol, and inulin is variable, depending on the method applied (Zlamala et al., 2002a). Succinate is utilized as a carbon source. Malate utilization is positive or negative, depending on the method applied (Zlamala et al., 2002a). Acetate, aconitate, benzoate, citrate, formate, and tartrate are not utilized. Urea, xanthine, hypoxanthine, gelatin, adenine, tyrosine, and Tween 80 are not decomposed. Tyrosine and hippurate decomposition is negative or variable, depending on the method applied (Zlamala et al., 2002a). Cells are susceptible to ampicillin, chloramphenicol, ciprofloxacin, erythromycin, gentamicin, kanamycin, lincomycin, neomycin, nitrofuran, oxacillin, oxytetracyclin, penicillin G, polymyxin B, rifampin, and streptomycin. Cells are resistant to sulfonamide.

The predominant quinones are MK-11 and MK-12. The branched fatty acids $C_{15:0}$ anteiso, $C_{17:0}$ anteiso, $C_{16:0}$ anteiso, and $C_{15:0}$ iso predominate. The polar lipids comprise diphosphatidylglycerol, phosphatidylglycerol, two unknown glycolipids, and one unknown phospholipid. Additionally, three unknown lipids and two yellow pigment spots are visible after two-dimensional thin-layer chromatography (Wieser et al., 1999). The polyamine pattern is characterized by the predominance of spermine (Altenburger et al., 1997). The cell wall contains the amino acids alanine, glycine, threonine, diaminobutyrate, aspartate, and glutamate. The acyl type is acetyl. Whole-cell sugars are glucose, rhamnose, and minor amounts of ribose, mannose, galactose, and an unknown compound. Mycolic acids are absent.

Source: soil and sandstone surface.

DNA G+C content (mol%): 74 (HPLC).

Type strain: 2002-39/1, ATCC 700087, CCUG 35514, CIP 106528, DSM 9580, NBRC 16126, JCM 9950, NBRC 100415.

Sequence accession no. (16S rRNA gene): AM410679, X92492.

2. **Agrococcus baldri** Zlamala, Schumann, Kämpfer, Rosselló-Mora, Lubitz and Busse 2002a, 1215[VP]

bal′dri. N.L. gen. n. *baldri* of Baldr, ancient German god of light, referring to the photochromogenic behavior.

Cells are irregular spherical, ovoid, or rod-shaped. They vary in size from 0.7–1.0 × 1.1–1.7 µm. Cells occur singly, in pairs, or in small irregular clusters. They stain Gram-positive and are nonmotile. Non-spore-forming. Colonies are slightly convex and smooth. Colony diameters on TSA (tryptic soy broth agar) are in a range of 2–7 mm. The color of the colonies on nutrient agar is light yellow when grown in the light and white when grown in the dark. Aerobic. Catalase is produced. Oxidase is negative. Nitrate is not reduced to nitrite. Other biochemical characteristics are given in Table 142.

The cell-wall diamino acid is diaminobutyrate; additionally alanine, glycine, glutamate, and aspartate are found. Threonine is absent. The polar lipids are comprised of diphosphatidylglycerol, phosphatidylglycerol, two unknown glycolipids, and one unknown polar lipid. The predominant fatty acids are $C_{15:0}$ anteiso and $C_{17:0}$ anteiso, whereas $C_{16:0}$ iso and $C_{15:0}$ iso are present in significant amounts. The major menaquinones are MK-11 and MK-12. Spermine is the predominant polyamine.

Source: isolated from the air in the "Virgilkapelle" a medieval chapel, approximately 12 m underneath Stephansplatz the most famous place in the center of Vienna, Austria.

DNA G+C content (mol%): 73.8–74.9 (HPLC).

Type strain: V-108, CCM 4953, DSM 14215, JCM 12132, NBRC 103055.

Sequence accession no. (16S rRNA gene): AJ309928.

3. **Agrococcus casei** Bora, Vancanneyt, Gelsomino, Swings, Brennan, Cogan, Larpin, Desmasures, Lechner, Kroppenstedt, Ward and Goodfellow 2007, 95[VP]

ca.se′i. L. gen. n. *casei* of cheese, named because the organism was isolated from smear-ripened cheeses.

Aerobic, stains Gram-positive, non-spore-forming, nonmotile, catalase-positive actinomycete that forms irregular, ovoid to short rods. Cells are 0.3–0.4 × 0.8–1.0 µm and occur singly, in pairs or short chains, and in small irregular clusters. Circular, cream-colored, smooth colonies with entire edges are formed on tryptic soy agar. Grows at 20–30°C, but not at 4 or 42°C. Good growth occurs at pH 6.0–10.0. Starch and Tween 80 are hydrolyzed but esculin and Tween 20 are not. Tributyrin is not degraded. Acid is produced from glucose and mannose but not from cellobiose, fructose, maltose, mannitol, sucrose, or trehalose. Growth on a range of carbon sources is variable. The cell-wall diamino acid is diaminobutyrate; alanine, glycine, and glutamate are also found. The peptidoglycan type is B2. Whole-organism sugars are galactose, glucose, and mannose. The polar lipids comprise diphosphatidylglycerol, phosphatidylglycerol, an unknown glycolipid, unknown polar lipids, and four uncharacterized phospholipids. The predominant fatty acids are $C_{15:0}$ anteiso, $C_{17:0}$ anteiso, and $C_{15:0}$ iso. The main menaquinones are MK-11 and MK-12.

Source: the surface of Gubbeen cheese. Representative strains were isolated from the surfaces of smear-ripened cheeses.

DNA G+C content (mol%): 65 (HPLC).

Type strain: R-17892t2, DSM 18061, JCM 14916, LMG 22410.

Sequence accession no. (16S rRNA gene): DQ168427.

4. **Agrococcus citreus** Wieser, Schumann, Martin, Altenburger, Burghardt, Lubitz and Busse 1999, 1168[VP]

ci′tre.us. L. masc. adj. *citreus* of or pertaining to the citron-tree; intended to mean lemon-yellow, describing the lemon-yellow pigmentation.

Cells are irregular spherical, ovoid, or rod shaped. The cell sizes vary from 0.7–1.0 × 1.1–1.7 µm. Cells occur singly, in pairs, or in small irregular clusters. They stain Gram-positive and are nonmotile. Non-spore-forming. Colonies are slightly convex and smooth. Colony diameters are in a range of 2–4 mm. The color of colonies on nutrient agar is yellow. Aerobic and microaerophilic. Catalase is produced. Oxidase-negative. Nitrate is not reduced to nitrite. Acids are produced from arabinose, fructose, mannitol, rhamnose, ribose, and sorbitol. Acid is produced weakly from mannose, glycerol, and xylose. Acid is not produced from cellobiose, dextrin, galactose, glucose, glycerol, inulin, lactose, maltose, raffinose, salicin, sucrose, starch, and trehalose. Acetate and aconitate are utilized as carbon sources. Benzoate, citrate, formate, malate, succinate, and tartrate are not utilized. Starch, esculin, and tyrosine are decomposed. Adenine, casein, gelatin, hippurate, xanthine, hypoxanthine, urea, and Tween 80 are not decomposed. Cells are sensitive to ampicillin, chloramphenicol, ciprofloxacin, erythromycin, gentamicin, kanamycin, lincomycin, neomycin, oxacillin, tetracycline, penicillin G, polymyxin B, rifampin, and streptomycin and are resistant to nitrofuran.

The cell-wall diamino acid is diaminobutyrate (DAB); additionally, alanine, glycine, glutamic, and aspartate are found. Threonine is absent. The whole cell sugars are glucose, rhamnose, mannose, ribose, galactose, and tyvelose. The polar lipids comprise diphosphatidylglycerol, phosphatidylglycerol, two unknown glycolipids, and four uncharacterized polar lipids. The predominant fatty acids are $C_{15:0}$ anteiso, $C_{17:0}$ anteiso, and $C_{16:0}$ iso. $C_{15:0}$ iso, $C_{15:1}$ iso, $C_{17:0}$ iso and $C_{16:0}$ are present in minor amounts. The main menaquinones are MK-11 and MK-12. Spermine is the predominant polyamine.

Source: a medieval wall painting in the church of castle Herberstein in Styria, Austria.

DNA G+C content (mol%): 74 (HPLC).

Type strain: D-1/1a, ATCC 700859, CIP 106287, DSM 12453, JCM 12398.

Sequence accession no. (16S rRNA gene): AJ012826.

5. **Agrococcus jejuensis** Lee 2008, 2299[VP]

je.ju.en′sis. N.L. masc. adj. *jejuensis* of or belonging to Jeju, Republic of Korea, where the type strain was isolated.

Cells are aerobic, Gram-stain-positive, non-spore-forming, oxidase-negative, catalase-positive, nonmotile rods 1.3–1.5 µm × 0.3–0.4 µm. Colonies are circular, smooth, translucent, yellow-colored, and 1–2 mm in diameter. Growth occurs at 10–37°C, with an optimum at 30°C. No growth occurs at 4 or 42°C. pH for growth is in the range 6.1–12.1. Growth occurs in the presence of 0–3% NaCl, but not at 4%. Positive for gelatin hydrolysis, but negative for indole production and glucose fermentation. Nitrate is not reduced to nitrite. Positive for β-galactosidase, but negative for arginine dihydrolase and urease. Assimilates D-glucose, malate, maltose, D-mannitol, D-mannose, and *N*-acetyl-D-glucosamine, but not adipate, D-arabinose, caprate, citrate, gluconate, or phenyl acetate (API 20NE). Hydrolyzes casein and elastin, but not starch. Does not degrade hypoxanthine, DL-tyrosine, or xanthine. Acid is produced from melibiose and salicin. Acid is not produced from adonitol, D-arabinose, L-arabinose, 2,3-butanediol, dulcitol, *meso*-erythritol, D-galactose, glycerol, *myo*-inositol, inulin, D-lactose, melezitose, methyl-D-glucoside, methyl-D-mannoside, 1,2-propanediol, D-raffinose, L-rhamnose, D-sorbitol, L-sorbose, D-xylitol, or D-xylose. Acid production from L-ribose is weak. Positive for alkaline phosphatase, leucine arylamidase, glucosidase, esterase (C4) (weak), and esterase lipase (C8) (weak), but negative for lipase (C14), valine arylamidase, cysteine arylamidase, trypsin, α-chymotrypsin, acid phosphatase, naphthol-AS-BI-phosphohydrolase, galactosidase, β-glucuronidase, β-glucosidase, *N*-acetyl-β-glucosaminidase, mannosidase, and fucosidase (API ZYM). The diagnostic cell-wall diamino acid is 2,4-diaminobutyric acid; alanine, glycine, glutamic acid, threonine, and aspartic acid are also detected. The acyl type of the muramic acid is acetyl. Mycolic acids are not present. Predominant menaquinones are MK-10 and MK-9. Major fatty acids are $C_{15:0}$ anteiso (56.7%) and $C_{16:0}$ iso (13.2%). Phospholipids are diphosphatidylglycerol and phosphatidylglycerol.

Source: a dried seaweed sample collected on Samyang Beach, Jeju, Republic of Korea.

DNA G+C content (mol%): 73.0 (HPLC).

Type strain: SSW1-48, JCM 14256, KCTC 19198.

Sequence accession no. (16S rRNA gene): AM396260.

6. **Agrococcus lahaulensis** Mayilraj, Suresh, Schumann, Kroppenstedt and Saini 2006, 1809[VP]

la.haul.en′sis. N.L. masc. adj. *lahaulensis* of or belonging to Lahaul Valley, located in the Indian Himalayas, where the type strain was isolated.

The cells vary in size from 0.6–1.0 µm × 1.0–1.5 µm. Stain Gram-positive, non-acid-fast, aerobic, non-spore-forming, and nonmotile. On TSA, colonies are lemon yellow colored, circular, glistening, and opaque with an entire margin. Colony sizes are in the range of 0.7–3.0 mm. Cells are irregular spherical, occurring in pairs and in clusters. Mycelial growth is not observed.

Catalase-positive and oxidase-negative. Tolerates up to 7.0% NaCl and grows at temperatures 25–37°C, with an optimum temperature of 30°C. Growth occurs at pH 6–10 with an optimum at pH 8.0. Growth is not observed at pH 5.2. Decomposes casein, Tween 20, starch, and gelatin. It is negative for indole production from tryptophan, urease, hydrogen sulfide production, methyl red test, Voges–Proskauer reaction, citrate utilization, decomposition of esculin and tyrosine, and nitrate reduction. Acid is produced from D-rhamnose, D-fructose, and D-trehalose but not from D-glucose, L-arabinose, D-cellobiose, *meso*- inositol, D-mannitol, D-maltose, D-galactose, D-raffinose, glycerol, salicin, sucrose, and D-xylose. Utilization of compounds as

sole source of carbon is given in Table 142. In the Biolog GN test, reactions are positive for dextrin, maltotriose, L-rhamnose, γ-hydroxybutyric acid, glycerol, adenosine, 2′-deoxyadenosine, inosine, thymidine, L-arabinose, D-fructose, D-mannitol, D-ribose, and D-xylose and negative for D-gluconic acid, α-D-glucose, D-mannose, D-psicose, D-sorbitol, D-agatose, turanose, D-fructose 6-phosphate, L-alaninamide, α-cyclodextrin, β-cyclodextrin, glucogen, inulin, mannan, Tween 40, Tween 80, *N*-acetyl-D-glucosamine, *N*-acetyl-β-D-mannosamine, amygdalin, D-arabitol, arbutin, D-cellobiose, L-fucose, D-galactose, D-galacturonic acid, gentiobiose, *m*-inositol, α- D-lactose, lactulose, maltose, D-melezitose, D-melibiose, α-methyl-D-galactoside, β-methyl-D-galactoside, 3-methyl-glucose, α-methyl-D-glucoside, β-methyl-D-glucoside, α-methyl-D-mannoside, palatinose, D-raffinose, salicin, sedoheptulosan, stachyose, sucrose, D-trehalose, xylitol, acetic acid, α-hydroxybutyric acid, β-hydroxybutyric acid, *p*-hydroxy-phenylacetic acid, α-ketoglutaric acid, α-ketovaleric acid, lactamide, D-lactic acid methyl ester, L-lactic acid, D-malic acid, L-malic acid, pyruvatic acid methyl ester, succinic acid monomethyl ester, propionic acid, pyruvic acid, succinamic acid, succinic acid, *N*-acetyl-L-glutamic acid, D-alanine, L-alanine, L-alanyl-glycine, L-asparagine, L-glutamic acid, glycyl-L-glutamic acid, L-serine, L-pyroglutamic acid, putrescine, 2,3-butanediol, uridine, adenosine-5′-monophosphate, thymidine-5′-monophosphate, uridine-5′-monophosphate, α-D-glucose 1-phosphate, D-glucose 6-phosphate, and DL-α-glycerol phosphate.

The diagnostic cell-wall diamino acid is diaminobutyrate (DAB). The peptidoglycan also contains alanine, glycine, aspartate, glutamate, and threonine. The cell-wall sugars are glucose, rhamnose, ribose, mannose, and galactose. The polar lipids are diphosphatidylglycerol, phosphatidylglycerol, and two unknown glycolipids. Mycolic acids are absent. The abundant fatty acids are $C_{15:0}$ anteiso, $C_{17:0}$ anteiso, $C_{15:0}$ iso, and $C_{16:0}$ iso. The major menaquinones are MK-10, MK-11, and MK-12. The acyl type is acetyl.

Source: soil, 0.45 m below an ice glacier at 4200 m above sea level in Lahaul Valley, Himachal Pradesh, India.

DNA G+C content (mol%): 74.1 (T_m).

Type strain: K22-21, DSM 17612, JCM 14301, MTCC 7154.

Sequence accession no. (16S rRNA gene): DQ156908.

7. **Agrococcus versicolor** Behrendt, Schumann and Ulrich 2008a, 2837[VP]

ver.si′co.lor. L. masc. adj. *versicolor* color changing.

Cells are nonsporeforming, nonmotile, short to ovoid rods (0.5–0.6 × 0.8–1.2 µm) and occur singly or in pairs, sometimes forming small irregular clusters. Gram-stain-positive and strictly aerobic. Colonies on nutrient agar are dark pink to red in color and are smooth with regular margins. Young, solitary, growing colonies are yellow, turning dark pink with age. The optimal growth temperature is 21°C; no growth occurs at 4 or 37°C. Nitrate and nitrite are not reduced. Growth occurs at 1% and 2% NaCl but not at higher concentrations. Catalase activity is present, but oxidase, urease, L-phenylalanine deaminase, lysine and ornithine decarboxylase, arginine dihydrolase, and tryptophan deaminase activities are absent. β-Galactosidase activity is weak. Gelatin, casein, and esculin are hydrolyzed, but starch and tyrosine are not. Hydrogen sulfide is not produced. Indole production from L-tryptophan and the reaction in the Voges–Proskauer test are negative. Using Biolog microplates GP2, positive for oxidation of dextrin, *N*-acetyl-D-glucosamine, L-arabinose, cellobiose, D-fructose, D-gluconic acid, α-D-glucose, maltose, D-mannitol, D-mannose, palatinose, D-psicose, D-ribose, sucrose, D-tagatose, turanose, D-xylose, propionic acid, and glycerol. Oxidation of maltotriose, trehalose, α-hydroxybutyric acid, α-ketovaleric acid, L-alaninamide, and adenosine is observed after prolonged incubation. The following substrates are not oxidized: α- and β-cyclodextrin, glycogen, inulin, mannan, Tweens 40 and 80, *N*-acetyl-D-mannosamine, amygdalin, D-arabitol, arbutin, D-galactose, L-fucose, D-galacturonic acid, gentiobiose, *myo*-inositol, D-lactose, lactulose, melezitose, melibiose, methyl-D-galactoside, methyl β-D-galactoside, methyl-D-glucoside, methyl β-D-glucoside, methyl -D-mannoside, raffinose, L-rhamnose, salicin, sedoheptulosan, D-sorbitol, stachyose, xylitol, acetic acid, β-hydroxybutyric acid, α-hydroxybutyric acid, *p*-hydroxyphenylacetic acid, ketoglutaric acid, lactamide, D-lactic acid methyl ester, L-lactic acid, D- and L-malic acid, monomethyl succinate, succinamic acid, succinic acid, *N*-acetyl L-glutamic acid, D- and L-alanine, L-alanyl glycine, L-asparagine, L-glutamic acid, glycyl L-glutamic acid, L-pyroglutamic acid, L-serine, putrescine, 2,3-butanediol, 2′-deoxyadenosine, inosine, thymidine, uridine, adenosine 5′-monophosphate, thymidine 5′-monophosphate, uridine 5′-monophosphate, D-glucose 1-phosphate, D-glucose 6-phosphate, and DL-glycerol phosphate. Oxidative acid production with API 50 CH is positive for ribose and L-arabinose but only weakly positive for D-glucose, D-fructose, D-mannose, sucrose, and turanose. Acid production is negative for glycerol, erythritol, D-arabinose, L-xylose, D-adonitol, methyl β-D-xylopyranoside, D-galactose, L-sorbose, L-rhamnose, dulcitol, inositol, D-sorbitol, methyl-D-mannopyranoside, methyl-D-glucopyranoside, amygdalin, arbutin, salicin, D-lactose (bovine origin), melibiose, trehalose, inulin, melezitose, raffinose, starch, glycogen, xylitol, gentiobiose, D-lyxose, D-tagatose, D- and L-fucose, D- and L-arabitol, potassium gluconate, potassium 2-ketogluconate, and potassium 5-ketogluconate. Strain-specific reactions are shown for D-mannitol, *N*-acetylglucosamine, D-cellobiose, and maltose. The diagnostic cell-wall diamino acid is diaminobutyric acid; alanine, glycine, threonine, aspartic acid, and glutamic acid are also present. The major fatty acids are $C_{15:0}$ anteiso, $C_{15:0}$ iso, $C_{16:0}$, and $C_{17:1}$ anteiso, and minor fatty acids are $C_{17:0}$ anteiso, $C_{16:0}$ iso, $C_{14:0}$, $C_{17:0}$ iso, and $C_{14:0}$ iso. The major menaquinones are MK-10 and MK-11.

Source: the phyllosphere of potato plants in Dahnsdorf, Germany.

Type strain: K 114/01, DSM 19812, LMG 24386.

Sequence accession no. (16S rRNA gene): AM940157.

Genus IV. **Agromyces** Gledhill and Casida 1969a, 346[AL] emend. Zgurskaya, Evtushenko, Akimov, Voyevoda, Dobrovolskaya, Lysak and Kalakoutskii 1992, 638[VP]

Vladimir N. Akimov and Lyudmila I. Evtushenko

Ag.ro.my′ces. Gr. n. *agros* field or soil; N.L. masc. n. *myces* (from Gr. masc. n. *mukes -etis*) fungus; N.L. masc. n. *Agromyces* soil fungus.

Young cultures produce **thin (mostly 0.2–0.6 µm in diameter) branched vegetative hyphae, short branching filaments or irregular rods that subsequently break up into diphtheroid, rod-like to coccoid fragments.** Scant aerial hyphae may occur on rare occasions. Nonmotile. Nonsporeforming. **Gram-stain-positive type** of cell wall. Non-acid-fast. Lysozyme sensitive. Colonies on nutrient agar medium are generally **yellow or white**, circular, 1–2 mm in diameter, opaque, **often penetrating into the agar**. **Chemo-organotrophs**, having a respiratory type of metabolism. **Aerobic to microaerophilic**. Catalase and oxidase test reactions intensities vary among species. Most strains grow well on standard laboratory media at near neutral pH. Some species are nutritionally exacting. Use a wide range of organic compounds as sole sources of carbon for growth and energy. Mesophilic; optimal growth at ~24–30°C; growth range ~7–40°C.

The cell-wall peptidoglycan is a group B type, based upon 2,4-diaminobutyric acid (L-isomer predominating). **The muramic acid residue of the peptidoglycan is *N*-acetylated.** Menaquinones are the sole respiratory quinones; **the predominant component is typically an unsaturated menaquinone with 12 isoprene units (MK-12);** the second most common components are MK-13 and MK-11. **Major fatty acids are $C_{15:0}$ anteiso, $C_{17:0}$ anteiso, and $C_{16:0}$ iso.** Straight-chain saturated acids are present in small quantities. Mycolic acids are absent. Principal polar lipids are diphosphatidylglycerol, phosphatidylglycerol, and characteristic glycolipids. Polyamine patterns include putrescine as the predominant compound.

Usually occurring in soils; some species can be associated with plants or animals. *Agromyces* species or strains have never been isolated from unequivocally pathological material from humans, warm-blooded animals, or plants.

DNA G+C content (mol%): 65–73.

Type species: **Agromyces ramosus** Gledhill and Casida 1969a, 346[VP].

Further descriptive information

The genus *Agromyces* belongs to the family *Microbacteriaceae*, order *Micrococcales*, and, at the time of writing, includes 19 species and 2 subspecies (Table 144). The 16S rRNA gene sequence analysis shows that *Agromyces* species examined form a coherent phylogenetic cluster, with sequence similarity levels above 93.8%. At least five phylogenetic subclusters may be further distinguished within the genus (Figure 177).

Agromyces species vary in cell morphology, ranging from thin (mostly 0.2–0.6 µm in diameter), extensively branching vegetative hyphae to small (0.2–0.4 × 0.5–1.5 µm), straight or slightly curved rods as reported for *Agromyces allii* (Jung et al., 2007). On agar media, they typically display a distinct growth cycle. Branching hyphae or short filaments, produced in an early growth phase, undergo septation and fragmentation, often very rapidly, to yield diphtheroid and irregular rod-like nonmotile elements; a proportion of the cells may be coccoid. Aerial mycelia are usually absent, except for *Agromyces aurantiacus*. The latter produces scant, sparsely branching aerial hyphae on glycerol-asparagine agar (Li et al., 2003). The consistency and composition of the growth medium can affect the hyphal formation, fragmentation intensity, and the fragment size and shape. The cell diameter may also vary depending on growth medium. The formation of hyphae by *Agromyces ramosus* is enhanced in the presence of fructose, sucrose, or carbohydrates containing a terminal sucrose moiety (Gledhill and Casida, 1969a). The mean cell diameter of *Agromyces ramosus* in 5-d-old shaken culture in nutrient broth (0.3% beef extract and 0.5% glucose) is 0.66 µm, while the diameter of cells grown on some other media can be half or less of this value, down to 0.18 µm on Ashby's agar with 0.2% glucose (Gledhill and Casida, 1969a).

Visible colonies of most species usually appear on agar plates after 2–3 d of incubation. Colonies are circular, somewhat convex, smooth and may be rough, often penetrating into agar media. The colony color of most *Agromyces* strains is yellow or white; color intensity and shade vary depending on the species, culture age, and growth conditions. The color of colonies (substrate mycelium) of the type strain of *Agromyces aurantiacus* can be yellow, light orange-yellow to pink-white or pink-gray (Li et al., 2003). Pigmentation of *Agromyces ramosus* is usually not evident, but a pale yellow, alcohol-soluble pigment (a carotenoid) may be observed under some growth conditions (Gledhill and Casida, 1969a; Jones et al., 1970). The yellow pigments of *Agromyces bracchium*, *Agromyces cerinus*, *Agromyces fucosus*, *Agromyces hippuratus*, *Agromyces luteolus*, *Agromyces mediolanus*, and *Agromyces rhizospherae*) are C_{40}–C_{50}-carotenoids (Takano et al., 2006; Trutko et al., 2005; Weeks and Garner, 1967). The pigment biosynthesis in some species (*Agromyces cerinus*, *Agromyces fucosus*, *Agromyces hippuratus*, and *Agromyces mediolanus*) is light-induced (Liaaen-Jensen and Andrewes, 1972; Trutko et al., 2005). Pigmentation (but not growth) of all carotenoid-producing *Agromyces* strains tested up to date is inhibited by fosmidomycin at a low concentration of 0.05 mg/ml (Trutko et al., 2005), which is indicative of the nonmevalonate pathway of the pigment biosynthesis (Kuzuyama, 2002; Lichtenthaler et al., 2000). The data on complete sequence of decaprenoxanthin biosynthetic operon of *Agromyces mediolanus* ATCC 13930 (= JCM 1376 = K. Suzuki CNF 134) and enzymes involved in the biosynthesis are available from public databases.

Agromycetes are Gram-stain-positive in terms of cell-wall composition, but the Gram-staining test may be indistinct or negative, particularly at fragmentation stage (Gledhill and Casida, 1969a). The peptidoglycans of *Agromyces* species are based upon 2,4-diaminobutyric acid (Dab) and, if reported, contain Dab, glycine, glutamate, and alanine in a molar ratio close to 2:1:1:1 (Fiedler and Kandler, 1973; Zgurskaya et al., 1992; Sasaki et al., 1998; and recent descriptions of *Agromyces* species). The molar ratio of amino acids corresponds to the peptidoglycan B2γ type, [L-Dab]D-Glu–D-Dab, as reported for *Agromyces ramosus* and *Agromyces mediolanus* (Fiedler and Kandler, 1973; Schleifer and Kandler, 1972). Sasaki et al. (1998) also found that five *Agromyces* species (*Agromyces ramosus*, *Agromyces cerinus*, *Agromyces fucosus*, *Agromyces hippuratus*, *Agromyces mediolanus*) possess

TABLE 144. Characteristics differentiating species and subspecies of the genus *Agromyces*[a,b,c]

Characteristic	*A. ramosus*	*A. albus*	*A. allii*	*A. aurantiacus*	*A. bracchium*	*A. cerinus* subsp. *cerinus*	*A. cerinus* subsp. *nitratus*	*A. fucosus*	*A. hippuratus*	*A. humatus*	*A. italicus*	*A. lapidis*	*A. luteolus*	*A. mediolanus*	*A. neolithicus*	*A. rhizospherae*	*A. salentinus*	*A. subbeticus*	*A. terreus*	*A. ulmi*
Colony color[d]	White	White	Yellow	O, Y, P	Yellow	Yellow	Yellow	Yellow	Yellow	Yellow	Cream	Yellow	Yellow	Yellow	Beige	Yellow	Yellow	Yellow	Cream	White
Morphology in young culture[e]	BH	BH	IR	BH; A[f]	SF	BH	BH	BH, SF	BH	BH	BH	BH	SF	IR	BH	SF	BH	SF, IR	SF, IR	BH
Cell width (μm)	0.4–0.7	0.3–0.6	0.2–0.4	0.4–0.5	0.2–0.4	0.2–0.6	0.2–0.6	0.2–0.6	0.2–0.6	0.3–0.5	0.4–0.6	0.4–0.6	0.2–0.4	0.3–0.6	0.3–0.5	0.2–0.4	0.5–0.7	0.3–0.5	0.2–0.5	< 1.0
Predominant menaquinones[g]	12, 13	12, 11	11, 12	12, 13, 11	12	12, 13	12, 13	12, 13	12, 13	13, 12	12, 13	12, 13	12, 11	12, 11, 10	13, 12	12, 11	12, 11	12, 13	12, 11, 10	12, 11
Cell-wall sugars[h]	Rha, Gal, Xyl, Man[i]	Rha, Gal, Glu, Man	[Rha, Gal, Rib, (Xyl)]	[Rha, Gal, Glu, Man]	Rha, Glu, Man, Gal	Rha, Tyv, Gal, (Man)	Gal, Glu, Rib, Man[j]	Rha, Gal, (Fuc, Man)[j]	Rha, Gal, (Man)[j]	[Glu, Gal, Rha, Man]	[Gal, Rib, Glu, Man]	[Glu, Gal, Man, Rib]	Rha, Fru, Man, Glu	nd	[Glu, Gal, Man]	Rha, Man, Glu	[Rha, Glu, Gal, Ara, Rib]	[Rha, Glu, Gal, Man]	[Gal, Rib]	[Rha, Fuc, Glu]
DNA G+C content (mol%)	68.9[g, k]	69.0	71.2	72.8	70.0	70.5[k]	70.9[k]	70.6[k]	70.6[k]	70.6	70.8	70.4	71.1	72.3 [k]	65.3	71.2	72.3	71.2	71.1	72
Growth at 10°C	+	w	+	nd	−	w	+	+	+	−	w	w	−	w	−	−	+	+	+	−
Growth at 37°C	+	+	−	+	−	−	w	+	w	+	+	+	−	nd	+	−	+	w	−	+
Growth in 4% NaCl (w/v)	w	v	−	nd	+	−	v	v	v	−	+	+	+	+	−	+	w	+	+	nd
Microaerophilic growth	+	−	nd	nd	nd	−	+	+	nd	−	+	+	nd	+	+	nd	w	+	nd	nd
Catalase reaction	−	+	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−
Oxidase test	−	+	+	nd	nd	+	v	+	+	−	+	v	nd	v	v	nd	v	v	+	−
Nitrate reduction	v	−	+	−	−	−	+	v	+	+	+	+	−	v	+	−	−	−	+	−
H_2S production	+	+	−	−	nd	+	+	+	+	+	+	+	nd	nd	+	nd	+	+	−	nd
Decomposition/ hydrolysis of:																				
Casein	−	+	+	nd	nd	−	+	+	nd	+	+	+	nd	+	+	**nd**	+	+	−	+
Gelatin	−	+	+	−	+	−	−	+	−	+	+	v	+	−	+	+	−	w	+	+
Hypoxanthine	−	v	−	nd	nd	w	+	v	+	w	+	−	nd	+	−	nd	−	+	+	nd
Tyrosine	−	v	−	nd	nd	+	+	v	+	+	+	+	nd	+	−	nd	+	+	+	nd
Starch	+	+	−	+	+	+	+	+	+	+	+	+	+	d/−	+	+	+	+	+	−
Urea	−	v	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	−	−	−
Xanthine	−	−	−	nd	nd	−	−	+	−	−	+	−	nd	+	−	nd	−	+	−	nd
Acid production (API):[l]																				
Amygdalin	+	−	nd	−	+	+	+	+	nd	−	w	+	−	+	−	−	w	+	−	+
N-Acetyl-glucosamine	+	−	nd	nd	−	−	+	+	nd	v	−	+	−	−	w	−	−	v	−	+

(continued)

TABLE 144. (continued)

Characteristic	*A. ramosus*	*A. albus*	*A. allii*	*A. aurantiacus*	*A. bracchium*	*A. cerinus* subsp. *cerinus*	*A. cerinus* subsp. *nitratus*	*A. fucosus*	*A. hippuratus*	*A. humatus*	*A. italicus*	*A. lapidis*	*A. luteolus*	*A. mediolanus*	*A. neolithicus*	*A. rhizospherae*	*A. salentinus*	*A. subbeticus*	*A. terreus*	*A. ulmi*
D-Arabinose	+	w	nd	–	–	+	–	+	+	–	+	+	–	w	+	–	+	nd	+	nd
L-Arabinose	+	+	+	w	+	–	–	+	–	+	+	–	–	–	+	–	+	+	+	nd
Cellobiose	–	–	+	+	+	+	+	+	v	v	+	+	–	+	+	+	+	+	nd	+
L-Fucose	+	–	nd	–	nd	w	+	+	nd	–	+	+	nd	–	+	nd	+	+	–	nd
Galactose	–	w	+	+	+	+	+	+	nd	+	+	+	–	+	+	–	+	+	+	+
Gentiobiose	nd	nd	nd	–	+	nd	–	–	nd	–	–	–	–	nd	–	w	w	–	–	+
D-Glucose	–	w	+	+	+	+	+	+	nd	+	+	+	+	+	+	+	+	+	nd	+
Inulin	+	v	nd	+	–	–	–	w	–	+	–	+	–	–	–	–	+	+	+	+
Melibiose	–	–	+	–	+	+	–	–	–	–	w	–	+	+	–	v/–	–	w	–	+
Melezitose	–	+	+	+	+	+	+	+	–	+	+	+	+	nd	+	+	+	+	nd	+
Lactose	w	+	–	+	+	–	–	–	nd	+	–	–	w	–	+	–	–	+	nd	–
Maltose	–	w	+	+	+	+	+	+	nd	+	+	+	+	nd	+	+	+	+	+	+
Mannitol	nd	nd	–	+	w	nd	nd	nd	nd	–	–	–	–	nd	nd	–	nd	–	–	+
Mannose	–	w	+	+	+	–	–	–	nd	–	–	–	w	nd	+	–	–	–	nd	+
Melezitose	–	–	nd	nd	–	–	–	+	nd	–	–	+	–	–	–	–	–	+	–	+
Melibiose	+	w	–	+	+	–	–	+	–	+	–	w	–	–	+	–	w	+	w	+
Methylα-D-mannoside	+	+	+	+	w	+	+	+	+	+	–	–	w	+	–	–	+	+	+	+
D-Raffinose	–	w	+	–	+	–	–	w	–	–	–	–	–	+	–	–	+	–	–	+
Rhamnose	–	–	+	–	+	+	+	+	–	–	+	+	–	w	–	+	+	+	w	+
Ribose	+	w	+	+	+	v	–	+	v	+	–	+	w	+	v	–	+	+	+	+
Salicin	v	–	+	–	+	–	–	w	–	–	–	–	+	+	–	–	+	–	–	+
Sucrose	v	–	nd	w	+	–	–	–	–	–	–	–	–	w	–	–	+	–	–	nd
Trehalose	–	w	+	–	+	–	–	–	+	–	+	–	–	–	–	–	+	+	+	+
D-Turanose	v	–	w	+	–	–	–	–	–	–	–	–	w	–	–	+	–	–	nd	nd
D-Xylose	–	w	+	–	+	–	–	–	+	–	+	–	–	–	–	–	+	+	+	+
Enzymes (API ZYM):																				
N-Acetyl-β-glucosaminidase	–	+	nd	–	–	–	–	+	nd	–	–	–	–	+	+	–	+	+	w	nd
Alkalinephos-phatase	+	nd	–	–	–	+	+	+	nd	+	+	+	–	nd	+	–	+	+	–	nd
α-Chymotrypsin	–	–	+	nd	nd	–	–	–	nd	–	–	+	nd	–	+	nd	–	–	–	nd
α-Galactosidase	–	–	–	nd	nd	+	+	–	nd	–	–	–	nd	–	+	nd	–	–	–	nd
β-Galactosidase	–	+	+	w	–	+	+	+	nd	+	+	+	–	+	–	–	+	+	w	+
α-Glucosidase	–	+	+	+	nd	+	+	+	nd	+	+	+	nd	nd	nd	nd	nd	+	+	nd
β-Glucosidase	+	nd	+	nd	nd	+	+	+	nd	+	+	+	nd	nd	–	nd	+	+	nd	nd
β-Glucuronidase	–	–	+	–	–	–	–	–	nd	+	–	–	–	–	+	–	–	–	–	nd
α-Mannosidase	–	–	–	nd	nd	–	–	–	nd	+	–	–	nd	–	+	nd	–	–	nd	nd

Antibiotic susceptibility (amount per disk):																				
Ampicillin (10 mg)	+	+	–	nd	nd	+	–	–	nd	+	+	+	nd	–	+	nd	+	–	+	nd
Ciprofloxacin (5 mg)	+	+	nd	nd	nd	–	v	+	nd	+	+	+	nd	–	+	nd	+	nd	nd	nd
Kanamycin (30 mg)	+	+	+	nd	nd	+	+	+	nd	–	–	–	nd	–	–	nd	+	–	+	nd
Nalidixic acid (30 mg)	–	+	nd	nd	nd	–	–	–	nd	–	–	+	nd	–	–	nd	+	–	nd	nd
Norfloxacin (10 μg)	–	–	nd	nd	nd	–	–	–	nd	–	–	–	nd	–	+	nd	+	–	nd	nd
Novobiocin (5 μg)	v	+	–	nd	nd	+	+	+	nd	+	+	+	nd	–	+	nd	+	+	+	nd
Penicillin G (10 U)	+	–	–	nd	nd	–	–	–	nd	–	+	+	nd	–	+	nd	+	–	+ (20 U)	nd
Polymyxin B (300 U)	+	+	+	nd	nd	v	+	–	nd	+	–	–	nd	–	+	nd	+	–	+	nd
Streptomycin (10 μg)	+	+	nd	nd	nd	+	+	+	nd	+	–	–	nd	–	–	nd	+	+	+ (50 μg)	nd
Sulfonamide (200 μg)	–	–	nd	nd	nd	–	–	–	–	–	+	–	nd	–	–	nd	+	nd	nd	nd

[a]Data are complied from Gledhill and Casida (1969a); Zgurskaya et al. (1992); Suzuki et al. (1996); Groth et al. (1996); Takeuchi and Hatano (2001); Dorofeeva et al. (2003); Li et al. (2003); Rivas et al. (2004a); Ortiz-Martinez et al. (2004); Jurado et al. (2005a, 2005b, 2005c); Jung et al. (2007); and Yoon et al. (2008).

[b]Symbols: –, negative; +, positive; w, weakly positive or delayed reaction; d, different among strains within a species (reaction for type strain is given behind oblique stroke); v, variable between experiments; nd, not determined.

[c]Data for the type strains unless indicated.

[d]O, orange; Y, yellow; P, pink-white or pink-gray. Yellow color of *Agromyces* species may vary in intensity and shade depending on culture age and growth conditions.

[e]BH, branching hyphae; SF, short filaments (up to 6 μm and more) with rudimentary branching; IR, cells mostly display irregular rods in young cultures. Note: the length of hyphae and "shorter filaments" and fragmentation intensity may vary depending on the growth medium.

[f]Aerial mycelium was observed after 2–3 weeks on ISP 5 medium.

[g]The predominant menaquinone, followed by the most abundant second (third) component constituting >25% of the main peak (where available); see the species descriptions for details and references. Numerals indicate the numbers of isoprene units in the side chain.

[h]Ara, arabinose; Fru, fructose; Fuc, fucose; Gal, galactose; Glu, glucose; Man, mannose; Rha, ramnose; Rib, ribose; Tyv, tyvelose; Xyl, xylose. Sugars in parentheses were found only in some strains of a taxon, either in trace (mannose) or in significant amounts (xylose for *Agromyces allii*, fucose and glucose for *Agromyces fucosus*). Square brackets indicate sugars determined in whole cells. Order of sugars is ranged in accordance with decrease of their amounts (where available) or given in the same way as in original papers (see species descriptions for details).

[i]Different cell-wall sugar pattern (rhamnose and glucose) was reported for some other strains assigned to *Agromyces ramosus* (Gledhill and Casida, 1969a). Whole cells of the type strain were reported to contain glucose and ribose also (Zgurskaya et al., 1992).

[j]Glucose, ribose, mannose, fucose, and xylose in different combinations were also detected in whole cells of the indicated taxa (see species descriptions for details).

[k]Data from Suzuki et al. (1996).

[l]Data on acid production as determined using the API test systems, bioMérieux (Jurado et al., 2005a, 2005b, 2005c; Li et al., 2003; Takeuchi and Hatano, 2001; Yoon et al., 2008), with the exception of reactions for *Agromyces allii*, *Agromyces hippuratus*, and some data on *Agromyces ramosus* and *Agromyces cerinus* subsp. *cerinus* obtained by conventional methods (Gledhill and Casida, 1969a; Groth et al., 1996; Jung et al., 2007; Zgurskaya et al., 1992). The results of conventional and API tests may sometimes differ.

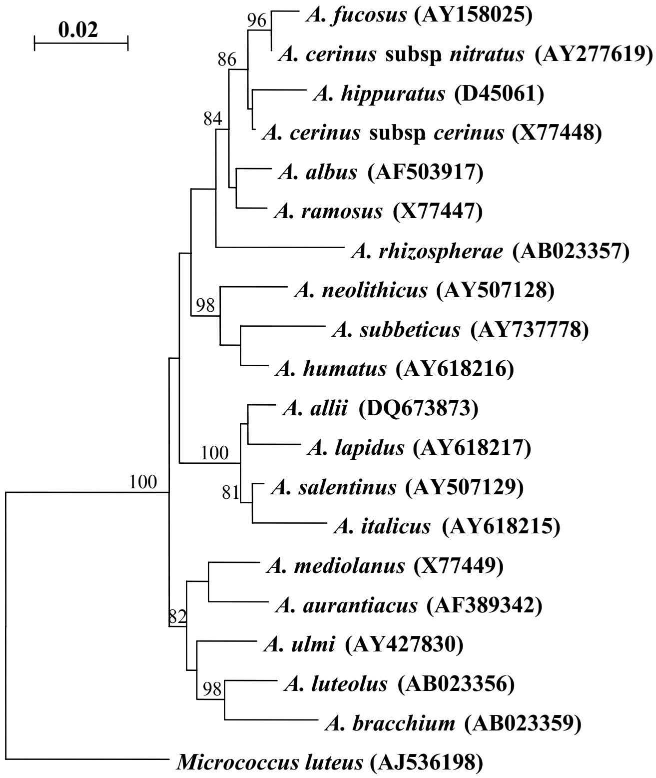

FIGURE 177. Phylogenetic dendogram based on 16S rRNA gene comparison of the type strains of the genus *Agromyces*. Bar = 0.02 inferred nucleotide substitution per nucleotide. Values at nodes indicate bootstrap values for 500 replicates.

almost exclusively the L-isomer of Dab, which suggests that their peptidoglycan differs from the B2γ type in that D-Dab is almost fully substituted by L-Dab. Although the peptidoglycan structure was not completely determined, these results suggest that it contains L-Dab in the interpeptide bridge (the peptidoglycan type [L-Dab]D-Glu–L-Dab). Muramic acid occurs in the *N*-acetyl form, as shown for several species (Jurado et al., 2005a, 2005b, 2005c).

Sugars determined in the cell wall or whole cells of most strains are rhamnose, galactose, and mannose (typically small quantities). In addition, glucose, arabinose, fucose, fructose, ribose, tyvelose, and xylose in various combinations have been reported for some species (Table 144). In several *Agromyces* strains tested so far (*Agromyces cerinus* subsp. *cerinus*, *Agromyces cerinus* subsp. *nitratus*, *Agromyces fucosus*, and *Agromyces hippuratus*) different cell-wall teichoic acids or related phosphorous-containing anionic polymers have been revealed (Gnilozub et al., 1994b; Naumova et al., 2001; Shashkov et al., 1993, 1995). No phosphorous-containing anionic polymers have been found in the cell walls of *Agromyces mediolanus* and a few other unnamed organisms of this genus. The tested type strains of *Agromyces cerinus* subsp. *cerinus*, *Agromyces cerinus* subsp. *nitratus*, *Agromyces fucosus*, and *Agromyces hippuratus* also contain lipoteichoic acids composed of poly(glycerolphosphate) and hydrophobic chains, fatty acids of which largely include $C_{17:0}$ iso (22–38%), $C_{15:0}$ anteiso (11–36%), $C_{16:0}$ iso (15–20%), and $C_{18:0}$ (6.0–17%) (Gnilozub et al., 1994a).

Most *Agromyces* species are aerobes; some also grow microaerophilically (Jurado et al., 2005a, 2005b, 2005c). *Agromyces ramosus* displays pronounced microaerophily; poor anaerobic growth has been reported for some strains (Gledhill and Casida, 1969a). In addition, this species has a low CO oxidizing activity (Bartholomew and Alexander, 1979); CO_2 is not required for growth (Gledhill and Casida, 1969a). The *Agromyces* species are mostly catalase-positive except for *Agromyces ramosus*, *Agromyces aurantiacus*, and *Agromyces ulmi* (Gledhill and Casida, 1969a; Li et al., 2003; Rivas et al., 2004a). *Agromyces ramosus* also shows negative reaction in the benzidine and oxidase tests (Gledhill and Casida, 1969a). Nevertheless, it produces cytochromes *b*, *c*, and aa_3 (where the latter is the cytochrome oxidase), suggesting the presence of a cytochrome electron transport system (Jones et al., 1970). Negative or variable reactions in oxidase tests have also been found in some other *Agromyces* species (Jurado et al., 2005c; Rivas et al., 2004a; Table 144), which suggests *Agromyces* species differ in the composition of terminal parts of respiratory chains. The major respiratory menaquinone of most *Agromyces* species is MK-12; the second most abundant component is MK-13 or MK-11. Minor or trace amounts of MK-7, MK-8, MK-9, MK-10, and MK-14 are also present (e.g. Collins and Jones, 1980; Suzuki et al., 1996; Takeuchi and Hatano, 2001; Rivas et al., 2004a).

Saturated anteiso- and iso-methyl branched fatty acids predominate in the cellular fatty acid profiles, and the components $C_{17:0}$ anteiso, $C_{15:0}$ anteiso, and $C_{16:0}$ iso may contribute up to 93–96% of the total FAMEs (Suzuki et al., 1996; Takeuchi and Hatano, 2001). $C_{17:0}$ anteiso or $C_{15:0}$ anteiso (and their sum) typically predominate. Polar lipids are represented mostly by diphosphatidylglycerol, phosphatidylglycerol, and one or two distinctive glycolipids (Collins, 1982; Collins and Jones, 1980; Jung et al., 2007; Jurado et al., 2005a, b; Jurado et al., 2005c). In addition, one to four minor unidentified phospholipids have been reported for some species (Jurado et al., 2005a, b; Jurado et al., 2005c; Zgurskaya, 1992). *Agromyces ramosus* contains two glycolipids with staining characteristics indicative of the possible presence of galactose and/or mannose within these lipids (Collins, 1982). The only glycolipid, designated G_2, which is most likely identical to one of the *Agromyces ramosus* glycolipids, has been detected in *Agromyces mediolanus* (Collins and Jones, 1980). It is noteworthy that this glycolipid is also present, along with 1–2 other characteristic glycolipids, in strains of *Clavibacter*, *Leifsonia*, and *Rathayibacter* (Collins and Bradbury, 1986; Collins and Jones, 1980). The glycolipid with the same chromatographic behavior and staining characteristics has also been found in strains of *Frigoribacterium* (Kämpfer et al., 2000), *Agreia*, and some other *Microbacteriaceae* (N.G. Vinokurova and L.I. Evtushenko, unpublished data).

Polyamines were determined for the type strains of *Agromyces cerinus* subsp. *cerinus*, *Agromyces cerinus* subsp. *nitratus*, *Agromyces fucosus*, *Agromyces hippuratus*, and *Agromyces mediolanus* (Altenburger et al., 1997). The total polyamine content is lower (0.21–0.28 μmol/g) than in most other representatives of the family *Microbacteriaceae*, with putrescine as the predominant component (0.11–0.21 μmol/g). The second most abundant polyamine is spermidine, spermine, or 1,3-diaminopropane, depending on the species; cadaverine, *sym*-homospermidine, and *sym*-norspermidine may occur in minor or trace amounts (Altenburger et al., 1997).

The majority of *Agromyces* strains grow well on standard nutrient media such as trypticase soy broth agar (TSBA)*, *Corynebacterium* (CB) medium†, rich (R) medium‡, peptone-yeast extract (PY) medium§, and some other media based on peptone, yeast extract, and glucose. Knowledge of minimal nutritional requirements of *Agromyces* species is incomplete. Some strains grow well on a basal mineral medium (e.g. ISP 9 medium**) with glucose as a carbon source and ammonium salt as a source of nitrogen (Li et al., 2003; Zgurskaya et al., 1992), whereas others exhibit growth on the same medium in the presence of 0.01% yeast extract (Zgurskaya et al., 1992).

Organic nitrogen is required for growth of some strains (Gledhill and Casida, 1969a; Zgurskaya et al., 1992). *Agromyces aurantiacus* grows on Czapek agar (nitrate as a nitrogen source), and some other simple, chemically defined media (Li et al., 2003). *Agromyces ramosus* is the most nutritionally exacting among the *Agromyces* species so far described. The study of nutritional requirements of this species (Gledhill and Casida, 1969a) showed that best growth is observed in the basal medium [0.04% K_2HPO_4, 0.05% $(NH_4)_2HP0_4$, 0.05% NaCl, 0.005% $MgSO_4{\cdot}7H_2O$, 0.001% $FeCl_3$, and 0.2% fructose, pH 7.0] supplemented with 0.5% vitamin-free Casamino Acids (Difco), 0.5% tryptone, and a 13 vitamin solution. The growth in the same basal medium supplemented with vitamin-free Casamino acids and vitamins is slower, and even weaker with vitamin-free Casamino acids only.

Members of the genus *Agromyces* use a wide range of organic compounds as sole sources of carbon for growth and possess various enzymic activities (Table 144). The published data (Dorofeeva et al., 2003; Gledhill and Casida, 1969a; Groth et al., 1996; Jung et al., 2007; Jurado et al., 2005a, b; Jurado et al., 2005c; Li et al., 2003; Ortiz-Martinez et al., 2004; Rivas et al., 2004a; Suzuki et al., 1996; Takeuchi and Hatano, 2001; Zgurskaya et al., 1992) show that all or the majority of the strains tested so far decompose esculin, casein, and starch, but not adenine and Tween 80. Most tested strains produce acids from starch, D-fructose, and glycogen, but not from adonitol, D- and L-arabitol, dulcitol, erythritol, inositol, D-lyxose, L-sorbose, D-tagatose, L-xylose, methyl-β-xyloside. Most strains do not assimilate aconitate, gluconate, 2- and 5-ketogluconate. Strains of the genus usually show activities (API ZYM) for esterase lipase(C8), leucine arylamidase, valine arylamidase, cystine arylamidase, acid phosphatase, naphthol-AS-BI-phosphohydrolase, but not for lipase (C14), trypsin, and α-fucosidase (except for *Agromyces cerinus* subsp. *nitratus*). Cultured strains typically do not produce indole and show negative reactions in the methyl red and Voges–Proskauer tests.

Agromyces strains also possess enzymic activities useful for biotechnological applications. Members of *Agromyces mediolanus*, *Agromyces cerinus* subsp. *cerinus*, *Agromyces fucosus*, and some other strains transform steroids (Donova, 2006; Ke et al., 2007; Mamoli, 1939; Spassov et al., 1983). Strains of *Agromyces mediolanus* assimilate aniline (Aoki et al., 1982; Suzuki et al., 1996) and degrade detergent compounds such as tallow-alkyl-sulfate, alkyl-ethoxylate-sulfate, and linear-alkyl-benzene-sulfonate (Goodnow and Harrison, 1972). *Agromyces ulmi* decomposes xylan (Rivas et al., 2004a). *Agromyces ramosus* possesses enzymic system able to destroy the cells of *Saccharomyces cerevisiae*, *Staphylococcus aureus*, and several Gram-stain-negative bacteria including *Escherichia coli*, *Agrobacterium tumefaciens*, and *Pseudomonas fluorescens* (Casida, 1983). Certain unnamed members of the genus *Agromyces* degrade nylon (6-aminohexanoate) oligomers (Yasuhira et al., 2007) and metabolize a range of organophosphonate compounds as sole phosphorus sources for growth and use phosphonoacetate as a sole carbon, energy, and phosphorus source for growth (O'Loughlin et al., 2006; Panas et al., 2007). Some strains of this genus are also capable of producing alcohol dehydrogenase (Llyod and Keene, 2008) and indole-3-acetic acid (Vasanthakumar and McManus, 2004).

Most *Agromyces* species grow optimally at near neutral pH; some species are able to initiate growth at pH values 5.0 and/or 9.5–10.0 (Jurado et al., 2005b; Jurado et al., 2005c; Yoon et al., 2008). Alkaliphilic representatives have also been reported (e.g. Yasuhira et al., 2007). Agromycetes are generally considered non-halophilic but some species, e.g. *Agromyces terreus*, grow optimally in the presence of 0.5–1.0% (w/v) NaCl (Yoon et al., 2008). Up to 8% NaCl is tolerated by other species, i.e. very weak growth is observed for *Agromyces brachium*, *Agromyces luteolus*, and *Agromyces rhizospherae* (Takeuchi and Hatano, 2001). Representatives of the genus can be resistant to high concentrations of heavy metals (up to 6 mM in the case of Zn) and mobilize metals (Zn and Cd) supposedly by the release of specific ligands (Kuffner et al., 2008). The exact mechanisms enabling the accumulation process are unclear so far.

The DNA base ratios of the majority of *Agromyces* species range from 68.9 mol% (the type strain of *Agromyces ramosus*) to 73.3 mol% (*Agromyces mediolanus* and *Agromyces rhizospherae*) as determined by Suzuki et al. (1996) and Takeuchi and Hatano (2001) using HPLC. Somewhat higher values for the type strain of *Agromyces ramosus* were reported in earlier works (Döpfer et al., 1982; Gledhill and Casida, 1969a). Among 14 tested strains assigned to *Agromyces cerinus* subsp. *cerinus*, *Agromyces cerinus* subsp. *nitratus*, *Agromyces fucosus*, and *Agromyces hippuratus* (Zgurskaya, 1992), a plasmid was found only in *Agromyces fucosus* VKM Ac-1346. The plasmid size, as determined by agarose gel electrophoresis, was approximately 67 kb.

The bacteriophages for *Agromyces ramosus* can be isolated from soil suspended in heart infusion broth (Difco) by shaking the suspension for 12 h followed by a diluting phage isolation procedure; the addition of *Agromyces ramosus* to the enrichment suspension is usually not necessary (Moore, 1974). It is

* Trypticase soy broth agar, TSA: trypticase soy broth (Difco), 30 g; agar, 15 g; distilled water, 1 liter.

† Corynebacterium medium, CB (DSMZ medium 53 (DSMZ, 2001): casein peptone, tryptic digest, 10 g; yeast extract, 5.0 g; glucose, 5 g; NaCl, 5 g; agar, 15 g; distilled water, 1 liter (pH 7.2–7.4).

‡ R medium (Suzuki et al., 1996; Yamada and Komagata, 1972b): Bacto peptone, 10 g; yeast extract, 5 g; malt extract, 5 g; Casamino acids, 5 g; beef extract, 2 g; glycerol, 2 g; Tween 80, 50 mg; $MgSO_4{\cdot}7H_20$, 1 g; agar, 15 g; water, 1 liter (pH 7.2).

§ Peptone-yeast extract (PY) medium (Takeuchi and Hatano, 2001): Polypeptone (Wako Pure Chemicals), 10 g; yeast extract, 2 g; $MgSO_4{\cdot}7H_2O$, 1 g; distilled water, 1 liter (pH 7.2).

** See Shirling and Gottlieb (1966) for the composition of ISP media cited here and in other sections of this chapter.

worth mentioning that significant differences in abundance of bacteriophages for *Agromyces* strains in some environments can be observed among sampling time period, as reported by Nakayama et al. (2007) for the floodwater of a Japanese paddy.

No serological cross-reactivity with sera raised against representatives of the genera *Actinomyces*, *Rothia*, *Bacterionema*, and *Ramibacterium* was shown for 24 strains assigned to *Agromyces ramosus* (Gledhill and Casida, 1969a).

The following antibiotics (µg per disk) inhibited *Agromyces* strains: tetracycline (30), chloramphenicol (100), imipenem (10), ofloxacin (10), oxytetracycline hydrochloride (30), rifampin (30), vancomycin hydrochloride (30) (Groth et al., 1996; Jurado et al., 2005a, b; Jurado et al., 2005c; Yoon et al., 2008). Sensitivity to several other antibiotics varied between strains or species (Table 144). Representatives of *Agromyces cerinus* subsp. *cerinus*, *Agromyces cerinus* subsp. *nitratus*, *Agromyces fucosus*, and *Agromyces hippuratus* are inhibited by antibacterial substances (termed bacteriocins in the original works) produced by the type strain of *Rathayibacter tritici*, some strains of *Clavibacter michiganensis* subsp. *tesselarius*, and *Clavibacter michiganensis* subsp. *sepedonicus* (Zgurskaya, 1992; Zgurskaya et al., 1992). The type strain of *Agromyces hippuratus* displays inhibitory activity against Gram-stain-positive bacteria including *Bacillus subtilis* and representatives of *Clavibacter michiganensis* subspecies (Zgurskaya, 1992; Zgurskaya et al., 1992). *Agromyces* species closely related to *Agromyces allii* and *Agromyces terreus* has been found to possess antifungal activity against *Mariannaea elegans* and *Rhizomucor variabilis* (Lauer et al., 2008).

The usual natural habitat of *Agromyces* species is soil of different origins, including polluted sites and plant rhizosphere. Like many other soil bacteria, agromycetes are probably involved in the turnover of organic matter in soil and may directly or indirectly contribute to the promotion of plant growth. Members of this genus are also found in other diverse environments including marine sediments (Gontang et al., 2007), mangrove rhizosphere (Takeuchi and Hatano, 2001), and the water phase of tropospheric clouds, 500,000 year-old glacial ice, and some other low temperature environments (Amato et al., 2007a, 2007b; Christner, 2002; Clocksin et al., 2007). Representatives of the genus have also been isolated from cyanobacterial biofilms on cave walls (Jurado et al., 2005b), plants and plant-derived materials (Dorofeeva et al., 2003; Rivas et al., 2004a), and occasionally among endophytic bacterial populations in plant roots (Conn and Franco, 2004; Zakhia et al., 2006). It is noteworthy in this context that *Agromyces* sp. ORS 1437H isolated from the root nodules of a legume species, *Argyrolobium uniflorum*, contains the dinitrogenase reductase-like gene (Zakhia et al., 2006). The determined *nifH* sequence (AJ968686) of this strain is almost identical to those found in *Microbacterium* species isolated from root nodules of *Ononis natrix* subsp. *falcate*, and shows close similarity to the *nifH* gene of *Sinorhizobium meliloti*. However, strain ORS 1437H, like the *Microbacterium* strains, did not induce nodule formation when tested on the wide-host-spectrum legume species *Macroptilium atropurpureum* and no *nodA* gene could be amplified by PCR (Zakhia et al., 2006). Agromycetes are also occasionally found in the soybean cyst nematode (Nour et al., 2003), among cutaneous bacteria of four-toed salamanders (*Hemidactylium scutatum*) (Lauer et al., 2008), and among the human skin microbiota (Grice et al., 2008). No species or strains pathogenic for humans, warm-blooded animals, and plants have been reported within this genus.

Enrichment and isolation procedures

Many isolation media, e.g. nutrient agar (Difco), R2A agar (Difco), peptone/yeast extract/brain heart infusion agar (PY-BHI) described by Yokota et al. (1993a), ten-fold diluted CB agar (DSMZ medium 53), and many other media based on peptone and yeast extract are suitable for isolation of aerobic species of the genus *Agromyces* by using dilution plating methods. Humic acid-vitamin (HV) agar medium, developed by Hayakawa and Nonomura (1987, 1989), was successfully used for isolation of *Agromyces* species from the rhizosphere of mangroves (Takeuchi and Hatano, 2001) and also from a soil sample (Li et al., 2003). The XED agar medium containing xylan and yeast extract was used for isolation of *Agromyces ulmi* from the decayed stump of an elm tree (Rivas et al., 2004a). The dilution frequency procedure for isolation of *Agromyces ramosus* was described in detail by Casida (1965, 1986) and Gledhill and Casida (1969b); the original plating procedure was reported by Labeda et al. (1974). *Agromyces ramosus* grows very slowly during the first passages and can be lost easily; adapted strains usually grow well on nutritionally rich media (e.g. heart infusion agar, Oxoid Ltd.).

Maintenance procedures

The agromycetes can be freeze-dried and preserved in liquid nitrogen by standard procedures.

Differentiation of the genus *Agromyces* from other genera

Agromyces strains form a phylogenetically coherent group based on 16S rRNA gene analysis that is clearly distinct from other genera of *Microbacteriaceae* (Figure 173). In young cultures, the production of well-developed branching hyphae, which may penetrate into agar media, is the most striking visible phenotypic feature that differentiates many *Agromyces* species from all other genera of the family *Microbacteriaceae*. Chemotaxonomic characteristics essential for delineation of agromycetes are listed in Table 140. The L-Dab-based peptidoglycan and fatty acid profile are the salient characteristics that differentiate *Agromyces* species from the related genus *Humibacter*, which has Dab in combination with ornithine in the cell wall and a significant amount of cyclohexyl fatty acid ($C_{17:0}$ Ch) (Vaz-Moreira et al., 2008b).

Taxonomic comments

The genus *Agromyces* was established by Gledhill and Casida (1969a) with the type species *Agromyces ramosus* to accommodate filamentous, branching, fragmenting, nutritionally fastidious, catalase and oxidase-negative soil isolates, considered to be intermediary forms to the facultative-to-anaerobic (*Actinomyces*) and aerobic (*Nocardia*) genera of the order *Actinomycetales*. *Agromyces ramosus* was the only recognized species in the genus till 1992, when Zgurskaya and co-authors described two new species, each with two subspecies, *Agromyces cerinus* subsp. *cerinus* and *Agromyces cerinus* subsp. *nitratus*, and *Agromyces fucosus* subsp. *fucosus* and *Agromyces* subsp. *hippuratus*, and emended the genus description (Zgurskaya et al., 1992). The 14 aerobic

soil isolates assigned by these authors to *Agromyces* possess the morphological and chemotaxonomic characteristics typical of the genus *Agromyces* but are able to grow on relatively simple media under aerobic conditions and show positive catalase and oxidase reactions. Suzuki and co-authors (1996) reclassified the familiar organisms *"Corynebacterium mediolanum"* JCM 3346 (= DSM 20152 = NCIMB 7206), *"Flavobacterium dehydrogenans"* JCM 1376 (= ATCC 13930), and some aniline-assimilating strains (Aoki et al., 1982) as *Agromyces mediolanus*. All other *Agromyces* species considered in this chapter were proposed within the period 2001–2008. Their descriptions are largely based on the type strains, except for *Agromyces allii* (Jung et al., 2007), *Agromyces rhizospherae* (Takeuchi and Hatano, 2001), and *Agromyces ulmi* (Rivas et al., 2004a).

The proposal of novel species within the genus *Agromyces* is justified by the results of 16S rRNA gene analysis and/or DNA–DNA hybridization studies, along with the phenotypic traits. Exceptions are the descriptions of *Agromyces cerinus* and *Agromyces fucosus*, which were done without analyzing 16S rRNA gene sequences (Zgurskaya et al., 1992). Moreover, the affiliation of strains to *Agromyces fucosus* (except for the type strain) and further division of this species into two subspecies is not supported by the DNA–DNA relatedness data and was based on phenotypic characters only (Zgurskaya et al., 1992). Suzuki et al. (1996) later found that the DNA–DNA similarity between the type strains of *Agromyces fucosus* subsp. *fucosus* and *Agromyces fucosus* subsp. *hippuratus* is 45–47%, and subsequent taxonomic revision of the latter resulted in its elevation to the species level (Ortiz-Martinez et al., 2004).

The taxonomic situation with *Agromyces cerinus* subsp. *nitratus* also invites comments. The type strains of *Agromyces cerinus* subsp. *cerinus* and *Agromyces cerinus* subsp. *nitratus* show a relatively high DNA–DNA similarity (68% and 60–62%), as reported by Zgurskaya et al. (1992) and Suzuki et al. (1996). According to Ortiz-Martinez et al. (2004), the strains exhibit the difference in the melting temperature (ΔT_m) equal to 4.1°C which is close to the borderline for species differentiation (Rosselló-Mora and Amann, 2001; Wayne et al., 1987). On the other hand, the 16S rRNA gene sequence identity between *Agromyces cerinus* subsp. *cerinus* and *Agromyces cerinus* subsp. *nitratus* is 99.4%, a value characteristic of many closely related species within the *Microbacteriaceae* and some other families of the order *Micrococcales*. The partial *gyrB* sequence similarity (Stackebrandt et al., 2007) between the type strains of these subspecies (91.5%) is also of the same level as between some closely related species of the family, e.g. *Microbacterium foliorum* and *Microbacterium phyllosphaerae*. The above data along with differences of the *Agromyces cerinus* subspecies in the composition of cell-wall polysaccharides (Shashkov et al., 1993, 1995), the polyamine patterns (Altenburger et al., 1997), and other phenotypic features suggest that *Agromyces cerinus* subsp. *nitratus* could be treated as a separate species in the future.

On compiling additional phenotypic data, strain *Agromyces mediolanus* JCM 9631 may also be classified in the future as a separate species. The strain shows only 46–47% DNA–DNA similarity to the type strain of *Agromyces mediolanus* and differs significantly from other *Agromyces mediolanus* strains in the 16S rRNA gene sequences (Suzuki et al., 1996).

Some of the strains assigned to *Agromyces ramosus* (Gledhill and Casida, 1969a) may also represent at least one novel species, taking into consideration their differences in cell-wall sugar composition and the heterogeneity of physiological and biochemical characteristics.

Differentiation of the species of the genus *Agromyces*

The characteristics useful for differentiation of *Agromyces* species at the phenotypic level are listed in Table 144 and outlined in the descriptions of species. These include, in particular, the colony color, menaquinone system, cell-wall sugar and polyamine patterns, as well as physiological and biochemical characters. It should be emphasized that the whole cells but not the purified cell wall were analyzed in some species for sugar content. Accordingly, some reported sugars may not be located in the cell wall, which should be taken into account while comparing the "cell-wall sugars." When determined under similar conditions, some species have quantitative differences in the predominant cellular fatty acids (Jurado et al., 2005a, 2005b, 2005c; Takeuchi and Hatano, 2001). Comparative analyses of polar lipids and polyamines performed under standardized growth conditions and analytical procedures may provide further chemotaxonomic information useful for differentiation (Altenburger et al., 1997; Jurado et al., 2005a, 2005b, 2005c). Partial sequence of 16S rRNA gene (about 900 bp from the 5′-end) of an isolate would greatly facilitate its species identification within the genus and species groups composing the phylogenetic subclusters (Figure 177). The DNA–DNA hybridization studies or analysis of other genomic characteristics for determination of inter- and intraspecies relatedness (Schumann et al., 2009) might be necessary to differentiate closely related species.

In general, *Agromyces ramosus* strains are easily recognizable among all other species of this genus due to their relatively weak growth, pronounced microaerophily, and requirement of nutritionally rich media. *Agromyces albus* and *Agromyces ulmi* are distinguishable among all other rapidly growing *Agromyces* species owing to their white colonies. Orange to pinkish colonies on some diagnostic media and the ability to form scant aerial hyphae are indicative of *Agromyces aurantiacus*. The species *Agromyces ramosus*, *Agromyces aurantiacus*, and *Agromyces ulmi* also exhibit a negative catalase reaction, in contrast to the remaining *Agromyces* species. Cream-colored to beige colonies are characteristic of the species *Agromyces italicus*, *Agromyces neolithicus*, and *Agromyces terreus*. The species *Agromyces allii* and *Agromyces mediolanus* are also readily recognized due to their cell morphology (usually irregular rods with no hyphal formation).

Acknowledgements

This work was supported by the program MCB RAS of the Russian Academy of Sciences.

List of species of the genus *Agromyces*

1. **Agromyces ramosus** Gledhill and Casida 1969a, 346[AL]

ra.mos′us. L. masc. adj. *ramosus* much-branched.

The characteristics are as described for the genus and listed in Table 144. Additional information is taken from the original description of Gledhill and Casida (1969a) unless indicated otherwise.

After initial growth on agar or in liquid media, microcolonies are composed of branched vegetative hyphae which subsequently undergo septation and fragment into irregular rod-like to coccoid elements. Microaerophilic to aerobic; in heart infusion agar (Difco) shake tubes, growth occurs primarily as a distinct microaerophilic band approximately 2.5 mm below the agar surface, with comparatively less growth above and below this region. Some strains have been reported to show poor growth under anaerobic conditions. Pigmentation is usually not evident under normal growth conditions, but the concentrated masses of cells may be pale yellow due to production of a carotenoid pigment (Jones et al., 1970).

All strains are catalase-negative and exhibit negative reactions with benzidine and tetramethyl-*p*-phenylenediamine. Sugars are oxidized without gas production. Organic nitrogen required for growth. Many biochemical characteristics of 60 soil isolates assigned to *Agromyces ramosus* by Gledhill and Casida (1969a) varied (see the original paper for details). Grow well at 20 and 37°C (optimum at 30°C); some strains show growth at 40°C. Optimal pH is 6.6–7.1. Growth on most agar media is relatively slow, requiring up to 7 d to produce visible colonies. The growth rate (and the pigment concentration) can be increased by culturing on fresh horse blood agar or on soil extract agar supplemented with bovine catalase or MnO_2 (Jones et al., 1970). The presence of other organisms can also stimulate growth and pigment formation (Casida, 1986). Growth in liquid media is poor, cells usually adhere to the glass surface and then settle to the bottom; growth could be increased by adding glass beads to provide additional surface area.

The major menaquinone determined for the type strain is MK-12 (56–62%), followed by MK-13 (14–15%); other menaquinones, MK-7 to MK-11 and MK-14, occur in minor (1–8%) amounts (Sasaki et al., 1998; Suzuki et al., 1996). The predominant cellular fatty acids determined for the type strain are $C_{15:0}$ anteiso (38%), $C_{17:0}$ anteiso (38%), and $C_{16:0}$ iso (16%) (Suzuki et al., 1996). Polar lipids are diphosphatidylglycerol, phosphatidylglycerol, and two characteristic glycolipids (Collins, 1982). Cell-wall sugars are rhamnose, galactose, xylose, and mannose.

Strains assigned to *Agromyces ramosus* were isolated from soils of different origin, ranging from fertile meadow to barren desert soils. *Agromyces ramosus* was reported to be 10- to 100 fold more numerous in many soil samples than the total microflora normally cultured on plates.

Source: soil.

DNA G+C content (mol%): 68.9 (HPLC).

Type strain: ATCC 25173, CIP 103037, DSM 43045, JCM 3108, LMG 16680, NBRC 13899, VKM Ac-1198.

Sequence accession no. (16S rRNA gene): X77447.

2. **Agromyces albus** Dorofeeva, Krausova, Evtushenko and Tiedje 2003, 1437[VP]

al′bus. L. masc. adj. *albus* white, referring to the white color of colonies.

The general characteristics are as described for the genus and listed in Table 144. Additional information is taken from the original description (Dorofeeva et al., 2003).

Colonies are white and may penetrate into agar media. After initial growth on agar, microcolonies are composed of branched vegetative hyphae which subsequently break up into diphtheroid and short, irregular rod-like fragments. Aerobic. Growth occurs between 7°C (weak) and 37°C; optimum is ~26–28°C. Adonitol, cellobiose, D-fucose, inositol, lyxose, maltose, D-mannitol, D-mannose, melibiose, L-rhamnose, raffinose, salicin, L-sorbose, sucrose, trehalose, turanose, and D-xylose are used as carbon sources for growth on basal medium (Shirling and Gottlieb, 1966) supplemented with 0.1% (w/v) yeast extract and 0.1% (w/v) casitone. Sensitive to the following antibiotics (μg/ml): ampicillin (10), cefazolin (10), doxycycline (5), erycycline (10), gentamicin (30), karbonicillin (30), levomycetin (10), metacycline (10), oletetrin (10), oxacillin (30), and rifampin (10). Tolerates amikacin (30), clindamycin (30), lincomycin (30), rubomycin (30), and streptomycin (30).

Predominant menaquinone is MK-12, with a smaller amount of MK-11. The major cellular fatty acids are $C_{15:0}$ anteiso (37%), $C_{17:0}$ anteiso (24%), and $C_{16:0}$ iso (20%). Cell-wall sugars include rhamnose as the predominant component; glucose, galactose, and mannose occur in smaller amounts. For further descriptive information see Dorofeeva et al. (2003) and Jurado et al. (2005c).

Source: a sample of leaves and inflorescence of *Androsace* sp. (the family Primulaceae), collected in the Central-Chernozem Biosphere Park, Belgorod region, Russia. The plant material was not subjected to surface sterilization.

DNA G+C content (mol%): 69.0 (T_m).

Type strain: UCM Ac-623, CIP 108259, DSM 15934, JCM 13565, NBRC 103057, VKM Ac-1800.

Sequence accession no. (16S rRNA gene): AF503917.

3. **Agromyces allii** Jung, Lee, Oh and Yoon 2007, 591[VP]

al′li.i. N.L. gen. n. *allii* of *Allium*, referring to the source of isolation of the microorganisms, the rhizosphere of *Allium victorialis* var. *platyphyllum*.

The general characteristics are as described for the genus and listed in Table 144. Additional information is taken from the original description (Jung et al., 2007).

Colonies are yellow-pigmented. Cells usually display irregular, slightly curved rods (0.2–0.4 × 0.5–1.5 μm). No growth occurs under anaerobic conditions on nutrient agar (Difco), with or without nitrate. Growth is observed between 4 and 33°C, with an optimum at 30°C. Optimal pH for growth is 6.5–7.5; no growth occurs at pH 5.0 and at 3% (w/v) NaCl. No activities for arginine decarboxylase, lysine decarboxylase, ornithine decarboxylase, and tryptophan deaminase. D-Glucose, D-galactose, D-cellobiose,

D-mannose, D-trehalose, D-xylose, L-arabinose, sucrose, maltose, and salicin are used for growth as determined by conventional methods.

Predominant menaquinones are MK-11 and MK-12 (peak area ratios of approximately 45–47% and 35–38%, respectively). The major cellular fatty acids reported for the type strain and the second strain (UMS-101) are $C_{15:0}$ anteiso (39 and 34%), $C_{16:0}$ iso (29 and 12%), $C_{17:0}$ anteiso (18 and 17%), and $C_{16:0}$ (6 and 24%). Major polar lipids are diphosphatidylglycerol, phosphatidylglycerol, and an unidentified glycolipid. Whole-cell sugars include rhamnose, ribose, and galactose; xylose has been detected in the type strain only.

The two described strains of this species have almost identical 16S rRNA gene sequences and show a mean DNA–DNA hybridization of 91%. For further descriptive information see Jung et al. (2007).

Source: the rhizhosphere of *Allium victorialis* var. *platyphyllum*, a type of wild edible greens grown on Ulleung Island, Korea.

DNA G+C content (mol%): 71.1–71.3 (HPLC).

Type strain: strain UMS-62, CIP 109546, JCM 13584, KCTC 19181.

Sequence accession no. (16S rRNA gene): DQ673873.

4. **Agromyces aurantiacus** Li, Zhang, Xu, Cui, Xu, Zhang, Schumann, Stackebrandt and Jiang 2003, 306[VP]

au.ran.ti.a′cus. N.L. masc. adj. *aurantiacus* orange-colored.

The general characteristics are as described for the genus and listed in Table 144. Additional information is taken from the original description (Li et al., 2003).

Colonies are orange-yellow of different intensity on inorganic salts-starch agar (ISP 4) and yeast extract-malt extract agar (ISP 2), pink-gray on oatmeal agar (ISP 3), and pink-white on Czapek agar and glycerol-asparagine agar (ISP 5). Young cultures on agar media produce branched vegetative hyphae which break up into diphtheroid and rod-like, irregular fragments at a later growth stage. Light yellow-brown aerial mycelium is observed in 2–3-weeks cultures on ISP5 agar medium. Aerobic. Both young (16 h) and older (4 d) cultures on tryptone soy broth agar (Oxoid) show negative catalase reaction. Optimal growth temperature is 28°C. The following carbon sources are used for growth as determined by conventional methods (Shirling and Gottlieb, 1966; Williams et al., 1989): glucose, galactose, fructose, sucrose, xylose, raffinose, D-arabinose, inulose, sorbitol, mannitol, and inositol; lactose and rhamnose are not utilized.

Predominant menaquinone is MK-12; minor components are MK-13 and MK-11. Major cellular fatty acids include $C_{15:0}$ anteiso (37%), $C_{17:0}$ anteiso (24%), and $C_{16:0}$ iso (20%). Principal phospholipids are phosphatidylglycerol and diphosphatidylglycerol. Whole cell sugars include rhamnose and small quantities of galactose, glucose, and mannose. For further descriptive information see Li et al. (2003).

Source: a soil sample collected from Xishuangbanna in Yunnan Province, China.

DNA G+C content (mol%): 72.8 (T_m).

Type strain: YIM 21741, AS 4.1717, CCTCC 001012, DSM 14598, JCM 12113.

Sequence accession no. (16S rRNA gene): AF389342.

5. **Agromyces bracchium** Takeuchi and Hatano 2001, 1536[VP]

brac′chi.um. L. n. *brachium* a twig, referring to the twig-like morphology.

The general characteristics are as described for the genus and listed in Table 144. Additional information is taken from the original description (Takeuchi and Hatano, 2001), unless indicated.

Colonies are yellow and sometimes penetrate into agar media. After initial growth on CB agar, microcolonies are composed of rudimentary branching filaments which relatively rapidly (18–24 h) fragment into short irregular rods to coccoid cells (recent observations). In liquid PY medium, filamentous and occasionally branching cells (approximately 5.0–6.0 µm or more in length) are observed in the early growth phase. These fragment into irregular rods (1.5–3.0 µm) during exponential growth stage.

Aerobic. Grows optimally at 20–30°C. Good growth occurs in the presence of 5% (w/v) NaCl; very weak growth may be observed at 8%.

The major menaquinone is MK-12 (78%) with minor amounts of MK-13 (14%) and MK-11 (8%). Predominant cellular fatty acids are $C_{15:0}$ anteiso (42%), $C_{17:0}$ anteiso (26%), and $C_{16:0}$ iso (28%). Cell-wall sugars include rhamnose (80%), glucose (7%), mannose (7%), and galactose (6%). For further descriptive information see Takeuchi and Hatano (2001).

Source: soil of mangrove rhizosphere (*Bruguera gymnorrhiza*) on Iriomote Island, Japan.

DNA G+C content (mol%): 70.0 (HPLC).

Type strain: 65, DSM 14596, NBRC 16238, JCM 11433, VKM Ac-2088.

Sequence accession no. (16S rRNA gene): AB023359.

6. **Agromyces cerinus** Zgurskaya, Evtushenko, Akimov, Voyevoda, Dobrovolskaya, Lysak and Kalakoutskii 1992, 639[VP]

ce′ri.nus. L. masc. adj. *cerinus* wax-colored, yellow like wax.

Colonies are typically whitish yellow to yellow and penetrate into agar media. The pigment production is light-induced (Trutko et al., 2005). After initial growth on agar, microcolonies are composed of branching vegetative hyphae which are occasionally septated. As growth proceeds, the hyphae undergo further septation and fragment into irregular rod-like to coccoid cells in 3–4 d culture. Optimal growth temperature is 26–28°C.

Two subspecies were described within this species (Zgurskaya et al., 1992). *Agromyces cerinus* subsp. *cerinus* includes strains that show DNA similarity levels ranging from 71–92%, containing tyvelose and rhamnose in their cell walls, and clustering together based on numerical analysis. Strain VKM Ac-1351 attributed to *Agromyces cerinus* subsp. *nitratus* exhibits a level of 60–68% DNA similarity with the type strain of *Agromyces cerinus* subsp. *cerinus* (Suzuki et al., 1996; Zgurskaya et al., 1992) but differs from the other strains of *Agromyces cerinus* in its cell-wall sugars and in a number of biochemical characteristics. Considering other genotypic and chemotaxonomic features revealed subsequently and discussed in the Taxonomic comments section, it is quite likely that *Agromyces cerinus* subsp. *nitratus* will be elevated to the species level in future.

DNA G+C content (mol%): 71.5–71.9 (HPLC).

Type strain: ATCC 51762, CIP 103632, DSM 8595, NBRC 15780, JCM 9083, LMG 16155, VKM Ac-1340.

Sequence accession no. (16S rRNA gene): AM410680, D45060, X77448.

6a. **Agromyces cerinus subsp. cerinus** Zgurskaya, Evtushenko, Akimov, Voyevoda, Dobrovolskaya, Lysak and Kalakoutskii 1992, 640[VP]

The general characteristics are as described for the genus and species and listed in Table 144. Additional information is taken from the original description unless indicated otherwise.

Most strains grow on medium containing *meso*-inositol as a carbon source. Organic nitrogen is not required for good growth. Elastin, guanine, pectin, and Tween 80 are not decomposed. The purified cell wall of the type strain contains (μg/mg dry wt) rhamnose (29), tyvelose (29), galactose (9.9), and mannose (7.3) (Zgurskaya, 1992). The same predominant cell-wall sugars (rhamnose, tyvelose, and galactose) in varying amounts are found in the other strains of this subspecies, while mannose is absent or occurs in minor or trace quantities. Whole cells were reported to include (μg/mg, dry wt): rhamnose (10.8–29.5), glucose (7.1–18.1), galactose (5.3–11.9), ribose (5.1–9.1), tyvelose (1.0–9.8), mannose (2.8–8.2), and xylose (0.4–7.3). Five strains of *Agromyces cerinus* subsp. *cerinus*, including the type strain, contain an unusual poly(arabitol phosphate) teichoic acid in the cell wall (Shashkov et al., 1995). The overall polyamine content of the type strain is 0.26 μmol/g (dry wt) and includes putrescine (42%), 1,3-diaminopropane (39%), cadaverine (7.7%), spermidine (7.7%), and spermine (3.8%) (Altenburger et al., 1997). The major menaquinone of the type strain is MK-12 (60%) followed by MK-13 (21%); other components MK-11, MK-10, MK-9, and MK-14 occur in small quantities (1–8%) or in a trace amount (MK-7) (Suzuki et al., 1996). Predominant cellular fatty acids are $C_{15:0}$ anteiso, $C_{17:0}$ anteiso, and $C_{16:0}$ iso. Principal phospholipids are diphosphatidylglycerol and phosphatidylglycerol; an unidentified minor phospholipid has been detected in the type strain. For further descriptive information, see Zgurskaya et al. (1992), Groth et al. (1996), Suzuki et al. (1996), and Jurado et al. (2005a, 2005b, 2005c).

Source: soil in a grove of birches, Moscow, Russia.

DNA G+C content (mol%): 72.0 (T_m); 70.5 (HPLC).

Type strain: ATCC 51762, CIP 103632, DSM 8595, NBRC 15780, JCM 9083, LMG 16155, VKM Ac-1340.

Sequence accession no. (16S rRNA gene): AM410680, D45060, X77448.

6b. **Agromyces cerinus subsp. nitratus** Zgurskaya, Evtushenko, Akimov, Voyevoda, Dobrovolskaya, Lysak and Kalakoutskii 1992, 640[VP]

ni.tra′tus. N.L. n. *nitras -atis* nitrate; N.L. masc. adj. *nitratus* pertaining to the ability to reduce nitrate.

The general characteristics are as described for the genus and species and listed in Table 144. Additional information is taken from the original description unless indicated otherwise.

L-Arabinose, melibiose, and starch are used as carbon sources on ISP 9 (Shirling and Gottlieb, 1966) as basal medium supplemented with 0.01% (w/v) yeast extract. Organic nitrogen is required for good growth. Nitrate is reduced to nitrite. Cell-wall sugars detected in the purified cell wall are galactose and smaller quantities of glucose and ribose (molar ratio, 1.0:0.3:0.2) and traces of mannose. Whole cell sugars detected include galactose, glucose, ribose, xylose, and mannose. An unusual anionic poly(ribofuranosylribitol phosphate) polymer is present in the cell wall (Shashkov et al., 1993). The overall polyamine content is 0.22 μmol/g (dry wt) and includes putrescine (64%), spermine (18%), spermidine (14%) and cadaverine (4.5%) (Altenburger et al., 1997). The major menaquinone is MK-12 (61%), and the next most abundant component is MK-13 (24%); other menaquinones, i.e. MK-11, MK-10, MK-14, and MK-7 occur in small quantities (1–7%) or in a trace amount (MK-9) (Suzuki et al., 1996). Predominant cellular fatty acids are $C_{15:0}$ anteiso, $C_{17:0}$ anteiso, and $C_{16:0}$ iso. Principal phospholipids are diphosphatidylglycerol and phosphatidylglycerol.

Agromyces cerinus subsp. *nitratus* is readily distinguished from *Agromyces cerinus* subsp. *cerinus* by the ability to reduce nitrate, the absence of rhamnose and tyvelose both in the whole cells and in the purified cell walls, and the polyamine profile with putrescine, spermine, and spermidine as the major components.

For further descriptive information, see Zgurskaya et al. (1992), Suzuki et al. (1996), and Jurado et al. (2005a, 2005b).

Source: soil in a grove of birches, Moscow, Russia.

DNA G+C content (mol%): 72.0 (T_m); 70.9 (HPLC).

Type strain: ATCC 51763, CIP 103634, DSM 8596, NBRC 15783, JCM 9084, LMG 16157, VKM Ac-1351.

Sequence accession no. (16S rRNA gene): AM410681, AY277619.

Additional comments: the phenotypic and genotypic features of *Agromyces cerinus* subsp. *nitratus* suggest that this subspecies could be treated as a separate species in the future (see the *Taxonomic comments* section for details).

7. **Agromyces fucosus** Zgurskaya, Evtushenko, Akimov, Voyevoda, Dobrovolskaya, Lysak and Kalakoutskii 1992, 640[VP] emend. Ortiz-Martinez, Gonzalez, Evtushenko, Jurado, Laiz, Groth and Saiz-Jimenez 2004, 1555

fu.co′sus. N.L. n. *fucosum* fucose; N.L. masc. adj. *fucosus* (*sic*), containing fucose in the cell wall.

The general characteristics are as described for the genus and species and listed in Table 144. Additional information is taken from Zgurskaya et al. (1992) and Ortiz-Martinez et al. (2004) unless indicated otherwise.

Colonies are whitish yellow to intense yellow and occasionally penetrate into the agar media. The pigment biosynthesis is light-induced (Trutko et al., 2005). After initial growth on CB agar, microcolonies are composed of branching vegetative hyphae and short filaments. As growth

proceeds, they undergo septation and fragment into irregular rod-like to coccoid cells in 2–3 d cultures.

Optimum growth is at 26–30°C. Strains are able to grow at 7°C; growth at 37°C is variable between experiments. D-Arabinose, cellobiose, D-fructose, D-galactose, D-glucose, maltose, D-mannose, melezitose, methyl D-glucoside, L-rhamnose, salicin, sucrose, trehalose, and D-xylose are used for growth as sole carbon sources on basal medium ISP 9 supplemented with 0.01% (w/v) yeast extract.

Arbutin and esculin are hydrolyzed; adenine, elastin, guanine, pectin, and testosterone are not decomposed or hydrolyzed. Hippurate, hypoxanthine, Tween 40, and tyrosine are decomposed by some strains, including the type strain. Casein is not hydrolyzed (except for by the type strain). Production of H_2S from peptone is variable among strains; the type strain shows a positive reaction. Nitrate reduction is negative or weak. No growth occurs on agar media supplemented with 5% NaCl.

Purified cell walls of four strains contain varying quantities of rhamnose, galactose, and fucose, and trace amounts of mannose in some strains, including the type strain. Whole cells of the examined strains include rhamnose, galactose, glucose, mannose, ribose, and fucose. Cell wall contains 1,3-poly(glycerol phosphate) teichoic acid with *N*-acetylglucosamine substituents (Malysheva, 1994; Naumova et al., 2001). Overall polyamine content determined for the type strain is 0.21 μmol/g (dry wt), and includes putrescine (57%), 1,3-diaminopropane (24%), spermidine (9.5%), and spermine (4.8%) (Altenburger et al., 1997). The major menaquinone determined in the type strain is MK-12 (67%); the next most abundant menaquinone is MK-13 (15%); other components, i.e. MK-11, MK-10, MK-9, and MK-14 occur in small quantities (1–9%) (Suzuki et al., 1996). Predominant cellular fatty acids are $C_{15:0}$ anteiso (47%), $C_{17:0}$ anteiso (28%), and $C_{16:0}$ iso (15%). Principal phospholipids are diphosphatidylglycerol and phosphatidylglycerol. For further descriptive information see Zgurskaya et al. (1992), Groth et al. (1996), Suzuki et al. (1996), and Jurado et al. (2005a, 2005b, 2005c).

Source: soil in a grove of birches, Moscow, Russia.

DNA G+C content (mol%): 70–72 (T_m); 70.6 (HPLC).

Type strain: ATCC 51764, CCUG 35506, CIP 103633, DSM 8597, NBRC 15781, IMET 11529, JCM 9085, LMG 16156, VKM Ac-1345.

Sequence accession no. (16S rRNA gene): AJ784791.

8. **Agromyces hippuratus** (Zgurskaya, Evtushenko, Akimov, Voyevoda, Dobrovolskaya, Lysak and Kalakoutskii 1992) Ortiz-Martinez, Gonzalez, Evtushenko, Jurado, Laiz, Groth and Saiz-Jimenez 2004, 1555[VP] (*Agromyces fucosus* subsp. *hippuratus* Zgurskaya, Evtushenko, Akimov, Voyevoda, Dobrovolskaya, Lysak and Kalakoutskii 1992, 640)

hip.pu.ra′tus. N.L. n. *hippuras -atis* hippurate; N.L. masc. adj. *hippuratus* pertaining to the ability to decompose hippurate.

The general characteristics are as described for the genus and species and listed in Table 144. Additional information is taken from Zgurskaya et al. (1992) and Ortiz-Martinez et al. (2004) unless indicated otherwise.

Colonies are whitish yellow to intense yellow and occasionally penetrate into the agar. Produces a yellow carotenoid pigment, biosynthesis of which is light-induced (Trutko et al., 2005). After initial growth on agar, microcolonies are typically composed of branched and occasionally septated vegetative hyphae. As growth proceeds, these undergo further septation and fragment into irregular rod-like to coccoid cells in 3–4 d cultures.

Optimum growth is at 26–30°C. Strains are able to grow at 7°C; weak or no growth occurs at 37°C. D-Arabinose, cellobiose, D-fructose, D-galactose, D-glucose, maltose, D-mannose, melezitose, melibiose, L-rhamnose, salicin, sucrose, trehalose, and D-xylose are used for growth as sole carbon sources on ISP 9 (Shirling and Gottlieb, 1966) as basal medium supplemented with 0.01% (w/v) yeast extract. No growth occurs on media supplemented with 5% NaCl.

The major menaquinone revealed in the type strain is MK-12 (67%) followed by MK-13 (15%); other components, i.e. MK-11, MK-10, MK-9, and MK-14 occur in small quantities (1–9%) (Suzuki et al., 1996). Predominant cellular fatty acids are $C_{15:0}$ anteiso (52%), $C_{17:0}$ anteiso (18%), and $C_{16:0}$ iso (14%). Principal phospholipids detected are diphosphatidylglycerol and phosphatidylglycerol; strain VKM Ac-1353 contains an unidentified phospholipid. The total polyamine content determined in the type strains is 0.24 μmol/g (dry wt) and composed of putrescine (58%), spermidine (29%), spermine (13%), and trace amounts of 1,3-diaminopropane, *sym*-homospermidine, and *sym*-norspermidine (Altenburger et al., 1997). Cell walls of both described strains of this species (VKM Ac-1352 and VKM Ac-1353) contain 1,5-poly(ribitol phosphate) teichoic acid with tetrasaccharide substituents consisted of residues of rhamnose and *N*-acetylglucosamine. The predominant sugar in purified cell walls is rhamnose. Minor amounts of galactose and traces of mannose may occasionally occur. Whole cells of the above strains contain rhamnose, galactose, glucose, ribose, mannose, and trace amounts of xylose and fucose. For further descriptive information see Zgurskaya et al. (1992) and Ortiz-Martinez et al. (2004).

Source: soil in a grove of birches, Moscow, Russia.

DNA G+C content (mol%): 71–72 (T_m); 70.8 (HPLC).

Type strain: ATCC 51765, CIP 103635, DSM 8598, NBRC 15782, JCM 9086, LMG 16158, VKM Ac-1352.

Sequence accession no. (16S rRNA gene): not available.

9. **Agromyces humatus** Jurado, Groth, Gonzalez, Laiz, Schuetze and Saiz-Jimenez 2005c, 874[VP]

hu.ma′tus. L. masc. part. adj. *humatus* buried.

The general characteristics are as described for the genus and listed in Table 144. Additional information is taken from the original description (2005c).

Colonies are yellow-pigmented. Young cultures on agar media produce branching vegetative hyphae which subsequently break up into irregular rod-like and diphtheroid fragments. Aerobic. Growth occurs at 15–37°C (optimal growth at 28°C) and at pH 5–9.5. NaCl is tolerated up to 2% (w/v).

Major menaquinones are MK-13 and MK-12. Predominant fatty acids include $C_{15:0}$ anteiso (42%), $C_{17:0}$ anteiso (34%), and $C_{16:0}$ iso (16%). Polar lipids are phosphatidylglycerol, phosphatidylglycerol, one unidentified phospholipid,

and two unidentified glycolipids. Whole cell sugars include glucose, galactose, rhamnose, and mannose. For further descriptive information see Jurado et al. (2005c).

Source: the wall of a tomb located in the Little Apostle cubicle, Domitilla Catacombs, Rome, Italy.

DNA G+C content (mol%): 70.6 (T_m).

Type strain: strain CD5, CIP 108741, DSM 16389, HKI 0327, JCM 14319, NCIMB 14012.

Sequence accession no. (16S rRNA gene): AY618216.

10. **Agromyces italicus** Jurado, Groth, Gonzalez, Laiz, Schuetze and Saiz-Jimenez 2005c, 874[VP]

i.ta′li.cus. L. masc. adj. *italicus* of or belonging to Italy, the origin of the type strain.

The characteristics are as described for the genus and listed in Table 144. Additional information is taken from the original description (Jurado et al., 2005c).

Colonies are cream-colored. Young cultures on agar media produce branching vegetative hyphae which subsequently break up into irregular rod-like and diphtheroid fragments. Aerobic to microaerophilic. Growth occurs at 10–37°C (optimal growth at 28°C) and at pH 5–9.5. NaCl is tolerated up to 4% (w/v), no growth is observed at 6% NaCl.

Major menaquinones are reported to be MK-12 and MK-13. Predominant fatty acids are $C_{15:0}$ anteiso (51%), $C_{17:0}$ anteiso (15%), $C_{16:0}$ iso (15%), and $C_{15:0}$ iso (14%). Polar lipids are diphosphatidylglycerol, phosphatidylglycerol, two unidentified phospholipids, and an unidentified glycolipid. Whole cell sugars include galactose, ribose, glucose, and mannose. For further descriptive information see Jurado et al. (2005c).

Source: the wall of a tomb located in the Little Apostle cubicle, Domitilla Catacombs, Rome, Italy.

DNA G+C content (mol%): 70.8 (T_m).

Type strain: strain CD1, DSM 16388, HKI 0325, IAM 15273, JCM 14320, NCIMB 14011.

Sequence accession no. (16S rRNA gene): AY618215.

11. **Agromyces lapidis** Jurado, Groth, Gonzalez, Laiz, Schuetze and Saiz-Jimenez 2005c, 874[VP]

la.pi′dis. L. gen. n. *lapidis* of a stone.

The characteristics are as described for the genus and listed in Table 144. Additional information is taken from the original description (Jurado et al., 2005c).

Colonies are yellow-colored. Young cultures on agar media are composed of branching vegetative hyphae which subsequently break up into irregular rod-shaped and diphtheroid fragments. Aerobic to microaerophilic. Growth occurs at 15–37°C (with an optimum temperature of 28°C) and at pH 5–9.5. NaCl is tolerated up to 4% (w/v); no growth is observed at 6% NaCl.

Major menaquinones are MK-12 and MK-13. Predominant fatty acids are $C_{15:0}$ anteiso (48%), $C_{16:0}$ iso (17%), $C_{17:0}$ anteiso (13%), and $C_{15:0}$ iso (11%). Polar lipid profile includes diphosphatidylglycerol, phosphatidylglycerol, one unidentified phospholipid, and two unidentified glycolipids. Major menaquinones are MK-12 and MK-13. Whole cell sugars detected are glucose, galactose, mannose, and ribose. For further descriptive information, see Jurado et al. (2005c).

Source: a carved stone wall of the Domitilla Catacombs, Rome, Italy.

DNA G+C content (mol%): 70.4 (T_m).

Type strain: CD55, DSM 16390, HKI 0324, IAM 15275, JCM 14321, NCIMB 14013.

Sequence accession no. (16S rRNA gene): AY618217.

12. **Agromyces luteolus** Takeuchi and Hatano 2001, 1535[VP]

lu.te.o′lus. L. dim. masc. adj. *luteolus* yellowish, somewhat yellow.

The characteristics are as described for the genus and listed in Table 144. Additional information is taken from the original description (Takeuchi and Hatano, 2001), unless indicated.

Colonies are yellow to whitish yellow and occasionally penetrate into agar media. After initial growth on CB agar, microcolonies are composed of rudimentary branching vegetative hyphae and short filaments which fragment as growth proceeds and display diphtheroid, irregular shaped rods and coccoid cells in 3–4 d culture (recent observations). In liquid PY medium, filamentous or elementary branching cells (up to 5.0 µm or more in length) are produced in the early growth phase, which fragment into irregular rods (0.2–0.4 × 1.5–3.0 µm) during exponential growth phase.

Grows optimally at 20–30°C. Good growth is observed in the presence of 5% (w/v) NaCl; very weak growth occurs at 8% NaCl.

Major menaquinones are MK-12 (65%) and MK-11 (35%). Predominant cellular fatty acids include $C_{16:0}$ iso (48%), $C_{15:0}$ anteiso (33%), and $C_{17:0}$ anteiso (9.4%). Cell-wall sugars are rhamnose (67%), fructose (25%), mannose (6%), and glucose (2%). For further descriptive information, see Takeuchi and Hatano (2001).

Source: the surface of root of mangrove (*Sonneratia alba*) on Iriomote Island, Japan.

DNA G+C content (mol%): 71.1 (HPLC).

Type strain: strain 8, DSM 14595, JCM 11431, NBRC 16235, NCIMB 13882, VKM Ac-2085.

Sequence accession no. (16S rRNA gene): AB023356.

13. **Agromyces mediolanus** (*ex* Mamoli 1939) Suzuki, Sasaki, Uramoto, Nakase and Komagata 1996, 92[VP] ("*Corynebacterium mediolanum*" Mamoli 1939, 1863)

me.di.o′la.nus. N.L. masc. adj. *mediolanus* (*sic*) of or belonging *Mediolanum*, the old name of Milan, Italy.

The characteristics are as described for the genus and listed in Table 144. Additional information is taken from the original description (Suzuki et al., 1996) unless indicated otherwise.

Colonies are whitish yellow to yellow due to carotenoid pigment, biosynthesis of which is light-induced (Trutko et al., 2005; Weeks and Garner, 1967). After initial growth on agar, cells are typically composed of irregular rods (0.3–0.6 × 1.4–3.0 µm) or short filaments; a rudimentary mycelium may occasionally be formed on some media (recent observations). Week-old cultures are composed mainly of short irregular rods, but a proportion of the cells may be coccoid. Aerobic to microaerophilic (Jurado et al., 2005c). Optimal growth temperature is 28–30°C.

Strains of this species hydrolyze starch (except for the type strain) and assimilate fumarate, hippurate (except for the type strain), propionate, and urate as determined by conventional methods. DNase-negative. The following characters are variable among strains: nitrate reduction, assimilation of glyoxalate, acid production from mannose, ribose, salicin, and sucrose (the type strain is positive in the listed activities). Acid is not produced by the type and some other strains from L-rhamnose, fructose, maltose, and amygdalin.

The major menaquinone is MK-12 (54–61%); the next most abundant menaquinones are MK-11 (14–20%) and MK-10 (12–15%); other components, i.e. MK-7, MK-8, MK-9, and MK-13 occur in minor (1–5%) or trace amounts (Sasaki et al., 1998; Suzuki et al., 1996). Predominant cellular fatty acids are $C_{15:0}$ anteiso (26–33%), $C_{17:0}$ anteiso (37–46%), and $C_{16:0}$ iso (17–28%) (Collins and Jones, 1980; Suzuki et al., 1996). The polar lipid profiles of two strains (the type strain and "*Flavobacterium dehydrogenans*" NCIMB 872) were almost identical and included diphosphatidylglycerol, phosphatidylglycerol (in very small quantities), and one characteristic glycolipid (Collins and Jones, 1980). The overall polyamine content is 0.28 μmol/g (dry wt); putrescine (75%) and 1,3-diaminopropane (25%) are the major compounds; cadaverine, spermine, spermidine, *sym*-homospermidine, and *sym*-norspermidine occur in trace amounts (Altenburger et al., 1997).

The levels of DNA–DNA hybridization of the type strain JCM 3346 and three other strains assigned to *Agromyces mediolanus* (JCM 1376, JCM 9632 and JCM 9633) are very high (80–99%), while the DNA–DNA hybridization between strain JCM 9631 and the above four strains ranged from 46–65%. This relationship coincides with a lower 16S rRNA gene sequence similarity determined among these strains. Suzuki et al. (1996) suggested that strain JCM 9631 is distinct from the other four strains at least at the subspecies level, but no clear differentiating phenotypic characters, except for acid production from mannitol, were observed. Therefore, strain JCM 9631 was not reclassified to a novel species.

Source: yeasts, pressed yeast cake, soil, and water filter (Aoki et al., 1982; Arnaudi, 1942; Cummins et al., 1974; Mamoli, 1939; Suzuki et al., 1996).

DNA G+C content (mol%): 72.3 (HPLC).

Type strain: ATCC 14004, CIP 104860, DSM 20152, JCM 3346, NBRC 15704, NCIMB 7206, VKM Ac-1388.

Sequence accession no. (16S rRNA gene): HM641754, X77449.

14. **Agromyces neolithicus** Jurado, Groth, Gonzalez, Laiz and Saiz-Jimenez 2005a, 157[VP]

ne.o.li′thi.cus. N.L. masc. adj. *neolithicus* neolithic, referring to the origin of the neolithic paintings in Grotta dei Cervi, the source of the soil from which the organism was isolated.

The characteristics are as described for the genus and listed in Table 144. Additional information is taken from the original description (Jurado et al., 2005a).

Colonies are beige-colored. Young cultures are composed of branching vegetative hyphae which subsequently break up into irregular rod-shaped and diphtheroid fragments. Aerobic to microaerophilic. Growth occurs at 15–37°C, with an optimum at 28°C.

Major menaquinones are MK-13 and MK-12. Predominant cellular fatty acids include $C_{15:0}$ anteiso (38%), $C_{17:0}$ anteiso (32%), $C_{16:0}$ iso (13%), and $C_{15:0}$ iso (13%). Major polar lipids are diphosphatidylglycerol, phosphatidylglycerol, two unidentified phospholipids, and an unidentified glycolipid. Whole cell sugars include glucose, galactose, and mannose. For further descriptive information see Jurado et al. (2005a).

Source: a soil sample of the Grotta dei Cervi, Italy.

DNA G+C content (mol%): 65.3 (T_m).

Type strain: strain 23-23, CIP 108987, DSM 16197, HKI 0321, JCM 4322, NCIMB 13990.

Sequence accession no. (16S rRNA gene): AY507128.

15. **Agromyces rhizospherae** Takeuchi and Hatano 2001, 1536[VP]

rhi.zo.sphe′ra.e. N.L. *rhiza* root; Gr. n. *sphaira* sphere; N.L. gen. n. *rhizospherae* of the sphere of the root.

The characteristics are as described for the genus and listed in Table 144. Additional information is taken from the original description (Takeuchi and Hatano, 2001), unless indicated.

Colonies are yellow-colored and occasionally penetrate into agar media. After initial growth on CB agar, microcolonies are composed of rudimentary branched vegetative hyphae or short filaments which relatively rapidly (1–2 d) fragment into short irregular rods to coccoid cells; older cultures (1–2 weeks) usually display coccobacillaric forms (recent observation). In a liquid culture in PY medium, cells display irregular rods (0.2–0.4 × 1.5–3.0 μm) or short, occasionally branching filaments in the early growth phase, which fragment into short rods (mean 0.2–0.4 × 0.8–1.5 μm) to coccoid cells during exponential growth phase.

Aerobic. Grows optimally at 20–30°C. Good growth occurs in the presence of 5% (w/v) NaCl; very weak growth is also observed at 8%.

The major menaquinones determined for the type strain are MK-12 (52%) and MK-11 (48%), while the second strain (IFO 16237) contains MK-12 as the predominant component (81%), with small amounts of MK-11 (19%). Predominant cellular fatty acids reported for the two strains are $C_{16:0}$ iso (54 and 44%), $C_{15:0}$ anteiso (26 and 27%), and $C_{17:0}$ anteiso (13 and 19%). Cell-wall sugars are rhamnose (89%), glucose (4–5%), and mannose (6–7%).

The two described strains of this species share 99.2% 16S rRNA gene sequence similarity and show 69–73% DNA–DNA hybridization. For further descriptive information see Takeuchi and Hatano (2001).

Source: soil of rhizosphere of mangroves on Iriomote Island, Japan; the type strain was isolated from the rhizosphere of *Sonneratia alba.*

DNA G+C content (mol%): 71.2–73.3 (HPLC).

Type strain: strain 14, DSM 14597, NBRC 16236, JCM 11432, VKM Ac-2086.

Sequence accession no. (16S rRNA gene): AB023357.

16. **Agromyces salentinus** Jurado, Groth, Gonzalez, Laiz and Saiz-Jimenez 2005a, 156[VP]

sa.len.ti′nus. N.L. masc. adj. *salentinus* of or belonging to Salentine Peninsula, the location of Grotta dei Cervi, the area from which the organism was isolated.

The characteristics are as described for the genus and listed in Table 144. Additional information is taken from the original description (Jurado et al., 2005a).

Colonies are yellow-pigmented. Young cultures are composed of branching vegetative hyphae which subsequently break up into diphtheroid and irregular rod-like fragments. Aerobic to microaerophilic. Optimal growth temperature is 20–28°C.

Predominant menaquinone is MK-12 (78%), with minor amounts of MK-13 (14%) and MK-11 (8%). Major cellular fatty acids are $C_{15:0}$ anteiso (52%), $C_{17:0}$ anteiso (25%), and $C_{16:0}$ iso (13%). Polar lipids are diphosphatidylglycerol, phosphatidylglycerol, an unidentified glycolipid, and four unidentified phospholipids. Whole cell sugars include rhamnose, glucose, galactose, arabinose, and ribose. For further descriptive information see Jurado et al. (2005a).

Source: a soil sample of the Grotta dei Cervi, Italy.

DNA G+C content (mol%): 72.3 (T_m).

Type strain: strain 20–5, DSM 16198, HKI 0320, JCM 14323, NCIMB 13989.

Sequence accession no. (16S rRNA gene): AY507129.

17. **Agromyces subbeticus** Jurado, Groth, Gonzalez, Laiz and Saiz-Jimenez 2005b, 1900[VP]

sub.be′ti.cus. N.L. masc. adj. *subbeticus* of or belonging to the Subbetic Mountain Range, Southern Spain, where the Cave of Bats is located.

The characteristics are as described for the genus and listed in Table 144. Additional information is taken from the original description (Jurado et al., 2005b).

Colonies are intense yellow. After initial growth on agar, cells are composed of irregular rods or short branching filaments (2.5–4.0 µm in length), that subsequently fragment into short, rod-shaped and diphtheroid elements. Aerobic to microaerophilic. Growth occurs at 6–37°C (optimally at 28°C) and at pH values of 5–9.5. NaCl is tolerated up to 4% (w/v).

Major menaquinones are MK-12 (58%) and MK-13 (35%). Predominant cellular fatty acids are $C_{15:0}$ anteiso (46%), $C_{17:0}$ anteiso (20%), $C_{15:0}$ iso (16%), and $C_{16:0}$ iso (12%). Polar lipids include diphosphatidylglycerol, phosphatidylglycerol, an unidentified phospholipid, and two unidentified glycolipids. Whole cell sugars detected are rhamnose, glucose, galactose, and mannose. For further descriptive information see Jurado et al. (2005b).

Source: a blue-gray cyanobacterial biofilm covering the walls of the Cave of Bats, Zuheros, Cordoba, Southern Spain.

DNA G+C content (mol%): 71.2 (T_m).

Type strain: strain Z33, DSM 16689, HKI 0340, JCM 14324, NCIMB 14025.

Sequence accession no. (16S rRNA gene): AY737778.

18. **Agromyces terreus** Yoon, Schumann, Kang, Park and Oh 2008, 1311[VP]

ter′re.us. L. masc. adj *terreus* of the earth.

Colonies on TSA are cream-colored. Cells are usually curved or straight irregular rods or short filaments (0.2–0.5 × 0.7–5.0 µm). No growth is observed under anaerobic conditions on nutrient agar (NA, Difco) or on na supplemented with nitrate. Growth occurs at 10–35°C with an optimum temperature of 25°C. Growth is observed at pH 5.0 and 10.0 but not at pH 4.5 or 10.5; optimal pH for growth is 7.0–8.0. Growth occurs at 0–6.0% (w/v) NaCl; best growth is in the presence of 0.5–1.0% (w/v) NaCl (tested in tripticase soy broth prepared according to the formula of the Difco medium). Tweens 20, 40, and 60 are hydrolyzed. Negative for arginine decarboxylase, lysine decarboxylase, ornithine decarboxylase, and tryptophan deaminase. Susceptible to the following antibiotics (amount per disk): polymyxin B (100 U), streptomycin (50 µg), penicillin G (20 U), cephalothin (30 µg), gentamicin (30 µg), carbenicillin (100 µg), neomycin (30 µg), and oleandomycin (15 µg), but not to lincomycin (15 µg).

The menaquinones detected include MK-11 (49%), MK-12 (31%), and MK-10 (16%). The predominant cellular fatty acids are $C_{15:0}$ anteiso (41%), $C_{17:0}$ anteiso (21%), $C_{16:0}$ iso (19%), and $C_{15:0}$ iso (12%). Major polar lipids are diphosphatidylglycerol, phosphatidylglycerol, and an unidentified glycolipid. Sugars detected in whole cells are galactose and ribose. For further descriptive information see Yoon et al. (2008).

Source: soil the Dokdo island, Korea.

DNA G+C content (mol%): 71.1 (T_m).

Type strain: strain DS-10, JCM 14581, KCTC 19216.

Sequence accession no. (16S rRNA gene): EF363711.

19. **Agromyces ulmi** Rivas, Trujillo, Mateos, Martinez-Molina and Velazquez 2004a, 1989[VP]

ul′mi. L. fem. gen. n. *ulmi* of the elm tree, referring to the isolation source of this microorganism.

The characteristics are as described for the genus and listed in Table 144. Additional information is taken from the original description (Rivas et al., 2004a).

Colonies are usually white. After initial growth on agar, cells are composed of branching vegetative hyphae which, after 15 h, break up into diphtheroid and irregular-shaped fragments (0.8–1.0 × 1.3–2.4 µm). Aerobic, albeit showing negative catalase and oxidase reactions. Optimal growth temperature is 28°C. Cellulose, gentiobiose, and xylan are used as carbon sources. Acids are produced from D-lyxose, L-sorbose, and xylitol.

The menaquinone system of the type strain includes MK-12, MK-11, MK-10, MK-13, and MK-9 (50:35:11:2:2), whereas the second described strain (XIL05) of this species contains these components in a slightly different proportion (61:23:3:7:4). The major fatty acids found for the two strains were similar: $C_{15:0}$ anteiso (46%), $C_{16:0}$ iso (24–25%), and $C_{17:0}$ anteiso (20–22%). Polar lipids reported for the type strain are diphosphatidylglycerol, phosphatidylglycerol, and an unidentified glycolipid. Cell-wall sugars include rhamnose, fucose, and glucose. The two described strains of this species have almost identical 16S rRNA gene sequences and show 100% DNA–DNA hybridization. For further descriptive information see Rivas et al. (2004a).

Source: the decayed stump of an elm tree, *Ulmus nigra.*

DNA G+C content (mol%): 72.0 (T_m).

Type strain: strain XIL01, DSM 15747, JCM 13315, LMG 21954.

GenBank accession number. (16S rRNA gene): AY427830.

Genus V. **Clavibacter** Davis, Gillaspie, Vidaver and Harris 1984, 113[VP]

Gerard S. Saddler and Ellen M. Kerr

Cla.vi.bac′ter. L. n. *clava* cudgel; N.L. masc. n. *bacter* rod; N.L. masc. n. *Clavibacter* club-shaped rod.

Pleomorphic rods (0.4–0.75 × 0.8–2.5 μm), often in angular, **V-formations** with no marked rod–coccus development cycle. Gram-stain-positive, non-acid-fast, nonmotile, and nonsporeforming. Obligate aerobes; growth factors are required for growth. **Acid production from carbohydrates is slow and weak**; acid is produced aerobically from glucose and glycerol. No acid is produced from adonitol, esculin, *meso*-inositol, melezitose, β-methyl-D-glucoside, raffinose, rhamnose, or ribose. Nitrate and nitrite are not reduced or utilized; casein is not hydrolyzed. Catalase-positive; oxidase, lipase, tyrosinase, and urease-negative. Optimum growth temperature 20–29°C; maximum temperature for growth is 35°C. **The cell-wall peptidoglycan, based on the presence of 2,4-diaminobutyric acid (DAB), is type B2γ**. Mycolic acids are not found. Nonhydroxylated fatty acids, specifically iso-methyl and anteiso-methyl branched chains fatty acids, predominate. Phosphatidylglycerol, diphosphatidylglycerol, and some unknown glycosyldiacylglycerols are the major polar lipids. Spermidine and spermine are the major polyamines with minor amounts of putrescine. **Isoprenoid quinones, comprising menaquinones with 9 isoprene units (MK-9) predominate.**

DNA G+C content (mol%): 65–75.

Type species: **Clavibacter michiganensis** corrig. (Smith 1910) Davis, Gillaspie, Vidaver and Harris 1984, 113[VP] (*Bacterium michiganense* Smith, 1910, 794; C*orynebacterium michiganense* Jensen 1934, 47).

Further descriptive information

The genus *Clavibacter* is a member of the family *Microbacteriaceae*, alongside other phytopathogenic coryneforms currently included in the genera *Curtobacterium*, *Leifsonia*, and *Rathayibacter*. Historically, all phytopathogenic coryneforms were contained within *Corynebacterium* principally on the basis of morphological and staining characteristics (Keddie and Jones, 1981). Subsequently, a subgroup, all of which contained 2,4-diaminobutyric acid (DAB) in their cell-wall peptidoglycan (type B2γ of Schleifer and Kandler, 1972), were assigned to the genus *Clavibacter* by Davis and co-workers (1984). When originally described, *Clavibacter* encompassed five species: *Clavibacter michiganense* [*sic*], *Clavibacter iranicum*, *Clavibacter rathayi*, *Clavibacter tritici*, and *Clavibacter xyli*. Additional taxonomic revision resulted in the present classification of these taxa, whereby they were redistributed across three genera: *Clavibacter*, *Leifsonia* (Evtushenko et al., 2000), and *Rathayibacter* (Zgurskaya et al., 1993). Differences in menaquinone profiles were highly significant; membership of *Clavibacter* was restricted to the species *Clavibacter michiganensis* which contains major amounts of MK-9 and, to a lesser extent, MK-7, MK-8, and MK-10. In contrast, MK-10 predominates in *Rathayibacter iranicus*, *Rathayibacter rathayi*, and *Rathayibacter tritici* (Carlson and Vidaver, 1982; Collins, 1983; Collins and Jones, 1980), and MK-11 in *Leifsonia xyli* (Sasaki et al., 1998). Analysis of D- to L-DAB isomers found in the peptide subunit and the interpeptide bridge of the peptidoglycan added further weight to the current classification. All members of the genus *Clavibacter* have D- and L-DAB in almost equal proportions, in contrast to *Rathayibacter* which possesses the L-isomer almost exclusively (Sasaki et al., 1998). Cell-wall sugars comprise rhamnose, galactose, and mannose; arabinose is not found and some strains contain fucose (Davis et al., 1984). Principal phospholipids are phosphatidylglycerol, diphosphatidylglycerol, and some unknown glycosyldiacylglycerols (Collins et al., 1980; Collins and Jones, 1980). Spermidine and spermine are the major polyamines, with minor amounts of putrescine, in contrast to *Curtobacterium*, where putrescine is absent (Altenburger et al., 1997). Nonhydroxylated, branched-chain fatty acids predominate, specifically 14-methylhexadecanoic ($C_{17:0}$ anteiso) and 12-methyltetradecanoic acids ($C_{15:0}$ anteiso; Collins and Jones, 1980; Kämpfer and Kroppenstedt,1996). Significant quantities of 14-methylpentadecanoic ($C_{16:0}$ iso) and octadecenoic acids ($C_{18:1}$) are also found. Kämpfer and Kroppenstedt (1996) concluded that it was not possible to differentiate *Clavibacter michiganensis* subspecies by fatty acids profiles, contrasting with the view of Henningson and Gudmestad (1991) who suggested that the ratios between key, minor fatty acid components could be used to differentiate subspecies and even suggested that the differences were such that the subspecific taxa merit elevation to the rank of species. The fatty acid profiles from a range of isolates of *Clavibacter michiganensis* subsp. *michiganensis* were typical for the genus with little variation found between isolates. Saturated, straight-chain fatty acids, namely dodecanoic ($C_{12:0}$) and hexadecanoic acids ($C_{16:0}$), were found in significant amounts (Gitaitis and Beaver, 1990) and the presence of the unsaturated, branched-chain acid, 12-methyltetradecenoic acid ($C_{15:1}$ anteiso), was considered diagnostic for this subspecies.

In general, members of the genus *Clavibacter* produce irregular rods which vary in size and shape (0.4–0.75 × 0.8–2.5 μm) and are arranged in V-formations. There is no marked rod–coccus cycle and branching does not occur (Davis, 1986). The mode of cell division has been considered to be of taxonomic significance, with all the *Clavibacter* subspecies examined dividing by bending division (Komagata et al., 1969), resulting in the V-form cell arrangements. Carlson and Vidaver (1982) found that colony color was a useful diagnostic characteristic; subspecies *nebraskensis* and *tessellarius* produce orange-pigmented colonies, while subsp. *sepedonicus* is usually nonpigmented and subspp. *insidiosus* and *michiganensis* are yellow when grown on NBY* Agar (Vidaver, 1967). Colonial variants are evident in some *Clavibacter michiganensis* subspecies. Smidt and Vidaver (1987) noted that *Clavibacter michiganensis* subsp. *nebraskensis* produced four morphological types: orange fluidal colonies typical of the subspecific description; a non-fluidal, dark-orange variant; a fluidal, yellow variant; and a fluidal, slightly orange-pigmented variant that, in younger colonies, appeared almost white. Variation has also been found among strains of *Clavibacter michiganensis* subsp. *sepedonicus* where both the fluidal, wild-type and afluidal variants are commonly found (Kokošková et al., 2005; Mills et al., 2007).

*NBY Medium (g/liter): Nutrient broth (Difco), 8.0; yeast extract (Difco), 2.0; K_2HPO_4, 2.0; KH_2PO_4, 0.5; agar, 15.0. Added separately: 50 ml of sterile glucose (50%, w/v aq.) and 1 ml of sterile 1 M $MgSO_4 \cdot 7H_2O$.

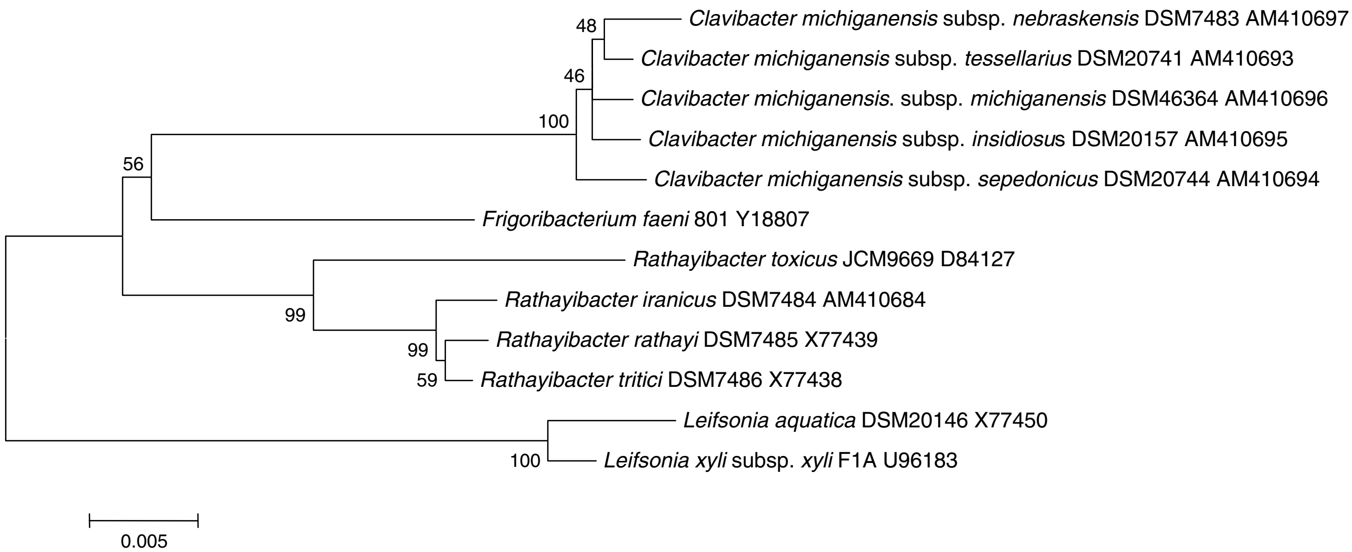

FIGURE 178. Phylogeny of the genus *Clavibacter* and close relatives drawn from the family *Microbacteriaceae* including representatives of the genera *Frigoribacterium*, *Rathayibacter*, and *Leifsonia*. Maximum composite likelihood with clustering achieved by the neighbor joining method to produce a bootstrap consensus tree was performed using MEGA4. Bar = 0.5% difference in the 16S rRNA gene sequences. Five hundred bootstrap trees were generated, and bootstrap confidence levels (shown as percentages above nodes) were determined.

Clavibacter strains are all chemo-organotrophs with a respiratory metabolism, producing acid weakly from a range of carbohydrates. Growth is relatively slow and B-vitamins are required (Collins and Bradbury, 1986). All strains produce catalase but not oxidase, tyrosinase, urease, and lipase (Davis et al., 1984). The complete genome sequence of *Clavibacter michiganensis* subsp. *michiganensis* has confirmed that the pathways for glycolysis, the pentose phosphate pathways and gluconeogenesis are all present and complete (Gartemann et al., 2008).

Li and De Boer (1995) found a high degree of 16S rRNA gene similarity between *Clavibacter michiganensis* subspecies; the subspecies displayed less than 1% dissimilarity. In this study, *Clavibacter michiganensis* was found to be closely related to *Leifsonia xyli* (basonym *Clavibacter xyli*), but more distantly related to members of the family *Corynebacteriaceae*, *Mycobacteriaceae*, and *Nocaridaceae*. A more comprehensive subsequent analysis shows that the genera *Clavibacter* and *Rathayibacter* form a discrete clade in which four major phylogenetic groups can be recognized (Lee et al., 1997); I, *Clavibacter michiganensis*; II, *Clavibacter xyli*; III, *Rathayibacter iranicus* and *Rathayibacter tritici*; and IV, *Rathayibacter rathayi*. On the basis of these results, a new classification was proposed, specifically that *Rathayibacter tritici/Rathayibacter iranicus* should be assigned to a different genus from that of *Rathayibacter rathayi* and that *Clavibacter xyli* should be encompassed within a separate genus. While the former suggestion has yet to be acted upon, the latter has seen *Clavibacter xyli* assigned to the genus *Lieifsonia* (Evtushenko et al., 2000). Circumscription of the genus *Frigoribacterium* on the basis of 16S rRNA gene sequence comparison (Kämpfer et al., 2000) reveals a high degree of relatedness to both *Rathayibacter* and *Clavibacter*, the former was found to be 96.1–97.1% similar, while the latter 96.9–97.1%. A comprehensive analysis of the family *Microbacteriaceace* encompassing representatives of 15 genera reaffirmed the close relationship between *Clavibacter*, *Frigoribacterium*, *Rathayibacter*, and *Curtobacterium* (Evtushenko and Takeuchi, 2006), with *Leifsonia* more distantly related. These relationships are demonstrated clearly in Figure 178 where the close relationship between *Clavibacter*, *Frigoribacterium*, and *Rathayibacter* is evident as is the more distant relationship to *Leifsonia*.

DNA–DNA hybridization studies show that *Clavibacter michiganensis* subspp. *michiganensis* and *insidiosus* are more closely related to each other (73–74% DNA–DNA relatedness) than they are to subspp. *sepedonicus* (51–56%) and *nebraskensis* (45–46%; Döpfer et al., 1982). Subsequently, *Clavibacter michiganensis* subsp. *tessellarius* was found to be 56% related to *Clavibacter michiganensis* subsp. *michiganensis* (Zgurskaya et al., 1993), Hence, the subspecies *sepedonicus*, *nebraskensis*, and *tessellarius* could be given species status if the threshold value of 70% for species differentiation is to be applied.

The phylogeny of the family *Microbacteriaceae* based on sequencing DNA of gyrase subunit B (*gyrB*), RNA-polymerase subunit B (*rpoB*), recombinase A (*recA*), and polyphosphate kinase (*ppk*) genes shows that although suprageneric relationships vary between each gene phylogeny, the genus *Clavibacter* remains a coherent entity (Stackebrandt et al., 2007). One of the significant findings of this study is that in none of the 16 trees generated did the topologies coincide with the key taxonomic marker for this group, namely the diagnostic diamino acid of the peptidoglycan, suggesting that this characteristic may have evolved independently several times or that it has regularly been subject to horizontal gene transfer. The complete genome sequence of *Clavibacter michiganensis* subspp. *michiganensis* (Gartemann et al., 2008) and *sepedonicus* (Bentley et al., 2008) has revealed a number of similarities but also many striking differences between these subspecies. *Clavibacter michiganensis* subsp. *michiganensis* has a circular chromosome of 3.298 Mb

comprising 2,984 predicted coding sequences, along with two circular plasmids, pCM1 (27.4 kb) and pCM2 (70 kb), which are essential for pathogenicity (Gartemann et al., 2008). Earlier studies have shown that curing plasmids from *Clavibacter michiganensis* subsp. *michiganensis* causes a loss of pathogenicity (Bermpohl et al., 1996). Similarly, *Clavibacter michiganensis* subsp. *sepedonicus* has a circular chromosome of 3.259 Mb comprising 3,058 predicted coding sequences along with two plasmids essential for pathogenicity, pCS1 (50.4 kb; Mogen et al., 1988) and pCSL1 (94.8 kb; Bentley et al., 2008). However, *Clavibacter michiganensis* subsp. *michiganensis* lacks complete insertion elements and transposons with no evidence of genome reduction, as has been found in other xylem-limited pathogens. Indeed, *Clavibacter michiganensis* subsp. *michiganensis* was found to possess a similar number of transporters and regulators as can be found in many soil-inhabiting bacteria. In contrast, *Clavibacter michiganensis* subsp. *sepedonicus* contains 106 insertion sequence elements which are implicated in the extensive chromosomal rearrangement observed. Functional disruption of many genes involved in carbohydrate metabolism, transcriptional regulation, and pathogenicity all suggest a recent adaptation to life in a more restricted niche and the general relaxation of the selective pressures that would normally be found applied to a more generalized, soil-inhabiting lifestyle.

A number of monoclonal antibodies have been raised against *Clavibacter michiganensis* subsp. *sepedonicus* (De Boer and Wieczorek, 1984); two of them show no cross-reactivity with other *Clavibacter michiganensis* subspecies or saprophytes isolated from potato. One of these monoclonals, 9A1 (McAb 5; De Boer and Wieczorek, 1984), reacted poorly in ELISA, resulting in the discovery of new more effective ELISA-monoclonal, 1H3 (De Boer et al., 1988). Monoclonal 9A1 has been used effectively for immunofluorescence testing (De Boer et al., 1994) and in conjunction with *in situ* hybridization (Li et al., 1997). Specificity of the monoclonal antibody (MAb) Cmm1 to *Clavibacter michiganensis* subsp. *michiganensis* was assessed (Kaneshiro et al., 2006) by screening 236 strains of the subspecies and representative saprophytes likely to be found on the tomato host. Of the *Clavibacter michiganensis* subsp. *michiganensis* strains tested, 99% reacted with this antibody with no cross-reactivity to common saprophytes. Polyclonals have been used successfully in conjunction with immunomagnetic separation to enhance the sensitivity of detection of *Clavibacter michiganensis* subsp. *michiganensis* from naturally infected tomato seeds (de Leon et al., 2006, 2008).

Phage have been described from a number of subspecies of *Clavibacter*, specifically for subspp. *insidiosus* (Cook and Katznelson, 1960), *michiganensis* (Echandi and M. Sun, 1973), and *nebraskensis* (Shirako et al., 1986; Smidt and Vidaver, 1987). Members of the genus *Clavibacter* are known to produce bacteriocins (Echandi, 1976; Gross and Vidaver, 1979a; Smidt and Vidaver, 1987). One such peptide bacteriocin, michiganin A, from *Clavibacter michiganensis* subsp. *michiganensis*, and active against *Clavibacter michiganensis* subsp. *sepedonicus*, has been recently characterized as a type B lantibiotic (Holtsmark et al., 2006), a compact, globular molecule which shows similarities to actagardine, produced by *Actinoplanes liguriae*. In addition, a 14 kDa antimicrobial protein and a low molecular mass antimicrobial putatively identified as a tunicamycin have been detected (Holtsmark et al., 2007).

The extracellular polysaccharide (EPS) composition of *Clavibacter michiganensis* subsp. *michiganensis* has been determined as fucose, glucose, galactose (2:1:1; molar ratio; Bermpohl et al., 1996). It is thought that EPS plays only a minor role in symptom expression as EPS$^-$ mutants maintain virulence, while plasmid-cured strains that produce EPS do not. Current thinking, based on the model of van Alfen and co-workers (1987), suggests that EPS interferes with water transport by physically plugging xylem vessels, but that it is not directly involved in virulence (Eichenlaub et al., 2006). Analysis of the genome of *Clavibacter michiganensis* subsp. *michiganensis* reveals at least four gene clusters implicated in EPS production, and that mutants specifically generated in one of these clusters will lead to the production of rough colonies from the mucoid, wild-type (Eichenlaub et al., 2006). *Clavibacter michiganensis* subsp. *michiganensis* is known to induce a hypersensitive-like reaction (HR) in the non-host plant, four-o'clock flower (*Mirabilis jalapa* L.; Bermpohl et al., 1996). Analysis of the HR-inducing activity revealed that it was heat-stable and sensitive to protease digestion and, in this respect, similar to harpin and popA described previously in Gram-stain-negative phytopathogens (Alarcón et al., 1998). Similar findings have been reported for *Clavibacter michiganensis* subsp. *sepedonicus*, in which HR-induction occurs in tobacco (Nissinen et al., 2001). It has been demonstrated that HR-induction and cellulase production are required to cause disease. A plasmid-free isolate of *Clavibacter michiganensis* subsp. *sepedonicus* that could induce HR on tobacco, but was unable to produce cellulose, was able to colonize the host plant efficiently but could only produce weak symptoms. In contrast, a strain that was unable to induce HR but could produce cellulase was impaired in its ability to colonize the host and caused no symptoms. Co-inoculation of both strains into the host restored disease development to its typical state (Nissinen et al., 2001). The significance of plant cell-wall degrading enzymes in the pathogenicity of *Clavibacter michiganensis* subsp. *michiganensis* has also been demonstrated (Eichenlaub et al., 2006; Evtushenko and Takeuchi, 2006). A 78 kDa CelA protein with similarity to endo-β-1,4-glucanases has been found to play a major role in pathogenicity (Jahr et al., 2000), although it is clear that this endoglucanase alone is insufficient to express the virulent wild-type phenotype (Gartemann et al., 2003; Jahr et al., 1999). It is likely that a second pathogenic determinant, the product of the *pat-1* gene, a serine protease-like enzyme (Dreier et al., 1997), is also required for pathogenicity. Recent work on *Clavibacter michiganensis* subsp. *sepedonicus* has demonstrated that virulence/pathogenicity in this subspecies is not just dependent on CelA and an, as yet uncharacterized, HR-inducing protein (Holtsmark et al., 2008). In this study, the expression of CelA and eight putative virulence factors were examined including CelB, another cellulase-encoding gene; a pectate lyase; a xylanase; and five homologs of the *Clavibacter michiganensis* subsp. *michiganensis pat-1* gene. Although further work is required, it is evident that additional proteins are likely to be involved in symptom expression in *Clavibacter michiganensis* subsp. *sepedonicus*.

Members of the genus *Clavibacter* are exclusively plant pathogens that primarily cause vascular disease in a variety of significant crops, specifically: alfalfa (*Clavibacter michiganensis* subsp. *insidiosus*), maize (*Clavibacter michiganensis* subsp. *nebraskensis*), potato (*Clavibacter michiganensis* subsp. *sepedonicus*), tomato (*Clavibacter*

michiganensis subsp. *michiganensis*), and wheat (*Clavibacter michiganensis* subsp. *tessellarius*). Three of the subspecies: subspp. *insidiosus*, *michiganensis*, and *sepedonicus* are subject to quarantine restrictions due to the losses they can induce (Eichenlaub et al., 2006). *Clavibacter michiganensis* subsp. *michiganensis* is primarily spread by movement of asymptomatic seedlings or carried on seed. Infection can spread rapidly under controlled conditions, e.g. in greenhouses, and spreads in irrigation waters and through cultural practices (trimming, harvesting etc.; EPPO, 2005). *Clavibacter michiganensis* subsp. *sepedonicus* is carried as a latent infection on potato tubers and can persist for long periods of time as dried slime in stores, packing material, and on farm machinery. In Northern Europe, *Clavibacter michiganensis* subsp. *sepedonicus* may persist for a limited period in the field in common weeds and field crops, in contrast to North America where the weed *Solanum rostratum* has the potential to serve as a disease-reservoir (van der Wolf et al., 2005). *Clavibacter michiganensis* subsp. *sepedonicus* is unable to survive in surface waters for extended periods (van der Wolf and Beckhoven, 2004), nor can it survive or spread in soil (Kaemmerer et al., 2007); plant-to-plant transmission in the field is thought to be insignificant (Mansfeld-Giese, 1997). Typically for all subspecies, survival away from the principal host is limited and is only likely to occur in soil and water when the bacteria are associated with debris from previously infected host plants.

Enrichment and isolation procedures

The recovery of *Clavibacter* isolates from the environment is hampered by their general slow growth, frequently resulting in swamping/overgrowth by associated saprophytes. In many cases the bacteria are frequently present as asymptomatic infections in host plants in very low numbers, making isolation especially difficult. In cases where disease symptoms are expressed, the bacteria are likely to be present in significant amounts and direct isolation from the affected tissues onto media designed to support good growth can be effective. Media such as NBY*, YGM*, GYCA† (Dye, 1962), and Doppel's Medium‡ (Lelliott and Stead, 1987) are all suitable for the cultivation of *Clavibacter*. In situations where asymptomatic infections are suspected or where problems with overgrowth have been encountered, semi-selective media are required. CNS medium§ (Gross and Vidaver, 1979a) can be used to selectively isolate *Clavibacter michiganensis* subspp. *michiganensis*, *nebraskensis* and *tessellarius*. A modified semi-selective medium, mSCM** (Waters and Bolkan, 1992), modified from the earlier SCM medium of Fatmi and Schaad (1988), can also be used to isolate *Clavibacter michiganensis* subsp. *michiganensis*. On this medium it produces mucoid colonies with yellow flecks and is easily distinguishable from saprophytes which tend to be much smaller in size. A semi-selective medium, MTNA††, which contains mannitol, trimethoprim, nalidixic acid, and amphotericin B, has proven effective at recovering *Clavibacter michiganensis* subsp. *sepedonicus* from artificially inoculated and naturally infected symptomless tubers (Jansing and K. Rudolph, 1998), as has NCP-88‡‡ (de la Cruz et al., 1992).

Maintenance procedures

Standard methods of preservation can all be applied to *Clavibacter*, though there is evidence that freeze-drying may result in a loss of virulence (Evtushenko and Takeuchi, 2006). Most cultures can be stored on solid media at 6°C for several months, and survival can be achieved in the medium-term (several years) by storage at −70 to −80°C in 15–25% glycerol (v/v) (Collins and Bradbury, 1986; Jacobs and Sundin, 2001; Kaneshiro et al., 2006).

Differentiation of the genus *Clavibacter* from other genera

Identification to the genus can be readily achieved using chemotaxonomic methods to identify DAB in the peptidoglycan, the presence of MK-9 in the cell wall, and spermidine and spermine as the major the polyamines. Sequencing 16S rRNA gene is also reliable for differentiating *Clavibacter* from other members of the family *Microbacteriaceae* (Evtushenko and Takeuchi, 2006; Stackebrandt et al., 2007) as are other genes, namely DNA gyrase subunit B (*gyrB*), RNA-polymerase subunit B (*rpoB*), recombinase A (*recA*), and polyphosphate kinase (*ppk*) (Stackebrandt et al., 2007). Further characteristics are listed in Table 145.

Taxonomic comments

Clavibacter is a well-defined, homogeneous genus that contains only one species, *Clavibacter michiganensis* encompassing five subspecies (subspp. *michiganensis*, *insidosus*, *nebraskensis*, *sepedonicus*, and *tessellarius*). Biochemical characteristics useful in distinguishing these subspecies are listed in Table 146. Commercial identification systems such as Biolog have been used to identify *Clavibacter* strains. Using an early version of the identification library (Biolog, release 3.5), Harris-Baldwin and Gudmestad (1996) found that results were highly variable with as few as 27% of *Clavibacter michiganensis* subsp. *nebraskensis* strains being correctly identified to the genus. Further, many strains were misidentified to *Curtobacterium*. More recent studies have shown that all virulent *Clavibacter michiganensis* subsp. *michiganensis* could be correctly identified to the species using Biolog, though hypovirulent and avirulent strains proved more problematic (Kaneshiro et al., 2006). Essentially similar results were obtained in a comprehensive study of *Clavibacter michiganensis* subsp. *sepedonicus*,

*YGM medium (g/liter): Bacto yeast extract (Difco), 2.0; D(+)-glucose (monohydrate), 2.5; K_2HPO_4, 40.25; KH_2PO_4, 0.25; $MgSO_4{\cdot}7H_2O$, 0.1; $MnSO_4{\cdot}H_2O$ 0.015; NaCl, 0.05; $FeSO_4{\cdot}7H_2O$ 0.005; Bacto agar (Difco), 18.

†GYCA medium (g/liter): glucose, 5.0; yeast extract, 5.0; $CaCO_2$ (finely ground), 41.0: agar, 15.0. pH 7.2.

‡Doppel's medium (g/liter): glucose, 10.0, yeast extract, 8.0; casein hydrolysate (not vitamin-free), 8.0; K_2HPO_4, 2.0; $MgSO_4{\cdot}7H_2O$, 0.3; agar, 12. pH 7.2.

§CNS medium: NBY* supplemented with lithium chloride, 10.0; nalidixic acid, 25 mg (freshly dissolved in 0.1 M NaOH, 10 $mg.ml^{-1}$); polymyxin $B.SO_4$, 32 mg (added as a 10 $mg.ml^{-1}$ freshly prepared aq. solution); actidione/cycloheximide, 40 mg (added as a 10 $mg.ml^{-1}$ aq. stock solution); chlorothalonil/Bravo 6F, 0.0625 ml (diluted 1:50 from a stock solution of tetrachloro-isophthalonitrile).

**mSCM (g/liter): pourite 0.013; $K_2HPO_4{\cdot}3H_2O$, 2.62; KH_2PO_4, 0.5; $MgSO_4{\cdot}7H_2O$, 0.25; boric acid, 1.5; mannose, 10.0; yeast extract, 0.1; nalidixic acid (Na salt), 0.03; nicotinic acid, 0.1; cycloheximide, 0.2; agar, 12.

††MTNA medium (g/liter): yeast extract (Difco), 2.0; mannitol, 2.5; K_2HPO_4, 0.25; KH_2PO_4, 0.25; NaCl, 0.05; $MgSO_4{\cdot}7H_2O$, 0.1; $MnSO_4{\cdot}H_2O$, 0.015; $FeSO_4{\cdot}7H_2O$, 0.005; agar (Oxoid No. 1), 16.0. pH 7.2. After autoclaving, add trimethoprim, 0.06 (5 $mg.ml^{-1}$ in 96% methanol); nalidixic acid, 0.002 (5 $mg.ml^{-1}$ in 96% methanol); amphotericin B, 0.01 (1 $mg.ml^{-1}$ in dimethyl sulfoxide).

‡‡NCP-88 medium (g/liter): Nutrient Agar (Difco), 23.0; yeast extract (Difco), 2.0; D-mannitol, 5.0; K_2HPO_4, 2; KH_2PO_4, 0.5; $MgSO_4{\cdot}7H_2O$, 0.25. pH to 7.2. After autoclaving add polymyxin B sulphate, 0.003 (aq.); nalidixic acid, 0.008 (stock solution prepared in 0.01 M NaOH); cycloheximide, 0.2 (stock solution prepared in 50% ethanol).

TABLE 145. Characteristics differentiating *Clavibacter* from other plant-associated genera of the family *Microbacteriaceae*[a,b]

Characteristic	*Agreia*	*Clavibacter*	*Curtobacterium*	*Frigoribacterium*	*Leifsonia*	*Okibacterium*	*Plantibacterium*	*Rathayibacter*
Colony color	O/Y	O/W/Y	I/OY	Y	I/R/Y/W	Y	Y	Y
Motility	+[c]	−	D	+	+	−	−	
Maximum growth temperature (°C)	37	35	37	25, 35[d]	42	37	35	35
Optimum growth temperature (°C)	24–26	20–29	24–26	4–10, 24–26[c]	24–28	24–26	25	24–28
Peptidoglycan amino acid	L-DAB, D-Orn, Hyg	DL-DAB	D-Orn	L-Lys	DL-DAB	Lys	L-DAB	L-DAB
Peptidoglycan type[e]	B	B2γ	B2β	B2β	B2γ	B	B2γ	B2γ
Menaquinones	MK-10	MK-9	MK-9	MK-9	MK-11	MK-10, 11	MK-9, 10	MK-10
Fatty acids	$C_{15:0}$ anteiso, $C_{16:0}$ iso, $C_{17:0}$ anteiso	$C_{15:0}$ anteiso, $C_{16:0}$ iso, $C_{17:0}$ anteiso	$C_{15:0}$ anteiso, $C_{16:0}$ iso, $C_{17:0}$ anteiso	$C_{15:0}$ anteiso, $C_{16:0}$ iso	$C_{15:0}$ anteiso, $C_{16:0}$ iso, $C_{17:0}$ anteiso	$C_{15:0}$ anteiso, $C_{17:0}$ anteiso	$C_{15:0}$ anteiso, $C_{16:0}$, $C_{16:0}$ iso, $C_{17:0}$ anteiso	$C_{15:0}$ anteiso, $C_{16:0}$ iso, $C_{17:0}$ anteiso
Polar lipids[f]	PG, DPG	PG, DPG, GL	PG, DPG, GL	PG, DPG, GL	PG, DPG, GL	PG, DPG, GL	PG, DPG, GL	PG, DPG, GL
Polyamines[g]	SPM[g]	SPD, SPM, (PUT, CAD)	SPD, SPM	SPD, SPM[h]	nd	nd	nd	SPD, SPM, (PUT, CAD)
DNA G+C content (mol%)	67.0	65–75	68–71	67–71	66–71	67	68–70	60–69

[a]Symbols: +, >85% positive; −, 0–15% positive; D, different reactions occur in different taxa (species of a genus); nd, not determined; I, ivory: O, orange; R, red; Y, yellow; W, white.

[b]Data from: Bradbury (1986); Dastager et al. (2008d); Evtushenko et al. (2001, 2002); Evtushenko and Takeuchi (2006); Lin and Yokota (2006); and Qiu et al. (2007).

[c]Weakly positive.

[d]The genus *Frigoribacterium* contains psychrophilic and mesophilic species. See Dastager et al. (2008d) and Kämpfer et al. (2000).

[e]After Schleifer and Kandler (1972).

[f]PG, phospatidylglycerol; DPG, diphospatidylglycerol; GL, glycolipids.

[g]SPD, spermidine; SPM, spermine, CAD, cadaverine, PUT, putrescine. Polyamines in parentheses are only found in small amounts. Data from Altenburger et al. (1997).

[h]H.-J. Busse, unpublished results.

TABLE 146. Characteristics differentiating subspecies of *Clavibacter michiganensis*[a,b]

Characteristic	*C. michiganensis* subsp. *michiganensis*	*C. michiganensis* subsp. *insidiosus*	*C. michiganensis* subsp. *nebraskensis*	*C. michiganensis* subsp. *sepedonicus*	*C. michiganensis* subsp. *tessellarius*
Acid produced from:					
Mannitol	−	−	−	+	+
Sorbitol	−	−	+	+	+
Pigmentation on NBY	Y/V_1	Y/B	O/V_2	W	O
Growth on CNS	+	−	+	−	+
Growth temperature (°C)	34–35[c]	31–32[c]	34[c]	34[c]	24–28[d]
Utilization of:					
Acetate	+	−	+	+	−
Lactate	d	−	+	−	nd
Propionate	−	−	+[e]	−	−
Succinate	+	−	+	+	+
Hydrolysis of:					
Gelatin	+[e]	−	−	−	−
Starch	d	−	d	d	+
Methyl red	−	+	d	−	−
H_2S from peptone	+	−	d	−	−
Plant host	Tomato (*Lycopersicum esculentum*)	Alfalfa (*Medicago sativa*)	Maize (*Zea mays*)	Potato (*Solanum tubersoum*)	Wheat (*Triticum aestivum*)

[a]Symbols: +, >85% positive; d, different strains give different reactions (16–84% positive); −, 0–15% positive; D, different reactions occur in different taxa (species of a genus); nd, not determined; Y, yellow; V_1, various pigments (occasionally pink, red, orange and white, or colorless); B, blue; O, orange; V_2, occasional yellow variants; W, white.

[b]Data from: Carlson and Vidaver (1982); Collins and Bradbury (1986); Evtushenko and Takeuchi (2006); Eichenlaub et al. (2006); and Davis and Vidaver (2001).

[c]Maximum growth temperature.

[d]Optimum growth temperature.

[e]Weakly positive.

where fluidal, wild-type strains could be more reliably identified than afluidal and intermediate strains (Kokošková et al., 2005). API ZYM has been used successfully to distinguish *Clavibacter michiganensis* subsp. *sepedonicus* from the other subspecies in the genus (De Bruyne et al., 1992). In contrast, *Clavibacter michiganensis* subspp. *insidiosus*, *michiganensis*, and *nebraskensis* show greater heterogeneity and are difficult to differentiate from each other. Similar findings have been made using API 50CH and API ZYM, and *Clavibacter michiganensis* subsp. *sepedonicus* has been found to be highly homogeneous and easily distinguishable from the other *Clavibacter michiganensis* subspecies (Palomo et al., 2006).

It should be noted that, in much of the recent literature, the specific/subspecific epithets have been altered to the masculine form, subsequent to Davis and co-workers original description of the genus (1984), because generic names which end in *-bacter* are regarded as having the masculine gender (Bradbury, 1986; Collins and Bradbury, 1986; Zgurskaya et al., 1993). Regarding possible future changes to the current classification, Collins and Bradbury (1986) reviewed data from a wide variety of studies and concluded that the taxa now contained within the genus *Clavibacter* may warrant recognition at the species level, a view expressed more recently by Henningson and Gudmestad (1991), Evtushenko and Takeuchi (2006), and Eichenlaub and co-workers (2006). Such a move would derive the additional benefit of enabling the formal description of nonpathogenic or saprophytic strains, as the current classification places a major emphasis on phytopathogenicity.

List of species of the genus *Clavibacter*

1. **Clavibacter michiganensis** corrig. (Smith 1910) Davis, Gillaspie, Vidaver and Harris 1984, 113^{VP} (*Bacterium michiganense* Smith 1910, 794; C*orynebacterium michiganense* Jensen 1934, 47)

mi.chi.gan.en′sis. N.L. masc. adj. *michiganensis* of or pertaining to Michigan (USA).

The description is as for the genus. Acid is produced aerobically from glucose, glycerol, and mannitol, but not from adonitol, esculin, *meso*-inositol, melezitose, β-methyl-D-glucoside, raffinose, rhamnose, or ribose. Nitrate is not reduced and casein is not hydrolyzed. Catalase-positive; oxidase, lipase, tyrosinase, and urease-negative. Optimum growth temperature is 20–29°C.

DNA G+C content (mol%): 72–74 (Bd).

Type strain: CFBP 2352, CIP 104846, DSM 46364, ICMP 2550, JCM 9665, LMG 7333, NCPPB 2979, VKM Ac-1403.

Sequence accession no. (16S rRNA gene): AM410696, U09762, X77435.

1a. **Clavibacter michiganensis subsp. michiganensis** corrig. (Smith 1910) Davis, Gillaspie, Vidaver and Harris 1984, 113^{VP} (*Bacterium michiganense* Smith, 1910, 794; C*orynebacterium michiganense* Jensen 1934, 47)

mi.chi.gan.en′sis. N.L. masc. adj. *michiganensis* pertaining to Michigan, USA.

A pathogen of tomato (*Lycopersicon esculentum*) causing vascular wilt, canker, leaf, and fruit spot. The description is as for the genus and diagnostic tests are listed in 1. In addition, colonies are yellow on NBY agar; growth occurs on CNS agar. Rhamnose and fucose are present in the cell wall. Maximum growth temperature 34–35°C.

DNA G+C content (mol%): 73 (Bd).

Type strain: CFBP 2352, CIP 104846, DSM 46364, ICMP 2550, JCM 9665, LMG 7333, NCPPB 2979, VKM Ac-1403.

Sequence accession no. (16S rRNA gene): AM410696, U09762, X77435.

1b. **Clavibacter michiganensis subsp. insidiosus** corrig. (McCulloch 1925) Davis, Gillaspie, Vidaver and Harris 1984, 113^{VP} ("*Aplanobacter insidiosum*" McCulloch 1925, 497; *Corynebacterium insidiosum* Jensen 1934, 41)

in.si.di.o′sus. L. masc.adj. *insidiosus* deceitful, insidious.

A pathogen of alfalfa (*Medicago sativa*) causing wilt; plants are stunted and yellow with vascular discoloration of the roots. The description is as for the genus and diagnostic tests are listed in Table 145. In addition, colonies are yellow on NBY agar, many strains produce a dark blue-gray diffusible pigment (indigoidine). Growth does not occur on CNS agar. Rhamnose and fucose are present in the cell wall. Maximum growth temperature 31–32°C.

DNA G+C content (mol%): 73 (Bd).

Type strain: CCUG 38895, CFBP 2404, CIP 105048, CIP 105363, DSM 20157, ICMP 2621, LMG 3663, JCM 9664, NCPPB 1109, VKM Ac-1402.

Sequence accession no. (16S rRNA gene): AM410695, U09761.

1c. **Clavibacter michiganensis subsp. nebraskensis** corrig. (Vidaver and Mandel 1974) Davis, Gillaspie, Vidaver and Harris 1984, 113^{VP} (*Corynebacterium nebraskense* Vidaver and Mandel 1974, 482)

ne.bras.ken′sis. N.L. masc. adj. *nebraskensis* of or pertaining to Nebraska (USA).

A pathogen of maize (*Zea mays*) which gives rise to leaf spots and wilt, frequently referred to as "Goss's bacterial wilt." The description is as for the genus and diagnostic tests are listed in Table 145. In addition, colonies are orange on NBY agar; growth occurs on CNS agar. Rhamnose and fucose are present in the cell wall. Maximum growth temperature is 34°C.

DNA G+C content (mol%): 73.5 (Bd).

Type strain: ATCC 27794, CCUG 38894, CIP 105362, DSM 7483, ICMP 3298, JCM 9666, LMG 3700, LMG 5627, LMG 7223, NCPPB 2581, VKM Ac-1404.

Sequence accession no. (16S rRNA gene): AM410697, U09763, X77434.

1d. **Clavibacter michiganensis subsp. sepedonicus** corrig. (Spieckermann and Kotthoff 1914) Davis, Gillaspie, Vidaver and Harris 1984, 113^{VP} (*Bacterium sepedonicum* Spieckermann and Kotthoff 1914, 692; *Corynebacterium sepedonicum* Skaptason and Burkholder 1942, 441)

se.pe.don′i.cus. Gr. n. *sepedon* rottenness, decay; L. masc. suff. *-icus*, suffix used with the sense of pertaining to; N.L.

masc. adj. *sepedonicus*, pertaining to decay, intended to mean leading to decay.

A pathogen of potato (*Solanum tuberosum*) causing a vascular wilt and frequently leading to tuber rot, commonly referred to as "ring rot." The description is as for the genus and diagnostic tests are listed in Table 145. In addition, colonies are white on NBY agar; growth does not occur on CNS agar. Rhamnose, but not fucose, is present in the cell wall. Maximum growth temperature is 34°C.

DNA G+C content (mol%): 72 (Bd).

Type strain: ATCC 33113, CCUG 23908, CIP 104844, CFBP 2049, DSM 20744, ICMP 2535, JCM 9667, LMG 2889, NCPPB 2137, VKM Ac-1405.

Sequence accession no. (16S rRNA gene): AM410694, U09764.

1e. **Clavibacter michiganensis subsp. tessellarius** corrig. (Carlson and Vidaver 1982) Davis, Gillaspie, Vidaver and Harris 1984, 113[VP] (*Corynebacterium michiganense* subsp. *tessellarius* Carlson and Vidaver 1982, 322)

tes.sel.la′ri.us. L. masc. n. *tessellarius* a mosaic stone maker.

A pathogen of wheat (*Triticum aestivum*) which causes a mottling or mosaic of leaves. The description is as for the genus and diagnostic tests are listed in Table 145. In addition, colonies are orange on NBY agar; growth occurs on CNS agar. Rhamnose and fucose are present in the cell wall. Optimum temperature for growth 24–28°C. Grows at 10°C but not at 37°C.

DNA G+C content (mol%): 74 (Bd).

Type strain: 78181, ATCC 33566, CCUG 38896, CIP 105364, DSM 20741, ICMP 7221, JCM 9668, LMG 7294, NCPPB 3664, PDDCC 7221, VKM Ac-1406.

Sequence accession no. (16S rRNA gene): AM410693, U96181, U30254.

Genus VI. **Cryobacterium** Suzuki, Sasaki, Uramoto, Nakase and Komagata 1997, 477[VP] emend. Dastager, Lee, Ju, Park and Kim 2008a, 1243[VP]

Ken-ichiro Suzuki

Cry.o.bac.te′ri.um. Gr. n. *kruos* icy cold; L. neut. n. *bacterium* a small rod; N.L. neut. n. *Cryobacterium* a cold (preferring) rod.

Pleomorphic, **motile or nonmotile rod.** Nonsporeforming. Branching occurs in the early growth phase. **Gram-stain-positive, sometimes variable.** Rod forms occur in old culture. Optimal growth temperature ranges from 9–28°C. **Strictly aerobic.** Catalase-positive. Starch is hydrolyzed, sometimes weak reaction. Gelatin not hydrolyzed. Hydrolysis of casein and DNA are variable among species. Acid is produced from several sugars. The amino acids of cell-wall peptidoglycan are **L-2,4-diaminobutyric acid**, alanine, glycine, and glutamic acid. Rhamnose and fucose are characteristic cell-wall sugars; cell-wall acyl type is acetyl (*Cryobacterium psychrophilum*). Glucose and ribose are the characteristic whole cell sugars (*Cryobacterium mesophilum* and *Cryobacterium psychrophilum*). The main cellular fatty acids are iso- and anteiso-branched acids, predominantly 12-methyl tetradecanoic acid ($C_{15:0}$ anteiso). The major menaquinone(s) are **MK-10 or MK-11 and MK-12**. Mycolic acids are absent. The polar lipids are diphosphatidylglycerol, phosphatidylglycerol, and one or more unidentified glycolipids.

DNA G+C content (mol%): 65–70.

Type species: **Cryobacterium psychrophilum** (*ex* Inoue and Komagata 1976) Suzuki, Sasaki, Uramoto, Nakase and Komagata 1997, 477 (*"Curtobacterium psychrophilum"* Inoue and Komagata 1976, 166).

Further descriptive information

Chemotaxonomy. The presence of 2,4-diaminobutyric acid is one of the essential phenotypic characteristics of the genus *Cryobacterium* and the analysis requires purification of cell-wall peptidoglycan from biomass (Komagata and Suzuki, 1987). The B type peptidoglycan structure characteristic for the family *Microbacteriaceae* and B2γ are estimated from the amino acid composition, namely glutamic acid:alanine:glycine:2,4-diaminobutyric acid is 1:1:1:2 as shown in Figure 179. However, the actual proportion of amino acids is variable in the B-type structure. The molar ratios of glutamic acid -glycine - alanine - 2,4-diaminobutyric acid are 1.0:1.0:0.6:1.3 for *Cryobacterium psychrophilum* (Suzuki et al., 1997), 1.0:0.8:1.0:1.6 for *Cryobacterium mesophilum* (Dastager et al., 2008a), and 1.0:0.9:0.5:1.1 for *Cryobacterium psychrotolerans.* In addition, two varieties of isomer composition of 2,4-diaminobutyric acid are known as a useful

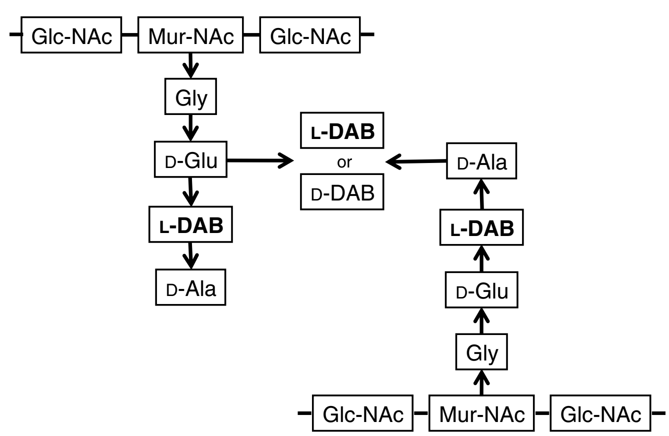

FIGURE 179. Variety of peptidoglycan containing 2,4-diaminobutyric acid. 2,4-Diaminobutyric acid of the peptidoglycan of the genus *Cryobacterium* contains exclusively L-isomers. GlcNAc, *N*-acetyl glucosamine; Mur, muramic acid; DAB, 2,4-diaminobutyric acid. *Arrows* indicate the direction from N-terminal to C-terminal.

TABLE 147. Characteristics differentiating species of the genus *Cryobacterium*[a]

Characteristic	*C. psychrophilum*[b]	*C. mesophilum*[c]	*C psychrotolerans*[d]	*C. roopkundense*[e]
Colony color	Pink	Cream to pale yellow	Yellow	Pink
Cell size (μm)	0.5–0.7 × 1.0–1.8	0.3–0.6 × 1.0–1.4	0.5–0.8 × 1.8–3.6	0.8 × 2.5
Motility	–	+	+	+
Growth temperature (°C)				
Optimum	9–12	25–28	20–22	15–18
Range	4–17	20–28	4–27	0–18
Cell-wall sugar (*whole-cell sugar)	Rhamnose and fucose	Glucose and ribose*	Glucose and ribose*	nd
Menaquinones	MK-10 (50%), MK-11 (17%), MK-8 (14%), MK-9 (11%)	MK-11(48%), MK-12 (37%)	MK-11 (39%), MK-12 (38%), MK-10 (16%)	MK-11 (53%), MK-10 (26%), MK-12 (21%)
Cellular fatty acids (cultivation temperature)	$C_{15:0}$ anteiso (34%), $C_{15:1}$ anteiso (20%), $C_{15:0}$ iso (18%) (at 10°C)	$C_{15:0}$ anteiso (35%), $C_{16:0}$ iso (35%), $C_{17:0}$ anteiso (17%) (at 28°C)	$C_{15:0}$ anteiso (40%), $C_{15:1}$ anteiso (17%), $C_{16:0}$ iso (14%) (at 20°C)	$C_{15:0}$ anteiso (20%), $C_{15:1}$ anteiso (20%) (at 15°C)
Polar lipids[f]	nd	DPG, PG, UGLs	DPG, PG, UGL	DPG, PG, UGL
DNA G+C content (mol%)	65 (HPLC)	70 (HPLC)	67 (T_m)	65
Nitrate reduction	–	–	+	+
Hydrolysis of:				
Casein	–	+	+	–
DNA	+	–	–	–
Starch	w	+	+	–
Acid from:				
L-Arabinose	–	+	+	–
Fructose	+	–	–	–
Galactose	+	+	–	–
Maltose	–	–	+	–
Mannose	+	–	–	–
Sucrose	+	–	–	–
Assimilation of:				
L-Arabinose	nd	–	+	–
Fructose	nd	–	+	–
Maltose	nd	–	+	–
Ribose	nd	–	+	–
Malate	+	+	–	–
Pyruvate	+	+	–	–

[a]Symbols: +, >85% positive; –, 0–15% positive; nd, not determined. The data for species are those of the type strains.

[b]Suzuki et al. (1997).

[c]Dastager et al. (2008a).

[d]Zhang et al. (2007a).

[e]Reddy et al. (2010).

[f]DPG, diphosphatidyl glycerol ; PG, phosphatidyl glycerol ; UGL, unknown glycolopid ; UL, unknown lipid.

taxonomic marker among the genera containing 2,4-diaminobutyric acid in the family *Microbacteriaceae*. Only L-form 2,4-diaminobutyric acid is found in the cells of *Cryobacterium psychrophilum* as in those of *Agrococcus*, *Agromyces*, *Leucobacter*, and *Rathayibacter* (Sasaki et al., 1998). In contrast, an almost equimolar mixture of the L-form and D-form of 2,4-diaminobutyric acid is found in the cells of representatives of the genera *Clavibacter*, *Leifsonia*, *Plantibacter*, *Pseudoclavibacter*, *Subtercola*, and *Yonghaparkia* (Schumann et al., 2009). The variation is possibly derived from the D-form 2,4-diaminobutyric acid present in the interpeptide bridge, while 2,4-diaminobutyric acid of the 3rd position of the peptide subunit is commonly L-2,4-diaminobutyric acid (Figure 179).

The members of the family *Microbacteriaceae* contain exclusively menaquinones with unsaturated isoprenoid chains from MK-8 to MK-14. The menaquinone profile of the genus *Cryobacterium* is around middle length from MK-10 to MK-12 and variable for the different species (Table 147). Small amounts of MK-8 and MK-9 were also found in the cells of *Cryobacterium psychrophilum*.

Major components of the cellular fatty acids of the genus *Cryobacterium* are iso- and anteiso-branched acids with 12-methyl tetradecanoic acid ($C_{15:0}$ anteiso) occurring predominantly. *Cryobacterium psychrophilum* is adapted to growth at low temperature and contains an increased amount of anteiso-branched unsaturated fatty acids in the membrane, especially 12-methyl tetradecenoic acid ($C_{15:1}$ anteiso). Unsaturated fatty acids such as 12-methyl tetradecenoic acid contribute to maintaining the fluidity of the cell membrane due to the low-melting-point. It is interesting that these psychrophilic bacteria such as *Cryobacterium psychrophilum* and *Cryobacterium psychrotolerans* contain unsaturated anteiso acids in the cells. *Cryobacterium psychrophilum*

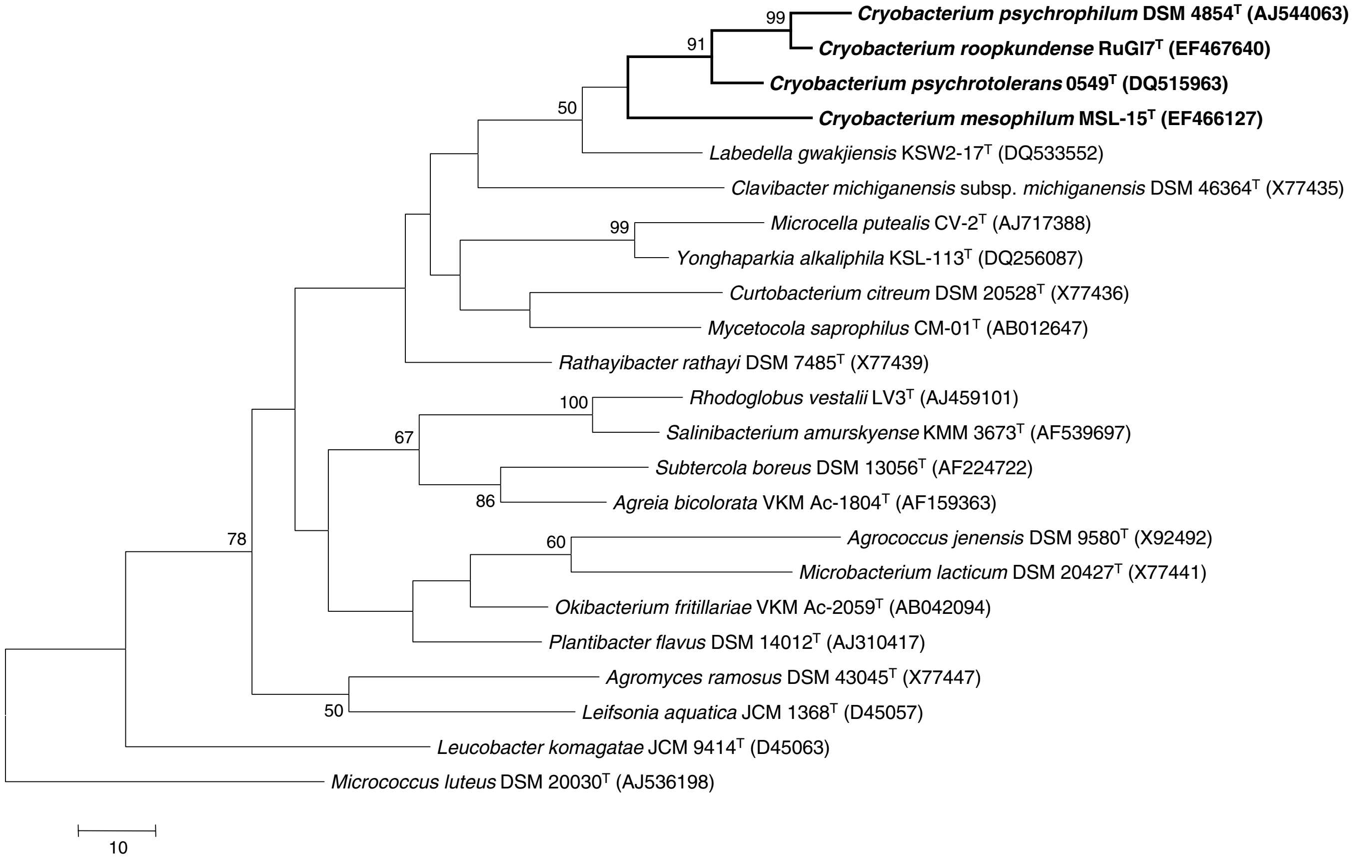

FIGURE 180. Phylogenetic tree derived from 16S rRNA gene sequences of the genus *Cryobacterium* and its taxonomic neighbors, constructed with the maximum parsimony method. The numbers at the branching points are the bootstrap values (>50%). Bar = 10 substitutions. (Tree courtesy of M. Hamada.)

increases the amount of the unsaturated acids in the cells grown under low temperatures (Suzuki et al., 1997).

Phylogeny. A phylogenetic tree based on the 16S rRNA gene sequences indicates a clear differentiation of the genus *Cryobacterium* from the other genera of the family *Microbacteriaceae* (Figure 180). However, there is no clear correlation between the phylogenetic position and the chemotaxonomic characteristics found for the most closely related genera.

Biochemical and physiological characteristics. Biochemical and physiological tests of members of the genus *Cryobacterium* show a wide variety among the species. The tests were carried out according to the methods described by Cowan and Steel (1965), Yamada and Komagata (1972b), and Kämpfer et al. (1991). The differences between the species are indicated in Table 147.

Enrichment and isolation procedures

The strains of the four species of the genus *Cryobacterium* were all isolated from soil. *Cryobacterium psychrophilum* was isolated aerobically at 0°C on PYG agar containing 1% peptone, 0.5% yeast extract, 0.3% glucose, and 1.5% agar (pH 7.2), paying attention to keep the process below 5°C for isolation of obligate psychrophiles (Inoue, 1976). *Cryobacterium psychrotolerans* and *Cryobacterium roopkundense* were isolated by cultivating a sample at 4°C on PYG medium (different composition from the above PYG) containing 0.5% polypeptone, 1% yeast extract, 1% glucose, and 40 ml salt solution which was composed of 0.02% $CaCl_2$, 0.04% $MgSO_4 \cdot 7H_2O$, 0.1% K_2HPO_4, 0.1% KH_2PO_4, 1% $NaHCO_3$, and 0.2% NaCl (pH 7.2) (Zhu et al., 2003) and ABM composed of 0.5% peptone, 0.2% yeast extract, and 1.5% agar (Reddy et al., 2010), respectively.

In contrast to these three species which were isolated under low temperature conditions, *Cryobacterium mesophilum* was isolated at 28°C by using ten-fold-diluted R2A agar (Difco).

Maintenance procedures

Strains are preserved by freezing at −80°C or lower or by lyophilization. The type strain of *Cryobacterium psychrophilum* can be lyophilized for long-term preservation. Although the strain does not grow at 18°C or higher, the lyophilized cells can be revived even after exposure to 37°C for 2 weeks. For lyophilization, it is recommended to harvest the cells at the late-exponential growth phase and to suspend them in 10% skim milk with 1% monosodium glutamate prior to freezing. Cells are suspended in 10–30% glycerol for freezing preservation.

Differentiation of the genus *Cryobacterium* from related genera

A phylogenetic analysis based on 16S rRNA gene sequences is the first step for allocation of isolates to this genus. The genus *Cryobacterium* is a member of the family *Microbacteriaceae* that

is characterized by B type peptidoglycan structure and unsaturated menaquinones with relatively long isoprenoid side chains. *Cryobacterium* species have 2,4-diaminobutyric acid as the characteristic diamino acid in the peptidoglycan which is found only in representatives of the family *Microbacteriaceae*. Twelve genera are known to contain 2,4-diaminobutyric acid in the peptidoglycan (Schumann et al., 2009). Differences in the occurrence of the isomers of 2,4-diaminobutyric acid enables a separation of these 12 genera into two groups (See subchapter chemotaxonomy above). The menaquinone systems of the genus *Cryobacterium* are variable ranging from MK-10 to MK-12 (Table 147) and do not allow unambiguous differentiation of the genus *Cryobacterium* from other 2,4-diaminobutyric acid containing genera. Species of the genus *Cryobacterium* can grow at temperatures from 4–28°C which distinguishes them from several 2,4-diaminobutyric acid contain taxa such as *Agrococcus*, *Agromyces*, and *Leucobacter*.

Taxonomic comments

Initially Inoue and Komagata (1976) proposed a new species of the genus *Curtobacterium* for the isolate strain 27-0-b on the basis of the presence of ornithine in the cell wall, although the name was not validated. Later, Suzuki et al. (1997) established the genus *Cryobacterium* for this strain based on the phylogenetic analysis of 16S rRNA gene sequence. Re-examination of the chemotaxonomy revealed that the strain contained 2,4-diaminobutyric acid in the cell wall and not ornithine. The other two species published later also contain 2,4-diaminobutyric acid in the cell wall demonstrating that the presence of this diamino acid is a common feature of the genus. The type strain of the type species, *Cryobacterium psychrophilum*, is known to contain exclusively the L-form of 2,4-diaminobutyric acid (Sasaki et al., 1998), but no information is available concerning the isomer of 2,4-diaminobutyric acid in the other three species. The cell-wall acyl type of *Cryobacterium psychrophilum* is acetyl, and sugars detected in the cell-wall hydrolysate are fucose and rhamnose, whereas no information is available for the other three species. However, glucose and ribose were detected in whole cell hydrolysate of *Cryobacterium psychrotolerans* and *Cryobacterium mesophilum*.

Differentiation of species of the genus *Cryobacterium*

The cell-wall peptidoglycan shows a common feature in the genus, while the menaquinone composition and the G+C content of genomic DNA are slightly variable. The range of growth temperatures is characteristic for the different species (Table 147). The biochemical and physiological features of the species are shown in Table 147. All species of the genus *Cryobacterium* have been described on the basis of one strain, each representing the type strain of the corresponding species. Sequence similarities of 16S rRNA genes of *Cryobacterium mesophilum* to *Cryobacterium psychrophilum* and *Cryobacterium psychrotolerans* are 94.8 and 96.6%, respectively. The DNA–DNA relatedness between the type strains of *Cryobacterium psychrotolerans* and *Cryobacterium psychrophilum* is 34% (Zhang et al., 2007a).

List of species of the genus *Cryobacterium*

1. **Cryobacterium psychrophilum** (*ex* Inoue and Komagata 1976) Suzuki, Sasaki, Uramoto, Nakase and Komagata 1997, 477[VP] (*"Curtobacterium psychrophilum"* Inoue and Komagata 1976, 166)

psy.chro′phi.lum. Gr. adj. *psuchros* cold; N.L. neut. adj. *philum* (from Gr. neut. adj. *philon*) loving; N.L. neut. adj. *psychrophilum* cold loving.

Nonmotile pleomorphic rods, 0.5–0.7 × 1.0–1.8 μm. Obligately psychrophilic. Growth occurs at 4–16°C, but optimal growth is observed at 9–12°C. No growth at 18°C and higher. Circular, opaque pink colonies are formed on R agar after 5 d at 10°C. Occasionally Gram-stain-variable. Nitrate is not reduced. DNA is hydrolyzed but casein is not. Starch is hydrolyzed weakly. Acid is produced from fructose, galactose, glucose, mannose, and sucrose but not from arabinose, glycerol, inulin, D-lactose, maltose, ribose, salicin, and D-xylose. Fumarate, glyoxylate, lactate, malate, propionate, pyruvate, and succinate are utilized, but *n*-butyrate, citrate, formate, glutalate, hippurate, maleate, tartrate, and uric acid are not utilized. 2,4-Diaminobutyric acid, alanine, glycine, and glutamic acid are the amino acids of cell-wall peptidoglycan (peptidoglycan type B2γ). Predominant menaquinones are MK-10 (50%) and MK-11 (17%). MK-8 (14%) and MK-9 (11%) are also found. Major cellular fatty acids are $C_{15:0}$ anteiso, $C_{15:0}$ iso, $C_{17:0}$ anteiso, and $C_{16:0}$ iso. Cells grown at lower than 10°C contain 20% or higher proportion of $C_{15:1}$ anteiso.

Source: soil of Antarctica.

DNA G+C content (mol%): 65 (HPLC).

Type strain: 27-O-b, ATCC 43563, CCM 3311, CGMCC 1.1912, CIP 105227, DSM 4854, IAM 12024, JCM 1463, KCTC 3487, LMG 23845, NBRC 15735, NCIMB 2068, VKM Ac-2100.

Sequence accession no. (16S RNA gene): AJ544063, D45058.

2. **Cryobacterium mesophilum** Dastager, Lee, Ju, Park and Kim 2008a, 1243[VP]

me.so′phi.lum. Gr. adj. *mesos* middle; N.L. neut. adj. *philum* (from Gr. neut. adj. *philon*) loving; N. L. neut. adj. *mesophilum* middle (temperature) loving, mesophilic.

Cells are motile, irregular rods, 0.3–0.6 × 1.0–1.4 μm. Colonies are light yellow, smooth, circular, and convex with entire margins. Mesophilic; grows at 20–28°C; optimal growth at 28°C. Growth occurs at 0–3% NaCl and pH 6.0–11.0 (optimally 7.0–8.0). Nitrate is reduced. Starch and casein are hydrolyzed, but esculin and DNA are not. Acid is produced from arabinose and galactose but not from fructose, maltose, mannose, and sucrose. Galactose, glucose, inositol, D-lactose, mannitol, mannose, melibiose, D-raffinose, and L-rhamnose are used as sole carbon and energy sources, but arabinose, fructose, maltose, and ribose are not. The amino acids of the cell-wall peptidoglycan are 2,4-diaminobutyric acid, alanine, glycine, and glutamic acid. Predominant menaquinones are MK-11 (48%) and MK-12 (37%). Major cellular fatty acids are $C_{15:0}$ anteiso, $C_{16:0}$ iso, and $C_{17:0}$ anteiso. Major whole cell sugars are glucose and ribose.

Source: soil of Bigeum Island, South Korea.
DNA G+C content (mol%): 70.0 (HPLC).
Type strain: MSL-15, DSM 19267, JCM 16539, KCTC 19270.
Sequence accession no. (16S rRNA gene): EF466127.

3. **Cryobacterium psychrotolerans** Zhang, Wang, Cui, Yang, Liu, Dong and Zhou 2007a, 868[VP]

psy.chro.to′le.rans. Gr. adj. *psuchros* cold; L. pres. part. *tolerans* tolerating; N.L. part. adj. *psychrotolerans* cold tolerating.

Cells are motile irregular rods, 0.5–0.8 × 1.8–3.6 µm. Yellow, smooth, circular, and convex colonies with entire margins are formed. Growth occurs at 4–27°C and pH 5.0–10.0, optimally at 20–22°C and pH 7.0–8.0. Growth occurs at 0–4% (w/v) NaCl. Nitrate is reduced. Casein and starch are hydrolyzed, but DNA is not. The Simmons' citrate and Voges–Proskauer tests are negative. L-Arabinose, D-cellobiose, fructose, glycerol, galactose, glucose, inulin, maltose, D-mannose, melezitose, ribose, salicin, xylose, glutamate, lactose, succinate, and acetate are utilized, but erythritol, galactitol, mannitol, raffinose, L-rhamnose, sorbinose, sorbitol, sucrose, citrate, gluconate, fumalate, hippurate, malate, pyruvate, and tartrate are not utilized. Acid is produced from arabinose, glucose, maltose, and xylose. The amino acids of the cell-wall peptidoglycan are 2,4-diaminobutyric acid, alanine, glycine, and glutamic acid. The predominant menaquinones are MK-11 (39%), MK-12 (38%), and MK-10 (16%). Major cellular fatty acids are $C_{15:0}$ anteiso, $C_{15:1}$ anteiso, $C_{16:0}$ iso, $C_{15:0}$ iso, and $C_{17:0}$ anteiso. Whole cell sugars are glucose and ribose predominantly.

Source: soil of China No. 1. Glacier (Xinjiang Uygur Autonomous Region).
DNA G+C content (mol%): 67 (T_m).
Type strain: 0549, CGMCC 1.5382, DSM 21575, JCM 13925.
Sequence accession no. (16S rRNA gene): DQ515963.

4. **Cryobacterium roopkundense** Reddy, Pradhan, Manorama and Shivaji 2010, 869[VP]

ro.op.kun.den′se. N.L. neut. adj. *roopkundense* of or pertaining to Lake Roopkund, the area from where the type strain was isolated.

Cells are Gram-stain-positive, aerobic motile rods, 0.8 × 2.5 µm. Colonies are convex, shiny pink colored, and 2–3 mm in diameter on LB agar. Optimum growth occurs at 15–18°C and pH 7.0. Catalase-positive and oxidase-negative. Nitrate is reduced. Gelatin is hydrolyzed, but casein, starch, and DNA are not. The Simmons' citrate and Voges–Proskauer tests are negative. Lysine, arginine, and ornithine decarboxylases, urease, phosphatase, and β-galactosidase are negative. Acid is not produced from erythritol, *myo*-inositol, inulin, D-melibiose, ribose, D-sorbitol, sorbose, trehalose, or D-xylose. Citrate and malonate are utilized, but acetate, adonitol, D-arabinose, L-arabinose, cellobiose, dulcitol, D-fructose, galactose, gluconate, D-glucose, glucosamine, glycereol, *myo*-inositol, inulin, lactose, maltose, D-mannitol, D-mannose, melibiose, melezitose, methyl α-D-glucoside, methyl α-D- mannoside, raffinose, D-rhamnose, D-ribose, salicin, sorbitol, L-sorbose, sucrose, trehalose, xylitol, and D-xylose are not.

The cell-wall peptidoglycan contains 2,4-diaminobutyric acid as the diagnostic diamino acid. The predominant menaquinones are MK-11 (53%), MK-10 (26%), and MK-12 (21%). Major cellular fatty acids are $C_{15:0}$ anteiso and $C_{15:1}$ anteiso in the cells grown on LB agar at 15°C for 7 d.

Source: soil sample from the banks of Lake Roopkund, Himalayan region, India.
DNA G+C content (mol%): 64.7.
Type strain: strain RuGl7, DSM 21065, JCM 15131.
Sequence accession no. (16S rRNA gene): EF467640.

Genus VII. **Curtobacterium** Yamada and Komagata 1972a, 425[AL]

Gerard S. Saddler and Patricia Messenberg Guimarães

Cur.to.bac.te′ri.um L. adj. *curtus* shortened; L. neut. n. *bacterium* a small rod; N.L. neut. n. *Curtobacterium* a short rodlet

Short irregular rods (0.3–0.6 × 0.5–3.0 µm); pleomorphism is not distinctive. Cells become shorter to coccoid in older cultures. Branching is not found, **bending type cell division**. Nonsporeforming. Not all species are motile; generally motile species show peritrichous flagellation. Gram-stain-positive, but staining properties can be lost with age. Non-acid-fast. Colonies are generally ivory, yellow, or orange. Strictly aerobic chemoorganotrophs. Acid is produced weakly from glucose, fructose, and some other carbohydrates. Acetate, pyruvate, and lactate are assimilated in addition to other organic acids. Catalase and DNase-positive; gelatin and esculin are hydrolyzed. Nitrate is not reduced; urease is not produced. **The cell-wall peptiodoglycan, based on the presence of D-ornithine, is type B2β. Acetyl is the peptidoglycan acyl type**. Mycolic acids are not found. Nonhydroxylated fatty acids, specifically **anteiso-methyl branched chains fatty acids predominate**; ω-cyclohexyl undecanoic acid is found in some species. Phosphatidylglycerol, diphosphatidylglycerol, and some glycosyldiacylglycerols are the major polar lipids. Spermidine and spermine are the major polyamines; putrescine and cadaverine are absent. **Isoprenoid quinones, comprising menaquinones with 9 isoprene units (MK-9), predominate.**

DNA G+C content (mol%): 65.8–75.2 (T_m).

Type species: **Curtobacterium citreum** (Komagata and Iizuka, 1964) Yamada and Komagata 1972a, 425 (*Brevibacterium citreum* Komagata and Iizuka 1964, 498).

Further descriptive information

The genus *Curtobacterium*, as described by Yamada and Komagata (1972a), encompasses coryneform bacteria that contain

D-ornithine in their cell-wall peptidoglycan (type B2β of Schleifer and Kandler, 1972). Additional distinguishing characters include the mode of cell division, G+C content, and a number of biochemical and physiological characteristics, principally the weak, slow production of acid from a range of carbohydrates (Yamada and Komagata, 1972a). The genus currently comprises eight species: *Curtobacterium citreum*, *Curtobacterium albidum*, *Curtobacterium ammoniigenes*, *Curtobacterium flaccumfaciens*, *Curtobacterium ginsengisoli*, *Curtobacterium herbarum*, *Curtobacterium luteum*, and *Curtobacterium pusillum*. There is a ninth species, *Curtobacterium plantarum* (Dunleavy, 1989), but genus membership is uncertain as the type strain is almost certainly a member of the family *Enterobacteriaceae* on the basis of biochemical tests (Funke et al., 2005), 16S rRNA gene sequencing, and fatty acid analysis (Evtushenko and Takeuchi, 2006).

Curtobacterium forms a coherent group on the basis of 16S rRNA gene sequencing and is distinct from other Group-B peptidoglycan genera (Rainey et al., 1994). However, the close relationship to *Aureobacterium* uncovered by Park and co-workers (1987), based on 5S rRNA sequencing, is not confirmed by 16S rRNA gene sequence data. Indeed, in a comprehensive analysis of the family *Microbacteriaceace*, based on 16S rRNA gene sequencing and encompassing representatives of 15 genera, *Curtobacterium* was found to be more closely related to *Clavibacter*, *Frigoribacterium*, and *Rathayibacter* (Evtushenko and Takeuchi, 2006). Essentially similar results from a subsequent study confirm this finding; the close relationship between *Curtobacterium* and *Clavibacter* is particularly marked (Chen et al., 2007). The relationship between *Curtobacterium* and its close relatives within the family *Microbacteriaceae*, based on 16S rRNA gene sequence data, is shown Figure 181. It is interesting to note that certain

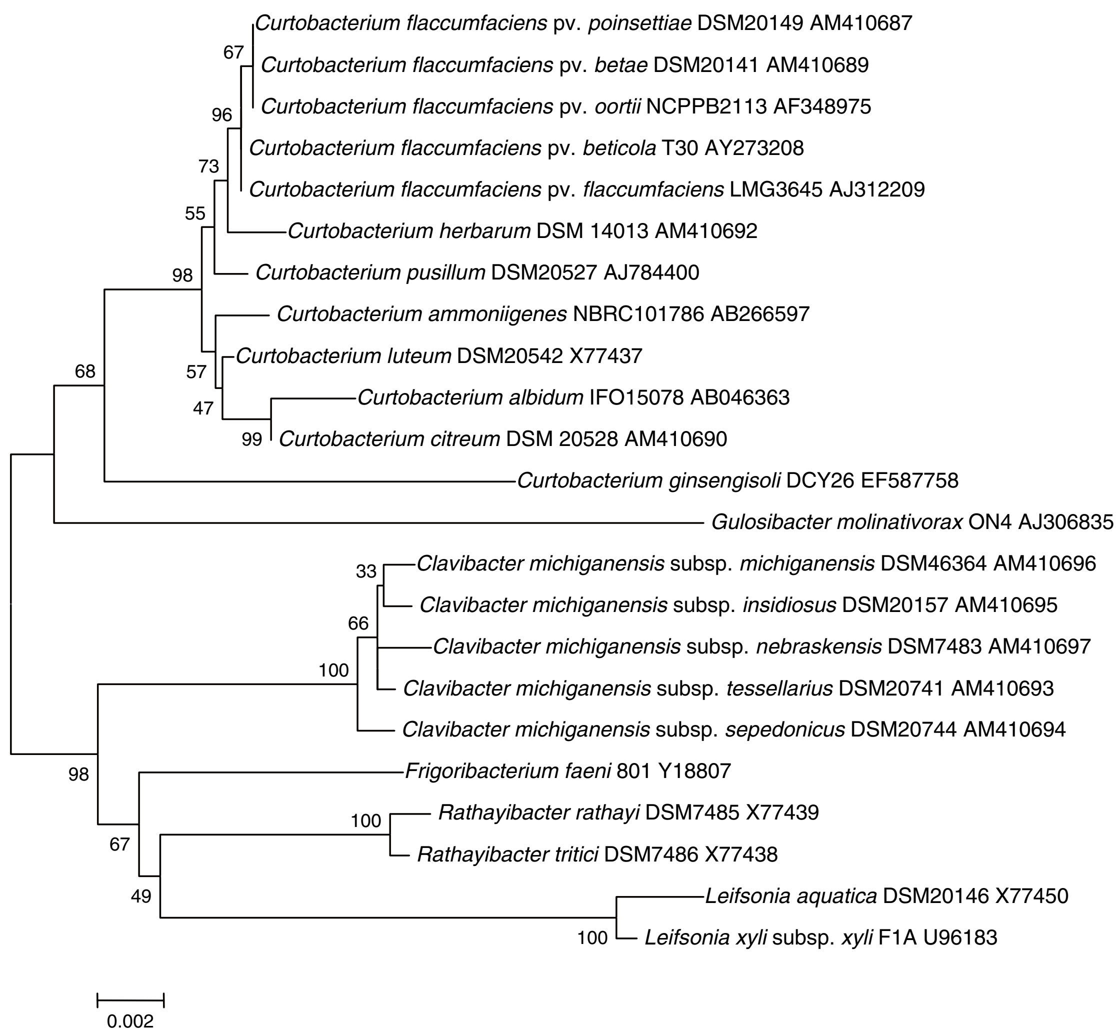

FIGURE 181. Phylogeny of *Curtobacterium* and close relatives drawn from the family *Microbacteriaceae*. Maximum composite likelihood with clustering achieved by the neighbor-joining method to produce a bootstrap consensus tree was performed using MEGA4. Bar = 0.2% difference in the 16S rRNA gene sequences. 500 Bootstrap trees were generated and bootstrap confidence levels (shown as percentages above nodes) were determined.

species within *Curtobacterium*, specifically *Curtobacterium albidum*, *Curtobacterium citreum*, and *Curtobacterium pusillum*, exhibit marked heterogeneity in their multi-copy 16S rRNA gene sequences as determined by temperature-gradient gel electrophoresis (TGGE). As a consequence, there is the potential to distort phylogenetic inference (Felske et al., 1999).

The phylogeny of the family *Microbacteriaceae* has also been studied using alternative genetic markers, namely DNA gyrase subunit B (*gyrB*), RNA-polymerase subunit B (*rpoB*), recombinase A (*recA*), and polyphosphate kinase (*ppk*), in addition to 16S rRNA (Stackebrandt et al., 2007). Analysis of 16S rRNA gene sequence in this study again highlights the close relationship between *Clavibacter* and *Curtobacterium*, but sequence data from *gyrB* and *rpoB* infers a closer relationship to *Frigoribacterium*. Although *Curtobacterium* remains a coherent entity in each phylogenetic tree, the suprageneric relationships vary considerably. One of the significant findings of this study is that in none of the 16 trees generated did the topologies coincide with the key taxonomic marker for this group, specifically the diagnostic diamino acid of the peptidoglycan, suggesting that this characteristic may have evolved independently several times or that it has regularly been subject to horizontal gene transfer. The DNA G+C composition ranges from 65.8–75.2 mol% (T_m) (Aizawa et al., 2007; Behrendt et al., 2002; Chen et al., 2007; Döpfer et al., 1982; Kim et al., 2008b). DNA–DNA hybridizations among representatives of the genus *Curtobacterium* revealed very high relatedness values (≥73%) between pathovars of the species *Curtobacterium flaccumfaciens*, while indices between *Curtobacterium flaccumfaciens* and other members of the genus were 48–54% (Döpfer et al., 1982).

Members of the genus *Curtobacterium* in general produce Gram-stain-positive pleomorphic rods (0.3–0.6 × 0.5–3.0 µm) that show a bending-type cell division, frequently producing V- and Y-pallisade arrangements (Collins and Jones, 1983; Yamada and Komagata, 1972a). Staining properties tend to be lost in older cultures. Rod forms are observed in young cultures, while coccoid forms predominate in older cultures, but there is no marked rod–coccus growth cycle (Komagata and Suzuki, 1986a). Branching is not observed. Motility is facilitated in some species by peritrichous flagella.

Curtobacterium strains possess D-ornithine in the interpeptide bridge and are therefore classed as type B2β, as described by Schleifer and Kandler (1972). Acetyl is the peptidoglycan acyl type. Principal phospholipids are phospatidylglycerol and diphospatidylglycerol, with a number of unidentified glycolipids (Collins et al., 1980). Later, the two major glycolipids were identified in *Curtobacterium flaccumfaciens* pv. *betae* as di- and trimannosyl glyceride (Mordarska et al., 1992). Spermidine and spermine are the major polyamines; putrescine and cadaverine are absent, which contrasts with *Clavibacter* and *Rathayibacter* where minor amounts of the latter two polyamines are present, albeit in small amounts (Altenburger et al., 1997). Anteiso isomers, specifically 14-methylhexadecanoic ($C_{17:0}$ anteiso) and 12-methyltetradecanoic acids ($C_{15:0}$ anteiso), are the predominant components of the fatty acid profile in *Curtobacterium*, comprising approximately 80% of the total (Collins et al., 1980; Henningson and Gudmestad, 1991; Kämpfer and Kroppenstedt, 1996). Significant quantities of 14-methylpentadecanoic ($C_{16:0}$ iso) are also found. Kämpfer and Kroppenstedt (1996) found that it was not possible to separate *Curtobacterium* from *Clavibacter* on the basis of fatty acids, contrasting with the view of Henninson and Gudmestad (1991) who suggested that the ratios between key, minor fatty acid components could even be used to differentiate pathovars of the species *Curtobacterium flaccumfaciens*. Certainly using a commercial identification system based on fatty acids in conjunction with a bespoke identification library, an accuracy of identification of 87.5% was obtained for *Curtobacterium flaccumfaciens* pv. *flaccumfaciens* (Stead et al., 1992). Where misidentifications occurred it was only to other pathovars of the species. *Curtobacterium pusillum* contains an unusual fatty acid, ω-cyclohexyl undecanoic acid (ch-$C_{17:0}$), as its major component (Suzuki et al., 1981). This fatty acid accounts for 26–96% of the cellular fatty acid composition, is strain dependant, and increases in relative amount with increasing growth temperature and glucose concentration. Up until recently it had only ever been found in one other, as yet unnamed, *Curtobacterium* strain (CNF 165), previously "*Brevibacterium helvolum*" isolated from oil-brine (Suzuki and Komagata, 1983), the source of *Curtobacterium pusillum* itself. However, with the recent description of *Curtobacterium ammoniigenes*, isolated from the stem of an aquatic weed, all strains of this species are reported to possess significant amounts of 11-cyclohexyl undecanoic acid (ch-$C_{17:0}$) with more than 80% of the fatty acid profile made up of this single fatty acid (Aizawa et al., 2007). As with previous findings, this component varies with changing growth conditions; the relative concentration of this fatty acid was observed to fall with decreasing pH. These observations highlight the importance of standardizing the growth conditions (temperature, medium composition, pH) if fatty acids are to be analyzed for classification of curtobacteria. All *Curtobacterium* strains possess menaquinones with nine isoprene units (MK-9) (Aizawa et al., 2007; Behrendt et al., 2002; Collins et al., 1980); MK-10 has not been found, in contrast to *Clavibacter*, where minor amounts of this menaquinone have been reported (Sasaki et al., 1998).

Curtobacterium strains grow well on nutrient agar at 25–30°C, however, *Curtobacterium ginsengisoli* has an optimum growth temperature of 30–37°C (Kim et al., 2008b). They are obligate aerobes which may require some amino acids or vitamins for growth (Komagata and Suzuki, 1986a). On nutrient agar, colonies are predominantly yellow/orange, circular, smooth, slightly convex, and glistening (Evtushenko and Takeuchi, 2006). Some strains of *Curtobacterium flaccumfaciens* pv. *flaccumfaciens* produce a purple diffusible pigment on Bacto Soytone Agar (Difco) supplemented with 10% (w/v) glucose (BSG agar) or lactose (BSL agar; Schuster et al., 1968). Strains of *Curtobacterium* produce acid slowly and weakly from various kinds of sugars (Yamada and Komagata, 1972a). All *Curtobacterium* strains produce acid from glucose and fructose, with the exception of *Curtobacterium ginsengisoli* which does not produce acid from glucose (Kim et al., 2008b). Some organic acids are assimilated in addition to acetate, pyruvate, and lactate. *Curtobacerium* strains, which remain to be fully characterized, are known to produce chondroitinase (Takegawa et al., 1991), β-glucuronidase (Tör et al., 1992), and phosphonoacetate hydrolases (McGrath et al., 1999).

Limited information exists on the development of antibodies suitable for the detection of members of the genus *Curtobacterium*. What little there is, focuses primarily on the plant pathogens within the genus and specifically *Curtobacterium flaccumfaciens* pv. *flaccumfaciens*, the causal agent of bacterial wilt of

beans. A comprehensive study reports two monoclonal antibodies raised against *Curtobacterium flaccumfaciens* pv. *flaccumfaciens* (MAb 1527 and 1535), both of which failed to react to all strains of the pathovar and cross-reacted with strains from other *Curtobacterium flaccumfaciens* pathovars (McDonald and Wong, 2000). Similar results were also found with a polyclonal antibody which failed to react with all strains of *Curtobacterium flaccumaciens* pv. *flaccumfaciens*, confirming the earlier findings of Calzolari and co-workers (1987). In contrast, two out of three monoclonal antibodies raised against an isolate of *Curtobacterium flaccumfaciens* pv. *flaccumfaciens* recovered from mungbean were able to detect all strains of the pathovar and showed no cross-reaction to any other organism tested (Diatloff et al., 1993). Members of the genus *Curtobacterium* are known to produce bacteriocins (Gross and Vidaver, 1979a). In this comprehensive study, a wide range of coryneform phytopathogens now members of the genera *Clavibacter*, *Curtobacterium*, *Rathayibacter*, and *Rhodococcus* were examined. A strain of *Curtobacterium flaccumfaciens* pv. *oortti* was found to inhibit a strain of *Curtobacterium flaccumfaciens* pv. *flaccumfaciens*, while *Curtobacterium flaccumfaciens* pv. *flacumfaciens* CV6 showed inhibitory effects on *Curtobacterium flaccumfaciens* pvs *betae*, *oortii* and *poinsettae* as well as other members of *Curtobacterium flaccumfacciens* pv. *flaccumfaciens*. The majority of the bacteriocins produced were resistant to heat but sensitive to proteolytic enzymes.

Most members of the genus *Curtobacterium* have been isolated from plants and their habitats. *Curtobacterium citreum*, *Curtobacterium albidum*, and *Curtobacterium luteum* were isolated from rice paddies (Komagata and Iizuka, 1964), *Curtobacterium ginsengisoli* from soil in a ginseng field (Kim et al., 2008b), while *Curtobacterium herbarum* was isolated from the litter layer of grass (Behrendt et al., 2002), and *Curtobacterium ammoniigenes* was recovered from the stem of an aquatic weed inhabiting acidic swamps in Vietnam (Aizawa et al., 2007). None are known to be phytopathogenic. In contrast, *Curtobacterium flaccumfaciens* is the only species regarded as a plant pathogen and is further subdivided into six pathovars: *Curtobacterium flaccumfaciens* pv. *flaccumfaciens* (*Phaseolus* species and *Vigna* species, beans); *Curtobacterium flaccumfaciens* pv. *betae* (*Beta vulgaris* var. *rubra*, red beet); *Curtobacterium flaccumfaciens* pv. *beticola* (*Beta vulgaris* var. *saccharifera*, sugar beet); *Curtobacterium flaccumfaciens* pv. *ilicis* (*Ilex opaca*, American holly); *Curtobacterium flaccumfaciens* pv. *oortii* (*Tulipa* species, tulips); and *Curtobacterium flaccumfaciens* pv. *poinsettiae* (*Euphorbia pulcherrima*, poinsettia). The only species in the genus not isolated from, or associated with, plants is *Curtobacterium pusillum*, isolated from oil brine in a Japanese oilfield (Iizuka and Komagata, 1965). It is likely that additional *Curtobacterium* species will be described in due course as there are numerous citations in the literature of partially characterized curtobacteria isolated from variety sources, namely agronomic crops and prairie plants (Zinniel et al., 2002), citrus plants (Lacava et al., 2004), apple phyllosphere (Kucheryava et al., 1999), clinical specimens (Funke et al., 2005), reed communities in shallow lakes (Ács et al., 2003), and waste waters (McGrath et al., 1999).

Enrichment and isolation procedures

Few selective media exist for curtobacteria; of these, the majority are used to recover *Curtobacterium flaccumfaciens* strains from suspect, infected plant materials. In situations where asymptomatic infections are suspected, or where problems with overgrowth have been encountered, semi-selective media are required. CNS medium* (Gross and Vidaver, 1979a), which was primarily developed for *Clavibacter michiganensis* subsp. *nebraskensis*, works well with some pathovars of *Curtobacterium flaccumfaciens*. Alternately, a semi-selective media MSCFF† has been reported to allow the growth of a wide variety of *Curtobacterium flaccumfaciens* pv. *flaccumfaciens* strains while inhibiting many saprophytic bacteria commonly found on bean seeds (Maringoni et al., 2006).

Maintenance procedures

Most curtobacteria can be stored on nutrient agar slopes at 5°C for 6 months. However, as with many bacteria, freeze-drying or freezing as a glycerol suspension is recommended for long-term preservation of more than one year (Komagata and Suzuki, 1986a).

Differentiation of the genus *Curtobacterium* from other genera

Curtobacterium is a well-defined genus, with good chemotaxonomic and molecular markers that can be exploited to distinguish it from other menaquinone MK-9 containing genera in the family *Microbacteriaceae* (Table 148). Identification to the genus can be readily achieved using chemotaxonomic methods to identify the presence of D-ornithine in the peptidoglycan of the cell wall. Both *Curtobacterium* and species of the genus *Aureobacterium*, which are all now contained within *Microbacterium*, share a group B peptidoglycan based on D-ornithine (Schleifer and Kandler, 1972). However, *Curtobacterium* species possess a single D-ornithine in the inter-peptide bridge while *Aureobacterium* species contain glycine in addition to the D-ornithine residue. Further, *Microbacterium* strains contain MK-10, MK-11, MK-12, and MK-13. *Gulosibacter*, which also contains D-ornithine and MK-9, can be differentiated from *Curtobacterium* on the basis of a positive oxidase reaction, a higher optimal temperature for growth, and a lower mol% G+C of the DNA (Manaia et al., 2004). Sequence analysis of 16S rRNA genes is also reliable for differentiating *Curtobacterium* from other members of the family *Microbacteriaceae* (Evtushenko and Takeuchi, 2006; Manaia et al., 2004; Stackebrandt et al., 2007). Other genes including; DNA gyrase subunit B (*gyrB*), RNA-polymerase subunit B (*rpoB*), recombinase A (*recA*), and polyphosphate kinase (*ppk*) have also been used successfully to differentiate *Curtobacterium* from other genera within the family (Stackebrandt et al., 2007). Only a small number of studies have been undertaken developing alternative molecular approaches for application in the differentiation and identification of curtobacteria; the bulk of these approaches have focused on *Curtobacterium flaccumfaciens* pv. *flaccumfaciens*. Fingerprinting methods such as RAPD

*CNS Medium (g/liter): Nutrient broth (Difco), 8.0; yeast extract (Difco), 2.0; K_2HPO_4, 2.0; KH_2PO_4, 0.5; agar, 15.0. Added separately: 50 ml of sterile glucose (50%, w/v aq.) and 1 ml of sterile 1M $MgSO_4{\cdot}7H_2O$; lithium chloride, 10.0; nalidixic acid, 25 mg (freshly dissolved in 0.lM NaOH, 10 $mg.ml^{-1}$), polymyxin $B.SO_4$ 32 mg (added as a 10 $mg.ml^{-1}$ freshly prepared aq. solution); Actidione/cycloheximide, 40 mg (added as a 10 $mg.ml^{-1}$ aq. stock solution); Chlorothalonil/Bravo 6F, 0.0625ml. (diluted 1:50 from a stock solution of tetrachloro-isophthalonitrile).

†MSCFF Medium (g/liter): Peptone, 5.0; meat extract, 3.0; sucrose, 5.0; agar, 15.0. Added after autoclaving to the basal medium: skim milk powder, 5.0; Congo red, 0.05; chlorothalonil, 0.01; thiophanate methyl, 0.01; nalidixic acid, 0.01; nitrofurantoin, 0.01; oxacillin, 0.001; sodium azide, 0.001.

TABLE 148. Characteristics differentiating *Curtobacterium* from other menaquinone MK-9 containing genera in the family *Microbacteriaceae*[a]

Characteristic	*Curtobacterium*	*Clavibacter*	*Frigoribacterium*	*Gulosibacter*	*Pseudoclavibacter*
Colony color	I/O/Y	O/W/Y	Y	W	Y
Motility	D	–	+	–	–
Optimum growth temperature (°C)	24–26, 30–37[b]	20–29	4–10, 24–26[c]	35–37	28–30
Peptidoglycan amino acid	D-Orn	DL-DAB	L-Lys	D-Orn	DL-DAB
Fatty acids	$C_{15:0}$ anteiso, $C_{16:0}$ iso, $C_{17:0}$ anteiso	$C_{15:0}$ anteiso, $C_{16:0}$ iso, $C_{17:0}$ anteiso	$C_{15:0}$ anteiso, $C_{16:0}$ iso	$C_{15:0}$ anteiso, $C_{16:0}$ iso, $C_{17:0}$ anteiso	$C_{15:0}$ anteiso, $C_{16:0}$ iso, $C_{17:0}$ anteiso
Polyamines[d]	SPD, SPM	SPD, SPM, (PUT, CAD)	(SPD, SPM, HSPD[e])	(SPD, SPM[e])`	DAP, CAD
DNA G+C content (mol%)	65.8–71	65–75	67–71	64.5	67

[a]Symbols: +, >85% positive; –, 0–15% positive; D, different reactions occur in different taxa (species of a genus or genera of a family); I, ivory: O, orange; Y, yellow; W, white. Data from: Dastager et al. (2008d); Evtushenko and Takeuchi (2006); Kämpfer et al. (2000); Manaia et al. (2004); Matsumoto et al. (2008); and Takeuchi and Hatano (1998b).

[b]Temperature optimum of *Curtobacterium ginsengisoli*. See Kim et al. (2008b).

[c]The genus *Frigoribacterium* contains psychrophilic and mesophilic species. See Dastager et al. (2008d) and Kämpfer et al. (2000).

[d]CAD, cadaverine; DAP, 1,3-diaminopropane; PUT, putrescine; SPD, spermidine; HSPD, *sym*-homospermidine; SPM, spermine. Polyamines in brackets are only found in small amounts. Data from Altenburger et al. (1997).

[e]H.-J. Busse, unpublished results.

(Chen et al., 2007), rep-PCR (McDonald and Wong, 2000), AFLP and pulsed-field gel electrophoresis (Guimarães et al., 2003), highlight differences between *Curtobacterium* and other phytopathogenic members of the family *Microbacteriaceae*, in addition to differentiating pathovars within species and uncovering heterogeneity within the pathovars themselves. Many of the phytopathogenic members of the species *Curtobacterium flaccumfaciens* can be readily detected using a number of different PCR assays. Most of the assays can be applied directly to the suspect plant material, with no cross-reaction to common saprophytes. In the case of *Curtobacterium flaccumfaciens* pv. *flaccumfaciens*, PCR assays have been designed from repetitive sequences (Tegli et al., 2002) or after a process of subtractive hybridization (Guimarães et al., 2001).

Taxonomic comments

Biochemical characteristics useful in distinguishing *Curtobacterium* species are listed in Table 149. Commercial identification systems such as Biolog have been used to identify *Curtobacterium* strains. Using an early version of the identification library (Biolog, release 3.5), Harris-Baldwin and Gudmestad (1996) found that results were highly variable, although 100% of *Curtobacterium flaccumfaciens* pvs *betae*, *flaccumfaciens*, and *oortii* strains were correctly identified to the genus, with this figure dropping dramatically for correct species or pathovar identifications. Indeed, no strains of *Curtobacterium flaccumfaciens* pv. *oortii* were correctly identified to the pathovar even after supplementing the database with additional data in an attempt to improve its accuracy. As the host range of *Curtobacterium flaccumfaciens* pathovars is restricted, it may still be possible to make an effective identification on the basis of knowledge of origin and species-level identification using this method. However, results obtained using API ZYM highlight a great deal of overlap between strains of the genus *Clavibacter* and *Curtobacterium*, with the bulk of curtobacteria indistinguishable from *Clavibacter* using this method (De Bruyne et al., 1992).

An area in need of attention within the genus is the continuing uncertainty over the legitimacy of the species *Curtobacterium plantarum*. On the basis of biochemical tests (Funke et al., 2005), 16S rRNA gene sequencing, and fatty acid analysis (Evtushenko and Takeuchi, 2006), it is evident that the type strain, ATCC 49174, is not a member of the genus *Curtobacterium*, and indeed it is currently listed as *Pantoea agglomerans* by the ATCC. It is possible, however, to identify discrepancies in characteristics as reported in the original description (Dunleavy, 1989) and by Funke and co-workers (2005). The logical conclusion is that an error may have occurred either selecting/depositing the type strain or subsequently. Certainly, it appears from the original description, that other strains assigned to the species may be authentic members of the genus (Evtushenko and Takeuchi, 2006). It is not clear whether these strains are still in existence, and it may be that the only possible solution will be to reject the name *Curtobacterium plantarum* and declare it *nomen dubium*. Since the type strain of *Curtobacterium plantarum* is not a member of the genus *Curtobacterium*, a neotype must be proposed and a request for an opinion submitted to the Judicial Commission of the International Committee for the Systematics of Prokaryotes.

A continuing area of debate also revolves around the subspecific classification within *Curtobacterium flaccumfaciens*. Carlson and Vidaver (1982) argued that differences in biochemical tests, bacteriocin production, and DNA–DNA reassociation experiments, independent of pathogenic specificity, merited recognition at the subspecific-level. This was in marked contrast to subsequent work that challenged the DNA–DNA reassociation experiments and failed to place the same emphasis on other differences suggesting rather that circumscription of these taxa within pathovars was more appropriate (Collins and Jones,

TABLE 149. Characteristics differentiating species of the genus *Curtobacterium*[a]

Characteristic	*C. citreum*	*C. albidum*	*C. ammoniigenes*[b]	*C. flaccumfaciens*[c]	*C. ginsengisoli*[d]	*C. herbarum*	*C. luteum*	*C. pusillum*
Motility	+	−	−	d	−	+	+	+
Colony color	Y	I	PY	Y/O	Y	O	PY	Y
Oxidase[d]	−	−	−	−	+	−	−	−
Acid production from:								
Glucose[d]	+	+	nd	+	−	+	+	+
D-Melezitose	+	+	−	+	nd	+	+	+
Raffinose	−	+	−	+	nd	+	−	+
L-Rhamnose	+[e]	+	+	d	nd	d	+	+
Ribose	+	+[e]	+	+[e]	nd	+	+	+
D-Sorbitol	−	−	+	+[e]	nd	+	−	−
Unusual cell-wall fatty acid:[b,f,g]								
ω-Cyclohexyl undecanoic acid	−	−	+	−	−	−	−	+
Optimal pH	7	7	4	7	nd	nd	7	7
Source	Rice paddy	Rice paddy	Creeping waterprimrose (*Ludwiga adscendens*)	Beans (*Phaseolus* species and *Vigna* species), red beet (*Beta vulgaris* var. *rubra*), sugar beet (*Beta vulgaris* var. *saccharifera*), American holly (*Ilex opaca*), Tulips (*Tulipa* species), and poinsettia (*Euphorbia pulcherrima*)	Ginseng field soil	Grass phyllosphere	Rice paddy	Oil brine

[a]Symbols: +, >85% positive; d, different strains give different reactions (16–84% positive); −, 0–15% positive; nd, not determined; I, ivory; PY, pale/light yellow; Y, yellow; O, orange. Unless otherwise stated, data from Behrendt et al. (2002).

[b]Data from Aizawa et al. (2007).

[c]Diagnostic tests for pathovars of *Curtobacterium flaccumfaciens* shown in Table 150.

[d]Data from Kim et al. (2008b).

[e]Conflicting results between Behrendt et al. (2002) and Aizawa et al. (2007).

[f]Data from Collins et al. (1980).

[g]Data from Suzuki et al. (1981).

1983). There is considerable support for the view of Collins and Jones (1983) as numerous subsequent studies have indicated the taxa overlap and differentiating between all strains on the basis of biochemical or chemotaxonomic characters alone is at best problematic. More recently, two strains held in the International Collection of Microorganisms from Plants (ICMP) which represent the type strain of *Arthrobacter ilicis*, a pathogen of American holly, were found not to be identical and that the designated type strain among these two strains was not representative of the pathogen (Young et al., 2004b); one strain, ICMP 2608, which could be sourced back to the original description did appear to be authentic, although it was not a member of the genus *Arthrobacter* based on an earlier comprehensive analysis of biochemical and physiological characteristics (Dye and Kemp, 1977). It was therefore proposed that the description and authority for *Arthrobacter ilicis* be amended to recognize it as novel species, albeit not a plant pathogen. *Curtobacterium flaccumfaciens* pv. *ilicis* was proposed for the pathogen of American holly, although no formal description was given. The description is taken from the earlier work of Dye and Kemp (1977) and lacks details on chemotaxonomic characteristics. Similarly, no 16S rRNA gene sequence data are available for the proposed taxon as yet.

List of species of the genus *Curtobacterium*

1. **Curtobacterium citreum** (Komagata and Iizuka 1964) Yamada and Komagata 1972a, 425[AL] (*Brevibacterium citreum* Komagata and Iizuka 1964, 498)

ci′tre.um. L. neut. adj. *citreum* of or pertaining to the citrus-tree, intended to mean lemon-colored.

The description is as for the genus, and diagnostic tests are listed in Table 149. Dull yellow colonies produced on nutrient agar. Cells are motile. Esculin is hydrolyzed, but casein, gelatin, starch, and Tween 80 are not. Optimum growth at pH 7.

Source: rice paddies.

DNA G+C content (mol%): 70.5–75.2 (T_m).

Type strain: ATCC 15828, CCUG 12163, CCUG 28999, CIP 81.26, DSM 20528, NBRC 12677, JCM 1345, LMG 8786, VKM B-1207.

Sequence accession no. (16S rRNA gene): X77436, AM410690.

TABLE 150. Characteristics differentiating pathovars of the species *Curtobacterium flaccumfaciens*[a]

Characteristic	*C. f.* pv. *flaccumfaciens*	*C. f.* pv. *betae*	*C. f.* pv. *beticola*	*C. f.* pv. *ilicis*	*C. f.* pv. *oortii*	*C. f.* pv. *poinsettiae*
Motility	d	d	+[c]	d	+	d
Colony color on NBY	Y/O[b]	Y[b]	Y[c]	nd	Y[b]	O[b]
Acid produced from: [d]						
Lactose	+/d	+/+	+[c]	+/d	+/+	+/+
β-Methyl-D-glucoside	+/d	+/+	nd	+/d	+/+	+/−
Rhamnose	+/+	+/+	+[c]	+/−	+/+	+/−
Sorbitol	d/d	d/−	−[c]	+/d	−/−	d/−
Utilization of:						
Acetate	+	+	+[c]	+	−	+
Fumarate	+	−	nd	−	−	−
Plant host	Beans (*Phaseolus* spp. and *Vigna* spp.)	Red beet (*Beta vulgaris* var. *rubra*)	Sugar beet (*Beta vulgaris* var. *saccharifera*)	American holly (*Ilex opaca*)	Tulips (*Tulipa* spp.)	Poinsettia (*Euphorbia pulcherrima*)

[a]Symbols: +, >85% positive; d, different strains give different reactions (16–84% positive); −, 0–15% positive; nd, not determined; Y, yellow; O, orange. Unless otherwise stated, data from Dye and Kemp (1977).

[b]Data from Vidaver and Davis (1988).

[c]Data from Chen et al. (2007).

[d]Results obtained with two basal media, hence two results are given for each characteristic, see Dye and Kemp (1977) for details.

2. **Curtobacterium albidum** (Komagata and Iizuka 1964) Yamada and Komagata 1972a, 425[AL] (*Brevibacterium albidum* Komagata and Iizuka 1964, 500)

al′bi.dum. L. neut. adj. *albidum* white.

The description is as for the genus, and diagnostic tests are listed in Table 149. Colonies on nutrient agar are an ivory color. Cells are nonmotile. Esculin, gelatin, and Tween 80 are hydrolyzed; starch is not. Optimum growth at pH 7.

Source: rice paddies.

DNA G+C content (mol%): 70.0–72.0 (T_m).

Type strain: ATCC 15831, CIP 102693, DSM 20512, HAMBI 2039, NBRC 15078, JCM 1344, VKM B-1206.

Sequence accession no. (16S rRNA gene): AB046363, AM042692, AM410691.

3. **Curtobacterium ammoniigenes** Aizawa, Ve, Kimoto, Iwabuchi, Sumida, Hasegawa, Sasaki, Tamura, Kudo, Suzuki, Nakajima and Sunairi 2007, 1451[VP]

am.mo.ni.i′ge.nes. N.L. n. *ammonia* ammonia; Gr. v. *gennâo* to produce; N.L. part. adj. *ammoniigenes* ammonia-producing.

The description is as for the genus, and diagnostic tests are listed in Table 149. Colonies are pale yellow, smooth, convex, and round with entire margins. Esculin is hydrolyzed; gelatin, and Tween 80 are not. Growth occurs between 15 and 37°C, but not at 4°C or 45°C. The pH range for growth is 3.5–8.0, with optimum growth at pH 4. Ammonia is produced in 10% tryptic soy broth (pH 4.0). The predominant cellular fatty acid is 11-cyclohexyl undecanoic acid (ch-$C_{17:0}$).

Source: creeping waterprimrose (*Ludwigia adscendens*) inhabiting acidic swamps in actual acid sulfate soil areas in Vietnam.

DNA G+C content (mol%): 68.8.

Type strain: B55, JCM 14609, NBRC 101786, VTCC D6-11.

Sequence accession no. (16S rRNA gene): AB266597.

4. **Curtobacterium flaccumfaciens** (Hedges 1922) Collins and Jones 1984, 270[VP] (Effective publication: Collins and Jones 1983, 3546.) (*Bacterium flaccumfaciens* Hedges 1922, 433; *Corynebacterium flaccumfaciens* (Hedges 1922) Dowson 1942, 313)

flac.cum.fa′ci.ens. L. adj. *flaccus* flabby; L. part. adj. *faciens* making; N.L. part. adj. *flaccumfaciens* wilt-making.

The description is as for the genus, and diagnostic tests are listed in Table 149. The colonies are yellow or orange on NBY agar. Some variants may lose pigmentation, and some may produce a blue to purple water-soluble pigment. Generally motile. Optimum growth temperature is 24–27°C and maximum temperature 35–37°C.

DNA G+C content (mol%): 67.5–73.7 (T_m).

Type strain: CIP 107085, ICMP 2584, JCM 9670, LMG 3645, NCPPB 1446.

Sequence accession no. (16S rRNA gene): AJ312209, AF348973, AM410688.

4a. **Curtobacterium flaccumfaciens pv. flaccumfaciens** (Hedges 1922) Collins and Jones 1983, 3547 (*Corynebacterium flaccumfaciens* pv. *flaccumfaciens* Dowson 1942; *Corynebacterium flaccumfaciens* subsp. *flaccumfaciens* Carlson and Vidaver 1982, 322.)

The description is as for the species, and diagnostic tests are listed in Table 150. Colonies can be yellow or orange. Some strains produce a purple diffusible pigment. Casein and gelatin are hydrolyzed; esculin and Tween 80 give variable results. Optimum pH for growth is 7. This organism was isolated from bean (*Phaseolus* species and *Vigna* species), where it causes a vascular wilt.

DNA G+C content (mol%): 72.2 (T_m).

Type strain: ATCC 6887, DSM 20129, ICMP 2584, IFO 12156, JCM 1347, LMG 3645, NBRC 12156, NCPPB 1446, NCTC 4758.

Sequence accession no. (16S rRNA gene): AJ312209, AF348973, AM410688.

4b. **Curtobacterium flaccumfaciens pv. betae** (Keyworth et al. 1956) Collins and Jones 1983, 3547 (*Corynebacterium betae* Keyworth, Howell and Dowson 1956; *Corynebacterium*

flaccumfaciens pv. *betae* Dye and Kemp 1977; *Corynebacterium flaccumfaciens* subsp. *betae* Carlson and Vidaver 1982, 322.)

The description is as for the species, and diagnostic tests are listed in Table 150. Colony color is yellow. Esculin is hydrolyzed; starch and Tween 80 give variable results. Casein and gelatin are not hydrolyzed. This organism was isolated from red beet (*Beta vulgaris* var. *rubra*), where it causes a vascular wilt and leaf spot.

DNA G+C content (mol%): 73.7 (T_m).

Type strain: DSM 20141, ATCC 13437, CFBP 2402, ICMP 2594, LMG 3596, NCPPB 374.

Sequence accession no. (16S rRNA gene): AF348974, AM410689.

4c. **Curtobacterium flaccumfaciens pv. beticola** Chen, Yin, Zhang and Guo 2007, 682

The description is as for the species, and diagnostic tests are listed in Table 150. Colonies are pale yellow with an entire margin, convex, and butyrous. Optimum growth occurs at 24–27°C, but not at 4°C or 37°C. No growth occurs at 10% NaCl with limited growth at 5%. Esculin, casein, starch, and Tween 80 are all hydrolyzed.

Source: sugar beet (*Beta vulgaris* var. *saccharifera*), where it causes bacterial leaf spot.

DNA G+C content (mol%): 67.5 (T_m).

Type strain: ATCC BAA-144.

Sequence accession no. (16S rRNA gene): AY273208.

4d. **Curtobacterium flaccumfaciens pv. ilicis** (Mandel et al. 1961) Young, Watson and Dye 2004b, 303 (*Corynebacterium ilicis* Mandel, Guba and Litsky 1961)

The description is as for the species, and diagnostic tests are listed in Table 150. Esculin is hydrolyzed; starch is not. This organism was isolated from and is a pathogen of American holly (*Ilex opaca*).

DNA G+C content (mol%): not available.

Type strain: ICMP 2608.

Sequence accession no. (16S rRNA gene): not available.

4e. **Curtobacterium flaccumfaciens pv. oortii** (Saaltink and Maas Geesteranus 1969) Collins and Jones 1983, 3547 (*Corynebacterium oortii* Saaltink and Maas Geesteranus 1969; *Corynebacterium flaccumfaciens* pv. *oortii* Dye and Kemp 1977; *Corynebacterium flaccumfaciens* subsp. *oortii* Carlson and Vidaver 1982)

The description is as for the species, and diagnostic tests are listed in Table 150. Colony color is yellow. Casein, esculin, and gelatin are hydrolyzed; starch, and Tween 80 give variable results. This organism was isolated from tulips (*Tulipa* species) where it causes a vascular disease and leaf and bulb spot.

DNA G+C content (mol%): 72.2 (T_m).

Type strain: ATCC 25283, CFBP 1384, ICMP 2632, LMG 3702, NCPPB 2113.

Sequence accession no. (16S rRNA gene): AF348975.

4f. **Curtobacterium flaccumfaciens pv. poinsettiae** (Starr and Pirone 1942) Collins and Jones 1983, 3547 (*Corynebacterium poinsettiae* Burkholder 1948; *Corynebacterium flaccumfaciens* pv. *poinsettiae* Dye and Kemp 1977; *Corynebacterium flaccumfaciens* subsp. *poinsettiae* Carlson and Vidaver 1982

The description is as for the species, and diagnostic tests are listed in Table 150. Colony color is orange. Esculin and Tween 80 are hydrolyzed; casein, gelatin, and starch give variable results.

Source: isolated from and is the cause of a stem canker and leaf spot of the poinsettia (*Euphorbia pulcherrima*).

DNA G+C content (mol%): 72.5 (T_m).

Type strain: ATCC 9682, CFBP 2403, ICMP 2566, LMG 3715, NCPPB 854.

Sequence accession no. (16S rRNA gene): AF348976, AM410687.

5. **Curtobacterium ginsengisoli** Kim, Kim, Kim, Kim, Yi and Yang 2008b, 2394[VP]

gin.sen.gi.so′li. N.L. n. *ginsengum* ginseng; L. n. *solum* soil; N.L. gen. n. *ginsengisoli* of soil of a ginseng field, the source of the type strain.

The description is as for the genus, and diagnostic tests are listed in Table 149. Colonies are pale yellow. Cells are nonmotile short rods. Optimum growth temperature is 30–37°C.

Source: soil of a ginseng field in South Korea.

DNA G+C content (mol%): 65.8 (HPLC).

Type strain: DCY26, JCM 14773, KCTC 13163.

Sequence accession no. (16S rRNA gene): EF587758.

6. **Curtobacterium herbarum** Behrendt, Ulrich, Schumann, Naumann and Suzuki 2002, 1452[VP]

her.ba′rum. L. gen. pl. fem. n. *herbarum* of plants.

The description is as for the genus, and diagnostic tests are listed in Table 149. Colonies are orange, shiny, slightly convex, and round with entire margins. Cells are motile. Esculin and Tween 80 are hydrolyzed; casein and gelatin give variable results; starch is not hydrolyzed. Optimum growth temperature is 25°C.

Source: the phyllosphere of grasses and from the litter layer after mulching the sward.

DNA G+C content (mol%): 71 (HPLC).

Type strain: P 420/07, DSM 14013, JCM 12140, LMG 19917, NBRC 103064.

Sequence accession no. (16S rRNA gene): AJ310413, AM410692.

7. **Curtobacterium luteum** (Komagata and Iizuka 1964) Yamada and Komagata 1972a, 425[AL] (*Brevibacterium luteum* Komagata and Iizuka 1964, 499)

lu′te.um. L. neut. adj. *luteum* saffron or golden yellow.

The description is as for the genus, and diagnostic tests are listed in Table 149.

Colonies on nutrient agar are dark yellow. Cells are motile. Tween 80 is hydrolyzed; casein, esculin, gelatin, and starch are not. Optimum pH for growth is 7.

Source: rice paddies.

DNA G+C content (mol%): 69.8–74.9 (T_m).

Type strain: ATCC 15830, CCUG 23848, CIP 102694, DSM 20542, IAM 1623, NBRC 12676, JCM 1480, LMG 8787, VKM B-1210.

Sequence accession no. (16S rRNA gene): X77437.

8. **Curtobacterium pusillum** (Iizuka and Komagata 1965) Yamada and Komagata 1972a, 425[AL]

(*Brevibacterium*, *pusillum* Iizuka and Komagata 1965, 2)

pu′sil.lum. L. neut. adj. *pusillum* very small.

The description is as for the genus, and diagnostic tests are listed in Table 149.

Colonies on nutrient agar are pale yellow. Cells are motile. Esculin and gelatin are hydrolyzed; casein, starch, and Tween 80 are not. The predominant cellular fatty acid is 11-cyclohexyl undecanoic acid (ch-$C_{17:0}$).

Source: oil brine.

DNA G+C content (mol%): 69.0–74.2 (T_m).

Type strain: ATCC 19096, CIP 81.24, DSM 20527, NBRC 12674, JCM 1350, LMG 8788, VKM Ac-2099.

Sequence accession no. (16S rRNA gene): AJ784400.

Genus VIII. **Frigoribacterium** Kämpfer, Rainey, Andersson, Nurmiaho Lassila, Ulrych, Busse, Weiss, Mikkola and Salkinoja-Salonen 2000, 362[VP]

Peter Kämpfer

Fri.go.ri.bac′te.ri.um. L. n. *frigor* frost, cold; L. neut. n. *bacterium* small rod; N.L. neut. n. *Frigoribacterium* a small rod growing in the cold.

Gram-stain-positive, nonsporeforming, motile, irregular-shaped rods, the **cell division zone being thicker than the poles of the cell**. No mycelium is produced. Catalase-positive. Oxidase-negative. Growth on complex media is observed in a temperature range of 2–25°C. Optimum 4–10°C. **The cell-wall peptidoglycan contains D-lysine as a diamino acid**. The **glycan moiety of the peptidoglycan contains acetyl residues**. The **major isoprenoid quinone is menaquinone MK-9**. Mycolic acids are not present. **Diphosphatidylglycerol, phosphatidylglycerol, and an unknown glycolipid** were detected in the polar lipid extracts. The main cellular fatty acids are $C_{15:0}$ anteiso, $C_{16:0}$ iso, and $C_{16:0}$).

In addition, **1,1 dimethoxy-anteiso-pentadecane ($C_{15:0}$ anteiso DMA = dimethylacetal) is produced in considerable amounts (10–30% of the amount of whole cell fatty substances).** Using standardized fatty acid methyl ester (FAME) analysis, the retention time of this compound is almost identical with that of $C_{14:0}$ 2-OH.

DNA G+C content (mol%): ~71.

Type species: **Frigoribacterium faeni** Kämpfer, Rainey, Andersson, Nurmiaho Lassila, Ulrych, Busse, Weiss, Mikkola and Salkinoja-Salonen 2000, 362[VP].

Further descriptive information

One species, *Frigoribacterium faeni*, has been described so far. Four isolates were obtained during an extensive study of bacteria collected from dust samples and animal sheds (Andersson et al., 1999). An additional strain was isolated from the air in the museum Sainsbury Centre for Visual Arts in Norwich, England. All these isolates showed initially good growth at 2–10°C but only moderate growth at 25°C.

Comparison of the almost complete 16S rRNA gene sequences of 1458–1467 nucleotides in length (which were determined for four isolates) and subsequent phylogenetic analyses based on a dataset comprising 1310 unambiguous nucleotides between positions 38 and 1478 (*Escherichia coli* positions; Brosius et al., 1978) showed that these isolates clustered together as a distinct lineage within the radiation of the actinomycete genera of the family *Microbacteriaceae* (Stackebrandt et al., 1997) (Figure 182). The five strains share 99.3–100% 16S rRNA gene sequence similarity. Highest 16S rRNA gene sequence similarity was shown with species of the genera *Frondicola* (98%), *Yonghaparkia* (97.0%), and *Microcella* (96.8%).

The chemotaxonomic features of *Frigoribacterium* are summarized in comparison to the other genera of the family *Microbacteriaceae* in Table 140. The amino acids found in the cell-wall hydrolysate were glycine, alanine, homoserine, and lysine (molar ratios of 1:1:1:1). The peptidoglycan belongs to the B2β group according to Schleifer and Kandler (1972). It is similar to that found in *Curtobacterium*, but D-lysine is detected instead of ornithine. This type of peptidoglycan is unique among the genera belonging to the B-peptidoglycan group.

Acid hydrolysates of bacterial cells revealed no glycolate, which suggested that muramic acid occurs in the *N*-acetyl form. The major isoprenoid quinone is MK-9. The polar lipid profile consists of diphosphatidylglycerol, phosphatidylglycerol, and an unknown glycolipid which is similar to that designated by Collins and Jones (1980) as glycolipid 2 (G_2). In addition, three unknown lipids were found which were not stained by any of the spray reagents, α-naphthol, molybdenum blue, or ninhydrin. The absence of additional glycolipids, which are found in strains of the phylogenetic neighbors *Clavibacter* and *Rathayibacter* (Collins and Jones, 1980), clearly distinguishes the genus from the representatives of these two genera. The predominant fatty acids were 12-methyltetradecanoic acid ($C_{15:0}$ anteiso), 14-methylpentadecanoic acid ($C_{16:0}$ iso), and hexadecanoic acid ($C_{16:0}$) obtained from cells grown near their maximum tolerable temperature, 28°C. At lower temperatures, a decrease of their contents of $C_{15:0}$ anteiso and (more pronounced) that of $C_{16:0}$ (to <4% at <4°C) was observed.

Fatty acid analysis by the MIDI system resulted in an identification of one peak in all strains as the hydroxylated fatty acid $C_{14:0}$ 2-OH. This peak was found in relatively high amounts (10–26%) of the fatty compounds. Detailed mass spectrometric investigations revealed that this component was 1,1-dimethoxy-12-methyl pentadecane ($C_{15:0}$ anteiso DMA). In spite of the great similarity between the strains in the physiological and chemotaxonomic properties, DNA–DNA reassociation studies showed that the levels of hybridization rates between strain 801 DNA and the DNAs of strains 227 and 301 were 37 and 44%, respectively, and the level of hybridization rate between strain 227 DNA and the 301 DNA was 52%. These data indicate that the strains are similar genomovars of one species.

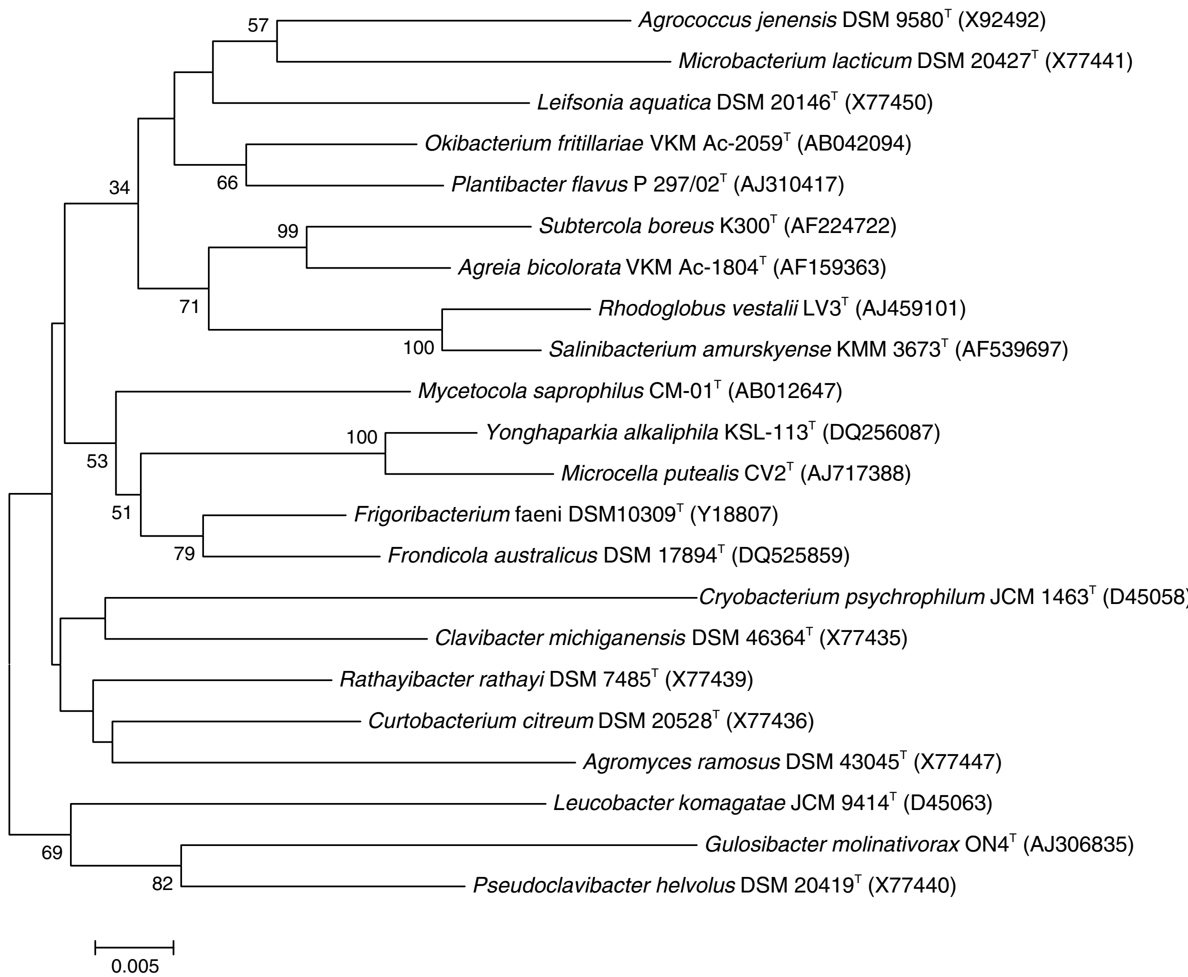

FIGURE 182. Phylogenetic analysis of *Frigoribacterium* and related genera based on 16S rRNA gene sequences available from the European Molecular Biology Laboratory data library (accession numbers are given in brackets) constructed after multiple alignments of data. Distances (distance options according to the Kimura-2 model) and clustering with the neighbor joining method was performed by using the software package MEGA (Molecular Evolutionary Genetics Analysis) version 2.1. Bootstrap values based on 1000 replications are listed as percentages at the branching points. Bar = 0.005 nucleotide substitutions per nucleotide position.

Enrichment and isolation procedures

No specific isolation medium has been described so far. Good growth occurs on complex media like PYES medium (Altenburger et al., 1996) as well as on nutrient agar and TS agar (both BBL Microbiology Systems) at 4–10°C. Growth is observed at 2–25°C.

Maintenance procedures

Frigoribacterium cultures may be lyophilized by common procedures used for many bacteria. In addition, cultures can be maintained by serial transfers on solid complex media. Growth on agar slants in screw-capped tubes can be kept at 4°C for about 2–4 weeks. Long-term preservation of liquid cultures supplemented with 5% dimethylsulfoxide is recommended in the vapor phase of liquid nitrogen (−140°C).

Differentiation of the genus *Frigoribacterium* from other genera

Frigoribacterium may be distinguished from other genera in the family *Microbacteriaceae* by cell-wall peptidoglycan, the menaquinone type, the fatty acid composition, the colony pigmentation, the G+C content, and the growth at low temperatures (Table 140).

List of species of the genus *Frigoribacterium*

1. **Frigoribacterium faeni** Kämpfer, Rainey, Andersson, Nurmiaho Lassila, Ulrych, Busse, Weiss, Mikkola and Salkinoja-Salonen 2000, 362[VP]

fae′ni. L. n. *faenum* hay; L. gen. n. *faeni* of hay.

Cells are Gram-stain-positive, irregular rods that are 0.2–0.3 μm × 1.0–1.5 μm. The cells are nonsporeforming and motile in 2-d-old cultures on nutrient agar. No mycelium is produced. The morphology of the species and its chemotaxonomic characteristics are the same as described for the genus. Aerobic. Good carbon sources are sugars including *N*-acetyl-D-glucosamine, L-arabinose, *p*-arbutin, D-cellobiose, D-fructose, D-galactose, gluconate, D-glucose, D-mannose, D-maltose, L-rhamnose, D-ribose, sucrose, D-trehalose, and D-xylose. Only few organic acids were utilized as sole source of carbon (citrate, fumarate). Details of other physiological properties are given in Table 151.

Because of the DNA–DNA similarity data, the genus contains at least two further genomovars. The reference strain for genomovar 2 is 227 (=DSM 10310), the reference strain for genomovar 3 is 301 (= DSM 10311). Details of the physiological chemotaxonomic and properties of representatives of these genomovars are given in Table 151.

Source: hay dust.

DNA G+C content (mol%): 71 (HPLC).

Type strain: 801, ATCC BAA-3, DSM 10309, JCM 11265, NBRC 103066.

Sequence accession no. (16S rRNA gene): AM410686, Y18807.

TABLE 151. Physiological characteristics of *Frigoribacterium* strains[a,b]

Characteristic	801[T]	227	301	312	NS 4
Acid produced from:[c]					
Adonitol	(+)	–	–	–	–
Glucose	(+)	–	–	–	–
Assimilation of:					
Adonitol	+	+	–	–	–
cis-Aconitate	–	(+)	(+)	(+)	–
L-Aspartate	+	(+)	+	–	(+)
Citrate	+	+	+	–	–
Fumarate	+	+	+	–	(+)
i-Inositol	–	+	+	+	+
L-Ornithine	+	(+)	+	–	–
L-Proline	(+)	(+)	(+)	–	–
Putrescine	–	+	–	–	–
L-Rhamnose	+	+	–	+	–
D-Sorbitol	+	+	+	–	–

[a]Symbols: +, positive; –, negative; (+) weak positive; test results given in the table were read after 72 h of incubation at 20°C.

[b]Data from Kämpfer et al. (2000).

[c]Acid formation from the carbohydrates lactose, sucrose, D-mannitol, dulcitol, salicin, inositol, sorbitol, L-arabinose, raffinose, rhamnose, maltose, D-xylose, trehalose, cellobiose, methyl-D-glucoside, erythritol, melibiose, D-arabitol, and D-mannose was negative for all strains. All strains utilized the following substrates as sole source of carbon: *N*-acetylglucosamine, L-arabinose, *p*-arbutin, D-cellobiose, D-fructose, D-galactose, gluconate, D-glucose, D-mannose, D-maltose, α-D-melibiose (weak), D-ribose, sucrose, salicin, D-trehalose, D-xylose, maltitol, and D-mannitol. All strains were negative for the utilization of acetate, propionate, *trans*-aconitate, adipae, 4-aminobutyrate, azelate, glutarate, 3-hydroxybutyrate, itaconate, DL-lactate, L-malate, mesaconate, oxoglutarate, pyruvate, suberate, L-alanine, β-alanine, L-histidine, L-leucine, L-phenylalanine, L-serine, L-tryptophan, 3-hydroxybenzoate, 4-hydroxybenzoate, and phenylacetate. All strains were positive for hydrolysis of esculin, pNP-β-D-galactopyranoside (weak), pNP-β-D-glucopyranoside, pNP-α-D-glucopyranoside, pNP-β-D-xylopyranoside, L-alanine-pNA, and L-proline-pNA. None of the strains hydrolyzed pNP-β-D-glucuronide, bis-pNP-phosphonate, *p*NP-phosphoryl-choline, 2-deoxythymidine-5′- pNP phosphate, and L-glutamate-γ-3-carboxy-pNA (pNP, *para*-nitrophenyl; pNA, *para*-nitroanilide).

Genus IX. **Frondihabitans** Greene, Euzéby, Tindall and Patel 2009, 448[VP]

Anthony C. Greene and Bharat K. C. Patel

Fron.di.ha′bi.tans. L. n. *frons*, *frondis* a leaf, foliage; L. part. adj. *habitans* inhabiting; N.L. part. adj. used as a masc. n. *Frondihabitans* inhabitant of leaves, leaf dweller.

Gram-stain-positive **short irregular-shaped rods.** Nonsporeforming. No mycelium is produced. **Aerobic. Mesophilic**. Temperature range for growth **15–37°C**. **Catalase-positive** and oxidase-negative. Cells are nonmotile with no flagella. A range of carbohydrates, organic acids, and amino acids are metabolized. The cell-wall peptidoglycan **type is B2β and contains L-ornithine. The major cellular fatty acid is $C_{18:1}$ ω7*c*, and menaquinones MK-7 and MK-8** are present. Glycolipids, disphosphatidylglycerol, phosphatidylglycerol, and three unidentified phospholipids are present.

DNA G+C content (mol%): 71–72.

Type species: **Frondihabitans australicus** Greene, Euzéby, Tindall and Patel 2009, 448[VP].

Further descriptive information

Phylogenetic analysis (Figure 183) places *Frondihabitans* (formerly *Frondicola* Zhang et al. 2007c, which is illegitimate because it is a later homonym of a fungal genus name *Frondicola* Hyde 1992) most closely to the psychrophilic genus *Frigoribacterium* in the phylum *Actinobacteria*, class *Actinobacteria*, order *Micrococcales*, and family *Microbacteriaceae*. *Frondihabitans* has a number of significant chemotaxonomic differences from *Frigoribacterium*, particularly in the cell wall and cell membranes and the type of substrates metabolized (Table 152). Furthermore, *Frondihabitans* is unable to grow at psychrophilic temperatures and has a higher optimum pH for growth than *Frigoribacterium*.

Frondihabitans australicus possesses traits that are atypical and have so far not been reported for any of the genera within the family *Microbacteriaceae* These include a high concentration of the mono-unsaturated fatty acid $C_{18:1}$ ω7*c* (78%), cell-wall type B2β peptidoglycan, MK-8 and MK-7 as the major menaquinones, the glycolipids disphosphatidylglycerol, phosphatidylglycerol, and three unidentified phospholipids (Table 152).

Enrichment and isolation procedures

Frondihabitans was isolated from moist leaf litter. It has not been established if this is the only habitat. Isolation can be accomplished by mixing a leaf litter sample with sterile distilled water, serially diluting ten-fold, and spreading aliquots on Trypticase Soy Broth (TSB)-Gelrite plates*. After 6 d incubation at 25°C,

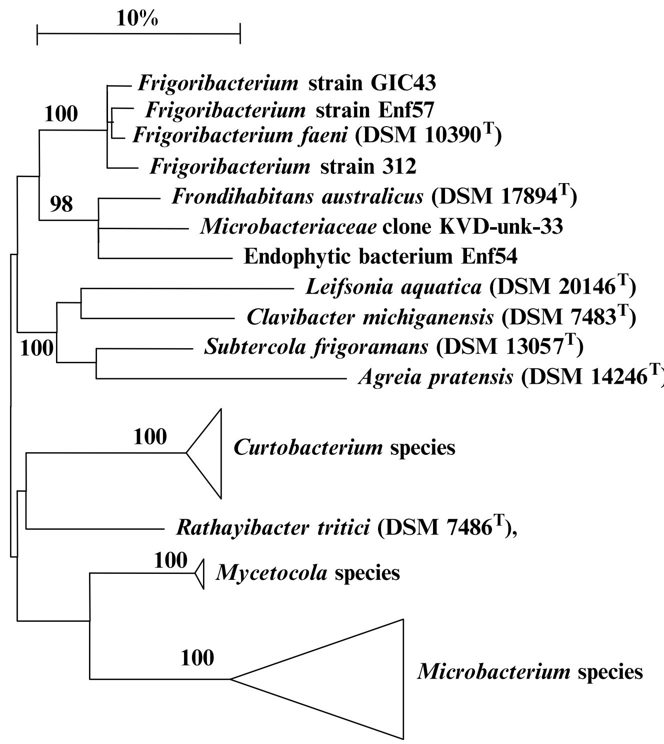

FIGURE 183. Phylogenetic tree showing the position of *Frondihabitans australicus* (DSM 17894[T]) within the family *Microbacteriaceae*. Clusters of members of the same genus are represented as *triangles* and include *Mycetocola saprophilus* (DSM 15178[T]) and *Mycetocola tolaasinivorans* (DSM 15179[T]) for the genus *Mycetocola*, *Microbacterium imperiale* (DSM 20530[T]), *Microbacterium phyllosphaerae* (DSM 13468[T]), *Microbacterium liquefaciens* (DSM 20638[T]), and *Microbacterium thalassium* (DSM 12511[T]) for the genus *Microbacterium*, and *Curtobacterium citreum* (DSM 20528[T]) and *Curtobacterium flaccumfaciens* (LMG 3645[T]) for the genus *Curtobacterium*. The following abbreviations have been used: T, Type culture; DSM, Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH; LMG, Collection of the Laboratorium voor Microbiologie en Microbiele Genetica. Bootstrap values above 95% from 1000 replicates are shown. Bar = 10 nucleotide changes per 100 nucleotides.

cream-colored, round, smooth colonies are picked and individually restreaked onto fresh TSB-Gelrite plates. The purity of the isolate is checked microscopically. Colonies from pure cultures were resuspended in sterile preservation medium (TSB, 0.3 g; glycerol, 15 ml; distilled water, 85 ml) and stored at −80°C.

*(TSB)-Gelrite plates contain (per liter distilled H_2O): TSB, 3 g; $MgCl_2$×$6H_2O$, 2.033 g; $CaCl_2$×$2H_2O$, 0.882 g; Gelrite, 20 g. After pH is adjusted to 7.2, medium is autoclaved and poured into Petri dishes.

TABLE 152. Characteristics differentiating genera of the family *Microbacteriaceae*[a]

Characteristic	*Frondihabitans australicus*	*Agreia*	*Agrococcus*	*Agromyces*	*Frigoribacterium*	*Clavibacter*	*Cryobacterium*	*Curtobacterium*	*Leifsonia*	*Leucobacter*	*Microbacterium*	*Mycetola*	*Okibacterium*	*Plantibacterium*	*Rathayibacter*	*Rhodoglobus*	*Subtercola*	*Zimmermannella*
Colony color[a]	W	Y, O	W, Y, O	Y	Y	W, Y, O, P, BL	nr	Y, O, W, P	Y, W	B	W, O	YW	Y	Y	nr	R	Y	W
Growth temperature range (°C)	15–37	Up to 37	Up to 37	Up to 37	0–28	Up to 35	<81	Up to 35	7–37	Up to 37	10–40	>33–4	7–37	nr	<73	-2–21	-2–28	nr
Growth optimum (°C)	25	24–26	28	26–30	4–10	21–26	9–12	24–27	24–28	28	30	25	24–27	25	24–28	18	15–17	30
DNA G+C (mol%)	71±1	67	74	70–72	72	67–78	65	68–75	66–73	66	65–75	64–65	67	68–70	63–72	62	64–68	62–68
Diamino acid[b]	Orn	DAB/ Orn	DAB	DAB	Lys	DAB	DAB	Orn	DAB	DAB	Lys/Orn	Lys	Lys	DAB	DAB	Orn	DAB	DAB
Major fatty acids (≥ 5%)[c]	$C_{18:1}$ ω7*c*, $C_{14:0}$ 2-OH	$C_{15:0}$ ante, $C_{16:0}$ iso, $C_{17:0}$ ante	$C_{15:0}$ ante, $C_{16:0}$ iso, a-$C_{17:0}$ ante, $C_{15:1}$ ante,	ante, iso,	$C_{15:0}$ ante, $C_{16:0}$ iso, $C_{16:0}$, $C_{15:1}$ ante	$C_{15:0}$ ante, $C_{16:0}$ iso, $C_{17:0}$ ante	$C_{15:0}$ ante, $C_{16:0}$ iso, $C_{17:0}$ ante, $C_{15:0}$ iso, $C_{15:1}$ ante	$C_{15:0}$ ante, $C_{16:0}$ iso, $C_{17:0}$ ante, $C_{15:1}$ ante, ch_{17}	$C_{15:0}$ ante, $C_{16:0}$ iso, $C_{17:0}$ ante	$C_{15:0}$ ante, $C_{16:0}$ iso, $C_{17:0}$ ante	$C_{15:0}$ ante, $C_{16:0}$ iso, $C_{17:0}$ ante, $C_{15:0}$ iso	$C_{15:0}$ ante, $C_{16:0}$ iso, $C_{17:0}$ ante	$C_{15:0}$ ante, $C_{16:0}$ iso, $C_{17:0}$ ante, $C_{15:0}$ iso	$C_{15:0}$ ante, $C_{16:0}$ iso, $C_{17:0}$ ante, $C_{15:0}$ iso	$C_{15:0}$ ante, $C_{16:0}$ iso, $C_{17:0}$ ante	$C_{15:0}$ ante, $C_{16:0}$ iso, $C_{17:0}$ ante, $C_{15:1}$ ante	$C_{15:0}$ ante, $C_{16:0}$ iso, $C_{17:0}$ ante, $C_{14:0}$ iso, $C_{15:1}$ ante	$C_{15:0}$ ante, $C_{16:0}$ iso, $C_{16:0}$, $C_{17:0}$ ante
Polar lipids[d]	PG, DPG, GL, PL	PG, DPG	PG, GL	PG, GL	PG, DPG	PG, GL	PG, GL	PG, GL	PG, DPG	PG, GL, DPG	PG, GL	nr	PG, DPG	nr	PG, GL	nr	PG, GL, DPG	nr
Major MK	7, 8	10	11, 12	12, 13	9	9, 10	10	9	10, 11	11	11, 12, 13, 14	9, 10	11, 12	10, 11	10	11, 12	9, 10	8, 9, 10

[a]R, Red; W, white; Y, yellow; O, orange; P, pink; BL, blue; B, brown; YW, yellowish-white.

[b]Orn, Ornithine; Lys, lysine; DAB, diaminobutyric acid.

[c]ch, ω-Cyclohexyl acid. Except for *Agrococcus jenensis* ST54, mono-unsatured 12-methyle-tetradecenoic acid ($C_{15:1}$ ante) represents 5% or more of the total only when the strains were grown at lower temperatures (EMBED Equation 3 10·C).

[d]PG, phosphatidylglycerol; DPG disphosphatidylglycerol; GL, Glycolipids; PL unidentified phospholipids; nr, not reported.

List of species of the genus *Frondihabitans*

1. **Frondihabitans australicus** Greene, Euzéby, Tindall and Patel 2009, 448[VP]

aus.tra′ li.cus. N.L. masc. adj. *australicus* of or pertaining to Australia.

Gram-stain-positive short irregular rods 0.5–1.0 × 0.2–0.4 μm. Nonsporeforming. Motility is not evident. Aerobic. Mesophilic. Optimum temperature 25°C (range 15–37°C); pH 9.1 (range pH 6–9.5). Dextrin, Tween 40, Tween 80, maltose, D-melibiose, α-D-glucose, sucrose, D-trehalose, turanose, succinic acid mono-methyl-ester, α-ketobutyric acid, α-ketoglutaric acid, α-ketovaleric acid, DL-lactic acid, L-glutamic acid, L-pyroglutamic acid, and urocanic acid are metabolized. α-Cyclodextrin, glycogen, *N*-acetyl-D-galactosamine, *N*-acetyl-D-glucosamine, adonitol, L-arbinose, D-arbitol, D-cellobiose, *i*-Erythritol, D-fructose, L-fucose, D-galactose, gentiobiose, *m*-inositol, α-D-lactose, lactulose, D-mannitol, D-mannose, β-methyl-D-glucoside, D-psicose, D-raffinose, L-rhamnose, D-sorbitol, xylitol, pyruvic acid methyl ester, acetic acid, *cis*-aconitic acid, citric acid, formic acid, D-galactonic acid lactone, D-galacturonic acid, D-gluconic acid, D-glucosaminic acid, D-glucuronic acid, α-hydroxybutyric acid, β-hydroxybutyric acid, γ-hydroxybutyric acid, *p*-hydroxyphenylacetic acid, itaconic acid, malonic acid, propionic acid, quinic acid, D-saccharic acid, sebacic acid, succinamic acid, glucuronamide, L-alaninamide, L-alanine, D-alanine, L-alanyl-glycine, L-asparagine, L-aspartic acid, glycyl-L-glutamic acid, L-histidine, hydroxy-L-proline, L-leucine, L-ornithine, L-phenylalanine, L-proline, L-serine, D-serine, L-threonine, DL-carnitine, γ-amino butyric acid, inosine uridine, thymidine, phenyethylamine, 2-aminoethanol, 2,3-butanediol, glycerol, DL-α-glycerol phosphate, α-D-glucose 1-phosphate, and D-glucose 6-phosphate are not metabolized when using the Biolog system (GN2 microplates). The type B2β {Gly} [L-Hsr] D-Glu–D-Orn peptidoglycan contains the amino acids ornithine, alanine, glycine, homoserine, and glutamic acid in a molar ratio of 1.0: 0.9: 1.7: 0.5: 1.0, respectively. The major cellular fatty acids are mono-unsaturated acids $C_{18:1}$ ω7*c* (78%) and $C_{17:1}$ ω9*c* (4%) acids; others include $C_{14:0}$ 2-OH (6%) and $C_{14:0}$ iso 3-OH (3%) acids, saturated $C_{16:0}$ (2%) acids, and saturated branched $C_{15:0}$ iso, $C_{15:0}$ anteiso, and $C_{16:0}$ iso (all 1%) acids. Major menaquinones detected are MK-8 (76%) and MK-7 (24%).

Source: decaying leaf litter of a slash pine forest located in southeast Queensland, Australia.

DNA G+C content (mol%): 71±1 (T_m).

Type strain: E1HC-02, DSM 17894, JCM 13598.

Sequence accession no. (16S rRNA gene): DQ525859.

Genus X. **Gulosibacter** Manaia, Nogales, Weiss and Nunes 2004, 786[VP]

Célia M. Manaia, Balbina Nogales and Olga C. Nunes

Gu.lo.si.bac′ter. L. adj. *gulosus* gluttonous, luxurious, dainty, fond of tidbits; N.L. masc. n. *bacter* rod; N.L. masc. n. *Gulosibacter* rod fond of tidbits.

Irregular rod-shaped cells with tendency to form short filaments and branching. Gram-stain-positive. Nonsporeforming. **Nonmotile**. **Obligately aerobic**. Mesophilic. Catalase and oxidase-positive. **Chemo-organotrophic**. Few organic compounds, including amines and nitrogen bases, are used as sole source of carbon and energy. **The diagnostic diamino acid of the peptidoglycan is D-Orn. The major respiratory quinone is MK-9**. The polar lipid pattern is composed of diphosphatidylglycerol, phosphatidylglycerol, and minor amounts of an unknown glycolipid. Major cellular fatty acids are 12-methyl-tetradecanoic acid ($C_{15:0}$ anteiso), 14-methyl-pentadecanoic acid ($C_{16:0}$ iso), and 14-methyl-hexadecanoic acid ($C_{17:0}$ anteiso); $C_{15:0}$ anteiso and $C_{16:0}$ iso are predominant.

DNA G+C content (mol%): 65 (HPLC).

Type species: **Gulosibacter molinativorax** Manaia, Nogales, Weiss and Nunes 2004, 787[VP].

Further descriptive information

Gulosibacter molinativorax is the only species of the genus and is represented by a single strain, *Gulosibacter molinativorax* ON4^T (Manaia et al., 2004). Phylogenetic analysis based on the 16S rRNA gene sequence demonstrates that *Gulosibacter* forms, with *Pseudoclavibacter*, a distinct lineage branching deeply within the family *Microbacteriaceae* (Figure 184). Based on the 16S rRNA gene sequence analysis, the closest relatives of *Gulosibacter molinativorax* ON4^T are members of the genera *Zimmermannella*, *Pseudoclavibacter*, and *Curtobacterium*. *Gulosibacter molinativorax* ON4^T exhibits highest 16S rRNA gene sequence similarities to the type strains of *Zimmermannella bifida* and *Zimmermannella faecalis* (95.7–96.1%) and lower similarity values (~95%) to *Pseudoclavibacter helvolus* (94.6%), *Zimmernannella alba* (94.4%), and *Curtobacterium flaccumfaciens* pv. flaccumfaciens (94.1%). However, the genus *Zimmermannella* (Lin et al., 2004) has to be considered a later homotypic synonym of the genus *Pseudoclavibacter* (Euzéby, 2005) because the type strains of the type species of the genera *Pseudoclavibacter* and *Zimmermannella* are identical, and the validation of the genus *Zimmermannella* was published later than that of *Pseudoclavibacter*.

Gulosibacter molinativorax cells are irregularly rod-shaped, with tendency to branch and to form short filaments (Figure 185 and Figure 186). The cells stain Gram-positive, are non-acid-fast, nonsporeforming, nonmotile, with no visible deposits of poly-beta-hydroxybutyrate (PHB).

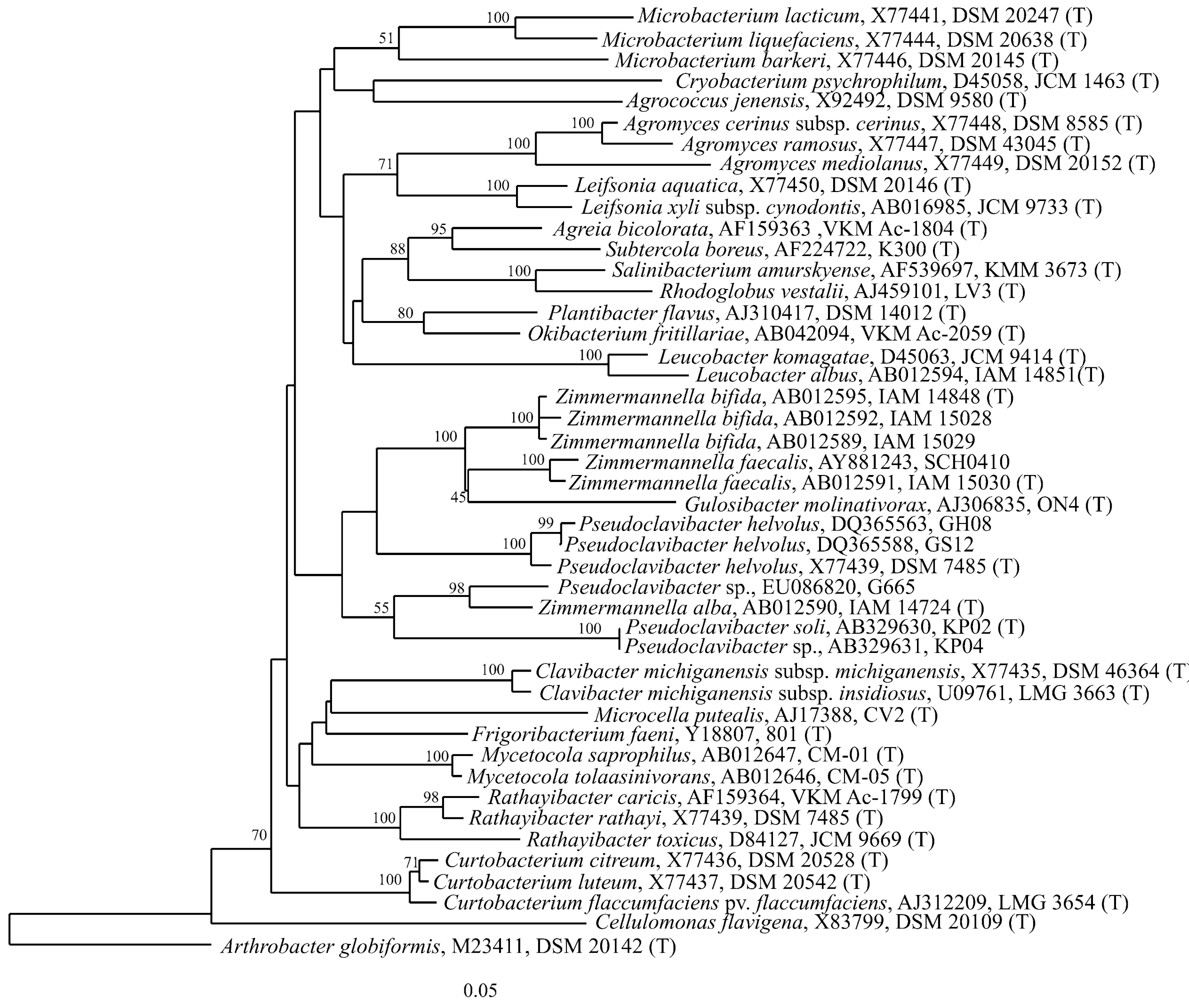

FIGURE 184. Phylogenetic relationships of the 16S rRNA gene sequences of the genera *Gulosibacter* and *Pseudoclavibacter* with sequences of species of related genera within the family *Microbacteriaceae*. Evolutionary distance matrices were calculated using the correction of Jukes and Cantor (1969). The dendrogram was constructed with the neighbor joining algorithm (Saitou and Nei, 1987), using a 50% homology conservation filter. The total number of positions used was 1330 nucleotides. Bootstrap values higher than 50% are indicated in the tree (1000 replicates). The 16S rRNA gene sequence of *Arthrobacter globiformis* DSM 20124^T was used as the outgroup.

The cell-wall peptidoglycan of *Gulosibacter* is of type B, with the diagnostic diamino acid D-ornithine. The major respiratory quinone of *Gulosibacter* is a menaquinone with nine isoprene units (MK-9), with a minor proportion of MK-8. The polar lipid pattern of *Gulosibacter* is composed of diphosphatidylglycerol, phosphatidylglycerol, and minor amounts of an unknown glycolipid. Major cellular fatty acids are 12-methyl-tetradecanoic acid ($C_{15:0}$ anteiso), 14-methyl-pentadecanoic acid ($C_{16:0}$ iso), and 14-methyl-hexadecanoic acid ($C_{17:0}$ anteiso) with $C_{15:0}$ anteiso and $C_{16:0}$ iso predominating. The polyamine pattern of *Gulosibacter* contains spermine and spermidine as predominant compounds (4.4 and 1.1 µmol/g dry wt, respectively) while 1,3-diaminopropane, putrescine, and cadaverine are present in very low amounts (0.2-< 0.1 µmol/g dry wt) (Busse, unpublished results). This polyamine pattern is quite similar to those found in species of *Clavibacter*, *Rathayibacter*, and *Curtobacterium* but, like in *Clavibacter* and *Rathayibacter*, the overall polyamine content is significantly higher than in species of the latter genus (Altenburger et al., 1997).

On complex agar media such as Luria–Bertani agar or Plate Count Agar, *Gulosibacter* forms opaque, white, circular, and convex colonies, 1.0 mm in diameter after 2–3 d of growth.

Gulosibacter is strictly aerobic and chemoorganothrophic. Nitrate is reduced under aerobic or anaerobic conditions, although growth does not occur without oxygen. In complex medium, growth occurs at 10–41°C, pH 5.5–10.5, and 0–7% (w/v) NaCl. *Gulosibacter molinativorax* ON4^T presents a restricted nutritional profile, assimilating few compounds as the only source of carbon and energy. Substrates that support growth include nitrogenated compounds. The Biolog and API 50CH profiles of *Gulosibacter molinativorax* ON4^T revealed that putrescine, L-glutamic acid, nitrogenated bases, 2,3-butanediol, pyruvic acid, methyl pyruvate, and *p*-hydrophenylacetic acid are assimilated by this strain, although Biolog does not directly examine assimilation but

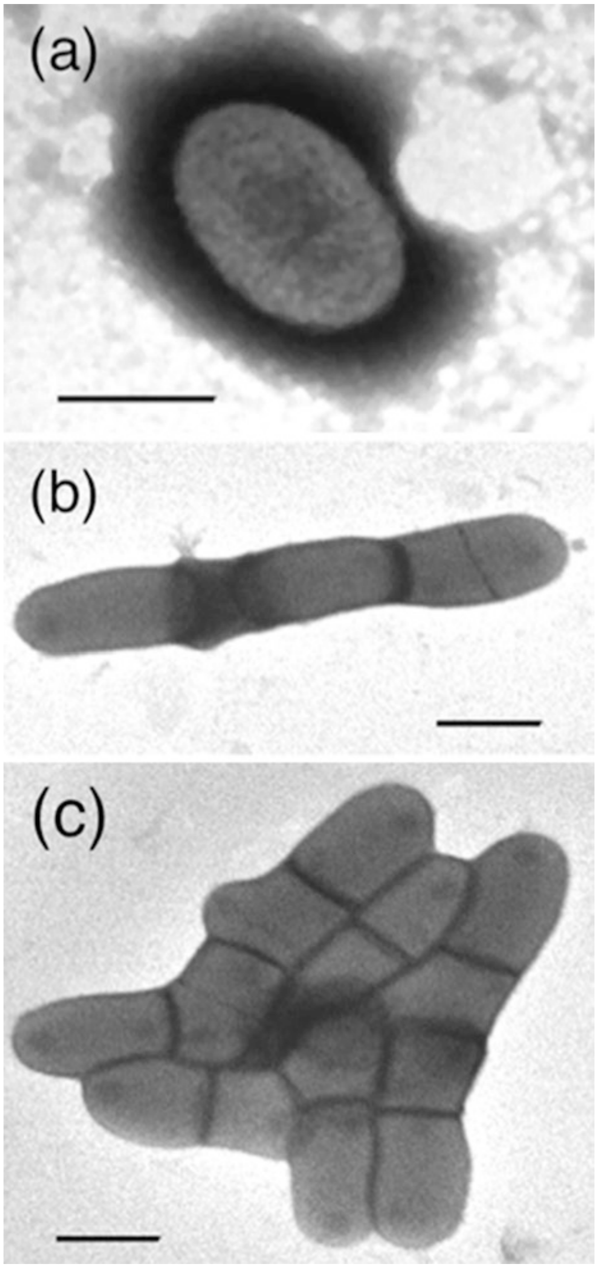

FIGURE 185. Transmission electron micrographs of negatively stained cells of *Gulosibacter molinativorax* ON4^T (a–c) grown on LB medium at 30°C for 24 h. Bar = 0.5 µm. (Reprinted with permission from Manaia et al., 2004. Int. J. Syst. Evol. Microbiol. 54: 783–789; supplementary figure in IJSEM Online.)

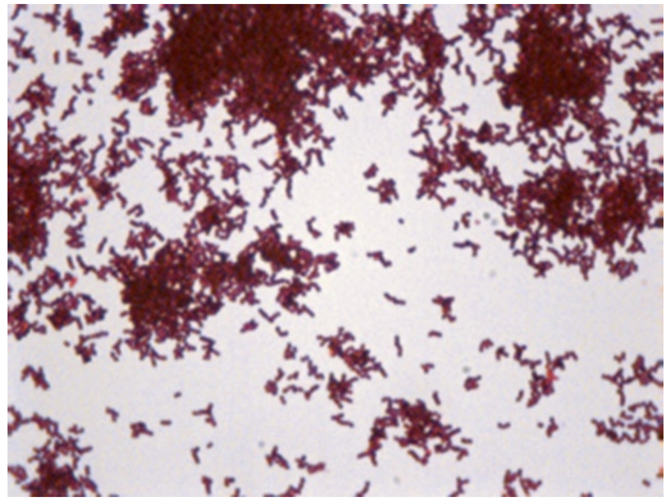

FIGURE 186. Gram-stained smear from a 3-d-old culture of *Gulosibacter molinativorax* ON4^T on Luria–Bertani agar medium at 30°C. Bright field (1000×). (Printed with permission from O.C. Nunes.)

oxidation of the carbon source tested. This microorganism is capable of growth on mineral medium with 2 mM molinate (azepan-1-yl-ethylsulfanyl-methanone), a thiocarbamate herbicide, as the sole source of carbon, energy, and nitrogen (Barreiros et al., 2003). However, other thiocarbamate herbicides such as EPTC (1-ethylsulfanyl-*N*,*N*-dipropyl-formamide), thiobencarb (1-[(4-chlorophenyl)methylsulfanyl]-*N*,*N*-diethyl-methanamide), cycloate (*N*-cyclohexyl-*N*-ethyl-1-ethylsulfanyl-methanamide), and vernolate (*N*,*N*-dipropyl-1-propylsulfanyl-methanamide) are not utilized nor degraded by *Gulosibacter molinativorax* ON4^T. This organism is responsible for the breakdown of the thioester bond of molinate, releasing ethanethiol, which accumulates in the medium, and an azepane moiety derivative of molinate (azepane-1-carboxylic acid) that support its growth (Barreiros et al., 2008). Due to the toxicity of the ethanethiol accumulated in the medium, no growth occurs at molinate concentrations above 3 mM. This toxic effect can be avoided when *Gulosibacter molinativorax* ON4^T grows in a defined mixed culture containing microorganisms co-isolated with strain ON4^T (Barreiros et al., 2003). *Gulosibacter molinativorax* ON4^T produces catalase and cytochrome *c* oxidase. The API ZYM profile shows that this organism also produces acidic and alkaline phosphatase, esterase and lipase (C_4, C_8 and C_{14}), leucine-, valine-, and cystine-arylamidase, and naphthol-AS-BI-phosphohydrolase.

Gulosibacter molinativorax ON4^T is affiliated with the family *Microbacteriaceae* according to phylogenetic analysis based on 16S rRNA gene sequence, constituting a deeply branching group together with the sequences of *Pseudoclavibacter* and *Zimmermannella* species. Irrespective of the treeing method used, and supported by high bootstrap values, the 16S rRNA gene sequence of *Gulosibacter molinativorax* forms a subgroup with *Zimmermannella faecalis* and *Zimmermannella bifida*, and more distant from *Pseudoclavibacter helvolus* and *Zimmermannella alba* (Figure 184). However, *Gulosibacter molinativorax* exhibits a distinctive phenotype including chemotaxonomic characteristics that support its status as a separated genus.

Gulosibacter molinativorax ON4^T was isolated from a microbial mixed culture enriched in molinate. The enrichment culture was obtained from a mixture of soil and water collected at a site where a molinate-containing effluent of a Portuguese pesticide-producing industry was discharged for several years. Several attempts were made to isolate other strains from pesticide contaminated soils (e.g. rice fields). However, *Gulosibacter molinativorax* ON4^T is still the only strain so far isolated and characterized, suggesting that it may be present in low numbers or be a slow grower in such habitats.

TABLE 153. Characteristics differentiating the genus *Gulosibacter* from closely related genera of the family *Microbacteriaceae*[a]

Characteristic	*Gulosibacter*	*Clavibacter*	*Curtobacterium*	*Pseudoclavibacter*[b]
Optimal growth temperature (°C)[c]	35–37	21–26	25–37	30
Oxidase test[c]	+	–	D	+
Utilization of:[c]				
m-Inositol	–	D	D	w
α-D-Lactose	–	D	+	+
D-Maltose	–	D	D	+
D-Melezitose	–	–	+	–
D-Melibiose	–	–	+	–
Molinate	+	na	na	–
D-Raffinose	–	–	–	+
D-Ribose	–	D	D	–
Tween 80	–	–	D	+
Diamino acid of peptidoglycan[c]	Orn	DAB	Orn	DAB
Isoprene units of major menaquinone[c]	9	9, 10	9	9
DNA G+C content (mol%)[c]	65	67–78	68–75	67
Major polyamines[d]	SPD, SPM[e]	SPD, SPM	SPD, SPM	CAD, DAP

[a]Symbols: +, positive result; –, negative result. na, no data available; w, weak; D, different reactions in different species of the genus; DAB, diaminobutyric acid; Orn, ornithine; DAP, 1,3-diaminopropane; CAD, cadaverine; SPD, spermidine; SPM, spermine.

[b]Characteristics for type strain of the type species of *Pseudoclavibacter* (*Pseudoclavibacter helvolus*).

[c]Data from Collins and Bradbury (1992), Sasaki et al. (1998), Barreiros et al. (2003), Manaia et al. (2004), Aizawa et al. (2007), and Kim et al. (2008b).

[d]Data from Altenburger et al. (1997).

[e]H.-J. Busse, unpublished results.

Enrichment and isolation procedures

Although *Gulosibacter molinativorax* ON4[T] grows well in nutritive medium, isolation from environmental samples seems to be difficult. Originally, it was purified from an enrichment culture of a mixture of soil and water contaminated with pesticides. For the enrichment, the sample (10%, v/v) was initially incubated in mineral medium with molinate as carbon and energy source and ammonium sulfate and potassium nitrate as additional nitrogen sources*. After ten successive transfers at 8-d intervals to fresh medium, it was cultivated in a different mineral medium with molinate as the only source of carbon, energy and nitrogen†. The mixed bacterial culture, obtained after three successive transfers in this medium, presented five different morphotypes which were further identified as members of the genera *Pseudomonas* (two isolates), *Achromobacter*, and *Stenotrophomonas*, and the species *Gulosibacter molinativorax* (Barreiros et al., 2003).

Maintenance procedures

The bacteria can be maintained in the laboratory by subculturing on nutritive medium (e.g. Luria–Bertani Agar, Plate Count Agar) or on the same medium supplemented with 1 mM molinate, after 8–10 d at 4°C. Deep freezing (–75°C) is recommended for long-term preservation in 15% glycerol and nutrient broth.

Procedures for testing special characters

Gulosibacter molinativorax ON4[T] is the single organism described as being able to grow and to degrade the thiocarbamate herbicide molinate in complex or in mineral medium with the accumulation of ethanethiol. Molinate depletion may be followed by high performance liquid chromatography (HPLC)‡ (Barreiros et al., 2003).

Differentiation of the genus *Gulosibacter* from other genera

Table 153 shows the characteristics useful to distinguish *Gulosibacter* from other closely related genera of the family *Microbactereaceae*. The major phenotypic and chemotaxonomic characteristics that distinguish the genera within this family include the diagnostic diamino acid of the peptidoglycan and the isoprene chain length of the respiratory menaquinones (Behrendt et al., 2002; Collins and Bradbury, 1992). *Gulosibacter* shares these characteristics with *Curtobacterium*, but can be differentiated from this genus by a lower G+C content, a higher growth temperature, a positive oxidase reaction, and the inability to use several sugars as sole source of carbon. This phenetic differentiation is supported by the 16S rRNA gene sequence based phylogenetic relationship between both genera.

*Mineral medium with 2 mM molinate as carbon and energy source, $(NH_4)_2SO_4$ (3.8 mM), KNO_3 (1.02 mM), $NaNO_3$ (8.21 mM), Na_2HPO_4 (6.0 mM), KH_2PO_4 (4.0 mM), $CaCl_2 \times H_2O$ (0.47 mM), NaCl (0.14 mM), $MgSO_4 \times 7H_2O$ (0.41 mM), nitrilotriacetate (0.52 mM), $FeSO_4 \times 7H_2O$ (2 mg/l), $ZnSO_4 \times 7H_2O$ (0.1 mg/l), $MnSO_4 \times H_2O$ (0.03 mg/l), H_3BO_3 (0.3 mg/l), $CoSO_4 \cdot 7H_2O$ (0.24 mg/l), $CuSO_4 \cdot 5H_2O$ (0.01 mg/l), $NiSO_4 \cdot 7H_2O$ (0.02 mg/l), $NaMoO_4 \times 2H_2O$ (0.03 mg/l), $Ca(OH)_2$ (0.5 mg/l), and EDTA (5 mg/l).

†Mineral medium with 4 mM molinate as carbon, nitrogen, and energy source, phosphate buffer (27 mM, pH 7.2), $CaCl_2 \cdot 2H_2O$ (0.2 mM), NaCl (7.56 mM), $MgCl_2 \cdot 6H_2O$ (0.81 mM), $FeCl_2 \cdot 4H_2O$ (5.19 µM), HCl (1.3 µl, 25%), $ZnCl_2$ (0.07 mg/l), $MnCl_2 \cdot 4H_2O$ (0.1 mg/l), H_3BO_3 (0.062 mg/l), $CoCl_2 \cdot 6H_2O$ (0.19 mg/l), $CuCl_2 \cdot 2H_2O$ (0.017 mg/l), $NiCl_2 \cdot 6H_2O$ (0.024 mg/l), and $NaMoO_4 \cdot 2H_2O$ (0.036 mg/l).

‡Molinate is quantified using a High Performance Liquid Chromatograph with an UV-VIS detector (210 nm), a Lichrosphere 5 µm RP-18 column, and a mixture of methanol:water (80:20, v/v) as mobile phase (flow rate of 0.8 ml/min).

Taxonomic comments

The description of genus *Gulosibacter* was based on the characterization of *Gulosibacter molinativorax* ON4^T. The distinctive chemotaxonomic and physiological characteristics, which include cell-wall composition, menaquinones, optimal growth temperature, and a very restricted nutritional pattern observed for strain ON4^T, along with the low values of 16S rRNA gene sequence similarity with validly named taxa, support the definition of the genus *Gulosibacter*.

Acknowledgements

We are grateful to Dr H-.J. Busse, Institute of Bacteriology, Mycology and Hygiene, University of Veterinary Medicine, Vienna, Austria, for the polyamine pattern information. We also acknowledge IUMS copyright holder (which has authorized SGM to act on their behalf in this matter) for permission to reproduce published material.

List of species of the genus *Gulosibacter*

1. **Gulosibacter molinativorax** Manaia, Nogales, Weiss and Nunes 2004, 787^{VP}

mo.li.na.ti.vo′rax. N.L. masc. n. *molinas -atis* molinate (a herbicide); L. adj. *vorax* devouring, ravenous, voracious; N.L. masc. adj. *molinativorax* molinate-degrading.

General characteristics are those listed in the genus description and in Table 153. Forms irregular rod-shaped cells, 0.8–1.0 µm × 0.5–0.6 µm. The maximal growth rate is observed in the presence of 1% (w/v) NaCl; 8% (w/v) NaCl inhibits growth.

Produces acid phosphatase, alkaline phosphatase, catalase, cystine arylamidase, esterase (C_4), esterase lipase (C_8), leucine arylamidase, lipase (C_{14}), naphthol-AS-BI-phosphohydrolase, and valine arylamidase, but not amylase, chymotrypsin, trypsin, α-fucosidase, α-galactosidase, α-glucosidase, α-mannosidase, β-galactosidase, β-glucosidase, β-glucuronidase, and *N*-acetyl-β-glucosaminidase. Degrades and grows at the expense of the herbicide molinate. Utilizes 2,3- butanediol, 2′-deoxyadenosine, adenosine, adenosine-5′-monophosphate, inosine, L-glutamic acid, *p*-hydroxyphenyl acetic acid, pyruvic acid, methyl pyruvate, putrescine, thymidine, thymidine-5′-monophosphate, uridine, and uridine-5′-monophosphate. Oxidation of α-D-glucose gives a weak reaction. The carbon sources 3-methyl-D-glucose, acetic acid, adonitol, esculin, alaninamide, amygdalin, arbutin, cellobiose, D-alanine, D-arabinose, D-arabitol, D-fucose, dextrin, D-fructose, D-galactose, D-galacturonic acid, D-gluconic acid, D-lactic acid methyl ester, D-lyxose, DL-α-glycerol phosphate D-malic acid, D-mannitol, D-mannose, D-psicose, D-sorbitol, D-tagatose, D-trehalose, dulcitol, D-xylose, erythritol, gentiobiose, glucose 1-phosphate, glucose 6-phosphate, glycerol, glycogen, glycyl-L-glutamic acid, inulin, lactamide, lactulose, L-alanine, L-alanyl-glycine, L-arabinose, L-arabitol, L-asparagine, L-fucose, L-lactic acid, L-malic acid, L-pyroglutamic acid, L-rhamnose, L-serine, fructose 6-phosphate, L-sorbose, L-xylose, maltotriose, mannan, methyl-α-D-galactoside, methyl-α-D-glucoside, methyl-α-D-mannoside, methyl-β-D-galactoside, methyl-β-D-glucoside, methyl-β-xyloside, mono-methyl-succinate, *N*-acetyl-L-glutamic acid, *N*-acetyl-D-glucosamine, *N*-acetyl-D-mannosamine, palatinose, propionic acid, salicin, sedoheptulosan, stachyose, succinamic acid, succinic acid, sucrose, turanose, Tween 40, xylitol, α-cyclodextrin, α-hydroxybutyric acid, α-ketoglutaric acid, α-ketovaleric acid, β-cyclodextrin, β-hydroxybutyric acid, and γ-hydroxybutyric acid are not used.

The major respiratory quinone is MK-9 (95%), with MK-8 as a minor component. The predominant cellular fatty acids are $C_{15:0}$ anteiso (46.9%), $C_{16:0}$ iso (33.2%), and $C_{17:0}$ anteiso (9.7%). Major polyamines are spermine and spermidine.

Source: a mixture of soil and water collected near a molinate-producing industry in Southern Portugal.

DNA G+C content (mol%): 64.5 (HPLC).

Type strain: ON4, CCUG 49965, CIP 108515, DSM 13485, JCM 13320, LMG 21909.

Sequence accession no. (16S rRNA gene): AJ306835.

Genus XI. **Humibacter** Vaz-Moreira, Nobre, Ferreira, Schumann, Nunes and Manaia 2008b, 1016^{VP}

Olga C. Nunes and Célia M. Manaia

Hu.mi.bac′ter. L. masc. n. *humus* earth, soil and, in earth sciences or agriculture, humus; N.L. masc. n. *bacter* rod; N.L. masc. n. *Humibacter* rod living in the humus.

Cells are motile Gram-stain-positive short rods (0.6 × 1.3 µm), forming coryneform V-shape cells only during the early stages of growth. Mesophilic and strictly aerobic chemo-organotroph. Reacts positively for **catalase**, **β-galactosidase**, **urease**, and **Voges–Proskauer** tests. Is able to **hydrolyze esculin** and to **reduce nitrate** but not nitrite. Cytochrome *c* **oxidase** tests **negative**. The peptidoglycan contains the diamino acid **ornithine** in combination with **2,4-diaminobutyric acid**. The major quinones are the **menaquinones 11** and **12**. The 16S rRNA gene sequence analysis positions this genus in a group of Gram-stain-positive bacteria within the family *Microbacteriaceae*. Isolated from sewage sludge compost produced in a municipal wastewater treatment plant in Northern Portugal.

DNA G+C content (mol%): 67.6.

Type species: **Humibacter albus** Vaz-Moreira, Nobre, Ferreira, Schumann, Nunes and Manaia 2008b, 1016^{VP}.

TABLE 154. Relevant chemotaxonomic characteristics differentiating *Humibacter* from closely related genera[a]

Characteristic	*Humibacter*[b]	*Agromyces*[c]	*Curtobacterium*[d]	*Leifsonia*[e]
Major menaquinones (>30%)	MK-11, MK-12	MK-11[f], MK-12, MK-13[f]	MK-9	MK-10, MK-11, MK-12[f]
Diamino acid of peptidoglycan	Orn, DAB	DAB	Orn	DAB
Predominant fatty acids	S, A, I, CH	S, A, I	S, A, I, CH[g]	S, A, I, CH[g]
DNA G+C content (mol%)	68	65–73	68–75	66–73

[a]Symbols: S, saturated; A, anteiso; I, iso; CH, cyclohexyl fatty acids; Orn, ornithine; DAB, 2,4-diaminobutyric acid.

[b]Vaz-Moreira et al. (2008b).

[c]Casida (1986); Takeuchi and Hatano (2001); Behrendt et al. (2002); Jurado et al. (2005a, 2005b, 2005c); and Jung et al. (2007).

[d]Komagata and Suzuki (1986a) and Behrendt et al. (2002).

[e]Suzuki et al. (1999) and Evtushenko et al. (2000).

[f]Only in some species.

[g]Cyclohexyl fatty acids are found only in some species (see text for details).

Further descriptive information

Good growth is observed on Brain Heart Infusion Agar (BHIA, LAB M, United Kingdom); growth is slower and poorer on other media such as Plate Count Agar (PCA, Conda-Pronadisa, Spain) and Luria–Bertani Agar (LBA)*. On BHIA, forms white, opaque, and convex colonies, after 48 h at 30°C. *Humibacter* contains the following major fatty acids: $C_{15:0}$ anteiso, $C_{17:0}$ anteiso, $C_{16:0}$ iso, and cyclohexyl $C_{17:0}$. The peptidoglycan is of acetyl type and contains, respectively, ornithine, 2,4-diaminobutyric acid, glycine, alanine, and glutamic acid in a molar ratio of 0.8:0.4:1.0:0.5:1.0. The major respiratory quinones are the menaquinones MK-11 (56%) and MK-12 (32%). MK-10 is a minor component (12%).

The only species in the genus was isolated from final compost produced from anaerobically digested sewage sludge of a domestic wastewater treatment plant. During this process, the anaerobic digest of decanted activated sludge is mixed with granular pine bark and submitted to windrow composting with additional induced aeration for 20 d. Temperatures of about 60°C are reached during this composting process.

Enrichment and isolation procedures

The type strain was isolated from a serial dilution of a suspension containing 10 g of compost per 90 ml of saline solution. The original suspension was shaken for 30 min and 1 ml of each serial dilution was filtered through a membrane of cellulose nitrate with 0.45 µm of pore size. The membranes were placed onto PCA and incubated at 30°C for 24 h. The organism represented a single morphotype in the culture at an estimate of about 7×10^5 colony forming units per gram of compost.

Maintenance procedures

The type strain is maintained in BHIA at 4°C for periods of less than 2 weeks and in nutrient broth with 15% glycerol at −80°C, for long-term storage. Cultures may also be lyophilized.

Differentiation of the genus *Humibacter* from other genera

Table 154 lists characteristics differentiating *Humibacter* from other closely related members of the family *Microbacteriaceae*. The presence of MK-11 as major menaquinone distinguishes *Humibacter* from *Curtobacterium*. The presence of ornithine in combination with 2,4-diaminobutyric acid distinguishes *Humibacter* from *Agromyces*, *Curtobacterium*, and *Leifsonia*. When compared with members of those genera, *Humibacter* presents a lower percentage (more than two times lower) of the fatty acid $C_{15:0}$ anteiso. The presence of cyclohexylundecanoic acid (cyclohexyl $C_{17:0}$), the major fatty acid in the type strain, is another distinctive feature to other taxa, although this same component has been detected in other species of *Curtobacterium* and *Leifsonia*. The presence of cyclohexyl $C_{17:0}$ in the distantly related species *Curtobacterium pusillum*, *Curtobacterium flaccumfaciens* pv. *flaccumfaciens*, *Curtobacterium ammoniigenes* (Aizawa et al., 2007), and in *Leifsonia ginsengi* (Qiu et al., 2007), but not in other members of the respective genera, limits the use of this component as chemotaxonomic marker until more representatives of *Humibacter* are isolated and characterized.

Taxonomic comments

Based on the 16S rRNA gene sequence analysis, the closest related genera are *Agromyces*, *Curtobacterium*, and *Leifsonia*, particularly the species *Leifsonia xyli*, *Leifsonia shinshuensi*, *Leifsonia naganoensis*, *Leifsonia aquatica*, *Agromyces ramosus*, and *Curtobacterium citreum* which have sequence similarities of 95–96%.

List of species of the genus *Humibacter*

1. **Humibacter albus** Vaz-Moreira, Nobre, Ferreira, Schumann, Nunes and Manaia 2008b, 1016[VP]

   al′bus. L. masc. adj. *albus* white.

Colonies on BHIA are white, opaque and 1–2 mm diameter. Growth occurs at 22–36°C, pH 5.5–8.0, and in the presence of 3% NaCl, with optima below 30°C, pH around 7, and less than 1% NaCl. No growth occurs at 15°C or 40°C, at pH 9, or in the presence of 5% NaCl. The type strain assimilates and produces acid from *N*-acetylglucosamine, amygdalin, L-arabinose, D-cellobiose, D-fructose, D-galactose,

*LBA (g/l distilled water): yeast extract, 5.0; tryptone, 10.0; NaCl, 10.0; agar, 15.0.

gentiobiose, glucose, D-lactose, D-maltose, D-mannitol, D-mannose, D-melibiose, methyl-β-D-xylopyranoside, L-rhamnose, D-sucrose, salicin, D-trehalose, and D-xylose. Additionally, inositol, turanose, and D-arabitol are assimilated, and D-arabinose and D-ribose support acid production. Growth occurs in the presence of ceftazidime (30 μg) but not in the presence of amoxicillin (25 μg), gentamicin (10 μg), ciprofloxacin (5 μg), tetracycline (30 μg), sulfamethoxazole (25 μg), cephalothin (30 μg), streptomycin (10 μg), ticarcillin (75 μg), meropenem (10 μg), or colistin sulphate (50 μg).

DNA G+C content (mol%): 67.6 (HPLC).

Type strain: SC-083, CCUG 54538, DSM 18994, LMG 23996.

Sequence accession no. (16S rRNA gene): AM494541.

Genus XII. **Labedella** Lee 2007, 2500[VP]

António Veríssimo

La.be.del′la. N.L. fem. dim. n. *Labedella* named in honor of David P. Labeda, who has made significant contributions to the area of actinomycete taxonomy.

Cells are rod-shaped (0.3–0.38 × 1.0–4.4 μm), **Gram-stain-positive**, nonmotile, and nonsporeforming. Aerobic, oxidase-negative, and catalase-positive. Chemo-organotrophic. Mesophilic. The cell-wall peptidoglycan is of the **B-type containing ornithine** as the diagnostic amino acid. Fatty acids are predominantly iso- and anteiso-branched, namely $C_{15:0}$ anteiso, $C_{16:0}$ iso, and $C_{17:0}$ anteiso. Mycolic acids are not present. The respiratory quinones are unsaturated **menaquinones, predominantly MK-10 (44%) and MK-11 (31%), but MK-9 and MK-7 are also present in lesser amounts.** The only strain was isolated from dried seaweed.

DNA G+C content (mol%): 68.0.

Type species: **Labedella gwakjiensis** Lee 2007, 2501[VP].

Further descriptive information

Phylogenetic analysis based on the 16S rRNA gene sequences positions the genus *Labedella* as a distinct branch within the radiation of the family *Microbacteriaceae*, forming a coherent cluster with members of genus *Cryobacterium* (Dastager et al., 2008a; Suzuki et al., 1997; Zhang et al., 2007b), *Microcella* (Tiago et al., 2006), and *Yonghaparkia* (Yoon et al., 2006b).

Cells are small and rod shaped. *Labedella* isolates were routinely cultured in TSA, forming translucent and yellow-pigmented colonies. The cellular fatty acids profile, obtained from cultures on TSB for 3 d at 30°C, is characterized by the predominance of $C_{15:0}$ anteiso (49.4%), $C_{16:0}$ iso (20.5%), and $C_{17:0}$ anteiso (11.4%) and includes, as a minor component, a small amount (1.8%) of tuberculostearic acid ($C_{18:0}$ 10 methyl) that has not been found in other species of *Microbacteriaceae*.

Labedella isolates are strictly aerobic mesophiles, and are able to utilize several sugars as sole carbon and energy sources.

The only strain belonging to *Labedella* was isolated from dried seaweed collected at Gwakji Beach in Jeju, Republic of Korea.

Enrichments and Isolation Procedures

The type strain was isolated by direct inoculation of dried seaweed on WAT-SW medium (Lee, 2006) and, after incubation at 30°C for 14 d, then subculturing onto TSA-SW medium. TSA-SW medium is Trypticase Soy Agar in a mixture of 60% (v/v) natural sea water and 40% (v/v) distilled water.

Maintenance procedures

Labedella is maintained in 20% glycerol suspension supplemented with 60% natural sea water at −80°C. Lyophilized cultures are also used.

Differentiation of the genus *Labedella* from closest related taxa

Chemotaxonomic characteristics such as the diagnostic diamino acid ornithine present in the peptidoglycan and the menaquinone composition characterized by the predominance of MK-10 and MK-11 are important distinctive features differentiating *Labedella* from the closest related taxa. Furthermore, growth temperature and some biochemical characters may also be useful differential characteristics.

Table 155 lists the main characteristics differentiating *Labedella* from the closest related *Microbacteriaceae*.

TABLE 155. Characteristics differentiating *Labedella* and closest related genera[a]

Characteristic	*Labedella*	*Cryobacterium*	*Microcella*	*Yonghaparkia*
Motility	−	+/−	−	−
Growth temperature, °C	10–37	4–28	15–40	10–37
Diamino acids	Orn	DAB	Lys, Orn	DAB
Major menaquinones (MK)	10, 11	10, 11, 12	12, 13, 14	12
DNA G+C content (mol%)	68.0	65.0–70.0	67.1–70.7	71.1–71.6

[a]Symbols: +, >85% positive −, 0–15% positive; Orn, Ornithine; Lys, lysine; DAB, diaminobutyric acid.

List of species of the genus *Labedella*

1. **Labedella gwakjiensis** Lee 2007, 2501[VP]

gwak.ji.en′sis. N.L. fem. adj. *gwakjiensis* of or pertaining to Gwakji Beach, Jeju, Republic of Korea, where the type strain was isolated.

The characteristics are as described for the genus with the following additional features. Growth occurs between 10 and 37°C. No growth occurs at 4 or 45°C. The pH range for growth is pH 5.1–10.1, with optimum growth at pH 7.1. Growth occurs in the presence of up to 5% NaCl. Nitrate is not reduced to nitrite. The type strain of this species hydrolyzes esculin, DNA, gelatin, and starch; casein and elastin are not hydrolyzed. Produces H_2S but not indole. D-Glucose, D-arabinose, D-mannose, D-mannitol, and *N*-acetyl-D-glucosamine are utilized as sole carbon and energy sources, but utilization of maltose, gluconate, caproate, adipate, malate, citrate, and phenylacetate is not observed. Under aerobic conditions, acid is produced from: L-arabinose, D-cellobiose, D-fructose, D-galactose, D-glucose, inulin, D-mannose, D-melezitose, methyl a-D-mannoside, D-raffinose, L-rhamnose, salicin, sucrose, D-xylose, glycerol, *myo*-inositol, D-mannitol, and D-sorbitol. Activity of enzymes urease, leucine arylamidase, α-galactosidase, β-galactosidase, α-glucosidase, and β-glucosidase is detected.

Major cellular fatty acids detected after incubation on TSB for 3 d at 30°C are: $C_{15:0}$ anteiso (49.4%), $C_{16:0}$ iso (20.5%), and $C_{17:0}$ anteiso (11.4%). Minor components detected are: $C_{16:0}$ (5.8%), $C_{18:0}$ (1.7%), $C_{14:0}$ iso (2.0%), $C_{15:0}$ iso (2.0%), $C_{18:1}$ ω9*c* (1.8%), $C_{18:0}$ 10-methyl (tuberculostearic acid, 1.8%), and a mixture of $C_{15:0}$ iso 2-OH and/or $C_{16:1}$ ω7*c* (1.6%).

Polar lipids include phosphatidylglycerol and diphosphatidylglycerol. Glycolipids or other phospholipids, namely phosphatidylcholine and ninhydrin-positive phospholipids, were not detected.

Source: dried seaweed collected at Gwakji Beach in Jeju, Republic of Korea.

DNA G+C content (mol%): 68.0 (HPLC).

Type strain: KSW2–17, JCM 14008, KCTC 19176.

Sequence accession no. (16S rRNA gene): DQ533552.

Genus XIII. **Leifsonia** Evtushenko, Dorofeeva, Subbotin, Cole and Tiedje 2000, 377[VP]

Lyudmila I. Evtushenko

Leif.so′ni.a. N.L. fem. n. *Leifsonia* named after Einar Leifson, an American microbiologist who isolated and described the first organism of this genus.

Young cultures produce **slender (mostly 0.3–0.6 µm in diameter) irregular rods or filaments**. **Primary branching and V-forms typically occur.** Cultures a week or more old usually contain shorter rods, but some cells may be coccoid. No aerial mycelium is produced. Nonsporeforming. Some species are **motile by means of peritrichous flagella**. **Gram-stain-positive** cell-wall architecture. **Non-acid-fast. Chemo-organotrophs**, metabolism is primarily respiratory with oxygen as the terminal electron acceptor. Mostly **catalase-positive**. Oxidase reaction varies with strains or experiments. Acids are produced oxidatively rather weakly from carbohydrates. Most species grow well on standard laboratory media at near neutral pH. Some species are nutritionally very exacting. Mesophilic, optimum temperature ~24–30°C; growth range ~4–42°C.

Cell-wall peptidoglycan contains 2,4-diaminobutyric acid as the diamino acid, the structural type of the peptidoglycan is **B2γ, [L-Dab]D-Glu–D-Dab. The muramic acid residue of the peptidoglycan is *N*-acetylated. Menaquinones** are the sole respiratory quinones detected; the predominant component is a **menaquinone with 11 unsaturated isoprene units (MK-11), the second most common major menaquinone is MK-10 or MK-12. Rhamnose** is typically present in the cell-wall polysaccharide(s). The major fatty acids are $C_{15:0}$ anteiso, $C_{17:0}$ anteiso, and $C_{16:0}$ iso. Straight-chain saturated or unsaturated acids are present in small quantities. Mycolic acids are absent. The principal polar lipids are diphosphatidylglycerol, phosphatidylglycerol, and characteristic glycolipids.

Occur in various environments and can be associated with plants, animals, and clinical specimens. Representatives of some species are plant pathogens.

DNA G+C content (mol%): 66–70.7.

Type species: **Leifsonia aquatica** (*ex* Leifson 1962) Evtushenko, Dorofeeva, Subbotin, Cole and Tiedje 2000, 377[VP] ("*Corynebacterium aquaticum*" Leifson 1962, 161).

Further descriptive information

Phylogenetic heterogeneity of the genus *Leifsonia*. The genus *Leifsonia* belongs to the family *Microbacteriaceae*, order *Micrococcales*, and, at the time of writing, includes 11 species and two subspecies with validly published names indicated in Figure 187. The 16S rRNA gene sequence similarities between the species described under the generic name *Leifsonia* range from ~94 to 99.8%, and the inferred phylogenies show that the genus comprises a rather heterogeneous group. The type species *Leifsonia aquatica* together with *Leifsonia naganoensis*, *Leifsonia poae*, *Leifsonia shinshuensis*, and *Leifsonia xyli* with two subspecies (*Leifsonia sensu stricto* species group) compose a coherent cluster with the 16S rRNA gene sequence similarity values exceeding 97.5%. The close phylogenetic relatedness of these species is also supported by analysis of the 16S–23S rRNA intergenic spacer (ITS) region sequences and the partial 23S rRNA gene sequences (approx. 200 bases) available in GenBank. The remaining species (*Leifsonia aurea*, *Leifsonia rubra*, *Leifsonia bigeumensis*, *Leifsonia ginsengi*, *Leifsonia kribbensis*, and *Leifsonia pindariensis*) form

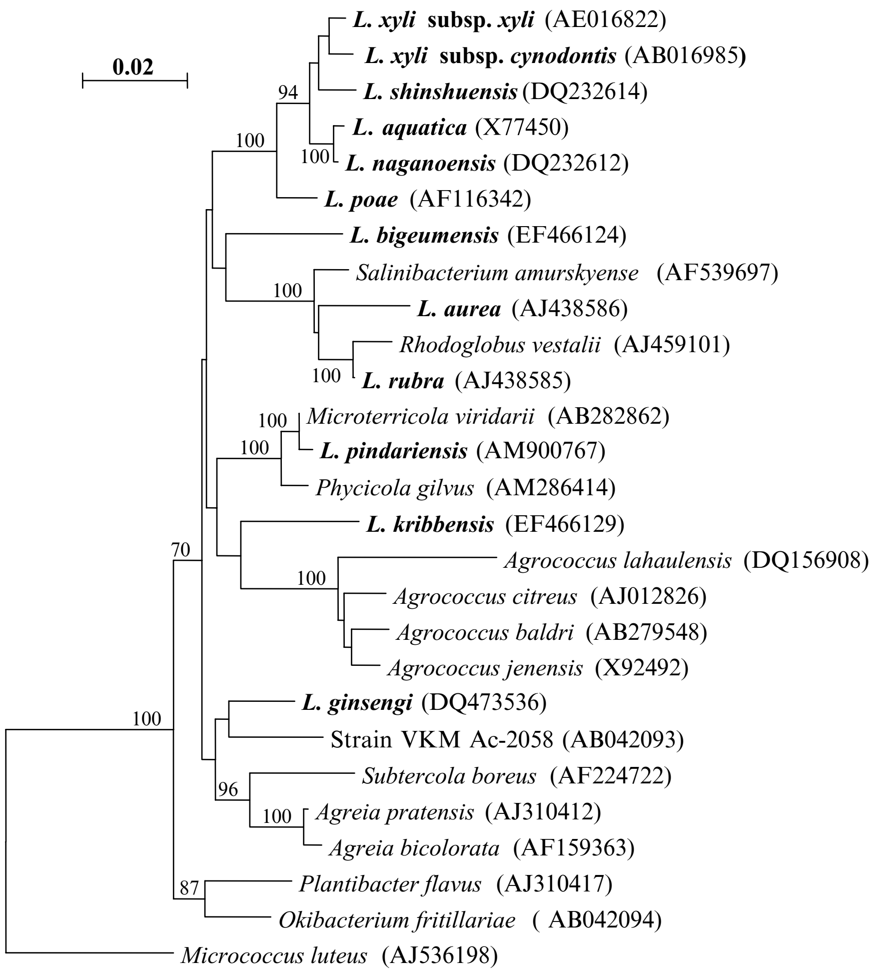

FIGURE 187. Phylogenetic relationship of *Leifsonia* species and related genera based on 16S rRNA gene analysis. The neighbor joining method was used for tree construction. Bar = 0.02 inferred nucleotide substitution per nucleotide. Values at nodes indicate bootstrap values for 500 replicates.

separate lineages outside the coherent *Leifsonia sensu stricto* species group, and the 16S rRNA gene sequence similarity values indicate their approximately equidistant or closer relationship to members of other genera or as yet unclassified microorganisms of the family *Microbacteriaceae*. In the opinion of the author of this chapter, the generic assignment of these six species should be re-examined (see the Taxonomic Comments section). Until their taxonomic status has been clarified, they are dealt with as species *insertae sedis*. The data provided in the genus description and in this section below concern the *Leifsonia sensu stricto* species only.

Cell morphology and motility. Cell morphology of the *Leifsonia* species in young cultures ranges from irregular rods tending to form primary branched cells to the relatively long, occasionally branching filaments resembling rudimentary mycelium of some *Agromyces* species (Figure 188). The mean cell width is 0.3–0.6 μm. Some cells may become thicker on some media and reach up to 0.8–0.9 μm in diameter. The rods or filaments are divided by septum formation, and the rapidly growing cultures a week or more old are usually composed of short rods and coccoid cells. A marked rod–coccoid cycle resembling that in *Arthrobacter* species may be observed in some

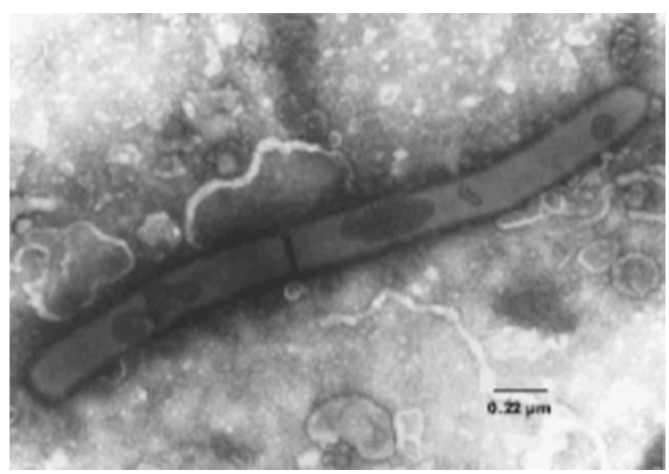

FIGURE 188. Electron micrograph of dividing *Leifsonia xyli* subsp. *xyli* cells isolated from nodal exudates from sugarcane, containing 4 distinct mesosomes. The cell size is most likely smaller than in a living bacterium as the sample was dried during the fixation process. (Reproduced with permission from Brumbley et al., 2006. Austr. Plant Pathol. *35*: 681–689.)

strains during growth on complex agar media, especially in synchronized cultures. No aerial mycelium is produced. Cells of some species are motile by means of 1–3 peritrichous flagella (length, ~10 µm or more) as measured for *Leifsonia aquatica* (Leifson, 1962). It is worth noting that *Leifsonia xyli* subsp. *xyli* reported to be nonmotile (Davis et al., 1984) has homologs of flagellar operons in the genome (Brumbley et al., 2006; Monteiro-Vitorello et al., 2004).

Colony morphology and pigments. Colonies are typically circular, somewhat convex, glossy, opaque, with entire margins. Penetration into agar media was not observed. Most of the *Leifsonia* species usually produce white to light yellow colonies in early growth phase; the color becomes bright yellow to pale brown with age in some species. The yellow pigments of two species (*Leifsonia aquatica* and *Leifsonia poae*) are C_{40}-carotenoids; the pigment biosynthesis is induced by light (Trutko et al., 2005). The pigment production in these two species is inhibited by fosmidomycin at concentrations lower than those inhibiting growth (Trutko et al., 2005) suggesting the nonmevalonate pathway of pigment biosynthesis (Kuzuyama, 2002; Lichtenthaler, 2000). No distinct pigmentation of *Leifsonia xyli* subsp. *xyli* has been reported, but genes for carotenoid biosynthesis (Armstrong, 1997) were identified in the genome of strain *Leifsonia xyli* subsp. *xyli* CTCB07 (Brumbley et al., 2006; Monteiro-Vitorello et al., 2004). The genome of this strain also harbors a gene encoding a member of the delta fatty acid desaturase family which could redirect the carotenoid biosynthetic pathway to the biosynthesis of the plant hormone abscisic acid (Bartley and Scolnik, 1995; Kende and Zeevaart, 1997; Ventura et al., 2007).

Chemotaxonomy. Peptidoglycan is of the B2γ type, [L-Dab]D-Glu–D-Dab, as determined for *Leifsonia aquatica* (Fiedler et al., 1970; Schleifer and Kandler, 1972). All other *Leifsonia sensu stricto* species contain 2,4-diaminobutyric acid (Dab), glycine, glutamic acid, and alanine in a molar ratio close to 2:1:1:1 (Evtushenko et al., 2000; Suzuki et al., 1999). The polymers of *Leifsonia naganoensis*, *Leifsonia shinshuensis*, and *Leifsonia xyli* subsp. *cinodontis* studied in more detail have both D- and L-isomers of Dab in almost equal proportions like that in *Leifsonia aquatica* (Suzuki et al., 1999). The muramic acid is *N*-acetylated (the glycolate test is negative) (Suzuki et al., 1999). The whole cell sugars in *Leifsonia aquatica* and *Leifsonia xyli* subsp. *cinodontis* are similar (glucose, galactose, mannose, fucose, ribose, rhamnose, and traces of xylose). According to Davis et al. (1984), the subspecies *Leifsonia xyli* subsp. *cynodontis* and *Leifsonia xyli* subsp. *xyli s*trains have similar cell-wall sugars (rhamnose, fucose, and trace of galactose). Somewhat different pattern of cell-wall sugars has been revealed in *Leifsonia xyli* subsp. *cinodontis* (rhamnose, galactose, glucose, and a lower content of fucose and mannose) by other authors (Evtushenko et al., 2000), probably due to different method of cell-wall preparation and purification. Cell-wall teichoic acids and related anionic phosphorous-containing polysaccharides have not been found in the type strains of *Leifsonia aquatica* and *Leifsonia poae* (Evtushenko et al., 2000). The major respiratory menaquinone is MK-11; the second most common major component is MK-10 or MK-12 depending on the species (Collins et al., 1979; Evtushenko et al., 2000; Suzuki et al., 1999; Yamada et al., 1976). Concentration of polyamines, as determined in strain *Leifsonia poae* VKM Ac-1401^{T} (DL 89), is low**;** putrescine is the predominant component (Altenburger et al., 1997).

The major fatty acids are represented by $C_{15:0}$ anteiso (24.1–47.3%), $C_{17:0}$ anteiso (32.6–54.4%), and $C_{16:0}$ iso (10–21%) as determined in cells grown at optimal temperatures (Collins and Jones, 1980; Evtushenko et al., 2000; Suzuki and Komagata, 1983; Suzuki et al., 1999). The content of $C_{15:0}$ anteiso may be significantly higher at lower (20°C) growth temperature (Suzuki and Komagata, 1983). The principal polar lipids, as determined in *Leifsonia aquatica*, are diphosphatidylglycerol, phosphatidylglycerol, and two characteristic glycolipids (glycosyldiacylglycerols), G_1 (minor) and G_2 (predominant), both giving green coloration with the anisaldehyde/sulfuric acid reagent indicating the possible presence of mannose (Collins and Jones, 1980). It is worth noting that the glycolipid G_2 as the only glycolipid also occurs in *Agromyces mediolanus* and, along with 1–2 other glycolipids, in some, but not all, members of the family *Microbacteriaceae* (Collins and Jones, 1980; Kämpfer et al., 2000; N.G. Vinokurova and L.I. Evtushenko, unpublished). Khuller and Brennan (1972) reported diphosphatidylglycerol, phosphatidylglycerol, phosphatidylinositol monomannoside, and dimannosyl diacylglycerol in *"Corynebacterium aquaticum"* B2252. Yanagi et al. (2000) identified two glycoglycerolipids, 1-[α-glucopyranosyl-(1α-3)-(6-*O*-acyl-α-mannopyranosyl)]-3-*O*-acylglycerol and 1-[α-mannopyranosyl-(1α-3)-(6-*O*-acyl-α-mannopyranosyl)]-3-*O*-acylglycerol, in two *"Corynebacterium aquaticum"* strains, S361 and S365, respectively. However, it is not quite clear whether the strains investigated by these authors are actually members of the genus *Leifsonia*.

Nutrition, growth conditions, and metabolic activities. *Leifsonia* species, with exception of xylem-invading *Leifsonia xyli*, usually grow well on standard nutrient media based on yeast-extract and peptone. Knowledge of nutritional requirements of *Leifsonia* species is incomplete. The type strain of *Leifsonia aquatica* can grow readily in simple vitamin-free Casamino acids (Difco) or peptone broth (Leifson, 1962), but not in a basal mineral medium (e.g. Shirling and Gottlieb, 1966) with glucose as a carbon source and ammonium salt as a source of nitrogen. This strain and some other representatives of the genus show growth in the same basal medium with traces of yeast extract. It therefore appears that some growth factors, such as amino acids and probably vitamins, are necessary for certain species. Strains of *Leifsonia xyli* are nutritionally very exacting and require more complex media containing, e.g. bovine serum albumin, bovine hemin chloride, and cysteine (Davis et al., 1984). Extracts of Bermuda grass and maize, as well as xylem fluid from maize, enhance the growth of *Leifsonia xyli* subsp. *cynodontis* (Haapalainen et al., 2000).

Representatives of the genus *Leifsonia* use a wide range of organic compounds as sole sources of carbon for growth and energy and possess various enzymic activities (Table 156 and Table 157). Although *Leifsonia xyli* subsp. *xyli* shows negative reactions in many conventional utilization tests (Davis et al., 1984), strain *Leifsonia xyli* subsp. *xyli* CTCB07 was found to possess a large number of ABC transporter homologs, indicating the potential ability to utilize a broad range of carbohydrates including glucose, arabinose, fructose, galactose, lactose, maltose, maltotriose, ribose, trehalose, and xylose; this strain has also the potential to degrade some organic compounds includ-

TABLE 156. Characteristics differentiating *Leifsonia sensu stricto* species[a,b]

Characteristic	*L. aquatica*[c,d]	*L. naganoensis*[c]	*L. poae*[d]	*L. shinshuensis*[c]	*L. xyli* subsp. *xyli*[e]	*L. xyli* subsp. *cynodontis*[c,d,e]
Colony color[f]	White to yellow	White to pale brown	White to yellow	White to pale brown	White	White to yellow
Visible growth, day	2–3	2–3	2–3	2–3	7–10	5–7
Morphology (2-d culture)	Irregular rods	Irregular rods	Filaments or irregular rods	Irregular rods	Irregular rods	Irregular rods
Motility	+	–	+	–	–	–
Growth on/at:						
R agar, CB agar	+	+	+	+	–	–
32°C	+	+	+	+	–	+
37°C	+	w	+	+	–	–
42°C	–	–	–	+	–	–
Oxidase reaction	v	–	v	–	–	–
Nitrate reduction	+	–		–	–	–
Production of H_2S	+	–	w	–	–	–
Hydrolysis of:						
Casein	–	w	–	–	–	–
Starch	+	+	–	+	–	+
Tween 40	+	+	–	w		–
Acid from:[g]						
D-Glucose	+	+	+	–	+	+
D-Galactose	+	+	+	–	–	–
D-Mannose	+	+	+	–	–	+
Utilization of:[g]						
Citrate	+	–	–	–	–	+
Fumarate	v	+	–	+	–	–
Malate	+	+	+	+	–	+
Menaquinones (%):[h]						
MK-8	8.5	2.0	–	–		–
MK-9	13.8	6.7	–	3.8		<0.5
MK-10	32.3	35.8	++	13.0		17.1
MK-11	35.3	42.6	+++	49.1		44.2
MK-12	8.4	8.9	+	29.5		38.7
Cell-wall sugars[i]	Rha, Fuc, Glu, Gal, Man		Rha, Glu, Gal, Man		Rha, Fuc, Gal	Rha, Fuc, Gal (Glu, Man)
DNA G+C content (mol%)	70.3 (HPLC)/ 69.3 (T_m)[j]	70.5 (HPLC)	69.5 (T_m)	70.7 (HPLC)	66 (T_m)/67.7[k]	66 (T_m)/69.4 (HPLC)
Source of isolation	Distilled water	Soil	Nematode gall on *Poa annua* roots	Soil	*Saccharum*, interspecific hybrid	*Cynodon dactylon*

[a]Symbols: +, positive or present; –, negative or absent; w, weak reaction; v, variable between experiments.

[b]Data for the type strains unless indicated.

[c]Data from Suzuki et al. (1999).

[d]Data from Evtushenko et al. (2000).

[e]Data from Davis et al. (1984).

[f]Colonies are typically white or yellowish white in young cultures and become yellow or pale brown with age.

[g]According to conventional tests (Davis et al., 1984; Evtushenko et al., 2000; Suzuki et al., 1999).

[h]Menaquinones MK-7 (0.7%) and MK-13 (<0.5%) for *Leifsonia aquatica*, MK-13 (<0.5%) for *Leifsonia naganoensis*, and MK-13 (1.2%) for *Leifsonia shinshuensis* were also reported.

[i]Gal, galactose; Glc, glucose; Man, mannose; Rha, rhamnose; Xyl, xylose. Sugars are listed in order of decreasing amounts. Sugars in parentheses were also reported (Evtushenko et al., 2000).

[j]Data from Yamada and Komagata (1970).

[k]Data for reference strain *Leifsonia xyli* subsp. *xyli* CTCB07 from the genome sequence (Monteiro-Vitorello et al., 2004).

ing heteropolysaccharides and peptides (Monteiro-Vitorello et al., 2004; Ventura et al., 2007).

Acids are produced oxidatively rather weakly from carbohydrates; the test results vary with experiments (Evtushenko et al., 2000; Leifson, 1962; Suzuki et al., 1999; Yamada and Komagata, 1972b). No acidification of glucose or other carbohydrates was observed under anaerobic conditions for *Leifsonia aquatica* (Leifson, 1962).

Strains of *Leifsonia naganoensis* and *Leifsonia shinshuensis* were reported to be amine-decomposing bacteria (Suzuki et al., 1999). The type strain of *Leifsonia aquatica* destroys detergent compounds such as tallow-alkyl-sulfate, alkyl-ethoxylate-sulfate,

TABLE 157. Oxidation of various carbon sources by *Leifsonia sensu stricto* species according to the BIOLOG test system[a,b]

Carbon source[c]	*L. aquatica*	*L. naganoensis*	*L. poae*	*L. shinshuensis*	*L. xyli* subsp. *cynodontis*
Acetic acid	w	−	−	w	−
N-Acetyl-D-glucosamine	−	w	w	−	−
N-Acetyl-L-glutamic acid	w	−	−	w	−
Adenosine	−	−	+	−	−
Adenosine 5′-monophosphate	−	−	+	−	−
L-Alaninamide	−	−	+	−	−
L-Alanine	−	−	w	w	−
L-Alanyl glycine	w	w	+	w	−
Amygdalin	+	+	+	+	−
Arbutin	w	+	+	w	−
L-Asparagine	w	−	+	+	−
2,3-Butanediol	−	−	w	−	−
D-Cellobiose	w	+	+	−	−
2′-Deoxyadenosine	−	−	+	w	−
Dextrin	+	+	+	w	−
D-Fructose	+	+	+	+	w
D-Fructose 6-phosphate	−	−	−	−	−
L-Fucose	w	w	−	w	w
D-Galactose	+	+	+	w	−
D-Galacturonic acid	−	w	w	−	−
Gentiobiose	+	+	+	−	−
D-Gluconic acid	w	w	−	w	−
D-Glucose	+	+	+	−	w
L-Glutamic acid	−	−	+	w	−
Glycerol	+/w	+/w	+	+	+/w
Glycogen	w	w	−	w	−
α-Hydroxybutyric acid	w	−	−	w	w
β-Hydroxybutyric acid	w	−	−	−	w
γ-Hydroxybutyric acid	w	−	−	w	w
p-Hydroxyphenylacetic acid	w	w	−	w	w
Inosine	−	−	+	−	−
m-Inositol	−	w	−	+	−
α-Ketoglutaric acid	−	−	−	w	−
α-Ketovaleric acid	w	w	w	w	−
L-Lactic acid	−	−	w	−	−
D-Lactose	−	+	−	−	−
Lactulose	−	w	−	−	−
L-Malic acid	−	−	−	w/−	−
Maltose	+	+	+	w	−
Maltotriose	w	+	+	w	−
Mannan	+	+	−	−	−
D-Mannitol	+	+	+	+	w
D-Mannose	+	+	+	+/w	+/w
D-Melezitose	+	w	+	w	−
D-Melibiose	w	w	−	w	−
α-Methyl D-galactoside	w	+/w	+	w/−	−
β-Methyl D-galactoside	w	+	+	−	−
3-Methyl glucose	w	+	+	−	−
α-Methyl D-glucoside	−	−	+	−	−
β-Methyl D-glucoside	−	−	+	−	−
α-Methyl D-mannoside	w	−	w	w	−
Palatinose	w	+	+	w	−
Propionic acid	w	w	−	w	w
D-Psicose	−	w	w	+	−
Putrescine	+	+	w	w	−
Pyruvic acid	+/w	+/w	+	+/w	+/w
Pyruvic acid methyl ester	+	+	+	+	w
D-Raffinose	−	−	w	−	−
L-Rhamnose	w	−	w	w	−
D-Ribose	w	w	+	w	−
Salicin	+	+	+	+	−

(continued)

TABLE 157. (continued)

Carbon source[c]	*L. aquatica*	*L. naganoensis*	*L. poae*	*L. shinshuensis*	*L. xyli* subsp. *cynodontis*
L-Serine	−	−	+	w	−
D-Sorbitol	w	+	+	w	w
Succinic acid	−	w	−	w	−
Sucrose	w	+	+	+	−
Thymidine	−	−	+	w	−
Thymidine 5′-monophosphate	−	−	+	−	−
D-Trehalose	w	w	+	w	−
Turanose	+	+	+	w	−
Tween 40	w	+	+	w	−
Tween 80	w	+	+	w	−
Uridine	−	−	+	−	−
Xylitol	w	w	−	w	−
D-Xylose	w	w	+	w	−

[a]Data for the type strains; taken from Suzuki at al. (1999) and Qiu et al. (2007).

[b]Symbols: +, positive test reaction; w, weakly positive; −, negative; +/w or w/−, different between experiments.

[c]A negative reaction was observed for all strains with the following carbon sources: L-arabinose, D-arabitol, stachyose, D-tagatose, α- and β-cyclodextrin, *N*-acetyl-β-D-mannosamine, D-alanine sedoeptulosan, α-D-glucose 1-phosphate, D-glucose 6-phosphate, uridine 5′-monophosphate, inulin, lactamide, D-lactic acid methyl ester, D-malic acid, DL-α-glycerol phosphate, glycyl L-glutamic acid, L-pyroglutamic acid, succinamic acid.

and linear-alkyl-benzene-sulfonate (Goodnow and Harrison, 1972). *Leifsonia* species strain S749, reduces phenyl trifluoromethyl ketone to (*S*)-1-phenyltrifluoroethanol (due to production of a novel alcohol dehydrogenase) (Inoue et al., 2005). *Leifsonia* species IS1 (= ATCC BAA-584) isolated from acidic soil is able to grow with naphthalene as a sole carbon source at pH 5.0 and detoxify mercury (Dore et al., 2003).

Two catalase-negative *Leifsonia* strains (ATSB20 and ATSB24) closely related to *Leifsonia shinshuensis* grow on mineral salts medium with D-glucose and some other single carbon sources including methanol (Anandham et al., 2008). The strains are also able to grow chemolithotrophically in a thiosulfate-containing medium, exhibiting growth coupled with thiosulfate oxidation. They accumulate sulfur, sulfite, and trithionate in the spent medium during the time-course of thiosulfate oxidation, and these compounds are finally oxidized into sulfate. The strains possess thiosulfate-metabolizing enzymes such as rhodanese, thiosulfate oxidase, sulfite oxidase, and trithionate hydrolase, suggesting that they use the “S4 intermediate” (S4I) pathway for thiosulfate oxidation which is characteristic of certain obligate and facultative chemolithotrophic S-oxidizing bacteria (Anandham et al., 2008). These examples and some other data available suggest that at least certain representatives of the genus *Leifsonia* are aerobic chemo-organotrophs with a potential for mixotrophy and metabolic flexibility.

Antibiotic susceptibility. The *Leifsonia* species tested to date are sensitive to rifampin (30 μg/ml) (type strain of *Leifsonia aquatica*), doxycycline (5 μg/ml), and erycycline (10 μg/ml) (type strain of *Leifsonia poae*). Both type strains are resistant to the following antibiotics (μg/ml): ampicillin (50), chloramphenicol (10), gentamicin (50), gramicidin G (10), lincomycin (50), neomycin (50), penicillin G (50), rifampin (30), streptomycin (50), and tetracycline (10).

Genomic characteristics. The DNA base ratios reported for *Leifsonia* species ranges from 66 mol% (Davis et al., 1984) to 70.7 mol% (Suzuki et al., 1999). The DNA G+C mol% content for the type strain of *Leifsonia aquatica* is 70.3 as determined by HPLC (Suzuki et al., 1999); in early works, the values 69.3 mol% (T_m) (Yamada and Komagata, 1970) and 73.2 mol% (T_m) (Döpfer et al., 1982) were reported for it. The minimal G+C content, i.e. 66 mol% (T_m), was determined for the type strain of *Leifsonia xyli* subsp. *xyli* (Davis et al., 1984), and 67.7% was calculated for strain *Leifsonia xyli* subsp. *xyli* CTCB07 from the genome sequence (Monteiro-Vitorello et al., 2004). The DNA–DNA hybridization values between the type strains of *Leifsonia* species and subspecies (except for *Leifsonia xyli* subsp. *xyli* for which the data are absent) vary with the technique used, but clearly indicate their separate species status (Evtushenko et al., 2000; Suzuki et al., 1999).

The genome of strain *Leifsonia xyli* subsp. *xyli* CTCB07 consists of a single circular chromosome of 2.58 Mb, of which 70.6% possesses a protein-coding capacity (Monteiro-Vitorello et al., 2004; Ventura et al., 2007). The genome harbors 307 predicted pseudogenes (i.e. gene fragments arising from frame shifts or deletions or caused by point mutations that introduce or remove stop codons, or incomplete genes), which is larger than the numbers of pseudogenes revealed in other actinobacteria, except for *Mycobacterium leprae* (Cole et al., 2001) and all bacterial plant pathogens sequenced to date. The 105 genes for virulence and pathogenicity found in *Leifsonia xyli* subsp. *xyli* present is, by far, the smallest number found in any of the plant-pathogenic bacteria sequenced (Brumbley et al., 2006; Monteiro-Vitorello et al., 2004). The genome of *Leifsonia xyli* subsp. *xyli* CTCB07 has four likely horizontally acquired DNA regions (genomic islands) with deviant G+C contents, codon biases, and dinucleotide signatures, and 3.5% of the genome is occupied by IS-elements of five distinct IS families and transposases (Monteiro-Vitorello et al., 2004; Ventura et al., 2007).

Sequencing of approximately 50% of the *Leifsonia xyli* subsp. *cynodontis* genome identified 56 copies of five distinct IS

elements, which shows that the chromosome contains a larger number of IS copies than the *Leifsonia xyli* subsp. *xyli* genome. Each genome has an unique set of elements although related to the same IS families when considering features such as similarity among transposases, inverted and direct repeats, and element size (Zerillo et al., 2008). A comparative analysis of *Leifsonia xyli* subsp. *cynodontis* and *Leifsonia xyli* subsp. *xyli* IS elements and their detailed characterization have been made by Zerillo et al. (2008). Further information on the *Leifsonia xyli* subsp. *xyli* CTCB07 genome is available from Monteiro-Vitorello et al. (2004); Brumbley et al. (2006), Sutcliffe and Hutchings (2007) and Ventura et al. (2007). Some genomic characteristics of *Leifsonia xyli* subsp. *xyli* related to plant pathogenicity are outlined in the respective section below.

Plasmids. The only known cryptic *Leifsonia* plasmid, pCXC100 (51 kb), has been found in some *Leifsonia xyli* subsp. *cynodontis* strains tested (Li et al., 2004a; Metzler et al., 1992; Taylor et al., 1993). The 5-kb replicon of pCXC100 encodes two proteins essential for plasmid replication and stability; the *RepA* encoding protein is homologous to that of the plasmids in the mycobacterial pLR7 family (Li et al., 2004a; Yin et al., 2006).

Plant pathogenicity and related matters. *Leifsonia xyli* subsp. *xyli* invades vascular systems and causes ratoon stunting (growth-hindering) disease of sugarcane (*Saccharum* interspecific hybrids) worldwide (Davis et al., 1984; Vidaver and Davis, 1988; Young et al., 2006). Infected sugarcane plants show slower growth, look stunted, and have fewer and thinner stalks, while no other reliable external or internal symptoms are typically observed (Davis et al., 1984; Gillaspie and Teakle, 1989). The ratoon stunting disease is difficult to detect in the field, since stunting can be caused by a range of environmental factors (Brumbley et al., 2006; Gillaspie and Teakle, 1989). The disease is considered to be transmitted mechanically or through infected plant materials (Davis et al., 1984; Taylor et al., 1988). This bacterium has not been found to naturally colonize any other grass or to occur free-living in the environment (Brumbley et al., 2006; Gillaspie and Teakle, 1989). In experimental systems, the bacterium was reported to colonize at least 17 plant species, however, with the exception of several *Saccharum spontaneum* clones, these plants remained asymptomatic (Matsuoka, 1971; Mills et al., 2001; Roach and Jackson, 1992; Young et al., 2006). The comprehensive genetic studies of Young et al. (2006) revealed no genetic variation among a large collection of *Leifsonia xyli* subsp. *xyli* isolates, irrespective of sampling time, host cultivar, or country of origin, suggesting the worldwide distribution of a single pathogenic clone (Brumbley et al., 2006; Young et al., 2006).

Exact mechanisms of pathogenicity of *Leifsonia xyli* are largely unknown. The slow growth and other symptoms were believed to be the result of vascular plugging (Kao and Damann, 1978, 1980). It is unclear whether this plugging is caused by substances produced by the bacterium, is a plant defense response, or is a combination thereof (Brumbley et al., 2006). A number of the predicted pathogenicity genes (thought to have been acquired by lateral transfer) encoding, in particular, the cell-wall-degrading enzymes and desaturase, have been found in the genome of *Leifsonia xyli* subsp. *xyli* CTCB07 (Brumbley et al., 2006; Monteiro-Vitorello et al., 2004). The cell-wall-degrading enzymes may be involved in nutrient extraction from the xylem vessels and also may trigger the host defense, the production of the gelatinous material plugging xylem vessels of sugarcane plants infected with *Leifsonia xyli* subsp. *xyli* (Brumbley et al., 2006; Kao and Damann, 1978). A *celA* gene encodes a cellulase similar to CelA of *Clavibacter species*, in which the gene is a plasmid-borne virulence factor (Jahr et al., 2000). The desaturase is assumed to contribute to stunting seen in plants infected with *Leifsonia xyli* subsp. *xyli*, as it may be involved in the synthesis of abscisic acid, a growth inhibitor of plant tissues (Bartley and Scolnik, 1995; Kende and Zeevaart, 1997) which may play a role in the stunting (Brumbley et al., 2006; Ventura et al., 2007). One of the two prophage-like regions in the *Leifsonia xyli* subsp. *xyli* CTCB07 genome carries a homolog of the plasmid-located *pat-1* gene of *Clavibacter michiganensis* subsp. *michiganensis*, which plays a decisive causal role in plant wilting (Dreier et al., 1997). As with other plant pathogens, the *Leifsonia xyli* subsp. *xyli* CTCB07 genome has the potential to encode a number of enzymes (superoxide dismutase, catalase, iron dependent peroxidase, and alkyl hydroperoxide reductase) that would enable the bacterium to resist the reactive oxygen species synthesized as part of the host defense mechanisms (Monteiro-Vitorello et al., 2004). This bacterium also has genes encoding a multidrug efflux pump similar to the AlbF system that allows self-protection of *Xanthomonas albilineans* against the albicidin toxin, presumably aiding survival in the complex ecosystem inhabited by the two organisms (Monteiro-Vitorello et al., 2004).

Leifsonia xyli subsp. *cynodontis* naturally occurs in the xylem vessels of Bermuda grass [*Cynodon dactylon* (L.S. Per)] in Taiwan (Davis et al., 1980, 1984). Under experimental conditions, the bacterium was shown to be pathogenic for Bermudagrass, causing a disease named Bermudagrass stunting disease, which is not known to occur in nature (Davis et al., 1984; Liao and Chen, 1981). In experimental settings, *Leifsonia xyli* subsp. *cynodontis* and its recombinant colonize more than 80 plant species of 26 families without inducing stunting (Fahey et al., 1991; Kostka et al., 1988; Lampel et al., 1994) and may even promote the growth of infected rice plants (Li et al., 2007). *Leifsonia xyli* subsp. *cynodontis* or closely related bacteria identified by the fluorescent antibody direct count on filters were found to be common endophytes of many grasses in eastern Australia (Mills et al., 2001). Several isolates from *Chloris guyana* were shown to be genetically almost identical to the type strain of *Leifsonia xyli* subsp. *cynodontis* (Young et al., 2006).

Although *Leifsonia poae* was isolated from plant galls induced by the plant parasitic nematode *Subanguina radicicola*, there is no clear evidence of plant pathogenic properties of this bacterium per se.

Medically relevant *Leifsonia* species. The genus *Leifsonia* is considered to include some opportunistic pathogens, of which the species *Leifsonia aquatica* ("*Corynebacterium aquaticum*") is most frequently mentioned in publications (Bosshard et al., 2003; Coyle and Lipsky, 1990; D'Amico et al., 2005; Dempsey et al., 2007; Funke and Bernard, 2003; Funke et al., 1994, 1997). *"Corynebacterium aquaticum"* has been described in earlier publications as a cause of disease in humans, e.g. as an etiologic agent of bacteremia, endocarditis, meningitis, peritonitis, and urinary tract infection, or as a cause of pseudobacteremia due to contamination of blood collection tubes (for references, see Funke et al., 1997, 1994). However, many case reports claiming an association of "*Corynebacterium aquaticum*" with human

disease provide inconclusive results. As with other coryneform bacteria of low pathogenicity, the distinction between colonization and infection has not been made in every case (Funke et al., 1997). Further, as shown in numerous studies, both the conventional methods and biochemical panels used for identification give largely ambiguous results with *Leifsonia* species and phenotypically similar organisms (Adderson et al., 2008a; Funke et al., 1994, 1998; Grove et al., 1999; Lau et al., 2002). The assignment of clinical isolates to the genus *Leifsonia* has been supported by 16S rRNA gene analysis in a few cases only. These include clinical strains with 98.2% 16S rRNA gene sequence similarity to *Leifsonia poae* (Bosshard et al., 2003) and isolates from the surface of prosthetic hip joints that are phylogenetically close to *Leifsonia aquatica* and *Leifsonia shinshuensis* (Dempsey et al., 2007). In another case, the sequence determined showed 96.9% sequence similarity to *Leifsonia xyli* (Bosshard et al., 2003). Further taxonomic study of clinical isolates referred to as *Leifsonia* species is necessary to elucidate their generic and species affiliations. Further research is also required for examination of their supposed pathogenicity.

Habitats. Members of the genus *Leifsonia* occur in different environments. The natural habitats of *Leifsonia xyli* subsp. *xyli* and *Leifsonia xyli* subsp. *cynodontis*, as indicated above, are plants (Davis et al., 1984; Mills et al., 2001; Young et al., 2006). *Leifsonia poae* is also associated with plants (Evtushenko et al., 2000) but may occur in plant debris and soil. *Leifsonia aquatica* has been isolated from distilled water supplies and is supposed to occur in natural fresh water (Leifson, 1962). Strains of *Leifsonia naganoensis* and *Leifsonia shinshuensis* have been found in soil (Suzuki et al., 1999). A wide range of unnamed or preliminarily identified organisms exhibiting very high 16S rRNA gene sequence similarities to the recognized *Leifsonia* species have been discovered in various aquatic and terrestrial environments, either neutral, acidic, or alkaline (e.g. Fritz, 2000; Dore et al., 2003; Rickard et al., 2003; Tiago et al., 2004; Otsuka et al., 2008; Graff and Conrad, 2005; Inagaki et al.; (2006). They are also found in association with eukaryotic organisms (e.g. Nakabachi et al., 2003; Evtushenko at al. (2006); Wichels et al., 2006; Nishiwaki et al., 2007) and, as mentioned before, with clinical specimens (Bosshard et al., 2003; Dempsey et al., 2007; Funke et al., 1997).

Enrichment and isolation procedures

The rapidly growing *Leifsonia* strains can be isolated from various environments where they provisionally occur using the dilution plating technique and nutrient media containing peptone and/or yeast extract such as *Corynebacterium* (CB) agar (5 g glucose, 5 g yeast extract, 10 g casein peptone or Casamino acids (Difco), 5 g NaCl, and 15 g agar per liter; pH 7.2), R agar (Yamada and Komagata, 1972b), R2A agar (Difco), the medium developed for cultivation of *Caulobacter* (Leifson, 1962), and some other media. Antibiotics or other growth inhibitors, to which a certain *Leifsonia* species are resistant, may be added to the isolation medium to inhibit the development of other bacteria. Certain media and procedures have been described which were suitable for isolation of not yet identified members of the genus *Leifsonia* or closely related organisms (see, e.g. Janssen et al., 2002; Dore et al., 2003; Nakabachi et al., 2003; Tiago et al., 2004; Wichels et al., 2006; Nishiwaki et al., 2007; Anandham et al., 2008).

For isolation of the slowly growing xylem-associated *Leifsonia xyli*, certain general rules should be followed, among which the choice of suitable material and medium are of primary importance. If nonselective media are used, successful isolation of *Leifsonia xyli* subsp. *xyli* and *Leifsonia xyli* subsp. *cynodontis* requires surface sterilization of plant material and other precautions to avoid or reduce the growth of accompanying bacteria. The nonselective SC medium (Davis et al., 1980) or some selective media (Brumbley et al., 2002; Teakle and Ryan, 1992) can be employed. The following technique has been successfully used for isolation of *Leifsonia xyli* subsp. *xyli* from infected sugarcane (Young et al., 2006). Presumably infected sugarcane is scrubbed clean using scourers and detergent. Stalks are cut into three-eye sets and are immersed in 10% commercial bleach for 10 min, soaked for 10 min in distilled water, rinsed in distilled water, sprayed with 70% ethanol, and flame-sterilized. The end nodes are then excised and the xylem fluid from the central node and internodes is pressed out using positive sterile air pressure and collected in sterile sample tubes. A small amount of the xylem fluid is placed on a microscope slide and examined for *Leifsonia xyli* subsp. *xyli* cells using phase-contrast microscopy, their presence being taken as evidence of infection. Approximately 100 μl of xylem exudate is spread on modified M-SC plates (Brumbley et al., 2002) and incubated at 28°C for 4 weeks or longer. Any contaminants are excised daily and, once visible, *Leifsonia xyli* subsp. *xyli* colonies are restreaked onto fresh medium until only colonies of *Leifsonia xyli* subsp. *xyli* are present. Pure cultures are established by serial plating of single colonies as described by Brumbley et al. (2002). For isolation of *Leifsonia xyli* subsp. *cynodontis*, a selective complex SCMS medium has been developed (Davis and Augustin, 1984).

Maintenance procedures

Strains of the genus *Leifsonia* can be freeze-dried and preserved in liquid nitrogen by standard procedures. Different media, depending on growth requirement of certain species, are used. Pure cultures of *Leifsonia xyli* subsp. *xyli* grown in S8 broth (Davis et al., 1984; Haynes and Britz, 1989) with 0.05–0.1% (w/v) of glycine can be maintained in glycerol stocks at −80°C (Brumbley et al., 2002).

Differentiation of the genus *Leifsonia* from other genera

Phenotypic characteristics that delineate *Leifsonia sensu stricto* species from other genera of the family *Microbacteriaceae* are listed in Table 140. As the *Leifsonia* species are similar to some other genera of the family *Microbacteriaceae* in morphological and key chemotaxonomic characters, a phylogenetic analysis based on 16S rRNA gene sequences is necessary to enable differentiation of *Leifsonia* from other genera and consequently achieve clear-cut identification of an isolate. It should be emphasized that representatives of the genus *Leifsonia sensu stricto* are expected to fall into a coherent 16S rRNA-based phylogenetic cluster which includes the type species *Leifsonia aquatica*. When an isolate is found to be loosely associated with the *Leifsonia sensu stricto* cluster, identification can be achieved by the detailed comparative analysis of other conserved genes, e.g. encoding 23S rRNA or 16S–23S rRNA intergenic spacer sequences (ITS) and the phenotype including chemotaxonomic characteristics. The following chemotaxonomic characteristics are essential: the cell-wall peptidoglycan structure

(or at least the quantitative amino acid composition, including the ratio of D-and L-isomers of Dab), the composition of menaquinones, fatty acids, polar lipids, and, probably, polyamines. The analytical methods to determine such characteristics have been reviewed by Schumann et al. (2009).

Taxonomic comments

As indicated in the *Further descriptive information* section, the genus *Leifsonia* represents a phylogenetically rather heterogeneous taxon. Along with the *Leifsonia sensu stricto* species considered before, the genus includes six species (*Leifsonia aurea*, *Leifsonia bigeumensis*, *Leifsonia ginsengi*, *Leifsonia kribbensis*, *Leifsonia pindariensis* and, *Leifsonia rubra*) branching outside the phylogenetic radiation of *Leifsonia sensu stricto* (Figure 187 and Figure 173). The available data tend to suggest that these species are also distinct from *Leifsonia sensu stricto* at the phenotypic level (Table 158), although the currently available evidence for some species seems inconclusive. Thus, the above-mentioned six species might be regarded as species *insertae sedis* at present, until their taxonomic status has been re-examined. Some details concerned with the taxonomic and nomenclature issues, along with the history of the genus, are outlined below.

The first clearly documented representative of the genus *Leifsonia* was strain 150 (= ATCC 14665), described by E. Leifson (1962) as the type strain of a new species "*Corynebacterium aquaticum*". Subsequent extensive taxonomic studies (Collins et al., 1979; Collins and Jones, 1980; Döpfer et al., 1982; Komura et al., 1975; Park et al., 1987; Schleifer and Kandler, 1972; Yamada and Komagata, 1972a; Yamada et al., 1976) have shown its relatedness to organisms with group B peptidoglycan and hence assigned subsequently to the family *Microbacteriaceae* (Park et al., 1993, 1995). Based on 16S rRNA gene analysis, Rainey et al. (1994) suggested that this bacterium represents the nucleus of a novel genus. Takeuchi and Yokota (1994) and Sasaki et al. (1998) showed its close phylogenetic relatedness to *Clavibacter xyli* subsp. *cynodontis* which, along with *Clavibacter xyli* subsp. *xyli* and other plant-pathogenic bacteria with diamino butyric acid (Dab) in the cell wall, had been described as a member of the genus *Clavibacter* (Davis et al., 1984). The similarity of "*Corynebacterium aquaticum*" JCM 1368 and the type strain of *Clavibacter xyli* subsp. *cynodontis* in the cell-wall peptidoglycan amino acids (in particular, the presence of both L- and D-isomers of Dab) and the predominant menaquinones, which are in fairy good accord with the 16S rRNA phylogeny, led Sasaki and colleagues

TABLE 158. Characteristics differentiating species of the genus *Leifsonia*[a,b,c]

Characteristic	*Leifsonia* spp. (*sensu stricto*)	*L. aurea*	*L. bigeumensis*	*L. ginsengi*	*L. kribbensis*	*L. pindariensis*	*L. rubra*
Colony color	White to yellow or pale brown	Yellow	Yellow	White to yellow	Yellow	Yellow	Red or reddish
Cell morphology[d]	Short rods to filaments[e]	Curved rods	Short rods	Straight or curved rods	Straight to curved rods	Short rods	Curved rods
Motility	D+	−	+	−	+	+	−
Growth temperature (°C)[f]	26–30 (4–42)	22 (0–30)	28 (20–37)	26–30 (4–37)	28 (20–37)	~28	15 (0–22)
Catalase test	+	+	−	+	−	+	+
Salt requirement	−	−	+	−	−	−	−
Rhamnose in cell wall	+	−	+	+	+	+	+
Major menaquinones	MK-11, 10 or MK-11, 12	MK-11	MK-11	MK-11	MK-12, 11	MK-11	MK-11
Major (>10%) fatty acids:[g]							
$C_{15:0}$ anteiso	24.1–47.3[h]	43.7	36.0	19.6	19.7	54.3	37.5
$C_{17:0}$ anteiso	32.6–54.4[h]	28.5	18.2	10.5	(6.8)	19.1	20.3
$C_{16:0}$ iso	10–21	(5.0)	38.9	(8.3)	43.4	(6.8)	11.5
$C_{17:0}$ Ch				52.0			
Polar lipids[i]	GPG, PG, GLs		GPG, PG, GLs		DPG, PG, ULs	PG, DPG, UL	
DNA G+C content (mol%)	67.7–70.7[j]	64 (T_m)	69 (T_m)	66.4 (T_m)	73.4 (T_m)		66 (T_m)

[a]Data compiled from publications cited in Table 156 and from Dastager et al. (2008c, 2009); Qiu et al. (2007); and Reddy et al. (2008, 2003c).

[b]See also Figure 187 for phylogenetic clustering.

[c]Symbols: +, positive or present; −, negative or absent; D, different between species (character for the type species is indicated).

[d]Cell morphology is given in accordance with the species descriptions or the micrographs available (*Leifsonia kribbensis*).

[e]Cells or cell ends of some species become thicker and may reach up to 0.8–0.9 µm in diameter.

[f]Optimal temperature and temperature range for growth (in parentheses).

[g]See the species descriptions for details.

[h]Different amounts of $C_{15:0}$ anteiso (66%) and $C_{17:0}$ anteiso (15%) were reported for the type strain of *Leifsonia aquatica* grown at 20°C (below the temperature optimum).

[i]DPG, diphosphatidylglycerol; PG, phosphatidylglycerol; GLs, unknown glycolipids; UL(s), unknown lipid(s).

[j]See Table 156 for details.

(Sasaki et al., 1998) to the conclusion that a new genus should be established to accommodate these two strains. Besides, close phylogenetic relatedness of the two *Clavibacter xyli* subspecies, *Clavibacter xyli* subsp. *xyli* and *Clavibacter xyli* subsp. *cynodontis* was reported (Lee et al., 1997).

The description of the genus *Leifsonia* with the type species *Leifsonia aquatica* was almost simultaneously published by two research groups on the basis of comparative taxonomic study of different strains isolated from soil (Suzuki et al., 1999, 2000) and nematode-induced plant galls on *Poa annua* (Evtushenko et al., 2000). The genus name *Leifsonia* and binominal *Leifsonia aquatica* proposed by Evtushenko et al. (2000) were accepted as having priority according to the Bacteriological Code. Along with the proposal of *Leifsonia aquatica* and *Leifsonia poae*, Evtushenko at al. (2000) formally transferred *Clavibacter xyli* with two subspecies to the genus *Leifsonia* as new combinations (*Leifsonia xyli*, *Leifsonia xyli* subsp. *xyli*, and *Leifsonia xyli* subsp. *cynodontis*). Suzuki and colleagues (Suzuki et al., 1999) described the species *Leifsonia aquatica*, *Leifsonia naganoensis*, and *Leifsonia shinshuensis*, and reclassified *Clavibacter xyli* subsp. *cynodontis* as *Leifsonia cynodontis*. The reclassification was proposed in view of unavailability of the type strain of *Clavibacter xyli* (and *Clavibacter xyli* subsp. *xyli*) strain L1A^T [= NCPPB 3152 = PDDCC 7127 (ICMP 7127) = ATCC 33974 = LMG 7352] either from public culture collections or the authors of the species description (Davis et al., 1984).

Elevation of both subspecies of *Leifsonia xyli* to species level is not in disagreement with the 16S rRNA gene sequence similarity of *Leifsonia xyli* subsp. *xyli* and *Leifsonia xyli* subsp. *cynodontis*. Although rather high (>99.5% identity), similar values have been reported for many closely related species of the family *Microbacteriaceae*. As deduced from analysis of respective sequences available in GenBank, the similarity value (~85%) of the 16S–23S rRNA ITS region and the similarity value (~95.5%) of concatenate sequences of 16S rRNA gene sequences, 16S–23S rRNA ITS, and partial 23S rRNA gene sequences (total of ~2100 nucleotides) of these subspecies are also in the ranks of those found between other *Leifsonia* species. The *Leifsonia xyli* subspecies, according to Davis et al. (1984), are also distinguishable by their characteristic protein bands in polyacrylamide gels, some phenotypic characteristics (Table 156), and their pathogenic properties (each can induce symptoms in its natural host but not in the natural host of the other subspecies). Further taxonomic studies (paying attention to availability/unavailability of the nomenclatural type for *Leifsonia xyli*), could justify the separate species status of *Leifsonia xyli* subsp. *xyli* and *Leifsonia xyli* subsp. *cynodontis*. If the type strain cannot be recovered, a neotype strain of *Leifsonia xyli* has to be described according to the Bacteriological Code. A potential candidate for the neotype could be *Leifsonia xyli* subsp. *xyli* CTCB07 of which the complete genome sequence is now available (Monteiro-Vitorello et al., 2004) or another strain chosen among the genetically almost identical isolates (Young et al., 2006) which is better adapted for growth in culture.

Some other taxonomic and nomenclature comments are concerned with the species *Leifsonia aurea*, *Leifsonia bigeumensis*, *Leifsonia ginsengi*, *Leifsonia kribbensis*, *Leifsonia pindariensis*, and *Leifsonia rubra* which are here regarded as species *insertae sedis*. Among these species, *Leifsonia aurea*, *Leifsonia pindariensis*, and *Leifsonia rubra*, show more close phylogenetic relatedness to other genera: *Leifsonia aurea* and *Leifsonia rubra* are intermixed with species belonging to the genera *Rhodoglobus* (Sheridan et al., 2003) and *Salinibacterium* (Han et al., 2003), while *Leifsonia pindariensis* forms a very tight phylogenetic group with the genera *Microterricola* (Matsumoto et al., 2008) and *Phycicola* (Lee et al., 2008).

Different phenotypic characteristics recorded along with different interpretations of results of the 16S rRNA gene sequence comparisons, lead the authors of the descriptions of above-mentioned *Leifsonia* species and the closely related genera to discrepant taxonomic conclusions. In particular, Reddy et al. (2003c) found their strains to possess key chemotaxonomic characteristic of *Leifsonia* and described two novel species *Leifsonia aurea* and *Leifsonia rubra*. Sheridan et al. (2003), unaware of the above description, decided in favor of creating a novel genus and species, *Rhodoglobus vestalii*, for their isolate that is closely related to *Leifsonia rubra* (>99% 16S rRNA gene sequence identity). Sheridan et al. (2003) revealed a number of phenotypic characteristics that clearly distinguished *Rhodoglobus vestalii* from *Leifsonia* species and related genera. Those include small rods with unusual bulbous protuberances, the cell-wall peptidoglycan with ornithine as the diamino acid, and the menaquinone system with predominant MK-12 (65%) and MK-11 (35%) as well as a low G+C content (62 mol%). Both research groups also indicated the presence of a nearly identical 13 bp and 14 bp insertion in the 16S rRNA gene sequences of their strains that is absent in any of the type strains of *Leifsonia (sensu stricto)* species. They, however, ascribed different taxonomic significance to this fact.

Similarly, without being aware of the recent proposal of *Leifsonia pindariensis* and the emended description of the genus *Leifsonia* (Reddy et al., 2008), two other research groups independently established two different genera, *Phycicola* (Lee et al., 2008) and *Microterricola* (Matsumoto et al., 2008) to accommodate their soil and sewage isolates closely related to *Leifsonia pindariensis*. Along with phylogenetic distinction, the authors, who described the above-mentioned genera, stressed phenotypic differences of their strains from the *Leifsonia sensu stricto* species (Table 140). For instance, the major menaquinone MK-12 (69%), followed by MK-11 (21%) and MK-13 (10%) were indicated for *Microterricola viridari*, and an unusual life cycle with budding-like process and the polar lipid pattern with phosphatidylinositol and phosphatidylcholine were reported for *Phycicola gilvus*. However, re-examination of the polar lipids would be highly desirable because members of the *Microbacteriacea* are quite homogeneous in their polar lipid profiles and the presence of phosphatidylinositol and phosphatidylcholine is so far unique among members of the family. In addition, both *Microterricola viridari* and *Phycicola gilvus*, like *Leifsonia pindargiensis*, appear to differ from the *Leifsonia sensu stricto* species in the predominant fatty acids (increased amounts of $C_{15:0}$ anteiso and lower content of $C_{17:0}$ anteiso and $C_{16:0}$ iso). It is worth noting here that, although the quantitative fatty acid profiles are commonly considered to be unreliable for differentiation of closely related genera, the proportion of predominant fatty acids in *Leifsonia bigeumensis* and *Leifsonia kribbensis* are clearly beyond the range obtained for the *Leifsonia sensu stricto* species determined in cells grown at optimal temperature, irrespectively of the growth medium, culture age, and analytical procedure (Collins and Jones, 1980; Evtushenko et al., 2000; Suzuki and Komagata, 1983; Suzuki et al., 1999). Taken together, all the above data evidence that *Leifsonia aurea*, *Leifsonia pindariensis*,

and *Leifsonia rubra* will be reclassified as members of the phylogenetically closely related genera mentioned.

It should also be emphasized in this context that, according to An and Yokota (2007), the type strain of *Leifsonia rubra* is not available from any established culture collection nor from the authors who described this species (Reddy et al., 2003c). The strains received by An and Yokota (2007) from the culture collections (MTCC 4210, JCM 12417, CIP 107783, DSM 15304) and obtained from the authors (CMS 76r) belong to two different taxa. From the 16S rRNA gene sequence analyses, these are affiliated with *Arthrobacter roseus* (Reddy et al., 2002) and the *Gammaproteobacteria*. It was suggested that the Judicial Commission considers placing the name *Leifsonia rubra* on the list of rejected names if the strain is not found or a neotype is not proposed within 2 years (An and Yokota, 2007).

Note added in proof: after the Request for an Opinion by An and Yokota (2007), the original authors deposited the type strain in the BCCM/LMG culture collection as LMG 24410. According to the LMG, the strain LMG 24410 is authentic. The culture whose authenticity was confirmed by 16S rRNA gene sequencing by the JCM, was also deposited in JCM as JCM 15064. See An and Yokota (2007) and J.P. Euzéby's List of Prokaryotic Names with Standing in Nomenclature (http://www.bacterio.cict.fr/) for more details.

Each of the remaining *insertae sedis* species, i.e. *Leifsonia bigeumensis*, *Leifsonia ginsengi*, and *Leifsonia kribbensis*, does not form a robust 16S rRNA-based phylogenetic cluster with the genus *Leifsonia sensu stricto* or any other genus in the family *Microbacteriaceae*. The phylogenetic distinctness of these species from *Leifsonia sensu stricto* is accompanied by some distinct phenotypic properties, for instance, the negative catalase reaction (indicative of the presence of different factors preventing oxygen damage), major menaquinones, predominant fatty acids, polar lipid pattern, and the DNA base ratio (Table 158). The study of some other characteristics which are not yet determined for *Leifsonia bigeumensis*, *Leifsonia ginsengi*, and *Leifsonia kribbensis*, might provide additional differentiating traits. These include the cell-wall peptidoglycan structure (or at least the quantitative amino acid composition, including the ratio of D-and L-isomers of diaminobutyric acid), acyl type of muramic acid (glycolate test), polyamine amount and composition, detailed polar lipid and menaquinone patterns, and probably some other characteristics at the epigenetic level that delineate genera in other bacterial groups.

It is thus apparent that the current taxonomy of the genus *Leifsonia* is in need of extensive revision, aiming at improvement of phylogenetic and phenotypic coherency in clustering of newly described species. One may note here that the main trend in improving classification schemes of many bacterial groups, including genera of the order *Actinomycetales*, involves the dissecting of polyphyletic and phenotypically heterogeneous genera into monophyletic genera accompanied by more focused circumscription using genomic and epigenetic characteristics (Stackebrandt, 2006, and more recent publications in IJSEM).

Differentiation of species of the genus *Leifsonia*

The phenotypic characteristics useful for the differentiation of the *Leifsonia sensu stricto* species and subspecies are listed in Table 156 and Table 157. The source of isolation (sugarcane with the typical symptoms of stunting disease or other plants where they provisionally occur) and special techniques of purposeful isolation of the xylem-inhabiting *Leifsonia xyli* strains are prerequisites for the assignment of an isolate to *Leifsonia xyli*. Slow growing, slender, elongated Gram-stain-positive rods which show a tendency to coryneform morphology and do not grow on standard nutrient media, e.g. R medium (Suzuki et al., 1999), can be preliminarily and tentatively identified as members of *Leifsonia xyli* subsp. *xyli* or *Leifsonia xyli* subsp. *cynodontis*. The 16S–23S ITS sequence analysis (Fegan et al., 1998; Pan et al., 1998; Young et al., 2006) and the BOX and ERIC PCR fingerprinting (Young et al., 2006) are very useful to achieve a further sharper differentiation of *Leifsonia* species and subspecies. Some techniques and primers developed to detect the ratoon stunting disease of sugarcane (see Brumbley et al., 2006, for references) can be also employed to differentiate *Leifsonia xyli* subsp. *xyli* and *Leifsonia xyli* subsp. *cynodontis*.

The phenotypic characters differentiating the *Leifsonia insertae sedis* species from each other and from the *Leifsonia sensu stricto* group are listed in Table 158. Considering the descriptions of the genera *Microterricola*, *Phycicola*, *Rhodoglobus*, and *Salinimicrobium* is advisable when an isolate is phylogenetically close to *Leifsonia aurea*, *Leifsonia pindariensis*, or *Leifsonia rubra*.

Acknowledgements

This work was supported by the program MCB of the Russian Academy of Sciences. The contribution of Dr V.N. Akimov to the phylogenetic analysis of *Leifsonia* is deeply appreciated.

List of species of the genus *Leifsonia*

1. **Leifsonia aquatica** (*ex* Leifson 1962) Evtushenko, Dorofeeva, Subbotin, Cole and Tiedje 2000, 377[VP] (*Corynebacterium aquaticum* Leifson 1962, 161)

a.qua′ti.ca. L. fem. adj. *aquatica* pertaining to water, the source of isolation of the type strain of this species.

The description is based on information taken from Leifson (1962), Suzuki et al. (1999), and Evtushenko et al. (2000) unless indicated otherwise.

Cells are irregular rods (generally 0.4–0.5 × 1.2–2.5 µm) in 2-d cultures, tending to form primary branching and V-arrangements. In older cultures, short rods and coccoid cells predominate and occur singly, in pairs, or sometimes in short chains or clumps. A distinct rod–coccoid cycle may be observed on some media. Motile by long peritrichous flagella (10 µm or more). Colonies are circular, somewhat convex, glossy, and opaque, with entire margin. The colony color is white to yellowish white in young culture, becoming pale yellow on R agar or deep yellow on CB agar with age due to production of a light-enhanced carotenoid pigment (Trutko et al., 2005).

Obligatory aerobe. Catalase-positive; oxidase reaction is variable. Growth occurs at 7–37°C; optimum is ~26–30°C. Adonitol, cellobiose, dulcitol, D-fructose, D-galactose, D-glucose, glycerol, inositol, lactose, maltose, D-mannitol,

D-mannose, melezitose, melibiose, L-rhamnose, salicin, L-sorbose, sucrose, trehalose, turanose, and D-xylose are used for growth as carbon sources on basal mineral medium (Shirling and Gottlieb, 1966) supplemented with 0.1% (w/v) yeast extract and 0.1% (w/v) casitone. D-Arabinose, D-fucose, inulin, lyxose, *meso*-erythritol, raffinose, ribose, and sorbitol are not used as carbon sources on the same medium. Acids are produced from D-arabinose, D-fructose, D-glucose D-galactose, D-mannose, sucrose, D-xylose, and sorbitol (weakly) but not from D-lactose, melibiose, D-raffinose, tagatose, inositol, and inulin. The acid production from some carbohydrates is rather weak and the test results vary with experiments (Evtushenko et al., 2000; Leifson, 1962; Suzuki et al., 1999; Yamada and Komagata, 1972b). Acetate, citrate, malate, and pyruvate are assimilated, but formate, succinate, oxalate, and tartrate are not. H_2S is produced from peptone. Nitrate is reduced to nitrite. DNA, Tween 40, and starch are hydrolyzed; casein, cellulose, gelatin, hypoxanthine, xanthine, tyrosine, Tween 60, Tween 80, and urea are not hydrolyzed or decomposed. Esculin is decomposed, but the test result may vary with the method used (Evtushenko et al., 2000; Funke et al., 1994; Suzuki et al., 1999). Methyl red and Voges–Proskauer tests are negative. The type strain tolerates up to 5% (w/v) NaCl and 0.02% (w/v) potassium tellurite. The data on utilization of various carbon sources with the Biolog test system are listed in Table 157.

The major menaquinones are MK-11 and MK-10. Cell-wall sugars include a predominant amount of rhamnose and minor amounts of fucose, mannose, glucose, and galactose. The principal polar lipids are diphosphatidylglycerol, phosphatidylglycerol, and two characteristic glycolipids (glycosyldiacylglycerols) (Collins and Bradbury, 1986; Collins and Jones, 1980).

Source (type strain): distilled water.

DNA G+C content (mol%): 70.3 (HPLC), 69.3 (T_m).

Type strain: 150, ATCC 14665, CCUG 27700, CIP 64.13, DSM 20146, JCM 1368, NBRC 15710, NCIMB 9460, VKM Ac-1400.

Sequence accession no. (16S rRNA gene): D45057X77450.

2. **Leifsonia naganoensis** Suzuki, Suzuki, Sasaki, Park and Komagata 2000, 1415[VP] (Effective publication: Suzuki, Suzuki, Sasaki, Park and Komagata 1999, 260.)

na.ga.no.en′sis. N.L. fem. adj. *naganoensis* pertaining to the Nagano Prefecture, Japan, the geographical origin of the type strain of this species.

Cells are irregular rods (generally 0.3–0.5 × 1.8 to 3.0 µm in a 2-d culture). Colonies on R agar are glossy, smooth, with entire margin. White colonies of a 2-d culture become pale brown in old culture at 30°C.

Obligatory aerobe. Catalase-positive; oxidase-negative. Weak growth occurs at 37°C and no growth at 42°C. Acid is produced from glucose, fructose, galactose, maltose, mannose, L-rhamnose, melibiose, sucrose, D-trehalose, D-xylose, glycerol, mannitol, salicin, and amygdalin, but not from inulin. Fumarate, lactate, malate, *n*-butyrate, and pyruvate are assimilated, but formate, citrate, succinate, and propionate are not. Starch and DNA are hydrolyzed. Gelatin is not hydrolyzed. Nitrate is not reduced to nitrite. No growth is observed on R agar containing 5% NaCl. The data on utilization of various carbon sources with the Biolog test system are presented in Table 157. The predominant menaquinones are MK-11 and MK-10.

Source: a forest soil sample, Japan.

DNA G+C content (mol%): 70.5 (HPLC).

Type strain: DB103, CCUG 51931, CIP 106799, DSM 15166, JCM 10592, NBRC 103131.

Sequence accession no. (16S rRNA gene): DQ232612.

3. **Leifsonia poae** Evtushenko, Dorofeeva, Subbotin, Cole and Tiedje 2000, 378[VP]

po′a.e. N.L. gen. n. *poae* of *Poa*, generic name of the annual meadow grass *Poa annua*, the source of the type strain of this species.

The description is based on information taken from Evtushenko et al. (2000) and recent observations unless indicated otherwise.

The young culture (16–24 h) on CB agar displays slender, straight or slightly curved long cells or filaments which are occasionally branching and resemble rudimentary mycelium of some *Agromyces* species. The cell width is generally 0.4–0.6 µm, but sometimes cells appear polymorphic and with swells in the middle or at the ends and reach up to 0.8–0.9 µm in diameter. After 3–4 d cultivation, they break up into shorter irregular fragments (length 2.0–2.5 µm) which are usually motile. The motion type is similar to that of *Leifsonia aquatica*, assuming that *Leifsonia poae* also has peritrichous flagella. In older cultures, short pleomorphic rods are predominant and occur singly, in pairs, or in short chains with diphtheroid arrangements. Colonies are circular, somewhat convex, glistening, opaque, and with entire margins on CB agar. The colony color is white or yellowish white in young cultures to deep yellow with age due to production of a light-enhanced carotenoid pigment (Trutko et al., 2005). Growth occurs at 4–37°C; optimum growth is at 24–28°C.

Aerobic. Catalase-positive; oxidase reaction is variable between experiments (negative or weakly positive). D-Glucose, D-arabinose, cellobiose, D-fructose, D-galactose, lactose, maltose, D-mannose, raffinose, L-rhamnose, sucrose, D-xylose, glycerol, dulcitol, D-mannitol, methyl-α-D-glucopyranoside, and salicin are used as carbon sources on basal mineral medium (Shirling and Gottlieb, 1966) supplemented with 0.1% (w/v) yeast extract and 0.1% (w/v) casitone. D-Fucose, lyxose, melezitose, sorbose, ribose, trehalose, adonitol, *meso*-erythritol, inositol, sorbitol, dextran, and inulin are not assimilated on the same medium. In conventional tests, acids are produced from D-arabinose, D-fructose, D-galactose, mannose, and sucrose, but not from melibiose. Malate, malonate, and propionate are utilized, but no alkaline reaction is registered with citrate, formate, fumarate, gluconate, oxalate, succinate, or tartrate. H_2S is produced from cysteine but not from peptone. Esculin and gelatin (weakly) are decomposed. Casein, hypoxanthine, xanthine, starch, tyrosine, Tweens 40, 60, and 80 are not hydrolyzed. The data on utilization of various carbon sources with the Biolog test system (Qiu et al., 2007) are presented in Table 157. No growth is observed in the presence of up to 5% (w/v) NaCl; tolerant to potassium tellurite at 0.02% (w/v).

Cell-wall sugars are predominantly rhamnose and minor amounts of glucose, galactose, and mannose. The major menaquinone is MK-11 followed by MK-10. The polyamine

pattern includes putrescine (51.6%) as a predominant component and minor amounts of spermine (18.6%), tyramine (12.5%), spermidine (10.9%), and cadaverine (3.1%) (Altenburger et al., 1997).

Source: Poa annua root gall induced by the grass root gall nematode *Subanguina radicicola*.

DNA G+C content (mol%): 69.5 (T_m).

Type strain: DL89, CCUG 47140, CIP 106654, DSM 15202, JCM 11952, NBRC 103069, VKM Ac-1401.

Sequence accession no. (16S rRNA gene): AF116342, AM410682.

4. **Leifsonia shinshuensis** Suzuki, Suzuki, Sasaki, Park and Komagata 2000, 1415[VP] (Effective publication: Suzuki, Suzuki, Sasaki, Park and Komagata 1999, 260.)

shin.shu.en′sis. N.L. fem. adj. *shinshuensis* of or belonging to Shinshu, the old name of Nagano Prefecture, Japan, the geographical origin of the type strain of this species.

Cells are irregular rods, generally 0.3–0.4 × 2.5–3.0 µm in a 2-d culture. Colonies on R agar are glossy, smooth, and with entire margins, having white color in a 2-d culture and becoming pale brown in older culture at 30°C.

Obligatory aerobe. Catalase-positive; oxidase-negative. Growth occurs at 42°C but not at 45°C. Nitrate is not reduced to nitrite. Acid is produced from fructose, glycerol, and inulin, but not from D-glucose, galactose, maltose, mannose, melibiose, L-rhamnose, sucrose, D-trehalose, D-xylose, mannitol, salicin, and amygdalin. Lactate, formate, fumarate, malate, pyruvate, succinate, and propionate are utilized, but citrate and *n*-butyrate are not. Starch is hydrolyzed but casein, gelatin, and DNA are not. The data on utilization of various carbon sources with the Biolog test system are presented in Table 157. The predominant menaquinones are MK-11 and MK-12.

Source: forest soil, Japan.

DNA G+C content (mol%): 70.7 (HPLC).

Type strain: DB 102, CIP 106805, DSM 15165, JCM 10591, NBRC 103132.

Sequence accession no. (16S rRNA gene): DQ232614.

5. **Leifsonia xyli** (Davis et al. 1984) Evtushenko, Dorofeeva, Subbotin, Cole and Tiedje 2000, 378[VP] *(Clavibacter xyli* Davis, Gillespie, Vidaver and Harris 1984, 114)

xy′li. Gr. n. *xulon* wood; N.L. gen. n. *xyli* of wood, intended to mean of the xylem.

The description is based on information taken from Davis et al. (1984) unless indicated otherwise.

Cells are typically slender, straight or slightly curved rods, frequently arranged in V-like forms. Colonies are colorless to yellow on SC agar. Obligate aerobes. Catalase-positive; oxidase-negative. Optimal growth occurs at 26–30°C; no growth is observed at 37°C. Nutritionally very exacting. Acids are produced from oxidative fermentation of glucose and some other carbohydrates. Shows weak hydrolytic activities in conventional tests.

Naturally occurs in sugarcane (*Saccharum* interspecific hybrids), Bermudagrass [*Cynodon dactylon* (L.) Pers.], and some other plants (Davis et al., 1984; Young et al., 2006). Strains of this species are known to be casual agents of stunting (growth-hindering) diseases in their natural host plants.

The species *Leifsonia xyli* is subdivided into the two subspecies, *Leifsonia xyli* subsp. *xyli* and *Leifsonia xyli* subsp. *cynodontis*.

DNA G+C content (mol%): 66 (T_m) – 69 (HPLC).

Type strain: L1A, ATCC 33974, ICMP 7127, LMG 7352, NCPPB 3152, PDDCC 7127.

Sequence accession no. (16S rRNA gene): not available.

Additional comments: according to Suzuki et al. (1999) the type strain of this species and subspecies is not available from the ATCC, ICMP, LMG, and NCPPB collections or from the authors who described this species (see the *Taxonomic comments* section).

5a. **Leifsonia xyli subsp. xyli** (Davis et al. 1984) Evtushenko, Dorofeeva, Subbotin, Cole and Tiedje 2000, 378[VP] (*Clavibacter xyli* subsp. *xyli* Davis, Gillespie, Vidaver and Harris 1984, 114)

xy′li. Gr. n. *xulon* wood; N.L. gen. n. *xyli* of wood, intended to mean of the xylem.

The description is based on information taken from Davis et al. (1984) unless indicated otherwise.

Slender, straight or slightly curved rods, approximately 0.2 µm in diameter and 1.2–2.0 µm in length as measured on electron micrographs (Brumbley et al., 2006; Davis et al., 1984). A living bacterium has most likely slightly larger cells because samples for electron microscopy were dried as part of the fixation process (Brumbley et al., 2006). Longer cells, up to 5–6 µm, have been observed (Davis et al., 1984). These probably consist of divided cells remaining together after septum formation as shown on the micrograph provided by Brumbley et al. (2006) and our own microscopic observation of the other subspecies, *Leifsonia xyli* subsp. *cynodontis*. Cells can have swelling on one end and show V-arrangements. Motility by means of flagella is not observed. Colonies are colorless on SC agar, glossy, smooth, and circular with entire margins.

Obligately aerobic; aerobic growth is not stimulated by increased CO_2 concentration. Catalase-positive; oxidase-negative. Nitrate and nitrite are not reduced. Optimal growth occurs at 26–30°C; no growth is typically observed at 32°C. Acid is produced oxidatively from glucose, maltose, starch, and dextrin in conventional tests; no acid production from arabinose, cellobiose, fructose, galactose, lactose, melibiose, melezitose, raffinose, rhamnose, sucrose, xylose, adonitol, dulcitol, geraniol, inositol, sorbitol, ethanol, propanol, β-methyl-D-glucoside, esculin, arbutin, inulin, and salicin. Indistinct results (weak reactions) were observed with mannose, trehalose, glycerol, and α-methyl-D-glucoside. Casein, corn oil, esculin, gelatin, starch, Tween 20, Tween 80, tyrosine, and urea are not decomposed. KCN is tolerated at a concentration of 0.0075%. Acetoin is produced, but H_2S, indole and ammonium from peptone are not formed. Acetate, acetamide, anthranilate, benzoate, citrate, citroaconate, formate, fumarate, galacturonate, gluconate, glucuronate, glycolate, *p*-hydroxybenzoate, β-hydroxybutyrate, lactate, levulinate, malate, maleic acid, malonate, oxalate, propionate, saccharate, succinate, *m*-tartrate, and tryptamine are not utilized.

Invades vascular systems of sugarcane (*Saccharum* interspecific hybrids) and causes ratoon stunting disease of this

plant. Colonizes many other plants in experimental settings, occasionally inducing similar disease in *Saccharum* species (Gillaspie and Teakle, 1989; Matsuoka, 1971; Mills et al., 2001; Roach and Jackson, 1992; Young et al., 2006).

Source: sugarcane (*Saccharurn* interspecific hybrids), Louisiana, USA.

DNA G+C content (mol%): 66 (T_m).

Type strain: L1A, ATCC 33974, ICMP 7127, LMG 7352, NCPPB 3152, PDDCC 7127.

Sequence accession no. (16S rRNA gene): not available.

Additional comments: according to Suzuki et al. (1999) the type strain of this species and subspecies is not available from the ATCC, ICMP, NCPPB, and LMG collections or from the authors who described this species (see the *Taxonomic comments* section).

5b. **Leifsonia xyli subsp. cynodontis** (Davis et al. 1984) Evtushenko, Dorofeeva, Subbotin, Cole and Tiedje 2000, 378[VP] (*Clavibacter xyli* subsp. *cynodontis* Davis, Gillaspie, Vidaver and Harris 1984, 114)

cy.no.don′tis. N.L. n. *Cynodon* genus of Bermudagrass; N.L. gen. n. *cynodontis* of Bermudagrass.

The description is based on information taken from Davis et al. (1984), Suzuki et al. (1999), Evtushenko et al. (2000), and recent observations, unless indicated otherwise.

Slender, straight or slightly curved rods, generally, 0.4 × 3.0–4.0 µm in a 2-d culture, tending to form primary branching. In older cultures, shorter cells (approximately 1.5–2.0 µm) predominate and occur singly or in pairs, or can be disposed at an angle to each other, giving V-forms. Divided cells frequently remain together after septum formation and thus may look appreciably longer (up to 5–6 µm). Cells can have swelling on one end. Colonies on SC agar are glossy, smooth, circular, with entire margins, and pale yellow in a weak culture at 28–30°C. The pigmentation intensifies with the age of culture.

Obligate aerobes. Growth is not stimulated by increased CO_2 concentration. Catalase-positive. Oxidase-negative. Optimal growth occurs at 26–30°C; no growth is observed at 37°C. Oxidative acid production from glucose, mannose, maltose, trehalose, glycerol, mannitol, dextrin, and starch; no acid production was observed in conventional tests from arabinose, cellobiose, fructose, galactose, lactose, melibiose, melezitose, raffinose, rhamnose, ribose, sucrose, xylose, adonitol, dulcitol, inositol, sorbitol, ethanol, propanol, propylene glycol, α-methyl-D-glucoside, β-methyl-D-glucoside, esculin, arbutin, inulin, and salicin. Starch is hydrolyzed, but casein, corn oil, esculin, gelatin, Tween 20, Tween 80, tyrosine, and urea are not decomposed. KCN is tolerated at a concentration of 0.0075%. Nitrate and nitrite are not reduced. Acetoin is produced, but production of H_2S, indole, or ammonium from peptone is not observed. Citrate and malate are utilized, but acetamide, anthranilate, benzoate, citroaconate, *p*-hydroxybenzoate, β-hydroxybutyrate, formate, fumarate, galacturonate, gluconate glucuronate, glycolate, lactate, levulinate, malate, maleic acid, malonate, oxalate, propionate, saccharate, succinate, *m*-tartrate, and tryptamine are not utilized. The data on utilization of various carbon sources revealed by the Biolog test system are listed in Table 157.

Cell-wall sugars are rhamnose, fucose, and trace amount of galactose (Davis et al., 1984); somewhat different cell-wall sugar pattern, i.e. rhamnose, galactose, glucose, and small quantities of fucose and mannose, was determined using different methods of cell-wall preparation and analysis (Evtushenko et al., 2000). The predominant menaquinones are MK-11 and MK-12.

Naturally occurring in Bermudagrass [*Cynodon dactylon* (L.) Pers.] and probably some other plants (Fahey et al., 1991; Kostka et al., 1988; Lampel et al., 1994; Li et al., 2007). Under experimental settings induces stunting disease in Bermudagrass and colonizes many other plants without producing clear disease symptoms (Davis et al., 1984; Young et al., 2006).

Source: Bermuda grass (*Cynodon dactylon*), Taiwan.

DNA G+C content (mol%): 69.4 (HPLC).

Type strain: TB1A, ATCC 33973, CIP 104615, DSM 46306, ICMP 8790, JCM 9733, LMG 7552, NCIMB 11927, NCPPB 3151, PDDCC 7126, VKM Ac-2041.

Sequence accession no. (16S rRNA gene): AB016985.

Species *incertae sedis*

1. **Leifsonia aurea** Reddy, Prakash, Srinivas, Matsumoto and Shivaji 2003c, 982[VP]

au′re.a. L. fem. adj. *aurea* golden, referring to the yellowish pigment that the bacterium produces.

Cells are curved, nonmotile rods. Colonies are circular, convex, smooth, butyrous, opaque, and yellow-pigmented. The pigment is supposedly a carotenoid (exhibits four peaks at 390, 414, 438, and 468 nm in methanol solution). Gram-stain-positive. Aerobic; catalase-positive. Oxidase-negative (Kovacs, 1956). The temperature range for growth is 0–30°C, optimum 22°C, and the pH range is 6–11, optimum pH 7 (initial pH values). Strains grow at salt concentrations up to 3% NaCl. Nitrate is reduced to nitrite.

The following carbon sources are used for growth and energy on minimal medium: D-glucose, D-fructose, D-galactose, D-mannose, L-rhamnose, D-ribose, D-sorbose, trehalose, glucosamine, erythritol, glycerol, acetate, pyruvate, L-arginine, L-glutamic acid, and L-phenylalanine. Does not grow on the same medium supplemented with L-arabinose, D-cellobiose, cellulose, lactose, D-maltose, D-melibiose, D-raffinose, sucrose, D-xylose, dextran, glycogen, inulin, adonitol, dulcitol, inositol, D-mannitol, melezitol, sorbitol, acetate, citrate, β-hydroxybutyrate, lactate, myristic acid, succinate, L-alanine, L-glycine, L-lysine, L-methionine, L-serine, L-tyrosine, L-tryptophan, L-glutamine, creatinine, and polyethylene glycol phthalate. Does not produce acid from L-arabinose, D-fructose, D-galactose, D-glucose, lactose, D-maltose, D-mannose, L-rhamnose, sucrose, or D-xylose. Strains show positive reactions in the tests for production of acetoin and β-galactosidase; negative for production of H_2S, lipase, phosphatase,

urease, and indole and hydrolysis of esculin, gelatin, and starch. Methyl red test is negative.

Strains are sensitive to the following antibiotics (amount per disk): ampicillin (10 μg), kanamycin (40 μg), novobiocin (30 μg), penicillin (10 U), rifampin (10 μg), streptomycin (10 μg), tetracycline (30 μg) tobramycin (15 μg), vancomycin (30 μg), chloramphenicol (30 μg), as well as amikacin, cefaperazone, cephatoxime, cefazoline, cefuroxime, amoxycillin, ciprofloxacin, and roxithromycin (unknown concentrations). The strains resisted erythromycin (15 μg) and also colistin, nitrofurantoin, cotrimoxazole, nor-floxacin, and lincomycin (unknown concentrations).

The peptidoglycan has been reported to contain DL-diaminobutyric acid as the diamino acid, along with glycine, glutamic acid, and alanine. The sugars determined by the method of Staneck and Roberts (1974) are galactose, glucose, and ribose. The fatty acid profile displays $C_{15:0}$ anteiso (43.7%), $C_{17:0}$ anteiso (28.2%), $C_{16:0}$ iso (5.0%), $C_{15:0}$ iso (3.3%), $C_{17:0}$ iso (6.3%), $C_{14:0}$ iso (0.2%), $C_{14:0}$ (0.2%), $C_{15:0}$ (1.6%), $C_{16:0}$ (1.4%), $C_{18:0}$ (4.3%), and $C_{18:1}$ (4.1%).

Six strains, including the type strain were isolated from a cyanobacterial mat sample, the Wright Valley in McMurdo, Antarctica, by using dilution plating method on Antarctic bacterial medium (ABM) containing 0.5% (w/v) peptone, 0.2% (w/v) yeast extract, and 1.5% (w/v) agar; pH 6.9 with incubation at 5°C.

Source: a cyanobacterial mat sample, the Wright Valley in McMurdo, Antarctica.

DNA G+C content (mol%): 64.0 (T_m).

Type strain: CMS 81y, CIP 107785, DSM 15303, JCM 12762, MTCC 4657.

Sequence accession no. (16S rRNA gene): AJ438586.

Additional comments: the 16S rRNA gene sequence analyses show that *Leifsonia aurea* is clustered with *Leifsonia rubra* and the genera *Rhodoglobus* (Sheridan et al., 2003) and *Salinibacterium* (Han et al., 2003). Based on the studies of nucleic sequences Sheridan et al. (2003) suggested that *Leifsonia aurea* may be included in the genus *Rhodoglobus*. Further studies are required to clarify its phenotypic, including chemotaxonomic, characteristics and taxonomic position.

2. **Leifsonia bigeumensis** Dastager, Lee, Ju, Park and Kim 2008c, 1936[VP]

bi.ge.um.en′sis. N.L. fem. adj. *bigeumensis* of or belonging to Bigeum Island, Korea, the geographical origin of the type strain of this species.

Cells are short, motile rods. Colonies are circular, convex, smooth, butyrous, opaque yellowish-white. Aerobic; catalase and oxidase-negative. Growth of the type strain was reported to occur within a relatively narrow temperature range (20–37°C), with an optimal growth temperature of 28°C, and at pH 6–12, with optimum pH 8 (initial pH values). Slightly halophilic, growing at 3% NaCl, with no growth in the absence of NaCl. Nitrate is reduced to nitrite. Utilizes D-cellobiose, D-melibiose, D-raffinose, D-rhamnose, trehalose, *m*-inositol, and D-mannitol as a sole source of carbon and fails to utilize L-arabinose, D-fructose, D-galactose, D-glucose, D-mannose, and D-ribose. Does not produce acid from L-arabinose, D-fructose, D-galactose, D-glucose, D-maltose, and D-mannose. Hydrolyzes gelatin and starch but not esculin. Negative for urease and H_2S production.

The peptidoglycan contains DL-diaminobutyric acid as the diamino acid, along with alanine, glycine, and glutamic acid. The cell-wall sugars are galactose, glucose, mannose, and rhamnose. The major menaquinone is MK-11. The polar lipids are diphosphatidylglycerol, phosphatidylglycerol, and unidentified glycolipids. The predominant cellular fatty acids are $C_{16:0}$ iso (38.9%), $C_{15:0}$ anteiso (36.0%), and $C_{17:0}$ anteiso (18.2%); other fatty acids detected are $C_{15:0}$ iso (2.4%), $C_{14:0}$ iso (1.9%), $C_{16:0}$ (1.5%), and $C_{17:0}$ iso (1.1%).

The type strain was isolated from a soil sample from the Bigeum Island, Republic of Korea by the standard dilution plating technique at 30°C on ten-fold-diluted R2A agar (Difco) with pH adjusted to 8.0 using Na_2CO_3.

Source: a soil sample from the Bigeum Island, Republic of Korea.

DNA G+C content (mol%): 69 (T_m).

Type strain: MSL-27, DSM 19322, KCTC 19268.

Sequence accession no. (16S rRNA gene): EF466124.

Additional comments: the 16S rRNA-based phylogenetic analysis indicates *Leifsonia bigeumensis* to be phylogenetically distinct from the *Leifsonia sensu stricto* clade (Figure 187). This species is also distinguished by its negative catalase reaction, salt requirement for growth, and a number of other phenotypic characteristics (see Table 158, the Taxonomic Comments section, and the original description of Dastager et al., 2008c, for details). It seems quite likely that this species will be reclassified as a member of a new genus within the family *Microbacteriaceae*.

3. **Leifsonia ginsengi** Qiu, Huang, Sun, Zhang, Liu and Song 2007, 407[VP]

gin.sen′gi. N.L. gen. n. *ginsengi* of ginseng, the source of the type strain of this species.

Cells are straight or curved, nonmotile rods, 0.6–0.8 × 1.2–2.5 μm. Colonies on CB agar are white to yellow, circular, convex, glistening, opaque, butyrous. Aerobic; catalase-positive; oxidase-negative. Growth occurs at 4–37°C; optimum growth is at 26–30°C. Growth occurs at 1% but not at 3% NaCl. Distinct positive reactions are obtained in the Biolog GP test system with the following substrates: D-glucose, D-cellobiose, D-fructose, maltose, D-mannose, D-ribose, D-sorbitol, sucrose, turanose, D-xylose, glycerol, D-mannitol, γ-hydroxybutyric acid, pyruvic acid, pyruvic acid methyl ester, adenosine, 2′-deoxyadenosine, and thymidine. Weak reactions are observed with acetic acid, dextrin, 2,3-butanediol, and Tween 40. Positive for hydrolysis of starch and indole production, but negative for production of urease, acetoin, and hydrolysis of gelatin (results of conventional tests).

Susceptible to the following antibiotics (μg/ml): tetracycline (10), erythromycin (15), rifampin (30), and chloramphenicol (10); resistant to ampicillin (10), lincomycin (15), streptomycin (10), kanamycin (15), and penicillin (10 U/ml).

Cell-wall peptidoglycan has been reported to contain 2,4-diaminobutyric acid along with alanine, glutamic acid and glycine. Cell-wall sugars are galactose, glucose, mannose, and rhamnose. The menaquinone system includes MK-11 as the major menaquinone and minor amount of MK-10 and trace amount of MK-12. The fatty acid profile contains a large amount (52%) of ω-cyclohexyl-undecanoic ($C_{17:0}$ Ch) acid that is unusual for members of the genus *Leifsonia*, and

smaller but significant amounts of $C_{15:0}$ anteiso (19.6%), $C_{17:0}$ anteiso (10.5%), and $C_{16:0}$ iso (8.3%).

The type strain was isolated from a suspension of a surface-sterilized ginseng root using nitrogen-free agar medium (Von Bulow and Dobereiner, 1975) and diluting plating method.

Source: a suspension of a surface-sterilized ginseng root.

DNA G+C content (mol%): 66.4 (T_m).

Type strain: wged11, CGMCC 4.3491, CIP 109552, DSM 19088, JCM 13908.

Sequence accession no. (16S rRNA gene): DQ473536.

Additional comments: as deduced from the 16S rRNA gene sequence analysis, *Leifsonia ginsengi* forms a separate phyletic line outside the *Leifsonia sensu stricto* clade (Figure 187). Besides the fatty acid composition, this species also differs from the *Leifsonia sensu stricto* in many physiological and biochemical characteristics (see the original description of Qiu et al., 2007, for details). It is most likely that *Leifsonia ginsengi* will be reclassified as a member of a new genus within the family *Microbacteriaceae*.

4. **Leifsonia kribbensis** Dastager, Lee, Ju, Park and Kim 2009, 19[VP]

krib.ben′sis. N.L. fem. adj. *kribbensis* pertaining to KRIBB, an arbitrary adjective formed from the acronym of the Korea Research Institute of Bioscience and Biotechnology, where taxonomic studies of this species were performed.

Cells are generally straight to slightly curved rods (approximately 0.6 µm or more × 0.9–1.3 µm) occurring singly or in pairs. Gram-stain-positive (KOH test); non-acid-fast. Colonies are yellow-colored, circular, small (0.6–1.8 mm in diameter), glistening, opaque, and entire on diluted R2A medium. Aerobic. Catalase-negative. Oxidase reaction determined using *N,N,N,N* -tetramethyl-*p*-phenylene diamine dihydrochloride is negative. Nitrate is not reduced to nitrite.

Growth of the type strain was reported to occur within a relatively narrow temperature range (20–37°C), with an optimal growth temperature of 28°C. Growth was observed at initial pH values of 6.0–11.0; with optimum at 8.0. Tolerates up to 3.0% NaCl on tenfold diluted R2A medium.

The type strain uses D-cellobiose, D-melibiose, D-raffinose, D-rhamnose, and *m*-inositol as carbon sources, while D-glucose, D-fructose, D-galactose, D-mannose, and D-ribose are not used for growth. Acid is weakly produced from L-rhamnose and sucrose; no acid production from D-glucose, L-arabinose, D-fructose, D-galactose, D-maltose, and D-mannose. The type strain is positive for decomposition of casein, starch, Tween 80, and tyrosine; negative for degradation of cellulose, elastin, gelatin, pectin, and xylan. No H_2S production is observed. No growth occurs in the presence of phenol (0.3%).

The diagnostic cell-wall diamino acid is diaminobutyric acid. The whole cell sugars are glucose, rhamnose, ribose, and galactose. The major menaquinones are MK-12 and MK-11. The phospholipids detected are diphosphatidylglycerol and phosphatidylglycerol; some unidentified lipids additionally occur. The fatty acid profile includes $C_{16:0}$ iso (43.4%), $C_{15:0}$ anteiso (19.7%), $C_{17:1}$ anteiso ω9c (8.7%), $C_{14:0}$ iso (7.8%), $C_{17:0}$ anteiso (6.8%), $C_{15:0}$ iso (5.3%), and $C_{17:0}$ iso (2.1%).

The type strain was isolated from a soil sample, Bigeum Island, Korea, by the dilution-plating technique on ten-fold diluted R2A medium (Reasoner and Geldreich, 1985; Difco).

Source: a soil sample, Bigeum Island, Korea.

DNA G+C content (mol%): 73.4 (T_m).

Type strain: MSL-13, DSM 19272, KCTC 19267.

Sequence accession no. (16S rRNA gene): EF466129.

Additional comments: the 16S rRNA-based analysis indicates that *Leifsonia kribbensis* is phylogenetically distinct from the *Leifsonia sensu stricto* clade (Figure 187). This species is also distinguished from the *Leifsonia sensu stricto* due to its menaquinone composition, the fatty acid profile with predominant amounts of iso-branched acids making up to nearly 60% of the total, a slightly higher G+C content, and physiological and biochemical characteristics (see Table 158, the Taxonomic Comments section, and the original description of Dastager et al., 2008c, for details). It is most likely that *Leifsonia kribbensis* will be reclassified as a member of a new genus within the family *Microbacteriaceae*.

5. **Leifsonia pindariensis** Reddy, Prabagaran and Shivaji 2008, 2231[VP]

pin.da.ri.en′sis. N.L. fem. adj. *pindariensis* of or belonging to the Pindari glacier of Himalayan region, India, the geographical origin of the type strain of this species.

Cells are curved, short, and motile rods. Colonies are yellow pigmented, convex, and opaque. Gram-stain-positive. Aerobic; catalase-positive. Oxidase reaction is negative. Nitrate is not reduced to nitrite. The type strain of this species as determined by using conventional methods and commercial test systems (HiMedia, Mumbai, India) utilizes D-glucose, lactose, D-maltose, D-mannose, D-melibiose, D-ribose, L-sorbose, dulcitol, glycerol, erythritol, glucosamine, salicin, inulin, D-glucuronic acid, L-arginine, L-asparagine, L-cysteine, L-methionine, and L-valine, but not D- and L-arabinose, D-cellobiose, D-fructose, galactose, maltose, D-melezitose, D-raffinose, rhamnose, sucrose, trehalose, D- and L-xylose, *meso*-inositol, D-mannitol, sorbitol, xylitol, citrate, gluconate, malonate, α-methyl-D-glucoside, α-methyl-D-mannoside, L-alanine, L-aspartic acid, L-glutamine, L-histidine, L-isoleucine, L-leucine, L-lysine, L-phenylalanine, L-proline, L-serine, L-threonine, and L-tyrosine. Produces acid from D-maltose and D-mannose, but not from D-arabinose, D-fructose, D-galactose, D-glucose, D-melibiose, salicin, sucrose, and D-xylose. Shows positive reactions in the tests for phenylalanine deamination, and acetoine and indole production. Produces β-galactosidase and urease, but not phosphatase, ornithine decarboxylase, and lysine decarboxylase.

The type strain was reported to be sensitive to various antibiotics (µg per disc): amikacin (30), ampicillin (10), bacitracin (10), carbenicillin (100), cefazolin (30), cefoperazone (75), cefotaxime (30), cefuroxime (30), chloramphenicol (30), ciprofloxacin (5), co-trimoxazole (25), doxycycline (10), erythromycin (15), gentamicin G (30), kanamycin (30), leukomycin (30), lomefloxacin (30), nitrofurantoin (300), novobiocin (30), oleandomycin (15), penicillinG (10), rifampin (30), roxithromycin (30), spectinomycin (100), streptomycin mycin (10), tetracycline (30), and vancomycin (30). Resistant to colistin (10), nalidixic acid (30), norfloxacin (10), polymixin B (50), and tobramycin (10).

The cell-wall peptidoglycan is based upon 2,4-diaminobutyric acid. The cell-wall sugars include rhamnose and trace

amounts of glucose, galactose, and mannose. The menaquinones are MK-9 (minor), MK-10 and MK-11 (major). Polar lipids are phosphatidylglycerol and diphosphatidylglycerol, with a minor amount of an unknown lipid. The fatty acid profile displays $C_{15:0}$ anteiso (54.3%), $C_{17:0}$ anteiso (19.1%), $C_{16:0}$ iso (6.8%), $C_{15:0}$ iso (5.6%), $C_{16:0}$ (1.9%), and $C_{17:0}$ iso (2.0%) as well as the set of fatty acids constituting 0.5–0.9% ($C_{14:0}$, $C_{11:0}$ anteiso, $C_{12:0}$ anteiso, $C_{13:0}$ anteiso, $C_{14:0}$ iso, and $C_{15:1}$ isoG).

The type strain was isolated from a soil sample collected near the Pindari glacier, Himalayas, by the following procedure. Approximately 200 mg of the soil sample was suspended in 0.9% NaCl; the suspension, after shaking for 1 h, was allowed to settle and plated (100 µl) on agar medium containing 0.03 g of beef extract, 0.05 g of peptone, 0.08 g of NaCl, and 15 g of agar per liter.

Source: a soil sample collected near the Pindari glacier, Himalayas.

DNA G+C content (mol%): no data available

Type strain: PON10, LMG 24222, JCM 15132, MTCC 9128.

Sequence accession no. (16S rRNA gene): AM900767.

Additional comments: the 16S rRNA gene sequence of *Leifsonia pindariensis* is nearly identical to that of *Microterricola viridarii*, but these species exhibit clear difference in a wide range of phenotypical characteristics. It is most likely that *Leifsonia pindariensis* will be reclassified as a new species of the genus *Microterricola.*

6. **Leifsonia rubra** Reddy, Prakash, Srinivas, Matsumoto and Shivaji 2003c, 982[VP]

rub′ra. L. fem. adj. *rubra* reddish, referring to the reddish pigment produced by the bacterium.

Cells are nonmotile, curved rods. Colonies are circular, convex, smooth, butyrous, opaque, red-pigmented, and 1–2 mm in diameter. Catalase-positive. Negative for oxidase activity tested by the method of Kovacs (1956). Growth occurs at 0–22°C and at pH 6–12 (initial pH). Tolerant to 3% NaCl. Optimum growth is observed at 15°C, pH 7. Nitrate is reduced to nitrite. The following carbon sources were reported to be used for growth and energy: D-glucose, D-fructose, D-galactose, L-rhamnose, D-ribose, D-mannose, D-sorbose, trehalose, glycerol, L-glutamate, pyruvate, glucosamine, and L-phenylalanine as sole carbon sources. Fails to utilize L-arabinose, D-cellobiose, lactose, D-maltose, D-melibiose, D-raffinose, sucrose, D-xylose, cellulose, dextran, adonitol, dulcitol, *meso*-erythritol, inositol, D-mannitol, melezitol, sorbitol, citrate, β-hydroxybutyrate, lactate, myristic acid, phthalate, succinate, L-alanine, L-arginine, L-glycine, L-glutamine, L-lysine, L-methionine, L-serine, L-tyrosine, L-tryptophan, glycogen, inulin, polyethylene glycol, and creatinine. Does not produce acid from L-arabinose, D-fructose, D-galactose, glucosamine, lactose, D-maltose, D-mannose, L-rhamnose, sucrose, or D-xylose. Positive for β-galactosidase and negative for urease, lipase, indole production, in methyl red and Voges–Proskauer tests, H_2S production and does not decompose esculin, gelatin, or starch.

Sensitive to the following antibiotics (amount per disk): amikacin (10 µg), ampicillin (10 µg), kanamycin (40 µg), erythromycin (15 µg), novobiocin (30 µg), streptomycin (10 µg), tetracycline (30 µg), tobramycin (15 µg), rifampin (10 µg), and vancomycin (30 µg) as well as cefaperazone, cefazoline, cephatoxime, and lincomycin (unknown concentrations). Strains are resistant to colistin (10 µg), chloramphenicol (30 µg), and penicillin (10 U), as well as to cefuroxime, ciprofloxacin, cotrimoxazole, nitrofurantoin, norfloxacin, and roxithromycin (unknown concentrations).

The cell-wall peptidoglycan was reported to contain DL-diaminobutyric acid as the diamino acid, along with glycine, glutamic acid, and alanine. The whole cell sugars are galactose, glucose, ribose, and rhamnose. The fatty acid profile reported consists of $C_{15:0}$ anteiso (37.5%), $C_{17:0}$ anteiso (20.3%), $C_{16:0}$ iso (11.5%), $C_{15:0}$ iso (7.8%), $C_{17:0}$ iso (1.8%), $C_{14:0}$ iso (0.5%), $C_{14:0}$ (1.3%), $C_{15:0}$ (3.6%), $C_{16:0}$ (1.8%), $C_{18:0}$ (3.7%), and $C_{18:1}$ (5.0%).

The type strain of this species was isolated from a cyanobacterial mat sample, the Wright Valley in McMurdo, Antarctica, using dilution plating method. Isolation medium was Antarctic bacterial medium (ABM) containing 0.5% (w/v) peptone, 0.2% (w/v) yeast extract, and 1.5% (w/v) agar; pH 6.9 with incubation at 5°C.

Source: a cyanobacterial mat sample, the Wright Valley in McMurdo, Antarctica.

DNA G+C content (mol%): 66.0 (T_m).

Type strain: S. Shivaji CMS 76r, LMG 24410, JCM 15064.

Sequence accession no. (16S rRNA gene): AJ438585.

Additional remarks: Leifsonia rubra is closely related to *Rhodoglobus vestalii* (99.4% 16S rRNA gene sequence identity) and most likely belongs to the genus *Rhodoglobus* as suggested by Sheridan et al. (2003). Further studies could clarify its phenotypic, including chemotaxonomic, characteristics and taxonomic position.

Note added in proof: the type strain of *Leifsonia rubra* is now available from the LMG and JCM culture collections (see the *Taxonomic comments* section for details).

Genus XIV. **Leucobacter** Takeuchi, Weiss, Schumann and Yokota 1996, 970[VP]

Akira Yokota

Leu.co.bac′ter. Gr. adj. *leukos* clear, light; N.L. masc. n. *bacter* rod; N.L. masc. n. *Leucobacter* colorless rod.

Gram-stain-positive, nonsporeforming, nonmotile rods. No mycelium is produced. Catalase is produced, but oxidase, arginine dihydrolase, hydrogen sulfide, and urease are not produced. The cell-wall peptidoglycan contains **2,4-diaminobutyric acid (DAB)** as the characteristic diamino acid. **The peptidoglycan acyl type is acetyl.** The quinone system contains either menaquinones MK-10 and MK-11 (*Leucobacter tardus*) or MK-11 only as major isoprenoid quinone. Mycolic acids are not

present. The major cellular fatty acids are **$C_{15:0}$ anteiso, $C_{16:0}$ iso, and $C_{17:0}$ anteiso. Diphosphatidylglycerol, phosphatidylglycerol, and one unknown glycolipid** are present.

The genus *Leucobacter* is a member of the family *Microbacteriaceae*.

DNA G+C content (mol%): 62.8–69.5.

Type species: **Leucobacter komagatae** Takeuchi, Weiss, Schumann and Yokota 1996, 970[VP].

Further descriptive information

Phylogenetically, the genus *Leucobacter* belongs to the family *Microbacteriaceae* and presently comprises eleven recognized species (Figure 189). All species of the genus are characterized by 2,4-diaminobutyric acid (DAB) as the diagnostic amino acid in the peptidoglycan. However, some differences in the amino acid composition of the peptidoglycan have been reported; in addition to DAB, glutamic acid, alanine, and glycine, *Leucobacter komagatae*, *Leucobacter albus*, *Leucobacter chironomi*, and *Leucobacter salcicius* contain γ-aminobutyric acid (GABA), and *Leucobacter aerolatus*, *Leucobacter alluvii*, *Leucobacter chironomi*, *Leucobacter chromiireducens*, *Leucobacter iarius*, and *Leucobacter luti* contain threonine. Detailed analysis of the genes encoding these differences might reveal the evolutionary background of these differences.

All species contain MK-11 as the major menaquinone except *Leucobacter tardus* which exhibits a quinone system with MK-10 and MK-11 predominantly; MK-12, MK-10, and/or MK-9 are detectable in minor amounts.

The genus *Leucobacter* can be differentiated from closely related genera of the family *Microbacteriaceae* based on chemotaxonomic and phylogenetic data as shown in Table 159.

Isolation and maintenance procedures

Strains belonging to the genus *Leucobacter* occur in a variety of ecological niches such as airborne (Martin et al., 2010; Takeuchi et al., 1996), soil (Lin et al., 2004), river sediment (Morais et al., 2006b), activated sludge (Morais et al., 2004; Morais et al., 2006b), chironomid egg mass (Halpern et al., 2009), contaminant of *Caenorhabditis elegans* (Muir and Tan, 2007), nematode (Somvanshi et al., 2007), Korean fermented food (Yun et al., 2011), and phylosphere of potato plants (Behrendt et al., 2008b). The majority of *Leucobacter* strains recover well from cultures grown on nutrient agar, Luria–Bertani (LB) agar, or PY-BHI (peptone, 5 g; yeast extract, 1 g; brain heart infusion, 1 g; and water, 1 liter; pH 7.2) agar stored at 5°C for 2 months. Lyophilization or deep freezing (−80°C) in glycerol solution are recommended for long-term preservation.

Differentiation of species of the genus *Leucobacter*

Characteristics distinguishing *Leucobacter* species are presented in Table 160.

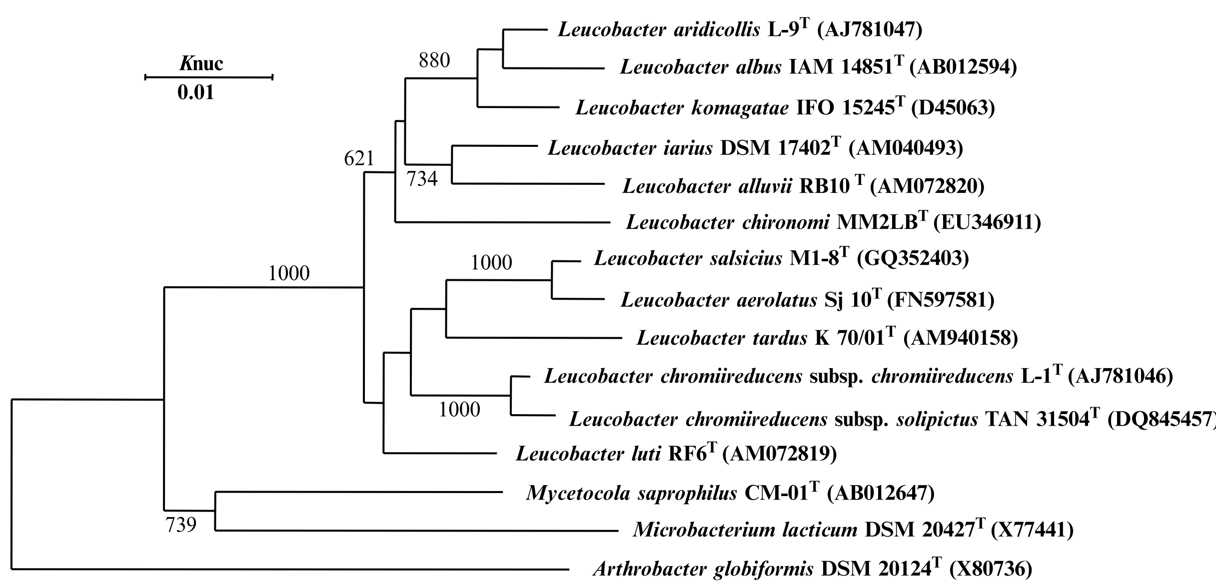

FIGURE 189. Phylogenetic relationships of the named species of the genus *Leucobacter*. The neighbor joining method was used for the tree construction. One thousand bootstrap trees were generated, and bootstrap values (>60%) are indicated at branch points.

TABLE 159. Characteristics differentiating the genus *Leucobacter* from DAB-containing genera of the family *Microbacteriaceae*[a]

Characteristic	*Leucobacter*	*Agreia*	*Agrococcus*	*Agromyces*	*Clavibacter*	*Cryobacterium*	*Humibacter*	*Leifsonia*	*Microterricola*	*Plantibacter*	*Pseudoclavibacter*	*Rathayibacter*	*Rhodoglobus*	*Schumannella*	*Subtercola*	*Yonghaparkia*
Diamino acid	L-DAB	L-DAB, Orn	L-DAB	L-DAB	L-DAB, D-DAB	L-DAB, D-DAB	Orn, DAB	L-DAB, D-DAB	DAB	L-DAB, D-DAB	L-DAB, D-DAB	L-DAB	DAB	DAB	L-DAB, D-DAB	L-DAB, D-DAB
Amino acid at position 3 of the peptide subunit	nd	nd	nd	D-Glu–L-DAB/[L-DAB]	D-Glu–L-DAB/[L-DAB]	D-Glu–L-DAB/[L-DAB]	nd	D-Glu–L-DAB/[L-DAB]	nd	D-Glu–L-DAB/[L-DAB]	D-Glu–L-DAB/[L-DAB]	D-Glu–L-DAB/[L-DAB]	nd	nd	D-Glu (Hyg)–D-DAB/[L-DAB]	D-Glu–D-DAB
Major menaquinone	MK-11, MK-10	MK-10, MK-11	MK-11, MK-12, MK-10	MK-12, MK-11, MK-13	MK-9, MK-10	MK-10, MK-12, MK-11	MK-11, MK-12	MK-11, MK-10, MK-12	MK-12, MK-11	MK-10, MK-11	MK-9	MK-10	MK-11, MK-12	MK-11, MK-10	MK-9, MK-10	MK-12, MK-11
Polar lipids[b]	DPG, PG, GL, PL	PG, DPG	PG, DPG, GL, PL	DPG, PG, GL	DPG, PG, GL	DPG, PG, GL	nd	PG, DPG, GL	nd	PG, DPG, GL	DPG, PG, GL	PG, DPG, GL	nd	nd	PG, DPG, PL, GL	PG, DPG, PL, GL
Cellular fatty acids[c]	$C_{15:0}$ anteiso, $C_{17:0}$ anteiso, $C_{16:0}$ iso	$C_{15:0}$ anteiso, $C_{16:0}$ iso, $C_{17:0}$ anteiso, $C_{15:0}$ anteiso-DMA	$C_{15:0}$ anteiso, $C_{16:0}$ iso, $C_{15:0}$ iso, $C_{17:0}$ anteiso	$C_{15:0}$ anteiso, $C_{17:0}$ anteiso, $C_{16:0}$ iso	$C_{15:0}$ anteiso, $C_{17:0}$ anteiso, $C_{16:0}$ iso	$C_{15:0}$ anteiso, $C_{15:1}$ anteiso, $C_{15:0}$ iso, $C_{17:0}$ anteiso, $C_{16:0}$ iso	$C_{17:0}$ Ch, $C_{17:0}$ anteiso, $C_{16:0}$ iso	$C_{15:0}$ anteiso, $C_{17:0}$ anteiso, $C_{16:0}$ iso, $C_{17:0}$ Ch	$C_{15:0}$ anteiso, $C_{17:0}$ anteiso	$C_{15:0}$ anteiso, $C_{16:0}$ iso, $C_{17:0}$ anteiso, $C_{15:0}$ iso	$C_{15:0}$ anteiso,- $C_{16:0}$ iso, $C_{17:0}$ anteiso	$C_{15:0}$ anteiso, $C_{17:0}$ anteiso	$C_{15:0}$ anteiso, $C_{16:0}$ iso, $C_{17:0}$ anteiso (at 2°C: $C_{15:1}$ anteiso)	$C_{15:0}$ anteiso, $C_{16:0}$ iso	$C_{15:0}$ anteiso, $C_{16:0}$ iso, $C_{17:0}$ anteiso, $C_{15:0}$ anteiso-DMA, $C_{16:0}$ iso-DMA	$C_{15:0}$ anteiso, $C_{15:0}$ iso, $C_{17:0}$ anteiso
DNA G+C content (mol%)	66–70	65–67	74–75	69–73	69–76	65–67	68	66–71	70	68–70	67	60–72	62	59	64–68	71–72

[a]nd, Not determined.

[b]PG, phosphatidylglycerol; DPG disphosphatidylglycerol; GL, Glycolipids; PL, unidentified phospholipids.

[c]DMA, Dimethoxy; Ch, cyclohexyl.

TABLE 160. Characteristics differentiating species of the genus *Leucobacter*[a]

Characteristic	*L. komagatae*	*L. aerolatus*	*L. albus*	*L. alluvii*	*L. aridicollis*	*L. chironomi*	*L. chromiireducens* subsp. *chromiireducens*	*L. chromiire-ducens* subsp. *solipictus*	*L. iarius*	*L. luti*	*L. salsicius*	*L. tardus*
Colony pigmentation	Whitish brown	White	White	Cream	Cream	Yellow	Cream	Yellow	White	Cream	Cream	Yellow
Urease	+	na	–	+	+	–	+	–	–	+	–	–
Gelatinase	+	na	na	+	–	–	–	–	–	–	–	–
pH optimum	7–9	na	na	7–8	7–8	6–8	7	5.5–9	na	7–9	7–8	na
Growth at:												
4°C	+	na	na	+	–	–	–	–	–	+	na	–
37°C	–	na	na	+	+	+	+	+	+	–	na	–
Optimal temperature (°C)	28	25–30	30	30	28	30	28	25	30	25	25–30	21–30
Growth in the presence of:												
7% NaCl	–	na	na	+	+	+	+	+	+	+	+	na
10% NaCl	–	na	na	–	+	–	–	–	–	–	–	na
Cell-wall amino acids (molar ratio):												
2,4-Diaminobutyric acid (DAB)	0.8	0.7	0.8	0.5	0.5	0.5	0.9	0.5	0.5	0.5	+	0.5
Alanine	1.9	2.1	1.8	2.4	2	2.5	1.9	1.8	1.5	0.5	+	3.2
Glycine	0.9	0.3	1.1	1.2	1.1	1.2	1.3	1	0.9	0.3	+	2.1
Glutamic acid	1	1	1	1	1	1	1	1	1	1	+	1
γ-Aminobutyric acid (GABA)	0.7	–	0.7	–	–	+	–	–	–	–	+	–
Threonine	–	0.8	–	0.7	–	0.9	0.7	0.6	0.7	0.6	–	–
Menaquinone(s) (MK-):												
Major amounts	11	11	11	11	11	11	11	11	11	11	11	10;11
Minor amounts	10;12	12;10	12;9	10	10	10;12	10	10	10;9;12	10	10;7	9
Acid production from (API 50CH):												
N-Acetylglu-cosamine	–		–	–	–	–	–		+	–	–	–
D-Adonitol	w		–	–	–	–	–		–	–	–	–
D-Arabinose	–		w	–	–	–	–		–	–	–	–
L-Arabinose	w		–	–	–	–	–		–	–	–	–
L-Fucose	–		+	–	–	–	–		–	–	–	–
Fructose	–		–	–	–	–	–		+	–	–	+
Glycerol	+		w	–	–	–	–		+	–	+	–
Inositol	–		–	–	–	–	–		w	w	+	–
5-Keto-gluconate	–		–	–	–	–	–		w	–	+	–
Melezitose	–		–	–	–	–	–		–	–	–	+
Salicin	–		–	–	–	–	–		w	–	+	–
Trehalose	–		+	–	–	–	–		–	–	+	–
Raffinose	–		–	–	–	–	–		–	–	–	+
L-Rhamnose	–		–	–	–	–	–		–	w	–	+

Ribose	+		+	−	−	−	−		+	+	+	−
Utilization of (Biolog GP2 MicroPlate):												
N-Acetyl-D-glucosamine	−	+	−	−	−	−	−	−	+	−		−
N-Acetyl-L-glutamic acid	−		+	−	−	−	−	−	−	w		+
Adenosine	−		w	+	+	−	+	−	+	−		+
Adenosine 5′-monophosphate	−		−	−	+	−	−	−	w	−		−
L-Alaninamide	+		+	+	+	−	+	+	+	−		+
L-Alanine	−	+	w	+	+	−	+	+	w	+		−
L-Alanylglycine	−		+	+	+	−	+/−	+	−	−		−
L-Arabinose	−	−	−	−	−	−	−	−	−	−		+
L-Asparagine	−		+	−	+	−	−		−	−		−
2,3-Butanediol	−		−	−	−	−	−	−	−	−		+
2′-Deoxyadenosine	+		+	−	+	−	+	−	−	w		+
Dextrin	−		−	+	−	−	−	−	−	−		−
D-Fructose	−	−	−	+	−	−	−	−	+	−		w
D-Fructose 6-phosphate	+		+	+	+	−	+/−	−	+	+		−
D-Gluconic acid	−	−	−	−	−	−	−	−	−	−		+
D-Glucose	−	+	−	+	−	−	−	−	−	−		+
D-Glucose 6-phosphate	−		−	−	−	−	−	−	−	−		+
L-Glutamic acid	w		+	−	+	−	+	+	w	w		−
Glycerol	+		+	+	+	+	−	+	+	−		+
DL-α-Glycerol phosphate	−		−	−	−	−	−	−	−	−		+
Glycyl L-glutamic acid	w		+	−	+	−	+	+	+	w		−
α-Hydroxybutyric acid	−		−	−	−	−	−	−	−	−		+
β-Hydroxybutyric acid	−	−	−	−	+	−	−	−	−	−		−
p-Hydroxyphenyl acetic acid	w		−	+	+	−	+	+	+	+		+
α-Ketovaleric acid	−		+	−	w	−	−	−	−	−		w
L-Lactic acid	−	+	−	+	−	−	−	−	−	−		+
Methyl α-D-galactoside	−		−	−	−	−	−	−	−	−		+
Methyl β-D-galactoside	−		−	−	−	−	−		−	−		+
3-Methyl glucose	−		−	−	−	−	−	−	−	−		+
Methylpyruvate	−		+	+	−	−	+	+	−	+		+
Putrescine	−	+	−	−	−	+	+/−	+	−	−		+
Pyruvic acid	−	+	−	−	−	−	−	−	−	−		+
D-Ribose	+	+	+	−	+	−	−	−	+	−		+
L-Serine	−	−	+	−	+	−	+/−	+	w	−		+

(continued)

TABLE 160. (continued)

Characteristic	*L. komagatae*	*L. aerolatus*	*L. albus*	*L. alluvii*	*L. aridicollis*	*L. chironomi*	*L. chromiireducens* subsp. *chromiireducens*	*L. chromiireducens* subsp. *solipictus*	*L. iarius*	*L. luti*	*L. salsicius*	*L. tardus*
D-Sorbitol	−	−	−	−	−	−	−	−	−	−		+
Succinamic acid	−		−	−	−	−	−	w	−	−		+
Succinic acid	−		−	−	−	−	−	−	−	−		+
Thymidine	−		+	+	+	−	+	−	+	−		+
Thymidine 5-monophosphate	−		+	−	+	−	−	−	+	−		−
Trehalose	−	+	+	−	−	−	−	−	−	−		−
Xylitol	−		−	+	−	−	−	−	−	−		−
D-Xylose	−	−	−	−	−	−	−	−	−	−		+
Uridine	−		+	−	+	−	+/−	−	+	−		+
Uridine 5′-monophosphate	−		w	−	+	−	−	−	+	−		−
Source of isolation	Agar plate contaminant	Airborne	Soil	River sediment	Activated sludge	Chironomid egg mass	Activated sludge	Contaminant of *Caenorhabditis elegans*	Nematode	Activated sludge	Korean fermented food	Phylosphere of potato plants

[a]Symbols: +, >85% positive; −, 0–15% positive; w, weak reaction; na, not available.

List of species of the genus *Leucobacter*

1. **Leucobacter komagatae** Takeuchi, Weiss, Schumann and Yokota 1996, 970[VP]

koma.ga′ta.e. N.L. gen. masc. n. *komagatae* of Komagata, named in honor of Kazuo Komagata, the Japanese microbiologist who first recognized this strain.

Cells are Gram-stain-positive, irregular rods that are 0.2–0.3 µm × 1.0–1.5 µm. The cells are nonsporeforming and nonmotile in 2-d-old cultures on PY-BHI agar. No mycelium is produced. Colonies are circular, entire, low convex, smooth, opaque, and whitish brown. Catalase is produced, but oxidase, arginine dihydrolase, hydrogen sulfide, and urease are not produced. Gelatin, starch, and esculin are not hydrolyzed, but Tween 20, Tween 40, Tween 60, and Tween 80 are hydrolyzed. Nitrate is not reduced. Growth occurs in the presence of 2.0–5.0% NaCl and at pH 10.0. Acetate, D- and L-lactate, malate, succinate, propionate, oxalate, and hippurate are assimilated, but citrate and formate are not assimilated. The G+C content of the DNA is 66 mol%. The cell-wall peptidoglycan contains L-2,4-diaminobutyric acid (L-DAB), alanine, glycine, γ-aminobutyric acid (GABA), and glutamic acid. The acyl type of the cell wall is an acetyl type. The major isoprenoid quinone is menaquinone MK-11. Mycolic acids are not present. The major cellular fatty acids are $C_{15:0}$ anteiso, $C_{16:0}$ iso, and $C_{17:0}$ anteiso. No diagnostic sugar is present in the cell wall. Diphosphatidylglycerol, phosphatidylglycerol, and one unknown glycolipid are present.

Source: a contaminant on an agar plate.

DNA G+C content (mol%): 66 (HPLC).

Type strain: CCUG 49676, CIP 105084, DSM 8803, IAM 1093, NBRC 15245, JCM 9414, VKM Ac-2073.

Sequence accession no. (16S rRNA gene): AB007419, AJ746337, AM42691, D17751, D45063.

2. **Leucobacter aerolatus** Martin, Lodders, Jäckel, Schumann and Kämpfer 2010, 2840[VP]

a.e.ro.la′tus. Gr. n. *aer* air; L. part. adj. *latus -a -um* carried; N.L. masc. part. adj. *aerolatus* airborne.

Cells are nonmotile, nonsporeforming rods approximately 2 µm in length. Gram-stain-positive, oxidase-positive (weak reaction), aerobic respiratory metabolism. Good growth occurs after 3 d of incubation on tryptone soy agar and nutrient agar at 25–30°C. On TSA, colonies are white and shiny with a diameter of approximately 2 mm. The peptidoglycan contains the amino acids 2,4-diaminobutyric acid (DAB), lysine, threonine, glycine, alanine, and glutamic acid. The quinone system is composed of menaquinone MK-11 as major component and minor amounts of MK-12 and MK-10. The polar lipid profile contains phosphatidylglycerol, diphosphatidylglycerol, one unknown phospholipid, one unknown glycolipid and one unknown aminoglycolipid. Major fatty acids are $C_{15:0}$ anteiso, $C_{16:0}$ iso, and $C_{17:0}$ anteiso.

The following carbon sources are utilized: *N*-acetyl-D-galactosamine, *N*-acetyl-D-glucosamine, D-galactose, D-glucose, L-rhamnose, D-ribose, D-trehalose, i-inositol, putrescine, 4-aminobutyrate, DL-lactate, pyruvate, L-alanine, L-aspartate, L-histidine,L-ornithine, L-proline, and weakly positive are D-maltose and oxoglutarate. Not utilized are L-arabinose, *p*-arbutin, D-cellobiose, D-fructose, gluconate, D-mannose, α-D-melibiose, sucrose, salicin, D-xylose, adonitol, maltitol, D-mannitol, D-sorbitol, acetate, propionate, *cis*-aconitate, *trans*-asconitate, adipate, citrate, fumarate, glutarate, DL-3-hydroxybutyrate, itaconate, L-malate, mesaconate, suberate, β-alanine, L-leucine, L-phenylalanine, L-serine, L-tryptophan, 3-hydroxybenzoate, 4- hydroxybenzoate, and phenylacetate. Following compounds are hydrolyzed (pNP- = *para*-nitrophenyl; oNP = *ortho*-nitrophenyl-; pNA = *para*-nitroanilide): 2-deoxythymidine-5′-pNP-phosphate, L-alanine-pNA and L-proline-pNA. The following compounds are not hydrolyzed: esculin, pNP-β-D-galactopyranoside, pNP-β-D-glucuronide, pNP-α-D- glucopyranoside, pNP-β-D-glucopyranoside, pNP-β-D-xylopyranosid, bis-pNP-phosphate, pNP-phenyl-phosphonate, pNP-phosphoryl-choline, and L-glutamate-γ-3-carboxy-pNA.

Source: the air in a duck barn in Germany.

DNA G+C content (mol%): not available.

Type strain: Sj 10, CCM 7705, DSM 22806.

Sequence accession no. (16S rRNA gene): FN597581.

3. **Leucobacter albus** Lin, Uemori, de Briel, Arunpairojana and Yokota 2004, 1675[VP]

al′bus. L. masc. adj. *albus* white.

Cells are Gram-stain-positive, aerobic rods, 0.3–0.4 × 0.5–1.1 µm, nonsporeforming, and nonmotile. Smooth white colonies are produced on PY-BHI agar. The optimal temperature for growth is 30°C. Catalase is produced, but oxidase is not. Acid is produced from glycerol, ribose, and L-fucose. The following tests are negative: reduction of nitrate or nitrite, nitrate respiration, liquefaction of gelatin, hydrolysis of esculin, urease, alkaline phosphatase, β-galactosidase, and α-glucosidase and acid production from D-glucose, D-fructose, D-mannose, rhamnose, inositol, and mannitol. The cell-wall peptidoglycan contains L-2,4-diaminobutyric acid (L-DAB) and γ-aminobutyric acid (GABA); the muramic acid of the cell wall is of the acetyl type. The major isoprenoid quinone is MK-11. The major fatty acids are $C_{15:0}$ anteiso, $C_{17:0}$ anteiso, and $C_{16:0}$ iso.

Source: soil.

DNA G+C content (mol%): 66 (HPLC).

Type strain: CCUG 50213, IAM 14851, JCM 12888, NBRC 103070, TISTR 1515.

Sequence accession no. (16S rRNA gene): AB012594.

4. **Leucobacter alluvii** Morais, Paulo, Francisco, Branco, Chung and da Costa 2006a, 2507[VP] (Effective publication: Morais, Paulo, Francisco, Branco, Chung and da Costa 2006b, 419.)

al.lu′vi.i. L. neut. gen. n. *alluvii* of an alluvial deposit.

Cells are Gram-stain-positive, irregularly shaped rods. Nonsporeforming; nonmotile in PY-BHI. Colonies are circular, entire, low raised, smooth, opaque, and cream-colored. Optimum growth temperature is 30°C; does not grow at 40°C. Optimum pH for growth is 7.0–8.0. Catalase and urease are produced, but oxidase and arginine dihydrolase are not. Esculin is not hydrolyzed. Tween 20, 40, 60, 80, and gelatin are hydrolyzed. Nitrate is not reduced. Growth occurs in

the presence of 8% NaCl; growth occurs in the presence of 3 mM Cr(VI). The cell-wall peptidoglycan contains alanine, glycine, threonine, 2,4-diaminobutyric acid (DAB), and glutamic acid. The major isoprenoid quinone is menaquinone MK-11. The major cellular fatty acids are $C_{15:0}$ anteiso, $C_{17:0}$ anteiso, $C_{16:0}$ and $C_{16:0}$ iso. Chemo-organotrophic, strictly aerobic, and nonfermentative. Growth is observed with D-glucose, D-fructose, D-mannose L-rhamnose, inositol, D-maltose, D-trehalose, and xylitol.

Source: River Alviela sediments, Portugal.

DNA G+C content (mol%): 68.9 (HPLC).

Type strain: RB10, CIP 108819, JCM 14919, LMG 23117.

Sequence accession no. (16S rRNA gene): AM072820.

5. **Leucobacter aridicollis** Morais, Francisco, Branco, Chung and da Costa 2005, 547[VP] (Effective publication: Morais, Francisco, Branco, Chung and da Costa 2004, 650.)

a.ri.di.col′lis. L. adj. *aridus* dry; L. masc. n. *collis* an elevation, high ground, hill; N.L. gen. n. *aridicollis* of a dry hill; referring to the town of Alcanena, which the Moors called al-Kinan, meaning a dry hill.

Cells are Gram-stain-positive, irregularly shaped rods. Nonsporeforming; nonmotile in PY-BHI. Colonies are circular, entire, low raised, smooth, opaque, and cream-colored. Optimum growth temperature is 28°C; does not grow at 40°C. Optimum pH is 7.0–8.0. Catalase and urease are produced, but oxidase and arginine dihydrolase are negative. Esculin and gelatin are not hydrolyzed. Nitrate is not reduced. Growth occurs in the presence of 10% NaCl and 5 mM Cr(VI). The cell-wall peptidoglycan contains 2,4-diaminobutyric acid (DAB), alanine, glutamic acid, and glycine. The major isoprenoid quinone is menaquinone MK-11. The major cellular fatty acids are $C_{15:0}$ anteiso, $C_{17:0}$ anteiso, $C_{16:0}$ iso, and $C_{16:0}$. Glucose is not fermented. Weak growth is obtained with D- and L-arabinose, D-fructose, D-mannose, L-sorbose, and D-maltose; D-ribose and L-rhamnose are not assimilated.

Source: activated sludge from chromium polluted wastewater treatment plant in Alcanena, Portugal.

DNA G+C content (mol%): 67.3 (HPLC).

Type strain: L-9, CIP 108388, JCM 13321, LMG 22507.

Sequence accession no. (16S rRNA gene): AJ781047.

6. **Leucobacter chironomi** Halpern, Shakéd, Pukall and Schumann 2009, 667[VP]

chi.ro′no.mi. N.L. gen. n. *chironomi* of *Chironomus*, named after the non-biting midge insect of the genus *Chironomus* (Chironomidae: Diptera) from which the type strain was isolated.

Cells are aerobic, Gram-stain-positive, nonmotile rods measuring 0.5–0.75 μm × 0.95–1.4 μm. Cells occur singly. After 48 h incubation on LB agar at 30°C, colonies are opaque, circular with entire margins, and yellow-colored. Growth is not observed under anaerobic conditions even after 8 d incubation. Growth is observed after 48 h incubation at 17–37°C (optimum 30°C), in the presence of 0–7.0% NaCl (optimum 0–1.0% NaCl) and at pH 4.0–9.5 (optimum pH 6.0–8.0). Good growth occurs on marine agar after 48 h incubation. Growth is not observed on MacConkey or cetrimide agars. Oxidase reaction is negative; catalase reaction is weakly positive. Although the type strain was not isolated from a chromium-enriched environment, it is able to grow in the presence of up to 18.0 mM Cr(VI). On API 50CH strips incubated for 48 h at 30°C, acid is not produced from any of the substrates tested and on API 20NE strips incubated for 48 h at 30°C; all tests are negative. The following results are obtained from API 20E strips after 48 h incubation at 30°C: acetoin is produced; nitrate is reduced to nitrogen; H_2S and indole are not produced; urea and gelatin are not hydrolyzed; citrate is not utilized. Activities of β-galactosidase, arginine dihydrolase, lysine decarboxylase, ornithine decarboxylase, and tryptophan deaminase are detectable. Putrescine and glycerol are oxidized in the Biolog test. Cell-wall amino acids are alanine, glycine, threonine, 2,4-diaminobutyric acid (DAB), γ-aminobutyric acid (GABA), and glutamic acid. The peptidoglycan is of B-type. The major menaquinone is MK-11; MK-10 and MK-12 occur in minor amounts (<10% each). The dominant cellular fatty acids are $C_{15:0}$ anteiso, $C_{17:0}$ anteiso, and $C_{16:0}$ iso. The following fatty acids are present as minor components (<5%): $C_{16:0}$ and $C_{15:0}$ iso.

Source: a chironomid egg mass in Israel.

DNA G+C content (mol%): not available.

Type strain: MM2LB, DSM 19883, LMG 24399.

Sequence accession no. (16S rRNA gene): EU346911.

7. **Leucobacter chromiireducens** Morais, Francisco, Branco, Chung and da Costa 2005, 547[VP] emend. Muir and Tan 2007, 2774 (Effective publication: Morais, Paulo, Francisco, Brancob, Chung and da Costa 2004, 650.)

chro.mi.i.re.du′cens. N.L. neut. n. *chromium* chromium; L. v. *reducere* to draw backwards, bring back to a state or condition and, in chemistry, converting to a reduced oxidation state; N.L. part. adj. *chromiireducens* chromium reducing.

Gram-stain-positive, irregular rod-shaped cells. Nonsporeforming; nonmotile after growth on PY-BHI. Colonies are circular, entire, low convex, smooth, opaque, and cream-colored. Optimum growth temperature is about 28°C; does not grow at 40°C. Optimum pH is about 7.0. Oxidase-negative and catalase-positive; urease is produced, arginine dihydrolase is not produced. Starch is hydrolized, but esculin and gelatin are not. Nitrate is not reduced. Growth occurs in with the presence of 9% NaCl and 5 mM Cr(VI). The cell-wall peptidoglycan contains 2,4-diaminobutyric acid (DAB), alanine, glycine, glutamic acid, and threonine. The major isoprenoid quinone is menaquinone MK-11. The major cellular fatty acids are $C_{15:0}$ anteiso, $C_{17:0}$ anteiso, $C_{16:0}$ iso, and $C_{16:0}$. Glucose is not fermented. L-Rhamnose is assimilated; D-galactose, D-sorbitol, D-cellobiose, D-melezitose, D-raffinose are weakly assimilated. Other carbohydrates are not utilized.

Source: activated sludge from chromium polluted wastewater treatment plant in Alcanena, Portugal.

DNA G+C content (mol%): 66.7 (HPLC).

Type strain: L-1, CIP 108389, JCM 13322, LMG 22506.

Sequence accession no. (16S rRNA gene): AJ781046.

7a. **Leucobacter chromiireducens subsp. chromiireducens** Morais, Francisco, Branco, Chung and da Costa 2005, 547[VP] emend. Muir and Tan 2007, 2774 (Effective publication: Morais, Paulo, Francisco, Branco, Chung and da Costa 2004, 650.)

See species description.

Source: activated sludge from chromium polluted wastewater treatment plant in Alcanena, Portugal.

DNA G+C content (mol%): 66.7 (HPLC).

Type strain: L-1, CIP 108389, JCM 13322, LMG 22506.

Sequence accession no. (16S rRNA gene): AJ781046.

7b. **Leucobacter chromiireducens subsp. solipictus** Muir and Tan 2007, 2774[VP]

so.li.pic′tus. L. masc. n. *sol* the sun; L. masc. part. adj. *pictus* painted; N.L. masc. part adj. *solipictus* painted by the sun.

Cells are Gram-stain-positive-staining, aerobic, nonmotile, nonsporeforming irregular rods that are 0.4–0.6 µm × 0.8–1.4 µm when grown at 25°C in liquid LB medium and 0.5–0.7 µm × 0.8–4.3 µm when grown at 25°C on solid LB agar for 1–2 d. Cells produce an extracellular material. Colonies are circular, entire, convex, small (0.5–1 µm in diameter), smooth, glistening, yellow-pigmented when grown in the light or cream-colored when grown in the dark (photochromogenic), and opaque on LB agar after 2–5 d growth at 25°C. The light-induced pigment is soluble in methanol and displays characteristic peaks in absorption at 413, 436, and 466 nm. Pigment production is dependent on light quality and temperature. Depigmentation of cells occurs in the combined presence of 0.5 mM fosmidomycin and 0.5 mM mevinolin. Growth occurs at 10–37°C, pH 5.5–9.0, in LB containing 0–8% NaCl. Growth occurs at 4°C, but does not at 40°C, pH 5.0, or in the presence of >9% NaCl. Optimum growth occurs in the range between 25–32°C at pH 7.5–8.5 in aerated LB lacking salt. After 2–5 d incubation at 25°C, growth is observed on solid LB agar containing each of the following metals: 1 mM arsenite, 80 mM cadmium, and 5 mM hexavalent chromium, however, cells are unable to reduce hexavalent chromium to trivalent chromium. Cells are resistant to 50 µg kanamycin/ml, 12.5 µg/ml tetracycline, 10 µg/ml gentamicin, 300 µg/ml streptomycin, and 20 µg/ml chloramphenicol and sensitive to 100 µg/ml ampicillin and 100 µg/ml rifampin. Positive for catalase and alkaline phosphatase activities, but not for cytochrome c oxidase, nitrate reductase, β-galactosidase, urease, gelatinase, amylase, cysteine desulfurase, tryptophanase, phenylalanine deaminase, hemolytic, or lipase activities. No endogenous plasmids are present. After 2–5 d incubation at 25°C, growth can be seen on solid LB agar containing each of the following individually: 1 mM arsenite, 80 µM cadmium, 50 µg kanamycin/ml, 12.5 µg/ml tetracycline, 10 µg/ml gentamicin, and 20 µg/ml chloramphenicol; cells are sensitive to 50 µg/ml streptomycin, 100 µg/ml ampicillin, and 100 µg/ml rifampin. Negative for β-galactosidase, urease, amylase, cysteine desulfurase, tryptophanase, phenylalanine deaminase, hemolytic, and lipase activities. No endogenous plasmids are present.

Cell-wall peptidoglycan contains 2,4-diaminobutyric acid (DAB), alanine, glutamic acid, glycine, and threonine. Galactose is the only cell-wall sugar detected. Major isoprenoid quinones are MK-11 and MK-10. Major cellular fatty acids are $C_{15:0}$ anteiso, $C_{17:0}$ anteiso, $C_{16:0}$ iso, and linear $C_{16:0}$. Polar lipids include diphosphatidylglycerol, phosphatidylglycerol, and an unknown glycolipid.

Source: a contaminant from *Caenorhabditis elegan* in Stanford, CA, USA.

DNA G+C content (mol%): 69.5 (HPLC).

Type strain: TAN 31504, ATCC BAA-1336, DSM 18340, JCM 15573.

Sequence accession no. (16S rRNA gene): DQ845457.

8. **Leucobacter iarius** Somvanshi, Lang, Schumann, Pukall, Kroppenstedt, Ganguly and Stackebrandt 2007, 685[VP]

i.a′ri.us. N.L. masc. adj. *iarius* (arbitrary adjective) formed from the acronym IARI (Indian Agricultural Research Institute, New Delhi, India) to commemorate its centenary year.

Gram-stain-positive, straight, nonmotile rods (1.66–2.91 × 0.37–0.49 µm). Neither mycelium nor spores are detected. Colonies are round, 0.4–1 mm in diameter with smooth edges, low-convex, opaque, whitish in color, finely granular and odorless. Optimum temperature for growth is 30°C; growth does not occur at temperatures higher than 37°C or at 4°C. Growth occurs at pH 5.0–9.0. Growth is reduced in the presence of 5–7% NaCl; no growth occurs at 10% NaCl. Urease-negative, nitrate is not reduced, D-glucose is not fermented, and esculin and PNPG (β-galactosidase) are positive in the API 20NE test. Gelatinase-negative. Utilizes *N*-acetylglucosamine and weakly utilizes 5-keto-gluconate, inositol, and salicin. MK-11 is the major menaquinone; MK-10 and MK-9 are present in minor amounts while MK-12 occurs in trace amounts only. Polar lipids are phosphatidylglycerol and diphosphoglycerol. Several unidentified components occur, i.e. a phospholipid, a glycolipid, and an aminophospholipid. Cross-linkage of peptidoglycan is of the B-type; cell-wall amino acids are L-2,4-diaminobutyric acid (L-DAB), alanine, glycine, threonine, and glutamic acid. γ-Aminobutyric acid (GABA) is absent. Major fatty acids are $C_{15:0}$ anteiso, $C_{17:0}$ anteiso, and $C_{16:0}$ iso; $C_{15:0}$ iso, and $C_{16:0}$ are present in minor amounts.

Source: crushed infective juveniles of *Steinernema thermophilum* collected from soil of the Indian Agricultural Research Institute, New Delhi, India.

DNA G+C content (mol%): not available.

Type strain: 40, CIP 108831, DSM 17402, JCM 14736.

Sequence accession no. (16S rRNA gene): AM040493.

9. **Leucobacter luti** Morais, Paulo, Francisco, Branco, Chung and da Costa 2006a, 2507[VP] (Effective publication: Morais, Paulo, Francisco, Branco, Chung and da Costa 2006b, 419.)

lu.ti. L. gen. n. *luti* of mud.

Cells are Gram-stain-positive, irregular rod-shaped. Nonsporeforming; nonmotile on PY-BHI. Colonies are circular, entire, low convex, smooth, opaque, and cream-colored. Optimum growth temperature is about

25°C; does not grow at 37°C. Optimum pH is 7.0–9.0. Oxidase-negative and catalase-positive; urease is produced, arginine dihydrolase is not produced. Starch is hydrolized, but esculin, gelatin, and Tween 20, 40, 60, and 80 are not. Nitrate is not reduced. Growth occurs in the presence of 8% NaCl and in the presence of 3 mM Cr(VI).

The cell-wall peptidoglycan contains alanine, glycine, threonine, DAB, and glutamic acid. The major isoprenoid quinone is menaquinone MK-11. The major cellular fatty acids are $C_{15:0}$ anteiso, $C_{17:0}$ anteiso, $C_{16:0}$ iso, and $C_{16:0}$. Chemo-organotrophic, strictly aerobic and nonfermentative. D-Ribose, L-rhamnose, and inositol are assimilated; glycerol is weakly assimilated.

Source: activated sludge from a chromium polluted wastewater treatment plant in Alcanena, Portugal.

DNA G+C content (mol%): 68.8 (HPLC).

Type strain: RF6, CIP 108818, JCM 14920, LMG 23118.

Sequence accession no. (16S rRNA gene): AM072819.

10. **Leucobacter salsicius** Yun, Roh, Kim, Jung, Park, Shin, Nam and Bae 2011, 505[VP]

sal.si′ci.us. L. masc. adj. *salsicius* salted, salty.

The strain forms circular, cream-colored colonies with entire margins. Cells are aerobic, Gram-stain positive, nonmotile (on semi-solid agar medium) rods that are 1.0–1.5 µm in length. Cells are catalase-positive and oxidase-negative. Optimal growth occurs at 25–30°C and pH 7.0–8.0 and in the presence of 0–4% (w/v) NaCl. The strain can tolerate up to 10.0 mM Cr (VI). In the API ZYM test, positive for esterase C4, leucine arylamidase, valine arylamidase, cystine arylamidase, naphthol-AS-BI-phosphohydrolase, and β-glucosidase. It does not produce alkaline phosphatase, esterase lipase C8, lipase C14, trypsin, α-chymotrypsin, acid phosphatase, α-galactosidase, β-galactosidase, β-glucuronidase, α-glucosidase, *N*-acetyl-β-glucosaminidase, α-mannosidase, or α-fucosidase. The strain is negative for reduction of nitrate to nitrite. It does not produce indole. The strain hydrolyzes casein, esculin, Tweens 20, 40, 60, and PNPG (*p*-nitrophenyl-β-D-galactopyranoside). It does not hydrolyze starch, urea, gelatin, or Tween 80. It is negative for D-glucose fermentation, L-arginine dihydrolase, and urease. $C_{15:0}$ anteiso, $C_{17:0}$ anteiso, and $C_{16:0}$ iso are the dominant cellular fatty acids. The cell-wall peptidoglycan of the strain contains 2,4-diaminobutyric acid (DAB), alanine, glycine, glutamic acid, and γ-aminobutyric acid (GABA). Polar lipids include diphosphatidylglycerol and an unknown glycolipid.

Source: a Korean salt-fermented food containing tiny shrimp.

DNA G+C content (mol%): 62.8 (T_m).

Type strain: M1-8, KACC 21127, JCM 16362.

Sequence accession no. (16S rRNA gene): GQ352403.

11. **Leucobacter tardus** Behrendt, Ulrich and Schumann 2008b, 2577[VP]

tar′dus. L. masc. adj. *tardus* slow, pertaining to the slow reactions in several physiological tests.

Nonsporeforming, nonmotile, short rods (0.4–0.6 × 0.8–1.6 µm), which occur singly or in pairs, sometimes forming small irregular clusters. Gram-stain-positive and strictly aerobic. The lemon-yellow colonies found on nutrient agar are smooth with regular margins. Optimal temperature for growth is 21–30°C; no growth at 4 or 37°C. Catalase activity is present, but urease, β-galactosidase, arginine dihydrolase, lysine decarboxylase, and ornithine decarboxylase activities are absent. Citrate utilization, H_2S production from sodium thiosulfate, tryptophan deaminase, indole production from L-tryptophan and reduction of nitrate are negative. Does not produce acetoin from sodium pyruvate. No growth on MacConkey agar. Weak oxidase reaction. Hydrolyses esculin, but not starch, casein, or gelatin. Oxidative acid production occurs on API 50CH and API 20E substrates for D-fructose, melezitose, raffinose, and L-rhamnose, but not for *N*-acetylglucosamine, D-adonitol, amygdalin, D- or L-arabinose, D- or L-arabitol, arbutin, D-cellobiose, dulcitol, erythritol, D- or L-fucose, D-galactose, gentiobiose, D-glucose, glycerol, glycogen, inositol, inulin, D-lactose, D-lyxose, maltose, D-mannitol, D-mannose, melibiose, methyl α-D-glucopyranoside, methyl α-D-mannopyranoside, methyl β-D-xylopyranoside, potassium gluconate, potassium 2-keto-gluconate, potassium 5-keto-gluconate, D-ribose, sucrose, salicin, D-sorbitol, L-sorbose, starch, D-tagatose, trehalose, turanose, xylitol, or D- or L-xylose. Utilizes *N*-acetyl-L-glutamic acid, adenosine, L-alaninamide, L-arabinose, 2,3-butanediol, 2′-deoxyadenosine, D-gluconic acid, α-D-glucose, D-glucose 6-phosphate, glycerol, DL-α-glycerol phosphate, α-hydroxybutyric acid, *p*-hydroxyphenylacetic acid, L-lactic acid, methyl α-D-galactoside, methyl β-D-galactoside, 3-methyl glucose, methyl α-D-glucoside, methyl pyruvate, putrescine, pyruvic acid, D-ribose, L-serine, D-sorbitol, succinamic acid, succinic acid, thymidine, Tweens 40 and 80, uridine and D-xylose on Biolog GP2 MicroPlates. Weak utilization is observed for D-fructose, *myo*-inositol, α-ketovaleric acid, D-mannitol, palatinose, D-psicose, and D-tagatose. Acetic acid, *N*-acetyl-D-glucosamine, *N*-acetyl-β-D-mannosamine, AMP, D- and L-alanine, L-alanyl glycine, amygdalin, D-arabitol, arbutin, L-asparagine, D-cellobiose, α- and β-cyclodextrin, dextrin, D-fructose 6-phosphate, L-fucose, D-galactose, D-galacturonic acid, gentiobiose, α-D-glucose 1-phosphate, L-glutamic acid, glycogen, glycyl L-glutamic acid, β- and γ-hydroxybutyric acids, inosine, inulin, α-ketoglutaric acid, lactamide, D-lactic acid methyl ester, α-D-lactose, lactulose, D- and L-malic acid, maltose, maltotriose, mannan, D-mannose, melezitose, melibiose, methyl β-D-glucoside, methyl α-D-mannoside, monomethyl succinate, propionic acid, L-pyroglutamic acid, raffinose, L-rhamnose, salicin, sedoheptulosan, stachyose, sucrose, TMP, trehalose, turanose, UMP, and xylitol are not assimilated. Cell-wall amino acids are 2,4-diaminobutyric acid (DAB), alanine, glycine, and glutamic acid. The main menaquinones are MK-10 and MK-11, and MK-9 is present in minor amounts. The fatty acid profile consists predominantly of $C_{15:0}$ anteiso, $C_{17:0}$ anteiso, and $C_{16:0}$ iso; $C_{16:0}$, $C_{15:0}$ iso, and $C_{14:0}$ iso are present in minor amounts.

Source: phyllosphere of potato plants in Dahnsdorf (Germany).

DNA G+G content (mol%): not available.

Type strain: K 70/01, DSM 19811, LMG 24388.

Sequence accession no. (16S rRNA gene): AM940158.

Genus XV. **Microcella**, Tiago, Pires, Mendes, Morais, da Costa and Veríssimo 2005a, 1743[VP] (Effective publication: Tiago, Pires, Mendes, Morais, da Costa and Veríssimo 2005b, 484.) emend. Tiago, Morais, da Costa and Veríssimo 2006, 2315[VP]

António Veríssimo

Mi.cro.cel′la. Gr. adj. *mikros* small; L. fem. n. *cella* chamber and in biology a cell; N.L. fem. n. *Microcella* a small cell.

Cells are, rod shaped (0.3–0.4 × 1.6–4 µm), **Gram-stain-positive**, and nonmotile. Nonsporeforming. Aerobic, oxidase-negative, and catalase-positive. Chemo-organotrophic. Mesophilic. Colonies are small and yellow-pigmented. The cell-wall peptidoglycan **is of the B-type containing lysine or ornithine** as the diagnostic amino acid. Fatty acids are predominantly iso- and anteiso-branched, namely $C_{15:0}$ anteiso, $C_{16:0}$ iso, $C_{14:0}$ iso, and $C_{15:0}$ iso. Mycolic acids are not present. The respiratory quinones are unsaturated **menaquinones with 11–14 isoprene** units, with predominance of MK-12 and MK-13. Phylogenetic analysis based on the16S rRNA gene sequences places the genus *Microcella* in the family *Microbacteriaceae*. The closest phylogenetic neighbor is *Yonghaparkia alkaliphila*. The species of *Microcella* are **alkaliphilic** and the strains were isolated from a non-saline highly alkaline (pH 11.4) groundwater located in Southern Portugal.

DNA G+C content (mol%): 67.1–70.7.

Type species: **Microcella putealis** Tiago, Pires, Mendes, Morais, da Costa and Veríssimo 2005a, 1743[VP] (Effective publication: Tiago, Pires, Mendes, Morais, da Costa and Veríssimo 2005b, 484.).

Further descriptive information

Phylogenetic analysis based on the 16S rRNA gene sequences places the genus *Microcella* within the family *Microbacteriaceae*. The lineage including members of genus *Microcella* is closely related with the type species of genera *Yonghaparkia* and *Frigoribacterium*, regardless of the algorithm used to reconstruct the phylogenetic tree.

The 16S rRNA gene sequence of the type strain *Microcella putealis*, shows the highest pairwise similarity (98.2%) with the type strain of *Yonghaparkia alkaliphila* (Yoon et al., 2006b) and 95.5% with the type strain of *Frigoribacterium faeni* (Kämpfer et al., 2000).

Cells are small and rod shaped. The cell-wall peptidoglycan structure found in members of *Microcella* is of the B-type as in all of the members of the family. However, whereas in *Microcella alkaliphila* the peptidoglycan structure is of the B2β type with D-ornithine as the diagnostic diamino acid, in *Microcella putealis*, a novel B-type peptidoglycan structure was found, with lysine as the diagnostic diamino-acid, but the detailed structure remains unknown. The major respiratory quinones found in *Microcella* strains are unsaturated menaquinones with 11 to 14 isoprene units, but the two species differ in their relative amounts. The cellular fatty acid compositions of *Microcella putealis* and *Microcella alkaliphila* are similar and characterized by the predominance of $C_{15:0}$ anteiso (27.4–32.6%), $C_{16:0}$ iso (34.6–30.8%), $C_{14:0}$ iso (12.1–12.5%), and $C_{15:0}$ iso (10.4–9.8).

Microcella strains were isolated and routinely cultured in Alkaline Buffered Medium 2 (ABM2) adjusted to pH 9.0 (Tiago et al., 2004). ABM2 contains the following components per liter of media: yeast extract (Difco), 5.0 g; triptone (Difco), 5.0 g; α-ketoglutaric acid monopotassium salt (Sigma), 1.0 g; agar (Difco), 15.0 g; 100 ml of a macronutrients solution 10× concentrated; 10 ml of a micronutrients solution 100× concentrated; and 100 ml of a specific buffer solution (listed below) at a concentration of 1 M, autoclaved separately and mixed after cooling. The 10× concentrated macronutrients solution contains per liter: nitrilotriacetic acid, 1.0 g; $CaSO_4 \cdot 2H_2O$, 0.6 g; $MgSO_4 \cdot 7H_2O$, 1.0 g; NaCl, 0.8 g; KNO_3, 1.03 g; $NaNO_3$, 6.89 g; $NaHPO_4$, 1.11 g. The 100× concentrated micronutrients solution contains per liter: $MnSO_4 \cdot H_2O$, 0.22 g; $ZnSO_4 \cdot 7H_2O$, 0.05 g; H_3BO_3, 0.05 g; $CuSO_4 \cdot 5H_2O$, 0.0025 g; $Na_2MoO_4 \cdot 2H_2O$, 0.0025 g; $CoCl_2 \cdot 6H_2O$, 0.0046 g.

The following buffer solutions are used to adjust the medium to different pH values: citrate buffer is used to adjust the pH to 6.5, phosphate buffer is used to adjust the pH to 7.0 and 7.5, tris (hydroxymethyl) aminomethane – Tris buffer is used to adjust pH between 8.0 and 8.5, carbonate-bicarbonate buffer is used to adjust pH between 9.0 and 10.0, and carbonate-KOH is used to adjust pH values higher than 10.0.

Strains belonging to *Microcella* are mesophiles and strictly aerobes, do not reduce nitrate, and are able to utilize several sugars and proteinaceous substances. The known members of the genus *Microcella* are alkaliphilic, growth only occurs at pH above 7, and optimum pH for growth varies from 8.5–9.5.

The type strains of *Microcella putealis* and *Microcella alkaliphila* show a close phylogenetic relationship and a high 16S rRNA gene sequence similarity (99.2%), but the DNA–DNA reassociation value is low.

Strains from both species were isolated from non-saline alkaline groundwater located in Cabeço de Vide in Southern Portugal and constitute the major cultivable population. This groundwater is characterized by a high alkalinity (pH 11.4) associated with an extremely low ionic concentration where Ca^{2+} and OH^- are major chemical constituents. The genesis of this particular environment depends on a complex geological context leading to a geochemical process known as serpentinization (Tiago et al., 2004).

Maintenance procedures

Microcella strains are maintained at −70°C in media used for routine culture (ABM2 adjusted to pH 9.0) supplemented with 15% glycerol. Lyophilized cultures are also used.

Differentiation of the genus *Microcella* from closest related genera

Chemotaxonomic characteristics such as the diagnostic diamino acid (lysine or ornithine) present in the peptidoglycan and the menaquinone composition (MK-11 to MK-14) are important distinctive features to differentiate *Microcella* isolates from the closest related taxa. Furthermore, growth temperature, pH range for growth, and some biochemical characters may also be useful differential characteristics.

Table 161 lists the main characteristics differentiating *Microcella* from closest related *Microbacteriaceae*.

TABLE 161. Characteristics differentiating *Microcella* from closest related bacteria[a]

Characteristic	*Microcella*	*Frigoribacterium faeni*	*Yonghaparkia alkaliphila*
Motility	–	+	–
Growth temperature (°C)	15–40	2–25	10–37
Optimal temperature (°C)	35	4–10	30
Optimal pH	8.5–9.5	nd	9
Oxidase	–	–	+
Diamino acids	Lys, Orn	Lys	DAB
Major menaquinones	MK-12, MK-13, MK-14	MK-9	MK-12
DNA G+C content (mol%)	67.1–70.7	71	71.1–71.6

[a]Symbols: +, >85% positive; –, 0–15% positive; nd, not determined; Orn, Ornithine; Lys, lysine; DAB, diaminobutyric acid.

List of species of the genus *Microcella*

1. **Microcella putealis** Tiago, Pires, Mendes, Morais, da Costa and Veríssimo 2005a, 1743[VP] (Effective publication: Tiago, Pires, Mendes, Morais, da Costa and Veríssimo 2005b, 484.)

pu.te.a′lis. L. fem. adj., *putealis* of or belonging to a well.

Cells are very small, rod shaped (0.4 µm × 0.8–1.6 µm),and nonmotile. Gram-stain-positive. Nonsporeforming. Aerobic and heterotrophic. Colonies are small, smooth, convex, and yellow. Oxidase-negative; catalase-positive.

The diamino acid of peptidoglycan is lysine. Major respiratory quinones are menaquinones 12 (MK-12) and 13 (MK-13), but MK-14 and MK-11 are also present. The predominant fatty acids are $C_{16:0}$ iso, $C_{15:0}$ anteiso, $C_{14:0}$ iso, and $C_{15:0}$ iso comprising about 85% of the total fatty acids.

The optimum temperature for growth is 35°C; no growth occurs at 10°C or 45°C. The optimum pH is between 8.5 and 9.0; no growth occurs at pH 7.0 or 10.5. Growth rate is higher without added NaCl, but grows in media containing up to7.0% NaCl.

The strains of this species do not hydrolyze elastin, esculin, or hippurate, however, they hydrolyze starch, gelatin, arginine, and arbutin. Urease, β-galactosidase, and DNase are present, but xylanase is not. Nitrate is not reduced. The type strain of this species assimilates glycerol, L-arabinose, ribose, D-xylose, galactose, glucose, fructose, mannose, mannitol, cellobiose, maltose, sucrose, inulin, starch, glycogen, gluconate, and 5-ketogluconate.

DNA G+C content (mol%): 68.8.

Type strain: CV-2, CIP 108471, LMG 22692.

Sequence accession no. (16S rRNA gene): AJ717388.

2. **Microcella alkaliphila** Tiago, Morais, da Costa and Veríssimo 2006, 2315[VP]

al.ka.li′phi.la. Arabic article *al* the; Arabic n. *qaliy* ashes of saltwort; N.L. n. *alkali* alkali; N.L. adj. *philus -a -um* (from Gr. adj. *philos -ê -on*) friend, loving; N.L. fem. adj. *alkaliphila* loving alkaline environments.

Cells are small, rod shaped (0.3 µm × 3.0 to 4.0 µm), and nonmotile. Gram-stain-positive. Nonsporeforming. Aerobic and heterotrophic. Colonies are small, smooth, convex, and yellow. Oxidase-negative; catalase-positive.

Peptidoglycan is type B2β with D-Orn as the diagnostic diamino acid. The major respiratory quinones are MK-13 and MK-14, but MK-12 is also present. The predominant fatty acids are $C_{15:0}$ anteiso, $C_{16:0}$ iso, $C_{14:0}$ iso, and $C_{15:0}$ iso comprising more than 85% of the total fatty acids.

The optimum temperature for growth is 35°C; no growth occurs at 20°C or 45°C. The optimum pH is 9.5; no growth occurs at pH 7.5 or 10.5. Growth rate is higher without added NaCl, but grows in medium containing up to 8.0% NaCl.

The type strain of this species does not hydrolyze elastin, esculin, gelatin, arbutin, or casein. Hydrolyzes starch and arginine. Urease, β-galactosidase, and DNase are present; xylanase is not detected. Nitrate is not reduced. The type strain of this species assimilates glycerol, arabinose, ribose, D-xylose, galactose, glucose, fructose, L-rhamnose, mannitol, cellobiose, maltose, sucrose, D-turanose, and 2-ketogluconate.

DNA G+C content (mol%): 67.1 (HPLC).

Type strain: AC4r, CIP 108473, LMG 22690

Sequence accession no. (16S rRNA gene): AJ717385.

Genus XVI. **Microterricola** Matsumoto, Yamada, Ōmura and Takahashi 2008, 1021[VP]

Atsuko Matsumoto and Yōko Takahashi

Mic.ro.ter.ri′co.la. Gr. adj. *mikros* small; L. fem. n. *terricola* dweller in soil; N.L. fem. n. *Microterricola* a small dweller in soil.

Irregular rod-shaped cells, 0.4-0.5 × 0.6-1.2 µm and longer in the early cultures. Gram-stain-positive. **Cells with long flagella have motility** (Figure 190, Figure 191). The color of colonies is pale yellow. Catalase-positive; oxidase-negative. Aerobic. Mesophilic. **The diamino acid in cell wall is diaminobutyric acid (DAB)**. The predominant menaquinone is MK-12. 16S rRNA gene sequence analysis shows that this genus belongs to the family *Microbacteriaceae.*

DNA G+C content (mol%): 70.

Type species: **Microterricola viridarii** Matsumoto, Yamada, Ōmura and Takahashi 2008, 1021[VP].

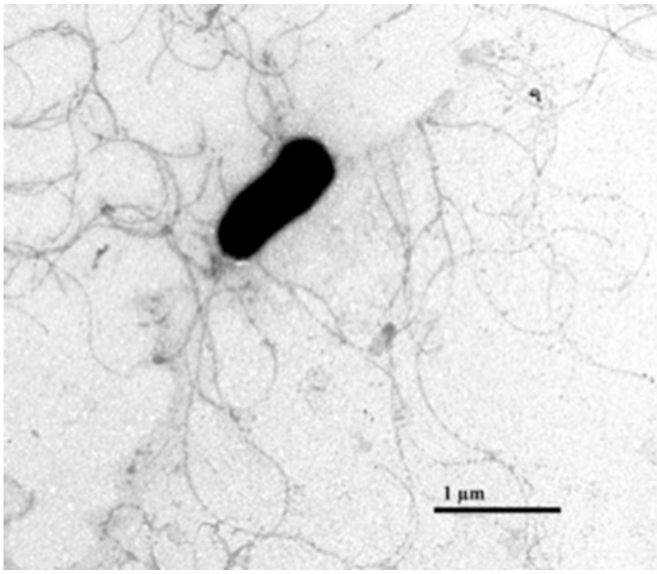

FIGURE 190. Electron micrograph of *Microterricola viridarii* KV-677^T that was negatively stained. Cells cultured on nutrient agar for 2 d at 27°C show long flagella and motility.

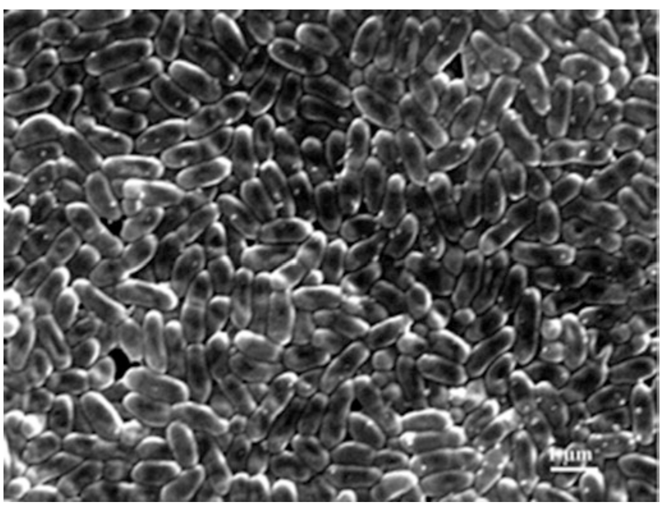

FIGURE 191. Scanning electron micrograph of *Microterricola viridarii* KV-677^T cells cultured on nutrient agar for 10 d at 27°C. Cells are irregular rods and longer in early cultures.

Further descriptive information

The cell-wall peptidoglycan contains DAB, glycine, alanine, and glutamic acid. The acyl type of muramic acid is acetyl. The predominant menaquinone is MK-12. MK-11 and MK-13 are minor menaquinones, however, the amount of total menaquinones in this strain is significantly lower than in other members of the family *Microbacteriaceae*. Mycolic acids are not detected. The dominant cellular fatty acids are $C_{15:0}$ anteiso and $C_{17:0}$ anteiso, and their total accounts for over 80% in type strain of *Microterricola viridarii*.

Enrichment and isolation procedures

The type strain was isolated using GPM agar* containing 0.002% Benlate (Dupont) and 0.0025% nalidixic acid (Sigma) after incubation for 1 week at 27°C from soil collected at a park in the Tokyo metropolitan area, Japan. The organisms grow well on nutrient agar for 2 d at 27°C.

Maintenance procedures

The type strain is maintained by lyophilization. Cultures may be also stored for several years at −80°C with 15% glycerol.

Differentiation of the genus *Microterricola* from other genera

Microterricola has long flagella and shows motility. Table 162 lists the chemotaxonomic differentiations of *Microterricola* and other genera with cell motility in the family *Microbacteriaceae*. *Microterricola* is distinguished from *Agreia* (Evtushenko et al., 2001), *Curtobacterium* (Yamada and Komagata, 1972b), *Frigoribacterium* (Kämpfer et al., 2000), *Humibacter* (Vaz-Moreira et al., 2008b), *Microbacterium* (Takeuchi and Hatano, 1998b), and *Rhodoglobus* (Sheridan et al., 2003) by the presence of DAB as the sole cell-wall diamino acid. The major menaquinone of *Microterricola* is different from those of *Agreia*, *Curtobacterium*, *Frigoribacterium*, *Leifsonia* (Evtushenko et al., 2000), and *Phycicola* (Lee et al., 2008).

Taxonomic comments

Microterricola is very closely related to *Phycicola* in 16S rRNA gene sequence analysis, and the similarity value is also high (>99%). They are distinguished by the ratio of menaquinones; the type species of *Microterricola* contains MK-12 (69%), MK-11 (21%), and MK-13 (10%), and *Phycicola* contains MK-11 (71%), MK-10 (15%), MK-12 (9%), and MK-9 (5%) (Lee et al., 2008).

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*GPM agar: D-glucose, 10 g; peptone, 5 g; meat extract, 5 g; NaCl, 3 g; agar, 12 g; pH 7.

TABLE 162. Characteristics differentiating *Microterricola* from genera containing species with cell motility in the family *Microbacteriaceae*

Chemotaxonomic characteristic	*Microterricola*	*Agreia*	*Curtobacterium*	*Frigoribacterium*	*Humibacter*	*Leifsonia*	*Microbacterium*	*Phycicola*	*Rhodoglobus*
Diamino acid(s)[a]	DAB	DAB and Orn	Orn	Lys	DAB and Orn	DAB	Orn or Lys	DAB	Orn
Acyl type	Acetyl	Acetyl	Acetyl	Acetyl	Acetyl	Acetyl	Glycolyl	Acetyl	Acetyl
Major menaquinone(s)	MK-12	MK-10	MK-9	MK-9	MK-11, MK-12	MK-11, MK-10	MK-11, MK-12, MK-13	MK-11	MK-12, MK-11

[a]Symbols: DAB, diaminobutyric acid; Orn, ornithine; Lys, lysine.

List of species of the genus *Microterricola*

1. **Microterricola viridarii** Matsumoto, Yamada, Ōmura and Takahashi 2008, 1021[VP]

vi.ri.da′ri.i. L. gen. n. *viridarii* of a pleasure-garden, the place where the type strain was isolated.

The color of colonies is pale yellow. Cell size is about 0.4-0.5 × 0.6-1.2 µm. The temperature range for growth is 10-38°C, and the optimum range is 15-30°C. Growth occurs at initial pH 6-11. Glycerol, L-arabinose, D-ribose, esculin, D-maltose, and glycogen are utilized as carbon sources. No growth occurs in the presence of 4% (w/v) NaCl.

In disc susceptibility tests, cells are susceptible to tetracycline (30 µg/disc), polymyxin B (300 U/disc), amikacin (30 µg/disc), chloramphenicol (30 µg/disc), erythromycin (15 µg/disc), norfloxacin (10 µg/disc), ciprofloxacin (5 µg/disc), tobramycin (10 µg/disc), vancomycin (30 µg/disc), kanamycin (30 µg/disc), gentamicin (10 µg/disc), ampicillin (10 µg/disc), imipenem (10 µg/disc), and ceftazidime (30 µg/disc).

The predominant components of cellular fatty acid are $C_{15:0}$ anteiso and $C_{17:0}$ anteiso, and other fatty acids are $C_{16:0}$ iso, $C_{15:0}$ iso, and $C_{16:0}$.

DNA G+C content (mol%): 70 (HPLC).

Type strain: KV-677, JCM 15926, NBRC 102123, NRRL B-24538.

Sequence accession no. (16S rRNA gene): AB282862.

Genus XVII. **Mycetocola** Tsukamoto, Takeuchi, Shida, Murata and Shirata 2001, 941[VP]

Takanori Tsukamoto

My.ce.to′co.la. Gr. n. *mukês -etis* a mushroom, fungus; L. suff. *-cola* (from L. masc. or fem. n. *incola*), dweller, inhabitant; N.L. masc. n. *Mycetocola* fungus-dweller.

Aerobic, Gram-stain-positive, nonsporeforming, nonmotile actinomycetes which form irregular rods (0.2–1.1 × 1.5–3.5 µm). Colonies are circular, convex, and smooth on nutrient agar. Growth occurs between 20–30°C, optimally at 25°C, but not at 4°C or 37°C. Catalase-positive, but oxidase-negative. Esculin is hydrolyzed and tributyrin and Tween 40 degraded. Acid is produced from a broad range of sugars and diverse compounds used as sole carbon sources. **Lysine is the diamino acid in the cell-wall peptidoglycan**. **Muramic acid moieties are *N*-acetylated. The polar lipid pattern consists of two phospholipids, a major diphosphatidylglycerol spot, a prominent glycolipid, and a faint phosphatidylglycerol spot; unsaturated menaquinones with ten isoprene units are the predominant isoprenologues. The major fatty acids are $C_{15:0}$ anteiso and $C_{17:0}$ anteiso.** The phylogenetic position of *Mycetocola*, as determined by 16S rRNA gene sequence analysis, is in the family *Microbacteriaceae*.

Isolated from rotting fruiting bodies of some cultivated or wild *Agaricales* fungi, such as *Lepista nuda* and *Pleurotus ostreatus*, and from the surfaces of Reblochon cheeses.

DNA G+C content (mol%): 64–70.

Type species: **Mycetocola saprophilus** Tsukamoto, Takeuchi, Shida, Murata and Shirata 2001, 942[VP].

Further descriptive information

Phylogeny. The genus *Mycetocola* is a member of the family *Microbacteriaceae* of the order *Micrococcales* and forms an independent phyletic line in the 16S rRNA *Microbacteriaceae* gene tree (Figure 13 in the Road Map to the present volume), a position supported by a high bootstrap value and several tree-making algorithms (Bora et al., 2008). Phylogenetic trees generated from 16S rRNA gene sequences and those from less conserved genes (*gyr*β, *ppk*, *recA* and *rpoB*) show that the genus is most closely related to the genera *Microbacterium* and *Okibacterium* (Evtushenko and Takeuchi, 2006; Stackebrandt et al., 2007).

Cell morphology. *Mycetocola lacteus*, *Mycetocola saprophilus*, and *Mycetocola tolaasinovorans* strains form irregular rods (0.2–0.4 × 2.0–3.5 µm) when grown in PS broth [$Ca(NO_3)_2$× $4H_2O$, 0.5 g; $Na_2HPO_4 \cdot 12H_2O$, 2 g; peptone, 5 g; sucrose, 15 g; decoction of 300 g potato tuber slices, 1 l] for 24 h at 28°C (Tsukamoto et al., 2001). Similarly, *Mycetocola reblochoni* strains form rod-shaped elements (0.3–1.1 × 1.5 µm) on PY agar after 2 d at 30°C (Bora et al., 2008).

Chemotaxonomy. Cell-wall hydrolysates of *Mycetocola* strains contain major amounts of lysine as the diagnostic

TABLE 163. Fatty acid and menaquinone composition (%) of the type strains of *Mycetocola*[a,b]

Component	*M. saprophilus*	*M. lacteus*	*M. reblochoni*	*M. tolaasinivorans*
Fatty acids:				
$C_{13:0}$ anteiso	–	–	–	0.09
$C_{14:0}$ iso	0.59	0.56	0.42	0.83
$C_{14:0}$	0.85	0.81	–	2.14
$C_{15:1}$ anteiso	–	–	0.92	–
$C_{15:0}$ iso	2.03	1.90	0.74	0.77
$C_{15:0}$ anteiso	48.71	47.88	44.48	50.31
$C_{15:0}$	1.09	1.02	–	2.07
$C_{16:0}$ iso	11.18	11.20	10.23	9.73
$C_{16:0}$	17.65	17.23	14.26	20.31
$C_{17:0}$ iso	0.90	0.95	0.51	0.22
$C_{17:0}$ anteiso	16.29	17.61	28.44	12.76
$C_{17:0}$	0.46	0.48	–	0.52
$C_{18:0}$	0.25	0.31	–	0.25
Menaquinones:				
MK-8	7.2	7.4	–	9.8
MK-9	30.4	30.7	2.0	30.9
MK-10	53.3	53.2	95.0	50.8
MK-11	9.1	8.7	3.0	8.5

[a]Symbols: –, 0–15% positive.

[b]Data taken from Bora et al. (2008).

diamino acid (Bora et al., 2008; Tsukamoto et al., 2001). The type strains of the four species contain lysine, alanine, glycine, and glutamic acid in the molar ratios of 1.0:0.6:1.1:1.6 (*Mycetocola reblochoni*), 1.0:0.6:1.0:1.6 (*Mycetocola lacteus*), 1.0:0.5:0.8:1.7 (*Mycetocola saprophilus*), and 1.0:0.7:1.0:1.7 in the case of *Mycetocola tolaasinivorans* (Bora et al., 2008). These workers found that the type strains of *Mycetocola saprophilus*, *Mycetocola reblochoni*, and *Mycetocola tolaasinovorans* had alanine as the *N*-terminal amino acid of the interpeptide bridge. They also found that the peptide patterns of partially hydrolyzed peptidoglycan of representative *Mycetocola reblochoni* strains were very similar, consisting of the dipeptides L-alanine-D-glutamic acid, glycine-D-glutamic acid, and L-lysine-D-alanine, data which justified the conclusion that the strains have a β-type peptidoglycan (Schleifer, 1985), a finding in line with the phylogenetic position of the organisms.

The type strains of the four *Mycetocola* species have *N*-acetylated muramic acid residues, fatty acid profiles rich in hexadecanoic ($C_{16:0}$), 12-methyltetradecanoic ($C_{15:0}$ anteiso), and 14-methylpentadecanoic ($C_{16:0}$ iso) acids, and major amounts of unsaturated menaquinones with ten isoprene units (Bora et al., 2008; Tsukamoto et al., 2001). These investigators also reported quantitative differences in fatty acid and menaquinone components, as is evident from Table 163. In addition, the type strains share simple polar lipid patterns (Bora et al., 2008), as given in the genus description.

Colony morphology and cultural conditions. *Mycetocola lacteus*, *Mycetocola saprophilus*, and *Mycetocola tolaasinivorans* strains grow optimally at 25°C, but do not grow above 33°C or at 4°C, and form circular, convex, smooth, yellowish-white colonies on PS agar (Tsukamoto et al., 2001). Similarly, *Mycetocola reblochoni* strains grow between 20 and 30°C on PY medium (peptone, 10 g; yeast extract, 2 g; D-glucose, 2 g; NaCl, 2 g; distilled water, 1 liter; Tsukamoto et al., 2001), but not at 37°C (Bora et al., 2008). These workers reported that members of *Mycetocola* species produced smooth, circular, convex colonies on brain heart infusion agar (Difco).

Metabolism. Little is known about the metabolic properties of *Mycetocola* strains though they are obligate aerobes and form acid from a broad range of sugars including cellobiose, fructose, glucose, glycerol, maltose, mannitol, and ribose (Bora et al., 2008; Tsukamoto et al., 2001). They use diverse carbon compounds, such as adonitol, dulcitol, ethanol, fucose, glycogen, melibiose, rhamnose, turanose, xylitol, and xylose (all at 1%, w/v), and sodium salts, including acetate, citrate, pyruvate, and valerate (all at 0.1%, w/v), as well as some sole carbon and nitrogen compounds (Bora et al., 2008). These investigators also noted that *Mycetocola* strains hydrolyzed esculin, but not allantoin, and produced esterase lipase (C8) and naphthol-AS-B1 phosphohydrolase, but not alkaline phosphatase, *N*-acetyl-β-glucosamidase, α-fucosidase, β-glucuronidase, lipase (C14), or α-mannosidase. Similarly, they degraded tributyrin and Tween 40, but not elastin, gelatin, guanine, hypoxanthine, uric acid, or xylan.

Strains of *Mycetocola lacteus*, *Mycetocola saprophilus*, and *Mycetocola tolaasinivorans* grow in peptone water but not in Cohn's, Fermi's, or Uschinsky's solutions, and do not produce hydrogen sulfide, indole, 3-ketolactose or 2-ketogluconate, macerate potato tubers, invoke hypersensitivity reactions in tobacco leaves, or form levan from sucrose, and they are negative for the methyl red and Voges–Proskauer tests (Tsukamoto et al., 2001). In addition, strains of these species detoxify tolaasins, pathogenic toxins (Nutkins et al., 1991; Shirata et al., 1995) which are produced extracellularly by *Pseudomonas tolaasii* (Suyama and Fujii., 1993; Tolaas, 1915) and cause brown blotch disease in the economically important cultivated mushrooms, *Agaricus*

bispora and *Pleurotus ostreatus* (Murata et al., 1998; Nutkins et al., 1991; Rainey et al., 1993; Shirata et al., 1995).

Ecology. The type strains of *Mycetocola lacteus*, *Mycetocola saprophilus*, and *Mycetocola tolaasinivorans*, and additional members of the first two species, were isolated from a rotting fruiting body of *Pleurotus ostreatus* and shown to markedly reduce the levels of extracellular toxins, tolaasins, produced by *Pseudomonas tolaasii*, the most destructive pathogen of cultivated mushrooms (Tsukamoto et al., 1998, 2001). Representatives of these organisms, including the type strains, were shown to suppress the development of brown blotch disease of cultivated mushrooms caused by *Pseudomonas tolaasii* (Tsukamoto et al., 2002). In contrast, *Mycetocola reblochoni* strains were isolated from the surfaces of Reblochon cheeses at the late stage of ripening (Bora et al., 2008).

Tsukamoto et al. (1998) showed that brown blotch disease did not develop on the fruiting bodies of cultivated mushrooms when they were sprayed with *Mycetocola tolaasinivorans* strain 9405^T (= CM-05^T) 1–2 d prior to inoculation with *Pseudomonas tolaasii*. Effective control of the disease required a ratio of 1:10 of the initial cell number of the type strain against those of the pathogen. This positive effect was considered to be due to inactivation of tolaasin by the live cells of the type strain, not to metabolites secreted from the cells into the culture media.

The effect of *Mycetocola tolaasinivorans* strain CM-05^T on *Pseudomonas tolaasii in vitro* was examined by Tsukamoto et al. (1998) who co-inoculated about 2×10^5 cells/ml of these bacteria at 24°C for 48 h on a rotary shaker. Cell pellets resuspended in sterile water were examined to determine viable cell numbers and culture supernatants were assayed for the level of tolaasin activity using a potato tuber slice method (Shirata et al., 1995) in which the presence or absence of strain CM-05^T, though supernatants from the mixed culture failed to induce blackening of tuber slices. The authors suggested that the suppression of the toxin occurred either through inhibition of tolaasin production in the *Pseudomonas tolaasii* strain or by inactivation of tolaasin activity in the medium, not through suppression of growth or viability of the pathogen. Strain CM-05^T was then cultured in PS-TOL broth, a medium which contained tolaasins, in order to establish whether the suppressive effect was due to the inactivation of tolaasin activity. To this end, culture supernatants were periodically harvested by centrifugation, serially diluted, and applied to potato tuber slices to determine tolaasin activity. The activity was found to sharply decrease to half the original level within 3 h of inoculation, with inactivation persisting for up to 24 h without any detectable level of toxicity even in the undiluted sample. *Pseudomonas tolaasii* was then cultured in PS-broth and in broth supplemented with spent culture supernatant of strain CM-05^T to establish whether detoxification could be attributed to secreted metabolites or was associated with live cells of strain CM-05^T. A significant level of tolaasin was detected irrespective of the presence or absence of the culture supernatants. In addition, two strains isolated from wild fungi belonging to the *Agaricales*, namely *Mycetocola lacteus* OM-A1 and *Mycetocola tolassinivorans* OM-F11, were found to detoxify tolaasin effectively and suppress the brown blotch disease of cultivated mushrooms (Tsukamoto et al., 2002).

Isolation procedures

The tolaasin-detoxifying *Mycetocola* species can be isolated on potato synthetic agar ($Ca(NO_3)_2 \cdot 4H_2O$, 0.5 g; $Na_2HPO_4 \cdot 12H_2O$, 2 g; peptone, 5 g; sucrose, 15 g; agar, 15 g; decoction of 300 g potato tuber slices, 1 liter; pH 7.0; Wakimoto, 1955) and T-PAF medium (Suyama et al., 1995); the latter formulation is based on *Pseudomonas* agar F (PAF) with the addition of tartrate, 2 g; 0.1% crystal violet, 5 ml; 0.2% BTB, 15 ml; cycloheximide, 100 μg in 1 liter (Suyama et al., 1995). *Mycetocola* strains form yellow colonies on these media, whereas those of *Pseudomonas tolaasii* are a distinctly blue color (Suyama et al., 1995; Wakimoto, 1955). Tolaasin-detoxifying mycetocolae can also be isolated by using the potato tuber slice method (Shirata et al., 1995). Target strains growing on isolation plates should be cultured aerobically at 28°C in PY agar or other media based on glucose, peptone, and yeast extract.

Bora et al. (2008) isolated *Mycetocola reblochoni* strains from Reblochon cheeses by suspending and homogenizing samples of the red smear surface in 2%, w/v, sodium citrate prior to plating tenfold dilutions onto plate count agar (PCA; Biokar Diagnostics) supplemented with 3% w/v sodium chloride. Purified cultures derived from the PCA plates were screened using a *rep*-PCR procedure and the *Mycetocola reblochoni* strains recognized by their distinctive banding patterns following agarose gel electrophoresis.

Maintenance procedures

Short-term preservation can be accomplished by serial transfer every few weeks in appropriate media, such as PS agar (Tsukamoto et al., 2001) and trypticase soy agar (Difco). Longer term preservation can be achieved as frozen stocks in 20%, v/v aqueous glycerol at −20°C and/or at 80°C, or by using standard lyophilization procedures.

Differentiation of the genus *Mycetocola* from other genera

Mycetocola strains form a distinct phyletic line in the 16S rRNA *Microbacteriaceae* gene tree (Figure 13 in the Road Map to the present volume). They can also be distinguished from other genera classified in this family using a combination of chemical markers (Table 163). In addition, peptidoglycan data distinguish them from all other known peptidoglycans based on lysine (Bora et al., 2008).

Differentiation of species of the genus *Mycetocola*

Mycetocola species can be distinguished from one another by using a combination of phenotypic properties (Table 164). They can also be separated by DNA:DNA relatedness data and by quantitative differences in fatty acid and menaquinone components (Bora et al., 2008; Tsukamoto et al., 2001).

TABLE 164. Characteristics differentiating the species of the genus *Mycetocola*[a,b]

Characteristics	*M. saprophilus*	*M. lacteus*	*M. reblochoni*	*M. tolaasinivorans*
Acid production from:				
Dextrin	+	+	−	−
Erythritol	−	+	+	−
Galactose	+	+	−	+
Lactose	+	+	−	−
Mannose	+	−	+	+
Melezitose	+	+	+	−
Raffinose	+	+	−	−
Salicin	+	+	+	−
Sucrose	+	−	+	+
Trehalose	+	+	+	−
Xylose	+	+	−	−+
Degradation of:				
Tween 80	−	+	nd	−
API ZYM:				
Acid phosphatase	−	+	−	+
α-Chymotrypsin	+	−	−	+
Cystine arylamidase	+	−	−	+
α-Galactosidase	−	+	−	+
β-Galactosidase	+	+	−	+
α-Glucosidase	+	−	+	+
β-Glucosidase	+	−	+	+
Leucine arylamidase	+	−	+	+
Trypsin	+	−	−	+
Valine arylamidase	+	−	−	+
Degradation of:				
Casein	−	+	−	−
DNA	−	−	+	−
Tween 60	+	−	−	−
Growth in the presence of 5% (w/v) NaCl	+	−	+	+
Hydrolysis of urea	+	−	+	+
Growth at pH 10.0	+	−	+	+
Reduction of nitrate	+	+	−	−
Utilization of sole carbon and nitrogen sources:				
L-Asparagine	−	−	+	−
L-Histidine	+	−	−	+

[a]Symbols: +, positive; −, negative; nd, not determined.

[b]Data taken from Tsukamoto et al. (2001) and Bora et al. (2008).

List of species of the genus *Mycetocola*

1. **Mycetocola saprophilus** Tsukamoto, Takeuchi, Shida, Murata and Shirata 2001, 942[VP]

sa.pro′phi.lus. Gr. adj. *sapros* putrid; N.L. masc. adj. *philus* (from Gr. masc. adj. *philos*) friend, loving; N.L. masc. adj. *saprophilus* putrid-loving.

Obligately aerobic, nonsporeforming, nonmotile, Gram-stain-positive actinomycetes which form irregular rods (0.2–0.4 × 2.0–3.5 μm) and smooth yellowish-white colonies on PS agar. Grows optimally at 25°C in PS broth. Maximum temperature for growth is 33°C, but does not grow at 4°C.

Additional phenotypic properties are cited either in the text or are shown in Table 164.

Forms a distinct phyletic line in the 16S rRNA gene tree and has chemotaxonomic properties typical of the genus *Mycetocola*.

Source: a rotting fruiting body of the cultivated mushroom *Pleurotus ostreatus*.

DNA G+C content (mol%): 65.2 (HPLC).

Type strain: CM-01, DSM 15178, NBRC 16274, JCM 11655, MAFF 211324, NRRL B-24119.

Sequence accession no. (16S rRNA gene): AB012647, AM410677.

2. **Mycetocola lacteus** Tsukamoto, Takeuchi, Shida, Murata and Shirata 2001, 942[VP]

lac.te′us. L. masc. adj. *lacteus* milky.

Obligately aerobic, nonsporeforming, nonmotile, Gram-stain-positive actinomycetes which form irregular rods (0.2–0.4 × 2.0–3.5 μm), and smooth yellowish-white colonies on PS agar. Grows optimally at 25°C in PS broth. Maximum temperature for growth is 33°C, but does not grow at 4°C.

Additional phenotypic properties are cited either in the text or are shown in Table 164.

Forms a distinct phyletic line in the 16S rRNA gene tree and has chemotaxonomic properties typical of the genus *Mycetocola*.

Source: a rotting fruiting body of the cultivated mushroom *Pleurotus ostreatus*.

DNA G+C content (mol%): 63.9–64.7 (HPLC).

Type strain: CM-10, DSM 15177, NBRC16278, JCM 11654, MAFF 211326, NRRL B-24121.

Sequence accession no. (16S rRNA gene): AB012648, AM410678.

3. **Mycetocola reblochoni** Bora, Vancanneyt, Gelsomino, Snauwaert, Swings, Jones, Ward, Chamba, Kroppenstedt, Schumann and Goodfellow 2008, 2691[VP]

re.blo.cho′ni. N.L. neut. n. *reblochonum* Reblochon cheese; N.L. gen. n. *reblochoni* of a Reblochon cheese, to denote that the first strains were isolated from the surface of Reblochon cheese.

Aerobic, Gram-stain-positive, nonsporeforming, rod-shaped actinomycetes (0.3 × 1.1–1.5 µm) which form circular, convex, smooth, shiny colonies on PY agar. Grows between 20–30°C, but not at 37°C, and from pH 7.0–10.0, but not at pH 12.0.

Additional phenotypic properties are cited either in the text or are shown in Table 164.

Forms a distinct phyletic line in the 16S rRNA gene tree and has chemotaxonomic properties typical of the genus *Mycetocola*.

Source: the surfaces of Reblochon cheeses at the late stage of ripening.

DNA G+C content (mol%): 70.0 (HPLC).

Type strain: R-20377, BRB-1L41, DSM 18580, LMG 22367.

Sequence accession no. (16S rRNA gene): DQ062097.

4. **Mycetocola tolaasinivorans** Tsukamoto, Takeuchi, Shida, Murata and Shirata 2001, 942[VP]

to.la.a.si.ni.vo′rans. N.L. neut. n. *tolaasinum* tolaasin, type of toxin produced by *Pseudomonas tolaasii*; L. part. adj. *vorans* devouring; N.L. part. adj. *tolaasinivorans* tolaasin-devouring (tolaasin-decomposing).

Obligately aerobic, nonsporeforming, nonmotile, Gram-stain-positive actinomycete which form irregular rods (0.2–0.4–2.0 × 3.5 µm), and smooth yellowish-white colonies on PS agar. Grows optimally at 25°C in PS broth. Maximum temperature for growth is 33°C, but does not grow at 4°C.

Additional phenotypic properties are cited either in the text or are shown in Table 164.

Forms a distinct phyletic line in the 16S rRNA gene tree and has chemotaxonomic properties typical of the genus *Mycetocola*.

Source: a rotting fruiting body of the cultivated mushroom *Pleurotus ostreatus*.

DNA G+C content (mol%): 65.1 (HPLC).

Type strain: CM-05, 9405, DSM 15179, NBRC 16277, JCM 11656, MAFF 211325, NRRL B-24120.

Sequence accession no. (16S rRNA gene): AB012646.

Genus XVIII. **Okibacterium** Evtushenko, Dorofeeva, Krausova, Gavrish, Yashina and Takeuchi 2002, 991[VP]

Lyudmila I. Evtushenko

O.ki.bac.te′ri.um. N.L. n. *Oka* the name of the river; L. neut. n. *bacterium* small rod and, in biology, a bacterium (so called because the first ones observed were rod-shaped); N.L. neut. n. *Okibacterium* a bacterium isolated from plants collected near the river Oka.

Small irregular rods (mean 0.4–0.6 × 0.7–1.5 µm). **In older cultures, ovoid or irregular coccoid cells predominate and occur mostly singly or in pairs.** A distinct rod–coccus growth cycle is not observed. No marked motility or primary branching. Cell-wall architecture is typical of **Gram-stain-positive** bacteria, **but cells are readily decolorized.** Nonsporeforming. Non-acid-fast. **Chemo-organotroph with a respiratory type of metabolism.** Catalase and oxidase-positive. Oxidative acid production from some carbohydrates. Mesophilic; optimum temperature ~24–27°C. **The cell-wall peptidoglycan contains lysine as the diamino acid** along with alanine, glycine, glutamic acid, **homoserine, and** the **glycolyl type of muramic acid. Major menaquinones are MK-10 and MK-11**. Saturated fatty acids with anteiso-methyl branched chains predominate (more than 70%). Mycolic acids are absent. Polar lipids comprise diphosphatidylglycerol, phosphatidylglycerol, and a variety of glycolipids.

DNA G+C content (mol%): 67.

Type species: **Okibacterium fritillariae** Evtushenko, Dorofeeva, Krausova, Gavrish, Yashina and Takeuchi 2002, 992[VP].

Further descriptive information

The genus *Okibacterium* belongs to the family *Microbacteriaceae*, order *Micrococcales*. Based on the 16S rRNA gene sequence analysis, *Okibacterium* forms a tight phylogenetic group with the genus *Plantibacter* (see Figure 173). Moreover, the genes encoding DNA gyrase subunit B (*gyrB*), RNA-polymerase subunit B (*rpoB*), recombinase A (*recA*), and polyphosphate kinase (*ppk*) of *Okibacterium fritillariae*, in the absence of the respective data on *Plantibacter* species, do not form robust clusters with representatives of 12 other genera of the family (Stackebrandt et al., 2007).

In young culture, cells are irregular, straight or sometimes slightly curved, short rods, mostly 0.4–0.6 µm × 0.7–1.5 µm (Figure 192). In older cultures (4–7 d), the cells become shorter rods or coccoid forms and usually occur singly or in pairs. A distinct rod–coccus growth cycle like that in *Arthrobacter* species is typically not observed. Reproduction is by fission with the formation of a transverse septum. The electron microscopic

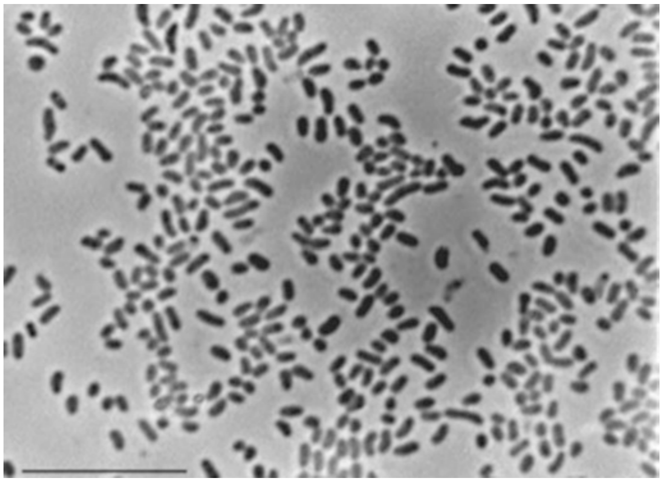

FIGURE 192. Cellular morphology of *Okibacterium fritillariae*. Cells from a 2-d-old slide culture on CB agar were imaged by phase-contrast microscopy. Bar = 10 μm. (Courtesy of N.E. Suzina.)

studies have shown that the cell-wall structure is typical of Gram-stain-positive bacteria. The wall contains a nearly homogeneous electron-dense thick coat (peptidoglycan) around the cytoplasmatic membrane and an irregular outer layer of less density. The tests with Gram-staining and KOH are indistinct or negative (cells frequently fail to retain the Gram stain; the KOH test also shows a reaction typical of Gram-stain-negative bacteria).

Cell-wall hydrolysates contain lysine, alanine, glycine, glutamate, and homoserine in a molar ratio close to 1:1:1:1:0.5 (the reduced content of homoserine is due to formation of the lactone from some portion of homoserine). The amino acid composition is similar to that of *Frigoribacterium faeni* which possesses the B2β type of peptidoglycan (Kämpfer et al., 2000). The glycan moiety of the peptidoglycan preferably contains *N*-glycolyl residues as determined by the method of Uchida and Aida (1977). The cell-wall sugars of both *Okibacterium frutillarim* strains examined are similar and include rhamnose as the predominant component and a minor quantity of glucose. The fatty acid profiles of the two *Okibacterium frutillarim* strains tested contain saturated $C_{15:0}$ anteiso (48% and 54%), $C_{17:0}$ anteiso (23% and 19%), and $C_{16:0}$ iso (29% and 20%) as predominant components; other branched ($C_{14:0}$ iso, $C_{15:0}$ iso, and $C_{17:0}$ iso), straight-chain, and unidentified fatty acids each make up 1–3% of the total. No ω-cyclohexyl or 10-methyl branched acids were detected. The principal polar lipids are diphosphatidylglycerol, phosphatidylglycerol, and a characteristic unknown predominant glycolipid; in addition, one unidentified minor and a few glycolipids in trace amounts have been revealed (N.G. Vinokurova and L.I. Evtushenko, recent observation). The predominant glycolipid showed the same chromatographic behavior as the glycolipid 3 (G_3) reported for representatives of *Rathayibacter* and *Clavibacter* (Collins and Jones, 1980). The major respiratory menaquinone is MK-10, followed by MK-11 and minor amounts of MK-9 and MK-8, as determined by mass spectrometry. Oxidase test with tetramethyl-*p*-phenylendiamine is positive. Both strains have the aa_3- types of cytochrome oxidase; oxidase *bd* is additionally synthesized under oxygen limitation (Trutko et al., 2003).

Colonies of *Okibacterium fritillariae* are yellow-pigmented due to the presence of C_{40}-carotenoids (Trutko et al., 2005). The suppression of pigmentation by fosmidomycin in the concentrations of 0.1–0.3 mg/ml, which are lower than those inhibitory for growth, suggests that the pigment is synthesized via the nonmevalonate pathway (Kuzuyama, 2002; Lichtenthaler, 2000).

Strains usually grow well on standard laboratory media based on yeast extract, peptone, and glucose at near neutral pH and use a wide range of carbon sources for growth and energy in a mineral medium supplemented with 0.1% (w/v) of yeast extract and 0.1% (w/v) of casitone.

Okibacterium fritillariae srains VKM Ac-2059 and VKM Ac-2062 are sensitive to the following antibiotics [inhibitory concentration (μg/ml) in parentheses]: erythromycin (15), levomycetin (30), neomycin (30), streptomycin (30), and tetracycline (30). Strain VKM Ac-2059 is also inhibited by kanamycin (30) and linkomycin (15), while VKM Ac-2062 tolerates these antibiotics.

Little information is available on the habitat and ecology of members of the genus *Okibacterium*. The bacteria are often associated with plants and can be endophytes. The two strains of *Okibacterium fritillariae* were isolated from nearly matured seeds removed aseptically from the seed pods of *Fritillaria ruthenica* Wikstr. and *Clematis recta* L. The seeds were not surface sterilized. On the basis of partial rRNA gene sequences, bacteria similar to *Okibacterium fritillariae* were revealed in endophytic bacterial communities in poplar grown under field conditions (Ulrich et al., 2008) and the shoots and rhizosphere of the Ni-hyperaccumulator *Thlaspi goesingense* (Idris et al., 2004). Most of these *Thlaspi goesingense*-associated strains formed siderophores and tolerated Ni at concentrations of 1–10 mM. Some strains produced 1-aminocyclopropane-1-carboxylic acid deaminase, an enzyme proposed to have no function in bacteria, but able to modulate ethylene levels in developing plants by reducing the amount of the precursor and lowering the levels of "stress" ethylene biosynthesized (Glick et al., 1998; Idris et al., 2004). *Okibacterium* sp. strain GIC 24y was recovered from a deep ice core from a Greenland glacier which was at least 120,000 years old. The strain had small yellow colonies, was able to grow at the temperature range of 2–25°C, and displayed the activities for amylases, β-galactosidases, and DNase (Miteva et al., 2004b). Representatives of the genus were also associated with sponge (*Petrosia ficiformis*) collected along the Mediterranean coast (Bourrain, M., R. Belbes, L. Intertaglia and P. Lebaron, GenBank accession no. AM990749) and with feces of giant panda (*Ailuropoda melanoleuca*) (Ley et al., 2008). There is also a clinical isolate among members of the genus *Okibacterium*. Strain VA8728_00 was discovered in the central venous catheter of a heart-transplant patient (Geissdoerfer, W., I. Wittmann, C. Schoerner, and M. Roellinghoff, GenBank accession no. AF306834).

Enrichment and isolation procedures

Several procedures and media commonly used for isolation of plant-associated bacteria can be used for the isolation of *Okibacterium*. For isolation of strains of *Okibacterium fritillariae*, the seeds of *Fritillaria ruthenica* and *Clematis recta* were cut into pieces, added to 1 ml of 0.85% NaCl (w/v) solution, and ground with a pestle. One drop of the suspension was plated onto CB agar (glucose, 5 g; peptone 5 g; Casamino acids, 3 g;

NaCl, 5 g; agar, 15 g, tap water 1 liter, pH 7.0) and incubated up to 3 weeks at room temperature (18–24°C) under daylight. Tenfold-diluted Trypticase soy agar (Difco) supplemented with $NiCl_2$ (up to 10 mM) and cycloheximide (100 mg/l) was used to isolate *Okibacterium*-like strains associated with the Ni-hyperaccumulator *Thlaspi goesingense* (Idris et al. (2004). Enrichment and subsequent isolation of *Okibacterium* strains can be carried out using MM1 medium supplemented with acetate as described by Miteva et al. (2004b).

Maintenance procedures

Okibacterium strains can be maintained on CB or R2A agar for up to 3 months at 10°C. For long-term preservation, strains can be lyophilized by standard procedures. The two strains of *Okibacterium fritillariae* have been safely maintained for 10 years in lyophilized state with skim milk as a cryoprotectant.

Differentiation of the genus *Okibacterium* from other genera

Characteristics useful for phenotypic delineation of *Okibacterium* from other genera of the family are discussed in the chapter on the family *Microbacteriaceae* (see Table 140). The salient characteristics which differentiate *Okibacterium* strains from the phylogenetically closest genus *Plantibacter* include the presence of lysine as the diamino acid in cell-wall peptidoglycan and the glycolyl type of muramic acid. *Okibacterium* is also clearly distinguished from *Plantibacter* and many other genera of the family by its cellular morphology, particularly in older cultures where coccobacillary forms dominate.

Acknowledgements

This work was supported by the program MCB of the Russian Academy of Sciences.

List of species of the genus *Okibacterium*

1. **Okibacterium fritillariae** Evtushenko, Dorofeeva, Krausova, Gavrish, Yashina and Takeuchi 2002, 992[VP]

fri.til.la.ri′a.e. N.L. gen. n. *fritillariae* of *Fritillaria* generic name of the plant, the source of isolation of the type strain of the species.

Visible growth is observed at 26°C after 3–4 d on CB medium. The colonies are yellow, circular, somewhat convex, glistening, and opaque. Pigment formation is not induced by daylight. No primary branching or distinct motility by flagella. Growth is observed at 7–37°C. The optimum is 24–27°C. H_2S is produced weakly from peptone. D-Arabinose, cellobiose, D-fructose, D-galactose, D-glucose, inositol, inulin, lyxose, maltose, D-mannitol, D-mannose, L-rhamnose, salicin, sorbitol, sucrose, trehalose, and D-xylose are used as carbon and energy sources. Adonitol, dextrin, dulcitol, *meso*-erythritol, and ribose are not used as carbon sources. Growth in the presence of D-melibiose and salicin is weak or absent. Acids are produced during aerobic growth from D-arabinose, D-glucose, mannose, L-rhamnose, sucrose, trehalose, and D-xylose, but not from D-melibiose, melezitose, L-rhamnose, raffinose, D-sorbose, and sorbitol. Starch and esculin are decomposed; hypoxanthine, tyrosine, xanthine, casein, Tween 40, Tween 60, and Tween 80 are not. Tolerates 6% (w/v) NaCl. The chemotaxonomic characteristics are listed in the genus description.

Source (type strain): seeds of *Fritillaria ruthenica* Wikstr. collected in Priosko-Terrasny Biosphere Park, Moscow Region, Russia.

DNA G+C content (mol%): 67 (T_m).

Type strain: DE-504, DSM 15271, NBRC 16404, JCM 12284, VKM Ac-2059.

Sequence accession no. (16S rRNA gene): AM410675.

Genus XIX. **Phycicola** Lee, Lee, Seo, Schumann, Kim and Lee 2008, 1321[VP]

António Veríssimo

Phy.ci′co.la. L. n. *phycos -i* seaweed; L. masc. suffix. *-cola* inhabitant; N.L. masc. n. *Phycicola* inhabitant of seaweed.

Cell morphology varies from rod-shaped (0.4–0.5 × 0.8–1.2 μm) to coccoid cells (0.18–0.33 μm in diameter). **Gram-stain-positive**. Rod-shaped cells are motile whereas the coccoid forms are nonmotile. Nonsporeforming. Chemo-organotrophic. Oxidase-negative and catalase-positive. The cell-wall peptidoglycan is of the **B-type containing 2,4-diaminobutyric acid** (DAB) as the diagnostic amino acid. Fatty acids are predominantly anteiso-branched, namely $C_{15:0}$ anteiso and $C_{17:0}$ anteiso. Mycolic acids are not present. The respiratory quinones are unsaturated **menaquinones, predominantly MK-11 (71%)**, but MK-10 (15%), MK-12 (9%), and MK-9 (5%) are also present in lesser amounts.

DNA G+C content (mol%): 69.8.

Type species: **Phycicola gilvus** Lee, Lee, Seo, Schumann, Kim and Lee 2008, 1321[VP].

Further descriptive information

Phylogenetic analysis based on the 16S rRNA gene sequences show that genus *Phycicola* is closely related with the type strain of *Microterricola viridarii* (Matsumoto et al., 2008), forming a coherent cluster within the radiation of the family *Microbacteriaceae*. The pairwise 16S rRNA gene sequence similarity between *Phycicola gilvus* and *Microterricola viridarii* is 99.5%.

Cell are rod shaped or coccoid depending on the culture age; while in young cultures rod-shaped cells are observed, in older cultures the coccoid forms are predominant. Colonies

are circular, smooth, entire, and the color is variable. The major fatty acids are $C_{15:0}$ anteiso (49.7%) and $C_{17:0}$ anteiso (27.3%) comprising about 77% of the total fatty acids. Polar lipids contain phosphatidylcholine, phosphatidylglycerol, phosphatidylinositol, and diphosphatidylglycerol.

Phycicola isolate is strictly aerobic and is able to utilize several sugars as sole carbon and energy sources.

The only strain belonging to *Phycicola* was isolated from a living seaweed collected at Samyang Beach in Jeju, Republic of Korea.

Maintenance procedures

Phycicola strain is maintained in 20% glycerol suspension supplemented with 60% natural seawater at −80°C. Lyophilized cultures are also used.

Differentiation of the genus *Phycicola* from other genera

The menaquinone composition characterized by the predominance of MK-11, the growth temperature, and some biochemical characteristics may be useful in differentiating *Phycicola* from the closest related taxa (Table 165).

TABLE 165. Characteristics differentiating *Phycicola* from closest related *Microbacteriaceae*[a]

Characteristic	*Phycicola*	*Microterricola*
Cell morphology	Rod/coccoid	Irregular
Motility	−/+	+
Growth temperature (°C)	4–30	10–38
Optimal temperature (°C)	25	15–30
Major menaquinones (MK)	11	12
DNA G+C content (mol%)	69.8	70.0

[a]Symbols: +, >85% positive; −, 0–15% positive.

Taxonomic comments

The close phylogenetic relationship between *Phycicola gilvus* and *Microterricola viridarii* confirmed by the formation of a very coherent cluster supported by bootstrap analysis at a confidence level of 100%, regardless the algorithm used to reconstruct the phylogenetic tree; the high pairwise 16S rRNA gene sequence similarity value (99.5%) between the two species; and the resemblance of some chemotaxonomic characteristics such as the diamino acid in the peptidoglycan, fatty acid profiles, and other physiological features, may raise doubts about the coherence of these two taxa as true distinct genera. An accurate comparison of the chemotaxonomic and biochemical characteristics of the two strains, as well as the determination of the DNA–DNA reassociation value, would be helpful to clarify the taxonomic position of these isolates.

List of species of the genus *Phycicola*

1. **Phycicola gilvus** Lee, Lee, Seo, Schumann, Kim and Lee 2008, 1321[VP]

gil′vus. L. masc. adj. *gilvus* pale yellow-colored.

The characteristics are as described for the genus with the following additional features.

The colony color is variable; colonies are white when cultures are incubated in the dark but yellow when incubated in the light. The optimum temperature for growth is 25°C, but growth occurs at 4–30°C, and no growth occurs at or above 37°C. The pH range for growth is pH 6.1–10.1 with optimum of pH 7.1–8.1. Growth occurs in the presence of up to 2% NaCl. Nitrate is reduced to nitrite. The type strain of this species hydrolyzes esculin, however, casein, elastin, starch, and gelatin are not hydrolyzed. Indole and H_2S are not produced. Citrate is not used. Acid is produced from same substrates such as L-arabinose, D-cellobiose, dextran, D-galactose, D-glucose, glycerol, maltose, L-rhamnose, D-ribose, salicin, and sucrose. Activity of the enzymes urease, alkaline phosphatase, leucine arylamidase, naphthol-AS-BI-phosphohydrolase, acid phosphatase, and α-glucosidase was detected

Major cellular fatty acids detected after incubation on TSA for 3 d at 30°C are $C_{15:0}$ anteiso (49.7%) and $C_{17:0}$ anteiso (27.3%), but $C_{16:0}$ iso (6.1%), $C_{16:0}$ (5.1%), $C_{18:0}$ (5.0%), $C_{15:0}$ iso (3.6%), and $C_{17:0}$ (2.6%) are also detected.

DNA G+C content (mol%): 69.8 (HPLC).

Type strain: SSWW-21, DSM 18319, KCTC 19185.

Sequence accession no. (16S rRNA gene): AM286414, DQ788720.

Genus XX. **Plantibacter** Behrendt, Ulrich, Schumann, Naumann and Suzuki 2002, 1451[VP]

Undine Behrendt, Andreas Ulrich and Peter Schumann

Plan.ti.bac′ter. L. fem. n. *planta* plant; N.L. masc. n. *bacter* rod; N.L. masc. n. *Plantibacter* rod of/from plants.

Pleomorphic cells which are sometimes arranged in an angle to give V-formations, 0.3–0.8 × 0.5–1.5 µm. **Nonmotile**. Nonsporeforming. Cells are **Gram-stain-positive**. Growth is **obligatory aerobic**. **Cell-wall peptidoglycan contains L-DAB, D-Glu, D-Ala, and Gly** corresponding to the **type B2γ**. The muramic acid is **acetylated**. Rhamnose is the predominant cell-wall sugar. **Fatty acid profiles** are characterized by nonhydroxylated fatty acids in which **anteiso- and iso-methyl-branched forms are predominant**. Minor amounts of straight-chain saturated acids are found. Predominant respiratory quinones are menaquinones MK-10 and MK-11, MK-10 or MK-10 and MK-9, depending on the species.

DNA G+C content (mol%): 66–70.

Type species: **Plantibacter flavus** Behrendt, Ulrich, Schumann, Naumann and Suzuki 2002, 1451[VP].

Further descriptive information

The genus comprises two established species, *Plantibacter flavus* (Behrendt et al., 2002) and *Plantibacter auratus* (Lin and Yokota, 2006). "*Plantibacter agrosticola*", "*Plantibacter elymi*", and "*Plantibacter cousiniae*" are three further species for which the names have not yet been validated (Evtushenko and Takeuchi, 2006). The analyses of the 16S rRNA gene sequences of the intended type strains reveal a clear clustering in the branch of the genus *Plantibacter* (Figure 193). Therefore, characteristics of the putative species are likewise considered in the definition of the genus.

The nature of the terminal oxidase of the respiratory chain in the genus *Plantibacter* was investigated by Trutko et al. (2003). It was shown that the studied species (Table 166) contains the aa_3-type cytochrome oxidase. Some species synthesize the quinole oxidases bo_3 or bb_3. Oxygen deficiency in the cultivation medium induces the synthesis of the *bd*-type oxidase. Another characteristic of the genus *Plantibacter* is its ability to synthesize isoprenoid pigments through the non-mevalonate pathway (Trutko et al., 2005). The spectral analysis of the pigments reveals that they are typically C_{40}-carotenoids. The suitability of polyamine patterns for the classification of actinobacterial taxa was studied by Altenburger et al. (1997). The investigations included two strains of the genus *Plantibacter* isolated from plant gall which were tentatively assigned to *Rathayibacter* species. In comparison with established *Rathayibacter* species, the polyamine content of both strains is relatively low and spermine is predominant. These characteristics support their classification separate from the genus *Rathayibacter*. One strain that belongs to the species "*Plantibacter agrosticola*" additionally contains 1,3-diaminopropane, whereas only traces of this polyamine was found in the type strain of "*Plantibacter cousiniae*".

Plantibacteria seem to be widely distributed in the environment, but plants and related sources are their main habitats. The species *Plantibacter flavus* has been isolated from the phyllosphere of grass and the surface litter from sward mulching (Behrendt et al., 2002), where it forms a significant portion of the actinobacterial community. Furthermore, it has been shown that strains of the genus *Plantibacter* are endophytic bacteria living on several plant species. Ulrich et al. (2008) found a multitude of endophytic strains inhabiting hybrid poplar clones. Zinniel et al. (2002) isolated an endophytic strain (LB030) from the above-ground plant tissue of agronomic plants. This strain, formerly classified as *Microbacterium* sp., showed a close relationship to species of *Plantibacter* in the 16S rRNA gene sequence comparison (data not shown). Endophytic

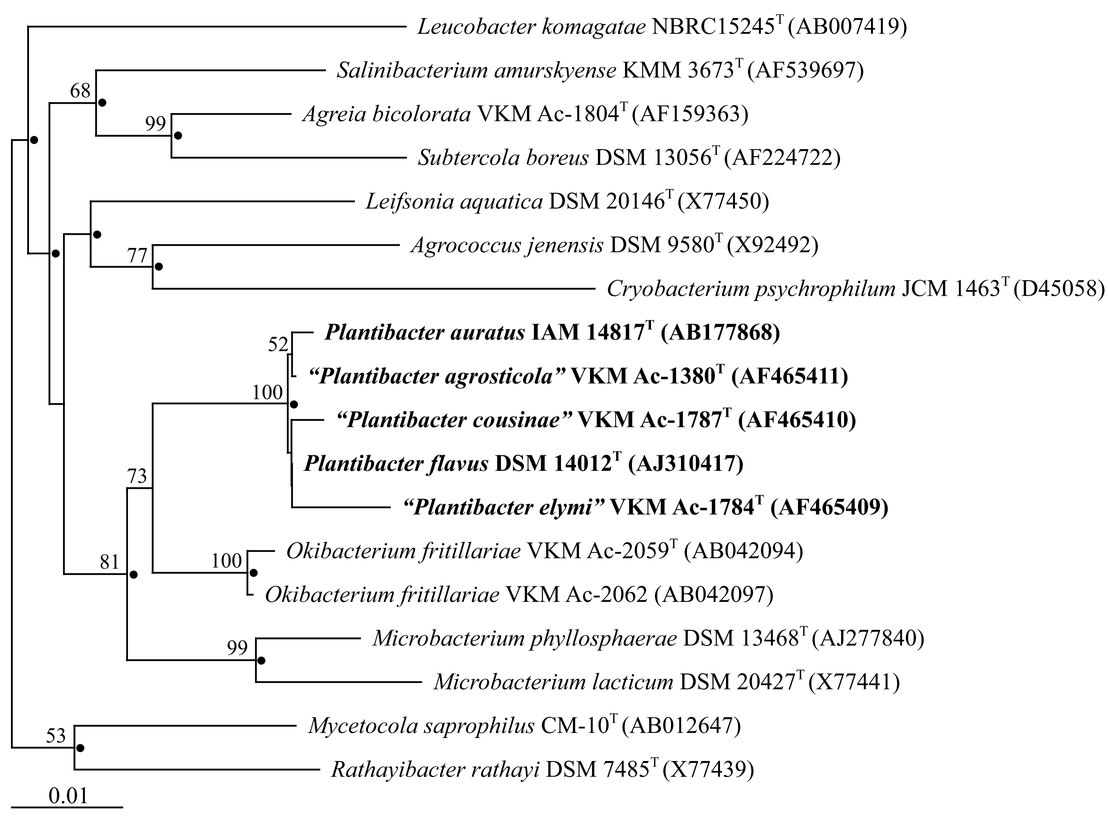

FIGURE 193. Phylogenetic tree showing the relationship of *Plantibacter flavus*, *Plantibacter auratus*, and the putative species "*Plantibacter agrosticola*", "*Plantibacter elymi*", and "*Plantibacter cousiniae*" within the circle of the closely related genera of the family *Microbacteriaceae*. The tree is based on a 1406 bp alignment of 16S rRNA gene sequences and was constructed using the neighbor joining method (Saitou and Nei, 1987). *Dots* indicate branches of the tree that were also formed using the maximum-likelihood method (Felsenstein, 1981). To estimate the root position of the tree, *Brevibacterium linens* (ATCC 9172T; X77451) was used as an outgroup. The values are the relative number that a branch appeared in 1000 bootstrap replications. Bar = relative sequence divergence.

plantibacteria isolated from flowering plants of the Ni hyperaccumulator *Thlaspi goesingense* Hálácsy are of particular interest (Idris et al., 2004). They are able to live in the shoots of *Thlaspi goesingense* despite the extreme high Ni concentrations. Endophytic bacteria are known for their beneficial effects on plant growth (Lodewyckx et al., 2002), and the functional analysis of the isolates accordingly reveals characteristics that potentially support the plant's uptake of heavy metal and the reduction of stress symptoms (Idris et al., 2004). A strain belonging to the genus *Plantibacter*, which displayed antagonistic properties against fungal pathogens, was isolated from *Sphagnum fallax* H. Klinggr. by Opelt et al. (2007) and demonstrates the potential of this bacterial group in the biological control of plant pathogens. An example of the association of plantibacteria with the rhizosphere of plants was shown by Heuer et al. (2002). They described an uncultured clone in the rhizosphere of potato plants that is highly related to the established species of the genus through 16S rRNA gene sequence analysis. The plant colonization of another group of *Plantibacter* species depends on the infection of the plant with highly specialized plant parasitic nematodes. As pointed out by Evtushenko and Takeuchi (2006), strains assigned to "*Plantibacter agrosticola*", "*Plantibacter elymi*", and "*Plantibacter cousiniae*" seem to be strictly associated with *Agrostis* sp. (probably *Agrostis capllaris* L.), *Elymus repens* (L.) Gould., and *Cousinia onopordioides* Ledeb, which are host plants for the nematodes *Anguina agropyri*, *Anguina agrostis*, and *Mesoanguina picridis*, respectively. The bacteria seem to only inhabit the inside of the galls, as they have not been recovered from samples outside of the galls, from plants without nematode infections, or from galls induced by other nematodes. Evtushenko and Takeuchi (2006) concluded from these findings that these *Plantibacter* species use plant parasitic anguinid species as vectors, similar to certain species of *Rathayibacter* (Riley, 1987). Examples of plant pathogenic properties of the strains belonging to the genus *Plantibacter* have not been convincingly demonstrated. Evtushenko and Takeuchi (2006) concluded from the phenotypic characteristics of "*Corynebacterium agropyri*" reported by O'Gara (1915), Cummins et al. (1974), and Riley et al. (1988), that the causative agent of gumming disease might be related to the species of *Plantibacter*.

A strain highly related to the species *Plantibacter auratus* was described after being isolated from an aquatic environment. Schulze et al. (2006) isolated this strain from oysters during a study of the bacterial diversity in a marine hatchery, whereas the type strain of *Plantibacter auratus* arose from a misidentified strain deposited in the National Collection of Industrial and Marine Bacteria (NCIMB) in 1964 (Lin and Yokota, 2006).

Pathogenicity. The clinical significance of the strains of the genus *Plantibacter* was demonstrated by Hirji et al. (2003) in the context of bacterial contamination of transplant products. A strain related by 16S rRNA gene investigations to *Plantibacter flavus* was isolated from peripheral blood or bone marrow stem cell products. The nosocomial outbreak of bacteremia in immunocompromised patients after reinfusion showed the pathogenic potential of otherwise harmless organisms.

Isolation and maintenance procedures

Strains of the species *Plantibacter flavus* can be isolated from plants and plant related sources by the approaches described by Behrendt et al. (1997), Heuer et al. (2002), Opelt et al. (2007), Ulrich et al. (2008), and Zinniel et al. (2002). The bacteria are able to grow on complex media such as Nutrient Agar II (Merck), Trypticase Soy Agar, R2A Agar (Difco), or Medium B from King et al. (1954)* without special requirements for growth factors. Furthermore, they can be cultured on CNS medium (Vidaver and Davis, 1994)†, a medium with selective properties for coryneform bacteria. This medium can be used for the semi-selective isolation of *Plantibacter*, accompanied by the growth of actinobacteria belonging to the genera *Curtobacterium*, *Microbacterium*, *Frigoribacterium*, *Agreia*, and *Clavibacter*, which are ubiquitous on plant material. A genus specific method for isolation has not been described so far.

The procedure for preparing a sample to isolate bacteria from plant galls was described by Evtushenko et al. (2001) and Evtushenko and Takeuchi (2006). The suspension of ground galls were plated on NWG Agar‡, *Corynebacterium* (CB) Agar§, or R2A Agar (Difco) for the first isolation. The media could also be used for further cultivation.

Stocks of the cultures could be maintained for a short period of about 4 weeks on agar slants at 4°C. The bacteria may be preserved for longer periods at subzero temperatures by various methods. There have been good results with preservation on glass beads at −80°C as described by Stead (1990) or the Microbank storage system (Pro-Lab Diagnostics). Recommended procedures for long-term preservation also include lyophilization.

Differentiation of the genus *Plantibacter* from other genera

The phylogenetic analyses on the basis of the 16S rRNA gene sequence comparison clearly demonstrate the separate genus status of *Plantibacter*. As shown in Figure 193, species of the genus constitute a monophyletic branch in the family *Microbacteriaceae*. The closest phylogenetic neighbors are the genera *Okibacterium* and *Microbacterium* which cluster with *Plantibacter* in both the maximum likelihood and the neighbor joining method supported by a high bootstrap value.

The 16S rRNA gene sequence analysis is the most frequently used method for studying the phylogenetic relationship between bacteria (Stackebrandt and Goebel, 1994). However, genes encoding proteins with higher sequence substitution rates than that of 16S rRNA genes are able to provide a better insight into intrageneric phylogenies. Richert et al. (2005) selected the gene encoding DNA gyrase B (*gyrB*) for testing the 16S rRNA gene phylogeny of members of the family *Microbacteriaceae* including the genus *Plantibacter*. It was shown that the nucleotide

*Medium B g/l aqua demin.: proteose-peptone (Difco), 20.0; agar, 15.0; glycerol, 10.0; KH_2PO_4, 1.5; and $MgSO_4 \cdot 7H_2O$, 1.5 (pH 7.2).

†CNS medium g/480 ml: nutrient broth, 8.0; yeast extract, 2.0; potassium phosphate (dibasic), 2.0; potassium phosphate (monobasic), 0.5; agar, 6.5 (pH 6.9 after autoclaving). Add the following ingredients to the medium (g) after autoclaving at 50°C: cycloheximide, 0.020 (2.0 ml of a 1%, w/v, solution); nalidixic acid, 0.0125 (1.25 ml of a solution of 0.1 g nalidixic acid in 7 ml aqua demineralized and 3 ml 1N NaOH); polymyxin B sulfate (8000 USP units/mg), 0.016 (1.6 ml of a solution of 1.0 g in 100 ml aqua demineralized); Daconil 2787-F (530 mg/ml chlorothalonil ml – the active ingredient is tetrachloroisophtalonitrile), 0.00048 (0.03 ml of a solution of 1.2 ml Daconil in 38.8 ml); glucose, 2.5 (25 ml of 10% w/v sterile solution); magnesium sulfate, 0.062 (0.25 ml of a 1.0 M sterile solution).

‡NWG Agar g/900 ml aqua demin.: peptone, 2.0; glucose, 1.0; casein peptone, 1.0; glycerol, 10 ml; wort (a liquid formed by soaking mash in hot water), 100 ml; $CaCO_3$, 5.0; agar, 15.0 (pH 7.2–7.4).

§Corynebacterium (CB) Agar g/l aqua demin.: glucose, 5.0; yeast extract, 5.0; Casamino acid, 10.0; NaCl, 5.0; agar, 15.0 (pH 7.2).

TABLE 166. Characteristics of diagnostic value in identifying the species of *Plantibacter*[a]

Characteristic	*P. flavus*[b,c]	*P. auratus*[d]	"*P. agrosticola*"[e]	"*P. elymi*"[e]	"*P. cousiniae*"[e]
Oxidase test	−	−	+	+	+
Cytochrome oxidase	aa_3	nt	aa_3	aa_3	aa_3
Quinole oxidase	bo_3	nt	bb_3	bo_3	−
Heme *O*	+	nt	−	+	−
Major menaquinone	MK-10, 11	MK-9, 10	MK-9, 10	MK-9, 10	MK-10
$C_{16:0}$ iso Me (%)	−	−	1.53	3.74	2.89
Hydrolysis of:					
Hypoxanthine	−	nt	−	d	+
Starch	d	−	d	+	+
Tween 80	+	+	+	d	−
Xanthine	−	nt	−	d	+
Acid production from:					
Adonitol	−	+	nt	nt	nt
Inositol	−	+	nt	nt	nt
Raffinose	+	−	nt	nt	nt
Ribose	+	−	nt	nt	nt
Methyl red test	−	−	−	d	+
Growth at 6% NaCl	−	nt	d	d	+
Fucose in cell wall	−	+	−	−	+
DNA G+C content (mol%)	68–70	68	67	66	68
Source	Phyllosphere of grass and surface litter	Deposited from V.I. Kudriavzev in 1964 (NCIMB 9991)	*Agrostis* sp.	*Elymus repens*	*Cousinia onopordioides*
Nematode vector	nt	nt	*Anguina agrostis*	*Anguina agropyri*	*Mesoanguina picridis*
Location of gall	nt	nt	Seed	Stem	Leaf

[a]Symbols: +, >85% positive; d, different strains give different reactions (16–84% positive); −, 0–15% positive; nt, not tested.

[b]Behrendt et al. (2002).

[c]Evtushenko (2006) (personal communication).

[d]Lin and Yokota (2006).

[e]Evtushenko and Takeuchi (2006) and Trutko et al. (2003).

substitution of *gyrB* is significantly higher than that of the 16S rRNA gene. Thus, the similarity of *Plantibacter* compared to the other genera on the basis of *gyrB* was reduced by approximately 10–15%, in comparison to its relatedness based on 16S rRNA gene sequences. However, it must be stated that a correlation between both gene similarities has not been elaborated and thus, sequence analyses of *gyrB* and 16S rRNA genes result in different insights into the phylogenetic relationship within the family *Microbacteriaceae*. Nevertheless, this higher heterogeneity enables one to discriminate between closely related strains.

A polyphasic approach for describing a taxon requires, in addition to the phylogenetic analysis, a convincing differentiation on the phenotypic level. Chemotaxonomic characteristics were widely used in the classification and identification of actinobacterial genera. Peptidoglycan typing is a prominent feature used for distinguishing the majority of genera from one another. Thus, the phylogenetically closely related genera *Okibacterium* and *Microbacterium* differ distinctly from *Plantibacter* in the structure of their peptidoglycan (Evtushenko et al., 2002; Takeuchi and Hatano, 1998b). The comparison of the peptidoglycan composition of *Plantibacter*, which corresponds to the type B2γ from Schleifer and Kandler (1972), with those of the remaining genera of the family *Microbacteriaceae* reveals its accordance to the genera given in Table 167. While *Clavibacter*, *Leifsonia*, *Pseudoclavibacter*, and *Yonghaparkia* represent a subgroup because they display the isomers L and D of DAB to almost equal amounts, the genera *Plantibacter*, *Rathayibacter*, *Cryobacterium*, *Agromyces*, and certain species of *Zimmermannella* share another subgroup in which the isomer L-DAB predominates (Behrendt et al., 2002; Evtushenko et al., 2000; Lin et al., 2004; Manaia et al., 2004; Sasaki et al., 1998). Within the latter subgroup, species of *Agromyces* are characterized by filamentous cells fragmenting into coccoid forms in older cultures and by the predominant menaquinones MK-12 and MK-11. Thus, they can easily be distinguished from the genus *Plantibacter* through phenotypic features without the need of phylogenetic studies. Another genus that contains L-DAB in its peptidoglycan is *Agrococcus* (Groth et al., 1996; Wieser et al., 1999). Threonine and aspartic acid were found in the peptidoglycan of certain *Agrococcus* species in addition to the amino acids determined in *Plantibacter*. The species *Agrococcus casei* (Bora et al., 2007) exhibit the same amino acid composition of the peptidoglycan as determined in the genus *Plantibacter* but differ in the occurrence of the main menaquinone MK-12. The different menaquinone composition is an additional feature for phenotypic distinction (Table 167). *Cryobacterium psychrophilum* is an obligatory psychrophile and can be differentiated from *Plantibacter* by a significant amount of $C_{15:1}$ anteiso in the fatty acid profile (Suzuki et al., 1997).

In contrast, the genus *Rathayibacter* is similar in its morphology and main chemotaxonomic characteristics to *Plantibacter* (Behrendt et al., 2002; Dorofeeva et al., 2002; Riley and Ophel, 1992; Zgurskaya et al., 1993) and thus, *Rathayibacter* isolates

TABLE 167. Characteristics differentiating the genera of the family *Microbacteriaceae* displaying the peptidoglycan type B2γ[a,b,c]

Characteristic	*Plantibacter*	*Agrococcus*	*Agromyces*	*Clavibacter*	*Cryobacterium*	*Leifsonia*	*Pseudoclavibacter/ Zimmermannella*	*Rathayibacter*	*Subtercola*	*Yonghaparkia*
Morphology	R	R,C	F, R	R	R	R, F	R	R	R	R
Motility	–	–	–	–	–	+	–	–	–	–
Growth temp. (°C):										
Range	–2 to nt	Up to 37	Up to 37	Up to 35	<18	7–37	nt	<37	–2 to 28	10–37
Optimum	25	28	26–30	21–26	9–12	24–28	30	24–28	15–17	30
Diagnostic diamino acid	L-DAB	L-DAB	L-DAB	DL-DAB	L-DAB	DL-DAB	DL-DAB/L-DAB	L-DAB	DAB	DL-DAB
Acyl type	Acetyl	Acetyl	Acetyl	Acetyl	nt	nt	Acetyl	nt	Acetyl	nt
DNA G+C content (mol%)	66–70	74	70–76	65–78	65	66–73	62–68	63–72	64–68	71–72
Predominant fatty acids (≥5%)	$C_{15:0}$ anteiso, $C_{16:0}$ iso, $C_{17:0}$ anteiso,	$C_{15:0}$ anteiso, $C_{16:0}$ iso, $C_{17:0}$ anteiso, $C_{15:0}$ iso	$C_{15:0}$ anteiso, $C_{17:0}$ anteiso, $C_{16:0}$ iso	$C_{15:0}$ anteiso, $C_{17:0}$ anteiso, $C_{16:0}$ iso	$C_{15:0}$ anteiso, $C_{15:1}$ anteiso, $C_{15:0}$ iso, $C_{17:0}$ anteiso, $C_{16:0}$ iso	$C_{15:0}$ anteiso, $C_{17:0}$ anteiso, $C_{16:0}$ iso	$C_{15:0}$ anteiso, $C_{16:0}$ iso, $C_{16:0}$, $C_{17:0}$ anteiso	$C_{15:0}$ anteiso, $C_{16:0}$ iso, $C_{17:0}$ anteiso,	$C_{15:0}$ anteiso, $C_{16:0}$ iso, $C_{17:0}$ anteiso,	$C_{15:0}$ anteiso, $C_{16:0}$ iso, $C_{17:0}$ anteiso,
Major MK	9, 10; 10; 10, 11	10, 11, 12; 11, 12	12, 13	9	10	11, 10; 11, 12	8, 9; 9; 10	10	9, 10	12, 11

[a]Symbols: C, cocci; F, filaments; R, rods; DAB, diaminobutyric acid; D and L, isomers of amino acids; anteiso, anteiso-branched acids; iso, iso-branched acids; MK, menaquinone; nt, not tested.

[b]Schleifer and Kandler (1972).

[c]Data obtained from following references: Behrendt et al. (2002); Davis et al. (1984); Evtushenko et al. (2000); Groth et al. (1996); Kämpfer et al. (2000); Lin et al. (2004); Mayilraj et al. (2006); Sasaki et al. (1998); Takeuchi et al. (1996); Takeuchi and Hatano (1998); Tsukamoto et al. (2001); Wieser et al. (1999); and Yoon et al. (2006b).

associated with grasses and nematodes in particular might be confused in their genus affiliation with *Plantibacter*. The application of the Fourier-transform infrared (FT-IR) spectroscopy, as a physico-chemical whole-cell fingerprint technique, in the classification of coryneform bacteria with special reference to plant-associated species shows that this method is reliable in differentiating members of *Plantibacter* and *Rathayibacter* effectively (Behrendt et al., 2002; Evtushenko and Takeuchi, 2006). Furthermore, Trutko et al. (2003) showed in a comparative study of the terminal oxidase composition, the potential of this parameter as a chemotaxonomic marker. Species of the genus *Plantibacter* produce cytochrome oxidase aa_3, while *Rathayibacter* species synthesize mainly cytochrome oxidase bb_3 or, depending on growth conditions, both bb_3 and aa_3.

The search by Trutko et al. (2005) for additional chemotaxonomic markers for differentiating actinobacterial genera on a phenotypic level reveals the taxonomic significance of pigment formation. The absorption spectra of pigments are virtually identical for the *Plantibacter* and *Okibacterium* strains tested and similar to *Rathayibacter*. However, the susceptibility to fosmidomycin, an inhibitor of the synthesis of isoprenoid pigments is different. At a concentration of 0.1 mg/ml fosmidomycin, the pigment synthesis in *Plantibacter* bacteria is suppressed, whereas, even at a concentration of 1.0 mg/ml, the growth of these bacteria is not inhibited. In the case of the investigated *Okibacterium* and *Rathayibacter* species, fosmidomycin inhibits not only the synthesis of pigments but also the growth of these bacteria in a concentration-dependent manner. These differences in their tolerance to fosmidomycin make it not only possible to differentiate between them, but also to formulate a selective medium for *Plantibacter* species on this basis. However, the latter will need to be proven in further studies.

Lastly, *Plantibacter* should be differentiated from the genus *Zimmermannella*, as proposed by Lin et al. (2004). The type species of the genus, *Zimmermannella helvola*, is a later homotypic synonym of *Pseudoclavibacter helvolus* and therefore, the genus *Zimmermannella* is illegitimate. However, Lin et al. (2004) described three novel species assigned to *Zimmermannella*. In aberrance to the type species, it was shown that their predominant isomer in the peptidoglycan is L-DAB, just as in the genus *Plantibacter*. The major isoprenoid quinones differ between species from MK-8 to MK-10 and thus, on this basis, only single species of *Plantibacter* and *Zimmermannella* could be differentiated. Fatty acid profiles are similar, but show differences in the amount of their straight-chain saturated fatty acid $C_{16:0}$. Since a clear discrimination based solely on a morphological and chemotaxonomic characterization appears to be difficult for certain genera, the analysis of the 16S rRNA gene sequences is an essential procedure for the clear affiliation of unknown isolates to the genus *Plantibacter*. However, the phenotypic characterization and differentiation is a prerequisite in taxonomic studies if new taxa are to be proposed.

Differentiation of the species of the genus *Plantibacter*

The differential characteristics of the species of *Plantibacter* are given in Table 166.

List of species of the genus *Plantibacter*

1. **Plantibacter flavus** Behrendt, Ulrich, Schumann, Naumann and Suzuki 2002, 1451[VP]

fla′vus L. masc. adj. *flavus* yellow, referring to the colony color.

Characteristics distinguishing the species from other organisms of the genus are shown in Table 166. Morphological and physiological description is based on five strains. Cells are Gram-stain-positive, strictly aerobic, nonsporeforming, nonmotile, irregularly shaped rods, which sometimes form V-shapes. Colonies are yellow, shiny, slightly convex, and round with entire margins. Voges–Proskauer reaction is negative, while catalase is positive. Esculin, DNA, Tween 60, and Tween 80 are hydrolyzed. The type strain is positive for the hydrolysis of starch, while the utilization of starch is strain dependent. None of the strains hydrolyzes casein, gelatin, or cellulose. Formation of levan from sucrose is negative. Strains produce oxidative acid from L-arabinose, D-fructose, D-galactose, D-glucose, lactose, D-maltose, D-mannitol, D-mannose, D-melezitose, raffinose, L-rhamnose, sucrose, D-trehalose, ribose, and D-xylose. Acid formation from inulin, melibiose, D-sorbitol, and L-sorbose is strain dependent while the type strain is negative for these carbohydrates. All strains are negative for adonitol and inositol. Optimum growth temperature is about 25°C. At 4°C, they grow slowly and at −2°C growth still occurs but much slower. The fatty acid profile of the type strain consists of the predominant compounds $C_{15:0}$ anteiso, $C_{16:0}$ iso, and $C_{17:0}$ anteiso, whereas $C_{16:0}$ and $C_{14:0}$ iso are present in minor amounts and $C_{14:0}$ iso in traces.

Source: the phyllosphere of grasses and from the litter layer from sward mulching. The type strain was isolated from the phyllosphere.

DNA G+C content (mol%): 68–70 (HPLC).

Type strain: P 297/02, DSM 14012, JCM 12144, LMG 19919, NBRC 103081.

Sequence accession no. (16S rRNA gene): AJ310417.

2. **Plantibacter auratus** Lin and Yokota, 2006, 2338[VP]

au.ra′tus L. masc. part. adj. *auratus* gold-colored, yellow.

Characteristics that differentiate the species from the other organisms of the genus are shown in Table 166. The morphology and cell-wall composition are as described for the genus. Colonies are circular, convex, smooth, and pale yellow on PY-BHI agar. The optimal temperature for growth is generally 30°C. Catalase and α- and β-galactosidase are produced. The hydrolysis of esculin is positive. Nitrate reduction and alkaline phosphatase are negative. Oxidative acid production from rhamnose, maltose, lactose, and melezitose is positive but negative for L-sorbose, sorbitol, melibiose, and inulin. The major fatty acids are $C_{15:0}$ anteiso, followed by $C_{17:0}$ anteiso, and $C_{16:0}$ iso, whereas minor amounts of $C_{16:0}$ and $C_{14:0}$ iso are present.

DNA G+C content (mol%): 68 (HPLC).

Type strain: IAM 14817, JCM 23192, NBRC 15702, NCDO2317, NCIMB 9991.

Sequence accession no. (16S rRNA gene): AB177868.

Other organisms

1. **"Plantibacter agrosticola"** Evtushenko and Takeuchi 2006

Characteristics differing from those of the other organisms of the genus are given in Table 166. Furthermore the utilization of DL-alanine and L-asparagine as nitrogen source is positive for 80% of the strains tested. Glutamic acid is not used as a nitrogen source. Hydrogen sulfide formation from cysteine is positive for 20% of the tested strains.

DNA G+C content (mol%): 67.

Type strain: VKM Ac-1380.

Sequence accession no. (16S rRNA gene): AF465411.

2. **"Plantibacter cousiniae"** Evtushenko and Takeuchi 2006

Characteristics that are different from the other organisms of the genus are given in Table 166. The utilization of DL-alanine, L-asparagine, and glutamic acid as nitrogen source is positive. Hydrogen sulfide formation is positive.

DNA G+C content (mol%): 68.

Type strain: VKM Ac-1787.

Sequence accession no. (16S rRNA gene): AF465410.

3. **"Plantibacter elymi"** Evtushenko and Takeuchi 2006

In addition to the differential characteristics given in Table 166, all tested strains are negative for the utilization of DL-alanine, L-aparagine, and glutamic acid as nitrogen sources. Formation of H_2S from cysteine is positive.

DNA G+C content (mol%): 66.

Type strain: VKM Ac-1784.

Sequence accession no. (16S rRNA gene): AF465409.

Genus XXI. **Pseudoclavibacter** Manaia, Nogales, Weiss and Nunes 2004, 787[VP]

Célia M. Manaia, Balbina Nogales and Olga C. Nunes

Pseu.do.cla.vi.bac′ter. Gr. adj. *pseudês* false; N. L. masc. n. *Clavibacter* a bacterial generic name; N.L. masc. n. *Pseudoclavibacter* false *Clavibacter*.

Rod-shaped cells. Gram-stain-positive. Nonsporeforming. **Nonmotile**. **Strictly aerobic**. The optimal growth temperature is 30°C. Catalase-positive. **Chemo-organotrophic**. Nitrate is not reduced. **The diamino acid 2,4-diaminobutyric acid (DAB) is present in the peptidoglycan**. The muramic acid of the peptidoglycan is of the acetyl type. **The major respiratory quinone is MK-9**. The major polar lipids are diphosphatidylglycerol and phosphatidylglycerol. The major cellular fatty acids are 12-methyl-tetradecanoic acid ($C_{15:0}$ anteiso), 14-methyl-pentadecanoic acid ($C_{16:0}$ iso), and 14-methyl-hexadecanoic acid ($C_{17:0}$ anteiso).

DNA G+C content (mol%): 67.

Type species: **Pseudoclavibacter helvolus** Manaia, Nogales, Weiss and Nunes 2004, 787[VP].

Further descriptive information

The genus *Pseudoclavibacter* was described based on *Pseudoclavibacter helvolus* DSM 20419[T] (Manaia et al., 2004), previously known as "*Brevibacterium helvolum*" (Zimmermann 1890) Lochhead 1955, and is an earlier homotypic synonym of *Zimmermannella helvola* Lin et al. 2004. Phylogenetic analysis based on the 16S rRNA gene sequence demonstrated that *Pseudoclavibacter* forms a distinct lineage adjacent to *Gulosibacter*, branching deeply within the family *Microbacteriaceae* (Figure 184). *Pseudoclavibacter helvolus* exhibits highest 16S rRNA gene sequence similarities to organisms belonging to the species *Zimmermannella alba* (95.6%), *Zimmermannella faecalis* (95.7%), and *Zimmermannella bifida* (95.6%) (Lin et al., 2004). The closest phylogenetic neighbors belonging to other genera are the species *Gulosibacter molinativorax* (~95%) and *Curtobacterium luteum* (~95%). The recently described species *Pseudoclavibacter soli* (Kim and Jung, 2009), shares with *Pseudoclavibacter helvolus* 93.7% 16S rRNA gene sequence similarity. The distant relatedness of *Pseudoclavibacter soli* to *Pseudoclavibacter helvolus* is also reflected by the quinone system, which, in addition to menaquinone MK-9, also contains major amounts of MK-8.

The cells of *Pseudoclavibacter helvolus* are Gram-stain-positive, irregular rods, non-acid-fast, and nonmotile (Figure 194). Endospores and granules of poly-β-hydroxybutyrate (PHB) are not produced. The cell-wall peptidoglycan of type B2γ, [L-DAB]-D-Glu–D-DAB was reported for *Pseudoclavibacter helvolus* (Schleifer and Kandler, 1972), with L- and D-DAB present in nearly equal proportions (Sasaki et al., 1998). In the species *Pseudoclavibacter helvolus*, the molar ratios of the amino acids glutamate, glycine, alanine, and DAB are approximately 1.0:1.0:0.8:1.8 (Sasaki et al., 1998); Lin et al. (2004) reported a similar composition, although only L-DAB was referred. The muramic acid in the peptidoglycan is of the acetyl type (Lin et al., 2004). In *Pseudoclavibacter soli*, the diagnostic diamino acid is also DAB; in addition, it contains alanine, glutamate, and glycine (Kim and Jung, 2009). The cell-wall sugars of *Pseudoclavibacter helvolus* are reported to be rhamnose and 6-deoxytalose (Lin et al., 2004) while *Pseudoclavibacter soli* contains rhamnose and ribose (Kim and Jung, 2009).

The peptidoglycan of all species of the genus *Zimmermannella* contains L-DAB as diamino acid and rhamnose as cell-wall sugar as well. In the species *Zimmermannella bifida* and *Zimmermannella alba*, the molar ratios of the amino acids glutamate, glycine, alanine, and L-DAB are similar to those found in *Pseudoclavibacter helvolus* (Lin et al., 2004). According to Lin et al. (2004), in the species *Zimmermannella faecalis* described based on strain ATCC 13722[T], the peptidoglycan contains glutamate, glycine, alanine, L-DAB, and homoserine in the molar ratios of 1.0:1.9:0.8:0.9:0.2. However, in a previous study the same strain was reported to exhibit a peptidoglycan composed of [L-Hsr]–D-Glu–D-DAB (Döpfer et al., 1982). Except for *Zimmermannella faecalis*, other sugars are also present in the cell wall of *Zimmermannella* species; *Zimmermannella bifida* contains glucose and *Zimmermannella alba* contains glucose and 6-deoxytalose.

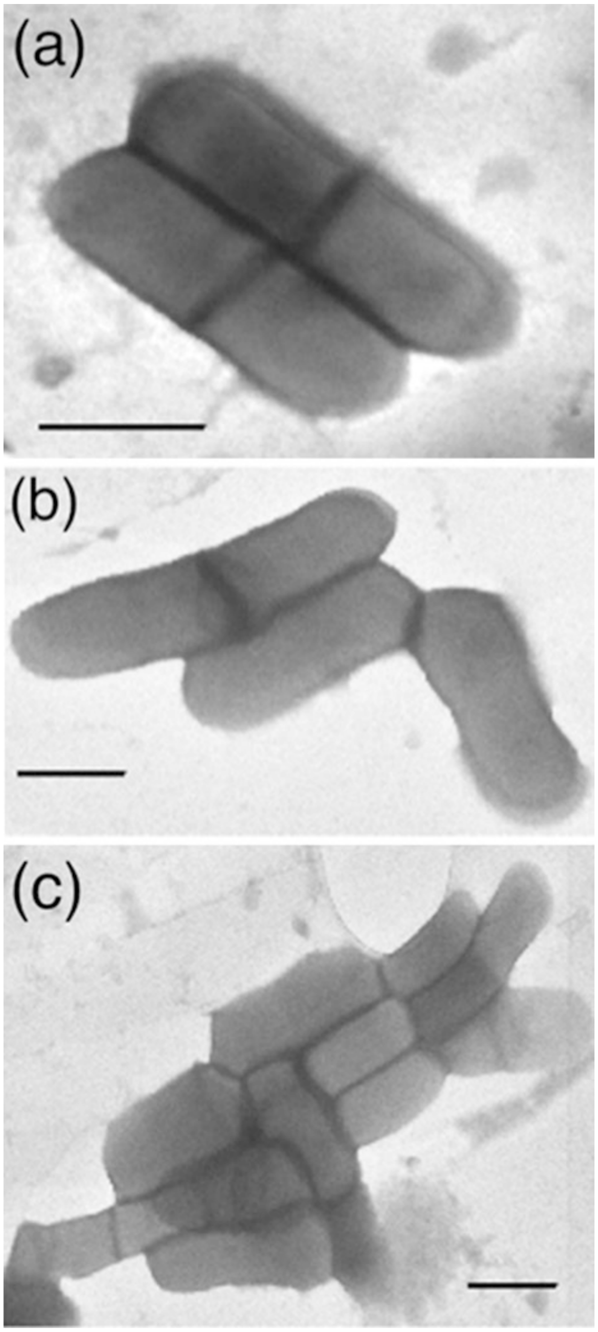

FIGURE 194. Transmission electron micrographs of negatively stained cells of *Pseudoclavibacter helvolus* DSM 20419[T] (a–c) grown on LB medium at 30°C for 24 h. Bar = 0.5 μm. (Reprinted with permission from Manaia et al., 2004. Int. J. Syst. Evol. Microbiol. 54: 783–789.)

The major respiratory quinones of *Pseudoclavibacter* are menaquinones. In *Pseudoclavibacter helvolus* MK-9 accounts for approximately 90%, whereas MK-8 and MK-10 are minor components (Lin et al., 2004; Manaia et al., 2004; Sasaki et al., 1998). In *Pseudoclavibacter soli*, both MK-9 and MK-8 are major components (Kim and Jung, 2009).

The quinone composition of *Zimmermannella faecalis* is similar to that of *Pseudoclavibacter helvolus*. In *Zimmermannella bifida*, MK-8 and MK-9 are in approximately equal proportions, with MK-7 as minor component. *Zimmermannella alba* is characterized by the predominance of MK-10, with minor proportions of MK-9, MK-8, and MK-7 (Lin et al., 2004).

The polyamine pattern of *Pseudoclavibacter helvolus* includes cadaverine as the predominant polyamine (74%), while 1,3-diamino propane is present in a minor proportion (23%) (Altenburger et al., 1997). Like in other members of the *Microbacteriaceae*, the major polar lipids of *Pseudoclavibacter helvolus* are diphosphatidylglycerol and phosphatidylglycerol. Minor amounts of an unknown glycolipid have been reported (Manaia et al., 2004). The major cellular fatty acids are 12-methyl-tetradecanoic acid ($C_{15:0}$ anteiso), 14-methyl-pentadecanoic acid ($C_{16:0}$ iso), and 14-methyl-hexadecanoic acid ($C_{17:0}$ anteiso) (Lin et al., 2004; Manaia et al., 2004). Slightly discrepant results for the relative proportions of hexadecanoic acid ($C_{16:0}$) were reported by Lin et al. (2004) and by Manaia et al. (2004), respectively 6% and 17%. This is probably due to differences in the growth conditions used to produce the biomass from which fatty acids were extracted. A similar fatty acid pattern is found in *Pseudoclavibacter soli* (Kim and Jung, 2009) and in *Zimmermannella* species (Lin et al., 2004). According to Lin et al. (2004), $C_{16:0}$ represents 10–31% of the total fatty acids in the *Zimmermannella* species.

On Luria–Bertani agar, *Pseudoclavibacter helvolus* DSM 20419[T] forms colonies that are yellow, circular, convex, with 2–3 mm in diameter after 2–3 d of growth (Manaia et al., 2004), and on peptone/yeast extract supplemented with brain heart infusion agar (PY-BHI agar) forms smooth white colonies (Lin et al., 2004). *Pseudoclavibacter soli* forms pale-yellow, circular, slightly convex colonies in R2A agar after 4 d of growth (Kim and Jung, 2009).

Pseudoclavibacter helvolus is aerobic and chemoorganothrophic. Nitrate is not reduced under aerobic or anaerobic conditions (Lin et al., 2004; Manaia et al., 2004). In nutritive medium, the range of growth of *Pseudoclavibacter helvolus* is 10–35°C, pH 6–10, and 0–6% (w/v) NaCl (Manaia et al., 2004). The optimal temperature of growth is 30°C (Lin et al., 2004; Manaia et al., 2004). In the characterization of the nutritional pattern, different methodologies were used by Manaia et al. (2004) and Lin et al. (2004). The first analyzed carbon source assimilation, whereas the latter tested acid production. Using the multi-test systems Biolog GP and API 50CH, *Pseudoclavibacter helvolus* presents a versatile nutritional profile, oxidizing a variety of organic compounds including nucleotides, amino acids, organic acids, alcohols, and sugars (Manaia et al., 2004). According to Lin et al. (2004), *Pseudoclavibacter helvolus* produces acid from rhamnose. *Pseudoclavibacter helvolus* is catalase and cytochrome *c* oxidase-positive (Lin et al., 2004; Manaia et al., 2004). The API ZYM profile of *Pseudoclavibacter helvolus* (Manaia et al., 2004) shows that this organism produces acid- and alkaline phosphatase, esterase, and lipase (C_4, C_8, and C_{14}), leucine-, valine-, and cystine-arylamidase, naphthol-AS-BI-phosphohydrolase, β-galactosidase, and α-glucosidase.

Analysis of 16S rRNA gene sequence permitted the affiliation of the genus *Pseudoclavibacter* to the family *Microbacteriaceae*, with *Zimmermannella*, *Gulosibacter*, and *Curtobacterium* as the closest relatives in terms of sequence similarities. Phylogenetic analysis including the sequences of representatives of the different gen-

era in the *Microbacteriaceae* indicates that the 16S rRNA gene sequences of *Pseudoclavibacter* form a deeply branching group within the family, also including *Zimmermannella* species and *Gulosibacter molinativorax*, which groups consistently with the sequences of *Zimmermannella bifida* and *Zimmermannella faecalis* (Figure 184).

Pseudoclavibacter helvolus and *Pseudoclavibacter soli* have high DNA G+C contents (67 and 64 mol%, respectively) (Kim and Jung, 2009; Lin et al., 2004; Manaia et al., 2004).

Pseudoclavibacter helvolus was isolated from butter, and *Pseudoclavibacter soli* was isolated from a ginseng field soil in South Korea (Kim and Jung, 2009).

Enrichment and isolation procedures

Scarce information about the isolation procedures of *Pseudoclavibacter* organisms is available.

Maintenance procedures

The microorganisms can be maintained in the laboratory on nutritive medium (e.g. Luria–Bertani agar* or PY-BHI agar†) for ~10 d at 4°C. Deep freezing (−75°C) or lyophilization are recommended for long-term preservation.

Differentiation of the genus *Pseudoclavibacter* from other genera

Characteristics useful for the differentiation of *Pseudoclavibacter* from other related organisms of *Microbacteriaceae* are indicated in Table 153. The majority of the genera of this family are characterized by having DAB as diamino acid and acetyl-type muramic acid in the peptidoglycan. *Pseudoclavibacter* can be differentiated from other closely related genera by a low phylogenetic relationship, the presence of both L- and D-DAB in the peptidoglycan (rare in *Microbacteriaceae*), the presence of MK-9 as major menaquinone, a distinctive polyamine pattern, and some physiological properties (Table 153 and Table 168).

Taxonomic comments

The circumscription of the genus *Pseudoclavibacter* was based on 16S rRNA gene sequence analysis and on the distinctive chemotaxonomic characteristics, which include cell wall, menaquinones, and polyamines composition. This genus comprises only two validly named species, *Pseudoclavibacter helvolus* and *Pseudoclavibacter soli*, although the 16S rRNA gene sequence-based phylogenetic analysis and the phenotypic and chemotaxonomic characterization indicate that *Pseudoclavibacter* and *Zimmermannella* are synonymic designations for a single genus. The taxonomic position of *Pseudoclavibacter* remained uncertain for a long time. Rainey et al. (1994) showed that strain DSM 20419^T, previously known as "*Brevibacterium helvolum*" (Zimmermann 1890) Lochhead (1955), formed a separate subline of descent within the family *Microbacteriaceae*. Based on cell-wall peptidoglycan type and menaquinones profile, Sasaki et al. (1998) argued that "*Brevibacterium helvolum*" 20419^T could be placed in the species *Clavibacter michiganensis*, whereas results from 16S rRNA gene sequence analysis does not support this affiliation (Sasaki et al., 1998).

The name *Pseudoclavibacter* was proposed by Manaia et al. (2004) based on a further characterization of "*Brevibacterium helvolum*" DSM 20419, and *Pseudoclavibacter helvolus* was proposed as the type species of the genus. A few months later, the genus *Zimmermannella*, with four distinct species and the type species *Zimmermannella helvola* (formerly "*Brevibacterium helvolum*" DSM 20419), was proposed by Lin et al. (2004). The description of *Zimmermannella alba* was based on the characterization of one strain (IAM 14724) which was isolated from human urine in France. The description of *Zimmermannella bifida* was based on three strains that were isolated from soil (Thailand; IAM 14848), from human blood (IAM 15028), and from a human wound (France; IAM 15029). *Zimmermannella faecalis* was described based on re-examination of *Corynebacterium bovis* ATCC 13722 (= IAM 15030). The latter species was listed as an addendum in the first edition of *Bergey's Manual of Systematic Bacteriology* (Collins and Cummins, 1986). In fact, the cell wall of *Corynebacterium bovis* ATCC 13722 contains DAB instead of the *meso*-diaminopimelic acid characteristic of corynebacteria and exhibits a higher mol% G+C content. Moreover, strain ATCC 13722 presents low $T_{m(e)}$ values of DNA-rRNA heteroduplexes with other members of *Corynebacterium* (Döpfer et al., 1982). Based on these features, Collins and Cummins (1986) suggested that *Corynebacterium bovis* ATCC 13722 should constitute the nucleus of a new taxon. Watts et al. (2000, 2001) supported Döpfer et al. (1982), reporting that *Corynebacterium bovis* ATCC 13722 shows highest 16S rRNA gene sequence similarity to "*Brevibacterium helvolum*" (95%) and a rep-PCR pattern different from all the other strains of *Corynebacterium bovis* tested.

Pseudoclavibacter soli Kim and Jung (2009) was described recently, based on the characterization of strain KCTC 19255^T which was isolated from soil in South Korea.

According to the *Bacteriological Code* (1990 Revision), Rule 51b, the genus name *Zimmermannella* Lin et al. 2004 and the species name *Zimmermannella helvola* Lin et al. 2004 are illegitimate. The name *Zimmermannella* is a later homotypic synonym of *Pseudoclavibacter* (Euzéby, 2005) and *Zimmermannella helvola* a later homotypic synonym of the name *Pseudoclavibacter helvolus* (Rule 24a). After formal proposal for combining these two taxa into *Pseudoclavibacter* according to Rule 37a, the species named as *Zimmermannella alba* Lin et al. 2004, *Zimmermannella bifida* Lin et al. 2004, and *Zimmermannella faecalis* Lin et al. 2004 cannot be maintained in the genus *Zimmermannella* because of lack of a type species.

Acknowledgements

We are grateful to Dr J.P. Euzéby, École National Vétérinaire, Toulouse, France, for helpful suggestions and recommendations. The authors acknowledge IUMS copyright holder (which has authorized SGM to act on their behalf in this matter) for permission to reproduce published material.

*Luria–Bertani agar contains yeast extract (5 g/l), tryptone (10 g/l), NaCl (10 g/l) and agar (15 g/l).

†PY-BHI agar contains yeast extract (2 g/l), peptone (1 g/l), NaCl (2 g/l), D-glucose (2 g/l), brain heart infusion (2 g/l), and agar (15 g/l), pH 7.0.

TABLE 168. Characteristics differentiating *Pseudoclavibacter* from *Zimmermannella* species[a]

Characteristic	*P. helvolus*[b,c]	*P. soli*[d]	*Z. alba*[b,d]	*Z. bifida*[b,d]	*Z. faecalis*[b,d]
Cell morphology	Irregular rods	Rods	Irregular rods	Irregular rods with branching	Irregular rods
Oxidase test	+[e]	–	–	–	+
Presence of:					
Alkaline phosphatase	–[c,f]	+	+	+	–
Acid production from:[d]					
D-Fructose	+	w	w	–	+
D-Glucose[b]	–	na	–	–	+
Inositol	w	–	–	–	+
5-Ketogluconate	–	–	w	+	w
D-Lactose	+	–	–	–	–
Maltose	w	–	–	–	+
Mannitol	+	–	–	–	+
D-Mannose	–	–	–	–	+
Rhamnose	w	–	–	d[b]	+
Ribose	w	–	w	–	+
Trehalose	+	–	–	–	+
D-Xylose	w	–	–	–	+
Assimilation of:[d]					
Citrate	–	+	–	–	w
Gluconate	+	–	–	+	–
D-Glucose	–	–	–	–	+
Maltose	–	–	–	+	–
D- Mannitol	–	–	–	–	+
D-Mannose	–	–	–	–	+
Phenyl acetate	–	–	–	–	+
Major menaquinone	MK-9	MK-8, MK-9	MK-10	MK-8, MK-9	MK-9
Cell-wall sugars[b,d,g]	Rha, 6-DT	Rha, rib	Rha, 6-DT, Glc	Rha, Glc	Rha
Amino acids in cell wall[b,d,h]	L-DAB, D-DAB	DAB	L-DAB	L-DAB	L-DAB, Hsr
DNA G+C content (mol%)	67	64	68	62	67

[a]Symbols: +, >89% of strains positive; d, 11–89% of strains are positive; –, <11% of strains are positive.
[b]Data from Lin et al. (2004).
[c]Data from Manaia et al. (2004).
[d]Data from Kim and Jung (2009).
[e]Weak reaction (Manaia et al., 2004).
[f]Positive result with API ZYM (Manaia et al., 2004).
[g]Rha, rhamnose; 6-DT, 6-deoxytalose; Rib, ribose; Glc, glucose.
[h]DAB, diaminobutyric acid; Hsr, homoserine.

List of species of the genus *Pseudoclavibacter*

1. **Pseudoclavibacter helvolus** Manaia, Nogales, Weiss and Nunes 2004, 787[VP]

hel′vo.lus. L. masc. adj. *helvolus* pale yellow, yellowish.

In addition to the characteristics described for the genus and listed in Table 153, Table 168, and Table 169, cells are 0.3–0.5 × 0.8–1.1 μm, and usually occur in pairs. Oxidase reaction is weakly positive. Maximal growth rate is observed in the presence of 1% (w/v) NaCl; 8% NaCl inhibits growth. The diamino acids of the cell-wall peptidoglycan are L- and D-DAB. The major respiratory quinone is MK-9. Major polyamines are cadaverine and 1,3-diaminopropane.

Source: butter.

DNA G+C content (mol%): 67 (HPLC).

Type strain: ATCC 13715, CCUG 43303, DSM 20419, IAM 14726, JCM 9491, NBRC 15775, TISTR 1509.

Sequence accession no. (16S rRNA gene): X77440.

2. **Pseudoclavibacter soli** Kim and Jung 2009, 838[VP]

so′li. L. neut. gen. n. *soli* of soil, the source of the organism.

In addition to the characteristics described for the genus and listed in Tables (Table 153, Table 168, and Table 169), cells are 0.3–0.3 × 0.3–1.5 μm. Oxidase reaction is negative. Maximal growth rate is observed at 30°C and pH 7. The diamino acid of the cell-wall peptidoglycan is DAB. The major respiratory quinones are MK-9 and MK-8.

Source: soil.

DNA G+C content (mol%): 64 (HPLC).

Type strain: KP02, JCM 15058, KCTC 19255.

Sequence accession no. (16S rRNA gene): AB329630.

TABLE 169. Other characteristics of species of the genera *Pseudoclavibacter* and *Zimmermannella*[a,b,c]

Characteristic	*P. helvolus*	*P. soli*	*Z. alba*	*Z. bifida*	*Z. faecalis*
Range of growth:					
Temperature (°C)	10–35	25–42	na	na	na
pH	6–10	5–8	na	na	na
NaCl (%, w/v)	0–6	na	na	na	na
Reduction of NO_3^-	–	–	–	–	–
Liquefaction of gelatin	–	–	–	–	–
Hydrolysis of esculin	–[d]	na	–	–	–
Acid production from:[e]					
L-Fucose	–	–	–	–	–
Glycerol	–	w	–	–	–
Ribose	–	–	–	d	–
Presence of:[c,e]					
Catalase	+	+	+	+	+
Acid phosphatase, cystine arylamidase, esterase (C_4), esterase lipase (C_8), leucine arylamidase, lipase (C_{14}), naphthol-AS-BI-phosphohydrolase, valine arylamidase	+	+	na	na	na
Chymotrypsin, trypsin, α-fucosidase, α-galactosidase, α-glucosidase[d], α-mannosidase, β-galactosidase[d], β-glucuronidase, N-acetyl-β-glucosaminidase	–[f]	–	na	na	na
Urease	–	–	–	–	–
Acid production from:[e]					
N-Acetylglucosamine	w	–	–	–	–
L-Arabinose	–	–	–	w	–
Erythritol	–	–	–	w	–
Glycerol	+	w	w	+	w
Sucrose	w	–	–	–	–
Assimilation of:[e]					
L-Malate	w	+	+	+	+
Menaquinone composition (ratio of peak areas):[b]					
MK-7	0	na	14	8	3
MK-8	6	+[g]	10	46	5
MK-9	88	+[g]	16	46	91
MK-10	6	na	60	0	1
MK-11	0	na	6	0	0
Cellular fatty acid pattern (>5%):[b]					
$C_{15:0}$ anteiso	41.6	64.7[e]	37.8	49.4	42.8
$C_{16:0}$ iso	17.9	6.9[e]	10.9	17.0	27.3
$C_{16:0}$	17.4	4.3[e]	30.5	17.6	10.4
$C_{17:0}$ anteiso	18.9	24.2[e]	15.7	13.3	15.6

[a]Symbols: +, positive reaction; –, negative reaction; w, weak reaction; na, no data available.

[b]Data from Lin et al. (2004).

[c]Data from Manaia et al. (2004).

[d]Positive result for assimilation with API 50CH (Manaia et al., 2004).

[e]Data from Kim and Jung (2009).

[f]Positive result with API ZYM (Manaia et al., 2004).

[g]Major compound.

Genus XXII. **Rathayibacter** Zgurskaya, Evtushenko, Akimov and Kalakoutskii 1993, 147[VP]

Lyudmila I. Evtushenko and Lubov V. Dorofeeva

Ra.thay.i.bac′ter. N.L. n. *Rathaya* Rathay, referring to E. Rathay, Australian plant pathologist who first isolated strains of the genus; N.L. masc. n. *bacter* rod; N.L. masc. n. *Rathayibacter* a rod isolated by Rathay.

Young cells are **irregular, straight to slightly curved rods**, most of which are 0.5–0.7 µm in diameter and ~1.1–2.0 µm in length, **with bluntly rounded ends**. **Cells are often disposed at an angle to each other, giving V-forms** or sometimes more complex angular arrangements. **In older cultures, coccobacillaric forms usually predominant**. Nonmotile. Nonsporeforming. Capsule is produced. Stains Gram-**positive** but not acid-fast. **Lysozyme resistant. Chemo-organotrophs. Obligately aerobic**, having a **respiratory type of metabolism** with oxygen as the terminal electron acceptor. Catalase-positive. **Oxidase reaction is usually negative** or weakly positive. **Nutritionally exacting**, most strains require vitamins and amino acids for growth. Growth moderate

to good on standard laboratory media based on peptone, yeast extract, and glucose at near neutral pH. Colonies are yellow or rose-orange to pink. Acids are produced oxidatively and rather weakly from carbohydrates. **Nitrate is not reduced to nitrite**; nitrite is not reduced. Mesophilic. Optimum temperature ~24–27°C; no growth at 7°C or 37°C.

The cell-wall peptidoglycan is a group B type containing L-**diaminobutyric acid** as the diamino acid. Characteristic cell-wall sugars are rhamnose, glucose, and mannose. Menaquinones are the sole respiratory quinones; the major component is an **unsaturated menaquinone with ten isoprene units (MK-10)**. Saturated anteiso- and iso-methyl-branched fatty acids predominate; 12-methyl-tetradecanoic ($C_{15:0}$ anteiso), 14-methylhexadecanoic ($C_{17:0}$ anteiso), and 14-methylpentadecanoic ($C_{16:0}$ iso) are the major fatty acids. Straight chain saturated acids are present in minor amounts. Mycolic acids absent. Principal polar lipids comprise phosphatidylglycerol, diphosphatidylglycerol, and some characteristic glycosyldiacylglycerols. Polyamine patterns include spermidine and spermine as the predominant compounds.

Most bacteria of the genus *Rathayibacter* so far isolated are plant pathogens causing gummosis in grasses and cereals that are transmitted by nematodes of the genus *Anguinina*.

DNA G+C (mol%): 60–69.

Type species: **Rathayibacter rathayi** (Smith 1913) Zgurskaya, Evtushenko, Akimov and Kalakoutskii 1993, 146[VP] ("*Aplanobacter rathayi*" Smith 1913, 926; "*Phytomonas rathayi*" Bergey, Harrison, Breed, Hammer and Hunton 1923, 192; *Corynebacterium rathayi* Dowson 1942, 313; "*Pseudobacterium rathayi*" Krasil'nikov 1949, 225; "*Corynebacterium michiganense* pv. *rathayi*" Dye and Kemp 1977, 578; *Clavibacter rathayi* Davis, Gillespie, Vidaver and Harris 1984, 113).

Further descriptive information

The genus *Rathayibacter* belongs to the family *Microbacteriaceae*, order *Micrococales*, and, at the time of writing, includes six species: *Rathayibacter rathayi*, *Rathayibacter caricis*, *Rathayibacter festucae*, *Rathayibacter iranicus*, *Rathayibacter tritici*, and *Rathayibacter toxicus*. Based on the 16S rRNA gene sequence analysis, *Rathayibacter* species form a common phylogenetic cluster in which *Rathayibacter toxicus* is the deepest branching species and also possesses the lowest 16S rRNA gene sequence similarities (approx. 96.5–97.5% sequence similarity) with other species of the genus. Comparison of less conserved gene sequences encoding DNA gyrase subunit B (*gyrB*), RNA polymerase subunit B (*rpoB*), recombinase A (*recA*), and polyphosphate kinase (*ppk*) in *Rathayibacter festucae*, *Rathayibacter iranicus*, and *Rathayibacter tritici* support the close relatedness of these species (Stackebrandt et al., 2007). The DNA–DNA similarity values for *Rathayibacter* species range from 15–19% to 50–59% for the pairs of *Rathayibacter rathay*–*Rathayibacter toxicus* and *Rathayibacter rathayi*–*Rathayibacter tritici*, respectively (Sasaki et al., 1998) as determined by the method of Ezaki et al. (1989). A somewhat lower DNA–DNA similarity value was obtained for *Rathayibacter rathayi* and *Rathayibacter tritici* (43%) using the membrane filter method (Zgurskaya et al., 1993).

Cells in young cultures (2–3 d) are straight to slightly curved rods or may be wedge-shaped (Figure 195, top); occurred singly, in pairs, or sometimes in palisade aggregates. The cells range from 0.4–0.8 μm in diameter but most are ~0.5–0.7 μm.

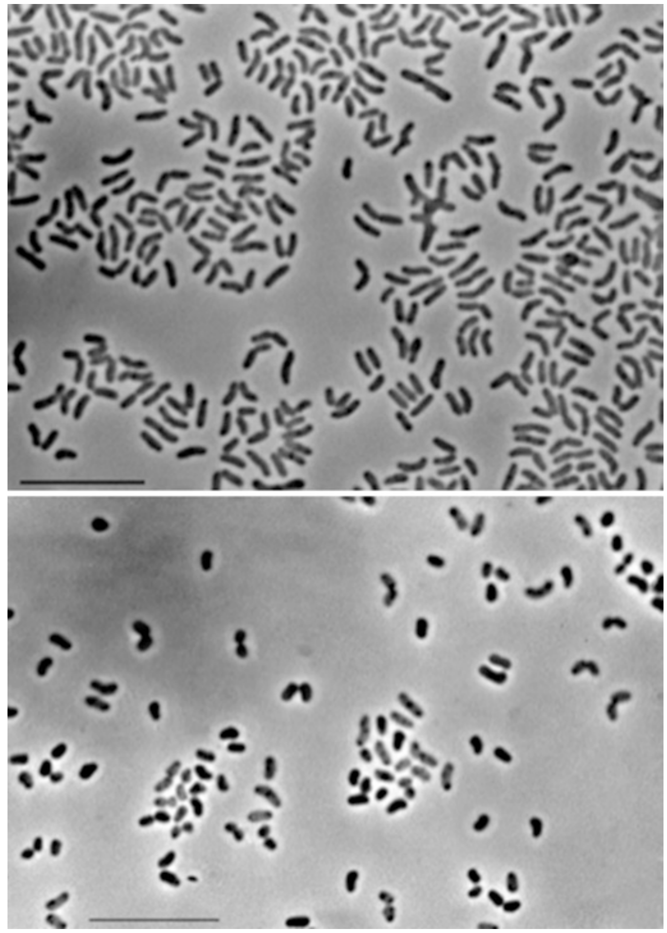

FIGURE 195. Cellular morphology of *Rathayibacter rathayi*, 3-d-old culture (top) and of *Rathayibacter tritici*, 6-d-old culture (bottom), on CB agar. Phase-contrast microscopy. Bars = 10 μm. (Courtesy of Dr N.E. Suzina).

Cells grown for 1 week or more are usually composed of shorter rods to coccoid cells (0.6–0.8 × 0.7–1.2 μm.) which are single or arranged in pairs with angular dispositions (Figure 195, bottom), sometimes forming short chains or clumps. On fresh agar medium, outgrowth of cells occurs by elongation from one or sometimes two (polar, subpolar, or lateral) parts of the cell (Figure 196 A-E). Reproduction is by fission with the formation of a transverse septum. Divided cells often fail to separate after septum formation. Thus, cells may appear quite variable in length and shape under the light microscope and sometimes resemble primary branching cells or hyphae (Figure 195, top; Figure 196 E, F). Larger globose or oval cells may occur occasionally, some of them 2–2.5-fold larger in diameter than the remainder. A distinct rod–coccoid cycle resembling that of *Arthrobacter* species may be observed in some strains during growth on complex agar media, especially in synchronized culture. Cells are surrounded by a capsule (Bird and Stynes, 1977; Bird, 1981, 1985; Ophel et al., 1993; Figure 197) approximately 0.1–0.2 μm or even more thick. Capsular material is usually structured and exhibits radial bundles (Figure 197a) or distinct extracellular outer layers (Figure 197b, arrows).

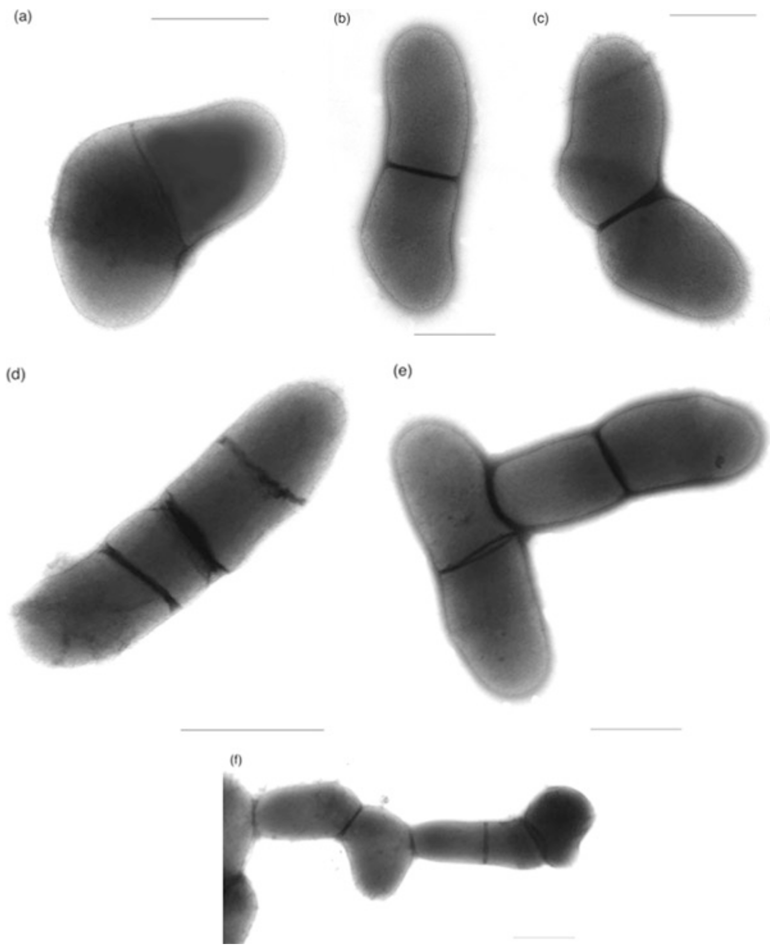

FIGURE 196. Various cell shapes of *Rathayibacter rathayi* (a–f), 1–3-d-old cultures on CB agar. Negative strained; transmission electron microscopy. The cell sizes are most likely smaller than in a living bacterium as the samples were dried during the fixation process. Bar = 0.5 µm. (Courtesy of Dr N.E. Suzina.)

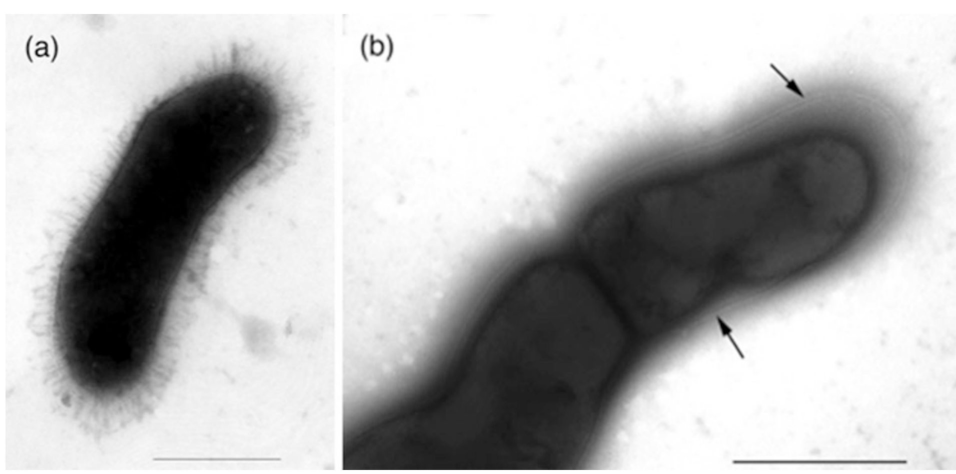

FIGURE 197. Cells of *Rathayibacter rathayi* with structured capsular material, radial bundles (a) and extracellular outer layers (b, *arrows*). 5-d-old culture on CB agar. Negatively stained; transmission electron microscopy. The sizes are most likely smaller than in a living bacterium as the sample was dried during the fixation process. Bar = 0.5 µm. (Courtesy of N.E. Suzina.)

TABLE 170. Characteristics differentiating species of the genus *Rathayibacter*[a,b]

Characteristic	*R. rathayi*	*R. caricis*	*R. festucae*	*R. iranicus*	*R. toxicus*	*R. tritici*
Colony color on CB agar	Y	Y	O, RO, P	Y	Y	Y
Time for visible growth on CB agar (d)	2–3	2	2	2	4–5	2
Cell-wall sugars:[c]						
Fucose	−	+	−	−	−[d]	−
Glucose, mannose, rhamnose	+	+	+	+	+[d]	+
Xylose	+	+	+	−	−[d]	+
Growth on carbon sources:[e]						
Erythrytol	−	−	+	−	−[d]	−
Inositol	−	+	+	−	−[d]	−
Melibiose	−	+	+	−	−[d]	−
Salicin	+	+	+	−	−[d]	+
Acid from:[f]						
Mannitol	+			−	−	+
Mannose	−			+	+	+
Hydrolysis of starch	d/−[g]	−	−	−	−	−
DNase[g]	+			−	−	−
H_2S from peptone	+	+	+	+	−	+
Levan formation	+			−	−	d/−[g]
Maximum NaCl (%)	3	5	<5	2	1	4–5
Tolerance to 0.03% tellurium acetate	+	−	−	+	−	+
Growth on CNS medium[h]	−			−		+
Growth inhibition by *R. iranicus*[i]	+			−	+[d]	d/−[j]
Production of corynetoxins	−			−	+	−
Plasmid size, kb	83/83, 37			−	−	50
DNA G+C content (mol%)	67[k]	68	68	66[k]	60.4[k]	69[k]
Source of isolation	*Dactylis glomerata*	*Carex* sp.	*Festuca rubra*	*Triticum aestivum*	*Lolium rigidum*[l]	*Triticum aestivum*
Normal nematode vector	Unnamed *Anguina* sp.	*Heteroanguina caricis* (?)[m]	*Anguina graminis*	*Anguina tritici*	*Anguina funesta*[l]	*Anguina tritici*

[a]Symbols: +, positive reaction/characteristic; −, negative reaction/characteristic; d, different results either between strains, test methods, or when repeated; w, weak reaction; Y, yellow; O, orange; RO, rose-orange; P, pink.

[b]Data compiled from: Sabet (1954); Gupta and Swarup (1972); Dye and Kemp (1977); Gross et al. (1979); Price (1979); Bradbury (1986); Riley and McKay (1990); Henningson and Cugmestad (1991); Riley and Ophel (1992); Zgurskaya et al. (1993); Sasaki et al. (1998); Behrendt et al. (2002); and Dorofeeva et al. (2002).

[c]Minor or trace amounts of galactose were additionally detected in some strains of *Rathayibacter rathayi* and *Rathayibacter tritici*.

[d]Data from Zgurskaya et al. (1993) for *Rathayibacter toxicus* ICMP 6307 (= WSM 188 = CS 28 = FH-138) originating from Western Australia (Agarkova et al., 2006; Riley and Ophel, 1992; Riley et al., 1988) and for strains ICMP 6306 (= WSM 163), ICMP 6308 (= WSM 189), ICMP 6309 (= WSM 195 = VKM Ac-1600), ICMP 6310 (= WSM 194), and ICMP 6311 (= WSM 190) (see *Taxonomic comments* section for details); no data are available for the type strain of *Rathayibacter toxicus*.

[e]Basal medium: ISP 9 (Shirling and Gottlieb, 1966) supplemented with yeast extract (0.1%) and Casamino acids (Difco) (0.1%).

[f]Method 1 of Dye and Kemp (1977).

[g]Data for type strain according to Behrendt et al. (2002).

[h]CNS agar medium (Gross and Vidaver, 1979b).

[i]Method of Gross and Vidaver (1979a).

[j]Data for type strain.

[k]According to Sasaki et al. (1998). Somewhat higher values were obtained by other authors using different methods (Riley and Ophel, 1992; Starr et al., 1975; Zgurskaya et al., 1993).

[l]Different plant sources (usually occurring in the sites with *Lolium rigidum* infested with *Anguina funesta*) and different nematode vectors were also reported; see the text for more information and references.

[m]Sedge species are host plants for the nematode *Heteroanguina caricis* (Krall, 1991).

Colonies are often variable in size, probably because of adhesive properties of the capsule (Riley and Ophel, 1992) and the presence of clumps of cells in the inoculum, which is especially pronounced with cells from agar media. Colony color is usually yellow to rose-orange or pink (Table 170); the color intensity and shade vary depending on the species, growth medium, culture age, and incubation temperature. The pigments are C_{40}-carotenoids, as seen both from the characteristic three absorption peaks in the spectrum and by mass spectroscopy (Trutko et al., 2005). Pigmentation (but not growth) is inhibited by fosmidomycin at concentrations of 0.1–0.5 mg/ml (Trutko et al., 2005), which suggests the non-mevalonate pathway of pigment biosynthesis (Kuzuyama, 2002; Lichtenthaler, 2000).

The cell-wall peptidoglycan of *Rathayibacter* species contains 2,4-diaminobutyric (DAB), glycine, glutamic acid, and alanine in the molar ratio close to 2:1:1:1 (Dorofeeva et al., 2002; Sasaki et al., 1998; Zgurskaya et al., 1993). When studied in more detail, the peptidoglycans of *Rathayibacter rathayi*, *Rathayibacter*

iranicus, *Rathayibacter tritici*, and *Rathayibacter toxicus* were found to contain predominantly the L-isomer of DAB (Perkins and Cummins, 1964; Sasaki et al., 1998). About a half of the L-DAB in *Rathayibacter tritici* has a free γ-amino group (Perkins, 1965). The peptidoglycan type is supposedly [L-DAB] D-Glu–L-DAB, in which L-DAB is located in the tetrapeptide, like that in many other DAB-containing bacteria (DSMZ, 2001; Schleifer and Kandler, 1972) and also in the interpeptide bridge. The polymer structure differs from that in some other DAB-containing genera (e.g. *Clavibacter*, *Leifsonia*, *Pseudoclavibacter*), which have the peptidoglycan type [L-DAB] D-Glu–D-DAB, variation B2γ, in accordance with the classification of Schleifer and Kandler (1972). Cell-wall mono-sugars of the *Rathayibacter* strains studied so far are glucose, rhamnose, and mannose; some species additionally contain galactose, fucose, or xylose, or their combinations (Davis et al., 1984; Dorofeeva et al., 2002; Zgurskaya et al., 1993). The cell walls of *Rathaybacter* species are resistant to lysozyme (Gross et al., 1979; Zgurskaya et al., 1993).

The major respiratory menaquinone is MK-10 (up to 73–83% of the peak area) (Sasaki et al., 1998); other menaquinones (MK-7, MK-8, MK-9, and MK-11) may be present in minor or trace amounts (Collins and Jones, 1980; Dorofeeva et al., 2002; Sasaki et al., 1998; Zgurskaya et al., 1993). The respiratory chain usually contains three oxidases in different proportions depending on the species, growth phase, and growth conditions: the bb_3- and aa_3- type cytochrome oxidases and non-heme cyanide-resistant oxidase. Under oxygen deficiency, *bd*-oxidase is also produced (Trutko et al., 2003). Oxidase reaction in the tetramethyl-*p*-phenylenediamine test is usually negative, however, some strains exhibit positive reaction (Dorofeeva et al., 2002). Saturated anteiso- and iso-methyl branched fatty acids dominate among cellular fatty acids, with anteiso-methyl branched fatty acids reaching 75–80% or more of the total; the major iso-branched acid is $C_{16:0}$ iso (up to ~40% in *Rathayibacter tritici*) (Collins and Jones, 1980; Dorofeeva et al., 2002; Henningson and Gudmestad, 1991; Zgurskaya et al., 1993). Principal polar lipids are phosphatidylglycerol, diphosphatidylglycerol, and 1–3 characteristic glycolipids, glycosyldiacylglycerols (Collins and Bradbury, 1986; Collins and Jones, 1980).

Polyamines were determined for strains of *Rathayibacter rathayi*, *Rathayibacter tritici*, and *Rathayibacter iranicus*. The overall polyamine content is 4.8–18.2 µmol/g, with spermine (56–71%) and spermidine (13–40%) as predominant components. Other compounds, such as 1,3-diaminopropane, putrescine, and cadaverine are present in minor quantities. Tyramine, *sym*-homospermidine, and *sym*-norspermidine may occur in minor or trace amounts in some species (Altenburger et al., 1997).

The data on minimal nutritional requirements of *Rathayibacter* species are incomplete. Strains grow well on salt mineral media with glucose or sucrose, supplemented with yeast extract (Riley and Ophel, 1992; Zgurskaya et al., 1993). Biotin alone is required for the growth of some *Rathayibacter rathayi* and *Rathayibacter tritici* strains including the type strains. For growth, the type strain of *Rathayibacter iranicus* requires a medium supplemented with biotin, calcium pantothenate, and nicotinic acid; the same vitamins do not satisfy the growth requirements of *Rathayibacter toxicus* (Riley and Ophel, 1992). Many strains grow in the presence of biotin, thiamine, and methionine (Zgurskaya et al., 1993). Glucose metabolism has been shown to take place in a strain of *Rathayibacter tritici*, primarily via the Embden–Meyerhof–Parnas pathway and, to a lesser extent, via the pentose-phosphate pathway (Collins and Bradbury, 1986; Zagallo and Wang, 1967). *Rathayibacter* strains utilize a wide range of carbohydrates and other compounds as carbon and energy sources on appropriate growth media (Carlson and Vidaver, 1982; Davis et al., 1984; Dorofeeva et al., 2002; Dye and Kemp, 1977; Jones, 1975; Locci et al., 1989; Riley and Ophel, 1992; Zgurskaya et al., 1993).

Acid production from various carbon sources is rather weak; test results may vary with the method used, with strains, or when repeated. Tests for acidification of the media supplemented with melibiose, raffinose, L-rhamnose, ribose, sorbose, adonitol, dulcitol, and inositol are negative both when tested with the conventional methods as well as with the API (bioMerieux) test systems (Behrendt et al., 2002). Methionine and phenylalanine can be used as sole sources of nitrogen (Zgurskaya et al., 1993). Most of the strains tested so far do not decompose casein, starch, elastin, hypoxanthine, xanthine, testosterone, Tween 65, urea, and hippurate. H_2S is typically produced from peptone (except for *Rathayibacter toxicus*) and cysteine. Growth is susceptible to 7% NaCl and 0.03% potassium tellurite (Carlson and Vidaver, 1982; Davis et al., 1984; Dorofeeva et al., 2002; Dye and Kemp, 1977; Locci et al., 1989; Zgurskaya et al., 1993). The numerical analysis of 85 strains, including 19 strains of *Rathayibacter* and other bacteria with DAB in the cell wall such as members of the genera *Clavibacter* and *Agromyces*, show that *Rathayibacter* strains form well-defined phenetic clusters in accordance with their species affiliation (Zgurskaya, 1992; Zgurskaya et al., 1993).

The genomes of toxigenic strains described in the literature under the species name *Rathayibacter toxicus* consist of a single linear chromosome (Agarkova et al., 2006). The genome sizes of strain FH-99 (belonging to the same genetic cluster as the type strain of *Rathayibacter toxicus*), strain FH-138 (= CS 28 = ICMP 6307), and FH-100 (= SE3) from two other genomic groups were estimated to be 2.21, 2.25, and 2.30 Mb, respectively (Agarkova et al., 2006).

Plasmids were found in all tested strains of *Rathayibacter rathayi* and *Rathayibacter tritici* except for *Rathayibacter tritici* ICMP 2628 (Gross et al., 1979; Zgurskaya, 1992; Zgurskaya et al., 1993). The sizes of plasmids, as determined by agarose gel electrophoresis, were ~50 kb in *Rathayibacter tritici* strains and 83 kb in *Rathayibacter rathay* strains, including the type strains. Two strains of *Rathayibacter rathayi* also contain a second plasmid of 37 kb. No plasmids were found in the type strain of *Rathayibacter iranicus* and in all tested toxigenic strains assigned to *Rathayibacter toxicus* (Agarkova et al., 2006; Gross et al., 1979; Zgurskaya et al., 1993).

Bacteriophages were isolated for several *Rathayibacter* strains (for early publications, see Collins and Bradbury, 1986). Later, bacteriophages lytic for *Rathayibacter toxicus* and related toxigenic strains but not for *Rathayibacter rathayi*, *Rathayibacter iranicus*, and *Rathayibacter tritici* have been described (McKay et al., 1993; Riley and Gooden, 1991). The phages associated with toxigenic rathayibacters are thought (McKay and Ophel, 1993; Ophel et al., 1993) to be involved in regulation of biosynthesis of the glycolipid toxins (tunicaminyluracil antibiotics) known as corynetoxins (Chatel et al., 1979; Finnie, 2006). However, Kowalski et al. (2007) analyzed bacterially infected galls from toxic grain of *Lolium rigidum* and demonstrated a positive correlation between the presence of the bacterium and corynetoxins

but not with the presence of the bacteriophage isolate NCPPB 3778 (Ophel et al., 1993; Riley and Gooden, 1991).

Out of 18 tested strains of *Rathayibacter rathayi*, *Rathayibacter tritici*, *Rathayibacter iranicus*, and *Rathayibacter toxicus*, production of certain antibacterial substances active against closely related taxa (termed originally bacteriocins) was revealed in the type strains of *Rathayibacter iranicus* and *Rathayibacter tritici* as well as *Rathayibacter tritici* ICMP 2624 (Gross and Vidaver, 1979a; Zgurskaya, 1992; Zgurskaya et al., 1993). An antibiotic with a wide activity spectrum produced by *Rathayibacter iranicus* was identified as a glycolipid (Gross et al., 1979; Gross and Vidaver, 1979a).

Earlier serological studies of plant-pathogenic bacteria, including *Rathayibacter tritici* and *Rathayibacter rathayi*, have been reviewed by Collins and Bradbury (1986) and Riley (1987). Immunodiffusion and enzyme-linked immunosorbent assays (ELISA) and polyclonal ELISA tests showed a close serological similarity among strains of the *Rathayibacter toxicus* group and differences from *Rathayibacter rathayi*, *Rathayibacter iranicus*, and *Rathayibacter tritici* (Riley, 1987). The immunodiffusion technique with antisera raised against *Rathayibacter rathayi*, *Rathayibacter iranicus*, and *Rathayibacter tritici* showed some cross-reactions, however, clear differences were found in the band patterns (Riley, 1987).

The main habitats of recognized *Rathayibacter* species, most of which are plant pathogens causing a gumming disease, are thought to be plants and related sources. *Rathayibacter iranicus* and *Rathayibacter tritici* usually occur in wheat (*Triticum aestivum*), and *Rathayibacter rathayi* is found in cocksfoot grass (*Dactylis glomerata*) (Bradbury, 1973a, 1973b; Collins and Bradbury, 1986, 1991; Gupta and Swarup, 1972; Postnikova et al., 2004; Sabet, 1954; Vidaver, 1982). Strains assigned to *Rathayibacter toxicus* are most commonly found in annual ryegrass (*Lolium rigidum*) and some related grasses (Chatel et al., 1979; McKay and Ophel, 1993; Riley and Ophel, 1992; Riley and Reardon, 1995). The type strains of *Rathayibacter festucae* and *Rathayibacter caricis* have been isolated from *Festuca rubra* and *Carex* species; their plant pathogenic properties are unknown (Dorofeeva et al., 2002). There are also reports of rathayibacters being found in some other sources, e.g. symptomless poplar (Ulrich et al., 2008), tomato leaf, barnyard dust, surface soil of short-grass steppe, cow rumen, a bark beetle (from the database entries of the 16S rRNA gene sequences AB242783, AM237343, AY150910, AY244990, EU379250, EU476035, and AY167853), as well as in ancient Siberian permafrost (Vishnivetskaya et al., 2006) and an ancient salt mine deposit (DQ358658). Representatives of *Rathayibacter* have also been isolated from airliner cabin air (Osman et al., 2008a) and detected among the human skin microbiota (Grice et al., 2009).

In nature, the plant pathogenic rathayibacters are carried into the grass by gall-forming plant parasitic nematodes of the genus *Anguina* (Krall, 1991; Subbotin et al., 2004), where they colonize the inflorescence and can inhabit specific galls induced by the nematodes in various organs of the plant host (Bird, 1981; Bradbury, 1986; Gupta and Swarup, 1972; McKay and Ophel, 1993; Pathak and Swarup, 1984; Postnikova et al., 2004; Price et al., 1979; Riley, 1992, 1995; Riley and McKay, 1991a; Riley and Reardon, 1995; Sabet, 1954). The bacteria show specific adhesion to the cuticle of *Anguina* species and are apparently carried to plants on the nematode surface (Bird, 1981; McClure and Spiegel, 1991; Riley and McKay, 1990). Under natural environmental conditions, the strains of each plant pathogenic *Rathayibacter* species are most commonly found in association with the normal vector nematode (Table 171). Different nematodes have also been shown to act as vectors for some *Rathayibacter* species under experimental conditions (Bradbury, 1973b; Riley, 1995; Sabet, 1954). It is suggested that the bacterial infection of a plant is largely influenced by the host specificity of the vector nematode, the receptiveness of nematodes to bacterial adhesion, and availability of a suitable vector nematode (Agarkova et al., 2006; Riley, 1992; Riley and McKay, 1990, 1991a; Riley and Reardon, 1995; Riley et al., 2001). The bacterial infection of a plant that is not a host of any known vector nematode is most probably related to non-specific invasion by the nematode of grasses, in which it is unable to reproduce (Agarkova et al., 2006; Riley and McKay, 1991b). On documented occasions, such plants were found in the sites with *Lolium rigidum* heavily infested with *Anguina funesta* (Chatel et al., 1979; Riley, 1987; Riley and McKay, 1991b). Some other toxigenic rathayibacters which differ from *Rathayibacter toxicus* at the genomic level can be associated with other vector nematodes (see the Taxonomic comments section).

The typical symptoms of gumming disease caused by plant pathogenic rathayibacters are yellow bacterial slime on developing seed heads, stems, and leaves of a plant and some distortion of these organs (Bradbury, 1986; Gupta and Swarup,

TABLE 171. Characteristics differentiating the genera *Rathayibacter* and *Plantibacter*[a,b]

Characteristic	*Rathayibacter*	*Plantibacter*
Colony color	Yellow, rose-orange, pink	Yellow
Cell diameter, μm	0.4–0.8	0.3–0.6
Cells in a week-old culture, CB agar	Cocco-bacillary	Polymorphic
Acid production from L-rhamnose	–	+
DNase	–/+	+
Resistance to lysozyme	+	–
Sensitivity to fosmidomycin (1 mg/ml)	+	–
Menaquinone composition:[c]		
MK-8	tr	0–12
MK-9	8–18	9–24
MK-10	73–83	55–60
MK-11	tr-18	8–16
Cytochrome oxidase	bb_3 or bb_3, aa_3	aa_3
Quinole oxidase	none	bo_3/bb_3/none
Polyamine, total amount (μmol/g dry wt)[d]	4.8–18.2	1.3–1.43
Predominant polyamines (%):[d]		
Spermine	55.5–71.1	60.8–86.9
Spermidine	13–40	3.5–4.6
1,3-Diaminopropane	0.4–6.4	tr–25.9
DNA G+C content (mol%)	60.4–69	67–70

[a]Symbols: +, positive reaction; –, negative reaction; tr, trace amounts.

[b]Data from Riley and Ophel (1992); Altenburger et al. (1997); Sasaki et al. (1998); Behrendt et al. (2002); Dorofeeva et al. (2002); Trutko et al. (2003, 2005); Evtushenko and Takeuchi (2006); Lin and Yokota (2006); and recent observations.

[c]Peak area.

[d]Data for *Plantibacter* are based on analyses of two strains, VKM Ac-1787 and VKM Ac-1789, supposedly belonging to two novel species.

1972; Riley and Ophel, 1992; Sabet, 1954; Scharif, 1961; Vidaver, 1982). The biochemical mechanisms of distortion have not been strictly elucidated but most likely involve glycolipids, enzymes, and other metabolites (Collins and Bradbury, 1986). *Rathayibacter toxicus* and related bacteria producing corynetoxins (tunicaminyluracil antibiotics) are responsible for toxicity of infected plants, which often results in fatal poisoning of grazing animals (Finnie, 2006; McKay et al., 1993; Riley et al., 2003, 2004b). Corynetoxins induce neurological disorder and are among the most lethal natural toxins (Cheeke, 1995; Finnie, 2006). The ability of rathayibacters to produce corynetoxins rapidly declines following isolation and subculturing (Riley and Ophel, 1992).

The bacteria within desiccated mature plant galls are highly resistant to environmental perturbations and are recoverable after several years from such sources (Bird, 1981; Bradbury, 1973a, 1973b). Strains of "*Corynebacterium agropyri*" have been isolated from 30- to 40-year-old herbarium specimens (Murray, 1986), however, there is no clear evidence for their affiliation with the genus *Rathayibacter*. The presence of a capsule with protective properties and finding of *Rathayibacter* species in some ancient samples suggests that rathayibacters are also able to withstand the hot and arid conditions characteristic of summer (Bird and Stynes, 1977) and some extreme environments in the absence of a host plant or residual plant materials.

Enrichment and isolation procedures

The parts of plants colonized by bacteria (visible bacterial slime or infected plant galls) are the preferable source of isolation of plant pathogenic rathayibacters. If the bacteria are isolated in the earlier phase of bacterial (nematode) infection, the developing seed heads and leaves are selected. Certain common rules of isolation of plant-pathogenic bacteria outlined by Collins and Bradbury (1986) should be followed to reduce contamination with saprophytic bacteria. A number of media based on glucose, peptone, yeast extract, and Casamino acids, e.g. the sterling or tenth-diluted CB* agar medium, NBY† agar, R2A agar (Difco), and some others, can be used for isolation and subculturing of the rathayibacters so far described. CNS‡ agar medium (Vidaver, 1967) enhances the chance of selective recovery of *Rathayibacter tritici*. *Rathayibacter toxicus* and closely related toxic species grow well on 523M agar medium§ (Riley and Ophel, 1992), R agar** (Sasaki et al., 1998), and CB agar.

Maintenance procedures

Rathayibacter strains can be maintained on CB agar, R2A agar, or other suitable growth media for up to 3 months at 4°C or 10°C. For long-term maintenance, strains may be lyophilized by standard procedures. The agar media outlined above are suitable for subculturing before lyophilization.

Differentiation of the genus *Rathayibacter* from other genera

Phenotypic characteristics that delineate *Rathayibacter* from other genera comprising the family *Microbacteriaceae* are listed in Table 170. The genus *Rathayibacter* is phenotypically distinguishable from most genera in the family *Microbacteriaceae* primarily due to the presence of both L-DAB in the cell wall and MK-10 as the predominant menaquinone. Chemotaxonomically, *Rathayibacter* species are similar to *Plantibacter* in the above characteristics. Like *Rathayibacter*, members of the genus *Plantibacter* also often occur in plants and related sources (Behrendt et al., 2002; Evtushenko and Takeuchi, 2006). Distinct gummosis symptoms of a specific plant host and the information on the associated vector nematode (Table 170) are generally indicative of the known plant pathogenic members of genus *Rathayibacter*. Rathayibacters are also distinguished from *Plantibacter* by cell morphology, especially in older (6–8 d) cultures; *Rathayibacter* cells appear cocco-bacillary (Figure 195, bottom), which are usually of similar density (CB agar), whereas cells of *Plantibacter* are markedly polymorphic (heterogeneous in size and density). Other characteristics useful to delineate these genera are listed in Table 171. In addition, Fourier-transform infrared (FT-IR) spectroscopy (Oberreuter et al., 2002) is helpful to differentiate the yellow-pigmented species of *Rathayibacter* and *Plantibacter* (Behrendt et al., 2002). Strains of these two genera can be readily delineated by restriction analysis of amplified 16S rRNA gene as described by Lee et al. (1997). Clear differentiation of *Rathayibacter* strains from *Plantibacter* and other genera is achieved by the 16S rRNA gene sequence analysis.

Taxonomic comments

The organisms of the genus *Rathayibacter*, originally described as "*Aplanobacter rathayi*" (Smith, 1913) and "*Pseudomonas tritici*" (Hutchinson, 1917), are among the oldest distinctly documented representatives of the family *Microbacteriaceae*. After several reclassifications, the above species were assigned to *Corynebacterium* (Burkholder, 1948; Cummins et al., 1974; Dowson, 1942). "*Corynebacterium iranicum*" was described in 1961 (Scharif, 1961). However, only *Corynebacterium rathayi* was included in the Approved Lists (Skerman et al., 1980). Carlson and Vidaver (1982) further supported the separate species status of *Corynebacterium iranicum* and *Corynebacterium tritici* based on analyses of the cellular proteins and previously performed phenotypic studies (Collins and Jones, 1980; Dye and Kemp, 1977; Gross and Vidaver, 1979b). Though the above plant pathogenic species significantly differed from *Corynebacterium sensu stricto* in many properties including cell chemistry (Collins and Jones, 1980; Goodfellow et al., 1976; Keddie and Cure, 1977, 1978; Minnikin et al., 1978; Schleifer and Kandler, 1972), the species were retained within the genus *Corynebacterium* pleading convenience and practicality (Carlson and Vidaver, 1982). In the first

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*Corynebacterium medium, CB (DSMZ medium 53): casein peptone, tryptic digest, 10 g; yeast extract, 5.0 g; glucose, 5 g; NaCl, 5 g; agar, 15 g; distilled water, 1 liter (pH 7.2–7.4).

†NBY agar (Vidaver, 1967): nutrient broth, 8 g; yeast extract, 2 g; K_2HPO_4, 2 g; KH_2PO_4, 0.5 g; agar, 15 g, water, 1 liter (pH 7.2).

‡CNS agar medium (Gross and Vidaver, 1979b): the CNS medium is based on NBY medium which is supplemented with 1% lithium chloride and the following antibiotics: nalidixic acid, 25 mg (2.5 ml freshly made 1% solution in 0.1 M NaOH); polymyxin B sulfate, 32 mg (3.2 ml of 1% aqueous solution of 8,000 USP units/mg, or a total of 256,000 units); cycloheximide, 40 mg (4 ml of 1% aqueous solution); and tetrachloro-isophthalonitrile, 0.66 mg (0.625 ml of Bravo 6F diluted 1:50).

§523M agar (Riley and Ophel, 1992): sucrose, 10 g; Casamino acids, 2 g; yeast extract, 2 g; K_2HPO_4, 2 g; $MgSO_4 \cdot 7\,H_2O$, 0.3 g; agar, 15 g, water, 1 liter (pH 7.2).

**R agar (Yamada and Komagata, 1972b): Bacto peptone, 10 g; yeast extract, 5 g; malt extract, 5 g; Casamino acids, 5 g; beef extract 2 g; glycerol 2 g; Tween 80, 50 mg; $MgSO_4 \cdot 7H_2O$, 1 g; agar, 15 g; water, 1 liter (pH 7.2).

edition of *Bergey's Manual of Systematic Bacteriology*, these and other plant pathogenic species characterized by DAB in the cell wall were described as the separate group "Plant pathogenic species of *Corynebacterium*", while stressing that none of them is an authentic *Corynebacterium* species (Collins and Bradbury, 1986). Almost at the same time, Davis et al. (1984) reclassified these species into the newly established genus *Clavibacter*.

The genus *Rathayibacter*, with the three species *Rathayibacter rathay*, *Rathayibacter tritici*, and *Rathayibacter iranicus*, was proposed by Zgurskaya et al. (1993) based on DNA–DNA hybridization, chemotaxonomic studies, and extensive phenotypic studies followed by numerical analysis as well as the relevant information about the group published by that time. This information included, in particular, the data on chemotaxonomy (Collins, 1983; Collins and Jones, 1980), serology (Riley, 1987), multilocus enzyme electrophoresis (Riley et al., 1988), and protein patterns (Carlson and Vidaver, 1982). The proposed generic affiliation of the strains was also consistent with associated diseases, host plants, and transfer by vector nematodes (Bradbury, 1986; Collins and Bradbury, 1991; Vidaver, 1982). The separate generic status of *Rathayibacter* species and their differences from *Clavibacter* was subsequently supported by the analyses of 16S rRNA gene sequences (Rainey et al., 1994; Takeuchi and Yokota, 1994). A little later, Sasaki et al. (1998) reclassified *Clavibacter toxicus* (Riley and Ophel, 1992) as *Rathayibacter toxicus*. The species *Rathayibacter festucae* and *Rathayibacter caricis* were added to the genus more recently (Dorofeeva et al., 2002).

Although many strains of the genus *Rathayibacter* have been isolated and studied due to their practical importance, a relatively small number of 16S rRNA gene sequences of *Rathayibacter* strains or uncultured clones are available in public databases. As is suggested by particular 16S rRNA gene sequence comparisons, some of these organisms may represent novel species. On the other hand, some recognized *Rathayibacter* species are not genetically homogeneous (Agarkova et al., 2006; Johnston et al., 1996; Riley and Ophel, 1992; Riley and Reardon, 1995; Riley et al., 1988). Further study may justify specific or subspecific status of the strains representing separate genomic groups. A few examples regarding *Rathayibacter toxicus* and related toxigenic bacteria are considered below.

The *Rathayibacter toxicus* strains colonizing *Lolium rigidum* grasses in Australia are clustered by multilocus enzyme electrophoresis patterns in two main groups reflecting their origin from the two regions in Western or South Australia (the South Australian group includes the type strain) (Riley et al., 1988). The grouping correlates well with the clusters generated from AFLP and PFGE patterns of whole genomes of a larger set of strains (Agarkova et al., 2006), including strains isolated from different grasses (*Phalaris* sp., *Austrodanthonia caespitosa*, *Avena sativa*, and *Avena caespitosa*) found among infected *Lolium rigidum* plants (Agarkova et al., 2006). Recent data (L.N. Anan'ina and L.I. Evtushenko, unpublished) have shown that strain VKM Ac-1600 (= ICMP 6309 = WSM 195) from *Phalaris minor*, which is most probably a representative of the Western Australian group [like the strain ICMP 6307 (= CS 28 = WSM 188) (Agarkova et al., 2006; Riley and Ophel, 1992)], exhibits 99.4% 16S rRNA gene sequence similarity to the type strain of *Rathayibacter toxicus*. Such a value, although high, is similar to those reported for many closely related species of the family *Microbacteriaceae* whose validity was supported by the DNA–DNA hybridization experiments. Representatives of the two groups, however, exhibited very similar physiological and biochemical characteristic traditionally used to differentiate species (Riley and Ophel, 1992), which is not unusual for bacteria occupying a similar ecological niche and adapted to similar nutritional conditions (Behrendt et al., 2001, 2002; Forst and Nealson, 1996; Pukall et al., 1999). Strains from the South Australian and Western Australian groups, on the other hand, showed clear differences in the specificity of their adhesion to the cuticle of nematodes representing different *Anguina* species (Riley and McKay, 1990). This result suggests that the groups may differ in the cell surface chemistry, perhaps targeting specific nematode receptors and yet-unknown molecular mechanisms responsible for interactions.

The toxigenic *Rathayibacter* strains from *Polypogon monspeliensis* and *Lachnagrostis filiformis* (syn. *Agrostis avenacea*) associated with closely related unnamed nematodes of the genus *Anguina* (Powers et al., 2001) differ by multilocus enzyme electrophoresis patterns from each other and from the type strain and all other strains assigned to *Rathayibacter toxicus* (Johnston et al., 1996; McKay et al., 1993). Strain FH-100 (= SE 3) from *Polypogon monspeliensis* was further shown to form a separate cluster by AFLP and PFGE analyses of whole genomes (Agarkova et al., 2006). It is worth noting that the regions of isolation of these strains are geographically separated from the area of the *Lolium rigidum* distribution and *Anguina funesta* infestation, and *Lachnagrostis filiformis* is an Australian native, in contrast to *Lolium rigidum* and *Polypogon monspeliensis* which are of Mediterranean origin (Agarkova et al., 2006; McKay et al., 1993; Riley et al., 2004b; Riley and Ophel, 1992).

The recently described toxigenic bacteria associated with *Anguina woodi*, a leaf gall nematode of dune grass *Ehrharta villosa* var. *villosa* in South Africa, possess some phenotypic characteristics consistent with those of *Rathayibacter* (Riley et al., 2004a). They also adhere to the juveniles of *Anguina woodi* but not *Anguina funesta*, and display distinct AFLP patterns, which are indicative of a novel species (Riley et al., 2004a).

The toxigenic bacteria associated with old nematode seed-galls in *Festuca nigrescens* from New Zealand (NZ) and New Jersey, USA, may represent another novel species or geographical variants (subspecies) of *Rathayibacter toxicus* (Riley et al., 2003, 2004b). The corynetoxin-like tunicaminyluracil-glycolipids detected in the NZ and USA galls differed from similar toxins produced by *Rathayibacter toxicus* (Anderton et al., 2004; Riley et al., 2004b). The NZ galls also gave a positive response to a *Rathayibacter toxicus*-specific monoclonal antibody assay, although a considerably weaker response than that seen with Australian *Rathayibacter toxicus* galls; the older USA galls were negative, probably due to deterioration of the antigen (Riley et al., 2003, 2004b). These bacteria may belong to a species thought to be responsible for the toxicity of *Festuca nigrescens* (syn. *Festuca rubra* subsp. *commutata*) on sites infested with *Anguina* species (*Anguina agrostis*) in Oregon, USA, in the 1940s to 1960s (Bird, 1981; Galloway, 1961). In this connection, it should be noted that in some earlier studies there is uncertainty

related to synonymy of names used for some nematodes and thereafter inadequate identification of associated bacteria. In particular, in the past the nematodes *Anguina graminis*, *Anguina funesta*, and the unnamed nematode infesting *Dactylis glomerata* have been synonymized with *Anguina agrostis* by some authors, and several grasses, including *Festuca*, *Lolium*, *Phalaris*, *Dactylis*, etc., have been listed as hosts for *Anguina agrostis* (see Southey et al., 1990, and Subbotin et al., 2004, for details). Accordingly, *Rathayibacter toxicus* and related toxigenic strains have been described under the name *Corynebactrium rathayi* in some of the earlier works (e.g. Bird and Stynes, 1977; Stynes et al., 1979; Bird, 1981; Vidaver, 1982).

Further study is required to determine the taxonomic position of strains assigned to the species "*Corynebacterium agropyri*" (O'Gara, 1915), which cause the gumming disease of *Agropyron smithii*. This species was not included in the *Approved Lists of Bacterial Names* because extant cultures were not available, and "*Corynebacterium agropyri*" and *Corynebacterium rathayi* (*Rathayibacter rathayi*) could not be clearly distinguished on the basis of their descriptions (Cummins et al., 1974; Murray, 1986; Young et al., 1978). The strains identified as "*Corynebacterium agropyri*" were afterwards isolated from old herbarium specimens of wheat grasses that were naturally infected at the time of collection (Murray, 1986). Strain "*Corynebacterium agropyri*" CS 35 (= T.D. Murray, CA-1) from *Agropyron smithii* exhibited rod-shaped cells (0.36–0.60 × 0.73–1.23 µm) and positive Gram-stain reaction, contained DAB in the cell wall, did not reduce nitrates (Murray, 1986), possessed specific adhesion to some *Anguina* species, and differed significantly from *Rathayibacter* species (*Rathayibacter rathayi*, *Rathayibacter iranicus*, *Rathayibacter tritici*, and *Rathayibacter toxicus*) in the multilocus enzyme electrophoresis patterns (Johnston et al., 1996; McKay et al., 1993; Riley et al., 1988). Attempts to determine the pathogenicity of cultured "*Corynebacterium agropyri*" in the absence of nematode vector were unsuccessful (Murray, 1986).

Differentiation of the species of the genus *Rathayibacter*

A familiar plant host and distinct symptoms of the gumming disease, along with information on the vector nematode and production of corynetoxins (tunicaminyluracil antibiotics) have a predictive value in identification of plant pathogenic *Rathayibacter* species. Some characteristics useful in distinguishing six species of the genus *Rathayibacter* are listed in Table 170. In addition, multilocus enzyme electrophoresis successfully differentiates strains of *Rathayibacter rathayi*, *Rathayibacter tritici*, *Rathayibacter iranicus*, and *Rathayibacter toxicus* (Riley et al., 1988). Strains of *Rathayibacter toxicus* are also distinguishable from other plant pathogenic *Rathayibacter* species by serology (Riley, 1987) and bacteriophage sensitivity (Riley and Gooden, 1991). PCR-based assays for identification of *Rathayibacter toxicus* and the bacteriophage isolate NCPPB 3778 have been described (Kowalski et al., 2007). The data on *gyrB*, *rpoB*, *recA*, and *ppk* gene sequences (Stackebrandt et al., 2007) for three species (*Rathayibacter festucae*, *Rathayibacter iranicus*, and *Rathayibacter tritici*) show that the analysis of housekeeping genes can be useful to differentiate *Rathayibacter* species. The AFLP and PFGE analyses of whole genomes (Agarkova et al., 2006) also seem applicable for this purpose, especially upon consideration of further improvement of taxomic structure of the genus. The 16S rRNA gene sequence similarity 98–99% (and more) between the organism in question and the type strains of phylogenetically neighboring species is generally predictive of a novel species. The DNA–DNA hybridization studies are expedient to support delineation.

Acknowledgements

This work was supported by the program MCB of the Russian Academy of Sciences.

List of species of the genus *Rathayibacter*

1. **Rathayibacter rathayi** (Smith 1913) Zgurskaya, Evtushenko, Akimov and Kalakoutskii 1993, 146^{VP} ("*Aplanobacter rathayi*" Smith 1913, 926; "*Phytomonas rathayi*" Bergey, Harrison, Breed, Hammer and Hunton 1923, 192; *Corynebacterium rathayi* Dowson 1942, 313; "*Pseudobacterium rathayi*" Krasil'nikov 1949, 225; "*Corynebacterium michiganense* pv. *rathayi*" Dye and Kemp, 1977, 578; *Clavibacter rathayi* Davis, Gillespie, Vidaver and Harris 1984, 113)

   rath′ay.i. N.L. masc. gen. n. *rathayi* of Rathay, named for E. Rathay, Australian plant pathologist, who first isolated the organism.

   The characteristics are as described for the genus and given in Table 170 and Table 171 with the following additional information compiled from: Sabet (1954), Bradbury (1973a), Jones (1975), Dye and Kemp (1977), Gross et al. (1979b), Gross and Vidaver (1979a), Carlson and Vidaver (1982), Davis et al. (1984), Henningson and Gugmestad (1991), Riley and Ophel (1992), Zgurskaya et al. (1993), Altenburger et al. (1997), Sasaki et al. (1998), Behrendt et al. (2002), and Dorofeeva et al. (2002).

   The colonies on corynebacterium agar are bright yellow. D-Glucose, L-arabinose, cellobiose, fructose, galactose, lactose, maltose, mannose, melezitose, raffinose, sucrose, trehalose, turanose, D-xylose, and glycerol are used as a carbon sources for growth on mineral base medium ISP 9 (Shirling and Gottlieb, 1966) supplemented with 0.1% (w/v) yeast extract and 0.1% (w/v) Casamino acids. Adonitol, dulcitol, lyxose, L-rhamnose, and tagatose are typically not used for growth on the same basal medium. Growth with sorbitol is weak and strain-dependent. Acids are produced oxidatively from xylose and glycerol. Strains are able to produce acids from glucose, cellobiose, trehalose, maltose, mannitol, fructose, galactose, lactose, sucrose, and sorbitol, but the test results may vary either with the method used or the strain examined and are often not reproducible. Acid production

by the type strain from melezitose was not detected by conventional methods and the API (bioMerieux) test system. Citrate, fumarate, malate, and succinate are utilized; results with malonate vary dependent on strain examined or the test method. Glutamate, DL-ornithine, and DL-valine are utilized as sole nitrogen source. Arbutin, Tween 21, 40, and 85 are hydrolyzed; some strains hydrolyze starch. Strains of this species decompose arbutin, gelatin, and Tween 60, but the test results may vary either with the strain examined or the test method. Tween 80 is not lipolyzed. Typically sensitive to tobramycin (10 μg per disk). Some strains, including the type strain, are also sensitive to the following antibiotics (μg/disk): streptomycin (10), chloramphenicol (10), kanamycin (30), novobiocin (5), oleandomycin (15), gentamicin (10), tetracycline (10), bacitracin, and penicillin (both 10 IU per disk). Some strains produce antibacterial substances active against closely related taxa.

The cell-wall sugars are rhamnose, glucose, mannose, and xylose; galactose may be a minor component in some strains. Polyamine pattern includes spermine (56–65%) and spermidine (30–40%) as predominant components, along with minor amounts of 1,3-diaminopropane, putrescine, cadaverine, tyramine, and trace amounts of *sym*-homospermidine and *sym*-norspermidine.

Causes a gumming disease of seed heads of cocksfoot grass (*Dactylis glomerata* Gaud.) and probably some related grasses. Artificial infection of wheat, using the nematode vector *Anguina tritici*, was reported. The normal vector nematode is an unnamed *Anguina* species parasitizing *Dactylis glomerata*.

Source: Dactylis glomerata, New Zealand.

DNA G+C content (mol%): 67 (HPLC).

Type strain: CFBP 2406, CIP 104036, DSM 7485, ICMP 2574, JCM 9307, LMG 7288, NCPPB 2980, VKM Ac-1601.

Sequence accession no. (16S rRNA gene): D45062, X77439.

2. **Rathayibacter caricis** Dorofeeva, Evtushenko, Krausova, Karpov, Subbotin and Tiedje 2002, 1921[VP]

ca′ri.cis. L. n. *carex* sedge; L. gen. n. *caricis* of sedge where the type strain of this species was found.

The characteristics are as described for the genus and given in Table 170 and Table 171 with the following additional information reported by Dorofeeva et al. (2002).

Colonies on corynebacterium agar are bright yellow. D-Glucose, D-galactose, L-arabinose, cellobiose, D-fructose, D-lactose, maltose, mannose, mannitol, melezitose, melibiose, raffinose, L-rhamnose, sorbitol, trehalose, turanose, and D-xylose are used for growth as a carbon source on ISP 9 medium (Shirling and Gottlieb, 1966) supplemented with yeast extract (0.1%, w/v) and Casamino acids (0.1%, w/v). Dextran, fucose, lyxose, and ribose are not utilized on the same basal medium. Alkaline reaction is positive with fumarate, malate, maleinate, and succinate but negative with acetate, citrate, glutamate, formate, lactate, oxalate, propionate, stearate, and tartrate. Asparagine, glycine, leucine, proline, tyrosine are used as nitrogen sources for growth. Tween 40 is hydrolyzed; starch, Tween 60, and Tween 80 are not decomposed. Resistant to ampicillin, gentamicin, and rubomicin (10 μg/ml). Susceptible to karbonicillin, levomicetin, metacycline, rifampin, and streptomycin at the same concentration.

Predominant cell-wall sugars are rhamnose and mannose, with a lower content of fucose, glucose, and xylose.

Plant pathogenicity is unknown.

Source: the phyllosphere of asymptomatic sedge (*Carex* species), Belgorod region, Russia.

DNA G+C content (mol%): 68 (T_m).

Type strain: UCM Ac-618, CIP 108056, DSM 15933, JCM 13576, VKM Ac-1799.

Sequence accession no. (16S rRNA gene): AF159364.

3. **Rathayibacter festucae** Dorofeeva, Evtushenko, Krausova, Karpov, Subbotin and Tiedje 2002, 1921[VP]

fes.tu′ca.e. N.L. gen. n. *festucae* from *Festuca*, generic name of fescue, a host plant of this species.

The characteristics are as described for the genus and given in Table 170 and Table 171 with the following additional information taken from Dorofeeva et al. (2002).

Colonies are orange to rose-orange or pink. Oxidase test with tetramethyl-*p*-phenylenediamine is positive. D-Glucose, D-galactose, L-arabinose, cellobiose, fructose, maltose, mannose, raffinose, L-rhamnose, trehalose, turanose, D-xylose, and mannitol are used for growth as a carbon source on ISP 9 medium (Shirling and Gottlieb, 1966) supplemented with yeast extract (0.1%, w/v) and Casamino acids (0.1%, w/v). Dextran, lyxose, ribose, and sorbose are not used on the same basal medium. Acetate, citrate, fumarate, gluconate, α-keto-glutarate, malate, malonate, succinate, and tartrate are utilized but formate and propionate are not. Asparagine, glycine, proline, and tyrosine are used as nitrogen sources for growth. Starch, Tween 40, Tween 60, and Tween 80 are hydrolyzed. Resistant to the following antibiotics (10 μg/ml): ampicillin, gentamicin, oxacillin, and rubomicin. Susceptible to doxycycline, levomicetin, metacycline, rifampin, and streptomycin at the same concentration.

Cell-wall sugars are mannose, rhamnose, glucose, and xylose.

Plant pathogenicity *per se*, if any, is unknown.

Source: a leaf gall induced by the plant pathogenic nematode *Anguina graminis* on *Festuca rubra* L., Moscow region, Russia.

DNA G+C content (mol%): 68.2 (T_m).

Type strain: UCM Ac-619, CIP 108055, DSM 15932, JCM 13577, VKM Ac-1390.

Sequence accession no. (16S rRNA gene): AF159365, AM410683.

4. **Rathayibacter iranicus** (Carlson and Vidaver 1982) Zgurskaya, Evtushenko, Akimov and Kalakoutskii 1993, 146[VP] ("*Corynebacterium iranicum*" Scharif 1961, 21; "*Corynebacterium michiganense* pv. *iranicum*" Dye and Kemp 1977, 578; *Corynebacterium iranicum* (*ex* Scharif 1961) Carlson and Vidaver 1982, 322; *Clavibacter iranicus* Davis, Gillespie, Vidaver and Harris 1984, 113)

i.ran′i.cus. N.L. masc. adj. *iranicus* of or pertaining to Iran.

The characteristics are as described for the genus and given in Table 170 and Table 171 with the following additional information compiled from: Dye and Kemp (1977), Gross and Vidaver (1979a), Carlson and Vidaver (1982), Davis et al. (1984), Collins and Bradbury (1986), Riley and Ophel (1992), Zgurskaya et al. (1993), Altenburger et al.

(1997), Sasaki et al. (1998), Behrendt et al. (2002), and Dorofeeva et al. (2002).

Colonies on corynebacterium agar are bright yellow. D-Glucose, L-arabinose, cellobiose, fructose, galactose, lactose, maltose, mannose, melezitose, raffinose, salicin, sucrose, trehalose, turanose, D-xylose, glycerol, and sorbitol are used for growth as a carbon source on ISP 9 medium (Shirling and Gottlieb, 1966) supplemented with 0.1% (w/v) yeast extract and 0.1% (w/v) Casamino acids. Adonitol, dulcitol, lyxose, L-rhamnose, and tagatose are not used for growth on the same basal medium. Acid is produced from xylose, galactose, fructose, glycerol, and sorbitol (weakly). The type strain produces acid from cellobiose, lactose, maltose, mannose, mannitol, melezitose, trehalose, and inulin, but test reactions vary with the methods used. Acid is not produced from tagatose, erythritol, and salicin. Citrate, fumarate, malate, and succinate are utilized but lactate is not. Malonate is utilized, but the test results may vary with the method used and is often not reproducible. Asparagine, glutamic acid, and DL-ornithine are used as a nitrogen source. Tween 21, Tween 40, and Tween 85 are hydrolyzed. Some strains decompose Tween 60 and Tween 80, but the test results may vary with the method. Arbutin, gelatin, and starch are not hydrolyzed. *Rathayibacter iranicus* NCPPB 2253 produces a glycolipid with antibacterial activity against strains of some *Rathayibacter* species and all tested strains of *Clavibacter*.

The cell-wall sugars are rhamnose, glucose, and mannose. Polyamine pattern of the type strain includes spermine (71%) and spermidine (19%) as the predominant components, with a lesser amount of putrescine (8%) and minor quantities of 1,3-diaminopropane and cadaverine.

Causes a gumming disease of seed heads of wheat (*Triticum aestivum*) and probably some related plants. Normal vector nematode is *Anguina tritici*.

Source: Triticum aestivum, Iran.

DNA G+C content (mol%): 66 (HPLC).

Type strain: CCUG 23897, CFBP 807, CIP 104037, DSM 7484, ICMP 3496, JCM 9308, LMG 3677, NCPPB 2253, VKM Ac-1602.

Sequence accession no. (16S rRNA gene): AM410684.

5. **Rathayibacter toxicus** (Riley and Ophel 1992) Sasaki, Chijimatsu and Suzuki 1998, 409[VP]
(*Clavibacter toxicus* Riley and Ophel 1992, 67)

tox′i.cus. L. m. *toxicus* (*sic*) poison.

The characteristics are as described for the genus and given in Table 170 and Table 171 with the following additional information compiled from: Riley and Ophel (1992), Sasaki et al. (1998), and Behrendt et al. (2002), unless indicated.

Colonies on R agar are flat, smooth, and pale yellow in a 5-d culture at 30°C. Cells are irregular rods, generally 0.6–0.75 × 1.5 μm. Oxidase-negative. Acid is produced from galactose, mannose, and xylose; the test reactions with inulin may vary with the method used. Acid is not produced from lactose, D-maltose, and D-melezitose and many other sugars and polyols. Acetate, glutamate, malonate, succinate, and some other organic acids are typically not utilized, but some strains may show positive reaction with acetate. Does not hydrolyze gelatin, Tween 80, and cotton seed oil. Tyrosinase and acetoin are not produced. The maximum concentration of NaCl tolerated is 1% (w/v).

Strains are lysed by the bacteriophage NCPPB 3778 (Ophel et al., 1993; Riley and Gooden, 1991). Causes gumming disease of ryegrass (*Lolium rigidum* Gaud.) and, occasionally, some related grasses. Produces corynetoxins (tunicaminyluracil antibiotics) in colonized plant hosts. The most common nematode vector is *Anguina funesta*. Under experimental conditions, the bacterium has been shown to be transmitted by other *Anguina* species such as *Anguina tritici* and *Anguina australis* (Riley, 1992; Riley et al., 2001).

Source: Lolium rigidum Gaud., from a site in Murray Bridge, South Australia, where toxicity to grazing animals and infestation with the nematode *Anguina funesta* had been observed.

DNA G+C content (mol%): 60.4 (HPLC); 67 (T_m).

Type strain: CS14, ATCC 49908, CIP 104617, DSM 7488, ICMP 9525, JCM 9669, NCPPB 3552.

Sequence accession no. (16S rRNA gene): D84127.

Additional comments: there are at least two distinct genetic groups among strains of this species originating from the two regions in Western and South Australia (Agarkova et al., 2006; Riley and Ophel, 1992; Riley et al., 1988). On compiling additional genotypic and phenotypic data, the group from Western Australia may eventually be classified as a separate species or subspecies of *Rathayibacter toxicus* (see the Taxonomic comments section for details).

6. **Rathayibacter tritici** (Carlson and Vidaver 1982) Zgurskaya, Evtushenko, Akimov and Kalakoutskii 1993, 146[VP] ["*Pseudomonas tritici*" Hutchinson 1917, 174; "*Corynebacterium michiganense* pv. *tritici*" Dye and Kemp 1977, 578; *Corynebacterium tritici* (*ex* Hutchinson 1917) Carlson and Vidaver 1982, 324; *Clavibacter tritici* Davis, Gillespie, Vidaver and Harris 1984, 113]

tri′ti.ci. L. n. *triticum* wheat, and also the generic name of wheat (*Triticum*); L. gen. n. *tritici*, of wheat, of *Triticum*.

The characteristics are as described for the genus and given in Table 170 and Table 171 with the following additional information compiled from: Bradbury (1973b), Dye and Kemp (1977), Gross et al. (1979), Gross and Vidaver (1979a), Carlson and Vidaver (1982), Henningson and Gugmestad (1991), Riley and Ophel (1992), Zgurskaya et al. (1993), Altenburger et al. (1997), Sasaki et al. (1998), Behrendt et al. (2002), and Dorofeeva et al. (2002).

Colonies on corynebacterium agar are bright yellow. D-Glucose, L-arabinose, cellobiose, fructose, galactose, maltose, mannose, raffinose, salicin, sucrose, trehalose, turanose, D-xylose, mannitol, and glycerol are used for growth as a carbon sources on salt medium supplemented with 0.1% of yeast extract (w/v) and 0.1% of Casamino acids (w/v). Growth with lactose and sorbitol is variable. Adonitol, dulcitol, erythritol, inositol, lyxose, melibiose, L-rhamnose, and tagatose are not used for growth on the same basal medium. Acid is produced from glucose, fructose, xylose, and glycerol. Strains are able to produce acid from cellobiose, galactose, lactose, maltose, mannose, sucrose, trehalose, mannitol and sorbitol, but the test reactions vary with the strain examined and the test method and are often not reproducible. Acid is not produced from melezitose and salicin. Utilizes citrate, fumarate, and succinate; the test results with acetate, malate, and malonate vary with the method and are often not reproducible. Glutamic acid and ornithine are used as a sole nitrogen source.

Tweens 21, 40, 60, and 85 are hydrolyzed; some strains are able to decompose arbutin and Tween 80, but the test results vary either with the strains or the test conditions.

The cell-wall sugars are rhamnose, glucose, mannose, and xylose; galactose is a minor component in some strains. Polyamine pattern includes spermine (64–67%) and spermidine (13–28%) as predominant components, along with minor amounts of 1,3-diaminopropane, putrescine, cadaverine, and *sym*-homospermidine.

Causes a gumming disease of wheat (*Triticum aestivum*) and some related plants. Artificial infection of *Triticum dicoccum*, *Triticum durum*, and *Triticum pyramidale* using the nematode vector *Anguina tritici* has been reported. *Dactylis glomerata* and *Secale cereale* can also be infected under experimental conditions. Normal vector nematode is *Anguina tritici*.

Source: Triticum aestivum, Egypt.

DNA G+C content (mol%): 69 (HPLC).

Type strain: ATCC 11403, CCUG 23914, CFBP 1385, CIP 104038, DSM 7486, ICMP 2626, JCM 9309, LMG 3728, NCPPB 1857, VKM Ac-1603.

Sequence accession no. (16S rRNA gene): X77438, AM410685.

Genus XXIII. **Rhodoglobus** Sheridan, Loveland-Curtze, Miteva and Brenchley 2003, 992[VP]

Peter P. Sheridan

Rho.do.glo′bus. Gr. n. *rhodon* the rose; L. masc. n. *globus* ball; N.L. masc. n. *rhodoglobus* red ball.

Slender thin rods (0.15–0.2 × 1–1.2 µm) **with bulbous protuberances are seen at all stages of growth**. The protuberances are of a mean diameter of 0.8 µm. **Cells are** aerobic, **Gram-stain-positive**, easily decolorized, **motile, nonsporeforming, and grow aerobically between −2 and 21°C, with optimal growth at 18°C.** They do not exhibit a marked rod–coccus cycle. Colonies are smooth, round, convex, non-slimy, **translucent red**, and small in size (2–3 mm in diameter). Positive enzymic activity is shown for alkaline phosphatase (weak), catalase, β-galactosidase (weak), α- and β-glucosidase, pyrazimidase, and pyrrolidonylarylamidase, but are negative for β-glucuronidase, *N*-acetyl-β- glucosidase, nitrate reductase, and urease. Gelatin is not hydrolyzed, nor is acid formed from glucose, glycogen, lactose, maltose, mannitol, ribose, sucrose, or xylose. **Cell walls contain ornithine as the diagnostic amino acid. The major menaquinones are MK-11 and MK-12. The predominant fatty acids are $C_{15:1}$ anteiso, $C_{15:0}$ anteiso, $C_{17:0}$ anteiso, and $C_{16:0}$ iso.** Phylogenetic analysis of the 16S rRNA gene sequence indicates that the genus *Rhodoglobus* is a member of the family *Microbacteriaceae* and related to, but distinct from, organisms belonging to the genera *Agreia*, *Leifsonia*, and *Subtercola*. **Alignments of the 16S rRNA gene sequences contain a 13 base pair insertion in the *Rhodoglobus* sequence** that is found only in the gene sequences of a few related organisms (*Leifsonia aurea*, *Leifsonia rubra*, and *Subtercola frigoramans*).

DNA G+C content (mol%): 62.

Type species: **Rhodoglobus vestalii** Sheridan, Loveland-Curtze, Miteva and Brenchley 2003, 992[VP].

Further descriptive information

The 16S rRNA gene sequence amplified from *Rhodoglobus vestalii* was analyzed using maximum- parsimony, maximum-likelihood, and distance methods. The alignments were based on 1,549 nucleotide positions and the results of all three analyses were congruent. The distance analysis results found using the Jukes–Cantor model with equal rates for variable sites (Figure 198) show that *Rhodoglobus vestalii* is related to, but phylogenetically distinct from, organisms belonging to the *Agreia*, *Leifsonia*, and *Subtercola* genera. The grouping of *Rhodoglobus vestalii* with *Leifsonia aurea* and *Leifsonia rubra* was supported by high bootstrap values (Figure 198). A distance matrix table

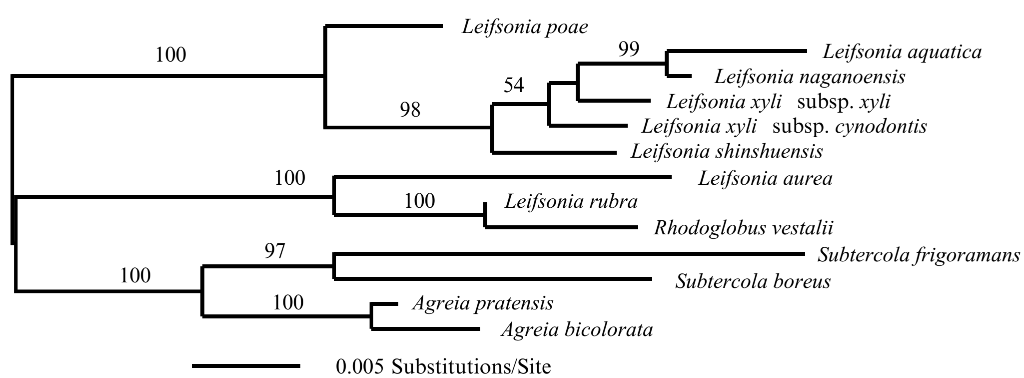

FIGURE 198. Phylogenetic tree based on an alignment of 1549 nucleotides from the 16S rRNA gene sequences of *Rhodoglobus vestalii* and closely related taxa. Phylogenetic analysis was performed using the distance method with Jukes–Cantor correction (equal rates for variable sites). Bootstrap values are based on 10,000 replicates, with only values >50 reported.

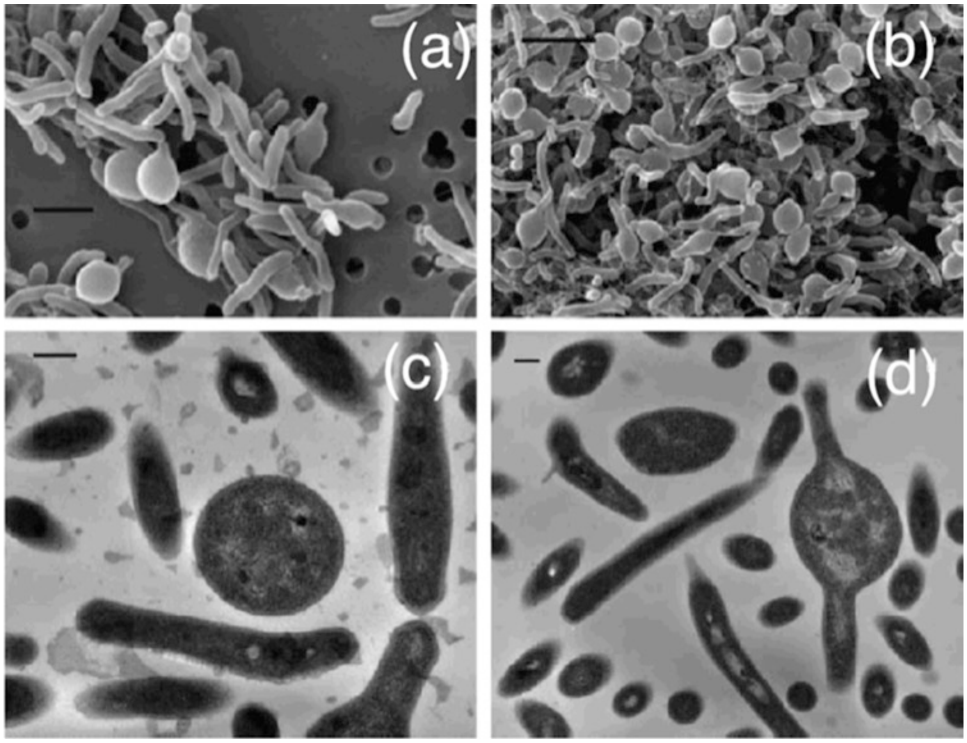

FIGURE 199. Scanning electron (a and b; bars = 1 μm) and transmission electron (c and d; bars = 200 nm) micrographs of *Rhodoglobus vestalii*. Photo credit: Vanya Miteva and Rosemary Walsh, Pennsylvania State University. (Reprinted with permission from Sheridan et al., 2003. Int. J. Syst. Evol. Microbiol. *53*: 985–994.)

generated using the Jukes-Cantor model showed that the evolutionary distances between the *Rhodoglobus vestalii* 16S rRNA gene sequence and the sequences of the closest type strains (*Agreia bicolorata*, *Agreia pratensis*, *Leifsonia aquatica*, *Leifsonia aurea*, *Leifsonia naganoensis*, *Leifsonia poae*, *Leifsonia rubra*, *Leifsonia shinshuensis*, *Leifsonia xyli* subspecies *cynodontis*, *Leifsonia xyli* subspecies *xyli*, *Subtercola boreus*, and *Subtercola frigoramans*) are 5.07, 4.84, 5.90, 2.79, 5.36, 5.21, 0.71, 6.35, 6.05, 5.75, 5.42, and 6.31% percentage different, respectively. These values are either borderline or exceed the accepted values for a genus, with the exception of the distances for *Leifsonia aurea* and *Leifsonia rubra*. Both *Leifsonia aurea* and *Leifsonia rubra* were isolated from a pond in the Wright Valley, Antarctica, which is within 100 miles of the lake from which the sample containing *Rhodoglobus vestalii* was collected.

The 16S rRNA gene sequence of *Rhodoglobus vestalii* contains a 13 base pair insert that is only present in a few related 16S rRNA genes. Inserts with almost the exact same sequence are found in *Leifsonia aurea* and *Leifsonia rubra*, although *Subtercola boreus* and *Subtercola frigoramans* have smaller inserts (4 and 10 base pairs, respectively) of dissimilar sequence. Based on the evolutionary distances between *Rhodoglobus vestalii*, *Leifsonia aurea*, and *Leifsonia rubra* and other members of the genus *Leifsonia*, as well as the presence of the 13 base pair insertion in the 16 rRNA gene sequences of these three organisms which is distinct from the other members of the genus *Leifsonia*, it is possible that *Leifsonia aurea* and *Leifsonia rubra* may need to be reclassified as members of the genus *Rhodoglobus*.

Cells of *Rhodoglobus* are pleomorphic short, slender rods about 0.15–0.2 × 1.0–1.2 μm (Figure 199). There is no marked rod–coccus cycle as occurs in organisms classified in the genera *Arthrobacter*, *Frigoribacterium*, and *Leifsonia*. Cells from freshly inoculated cultures are longer than those from older cultures, however, no true rod–coccus cycle is observed. Clumps of cells often form in broth cultures. In addition, cells frequently appear swollen at the ends or in the middle. Scanning electron micrographs show extremely slender cells with enlarged, bulbous protuberances approximately 0.8 μm in diameter (Figure 199 A and B). Some cells are slightly curved but lack the typical fragmented shapes often seen with members of the genus *Arthrobacter*. Most protuberances appear to be in the central part of the cells and occur at all growth stages and under all culture conditions.

The amino acids present in *Rhodoglobus* cell walls contain ornithine as the diamino acid in the peptidoglycan. Significant amounts of alanine, glycine, and glutamic acid are also found, although only small amounts of lysine are detected. The molar ratio of glutamic acid to glycine to alanine to ornithine is 1:1.5:1.1:1.4. The predominant fatty acids in *Rhodoglobus* cells cultured at 18°C are branched-chain saturated fatty acids, $C_{15:0}$ anteiso, $C_{16:0}$ iso, and $C_{17:0}$ anteiso. When *Rhodoglobus* is cultured at −2°C, the amount of $C_{15:0}$ anteiso decreases by 1.5 fold and the unsaturated fatty acid $C_{15:1}$ anteiso increases tenfold. These results are consistent with changes in the unsaturated and saturated fatty acid ratios found in other Gram-stain-positive nonsporeforming rods grown at different temperatures.

Rhodoglobus forms smooth, round, convex, non-slimy, reddish, small (2–3 mm in diameter) colonies on solid media. The organism grows aerobically on most agar media, with the exception of Nutrient Agar (na), at temperatures of 2, 10, and 18°C, but does not grow at 25°C or higher. Cells reach a higher density when grown in Tryptic Soy Broth (TSB) or Luria Broth (LB) than when grown in R2 broth. The generation time in TSB is estimated to be 10 h at 18°C, 20 h at 10°C, and 29 h at 5°C. Cells grow more slowly at 21°C than they do at 18°C, indicating that 18°C is the optimal temperature for growth. The upper temperature limit of growth is 22°C. Cells of *Rhodoglobus* will grow at −2°C even though there is an extended lag phase independent of the growth temperature of the inoculum. Formation of the reddish intracellular pigment appears soon after the beginning of the exponential phase in all cultures. Cells also reach a high cell density and remain viable after prolonged incubation, with over 10^{10} colony forming units per milliliter (c.f.u./ml) remaining after 4 months in TSB at −2 and 10°C.

Rhodoglobus shows positive enzymic activity for alkaline phosphatase (weak), catalase, β-galactosidase (weak), α- and β-glucosidase, pyrazimidase, and pyrrolidonylarylamidase. Enzyme activity is not shown for *N*-acetyl-β-glucosidase, β-glucuronidase, nitrate reductase, and urease. *Rhodoglobus* does not hydrolyze gelatin nor does it form acid from glucose, glycogen, lactose, maltose, mannitol, ribose, sucrose, or xylose. It does not grow anaerobically or in the presence of 2.5, 5.0 or 10.0% NaCl, and does not form spores. The G+C mol% of *Rhodoglobus* is calculated to be 62 mol% based on the melting temperature of *Rhodoglobus* DNA. A plasmid of approximately 900 base pairs has been found in *Rhodoglobus vestalii*, although its function has not been determined.

Rhodoglobus vestalii was isolated from a cyanobacterial mat sample from a meromictic pond (informally called "Lake Vestal" in honor of the late J. Robie Vestal) located south of the Miers and Adams glaciers near Bratina Island, on the McMurdo Ice Shelf, Antarctica. Organisms determined to be closely related to *Rhodoglobus vestalii* by 16S rRNA gene sequence identity (*Leifsonia aurea* and *Leifsonia rubra*) were isolated from an Antarctic Dry Valley pond in the Wright Valley, Antarctica, approximately 100 miles from "Lake Vestal."

Enrichment and isolation procedures

A cyanobacterial mat sample was collected in January 1993 from a meromictic pond (informally called "Lake Vestal") located South of the Miers and Adams glaciers near Bratina Island, on the McMurdo Ice Shelf, Antarctica. The sample was kept frozen at −80°C until an approximately 2 g sample was inoculated into 5 ml of Instant Ocean broth (Loveland-Curtze et al., 1999) and incubated at 10°C until the culture became turbid. Organisms were isolated on Instant Ocean agar using streak plating techniques, and colonies were passed at least three times to ensure purity.

Maintenance procedures

Growth of *Rhodoglobus vestalii* has been tested on a variety of media. The organism grows aerobically on most agar media, except na, at temperatures of 2, 10, and 18°C, but does not grow at 25°C or higher. Cells reach a higher density when grown in LB or TSB than when grown in R2 broth. The generation time in TSB was estimated to be 10 h at 18°C, 20 h at 10°C, and 29 h at 5°C. Cells grow more slowly at 21°C than they do at 18°C. The upper temperature limit of growth is 22°C. Cells grow at −2°C even though there is an extended lag phase independent of the growth temperature of the inoculum. Formation of the reddish intracellular pigment appears soon after the beginning of the exponential phase in all cultures. Cells reach a high cell density and remain viable after prolonged incubation with over 10^{10} c.f.u./ml remaining after 4 months in TSB at −2 and 10°C. Cells can be stored via either lyophilization or as 20% glycerol stocks at either −20°C or −80°C.

Procedures for testing special characteristics

The 16S rRNA gene sequence was amplified from a genomic DNA preparation with the Bacterial Domain-specific primer 8 Forward and the Universal primer 1492 Reverse (Pace et al., 1986; Weisburg et al., 1991) using the following conditions: 10′ at 95°C, followed by 30 cycles of (1′ at 94°C, 1′ at 53°C, 2′30″ at 72°C), followed by 7′ at 72°C, followed by a 4°C "hold."

Differentiation of the genus *Rhodoglobus* from other genera

Based on 16S rRNA gene sequence analysis, as well as the presence of a similar 13 base pair insert in their 16S rRNA gene sequences, the taxa most closely related to *Rhodoglobus vestalii* are *Leifsonia aurea* and *Leifsonia rubra*. *Rhodoglobus vestalii* can be distinguished from these organisms not only by sequencing of the 16S rRNA gene, but also through the presence of $C_{15:1}$ anteiso fatty acids in *Rhodoglobus vestalii*; these are not found in *Leifsonia aurea* and *Leifsonia rubra* (Table 172). Moreover, *Rhodoglobus vestalii* is motile, while *Leifsonia aurea* and *Leifsonia rubra* are not. In addition, the major menaquinones are MK-11 and MK-12 in *Rhodoglobus vestalii*, while the major menaquinone is MK-11 in *Leifsonia aurea* and *Leifsonia rubra*. The cell walls of *Rhodoglobus vestalii* contain ornithine as the diamino acid, while DL-diaminobutyric acid is the diamino acid in the cell walls of *Leifsonia aurea* and *Leifsonia rubra*. Cells of *Rhodoglobus vestalii* exhibit bulbous protuberances at all stages of growth, but these structures have not been reported for either *Leifsonia aurea* or *Leifsonia rubra*. Less significantly, the minimum growth temperature for *Rhodoglobus vestalii* is −2°C, but is 0°C for *Leifsonia aurea* and *Leifsonia rubra*, and *Rhodoglobus vestalii* is unable to grow in NaCl concentrations above 2.5%, although the upper limit of NaCl tolerated by *Leifsonia aurea* and *Leifsonia rubra* is 3%.

Taxonomic comments

The genus *Rhodoglobus* was first described in 2003 by Sheridan and co-authors (Sheridan et al., 2003). The type strain, *Rhodoglobus vestalii*, was isolated from a cyanobacterial mat sample collected from a pond near the Miers and Adams glaciers in the Dry Valleys of Antarctica. Amplification and sequencing of the isolate's 16S rRNA gene sequence indicates that the organism is a member of the family *Microbacteriaceae* and related to, but distinct from, organisms belonging to the genera *Agreia* (Evtushenko et al., 2001; Schumann et al., 2003), *Leifsonia* (Evtushenko et al., 2000; Suzuki et al., 1999), and *Subtercola* (Behrendt et al., 2002; Männistö et al., 2000). Analysis of the 16S rRNA gene sequences of the validly published organisms within the family *Microbacteriaceae* indicate that *Rhodoglobus vestalii* contains a 13 base pair insertion that is only found in a few related organisms (*Leifsonia rubra*, *Leifsonia aurea*, and *Subtercola frigoramans*). Electron micrographs of *Rhodoglobus vestalii* show the presence of

TABLE 172. Characteristics distinguishing between *Rhodoglobus vestalii* and related taxa classified in the family *Microbacteriaceae*[a]

Characteristic	*Rhodoglobus vestalii*	*Leifsonia rubra*	*Leifsonia aurea*	*Leifsonia aquatica*	*Leifsonia poae*	*Leifsonia xyli* subsp. *xyli*	*Leifsonia xyli* subsp. *cynodontis*	*Leifsonia shinshuensis*	*Leifsonia nagaoensis*	*Subtercola boreus*	*Subtercola frigoramans*	*Agreia bicolorata*	*Agreia pratensis*
Gram reaction	+	+	+	+	+	+	+	+	+	+	+	+	+
Cell size:													
Width (μm)	0.15–0.2	nr	nr	0.4–0.7	0.6–0.9	0.2	0.2–0.3	0.3–0.4	0.3–0.5	0.2–0.3	0.3–0.4	0.4–0.5	nr
Length (μm)	1.0–1.2	nr	nr	1.2–2.5	8.0–15.0	5.0	3.0–6.0	2.5–3.0	1.8–3.0	0.6–1.0	0.9–1.5	1.2–2.5	nr
Growth temperature range (°C)	−2 to 22	0–22	0–30	7–37	7–37	7–31	7–35	7–42	7–37	2–28	−2 to 28	Mesophilic	Mesophilic
Optimal growth temperature (°C)	18	15	22	24–28	24–28	nr	nr	nr	nr	15–17	15–17	24–26	25
Aerobic growth	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Anaerobic growth	No	No	No	No	No	No	No	No	No	No	No	No	No
Spore production	No	No	No	No	No	No	No	No	No	No	No	No	No
Colony color	Red	Red	Yellow	Yellow	Yellow	White	Yellow	White	White	Pale to bright Yellow	Pale to bright Yellow	Yellow/Yellow-Orange	Yellow
Motility	Yes	No	No	Yes	Yes	No	No	No	No	nr	nr	Weak	No
Presence of protuberances	Yes	No	No	No	No	No	No	No	No	No	No	No	No
DNA G+C content (mol%)	62	66	64	70	nr	66	69	71	71	68	64	67	65
Diamino acid[b]	Ornithine	DAB	DAB	DAB	DAB	DAB	DAB	DAB	DAB	DAB	DAB	DAB and ornithine	DAB and Ornithine
Major menaquinone	MK-11, MK-12	MK-11	MK-11	MK-11, MK-10	MK-11	nr	MK-11, MK-12	MK-11, MK-12	MK-11, MK-10	MK-9, MK-10	MK-9, MK-10	MK-10, MK-9	MK-10, MK-11
Gelatin hydrolysis	−	−	−	−	+	−	−	−	−	nr	nr	nr	−
Growth in presence of NaCl	<5.2%	up to 3%	up to 3%	+ in 5%	− in 5%	− in 5%	− in 5%	nr	nr	nr	nr	<6%	nr
Enzymic activities:													
N-Acetyl-β-glucosidase	−	nr	nr	nr	nr	nr	nr	nr	nr	−	−	nr	nr
Alkaline phosphatase	+ (weak)	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr
Catalase	+	+	+	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr
β-Galactosidase	+ (weak)	+	+	nr	nr	nr	nr	nr	nr	−	−	nr	nr
α-Glucosidase	+	nr	nr	nr	nr	nr	nr	nr	nr	+	+	nr	nr
β-Glucosidase	+	nr	nr	nr	nr	nr	nr	nr	nr	+	+	nr	nr
β-Glucuronidase	−	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr
Nitrate reductase	−	+	+	+	nr	nr	nr	−	−	nr	nr	nr	nr
Pyrazinamidase	+	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr
Pyrrolidonyl α-arylamidase	+	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr
Urease	−	−	−	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr
Acid production from:													
Glucose	−	nr	nr	+	−	+	+	−	+	nr	nr	nr	nr
Glycogen	−	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr
Lactose	−	−	−	−	nr	nr	−	nr	nr	nr	nr	nr	−
Maltose	−	−	−	−	−	+	+	−	+	nr	nr	nr	+
Mannitol	−	nr	nr	−	nr	nr	+ (weak)	−	+	nr	nr	nr	nr
Ribose	−	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	−

(continued)

TABLE 172. (continued)

Characteristic	*Rhodoglobus vestalii*	*Leifsonia rubra*	*Leifsonia aurea*	*Leifsonia aquatica*	*Leifsonia poae*	*Leifsonia xyli* subsp. *xyli*	*Leifsonia xyli* subsp. *cynodontis*	*Leifsonia shinshuensis*	*Leifsonia nagaoensis*	*Subtercola boreus*	*Subtercola frigoramans*	*Agreia bicolo-rata*	*Agreia pratensis*
Sucrose	–	–	–	+	+	–	–	–	+	nr	nr	nr	nr
Xylose	–	–	–	+	nr	nr	nr	–	+	nr	nr	nr	nr
Major fatty acids:													
$C_{15:0}$ anteiso	+	+	+	+	+	nr	+	+	+	+	+	+	+
$C_{16:0}$ iso	+	+	+	+	+	nr	+	+	+	+	+	+	+
$C_{17:0}$ anteiso	+	+	+	+	+	nr	+	+	+	+	+	+	+
$C_{15:1}$ anteiso	+	–	–	–	–	nr	–	–	–	–	–	–	–

[a]Symbols: +, >85% positive; –, 0–15% positive; w, weak reaction; nr, not reported.

[b]DAB, Diaminobutyric acid.

large, bulbous protuberances that are present at all stages of growth. Detailed inspection of the protuberances indicate that they are contiguous with the portions of the cell that were rods. The evolutionary distances from described taxa, along with the 16S rRNA gene insertion and the presence of ornithine as the diamino acid in the cell wall and of MK-11 and MK-12 as the major menaquinones are deemed sufficient to warrant the creation of the novel genus *Rhodoglobus*.

The organisms *Leifsonia aurea* and *Leifsonia rubra* were being described at the same time in 2003, and the phylogenetic analyses of the 16S rRNA gene sequence data indicate a close relationship to the proposed novel organism *Rhodoglobus vestalii* (evolutionary distances of 2.79 and 0.71, respectively). It was proposed by Sheridan (2003) that perhaps *Leifsonia aurea* and *Leifsonia rubra* should be included within the novel genus *Rhodoglobus*, as they had not been validly published as members of the genus *Leifsonia*. However, a number of significant differences between *Rhodoglobus vestalii* and *Leifsonia aurea* and *Leifsonia rubra* argue for their exclusion from the genus *Rhodoglobus*. *Rhodoglobus vestalii* is distinct from the two *Leifsonia* species in that it has $C_{15:1}$ anteiso fatty acids. Furthermore, *Rhodoglobus vestalii* is motile, while *Leifsonia aurea* and *Leifsonia rubra* are not. Additionally, the major menaquinones in *Rhodoglobus vestalii* are MK-11 and MK-12, while the major menaquinone is MK-11 in *Leifsonia aurea* and *Leifsonia rubra*. The cell walls of *Rhodoglobus vestalii* contain ornithine as the diamino acid, while DL-diaminobutyric acid is the diamino acid in the cell walls of *Leifsonia aurea* and *Leifsonia rubra*. Finally, cells of *Rhodoglobus vestalii* exhibit bulbous protuberances at all stages of growth; these structures have not been reported for either *Leifsonia aurea* or *Leifsonia rubra*. Therefore, inclusion of *Leifsonia aurea* and *Leifsonia rubra* in the genus *Rhodoglobus* is not warranted, although the significant differences between *Leifsonia aurea* and *Leifsonia rubra* and other members of the genus *Leifsonia* indicate that these organisms should be placed within their own genus distinct from both *Leifsonia* and *Rhodoglobus*.

Acknowledgements

I wish to thank my colleagues Jean Brenchley, Jennifer Loveland-Curtze, and Vanya Miteva for their work in the original characterization and description of the genus *Rhodoglobus* and the organism *Rhodoglobus vestalii*. Additional thanks to Vanya Miteva and Rosemary Walsh for the preparation of the electron micrographs.

List of species of the genus *Rhodoglobus*

1. **Rhodoglobus vestalii** Sheridan, Loveland-Curtze, Miteva and Brenchley 2003, 992[VP]

ves.ta′.li.i. N.L. gen. masc. n. *vestalii* of Vestal, in honor of J. Robie Vestal, who studied the ecology and physiology of Antarctic microorganisms.

Colonies are smooth, round, convex, non-slimy, translucent red, and small in size (2–3 mm in diameter). Cells are slender thin rods (0.15–0.2 × 1–1.2 µm) with bulbous protuberances seen at all stages of growth. The protuberances are of a mean diameter of 0.8 µm. Cells are Gram-stain-positive, easily decolorized, nonsporeforming, and motile. They grow aerobically between −2 and 21°C, with optimal growth rate at 18°C, but do not exhibit a marked rod–coccus cycle. Cell walls contain ornithine as the diagnostic amino acid. The major menaquinones are MK-11 and MK-12. The predominant fatty acids are $C_{15:1}$ anteiso, $C_{15:0}$ anteiso, $C_{17:0}$ anteiso, and $C_{16:0}$ iso. Cells do not hydrolyze gelatin and do not form acid from glucose, glycogen, lactose, maltose, mannitol, ribose, xylose, or sucrose, but show positive enzymic activity for alkaline phosphatase (weak), catalase, β-galactosidase (weak), α-and β-glucosidase, pyrazimidase, and pyrrolidonylarylamidase but are negative for β-glucuronidase, *N*-acetyl-β- glucosidase, nitrate reductase, and urease.

Source: an Antarctic Dry Valley lake located south of the Miers and Adams glaciers near Bratina Island, on the McMurdo Ice Shelf, Antarctica.

DNA G+C content (mol%): 62 (T_m).

Type strain: LV3, ATCC BAA-534, CIP 107482, JCM 12695, NBRC 103084.

Sequence accession no. (16S rRNA gene): AJ45910.

Genus XXIV. **Salinibacterium** Han, Nedashkovskaya, Mikhailov, Kim and Bae 2003, 2064[VP]

Seung Bum Kim and Olga I. Nedashkovskaya

Sa.li.ni.bac.te′ri.um. L. n. *salinum* salt-cellar; L. neut. n. *bacterium* a small rod and, in biology, a bacterium (so called because the first ones observed were rod-shaped); N.L. neut. n. *Salinibacterium* a saline bacterium.

Gram-stain-positive, **nonmotile, aerobic, nonsporeforming, irregular rods**. Mycolic acid is absent. Metabolism is respiratory, but acids can also be formed from some sugars. Arginine dihydrolase, oxidase, and urease-negative, but catalase-positive. **Grows in the presence of NaCl**. Grows at mesophilic temperatures and around neutral pH. **Cell-wall peptidoglycan is type B; lysine and ornithine are the diagnostic diamino acids**. **Muramic acid contains acetyl residues**. Principal phospholipids are phosphatidylglycerol and diphosphatidylglycerol (**phospholipid type I**). Cellular fatty acids consist of branched, saturated species; major components are 12-methyltetradecanoic acid ($C_{15:0}$ anteiso) and 14-methylpentadecanoic acid ($C_{16:0}$ iso). **Predominant menaquinone is MK-11**, and a smaller amount of MK-10 is also present.

DNA G+C content (mol%): 61.0–63.5.

Type species: **Salinibacterium amurskyense** Han, Nedashkovskaya, Mikhailov, Kim and Bae 2003, 2065[VP].

Further descriptive information

Salinibacterium is one of the 26 genera belonging to the family *Microbacteriaceae* and currently contains two species, *Salinibacterium amurskyense* (Han et al., 2003) and *Salinibacterium xinjiangense* (Zhang et al., 2008). The level of DNA–DNA relatedness between *Salinibacterium amurskyense* and *Salinibacterium xinjiangense* is reported to be 46.7%, and the 16S rRNA gene sequence similarity is 97.4% (Zhang et al., 2008). The differential phenotypic and chemotaxonomic properties between the two species are listed in Table 173. *Rhodoglobus* is the closest neighbor of *Salinibacterium*, and two species of *Leifsonia*, namely *Leifsonia aurea* and *Leifsonia rubra*, are also closely related to the genus (Figure 200). The relationship among *Salinibacterium*, *Rhodoglobus*, and two *Leifsonia* species is discussed in the Taxonomic Comments section.

The genus has common morphological features shared by the members of *Microbacteriaceae*, such as irregular cell shapes (Figure 201), aerobic respiratory metabolism, unsaturated menaquinones, type I phospholipids (major amount of phosphatidylglycerol), and branched/saturated fatty acids as the major components. The colonies of *Salinibacterium* are yellow-colored and smooth-surfaced. Good growth occurs on marine agar plates at mesophilic temperatures and neutral pH. All strains of *Salinibacterium* are halophilic, although salt is not required for growth.

Cell-wall hydrolysates contain major amounts of lysine and ornithine as diagnostic cell-wall diamino acids, and the molar ratio of alanine: glycine: glutamic acid: lysine: ornithine of *Salinibacterium amurskyense* was reported as 1.0:1.0:0.4:0.25:1.0. The membrane fatty acids consist of 12-methyltetradecanoic acid ($C_{15:0}$ anteiso, 40.4–58.4% of the total), 14-methylpentadecanoic acid ($C_{16:0}$ iso, 21.1–34.7%), 12-methyltridecanoic acid ($C_{14:0}$ iso, 10.3–14.7%), 13-methyltetradecanoic acid ($C_{15:0}$ iso, 0.5–6.6%) and 14-methylhexadecanoic acid ($C_{17:0}$ anteiso, 2.8–7.2%). A considerable degree of difference can be observed between

TABLE 173. Characteristics differentiating the species of the genus *Salinibacterium*[a]

Characteristic	*S. amurskyense*	*S. xinjiangense*
Degradation of:		
Gelatin	+	–
Starch	–	+
Tween 20	–	+
Acid production from:		
Galactose	–	+
Glucose	–	+
Nitrate reduction	–	+
Growth temperature (°C) (optimum)	4–37 (25–28)	4–23 (18–19)
NaCl range for growth (%)	0–10	0–14
Menaquinones	MK-11, MK-10	MK-10, MK-11
Fatty acids	$C_{15:0}$ anteiso, $C_{16:0}$ iso, $C_{14:0}$ iso, $C_{15:0}$ iso, $C_{17:0}$ anteiso	$C_{15:0}$ anteiso, $C_{16:0}$ iso, $C_{14:0}$ iso, $C_{17:0}$ anteiso, $C_{15:1}$ anteiso
DNA G+C content (mol%)	61	63.5

[a]Symbols: +, >85% positive; –, 0–15% positive.

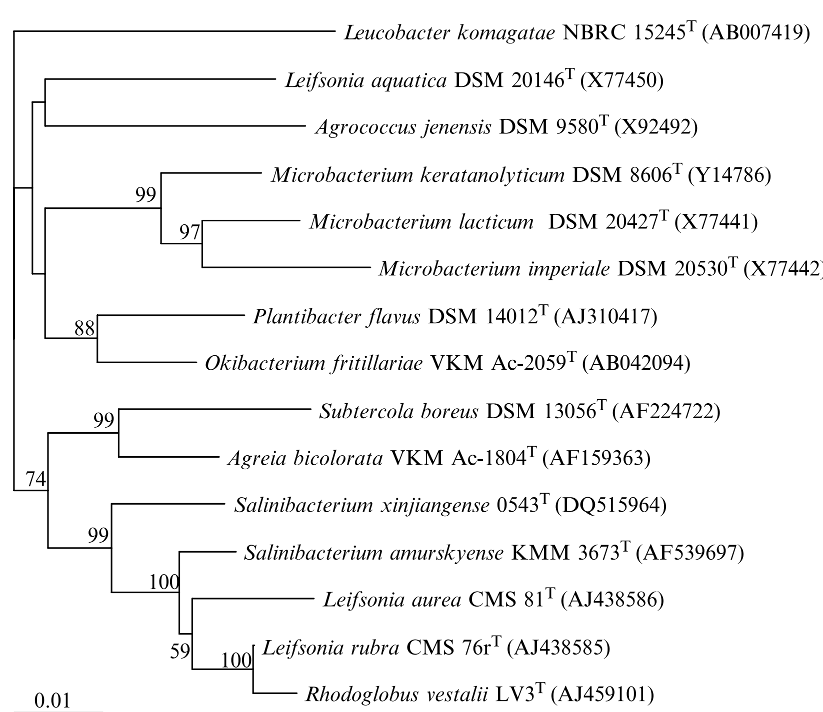

FIGURE 200. Phylogenetic position of *Salinibacterium*. Bar = 0.01 substitutions per nucleotide position.

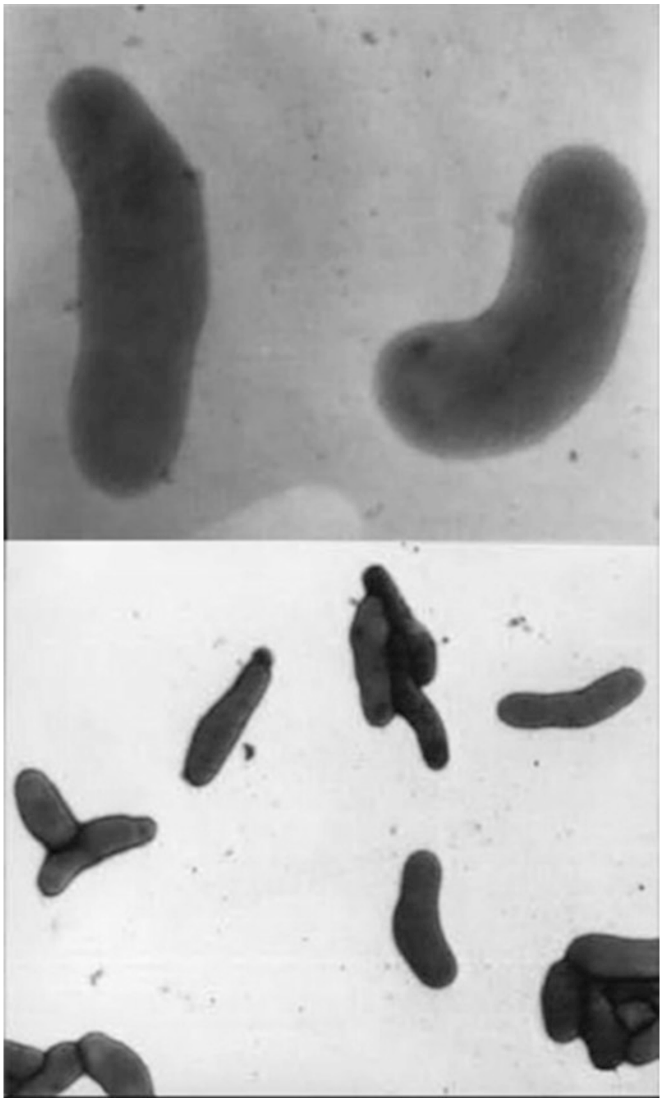

FIGURE 201. Transmission electron photomicrographs of cells of *Salinibacterium xinjiangense* 0543^T grown on PYG medium. (Reproduced with permission from Zhang et al., 2008. Int. J. Syst. Evol. Microbiol. *58*: 2739–2742.)

the two species in the proportion of each major constituent (Zhang et al., 2008). MK-11 is the major menaquinone and smaller amount of MK-10 is present in *Salinibacterium amurskyense* (Han et al., 2003), but MK-10 (54.4%) is the major component and smaller amounts of MK-11 (28.9%), MK-9 (6.8%), MK-12 (5.4%), and MK-8 (4.3%) are present in *Salinibacterium xinjiangense* (Zhang et al., 2008). The major chemotaxonomic profiles of *Salinibacterium* are consistent with their classification in the family *Microbacteriaceae*.

Salinibacterium amurskyense was the first species of *Microbacteriaceae* reported from a marine environment. A marine environment is not a common habitat for the members of *Microbacteriaceae*, and only a few cases of taxa isolated from a marine environment are known - *Labedella gwakjiensis* and *Phycicola gilvus* isolated from seaweed (Lee et al., 2008; Lee, 2007) and some species of *Microbacterium* isolated from seawater and marine sediment (Kageyama et al., 2007b; Lee et al., 2006; Shivaji et al., 2007). There are currently 22 entries of *Salinibacterium* sequences available in the GenBank database, and 12 out of 19 entries for which the sources are known originated from the marine environment. The sources include seawater, the Antarctic Ocean, estuary, marine bacterioneuston, marine sediment, permafrost ice wedge, glacier soil, and soils contaminated by chlorinated pesticides.

Enrichment and isolation procedures

The strains of *Salinibacterium* can be isolated using marine agar or PYG medium (Han et al., 2003; Zhang et al., 2008). For the enrichment of *Salinibacterium xinjiangense*, the glacier soil samples were resuspended in PYG broth, incubated at 4°C, and then spread on PYG agar plates for isolation. Since the organisms are halophilic, addition of NaCl or seawater to the isolation medium would enhance their growth. Growth temperature may vary depending on the nature of the sample. For the isolation of *Salinibacterium amurskyense*, 28°C was used, whereas *Salinibacterium xinjiangense* was isolated at 18°C. The optimal pH range is between 7.0 and 7.5.

Maintenance procedures

Strains can be cultivated on marine agar 2216 (Difco) or PYG medium (0.5% Bacto peptone, 0.02% yeast extract, 0.5% glucose, 0.3% beef extract, 0.05% NaCl, and 0.15% $MgSO_4 \cdot 7H_2O$). The cultures can be maintained at 25–28°C for mesophilic strains, and at 18–19°C for psychrophilic strains. For long-term preservation, storage of the cell suspensions in 20–30% (v/v) glycerol at −20 to −80°C, or lyophilization is recommended.

Differentiation of the genus *Salinibacterium* from other genera

Salinibacterium can be differentiated from the related genera of the family *Microbacteriaceae* based on the chemotaxonomic properties, including cell-wall diamino acids, isoprenoid quinones, acyl type, fatty acid profiles, and DNA G+C content (Table 140 for *Microbacteriaceae*). *Salinibacterium* contains lysine and ornithine as the diagnostic diamino acid of the cell wall, which separates the genus from other genera containing diaminobutyric acid including *Agreia*, *Agrococcus*, *Agromyces*, *Clavibacter*, *Cryobacterium*, *Leifsonia*, *Leucobacter*, *Microterricola*, *Plantibacter*, *Pseudoclavibacter*, *Rathayibacter*, *Rhodoglobus*, *Subtercola*, *Yonghaparkia*, and *Zimmermannella* (Lin et al., 2004; Matsumoto et al., 2008; Zhang et al., 2007c). The major menaquinone of *Salinibacterium* is either MK-10 or MK-11, which is different from those of *Clavibacter* (MK-9), *Curtobacterium* (MK-9), *Frigoribacterium* (MK-9), *Frondihabitans* (MK-8), *Gulosibacter* (MK-9), *Microcella* (MK-12 and 13), *Microterricola* (MK-12), *Pseudoclavibacter* (MK-9), *Rhodoglobus* (MK-12 and MK-11); *Subtercola* (MK-9 and 10), *Yonghaparkia* (MK-12), and *Zimmermannella* (MK-8, 9 and 10) (Lin et al., 2004; Matsumoto et al., 2008; Zhang et al., 2007c). *Salinibacterium* has acetyl type peptidoglycan (Han et al., 2003) which separates the genus from *Microbacterium* and *Okibacterium*, both having glycolyl type (Evtushenko et al., 2002).

Taxonomic comments

Salinibacterium was described in 2003 (Han et al., 2003) when the description of *Rhodoglobus*, the mostly related genus to the former, was also published (Sheridan et al., 2003). The 16S

rRNA gene sequence similarity clearly indicates close relationship between the two genera, as the similarity values are 95.8–97.1% based on the BLAST results (http://blast.ncbi.nlm.nih.gov/blast.cgi). *Rhodoglobus vestalii*, isolated from a lake in Antarctica, shows many properties similar to the species of *Salinibacterium*; growth between 2 and 21°C with an optimal temperature of 18°C, 62 mol% molar G+C content of the genomic DNA, and $C_{15:0}$ anteiso, $C_{16:0}$ iso, and $C_{17:0}$ anteiso as the major fatty acids. In contrast, red-pigmented colonies, small rods with unusual bulbous protuberances during all phases of growth, MK-12 as the major menaquinone with smaller amount of MK-11, absence of lysine in the cell wall, and lack of growth in the presence of 2.5% and higher concentration of NaCl, are obvious differences between *Rhodoglobus* and *Salinibacterium*. Considering the high level of 16S rRNA gene sequence similarity, the taxonomic status of the two genera will have to be further examined.

The two species of *Leifsonia*, *Leifsonia rubra* and *Leifsonia aurea* have also been constantly clustered with *Salinibacterium* (Lee et al., 2008; Lee, 2007; Zhang et al., 2008). The level of 16S rRNA gene sequence similarities between the *Salinibacterium* species and two *Leifsonia* species range from 95.1–97.6%. Coincidentally, the two *Leifsonia* species were also described in 2003 (Reddy et al., 2003b), and thus proper comparison between *Salinibacterium amurskyense* and *Leifsonia* species could not be performed then. Zhang et al. (2008) pointed out such problems regarding the taxonomic positions of five taxa, namely the two species of *Salinibacterium*, *Rhodoglobus vestalii*, and the two *Leifsonia* species. Since the level of 16S rRNA gene sequence similarity ranges between 95.1– 99.4% based on the BLAST results, it is likely that the five taxa form a single genus. Further investigation will be able to clarify their taxonomic status.

List of species of the genus *Salinibacterium*

1. **Salinibacterium amurskyense** Han, Nedashkovskaya, Mikhailov, Kim and Bae 2003, 2065[VP]

a.mur.sky.en′se. N.L. neut. adj. *amurskyense* of or belonging to Amursky Bay, the geographical location where the organism was first isolated.

General morphological and chemotaxonomic properties are as given in the genus description. Grows between 4 and 37°C (with optimal growth at 25–28°C), between pH 4.5 and 11.0 (with optimal growth around pH 7.2–7.5), and in the presence of 0–10% NaCl (with optimal growth at 1–3%). Gelatin and DNA are degraded, but agar, casein, starch, cellulose, alginate, chitin, and Tweens 20 and 40 are not. Acid is produced from rhamnose and glycerol. Acid is not produced from acetate, *N*-acetylglucosamine, adonitol, cellobiose, dulcitol, fumarate, galactose, glucose, inositol, lactose, malate, maltose, melibiose, raffinose, sorbitol, sorbose, sucrose, or xylose. Susceptible to ampicillin (10 μg per disc), carbenicillin (100 μg), and streptomycin (10 μg), but resistant to gentamicin (10 μg), kanamycin (30 μg), neomycin (30 μg), polymyxin B (300 U), and tetracycline (30 μg).

Source: sea water.

DNA G+C content (mol%): 61.0 (T_m).

Type strain: JCM 12362, KCTC 9931, KMM 3673, NBRC 100448.

Sequence accession no. (16S rRNA gene): AF539697.

2. **Salinibacterium xinjiangense** Zhang, Liu, Xin, Yu, Zhou and Zhou 2008, 2741[VP]

xin.jiang.en′se. N.L. neut. adj. *xinjiangense* of or pertaining to Xinjiang, where the type strain was isolated.

Psychrophilic, nonsporeforming, nonflagellated, and nongliding irregular rods. Cells are 1.4–2.3 μm long and 0.5–0.8 μm wide. Colonies are yellow, smooth, circular, and convex with entire margins. Grows at 4–23°C (optimal temperature 18–19°C) and also at pH 5.0–9.0 (approximate optimal pH 6.0–8.0). Grows in the presence of 0–14% (w/v) NaCl. Catalase-positive but oxidase-negative. Starch and Tweens 20, 60, and 80 are hydrolyzed, but gelatin or casein are not. Nitrate is reduced. Tests for naphthol-AS-BI-phosphohydrolase, amannosidase, esterase (C4), esterase lipase (C8), cystine arylamidase, leucine arylamidase, and valine arylamidase are positive. Tests for arginine dihydrolase, lysine decarboxylase, ornithine decarboxylase, alkaline phosphatase, acid phosphatase, indole production, α-chymotrypsin, trypsin, urease, α-galactosidase, β-galactosidase, β-glucuronidase, β-glucosidase, α-glucosidase, and α-fucosidase are negative. Arabinose, cellobiose, fructose, galactose, glucose, maltose, D-mannose, melibiose, and sucrose can be utilized as sole carbon sources. Acids are produced from glucose, fructose, D-mannose, and galactose. Erythritol, fumarate, hippurate, melezitose, pyruvate, L-rhamnose, D-sorbitol, sorbose, succinate, tartrate, and uric acid are not utilized as sole carbon sources. The major menaquinone is MK-10, and a smaller amount of MK-11 is also present. The polar lipids are diphosphatidylglycerol and phosphatidylglycerol. The peptidoglycan contains ornithine and lysine as major diamino acids, and alanine, glycine, and glutamic acid are also present. The major cellular fatty acids are $C_{15:0}$ anteiso, $C_{16:0}$ iso, $C_{14:0}$ iso, and $C_{17:0}$ anteiso.

Source: glacier soil.

DNA G+C content (mol%): 63.5 (T_m).

Type strain: 0543, CGMCC 1.5381, JCM 13926.

Sequence accession no. (16S rRNA gene): DQ515964.

Genus XXV. **Subtercola** Männistö, Schumann, Rainey, Kämpfer, Tsitko, Tiirola and Salkinoja-Salonen 2000, 1737[VP]

Peter Kämpfer

Sub.ter'co.la. L. prep. *subter* below, underneath; L. masc. suff. *-cola* inhabitant; N.L. masc. n. *subtercola* the one who lives underneath.

Short irregular rods, 0.2–0.4 × 0.6–1.6 μm, often occurring singly or in v-forms. **Gram-stain-positive**. Nonencapsulated. Nonmotile. Nonsporeforming. Aerobic. The colonies are circular, convex, smooth, and pale to bright yellow, depending on the medium. **Growth occurs best at 15–17°C, but is also observed in the temperature range 2–28°C.** The cell-wall diamino acid is **diamino butyric acid (DAB)**. **The main cell-wall amino acids are alanine, glycine**, ***threo*-3-hydroxy-glutamic acid, and DAB**. The peptidoglycan type is B2γ. The polar lipids are phosphatidylglycerol, diphosphatidylglycerol, one unknown phospholipid, and two glycolipids. The main cellular fatty acids are 12-methyltetradecanoic acid ($C_{15:0}$ anteiso), 14-methylpentadecanoic acid ($C_{16:0}$ iso), and 14-methylhexadecanoic acid ($C_{17:0}$ anteiso). **The whole-cell methanolysates of *Subtercola* species contain 1,1-dimethoxy-anteiso-pentadecane ($C_{15:0}$ anteiso DMA) and 1,1-dimethoxy-iso-hexadecane ($C_{16:0}$ iso DMA) as major components in amounts comparable to those of fatty acids.** No mycolic acids are present. The major isoprenoic quinones are MK-9 and MK-10.

DNA G+C content (mol%): 64–68.

Type species: **Subtercola boreus** Männistö, Schumann, Rainey, Kämpfer, Tsitko, Tiirola and Salkinoja-Salonen 2000, 1737[VP].

Further descriptive information

Isolated from Finnish groundwater (Männistö et al., 2000). Belong to the few psychrophilic or pychrotolerant actinobacteria of the family *Microbacteriaceae*. Two species have been described so far, *Subtercola boreus* and *Subtercola frigoramans* (Männistö et al., 2000). An additional species, *Subtercola pratensis*, was proposed by Behrendt et al. (2002), however, after the description of the genus *Agreia* (Evtushenko et al., 2001), *Subtercola pratensis* was transferred to the genus *Agreia* (Schumann et al., 2003).

Phylogenetic analyses based on a dataset comprising 1330 unambiguous nucleotides between positions 38 and 1478 (but excluding positions 97–193 due to the absence of this region in the reference sequence of *Leucoacter komagatae* D17751, *Escherichia coli* positions; Brosius et al., 1978) showed that the new isolates cluster together as distinct lineages within the radiation of the actinomycete genera with group-B-peptidoglycan that comprises the family *Microbacteriaceae* (Stackebrandt et al., 1997). Both species show the highest levels of 16S rRNA gene sequence similarity with species of the genera *Clavibacter* (95.1–97.1%), *Frigoribacterium* (95.5–96.7%), and *Rathayibacter* (94.3–96.7%). The 16S rRNA gene sequences (1465 bp) of the type strains of both species show a similarity of 96.4%.

The two established species share major chemotaxonomic characteristics including peptidoglycan type B2γ (Schleifer and Kandler, 1972), murein type, quinone system with MK-9 and MK-10 (in almost equal proportions), a similar polar lipid profile with phosphatidylglycerol, diphosphatidylglycerol, one unknown phospholipid and two glycolipids, and a fatty acid profile with almost exclusively branched acids and the predominant $C_{15:0}$ anteiso. Significant quantitative differences in the presence of certain fatty acids and several physiological traits (Table 174 and Table 175) are found.

TABLE 174. Characteristics differentiating *Subtercola boreus* from *Subtercola frigoramans*[a,b]

Characteristic	*S. frigoramans* K265[T]	*S. boreus* K300[T]
Assimilation of:		
p-Arbutin, salicin	+	−
L-Rhamnose, D-ribose, i-inositol, citrate, 3-hydroxybenzoate, acetate, propionate, *cis*-aconitate	−	+
Hydrolysis of:		
*p*NP-β-D-Glucuronide, Bis-*p*NP-phosphate	−	+
*p*NP-β-D-Xylopyranoside	+	−

[a]Symbols: +, >85% positive; d, different strains give different reactions (16–84% positive); −, 0–15% positive; D, different reactions occur in different taxa (species of a genus or genera of a family); w, weak reaction; nd, not determined; nr, not reported. *p*NP, para-nitrophenyl.

[b]Data from Männistö et al. (2000).

The principal phospholipids of *Subtercola boreus* and *Subtercola frigoramans* are similar to those of *Clavibacter* (Collins and Jones, 1980), *Rathayibacter* (Zgurskaya et al., 1993), *Cryobacterium* (Suzuki et al., 1997), and *Frigoribacterium* (Kämpfer et al., 2000). The whole-cell methanolysates of both species contain predominantly iso- and anteisobranched fatty acids and 1,1-dimethyl acetals. The whole-cell methanolysates of *Subtercola* species are quite similar to that of *Frigoribacterium faeni* (Table 175), but the peptidoglycan type of *Frigoribacterium* is B2β, whereas that of *Subtercola* is B2γ. The amino acid ratio in the peptidoglycan of *Subtercola* is different from that of other DAB containing genera (Table 175) in the low levels of glutamic acid. In both *Subtercola* species, nearly all of the glutamic acid residues at position 2 of the peptide subunit were replaced by hydroxyglutamic acid. The optimal growth temperatures of both species are 15–17°C. These temperatures are approximately 10 degrees, down to −2°C for *Subtercola frigoramans*, lower than those in the genera *Clavibacter* (Davis et al., 1984) and *Rathayibacter* (Zgurskaya et al., 1993). The new genus *Frigoribacterium* (Kämpfer et al., 2000), recently described and isolated from a Finnish farming environment, showed a similar growth-temperature range, whereas *Cryobacterium* (Suzuki et al., 1997) showed no growth above 18°C. A strong influence of temperature decrease was observed both for the fatty acid and dimethyl acetal compositions of *Subtercola boreus* and *Subtercola frigoramans* (Table 175).

The production of 1,1-dimethyl acetals has been observed by the methanolysis of alk-1′-enyl glyceryl ethers (plasmalogens) (Jantzen and Hofstad, 1981). These plasmalogens are known

TABLE 175. Diagnostic and differentiating characteristics of the type strains of both *Subtercola* species and the genera *Clavibacter*, *Cryobacterium*, *Frigoribacterium*, and *Rathayibacter*[a]

Characteristic	*S. boreus*[b] K300^T	*S. frigoramans*[b] K265^T	*Clavibacter*[c]	*Cryobacterium*[d]	*Frigoribacterium*[e]	*Rathayibacter*[f]
Peptidoglycan type	B2γ	B2γ	B2γ	B2γ	B2β	B2γ
Cell-wall diamino acid	DAB	DAB	DAB	DAB	D-Lysine	DAB
Major menaquinone(s)	MK-9, MK-10	MK-9, MK-10	MK-9	MK-10	MK-9	MK-10
G+C content (mol%)	68	64	67–78	66.5	71	67–78
Main cell-wall sugars:						
Galactose	–	–	–	–	nr	D
Glucose	+	–	–	–	nr	+
Fucose	–	–	–	+	nr	–
Mannose	–	+	+	–	nr	+
Rhamnose	+	+	+	+	nr	+
Ribose	–	+	–	–	nr	–
Xylose	+	+	–	–	nr	D
Major fatty acid types	A.I	A.I	A.I.S	A.I.S	A.I.S	A.I.S
Major 1.1-dimethyl acetales	$C_{15:0}$ anteiso, $C_{16:0}$ iso, $C_{15:0}$ iso, $C_{17:0}$ anteiso, $C_{16:0}$	$C_{15:0}$ anteiso, $C_{16:0}$ iso, $C_{17:0}$ anteiso	–	–	$C_{15:0}$ anteiso	–
Growth temperature range (°C)	2–28	−2 to 28	nr	<81	0–28	nr
Optimal growth temperature (°C)	15–17	15–17	20–29	9–12	nr	24–28
Isolation source	Boreal groundwater	Boreal groundwater	Plant material	Antarctic soil	Farm air	Plant material

[a]Symbols: +, >85% positive; –, 0–15% positive; D, different reactions occur in different taxa (species of a genus); nr, not reported; A, anteiso-branched; I, iso-branched; S, straight-chain saturated; DAB, diamino butyric acid.

[b]Data from Männistö et al. (2000).

[c]Data from Groth et al. (1996).

[e]Data from Kämpfer et al. (2000).

[d]Data from Suzuki et al. (1997).

[f]Data from Zgurskaya et al. (1993).

to occur as significant components of animal cell membranes and in many obligately anaerobic bacteria such as *Fusobacterium* species (Jantzen and Hofstad, 1981), clostridia (Johnston and Goldfine, 1994), *Megasphaera elsdenii* (Kaufman et al., 1990), and *Eubacterium lentum* (Verhulst et al., 1987). They are also present in the anaerobic actinobacterial species *Propionibacterium freudenreichii* and *Propionibacterium jensenii* and also in *Frigoribacterium faeni* (Kämpfer et al., 2000).

The biological function of plasmalogens is not known, but Kaufman et al. (1990) showed that membranes of *Megasphaera elsdenii* (with high plasmalogen content) were more ordered than membranes devoid of plasmalogens. This may indicate that the changes in the plasmalogen derived 1,1-dimethyl acetal composition may reflect a role in membrane liquidity.

Enrichment and isolation procedures

No specific isolation medium has been described so far. Good growth occurs on R2A agar and also PYGV agar (Staley, 1968) as well as on TS agar at 15–17°C. Growth is observed at −2°C–25°C.

Maintenance procedures

Subtercola cultures may be lyophilized by common procedures used for many bacteria. In addition, cultures can be maintained by serial transfers on solid complex media. Growth on agar slants in screw-capped tubes can be kept at 4°C for about 2–4 weeks. Long-term preservation of liquid cultures supplemented with 5% dimethylsulfoxide is recommended in the vapor phase of liquid nitrogen (−140°C).

Differentiation of the genus *Subtercola* from other genera

Subtercola may be distinguished from other genera in the family *Microbacteriaceae* by cell-wall peptidoglycan, the menaquinone type, the colony pigmentation, presence of DMA, the G+C content, and the growth at low temperatures (Table 175).

List of species of the genus *Subtercola*

1. **Subtercola boreus** Männistö, Schumann, Rainey, Kämpfer, Tsitko, Tiirola and Salkinoja-Salonen 2000, 1737[VP]

bo.re′us. L. masc. adj. *boreus* Northern, referring to the boreal groundwater aquifer in Finland, from which the organism was isolated.

Cells are short, irregular rods 0.2–0.3 × 0.6–1.0 µm. Colonies are yellow pigmented, circular, convex, and smooth. Growth occurs at 2–28°C. The major cellular fatty acids are 12-methyltetradecanoic acid ($C_{15:0}$ anteiso), 14-methylpentadecanoic acid ($C_{16:0}$ iso), 14-methylhexadecanoic ($C_{17:0}$ anteiso) acid, and 13-methyltetradecanoic acid ($C_{15:0}$ iso). 1,1-Dimethoxy-iso-pentadecane ($C_{15:0}$ iso DMA), 1,1-dimethoxy-anteiso-heptadecane ($C_{17:0}$ anteiso DMA), and 1,1-dimethoxy-hexadecane ($C_{16:0}$ DMA) are found in whole-cell methanolysates. The cell wall contains glucose, rhamnose, and xylose. Other chemotaxonomic characteristics are as described for the genus. The following carbon sources are utilized: L-arabinose, D-cellobiose, D-fructose, D-galactose, gluconate, D-glucose, D-mannose, D-maltose, L-rhamnose, D-ribose, sucrose, D-trehalose, D-xylose, adonitol, *i*-inositol, D-mannitol, acetate, propionate, *cis*-aconitate, citrate, fumarate, DL-3-hydroxybutyrate, 3-hydroxybenzoate, and 4-hydroxybenzoate. Amino acids are mostly not utilized.

Source: groundwater.

DNA G+C content (mol%): 68 (HPLC).

Type strain: K300, ATCC BAA-168, CCUG 43135, CIP 106947, DSM 13056, JCM 11267, NBRC 103085.

Sequence accession no. (16S rRNA gene): AF224722, AM410674.

2. **Subtercola frigoramans** Männistö, Schumann, Rainey, Kämpfer, Tsitko, Tiirola and Salkinoja-Salonen 2000, 1737[VP]

fri.gor.a′mans. L. neut. n. *frigus -oris* the cold; L. part. pres. *amans* loving; N.L. part. adj. *frigoramans* loving the cold.

Cells are pleomorphic, irregular rods 0.3–0.4 × 0.9–1.5 µm. In liquid culture, the cells may show v- or y-forms. Colonies appear pale yellow to bright yellow depending on the culture medium. Large, mucoid colonies are formed on glucose-containing media. Growth occurs at −2°C to 28°C. The major cellular fatty acids are 12-methyltetradecanoic acid ($C_{15:0}$ anteiso), 14-methylpentadecanoic acid ($C_{16:0}$ iso), 14-methylhexadecanoic acid ($C_{17:0}$ iso), and 12-methyltridecanoic acid ($C_{14:0}$ iso). 1,1-Dimethoxy-anteiso-heptadecane ($C_{17:0}$ anteiso DMA) is found in whole-cell methanolysates. The cell wall contains rhamnose, ribose, xylose, and mannose. The main isoprenoid quinones are MK-10 and MK-9.

Other chemotaxonomical characteristics are the same as described for the genus. The following carbon sources are utilized: L-arabinose, *p*-arbutin, D-cellobiose, D-fructose, D-galactose, gluconate, D-glucose, D-mannose, D-maltose, sucrose, salicin, D-trehalose, D-xylose, adonitol, and D-mannitol. Acetate, propionate, aconitate, and most amino acids are not utilized as sole source of carbon. Fumarate, but not citrate, is assimilated.

Source: groundwater.

DNA G+C content (mol%): 64 (HPLC).

Type strain: K265, ATCC BAA-169, CCUG 43136, CIP 106948, DSM 13057, JCM 11268, NBRC 103086.

Sequence accession no. (16S rRNA gene): AF224723, AM410673.

Genus XXVI. **Yonghaparkia** Yoon, Kang, Schumann and Oh 2006b, 2418[VP]

Jung-Hoon Yoon

Yong.ha.park′i.a. N.L. fem. n. *Yonghaparkia* named after Yong-Ha Park, a Korean microbiologist who proposed the family *Microbacteriaceae* and has contributed significantly to bacterial systematics.

Cells are short rods or rods, 0.2–0.4 × 0.6–1.4 mm under optimal growth conditions. Gram-stain-positive. Nonsporeforming. Nonmotile. Strictly aerobic. The cell-wall peptidoglycan type is B2γ and contains the dipeptide D-Glu–D-2,4-diaminobutyrate (D-DAB) with Gly in position 1 and L-DAB in position 3. The predominant menaquinone is MK-12. A significant amount of MK-11 is also present. The fatty acid profile consists of branched and straight-chain fatty acids; predominant fatty acids are $C_{15:0}$ anteiso, $C_{16:0}$ iso, and $C_{17:0}$ anteiso.

DNA G+C content (mol%): 71.1–71.6.

Type species: **Yonghaparkia alkaliphila** Yoon, Kang, Schumann and Oh 2006b, 2418[VP].

Further descriptive information

Growth is alkaliphilic and moderately oligotrophic. The optimal pH for growth is 9.0. Growth does not occur at pH 7.0. Good growth occurs on half strength nutrient agar with the pH adjusted to 9.0, with Na_2CO_3 but growth is poor on nutrient agar and does not occur on trypticase soy agar. Growth occurs in the absence of additional NaCl. Phylogenetically, the genus *Yonghaparkia* belongs to the family *Microbacteriaceae* of the order *Micrococcales* and is phylogenetically most closely related to the genus *Microcella*.

Enrichment and isolation procedures

The type of the genus *Yonghaparkia*, *Yonghaparkia alkaliphila*, was isolated from an alkaline serpentinite soil (approximate pH 10.0) from Kwangkchun, Korea. It is not known whether members of this genus can be enriched from similar samples from other natural habitats. However, two potential species of the genus with 99.1 and 99.6% 16S rRNA gene similarity to *Yonghaparkia alkaliphila* KSL-133[T], were isolated from another alkaline soil on Luria–Bertani (LB) agar (pH 9.0) under an atmosphere of 5% CO_2 (Ueda et al., 2008). This observation supports the

view that this genus is comprised of alkalophiles. When present, *Yonghaparkia* may be isolated by standard dilution plating techniques using 2–10× diluted nutrient agar, pH 9.0–10.0.

Maintenance procedures

For short-term preservation, serial transfer on agar slants (e.g. half-strength nutrient agar, pH 9.0) is recommended. Agar slants can be stored at 4°C for at least 2 months. For long-term preservation, lyophilization, storage in liquid nitrogen, and frozen glycerol suspensions are suitable. For lyophilization, the cells are collected by centrifugation, and the cells are suspended in 20% (w/v) skim milk prior to lyophilization. For storage in liquid nitrogen, cells are harvested from agar plates by suspending in 1 ml sterile water and transferred to cryo-tubes containing 1 ml of 20% (w/v) glycerol solution. The tube is stored at −20°C or −70°C.

Differentiation of the genus *Yonghaparkia* from other genera

The genus *Yonghaparkia* is distinguished from members of the family *Microbacteriaceae* by differences in chemotaxonomic properties such as predominant menaquinone(s) and the diamino acids in their cell walls. It is distinguishable from the genera *Microcella*, *Frigoribacterium*, *Frondihabitans*, and *Mycetocola*, the closest phylogenetic neighbors, by differences of the predominant menaquinone, the fatty acid profile, and the cell-wall composition. The genus *Yonghaparkia* contains MK-12 as the predominant menaquinone, whereas the genera *Microcella*, *Frigoribacterium*, *Frondihabitans*, and *Mycetocola* contain MK-12 and MK-13, MK-9, MK-7 and MK-8, or MK-10, respectively (Table 176). Likewise, while the fatty acids abundant in the genus *Yonghaparkia* are found in other closely related genera, the profile is readily distinguishable from that of the genera *Microcella*, *Frigoribacterium*, *Frondihabitans*, and *Mycetocola* (Table 176). The genus *Yonghaparkia* is further distinguished by cell walls containing 2,4-diaminobutyric acid (DAB) as the diamino acid in position 3 of the peptidoglycan. In contrast, cell walls of the genera *Microcella*, *Frigoribacterium*, and *Mycetocola* and the genus *Frondihabitans* contain lysine and D-ornithine, respectively (Table 176). The genus *Yonghaparkia* is further differentiated from the above-mentioned genera by some other phenotypic characteristics which are listed in Table 176. Although the genus *Yonghaparkia* is similar to the genus *Agromyces* in the chemotaxonomic properties, it forms an independent lineage in the phylogenetic tree based on 16S rRNA gene sequences.

TABLE 176. Phenotypic characteristics differentiating *Yonghaparkia* and some phylogenetically related genera of the family *Microbacteriaceae*[a]

Characteristic	*Yonghaparkia*[b]	*Cryobacterium*[c]	*Frigoribacterium*[d]	*Frondihabitans*[e]	*Labedella*[f]	*Microcella*[g]	*Mycetocola*[h]
Cell size (μm)	0.2–0.4 × 0.6–1.4	0.3–0.8 × 1.0–3.6	0.2–0.4 × 1.0–1.5	0.2–0.4 × 0.5–1.0	0.3–0.4 × 1.0–4.4	0.3–0.4 × 0.8–4.0	0.2–0.4 × 1.1–3.5
Motility	−	v(−)	+ or nd	−	−	− or nd	−
Optimal temperature for growth (°C)	30	9–12, 20–22 or 28	4–10 or 20–28	25	25–30	35	25
Oxidase activity	+	− or nd	−	−	−	−	− or nd
Growth at:							
4°C	−	v(+)	v(+)	−	−	−	−
30°C	+	−	v(−)	+	+	+	+
Hydrolysis of:							
Casein	+	v(−)	nd	nd	−	v()	v(−)
Esculin	+	v(nd)	v(+)	nd	+	−	+
Gelatin	−	−	nd	nd	+	−	−
Utilization of:							
L-Arabinose	−	v(nd)	+	−	nd	+	nd
D-Cellobiose	−	+ or nd	+	−	nd	+	nd
Citrate	−	−	v(+)	−	−	nd	+
D-Fructose	−	v(nd)	v(+)	−	nd	+	nd
D-Galactose	v()	+ or nd	+	−	nd	v()	nd
D-Glucose	−	+ or nd	+	+	+	+	nd
Maltose	−	v(nd)	+	+	−	+	nd
D-Mannose	−	+ or nd	+	−	+	+ or nd	nd
Pyruvate	+	v(+)	− or nd	−	nd	nd	+
Sucrose	−	+ or nd	+	+	nd	+	nd
D-Trehalose	−	nd	+	+	nd	v(−)	nd
D-Xylose	−	+ or nd	+	nd	nd	v()	nd
Diamino acid	2,4-Diaminobutyric acid (DAB)	2,4-Diaminobutyric acid	D-Lysine or 2,4-diaminobutyric acid	D-Ornithine	Ornithine	Lysine or D-ornithine	Lysine

(continued)

TABLE 176. (continued)

Characteristic	*Yonghaparkia*[b]	*Cryobacterium*[c]	*Frigoribacterium*[d]	*Frondihabitans*[e]	*Labedella*[f]	*Microcella*[g]	*Mycetocola*[h]
Predominant menaquinone(s)	MK-12	MK-10 or MK-11 and MK-12	MK-9	MK-7, MK-8	MK-10,	MK-13 and MK-12 or MK-14	MK-10
Major fatty acids (>10% of total fatty acids)[b]	$C_{15:0}$ anteiso, $C_{16:0}$ iso, $C_{17:0}$ anteiso	$C_{15:0}$ anteiso, $C_{17:0}$ anteiso, $C_{16:0}$ iso	$C_{15:0}$ anteiso, $C_{16:0}$ iso, $C_{17:0}$ anteiso, $C_{15:0}$ anteiso DMA[i] or $C_{18:1}$ ω7*c*	$C_{18:1}$ ω7*c*[j]	$C_{15:0}$ anteiso, $C_{16:0}$ iso, $C_{17:0}$ anteiso	$C_{16:0}$ iso, $C_{15:0}$ anteiso, $C_{14:0}$ iso, $C_{15:0}$ iso	$C_{15:0}$ anteiso, $C_{17:0}$ anteiso, $C_{16:0}$
DNA G+C content (mol%)	71.1–71.6	65.0–70.0	67.5–71.0	71	68	68.8–70.7	63.9–70.0

[a]Symbols: +, positive; –, negative; v, variable (results for the type strain of the type species are in parentheses); nd, not determined. All genera are positive for Gram stain and catalase activity.

[b]Data from Yoon et al. (2006b).

[c]Data from Dastager et al. (2008a); Suzuki et al. (1997); and Zhang et al. (2007b).

[d]Data from Dastager et al. (2008d) and Kämpfer et al. (2000).

[e]Data from Greene et al. (2009) and Zhang et al. (2007c).

[f]Data from Lee (2007).

[g]Data from Tiago et al. (2006, 2005b).

[h]Data from Bora et al. (2008) and Tsukamoto et al. (2001).

[i]$C_{15:0}$ anteiso DMA, 1,1-dimethyl-anteiso-pentadecane.

[j]Though reported in the original description to be present along with $C_{14:0}$ 2-OH (Zhang et al., 2007c), it is most likely that the fatty acid data were not obtained from the same bacterium which was also subjected to the other examinations for characterization and then described as *Frondihabitans australicus.* The fatty acid profile suggests no affiliation of the corresponding bacterium with the *Microbacteriaceae* but with the Gram-negative family *Sphingomonadaceae* within the *Alphaproteobacteriaceae.* So far, all taxa of the *Microbacteriaceae* were reported to contain predominantly iso- and anteiso-methylbranched fatty acids and most of them contain $C_{15:0}$ anteiso, $C_{16:0}$ iso, and $C_{17:0}$ anteiso fatty acids as the major compounds. No other taxon except *Frondihabitans* was reported to contain $C_{18:1}$ ω7*c*.

List of species of the genus *Yonghaparkia*

1. **Yonghaparkia alkaliphila** Yoon, Kang, Schumann and Oh 2006b, 2418[VP]

al.ka.li′phi.la. N.L. n. *alkali* alkali; N.L. fem. adj. *phila* (from Gr. fem. adj. *philê*) loving; N.L. fem. adj. *alkaliphila* loving alkaline conditions.

Cells are short rods or rods, 0.2–0.4 × 0.6–1.4 mm, under optimal growth conditions. Gram-stain-positive. Nonspore-forming. Nonmotile. Strictly aerobic. Colonies are circular, slightly convex, smooth, glistening, yellow in color, and 1.5–2.0 mm in diameter after incubation for 10 d at 30°C on one-half strength nutrient agar, pH 9.0. Growth occurs at 10 and 37°C, but not at 4 and 38°C. Optimal pH for growth is 9.0. Growth occurs at 0–2% (w/v) NaCl, with an optimum at 1% but not at 3% NaCl. Growth does not occur under anaerobic conditions on half-strength nutrient agar (pH 9.0) in the absence or presence of 0.1% nitrate (w/v). Urease-negative. Starch and Tween 40 are hydrolyzed. Hypoxanthine, xanthine, L-tyrosine, Tween 20, Tween 60, and Tween 80 are not hydrolyzed. Nitrate is not reduced to nitrite. In assays with the API ZYM system, esterase (C 4), esterase lipase (C 8), leucine arylamidase, β-glucuronidase, and α-glucosidase are present, but alkaline phosphatase, lipase (C 14), valine arylamidase, cystine arylamidase, trypsin, α-chymotrypsin, acid phosphatase, naphthol-AS-BI-phosphohydrolase α-galactosidase, β-galactosidase, β-glucosidase, *N*-acetyl-β-glucosaminidase, α-mannosidase, and α-fucosidase are absent. Acetate, succinate, benzoate, L-malate, formate, and L-glutamate are not utilized as sole carbon and energy sources. Utilization of salicin is variable (positive for type strain). Susceptible to polymyxin B, streptomycin, chloramphenicol, gentamicin, novobiocin, lincomycin, oleandomycin, neomycin, and carbenicillin, but not to penicillin G, ampicillin, tetracycline, and kanamycin. The major fatty acids are $C_{15:0}$ anteiso, $C_{16:0}$ iso, and $C_{17:0}$ anteiso. The major polar lipids are diphosphatidylglycerol, phosphatidylglycerol, unidentified phospholipids, and unidentified glycolipids.

Source: an alkaline soil.

DNA G+C content (mol%): 71.1–71.6 (HPLC).

Type strain: KSL-113, CIP 108920, JCM 15138, KCTC 19126.

Sequence accession no. (16S rRNA gene): DQ256087.

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Family XII. **Promicromonosporaceae** Rainey, Ward-Rainey and Stackebrandt 1997, 484[VP] emend. Zhi, Li and Stackebrandt 2009, 598

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Pro.mi.cro.mo.no.spo.ra.ce′a.e. N.L. fem. n. *Promicromonospora* type genus of the family; L. suff. *-aceae* ending to denote a family; N.L. fem. pl. n. *Promicromonosporaceae* the *Promicromonospora* family.

The pattern of 16S rRNA gene sequence signatures consists of nucleotides at positions: 120 (A), 131:231 (A–G), 196 (U), 342:347 (C–G), 444:490 (A–U), 580:761 (C–G), 602:636 (G–U), 670:736 (A–U), 822:878 (G–C), 823:877 (G–C), 826:874 (C–G), 827 (U), 843 (U), 950:1231 (U–A), 1047:1210 (G–C), 1109 (C), 1145 (G), 1309:1328 (G–C), 1361 (G), and 1383 (C). Chemotaxonomically, the family is defined by peptidoglycan type A4α, MK-9(H_4) as predominant menaquinone and iso- and anteiso-branched cellular fatty acids. The family *Promicromonosporaceae* belongs to the order *Micrococcales* (formerly suborder *Micrococcineae* Rainey, Ward-Rainey and Stackebrandt (1997) of the class *Actinobacteria* Stackebrandt, Rainey and Ward-Rainey (1997).

Type genus: **Promicromonospora** Krasil'nikov, Kalakoutskii and Kirillova 1961, 107[AL].

Taxonomic comments

The family *Promicromonosporaceae* Rainey, Ward-Rainey and Stackebrandt (1997) was established to accommodate *Promicromonospora* as the only genus because of its distinct phylogenetic position within the suborder *Micrococcineae* and the presence of a taxon-specific set of 16S rRNA signature nucleotides (Stackebrandt et al., 1997). This pattern has been revised in view of novel members added to the family (Zhi et al., 2009). Taxonomic investigations of cellulolytic and xylanolytic isolates and reclassifications of validly named species have resulted in the proposals of six additional genera of this family: *Cellulosimicrobium* (Schumann et al., (2001), emend. Brown et al., 2006), *Isoptericola* (Stackebrandt et al., 2004), *Myceligenerans* (Cui et al., 2004), *Xylanibacterium* (Rivas et al., 2004), *Xylanimicrobium* (Stackebrandt and Schumann, 2004), and *Xylanimonas* (Rivas et al., 2003). The chemotaxonomic properties characterizing these genera are indicated in Table 177. Many of the representatives of the family can degrade polysaccharides such as cellulose or xylan. A phylogenetic dendrogram of members of the family *Promicromonosporaceae* is shown in Figure 202.

Genus I. **Promicromonospora** Krasil'nikov, Kalakoutskii and Kirillova 1961, 107[AL]

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Pro.mi.cro.mo.no.spo′ra. Gr. pref. *pro* before, primitive; Gr. adj. *mikros* small; Gr. adj. *monos* single, solitary; Gr. fem. n. *spora* a seed, and in microbiology a spore; N.L. fem. n. *Promicromonospora* the genus name was coined to reflect the combination of traits then thought to be characteristic of the actinomycete form-genera "*Proactinomyces*" (the tendency of the mycelium to fragment) and *Micromonospora* (the formation of single spores on the substrate mycelium).

Branching septate **hyphae** (0.5–1.0 µm in diameter) growing on the surface of and penetrating into the agar, which **break up into fragments** of various size and shape. Fragmentation results in **nonmotile**, Y- or V-shaped, rodlike, coccoid, chlamydospore-like, and other spore-shaped elements. All of them may give rise to new mycelia. **Growth pasty to leathery**. Aerial hyphae in different strains may vary in abundance (sometimes discernible only microscopically). These are straight to curved, sometimes sparsely branched, usually fragmented into rodlike or elongated coccoid elements. **Gram-stain-positive, non-acid-fast, catalase-positive. Aerobic.** Chemo-organotrophic. Glucose metabolized oxidatively, rarely also fermentatively. **Mesophilic. Peptidoglycan type A4α, variation L-Lys←L-Ala←D-Glu**. Mycolic acids and wall teichoic acids are lacking. Branching fatty acids of the iso- and anteiso- types ($C_{15:0}$ iso and $C_{15:0}$ anteiso) predominate. Diagnostic phospholipids are represented by phosphatidylglycerol and an unidentified glucosamine-containing phospholipid. Major **menaquinone** is **MK-9(H_4)**. Mainly found in soils. The genus *Promicromonospora* is the type genus of the family *Promicromonosporaceae* of the order *Micrococcales*, class *Actinobacteria*.

DNA G+C content (mol%): 70–75 (T_m).

Type species: **Promicromonospora citrea** Krasil'nikov, Kalakoutskii and Kirillova 1961, 107[AL].

Further descriptive information

The genus *Promicromonospora* was established by Krasil'nikov et al. (1961) to harbor at that time the only species *Promicromonospora citrea* which embraced approximately 30 soil isolates. Six additional species were described later, *Promicromonospora enterophila* (Jáger et al., 1983), *Promicromonospora sukumoe* (Takahashi et al., 1987), *Promicromonospora pachnodae* (Cazemier et al., 2003), *Promicromonospora aerolata* (Busse et al., 2003), *Promicromonospora vindobonensis* (Busse et al., 2003), and *Promicromonospora kroppenstedtii* (Alonso-Vega et al., 2008). Two of them (*Promicromonospora enterophila* and *Promicromonospora pachnodae*) have been reclassified as species of other genera (see *Taxonomic comments*). Members of the genus *Promicromonospora* are characterized by the development of mycelia which fragment into nonmotile, coccoid, Y- or V-shaped, rod-like, diphtheroid or chlamydospore-like elements. Aerial

TABLE 177. Differential characteristics of members of the family *Promicromonosporaceae*[a,b]

Characteristic	*Promicromonospora* species	*Xylanimicrobium pachnodae* DSM 12657^T	*Xylanibacterium ulmi* XIL08^T	*Xylanimonas cellulosilytica* XIL07^T	*Isoptericola* species	*Myceligenerans* species	*Cellulosimicrobium* species
Shape of cells	Short rods, V- or Y-shaped or coccoid	Irregular shaped, single or in pairs	Small rods	Coccoid	Short rods, V-shaped or coccoid	Irregular nonmotile rods and cocci in one species	Rods, short rods or coccoid
Mycelium	Mycelium	Mycelia-like fringes	–	–	Primary mycelium	Primary mycelium	Fragmenting primary mycelium
Aerial hyphae	+	–	–	–	–	–	–
Fermentation	Rarely	+	+	w	+	w or –	+
Peptidoglycan structure	L-Lys←L-Ala←D-Glu	L-Lys←L-Ser←D-Glu	L-Lys←L-Ala←D-Glu	L-Lys←D-Asp	L-Lys←D-Asp or L-Lys←D-Glu	L-Lys←L-Thr←D-Glu	L-Lys←D-Ser←D-Asp
Cell-wall sugars	Rha, Gal, Glc or Glc, Gal, Xyl	Rha, Gal, Glc	Rha, Fuc, Man, Gal, Ara, Glc	Gal, Rha	Rha, Gal, Glc or Rha, Rib, Glu may occur	Glu, Gal, Man	Gal, Rha, Fuc, or Gal, Rib
Phospholipid composition	PG, DPG, 2 GLs, PGLs, PL or PG, DPG, PI	PG, DPG, PI, PLs	PG, DPG, PI, PIM	PG, DPG, PI, PIM	PG, DPG, PI, 2 PLs, or PG, DPG, PLs, PIM may occur	PG, DPG, or PG, DPG, PI, PL, GL	nd
Major menaquinones	MK-9(H_4)	MK-9(H_4)	MK-9(H_4), MK-8(H_4)	MK-9(H_4), MK-8(H_4)	MK-9(H_4), or MK-9(H_2), MK-9	MK-9(H_8), MK-9(H_4), MK-9(H_6) and MK-9(H_2), or MK-9(H_4) and MK-9(H_6)	MK-9(H_4), MK-9(H_2), MK-8(H_4), MK-7(H_4)
Predominant fatty acids	$C_{15:0}$ anteiso	$C_{15:0}$ anteiso, $C_{15:0}$ iso, $C_{16:0}$, $C_{14:0}$	$C_{15:0}$ anteiso, $C_{15:0}$ iso, $C_{16:0}$, $C_{14:0}$	$C_{15:0}$ anteiso, $C_{15:0}$ iso	$C_{15:0}$ anteiso, $C_{15:0}$ iso, $C_{16:0}$, $C_{14:0}$, $C_{16:0}$ iso, $C_{17:0}$ anteiso, $C_{14:0}$ iso may occur	$C_{15:0}$ anteiso, $C_{15:0}$ iso, $C_{16:0}$ iso	$C_{15:0}$ anteiso, $C_{16:0}$ iso, $C_{16:0}$, $C_{15:0}$ iso

[a]Abbreviations and symbols: Ara, arabinose; Gal, galactose; Rha, rhamnose; Fuc, fucose; Man, mannose; Glc, glucose; Xyl, xylose; PG, phosphatidylglycerol; DPG, diphosphatidylglycerol; PI, phosphatidylinositol; PIM, phosphatidylinositol mannosides; PL, unknown phospholipid; PGL, unknown phosphoglycolipid; GL, unknown glycolipid; –, negative; w, weak; +, positive; nd, not determined.

[b]Data from Rivas et al. (2003), Rivas et al. (2004), Busse et al. (2003), Stackebrandt and Schumann (2004), Cui et al. (2004), Stackebrandt et al. (2004), Zhang et al. (2005), Groth et al. (2005), Groth et al. (2006) and Yoon et al. (2006).

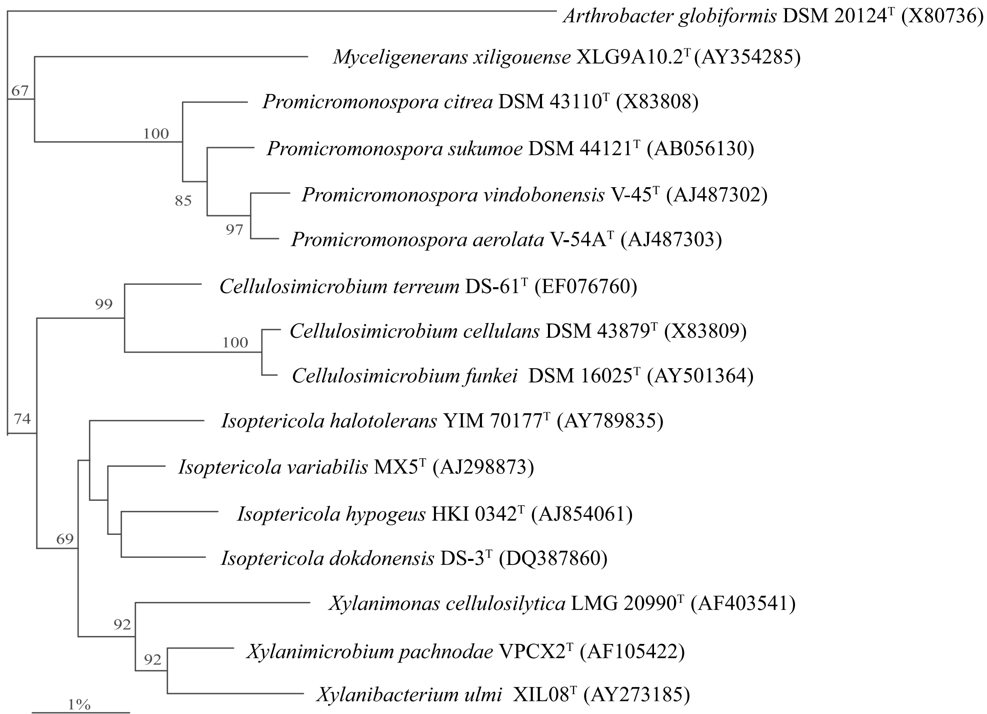

FIGURE 202. Distance matrix dendrogram of 16S rRNA gene sequence relationship (DeSoete, 1983), displaying the relatedness of members of the family *Promicromonosporaceae*. Bar = 1 inferred nucleotide change per 100 substitutions. Numbers at branch points are bootstrap values (500 resamplings) (Felsenstein, 1985). The sequence of *Arthrobacter globiformis* served as the root for the dendrogram.

hyphae are produced after 2–4 d and are straight to curved and only sparsely branched or not branched at all. The length of hyphae, the extent of branching, and the tendency to fragmentation depend on the media and cultivation conditions but are also strain-specific. The aerial hyphae are usually sparse and short, and only a few strains produce distinct aerial mycelium.

Fragmentation may begin after 8 h of cultivation and occurs preferably in shaken liquid cultures of complex media but to a lesser extent on mineral salt agar media. Spore-shaped elements, observed for *Promicromonospora citrea* in solid surface cultures, were often located at the end of short side branches (Krasil'nikov et al., 1961). Chlamydospore-like forms were observed for *Promicromonospora kroppenstedtii* (Alonso-Vega et al., 2008). The formation of monospores by *Promicromonospora citrea* could not be confirmed by Lechevalier and Lechevalier (1981) and spores were not reported for *Promicromonospora aerolata*, *Promicromonospora sukumoe*, and *Promicromonospora vindobonensis* (Busse et al., 2003; Takahashi et al., 1987).

Ambiguous data about the peptidoglycan structure of members of the genus *Promicromonospora* have been reported in the literature. Evtushenko et al. (1984) reported the peptidoglycan type A4α Lys←Ala←Glu, whereas Stackebrandt et al. (1983) found a molar ratio of amino acids which suggested the type A3α Lys←Ala←Ala for *Promicromonospora citrea*. Busse et al. (2003) proposed the peptidoglycan type A3α Lys←Gly←Ala for *Promicromonospora aerolata* and *Promicromonospora vindobonensis*, and only the occurrence of lysine and the absence of glycine and aspartic acid were reported for *Promicromonospora sukumoe* (Takahashi et al., 1987). The reinvestigation of the peptidoglycan structure of all present members of the family *Promicromonosporaceae* by supplementing the methods of Schleifer (1985) with gas-chromatographic and mass spectrometric techniques (MacKenzie, 1984, 1987) revealed that all members display the peptidoglycan type A4α and that the type strains of *Promicromonospora citrea*, *Promicromonospora aerolata*, *Promicromonospora sukumoe*, and *Promicromonospora vindobonensis* all display the peptidoglycan type A4α L-Lys←L-Ala←D-Glu (Schumann et al., 2004). Analytical data reported for *Promicromonospora kroppenstedtii* (Alonso-Vega et al., 2008) suggest the occurrence of the same peptidoglycan structure.

Colonies vary from yellow to white in color and are usually concave to wrinkled, but some strains may also have smooth and pasty colonies.

Strains of the genus *Promicromonospora* grow under aerobic conditions at 28–30°C on organic medium 79 (Prauser and Falta, 1968; 10 g of glucose, 10 g of Bacto peptone, 2 g of Casamino acids, 2 g of yeast extract, 6 g of NaCl, 15 g of agar, and 1000 ml of tap water, pH 7.5), Czapek peptone agar (30.00 g of sucrose, 3.00 g of $NaNO_3$, 1.00 g of K_2HPO_4, 0.50 g of $MgSO_4 \cdot 7H_2O$, 0.50 g of KCl, 0.01 g of $FeSO_4 \cdot 7H_2O$, 2.00 g of yeast extract, 5.00 g of peptone, 15.00 g of agar, and 1000 ml of distilled water, pH 7.3), trypticase soy yeast extract medium (30.0 g of trypticase soy broth, 3.0 g of yeast extract, 15.0 g of agar, and 1000 ml of distilled water pH 7.0–7.2), or yeast starch agar (15.0 g of soluble starch, 4.0 g of yeast extract, 0.5 g of K_2HPO_4, 0.5 g of $MgSO_4 \cdot 7H_2O$, 15.0 g of agar, and 1000 ml of distilled water, pH 7.4).

Four phages are available which cause true lysis and/or clearing effects on strains of *Promicromonospora citrea* and *Promicromonospora sukumoe* (Stackebrandt and Prauser, 1991): P1 (=DSM 49141), P2 (=DSM 49106), P3 (=DSM 49107), and P4 (=DSM 49108). *Promicromonospora citrea* strains are not susceptible to any other phages, including those specific for *Oerskovia* species, nocardioforms, and sporoactinomycetes. None of the *Promicromonospora* phages were effective against strains of other taxa investigated at that time (Prauser, 1984; Prauser and Falta, 1968). The phage susceptibility of *Promicromonospora* species other than *Promicromonospora citrea* and *Promicromonospora sukumoe* have not been examined yet.

Members of the genus *Promicromonospora* were mainly isolated from soils or from air and were considered nonpathogenic. The susceptibility of *Promicromonospora citrea* and *Promicromonospora sukumoe* to antimicrobial agents was reported by Takahashi et al. (1987).

Promicromonospora citrea, *Promicromonospora aerolata*, *Promicromonospora sukumoe*, *Promicromonospora vindobonensis*, and *Promicromonospora kroppenstedtii* share approximately 99% 16S rRNA gene sequence similarity (Alonso-Vega et al., 2008; Busse et al., 2003). The ranges of DNA–DNA similarity values between *Promicromonospora vindobonensis* and *Promicromonospora citrea*, *Promicromonospora aerolata*, and *Promicromonospora sukumoe* are below 55%, while those between *Promicromonospora aerolata* and *Promicromonospora citrea*, *Promicromonospora vindobonensis*, and *Promicromonospora sukumoe* are below 48% (Busse et al., 2003). Species of the genus *Promicromonospora* cannot be differentiated unambiguously by chemotaxonomic characteristics such as major menaquinone, cellular fatty acid profile, polar lipids (Busse et al., 2003), and peptidoglycan structure (Schumann et al., 2004). *Promicromonospora sukumoe* differed from the other species in having a lower concentration of major polyamines (Busse et al., 2003). *Promicromonospora sukumoe* is the only member of the genus for which the production of an antibiotic (7-hydro-8-methylpteroylglutamylglutamic acid; Murata et al., 1987) was reported. The type strains of all five *Promicromonospora* species can clearly be differentiated by their metabolic profiles (Alonso-Vega, et al., 2008; Busse et al., 2003; see Table 178). Owing to the application of different test methods and non-standardized criteria for evaluation of results, some of the reported physiological data differ between laboratories (Alonso-Vega et al., 2008; Busse et al., 2003; Kalakoutskii et al., 1986; Takahashi et al., 1987).

Enrichment and isolation procedures

The strains of *Promicromonospora citrea*, *Promicromonospora sukumoe*, and *Promicromonospora kroppenstedtii* were isolated from soils or aluminum hydroxide gel antacid (Alonso-Vega et al., 2008; Lechevalier, 1972; Takahashi et al., 1987). Among the various isolation media were several kinds of soil extract agar, oatmeal agar, and peptone-corn extract agar (Kalakoutskii et al., 1986). The growth of contaminating bacilli and hyphal fungi can be successfully suppressed by supplementing the isolation media with nalidixic acid (20–50 mg/l) and cycloheximide (50 mg/l) since promicromonosporae are not susceptible to these compounds. The use of double-layer agar plates is recommended for isolation (Stackebrandt and Prauser, 1991). *Promicromonospora aerolata* and *Promicromonospora vindobonensis* were isolated as airborne bacteria using an air-sampler and subcultivation on PYES agar (Zlamala et al., 2002).

Maintenance procedures

For preservation, cells are serially transferred at 3-month intervals, maintained at 4°C, and stored as 20% (w/v) glycerol suspensions at −20°C and at −80°C. Long-term preservation methods are freeze drying in skim milk and maintenance in liquid nitrogen at −196°C.

Procedures for testing special characters

Since the application of other methods for determination of the peptidoglycan structure of members of the genus *Promicromonospora* lead in some cases to unambiguous results, the procedure of Schleifer (1985) in combination with methods for quantitative amino-acid analysis (e.g. the method of MacKenzie (1987) is recommended.

Differentiation of the genus *Promicromonospora* from other genera

Authentic members of the genus *Promicromonospora* represent a distinct cluster within the phylogenetic tree of the family *Promicromonosporaceae* (Figure 203). The genus *Promicromonospora* can be differentiated from all other genera of its family except *Xylanibacterium* by the occurrence of the peptidoglycan type A4α, variation A4α L-Lys←L-Ala←D-Glu (see the family *Promicromonosporaceae*, above). *Promicromonospora* strains show a nocardioform life cycle and differ from the type strain of *Xylanibacterium* by occurring invariably as small rods that have catalase activity and lack phosphatidylinositol mannosides.

Taxonomic comments

The family affiliation of the nocardioform actinomycete genus *Promicromonospora* has caused problems during the pre-molecular era of bacterial systematics, and the genus has been regarded as "in search of a family" (Lechevalier and Lechevalier, 1981). The genus *Promicromonospora* was considered a relative of the genera *Cellulomonas* and *Oerskovia* because of common chemotaxonomic characteristics such as menaquinone MK-9(H_4), iso/anteiso branched chain fatty acids, and similarity with *Oerskovia* spp. in the occurrence of lysine in the peptidoglycan and a nocardioform life cycle (Prauser, 1986). As a consequence of a phylogenetic study (Stackebrandt et al., 1983), the genus *Promicromonospora* was united with these two genera and the genus *Jonesia* in the family *Cellulomonadaceae* (Stackebrandt and Prauser, 1991). Later, the genus *Jonesia* was excluded from the family *Cellulomonadaceae* on the basis of obvious differences in chemotaxonomic characteristics and 16S rRNA gene sequence data (Rainey et al., 1995), and in the present volume it is classified in its own family, *Jonesiaceae*. The occurrence of a characteristic set of 16S rRNA signature nucleotides revealed that the genus *Promicromonospora* represents a family of its own, the family *Promicromonosporaceae* (Stackebrandt et al., 1997). Whereas the species *Promicromonospora citrea*, *Promicromonospora aerolata*, *Promicromonospora kroppenstedtii*, *Promicromonospora sukumoe*, and *Promicromonospora vindobonensis* form a well distinguished cluster within the phylogenetic tree of the family *Promicromonosporaceae* (Figure 203), the species *Promicromonospora enterophila* and

TABLE 178. Differentiating biochemical characteristics of *Promicromonospora* species[a,b]

Characteristic	*P. citrea* NBRC 12397^T	*P. aerolata* V54A^T	*P. kroppenstedtii* RS16^T	*P. sukumoe* NBRC 14650^T	*P. vindobonensis* V45^T
API Coryne tests:					
Nitrate reductase	–	–	+	+	+
Pyrrolidonyl arylamidase	(+)	+	–	(+)	+
Urease	(+)	–	–	+	–
Alkaline phosphatase	–	–	+	–	–
Pyrazinamidase	+	+	–	+	+
α-Glucosidase	–	–	+	–	–
β-Galactosidase	–	–	+	–	–
N-Acetyl-β-glucosaminidase	–	–	+	–	–
Fermentation of:					
Glucose	–	+	+	–	–
Maltose	–	+	–	–	–
Sucrose	–	+	(+)	–	–
Glycogen	–	+	–	–	–
Xylose	–	–	+	–	–
Acid production from (API 50CH):					
Glycerol, methyl α-glucoside, salicin, sucrose, arbutin, maltose, turanose, amygdalin, melezitose, D-mannose, galactose, D-glucose, trehalose, and glycogen	–	+	nd	–	–
D-Fructose	–	+	nd	–	+
Cellobiose	–	+	nd	+	–
Mannitol, *N*-acetylglucosamine	–	(+)	nd	–	–
Assimilation of:[c]					
Acetate	+	(+)	nd	+	+
D-Fructose	+	+	–	+	+
D-Glucose, D-xylose	+	–	+	+	+
D-Maltose	+	–	–	+	+
D-Ribose, sucrose, and D-sorbitol	–	–	+	+	(+)
D-Mannose, salicin	–	–	–	+	(+)
L-Arabinose	+	–	+	+	–
L-Aspartate, L-histidine	+	–	nd	+	–
Pyruvate	(+)	(+)	nd	+	(+)
L-Proline	(+)	(+)	nd	+	+
DL-3-Hydroxybutyrate, DL-lactate, and L-malate	+	(+)	nd	+	(+)
Fumarate	+	–	nd	+	(+)
L-Alanine	(+)	–	nd	+	–
L-Rhamnose	+	(+)	+	–	(+)
Glutarate	–	(+)	nd	–	(+)
D-Melibiose, *cis*-aconitate, and citrate	–	–	nd	+	–
Propionate	–	–	nd	–	+
Hydrolysis of:[c]					
pNP phenylphosphonate, L-proline pNA, L-alanine pNA, pNP β-D-xylopyranoside, and pNP β-D-glucopyranoside	+	–	nd	+	+
Esculin	+	–	nd	+	(+)
bis-pNP phosphate	+	–	nd	+	–
pNP β-D-galactopyranoside	(+)	–	nd	(+)	–

[a]Symbols and abbreviations: +, Positive; (+), weakly positive; –, negative; nd, no data available; pNA, *p*-nitroanilide; pNP, *p*-nitrophenyl. All strains are positive or weakly positive for esculin (β-glucosidase), gelatinase, and catalase (API Coryne) and assimilation of *N*-acetyl-D-glucosamine, D-trehalose, and D-cellobiose. All strains are negative for β-glucuronidase, fermentation of ribose, mannitol, and lactose and production of acid (no data available for *Promicromonospora kroppenstedtii*) from erythritol, D-arabinose, L-arabinose, ribose, xylose, adonitol, methyl-β-xyloside, sorbose, rhamnose, dulcitol, inositol, sorbitol, methyl-α-mannoside, lactose, melibiose, inulin, raffinose, starch, xylitol, gentiobiose, lyxose, tagatose, fucose, arabitol, gluconate, 2-ketogluconate, and 5-ketogluconate (API 50CH).

[b]Data from Busse et al. (2003); data for *Promicromonospora kroppenstedtii* from Alonso-Vega et al. (2008).

[c]For *Promicromonospora citrea*, *Promicromonospora sukumoe*, *Promicromonospora vindobonensis*, and *Promicromonospora aerolata* determined according to Kämpfer et al. (1991); for *Promicromonospora kroppenstedtii* determined by using API 50 CH.

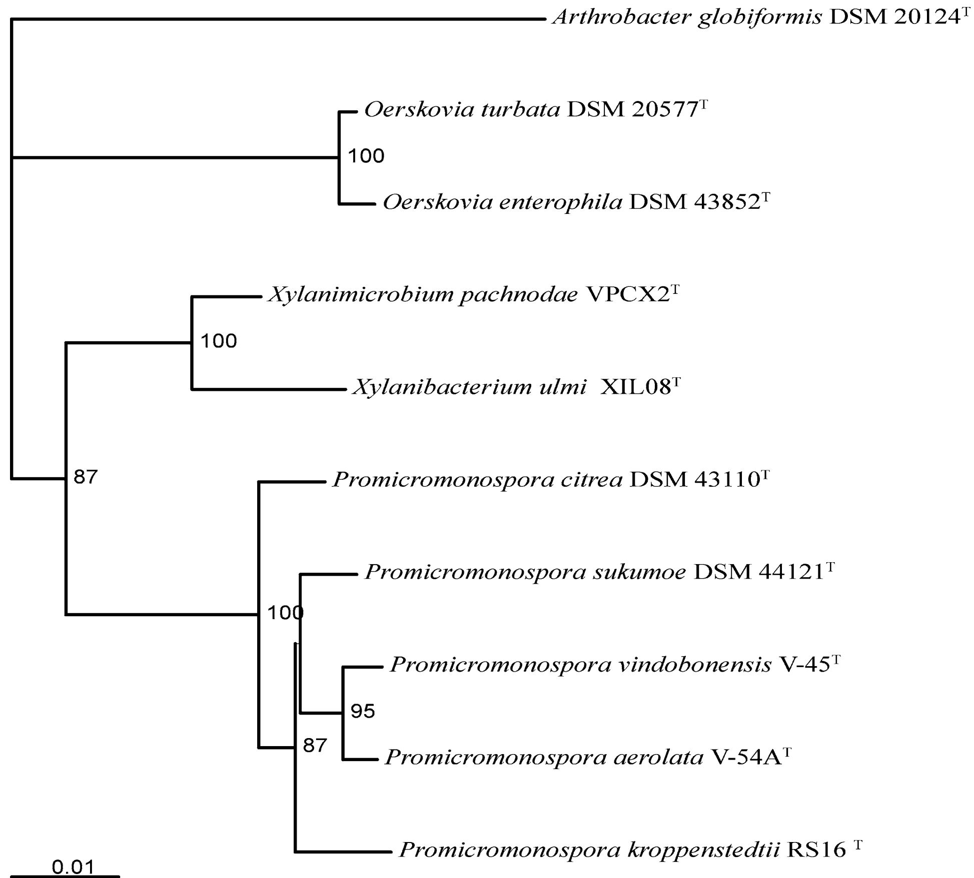

FIGURE 203. 16S rRNA gene sequence neighbor joining tree (Felsenstein, 1993) of *Promicromonospora citrea*, *Promicromonospora aerolata*, *Promicromonospora kroppenstedtii*, *Promicromonospora sukumoe*, *Promicromonospora vindobonensis*, *Oerskovia* (*Promicromonospora*) *enterophila*, *Xylanimicrobium* (*Promicromonospora*) *pachnodae*, and related species, represented by their type strains. Bootstrap values (>80%) of 1000 resamplings (Felsenstein, 1985) are indicated at nodes. *Arthrobacter globiformis* served as the root. Bar = 1 nt substitution per 100 nt.

Promicromonospora pachnodae (shown as *Oerskovia enterophila* and *Xylanimicrobium pachnodae*, respectively) represent separate phylogenetic lineages (Figure 203). The species *Promicromonospora enterophila* (Jáger et al., 1983) was already considered a species *incertae sedis* by Kalakoutskii et al. (1986) because of its susceptibility to *Oerskovia*- rather than to *Promicromonospora*-specific phages, occurrence of threonine in the peptidoglycan, lack of aerial mycelium, motility of two strains, and colony appearance (Prauser, 1986). On the basis of phylogenetic and chemotaxonomic evidence, *Promicromonospora enterophila* was reclassified as member of the emended genus *Oerskovia*, as *Oerskovia enterophila* (Stackebrandt et al., 2002).

The species *Promicromonospora pachnodae* (Cazemier et al., 2003) did not develop aerial hyphae, showed only 95.8–96.7% 16S rRNA gene sequence similarity (calculated by using the EzTaxon server; Chun et al., 2007) to authentic species of the genus *Promicromonospora*, and differed from them in its peptidoglycan type A4α, variation L-Lys←L-Ser←D-Glu. Since *Promicromonospora pachnodae* differed from its closest phylogenetic neighbor *Xylanibacterium ulmi* (98.5% 16S rRNA gene sequence similarity) in the composition of the peptidoglycan and in additional chemotaxonomic characteristics as well as in morphology, the new genus *Xylanimicrobium* was established and *Promicromonospora pachnodae* was reclassified as *Xylanimicrobium pachnodae* (Stackebrandt and Schumann, 2004).

Further reading

Stackebrandt, E., P. Schumann and H. Prauser. 2006. The family *Cellulomonadaceae*. *In* The Prokaryotes: a Handbook on the Biology of Bacteria, 3rd edn, vol. 3, *Archaea*, *Bacteria*, *Firmicutes*, Actinomycetes (edited by Dworkin, Falkow, Rosenberg, Schleifer and Stackebrandt). Springer, New York, pp. 983–1001.

List of species of the genus *Promicromonospora*

1. **Promicromonospora citrea** Krasil'nikov, Kalakoutskii and Kirillova 1961, 107[AL]

ci'tre.a. L. fem. adj. *citrea* of or pertaining to the citron-tree; intended to mean lemon-yellow.

In addition to the properties given in the genus description and in Table 178, this species has the following characteristics. Life cycle nocardioform; primary and aerial hyphae fragmenting. Colors of colonies on oatmeal agar and inorganic salts-starch agar citron-yellow or white to whitish; on peptone-corn extract agar citron-yellow or cream; on the latter media in exceptional cases brown to orange-brown.

Aerial hyphae are only microscopically visible in some strains, but well developed in others; in the latter case these are thin, white, and chalky.

Optimal temperature 28°C; growth occurs between 6°C and 42°C. The following features were found to be of value in presumptive differentiation of *Promicromonospora citrea* from related strains: utilization of malate, malonate, and succinate; production of acid from rhamnose and raffinose, and absence of clearing zones on yeast-peptone agar with $CaCO_3$.

Susceptible to taxon-specific phages.

Source: mainly from soil, also found on aluminum hydroxide gel antacid.

DNA G+C content (mol%): >70 (T_m; strain VKM Ac 665^T); 73.6 (Evtushenko et al., 1984).

Type strain: ATCC 15908, DSM 43110, NBRC 12397, INMI 18, JCM 3051, KCC A 0051, LMG 4076, NRRL B-3485, RIA 562, VKM Ac-665.

Sequence accession no. (16S rRNA gene): X83808.

2. **Promicromonospora aerolata** Busse, Zlamala, Buczolits, Lubitz, Kämpfer and Takeuchi 2003, 1505[VP]

a.e.ro.la'ta. Gr. n. *aer aeros* air; L. part. adj. *latus, -a, -um* carried; N.L. part. fem. adj. *aerolata* airborne.

In addition to the properties given in the genus description and in Table 178, this species has the following characteristics. Cells show branching hyphae that are 0.3–0.5 µm in diameter after 18 h of growth on PY agar. After 3 d of growth, the mycelium fragments into nonmotile, Y- or V-shaped, rod-like, coccoid elements, 0.3–0.5 × 0.6–1.5 µm. No sessile, oval spores, chlamydospore-like or other spore-like elements are observed. Colonies are light yellow, translucent, and reach approx. 1 mm in diameter. Cell-wall sugars are glucose, galactose, and rhamnose. Diphosphatidylglycerol, phosphatidylglycerol, two unknown glycolipids, two unknown phosphoglycolipids, and one unknown phospholipid are present in the polar lipid profile. $C_{16:0}$ and $C_{17:0}$ anteiso are present in moderate amounts. Spermidine is the predominant cellular polyamine and spermine is present in moderate amounts.

Source: air in the "Virgilkapelle" chapel in Vienna.

DNA G+C content (mol%): 70 (HPLC).

Type strain: V54A, NBRC 16526, CCM 7043, DSM 15943, JCM 14119.

Sequence accession no. (16S rRNA gene): AJ487303.

3. **Promicromonospora kroppenstedtii** Alonso-Vega, Santamaría, Martínez-Molina and Trujillo 2008, 1480[VP]

krop.pen.stedt'i.i. N.L. gen. masc. n. *kroppenstedtii* of Kroppenstedt, named in honor of Reiner M. Kroppenstedt for his valuable contribution to the chemotaxonomy and systematics of actinobacteria.

Gram-stain-positive, aerobic, chemo-organotrophic actinobacterium. Colonies are cream to yellow in ISP 2, Bennett's, and nutrient agar. Well developed branched septate hyphae that break into nonmotile, V and Y forms. Chlamydospores observed in ISP 2 broth. Aerial mycelium or diffusible pigments are not observed. Temperature growth range 12–37°C with an optimum at 28°C. Oxidase- and catalase-positive. NaCl tolerance up to 8%. Other physiological characteristics are described in Table 178. The peptidoglycan contains alanine, glutamic acid, and lysine. Cell-wall sugars galactose and rhamnose are present. The main menaquinone component is MK-9(H_4), in addition RS16^T also contains MK-8(H_4). The polar lipid pattern includes phosphatidylglycerol, diphosphatidylglycerol, and phosphatidylinositol. Major fatty acids are $C_{15:0}$, $C_{15:0}$ iso, and $C_{16:0}$ iso.

Source: sandy soil.

DNA G+C content (mol%): 70.8 (HPLC).

Type strain: RS16, DSM 19349, LMG 24382.

Sequence accession no. (16S rRNA gene): AM709608.

4. **Promicromonospora sukumoe** Takahashi, Tanaka, Iwai and Ōmura 1988, 449[VP] (Effective publication: Takahashi, Tanaka, Iwai and Ōmura 1987, 512.)

su.ku'mo.e. N.L. fem. adj. *sukumoe* (*sic*) of or belonging to Sukumo City, Kochi Prefecture in Japan, where the soil sample was collected from which strain SK-2049^T was isolated.

In addition to the properties given in the genus description and in Table 177, this species has the following characteristics. Grows well on synthetic and organic agar media under aerobic conditions. Filamentous. The substrate mycelia fragment into bacillary and coccoid elements. Poor aerial mycelia without spores are produced on a few agar media. In general, the colonies are yellow in color; leathery on synthetic media, but bacteroid on organic ones. No growth occurs under anaerobic conditions.

The characteristic whole cell sugars are glucose, ribose, and rhamnose. Phospholipids are of the P I or P IV type. Mycolic acids are absent. The *N*-acylmuramic acid in the cell wall is of the acetyl type.

Soluble pigments and melanins are not produced. Nitrate reduction is positive. The range of growth temperature is 10–35°C.

Source: soil.

DNA G+C content (mol%): not available.

Type strain: SK-2049, JCM 6845, NBRC 14650, DSM 44121, JCM 6845, VKM Ac-1966.

Sequence accession no. (16S rRNA gene): AB023375.

4. **Promicromonospora vindobonensis** Busse, Zlamala, Buczolits, Lubitz, Kämpfer and Takeuchi 2003, 1505[VP]

vin.do.bo.nen'sis, L. fem. adj. *vindobonensis* of Vindobona, the Roman name for Vienna, where the type strain was isolated.

In addition to the properties given in the genus description and in Table 177, this species has the following characteristics. Cells show branching hyphae that are 0.3–0.5 µm in diameter after 18 h of growth on PY agar. After 3 d of growth,

the mycelium fragments into nonmotile, Y- or V -shaped, rod-like, coccoid elements, 0.3–0.5 × 0.6–1.5 μm. No sessile spores, oval spores, chlamydospore-like elements, or other spore-like elements are observed. On PY agar, colonies are whitish, translucent, convex, glistening, and approx. 1 mm in diameter with an entire edge. Cell-wall sugars are glucose, galactose, and rhamnose. Diphosphatidylglycerol, phosphatidylglycerol, two unknown glycolipids, three unknown phosphoglycolipids, one unknown phospholipid, and one unknown polar lipid are present in the polar lipid profile. $C_{16:0}$ and $C_{17:0}$ anteiso are present in moderate amounts. Spermidine is the predominant cellular polyamine and spermine is present in moderate amounts.

Source: air in the "Virgilkapelle" chapel in Vienna.

DNA G+C content (mol%): 70 (HPLC).

Type strain: V45, NBRC 16525, CCM 7044, DSM 15942, JCM 14120.

Sequence accession no. (16S rRNA gene): AJ487302.

Genus II. **Cellulosimicrobium** Schumann, Weiss and Stackebrandt 2001, 1009[VP] (emend. Brown, Steigerwalt, Morey, Daneshvar, Romero and McNeil 2006, emend. Yoon, Kang, Schumann and Oh 2007)

Peter Schumann and Erko Stackebrandt

Cel.lu.lo.si.mi.cro′bi.um. N.L. n. *cellulosum* cellulose; N.L. neut. n. *microbium*, microbe; N.L. neut. n. *Cellulosimicrobium* cellulose microbe.

Gram-stain-positive, but cells are very readily decolorized. In young cultures, a substrate mycelium is produced which fragments later into irregular, curved, and club-shaped rods which may be arranged in V forms. After exhaustion of the medium, the rods are transformed into shorter rods or even spherical cells. Do not form endospores. Not acid-fast. Motile or nonmotile. Chemo-organotrophic; metabolism respiratory and facultatively anaerobic. Acid produced from several carbohydrates. Catalase positive. Nitrate reduction is positive or negative. **Cellulolytic**. **Peptidoglycan contains L-lysine; the interpeptide bridge consists of D-Ser–D-Asp or L-Thr–D-Asp, type A4α. Major menaquinone is MK-9(H_4), and predominating fatty acids are $C_{15:0}$ anteiso, $C_{15:0}$ iso, $C_{16:0}$ iso, and $C_{17:0}$ anteiso.** Whole-cell sugars are galactose, rhamnose, glucose, fucose, and mannose or galactose and ribose; one type strain contains only galactose. Phylogenetically, a member of the family *Promicromonosporaceae.*

DNA G+C content (mol%): 72.9–76.5 (T_m).

Type species: **Cellulomicrobium cellulans** (Metcalfe and Brown 1957) Schumann, Weiss and Stackebrandt, 2001, 1009[VP] (*Nocardia cellulans* Metcalfe and Brown 1957).

The description is based on the description of the species *Nocardia cellulans* DSM 43879[T] (Schumann et al., 2001; Stackebrandt et al., 1980), *Cellulomonas cartae* (Stackebrandt and Kandler, 1980), considered a subjective synonym of *Cellulomonas cellulans* (Stackebrandt and Keddie, 1986), basonym *Nocardia cellulans* and on the descriptions of *Cellulosimicrobium funkei* (Brown et al., 2006) and *Cellulosimicrobium terreum* (Yoon et al., 2007). The fatty acid composition has been determined by Funke et al. (1995) for *Oerskovia xanthineolytica* CIP 81.28.

Further descriptive information

The observation that *Cellulomonas cellulans* clustered outside the genus *Cellulomonas* as revealed by phylogenetic comparison of 16S rRNA gene sequences of the current members of the order *Micrococcales* and the detection of its peptidoglycan type A4α (instead of type A4β as in *Cellulomonas* species), led to the proposal of the new genus *Cellulosimicrobium* and reclassification of *Cellulomonas cellulans* as *Cellulosimicrobium cellulans* (Schumann et al., 2001). The closest phylogenetic neighbors of *Cellulosimicrobium cellulans* are strains of *Cellulosimicrobium funkei* (Brown et al., 2006) sharing 99.5–99.8% 16S rRNA gene sequence similarities. The intraspecies relatedness of strains of *Cellulosimicrobium funkei*, determined by the hydroxyapatite method of DNA–DNA reassociation, ranges between 76% and 79%; the gene sequence similarities of these strains range between 99.9% and 100%. The type strains of *Cellulosimicrobium cellulans* and *Cellulosimicrobium funkei* share 47% DNA–DNA similarity (Brown et al., 2006).

Phylogenetic neighbors of *Cellulosimicrobium* are the genera *Isoptericola*, *Xylanimonas*, *Xylanimicrobium*, and *Xylanibacterium* of the family *Promicromonosporaceae* (see Figure 202 in the chapter on *Xylanimicrobium*). Members of the genus *Cellulosimicrobium* form a primary mycelium which fragments into irregular, curved, and club-shaped rods. Rods are transformed into shorter rods or even spherical cells after exhaustion of the medium. Cells of *Cellulosimicrobium funkei* are motile by one to five polar and/or lateral flagella (Sottnek et al., 1977), whereas *Cellulosimicrobium cellulans* is nonmotile (Schumann et al., 2001). The peptidoglycan of members of the genus *Cellulosimicrobium* is of the type A4α, variation L-Lys←D-Ser←D-Asp (A11.37 according to http://www.peptidoglycan-types.info). *Cellulosimicrobium cellulans* contains galactose, rhamnose, glucose, fucose, and mannose (Stackebrandt et al., 2004) whereas galactose is the only whole-cell sugar in *Cellulosimicrobium funkei* (Brown et al., 2006). On peptone-yeast extract-glucose agar, the colonies of *Cellulosimicrobium cellulans* are circular, convex, yellow-whitish, and glistening with entire edges. On other media, colonies of *Cellulosimicrobium cellulans* are cream in color (Metcalfe and Brown, 1957). On trypticase soy agar (BBL) and heart infusion agar (Difco), colonies of *Cellulosimicrobium funkei* have substrate hyphae and vary from 1 mm to 2 mm in diameter after 24 h. Young colonies were filamentous with distinct centers, and mature colonies develop dense centers with a filamentous fringe that is so short that it is barely visible. No aerial mycelia occur and a pale yellow, nonsoluble pigment is formed (Sottnek et al., 1977). *Cellulosimicrobium cellulans* DSM 43879[T] and *Cellulosimicrobium terreum* grow at 25–28°C on medium No. 65 (www.dsmz.de) containing 4 g of glucose, 4 g of yeast extract, 10 g of malt extract, 2 g of $CaCO_3$, and 12 g of agar per 1000 ml of distilled water (pH 7.2). Cells grow well on nutrient agar

(Difco) or tryptic soy agar (Difco). *Cellulosimicrobium funkei* W6122^T is cultivated at 35°C on heart infusion agar and trypticase soy agar.

The organisms are chemo-organotrophic with aerobic and facultatively anaerobic metabolism. Nitrate is reduced to nitrite by *Cellulosimicrobium cellulans* (Metcalfe and Brown, 1957) but not by *Cellulosimicrobium funkei* (Sottnek et al., 1977). *Cellulosimicrobium* (*Nocardia*) *cellulans* is reported to fix atmospheric nitrogen and to decompose cellulose (Metcalfe and Brown, 1957). However, serial transfer on media such as yeast-extract peptone results in a very rapid loss of fixing ability and of the ability to decompose cellulose. Passage through sterile soil may restore these properties occasionally. The organism survives and retains both properties over a period of years in damp or air-dry sterile soil (Metcalfe and Brown, 1957).

Strains of *Cellulosimicrobium cellulans* are hosts of the actinophages O5 (=DSM 49112; host strain DSM 43881; Stackebrandt and Prauser, 1991) and O13 (=DSM 49139; host strain DSM 43879^T; Prauser, 1986).

All hitherto known strains of *Cellulosimicrobium funkei* and the type strain of *Cellulosimicrobium cellulans* are resistant to amikacin (MIC ≥64 μg/ml) and ciprofloxacin (MIC ≥4 μg/ml). All strains of *Cellulosimicrobium funkei* are sensitive to imipenem (MIC ≥16 μg/ml). *Cellulosimicrobium cellulans* ATCC 51964^T is resistant to this antibiotic. The type strain of *Cellulosimicrobium cellulans* is sensitive to trimethoprim-sulfamethoxazole (MIC ≥4–76 μg/ml), whereas the strains of *Cellulosimicrobium funkei* are resistant to it (Brown et al., 2006).

Strains of *Cellulosimicrobium cellulans* were isolated from fetal tissues or placentas from cases of equine abortion, premature birth, and term pregnancies of horses, suggesting the causative role of these bacteria (Bolin et al., 2004). *Cellulosimicrobium cellulans* was discussed as etiological agent of chronic tongue ulcerations in an immunocompromised patient (Heym et al., 2005). The strains of *Cellulosimicrobium funkei* are isolates from human specimens sent to the Special Bacteriology Section and the Actinomycete Reference Laboratory at the Center for Disease Control (Atlanta, Georgia, USA) between 1957 and 1977 and classified earlier as strains of *Oerskovia turbata* (McNeil et al., 2004). The type strain W6122^T and strain W4083 were isolated from blood from patients in Colorado and California, respectively, and the lung isolate W2796 and strain W6123 originate from patients in New York. Although the isolation sites were suggestive of invasive infections, the clinical significance of these strains could not be elucidated since no satisfactory case histories were available. The only strain of *Cellulosimicrobium terreum* was isolated from a Korean soil sample (Yoon et al., 2007).

Enrichment and isolation procedures

For isolation of *Cellulosimicrobium* (*Nocardia*) *cellulans* DSM 43879^T the following method was used (Metcalfe and Brown, 1957): Silica gel was impregnated with basal medium (2.5 g of KNO_3; 1.0 g of K_2HPO_4; 0.1 g of $CaCl_2$; 0.3 g of $MgSO_4 \cdot 7H_2O$; 0.1 g of NaCl; 0.01 g of $FeCl_3$, 1000 ml of distilled water; pH 7.0–7.2). Washed filter paper placed on the surface of the gel was seeded with minute grains of chalk grassland soil. After incubation at 25°C for 2–3 d, further plates were inoculated from the areas of decomposition around the grains. *Cellulosimicrobium* (*Nocardia*) *cellulans* was isolated as one of the most vigorous strains of mixed cultures by repeated plating on yeast-extract peptone agar. The type strain W6122^T of *Cellulosimicrobium funkei* was identical to the clinical isolates sent to the Center for Disease Control between 1972 and 1974 (Reller et al., 1975). Details of the isolation procedure were not reported. *Cellulosimicrobium terreum* was isolated by means of standard dilution plating techniques at 25°C on 10× diluted nutrient agar (Difco).

Maintenance procedures

For preservation, cells are serially transferred at 4-week intervals using cultivation conditions specified above, maintained at 4°C, and stored as 20% (w/v) glycerol suspensions at −20°C and at −80°C. Long-term preservation methods include freeze drying in skim milk and maintenance in liquid nitrogen at −196°C.

Differentiation of the genus *Cellulosimicrobium* from other genera

The genus *Cellulosimicrobium* shares with its closest phylogenetic neighbors *Isoptericola*, *Xylanimonas*, *Xylanimicrobium*, and *Xylanibacterium* the major menaquinone MK-9(H_4), the occurrence of mainly *iso*- and *anteiso*- branched cellular fatty acids, the ability to decompose polysaccharides and to grow as an aerobe or facultative anaerobe, as well as the occurrence of one or several cell-wall sugars. Members of the genus *Cellulosimicrobium* can be differentiated from all genera of the family *Promicromonosporaceae* by their peptidoglycan variation (L-Lys←D-Ser/L-Thr←D-Asp). With respect to morphological features, members of the genus *Cellulosimicrobium* differ from those of the genera *Isoptericola*, *Xylanimonas*, *Xylanimicrobium*, and *Xylanibacterium* by the development of primary mycelia which fragment later into irregular rods. Fucose as cell-wall sugar was detected only in *Cellulosimicrobium* and *Xylanibacterium* (see Table 177).

Taxonomic comments

Nocardia cellulans (Metcalfe and Brown, 1957) and *Oerskovia xanthineolytica* were proposed among other taxa as synonyms of *Cellulomonas cartae* (Stackebrandt and Kandler, 1980), a species with an illegitimate name since the specific epithet *cellulans* antedates the epithet *cartae* (Rules 38 and 42 of the Bacteriological Code; Lapage et al., 1992). As *Nocardia cellulans* was formally reclassified as *Cellulomonas cellulans* (Stackebrandt and Keddie, 1986), the nonmotile strains of *Nocardia cellulans* were combined with the motile strains of *Oerskovia xanthineolytica* into one and the same taxon. *Cellulomonas cellulans* embraces the additional organisms *Brevibacterium lyticum* (Takayama et al., 1960), *Brevibacterium fermentans* (Chatelain and Second, 1966), "*Arthrobacter luteus*" (Kaneko et al., 1969), "*Corynebacterium manihot*" (Collard, 1963), and *Cellulomonas cartae* (Stackebrandt et al., 1978; Stackebrandt and Kandler, 1980). These organisms were united because they share the peptidoglycan variation L-Lys←D-Ser←D-Asp (Seidl et al., 1980), similar menaquinone and fatty acid profiles, and show a close genetic relationship (Stackebrandt and Keddie, 1986). The description of several new genera between 1997 and 2000 provided new insight into the phylogenetic structure of the suborder *Micrococcineae* and revealed that *Cellulomonas cellulans* clustered outside the authentic genus *Cellulomonas*. This observation and the

TABLE 179. Phenotypic characteristics which differentiate *Cellulosimicrobium cellulans*, *Cellulosimicrobium funkei*, and *Cellulosimicrobium terreum*[a,b]

Characteristic	*C. cellulans* ATCC 12830[T]	*C. funkei* W6122[T]	*C. terreum* DS-61[T]
Peptidoglycan type	L-Lys←D-Ser←D-Asp	nd	L-Lys←L-Thr←D-Asp
Major cell-wall sugar	Rhamnose	Galactose	Galactose
Nitrate reduction	+	v(−)	+
Hydrolysis of or activity of:			
Gelatin	+	+	−
β-Galactosidase	+	+	−
β-Glucodidase	−	w	−
N-Acetyl-β-glusosaminidase	+	+	−
Acid production from:			
Melibiose	+	v(−)	−
L-Arabinose	−	−	nd
Resistance to ampicillin	+	+	−
Motility	v	+	nd

[a]Symbols and abbreviations: −, negative; (+), weak positive; +, positive; v, variable; w, weak; nd, no data available.

[b]Data from Schumann et al. (2001), Bakalidou et al. (2002), Stackebrandt et al. (2004), Yoon et al. (2007) and Brown et al. (2006).

[c]Data in parentheses are for the type strain.

TABLE 180. Percentage of cellular fatty acids of type strains of *Cellulosimicrobium cellulans*, *Cellulosimicrobium funkei*, *Cellulosimicrobium terreum*, and *Isoptericola variabilis* (*Cellulosimicrobium variabile*) as analyzed by using the Sherlock Microbial Identification System (MIDI) after cultivation on tryptic soy agar at 28°C for 24 h[a]

Type strain	$C_{14:0}$	$C_{14:0}$ iso	$C_{15:0}$	$C_{15:0}$ iso	$C_{15:0}$ anteiso	$C_{16:0}$	$C_{16:0}$ iso	$C_{17:0}$ iso	$C_{17:0}$ anteiso
C. cellulans DSM 43879[T]	4.6	2.8	1.1	16.5	35.7	6.9	12.1	4.5	14.8
C. funkei DSM 16025[T]	1.9	−	1.0	21.9	44.4	6.6	5.3	2.9	15.1
C. terreum DS-61[Tb]	3.3	4.0	1.3	14.5	52.5	5.5	8.4	0.8	7.8
I. variabilis (*C. variabile*) DSM 10177[Tc]	6.6	1.5	0.5	17.0	53.6	7.2	6.0	0.6	7.0

[a]Values below 0.5% were omitted.

[b]Data from Yoon et al. (2007).

[c]Data from Stackebrandt et al. (2004).

difference in peptidoglycan type between these and all other representatives of the genus *Cellulomonas* led to the proposal of the genus *Cellulosimicrobium* and reclassification of *Cellulomonas cellulans* as *Cellulosimicrobium cellulans* (Schumann et al., 2001). The second species *Cellulosimicrobium variabile* was described for a cellulolytic bacterium isolated from the hindgut of an Australian termite (Bakalidou et al., 2002). However, its phylogenetic distance to *Cellulosimicrobium cellulans* (see Figure 202 in the chapter on *Xylanimicrobium*), the re-examination of its peptidoglycan structure which revealed the variation L-Lys←D-Asp, and some additional differentiating phenotypic characteristics (Table 177, Table 179, and Table 180) led to the proposal of the genus *Isoptericola* and to the reclassification of *Cellulosimicrobium variabile* as *Isoptericola variabilis* (Stackebrandt et al., 2004). The inclusion of "nonmotile organism" in the definition of the genus *Cellulosimicrobium* by Schumann et al. (2001) did not account for motile strains of *Cellulomonas cellulans* earlier classified as *Oerskovia xanthineolytica*. Studies on motile clinical isolates have revealed recently that these organisms represent a new species of the genus *Cellulosimicrobium*, *Cellulosimicrobium funkei* (Brown et al., 2006). The genus *Cellulosimicrobium* (Schumann et al., 2001) was emended consequently to include motile organisms and strains that are unable to reduce nitrate (Brown et al., 2006).

Differentiation of the species of the genus *Cellulosimicrobium*

Characteristics that differentiate the species *Cellulosimicrobium cellulans* and *Cellulosimicrobium funkei* from one another and from related taxa are listed in Table 177, Table 179, and Table 180.

Further reading

Stackebrandt, E., P. Schumann and H. Prauser. 2002. The family *Cellulomonadaceae*. *In* The Prokaryotes: an Evolving Electronic Resource for the Microbiological Community, 3rd edn (edited by Dworkin, Falkow, Rosenberg, Schleifer and Stackebrandt). Springer, New York, p. 983.

Acknowledgements

The authors thank Jennifer Gregor and Marlen Jando (both of DSMZ) for technical assistance in chemotaxonomic analyses.

List of species of the genus *Cellulosimicrobium*

1. **Cellulosimicrobium cellulans** (Metcalfe and Brown 1957) Schumann, Weiss and Stackebrandt 2001, 1009[VP] (*Nocardia cellulans* Metcalfe and Brown 1957).

cel′lu.lans. N.L. part. adj. *cellulans* cell-making. Basonym *Nocardia cellulans*.

The following description is based on that of *Nocardia cellulans* DSM 43879[T] (Funke et al., 1995; Metcalfe and Brown, 1957; Schumann et al., 2001; Stackebrandt et al., 1980; Stackebrandt and Keddie, 1986) *Cellulomonas cartae* DSM 20106, "*Corynebacterium manihot*" DSM 20155, *Brevibacterium fermentans* CIP 6611, and *Brevibacterium lyticum* ATCC 15921 (Stackebrandt and Kandler, 1980; Stackebrandt et al., 1982), and that of *Oerskovia xanthineolytica* (Funke et al., 1995; Stackebrandt et al., 1980). In addition to the properties given in the genus description, this species has the following characteristics. A primary mycelium is produced which fragments later in the growth cycle. After exhaustion of the medium, the rods transform into shorter rods or even spherical cells. On peptone-yeast extract-glucose agar, colonies are circular, 0.9–5 mm in diameter, convex, yellow-whitish, and glistening, with entire edge. Catalase is produced. Nitrite is produced from nitrate. Aerobic and facultatively anaerobic. Alkaline phosphatase, esterase (C4), esterase lipase (C8), acid phosphatase, naphthol-AS-BI-phosphohydrolase, and α-glucosidase are present. Casein, gelatin, hypoxanthine, xanthine, and starch are hydrolyzed. Negative for hydrolysis of tyrosine, acid production from L-rahmnose, D-mannitol, inositol and D-sorbitol, utilization of L-malate, lipase (C14) leucine arylamidase, valine arylamidase, cystine arylamidase, trypsin, α-chymotrypsin, α-galactosidase, β-glucoronidase, α-mannosidase, and α-fucosidase (Yoon et al., 2007). Glucose, mannose, maltose, sucrose, D-xylose, L-arabinose, D-ribose, D-xylose, *m*-inositol, glycerol, cellobiose, lactose, sucrose, gluconate, mannitol, D-mannose, L-lactate, acetate, pyruvate, propionate, pentanoate, capronate, heptanoate, caprylate, glyoxylate, proline, asparagine, aspartate, and histidine but not raffinose, DL-malate, and D-lactate are used as sole carbon sources. Acid is produced from glucose, mannose, sucrose, D-xylose, L-arabinose, ribose, gluconate, glycerol, lactose, pyruvate, and cellobiose. Acetic acid is the main acidic intermediary product of aerobic glucose dissimilation. Resting cells ferment glucose anaerobically predominantly to CO_2, acetic acid, and L-lactic acid. Ethanol and formic acid are minor end products. The major cell-wall sugar is rhamnose, while fucose and galactose are minor components. The major menaquinone is MK-9(H_4); the minor menaquinones are MK-9(H_2), MK-8(H_4), and MK-7(H_4). The predominant fatty acids are $C_{15:0}$ anteiso, $C_{16:0}$ iso, $C_{16:0}$, and $C_{15:0}$ iso.

Source: the soil of chalk grassland.

DNA G+C content (mol%): 74 (T_m).

Type strain: ATCC 12830, CCUG 50776, CFBP 4267, CIP 103404, DSM 43879, HAMBI 95, NBRC 15516, JCM 9965, LMG 16221, NRRL B-2768, VKM Ac-1412.

Sequence accession no. (16S rRNA gene): X83809.

2. **Cellulosimicrobium funkei** Brown, Steigerwalt, Morey, Daneshvar, Romero and McNeil 2006, 804[VP]

fun′ke.i. N.L. gen. masc. n. *funkei* of Funke, to honor the contributions of Guido Funke, a distinguished microbiologist, who recognized the heterogeneity within the yellow-pigmented coryneform bacteria.

Cells are short (1 µm), thin, Gram-stain-positive and non-sporulating rods. Motile by polar and/or lateral flagella (Sottnek et al., 1977). Growth occurs at 35°C and 45°C, but not at 25°C. Pale-yellow pigment. Substrate hyphae are present. Catalase-positive. Esculin, adenine, casein, gelatin, hypoxanthine, urea, and xanthine are decomposed. Nitrate is not reduced to nitrite (Sottnek et al., 1977). Catalase is produced. Aerobic and facultatively anaerobic. Alkaline phosphatase, esterase (C4), esterase lipase (C8), acid phosphatase, naphthol-AS-BI-phosphohydrolase, and α-glucosidase are present. Casein, gelatin, hypoxanthine, xanthine, and starch are hydrolyzed. Negative for hydrolysis of tyrosine, acid production from L-rhamnose, D-mannitol, inositol and D-sorbitol, utilization of L-malate, lipase (C14) leucine arylamidase, valine arylamidase, cystine arylamidase, trypsin, α-chymotrypsin, α-galactosidase, β-glucoronidase, α-mannosidase, and α-fucosidase. Utilization of D-glucose, D-fructose, D-galactose, D-cellobiose, D-mannose, D-trehalose, D-xylose, L-arabinose, sucrose, maltose, and salicin (Yoon et al., 2007). Acid is produced from L-arabinose, cellobiose, fructose, D-galactose, D-glucose, glycerol, lactose, maltose, mannose, salicin, sucrose, D-trehalose, and D-xylose but not from adonitol, dulcitol, *i*-erythritol, *myo*-inositol, inulin, D-mannitol, melezitose, melibiose, raffinose, L-rhamnose, or D-sorbitol. Predominant fatty acids are 12-methyltetradecanoic acid ($C_{15:0}$ anteiso), 14-methylpentadecanoic acid ($C_{16:0}$ iso), hexadecanoic acid ($C_{16:0}$), and 13-methyltetradecanoic acid ($C_{15:0}$ iso). The characteristic whole-cell sugar is galactose; ribose is present.

Source: blood of an endocarditis patient and from homograft valves.

DNA G+C content (mol%): 74.5 (T_m).

Type strain: W6122, ATCC BAA-886, DSM 16025, CCUG 50705, JCM 14302.

Sequence accession no. (16S rRNA gene): AY501364.

3. **Cellulosimicrobium terreum** Yoon, Kang, Schumann and Oh 2007, 2496[VP]

ter′re.um. L. neut. adj. *terreum* of the earth.

Cells are Gram-stain-positive, non-spore-forming rods or cocci (0.4–0.8 µm × 0.4–2.0 µm); in older cultures, cells are Gram-stain-variable and most cells are cocci. Colonies on TSA are circular, convex, smooth, glistening, yellow in color, and 1.5–2.0 mm in diameter after 7 d incubation at 25°C. Substrate hyphae are present. Optimal temperature for growth is 25°C. Growth occurs at 4°C and 34°C, but not at 35°C. Optimal pH for growth is 6.5–7.5; growth occurs at pH 6.0 and 9.0, but not at pH 5.5 and 9.5. Growth occurs in the presence of 0–9 % (w/v) NaCl; optimal growth occurs in the presence of 1.0% (w/v) NaCl. Anaerobic growth does not occur on TSA and on TSA supplemented with nitrate. Oxidase-negative. Tweens 20, 40, 60, and 80 are hydrolyzed. H_2S and indole are not produced. L-Glutamate is utilized as a sole carbon and energy sources, but succinate, benzoate, and formate are not. Alkaline phosphatase, esterase (C4), esterase lipase (C8), acid phosphatase, naphthol-AS-BI-phosphohydrolase, and α-glucosidase are present. Esculin, casein,

hypoxanthine, xanthine, and starch are hydrolyzed. Negative for arginine dihydrolase, lysine decarboxylase, ornithine decarboxylase, tryptophan deaminase, hydrolysis of tyrosine, acid production from L-rhamnose, D-mannitol, inositol, and D-sorbitol, utilization of citrate, L-malate, lipase (C 14) leucine arylamidase, valine arylamidase, cystine arylamidase, trypsin, α-chymotrypsin, α-galactosidase, β-glucoronidase, α-mannosidase and α-fucosidase. Utilization of D-glucose, D-fructose, D-galactose, D-cellobiose, D-mannose, D-trehalose, D-xylose, L-arabinose, acetate, pyruvate, sucrose, maltose, and salicin. Acid production from D-glucose and sucrose. Susceptible to cephalothin, chloramphenicol, neomycin, novobiocin, oleandomycin, penicillin G, streptomycin, tetracycline, but not to carbenicillin, gentamicin, kanamycin, lincomycin, and polymyxin B. The cell-wall peptidoglycan type is L-Lys←L-Thr←D-Asp. The whole-cell sugar is galactose. The predominant menaquinone is MK-9(H_4). The major fatty acids are $C_{15:0}$ anteiso and $C_{15:0}$ iso. Major polar lipids are diphosphatidylglycerol, phosphatidylglycerol, phosphatidylinositol, and an unidentified phospholipid.

Source: soil from Dokdo, Korea.

DNA G+C content (mol%): 72.9 (HPLC).

Type strain: DS-61, KCTC 19206, JCM 15619, DSM 18665.

Sequence accession no. (16S rRNA gene): EF076760.

Genus III. **Isoptericola** Stackebrandt, Schumann and Cui 2004, 686[VP]

Erko Stackebrandt and Peter Schumann

I.sop.te.ri′co.la. N.L. n. *Isoptera* order of termites; L. masc. or fem. suff. *-cola* (from L. masc. or fem. n. *incola*), inhabitant; N.L. masc. n. *Isoptericola* inhabitant of termites.

Gram-stain-positive. Cells rod-shaped, V-shaped, or coccoid and **nonmotile**. **Primary mycelium may be formed**. **Cellulolytic and xylanolytic**; facultatively anaerobic, acid is produced from some carbohydrates. **The peptidoglycan type is A4α, L-Lys←D-Asp, variation A11.31.** *N*-glycolylmuramic acid, mycolic acids, and hydroxy fatty acids are absent. The acyl type is acetyl. Whole cell sugars are galactose, rhamnose, and glucose (ratio 4:2:1); ribose and minor amounts of mannose may occur. **The main menaquinones are MK-9(H_2) and/or MK-9(H_4). Major fatty acids are $C_{15:0}$ anteiso and $C_{15:0}$ iso**. Some strains have in addition $C_{16:0}$, $C_{17:0}$ anteiso, and $C_{14:0}$ and in some strains $C_{16:0}$ iso. Smaller amounts of $C_{14:0}$ iso, $C_{17:0}$ iso, and $C_{15:0}$ are present. The **phospholipids include phosphatidylglycerol, diphosphatidylglycerol, and phosphatidylinositol**; unknown glycolipids and/or phospholipids may occur.

DNA G+C content (mol%): 70.0–74.1 (HPLC).

Type species: **Isoptericola variabilis** (*Cellulosimicrobium variabile* Bakalidou, Kämpfer, Berchtold, Kuhnigk, Wenzel and König 2002, 1189[VP]) Stackebrandt, Schumann and Cui 2004, 686[VP].

Further descriptive information

Isoptericola embraces four species, *Isoptericola variabilis*, *Isoptericola halotolerans, Isoptericola hypogeus*, and *Isoptericola dokdonensis*, which share 98–98.5% 16S rRNA gene sequence similarity. The nearest phylogenetic neighbors (Figure 204) are *Xylanimicrobium* [*Promicromonospora*] *pachnodae* (Cazemier et al., 2004; Stackebrandt and Schumann, 2004), *Xylanimonas cellulosilytica* (Rivas et al., 2003), and *Xylanibacterium ulmi* (Rivas et al., 2004), sharing 97.0–97.3% gene sequence similarities: less than 97.6%

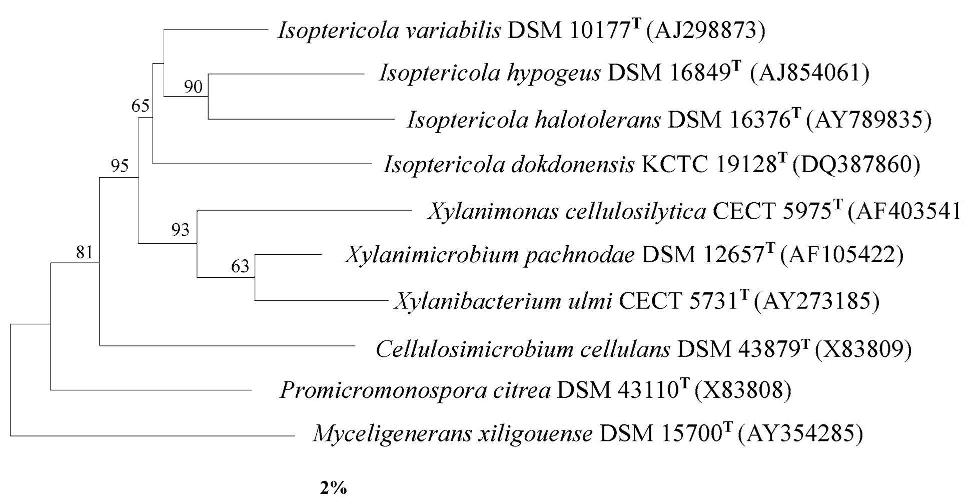

FIGURE 204. Distance matrix dendrogram of 16S rRNA gene sequence relationship (DeSoete, 1983), displaying the relatedness of the four *Isoptericola* species with their nearest neighbors. Bar = 2 inferred changes per hundred nucleotides. Numbers at the branch points are bootstrap values (on the basis of 1000 samplings) (Felsenstein, 1985).

are shared between *Isoptericola* type strains and members of other genera of the order *Micrococcales*.

The DNA–DNA relatedness between the type strains *Isoptericola variabilis* DSM 10177^T and *Isoptericola hypogeus* DSM 16849^T, and between *Isoptericola variabilis* DSM 10177^T and *Isoptericola halotolerans* DSM 16376^T were 31.7% and 27.4%, respectively, as determined by the spectroscopic method (Huss et al., 1983).

Chemotaxonomically the strains are similar (with only small differences in menaquinone and fatty acid composition) as expected from their affiliation to the same genus. However, their growth characteristics differ: *Isoptericola variabilis* DSM 10177^T is a facultative anaerobic organism (Bakalidou et al., 2002), while *Isoptericola hypogeus* DSM 16849^T (Groth et al., 2005) and *Isoptericola halotolerans* DSM 16376^T (2005) are aerobic to microaerophilic organisms that do not grow in an anaerobic atmosphere. *Isoptericola dokdonensis* DS-3^T grows anaerobically on tryptic soy agar (TSA) and on TSA supplemented with nitrate (Yoon et al., 2006). Morphologically, *Isoptericola hypogeus* forms wrinkled, circular, smooth, and pale yellow colonies on medium 79 of about 1–2 mm in diameter. In young cultures (8–24 h), a well developed primary mycelium (width 0.6–0.7 µm) was observed to fragment in the stationary growth phase (48–72 h) into irregular nonmotile short rods and cocci (diameter 0.8–1.0 µm). In contrast to stationary cultures of *Isoptericola variabilis*, both cell types were mainly arranged in short flexible chains or in clusters. A primary mycelium was also observed in *Isoptericola halotolerans* and *Isoptericola dokdonensis*. *Isoptericola variabilis* and *Isoptericola hypogeus* have been characterized in detail (Bakalidou et al., 2002; Groth et al., 2005), while less information on morphological properties is available for *Isoptericola halotolerans* (Zhang et al., 2005) and *Isoptericola dokdonensis* (Yoon et al., 2006).

The type strains of *Isoptericola variabilis* and *Isoptericola hypogeus* are resistant to ampicillin 10 µg, ciprofloxacin 5 µg, kanamycin 30 µg, lincomycin 2 µg, methicillin 5 µg, norfloxacin 10 µg, and penicillin G 10 IU. All type strains are susceptible to neomycin (30 µg), tetracycline (30 µg), and chloramphenicol (100 µg). *Isoptericola hypogeus* but not *Isoptericola variabilis* and *Isoptericola dokdorensis* is susceptible to 10 µg streptomycin. No information on antibiotic sensitivity and resistance is available for *Isoptericola halotolerans*.

Enrichment and isolation procedures

Isoptericola [*Cellulosimicrobium*] *variabilis* was isolated from the hindgut of the Australian termite *Mastotermes darwiniensis* (Froggatt). *Isoptericola hypogeus* CD12-119^T was isolated from a sample of tufa collected in the burial chamber of the first arcosolium behind the entrance of the catacomb Domitilla using PY-BHI agar (Yokota et al., 1993) and a standard dilution plate procedure. *Isoptericola halotolerans* YIM 70177^T was isolated from saline soil by the dilution plating method on modified Horikoshi medium (Horikoshi and Grant, 1998; Zhang et al., 2005). *Isoptericola dokdonensis* DS-3^T is a soil isolate from Dokdo, Korea.

Maintenance procedures

Stock cultures of strains in liquid organic DSM medium 92 (DSMZ, 2001) supplemented with 5% DMSO were maintained in either the vapor phase of liquid nitrogen or at −80°C by adding a glycerol-medium (1:1) that consisted of K_2HPO_4 (1.26%), KH_2PO_4 (0.36%), $MgSO_4$ (0.01%), Na-citrate (0.09%), $(NH_4)_2SO_4$ (0.18%), and glycerol (8.8%).

Differentiation from closely related taxa

Table 177 lists the characteristics useful to distinguish *Isoptericola* from other genera of the family *Promicromonosporaceae*. A comparison of phenotypic properties between *Isoptericola variabilis* DSM 10177^T, *Myceligenerans xiligouense* XLG9A10.2^T, members of *Promicromonospora*, *Xylanimicrobium* (*Promicromonospora*) *pachnodae* DSM 12657^T, and *Xylanimonas cellulosilytica* $XIL07^T$ has been published by Cui et al. (2004).

Taxonomic comments

As already noted in the original publication (Bakalidou et al., 2002), the type strain of the species *Cellulosimicrobium variabile* DSM 10177^T does not phylogenetically cluster unambiguously with the type species *Cellulosimicrobium cellulans* DSM 43879^T (96.6% 16S rRNA gene sequence similarity). The type strains of the two *Cellulosimicrobium* species resembled each other in morphology, the composition of fatty acids, base composition of DNA, phospholipids, and the presence of lysine in position 3 of the peptide subunit of peptidoglycan. They differed from each other in cell-wall sugars and in the amino acid composition of the A4α-type peptidoglycan, which contains serine and aspartic acid in the interpeptide bridge of *Cellulosimicrobium cellulans*, and only aspartic acid in the interpeptide bridge of *Cellulosimicrobium variabile*. As the presence of different peptidoglycan variations is rarely encountered in genera of the order *Micrococcales* (formerly suborder *Micrococcineae* (Stackebrandt and Schumann, 2004), the peptide structure of *Isoptericola* [*Cellulosimicrobium*] *variabilis* was reinvestigated. As published originally, the peptidoglycan contained L-lysine, aspartic acid, glutamic acid, and alanine in the molar ratio 1:0.9:2:2, which raised the question about the position of the second molecule of glutamic acid (one molecule is positioned at pos. 2 of the peptide subunit). The reanalysis of purified peptidoglycan revealed the ratio of L-lysine:aspartic acid:glutamic acid:alanine to be 1:0.9:1:2, and analysis of partial hydrolysis fragments (Schleifer and Kandler, 1972) indicated the presence of the peptide L-Lys←Asp. Thus, the A4α type of variation A11.31 (http://www.peptidoglycan-types.info) was proposed for *Isoptericola variabilis* DSM 10177^T in the formal reclassification. Several new members of the family *Promicromonospora* were described in the following years, which demonstrated strain DSM 10177^T to be moderately related to *Xylanimonas cellulosilytica*, *Xylanimicrobium pachnodae*, and *Xylanibacterium ulmi*, forming a lineage that forms a sister lineage of the *Promicromonospora*-*Myceligenerans* lineage.

Differentiation of the species of the genus *Isoptericola*

Differential characters of the type strains of the four type strains are shown in Table 181.

TABLE 181. Differential physiological characteristics of *Isoptericola type strains*[a,b]

Characteristic	*I. variabilis* DSM 10177^T	*I. hypogeus* DSM 16849^T	*I. halotolerans* YIM 70177^T	*I. dokdonensis* DS-3^T
Oxidase	–	–	–	+
Growth at 10°C	–	+	+	+
Growth at 42°C	+	–	+	–
Growth in the presence of 10% NaCl	+	–	+	–
Hydrolysis of:				
Starch	+[b]	+	–	–
Casein	+[b]	+	–	–
Gelatin	+	+	–	+
Tyrosine	+	+	+	–
Hypoxanthine	+	+	–	–
Urea	+[c]	–	–	+
Xanthine	+	+	–	–
Utilization of:				
D-Fructose	+	+	+	–
D-Trehalose	+	–	+	+
Sucrose	+	–	+	+
Maltose	+	w	+	+
Acetate	+	–	nd	–
L-Glutamate	+	–	nd	–
API ZYM tests:				
Alkaline phosphatase	+	+	–	–
Lipase (C14)	w	–	–	–
Valine arylamidase	w	w	–	–
Cystine arylamidase	+	+	–	–
Acid phosphatase	w	+	–	–
α-Galactosidase	+	–	–	+
N-Acetyl-β-glucosaminidase	+	+	–	+

[a]Symbols and abbreviations: –, negative; +, positive; w, weak; nd, not determined.

[b]Data from Yoon et al. (2006).

[c]Data from Groth et al. (2005); the opposite result was reported by Zhang et al. (2005).

List of species of the genus *Isoptericola*

1. **Isoptericola variabilis** (Bakalidou, Kämpfer, Berchtold, Kuhnigk, Wenzel and König 2002) Stackebrandt, Schumann and Cui 2004, 686[VP]

va.ri.a′bi.lis. L. masc. adj. *variabilis* variable, because cells are rods or cocci.

Gram-stain-positive. Cells are rod-shaped (0.3 × 0.7 μm) V-shaped or coccoid (diameter 0.25 μm) and nonmotile. Yellow, smooth colonies on TSA (Difco) about 2 mm in diameter after 48 h at 28°C. Catalase-positive, oxidase-negative. Nitrate reduction and esculin hydrolysis positive. Cellulolytic and xylanolytic. Utilizes of D-glucose, D-galactose, gentobiose, D-gluconic acid, D-cellobiose, D-lactose, lactulose, D-mannose, maltose, maltotriose, mannan, D-mannitol, D-xylose, L-arabinose, acetate, citrate, propionic acid, pyruvic acid, D-arabitol, glycerol, adenosine, dextrin, aconitate, starch, glycogen, amygdalin, arbutin, D-galactose, D-psicose, D-ribose, *N*-acetylglucosamine, D-melezitose D-melibiose, methyl α-D-galactoside, methyl-β-D-galactoside, 3-methylglucose, D-raffinose, stachyose, D-trehalose, sucrose, L-asparagine, L-glutamic acid, L-pyroglutamic acid, L-serine, 2′-deoxyadenosine, inosine, and salicin. Acid produced from D-glucose, L-arabinose, raffinose, L-rhamnose, D-xylose, D-cellobiose, D-melibiose, D-arabitol, and D-mannose. No acid produced from sucrose, D-mannitol, dulcitol, salicin, adonitol, inositol, sorbitol, maltose, methyl β-D-glucose, or erythritol. According to the substrate panels of commercial systems the following reactions are positive: peptonization of milk, adenine, casein, gelatin, hypoxanthine, potato, starch, tyrosine, xanthine, xylan, succinate, alkaline phosphatase, esterase (C4), esterase lipase (C8), leucine, arylamidase, cystine arylamidase, urease, naphthol-AS-BI-phosphohydrolase, β-galactosidase, α-glucosidase, β-glucosidase, and *N*-acetyl-β-glucosamidase, α-galactosidase, methyl α-D-mannoside, and β-gentiobiose. Other properties are indicated in Table 181. Susceptible to imipenem (10 μg), ofloxacin (10 μg), oxytetracycline (30 μg), polymyxin B 300 IU, rifampin (30 μg), sulfonamide (200 μg), vancomycin (30 μg), neomycin (30 μg), tetracycline (30 μg), and chloramphenicol (100 μg).

Source: the hindgut of the Australian termite *Mastotermes darwiniensis* (Froggatt).

DNA G+C content (mol%): 70–72 (T_m).

Type strain: MX5, DSM 10177, ATCC BAA-303, JCM 11754.

Sequence accession no. (16S rRNA gene): AJ298873.

2. **Isoptericola dokdonensis** Yoon, Schumann, Kang, Jung and Oh 2006, 2896[VP]

dok.do.nen′sis. N.L. masc. adj. *dokdonensis* of Dokdo, Korea, where the organism was first isolated.

Cells are Gram-stain-positive, nonmotile rods or cocci (0.8–1.1 × 0.8–4.5 μm). Primary mycelium is formed. Colonies on TSA are circular, convex, smooth, yellow in color,

and 1.0–2.0 mm in diameter after 3 d of incubation at 30°C. Optimal temperature for growth is 30°C. Growth occurs at 10°C and 40°C, but not at 4°C or 41°C. Optimal pH for growth is between 7.0 and 8.0; growth occurs at pH 6.5, but not at pH 6.0. Growth occurs in the presence of 0–9 % (w/v) NaCl. Catalase- and oxidase-positive. Nitrate reduction and esculin hydrolysis positive. Anaerobic growth occurs on TSA and on TSA supplemented with nitrate. Tweens 20, 40, and 60 are not hydrolyzed. H_2S and indole are not produced. D-Glucose, D-galactose, D-cellobiose, D-mannose, D-xylose, L-arabinose, and salicin are utilized. Esterase (C4), esterase lipase (C8), leucine arylamidase, naphthol-AS-BI-phosphohydrolase, β-galactosidase, α-glucosidase, and β-glucosidase are present. Arginine dihydrolase, lysine decarboxylase, ornithine decarboxylase, and tryptophan deaminase are absent. Pyruvate but not benzoate, citrate, L-malate, succinate, or formate is utilized as a carbon source. Other phenotypic characteristics are given in Table 181. The whole-cell sugars are galactose, glucose, rhamnose, and ribose. The predominant menaquinone is MK-9(H_4). The major fatty acids (>10% of total fatty acids) are $C_{15:0}$ anteiso and $C_{15:0}$ iso. The major polar lipids are diphosphatidylglycerol, phosphatidylglycerol, phosphatidylinositol, and two unidentified glycolipids. Resistant to polymyxin B, 100 U; streptomycin, 50 µg; penicillin G, 20 U; ampicillin, 10 µg; cephalothin, 30 µg; gentamicin, 30 µg; novobiocin, 5 µg; kanamycin, 30 µg; lincomycin, 15 µg; oleandomycin, 15 µg; carbenicillin, 100 µg. Susceptible to neomycin, 30 µg; tetracycline, 30 µg; chloramphenicol, 100 µg.

Source: soil in Dokdo, Korea.

DNA G+C content (mol%): 74.1 (HPLC).

Type strain: DS-3, KCTC 19128, CIP 108921, JCM 15137.

Sequence accession no. (16S rRNA gene): DQ387860.

3. **Isoptericola halotolerans** Zhang, Schumann, Li, Cheng, Tian, Stackebrandt, Xu and Jiang 2005, 1869[VP]

ha.lo.to′le.rans. Gr. n. *hals halos* salt; L. part. adj. *tolerans* tolerating; N.L. part. adj. *halotolerans* salt-tolerating, referring to the organism's ability to tolerate high salt concentrations.

Cells are Gram-stain-positive, coccoid or rod-shaped, nonmotile, and do not form spores. Primary mycelium is formed. Colonies are pale-yellow, circular, opaque, and approximately 1.0 mm in diameter after 24 h growth at 28°C. Optimal growth occurs at 10% NaCl, pH 8.0–9.0, and 28°C. Catalase-positive and oxidase-negative. Nitrate reduction and esculin hydrolysis positive. Milk coagulation, melanin production, H_2S production, Voges–Proskauer, and indole production negative. The following substrates are utilized: glucose, ribose, arabinose, maltose, sucrose, mannose, fructose, salicin, galactose, cellobiose, trehalose, sorbitol, lactose, xylose, and dextrin; acid is produced from maltose. Does not utilize rhamnose, acetamide, inositol, mannitol, adonitol, and sorbitol. Negative for Tween 20 and Tween 80 utilization, gelatin liquefaction, ammonia production, milk peptonization, and casein and starch hydrolysis. Trehalose is not utilized. Positive for esterase (C4), esterase lipase (C8), leucine arylamidase, naphthol-AS-BI-phosphohydrolase, α-glucosidase, β-glucosidase, β-galactosidase, α-maltosidase, and cellulose; negative for arginine dihydrolase, ornithine decarboxylase, lysine decarboxylase, and L-aspartic arylamidases. Other phenotypic characteristics are given in Table 181. The cell-wall sugar is galactose. Phospholipids are phosphatidylinositol, phosphatidylglycerol, and diphosphatidylglycerol. The predominant menaquinones are MK-9(H_4) and MK-9(H_2). Susceptible to neomycin, 30 µg; tetracycline, 30 µg; chloramphenicol, 100 µg.

Source: a saline soil sample collected from Qinghai Province, North-West China.

DNA G+C content (mol%): 72.8 (HPLC).

Type strain: YIM 70177, DSM 16376, JCM 13590, KCTC 19046.

Sequence accession no. (16S rRNA gene): AY789835.

4. **Isoptericola hypogeus** Groth, Schumann, Schütze, Gonzalez, Laiz, Saiz-Jiminez and Stackebrandt 2005, 1718[VP]

hy.po′ge.us. L. masc. adj. *hypogeus* underground, referring to the site of isolation.

Gram-stain-positive, aerobic to microaerophilic actinomycete with a well developed primary mycelium (diameter of hyphae 0.6–0.7 µm) that undergoes fragmentation into short irregular nonmotile rods and cocci (diameter 0.8–1.0 µm) in the stationary growth phase. Aerial mycelium is absent. Colonies on organic medium 79 are wrinkled, circular, smooth, and pale yellow (diameter about 1–2 mm). Growth between 10°C and 40°C (optimal growth at 28°C) and at pH values in the range of 6–9. NaCl in the culture medium is tolerated up to 10%. Catalase-positive and oxidase-negative. Nitrate reduction and esculin hydrolysis positive. The following reactions are distinctly positive: peptonization of milk, adenine, casein, gelatin, hypoxanthine, potato, starch, tyrosine, xanthine, xylan, succinate, and nitrate reduction. Utilizes acetate, propionic acid, pyruvic acid, glycerol, adenosine, dextrin, L-arabinose, arbutin, D-galactose, α-D-glucose, D-galactose, L-arabinose, salicin, D-mannose, malate, D-psicose, D-ribose, salicin, D-xylose, *N*-acetylglucosamine, amygdalin, arbutin, salicin, cellobiose, maltose, lactose, methyl α-D-mannoside, starch, glycogen, β-gentiobiose, D-arabitol. Negative for D-mannitol, D-melezitose, D-melibiose, methyl-α-D-galactoside, methyl-β-D-galactoside, 3-methyl-glucose, D-raffinose, stachyose, acetic acid, D-trehalose, sucrose, L-asparagine, L-glutamic acid, L-pyroglutamic acid, L-serine, 2′-deoxyadenosine, and inosine. Positive for alkaline phosphatase, esterase (C4), esterase lipase (C8), leucine, arylamidase, cystine, arylamidase, naphthol-AS-BI-phosphohydrolase, β-galactosidase, α-glucosidase, β-glucosidase, *N*-acetyl-β-glucosamidase, and α-galactosidase. Other phenotypic characteristics are given in Table 181. Susceptible to imipenem (10 µg), ofloxacin (10 µg), oxytetracycline (30 µg), polymyxin B (300 IU), rifampin (30 µg), sulfonamide (200 µg), and vancomycin (30 µg), neomycin (30 µg), tetracycline (30 µg), and chloramphenicol (100 µg).

Source: a tufa sample of a burial chamber in the catacomb Domitilla, Rome, Italy.

DNA G+C content (mol%): 73.8 (HPLC).

Type strain: CD12-119, HKI 0342, DSM 16849, JCM 14325, NCIMB 14033.

Sequence accession no. (16S rRNA gene): AJ854061.

Genus IV. **Myceligenerans** Cui, Schumann, Stackebrandt, Kroppenstedt, Pukall, Xu, Rohde and Jiang 2004, 1292[VP]

Erko Stackebrandt and Peter Schumann

My.ce.li.ge′ne.rans. N.L. neut. n. *mycelium* filamentous cell; L. part. adj. *generans* producing; N.L. neut. subst. *Myceligenerans* hyphae-forming microbe.

Gram-stain-positive, mycelium-and spore-forming organism. **Substrate mycelia are well developed and branched in and on the media**. Aerial mycelium absent. **The surface of substrate mycelium bears fragmented cells and spore chains with one or two spores at the tips of the mycelium. Spores are coccoid to rod-shaped, and nonmotile**. Aerobic to microaerophilic. **Peptidoglycan type is A4α, variation L-Lys←L-Thr←D-Glu**. The acyl type is acetyl. Cell-wall sugars are glucose, mannose, and galactose. **Major menaquinones are MK-9(H_4)** and MK-9(H_6); **predominant fatty acids are $C_{15:0}$ anteiso and $C_{15:0}$ iso**; C_{16} may occur. Cells contain phosphatidylglycerol, diphosphatidylglycerol, phospatidylinositol, three unidentified phospholipids, and one unidentified glycolipid. Mycolic acids are absent.

The 16S rRNA gene sequence analysis places the genus in the family *Promicromonosporaceae*.

DNA G+C content (mol%): 72–72.3.

Type species: **Myceligenerans xiligouense** Cui, Schumann, Stackebrandt, Kroppenstedt, Xu, Rohde and Jiang 2004, 1292[VP].

Further descriptive information

16S rRNA gene sequence analysis indicates that the two species of *Myceligenerans* constitute a distinct lineage within the family *Promicromonosporaceae*, sharing with other genera of this family between 93.0 and 95.0% similarity. The 16S rRNA gene sequences of the two *Myceligenerans* species contain the signature nucleotides of the family *Promicromonosporaceae* (Stackebrandt and Schumann, 2000) as emended by Cui et al. (2004). The relatedness between the two species *Myceligenerans xiligouense* and *Myceligenerans crystallogenes* is 97.9% 16S rRNA gene sequence similarity and 14% DNA–DNA reassociation similarity. The three strains of *Myceligenerans crystallogenes* shared almost identical riboprint patterns which affiliated them to the same ribogroup. The riboprint patterns of *Myceligenerans crystallogenes* show close similarity to that of *Myceligenerans xiligouense* (Groth et al., 2006).

The morphology of the two species is similar (Figure 205 for *Myceligenerans xiligouense*) but they differ in the colony pigmentation. In liquid culture *Myceligenerans crystallogenes* forms a well-developed primary mycelium within 8–24 h which undergoes fragmentation into irregular nonmotile rods and cocci after about 48 h of cultivation (Groth et al., 2006).

Differential characteristics for the two species are shown in Table 182. Strains grow at 10°C and 35°C and at pH 6 and 9. They do not grow at 42°C, and at pH 5 or 10. The strain from the salt marsh grows between 4°C and 50°C, between pH 4 and 13, and in up to 17.5% NaCl (w/v). The other strains grow in 2% or less NaCl. All strains decompose casein, xylan, esculin, gelatin, potato starch, and Tween 80, and they utilize acetate. Detailed analysis of physiological properties for the type strain strains of *Myceligenerans crystallogenes* and two additional strains and the type strain of *Myceligenerans xiligouense* are listed as supplementary data in the publication of Groth et al. (2006).

Enrichment and isolation procedures

The type strain of *Myceligenerans xiligouense* has been isolated from a pasture nearby an alkaline salt marsh in Qinghai province, western China, while strains of *Myceligenerans crystallogenes* originate from tufa and stone surface and from a fresco of two different Roman catacombs (Domitilla and San Callisto).

Strains were isolated by standard dilution plating. Strain DSM 15700[T] from the alkaline salt marsh was plated on Bacto Marine Broth agar, pH 7.2 at 28°C, while strains from stones and fresco were obtained on PY-BHI agar (Yokota et al., 1993), humic acid agar (Hayakawa and Nonomura, 1987), or on medium 79 (Prauser and Falta, 1968) at 28°C and neutral pH.

Maintenance procedures

Strains can be stored for some weeks on slants at 4°C and in 20% (w/v) glycerol suspensions at −20°C and at −80°C. Long-term preservation methods include freeze drying in skim milk and in liquid nitrogen at −196°C.

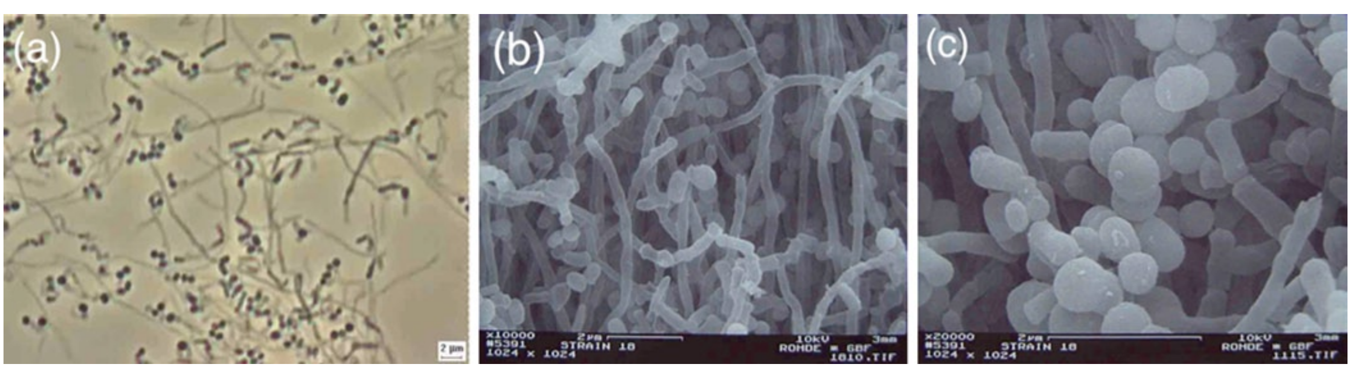

FIGURE 205. Micrographs of cells of *Myceligenerans xiligouense* DSM 15700[T] showing (a) the well-developed mycelia with abundant coccoid to rod-shaped spores on TSBA medium (7 d at 28°C) and (b) and (c) scanning electron micrographs. (Reprinted with permission from Cui et al., 2004. Int. J. Syst. Evol. Microbiol. *54*: 1287–1293.)

TABLE 182. Differential characteristics of the type strains of *Myceligenerans* species[a,b]

Characteristic	*M. xiligouense* DSM 15700^T	*M. crystallogenes* DSM 17134^T
Nitrate reduction	–	+
Decomposition of:		
Urea	–	+
Tyrosine	–	+*
Utilization of:		
Aconitate	–	+
Citrate	–	+
Substrate utilization Biolog GP2:		
Glycogen	+	–
Amygdalin	+	–
L-Arabinose	+	–
D-Arabitol	+	–
Arbutin	+	–
D-Fructose	+	–
D-Galactose	+	–
Gentobiose	+	–
α-D-Glucose	+	–
α-D-Lactose	+	–
Maltose	+	–
Methyl α-D-galactoside	+	–
Methyl α-D-galactoside	+	–
Palatinose	+	–
Salicin	+	–
D-Sorbitol	+	–
D-Trehalose	+	–
Turanose	+	–
L-Lactic acid	+	–
Monomethyl succinate	–	+
L-Alaninamide	+	–
2-Deoxyadenosine	+	–
Inosine	+	–
Uridine	+	–
Antibiotic susceptibility:		
Ampicillin (10 μg)	–	+
Penicillin G (10 IU)	–	+

[a]Symbols: reaction of other strains in parentheses, +, positive; –, negative; *, delayed reaction.

[b]Data from Groth et al. (2006).

Differentiation of the genus *Myceligenerans* from other genera

Table 177 of the chapter *Promicromonosporaceae* indicates the characters useful in the differentiation of members of the genus *Myceligenerans* from other genera of the family *Promicromonosporaceae*. The use of 16S rRNA gene sequence analysis is recommended to affiliate new members to the genus, followed by determination of chemotaxonomic properties that circumscribe the genus.

List of species of the genus *Myceligenerans*

1. **Myceligenerans xiligouense** Cui, Schumann, Stackebrandt, Kroppenstedt, Xu, Rohde and Jiang 2004, 1292[VP]

xi.li.gou.en′se. N.L. neut. adj. *xiligouense* pertaining to Xiligou, a location in China where the type strain was isolated.

Gram-positive, aerobic, nonmotile, mycelium- and spore-forming microorganism. Colonies are brilliant yellow light (about 1–5 mm in diameter) on Bacto Marine Broth agar and yellowish (about 1.5–5 mm in diameter) on Tryptone Soy Broth agar (Difco) media after 7 d incubation at 28°C. Mycelia are well developed in and on the surface of the agar media. The tip of the mycelia bear fragmented cells and one to two coccoid nonmotile spores (0.5 μm in diameter). Grows between 4°C and 50°C and optimally between 20°C and 30°C; grows between pH 4 and 13 with optimal growth at pH values between 7 and 9; grows at salt concentrations between 2% and 8% (NaCl, w/v), with optimum growth between 2% and 7%.

The following compounds were utilized by all strains in API 50CHE tests: glycerol, L-arabinose, rhamnose, D-xylose, mannitol, arbutin, salicin, melibiose, melezitose, galactose, D-glucose, D-fructose, D-mannose, amygdalin, esculin, cellobiose, lactose, saccharose, trehalose starch, glycogen, D-lyxose, D-tagatose, D-turanose, L-arabitol, and D-arabitol. The following substrates were not utilized: erythritol, D-arabinose, ribose, L-xylose, adonitol, methyl-β-xyloside, L-sorbose, methyl-D-mannoside, methyl-α-D-glucoside, *N*-acetylglucosamine,

D-raffinose, D-fucose, gluconate, and 2-ketogluconate. The following compounds were utilized by all strains in BIOLOG GP2 tests: β-cyclodextrin, glycogen, mannan, Tween 40, Tween 80, amygdalin, D-arabitol, D-galacturonic acid, D-gluconic acid, D-lactose, lactulose, D-mannitol, D-mannose, D-melizitose, methyl D-galactoside, methyl D-glucoside, 3-methyl glucose, D-psicose, D-raffinose, D-ribose, sedoheptulose, D-sorbotil, stachyose, D-tagatose, D-trehalose, acetic acid, *p*-hydroxyphenylacetic acid, L-lactic acid, methyl pyruvate, propionic acid, pyruvic acid, succinamic acid, succinic acid, *N*-acetyl-L-glutamic acid, L-alaninamide, L-serine, 2,3-butanediol, 2′-deoxyadenosine, inosine, uridine, fructose 6-phosphate, glucose 1-phosphate, glucose 6-phosphate, and D-L-α-glycerol phosphate. Chemotaxonomic characteristics are as described for the genus.

Source: a pasture near an alkaline salt marsh, Qinghai Province, western China.

DNA G+C content (mol%): 71.9 (HPLC),

Type strain: XLG9A10.2, DSM 15700, CGMCC 1.3458, JCM 14112.

Sequence accession no. (16S rRNA gene): AY354285.

2. **Myceligenerans crystallogenes** Groth, Schumann, Schütze, Gonzalez, Laiz, Suihko and Stackebrandt 2006, 286[VP]

crys.tal.lo.ge′nes. Gr. n. *krustallos* crystal; Gr. v. *gennaio* to produce; N.L. part. adj. *crystallogenes* producing crystals of iodinin (1,6-phenazine-diol 5,10-dioxide).

Gram-positive, aerobic to microaerophilic actinomycete with a well-developed primary mycelium (diameter of hyphae 0.5–0.7 μm) that undergoes fragmentation into short, irregular, non-motile rods and cocci in the stationary growth phase. Aerial mycelium is absent. Spore-like cells occur in the substrate mycelium. Colonies on organic medium 79 are wrinkled, circular, smooth, and white to cream (diameter about 1–4 mm). Grows between 10°C and 40°C (optimal growth is at 28°C) and at pH values in the range 6–9. NaCl in the culture medium is well tolerated up to 5%. No growth at 8% NaCl. The type strain utilizes dextrin, D-cellobiose, α-ketovaleric acid, methyl pyruvate, pyruvic acid, and thymidine. Does not utilize glycogen, inulin, D-arabitol, arbutin, methyl-α-D-galactoside, methyl-α-D-glucoside, 3 methyl glucose, D-sorbitol, D-tagatose, L-lactic acid, propionic acid, L-alaninamide, uridine, thymidine 5′-monophosphate, and α- and β-hydroxybutyric acid. The major menaquinones are MK-9(H_8), MK-9(H_4), MK-9(H_6), and MK-9(H_2), and the minor menaquinones are MK-8(H_4), MK-9, and MK-8(H_2).

Source: the catacomb of Domitilla in Rome, Italy.

DNA G+C content (mol%): 72.0–72.3 (72.3 for the type strain; HPLC).

Type strain: CD12E2-27, HKI 0369, JCM 14326, DSM 17134, NCIMB 14061, VTT E-032285.

Sequence accession no. (16S rRNA gene): AY928181.

Genus V. **Xylanibacterium** Rivas, Trujillo, Schumann, Kroppenstedt, Sánchez, Mateos, Martínez-Molina and Velázquez 2004, 560[VP]

Encarna Velázquez, Martha E. Trujillo and Eustoquio Martínez-Molina

Xylanibacterium (Xy.la.ni.bac.te′ri.um N.L. neut. n. *xylanum* xylan, a polysaccharide; L. neut. n. *bacterium* small rod; N.L. neut. n. *Xylanibacterium* xylan hydrolyzing small rod).

Gram-stain-positive, non-spore forming small rod-shaped bacterium**.** Aerial mycelium is not formed. **Aerobic or facultatively anaerobic**, chemo-organotrophic and **xylanolytic bacterium.** Optimum temperature 30°C and pH 7. Oxidase-positive and catalase-negative. Phylogenetically related to members of the family *Promicromonosporaceae* on the basis of 16S rRNA gene sequence. **Peptidoglycan type is A4α, L-Lys–L-Ala–D-Glu**. The cell-wall sugars are **rhamnose in great amounts**, fucose, mannose, galactose and traces of arabinose and glucose. Major menaquinones are **MK-9(H_4)** and **MK-8(H_4).** Predominant fatty acids are $C_{15:0}$ anteiso, $C_{15:0}$ iso, $C_{16:0}$, and $C_{14:0}$. Mycolic acids are absent. The polar lipids are phosphatidylglycerol (PG), diphosphatidylglycerol (DPG), phosphatidylinositol (PI), and phosphatidylinositol mannosides (PIM).

DNA G+C content (mol%): 72.3 (T_m).

Type species: **Xylanibacterium ulmi** (Rivas, Trujillo, Schumann, Kroppenstedt, Sánchez, Mateos, Martínez-Molina and Velázquez 2004, 560[VP].

Further descriptive information

The genus *Xylanibacterium* is currently represented by a single species, *Xylanibacterium ulmi*, which was isolated from a decayed tree (*Ulmus nigra*). Cells of *Xylanibacterium ulmi* typically appear as nonmotile and non-sporulated rods and are 0.9 μm × 0.3 μm.

The cell-wall composition of *Xylanibacterium* is recorded in Table 183 and constitutes the main source of differences among the genera from family *Promicromonosporaceae*. The peptidoglycan composition of *Xylanibacterium* corresponds to the type A4α, L-Lys–L-Ala–D-Glu and coincides with that reported for genus *Promicromonospora* by Evtushenko et al. (1984), although Kalakoutskii et al. (1989) did not report glutamic acid in the peptidoglycan composition for *Promicromonospora*. The peptidoglycan of *Xylanibacterium* differs from the remaining genera encompassed within the family *Promicromonosporaceae*. The cell-wall sugars present in *Xylanibacterium* are rhamnose in high amounts, fucose, mannose, galactose and traces of arabinose and glucose. This profile is different to that presented by the remaining genera in the family *Promicromonosporaceae* although all genera of this family contain high amounts of rhamnose and galactose. The major polar lipids of the genus *Xylanibacterium* are phosphatidylglycerol (PG), diphosphatidylglycerol (DPG), phosphatidylinositol (PI), phosphatidylinositol mannoside (PIM) and other unidentified phosphoglycolipids (PGL) This profile is basically identical to that obtained for the genus *Xylanimonas* (Rivas et al., 2003) and differs from that of the remaining

TABLE 183. Differential characteristics of the genus *Xylanibacterium* from related genera[a]

Characteristic	*Xylanibacterium*[b]	*Xylanimonas*[c]	*Promicromonospora*[d,e,f]	*Cellulosimicrobium*[e,h,i,j]	*Isoptericola*[f,k,l]	*Xylanimicrobium*[f,m]	*Myceligenerans*[n,o]
Shape	Small rods	Coccoid	Short rods, coccoid, Y or V shaped	Short rods	Short rods, V shaped or coccoid	Irregular shaped, single or in pairs	Coccoid, rod-shaped
Mycelium	–	–	Mycelium	Primary mycelium	Primary mycelium	Mycelia-like fringes	Substrate mycelium
Aerial hyphae	–	–	+	–	–	–	–
Catalase	–	w	+	+	+	+	nd
Fermentation of glucose	+	w	Rarely	+	+	+	+
Peptidoglycan composition[p]	L-Lys–L-Ala–D-Glu	L-Lys–D-Asp	L-Lys–L-Ala–D-Glu	L-Lys–D-Ser–D-Asp	L-Lys–D-Asp	L-Lys–L-Ser–D-Glu	L-Lys–L-Thr–D-Glu
Cell-wall sugars[q]	Rha, Fuc, Man, Gal, Ara, Glc	Gal, Rha	Glc, Gal, Xyl or Glc, Gal, Rha	Gal, Rha, Glc, Fuc or Gal, Rib	Rha. Gal, Glc	Rha. Gal, Glc	Glc, Gal, Man
Major menaquinones	MK-9(H_4), MK-8(H_4)	MK-9(H_4), MK-8(H_4)	MK-9(H_4)	MK-9(H_4)	MK-9(H_4)	MK-9(H_4)	MK-9(H_4), MK-9(H_6)
Cellular fatty acids	$C_{15:0}$ anteiso, $C_{15:0}$ iso, $C_{16:0}$, $C_{14:0}$	$C_{15:0}$ anteiso, $C_{15:0}$ iso, $C_{14:0}$	$C_{15:0}$ anteiso, $C_{15:0}$ iso	$C_{15:0}$ anteiso, $C_{15:0}$ iso, $C_{16:0}$, $C_{16:0}$ iso	$C_{15:0}$ anteiso, $C_{15:0}$ iso, $C_{14:0}$, $C_{16:0}$, $C_{16:0}$ iso, $C_{17:0}$ iso	$C_{15:0}$ anteiso, $C_{15:0}$ iso, $C_{16:0}$, $C_{16:0}$ iso	$C_{15:0}$ anteiso, $C_{15:0}$ iso, $C_{16:0}$ iso
Phospholipid composition[r]	PG, DPG, PI, PIM	PG, DPG, PI, PIM	PG, DPG, PGL, PL, GL	PG, DPG, PI, PL	PG, DPG, PI, PL	PG, DPG, PI, 3 PLs	PG, DPG, PI, PL,GL

[a]Symbols: +, positive result; –, negative result; w, weakly positive result; nd, no data.

[b]Rivas et al. (2004).

[c]Rivas et al. (2003).

[d]Evtuschenko et al. (1984).

[e]Stackebrandt et al. (2004).

[f]Stackebrandt and Schumann (2004).

[g]Busse et al. (2003).

[h]Schumann et al. (2001).

[i]Bakalidou et al. (2002).

[j]Brown et al. (2006).

[k]Groth et al. (2005).

[l]Zhang et al. (2005).

[m]Cazemier et al. (2003).

[n]Cui et al. (2004).

[o]Groth et al. (2006).

[p]Ala, alanine, Asp, aspartic acid; Glu, glutamic acid; Lys, lysine; Ser, serine.

[q]Gal, galactose; Rha, rhamnose; Fuc, fucose; Man, mannose; Glc, glucose; Ara, arabinose; Xyl: xylose, Rib: ribose.

[r]PG, phosphatidylglycerol; DPG, Diphosphatidylglycerol. PI, phosphatidylinositol; PIM, Phosphatidylinositol mannoside; PET, Phosphatidyl ethanolamine; GL: unknown glycolipid; PL: unknown phospholipid; PGL: unknow phosphoglycolipid.

genera of the family *Promicormonosporaceae*. Phosphatidylglycerol and diphosphatidylglycerol are present in all genera from this family but the presence of phosphatidylinositol mannoside was only reported in genus *Xylanimonas* (Rivas et al., 2003). The main fatty acid detected in *Xylanibacterium* is $C_{15:0}$ anteiso, followed by $C_{15:0}$ iso, $C_{14:0}$ and $C_{16:0}$ in lower amounts. According to the published data, this cellular fatty acid pattern is similar to that of *Xylanimicrobium pachnodae* (Stackebrandt and Schumann, 2004) but differs from that of the remaining genera of the family *Promicormonosporaceae*, although the main fatty acid in all these genera is also $C_{15:0}$ anteiso. The main menaquinone present in *Xylanibacterium* as well as in the remaining genera of family *Promicormonosporaceae* is MK-9(H_4), nevertheless MK-8(H_4) is also present in *Xylanibacterium* as well as in *Xylanimonas* (Rivas et al., 2003).

Cells of *Xylanibacterium ulmi* grow as white to cream colored colonies on XED (0.7% Xylan, 0.5% Yeast Extract, 2% Agar) medium that are smooth and mostly flat.

Xylanibacterium ulmi shows high xylanase and β-xylosidase activity, however no cellulase production has been observed. It utilizes a wide range of carbohydrates as carbon source which are listed in the species description.

Xylanibacterium is phylogenetically related to the family *Promicromonosporaceae* (Figure 202) and contains many of the nucleotide signatures as defined by Stackebrandt et al. (1997) and Stackebrandt and Schumann (2000). Nevertheless, *Xylanibacterium* presents five differences at nucleotide positions 384 (A for G), 648 (G for A), 615 (A for U), 624 (C for U), and 1263–1272 (G-C for A-U).

During the last decade *Ulmus nigra* trees were extensively affected by the Dutch elm disease in Spain. Consequently elm trees were eliminated from parks and gardens and the remaining stumps were reduced to a powdery material by the action of humidity. *Xylanibacterium ulmi* was isolated in Spain from one of these stumps.

Enrichment and isolation procedures

For isolation, a sample of wood decay from a stump of an elm tree was collected in aseptic conditions and 1 g was suspended in sterile water and stirred during 60 min. From this suspension, 100 μl were spread on XED medium (xylan, 0.7%; yeast extract, 0.3%; agar, 2.5%) and incubated at 28°C. This organism does not require special conditions to be cultivated although the best growth is obtained on solid media XED incubated at 28°C.

Maintenance procedures

Xylanibacterium ulmi can be maintained in the laboratory by transfer on the same medium used for isolation. For long-term conservation the recommended conditions are storage at −70°C in glycerol suspension (25%, v/v) or lyophilization in the presence of stabilizers such as 50% skim milk.

Procedures for testing special characters

Xylanases are detected on XED plates by direct visualization of hydrolysis haloes and cellulases are detected after 7 d incubation on plates containing 0.5% carboxymethylcellulose as the carbon source, 0.3% yeast extract and 1.5% agar. Plates are stained with a 1% Congo red water solution.

Differentiation of the genus *Xylanibacterium* from other genera

The genus *Xylanibacterium* can be differentiated from other genera of family *Promicromonosporaceae* by comparative analysis of 16S rRNA gene sequence as well as on the basis of chemotaxonomic characteristics recorded in Table 183.

Taxonomic comments

The genus *Xylanibacterium* was created to accommodate the single species *Xylanibacterium ulmi* which at the moment contains a single strain, XIL08 which is the type strain of this species (Rivas et al., 2004). The placement within the family *Promicromonosporaceae* is based on the 16S rRNA gene sequence signatures as well as chemotaxonomic analyses.

Acknowledgements

We thank June M. Brown and colleagues for providing information on the description of *Cellulosimicrobium funkei*.

List of species of the genus *Xylanibacterium*

1. **Xylanibacterium ulmi** Rivas, Trujillo, Schumann, Kroppenstedt, Sánchez, Mateos, Martínez-Molina and Velázquez 2004, 560[VP]

   ul′mi. L. masc. n. *ulmus* elm tree; L. gen. n. *ulmi* of the elm tree, referring to the isolation source of this micro-organism, rotten stump of an elm tree.

   Coccoid cells, 1.1 μm length and 0.8 μm in diameter. Nonmotile.

   Optimal growth in media containing 0.7% xylan as only carbon source. Optimal growth temperature 28°C.

   Xylanases, β-xylosidase and β-galactosidase are produced. Gelatinase production is weak. Esculin is hydrolysed. Nitrate reduction is negative. Arginine dehydrolase, caseinase, tryptophan deaminase or urease are not produced. Acetate, *N*-acetylglucosamine, L-arabinose, cellobiose, D-fructose, galactose, gentiobiose, gluconate, 2 and 5-ketogluconate, glycerol, L-lyxose, D-mannose, ribose, L-sorbose, sucrose, trehalose, D-turanose, xylitol, D-xylose and xylan are used as sole carbon sources. Adipate, adonitol, amygdalin, D-arabinose, D and L-arabitol, arbutin, caproate, citrate, dulcitol, erythritol, D and L-fucose, gluconate, glycogen, inositol, inulin, lactose, malate, maltose, mannitol, melibiose, melezitose, phenylacetate, raffinose, rhamnose, salicin, sorbitol, L-sorbose, starch, D-tagatose, L-xylose methyl α-D-glucoside, methyl α-D-mannoside, *N*-acetylglucosamine or methyl β-xyloside were not used as carbon sources.

   DNA G+C content (mol%): 73 (T_m).

   Type strain: XIL08, LMG 20990, CECT 5975.

   Sequence accession no. (16S rRNA gene): AY273185.

Genus VI. **Xylanimicrobium** Stackebrandt and Schumann 2004, 1385[VP]

Peter Schumann and Erko Stackebrandt

Xy.la.ni.mi.cro′bi.um. N.L. neut. n. *xylanum* xylan, a polysaccharide; N.L. neut. n. *microbium* microbe; N.L. neut. n. *Xylanimicrobium* xylan (-hydrolyzing) microbe.

Gram-stain-positive, non-spore-forming, irregular rod-shaped cells, nonmotile, occurring singly or in pairs. Aerial mycelium not formed. Catalase, oxidase, and aminopeptidase-positive. **Xylanolytic**; facultative anaerobic, acid is produced from some carbohydrates. The murein contains the amino acids lysine, glutamic acid, and serine, belonging to the **peptidoglycan type A4α, variation D-Lys←L-Ser←D-Glu**. *N*-glycolylmuramic acid, mycolic acids, and hydroxy fatty acids are absent. Whole cell sugars are rhamnose, galactose, and glucose. The main **menaquinone is MK-9(H_4). Major fatty acid is $C_{15:0}$anteiso**. **Phospholipids are phosphatidylglycerol, diphosphatidylglycerol, phosphatidylinositol**, and three unknown phospholipids. Phylogenetically, the genus *Xylanimicrobium* is a member of the family *Promicromonosporaceae* with close relationship to *Xylanibacterium* and *Xylanimonas*.

DNA G+C content (mol%): 70.

Type species: **Xylanimicrobium pachnodae (**Cazemier, Verdoes, Reubsaet, Hackstein, van der Drift and Op den Camp 2004) Stackebrandt and Schumann 2004, 1385[VP] (*Promicromonospora pachnodae* Cazemier, Verdoes, Reubsaet, Hackstein, van der Drift and Op den Camp 2004, 1).

Further descriptive information

The genus *Xylanimicrobium* was established as member of the family *Promicromonosporaceae* as consequence of the reclassification of *Promicromonospora pachnodae* Cazemier et al., (2004) as *Xylanimicrobium pachnodae* (Stackebrandt and Schumann, 2004), which in turn was due to only moderate phylogenetic relationship and phenotypic differences when compared to *Promicromonospora citrea*, *Promicromonospora aerolata*, *Promicromonospora sukumoe*, and *Promicromonospora vindobonensis*. *Xylanimicrobium pachnodae* shares with its phylogenetic neighbors *Xylanibacterium ulmi* and *Xylanimonas cellulosilytica* the ability to produce xylanases. Growth of *Xylanimicrobium pachnodae* occurs under aerobic and under anaerobic conditions. Under aerobic conditions in basal medium II (see *Enrichment and isolation procedures*, below) with glucose (5 g/l) as carbon source, nocardioform mycelium-like fringes were formed which were not detected under anaerobic conditions. In the exponential phase of growth, the cells were pleomorphic rods, while in the stationary phase (after 48 h) coccobacilli dominated (Cazemier et al., 2004). *Xylanimicrobium pachnodae* contains a peptidoglycan of type A4α, variation L-Lys←L-Ser←D-Glu (A11.48, according to http://www.peptidoglycan-types.info). The cellular fatty acid profile consists of $C_{15:0}$ anteiso (58.7%), $C_{16:0}$ iso (8.5%), $C_{14:0}$ (8.0%), $C_{15:0}$ iso (6.8%), $C_{16:0}$ (6.1%), $C_{14:0}$ (5.5%), $C_{14:0}$ iso (3.9%), and $C_{17:0}$ anteiso (1.9%) (Cazemier et al., 2004).

Enrichment and isolation procedures

Xylanimicrobium pachnodae VPCX2[T] was isolated after enrichment of bacteria originating from flora of the hindgut of the rose chafer *Pachnoda marginata* larvae in the following media under aerobic and anaerobic conditions (Cazemier et al., 2004):

Medium I: peptone, 5 g/l; yeast extract, 2 g/l; K_2HPO_4, 1 g/l; $MgSO_4 \cdot 7H_2O$, 0.2 g/l; $Na_2S \cdot 9H_2O$, 0.16 g/l; cysteine, 0.32 g/l; Na_2CO_3, 10 g/l (sterilized separately); pH 10.3.

Medium II, modified from Yamin (1978): K_2HPO_4, 1.9 g/l; KH_2PO_4, 0.94 g/l; $NaHCO_3$, 1.68 g/l; KC1, 1.6 g/l; NaCl, 1.43 g/l; NH_4Cl, 0.15 g/l; $MgSO_4 \cdot 7H_2O$, 0.037 g/l; $CaC1_2 \cdot 2H_2O$, 0.017 g/l; yeast extract, 0.1 g/l; $Na_2S \cdot 9H_2O$, 0.16 g/l, and cysteine, 0.32 g/l; 0.2 ml/l trace element solution (Vishniac and Santer, 1957); pH 7–7.2.

Resazurin (0.0001%, w/v) was added as a redox indicator to the anaerobic media. Anaerobic incubations were usually performed in 50-ml serum bottles closed with butyl rubber stoppers and aluminum caps. The gas phase was N_2/CO_2 (80/20, at 0.5 atm overpressure). For cultivation under aerobic conditions $Na_2S \cdot 9H_2O$, cysteine, and resazurin were omitted from media I and II. In addition, $NaHCO_3$ was not added to medium II under aerobic conditions. Cultures were incubated on a rotary shaker (100 r.p.m.) at 30°C.

The hindgut suspension in medium I was plated on solidified medium II containing xylan (1 g/l) or carboxymethylcellulose (10 g/l). Colonies showing cellulolytic or hemicellulolytic activity and penetrating into the agar surface were selected for restreaking on medium II plates containing xylan or carboxymethylcellulose. Incubation under aerobic conditions resulted in a pure culture of strain VPCX2[T].

Maintenance procedures

For preservation, cells are serially transferred at 4-week intervals on medium 92 (http://www.dsmz.de) consisting of 30 g of trypticase soy broth, 3 g of yeast extract, and 15 g of agar per 1000 ml distilled water at 28°C, maintained at 4°C, and stored as 20% (w/v) glycerol suspensions at −20°C and at −80°C. Long-term preservation methods include freeze drying in skim milk and maintenance in liquid nitrogen at −196°C.

Differentiation of the genus *Xylanimicrobium* from other genera

The 16S rRNA gene sequence similarities of the type strain of *Promicromonospora pachnodae* strain VPCX2[T] (Cazemier et al., 2003) to those of *Promicromonospora citrea*, *Promicromonospora aerolata*, *Promicromonospora sukumoe*, and *Promicromonospora vindobonensis* are only in the range 95.5–96.1% whereas the authentic members of the genus *Promicromonospora* share similarity values between 98.4 and 99.2%. The closest phylogenetic neighbors of *Xylanimicrobium pachnodae* are *Xylanibacterium ulmi* XIL08[T] (Rivas et al., 2004) and *Xylanimonas cellulosilytica* XIL07[T] (Rivas et al., 2003), showing 16S rRNA gene sequence similarity of >97.5% (see Figure 202). This observation gave rise to search for additional differentiating characteristics to support the separate generic status of strain VPCX2[T]. These are, besides morphological differences to separate them from authentic *Promicromonospora* members, facultative anaerobic growth, a significantly lower metabolic activity, the absence of the fatty acid $C_{17:0}$ iso as well as the occurrence of L-serine instead of L-alanine in the peptidoglycan interpeptide bridge of strain VPCX2[T]. The peptidoglycan variation L-Lys←L-Ser←D-Glu differentiates *Xylanimicrobium pachnodae* from all members of the family *Promicromonosporaceae*. *Xylanimicrobium pachnodae* differs from its closest phylogenetic

neighbors *Xylanibacterium ulmi* and *Xylanimonas cellulosilytica* additionally in morphological characteristics, the absence of phosphatidylinositol mannosides, the absence of MK-8(H_4) as a minor isoprenoid quinone component (Table 177), and utilization of *N*-acetylglucosamine but not 5-ketogluconate (Stackebrandt and Schumann, 2004). *Xylanimicrobium pachnodae* differs from members of *Myceligenerans* in the lack of a primary mycelium and the presence of serine instead of threonine in the interpeptide bridge of peptidoglycan. It differs from *Cellulosimicrobium* species in the lack of a fragmenting primary mycelium and in the presence of glutamic acid, rather than aspartic acid, in the interpeptide bridge of peptidoglycan. Also, *Xylanimicrobium pachnodae* lacks $C_{16:0}$ iso as a major fatty acid.

List of species of the genus *Xylanimicrobium*

1. **Xylanimicrobium pachnodae** (Cazemier, Verdoes, Reubsaet, Hackstein, van der Drift and Op den Camp 2004) Stackebrandt and Schumann 2004, 1385[VP] (Basonym: *Promicromonospora pachnodae* Cazemier, Verdoes, Reubsaet, Hackstein, van der Drift and Op den Camp 2004.).

pach.no′da.e. N.L. gen. n. *pachnodae* of *Pachnoda* referring to the source of the micro-organism, *Pachnoda marginata*.

In addition to the properties given in the genus description, the description is that of *Promicromonospora pachnodae*, and data included in the publication of Cazemier et al. (2004), supplemented with data of Stackebrandt and Schumann (2004). In the exponential phase of growth, cells are pleomorphic rods (0.4–0.6 × 0.6–3.0 μm), whereas, in the stationary phase, spherical cells dominate (diameter 0.5–0.7 μm). Under aerobic conditions in basal medium II with glucose as carbon source, nocardioform mycelia-like fringes predominate; these forms are absent under anaerobic conditions. In rich media, cells are more homogeneously distributed. Aerobic to facultatively anaerobic, fermenting glucose, xylose, maltose, lactose (weak), and sucrose; fermentation products of glucose or xylose are formate, lactate, ethanol, and acetate but not succinate. Aerobically, cells grow with high densities in media with beach litter, NaOH-pretreated beach litter, xylan, carboxymethylcellulose, cellobiose, glucose, xylose, and brain heart infusion medium. Optimum growth at pH 7.5 and 35°C. Similar pH and temperature optima are found under anaerobic conditions. Nitrate reduction is positive, gelatin is hydrolyzed. Major fatty acids are $C_{15:0}$ anteiso, $C_{16:0}$ iso, $C_{14:0}$, and $C_{15:0}$ iso. The following compounds of the API 50 CHE gallery were used: glycerol, L-arabinose, D-xylose, D-lyxose, galactose, D-fructose, D-mannose, esculin, cellobiose, sucrose, trehalose, β-gentiobiose, D-turanose, acetate, *N*-acetylglucosamine, amygdalin, lactose, maltose, and tagatose. The following reactions are observed in the Biolog GP2 microtiter plate substrate panel (only strong positive reactions that are not already indicated for the API 50 CHE test are listed): glycogen, mannan, Tween 40, D-glucose, lactulose, methyl β-D-galactoside, 3-methyl glucose, methyl-α-D-glucoside, palatinose, D-psicose, D-ribose, methyl pyruvate, 2′-deoxyadenosine, inosine, uridine, adenosine 5′-monophosphate, thymidine 5′-monophosphate, fructose 6-phosphate, glucose 1-phosphate, and glucose 6-phosphate.

Source: intestine of the rose chafer *Pachnoda marginata* (Scarabaeidae, Coleoptera).

DNA G+C content (mol%): 70.0 (HPLC).

Type strain: VPCX2, DSM 12657, JCM 13526, NCCB 100020.

Sequence accession no. (16S rRNA gene): AF105422.

Genus VII. **Xylanimonas** Rivas, Sánchez, Trujillo, Zurdo-Piñeiro, Mateos, Martínez-Molina and Velázquez 2003, 102[VP]

Encarna Velázquez, Raúl Rivas and Pedro F. Mateos

Xy.la.ni.mo′nas. N.L. n. *xylanum* xylan a polysaccharide; Gr. fem. n. *monas* a monad, unit; N.L. fem. n. *Xylanimonas* a monad from xylan.

Gram-stain-positive, non-spore forming coccoid cells. Aerial mycelium is not formed. **Aerobic or facultatively anaerobic**, chemo-organotropic, **and xylanolytic.** Optimum temperature 30°C and pH 7. Oxidase-positive. Catalase weak positive. Phylogenetically related to members of the family *Promicromonosporaceae* on basis of the 16S rRNA gene sequence. **Peptidoglycan type is A4α, L-Lys–D-Asp.** Cell-wall sugars are **galactose and rhamnose.** Major menaquinones are **MK-9(H_4)** and **MK-8(H_4)**. Predominant fatty acids are $C_{15:0}$ anteiso and $C_{15:0}$ iso. Mycolic acids are absent. The polar lipids detected were phosphatidylglycerol (PG), diphosphatidylglycerol (DPG), phosphatidylinositol (PI), and phosphatidylinositol mannoside (PIM).

DNA G+C content (mol%): 73.

Type species: **Xylanimonas cellulosilytica** Rivas, Sánchez, Trujillo, Zurdo-Piñeiro, Mateos, Martínez-Molina and Velázquez 2003, 102[VP].

Further descriptive information

The genus is currently represented by a single species, *Xylanimonas cellulosilytica*, which was isolated from a decayed tree (*Ulmus nigra*). *Xylanimonas cellulosilytica* typically appears as nonmotile and non-sporulated coccoid cells that are 1.1 μm × 0.8 μm.

The cell-wall composition of *Xylanimonas cellulosilytica* (Table 177) differs from that of other genera in the family *Promicromonosporaceae*. The peptidoglycan type of *Xylanimonas* is A4α, L-Lys–D-Asp, which is identical to that reported for the genus *Isoptericola* (Stackebrandt et al., 2004). Nevertheless, it differs from the composition reported for the remaining genera within this family. Although other genera in the family *Promicromonosporaceae* contain high amounts of rhamnose and galactose, these are the principal cell-wall sugars of *Xylanimonas*. The major polar lipids of the genus *Xylanimonas* are phosphatidylglycerol (PG), diphosphatidylglycerol (DPG), phosphatidylinositol (PI), phosphatidylinositol mannoside (PIM), and other unidentified phosphoglycolipids (PGL). This profile is basically identical to that obtained for the genus *Xylanibacterium* (Rivas et al., 2004) but not for the remaining genera of the family *Promicromonosporaceae*. PG and DPG are present in all genera of this family, but PIM is present only in

the genus *Xylanibacterium* (Rivas et al., 2004). The main fatty acids detected in *Xylanimonas* are $C_{15:0}$ anteiso, $C_{15:0}$ iso, and $C_{14:0}$. According to the published data, this cellular fatty acid pattern differs from that of the remaining genera of the family *Promicromonosporaceae*, although $C_{15:0}$ anteiso is the main fatty acid in all genera of the family. The main menaquinone in all genera of the family *Promicromonosporaceae* is MK-9(H_4), but MK-8(H_4) is also present in *Xylanimonas* and *Xylanibacterium* (Rivas et al., 2004).

Colonies of *Xylanimonas cellulosilytica* on XED medium (0.7% xylan, 0.5% yeast extract, and 2% agar) are circular, convex, white, opaque, and usually 1–3 mm in diameter within 7 d at 28°C. *Xylanimonas cellulosilytica* shows high xylanase and cellulase activity. It utilizes a wide range of carbohydrates as carbon sources listed in the species description.

Xylanimonas is phylogenetically related to the family *Promicromonosporaceae* (Please refer to Figure 202 in the chapter on *Promonomicrosporaceae*) and contains many of the nucleotide signatures as defined by Stackebrandt et al. (1997) and Stackebrandt and Schumann (2000).

Enrichment and isolation procedures

For isolation, a sample of sawdust from a decayed tree, *Ulmus nigra*, was collected under aseptic conditions. One gram was suspended in sterile water and stirred 30 min. This suspension (100 µl) was spread on XED medium (xylan 0.7%, yeast extract 0.3%, and agar 2.5%) and incubated at 28°C. Cultivation of this organism does not require special conditions although growth is better on solid XED medium after incubation at 28°C.

Maintenance procedures

The organism can be maintained in the same medium used for isolation. For long-term conservation, the recommended conditions are storage at −70°C in 25% (v/v) glycerol or lyophilization in the presence of stabilizers such as 50% skim milk.

Procedures for testing special characters

Xylanases are detected on XED plates by direct visualization of hydrolysis haloes, and cellulases are detected after 7 d incubation on plates containing 0.5% carboxymethylcellulose as the carbon source, 0.3% yeast extract, and 1.5% agar. Plates are stained with a 1% aqueous Congo red.

Differentiation of the genus *Xylanimonas* from other genera

This genus can be differentiated from other related genera of family *Promicromonosporaceae* by comparative analysis of 16S rRNA gene sequences as well as on basis of chemotaxonomic and physiological characteristics (Table 177).

List of species of the genus *Xylanimonas*

1. **Xylanimonas cellulosilytica** (Rivas, Sánchez, Trujillo, Zurdo-Piñeiro, Mateos, Martínez-Molina and Velázquez 2003, 102[VP]

cel.lu.lo.si.ly′ti.ca. N.L. n. *cellulosum* cellulose; N.L. adj. *lyticus -a -um* (from Gr. adj. *lutikos -ê -on*) able to loosen, able to dissolve; N.L. fem. adj. *cellulosilytica* cellulose-dissolving.

Coccoid cells, 1.1 µm in length and 0.8 µm in diameter. Nonmotile. Grows optimally in media containing 0.7% xylan as carbon source. Optimal growth temperature is 28°C.

Amylases, cellulases, gelatinase, xylanases, and β-galactosidase are produced. Esculin is hydrolyzed. Nitrate reduction and acetoin production (Voges–Proskauer medium) are positive. Arginine dihydrolase, caseinase, indole, lysine decarboxylase, ornithine decarboxylase, tryptophan deaminase, and urease are negative. Arabinose, carboxymethylcellulose, mannose, maltose, rhamnose, starch, and xylan are used as sole carbon sources. No growth in acetate, adonitol, D-arabinose, citrate, dulcitol, erythritol, gluconate, inositol, inulin, malate, mannitol, raffinose, sorbitol, L-sorbose, L-xylose, methyl α-D-glucoside, methyl α-D-mannoside, *N*-acetylglucosamine or methyl β-D-xyloside. Acid production from amygdalin, L-arabinose, arbutin, cellobiose, fructose, galactose, gentobiose, glucose, glycerol, glycogen, lactose, lyxose, maltose, mannose, melezitose, rhamnose, salicin, sucrose, trehalose, turanose, and D-xylose.

Source: a stump of *Ulmus nigra* affected by the Dutch elm disease in Spain.

DNA G+C content (mol%): 73 (T_m).

Type strain: XIL07, CECT 5975, JCM 12276, LMG 20990.

Sequence accession no. (16S rRNA gene): AF403541.

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Family XIII. **Rarobacteraceae** Stackebrandt and Schumann 2000, 1284^VP emend. Zhi, Li and Stackebrandt 2009, 598

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Ra.ro.bac.te.ra.ce′a.e. N.L. masc. n. *Rarobacter* type genus of the family; L. suff. *-aceae* ending to denote a family; N.L. fem. pl. n. *Rarobacteraceae* the *Rarobacter* family.

The family *Rarobacteraceae* belongs to the order *Micrococcales* (reclassified for this volume from suborder *Micrococcineae* Stackebrandt, Rainey and Ward-Rainey 1997 of the class *Actinobacteria* Stackebrandt, Rainey and Ward-Rainey 1997.

Chemotaxonomically, the family is defined by peptidoglycan type A4β, MK-9(H_4) as predominant menaquinone and iso- and anteiso-branched cellular fatty acids. The pattern of 16S rRNA gene sequence signatures consists of nucleotides at positions: 120 (A), 131:231 (C–G), 196 (A), 342:347 (C–G), 444:490 (A–U), 580:761 (C–G), 602:636 (G–U), 670:736 (A–U), 822:878 (G–C), 823:877 (G–C), 826:874 (C–G), 827 (U), 843 (U), 950:1231 (U–A), 1047:1210 (G–C), 1109 (C), 1145 (G), 1309:1328 (G–C), 1361 (G), 1383 (C) (Zhi et al., 2009).

Type genus: **Rarobacter** Yamamoto, Sato, Saito, Hasuo, Tadenuma, Suzuki, Tamaoka and Komagata 1988, 10^VP.

Taxonomic comments

The family *Rarobacteraceae* Stackebrandt and Schumann (2000) was established to accommodate *Rarobacter* as the only genus because of its distinct phylogenetic position within the suborder *Micrococcineae* and the presence of a taxon-specific set of 16S rRNA signature nucleotides (Stackebrandt and Schumann, 2000). Phylogenetically, it is most closely related to the family *Sanguibacteraceae* Stackebrandt and Schumann 2000.

Genus I. **Rarobacter** Yamamoto, Sato, Saito, Hasuo, Tadenuma, Suzuki, Tamaoka and Komagata 1988, 10[VP]

Peter Kämpfer

Ra.ro.bac′ter. L. adj. *rarus* curious; N.L. masc. n. *bacter* masculine form from Gr. neut. n. *baktron* a rod; N.L. masc. n. *rarobacter* curious rod.

Cells are irregular rods, 0.2–0.3 × 0.8–1.0 μm. **Gram-stain-positive, but readily decolorized. Not acid-fast. Motile by multitrichous flagella.** Nonsporeforming. Optimal temperature is 30°C, and optimal pH is 6–8 for growth. **Facultatively anaerobic.** Chemo-organotrophic. **Mesophilic. Requires hemin of hemoproteins for growth.** Ammonium salts are utilized as nitrogen source, but nitrate is not utilized. Acid is produced aerobically and anaerobically from D-glucose and D-mannose, but not from L-arabinose or L-rhamnose. Organic acids studied thus far are not assimilated. Starch, casein, and gelatin are hydrolyzed, but cellulose is not hydrolyzed. Oxidase-positive. **Diamino acid of cell wall is L-ornithine. Amino acid composition of the cell-wall peptidoglycan is D-Ala, L-Ala, D-Glu, and L-Orn (and D-Ser) corresponding to type A4β.** Mycolic acids and cell-wall teichoic acids are lacking. Branching fatty acids of the iso and anteiso types ($C_{16:0}$ iso and $C_{15:0}$ anteiso) predominate. Major **menaquinone** is **MK-9(H_4)**.

The genus *Rarobacter* is the type genus of the family *Rarobacteraceae* in the order *Micrococcales*.

DNA G+C content (mol%): 64.6–66.1.

Type species: **Rarobacter faecitabidus** Yamamoto, Sato, Saito, Hasuo, Tadenuma, Suzuki, Tamaoka and Komgata 1988, 10[VP].

Further descriptive information

The genus *Rarobacter* was established by Yamamoto et al. (1988) to harbor what was at that time the only species, *Rarobacter faecitabitus*. It was described on the basis of four yeast-lysing bacteria from wastewater. One additional species, *Rarobacter incanus*, was described later (Goto-Yamamoto et al., 1993).

Members of the genus are yeast-lysing, facultatively anaerobic bacteria which require hemin or hemoproteins as essential factors for aerobic growth and carbon dioxide for anaerobic growth. Members of the genus *Rarobacter* form motile, irregular rods. Sometimes V-shaped elements are observed. Cells are motile by multitrichous flagella. *Rarobacter* cells can adhere to and agglutinate yeast cells and subsequently lyse them to utilize the digested cells as nutrients. A number of lytic enzymes have been identified, one of which recognizes mannose chains of mannoproteins (Hasuo et al., 1984). Strains of *Rarobacter faecitabidus* were isolated from wastewater treatment systems of alcoholic beverage factories (Yamamoto et al., 1988). Species are considered nonpathogenic, and they are characterized by the peptidoglycan variation A4β with L-ornithine as diagnostic diamino acid (Schleifer and Kandler, 1972) and unsaturated menaquinones with nine isoprenoid units. Branching fatty acids of the iso- and anteiso- types ($C_{16:0}$ iso and $C_{15:0}$ anteiso) are predominant. Colonies are pale-grayish cream and are usually circular, and convex. *Rarobacter faecitabitus* grows under aerobic conditions well on YM- catalase-agar containing 5 g yeast extract, 5.0 g malt extract, 5.74 g K_2HPO_4, 205 mg $MgSO_4 \cdot 7H_2O$, 1.15 g $NH_4H_2PO_4$, 60 mg catalase C-10 (Sigma), and 15 g of agar (Yamamoto et al., 1988). Strains of *Rarobacter incanus* grow under aerobic conditions at 28–30°C on TYMC agar containing 5 g trypticase peptone, 3.0 g yeast extract, 3.0 g malt extract, 5.8 g K_2HPO_4, 1.15 g $MgSO_4 \cdot 7H_2O$, 0.12 g catalase C-10 (Sigma), and 15 g agar per liter. Members of the genus *Rarobacter* were isolated from wastewater treatment systems of alcoholic beverage factories.

Phylogenetically, they are most closely related to *Sanguibacter* with 16S rRNA gene sequence similaries ranging from 94.6–95.5%. *Rarobacter faecitabitus* and *Rarobacter incanus* share approximately 96.8% 16S rRNA gene sequence similarity. The ranges of DNA–DNA similarity values between both species are below 40%. Species of the genus *Rarobacter* can be differentiated unambiguously by chemotaxonomic characteristics such as the amino acid compositions of the cell-wall peptidoglycan, the G+C content of the DNA, and catalase activity.

Enrichment and isolation procedures

The strains of *Rarobacter faecitabitus* and *Rarobacter incanus* were isolated from the wastewater of wastewater treatment systems of alcoholic beverage factories on YM-catalase agar and TYMC agar. (Goto-Yamamoto et al., 1993; Yamamoto et al., 1988). They have a requirement for hemin of hemoproteins.

Maintenance procedures

Serial transfers at 3-months intervals followed by maintenance at 4°C and storage of cells as 20% (w/v) glycerol suspensions at −20°C and at −80°C were suitable provisions for preservation. Long term preservation methods are freeze-drying in skim milk and maintenance in liquid nitrogen at −196°C.

Differentiation of the genus *Rarobacter* from other genera

Authentic members of the genus *Rarobacter* represent a distinct cluster within the phylogenetic tree of the family *Rarobacteraceae* (Stackebrandt and Schumann, 2000). The genus can be differentiated from the most closely related genus *Sanguibacter* by several chemotaxonomic features (Table 184).

TABLE 184. Characteristics differentiating *Rarobacter* and *Sanguibacter*[a,b]

Characteristic	*Rarobacter*	*Sanguibacter*
Diamino acid	L-Orn	L-Lys
Interpeptide bridge	Ser–Glu or Glu	Ser–D-Glu
Major menaquinone	MK-9	MK-9(H_4)
Predominant cellular fatty acids	$C_{15:0}$ anteiso, $C_{16:0}$ iso, $C_{14:0}$ (aerobic)	$C_{16:0}$, $C_{15:0}$ anteiso, $C_{18:0}$
DNA G+C content (mol%)	65–66	69–70

[a]Abbreviations: L-Orn, L-ornithine; L-Lys, lysine; Ser, serine; Glu, glutamic acid.
[b]Data from Goto-Yamamoto et al. (1993), Fernández-Garayzábal et al. (1995), and Pascual et al. (1996).

List of species of the genus *Rarobacter*

1. **Rarobacter faecitabidus** Yamamoto, Sato, Saito, Hasuo, Tadenuma, Suzuki, Tamaoka and Komagata 1988, 10[VP]

fa.e.ci.ta′bi.dus. L.fem.n. *faex*, *faecis* dregs; L.adj. *tabidus* dissolving; N.L. masc. adj. *faecitabidus* dreg dissolving.

In addition to the properties given in the genus description, the species has the following characteristics. Facultative anaerobic. Requires hemin or hemoproteins besides biotin and thiamine as growth factors in the aerobic condition. Requires carbon dioxide but not hemin or hemoproteins for anaerobic growth. Colonies on nutrient-catalase are pale yellow, opaque, circular, convex, entire, and smooth. Acid is produced aerobically and anaerobically from D-glucose, D-fructose, D-mannose, maltose, cellobiose, dextrin, arbutin, and salicin, but not from L-arabinose, L-rhamnose, D-galactose, L-sorbose, lactose, trehalose, raffinose, inulin, glycerol, erythritol, adonitol, mannitol, dulcitol, D-sorbitol, inositol, esculin, or α-methyl-D-glucoside. Hydrogen sulfide is not produced. DNase and urease are negative. Catalase positive. Amino acid composition of cell-wall peptidoglycan is D-Ala, L-Ala, D-Glu, L-Orn, and D-Ser (1:1:2:1:1). Adheres to viable yeast cells of genera *Saccharomyces* and *Hansenula*, agglutinates with them, and lyses them.

DNA G+C content (mol%): 65.7–66.1 (HPLC).

Type strain: YLM-1, ATCC 49628, CIP 103380, DSM 4813, NBRC 14760, JCM 6097.

Sequence accession no. (16S rRNA gene): AB056127, Y17870.

2. **Rarobacter incanus** Goto-Yamamoto, Sato, Miki, Park and Tadenuma 1994, 370[VP] (Effective publication: Goto-Yamamoto, Sato, Miki, Park and Tadenuma 1993, 274.)

in.ca′nus. L. masc. adj. *incanus* grayish white.

In addition to the properties given in the genus description, the species has the following characteristics. Hemin or hemoproteins are required as growth factors under aerobic condition. Carbon dioxide but not hemin or hemoproteins are required for anaerobic growth. Colonies on nutrient-catalase agar are pale grayish cream, opaque, circular, convex, entire, and smooth. Acid is produced aerobically and anaerobically from D-glucose, D-fructose, D-mannose, maltose, dextrin, arbutin and salicin, but not from L-arabinose, L-rhamnose, D-galactose, L-sorbose, lactose, trehalose, raffinose, inulin, glycerol, erythritol, adonito, mannitol, dulcitol, D-sorbitol, or α-methyl-D-glucoside. Type strain does not produce hydrogen sulfide, and produces acid from cellobiose, however, some strains produce hydrogen sulfide, and do not produce acid from cellobiose. DNase, urease, and catalase were negative. Amino acid composition of cell-wall peptidoglycan is D-Ala–L-Ala–D-Glu–L-Orn (1:1:2:1 ratio). Adheres to viable yeast cells of the genera *Saccharomyces* and *Hansenula*, agglutinates with them, and lyses them. The other description of the type strain is the same as that of the species *Rarobacter incanus*.

DNA G+C content (mol%): 64.6–65 (HPLC).

Type strain: YLM-32, ATCC 51544, CIP 104132, DSM 10596, NBRC 16558, JCM 6350, NBRC 101357.

Sequence accession no. (16S rRNA gene): AB056129.

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Family XIV. **Ruaniaceae** Tang, Zhi, Wang, Wu, Lee, Kim, Lou, Xu and Li 2010, 2118[VP]

Shu-Kun Tang, Xiao-Yang Zhi and Wen-Jun Li

Ru.a.ni.a.ce′a.e. N.L. fem. n. *Ruania* type genus of the family; suff. -*aceae* ending to denote a family; N.L. fem. pl. n. *Ruaniaceae* the *Ruania* family.

The family *Ruaniaceae* was circumscribed for this volume based mainly on signature nucleotide patterns and phylogenetic criteria; the family contains the genera *Ruania* and *Haloactinobacterium*. Members of the family *Ruaniaceae* are Gram-stain-positive, aerobic and facultatively anaerobic, nonmotile, halophilic or halotolerant, and short rod or coccoid actinobacteria. MK-8(H_4) is the predominant menaquinone and A4α is the peptidoglycan type. The pattern of 16S rRNA signature nucleotides is shown in Table 185.

DNA G+C content (mol%): 68–70.

Type genus: **Ruania** Gu, Paściak, Luo, Gamian, Liu and Huang 2007, 811[VP].

Taxonomic comments

The genus *Ruania* was first proposed by Gu et al. (2007), but it was not classified within a family in the suborder *Micrococcineae*, now order *Micrococcales*. Another novel genus *Haloactinobacterium*, also now in the order *Micrococcales*, was established by Tang et al. (2010).

Representatives of known families in the order *Micrococcales* showed 16S rRNA gene sequence similarities to those of the genera *Ruania* and *Haloactinobacterium* of between 92.0 and 95.2%. Although the genera *Ruania* and *Haloactinobacterium* clearly belong to the order *Micrococcales*, they did not belong to any of the previously described families. Comparative 16S rRNA gene sequence analysis showed that the genera *Ruania* and *Haloactinobacterium* formed a deep branch that could be clearly distinguished from members of other described families (Figure 206). The 16S rRNA gene sequences of 15 families of the suborder *Micrococcineae* (Zhi et al., 2009), in this volume reclassified as the order *Micrococcales*, and the genera *Ruania* and *Haloactinobacterium* were scanned for the signature nucleotide patterns (Table 185). However, only one difference was found between the genera *Ruania* and *Haloactinobacterium*: at position 602:636 *Haloactinobacterium* has a C–G pair and *Ruania* has a G–U pair. However, members of both genera have many unique 16S rRNA gene signature nucleotides compared to the known family lineages (Table 185). There is only one unique signature: C–U at 444:490. All other signatures were found in at least one other family of the order *Micrococcales*. Higher hierarchical taxa in the class *Actinobacteria* are defined based mainly on signature nucleotide patterns and phylogenetic criteria (Stackebrandt et al., 1997; Zhi et al., 2009). Accordingly, the genera *Ruania* and *Haloactinobacterium* are phylogenetically closely related and are clearly distinct from members of other families in the order *Micrococcales*. Thus, the novel family *Ruaniaceae* was proposed to accommodate the genera *Ruania* and *Haloactinobacterium* (Tang et al., 2010).

TABLE 185. Patterns of 16S rRNA gene signature nucleotides detected in the family *Ruaniaceae* and the defined families of the order *Micrococcales*[a]

Position	*Ruaniaceae*	*Beutenbergiaceae*	*Bogoriellaceae*	*Brevibacteriaceae*	*Cellulomonadaceae*	*Dermabacteraceae*	*Dermacoccaceae*	*Dermatophilaceae*	*Intrasporangiaceae*	*Jonesiaceae*	*Microbacteriaceae*	*Micrococcaceae*	*Promicromonosporaceae*	*Rarobacteraceae*	*Sanguibacteraceae*	*Yaniella*[b]
120	A	A	A	A	A	A	A	A	A	A	A	W	A	A	A	U
131:231	A–G	C–G	A–G	C–G	C–G	C–G	A–G	Y–K	A–G	A–G	G–R	C–G	A–G	C–G	C–G	A–G
196	C	G	U	A	U	U	C	A	G	C	U	C	U	A	U	C
342:347	C–G	C–G	U–G	C–G	C–G	C–G	C–G	C–G	C–G	C–G	C–G	C–G	C–G	C–G	C–G	C–G
444:490	C–U	A–U	A–U	A–U	A–U	A–U	A–U	A–U	A–U	A–U	A–U	A–U	A–U	A–U	A–U	U–U
580:761	C–G	C–G	C–A	C–G	C–G	U–A	U–A	U–A	U–A	C–G	C–G	C–G	C–G	C–G	C–G	C–G
602:636	S–K	C–G	C–G	C–G	C–G	C–G	C–G	C–G	C–G	C–G	C–G	C–G	G–U	G–U	G–U	C–G
670:736	A–U	A–U	A–U	U–A	A–U	A–U	A–U	A–U	A–U	A–U	A–U	A–U	A–U	A–U	A–U	A–U
822:878	G–C	G–C	G–C	G–C	G–C	G–C	G–C	G–C	G–C	U–C	G–C	G–C	G–C	G–C	G–C	G–C
823:877	G–C	G–C	G–C	G–C	G–C	G–C	G–C	G–C	G–C	A–G	G–C	G–C	G–C	G–C	G–C	G–C
826:874	C–G	C–G	C–G	C–G	C–G	C–G	C–G	C–G	C–G	U–G	C–G	C–G	C–G	C–G	C–G	C–G
827	U	U	U	U	U	U	U	U	U	G	U	U	U	U	U	U
843	U	U	C	C	U	C	C	U	U	C	C	C	U	U	C	U
950:1231	U–A	U–A	U–A	U–A	U–A	U–G	U–A	U–A	U–A	U–A	U–A	U–A	U–A	U–A	U–A	U–A
1047:1210	G–C	G–C	G–C	G–U	G–C	G–C	G–C	G–C	G–C	G–C	G–C	G–C	G–C	G–C	G–C	G–C
1109	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	G
1145	G	G	G	A	G	G	G	G	G	G	R	G	G	G	G	G
1309:1328	G–C	G–C	A–U	G–C	G–C	G–U	G–C	G–C	G–C	G–C	G–C	G–C	G–C	G–C	G–C	G–C
1361	G	G	G	G	G	G	G	G	C	G	G	G	G	G	G	G
1383	U	U	U	C	Y	C	C	C	C	C	C	C	C	C	C	C

[a]Data from Tang et al. (2010).

[b]*Yaniella* is now classified within the *Micrococcaceae* and is shown for comparison.

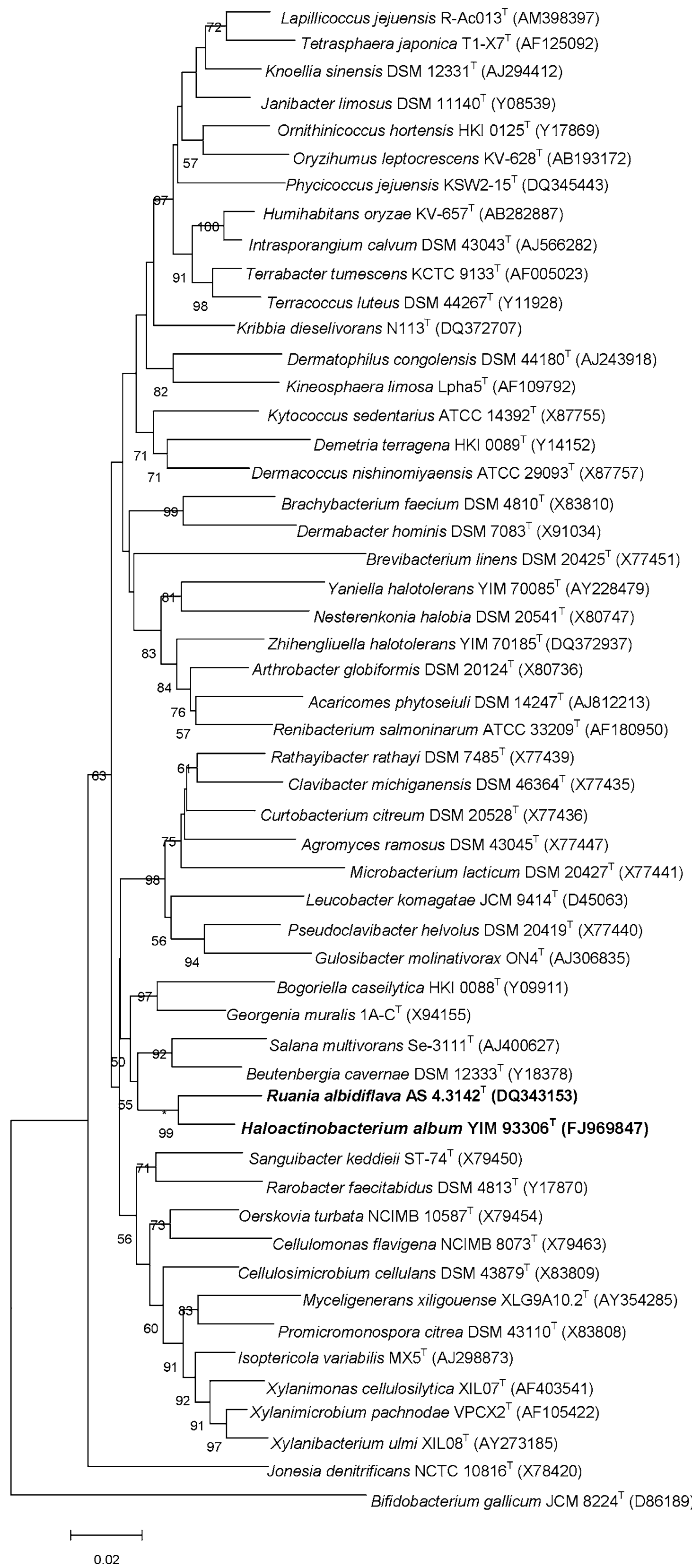

FIGURE 206. Phylogenetic dendrogram obtained by distance matrix analysis of 16S rRNA gene sequences showing the position of the genera *Ruania* and *Haloactinobacterium* and their phylogenetic neighbors. Asterisks indicate branches of the tree that were also found using the maximum-likelihood (Felsenstein, 1981) and maximum-parsimony (Fitch, 1971) tree-making algorithms. Numbers at nodes indicate levels of bootstrap support (%) based on a neighbor-joining analysis of 1000 resampled datasets (1000 resamplings, only values over 50% are given). The sequence of *Bifidobacterium gallicum* JCM 8224^{T} (D86189) was used as an outgroup. Bar = 0.2 substitutions per nucleotide position.

Genus I. **Ruania** Gu, Paściak, Luo, Gamian, Liu and Huang 2007, 811[VP]

Hans-Jürgen Busse

Ru.a.n′i.a. N.L. fem. n. *Ruania* named after Ji-Sheng Ruan, a Chinese microbiologist who has made great contributions to the development of actinomycete taxonomy in China.

Gram-stain-positive, aerobic, mesophilic, moderately halotolerant, non-acid-fast, nonmotile, non-spore-forming cocci. The rod–coccus life cycle is not detected. The **peptidoglycan type is A4α** with an **L-Lys–Gly–L-Glu–L-Glu interpeptide bridge**. The predominant menaquinone **is MK-8(H_4)**. The **main fatty acid is $C_{15:0}$ anteiso**. The major **polar lipids are phosphatidylglycerol, diphosphatidylglycerol**, and one **unknown glycolipid**. Mycolic acids are absent. Phylogenetically, the genus belongs to the order *Micrococcales*.

DNA G+C content (mol%): 69.8.

Type species: **Ruania albidiflava** Gu, Paściak, Luo, Gamian, Liu and Huang 2007, 812[VP].

Further descriptive information

The single species of the genus *Ruania*, *Ruania albidiflava* has been identified as a member of the suborder *Micrococcineae*, now order *Micrococcales* in the taxonomic outline to the present volume, based on its phylogenetic position (Gu et al., 2007) and the genera *Georgenia* and *Bogoriella* were identified as nearest related genera. The species shares with representatives of these two genera the peptidoglycan type A4α, which is based on the presence of the diamino acid L-lysine and an interpeptide bridge containing a dicarboxylic amino acid (L-glutamic acid), a quinone system with the predominant compound MK-8(H_4), a fatty acid profile with $C_{15:0}$ anteiso as the major compound, a polar lipid profile containing among other components diphosphatidylglycerol and phosphatidylglycerol, and a similar G+C content (70 mol%). Hence, this genus was initially considered as a member of the *Bogoriellaceae* (Stackebrandt and Schumann, 2000). Subsequent analyses suggested that it should be placed in a novel family, *Ruaniaceae* (Tang et al., 2010).

Differentiation of the genus *Ruania* from related genera

The genus *Ruania* is so far represented by a single species, *Ruania albidiflava*, which can be distinguished from its close phylogenetic relatives in the genera *Beutenbergia*, *Bogoriella*, *Georgenia*, and *Salana* by a set of signature nucleotides in the 16S rRNA coding gene (Table 185), differences in the interpeptide bridge of the peptidoglycan, and differences in the major fatty acid profile components (Table 186).

Enrichment and isolation procedures

Enrichment of *Ruania* may be done at approximately 28°C on R-agar* (Yamada and Komagata, 1972), nutrient agar, or trypticase soy agar. Isolation from soil can be done on yeast extract-starch agar (JCM medium no. 42) incubated for 1 week under humid conditions at 28°C.

Pathogenicity

No reports on pathogenicity have been found in the literature.

Maintenance procedures

Cultures of *Ruania albidiflava* can be maintained on nutrient agar slants at 4°C.

TABLE 186. Differential characteristics of the genera *Ruania*, *Beutenbergia*, *Borgoriella*, *Georgenia*, and *Salana*

Characteristic	*Ruania*	*Beutenbergia*	*Bogoriella*	*Georgenia*	*Salana*
Morphology	Coccoid	Rod-coccus cycle	Irregular rod or coccoid	Short rods or rod-coccus cycle	Rod, coccus, club-like
Interpeptide bridge	L-Lys–Gly–L-Glu–L-Glu	L-Lys–L-Glu	L-Lys–L-Ala–L-Ala–L-Glu	L-Lys–L-Glu	L-Ser/L-Orn–L-Glu
Polar lipids[a]	PG, DPG, 1GL	PI, DPG, 3PL	PI, PG, DPG, 1PL	PG, DPG, PIM, 2PL, 1GL	PG, DPG, PLs
Major fatty acid(s)	$C_{15:0}$ anteiso, $C_{17:0}$ anteiso, $C_{16:0}$ iso	$C_{15:0}$ iso, $C_{15:0}$ anteiso	$C_{15:0}$ anteiso	$C_{15:0}$ anteiso, $C_{14:0}$ iso, $C_{15:1}$ anteiso	$C_{16:0}$ iso, $C_{14:0}$, $C_{16:0}$
DNA G+C content (mol%)	70	71	70	70-73	75

[a]DPG, Diphosphatidylglycerol; *n*GL, *n* unknown glycolipid(s); PG, phosphatidylglycerol; PI, phosphatidylinositol; PIM, phosphatidylinositol mannoside; *n*PL, *n* unknown phospholipid(s).

*R-agar has the following composition (w/v): peptone, 1%; yeast extract, 0.5%; Casamino acids, 0.5%; meat extract, 0.2%; malt extract, 0.5%; glycerol, 0.2%; $MgSO_4 \cdot 7H_2O$, 0.1%; Tween 80, 0.005%; agar-agar, 1.5%; pH 7.2

List of species of the genus *Ruania*

1. **Ruania albidiflava** Gu, Paściak, Luo, Gamian, Liu and Huang 2007, 812[VP]

   al.bi.di.fla′va. L. adj. *albidus* white; L. fem. adj. *flava* yellow; N.L. fem. adj. *albidiflava* whitish yellow.

   Cells are 0.5–0.8 µm in diameter. Colonies are convex, moist, and pale yellow in color. Growth occurs at 20–37°C, with an optimum at 28°C, and at a wide initial pH range of 5.5–12.5, with an optimum at pH 6.5–10.5. Can tolerate up to 10% (w/v) NaCl. Catalase-positive, oxidase-negative. The methyl red test is positive, but indole and Voges–Proskauer reactions are negative. Nitrate is reduced to nitrite. H_2S is not produced. Potato starch is decomposed, but adenine, casein, esculin, gelatin, hippurate, hypoxanthine, tyrosine, urea, and xanthine are not. Acid is produced from arbutin, salicin, D-ribose, methyl β-D-xylopyranoside, D-fructose, esculin, maltose, D-arabinose, L-arabinose, D-xylose, L-rhamnose, D-lyxose, and L-fucose, but not from glycerol, erythritol, L-xylose, D-adonitol, D-galactose, D-glucose, D-mannose, L-sorbose, dulcitol, inositol, D-mannitol, D-sorbitol, methyl α-D-mannopyranoside, methyl α-D-glucopyranoside, *N*-acetylglucosamine, amygdalin, D-cellobiose, D-lactose, melibiose, sucrose, trehalose, inulin, melezitose, raffinose, starch, glycogen, xylitol, gentiobiose, D-turanose, D-tagatose, D-fucose, DL-arabitol, potassium 2-ketogluconate, or potassium 5-ketogluconate. Utilizes the following substrates as sole carbon sources: acetate, D-lactose, melezitose, L-alanine, L-cysteine, L-leucine, L-methionine, L-proline, methyl α-D-glucoside, oxalate, D-fructose, D-glucose, D-glutamic acid, maltose, D-ribose, D-sorbitol, trehalose, glycerol, L-arginine, L-fucose, and sucrose. The following substrates are not utilized: citrate, cellobiose, D-galactose, D-inulin, D-lactulose, D-mannitol, D-mannose, raffinose, D-rhamnose, D-sorbose, D-xylose, dulcitol, erythritol, glycogen, inositol, L-arabinose, L-leucinamide, L-ornithine, L-phenylalanine, L-tyrosine, L-valine, malate, malonate, nicotinamide, salicin, and succinate. In the API ZYM assay, tests are positive for cystine arylamidase, α-fucosidase, alkaline phosphatase, esterase lipase (C8), leucine arylamidase, valine arylamidase, acid phosphatase, naphthol-AS-BI-phosphohydrolase, α-galactosidase, β-galactosidase, α-glucosidase, β-glucosidase, *N*-acetyl-β-glucosaminidase, and α-mannosidase, but negative for esterase (C4), lipase (C14), trypsin, chymotrypsin, and β-glucuronidase. Cells are susceptible to ampicillin (10 µg), chloramphenicol (30 µg), ciprofloxacin (5 µg), erythromycin (15 µg), gentamicin (10 µg), kanamycin (30 µg), neomycin (30 µg), polymyxin B (300 IU), rifampin (15 µg), and streptomycin (10 µg). Not susceptible to nitrofurantoin (300 µg) or oxacillin (1 µg). Whole-cell sugars are galactose and glucose. Other chemotaxonomic characteristics are as described for the genus.

   Source: isolated from farmland soil collected in Shandong Province, China.

   DNA G+C content (mol%): 69.8 (T_m).

   Type strain: 3-6, CGMCC 4.3142, DSM 18029, JCM 13910, PCM 2644.

   Sequence accession no. (16S rRNA gene): DQ343153.

Genus II. **Haloactinobacterium** Tang, Zhi, Wang, Wu, Lee, Kim, Lou, Xu and Li 2010, 2118[VP]

Shu-Kun Tang, Xiao-Yang Zhi and Wen-Jun Li

Ha.lo.ac.ti.no.bac.te′ri.um. Gr. n. *hals*, *halos* salt; Gr. n. *actis*, *actinos* a ray; L. neut. n. *bacterium* a rod; N.L. neut. n. *Haloactinobacterium* a halophilic actinobacterium.

Short rods. Gram-stain-positive. Nonmotile. Moderately halophilic. Facultatively anaerobic in the presence of KNO_3. The peptidoglycan is type A4α with L-Lys–L-Glu interpeptide bridges.

DNA G+C content (mol%): 68–69.

Type species: **Haloactinobacterium album** Tang, Zhi, Wang, Wu, Lee, Kim, Lou, Xu and Li 2010, 2118[VP].

Further descriptive information

Phylogenetic analysis of the 16S rRNA gene sequence places the genus within the family *Ruaniaceae*, order *Micrococcales*. Its closest relative is *Ruania albidiflava*. The peptidoglycan is type A4α with L-Lys–L-Glu interpeptide bridges. The whole-cell sugars are glucosamine, arabinose, mannose, and two unknown sugars. The predominant menaquinone is MK-8(H_4). The polar lipids are diphosphatidylglycerol, phosphatidylglycerol, phosphatidylinositol, one unknown phosphoglycolipid, and one unknown phospholipid. The major fatty acids are $C_{15:0}$ iso, $C_{15:0}$ anteiso, and $C_{17:0}$ anteiso.

Isolation procedures

The only species in the genus was isolated from a soil sample collected from Qijiaojing Lake, which is a salt lake in Xinjiang province, north-west China (GPS coordinates for the sampling site are 43°26′48″N 91°29′13″E), after 3 weeks incubation at 37°C on glucose-tryptone-yeast (GTY) medium, consisting of 1.0 g/l glucose, 0.5 g/l tryptone, 2.0 g/l yeast extract, 1.0 g/l $CaCl_2$, 100.0 g/l NaCl, and 15.0 g/l agar, final pH not adjusted. The NaCl was sterilized separately before being added to the medium.

Maintenance procedures

The type strain was maintained on GTY agar slants at 4°C and in 20% (v/v) glycerol suspensions of well-grown liquid cultures and stored frozen at −80°C or in liquid nitrogen.

Differentiation of the genus *Haloactinobacterium* from other genera

Table 187 lists characteristics that differentiate between *Haloactinobacterium* and its relative *Ruania albidiflava*, which is the sole recognized species of the genus *Ruania*. *Haloactinobacterium* is a halophilic bacterium and this characteristic distinguishes it from *Ruania*. In addition, the whole cell sugars, interpeptide bridge of the peptidoglycan, polar lipids, and the major fatty acids further distinguish the two genera.

TABLE 187. Features differentiating *Haloactinobacterium* and *Ruania*[a]

Characteristic	*Haloactinobacterium album* YIM 93306[T]	*Ruania albidiflava* AS 4.3142[T]
Cell morphology	Rods	Coccoid
Color of colonies	Cream white	Pale yellow
Facultative anaerobe	+	–
Growth in NaCl:		
0%	–	+
15%	+	–
pH range	6–10	5.5–12.5
Growth temperature range (°C)	10–40	20–37
Methyl red test	–	+
API ZYM:		
Esterase (C4)	+	–
Alkaline phosphatase	–	+
Leucine arylamidase	–	+
Valine arylamidase	–	+
Naphthol-AS-BI-phosphohydrolase	–	+
α-Glucosidase	–	+
α-Galactosidase	–	+
α-Fucosidase	–	+
Acid production from (API 50CH):		
Arbutin	–	+
D-Adonitol	+	–
D-Cellobiose	+	–
D-Galactose	+	–
D-Glucose	+	–
D-Lyxose	–	+
D-Mannose	+	–
D-Turanose	+	–
D-Tagatose	+	–
Erythritol	+	–
Glycerol	+	–
N-Acetylglucosamine	+	–
Interpeptide bridge (A4α)	L-Lys–L-Glu	L-Lys–Gly–L-Glu–L-Glu
Whole-cell sugars	Glucosamine, arabinose, mannose, two unknown sugars	Galactose, glucose
Polar lipids	DPG, PG, PGL, PI, PL	DPG, PG, 1GL
Major fatty acid(s) (>10%)	$C_{15:0}$ anteiso (34.4%), $C_{15:0}$ iso (33.1%), $C_{17:0}$ anteiso (14.8%)	$C_{15:0}$ anteiso (37.3%), $C_{17:0}$ anteiso (14.7%), $C_{16:0}$ iso (14.3%)

[a]Data were taken from Gu et al. (2007) and Tang et al. (2010).

List of species of the genus *Haloactinobacterium*

1. **Haloactinobacterium album** Tang, Zhi, Wang, Wu, Lee, Kim, Lou, Xu and Li 2010, 2118[VP]

al′bum. L. neut. adj. *album* white.

Cells are short rods (0.3–0.4 × 0.6–0.7 µm). Colonies are cream white, circular, smooth, opaque, and non-pigmented on GTY agar containing 10% NaCl. Temperature, pH, and NaCl tolerances ranges for growth are 10–40°C, pH 6.0–10.0, and 2–16% (w/v), respectively, and optimal growth occurs at 37°C, pH 7.0–8.0, and 7–10% (w/v) NaCl. Catalase-positive. Oxidase, methyl red, and Voges–Proskauer tests are negative. Cellulose and esculin are hydrolyzed. Gelatin, casein, dextrin, starch, DNA, chitin, urea, and Tweens 20, 40, 60, and 80 are not hydrolyzed. H_2S, melanin, and indole production are negative. In the API ZYM system, positive for esterase (C4), esterase lipase (C8), cystine arylamidase, β-galactosidase, β-glucosidase, α-mannosidase, and *N*-acetyl-β-glucosaminidase, but negative for alkaline phosphatase, leucine arylamidase, valine arylamidase, trypsin, α-chymotrypsin, naphthol-AS-BI-phosphohydrolase, β-glucuronidase, α-glucosidase, lipase (C14), α-galactosidase, and α-fucosidase. The following substrates are oxidized on Biolog microplates GP2: D-fructose, L-fucose, methyl β-D-galactoside, D-psicose, α-D-glucose, maltose, D-mannose, D-ribose, sucrose, D-xylose, pyruvic acid, L-arabinose, D-galactose, pyruvic acid methyl ester, DL-α-glycerolphosphate, L-lactic acid, acetic acid, glycerol, and amygdalin. Acid is produced from glycerol, erythritol, DL-arabinose, D-ribose, D-xylose, D-adonitol, methyl β-D-xylopyranoside, D-galactose, D-glucose, D-fructose, D-mannose, L-rhamnose, *N*-acetylglucosamine, esculin, salicin, cellobiose, maltose, D-turanose, D-tagatose, and L-fucose, but not from amygdalin, DL-arabitol, arbutin, dulcitol, D-fucose, gentiobiose, glycogen, inositol, inulin, D-lactose, D-lyxose,

D-mannitol, melezitose, melibiose, raffinose, D-sorbitol, L-sorbose, starch, L-xylose, methyl α-D-mannopyranoside, methyl α-D-glucopyranoside, xylitol, sucrose, trehalose, potassium 5-ketogluconate, potassium gluconate, or potassium 2-ketogluconate. Cellular fatty acid profile contains branched fatty acids as major components and straight fatty acids as minor components: $C_{15:0}$ iso (33.1%), $C_{15:0}$ anteiso (34.4%), $C_{16:0}$ iso (2.3%), $C_{16:0}$ (2.1%), $C_{15:0}$ iso 3-OH (0.9%), $C_{15:0}$ iso 2-OH (0.9%), $C_{17:0}$ (8.7%), and $C_{17:0}$ anteiso (14.8%). Other fatty acids were present in amounts less than 0.5%. For all other characteristics, refer to the genus description.

Source: isolated from a soil sample collected from Qijiaojing Lake, a salt lake in Xinjiang province, north-west China.

DNA G+C content (mol%): 68–69 (HPLC).

Type strain: YIM 93306, DSM 21368, KCTC 19413, CCTCC AB 208069.

Sequence accession no. (16S rRNA gene): FJ969847.

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Family XV. **Sanguibacteraceae** Stackebrandt and Schumann 2000, 1284[VP]

Cristina Pascual Ramos and José Francisco Fernández-Garayzábal

San.gui.bac.te.ra.ce′a.e. N.L. masc. n. *Sanguibacter* type genus of the family; *-aceae* ending to denote a family; N.L. fem. pl. n. *Sanguibacteraceae* the *Sanguibacter* family.

Cells consist of regular or irregularly shaped **short rods**. **Gram-stain-positive**. Nonsporeforming. **Motile**. **Aerobic or facultatively anaerobic**. **Catalase-positive**.

The **cell-wall murein** is of the type **A4α** (L-Lys–Ser–D-Glu) and the **major menaquinone** is **MK-9(H_4)**. The cellular **fatty acid profile** consists mainly of **anteiso-methyl-branched chain and straight-chain saturated components**. Species have been isolated from animals and the environment; they have been not associated with diseases in humans or animals.

The pattern of 16S rRNA gene signatures that describe the above–mentioned family consists of nucleotides at position 69:99 (G–U), 140:223 (G–C),144:178 (C–G), 248:276 (C–G), 258:268 (A–U), 379:384 (C–G), 407:435 (A–U), 502:543 (G–C), 586:755 (C–G), 589:650 (U–A), 602:636 (G–U), 610 (U), 612:628 (U–A), 615:625 (A–U), 616:624 (G–C), 630 (C), 668:738 (U–A), 839:847 (U–A), 863 (U), 1133:1141 (A–U) and 1134:1140 (C–G).

DNA G+C content (mol%): 69.5–73.4.

Type genus: **Sanguibacter** Fernández-Garayzábal, Domínguez, Pascual, Jones and Collins 1995b, 619[VP] (Effective publication: Fernández-Garayzábal, Domínguez, Pascual, Jones and Collins 1995a, 73.).

Further descriptive information

The family *Sanguibacteraceae* contains the single genus *Sanguibacter*. It was proposed on the basis of 16S rRNA gene sequence data and the presence of specific signature nucleotides (Stackebrandt and Schumann, 2000). However, it should be noted that the recently described species *Sanguibacter antarcticus*, *Sanguibacter marinus*, and *Sanguibacter soli* do not display the signature nucleotides at positions 258:268 (G–C instead of A–U); *Sanguibacter marinus* and *Sanguibacter soli* do not display the signature nucleotides at positions 140:223 (C–G instead of G–C), 407:435 (A–C instead of A–U); *Sanguibacter antarcticus* and *Sanguibacter soli* do not display the signature nucleotide at position 863 (A instead of T).

The phylogenetic position of the family *Sanguibacteraceae* in relation to the *Cellulomonadaceae*/*Promicromonosporaceae* clade is not clear as demonstrated by low bootstrap values (see Figure 207). However, the close relatedness between these three families is also supported by chemotaxonomic traits such as quinone systems and cellular fatty acid profiles (see Table 188).

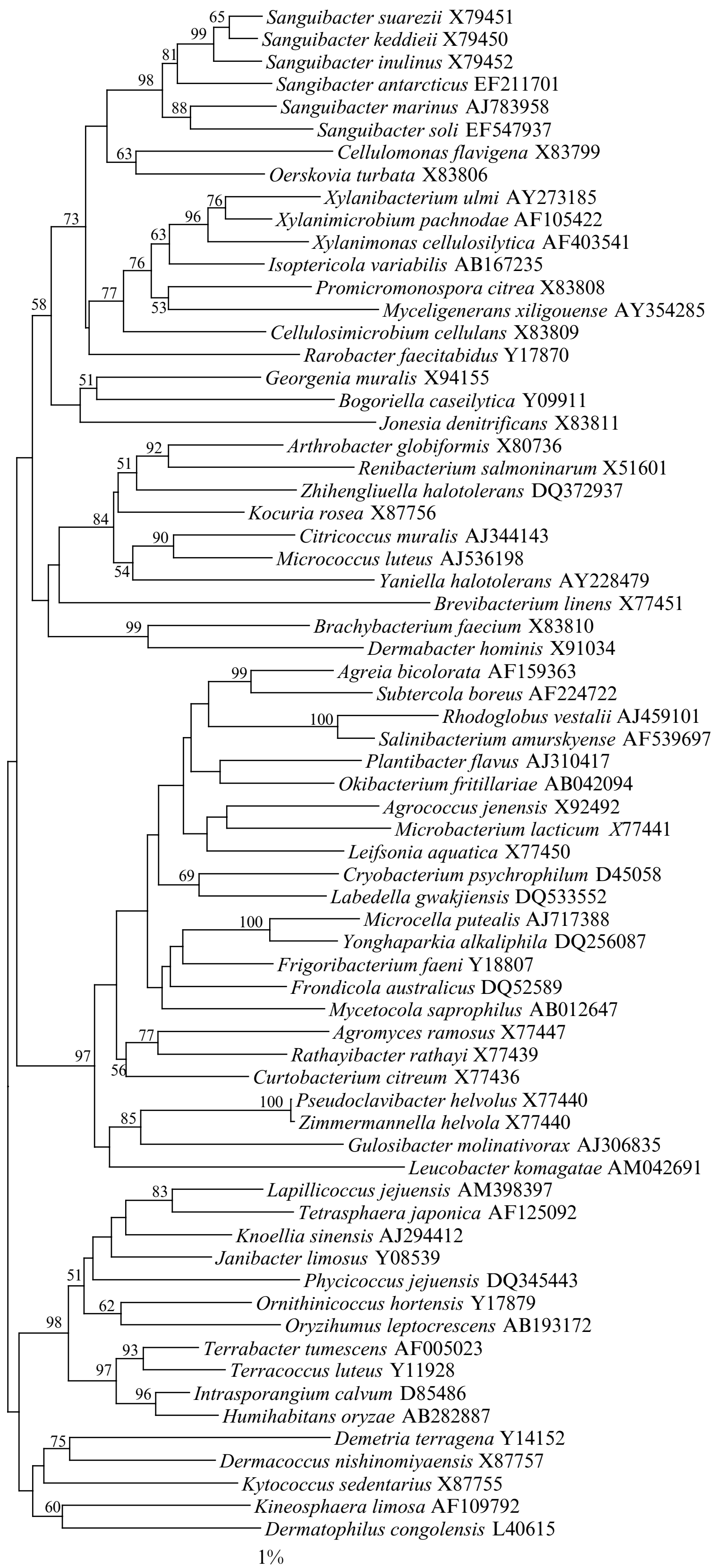

FIGURE 207. 16S rRNA gene sequence-based phylogenetic tree reconstructed from evolutionary distances by the neighbor-joining method (Saitou and Nei, 1987) showing the phylogenetic position of the family *Sanguibacteraceae* in relation to representatives of the order *Micrococcales*. Numbers at branching points refer to bootstrap values (1000 trees resampled); only values above 50% are indicated. Scale bar, 1 inferred nucleotide substitutions per 100 nucleotides.

TABLE 188. Characteristics differentiating the genus *Sanguibacter* from other phylogenetically related genera of the order *Micrococcales*

Characteristic	*Sanguibacter*/ *Sanguibacteraceae*[a]	*Cellulomonas*/ *Cellulomonadaceae*[b]	*Oerskovia*/ *Cellulomonadaceae*[c]	*Promicromonospora*/ *Promicromonosporaceae*[d]	*Cellulosimicrobium*/ *Promicromonosporaceae*[e]	*Isoptericola*/ *Promicromonosporaceae*[f]	*Myceligenerans*/ *Promicromonosporaceae*[g]	*Xylanibacterium*/ *Promicromonosporaceae*[h]	*Xylanimicrobium*/ *Promicromonosporaceae*[i]	*Xylanimonas*/ *Promicromonosporaceae*[j]
Cell morphology	Rods, short, irregular, motile	Irregular rods, motile or nonmotile	Cocci, some branching, vegetative hyphae, motile or nonmotile	Rod-shaped, coccoid, Y or V-shaped, hyphae	Short irregular rods, primary mycelium	Short rods, V-shaped or coccoid, primary mycelium	Coccoid to rod-shaped, hyphae, sporeforming	Small rods	Irregular shaped, single or in pairs, mycelia like fringes	Coccoid, rod-shaped, no aerial hyphae
Murein type	A4α	A4β	A4α	A4α	A4α	A4α	A4α	A4α	A4α	A4α
Major menaquinone(s)	MK-9(H_4)	MK-9(H_4)	MK-9(H_4)	MK-9(H_4)	MK-9(H_4)	MK-9(H_4), MK-9(H_2) or MK-9	MK-9(H_4), MK-9(H_6)	MK-9(H_4), MK-8(H_4)	MK-9(H_4)	MK-9(H_4), MK-8(H_4)
Predominant fatty acids[k]	S, A, I	S, A, I	S, A, I	A, I	S, A, I	S, A, I	A, I	A, I	A, I	A, I
DNA G+C content (mol%)	69.5–73.4	68.5–76	70.5–75	70	74	70–74	72	72	70	73

[a]Fernández-Garayzábal et al. (1995a, 1995b), Pascual et al. (1996), Huang et al. (2005), and Hong et al. (2008).

[b]Stackebrandt et al. (1995), An et al. (2005), Brown et al. (2005), and Jones et al. (2005).

[c]Stackebrandt et al. (2002).

[d]Kalakoutskii et al. (1989), Busse et al. (2003), and Cui et al. (2004).

[e]Schumann et al. (2001).

[f]Stackebrandt et al. (2004), Groth et al. (2005), and Zhang et al. (2005).

[g]Cui et al. (2004) and Groth et al. (2006).

[h]Rivas et al. (2004).

[i]Stackebrandt and Schumann (2004).

[j]Rivas et al. (2003).

[k]Symbols: S, straight-chain saturated; A, anteiso-methyl branched; I, iso-methyl branched; U, monounsaturated.

Genus I. **Sanguibacter** Fernández-Garayzábal, Domínguez, Pascual, Jones and Collins 1995b, 619[VP] (Effective publication: Fernández-Garayzábal, Domínguez, Pascual, Jones and Collins 1995a, 73.)

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San.gui.bac′ter. L. n. *sanguis*, *-inis* blood; N.L. masc. n. *bacter* a rod; N.L. masc. n. *Sanguibacter* (*sic*) a blood rod.

Species of the genus share the characteristics listed in the family description. Not hemolytic on sheep blood agar. Esculin is hydrolyzed. Acid is produced from glycogen, sucrose, D-cellobiose, D-fructose, D-glucose, L-arabinose, D-maltose, D-trehalose, and D-xylose. Acid is not produced from dulcitol, erythritol, inositol, xylitol, D-adonitol, D-arabitol, D-arabinose, D-fucose, D-melezitose, D-tagatose, and L-sorbose.

DNA G+C content (mol%): 69.5–73.4.

Type species: **Sanguibacter keddieii** Fernández-Garayzábal, Domínguez, Pascual, Jones and Collins 1995b, 619[VP] (Effective publication: Fernández-Garayzábal, Domínguez, Pascual, Jones and Collins 1995a, 74.).

Further descriptive information

Phylogeny. The genus *Sanguibacter* was proposed in 1995 on the basis of 16S rRNA gene sequence analysis (Fernández-Garayzábal et al., 1995a) and depending on the outgroups, the species, and/or the tree-making algorithms selected, it appeared phylogenetically related to the cellulomonad/oerskoviae/promicromonosporae clade or to the LL-diaminopimelic-containing genera *Terrabacter tumescens*, *Terracoccus luteus*, and *Intrasporangium calvum* (Fernández-Garayzábal et al., 1995a). With the inclusion of numerous genera in the rapidly growing suborder *Micrococcineae*, the genus *Sanguibacter* constitutes a tight monophyletic group (maximum 4.0% 16S rRNA gene sequence divergence) with chemotaxonomic coherence and a polyamine pattern unique among taxa of the suborder *Micrococcineae* (Busse and Schumann, 1999), elevated to order Microoccales in the taxonomic outline for the present volume, branching within the vicinity of the *Cellulomonas*/*Oerskovia*/*Pro micromonospora* clade.

Three species isolated from blood of apparently healthy cows (*Sanguibacter keddieii*, *Sanguibacter inulinus*, and *Sanguibacter suarezii*) are forming a subclade within the *Sanguibacter* branch (Figure 207). The species *Sanguibacter marinus* and *Sanguibacter soli* form another tight subclade, while *Sanguibacter antarcticus*, isolated from sea sand of Antarctica, constitutes a distinct line that branches at the periphery of the *Sanguibacter keddieii* subcluster.

Chemotaxonomy. *Sanguibacter* species display a cell-wall murein of the A4α type, variation L-Lys–Ser–D-Glu: the diamino acid and the amino acid at position 1 of the peptidoglycan peptide side chain is L-lysine and L-alanine, respectively, and the interpeptide bridge consists of serine and D-glutamic acid (Fernández-Garayzábal et al., 1995a). Mycolic acids are not detected. Polyamine analysis shows members of the genus *Sanguibacter* to possess spermine as the predominant compound and, when compared to members of the family *Intrasporangiaceae*, a significantly reduced amount of putrescine (Busse and Schumann, 1999). Similar to taxa of the genera *Cellulomonas*, *Oerskovia*, and *Promicromonospora*, *Sanguibacter* species contain MK-9(H_4) as the predominant isoprenoid quinone. Minor amounts of MK-9(H_6) are found in *Sanguibacter marinus* (Huang et al., 2005) whereas *Sanguibacter suarezii* exhibits minor amounts of MK-7 and MK-8 (Brown et al., 2005), and *Sanguibacter soli* contains minor amounts of MK-8 (Kim et al., 2008). *Sanguibacter suarezii* displays a whole cell sugar composition that consists of galactose, mannose, and ribose (Brown et al., 2005).

Predominant cellular fatty acids in most *Sanguibacter* species are of the straight-chain saturated and the anteiso-methyl-branched types. Significant levels of iso-methyl-branched fatty acids are also present. This cellular fatty acid pattern is also shared by species of the genera *Cellulomonas*, *Oerskovia*, and *Promicromonospora*. *Sanguibacter soli* has a different cellular fatty acid profile with two unidentified fatty acids (equivalent chain-length 13.961 and 14.966), $C_{17:0}$ anteiso, and $C_{18:0}$ iso as the major fatty acids (Kim et al., 2008).

Colonial and cultural characteristics. *Sanguibacter* strains are non-fastidious microorganisms and do not have special nutritional requirements. They grow perfectly on ordinary media such as Columbia blood or tryptone soy agars producing circular and convex colonies with entire edges and yellow or pale yellow color. The colonies are generally small with a diameter of 0.5–2.0 mm after 48 h of incubation at the optimum temperatures described in Table 189. No hemolysis is observed on sheep blood agar.

Genetics. In the mid-1990s, the genus *Sanguibacter* was examined by applying molecular genetic studies (Pascual et al., 1996). The results of the DNA–DNA hybridization experiments identified the genomically homogeneous ST-50 group of strains as a distinct species. Representative strains belonging to the ST-50 group were shown to exhibit more than 72% DNA reassociation with isolate ST-50 at 70°C with insignificant divergence (ΔT_m <2.5°C). The results provided by Pascual et al. (1996) indicate that isolate ST-50 is more closely related to *Sanguibacter keddieii* (36–41% DNA reassociation) than to *Sanguibacter suarezii* (14–23% DNA reassociation). Based on these results, the name *Sanguibacter inulinus* was proposed for the ST-50 group of strains. In addition, *Sanguibacter keddieii* and *Sanguibacter suarezii* were confirmed as genotypically homogeneous and highly related species. The level of reassociation (generally <41%) confirms their status as separate species.

At genomic level, so far only the gene *chit58* (encoding the enzyme chitinase) from *Sanguibacter* sp. C4 has been characterized (GenBank accession no. DQ282126).

Antibiotic sensitivity. Minimum inhibitory concentrations (MICs) of selected antimicrobial agents for *Sanguibacter suarezii* have been reported by Brown et al. (2005). Clarithromycin, clindamycin, imipenem, minocycline, rifampin, and vancomycin appear to be active against the type strain of *Sanguibacter suarezii* (ATCC 51766[T]).

Ecology and pathogenicity. With the exception of *Sanguibacter antarcticus*, *Sanguibacter marinus*, and *Sanguibacter soli*, the remaining three species of the genus *Sanguibacter* have been isolated from the blood of apparently healthy cows. *Sanguibacter antarticus* has been isolated from sea sand of Antarctica, *Sanguibacter marinus* from coastal sediment in China, and *Sanguibacter soli* from soil of a ginseng field in South Korea.

TABLE 189. Characteristics differentiating the species of the genus *Sanguibacter*[a]

Characteristic[b]	*S. keddieii*	*S. antarticus*	*S. inulinus*	*S. marinus*	*S. soli*	*S. suarezii*
Cell morphology	Irregular rods	Irregular rods	Irregular rods	Irregular rods	Rods	Irregular rods
Colony color	Yellow	Yellow	Pale yellow	Pale yellow	Yellow	Pale yellow
Growth temperature conditions:						
Optimum growth (°C)	25–30	23–26	25–30	25–30	37	25–30
Growth at 10°C	–	+	–	–	–	–
Growth at 15°C	+	+	+	+	–	+
Growth at 42°C	–	–	–	–	+	–
Oxidase	–	–	–	–	+	–
Nitrate reduction	–	+	d[d]	+	–	d
Fermentation of:[c]						
N-Acetylglucosamine	+	–	+	–	+	–
Amygdalin	+	–	+	–	–	+[d]
Gentiobiose	+	+	+	–	+	+
Gluconate	d	–	+	–	–	–
Glycerol	+	–	+	–	+[d]	+
Inulin	–	–	+	–	–	–
5-Ketogluconate	–	–	+	–	–	nr[e]
D-Lactose	+	–	+	+	+	+
D-Lyxose	+	–	+	–	–	+
D-Mannitol	–	+	d	–	–	–
D-Mannose	+	–	+	+	+	+
Melibiose	+	–	+	–	–	+
Methyl α-D-glucoside	+	–	+	+[d]	+	–
Methyl α-D-mannoside	+	–	+	–	–	–
Methyl β-D-xyloside	+	–	d	–	–	+
Raffinose	+	–	+	–	–	d
L-Rhamnose	+	–	+	–	–	+
Ribose	+	+	+	–	–	+
Salicin	+	–	+	+	+	+
D-Sorbitol	+	–	–	–	–	–
Turanose	+	+	+	–	+[d]	+

[a]Symbols: +, 90% or more of the strains are positive within incubation period; d, 11–89% of the strains are positive within incubation period); –, 10% or fewer of the strains are positive within incubation period.

[b]Phenotypic characteristics of *Sanguibacter keddieii*, *Sanguibacter antarticus*, *Sanguibacter inulinus Sanguibacter marinus*, *Sanguibacter soli*, and *Sanguibacter suarezii* are based on three strains, one strain, six strains, one strain, one strain, and three strains, respectively.

[c]Fermentation of carbohydrates was performed on API 50CH strips (bioMérieux) according to the manufacturer`s instructions.

[d]Weak or delayed reaction.

[e]Not reported.

Data provided with deposited 16S rRNA gene sequences in public databases indicate the occurrence of *Sanguibacter* strains in different habitats such as subsurface environments (oil reservoirs: *Sanguibacter* sp. SG-4, accession no. AB126692), mudflow deposits (*Sanguibacter* sp. 1161, accession no. AB054900), plants (tomato leaves: *Sanguibacter* sp 12AD12, accession no. AB242791); alpine subnival plants (*Sanguibacter* sp Enf10, accession no. DQ339590); potatoes (*Sanguibacter suarezii* iCTE629, accession no. DQ122334) and in invertebrates (gut of the millipede *Cylindrojulus boleti* (Julidae, Diplopoda): *Sanguibacter inulinus* dtb 62, accession no. AJ309914). In addition, recent studies in microbial diversity have genotypically identified *Sanguibacter* isolates in the roots of wheat *Triticum aestivum* L. (Conn and Franco, 2004), in maple sap (Lagacé et al., 2004), and in the pig gastrointestinal tract (Leser et al., 2002). Although the genus *Sanguibacter* seems to be widely disseminated in terrestrial and aquatic ecosystems as well as in animals, insight into their natural distribution is still limited.

Until now, *Sanguibacter* has never been associated with disease in animals or humans. However, a chitinase producing *Sanguibacter* strain, *Sanguibacter* sp. C4 isolated from soil and tested as biocontrol agent for insect pests, has been reported to be pathogenic to locusts (Yong et al., 2005).

Isolation and maintenance procedures

Sanguibacter strains were originally isolated on *Listeria* selective agar (Domínguez Rodríguez et al., 1984) containing nalidixic acid (40 mg/l) and acriflavin (6 mg/l). Selective media including these concentrations of antimicrobials may be used for primary isolation of *Sanguibacter* strains from natural environments.

Sanguibacter species can survive without loss of viability for about 2 months on nutrient agar or tryptone soy agar slants stored at 4°C. Long-term survival procedures include either lyophilization or frozen stocks maintained at −20°C/−80°C in nutrient broth containing 20% glycerol.

Differentiation of the genus *Sanguibacter* from other genera

The genus *Sanguibacter* can be differentiated from other phylogenetically related genera of the order *Micrococcales* by the

phenotypic characteristics depicted in Table 188. Two species, including the type species of the genus, can also be differentiated from genera of the family *Intrasporangiaceae* on the basis of their polyamine pattern. *Sanguibacter keddieii* and *Sanguibacter suarezii* have been found to display a low polyamine content when compared to members of the above-mentioned family and to exhibit spermine as the predominant compound and significantly reduced amount of putrescine (Busse and Schumann, 1999).

Taxonomic comments

In 1997, the genus *Sanguibacter* was placed in the newly introduced family *Intrasporangiaceae* based on 16S rRNA phylogeny and signature nucleotides within the 16S rRNA gene (Stackebrandt et al., 1997). However, using a polyphasic approach and considering chemotaxonomic as well as phylogenetic data, the inclusion of *Sanguibacter* in the above-mentioned family was seriously questioned (Busse and Schumann, 1999; Prauser et al., 1997). *Sanguibacter* was then reported as the deepest branching lineage of the family *Intrasporangiaceae* (Prauser et al., 1997) differing from members of the family in respect to their peptidoglycan diamino acid (L-lysine), their menaquinone [MK-9(H_4)], their fatty acid profile (see Table 188), and their polyamine pattern (spermine predominant; Busse and Schumann, 1999). Recently, the genus *Sanguibacter* was reclassified in the newly proposed family *Sanguibacteriaceae* (Stackebrandt and Schumann, 2000) on the basis of 16S rRNA phylogenetic analysis and signature nucleotides. Indeed, with the subsequent addition of novel taxa into the rapidly growing suborder *Micrococcineae* (now order *Micrococcales*), the phylogenetic coherence of the family *Instrasporangiaceae* was disrupted, leading to the appearance of the genus *Sanguibacter* as a novel deeply branching lineage equivalent to those of neighboring families.

Differentiation of the species of the genus *Sanguibacter*

Phylogenetically, the six established species of *Sanguibacter* form a distinct line of descent with 96.0–99.2% 16S rRNA gene sequence similarities among them**.** Their 16S rRNA gene sequences are deposited in public databases (GenBank, EMBL). Therefore, if sequencing facilities are available, preliminary allocation of isolates to the genus *Sanguibacter* could be accomplished if 16S rRNA gene sequence similarities to established species of the genus are higher than 96.0%. Phenotypic characteristics useful for differentiation of species of *Sanguibacter* are provided in Table 189. However, correct allocation of isolates to the newly described species *Sanguibacter marinus*, *Sanguibacter antarcticus*, and *Sanguibacter soli* based on the phenotype may be problematic since the phenotypic characterizations of these species are each based on a single strain and, hence, neither stable nor variable traits of the species are known.

List of species of the genus *Sanguibacter*

1. **Sanguibacter keddieii** Fernández-Garayzábal, Domínguez, Pascual, Jones and Collins 1995b, 619[VP] (Effective publication: Fernández-Garayzábal, Domínguez, Pascual, Jones and Collins 1995a, 74.)

ked.di.e′i.i. N.L. gen. masc. n. *keddieii* of Keddie, named in honor of R.M. Keddie, a British bacteriologist.

Cells consist of Gram-stain-positive, short, irregularly shaped, motile rods. Colonies are circular, convex with entire edges, and yellow in color. Colony sizes range from 1.0–2.0 mm in diameter after 48 h of incubation on Tryptone Soy Agar. Facultatively anaerobic. Oxidase negative. In addition to the features common to all members of the genus and characteristics listed in Table 189, strains of the species share the following biochemical characteristics. Voges–Proskauer test is negative. Casein, esculin, and gelatin are hydrolyzed. Cellulose and Tween 80 are not decomposed. Acid is produced from arbutin and D-galactose. Some strains produce acid from gluconate. Acid is not produced from L-arabitol and L-fucose. Predominant fatty acids consist of $C_{16:0}$ (43.9%), $C_{15:0}$ anteiso (17.0%), and $C_{18:0}$ (12.0%).

Source: blood samples of apparently healthy dairy cows.

DNA G+C content (mol%): 70 (T_m).

Type strain: ST-74, ATCC 51767, CCUG 36690, CECT 4540, CIP 106323, DSM 10542, JCM 11429, NBRC 16158, NCIMB 703025.

Sequence accession no. (16S rRNA gene): X79450.

2. **Sanguibacter antarcticus** Hong, Lee, Yim, Chun and Lee 2008, 51[VP]

an.tarc′ti.cus. L. masc. adj. *antarcticus* southern, pertaining to the Antarctic, the geographical origin of the type strain.

Cells consist of Gram-stain-positive, irregularly shaped motile rods with sparse peritrichous flagella. Colonies are yellow, circular, and convex with entire edges. Diffusible pigments are not produced. Facultatively anaerobic. Oxidase negative. Growth is observed in a wide range of temperatures (4–30°C). Growth occurs at pH 4–9 (optimum pH 5–6) and with 0–7% NaCl (optimum 2–5%). Nitrate is reduced to nitrite and nitrogen. In addition to the features common to all members of the genus and characteristics listed in Table 189, strains of the species exhibit the following biochemical characteristics. Citrate is utilized and Voges–Proskauer reaction is weak. Gelatin is not hydrolyzed. Acid is not produced from arbutin, D-galactose, L-arabitol, L-fucose, and 2-ketogluconate. Arginine dihydrolase, cytochrome oxidase, β-galactosidase, gelatinase, H_2S, indole, lysine decarboxylase, ornithine decarboxylase, tryptophan deaminase, and urease are not produced. The predominant fatty acids are $C_{15:0}$ anteiso (50.5%), $C_{16:0}$ (9.6%), $C_{13:0}$ anteiso (7.6%), $C_{16:0}$ iso (7.5%), and $C_{17:0}$ anteiso (6.4%).

Source: a sea sand sample from King George Island, Antarctica.

DNA G+C content (mol%): 69.5 (T_m).

Type strain: KOPRI 21702, DSM 18966, JCM 14623, KCTC 13143.

Sequence accession no. (16S rRNA gene): EF211071.

3. **Sanguibacter inulinus** Pascual, Collins, Grimont, Domínguez and Fernández-Garayzábal 1996, 812[VP]

i.nu.li′nus. N.L. n. *inulum* (*sic*) inulin; L. masc. suff. *-inus* suffix used with the sense of pertaining to; N.L. masc. adj. *inulinus* pertaining to inulin.

Cells consist of Gram-stain-positive, short, irregularly shaped, motile rods. Colonies are circular, convex with entire edges, and pale yellow in color. Colony sizes range from 0.5–1.0 mm in diameter after 48 h of incubation on Tryptone Soy Agar. Facultatively anaerobic. Oxidase negative. Some strains reduce nitrate. In addition to the features common to all members of the genus and characteristics listed in Table 189, strains of the species share the following biochemical characteristics. Strains are negative in the Voges–Proskauer test. Esculin is hydrolyzed. Cellulose and Tween 80 are not hydrolyzed. Acid is produced from arbutin and D-galactose. Some strains produce acid from D-mannitol and methyl-β-D-xyloside. Acid is not produced from L-sorbose and 2-keto-gluconate. The predominant fatty acids are $C_{15:0}$ anteiso (59.8%), $C_{17:0}$ anteiso (8.1%), and $C_{16:0}$ (7.3%).

Source: blood samples of apparently healthy dairy cows.

DNA G+C content (mol%): 70.5 (T_m).

Type strain: ST-50, CCUG 36689, CIP 104915, JCM 11442, NCIMB 703024.

Sequence accession no. (16S rRNA gene): X79451.

4. **Sanguibacter marinus** Huang, Dai, He, Wang, Wang, Liu and Liu 2005, 1756[VP]

ma.ri′nus. L. masc. adj. *marinus* pertaining to the sea, where the isolate was found.

Cells are Gram-stain-positive, nonsporeforming, short, irregular, motile rods with sparse peritrichous flagella. Colonies are circular, convex with entire edges, and pale yellow. Diffusible pigments are not produced. Facultatively anaerobic. Oxidase negative. Growth is observed in a wide range of temperatures (15–37°C), pH (5.5–9.0), and NaCl concentrations (0–7% (w/v)). No growth is detected at 42°C and 10% NaCl. In addition to the features common to all members of the genus and characteristics listed in Table 189, strain of the species exhibit the following biochemical reactions. Citrate is not utilized. Esculin, gelatin, and starch are hydrolyzed, but casein, cellulose, and Tween 80 are not decomposed. Methyl red and indole tests are positive, but Voges–Proskauer test is negative. Acid is produced from arbutin and D-galactose. Acid is not produced from L-arabitol, L-fucose, and 2-keto-gluconate. The predominant fatty acids are $C_{15:0}$ anteiso (46.7%), $C_{15:1}$ anteisoA (17.7%), $C_{16:0}$ (10.7%), and $C_{15:0}$ (9.3%).

Source: coastal sediment collected in the Fujian province of China.

DNA G+C content (mol%): 73.4 (T_m).

Type strain: 1-19, CGMCC 1.3457, JCM 12547.

Sequence accession no. (16S rRNA gene): AJ783958.

5. **Sanguibacter soli** Kim, Pulla, Kim, Yi, Soung and Yang 2008, 540[VP]

so′li. L. neut. gen. n. *soli* of soil, the source of the type strain.

Cells consist of Gram-stain-positive motile rods. Colonies are yellow pigmented and circular. Aerobic. Oxidase positive. Growth is observed in a wide range of temperatures (25–42°C). No growth is detected at 4°C. Growth occurs at pH 5–9. In addition to the features common to all members of the genus and characteristics listed in Table 189, strains of the species exhibit the following biochemical reactions. Acid is produced from arbutin and D-galactose but not from L-arabitol, L-fucose, and 2-keto-gluconate. Positive for *N*-acetyl-β-glucosaminidase, acid phosphatase, alkaline phosphatase, α-chymotrypsin, cystine arylamidase, esterase (C4), esterase (C8), β-galactosidase, α-glucosidase, β-glucosidase (esculin hydrolysis), β-glucuronidase, leucine arylamidase, lipase (C14), α-mannosidase, naphthol-AS-BI-phosphohydrolase, trypsin, and valine arylamidase. Arginine dihydrolase, α-fucosidase, α-galactosidase, protease (gelatin hydrolysis), and urease are not produced. Assimilates acetate, L-arabinose, D-glucose, maltose, D-mannose, sucrose, glycogen, and salicin, but not 3-hydroxybenzoate, 4-hydroxybenzoate, DL-3-hydroxybutyrate, 4-hydroxybenzoate, 2-ketogluconate, 5-ketogluconate, adipate, caprate, citrate, gluconate, itaconate, L-lactate, L-malate, malonate, phenyl acetate, propionate, suberate, n-valerate, L-fucose, melibiose, L-rhamnose, D-ribose, *myo*-inositol, D-mannitol, D-sorbitol, *N*-acetyl-D-glucosamine, L-alanine, L-histidine, L-proline, or L-serine. The predominant fatty acids are ECL 13.961 (45.81%), $C_{17:0}$ anteiso (23.46%), and $C_{18:0}$ iso (15.42%).

Source: soil of a ginseng field in South Korea.

DNA G+C content (mol%): 69.9 (T_m).

Type strain: DCY 22, JCM 14841, KCTC 13155.

Sequence accession no. (16S rRNA gene): EF547937.

6. **Sanguibacter suarezii** Fernández-Garayzábal, Domínguez, Pascual, Jones and Collins 1995b, 619[VP] (Effective publication: Fernández-Garayzábal, Domínguez, Pascual, Jones and Collins 1995a, 74.)

su.ar.é.zi.i. N.L. gen. masc. n. *suarezii* of Suarez, named in honor of G. Suarez, a Spanish veterinary microbiologist.

Cells are Gram-stain-positive, short, irregularly shaped, motile rods. Colonies are circular, convex with entire edges, and pale yellow in color. Colony sizes are approximately 0.5 mm in diameter after 48 h of incubation on Tryptone Soy Agar. Facultatively anaerobic. Oxidase-negative. Voges–Proskauer reaction is negative. Nitrate is reduced by most strains. In addition to the features common to all members of the genus and characteristics listed in Table 189, strains of the species share the following biochemical characteristics. Esculin is hydrolyzed. Casein, gelatin, cellulose, and Tween 80 are not hydrolyzed. Acid is produced from arbutin and D-galactose. Some strains produce acid from D-raffinose. Acid is not produced from L-arabitol and L-fucose. The predominant fatty acids are $C_{15:0}$ anteiso (40.5%), $C_{16:0}$ (34.3%), and $C_{14:0}$ (13.9%).

Source: blood samples of apparently healthy dairy cows.

DNA G+C content (mol%): 69.5 (T_m).

Type strain: ST-26, ATCC 51766, CCUG 36691, CECT 4539, CIP 106322, DSM 10543, JCM 11430, NBRC 16159, NCIMB 703023.

Sequence accession no. (16S rRNA gene): X79452.

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Order XI. **Micromonosporales** ord. nov.

Olga Genilloud

Mi.cro.mo.no.spo′ra.les. N.L. fem. n. *Micromonospora* type genus of the order; suff. *-ales* ending to denote a order; N.L. fem. pl. n. *Micromonosporales* the *Micromonospora* order.

The order was formed by elevation of the suborder *Micromonosporineae* Stackebrandt, Rainey and Ward-Rainey 1997, 486[VP] emend. Zhi, Li and Stackebrandt 2009, 599. It contains the family *Micromonosporaceae*. The recognition of this taxon is based on the distinct phylogenetic position of its constituent members as determined by 16S rRNA gene sequence analysis and by the presence of a family-specific pattern of 16S rRNA nucleotide positions. The pattern of 16S rRNA gene signature nucleotides for the order is as indicated for the family by Zhi et al. (2009).

Type genus: **Micromonospora** Ørskov 1923, 156[AL].

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Family I. **Micromonosporaceae** Krasil'nikov 1938, 272[AL] emend. Zhi, Li and Stackebrandt 2009, 599

Olga Genilloud

Mi.cro.mo.no.spo.ra.ce′a.e. N.L. fem. n. *Micromonospora* type genus of the family; suff. *-aceae* ending to denote a family; N.L. fem. pl. n. *Micromonosporaceae* the *Micromonospora* family.

Aerobic, **Gram-stain-positive**, **non-acid-fast organisms that form a non-fragmenting**, **branched, and septate mycelium that rarely carry scant aerial hyphae**. The family is a member of the order *Micromonosporales* and cannot be distinguished from other suprageneric groups of *Actinomycetales* by using a set of exclusive phenotypic characters. The family encompasses a chemotaxonomically and morphologically diverse group of filamentous bacteria that, at the time of writing, included 17 phylogenetically closely related genera that present distinctive morphological and chemotaxonomic characteristics. **The organisms are mesophilic, showing optimum growth between 20 and 30°C, and grow well at pH 7;** growth can be observed in some genera from10 to 40°C and from pH 4.5 to 12. Colonies on agar media are flat to elevated with smooth or wrinkled surfaces and show a large variety of pigments. **Most strains contain carotenoid mycelial pigments**, but some produce blue-green and maroon to purple pigments. Diffusible pigments may be formed depending on the media. **Single spores**, **short spore chains, or sporangia borne directly from the substrate hyphae may be formed**. Spores may be motile with tufts of polar flagella. With few exceptions, cell walls contain ***meso*-2,6-diaminopimelic acid and glycolated muramic acid**. Predominant menaquinones may include all types of the MK-9 and MK-10 series. **Phosphatidylethanolamine is the diagnostic phospholipid**. Mycolic acids are absent. Members of the family *Micromonosporaceae* are widely distributed in the environment and have been found in sediments, soils, rhizospheres, colonizing living and decaying plant material, and in freshwater and marine habitats.

DNA G+C content (mol%): 69–73 (T_m, HPLC).

Type genus: **Micromonospora** Ørskov 1923, 156[AL].

Further descriptive information

The family *Micromonosporaceae* currently comprises the genera *Micromonospora, Actinocatenispora, Actinoplanes, Asanoa, Catellatospora, Catenuloplanes, Couchioplanes, Dactylosporangium, Krasilnikovia, Longispora, Luedemannella, Pilimelia, Polymorphospora, Salinispora, Spirilliplanes, Verrucosispora*, and *Virgisporangium*. These genera can be distinguished on the basis of their chemotaxonomic, phenotypic, and phylogenetic characteristics.

Phylogeny. Members of the family *Micromonosporaceae* are phylogenetically related as determined by 16S rRNA gene sequence analysis and form a coherent cluster within the actinomycete subphylum of Gram-stain-positive bacteria that is well separated from other families classified in the order *Actinomycetales* (Embley and Stackebrandt, 1994). The 16S rRNA gene sequence similarity values for members within the family *Micromonosporaceae* are above 91% (Matsumoto et al., 2003). The distribution of genus-specific properties and signature nucleotides that define the genera correlate with the phylogenetic distinctness of each genus. The relationships between the constituent genera of the family are shown in Figure 208.

Cell morphology. Members of the family *Micromonosporaceae* show three major types of sporulating structures, namely single spores, spore chains, and sporangia. The type genus *Micromonospora* Ørskov 1923 is characterized by the production of nonmotile spores that are borne singly, sessile, or on short or long sporophores, often in clusters on extensively branched substrate hyphae. Spores are spherical, ovoid, or ellipsoidal with a thick wall that may carry blunt spiny ornamentations (Kawamoto, 1989). The genus *Salinispora* Maldonado et al. 2005 cannot be distinguished morphologically from members of the genus *Micromonospora. Salinispora* strains do not produce aerial hyphae, but form spores singly or in clusters; the spores have smooth surfaces and are sessile or borne on short sporophores. Salinisporae are abundant and widespread in marine sediments and other marine substrates and will only grow on 20–25% seawater or on a sodium-enriched medium. The genus *Verrucosispora* Rheims et al. 1998 does not produce aerial hyphae, but does form granular structures on the mycelial surface. Nonmotile spores are borne singly and may be sessile or carried on short sporophores. Initially, the spores are warty, but turn hairy in older cultures.

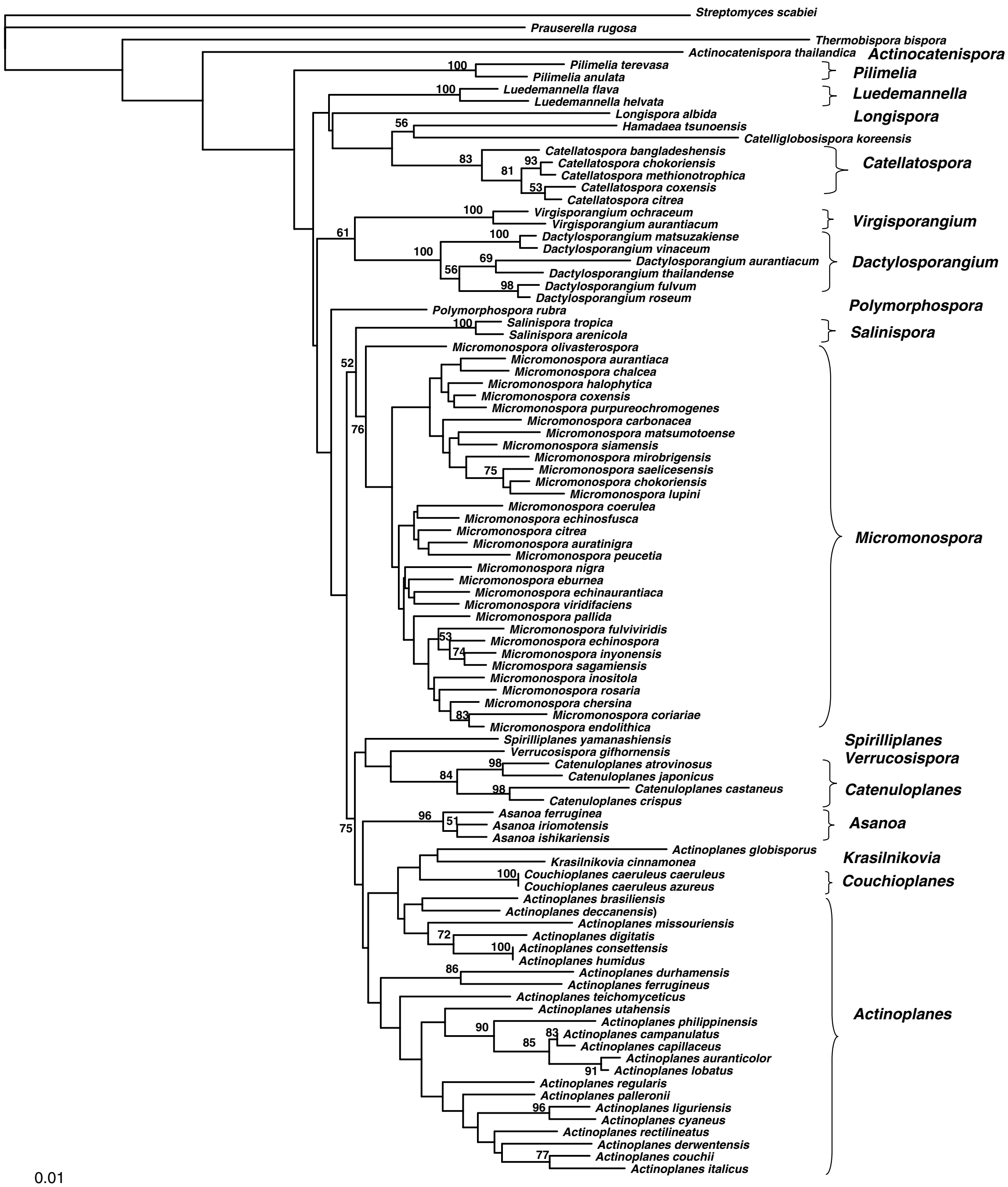

FIGURE 208. Neighbor-joining tree (Saitou and Nei, 1987) based on almost complete 16S rRNA gene sequences showing relationship between type strains classified in the family *Micromonosporaceae*. The tree is based on the Kimura two-parameter method with the confidence values of the branches determined by bootstrap analyses (Felsenstein, 1985) based on 1000 replicates. Only values >50% are shown at the nodes. Sequence accession numbers of type strains are given in the text. Bar = 0.01 substitutions per nucleotide position.

The genera *Actinoplanes* Couch 1950 emend. Stackebrandt et al. 1997, *Dactylosporangium* Thiemann et al. 1967, *Pilimelia* Kane 1966, and *Virgisporangium* Tamura et al. 2001 produce spore vesicles (sporangia) in which motile spores develop at the tips of short or long sporangiophores on the surface of substrate mycelia. The spores are formed within a sporangial envelope by fragmentation of branched or unbranched, straight or coiled sporogenous hyphae. The spore vesicles of the genera *Actinoplanes* and *Pilimelia* may be campanulate, cylindrical, digitate, lobate, ovoid, spherical, or irregular. Members of the genus *Pilimelia* can be differentiated from *Actinoplanes* strains by their keratinophilic character and their slow growth.

The genus *Dactylosporangium* produces two different types of spores: motile spores inside spore vesicles (sporangia) and non-motile spores borne singly on substrate hyphae. Motile spores are formed within finger-shaped to claviform, oligosporous spore vesicles that contain a single row of 2–5 spores. The sporangiospores are spherical, rod-shaped or oblong, ellipsoidal,

or ovoid to pyriform. The spores are motile with polar or lateral tufts of flagella. The spore walls are single-layered and are not ornamented. The nonmotile spores are spherical and are formed terminally on short branches of the substrate hyphae (Vobis, 1989b, 1991). Members of the genus *Virgisporangium* Tamura et al. 2001 produce rod-shaped spore vesicles that develop singly or in clusters on the substrate mycelium. Each spore vesicle contains a single row of six or more oval to short-rod-shaped motile spores. In contrast, members of the genus *Luedemannella* Ara and Kudo 2007d form spherical spore vesicles on short sporangiophores that arise singly or in clusters from the substrate mycelium; each sporangiophore carries several nonmotile spherical to oval spores.

Members of the genera *Actinocatenispora* Thawai et al. 2006a, *Asanoa* Lee and Hah 2002, *Catellatospora* Lee and Hah 2002, *Catenuloplanes* Yokota et al. 1993, *Couchioplanes* Tamura et al. 1994, *Krasilnikovia* Ara and Kudo 2007a, *Longispora* Matsumoto et al. 2003, *Polymorphospora* Tamura et al. 2006, and *Spirilliplanes* Tamura et al. 1997 are non-sporangiate and produce chains of spores. Members of the genus *Catellatospora* do not form aerial hyphae, but produce short chains of nonmotile spores that emerge singly or in tufts from the vegetative mycelium (Asano and Kawamoto, 1986; Koch et al., 1996a). *Couchioplanes* strains form chains of motile spores that, together with aerial mycelia, are often aggregated into clusters that resemble sporangia, though true spore vesicles are not observed. Members of the genus *Krasilnikovia* also form globose pseudosporangial structures (Ara and Kudo, 2007a) that contain nonmotile spores.

Catenuloplanes strains form motile arthrospores in short chains that are arranged in one or two spirals that are often aggregated with normally sparse aerial mycelia. The spores are rod-shaped, straight, or curved with smooth surfaces (Kudo et al., 1999; Tamura et al., 1995; Yokota et al., 1993). Members of the genus *Spirilliplanes* produce long spore chains that are in spirals of five to ten turns. Spores are oval or rod-shaped with smooth surfaces and are motile in the presence of 10% soil extract or distilled water (Tamura et al., 1997).

The genus *Asanoa* was described to accommodate two species that were initially assigned to the genus *Catellatospora*, but it was found subsequently that these species could be clearly differentiated from members of the genus *Catellatospora* and other genera with validly published names classified in the family *Micromonosporaceae*. *Asanoa* strains sporulate poorly on tap-water and glycerol/calcium malate agars (Lee and Hah, 2002). Members of the taxon do not produce aerial mycelia or globose bodies, which are observed in some *Catellatospora* species. Members of the genus *Longispora* Matsumoto et al. 2003 produce a sparse aerial mycelium and only sporulate when grown on gellan gum; spores are borne on short sporophores that arise from the vegetative mycelium. The spores are cylindrical, have smooth surfaces, and are carried in short straight chains of 20 or more spores. Members of the genus *Polymorphospora* Tamura et al. 2006 develop short chains of nonmotile spores of varying shapes that differentiate into rods. Finally, members of the genus *Actinocatenispora* Thawai et al. 2006a produce aerial hyphae on oatmeal-nitrate agar; these develop into cylindrical spore chains containing more than 10 spores.

Chemotaxonomy. The phenotypic heterogeneity of the family *Micromonosporaceae* is further increased when chemotaxonomic markers are considered, namely the amino acids of the peptidoglycan, whole-organism sugar patterns, phospholipid types, and menaquinone and fatty acid composition (Table 190). Most members of the family are characterized by a cell-wall chemotype II *sensu* Lechevalier and Lechevalier (1970a); the wall peptidoglycan contains *meso*-diaminopimelic acid (*meso*-A_2pm) and/or 3-hydroxy-A_2pm. The first amino acid of the peptide side chain is glycine in all members of the family, apart from *Pilimelia* which contains acetate. The genus *Pilimelia* can be differentiated from other members of the family as it contains *N*-acetylated, as opposed to *N*-glycolated, muramic acid (Koch et al., 1996a). Deviations from this wall chemotype have been observed in *Micromonospora* species that contain LL-A_2pm (Kawamoto et al., 1981) and in *Couchioplanes* and *Catenuloplanes* species that have L-lysine and D- and L-serine as diagnostic amino acids in the peptidoglycan rather than *meso*-A_2pm acid (Horan and Brodsky, 1986a; Tamura et al., 1994; Yokota et al., 1993).

Arabinose, galactose, and xylose are the major sugars present in whole-organism hydrolysates [sugar pattern D *sensu* Lechevalier and Lechevalier (1970a)] with variable amounts of other sugars. The diagnostic phospholipid of the cell membrane is phosphatidylethanolamine [phospholipid type II *sensu* Lechevalier et al. (1977)], but diphosphatidylglycerol, phosphatidylglycerol, and phosphatidylinositol may be present. However, *Catenuloplanes* strains also contain phosphatidylcholine (Yokota et al., 1993) and, hence, have a phospholipid type III (Table 190).

Members of the family *Micromonosporaceae* contain complex and highly variable fatty acids, although saturated iso- and anteiso-branched fatty acids are predominant in nearly all genera, apart from the genus *Pilimelia*. Unsaturated fatty acids may or may not be present, although they are major components in *Longispora* strains (Matsumoto et al., 2003). 10-Methyl-branched fatty acids are found in certain strains of *Micromonospora*; cyclic fatty acids are absent from all strains, as are mycolic acids. The menaquinone composition is heterogeneous. Major amounts of tetra-, hexa- and/or octahydrogenated menaquinones with 9, 10, and 12 isoprene units are distributed among genera and species (Koch et al., 1996a; Vobis, 1989b) (Table 190).

Genetics. A family-specific pattern of 16S rRNA gene signatures was defined by Stackebrandt et al. (1997) to distinguish members of the family *Micromonosporaceae* from all other actinomycetes. The 11 16S rRNA gene signatures detected in representatives of *Actinoplanes*, *Catellatospora*, *Couchioplanes*, *Catenuloplanes*, *Dactylosporangium*, *Micromonospora*, and *Pilimelia* consist of nucleotides at positions 66:103 (G–C), 127:234 (A–U), 153:168 (C–G), 502:543 (G–C), 589:650 (C–G), 747 (A), 811 (U), 840:846 (C–G), 952:1229 (C–G), 1116:1184 (C–G), and 1133:1141 (G–C) (Stackebrandt et al., 1997). Since then, genus-specific signature nucleotides have been described for *Actinocatenispora* [positions 502:543 (U–G), 747 (G), and 811 (C)], *Krasilnikovia* [positions 445:489 (C–G), 446:488 (C–G), 1011:1018 (U–A), and 1263:1272 (G–U)], *Longispora* [position 502 (A); Matsumoto et al., 2003], *Luedemannella* [positions 139:224 (U–A), 381 (A), 656:750 (A/G–C), and 999:1041 (C–U)], *Polymorphospora* at position 1244 (U) (Tamura et al., 2006), *Verrucosispora* [positions 1133:1141 (A–U); Rheims et al., 1998], and *Virgisporangium* [positions 502:543 (G–C) and 1116:1184 (C–G); Tamura et al., 2001].

Acknowledgements

I am especially grateful to Dr Oscar Salazar for the updated phylogenetic tree of the family *Micromonosporaceae*.

TABLE 190. Morphological and chemotaxonomic characteristics of members of the family *Micromonosporaceae*[a]

Characteristic	*Micromonospora*	*Actinocatenispora*	*Actinoplanes*	*Asanoa*	*Catellatospora*	*Catenuloplanes*	*Couchioplanes*	*Dactylosporangium*	*Krasilnikovia*	*Longispora*	*Luedemannella*	*Polymorphospora*	*Pilimelia*	*Salinispora*	*Spirilliplanes*	*Verrucosispora*	*Virgisporangium*
Single spores	+	−	−	−	−	−	−	+	−	−	−	−	−	+	−	+	−
Sporangia	−	−	+	−	−	−	−	+	−	−	+	−	+	−	−	−	+
Spore chains	−	+	−	+	+	+	+	−	+	+	−	+	−	−	+	−	−
Motile spores	−	−	+	−	−	+	+	+	−	−	−	−	+	−	+	−	+
Salt required	−	−	−	−	−	−	−	−	−	−	−	−	−	+	−	−	−
Cell-wall chemotype[b]	II	II	II	II	II	VI	VI	II	II	II	II	II	II	II	II	II	II
Whole-cell sugars[c]	Ara, Xyl	Gal, Xyl, Ara, Glu, Man, Rib	Ara, Xyl	Ara, Xyl, Gal	Ara, Xyl, Gal	Xyl	Ara, Xyl, Gal	Ara, Xyl	Gal, Man, Xyl, Ara, Rib, Glu	Ara, Xyl, Gal	Xyl, Gal, Man, Rha, Rib, Ara	Xyl	Ara, Xyl	Ara, Xyl, Gal	Xyl, Gal	Man, Xyl	Ara, Gal, Man, Rha, Xyl
Fatty acid type[d]	3b	3b	2d	2d	3b	2c	2c	3b	2d	2d	2d	2a	2d	3a	2d	2b	2d
Major menaquinones (MK-)	10(H_4, H_6) 9(H_4,H_6)	9(H_4, H_6)	9(H_4) 10(H_4)	10(H_6, H_8)	10(H_8, H_6) 9(H_4, H_6)	9(H_8)[e], 10(H_8)	9(H_4)	9(H_4, H_6, H_8)	9(H_6)	10(H_4, H_6)	9(H_4, H_6)	10(H_4, H_6) 9(H_4, H_6)	9(H_2, H_4)	10(H_4)	10(H_4)	9(H_4)	10(H_4, H_6, H_8)
Phospholipid type[f]	II	II	II	II	II	III	II	II	II	II	II	II	II	II	II	II	II
DNA G+C content (mol%)	71–73	72	72–73	71–72	71–72	71–73	70–72	72–73	71	70	71	70–71	70–72	70–73	69	70	71–72

[a]Except where marked, data are from Stackebrandt and Kroppenstedt (1987), Vobis (1989b), Goodfellow et al. (1990b), Yokota et al. (1993) Tamura et al. (2006, 1997, 2001, 1994), Rheims et al. (1998), Kudo et al. (1999), Lee et al. (2000b), Lee and Hah (2002), Matsumoto et al. (2003), Maldonado et al. (2005), Thawai et al. (2006a), and Ara and Kudo (2007a, 2007d). +, Positive; −, negative.

[b]Data from Lechevalier and Lechevalier (1970a).

[c]Ara, arabinose; Gal, galactose; Glu, glucose; Man, mannose; Rha, rhamnose; Rib, ribose; Xyl, xylose.

[d]Data from Kroppenstedt (1985).

[e]Reported to be MK-10(H_4) and MK-11(H_4) by Kudo et al. (1999).

[f]Data from Lechevalier et al. (1977).

Genus I. **Micromonospora** Ørskov 1923, 156^AL

Olga Genilloud

Mi.cro.mo.no.spo'ra. Gr. adj. *mikros* small; Gr. adj. *monos* single, solitary; Gr. fem. n. *spora* a seed and in biology a spore; N.L. fem. n. *Micromonospora* small, single-spored (organism).

Well-developed, branched, substrate mycelium (0.2–0.6 μm diameter). **Nonmotile spores are borne singly, sessile, or terminally on short sporophores**. Sporophore development is monopodial or in some cases sympodial. Spores are spherical to oval in shape (0.7–1.5 μm) and in most species have blunt spiny projections. The spores are often carried in branched clusters on short hyphae of the substrate mycelium. **Aerial mycelium is usually absent**, but some cultures develop sterile short aerial hyphae. Gram-stain-positive, mesophilic, non-acid-fast. Aerobic to microaerophilic. Chemo-organotrophic. Sensitive to pH below 5.0. Growth usually occurs between 20 and 40°C, but not above 50°C. **Cell wall contains *meso*-diaminopimelic acid and/or 3-OH-diaminopimelic acid**. **Arabinose and xylose are the characteristic sugars present in whole-organism hydrolysates of most species**, but variable amounts of galactose, glucose, mannose, and rhamnose can also be found depending on the species. **The major phospholipids are phosphatidylethanolamine, phosphatidylinositol, and phosphatidylinositol mannosides**. Saturated and unsaturated fatty acids are present, mostly $C_{15:0}$ iso, $C_{17:0}$ iso, $C_{15:0}$ anteiso, $C_{17:0}$ anteiso, $C_{17:0}$ 10-methyl, and $C_{17:0}$ 10-methyl. Mycolic acids are absent. Major menaquinones are MK-9(H_4), MK-9(H_6), MK-10(H_4), MK-10(H_6), or MK-12(H_6). Isolated from soil, plant materials, freshwater, and marine environments.

DNA G+C content (mol%): 68–75 (T_m, HPLC).

Type species: **Micromonospora chalcea** (Foulerton 1905) Ørskov 1923, 156^AL ("*Streptothrix chalcea*" Foulerton 1905, 1199).

Further descriptive information

The genus *Micromonospora* is well defined in terms of morphological, chemotaxonomic, and phylogenetic criteria (Kawamoto, 1989; Koch et al., 1996a; Kroppenstedt, 1985) that distinguish it from other members of the family *Micromonosporaceae* (Table 191). *Micromonospora* species are defined based on chemotaxonomic markers, physiological characteristics, and on phylogenetic relationships.

Phylogeny. The intrageneric structure of the genus *Micromonospora* was described first by Koch et al. (1996b) in an analysis of 16S rRNA gene sequences of representatives of 15 species with validly published names, four subspecies, and 19 species with names that have not been validly published. This and later phylogenetic studies based on 16S rRNA gene sequences of the type strains of the genus have shown that *Micromonospora* species form a tight clade within the family *Micromonosporaceae*; they show 16S rRNA similarity values within the range 96.7–99% (Koch et al., 1996b). These high values cannot be used to confirm the species status of closely related strains, not least because nucleotide substitutions in variable regions of RNA genes may induce errors when establishing their relative phylogenetic position. However, *Micromonospora* species tend to group consistently into subclusters, the relative positions of which are generally conserved in the topology of phylogenetic trees, although *Micromonospora olivasterospora* represents an unrelated line of descent (Koch et al., 1996b; Kroppenstedt et al., 2005a; Thawai et al., 2005b). Nevertheless, few internal relationships can be confirmed by bootstrap values higher than 50%. The phylogenetic relationships between *Micromonospora* species are presented in Figure 209.

Cell morphology. *Micromonospora* strains frequently form raised and folded colonies on agar media. Colonies are initially pale yellow to light orange and, depending on the strain, they develop a blue-green, brown, dark orange, purple, or red pigmentation with age. Soluble pigments may be formed. Older colonies tend to be covered by a black, brown-black, or green-black mucous mass of spores (Kawamoto, 1989). Spores often occur in dense clusters and may be produced in distinct areas on the surface or be completely embedded in the substrate. Mycelial pigments sensitive to pH changes have been observed in *Micromonospora coerulea* (blue-green) and *Micromonospora echinospora* (maroon-purple) (Kawamoto, 1989). Other species characterized by soluble pigments are *Micromonospora chalcea* (yellow), *Micromonospora halophytica* (red-brown), *Micromonospora olivasterospora* (olive-green), *Micromonospora purpureochromogenes* (dark-brown), and *Micromonospora rosaria* (wine-red) (Horan and Brodsky, 1986b; Kawamoto, 1989). Nevertheless, mycelial pigmentation, mycelial development, and sporophore morphology have little diagnostic value for the identification of *Micromonospora* species.

Micromonosporae often produce short sporophores which occur in branched clusters on short hyphae of the substrate mycelium (Luedemann and Casmer, 1973). The sympodial sporophore of *Micromonospora carbonacea* (Luedemann and Brodsky, 1964) has also been observed in other species. The formation of single spores on the substrate mycelium is the main morphological characteristic of the genus *Micromonospora*. Occasionally, spores have been found longitudinally in pairs or in larger groups of spores (Luedemann and Casmer, 1973) and a sporulation process resembling sporangial formation has been described in *Micromonospora purpureochromogenes* NRRL B-2671 (Stevens, 1975).

The spore surface ornamentations formed on the outer layer of the spore wall differ from those observed in the sheath of some *Streptomyces* spores (Luedemann and Casmer, 1973). Spore ornamentations of *Micromonospora* strains described originally on the basis transmission electron microcopy as "smooth", "warty", or "blunt-spiny" have been shown by scanning electron microscopy to have blunt-spiny surfaces with variable spine sizes, but this characteristic is not a diagnostic characteristic for the differentiation of *Micromonospora* species (Kawamoto, 1989) (Figure 210).

Sporogenesis involves the formation of a sporulation septum that is followed by spore maturation with a thickening of wall layers (Hardisson and Suarez, 1979; Luedemann and Casmer, 1973). Spore dehiscence is favored by a stretching of the hyphal wall near the sporulation septum. The thickened walls may explain the high refractility and the relative differential resistance of mature spores to physical and chemical treatments, such as sonication or heat treatment up to 75°C

TABLE 191. Differential characteristics of the genus *Micromonospora* and related taxa classified in the family *Micromonosporaceae*

Characteristic	*Micromonospora*	*Actinocatenispora*	*Actinoplanes*	*Asanoa*	*Catellatospora*	*Catenuloplanes*	*Couchioplanes*	*Dactylosporangium*	*Krasilnikovia*	*Longispora*	*Luedemannella*	*Pilimelia*	*Polymorphospora*	*Salinispora*	*Spirilliplanes*	*Verrucosispora*	*Virgisporangium*
Single spores	+	−	−	−	−	−	−	+	−	−	−	−	−	+	−	+	−
Sporangia	−	−	+	−	−	−	−	+	−	−	+	+	−	−	−	−	+
Spore chains	−	+	−	+	+	+	+	−	+	+	−	−	+	−	+	−	−
Motile spores	−	−	+	−	−	+	+	+	−	−	−	+	−	−	+	−	+
Salt requirement	−	−	−	−	−	−	−	−	−	−	−	−	−	+	−	−	−
Cell-wall chemotype[a]	II	II	II	II	II	VI	VI	II	II	II	II	II	II	II	II	II	II
Whole-organism sugars	Ara, Xyl	Gal, Xyl, Ara, Glu, Man, Rib	Ara, Xyl	Ara, Xyl, Gal	Ara, Xyl, Gal	Xyl	Ara, Xyl, Gal	Ara, Xyl	Gal, Man, Xyl, Ara, Rib, Glu	Ara, Xyl, Gal	Xyl, Gal, Man, Rha, Rib, Ara	Ara, Xyl	Xyl	Ara, Xyl, Gal	Xyl, Gal	Man, Xyl	Ara, Gal, Man, Rha, Xyl
Fatty acid type[b]	3b	3b	2d	2d	3b	2c	2c	3b	2d	2d	2d	2d	2a	3a	2d	2b	2d
Major menaquinones (MK-)	10(H_4, H_6) 9(H_4, H_6)	9(H_4, H_6)	9(H_4) 10(H_4)	10(H_6, H_8)	10(H_8, H_6) 9(H_4, H_6)	9(H_8), 10(H_8)	9(H_4)	9(H_4, H_6, H_8)	9(H_6)	10(H_4, H_6)	9(H_4, H_6)	9(H_2, H_4)	10(H_4, H_6) 9(H_4, H_6)	10(H_4)	10(H_4)	9(H_4)	10(H_4, H_6, H_8)
Phospholipid type[c]	PII	PII	PII	PII	PII	PIII	PII	PII	PII	PII	PII	PII	PII	PII	PII	PII	PII

[a]Lechevalier and Lechevalier (1970a, 1970b).

[b]Kroppenstedt (1985).

[c]Lechevalier et al. (1977).

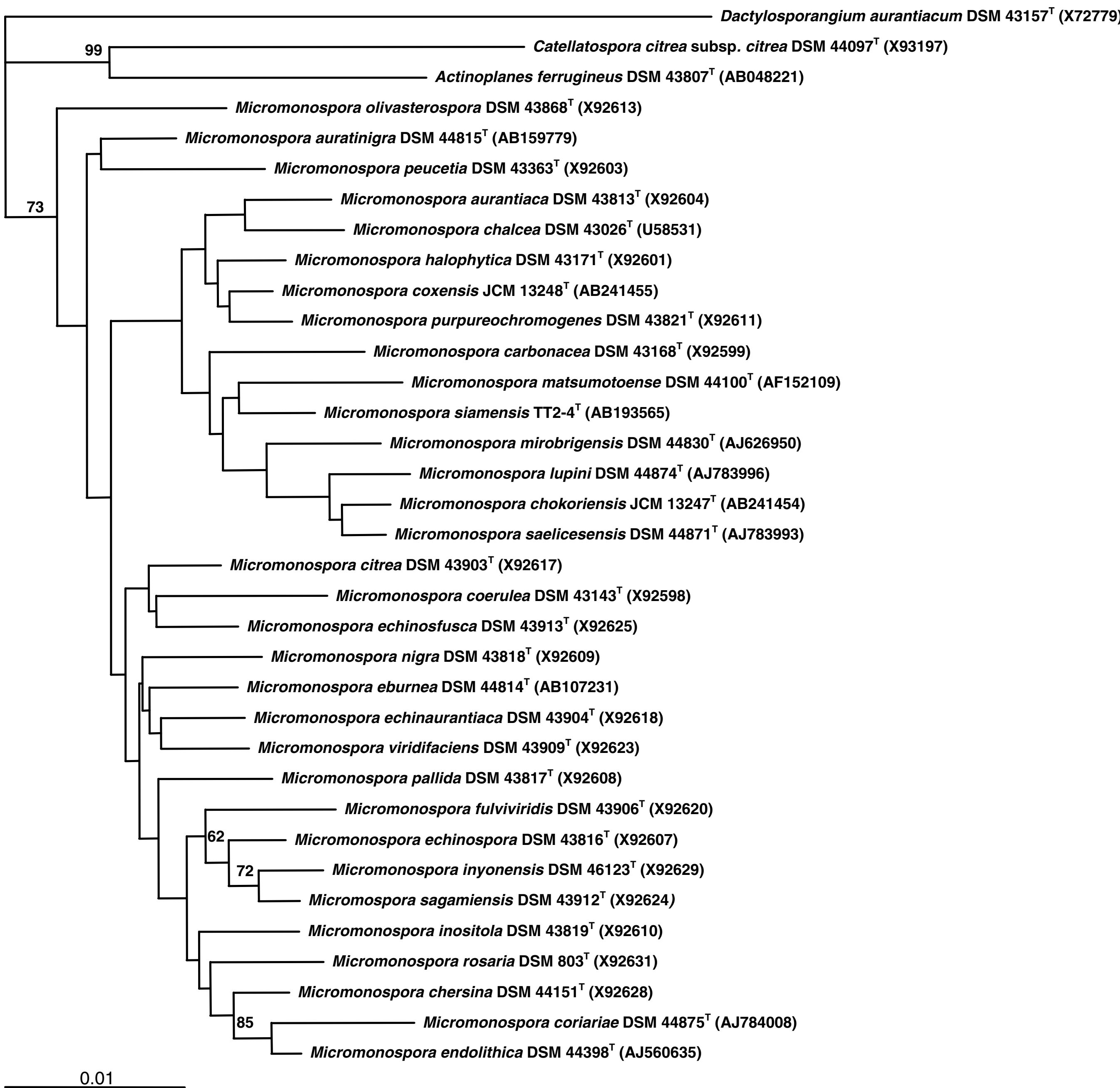

FIGURE 209. Neighbor-joining tree (Saitou and Nei, 1987) based on almost-complete 16S rRNA gene sequences showing relationships between type strains of the genus *Micromonospora*. The tree is based on the Kimura two-parameter method and confidence values of branches were determined by bootstrap analysis (Felsenstein, 1985) based on 1000 replicates. Only values >50% are shown at nodes. Bar = 0.01 substitutions per nucleotide position.

(Johnston and Cross, 1976; Kawamoto et al., 1982; Suárez et al., 1980). Spore viability is only markedly reduced at temperatures above 75°C, as shown in *Micromonospora chalcea* (Suárez et al., 1980). Spores are resistant to desiccation and remain viable in soil or in mud for extended periods (Cross and Attwell, 1974). Heat shock above 70°C induces spore germination (Ensign, 1982; Hoskisson et al., 2000) and dry heat treatments at 120°C clearly select for strains of this genus (Hayakawa et al., 1991b).

Micromonospora spores show some resistance to treatment with different chemical agents, such as acetone (Kawamoto et al., 1982), dimethylformamide, formamide, *tert*-butyl alcohol, and phenol (Hayakawa et al., 1991b). Spore viability is not affected within the pH range 6.0–8.0, but decreases at acidic pH values (Kawamoto et al., 1982). In general, the spore germination process in *Micromonospora* is slower but similar to that observed in other actinomycetes (Kawamoto, 1989).

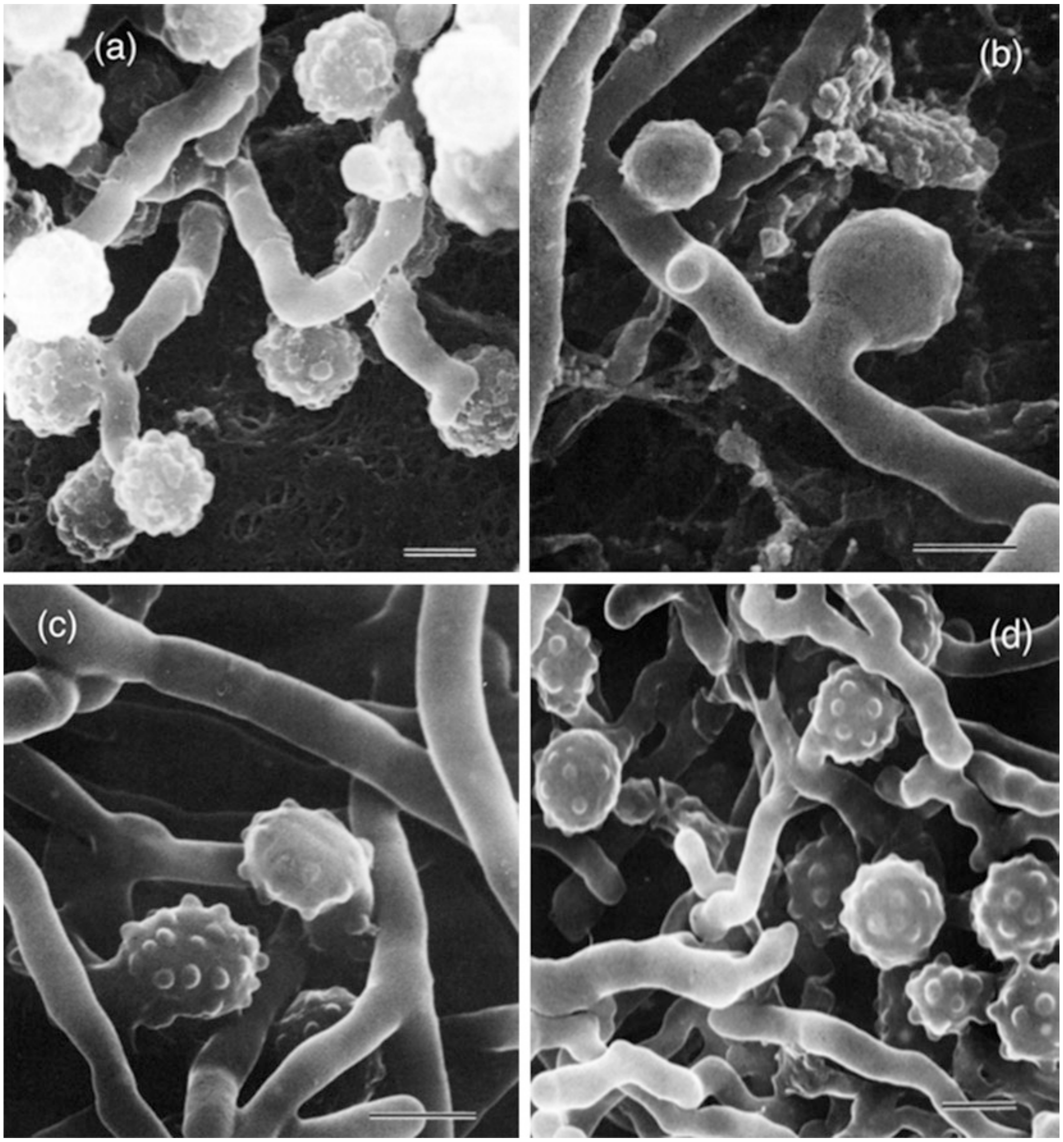

FIGURE 210. Scanning electron micrographs of: (a) *Micromonospora carbonacea* NRRL 2972^T; (b) *Micromonospora chalcea* ATCC 12452^T; (c) *Micromonospora purpureochromogenes* ATCC 27007^T; and (d) *Micromonospora echinospora* NRRL 2985^T. Bar = 0.5 µm.

Cell-wall composition. The genus *Micromonospora* is characterized by a cell-wall type II (Lechevalier and Lechevalier, 1970a, 1970b), a whole-organism sugar pattern D (Lechevalier and Lechevalier, 1970a, 1970b), a phospholipid type PII (Lechevalier et al., 1977), and a fatty acid pattern 3b (Kroppenstedt, 1985). Cell-wall hydrolysates may contain glycine, glutamic acid, diaminopimelic acid (A_2pm), including *meso*- and 3-OH-A_2pm, and D-alanine (Kawamoto et al., 1981). The presence of 3-OH-A_2pm is limited to certain species and its presence/absence divides *Micromonospora* species into two groups (Kawamoto, 1989). The acyl type of the cell-wall muramic acid is glycolyl and there is an almost equimolar ratio of glycolic acid and A_2pm (Kawamoto et al., 1981). Glycine is always present in the first position of the peptide subunit that is attached to *N*-glycolylmuramic acid. However, the peptidoglycan cannot always be hydrolyzed with lysozyme (β-*N*-acetylmuramidase) as observed in *Micromonospora olivasterospora* and *Micromonospora sagamiensis* (Kawamoto et al., 1981) or in strains where protoplasts cannot be effectively obtained (Szvoboda et al., 1980). The cell-wall sugars arabinose and xylose are constituents of the cell wall of most *Micromonospora* species, albeit in variable amounts. Glucose, galactose, mannose, and rhamnose can also be found in whole-organism hydrolysates, although amounts differ from species to species (Kawamoto et al., 1981); galactose, glucose, and rhamnose are found instead of arabinose in certain species (Kroppenstedt et al., 2005a; Trujillo et al., 2005).

Lipids and menaquinone composition. Characteristic phospholipids include diphosphatidylglycerol, phosphatidylethanolamine, phosphatidylinositol, and phosphatidylinositol mannosides (Lechevalier et al., 1977). Dassain et al. (1983) identified the glycolipids, monoglucosyldiglycerides, diglucosyldiglycerides, esters of trehalose, and fatty acids. Predominant

fatty acids are iso- and anteiso-branched components. Fatty acids patterns are of type 3b with saturated and unsaturated fatty acids including $C_{15:0}$ anteiso, $C_{17:0}$ anteiso, $C_{17:0}$ 10-methyl, $C_{18:0}$ 10-methyl, $C_{16:0}$ iso, $C_{15:0}$ iso, and $C_{17:0}$ iso acids (Kroppenstedt, 1985). Mycolic acids and cyclic fatty acids are not present. The menaquinone composition of members of the genus *Micromonospora* is complex and heterogeneous; strains can be divided into three groups based on the predominant menaquinone. The two major groups encompass species that have menaquinones with either nine (MK-9) or ten isoprene units (MK-10); the exception, *Micromonospora pallida*, has menaquinones with 12 units (MK-12) (Collins et al., 1984; Hirsch et al., 2004a; Kawamoto, 1989; Kroppenstedt et al., 2005a; Thawai et al., 2004b, 2005a, 2005b; Tomita et al., 1992b; Trujillo et al., 2005).

Growth conditions. The identification of *Micromonospora* species is supported by physiological characteristics, such as growth on special media, carbon utilization profiles, glycosidase activity, nitrate reduction, and NaCl tolerance (Kawamoto, 1989). Fructose, glucose, sucrose, and starch are utilized as sole carbon sources by almost all strains and most strains utilize cellobiose, galactose, mannose, trehalose, and xylose, but little, if any, growth occurs with glycerol, inositol, mannitol, rhamnose, ribose, or salicin. Carbohydrate utilization patterns can be affected by the basal medium and this can account for discrepancies between results reported by different authors (Kawamoto, 1989). *Micromonospora* strains show α- and β-glucosidase, β-galactosidase, and β-*N*-acetylglucosaminidase activities, but variable results are obtained for α-galactosidase, α-mannosidase, and β-xylosidase (Kawamoto, 1989). Inorganic ammonium salts and acidic and basic amino acids are better nitrogen sources than nitrate salts (Kawamoto et al., 1983). NaCl tolerance ranges from 1.5 to 5% (w/v). Micromonosporae do not grow below pH 5.0 or above pH 9.5 and most strains do not grow at 45°C.

Genetics. *Micromonospora* species are defined genomically by having total genomic DNA–DNA reassociation values lower than 70%; this contrasts with their high 16S rRNA similarity values. Indeed, 16S rRNA gene sequences are almost identical with similarities of 99.7–99.9% for members of *Micromonospora* species and subspecies that were later reclassified as synonyms in DNA–DNA hybridization studies (Koch et al., 1996b). These workers also noted that the composition of the different groups of *Micromonospora* species are supported by signature nucleotides between 16S rRNA gene positions 603 and 627 (Koch et al., 1996b). DNA–DNA reassociation studies have confirmed that the genus is overspeciated (Kasai et al., 2000). The protein-encoding gene *gyrB* has been used as an alternative gene to determine the phylogenetic position and relationships of *Micromonospora* type strains given the high consistency found in their 16S rRNA gene sequences (Kasai et al., 2000). The branching patterns in the *gyrB*-based phylogenetic tree of *Micromonospora* type strains representing 14 species were similar to those of the corresponding 16S rRNA-based tree (Kasai et al., 2000).

Genetics, phages, and plasmids. Various lytic and temperate phages, and phages with no characterized infection cycles have been described in *Micromonospora* species (Alexander et al., 2003; Caso et al., 1990; Kikuchi and Perlman, 1977, 1978; Li et al., 2004; Tilley et al., 1990). Plasmids occur frequently, with many of them being cryptic. Numerous host vectors and shuttle vector systems have been developed from *Micromonospora* plasmids and have allowed genetic recombination studies of important biosynthetic gene clusters (Hosted et al., 2005; Inouye et al., 1994; Li et al., 2003; Parag and Goedeke, 1984; Takada et al., 1994; Vukov and Vasiljevic, 1998).

Ecology. *Micromonospora* strains can be isolated easily from soil and aquatic environments, but almost all described type strains have a terrestrial origin. Micromonosporae are very common in alkaline and neutral soils (Jensen, 1930; Vobis, 1992) and although many strains have been shown to be sensitive to acid pH (Kawamoto, 1989), they have also been isolated from acid soils and plant substrates on acidified media (Zenova et al., 2004). *Micromonospora* spores are hydrophilic, highly resistant to heat treatment and to different chemical agents, and can survive for years in the environment (Cross, 1981b).

It was widely accepted for a long time that the principal habitats of micromonosporae were aquatic ecosystems (Cross, 1981a; Goodfellow and Haynes, 1984). *Micromonospora* strains have been isolated extensively from freshwater and marine aquatic environments (Cross, 1981a, 1981b; Goodfellow and Williams, 1983), are frequently present in water samples of streams and rivers (Burman, 1973), and are amongst the predominant actinomycetes in lake sediments and mud samples (Johnston and Cross, 1976). An active role in the degradation of biopolymers in sediments has been proposed given their ability to decompose cellulose, chitin, and xylan (Erikson, 1941; Hunter et al., 1981). Their tolerance to low oxygen tensions suggests that they may grow under the microaerophilic conditions found in alluvial soils, floodplain meadows, and wet soils of river ecosystems (Goodfellow and Williams, 1983; Vobis, 1992; Zenova and Zviagintsev, 2002). *Micromonospora* strains predominate in actinomycete communities isolated from plant litter, lichens, roots, and organic soil horizons (González et al., 2005; Zenova et al., 1994). Their widespread occurrence even in vegetated arid zones has been explained by their ability to degrade biopolymers (Zenova et al., 1994).

Micromonospora strains have been found to be constituents of endophytic actinobacterial populations recovered from plant tissues; they have been described as colonizing roots of *Casuarina* and *Triticum* species (Coombs and Franco, 2003; Valdés et al., 2005). A selective advantage has been suggested for their association with plants given their ability to suppress the fungal pathogens *Pythium* and *Phytophthora* (Coombs and Franco, 2003). An isolate most closely related to "*Micromonospora yulongensis*" (name not validly published) has been described from surface-sterilized wheat root tissue and culture-independent methods have identified clones of endophytic actinobacteria closely related to *Micromonospora endolithica* and *Micromonospora peucetica* in wheat roots suggesting the presence of a large diversity of *Micromonospora* species in plants (Conn and Franco, 2004; Coombs and Franco, 2003). More recently, surface-sterilized legume nodules of *Lupinus angustifolius*, *Pisum sativum*, and *Vicia sativa* have been shown to be extremely rich reservoirs for the isolation of novel *Micromonospora* strains (Trujillo et al., 2006, 2007).

Micromonospora species have been isolated from diverse marine habitats, including coastal areas (Watson and Williams, 1974), salt marshes (Hunter et al., 1981), and deep-sea sediments (Weyland, 1969, 1981). It was widely accepted for many

years that *Micromonospora* spores were washed from soils and accumulated as dormant spores in lake and sea sediments (Cross, 1981a, 1981b; Goodfellow and Haynes, 1984; Johnston and Cross, 1976; Weyland, 1969, 1981). Despite prior evidence suggesting the adaptation of actinomycetes to the marine environment and the existence of an indigenous marine population (Jensen et al., 1991; Magarvey et al., 2004; Mincer et al., 2002; Okami and Okazaki, 1978; Takizawa et al., 1993), it was only recently that active mycelial growth was demonstrated in sand particles (Jensen et al., 2005). In spite of this, many actinomycetes isolated using seawater-based media grow well in media prepared using distilled water.

Secondary metabolism. Micromonosporae, after streptomycetes, are one of the most prolific producers of bioactive secondary metabolites; they produce an extremely large diversity of chemical structures. Bérdy (2005) cited more than 740 *Micromonospora* strains as producers of different bioactive metabolites. They are particularly important producers of aminoglycosides (fortimicins, gentamicins, kanamycin, mannosidostreptomycin, neomycin B, paromamine, sagamicin, sisomicin, and verdamicin), ansamycins (halomicins, rifamycins), anthracyclines (daunorubicin, sibanomicin), anthraquinones (dynemicin), macrolides (antibiotic XK 41-B-2, erythromycins, juvenimicins, megalomycins, rosaramicin, and rustmicin), oligosaccharides (antlermicin and everninomicin), enediyne antitumor antibiotics (calicheamycin), and peptide antibiotics (actinomycin, bottromycin, microsporin, and thiocoraline) (Horan, 1999; Vobis, 1992; Wagman and Weinstein, 1980).

Enrichment and isolation procedures

Micromonosporae are easily isolated from soils and sediments by plating serial dilutions onto suitably nutritionally poor selective media that limit the growth of members of fast-growing bacterial species. The media most commonly used are arginine-glycerol-salts agar (Hunter et al., 1984), arginine-vitamin agar, starch-casein-nitrate agar supplemented with B vitamins (Shearer, 1987), cellulose-asparagine agar (Goodfellow and Haynes, 1984), colloidal chitin agar (Hsu and Lockwood, 1975), humic acid-vitamins agar (Hayakawa and Nonomura, 1987a), Kodoka's cellulose benzoate medium (Sandrak, 1977), and M3 medium (Rowbotham and Cross, 1977); inoculated plates are incubated at 28–30°C for at least 2–3 weeks. The addition of antibiotics such as gentamicin (1–10 µg/ml) (Ivanitskaia et al., 1978), nalidixic acid (20–30 µg/ml), novobiocin (25–50 µg/ml) (Sveshnikova et al., 1976), and tunicamycin (20–50 µg/ml) (Nonomura and Hayakawa, 1988; Wakisaka et al., 1982) to media promote the isolation of *Micromonospora* species. Different pretreatment regimes can also be used such as heating soil suspensions at 70°C for 10–30 min (Rowbotham and Cross, 1977; Sandrak, 1977), and dry heating of soil samples at 120°C for 60 min (Shearer, 1987). The pretreatment of soil suspensions with ammonia then chlorine for 10–30 min (Burman et al., 1969; Willoughby, 1969a) or with 1.5% (w/v) phenol followed by plating onto humic acid-vitamins agar supplemented with nalidixic acid (20 µg/ml) and tunicamycin (20 µg/ml) have been shown to be highly effective isolation methods (Hayakawa et al., 1991b).

The isolation of micromonosporae from plant materials, including roots, requires a preliminary sterilization of the material surface by successive treatments in 99% ethanol, 3.1% NaOCl, and 99% ethanol prior to plating (Coombs and Franco, 2003). Selective media prepared with artificial seawater are used in combination with specific methods to isolate micromonosporae from marine sediments and marine invertebrates. Dilution and heat shock treatments, stamping methods, and enrichments based on the incubation of sediments plated on membrane filters in humid chambers have been effective for the isolation of *Micromonospora* strains from sediments (Magarvey et al., 2004; Mincer et al., 2002). The slow growth of isolates frequently requires extended incubation for up to 10 weeks at lower temperatures (Goodfellow and Haynes, 1984).

Maintenance procedures

Sporulated cultures can be maintained for months on agar slopes or sealed plates at 4°C. Long-term storage can be achieved by lyophilization, by liquid drying, or by maintaining spores or liquid seed cultures in 10–15% glycerol at −80°C.

Differentiation of the genus *Micromonospora* from other genera

The morphological and chemotaxonomic characteristics that distinguish *Micromonospora* from other genera classified in the family *Micromonosporaceae* are given in Table 191. The genus can be differentiated from other members of the family on the basis of cell morphology, chemotaxonomy, and 16S rRNA gene sequence analysis.

Taxonomic comments

The description of *Micromonospora* species began with taxonomic studies on gentamicin-producing strains (Luedemann and Brodsky, 1964). The *Approved Lists of Bacterial Names* (Skerman et al., 1980) included 12 species (*Micromonospora aurantiaca*, *Micromonospora brunnea*, *Micromonospora carbonacea*, *Micromonospora chalcea*, *Micromonospora coerulea*, *Micromonospora echinospora*, *Micromonospora gallica*, *Micromonospora halophytica*, *Micromonospora inositola*, *Micromonospora purpurea*, *Micromonospora purpureochromogenes*, and *Micromonospora rhodorangea*) and seven subspecies as members of the genus *Micromonospora*, though the type strain of *Micromonospora gallica* is not extant (Kawamoto, 1989). *Micromonospora olivasterospora* (Kawamoto et al., 1983), *Micromonospora rosaria* (Horan and Brodsky, 1986b), and *Micromonospora chersina* (Tomita et al., 1992b) were described later and *Catellatospora matsumotoense* was transferred to the genus as *Micromonospora matsumotoense* (Lee et al., 1999).

DNA–DNA hybridization experiments and the study of the intrageneric relationships deduced from 16S rRNA and *gyrB* phylogenetic studies of *Micromonospora* type strains have confirmed the reclassification of many species and subspecies of the genus (Kasai et al., 2000; Koch et al., 1996b). *Micromonospora echinospora* subsp. *pallida* was reclassified as the novel species *Micromonospora pallida*, and the subspecies *Micromonospora halophytica* subsp. *nigra* as *Micromonospora nigra*. DNA–DNA reassociation studies on strains of *Micromonospora* species have confirmed that the taxa *Micromonospora echinospora* subsp. *ferruginea*, *Micromonospora purpurea*, and *Micromonospora rhodorangea* are synonyms of *Micromonospora echinospora* and that *Micromonospora brunnea* is a synonym of *Micromonospora purpureochromogenes* (Kasai et al., 2000; Szabó and Fernandez, 1984). In addition, the division of *Micromonospora carbonacea* into two subspecies, *Micromonospora carbonacea* subsp. *carbonacea* and *Micromonospora carbonacea* subsp. *aurantiaca*, originally established on the basis of the morphology of the spores, is not supported by DNA relatedness data; hence, the subspecies are considered as synonyms

of *Micromonospora carbonacea* (Kasai et al., 2000). The phylogenetic position of the type strain of *Micromonospora aurantiaca* within the genus has been confirmed by 16S rRNA analysis (Kasai et al., 2000), in spite of an early association of this taxon with the genus *Actinoplanes* (Kawamoto, 1989).

Descriptions of 18 novel *Micromonospora* species have been published recently: *Micromonospora auratinigra*, *Micromonospora chokoriensis*, *Micromonospora citrea*, *Micromonospora coriariae*, *Micromonospora coxensis*, *Micromonospora eburnea*, *Micromonospora echinaurantiaca*, *Micromonospora echinofusca*, *Micromonospora endolithica*, *Micromonospora fulviviridis*, *Micromonospora inyonensis*, *Micromonospora lupini*, *Micromonospora mirobrigensis*, *Micromonospora peucetia*, *Micromonospora saelicesensis*, *Micromonospora sagamiensis*, *Micromonospora siamensis*, and *Micromonospora viridifaciens* (Ara and Kudo, 2007b; Hirsch et al., 2004a; Kroppenstedt et al., 2005a; Thawai et al., 2004b, 2005a, 2005b; Trujillo et al., 2005, 2006, 2007).

Since submission of this chapter, the following *Micromonospora* species have been described: *Micromonospora chaiyaphumensis* (Jongrungruangchok et al., 2008b); *Micromonospora krabiensis* (Jongrungruangchok et al., 2008a); *Micromonospora marina* (Tanasupawat et al., 2010); *Micromonospora narathiwatensis* (Thawai et al., 2007); *Micromonospora pattaloongensis* (Thawai et al., 2008); *Micromonospora pisi* (Garcia et al., 2010); *Micromonospora rifamycinica* (Huang et al., 2008); and *Micromonospora tulbaghiae* (Kirby and Meyers, 2010).

Differentiation of species of the genus *Micromonospora*

Characteristics which can be used to differentiate between *Micromonospora* species are shown in Table 192 and Table 193.

Acknowledgements

Many thanks to Dr Oscar Salazar for his contribution to updating the phylogenetic analysis of *Micromonospora* species.

List of species of the genus *Micromonospora*

1. **Micromonospora chalcea** (Foulerton 1905) Ørskov 1923, 156[AL] ("*Streptothrix chalcea*" Foulerton 1905, 1199)

chal′ce.a. L. fem. adj. *chalcea* brazen, of brass.

Raised and folded reddish orange colonies that turn brown, olive-brown to dark-brown with age and eventually black upon sporulation. The substrate mycelium is covered by a moist to dry spore layer; a pale yellow fluorescent diffusible pigment is produced in yeast starch media. Does not form melanin pigments. Spores are produced abundantly on short or long sporophores. Dark brown occasionally sessile, oval to spherical spores (0.7–1.0 µm in diameter) are formed. Phase-contrast microscopy has shown that the spores have smooth surfaces.

Diagnostic carbohydrate utilization pattern is good growth on melibiose, but growth does not occur on mannitol or rhamnose. Grows on arabinose, cellobiose, fructose, glucose, galactose, lactose, levulose, mannose, soluble starch, sucrose, trehalose, and xylose as sole carbon sources, but not on glycerol, inositol, salicin, or ribose. Decomposes cellulose; negative for nitrate reduction; liquefies gelatin and digests milk. Good growth occurs between 27 and 37°C, but does not grow at 45°C. Maximum NaCl tolerance is 5% (w/v). Peptidoglycan contains *meso*-A_2pm; major menaquinones are MK-10(H_4) and MK-10(H_6).

Source: isolated from air, soil, and aquatic environments.

DNA G+C content (mol%): 71.9 (HPLC).

Type strain: ATCC 12452, DSM 43026, NBRC 13503, JCM 3031, NRRL B-2344, VKM Ac-822.

Sequence accession nos: U58531, X92594 (16S rRNA gene); AB014148 (*gyrB*).

2. **Micromonospora aurantiaca** Sveshnikova, Maksimova and Kudrina 1969, 758[AL]

au.ran.ti.a′ca. N.L. fem. adj. *aurantiaca* orange-colored.

Colonies grown on synthetic media produce light yellow, orange, or dark orange mycelium without a spore layer. On organic media, colonies present a pale orange to bright orange pigmentation of the substrate mycelium that turns grayish on sporulation on some media. Does not produce melanin pigments. Spores are formed in clusters, albeit moderately so.

Arabinose, cellobiose, fructose, galactose, melibiose, and xylose are used as sole carbon sources, but not glycerol, lactose, mannitol, rhamnose, ribose, or salicin. Decomposes cellulose; positive for nitrate reduction, milk peptonization, and decomposition of tyrosine; negative for starch hydrolysis.

Source: soil.

DNA G+C content (mol%): 71.6 (HPLC).

Type strain: ATCC 27029, DSM 43813, NBRC 16125, NBRC 16155, JCM 10878, NRRL B-16091, VKM Ac-1936.

Sequence accession nos: X92604 (16S rRNA gene); AB015621 (*gyrB*).

Additional remarks: according to Rule 12b of the *Bacteriological Code* (1990 Revision), Euzéby and Tindall (2004) requested the replacement of the specific epithet "*aurantiaca*" in *Micromonospora aurantiaca* by "*sandarakina*".

3. **Micromonospora auratinigra** corrig. Thawai, Tanasupawat, Itoh, Suwanborirux and Kudo 2004a, 1425[VP] [*Micromonospora aurantionigra* (*sic*)] (Effective publication: Thawai, Tanasupawat, Itoh, Suwanborirux and Kudo 2004b, 13.)

au.ra.ti.ni′gra. L. adj. *auratus -a -um* gold-colored; L. adj. *niger -gra -grum* black; N.L. fem. adj. *auratinigra* gold- and black-colored, referring to the color of colonies.

Colonies are vivid orange on yeast extract-malt extract agar (ISP medium 2) turning to brownish black or black after sporulation; a brown soluble pigment is produced. Aerial mycelium is absent. Does not produce melanin pigments. Single spores are formed on sporophores produced on substrate hyphae. Growth is observed between 20 and 30°C, but not above 40°C. Maximum NaCl concentration for growth is 2% (w/v); pH range for growth is 5–10.

Arabinose, cellobiose, fructose, galactose, glucose, lactose, melibiose, raffinose, ribose, salicin, and xylose are used as sole carbon sources, but not glycerol, mannitol, or

TABLE 192. Morphological and chemotaxonomic characteristics of *Micromonospora* species[a]

Characteristic	*M. chalcea* DSM 43026[T]	*M. aurantiaca* DSM 43813[T]	*M. auratinigra* DSM 44815[T]	*M. carbonacea* DSM 43168[T]	*M. chersina* DSM 44151[T]	*M. chokoriensis* JCM 13247[T]	*M. citrea* DSM 43903[T]	*M coerulea* DSM 43143[T]	*M. coriariae* DSM 44875[T]	*M. coxensis* JCM 13248[T]	*M. eburnea* DSM 44814[T]	*M. echinaurantiaca* DSM 43904[T]	*M. echinofusca* DSM 43913[T]	*M. echinospora* DSM 43816[T]
Substrate mycelium	Red-orange	Yellow orange to dark orange	Bright orange to brown black	Orange to black	Light orange-yellow	Golden brown to dark brown	Salmon to yellow orange	Blue-green	Orange	Light brown to cinnamon brown	Yellowish orange	Orange to light yellow	Brown orange	Dark brown to purple/red brown to orange
Diffusible pigment	Light yellow	None	Brown	None	Yellow	None	Yellow orange	None	None	None	Pale yellow	None	None	None
Diaminopimelic acid isomer	*meso*	*meso*	*meso* + 3-OH	*meso* + 3-OH	*meso*	*meso*	*meso*	*meso*	*meso*	*meso*	*meso*	*meso*	*meso*	*meso* + 3-OH
Whole-organism sugars[b]	nd	Xyl, Ara	Glu, Xyl, Ara, Gal, Man, Rib	nd	Glu, Man, Xyl, Ara	Rib, Man, Xyl, Gal, Glu, Ara	Xyl, Glu	nd	Glu, Man, Ara, Xyl, Rib	Glu, Rha, Man, Ara, Xyl, Gal, Rib	Xyl, Ara	Xyl, Glu, Rham	Xyl, Glu	nd
Major menaquinones	MK-10	nd	MK-9, MK-10	MK-9	MK-9, MK-10	MK-10	MK-9	MK-10	MK-10	MK-10	MK-9	MK-9	MK-9	MK-10
DNA G+C content (mol%)	71.9	71.6	72.8	73.3	72.9	71	nd	71.7	70.2	73	71.5	nd	nd	71.7
Maximum NaCl tolerance (%, w/v)	5	4	2	3	3	3	nd	1.5	1	3	4	nd	nd	3
Temperature growth range (°C)	27–45	nd	25–30	27–37	18–49	20–37	nd	24–41	12–37	15–37	25–45	nd	nd	27–37
Antibiotic produced				Everninomicin complex	Dynemicins									Gentamicin complex

[a]Data from: Ara and Kudo (2007b); Asano et al. (1989a); Hirsch et al. (2004a); Horan and Brodsky (1986b); Kasai et al. (2000); Kawamoto (1989); Kawamoto et al. (1974, 1983); Kroppenstedt et al. (2005a); Lee et al. (1999); Luedemann (1971); Luedemann and Brodsky (1964, 1965); Nara et al. (1977); Thawai et al. (2004b, 2005a, 2005b); Tomita et al. (1992b); Trujillo et al. (2005, 2006, 2007); Weinstein et al. (1968, 1970). nd, Not determined.

[b]Ara, Arabinose; Gal, galactose; Glu, glucose; Man, mannose; Rha, rhamnose; Rib, ribose; Xyl, xylose.

M. endolithica DSM 44398^T	*M. fulvoviridis* DSM 43906^T	*M. halophytica* DSM 43171^T	*M. inositola* DSM 43819^T	*M. inyonensis* DSM 46123^T	*M. lupini* DSM 44874^T	*M. matsumotoense* DSM 44100^T	*M. mirobrigensis* DSM 44830^T	*M. nigra* DSM 43818^T	*M. olivasterospora* DSM 43868^T	*M. pallida* DSM 43817^T	*M. peucetia* DSM 43363^T	*M. purpureochromogenes* DSM 43821^T	*M. rosaria* DSM 803^T	*M. saelicesensis* DSM 44871^T	*M. sagamiensis* DSM 43912^T	*M. siamensis* TT2-4^T	*M. viridifaciens* DSM 43909^T
Orange, olive to black	Salmon orange to light yellow	Orange brown	Bright orange	Yellow olive to red brown to yellow	Light orange	Red to brown orange	Orange	Orange olive to brown black	light brown to olive	light ivory brown to black	Deep orange to green	Dark brown	Orange brown to purple black	Orange	Coral red	Vivid orange	Pastel yellow to nut brown
None	None	Red-brown	None	Brown yellow	None	Red-brown	None	None	Olive green	None	None	Dark brown	Wine red	Orange brown-brown	None	Pale yellow	None
meso	*meso*	*meso* + 3-OH	*meso* + 3-OH	*meso*	*meso*	*meso* + 3-OH	*meso*	*meso* + 3-OH	*meso* + 3-OH	*meso* + 3-OH	*meso*	*meso*	*meso* + 3-OH	*meso*	*meso*	*meso*	*meso*
Xyl, Ara, Gal, Rib, Rham	Xyl, Glu	nd	nd	Xyl, Glu, Ara	Glu, Man, Ara, Xyl, Rha	Ara, Xyl, Gal, 3-OH-Met-Rham	Glu, Gal, Man, Xyl	nd	Xyl, Ara, Glu	nd	Xyl, Ara, Glu	nd	Xyl, Ara, Glu	Glu, Man, Ara, Xyl, Rib, Rha	Xyl, Glu	Xyl, Ara	Xyl, Glu, Rham
MK-10	MK-9	MK-9	MK-10	MK-9	MK-10	MK-10	MK-10	MK-9	MK-10	MK-12	MK-9	MK-10	MK-10	MK-10	MK-9	MK-10	MK-9
70	nd	72.5	71.4	nd	70.9	71	68.6	71.7	71.9	71.1	nd	73	72.9	71.6	nd	73	nd
2.5	nd	4	1.5	nd	2	2	3	4	3	3	nd	1.50	2	2	nd	5	nd
8–39	nd	18–40	25–40	nd	20–37	nd	20–37	18–40	28–38	nd	nd	nd	35–40	20–37	nd	20–40	nd
		Halomycin complex	Antibiotic XK-41 complex	Sisomicin				Halomycin complex	Fortimicin complex	Gentamicin complex	Adriamycin		Rosaramicin		Sagamicin (XK62-2)		Antibiotic 37505

TABLE 193. Utilization of carbohydrates and other physiological characteristics of *Micromonospora* type strains[a]

Characteristic	*M. chalcea* DSM 43026^T	*M. aurantiaca* DSM 43813^T	*M. auratinigra* DSM 44815^T	*M. carbonacea* DSM 43168^T	*M. chersina* DSM 44151^T	*M. chokoriensis* JCM 13247^T	*M. citrea* DSM 43903^T	*M. coerulea* DSM 43143^T	*M. coriariae* DSM 44875^T	*M. coxensis* JCM 13248^T	*M. eburnea* DSM 44814^T	*M. echinaurantiaca* DSM 43904^T	*M. echinofusca* DSM 43913^T	*M. echinospora* DSM 43816^T	*M. endolithica* DSM 44398^T	*M. fulviviridis* DSM 43906^T	*M. halophytica* DSM 43171^T	*M. inositola* DSM 43819^T	*M. inyonensis* DSM 46123^T	*M. lupini* DSM 44874^T	*M. matsumotoense* DSM 44100^T	*M. mirobrigensis* DSM 44830^T	*M. nigra* DSM 43818^T	*M. olivasterospora* DSM 43868^T	*M. pallida* DSM 43817^T	*M. peucetia* DSM 43363^T	*M. purpureochromogenes* DSM 43821^T	*M. rosaria* DSM 803^T	*M. saelicesensis* DSM 44871^T	*M. sagamiensis* DSM 43912^T	*M. siamensis* TT2-4^T	*M. viridifaciens* DSM 43909^T
Nitrate reduction	v	+	−	+	v	nd	nd	−	+	nd	+	nd	nd	v	−	nd	+	−	nd	−	+	−	+	+	+	nd	v	−	−	+	–	−
Peptonization of milk	+	+	w	+	-	nd	nd	−	nd	nd	+	nd	nd	+	nd	nd	+	−	nd	nd	v	nd	v	+	+	nd	w	+	nd	+	+	+
Starch hydrolysis	+	−	+	+	+	nd	nd	+	+	nd	+	nd	nd	+	+	nd	+	+	nd	+	+	+	+	+	+	nd	−	+	v	+	+	nd
Decomposition of tyrosine	−	+	−	nd	−	nd	nd	−	−	nd	−	nd	nd	−	+	nd	−	−	nd	−	−	nd	−	−	−	nd	−	+	−	nd	nd	−
Liquefaction gelatin	+	+	+	+	+	nd	nd	nd	nd	nd	+	nd	nd	+	+	nd	w	+	nd	+	+	+	v	+	+	nd	w	+	+	w	+	+
Carbohydrate utilization:																																
L-Arabinose	w	w	+	+	+	+	+	−	+	+	−	+	−	+	+	+	+	−	+	−	+	+	+	+	+	−	−	+	+	−	+	+
Cellobiose	+	+	+	+	+	nd	nd	+	+	nd	+	nd	nd	+	+	nd	+	+	nd	+	+	+	+	+	+	nd	+	+	+	nd	+	nd
D-Fructose	w	+	+	+	+	+	−	+	+	+	−	+	+	−	+	+	+	+	+	nd	+	nd	w	+	+	+	+	+	nd	−	w	+
D-Galactose	+	+	+	+	+	+	nd	+	−	+	w	nd	nd	−	+	nd	+	+	nd	+	+	+	+	+	−	nd	+	w	+	+	+	nd
Glycerol	−	−	−	−	-	−	nd	−	nd	+	+	nd	nd	−	nd	nd	−	−	nd	nd	−	nd	−	−	−	nd	+	−	nd	−	-	nd
L-Inositol	−	nd	nd	−	-	−	−	−	nd	−	nd	−	−	−	−	−	−	w	+	−	−	nd	−	−	−	−	−	nd	+	−	nd	+
Lactose	+	−	+	+	w	+	nd	+	nd	+	+	nd	nd	−	+	nd	+	+	nd	nd	+	nd	+	−	−	nd	w	+	nd	−	+	nd
D-Mannitol	−	−	−	−	−	−	−	+	nd	−	w	+	−	−	−	+	−	v	+	+	−	−	−	−	−	−	−	+	+	−	-	+
D-Mannose	+	nd	nd	+	+	+	nd	nd	+	+	nd	nd	nd	+	+	nd	+	+	nd	+	+	+	+	+	+	nd	nd	+	+	nd	nd	nd
Melezitose	−	nd	nd	−	-	nd	nd	nd	−	nd	nd	nd	nd	−	nd	nd	nd	nd	nd	nd	−	−	nd	−	−	nd	−	−	nd	nd	nd	nd
Melibiose	+	+	+	+	+	+	nd	+	+	nd	+	nd	nd	−	+	nd	+	+	nd	+	−	nd	+	−	−	nd	+	−	+	nd	+	nd
Raffinose	+	w	+	−	v	+	−	+	+	+	+	+	−	−	+	+	+	+	+	+	+	+	+	−	−	−	+	−	+	−	+	+
L-Rhamnose	−	−	−	−	−	−	−	−	+	w	+	−	−	+	−	−	−	−	+	−	−	−	−	−	+	−	−	+	−	−	+	+
D-Ribose	−	−	+	−	v	w	nd	−	+	+	−	nd	nd	v	−	nd	−	−	nd	nd	w	nd	−	+	w	nd	−	+	nd	nd	-	+
Salicin	−	−	+	v	nd	+	nd	w	+	+	+	nd	nd	−	+	nd	+	−	nd	nd	−	nd	+	−	−	nd	−	−	nd	nd	+	nd
Soluble starch	+	−	+	+	+	nd	nd	nd	+	nd	w	nd	nd	+	nd	nd	+	+	nd	nd	+	+	+	+	+	nd	nd	+	nd	nd	nd	+
Sucrose	+	nd	nd	+	+	+	+	nd	−	+	nd	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	+	nd	+
Trehalose	+	nd	nd	+	+	+	nd	nd	+	+	nd	nd	nd	+	+	nd	+	nd	nd	+	+	+	+	+	+	nd	nd	+	−	nd	nd	nd
D-Xylose	+	+	+	+	+	+	−	+	+	+	+	−	−	+	+	−	+	+	+	nd	+	nd	+	+	+	−	+	+	nd	+	+	+

[a]Data from: Ara and Kudo (2007b); Asano et al. (1989a); Hirsch et al. (2004a); Horan and Brodsky (1986b); Kasai et al. (2000); Kawamoto (1989); Kawamoto et al. (1974, 1983); Kroppenstedt et al. (2005a); Lee et al. (1999); Luedemann and Brodsky (1964, 1965); Luedemann (1971); Nara et al. (1977); Thawai et al. (2004b, 2005a, 2005b); Tomita et al. (1992b); Trujillo et al. (2005, 2007, 2006); Weinstein et al. (1968, 1970). nd, Not determined; v, variable response; w, weak response.

rhamnose. Positive for starch hydrolysis and gelatin liquefaction; weakly positive for milk peptonization; negative for nitrate reduction and for decomposition of adenine, hypoxanthine, tyrosine, and xanthine. Hydrogen sulfide is not produced.

Cell wall contains glutamic acid, glycine, alanine, and *meso*-A_2pm and 3-OH-A_2pm. Arabinose and xylose are the characteristic whole-organism sugars, but galactose, glucose, mannose, and ribose are also present. The phospholipid profile contains diphosphatidylglycerol, phosphatidylethanolamine, phosphatidylinositol, and phosphatidylinositol mannosides. Predominant menaquinones are MK-10(H_4), MK-9(H_4), and MK-10(H_6); the major fatty acids include $C_{15:0}$ iso and $C_{16:0}$ iso with small amounts of $C_{17:0}$ iso, $C_{17:0}$ anteiso, and $C_{15:0}$ anteiso. Does not contain mycolic acids. DNA–DNA relatedness with other *Micromonospora* type strains ranges between 32 and 53%.

Source: isolated from soil collected in a peat swamp forest, Thailand.

DNA G+C content (mol%): 72.8 (HPLC).

Type strain: TT1-11, JCM 12357, PCU 239, DSM 44815, TISTR 1532.

Sequence accession no. (16S rRNA gene): AB159779.

4. **Micromonospora carbonacea** Luedemann and Brodsky 1965, 51[AL]

car.bo.na′ce.a. L. n. *carbo -onis* coal, charcoal; L. suff. *-aceus* suffix of various meanings, but signifying in general made of or belonging to; N.L. fem. adj. *carbonacea* charcoal-like (referring to color of spores).

Colonies are raised, folded, and initially orange, but turn brown to black on sporulation. Spores are generally abundant. Characteristic blackish sporulating peripheral sectors are formed. Spore layer is moist or dry, but not viscid. Spores are oval to spherical (0.7–1.0 µm in diameter), and smooth-walled by light microscopy. Sporulation is observed in liquid media as sparsely dispersed clumps of mycelium, which consist mostly of unbranched, long, loosely woven, fine mycelial strands. Spores remain firmly attached to the sympodial type of sporophore, and are only free in older cultures. The mycelium does not degenerate into polymorphic bodies. Fair to good growth occurs on Czapek's sucrose agar supplemented with 0.1% $CaCO_3$. Melanin pigments are not produced. Good growth occurs between 27 and 37°C, but does not grow at 45°C.

Arabinose, cellobiose, fructose, glucose, galactose, lactose, levulose, mannose, melibiose, soluble starch, sucrose, trehalose, and xylose are used as sole carbon sources, but not mannitol or rhamnose. Poor growth is observed on arabinose, dulcitol, glycerol, inositol, raffinose, ribose, salicin, sorbose, and sorbitol. Gelatin is liquefied. Milk is digested. Positive for nitrate reduction, hydrolysis of tyrosine, and oxidase reaction. Tolerates salt up to 3% (w/v).

Major fatty acids are $C_{15:0}$ iso and $C_{17:1}$ ω8 and the predominant menaquinone is MK-9(H_4). Produces antibiotics of the everninomicin complex.

Source: isolated from a soil sample collected from Olean, New York.

DNA G+C content (mol%): 73.3 (HPLC).

Type strain: ATCC 27114, DSM 43168, NBRC 14108, JCM 3139, NRRL 2972.

Sequence accession nos: X92599 (16S rRNA gene); AB014147 (*gyrB*).

Additional remarks: in the original description, poor growth was reported on raffinose and salicin, but these sugars are utilized in a chemically defined agar medium (Kawamoto, 1989). *Micromonospora carbonacea* subsp. *carbonacea* Luedemann and Brodsky 1965[AL] and *Micromonospora carbonacea* subsp. *aurantiaca* Luedemann and Brodsky 1965[AL] are synonyms of *Micromonospora carbonacea* Luedemann and Brodsky 1965, 51[AL] (Kasai et al., 2000).

5. **Micromonospora chersina** Tomita, Hoshino, Ohkusa, Tsuno and Miyaki 1992a, 656[VP] (Effective publication: Tomita, Hoshino, Ohkusa, Tsuno and Miyaki 1992b, 25.)

cher.si′na. Gr. adj. *khersinos* living in dry land; N.L. fem. adj. *chersina* referring to the savanna vegetation from which this organism was isolated.

Light orange to yellow colonies turning light olive gray or black upon sporulation. Fluorescent yellow diffusible pigment produced on Czapek's sucrose-nitrate agar and inorganic salts-starch agar (ISP medium 4). Melanin pigments are not produced. Single spherical spores (1.2–1.8 µm) are sessile or borne on short or long monopodial sporophores developed from well-branched substrate hyphae (0.5 µm diameter). The spore surface carries short blunt spines. Short sterile aerial hyphae occasionally developed on some media. Moderate growth occurs on inorganic salts starch and yeast-malt extract agars, but poor growth occurs on many media, including Czapek's agar, and glycerol-asparagine and tyrosine agars. Grows between 18 and 49°C, and optimally between 37 and 44°C. pH range for growth is 5.5–10.5. Maximum NaCl tolerance is 3% (w/v).

Arabinose, cellobiose, fructose, galactose, glucose, mannose, melibiose, sucrose, trehalose, and xylose are used as sole carbon sources, but not arabinose, inositol, glycerol, mannitol, rhamnose, or ribose. Utilization of raffinose is variable depending on the medium. Positive for gelatin liquefaction and starch hydrolysis. Nitrate reduction is variable depending on the medium. Negative for tyrosinase activity. Positive for α- and β-glucosidases, but negative for α-mannosidase and β-xylosidase.

Cell wall contains *meso*-A_2pm and glycine. Whole-organism sugars include glucose and mannose, and traces of arabinose and xylose. Predominant menaquinones are MK-9(H_4), MK-9(H_6), MK-10(H_4), and MK-10(H_6). Major phospholipids are phosphatidylinositol and phosphatidylethanolamine. Produces antitumor antibiotic dynemicin.

Source: isolated from an Indian soil.

DNA G+C content (mol%): 72.9 (HPLC).

Type strain: M956-1, ATCC 53710, DSM 44151, NBRC 15963, JCM 9459.

Sequence accession nos: X92628 (16S rRNA gene); AB015622 (*gyrB*).

6. **Micromonospora chokoriensis** Ara and Kudo 2007, 1372[VP] (Effective publication: Ara and Kudo 2007b, 35.)

cho.ko.ri.en′sis. N.L. fem. adj. *chokoriensis* of or pertaining to Chokoria, Bangladesh, the origin of the soil from which the type strain was isolated

Golden brown to dark brown colonies are formed on yeast extract-starch agar without soluble pigments. Melanin pigments are not produced. Well-developed branched substrate mycelium bears single sessile spores. Spores are spherical with a rough to nodular spore surface. Grows at pH 5–9, at 20–37°C, and in 3% NaCl.

Arabinose, fructose, galactose, glucose, lactose, maltose, mannose, α-melibiose, raffinose, salicin, sucrose, trehalose, and xylose are used as sole carbon sources, but not adonitol, erythritol, fructose, glycerol, rhamnose, *myo*-inositol, or mannitol. Ribose is weakly utilized.

Cell wall contains *meso*-A_2pm. Whole-organism sugars include arabinose, galactose, glucose, mannose, ribose, and xylose. Contains diphosphatidylglycerol, phosphatidylethanolamine, phosphatidylinositol, and phosphatidylinositol mannosides as major polar lipids. Major fatty acids are $C_{15:0}$ iso, $C_{16:0}$ iso, $C_{17:0}$ iso, and $C_{15:0}$ anteiso. The predominant menaquinone is MK-10(H_4); minor amounts of MK-10(H_6), MK-9(H_4), and MK-9(H_6) are formed.

Source: isolated from sandy soil in forest-side waterfall.

DNA G+C content (mol%): 71 (HPLC).

Type strain: 2-19(6), JCM 13247.

Sequence accession no. (16S rRNA gene): AB241454.

7. **Micromonospora citrea** Kroppenstedt, Mayilraj, Wink, Kallow, Schumann, Secondini and Stackebrandt 2005b, 1743[VP] (Effective publication: Kroppenstedt, Mayilraj, Wink, Kallow, Schumann, Secondini and Stackebrandt 2005a, 333.)

ci′tre.a. L. fem. adj. *citrea* of or pertaining to the citrus-tree.

Colonies are pastel orange on yeast-malt extract agar (ISP medium 2), pastel yellow on inorganic salts-starch agar (ISP medium 4), and yellow orange on peptone-yeast extract-iron agar (ISP medium 6). A yellow orange soluble pigment is formed on peptone-yeast extract-iron agar. Aerial mycelium is absent. Single spores are formed on substrate mycelium.

Arabinose, glucose, and sucrose are used as sole carbon sources, but not fructose, inositol, mannitol, raffinose, rhamnose, or xylose. Positive for alkaline phosphatase, *N*-acetyl-β-glucosaminidase, esterase, α-and β-galactosidases, α-glucosidase, and acid phosphatase, but negative for α-fucosidase, β-glucosidase, β-glucuronidase, lipase, and α-mannosidase.

Peptidoglycan contains *meso*-A_2pm. Whole-organism diagnostic sugars are glucose and xylose. Major menaquinone is MK-9(H_4); phosphatidylethanolamine is the diagnostic phospholipid. Major fatty acids are $C_{15:0}$ iso, $C_{16:0}$ iso, and $C_{17:0}$ 10-methyl.

Source: isolated from lake mud, China.

DNA G+C content (mol%): not determined.

Type strain: DSM 43903, ATCC 35571, JCM 3256, NBRC 14025, NRRL B-16101.

Sequence accession no. (16S rRNA gene): X92617.

8. **Micromonospora coerulea** Jensen 1932, 177[AL]

coe.ru′le.a. L. fem. adj. *coerulea* dark blue, dark green.

Slow-growing colonies that require 3–5 weeks to develop. Pale-yellow-orange colonies turn yellow-green then dark blue-green to greenish black with age. pH-sensitive soluble pigment is produced. Spherical spores (0.8–1.5 µm in diameter) are borne on short or lateral sporophores. Mycelium fragmentation is observed rarely in old liquid cultures. Good growth occurs between 24 and 37°C.

Fructose, galactose, and lactose are used as sole carbon sources, but poor growth is seen on arabinose and arabinose. Starch is hydrolyzed.

Source: isolated from soil from Mt Haleakala, Maui Island, Hawaii.

DNA G+C content (mol%): 71.7 (HPLC).

Type strain: ATCC 27008, DSM 43143, NBRC 13504, JCM 3175, VKM Ac-661.

Sequence accession nos: X92598 (16S rRNA gene); AB014151 (*gyrB*).

9. **Micromonospora coriariae** Trujillo, Kroppenstedt, Schumann, Carro and Martínez-Molina 2006, 2384[VP]

co.ri.a.ri′a.e. N.L. gen. n. *coriariae* of *Coriaria*, pertaining to the isolation of the type strain from root nodules of *Coriaria myrtifolia*.

Raised, folded, intensively orange colonies turn darker with age, does not form diffusible pigments. Abundant growth on Bennett's SA1 and yeast extract-malt extract (ISP medium 2) agars. Single spores form at the tips of well-developed substrate hyphae. Optimum growth is at 28°C. Grows at pH 7–9, does not grow below pH 6.5.

Alanine, arabinose, arginine, cellobiose, fructose, gluconate, glucose, histidine, mannose, melibiose, pyruvate, raffinose, rhamnose, salicin, serine, starch, trehalose, and xylose are used as sole carbon sources, but not ascorbic acid, galactose, glutaric acid, lysine, melezitose, proline, propionic acid, sorbitol, sorbose, sucrose, tyrosine, valine, or xylitol. Positive for acid and alkaline phosphatases, esterases, α- and β-galactosidases, α- and β-glucosidases, and *N*-acetyl-β-glucosaminidase, but not for α-chymotrypsin, β-glucuronidase, or α-mannosidase. Oxidase- and catalase-positive. Degrades arbutin, casein, esculin, gelatin, starch, and Tween 80, but not tyrosine, urea, or Tween 20.

Whole-organism hydrolysates contain *meso*-A_2pm, and arabinose, glucose, mannose, ribose, and, xylose as characteristic sugars. Fatty acid profile is characterized by significant amounts of $C_{15:0}$ iso, $C_{16:0}$ iso, $C_{17:0}$, and $C_{17:0}$ 10-methyl. Major menaquinone is MK-10(H_4), with minor amounts of MK-10(H_6) and MK-9(H_4).

Source: the type strain was isolated from root nodules of *Coriaria myrtifolia*.

DNA G+C content (mol%): 70.2 (T_m).

Type strain: NAR01, DSM 44875, LMG 23557.

Sequence accession no. (16S rRNA gene): AJ784008.

10. **Micromonospora coxensis** Ara and Kudo 2007, 1372[VP] (Effective publication: Ara and Kudo 2007b, 36.)

cox.en′sis. N.L. fem. adj. *coxensis* pertaining to Cox's Bazaar, Bangladesh, the origin of the soil from which the type strain was isolated.

Colonies on yeast extract-starch agar are light brown to cinnamon brown without diffusible pigment. Melanin pigments are not produced. Well-developed substrate mycelium is formed with spherical single spores borne directly on substrate hyphae. Spore surface is nodular to warty. Grows at pH 5–9, at 15–37°C, and in 3% (w/v) NaCl.

Adonitol (weak), arabinose, fructose, galactose, glycerol, lactose, mannose, maltose, α-melibiose, raffinose, rhamnose, ribose, salicin, sucrose, trehalose, and xylose are used as sole carbon sources, but not erythritol, *myo*-inositol, or mannitol.

Whole-organism hydrolysates contain *meso*-A_2pm, and arabinose, galactose, glucose, mannose, rhamnose, ribose, and xylose as characteristic sugars. Fatty acid profile is characterized by significant amounts of $C_{17:0}$, $C_{17:1}$ iso, $C_{15:0}$ iso, $C_{17:0}$ anteiso, and $C_{16:1}$ iso. Major menaquinones are MK-10(H_6) and MK-10(H_8), with minor amounts of MK-10(H_4), MK-9(H_4), MK-9(H_6), and MK-9(H_8).

Source: isolated from sandy soil next to a forest-side waterfall.

DNA G+C content (mol%): 73 (HPLC).

Type strain: 2-30-b(28), JCM 13248, MTCC 8093.

Sequence accession no. (16S rRNA gene): AB241455.

11. **Micromonospora eburnea** Thawai, Tanasupawat, Itoh, Suwanborirux, Suzuki and Kudo 2005b, 55[VP]

e.bur′ne.a. L. fem. adj. *eburnea* of ivory, white as ivory, referring to the color of colonies.

Well-developed and branched substrate mycelium. Colonies have a characteristic yellowish white or dull orange color depending on the media. They turn grayish black upon sporulation on yeast extract-malt extract agar (ISP medium 2); a pale yellow soluble pigment is produced on this medium and on oatmeal (ISP medium 3) and nutrient agars. Nonmotile single spores (0.45 µm in diameter)

are borne on substrate hyphae. Aerial mycelium is not produced. Optimal temperature for growth is 25–30°C, does not grow above 45°C. Maximum NaCl concentration tolerated for growth is 4% (w/v).

Cellobiose, glucose, glycerol, lactose, melibiose, raffinose, rhamnose, salicin, and xylose are used as sole carbon sources, but not arabinose, fructose, or ribose. Positive for milk peptonization, starch hydrolysis, and nitrate reduction.

Cell-wall hydrolysates contain alanine, glycine, glutamic acid, and *meso*-A_2pm. Muramic acid is glycolated. Arabinose and xylose are the characteristic whole-organism sugars. Whole-organism hydrolysates contain galactose, glucose, mannose, and ribose. The characteristic phospholipids are phosphatidylethanolamine, phosphatidylglycerol, phosphatidylinositol, and phosphatidylinositol mannosides. Predominant menaquinones are MK-9(H_4), MK-10(H_4), and MK-9(H_6). Major fatty acids include $C_{15:0}$ iso, $C_{16.0}$, $C_{17:0}$ iso, $C_{15:0}$ anteiso, $C_{17:0}$, and $C_{17:0}$ anteiso. Mycolic acids are absent.

Source: isolated from a peat swamp forest soil in southern Thailand.

DNA G+C content (mol%): 71.5 (HPLC).

Type strain: LK2-10, DSM 44814, JCM 12345, PCU 238, TISTR 1531.

Sequence accession no. (16S rRNA gene): AB107231.

12. **Micromonospora echinaurantiaca** Kroppenstedt, Mayilraj, Wink, Kallow, Schumann, Secondini and Stackebrandt 2005b, 1743[VP] (Effective publication: Kroppenstedt, Mayilraj, Wink, Kallow, Schumann, Secondini and Stackebrandt 2005a, 333.)

e.chin.au.ran.ti.a′ca. Gr. n. *echinos* hedgehog, sea-urchin, N.L. fem. adj. *aurantiaca* orange-colored; N.L. fem. adj. *echinaurantiaca* spiny and orange-colored.

Orange-colored colonies. Aerial mycelium is not formed. Pigment of substrate mycelium ranges from bright orange on inorganic salts-starch agar (ISP medium 4), peptone-yeast extract-iron agar (ISP medium 6), and yeast extract-malt extract agar (ISP medium 2) to deep orange on oatmeal agar (ISP medium 3). Soluble pigments are not produced.

Arabinose, fructose, glucose, mannitol, sucrose, and raffinose are used as sole carbon sources, but not inositol, rhamnose, or xylose. Positive for alkaline phosphatase, *N*-acetyl-β-glucosaminidase, esterase, α-glucosidase, α- and β-galactosidase, and phosphatase acid, but not for β-glucosidase, β-glucuronidase, lipase, α-mannosidase, or α-fucosidase.

Peptidoglycan contains *meso*-A_2pm. Whole-organism diagnostic sugars are glucose and rhamnose. Major menaquinone is MK-9(H_4). Phosphatidylethanolamine is the diagnostic phospholipid. Major fatty acids include $C_{15:0}$ iso, $C_{16.0}$, and $C_{17:0}$ iso.

Source: isolated from a Chinese soil.

DNA G+C content (mol%): not determined.

Type strain: DSM 43904, ATCC 35572, JCM 3257, NBRC 14022, NRRL B-16102.

Sequence accession no. (16S rRNA gene): X92618.

13. **Micromonospora echinofusca** Kroppenstedt, Mayilraj, Wink, Kallow, Schumann, Secondini and Stackebrandt 2005b, 1743[VP] (Effective publication: Kroppenstedt, Mayilraj, Wink, Kallow, Schumann, Secondini and Stackebrandt 2005a, 333.)

e.chi.no.fus′ca. Gr. n. *echinos* hedgehog, sea-urchin; L. fem. adj. *fusca* brown-colored, N.L. fem. adj. *echinofusca* spiny and brown-colored.

Colonies are orange-colored ranging from deep brownish-orange on inorganic-salts-starch agar (ISP medium 4), and glucose-asparagine agar (ISP medium 5), to pastel orange on oatmeal agar (ISP medium 3), tyrosine agar (ISP medium 7), and yeast extract-malt extract agar (ISP medium 2). Aerial mycelium is absent. Poor growth occurs on peptone-yeast extract-iron agar (ISP medium 6). Soluble pigments are not formed.

Fructose, glucose, and sucrose are used as sole carbon sources, but not arabinose, inositol, mannitol, raffinose, rhamnose, or xylose. Positive for *N*-acetyl-β-glucosaminidase, alkaline phosphatase, α- and β-galactosidases, α- and β-glucosidases, and acid phosphatase, but not for α-fucosidase, glucuronidase, lipase, or α-mannosidase.

Peptidoglycan contains *meso*-A_2pm. Whole-organism diagnostic sugars are glucose and xylose. Major menaquinone is MK-9(H_4). Phosphatidylethanolamine is the diagnostic phospholipid. Major fatty acids are $C_{15:0}$ iso, $C_{16:0}$ iso, and $C_{17:1}$ ω8*c*.

Source: isolated from excrement of a chukar at Beijing Zoological Garden, China.

DNA G+C content (mol%): not determined.

Type strain: DSM 43913, JCM 3327, NBRC 14267.

Sequence accession no. (16S rRNA gene): X92625.

14. **Micromonospora echinospora** Luedemann and Brodsky 1964, 121[AL] emend. Kasai, Tamura and Harayama 2000, 131

e.chi.no.spo′ra. Gr. n. *echinos* hedgehog, sea-urchin; Gr. n. *spora* seed and in biology a spore; N.L. fem. n. *echinospora* spiny spore.

Colonies are orange-brown, maroon to dark purple and folded. Maroon to dark-purple pigments are pH-sensitive (red in the acid range and blue-green and precipitable in the basic range). Black spore layer is waxy to dry. Does not form melanin pigments. Sporulation is moderate, requiring 2–3 weeks for completion. Occasionally forms a sterile aerial mycelium in the form of a gray bloom. Spores are spherical (1.0–1.5 µm) and rough walled under phase-contrast microscopy. Sporophores are single or in small clusters on the same hyphae. Grows between 27 and 37°C, but not at 45°C.

Arabinose, cellobiose, glucose, mannose, sucrose, trehalose, soluble starch, and xylose are used as sole carbon sources, but not glycerol, inositol, raffinose, sorbitol, or salicin. Poor to fair growth occurs on galactose, lactose, and levulose. Diagnostic carbohydrate utilization pattern: good growth on rhamnose; poor growth on melibiose, mannitol, and ribose. Slow decomposition of cellulose. Gelatin is weakly liquefied. Milk is weakly digested.

Most strains produce antibiotics of the gentamicin complex.

Source: isolated from a soil sample collected from Jamesville, New York.

DNA G+C content (mol%): 71.7 (HPLC).

Type strain: ATCC 15837, DSM 43816, NBRC 13149, JCM 3073, NRRL 2985, VKM Ac-669.

Sequence accession nos: X92607 (16S rRNA gene); AB014154 (*gyrB*).

Additional remarks: earlier heterotypic synonyms are *Micromonospora rhodorangea* Wagman et al. 1974, *Micromonospora echinospora* subsp. *ferruginea* Luedemann and Brodsky 1964, and *Micromonospora purpurea* Luedemann and Brodsky 1964 (Skerman et al., 1980).

15. **Micromonospora endolithica** Hirsch, Mevs, Kroppenstedt, Schumann and Stackebrandt 2004b, 631[VP] (Effective publication: Hirsch, Mevs, Kroppenstedt, Schumann and Stackebrandt 2004a, 172.)

en.do.li′thi.ca. L. prep. *endo* in, within; Gr. n. *lithos* stone; L. suff. *-icus -a –um*, suffix used in adjectives with the sense of belonging to; N.L. fem. adj. *endolithica* (growing) within stone.

Orange, folded colonies that turn grayish-black, olive, or black upon sporulation. Soluble pigments are not produced. Vegetative hyphae (0.5–1.0 µm in diameter) often produce terminal "lemon-shaped" bodies. Spores (0.8–1.1 µm) are produced singly on short hyphae side branches and have short warty surfaces. Temperature range for growth is 8–39°C, with an optimal temperature of 27–29°C. Tolerates up to 7% salt, but optimum for growth is 2%.

Arabinose, cellobiose, fructose, galactose, glucose, lactose, maltose, mannitol (weak), mannose, melibiose, raffinose (weak), sucrose, trehalose, and xylose are used as sole carbon sources, but not *N*-acetylglucosamine, citrate, dextrin, inositol, oxalate, rhamnose, ribose, sorbitol, or sorbose. Catalase- and cytochrome oxidase-positive. Degrades adenine, casein, gelatin, starch, tyrosine, and xanthine, but not cellulose, chitin, or xylan. Hydrolyzes esculin.

Cell-wall amino acids include alanine, glycine, glutamic acid, and *meso*-A_2pm, and whole-organism sugars are arabinose, galactose, rhamnose, ribose, and xylose. Characteristic cell-wall sugars are arabinose and xylose. The characteristic phospholipids are phosphatidylethanolamine, phosphatidylglycerol, and phosphatidylinositol. Predominant menaquinones are MK-10(H_4) and MK-10(H_6). Major fatty acids include $C_{16:0}$ iso, $C_{17:1}$ ω8*c*, $C_{17:1}$ iso ω9*c*, and $C_{15:0}$ iso; $C_{18:0}$ 10-methyl is also present. Sensitive to 50 µg/ml lysozyme.

Source: isolated from sandstone in Antarctica.

DNA G+C content (mol%): 70.0 (T_m).

Type strain: AA-459, DSM 44398, JCM 12677, NRRL B-24248.

Sequence accession no. (16S rRNA gene): AJ560635.

16. **Micromonospora fulviviridis** Kroppenstedt, Mayilraj, Wink, Kallow, Schumann, Secondini and Stackebrandt 2005b, 1743[VP] (Effective publication: Kroppenstedt, Mayilraj, Wink, Kallow, Schumann, Secondini and Stackebrandt 2005a, 335.)

ful.vi.vi′ri.dis. L. adj. *fulvus* tawny, brown; L. adj. *viridis* green; N.L. fem. adj. *fulviviridis* brown-green.

Salmon orange to salmon pink colonies are formed on inorganic salts-starch (ISP medium 4), oatmeal (ISP medium 3), and yeast extract-malt extract (ISP medium 2) agars. Pastel yellow substrate mycelium is formed on glycerol-asparagine (ISP medium 5) and peptone-yeast extract (ISP medium 6) agars. Soluble pigments are not formed.

Arabinose, fructose, glucose, mannitol, raffinose, and sucrose are used as sole carbon sources, but not inositol, rhamnose, or xylose. Positive for alkaline phosphatase, α- and β-galactosidases, α- and β-glucosidases, and acid phosphatase, but not for *N*-acetyl-β-glucosaminidase, α-fucosidase, β-glucuronidase, or α-mannosidase.

Peptidoglycan contains *meso*-A_2pm. Whole-organism diagnostic sugars are glucose and xylose. Major menaquinone is MK-9(H_4). Phosphatidylethanolamine is the diagnostic phospholipid. Major fatty acids are $C_{15:0}$ iso, $C_{16:0}$, and $C_{17:0}$ iso.

Source: isolated from soil.

DNA G+C content (mol%): not determined.

Type strain: DSM 43906, ATCC 35574, JCM 3259, NRRL B-6104.

Sequence accession no. (16S rRNA gene): X92620.

17. **Micromonospora halophytica** Weinstein, Luedemann, Oden and Wagman 1968, 436[AL]

ha.lo.phy′ti.ca. N.L. fem. adj. *halophytica* growing under the influence of seawater.

Forms folded orange colonies that turn orange-brown with age. Sporulation is abundant, especially in older colonies. Light reddish brown diffusible pigment is produced in media containing galactose, lactose, levulose, mannose, raffinose, or trehalose. Melanin pigments are not produced. Grows between 18 and 40°C, but not at 50°C. Good growth within pH range 6.8–7.8. Spores are produced randomly along branching mycelium on short or long sporophores, but are occasionally sessile. Abundant dark colored spores occur in older cultures. Spores are spherical to ellipsoidal (up to 1.2 µm in diameter).

Arabinose, fructose, galactose, glucose, lactose, levulose, mannose, melibiose, raffinose, starch, sucrose, trehalose, and xylose are used as sole carbon sources. Poor growth occurs on adonitol, cellulose, rhamnose, inositol, mannitol, ribose, and sorbitol. Hydrolyzes starch, decomposes cellulose, reduces nitrate, liquefies gelatin, and digests milk. Produces antibiotics of the halomicin antibiotic complex.

Source: isolated from a salt pool in Syracuse, New York.

DNA G+C content (mol%): 72.5 (HPLC).

Type strain: ATCC 27596, DSM 43171, NBRC 14112, JCM 3125, NRRL 2998.

Sequence accession nos: X92601 (16S rRNA gene); AB014157 (*gyrB*).

18. **Micromonospora inositola** Kawamoto, Okachi, Kato, Yamamoto, Takahashi, Takasawa and Nara 1974, 495[AL]

i.no.si′to.la. N.L. fem. adj. *inositola* intended to mean that the bacterium is able to utilize inositol.

Folded, bright orange to orange colonies are formed on Bennett's, nutrient, and potato agars. Soluble pigments are not produced. Does not form a spore layer. Poor growth occurs on glucose-asparagine, inorganic starch, and oatmeal agars. Poor sporulation occurs on most media. Spores are oval to spherical (0.8–1.0 µm in diameter) and borne on short sporophores. Grows between 25 and 40°C and at pH 5.5–8.5.

Cellobiose, fructose, galactose, glucose, inositol (weak), lactose, mannose, raffinose, and xylose are used as sole carbon sources, but not arabinose, glycerol, mannitol, rhamnose, ribose, salicin, or sorbitol. Liquefies gelatin. Milk is slightly coagulated, but not peptonized. Negative for nitrate reduction. Cellulose is slightly decomposed. Starch is hydrolyzed. Produces antibiotics of the macrolide XK-41 complex.

Source: isolated from a soil sample from Hokkaido, Japan.

DNA G+C content (mol%): 71.4 (HPLC).

Type strain: ATCC 21773, DSM 43819, JCM 6239, NRRL B-16095.

Sequence accession nos: X92610 (16S rRNA gene); AB014158 (*gyrB*).

19. **Micromonospora inyonensis** Kroppenstedt, Mayilraj, Wink, Kallow, Schumann, Secondini and Stackebrandt 2005b, 1743[VP] (Effective publication: Kroppenstedt, Mayilraj, Wink, Kallow, Schumann, Secondini and Stackebrandt 2005a, 337.)

in.yo.nen′sis. N.L. fem. adj. *inyonensis* of or pertaining to Inyo County, California.

Viscid to poorly plicate colonies with fair to poor growth of substrate mycelium. Aerial mycelium is not formed. Substrate mycelium ranges from copper brown on inorganic salts-starch agar (ISP medium 4), to red-green on oatmeal agar (ISP medium 3), yellow on peptone-yeast extract-iron (medium ISP 6) and tyrosine (ISP medium 7), and yellow-olive on yeast extract-malt extract agar (ISP medium 2). Soluble brown yellow pigment is produced on ISP medium 6. Sparse growth occurs on glycerol-asparagine agar (ISP medium 5). Long branched mycelium is formed that is regular and nonseptate and with a mean diameter of 0.5 µm. Ovoid to spherical spores (1.0–1.5 µm in diameter) are rough walled and occasionally borne singly on sporophores.

Poor utilization of arabinose, fructose, glucose, inositol, mannitol, rhamnose, raffinose, sucrose, and xylose is observed. Positive for alkaline phosphatase, esterase, *N*-acetyl-β-glucosaminidase, α- and β-glucosidases, and acid phosphatase, but not for α-fucosidase, lipase, α- or β-galactosidase, β-glucuronidase, or α-mannosidase.

Peptidoglycan contains *meso*-A_2pm. Whole-organism diagnostic sugars are arabinose, glucose, and xylose. Major menaquinone is MK-9(H_4). Phosphatidylethanolamine is the diagnostic phospholipid. Major fatty acids are $C_{15:0}$ iso, $C_{16:0}$ iso, and $C_{17:1}$ iso. Produces the aminoglycoside sisomicin (also known as rickamicin or antibiotic 6640).

Source: isolated from soil.

DNA G+C content (mol%): not determined.

Type strain: DSM 46123, JCM 3188, ATCC 27600, NBRC 13156, NRRL 3292.

Sequence accession no. (16S rRNA gene): X92629.

20. **Micromonospora lupini** Trujillo, Kroppenstedt, Fernández-Molinero, Schumann and Martínez-Molina 2007, 2803[VP]

lu′pi.ni. L. gen. n. *lupini* of a lupin, referring to the isolation of the first strains from *Lupinus angustifolius*.

Light orange, folded, raised colonies without diffusible pigments are formed on yeast extract-malt extract agar. Produces well-developed branched hyphae (0.3–0.6 µm in diameter) with smooth-surfaced spores produced at hyphal tips. Grows at 20–37°C. Good growth occurs in 1% (w/v) NaCl, but growth is variable in 2% (w/v) NaCl.

Alanine, cellobiose, galactose, maltose, mannose, melibiose, raffinose, and trehalose are assimilated as carbon sources, but not arginine, histidine, lysine, proline, rhamnose, serine, sorbitol sorbose, tyrosine, valine, or xylitol. Catalase- and oxidase-positive. Nitrate is not reduced. Degrades arbutin, casein, esculin, gelatin, starch, and xylan, but not tyrosine. Positive for alkaline phosphatase, esterases and lipases, α- and β-galactosidases, α- and β-glucosidases, and *N*-acetyl-β-glucosaminidase, but not for urease, or β-glucuronidase.

Whole-organism hydrolysates contain *meso*-A_2pm and arabinose, glucose, mannose, rhamnose, and xylose as characteristic sugars. Diagnostic phospholipid is diphosphatidylethanolamine. Major fatty acids are $C_{16:0}$ iso and $C_{15:0}$ iso. Predominant menaquinone is MK-10(H_4). One of the strains of this species produces the antitumoral lupinamicins A and B.

Source: isolated from root nodules of *Lupinus angustifolius*.

DNA G+C content (mol%): 70.9 (T_m).

Type strain: Lupac 14N, DSM 44874, LMG 24055.

Sequence accession no. (16S rRNA gene): AJ783996.

21. **Micromonospora matsumotoense** (Asano, Masunaga and Kawamoto 1989a) Lee, Goodfellow and Hah 2000a, 3[VP] (Effective publication: Lee, Goodfellow and Hah 1999, 353.) (*Catellatospora matsumotoense* Asano, Masunaga and Kawamoto 1989a, 313)

mat.su.mo.to.en′se. L. deriv. *matsumotoense* (*sic*) of Matsumoto Nagago, Japan, the location of the soil sample from which the type strain was isolated.

Orange colonies are formed on oatmeal and yeast extract-malt extract agars and yellow colonies are formed on glucose-yeast extract and nutrient agars. A reddish-brown soluble pigment is produced on oatmeal agar. Does not require thiamine for growth. Grows in the presence of 2% (w/v) NaCl, but not in 3% (w/v) NaCl.

L-Arabinose, cellobiose, fructose, glucose, galactose, lactose, maltose, mannose, raffinose, ribose, starch, sucrose, trehalose, and xylose are used as sole carbon sources, but not adonitol, D-arabinose, dextran, gluconate, glycerol, mannitol, melezitose, melibiose, rhamnose, salicin, sorbitol, sorbose, or xylitol. Decomposes casein, elastin, gelatin, and starch. Resistant to neomycin (5 µg/ml), novobiocin (50 µg/ml), tetracycline (20 µg/ml), and vancomycin (20 µg/ml).

Cell walls contain *meso*- and 3-OH-A_2pm. Muramic acid is glycolated. Diagnostic cell-wall sugars are arabinose, galactose, 3-*O*-methylrhamnose, and xylose. Major menaquinones are MK-10(H_4), MK-10(H_6), and MK-10(H_8). Diphosphatidylglycerol, phosphatidylethanolamine, phosphatidylinositol, and phosphatidylglycerol are the predominant polar lipids. Major fatty acids include $C_{16:0}$ iso, $C_{18:1}$, $C_{17:1}$, $C_{16:1}$ iso, and $C_{15:0}$ iso.

Source: isolated from soil.

DNA G+C content (mol%): 71.0 (HPLC).

Type strain: 6393-C, ATCC 49364, CIP 106812, DSM 44100, NBRC 14550, JCM 9104, NRRL B-16490, VKM Ac-2009, MSNU 22003.

Sequence accession no. (16S rRNA gene): AF152109.

22. **Micromonospora mirobrigensis** Trujillo, Fernández-Molinero, Velázquez, Kroppenstedt, Schumann, Mateos and Martínez-Molina 2005, 879[VP]

mi.ro.bri.gen′sis. N.L. fem. adj. *mirobrigensis* of or belonging to Mirobriga, the region in Spain where the type strain was isolated.

Small, raised, and folded colonies. Colonies grow slowly on Bennett's, glucose-yeast extract, and nutrient agars, and are 2–3 mm in diameter after incubation for 2 weeks on SA1 medium. Initially, colonies are orange, but turn brownish black with age and upon sporulation. Produces a well developed substrate mycelium with single warty spores. Grows between 20–37°C, with an optimal temperature of 28°C, but does not grow at 45°C. Grows at pH 7.0 and tolerates NaCl up to 3% (w/v).

Arabinose, cellobiose, galactose, glucose, maltose, mannose, raffinose, sucrose, and trehalose are used as sole carbon sources, but not citrate, malate, mannitol, melezitose, rhamnose, sorbitol, sorbose, or xylitol. Hydrolyzes arbutin and esculin. Degrades casein, gelatin, starch, and xylan. Nitrate is not reduced. Shows resistance to ampicillin (2 μg). Positive for acid phosphatase, alkaline phosphatase, esterase lipase, α- and β-galactosidases, β-glucuronidase, α- and β-glucosidases, and *N*-acetyl-β-glucosaminidase.

Cell wall contains *meso*-A_2pm. Whole-organism sugars are galactose, glucose, mannose, and xylose. Major fatty acids are $C_{15:0}$ iso, $C_{16:0}$ iso, $C_{17:0}$ iso, and $C_{17:0}$ anteiso. Predominant menaquinones are MK-10(H_4) and MK-10(H_6). Major phospholipids are diphosphatidylglycerol, phosphatidylethanolamine, phosphatidylglycerol, and phosphatidylinositol.

Source: isolated from a water sample taken from a pond in the region of Mirobriga, Ciudad Rodrigo, Spain.

DNA G+C content (mol%): 68.6 (T_m).

Type strain: WA201, DSM 44830, LMG 22229.

Sequence accession no. (16S rRNA gene): AJ626950.

23. **Micromonospora nigra** (Weinstein, Luedemann, Oden and Wagman 1968) Kasai, Tamura and Harayama 2000, 131[VP] (*Micromonospora halophytica* subsp. *nigra* Weinstein, Luedemann, Oden and Wagman 1968, 437; Kawamoto 1989, 2446)

ni′gra. L. fem. adj. *nigra* black.

Orange colonies turn olive-brown to black upon sporulation. Diffusible pigments are not produced. A black spore layer is observed on media containing fructose, galactose, lactose, raffinose, or trehalose. Melanin pigments are not produced. Grows between 18 and 40°C, but not at 50°C.

Adonitol (weak), arabinose, cellulose (weak), fructose, galactose, lactose, salicin, and raffinose are used as sole carbon sources, but not glycerol, mannitol, or rhamnose. Starch is hydrolyzed, cellulose is decomposed, and nitrate is reduced. Gelatin liquefaction and milk digestion are variable. Produces antibiotics of the halomycin complex.

Source: isolated from a salt pool in Syracuse, New York.

DNA G+C content (mol%): 71.7 (HPLC).

Type strain: ATCC 33088, DSM 43818, NBRC 16103, JCM 8973, NCIMB 2225, NRRL 3097.

Sequence accession nos: X92609 (16S rRNA gene); AB014156 (*gyrB*).

24. **Micromonospora olivasterospora** Kawamoto, Yamamoto and Nara 1983, 110[VP]

o.li.va.ste.ro.spo′ra. L. n. *oliva* an olive; Gr. n. *aster* a star; Gr. n. *spora* a seed and in biology a spore; N.L. fem. n. *olivasterospora* olive-colored spore that looks like a star.

Light brown to dark yellow colonies that become covered with an olive to dark green waxy layer of spores with age. Olive-green soluble, non-pH-sensitive pigments are formed on oatmeal and yeast extract-malt extract agars. Good growth occurs on most organic media. Melanin pigments are not formed. Well-developed branched and septate substrate hyphae (0.5 μm diameter) are formed. Spores are oval to spherical (approx 1.0 μm diameter), and rough-surfaced as seen by phase-contrast microscopy. Spores are borne on short sporophores or sessile, occurring randomly or in clusters. Terminally or intercalary chlamydospore-like swellings are sometimes present. Grows at 28–38°C and pH 6.8–7.8.

Fructose, galactose, glycerol, mannose, maltose, ribose, starch, sucrose, trehalose, and xylose are used as sole carbon sources, but not arabinose, dulcitol, inositol, lactose, mannitol, raffinose, melezitose, α-melibiose, rhamnose, salicin, sorbitol, or sorbose. NH_4NO_3, $(NH_4)_2SO_4$, NH_4Cl, arginine, aspartic acid, glutamic acid, histidine, and serine are used as sole nitrogen sources. Starch is hydrolyzed. Skim milk is peptonized, but not coagulated. Weak decomposition of cellulose. Positive for α- and β-glucosidases, and *N*-acetyl-β-glucosaminidase, but not for α-fucosidase, α- or β-galactosidases, or α-mannosidase.

Cell walls contain D-alanine, 3-OH-A_2pm, glycine, and glutamic acid. Arabinose, glucose, and xylose are the predominant whole-organism sugars. Major polar lipids are diphosphatidylglycerol, phosphatidylethanolamine, phosphatidylinositol, and phosphatidylinositol mannosides. Major fatty acids are $C_{15:0}$ iso and $C_{16:0}$ iso. Produces antibiotics of the fortimicin complex.

Source: isolated from a soil sample from Hiroshima, Japan.

DNA G+C content (mol%): 71.9 (HPLC).

Type strain: MK-70, ATCC 21819, DSM 43868, NBRC 14304, JCM 7348, NRRL 8178, VKM Ac-1317.

Sequence accession nos: X92613 (16S rRNA gene); AB014159 (*gyrB*).

25. **Micromonospora pallida** (Luedemann and Brodsky 1964) Kasai, Tamura and Harayama 2000, 131^VP (*Micromonospora echinospora* subsp. *pallida* Luedemann and Brodsky 1964, 116)

pal′li.da. L. fem. adj. *pallida* pale.

Light ivory to light melon-yellow colonies, which turn brown to black upon sporulation. Does not produce purple mycelial pigments, or melanin pigments. Spores are spherical, dark brown to black. Sporophores are solitary or in small clusters.

Arabinose, cellobiose, glucose, levulose, mannose, soluble starch, sucrose, trehalose, and xylose are used as sole carbon sources. Good growth occurs on rhamnose, whereas slight growth is found on ribose with abundant sporulation. Poor growth occurs on salicin. Growth on and decomposition of cellulose is slow. Positive for nitrate reduction, gelatin liquefaction, and milk digestion. Good growth is observed at 27–37°C.

Possesses a different menaquinone (MK-12) to other *Micromonospora* species. Produces antibiotics of the gentamicin complex.

Source: isolated from soil collected in Jamesville, New York.

DNA G+C content (mol%): 71.1 (HPLC).

Type strain: ATCC 15838, DSM 43817, NBRC 16070, JCM 3133, NRRL 2996.

Sequence accession nos: X92608 (16S rRNA gene); AB014153 (*gyrB*).

26. **Micromonospora peucetia** Kroppenstedt, Mayilraj, Wink, Kallow, Schumann, Secondini and Stackebrandt 2005b, 1743^VP (Effective publication: Kroppenstedt, Mayilraj, Wink, Kallow, Schumann, Secondini and Stackebrandt 2005a, 337.)

peu.ce′ti.a. L. fem. adj. *peucetia* Peucetian, of Peucetia, Latin name of landscape in Apulia (Puglia), Southern Italy.

Bottle green colonies are formed on inorganic salts-starch agar (ISP medium 4), pine green colonies are formed on glycerol-asparagine (ISP medium 5) and tyrosine agars (ISP medium 7), pale green to beige red colonies are formed on oatmeal agar (ISP medium 3), deep orange colonies are formed on yeast extract-malt extract agar (ISP medium 2), and chrome green colonies are formed on peptone-yeast extract-iron agar (ISP medium 6). Aerial hyphae are absent. Single spores are produced on substrate mycelium. Soluble pigments are not formed.

Fructose, glucose, and sucrose are used as sole carbon sources. Positive for alkaline phosphatase, *N*-acetyl-β-glucosaminidase, esterase (C4), α-glucosidase, and acid phosphatase, but not for α-fucosidase, α- or β-galactosidases, lipase, β-glucosidase, β-glucuronidase, or α-mannosidase.

Peptidoglycan contains *meso*-A_2pm. Whole-organism diagnostic sugars are arabinose, glucose, and xylose. Major menaquinone is MK-9(H_4). Phosphatidylethanolamine is the diagnostic phospholipid. Major fatty acids are $C_{15:0}$ iso, $C_{16:0}$, and $C_{17:1}$ ω8.

Source: isolated from soil.

DNA G+C content (mol%): not determined.

Type strain: DSM 43363, JCM 12820.

Sequence accession no. (16S rRNA gene): X92603.

27. **Micromonospora purpureochromogenes** (Waksman and Curtis 1916) Luedemann 1971, 244^VP ("*Actinomyces purpureochromogenes*" Waksman and Curtis 1916, 113; *Micromonospora fusca* Jensen 1932, 173; *Micromonospora brunnea* Sveshnikova, Maksimova and Kudrina 1969, 762)

pur.pur.e.o.chro.mo′ge.nes. L. adj. *purpureus* purple colored; Gr. n. *chroma* color; Gr. v. *gennaio* to produce; N.L. part. adj. *purpureochromogenes* producing purple color (relating to the color of the diffusible pigment).

Dark brown colonies are formed on yeast extract-glucose agar after 21 days; a dark brown, diffusible, and water-soluble pigment is produced in most media. Very slow growth is seen on Czapek's sucrose agar. Spores (0.8–1.0 µm in diameter) are formed singly or in clusters. A monopodial branching system of sporulating hyphae is found, best observed at the periphery of colonies. Apparent dichotomous branching of sporulating hyphae is observed. Fragmentation of mycelium occurs in liquid cultures. Grows at 26–37°C, but not at 45°C.

Fructose, galactose, glucose, glycerol, lactose, levulose, melibiose, raffinose, sucrose, and xylose are used as sole carbon sources. Poor growth occurs on D- and L-arabinose. Milk is poorly peptonized. Limited hydrolysis of casein occurs and nitrate reduction is variable.

Source: isolated from adobe soil in California.

DNA G+C content (mol%): 73 (HPLC).

Type strain: ATCC 27007, IMRU 3343, ATCC 27334, DSM 43821, NBRC 13324, JCM 3156, NRRL B-2101, NRRL B-16094, VKM Ac-937.

Sequence accession nos: X92611 (16S rRNA gene); AB014161 (*gyrB*).

28. **Micromonospora rosaria** (*ex* Wagman, Waltz, Marquez, Murawski, Oden, Testa and Weinstein 1972) Horan and Brodsky 1986b, 478^VP ("*Micromonospora rosaria*" Wagman, Waltz, Marquez, Murawski, Oden, Testa and Weinstein 1972, 641)

ro.sa′ri.a. L. fem. adj. *rosaria* of roses, rose-. The epithet refers to the wine red diffusible pigment produced by the strain.

Raised, folded colonies are formed on most media with orange brown to purple black vegetative mycelial pigments. Produces characteristic wine red diffusible pigment on most media. Short, sterile aerial hyphae produce a gray white bloom in some media. Single, warty spores (1.5–1.8 µm) are formed on short sporophores or are sessile. Good growth occurs on most rich organic media. Good growth is found at 35–40°C; grows poorly at 42°C.

D- and L-Arabinose, fructose, galactose (weak), glucose, lactose, maltose, mannose, mannitol, rhamnose, ribose, sucrose, trehalose, and xylose are used as sole carbon sources, but not adonitol, dulcitol, glycerol, melibiose, melezitose, or raffinose. Degrades chitin, casein, starch, gelatin, tyrosine, and xylan. Hydrolyzes esculin. Nitrate reduction is negative.

Sensitive to acid. Resistant to clindamycin, erythromycin, everninomicin, gentamicin, lincomycin, novobiocin, penicillin G, and sisomicin, at concentrations of 50 µg/ml, but is sensitive to aminoglycosides, rifamycin, and tetracycline at this concentration.

Whole-organism hydrolysates contain *meso-* and 3-OH-A_2pm. Arabinose, galactose, and xylose are the characteristic sugars. Produces the macrolide antibiotic rosaramicin.

Source: isolated from soil.

DNA G+C content (mol%): 72.9 (HPLC).

Type strain: SCC 957, 67694, NRRL 3718, ATCC 29337, NBRC 13697, DSM 803, JCM 3159.

Sequence accession nos: X92631 (16S rRNA gene); AB014163 (*gyrB*).

29. **Micromonospora saelicesensis** Trujillo, Kroppenstedt, Fernández-Molinero, Schumann and Martínez-Molina 2007, 801[VP]

sa.e.li.ces.en′sis. N.L. fem. adj. *saelicesensis* of or pertaining to Saelices, the place where the plants from which the first strains were isolated were collected.

Orange colonies are formed on yeast extract-malt extract agar (ISP medium 2). Orange-brown to brown diffusible pigments are produced on oatmeal agar (ISP medium 3). Well-developed branched hyphae (0.3–0.6 µm diameter) are observed with smooth-surfaced spores formed at the tips of hyphae. Good growth is seen at 20–37°C. Grows in the presence of 2% (w/v) NaCl.

Arabinose, cellobiose, galactose, glutarate, histidine, inositol, maltose, mannose, melibiose, and raffinose are used as sole carbon sources, but not alanine, arginine, gluconate, lysine, proline, rhamnose, serine, sorbitol sorbose, sucrose, trehalose, valine, or xylitol. Catalase-positive and oxidase-variable. Nitrate is not reduced. Degrades arbutin, casein, gelatin, and xylan; starch degradation is variable. Esculin is hydrolyzed. Positive for acid and alkaline phosphatases, esterases and lipases, α- and β-galactosidase, α- and β-glucosidases, and *N*-acetyl-β-glucosaminidase, but not for β-glucuronidase or urease.

Whole-organism hydrolysates contain *meso*-A_2pm, and arabinose, glucose, mannose, ribose, rhamnose, and xylose as characteristic sugars. Diagnostic phospholipid is diphosphatidylethanolamine. Major fatty acids are $C_{15:0}$ iso, $C_{16.0}$, and $C_{17:1}$ ω8. Predominant menaquinone is MK-10(H_4).

Source: isolated from root nodules of *Lupinus angustifolius.*

DNA G+C content (mol%): 71.6 (T_m).

Type strain: Lupac 09, DSM 44871, LMG 24056.

Sequence accession no. (16S rRNA gene): AJ783993.

30. **Micromonospora sagamiensis** Kroppenstedt, Mayilraj, Wink, Kallow, Schumann, Secondini and Stackebrandt 2005b, 1743[VP] (Effective publication: Kroppenstedt, Mayilraj, Wink, Kallow, Schumann, Secondini and Stackebrandt 2005a, 338.)

sa.ga.mi.en′sis. N.L. fem. adj. *sagamiensis* of or belonging to Sagami Bay.

Salmon pink colonies are formed on inorganic salts-starch (ISP medium 4) oatmeal agar (ISP medium 3) and tyrosine agar (ISP medium 7), red colonies are formed on glycerol-asparagine agar (ISP medium 5), and pastel coral red colonies are formed on yeast extract-malt extract agar (ISP medium 2). Does not grow on peptone-yeast extract-iron agar (ISP medium 6). Aerial mycelium is not formed. Diffusible pigments are not produced. Oval or spherical spores with rough surfaces are produced singly on the substrate mycelium. Grows optimally at 30–40°C; optimum pH for growth is between 7.0 and 8.0.

Galactose, glucose, levulose, sucrose, raffinose (weak), and xylose are used as sole carbon sources, but not arabinose, glycerol, lactose, inositol, mannitol, or rhamnose. Positive for starch hydrolysis, nitrate reduction, and milk coagulation. Cellulose decomposition is weak. Positive for alkaline phosphatase, α- and β-galactosidases, α- and β-glucosidases, *N*-acetyl-β-glucosaminidase, and acid phosphatase, but not for α-fucosidase, β-glucuronidase, lipase, or α-mannosidase.

Peptidoglycan contains *meso*-A_2pm. Whole-organism diagnostic sugars are glucose and xylose. Major menaquinone is MK-9(H_4). Phosphatidylethanolamine is the diagnostic phospholipid. Major fatty acids are $C_{15:0}$ iso, $C_{16.0}$, $C_{17:1}$ iso, and $C_{17:0}$.

Produces sagamicin (XK62-2) and antibiotics of the gentamicin C complex.

Source: isolated from forest soil, Japan.

DNA G+C content (mol%): not determined.

Type strain: MK-65, DSM 43912, JCM 3310, ATCC 21826, NRRL 11334.

Sequence accession no. (16S rRNA gene): X92624.

31. **Micromonospora siamensis** Thawai, Tanasupawat, Itoh, Suwanborirux and Kudo 2006b, 2[VP] (Effective publication: Thawai, Tanasupawat, Itoh, Suwanborirux and Kudo 2005a, 233.)

si.am.en′sis. N.L. fem. adj. *siamensis* of or pertaining to Siam, the old name for Thailand, the origin of the soil from which the type strain was isolated.

Forms vivid orange colonies that turn brownish black upon sporulation, and a pale yellow soluble pigment on yeast extract-malt extract agar (ISP medium 2). Aerial mycelium is absent. Melanin pigments are not formed. Develops abundant branched substrate hyphae which carry single spores. Spores are nonmotile and smooth-surfaced. Grows optimally at 25–30°C, but not above 40°C. Tolerates up to 5% (w/v) NaCl.

L-Arabinose, cellobiose, fructose (weak), galactose, glucose, lactose, melibiose, raffinose, rhamnose, salicin, and xylose are used as sole carbon sources, but not glycerol, mannitol, or ribose. Positive for milk peptonization, starch hydrolysis, and gelatin liquefaction, but negative for nitrate reduction and H_2S production.

Peptidoglycan contains *meso*-A_2pm. Whole-organism diagnostic sugars are arabinose and xylose. Predominant menaquinones are MK-10(H_4) and MK-10(H_6). Diphosphatidylglycerol, phosphatidylethanolamine, phosphatidylinositol, and phosphatidylinositol mannosides are the major polar lipids. Rich in $C_{15:0}$ iso, $C_{16:0}$ iso, $C_{17:0}$ anteiso, and $C_{17:0}$ fatty acids.

Source: isolated from a peat swamp forest soil, Thailand.

DNA G+C content (mol%): 73.0 (HPLC).

Type strain: TT2-4, JCM 12769, PCU 266, TISTR 1554.

Sequence accession no. (16S rRNA gene): AB193565.

32. **Micromonospora viridifaciens** Kroppenstedt, Mayilraj, Wink, Kallow, Schumann, Secondini and Stackebrandt 2005b, 1744[VP] (Effective publication: Kroppenstedt, Mayilraj, Wink, Kallow, Schumann, Secondini and Stackebrandt 2005a, 338.)

vi.ri.di.fa′ci.ens. L. adj. *viridis* green; L. part. adj. *faciens* making, N.L. part. adj. *viridifaciens* green making.

Beige red colonies are formed on oatmeal agar (ISP medium 3), yellow colonies are formed on peptone-yeast extract-iron agar (ISP medium 6) and yellow to brown are formed colonies on yeast extract-malt extract-iron agar (ISP medium 2). Sparse growth is seen on inorganic salts-starch (ISP medium 4), glycerol-asparagine agar (ISP medium 5), and tyrosine agar (ISP medium 7).

Aerial mycelium is absent. Soluble pigments, including melanin, are not produced. Spores with smooth surfaces are formed singly on the substrate mycelium. Good growth occurs between 28 and 45°C.

Good utilization of ribose and starch is observed, but poorly utilizes arabinose, fructose, glucose, inositol, mannitol, rhamnose, raffinose, and sucrose. Positive for liquefaction of gelatin, and milk coagulation and peptonization, but negative for nitrate reduction and tyrosine degradation. Positive for alkaline phosphatase, α-glucosidase, and acid phosphatase; negative for α-fucosidase, lipase, *N*-acetyl-β-glucosaminidase, α- and β-galactosidase, β-glucosidase, β-glucuronidase, and α-mannosidase.

Peptidoglycan contains *meso*-A_2pm. Whole-organism diagnostic sugars are glucose, rhamnose, and xylose. Major menaquinone is MK-9(H_4). Phosphatidylethanolamine is the diagnostic phospholipid. Major fatty acids are $C_{15:0}$ iso and $C_{17:0}$ 10-methyl. Produces antibiotic 37505.

Source: isolated from soil sample, Japan.

DNA G+C content (mol%): not determined.

Type strain: DSM 43909, JCM 3267, ATCC 31146.

Sequence accession no. (16S rRNA gene): X92623.

Species *incertae sedis*

1. **Micromonospora gallica** (Erikson 1935; Waksman 1961) Kawamoto 1989

gal′li.ca. L. fem adj. *gallica* of or belong to the Gauls.

This species was included in the *Approved Lists of Bacterial Names* (Skerman et al., 1980), but the type strain NCTC 4582[T] is not extant (Kawamoto, 1989).

Genus II. **Actinocatenispora** Thawai, Tanasupawat, Itoh and Kudo 2006a, 1792[VP]

The Editorial Board

Ac.ti.no.ca.te.ni.spo′ra. Gr. n. *aktis -inos* ray; L. n. *catena* chain; Gr. n. *spora* seed and in biology a spore; N.L. fem. n. *Actinocatenispora* spore chain-producing ray (fungus).

Gram-stain-positive. Nonmotile. **Aerobic**. **Sporeforming** branching substrate hyphae; mycelia are yellow to vivid orange in color. Aerial hyphae or vegetative mycelium bear spore chains consisting of more than 10 spores. Cylindrical spores (0.3–0.4 × 0.5–1.0 µm) have a smooth surface. Cell wall contains glutamic acid, glycine, alanine, and *meso*-diaminopimelic acid. The *N*-acyl group of the cell-wall muramic acid is glycolyl. Characteristic whole-cell sugars are arabinose and xylose. Possess phospholipid pattern type II; cellular phospholipids are phosphatidylethanolamine, diphosphatidylglycerol, phosphatidylinositol, phosphatidylinositol mannosides, phosphatidylglycerol and unidentified ninhydrin-negative phospholipids. The predominant fatty acids are iso- and anteiso-methyl branched acids. Major menaquinone is MK-9(H_4). Mycolic acids are not detected.

DNA G+C content (mol%): 72.0–74.3.

Type species: **Actinocatenispora thailandica** Thawai, Tanasupawat, Itoh and Kudo 2006a, 1793[VP].

Further descriptive information

Phylogenetic analysis of the 16S rRNA gene positions the genus within the family *Micromonosporaceae*. The closest phylogenetic neighbor is *Phytohabitans suffuscus* (93.4%, accession no. AB490769) (Inahashi et al., 2010). So far, all the described species have all been isolated from soil. Environmental clones with high 16S rRNA gene sequence similarity have been detected in cellulosic waste from a simulated low-level-radioactive-waste site (99.2 %, accession no. GQ263629 and 99.0%, accession no. GQ263525). Environmental isolates identified as *Micromonospora* sp. and *Solwaraspora* sp. but with high 16S rRNA gene sequence similarity to *Actinocatenispora* have been cultivated from marine sediments (94% accession no. DQ448714 and ~93% accession nos AY552766–AY552773, respectively) (Gontang et al., 2007; Magarvey et al., 2004).

Enrichment and isolation procedures

Actinocatenispora thailandica strain TT2-10[T] was isolated from peat swamp forest soil in Pattaloong Province, Thailand. Samples were taken from the soil surface were cultivated on starch-casein nitrate agar for 21 d at 30°C. Isolated colonies were purified on yeast extract-malt extract agar (ISP medium no. 2) and maintained on the same medium as a working culture (Thawai et al., 2004b), and the pure culture was kept at 4–10°C on yeast extract-malt extract agar (ISP 2 medium) slants.

Maintenance procedures

Strains are maintained yeast extract-malt extract agar (ISP medium no. 2) or in 20% (v/v) glycerol at −20 °C or −70°C.

Differentiation of the genus *Actinocatenispora* from closely related genera

Spore motility and true aerial mycelium production differentiates *Actinocatenispora* from other genera of the family *Micromonosporaceae*, except for *Longispora*. *Actinocatenispora* can be differentiated from *Longispora* by differences in the menaquinone and fatty acid composition.

List of species of the genus *Actinocatenispora*

1. **Actinocatenispora thailandica** Thawai, Tanasupawat, Itoh and Kudo 2006a, 1793[VP]

thai.lan′di.ca. N.L. fem. adj. *thailandica* of or belonging to Thailand, where the type strain was isolated.

White aerial mycelia are formed on oatmeal-nitrate agar. Soluble yellow pigment present on oatmeal agar. Temperature range for growth 25–30°C, does not grow above 40°C. Does not grow below pH 4.5, maximum NaCl tolerance is 7%. Major menaquinones are MK-9(H_4) and MK-9(H_6); MK-9(H_2) and MK-9(H_8) are also produced. Whole-cell sugars are galactose, xylose, arabinose, glucose, mannose, and ribose. Utilizes D-glucose, D-mannitol, D-melibiose, D-raffinose, glycerol, *myo*-inositol, salicin, and cellobiose. Does not utilize D-ribose, L-rhamnose, lactose, D-galactose, L-arabinose or D-fructose. Reduces nitrate, weakly positive for peptonization of milk and gelatin liquefaction. Does not hydrolyze starch. Melanin and H_2S are not produced. The major cellular fatty acid components are $C_{16:0}$ iso (26%), $C_{17:0}$ anteiso (19%), $C_{15:0}$ iso (20%) and $C_{17:0}$ iso (11%).

For all other characteristics, refer to the genus description.

DNA G+C content (mol%): 72.0 (HPLC).

Type strain: TT2-10, JCM 12343, PCU 235, DSM 44816.

Sequence accession no. (16S rRNA gene): AB107233.

2. **Actinocatenispora rupis** Seo and Lee 2009, 3082[VP]

ru′pis. L. gen. n. *rupis* of a cliff, referring to the site from which the type strain was isolated.

Vegetative mycelium is well developed and pale to strong yellow in color. Hyphal swellings are observed on the tips of the vegetative mycelium. Growth temperature optimum is 37°C, range is 25–42°C; and pH optimum is 5.1–9.1, range is 5.1–12.1; NaCl tolerance up to 4%. Degrades DNA, elastin, esculin, casein, Tween 80 and chitin, but not cellulose, starch, hypoxanthine, tyrosine, urea or xanthine. Catalase- and oxidase-negative. Utilizes D-arabinose, dextran, D-galactose, lactose, maltose, D-mannose, methyl α-D-glucoside, sucrose, adonitol, *meso*-erythritol, D-sorbitol and citrate as sole carbon sources. Does not utilize inulin, melezitose, methyl α-D-mannoside, salicin, L-sorbose, dulcitol, D-xylitol, acetate, benzoate, formate, malate, succinate or tartrate. Does not reduce nitrate to nitrite. Major menaquinones are MK-9(H_4), MK-9(H_6) and MK-9(H_8). The whole-cell sugars are glucose, rhamnose, ribose, arabinose and xylose. The diagnostic polar lipids are phosphatidylethanolamine, phosphatidylinositol and phosphatidylglycerol. The predominant fatty acids are $C_{16:0}$ iso (39%), $C_{17:0}$ anteiso (15%) and $C_{16:1}$ iso (13%).

For all other characteristics, refer to the genus description.

DNA G+C content (mol%): 74.3 (HPLC).

Type strain: CS5-AC17, DSM 45178, NRRL B-24660.

Sequence accession no. (16S rRNA gene): AM980986.

3. **Actinocatenispora sera** Matsumoto, Takahashi, Fukumoto and Ōmura 2007, 2653[VP]

se′ra. L. fem. adj. *sera* late.

Growth temperature optimum is 18–25°C, range is 13–37°C. Growth occurs at pH 6–9. No growth at 5% NaCl. Melanoid pigments are not produced. Negative for the liquefaction of gelatin. Hydrolyzes starch. Positive for coagulation and peptonization of milk. Reduces nitrate. Utilizes adonitol, D-glucose, L-rhamnose, xylitol and D-xylose. Does not utilize L-arabinose, D-cellobiose, D-fructose, glycerol, *myo*-inositol, maltose, D-mannose, D-mannitol, melibiose, raffinose, D-ribose, trehalose and sucrose. Cellulose is not decomposed. Predominant cellular fatty acids are $C_{17:0}$ anteiso (25%), $C_{16:0}$ iso (22%), $C_{17:0}$ iso (16%) and $C_{15:0}$ iso (14%).

Isolated from soil in Niigata Prefecture, Japan.

For all other characteristics, refer to the genus description.

DNA G+C content (mol%): 72.0 (HPLC).

Type strain: KV-744, NRRL B-24477, NBRC 101916.

Sequence accession no. (16S rRNA gene): AB263096.

Genus III. **Actinoplanes** Couch 1950, 89[AL] emend. Stackebrandt and Kroppenstedt 1987, 112

Gernot Vobis, Jenny Schäfer and Peter Kämpfer

Ac.ti.no.pla′nes. Gr. n. *aktis aktinos* ray, beam; Gr. masc. n. *planes* a wanderer, roamer; N.L. masc. n. *Actinoplanes* literally, a ray wanderer (intended to signify an actinomycete with swimming spores).

Substrate mycelium is developed on various agar media. Hyphae are 0.2–1.2 µm in diameter, branched, and septated; fragmentation is very rare. Gram-stain-positive, although parts may be Gram-stain-negative. Non-acid-fast. Aerial mycelium is absent or scanty.

Sporangia are produced on the surface of the substrate, sessile on the agar or detached on short sporangiophores. In many cases, they are formed at the tip of so-called palisade hyphae. **The shape of the sporangia varies from spherical, subspherical to irregular** (mean diameter 7.0–16.0 µm), **or cylindrical, bell-shaped, lobate, or digitate** (mean dimensions: 5.0–12.0 µm wide and 8.0–20.0 µm long). Numerous spores are produced within the sporangium, arranged in coiled, parallel, or irregular chains. **Sporangiospores are globose, subglobose, oval** (mean diameter 1.1–1.6 µm), **oblong** (mean 0.8 × 1.6 µm) or **rod-shaped** (mean 0.8 × 3.0 µm); **motile by a polarly inserted tuft of flagella**.

Grows under aerobic conditions. Colonies on various complex agar media are flat, elevated, or convoluted with a smooth, wrinkled, or ridged surface. The characteristic color of substrate mycelium is orange; by loss or additional formation of pigments, nearly all modifications occur from ivory,

pale yellow to red, violet, green, brown and black. Most strains do not require organic growth factors. Chemo-organotrophic, mesophilic, in some cases moderately psychrophilic or thermotolerant. Grows well between 20 and 28°C.

The peptidoglycan of the cell wall contains glycine, *meso*-diaminopimelic acid and/or hydroxy-diaminopimelic acid. The diagnostic sugar of whole-cell hydrolysates is xylose; galactose and/or arabinose are also present. Phosphatidylethanolamine is the predominant phospholipid. Major menaquinone is MK-9(H_4). Iso/anteiso-branched and monounsaturated fatty acids and/or *cis*-9,10-octadecanoic acid (oleic acid) are the predominant fatty acids.

DNA G+C content (mol%): 69.0–73.0 (T_m).

Type species: **Actinoplanes philippinensis** Couch 1950, 89^{AL}.

Further descriptive information

Phylogeny. A phylogenetic study based on 16S rRNA gene sequence analysis assigned the genus *Actinoplanes* to the family *Micromonosporaceae*. This has been confirmed by other 16S rRNA gene sequence studies of this genus (Stackebrandt and Kroppenstedt, 1987; Stackebrandt et al., 1997; Tamura and Hatano, 2001). The taxon currently encompasses 28 species with validly published names. Representatives of species with validly published names show a high similarity in the 16S rRNA *Actinoplanes* gene tree (Figure 211), with the exception of *Actinoplanes globisporus*. Similarity values of the different species within the genus *Actinoplanes* range between 95.0 and 100%. The 16S rRNA gene sequence of *Actinoplanes globisporus* differs by 3.1–5.0% from those of other species of the genus *Actinoplanes* (similarity values between the sequences of *Actinoplanes globisporus* and those of other species of the genus *Actinoplanes* range from 95.0 to 96.9%).

Similarity values between *Actinoplanes* and the other genera within the family *Micromonosporaceae* (based on the type species) range from 97.7 to 94.4%. Based on distance calculations of the type species of the different genera, the nearest relatives are the genera *Spirilliplanes* (97.0%), *Polymorphospora* (96.9%), and *Micromonospora* (96.8%).

Cell morphology. Strains of *Actinoplanes* produce substrate mycelium on solid agar media. Substrate hyphae are fine, 0.2–1.0 µm in diameter, branched, and septated. Fragmentation is very rare, e.g. observable in *Actinoplanes couchii*, developing substrate mycelium that fragments in irregular rod-shaped cells when cultured on DSMZ medium 65 (Kämpfer et al., 2007). Strains may be morphogenetically characterized by specialized, vertically orientated hyphae directly under the surface of agar media (Couch, 1963). They are two to three times thicker than the usual hyphae and strongly arranged in parallel positions. In general, these so called palisade hyphae are identical to sporangiophores (Figure 213A), first described from the species *Actinoplanes philippinensis* (Couch, 1950).

The development of aerial mycelium in the genus *Actinoplanes* is not frequent and is observed only in some species, e.g. *Actinoplanes couchii*, *Actinoplanes ferrugineus*, *Actinoplanes liguriensis*,

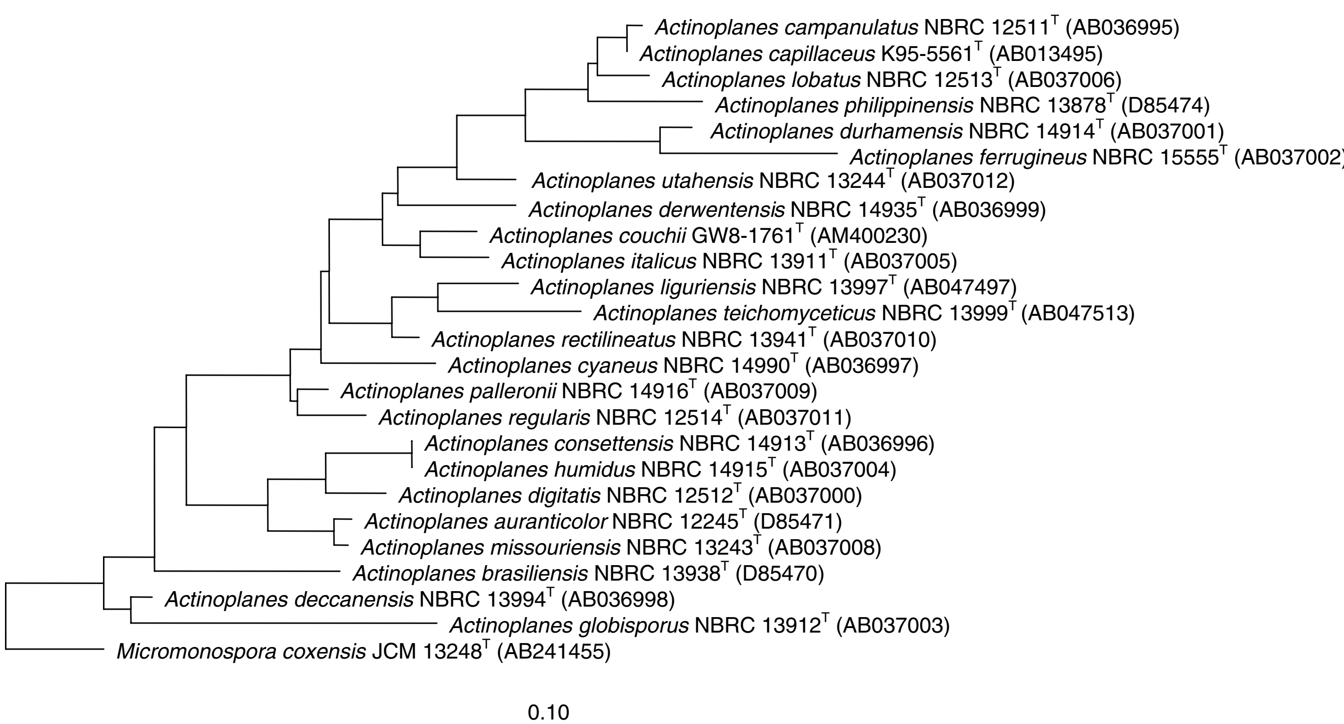

FIGURE 211. Phylogenetic analysis of *Actinoplanes* species based on 16S rRNA gene sequences available from EMBL (accession numbers are given in parentheses). Multiple alignment, distances (distance options according to the Kimura-2 model; Kimura, 1980) and clustering with the neighbor-joining method were performed by using the software packages MEGA (Molecular Evolutionary Genetics Analysis) version 4 (Tamura et al., 2007). Bootstrap values based on 1000 replications are given as percentages at the branching points. *Micromonospora coxensis* was used as an outgroup.

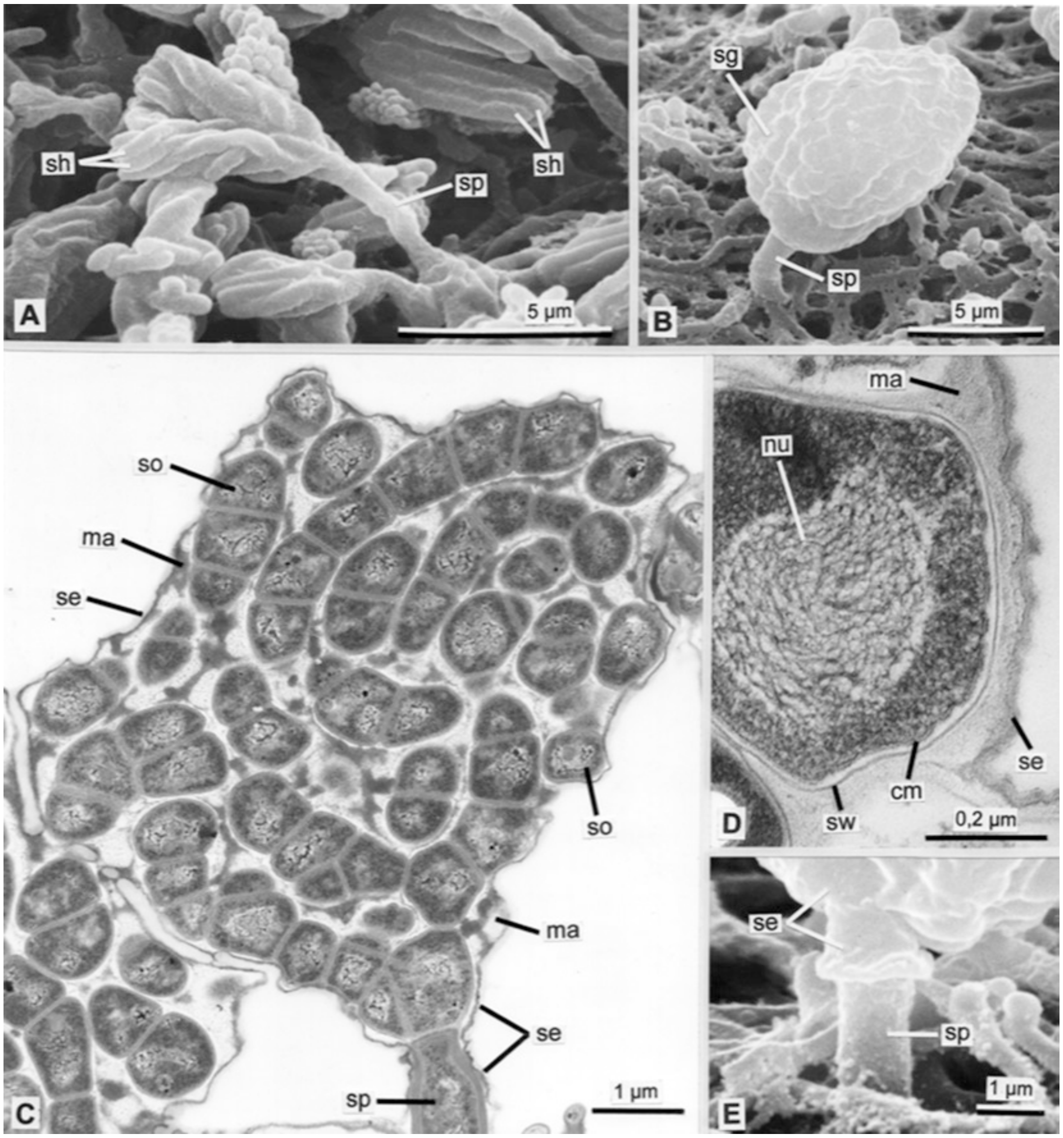

FIGURE 212. Multispored sporangia of *Actinoplanes*. A, "*Ampullariella*"-type with rows of sporogenous hyphae orientated in parallel (strain MB-VE 1144, SEM); B, subglobose sporangium developed on substrate mycelium (strain MB-SE 50, SEM); C, longitudinal section of a sporangium containing spores in chains (strain MB-SE 50, TEM); D, spore on the inner side of a thin, "membranous" sporangial envelope (strain ATCC 21983, TEM); E, sporangiophore in transition between humid substrate and air-exposed sporangium (strain MB-SE 50, SEM). Abbreviations: SEM, scanning electron microscope; TEM, transmission electron microscope; ma, intrasporangial matrix; se, sporangial envelope; sg, sporangium; sh, sporogenous hypha; so, spore; sp, sporangiophore; sw, spore wall.

Actinoplanes rectilineatus, and *Actinoplanes teichomyceticus*, if cultured on special media. In general, this kind of aerial mycelium is named rudimentary or sterile because it does not reach the dimensions of typical aerial mycelium and its hyphae are not involved in any production of sporangia or spores. Aerial mycelium is white and imparts to the colonies a powdery appearance (Lechevalier and Lechevalier, 1975; Parenti et al., 1978).

Sporangia are the characteristic reproductive structures of *Actinoplanes*, which are developed by substrate mycelium (Figure 212A, B, E). The sporangiophores are either more or less undifferentiated hyphae or palisade hyphae. They transgress the aqueous surroundings of the substrate. Once air-exposed, sporangia are additionally covered by a hydrophobic sheath, which passes over without interruption into the sporangial envelope (Figure 212C, E). The single steps of sporangial development are described elsewhere (Lechevalier and Holbert, 1965; Lechevalier et al., 1966; Vobis, 1997; Vobis and Kothe, 1985): inside the sporangial envelope, sporogenous hyphae are septated into spore-shaped segments, which are rounded off into individual spores (Figure 212C). In most species, the spore chains are arranged in coils or, in the case of the former "*Ampullariella*" strains, in parallel rows. Evidently, the external form of a sporangium depends on the internal arrangement of the sporogenous hyphae/spore chains (Figure 212A,

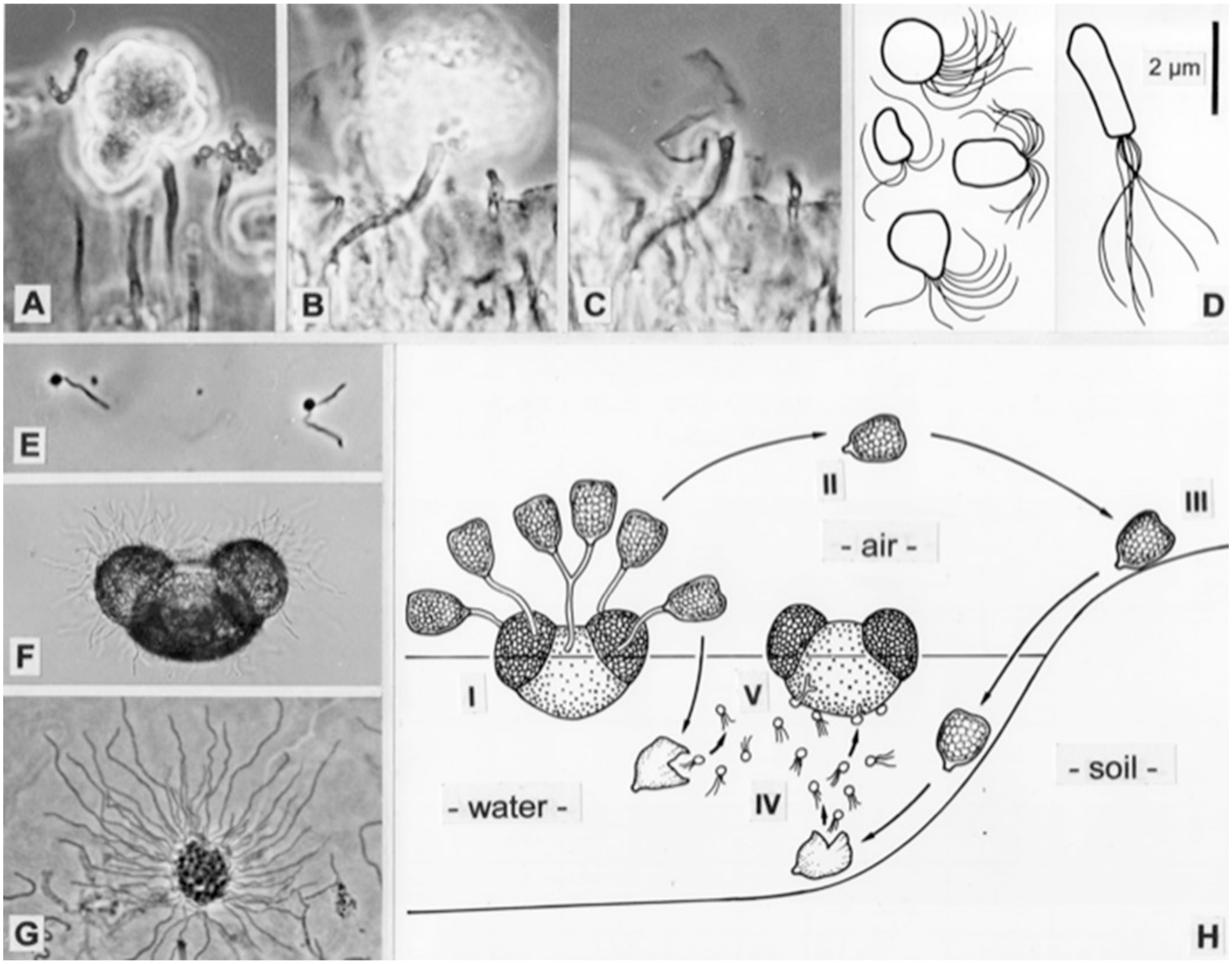

FIGURE 213. Release of sporangiospores (A, B, C), and aero-aquatic life cycle of *Actinoplanes* (H). A, Sporangium originated on palisade hypha, submersed in water; B, burst sporangium and swarming spores; C, left empty sporangial envelope; D, sporangiospores with a polarly inserted tuft of flagella, varying in size and shape; E, germinating spores; F, growth of mycelium on a pollen grain of *Pinus* sp.; G, germinated sporangium, developing new mycelium. H (I) Floating pollen grain with mycelium and sporangia; (II) dispersal of sporangia by air; (III) dry-resistant sporangia on soil; (IV) flagellated sporangiospores are released in water; (V) new substrate is colonized by germinating spores. Note: A, B, C, E, F, G (strain MB-SE 165; light microscope, phase-contrast; for dimensions compare Figure 212).

B). Very rarely, chains orientated in spirals can be observed (G. Vobis, unpublished). The surface of the sporangial envelope is generally smooth (Figure 212A–D). External ornamentation is described only for *Actinoplanes capillaceus*, including four invalidly proposed species of "*Ampullariella*" (Matsumoto et al., 2000; Miyadoh et al., 1997; Seino, 1983). In all these cases, the sporangia are covered with hair-like structures, resembling the hairy spores of *Streptomyces*.

The polysporous sporangia vary in shape and size (Vobis, 1997). In most species, the sporangia are spherical (globose), subspherical (subglobose), oval to irregular, occasionally wider than they are long, and rarely lobed. The smallest sporangia have a diameter of 4 μm, with the biggest being 25 μm (mean diameter of about 11 μm). As an extreme exception, a strain with sporangia of 47 μm in diameter has been observed (Vobis, 1992). The former "*Ampullariella*" species, including *Actinoplanes capillaceus* and *Actinoplanes rectilineatus*, have sporangial shapes from cylindrical or bottle-shaped to bell-shaped and digitate, frequently lobed and papillate (Vobis and Kothe, 1989). Extremely small sporangia may be found in *Actinoplanes digitatis* (3 μm wide and 6 μm long), and the biggest are found in *Actinoplanes regularis* (14 × 30 μm). Typical sporangia with parallel spore chains have a mean size of 5–12 × 8–20 μm.

The spores produced within the sporangia have a smooth surface and are actively motile by flagella (zoospores or planospores). With regard to their shape, they can be subdivided into two categories (Figure 213D). The typical globose spores vary from spherical (globose), subspherical (subglobose), to oval with a mean diameter of 1.35 μm (range: 0.8–2.0 μm). The sporangiospores of the former "*Ampullariella*" species are

distinct rod-shaped or bacilliform, four times longer than they are wide, with a mean width of 0.75 µm and mean length of 3.0 µm (range: from 0.5 × 2.0 µm to 1.0 × 4.0 µm). Oblong spores are twice as long as they are wide and are generally described as short rod-shaped; with a mean width of 0.8 µm and a mean length of 1.63 µm (range: from 0.5 × 1.0 µm to 1.2 × 2.3 µm), they may belong to the last category. Variations in the shape of spores of one strain (e.g. globose, subglobose, oval) are explained by slight distance deviations during the segmentation process of the branched sporogenous hyphae.

The motility of the spores is caused by means of a tuft of flagella, inserted at the apical end (Figure 213D). This polytrichous type of flagellation was first documented by light microscopy in *Actinoplanes philippinensis* (Couch, 1950) and confirmed by electron microscopy preparations in other species and strains (Bland, 1970; Hanton, 1968; Karwowski et al., 1988; Lechevalier and Holbert, 1965; Miyadoh et al., 1997; Palleroni, 1979; Schäfer, 1973). In *Actinoplanes rectilineatus*, the number of flagella ranges from 17 to 40; their bases are hooked (Lechevalier and Lechevalier, 1975). In general, the tuft is formed by less flagella. They are 2–6 µm in length (Couch and Bland, 1974b). The rod-shaped spores with their polar tuft of flagella can be named lophotrichous (Schäfer, 1973) and they are well documented in *Actinoplanes regularis* (Kane, 1966), *Actinoplanes campanulatus* (Higgins et al., 1967), and *Actinoplanes capillaceus* (Matsumoto et al., 2000). The number of flagella on these species ranges from 1 to 12 (Higgins et al., 1967) and they are 3.5–6.0 µm in length (Couch and Bland, 1974b). Peritrichous flagellation has also been reported occasionally, both in spherical spores (Ruan et al., 1976; Willoughby, 1968) and in rod-shaped spores (Ruan and Zhang, 1974). Nonomura et al. (1979) observed polar (lophotrichous and amphitrichous) and peritrichous flagella arrangement in 12 "*Ampullariella*" isolates. The flagella are up to 19.0 µm in length (mean of 6–12 µm).

Besides the characteristic sporangia producing zoospores, formation of nonmotile spores (conidia) may also occur. The conidiophores develop in a similar manner to the sporangia, but in the absence of the sporangial envelope, the sporogenous (conidiogenous) hyphae appear as brushlike bunches (Willoughby, 1966). Conidiophores originate at the margin of the colonies of *Actinoplanes regularis* (Couch, 1963). In "*Actinoplanes arizonaensis*", conidiogenous hyphae occur as digitate hyphae on the surface of the colony (Karwowski et al., 1988). They are interpreted as abortive sporangia. *Actinoplanes campanulatus* develops microconidia and *Actinoplanes lobatus* develops oval conidia in moniliform arrangement, which are produced by substrate mycelium (Couch, 1963). Microconidia are also formed in the substrate mycelium of *Actinoplanes auranticolor* (Hanton, 1968) and chlamydospores are formed in *Actinoplanes globisporus* (Thiemann, 1967).

Cell-wall composition. The peptidoglycan of the cell wall contains glycine and *meso-* and/or hydroxy-diaminopimelic acid, according to wall chemotype II of Lechevalier and Lechevalier (1970a). It has been demonstrated that the acyl groups of the muramyl residue in the cell-wall peptidoglycan are of the glucolyl type (Uchida and Seino, 1997).

The principal wall sugars are arabinose, galactose, glucose, mannose, and xylose. The latter is the diagnostic sugar of whole-cell hydrolysates (Tamura and Hatano, 2001). The presence of xylose and arabinose corresponds to the whole-cell sugar pattern type D of Lechevalier and Lechevalier (1970a). Galactose was not detected in *Actinoplanes regularis*, *Actinoplanes campanulatus*, or *Actinoplanes digitatis* (Stackebrandt and Kroppenstedt, 1987).

The polar lipid profile is characterized by the presence of phosphatidylethanolamine as diagnostic phospholipid and the absence of phosphatidylcholine and amino-containing phosphoglycolipid, corresponding to phospholipid type PII of Lechevalier et al. (1981, 1977). Many species also contain phosphatidylinositol, phosphatidylinositol mannoside, and phosphatidylglycerol (Table 194). The type strain of *Actinoplanes regularis* is of particular note as it lacks phosphatidylethanolamine (Stackebrandt and Kroppenstedt, 1987). The whole polar lipid composition may also contain a highly hydrophilic glycolipid and further unknown or uncharacterized lipids and glycolipids (Goodfellow et al., 1990b; Kämpfer et al., 2007).

Fatty acids. *Actinoplanes* species contain complex mixtures of straight-chain, branched-chain, and unsaturated fatty acids and the proportions of these vary considerably between the strains (Ara et al., 2010; Goodfellow et al., 1990b; Sun et al., 2009). Characteristic fatty acid patterns for *Actinoplanes* species include major amounts of iso/anteiso branched- and monosaturated fatty acids, as well as *cis*-9,10-octadecanoic acid (oleic acid, n-$C_{18:1}$), 14-methylpentadecanoic ($C_{16:0}$ iso), and 14-methylhexadecanoic ($C_{17:0}$ anteiso) acids (Ara et al., 2010; Kämpfer et al., 2007; Matsumoto et al., 2000; Stackebrandt and Kroppenstedt, 1987; Sun et al., 2009; Wink et al., 2006).

The predominant saturated straight-chain fatty acids of the *Actinoplanes* species are penta- ($C_{15:0}$), hexa- ($C_{16:0}$), and heptadecanoic ($C_{17:0}$) acids. Major saturated branched fatty acids are 13-methyltetradecanoic ($C_{15:0}$ iso) and 14-methylpentadecanoic ($C_{16:0}$ iso), as well as 12-methyltetradecanoic ($C_{15:0}$ anteiso) and 14-methylhexadecanoic ($C_{17:0}$ anteiso) acids.

The predominant components of the unsaturated fatty acids are *cis*-9-heptadecenoic acid ($C_{17:1}$ ω9*c*) and *cis*-9-octadecenoic acid ($C_{18:1}$ ω9*c*, oleic acid). However, some differences have been detected by Kämpfer et al. (2007), where only *cis*-8-heptadecenoic acid ($C_{17:1}$ ω8*c*) instead of *cis*-9-heptadecenoic acid were detected in the analyzed strains, e.g. *Actinoplanes philippinensis*, *Actinoplanes couchii*, *Actinoplanes italicus*, and *Actinoplanes rectilineatus*. Minor amounts of tetra- and octadecanoic ($C_{14:0}$, $C_{18:0}$) acids, as well as 15-methylhexadecanoic- ($C_{17:0}$ iso) and *cis*-9-hexadecenoic acid ($C_{16:1}$ ω9*c*) have been found in most species. In a few cases, 12-methyltridecanoic acid ($C_{14:0}$ iso), 10-methylheptadecanoic acid ($C_{17:0}$ 10-methyl), and *cis*-9,14-methylhexadecenoic ($C_{17:1}$ iso ω9*c*) acids (Ara et al., 2010; Kämpfer et al., 2007; Sun et al., 2009) were detected in *Actinoplanes* species.

No fatty acid data are available for *Actinoplanes cyaneus* and *Actinoplanes digitatis*. Most analyses of the fatty acid components have been made according to Sasser (1990); fatty acid analyses described by Goodfellow et al. (1990b) were done according to Miwa et al. (1960). The distribution of cellular fatty acids amongst *Actinoplanes* species is summarized in Table 194.

Actinoplanes species contain tetrahydrogenated menaquinones with nine isoprene units [MK-9(H_4)] as predominant isoprenologue, with moderate amounts of MK-9(H_2) and MK-9(H_6) (Goodfellow et al., 1990b; Stackebrandt and Kroppenstedt, 1987). Minor proportions of MK-9(H_8) can also be found (Goodfellow et al., 1990b; Kämpfer et al., 2007). Tetrahydrogenated menaquinones with 10 side chains [MK-10(H_4)] may occur additionally as characteristic components of the quinone

TABLE 194. Differential characteristics of *Actinoplanes* species[a]

Characteristic	1. *A. philippinensis*	2. *A. auranticolor*	3. *A. brasiliensis*	4. *A. campanulatus*	5. *A. capillaceus*	6. *A. consettensis*	7. *A. couchii*	8. *A. cyaneus*	9. *A. deccanensis*	10. *A. derwentensis*	11. *A. digitatis*	12. *A. durhamensis*	13. *A. ferrugineus*	14. *A. globisporus*	15. *A. humidus*	16. *A. italicus*	17. *A. liguriensis*	18. *A. lobatus*	19. *A. missouriensis*	20. *A. palleronii*	21. *A. rectilineatus*	22. *A. regularis*	23. *A. sichuanensis*	24. *A. teichomyceticus*	25. *A. toevensis*	26. *A. tereljensis*	27. *A. utahensis*	28. *A. xinjiangensis*
Shape of sporangia:																												
Globose (subglobose, oval, pyriform)	+	−	(+)	−	−	+	+	+	+	+	−	+	+	−	+	(+)	+	−	+	+	−	−	+	+	+	−	(+)	+
Irregular	−	+	+	+	−	−	−	−	−	−	−	−	+	+	−	+	−	+	(+)	−	−	−	−	−	−	+	+	−
Lobed	−	+	−	+	−	−	−	−	−	−	(+)	−	−	−	−	−	−	+	−	−	−	−	−	−	−	−	+	−
Umbrella	−	−	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
Campanulate	−	−	−	+	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
Digitate	−	−	−	−	−	−	−	−	−	−	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	−
Cylindrical	−	−	−	(+)	−	−	−	−	−	−	−	−	−	−	−	−	−	(+)	−	−	+	+	−	−	−	−	−	−
Spore arrangement within sporangia:																												
Coils	+	−	−	−	−	−	−	nd	+	−	−	−	+	+	−	+	+	−	+	−	−	−	nd	+	nd	nd	+	nd
Irregular	−	+	+	−	−	+	+	nd	−	+[r]	−	+	−	−	+	−	−	−	−	+	−	−	nd	−	nd	nd	−	nd
Parallel rows	−	−	−	+	+	−	−	nd	−	−	+	−	−	−	−	−	−	+	−	−	+	+	nd	−	nd	nd	−	nd
Shape of spores:																												
Globose (subglobose, oval)	+	−	(+)	−	−	nd	+	+	+	+	−	nd	+	+	nd	+	+	−	+	nd	+	−	nd	+	+	+	+	nd
Rods (oblong, short rod-like)	−	+	+	+	+	nd	−	−	−	−	+	nd	−	−	nd	−	−	+	−	nd	−	+	nd	−	−	−	−	nd
Aerial mycelium:																												
Absent	+	+	+	+	+	+	−	+	+	+	+	+	−	+	+	+	−	+	+	+	−	+	+	−	+	+	+	+
Rudimentary	−	−	−	−	−	−	+	−	−	−	−	−	+	−	−	−	+	−	−	−	−	−	−	−	−	−	−	−
Well developed	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	−	−	+	−	−	−	−
Color of substrate mycelium:																												
White	−	−	−	−	−	−	−	nd	−	−	−	−	−	+	−	−	−	+	−	−	+	−	−	−	−	−	−	+
Yellow	+	−	−	−	+	+	(+)	nd	−	−	(+)	−	(+)	−	(+)	−	(+)	−	(+)	+	+	(+)	−	+	(+)	−	−	−
Orange	+	+	+	+	−	−	+	nd	+	+	−	+	−	+	+	+	+	−	+	−	+	+	+	+	(+)	(+)	+	+
Red	+	−	(+)	+	+	−	(+)	nd	(+)	−	+	−	−	−	−	+	−	+	(+)	−	−	+	+	−	(+)	(+)	(+)	−
Brown	(+)	−	−	+	−	+	−	nd	−	−	+	−	+	−	+	+	−	−	−	+	−	(+)	−	(+)	(+)	+	−	−
Black	−	−	−	+	−	−	−	nd	−	−	(+)	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
Production of soluble pigments:																												
Yellow	(+)	+	−	(+)	−	−	−	−	−	−	+	−	−	−	−	(+)	+	(+)	−	−	(+)	(+)	−	+	−	−	(+)	−
Red	−	−	−	−	−	−	+	−	−	−	−	−	(+)	−	−	+	+	−	−	−	−	−	−	+	(+)	(+)	−	−
Green	−	−	−	(+)	−	−	−	−	−	−	(+)	−	−	−	−	−	−	(+)	−	−	−	(+)	−	−	−	−	−	−
Blue	−	−	−	−	−	−	−	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
Brown	+	−	−	(+)	−	−	+	−	−	−	+	−	+	−	+	−	−	(+)	−	−	−	+	−	−	+	+	(+)	−
Physiological tolerance to (%, w/v):																												
Brilliant green (0.001)	−	−	nd	+	nd	−	nd	nd	nd	−	−	−	nd	nd	−	−	nd	+	+	−	nd	−	nd	nd	nd	nd	−	nd

(continued)

TABLE 194. (continued)

Characteristic	1. *A. philippinensis*	2. *A. aurantiicolor*	3. *A. brasiliensis*	4. *A. campanulatus*	5. *A. capillaceus*	6. *A. consettensis*	7. *A. couchii*	8. *A. cyaneus*	9. *A. deccanensis*	10. *A. derwentensis*	11. *A. digitatis*	12. *A. durhamensis*	13. *A. ferrugineus*	14. *A. globisporus*	15. *A. humidus*	16. *A. italicus*	17. *A. liguriensis*	18. *A. lobatus*	19. *A. missouriensis*	20. *A. palleronii*	21. *A. rectilineatus*	22. *A. regularis*	23. *A. sichuanensis*	24. *A. teichomyceticus*	25. *A. toevensis*	26. *A. tereljensis*	27. *A. utahensis*	28. *A. xinjiangensis*
Lysozyme (0.005)	+	+	nd	–[b], +[j]	nd	d	nd	nd	nd	d	+	+	+	nd	d	–[b], +[j]	nd	+	+	d	+	+	nd	nd	nd	nd	+	nd
Lysozyme (0.01)	+	+	nd	–	nd	d	nd	nd	nd	d	+	+	nd	nd	d	–	nd	–	+	–	nd	–	nd	nd	nd	nd	+	nd
Potassium tellurite (0.001)	+	+	nd	+	nd	+	nd	nd	nd	d	+	+	nd	nd	+	–	nd	–	+	+	nd	+	nd	nd	nd	nd	+	nd
Potassium tellurite (0.005)	+	+	nd	+	nd	–	nd	nd	nd	d	+	+	nd	nd	d	–	nd	–	+	–	nd	–	nd	nd	nd	nd	+	nd
Potassium tellurite (0.01)	+	+	nd	–	nd	–	nd	nd	nd	d	+	+	nd	nd	–	–	nd	–	+	–	nd	–	nd	nd	nd	nd	–	nd
Sodium azide (0.0001)	+	+	nd	+	nd	d	nd	nd	nd	d	+	d	nd	nd	d	+	nd	+	+	d	nd	+	nd	nd	nd	nd	+	nd
Sodium azide (0.001)	+	–	nd	+	nd	d	nd	nd	nd	+	+	d	nd	nd	–	+	nd	–	+	d	nd	–	nd	nd	nd	nd	–	nd
Resistance to antibiotics (μg/ml):																												
Ampicillin (0.5)	+	+	nd	+	nd	+	nd	nd	nd	+	+	d	nd	nd	+	–	nd	+	+	+	nd	+	nd	nd	nd	nd	+	nd
Ampicillin (2.0)	+	+	nd	+	nd	+	nd	nd	nd	–	–	–	nd	nd	d	–	nd	+	+	d	nd	+	nd	nd	nd	nd	+	nd
Ampicillin (8.0)	–	+	nd	+	nd	–	nd	nd	nd	–	–	–	nd	nd	–	–	nd	–	+	–	nd	–	nd	nd	nd	nd	–	nd
Ampicillin (100.0)	+[j]	+	nd	+	nd	nd	nd	nd	nd	nd	–	nd	+	nd	nd	+[j]	nd	nd	nd	nd	+	+[j]	nd	nd	nd	nd	+[j]	nd
Cephaloridine hydrochloride (1.0)	+	+	nd	+	nd	d	nd	nd	nd	–	–	–	nd	nd	d	–	nd	+	+	–	nd	–	nd	nd	nd	nd	+	nd
Cephaloridine hydrochloride (2.0)	–	+	nd	+	nd	d	nd	nd	nd	–	–	–	nd	nd	d	–	nd	–	–	–	nd	–	nd	nd	nd	nd	–	nd
Chloramphenicol (25.0)	+	+	nd	–	nd	nd	nd	nd	nd	nd	–	nd	+	nd	nd	+	nd	nd	nd	nd	+	+	nd	nd	nd	nd	+	nd
Chlortetracycline hydrochloride (1.0)	+	–	nd	–	nd	d	nd	nd	nd	–	–	–	nd	nd	d	–	nd	–	+	–	nd	–	nd	nd	nd	nd	–	nd
Gentamicin sulfate (0.5)	d	+	nd	+	nd	d	nd	nd	nd	d	+	d	nd	nd	+	+	nd	+	+	–	nd	+	nd	nd	nd	nd	+	nd
Gentamicin sulfate (1.0)	d	–	nd	–	nd	–	nd	nd	nd	–	+	–	nd	nd	–	–	nd	–	+	–	nd	–	nd	nd	nd	nd	–	nd
Gentamicin sulfate (4.0)	–	–	nd	–	nd	–	nd	nd	nd	–	+	–	nd	nd	–	–	nd	–	–	–	nd	–	nd	nd	nd	nd	–	nd
Kanamycin (25.0)	d	+	nd	+	nd	nd	nd	nd	nd	nd	–	nd	+	nd	nd	+	nd	nd	nd	nd	+	+	nd	nd	nd	nd	+	nd
Lincomycin hydrochloride (2.0)	+	+	nd	+	nd	d	nd	nd	nd	d	+	d	nd	nd	+	+	nd	+	+	d	nd	+	nd	nd	nd	nd	+	nd
Lincomycin hydrochloride (8.5)	+	+	nd	–	nd	d	nd	nd	nd	d	–	–	nd	nd	d	+	nd	+	+	d	nd	–	nd	nd	nd	nd	–	nd
Metacycline hydrochloride (0.25)	d	+	nd	+	nd	+	nd	nd	nd	d	+	d	nd	nd	+	+	nd	+	+	–	nd	+	nd	nd	nd	nd	–	nd
Metacycline hydrochloride (2.0)	–	–	nd	–	nd	–	nd	nd	nd	–	–	–	nd	nd	d	–	nd	–	+	–	nd	–	nd	nd	nd	nd	–	nd
Neomycin sulfate (0.5)	+	+	nd	–	nd	–	nd	nd	nd	d	+	d	nd	nd	d	–	nd	–	+	–	nd	+	nd	nd	nd	nd	+	nd
Neomycin sulfate (2.0)	–	–	nd	–	nd	–	nd	nd	nd	–	+	–	nd	nd	–	–	nd	–	+	–	nd	–	nd	nd	nd	nd	–	nd
Rifampin (0.25)	+	+	nd	–	nd	+	nd	nd	nd	d	+	+	nd	nd	+	–	nd	+	+	+	nd	+	nd	nd	nd	nd	+	nd
Rifampin (2.0)	+	–	nd	–	nd	–	nd	nd	nd	–	–	–	nd	nd	–	–	nd	+	+	–	nd	+	nd	nd	nd	nd	–	nd
Streptomycin (50.0)	nd	nd	nd	nd	–	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	+	nd	nd	nd	nd	+

Tobramycin sulfate (0.5)	+	–	nd	–	nd	d	nd	nd	nd	d	+	+	nd	nd	d	–	nd	+	+	–	nd	–[b]	nd	nd	nd	nd	+	nd
Tobramycin sulfate (1.5)	+	–	nd	–	nd	d	nd	nd	nd	–	+	d	nd	nd	–	–	nd	+	+	–	nd	+[b]	nd	nd	nd	nd	–	nd
Antimicrobial activity against:																												
Aspergillus niger	–	–	nd	–	nd	d	nd	nd	nd	–	–	–	nd	nd	–	–	–	–	–	–	–	–	nd	–	nd	nd	–	nd
Bacillus subtilis	–	–	d	–	nd	nd	nd	nd	nd	d	–	d	nd	nd	d	–	+	–	–	–[b], +[h]	–	–[b], +[h]	nd	+	nd	nd	–	nd
Candida albicans	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	–	nd	nd	–	–	–	nd	–	nd	nd	nd	nd
Escherichia coli	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	–	nd	nd	–	–	–	nd	–	nd	nd	nd	nd
Micrococcus luteus	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	–	nd	nd	+	–	+	nd	–	nd	nd	nd	nd
Pseudomonas aeruginosa	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	–	nd	nd	–	–	–	nd	–	nd	nd	nd	nd
Saccharomyces cerevisiae	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	–	nd	nd	–	–	–	nd	–	nd	nd	nd	nd
Staphylococcus aureus	–	–	nd	–	nd	d	nd	nd	nd	d	–	+	nd	nd	d	–	+	–	–	+[b], –[h]	–	–[b], +[h]	nd	+	nd	nd	–	nd
Streptomyces murinus	d	–	nd	–	nd	–	nd	nd	nd	d	–	–	nd	nd	d	–	–	–	+	–	–	+	nd	+	nd	nd	–	nd
Major fatty acids:[o]	[c]	[n]	[m]	[n]	[n]	[b]	[c]		[m]	[b]		[m]	[m]	[m]	[b]	[c]	[h]	[n]	[b]	[b]	[c]	[b]	[n]	[h]	[m]	[m]	[b]	[n]
Saturated straight-chain																												
$C_{14:0}$	0.7	0.7		3.7	1.7	1.2	2.8			2.1			1.0		4.8	2.0	1.8	1.4	1.8	0.8	6.5	0.7	6.2				3.2	3.7
$C_{15:0}$	19.2	10.5	3.0	4.5	8.0	8.3	11.0			6.0		1.2	2.3	1.0	4.2	4.5	1.5	4.9	2.1	9.9	15.3	2.3	4.2	1.1		2.3	4.7	6.7
$C_{16:0}$	1.7	5.6	1.7	2.5	1.3	18.3	14.5		1.6	24.3		4.6	15.5	3.6	17.7	11.4	6.5	1.1	6.0	4.1	11.9	4.9	7.7	2.5	1.5	4.4	15.5	6.5
$C_{17:0}$	6.8	2.1	5.1	3.4	4.6	2.5	5.9		1.0	5.7		4.7	7.2	3.0	15.5	5.1	1.2	4.3	7.9	12.0	9.6	9.2	4.4	3.5	1.2	5.1	28.8	4.2
$C_{18:0}$	0.1		1.4	0.9	0.8	9.6	0.3		3.1	10.2		8.9	9.3	3.8	12.0	3.3	1.5	0.9	9.0	12.2	2.9	4.3		1.8	1.5	4.4	14.0	
$C_{19:0}$						1.3				0.5					9.0				26.4	0.5	0.5	2.4					5.6	
Saturated branched																												
$C_{14:0}$ iso	1.3	0.9	1.5	2.8	3.0		0.4					2.0	1.6			0.5	1.7	3.4						1.0		1.9		0.9
$C_{15:0}$ iso	9.5	15.7	10.1	17.6	16.7	12.0	4.1		12.6	5.2		7.3	8.4	17.6	6.6	19.8	26.1	22.1	6.5	15.8	7.0	10.6	22.2	15.1	12.6	10.6	4.1	10.8
$C_{15:0}$ anteiso	1.2	3.2	24.5	6.9	4.7	27.2	1.5		10.6	1.6		12.6	22.9	3.4	8.8	1.8	13.1	4.9	7.0	29.1	0.4	13.3	1.4	8.8	11.7	11.8	3.8	3.6
$C_{16:0}$ iso	25.1	19.2	17.1	14.9	14.1	7.2	10.5		20.5	22.2		19.8	7.7	20.1	10.4	13.1	12.1	14.6	18.3	6.2	1.9	15.0	15.3	20.7	21.6	18.6	8.5	16.0
$C_{17:0}$ iso	1.1	2.9	2.1	5.0	6.0	0.3	4.7		7.3	1.2		2.1	1.1	9.7	1.1	4.0	3.0	9.0	3.5	0.8	1.3	7.0	4.2	3.8	5.0	2.4	3.8	4.1
$C_{17:0}$ anteiso	1.8		18.8		5.8	11.8	8.6		34.0	20.5		20.4	13.4	13.4	7.2	3.8	1.0	5.1	7.8	5.9		20.8	3.1	19.8	24.9	15.3	6.3	4.7
$C_{17:0}$ 10-methyl	0.6			0.9	0.8											0.2												
Unsaturated																												
$C_{15:1}$ ω8*c*	0.3																											
$C_{16:1}$ iso	1.6															0.6								1.6				
$C_{16:1}$ ω9*c*		5.6		10.6	2.3							1.2	1.9					3.4			0.3		3.3	3.4				5.9
$C_{17:1}$ iso	0.7			6.9			1.7									1.9	1.9							2.6				
$C_{17:1}$ anteiso	0.5								1.1							0.3								2.0	1.7			
$C_{17:1}$ ω10*c*																	3.3											
$C_{17:1}$ ω9*c*		13.4	1.9	5.3	18.4							1.4	1.3	1.3			2.2	8.9					11.1	6.7	3.1	1.9		21.5
$C_{17:1}$ ω8*c*	19.2						9.9									9.7												
$C_{17:1}$ ω6*c*	1.9						1.5																					
$C_{18:1}$ ω7*c*	0.4															1.1												
$C_{18:1}$ ω9*c*	0.4	6.2	5.0	5.3	7.1				1.0			4.6	4.6	3.9		6.5	2.9	4.6					7.5	4.3	3.6	5.0		3.9
$C_{16:1}$ ω7*c* and/or $C_{15:0}$ iso 2-OH	2.2						17.5									9.4												
$C_{19:1}$ ω11*c* and/or $C_{19:1}$ ω9*c*																												
Menaquinones:[p]																												
MK-7(H_4)	–					MI				–		–			–	–		–	–	–		–					–	

(continued)

TABLE 194. (continued)

Characteristic	1. *A. philippinensis*	2. *A. aurantiicolor*	3. *A. brasiliensis*	4. *A. campanulatus*	5. *A. capillaceus*	6. *A. consettensis*	7. *A. couchii*	8. *A. cyaneus*	9. *A. deccanensis*	10. *A. derwentensis*	11. *A. digitatis*	12. *A. durhamensis*	13. *A. ferrugineus*	14. *A. globisporus*	15. *A. humidus*	16. *A. italicus*	17. *A. liguriensis*	18. *A. lobatus*	19. *A. missouriensis*	20. *A. palleronii*	21. *A. rectilineatus*	22. *A. regularis*	23. *A. sichuanensis*	24. *A. teichomyceticus*	25. *A. toevensis*	26. *A. tereljensis*	27. *A. utahensis*	28. *A. xinjiangensis*
$MK-7(H_6)$	MI					–				–		–			MI	–		MI	MI	–		MI					–	
$MK-7(H_8)$	–					–				–		–			MI	–		–	–	–		–					–	
$MK-8(H_2)$	–					MI				MI		–			–	–		–	–	MI		–					–	
$MK-8(H_4)$	–					–				–		–			–	–		MI	–	–		–					–	
$MK-8(H_6)$	–					–				MI		–			MI	MI		MI	MI	MI		MI					MI	
$MK-8(H_8)$	–					MI				–		–			–	–		–	–	–		–					–	
$MK-9(H_2)$	–[b], MO[k]	MI				–	MI			MI		–			MI	MI		MI	MI	MI		MI[b], MO[k]					MI	
$MK-9(H_4)$	PR[b] MO[k]	PR		PR	PR	PR	PR			PR	PR	PR			PR	PR[b] MO[k]	PR	PR	PR	PR		PR	PR	PR			PR	PR
$MK-9(H_6)$	–	MI			MI	–	MO			MO	MI	MI			MO	MO		–	MI	MO		–	MI		PR	PR	MO	
$MK-9(H_8)$	–					MI	MI			–		MI			–	–		–	–	MI		–			MI	MO	–	
$MK-10(H_2)$	–					–	MI			–		–			–	–		–	–	–		–					–	
$MK-10(H_4)$	–[b], MI[k]				MI	–				–		–			–	–[b], MI[k]	PR	–[b], MI[k]	–	–		–		PR			–[b], MI[k]	
$MK-10(H_6)$	–					–	tr			–		–			–	–		–	–	–		–	MI				–	MI
$MK-10(H_8)$	–					–				–		–			–	–		–	–	–		–					–	
Polar lipids[q]																												
DPG	+	+				+	+			+		+			+	+			+	+	+	+			+	+		
PG	–	+				+	+			+		+			+	+			+[k], (+)[l]	+	(+)	+			–	–		
PE	+	+	+[m]		+	+	+		+[m]	+		+	+[m]	+[m]	+	+	+		+	+	+	–	+	+	+	+		+
PI	+	+				+	(+)			+		+			+	+			+	+	+	+	+		+	+		
PIM	+	+					(+)									+			+		+	+						
PC	–	–			–		–									–	–		–		–	–		–	–	–		
AGP	–	–			–		–									–			–			–						
HL							(+)																					
PL							(+)																					
L							+																					
GL															+													

[a]Symbols: +, >85% positive; d, different strains give different reactions (16–84% positive); –, 0–15% positive; (+), weakly positive, moderate; D, different reactions occur in different taxa (species of a genus or genera of a family); w, weak reaction; nd, not determined; nr, not reported.

[b]Data compiled from Goodfellow et al. (1990a)

[c]Kämpfer et al. (2007)

[d]Matsumoto et al. (2000)

[e]Palleroni (1989)

[f]Thiemann (1967)

[g]Vobis and Kothe (Vobis and Kothe, 1989)

[h]Wink et al. (2006)

[i]Schäfer (1973)

[j]Kothe (1987)

[k]Stackebrandt and Kroppenstedt (1987)

[l]Lechevalier et al. (1977)

[m]Ara et al. (2010)

[n]Sun et al. (2009); deviations or incoherent data are indicated by superscript letters.

[o]Percentages of fatty acids are shown; for unsaturated fatty acids, the position of the double bond is located by counting from the methyl (ω) end of the carbon chain; *cis* isomers are indicated by the suffix *c*.

[p]Menaquinones: PR, predominant component (>50%); MO, moderate component (11–50%); MI, minor component (1–10%); tr, traces (<1%).

[q]Polar lipids: DPG, diphosphatidylglycerol; PG, phosphatidylglycerol; PE, phosphatidylethanolamine; PI, phosphatidylinositol; PIM, phosphatidylinositol mannoside; PC, phosphatidylcholine; APG, amino-containing glycophospholipid; HL, highly hydrophilic lipid; PL, unknown phospholipids; L, unknown polar lipid; GL, uncharacterized glycolipid.

[r]Occurs in spirals (G. Vobis, unpublished observation).

system (Kroppenstedt, 1985; Wink et al., 2006), but usually in minor amounts (Matsumoto et al., 2000; Stackebrandt and Kroppenstedt, 1987). Smaller amounts of MK-10(H_2) and traces of MK-10(H_6) are characteristic for *Actinoplanes couchii* (Kämpfer et al., 2007). The additional presence of MK-7(H_4, H_6, H_8) and MK-8(H_2, H_4, H_6, H_8) can be observed in some species, where they may occur in minor amounts (Goodfellow et al., 1990b). The distribution patterns of polar lipids and menaquinones for the species are given in Table 194.

Colonial characteristics. *Actinoplanes* strains grow well on various complex media, forming compact colonies up to about 3 cm in diameter after 4–6 weeks of incubation. They are characteristically elevated and convoluted, and frequently have protuberances in the center. The marginal areas can be ridged or flat. The colonies can be divided into sectors. Their consistency is generally hard, but may occasionally be soft, especially for "*Ampullariella*" strains. Colonies can be covered with a slight glittering whitish bloom if abundant sporangia are produced on the surface of the substrate mycelium. Aerial mycelium is rarely developed and generally only in a rudimentary state, giving the colonies a whitish gray surface layer.

Colonies are usually brightly colored, with orange being the basic color component. Szaniszlo (1968) has identified spectrophotometrically the orange pigment of various strains as being associated with carotenoids. Besides all shades of orange, a great variety of additional colors exists, depending on the individual strains: cream to yellow, brown, rusty brown, red, purple, violet, green, or black (Palleroni, 1989; Parenti and Coronelli, 1979; Vobis, 1987). Segments with different colors may even occur in a single colony (Vobis, 1992). Palleroni (1989) reported a red pigment analyzed from two strains of *Actinoplanes* that had the structure of an anthraquinone derivative. Methanolic extracts of *Actinoplanes ferrugineus* lack a characteristic carotenoid spectrum (Palleroni, 1979).

Soluble pigments are also produced in several species, ranging from yellowish, greenish, and auburn to dark brown (Couch, 1963). "*Ampullariella violaceochromogenes*" produces a violet to dark purple soluble pigment on various agar media (Nonomura et al., 1979). A cherry red pigment is the distinctive mark of *Actinoplanes italicus*, although this red pigment is not produced and substrate mycelium appears deep orange on exposure to light during growth (Beretta, 1973). The soluble blue pigment of *Actinoplanes cyaneus* has been shown to belong to the celocomycin-actinorodine group (Terekhova et al., 1977). Melanoid pigments are produced in *Actinoplanes digitatis* (Couch, 1963).

Production of secondary metabolites. *Actinoplanes* strains have shown a high capacity to produce antibiotics and other useful secondary metabolites. First reviews revealed about 20 antibiotics (Palleroni, 1983; Parenti and Coronelli, 1979). The number increased to more than 120, covering a wide range of chemical diversity including peptides, glycopeptides, anthracyclines, nucleosides, polyenes, and quinones (Okami and Hotta, 1988; Vobis, 1992). The amino acid derivatives are predominant and some are of clinical relevance (Lazzarini et al., 2000), e.g. teicoplanin, a glycopeptide from *Actinoplanes teichomyceticus* ATCC 31121^T (Bardone et al., 1978), actaplanin, a glycopeptide from *Actinoplanes missouriensis* ATCC 23342 (Debono et al., 1984), and ramoplanin, a glycolipodepsidpeptide from *Actinoplanes* sp. ATCC 33076 (Ciabatti and Cavalleri, 1989). Further examples of other interesting antibiotics are the friulimicins, lipopetides from "*Actinoplanes friuliensis*" DSM 7358 (Aretz et al., 2000), purpuromycin, a naphthoquinone from "*Actinoplanes ianthinogenes*" ATCC 21884 (Coronelli et al., 1974), the chloride-containing lipiarmycin from *Actinoplanes deccanensis* ATCC 21983^T (Parenti et al., 1975), and actagardine, an oligopeptide from *Actinoplanes liguriensis* ATCC 31048^T (Parenti et al., 1976; Vértesy et al., 1999). The list of "Practically used Antibiotics and Their Related Substances" (Sezaki and Miyadoh, 2001) also includes acarbose, a pseudotetrasaccharide produced by *Actinoplanes* sp. SE-50 (Truscheit et al., 1981), a very effective inhibitor of α-glucosidase, successfully applied in cases of diabetes mellitus (Creutzfeld, 1988).

Life cycle. The life cycle of *Actinoplanes* is characterized by an alternation between terrestrial and aquatic habitats (Figure 213H) (Vobis, 1987, 1992). The growth of vegetative mycelium on plant or animal debris or other adequate substrates (Figure 213F) culminates in the differentiation into sporangia (Figure 213H, I), which are generally produced on the surface of the substrate, directly in contact with the air (Bland and Couch, 1981). The sporangia can easily lose their connection to the degenerating mycelium and are disseminated as diaspores by the wind (Figure 213H, II), or by soil fauna such as mites, collembola, or arthropods. The sporangial envelope and the intrasporangial matrix hold together the mass of spores and shield the thin-walled spores (Figure 212C, D) from mechanical and physiological stress. The sporangia withstand prolonged desiccation (Figure 213H, III) and survive for many years (Makkar and Cross, 1982).

The sporangial envelope is usually hydrophobic. It can be rehydrated by sufficient moisture, e.g. during periods of fog or rain, and flagellated spores are released from the sporangia. Under laboratory conditions (Figure 213A, B, C), this process takes 10–60 min (Higgins, 1967; Vobis, 1987). The process can be facilitated by the addition of wetting agents like Tween 80 (Higgins, 1967). During immersion in water, the spores swell, becoming more clearly visible within the sporangium. In general, spores show motility inside the sporangium before they are released by disruption of the sporangial envelope (Lechevalier and Holbert, 1965). As Higgins (1967) has shown, both swelling and motility of the spores contribute to their release, thus contradicting the suggestion made by Couch (1963) that this may be a consequence of the swelling of the intersporal material. Of the two factors, swelling of the spores is the more important, as sometimes sporangium dehiscence gives nonmotile spores (Palleroni, 1989). In some instances, motility begins after spore release. Spores of *Actinoplanes brasiliensis* retain their motility in water or in diluted buffers for more than 1 d at room temperature (Palleroni, 1983). In baiting experiments simulating a natural aquatic microhabitat, pollen or other natural substrates are exposed to the surface of water. The zoospores, once released from the submerged sporangia, are able to swim to the surface (Figure 213H, IV), attach to the natural substrates, germinate (Figure 213E, H, V), and colonize them within several days (Figure 213F) (Couch, 1963; Vobis, 1987). This may be a result of aerotactic and chemotactic behavior of the spores (Cross, 1986). Although the chemotactic response is used effectively in the isolation method of Palleroni (1980), up to now the exact physiological explanation is not known. If the liquid contains nutrients, germination may ensue after a few hours and

the emergence of the filamentous stage completes the cycle of morphogenetic changes (Palleroni, 1989).

The state of swarming sporangiospores (Cross, 1986) may be skipped over during the life cycle. In nearly all cases, under culture conditions, it is possible to induce the spores to germinate, if they are still enclosed within the sporangia. Exposed on the surface of agar media, the spores germinate synchronously, and can develop directly into new mycelium (Figure 213G) (Vobis, 1987).

Metabolism and physiology. *Actinoplanes* strains are aerobic and mesophilic, growing between 10 and 35°C, with an optimal growth temperature of about 23–28°C. Some species, e.g. *Actinoplanes deccanensis*, show moderate thermotolerance, growing at a maximum temperature of 45°C (Parenti and Coronelli, 1979). No growth occurs at 50°C (Kothe, 1987; Parenti et al., 1978). The minimum growth temperature observed for many species is 4°C (Goodfellow et al., 1990b).

Species of *Actinoplanes* can be characterized as neutrophilic micro-organisms, growing well at pH 6.0–8.0. Goodfellow et al. (1990b) could not register growth at either pH 5.6 and lower or at pH 9.0 and higher for 13 species cultivated on modified Bennett's agar. In contrast, Kothe (1987) observed growth in acid conditions at pH 4.0 for four species and in alkaline conditions at pH 10.0 for nine species grown on yeast extract-starch agar (Table 195). These differences can probably be explained by the use of the two distinct agar media, perhaps having diverse buffer effects.

Strains of *Actinoplanes* can be cultured on most complex media generally used for actinomycetes, including various ISP media (Wink et al., 2006). Further recommendations and/or recipes of media suitable for isolation, vegetative growth, sporulation, and many kinds of physiological tests are given by Goodfellow et al. (1990b), Palleroni (1989), and Vobis (1992).

Isolates of *Actinoplanes* are able to grow on chemically defined agar media prepared with inorganic salts and a single compound as the sole source of carbon. In general, the test method follows the recommendations of Shirling and Gottlieb (1966) using about ten organic compounds (Schäfer, 1973; Thiemann et al., 1969; Wink et al., 2006). Other authors used different basal media and/or amplified the number of tested carbohydrates (Goodfellow et al., 1990b; Kämpfer et al., 2007; Kothe, 1987; Palleroni, 1989). D-Glucose and, to a lesser extent, L-arabinose, D-mannose, and maltose are used as carbon sources by all species (Table 195).

As typical saprophytic inhabitants of soil, *Actinoplanes* strains are able to degrade organic compounds (Table 195). Casein, chitin, gelatin, DNA, RNA, and lecithin are decomposed by most species, as well as starch as a representative polymer of plant origin. Pectin is decomposed by many species. Different results are reported for cellulose degradation. Goodfellow et al. (1990b) found that none of the 55 strains investigated were able to break down cellulose, whereas tests of Schäfer (1973) and Solans and Vobis (2003) presented positive results for 21 strains. Hemicellulose can be degraded and lignocellulose is used as a preferred substrate by various strains (Solans and Vobis, 2003). The compound guanine and the polymer keratin, both of typical animal origin, are not degraded. Further enzymic activities have been studied recently (Wink et al., 2006), specifically hydrolysis of chromogenic substances (Kämpfer et al., 2007) (Table 195).

Studies on other physiological features, including the ability to grow in media supplemented with sodium chloride, have been tested for 15 species with different results (Table 195): concentrations of up to 2.0% (w/v) were tolerated by six species; *Actinoplanes campanulatus* and one strain of *Actinoplanes palleronii* could grow at 3.0% (w/v) NaCl (Goodfellow et al., 1990b); and a concentration of 5% (w/v) sodium chloride was not tolerated by eight species including *Actinoplanes campanulatus* (Kothe, 1987).

Many species have been tested by Goodfellow et al. (1990b) for their capacity to grow in the presence of various other organic and inorganic chemical compounds at different concentrations, as well as their resistance to several antibiotics (Table 194). Aside from well-studied antibiotics produced by *Actinoplanes*, antimicrobial activities against *Aspergillus niger* and *Candida albicans*, as representatives of eukaryotic microorganisms, and against Gram-stain-negative and Gram-stain-positive bacteria have been carried out to characterize various species (Goodfellow et al., 1990b; Wink et al., 2006) (Table 194). Further biochemical tests like reduction of nitrate, production of urease and hydrogen sulfide, and coagulation and peptonization of milk are additional useful taxonomic markers (Table 195).

The production of sporangia occurs preferably on certain agar media, depending on the strain. In general, starch-casein and a minimal agar medium with certain carbon sources promotes sporangia formation (Palleroni, 1989). Humic acid and fulvic acid are possible factors to stimulate sporangiogenesis (Willoughby et al., 1968; Willoughby and Baker, 1969), as well as tea infusion (Parenti et al., 1978). Good results have been obtained when sections of mycelia, originally grown on nutrient-rich complex media, were transferred onto nutrient-poor media like artificial soil agar (Vobis, 1992; Vobis and Kothe, 1985).

The motile sporangiospores of *Actinoplanes* are, in general, vigorous swimmers, moving in a straight line. They interrupt the movement for moments, tumbling and "re-arranging" their flagella, and continue to swim in a different direction (Palleroni, 1983). Couch and Koch (1962) have observed that 1% Casamino acid induces the motility of sporangiospores of an "*Ampullariella*" strain that is a rather poor swimmer and that L-arginine hydrochloride (6 mM) and urea (0.01 M) greatly increase the motility. In spore-releasing studies on *Actinoplanes rectilineatus*, Higgins (1967) could demonstrate that flagellation and motility decreased in old spores, but motility can be regained if glucose is supplied as exogenous carbon source, which must be applied within 180 min of the initial wetting. The addition of amino acids or phosphate buffer permitted flagellation, but not motility. Deflagellation/reflagellation experiments indicated that functional flagella can be regenerated only in the presence of both amino acids and glucose. Inoperative flagella were formed in the presence of inhibitors of nucleic acid synthesis, such as 6-azauracil, but inhibitors of protein synthesis, such as chloramphenicol, did not interfere with reflagellation. Inhibitors such as sodium *p*-chlormercuribenzoate, sodium iodoacetate, 2-iodoacetamide, sodium azide, and 2,4-dinitrophenol inhibit the formation of flagella and also immobilized fully motile spores (Higgins, 1967). Carbonylcyanide-*m*-chlorophenylhydrazone, which is effective against oxidative phosphorylation, has the same physiological effect (Palleroni, 1983).

TABLE 195. Physiological and biochemical tests differentiating *Actinoplanes* species[a]

Characteristic	1. *A. philippinensis*	2. *A. auranticolor*	3. *A. brasiliensis*	4. *A. campanulatus*	5. *A. capillaceus*	6. *A. consettensis*	7. *A. couchii*	8. *A. cyaneus*	9. *A. deccanensis*	10. *A. derwentensis*	11. *A. digitatis*	12. *A. durhamensis*	13. *A. ferrugineus*	14. *A. globisporus*	15. *A. humidus*	16. *A. italicus*	17. *A. liguriensis*	18. *A. lobatus*	19. *A. missouriensis*	20. *A. palleronii*	21. *A. rectilineatus*	22. *A. regularis*	23. *A. sichuanensis*	24. *A. teichomyceticus*	25. *A. tereljensis*	26. *A. toevensis*	27. *A. utahensis*	28. *A. xinjiangensis*
Utilization of sole carbon sources:																												
Acetate	nd	nd	nd	nd	nd	nd	–	nd	nd	nd	nd	nd	nd	nd	nd	–	nd	nd	nd	nd	–	nd	+	nd	nd	nd	nd	+
N-Acetyl-D-galactosamine	nd	nd	nd	nd	nd	nd	–	nd	nd	nd	nd	nd	nd	nd	nd	–	nd	nd	nd	nd	–	nd	nd	nd	nd	nd	nd	nd
N-Acetyl-D-glucosamine	nd	nd	nd	nd	nd	nd	–	nd	nd	nd	nd	nd	nd	nd	nd	–	nd	nd	nd	nd	–	nd	nd	nd	nd	nd	nd	nd
trans-Aconitate	nd	nd	nd	nd	nd	nd	–	nd	nd	nd	nd	nd	nd	nd	nd	–	nd	nd	nd	nd	–	nd	nd	nd	nd	nd	nd	nd
Adipate	+	nd	–	nd	nd	nd	–	nd	nd	nd	nd	nd	–	nd	nd	+[e], –[c]	nd	nd	–	nd	–	nd	nd	nd	nd	nd	+	nd
Adonitol	–	–	nd	–	nd	–	–	nd	nd	–	–	–	nd	nd	–	–	nd	–	–	–	–	–	+	nd	nd	nd	–	+
β-Alanine	+	nd	–	nd	nd	nd	–	nd	nd	nd	nd	nd	+	nd	nd	+	nd	nd	–	nd	–	nd	nd	nd	nd	nd	+	nd
4-Aminobutyrate	nd	nd	nd	nd	nd	nd	–	nd	nd	nd	nd	nd	nd	nd	nd	(+)	nd	nd	nd	nd	–	nd	nd	nd	nd	nd	nd	nd
γ-Aminobutyrate	+	nd	+	nd	nd	nd	nd	nd	nd	nd	nd	nd	–	nd	nd	+	nd	nd	–	nd	nd	nd	nd	nd	nd	nd	–	nd
D-Arabinose	–	+	+	–	nd	d	nd	nd	nd	d	–	d	+	nd	+	–	nd	–	–	–	nd	–	+	nd	nd	nd	–	+
L-Arabinose	+	+	+	+	+	+	+	nd	+	+	+, d[g]	+	+	+	+	+	–[h], +[e]	+	+	d[b], –[h]	+	+	nd	+	nd	nd	+	nd
Arabitol	–	nd	+	nd	nd	nd	nd	nd	nd	nd	nd	nd	+	nd	nd	–	nd	nd	d	nd	nd	nd	nd	nd	nd	nd	–	nd
p-Arbutin	nd	nd	nd	nd	nd	nd	+	nd	nd	nd	nd	nd	nd	nd	nd	–	nd	nd	nd	nd	+	nd	nd	nd	nd	nd	nd	nd
L-Arginine	+	nd	–	nd	nd	nd	nd	nd	nd	nd	nd	nd	–	nd	nd	+	nd	nd	–	nd	nd	nd	nd	nd	nd	nd	+	nd
L-Asparagine	(+)	nd	+	nd	nd	nd	nd	nd	nd	nd	nd	nd	(+)	nd	nd	–	nd	nd	–	nd	nd	nd	nd	nd	nd	nd	(+)	nd
L-Aspartate	nd	nd	nd	nd	nd	nd	+	nd	nd	nd	nd	nd	nd	nd	nd	(+)	nd	nd	nd	nd	(+)	nd	nd	nd	nd	nd	nd	nd
Azelate	nd	nd	nd	nd	nd	nd	–	nd	nd	nd	nd	nd	nd	nd	nd	–	nd	nd	nd	nd	–	nd	nd	nd	nd	nd	nd	nd
Catechol	–	–	nd	–	nd	–	nd	nd	nd	–	–	–	nd	nd	–	–	nd	–	–	–	nd	–	nd	nd	nd	nd	–	nd
Cellobiose	–	–	nd	–	nd	–	nd	nd	nd	–	–	–	nd	nd	–	–	nd	–	–	–	nd	–	–	nd	nd	nd	–	–
Cellulose	+	–	+	nd	nd	nd	nd	nd	–	nd	nd	nd	–	–	nd	–	–	nd	–	nd	–	nd	nd	–	nd	nd	–	nd
Citrate	–	nd	–	nd	nd	nd	–	nd	nd	nd	nd	nd	–	nd	nd	+	nd	nd	–	nd	–	nd	+	nd	nd	nd	–	+
L-Citrulline	(+)	nd	–	nd	nd	nd	nd	nd	nd	nd	nd	nd	–	nd	nd	(+)	nd	nd	d	nd	nd	nd	nd	nd	nd	nd	+	nd
Coumarin	–	–	nd	–	nd	–	nd	nd	nd	–	–	–	nd	nd	–	–	nd	–	–	–	nd	–	nd	nd	nd	nd	–	nd
Dextrin	+	+	nd	+	nd	+	nd	nd	nd	+	+	+	nd	–	+	+	nd	+	+	d	nd	+	–	nd	nd	nd	+	–
Dulcitol	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	–	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Erythritol	–	–	nd	–	nd	–	nd	nd	nd	–	–	–	nd	nd	–	–	nd	–	–	–	nd	–	nd	nd	nd	nd	–	nd
D-Fructose	+	+	+	+	+	+	+	nd	–	+	+	+	(+)	+	+	+	–[h], +[e]	+	+	+	+	+	+	+	nd	nd	+	+
L-Fucose	–	nd	–	nd	nd	nd	nd	nd	nd	nd	nd	nd	+	nd	nd	–	nd	nd	d	nd	nd	nd	nd	nd	nd	nd	+	nd
Fumarate	nd	nd	nd	nd	nd	nd	–	nd	nd	nd	nd	nd	nd	nd	nd	+	nd	nd	nd	nd	+	nd	nd	nd	nd	nd	nd	nd
D-Galactose	+	+	nd	+	+	+	+	nd	nd	+	+	+	nd	+	+	+	nd	+	+	d	+	–	+	nd	nd	nd	+	–
Gluconate	nd	nd	nd	nd	nd	nd	+	nd	nd	nd	nd	nd	nd	nd	nd	+	nd	nd	nd	nd	+	nd	+	nd	nd	nd	nd	+
D-Glucose	+	+	+	+	+	+	+	nd	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
L-Glutamine	–	nd	+	nd	nd	nd	nd	nd	nd	nd	nd	nd	–	nd	nd	+	nd	nd	+	nd	nd	nd	nd	nd	nd	nd	+	nd
Glutarate	nd	nd	nd	nd	nd	nd	(+)	nd	nd	nd	nd	nd	nd	nd	nd	(+)	nd	nd	nd	nd	(+)	nd	nd	nd	nd	nd	nd	nd
Glycerol	nd	nd	nd	+	+	nd	nd	nd	nd	nd	+	nd	–	nd	nd	nd	nd	+	nd	nd	nd	+	nd	nd	nd	nd	nd	nd

L-Histidine	+	nd	–	nd	nd	nd	–	nd	nd	nd	nd	nd	–	nd	nd	+	nd	nd	d	nd	–	nd	nd	nd	nd	nd	–	nd
3-Hydroxybenzoate	nd	nd	nd	nd	nd	nd	–	nd	nd	nd	nd	nd	nd	nd	nd	–	nd	nd	nd	nd	–	nd	nd	nd	nd	nd	nd	nd
4-Hydroxybenzoate	nd	nd	nd	nd	nd	nd	–	nd	nd	nd	nd	nd	nd	nd	nd	–	nd	nd	nd	nd	–	nd	nd	nd	nd	nd	nd	nd
m-Hydroxybenzoic acid	d	+	nd	–	nd	–	nd	nd	nd	–	–	d	nd	nd	–	–	nd	–	–	d	nd	+	nd	nd	nd	nd	–	nd
o-Hydroxybenzoic acid	–	–	nd	–	nd	–	nd	nd	nd	–	–	–	nd	nd	–	–	nd	–	–	–	nd	–	nd	nd	nd	nd	–	nd
p-Hydroxybenzoic acid	–	–	nd	–	nd	–	nd	nd	nd	d	–	+	nd	nd	+	–	nd	–	+	–	nd	+	nd	nd	nd	nd	–	nd
β-Hydroxybutyrate	+	nd	+	nd	nd	nd	nd	nd	nd	nd	nd	nd	–	nd	nd	+	nd	nd	+	nd	nd	nd	nd	nd	nd	nd	+	nd
DL-3-Hydroxybutyrate	nd	nd	nd	nd	nd	nd	–	nd	nd	nd	nd	nd	nd	nd	nd	(+)	nd	nd	nd	nd	–	nd	nd	nd	nd	nd	nd	nd
Inositol[k]	+	+	+	–	–	–	+	nd	nd	+	+	d	–	+	d	+	+[e], –[h]	–	–	d[b], –[h]	+[h], –[c]	–	–	–	nd	nd	–	–
Inulin	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	+	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Itaconate	nd	nd	nd	nd	nd	nd	–	nd	nd	nd	nd	nd	nd	nd	nd	–	nd	nd	nd	nd	–	nd	nd	nd	nd	nd	nd	nd
α-Ketoglurate	–	nd	–	nd	nd	nd	nd	nd	nd	nd	nd	nd	–	nd	nd	+	nd	nd	d	nd	nd	nd	nd	nd	nd	nd	–	nd
DL-Lactate	+	nd	+	nd	nd	nd	–	nd	nd	nd	nd	nd	–	nd	nd	+	nd	nd	+	nd	–	nd	nd	nd	nd	nd	–	nd
D-Lactose	+	+	nd	–	nd	+	nd	nd	+	+	+	+	+	+	+	+	–	+	+	d	+	–	–	(+)	(+)	+	–	–
D-Malate	–	nd	–	nd	nd	nd	nd	nd	nd	nd	nd	nd	–	nd	nd	(+)	nd	nd	–	nd	nd	nd	nd	nd	nd	nd	+	nd
L-Malate	nd	nd	nd	nd	nd	nd	+	nd	nd	nd	nd	nd	nd	nd	nd	(+)	nd	nd	nd	nd	(+)	nd	nd	nd	nd	nd	nd	nd
Maltitol	nd	nd	nd	nd	nd	nd	–	nd	nd	nd	nd	nd	nd	nd	nd	(+)	nd	nd	nd	nd	–	nd	nd	nd	nd	nd	nd	nd
D-Maltose	+	+	nd	+	+	+	+	nd	nd	+	+	+	nd	+	+	+	nd	+	+	+	+	+	+	nd	+	+	+	–
D-Mannitol	+	+	+	+	+	+	nd	nd	–	+	–	+	+	+	+	+	–	+[g], –[b]	+	+[h], d[b]	+	–	–	+	–	–	+	–
D-Mannose	+	+	nd	+	+	+	+	nd	+	+	+	+	+	+	+	+	+	+	+	+	+	+	–	+	nd	nd	+	+
Melezitose	+	+	–	–	nd	d	nd	nd	d	nd	–	+	–	nd	d	+	nd	–	–	d	nd	+	nd	nd	nd	nd	–	nd
Melibiose (α-D-)[c]	–	nd	+	nd	–	nd	–	nd	nd	nd	nd	nd	–	nd	nd	–	nd	nd	–	nd	–	nd	–	nd	nd	nd	–	–
Mesaconate	nd	nd	nd	nd	nd	nd	–	nd	nd	nd	nd	nd	nd	nd	nd	–	nd	nd	nd	nd	–	nd	nd	nd	nd	nd	nd	nd
Methyl-α-D-glucoside	–	+	nd	–	nd	–	nd	nd	nd	–	–	d	nd	nd	d	–	nd	–	–	–	nd	–	–	nd	nd	nd	–	–
Methyl-β-D-glucoside	+	+	nd	+	nd	+	nd	nd	nd	+	–	+	nd	nd	+	+	nd	+	+	–	nd	–	nd	nd	nd	nd	+	nd
L-Ornithine	–	nd	+	nd	nd	nd	–	nd	nd	nd	nd	nd	–	nd	nd	(+)	nd	nd	–	nd	–	nd	nd	nd	nd	nd	–	nd
Phenylacetate	–	nd	–	nd	nd	nd	–	nd	nd	nd	nd	nd	(+)	nd	nd	–	nd	nd	d	nd	–	nd	nd	nd	nd	nd	–	nd
L-Proline	+	nd	+	nd	nd	nd	+	nd	nd	nd	nd	nd	+	nd	nd	+[e], (+)[c]	nd	nd	d	nd	–	nd	nd	nd	nd	nd	+	nd
Propionate	nd	nd	nd	nd	nd	nd	–	nd	nd	nd	nd	nd	nd	nd	nd	–	nd	nd	nd	nd	–	nd	nd	nd	nd	nd	nd	nd
Putrescine	nd	nd	nd	nd	nd	nd	–	nd	nd	nd	nd	nd	nd	nd	nd	(+)	nd	nd	nd	nd	–	nd	nd	nd	nd	nd	nd	nd
Pyruvate	nd	nd	nd	nd	nd	nd	+	nd	nd	nd	nd	nd	nd	nd	nd	–	nd	nd	nd	nd	(+)	nd	nd	nd	nd	nd	nd	nd
Quinate	+	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	–	nd	nd	–	nd	nd	–	nd	nd	nd	nd	nd	nd	nd	+	nd
Raffinose	+[e,f], –[b]	+	–	–	–	+	nd	nd	–	d	–[b], +[g]	+	–	–	d	–	–	–[b], +[g]	–	d[b], –[h]	–[e], +[h]	–	–	–	nd	nd	–	–
L-Rhamnose	+	+	+	+	+	+	+	nd	+	d	+	+	–	+	d	+	+	–[b], +[g]	+	d[b], +[h]	+	+	–	–	nd	nd	+	–
D-Ribose	+	+	–	+	–	–	–	nd	nd	–	–[b], +[g]	d	–	–	d	+	nd	+	–	–	–	+	+	nd	–	–	+	+
Salicin	nd	+	nd	nd	nd	nd	(+)	nd	nd	nd	nd	nd	+	nd	nd	–	–	nd	nd	nd	+	nd	–	(+)	nd	nd	nd	–
L-Serine	nd	nd	nd	nd	nd	nd	+	nd	nd	nd	nd	nd	nd	nd	nd	–	nd	nd	nd	nd	–	nd	nd	nd	nd	nd	nd	nd
Sodium acetate	+	–	nd	–	nd	d	nd	nd	nd	d	–	d	nd	nd	d	–	nd	–	+	d	nd	–	–	nd	nd	nd	–	–
Sodium fumarate	+	–	nd	–	nd	d	nd	nd	nd	+	–	–	nd	nd	d	–	nd	–	+	–	nd	–	nd	nd	nd	nd	–	nd
Sodium succinate	+	–	nd	–	nd	d	nd	nd	nd	d	–	–	nd	nd	d	–	nd	–	–	–	nd	–	nd	nd	nd	nd	–	nd
D-Sorbitol	(+)[e], +[b]	+	+	+	nd	d	–	nd	nd	+	–	d	–	–	+	+[b], (+)[e], –[c]	nd	+	+[e], –[b]	+	–	+	+	nd	nd	nd	(+)[e], +[b]	+
Sorbose	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	–	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Spermine	–	nd	–	nd	nd	nd	nd	nd	nd	nd	nd	nd	+	nd	nd	–	nd	nd	–	nd	nd	nd	nd	nd	nd	nd	–	nd

(continued)

TABLE 195. (continued)

Characteristic	1. *A. philippinensis*	2. *A. auranticolor*	3. *A. brasiliensis*	4. *A. campanulatus*	5. *A. capillaceus*	6. *A. consettensis*	7. *A. couchii*	8. *A. cyaneus*	9. *A. deccanensis*	10. *A. derwentensis*	11. *A. digitatis*	12. *A. durhamensis*	13. *A. ferrugineus*	14. *A. globisporus*	15. *A. humidus*	16. *A. italicus*	17. *A. liguriensis*	18. *A. lobatus*	19. *A. missouriensis*	20. *A. palleronii*	21. *A. rectilineatus*	22. *A. regularis*	23. *A. sichuanensis*	24. *A. teichomyceticus*	25. *A. tereljensis*	26. *A. toevensis*	27. *A. utahensis*	28. *A. xinjiangensis*
Suberate	nd	nd	nd	nd	nd	nd	–	nd	nd	nd	nd	nd	nd	nd	nd	–	nd	nd	nd	nd	–	nd	nd	nd	nd	nd	nd	nd
Sucrose	+	+	+	+	(+)	nd	+	nd	+	nd	+	nd	–	+	nd	+	–	+	+	+	+[h], –[e,c]	+	+	+	nd	nd	+	+
Syringaldehyde	–	–	nd	–	nd	nd	nd	nd	nd	–	–	–	nd	nd	–	–	nd	–	–	–	–	–	nd	nd	nd	nd	–	nd
Syringic acid	–	–	nd	–	nd	d	nd	nd	nd	–	–	–	nd	nd	d	–	nd	–	–	–	–	nd	nd	nd	nd	nd	–	nd
Trehalose	nd	nd	nd	nd	nd	nd	+	nd	nd	nd	nd	nd	nd	nd	nd	+	nd	nd	nd	nd	+	nd	–	nd	nd	nd	nd	nd
Vanillin	+	–	nd	–	nd	–	nd	nd	nd	–	–	–	nd	nd	–	–	nd	+	–	nd	nd	–	nd	nd	nd	nd	–	nd
Xylitol	–	nd	+	nd	nd	nd	nd	nd	nd	nd	nd	nd	–	nd	nd	–	nd	nd	–	nd	nd	nd	nd	nd	nd	nd	–	nd
D-Xylose	+	+	+	+	+	+	+	nd	+	+	+	+	+	+	+	+	–	–[b], +[g]	+	+[b], –[h]	+	–[h], +[b,g]	+	–	+	+	+	+
Degradation of:																							nd		nd	nd		nd
Adenine	nd	–	nd	nd	–	nd	+	nd	nd	nd	nd	nd	nd	nd	nd	+	nd	–	nd	nd	+	nd	–	nd	–	–	nd	–
Calcium malate	+	nd	+	nd	nd	nd	nd	nd	+	nd	nd	nd	+	–	nd	–	–	nd	+	nd	nd	nd	nd	–	–	–	+	nd
Casein	+	+	+	+	+	+	+	nd	–	+	+[b], –[i]	+	+	+[e], (+)[f]	+	+	+	+	+	+	+	+	–	+	(+)	+	+	–
Cellulose	–[b], +[i]	–	nd	–	d	–	nd	nd	nd	–	–	–	nd	nd	–	nd	nd	–	–	–	nd	–[b], +[i]	nd	nd	nd	nd	–	nd
Chitin	+	–	nd	+	nd	d	nd	nd	nd	d	+	d	nd	nd	d	+	nd	+	+	d	nd	+	nd	nd	nd	nd	+	nd
DNA	d	+	nd	+	nd	d	nd	nd	nd	d	+	d	nd	nd	+	+	nd	+	+	–	nd	+	nd	nd	nd	nd	+	nd
Elastin	+	–	nd	–	nd	+	nd	nd	nd	+	+	d	nd	nd	d	–	nd	+	+	–	nd	–	–	nd	nd	nd	+	–
Esculin	nd	nd	nd	nd	+	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Gelatin	+	+[e], –[i]	+	+[b], –[g]	–	+	nd	nd	+	+	+[b], v[g]	+	+	–	+	+	+[h], –[e]	+	+[b], –[e]	+	+	+	+	+	–	+	+	+
Guanine	–	–	nd	–	–	–	nd	nd	nd	–	–	–	nd	nd	–	–	nd	–	–	–	nd	–	–	nd	nd	nd	–	–
Hypoxanthine	–	–	nd	–	–	–	+	nd	nd	–	–	–	nd	nd	–	+[c], –[b]	nd	–	–	–	+	–	–	nd	–	–	–	–
Lecithin	+	–	nd	+	nd	+	nd	nd	nd	d	+	+	nd	nd	+	+	nd	+	+	+	nd	–	nd	nd	nd	nd	+	nd
Pectin	+	+	nd	–	nd	d	nd	nd	nd	d	–	–	nd	nd	d	+	nd	+	+	d	nd	–	nd	nd	nd	nd	+	nd
RNA	+	+	nd	+	nd	+	nd	nd	nd	d	+	d	nd	nd	+	+	nd	+	+	d	nd	+	nd	nd	nd	nd	+	nd
Starch	+	+	+	+	+	+	+	nd	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	–	+	+	–
Tyrosine	+	–	–	–	nd	d	+	nd	nd	d	+[b], –[g]	d	+	nd	d	+	–	+[b], –[g]	+	d	+	–	+	–	(+)	(+)	–[b], +[e]	–
Xanthine	–	–	nd	–	–	–	+	nd	nd	–	–	–	nd	nd	–	–[b], +[c]	nd	–	–	–	+	–	–	nd	–	–	–	–
Xylan	nd	nd	nd	nd	nd	nd	+	nd	nd	nd	nd	nd	nd	nd	nd	+	nd	nd	nd	nd	+	nd	nd	nd	nd	nd	nd	nd
Hydrolysis of:[c]																												
Esculin	nd	nd	nd	nd	nd	nd	+	nd	nd	nd	nd	nd	nd	nd	nd	+	nd	nd	nd	nd	+	nd	+	nd	nd	nd	nd	+
L-Alanine pNA[l]	nd	nd	nd	nd	nd	nd	(+)	nd	nd	nd	nd	nd	nd	nd	nd	–	nd	nd	nd	nd	–	nd	nd	nd	nd	nd	nd	nd
Arbutin[b]	–	+	nd	+	nd	+	nd	nd	nd	+	+	+	nd	nd	+	+	nd	+	+	d	nd	+	nd	nd	–	–	+	nd
2-Deoxythymidine-5′-pNP phosphate[m]	nd	nd	nd	nd	nd	nd	(+)	nd	nd	nd	nd	nd	nd	nd	nd	–	nd	nd	nd	nd	–	nd	nd	nd	nd	nd	nd	nd
L-Glutamate-γ-3-carboxy pNA	nd	nd	nd	nd	nd	nd	–	nd	nd	nd	nd	nd	nd	nd	nd	–	nd	nd	nd	nd	–	nd	nd	nd	nd	nd	nd	nd
oNP β-D-glucopyranoside[n]	nd	nd	nd	nd	nd	nd	+	nd	nd	nd	nd	nd	nd	nd	nd	–	nd	nd	nd	nd	+	nd	nd	nd	nd	nd	nd	nd
pNP α-D-glucopyranoside	nd	nd	nd	nd	nd	nd	+	nd	nd	nd	nd	nd	nd	nd	nd	+	nd	nd	nd	nd	+	nd	nd	nd	nd	nd	nd	nd

pNP β-D-glucopyranoside	nd	nd	nd	nd	nd	nd	+	nd	nd	nd	nd	nd	nd	nd	nd	+	nd	nd	nd	nd	+	nd	nd	nd	nd	nd	nd	nd
pNP β-D-glucuronide	nd	nd	nd	nd	nd	nd	−	nd	nd	nd	nd	nd	nd	nd	nd	−	nd	nd	nd	nd	−	nd	nd	nd	nd	nd	nd	nd
pNP phenylphosphate	nd	nd	nd	nd	nd	nd	+	nd	nd	nd	nd	nd	nd	nd	nd	+	nd	nd	nd	nd	−	nd	nd	nd	nd	nd	nd	nd
bis-pNP phosphate	nd	nd	nd	nd	nd	nd	+	nd	nd	nd	nd	nd	nd	nd	nd	+	nd	nd	nd	nd	−	nd	nd	nd	nd	nd	nd	nd
pNP phosphorylcholine	nd	nd	nd	nd	nd	nd	+	nd	nd	nd	nd	nd	nd	nd	nd	(+)	nd	nd	nd	nd	−	nd	nd	nd	nd	nd	nd	nd
pNP β-D-xylopyranoside	nd	nd	nd	nd	nd	nd	+	nd	nd	nd	nd	nd	nd	nd	nd	+	nd	nd	nd	nd	+	nd	nd	nd	nd	nd	nd	nd
L-Proline pNA	nd	nd	nd	nd	nd	nd	−	nd	nd	nd	nd	nd	nd	nd	nd	−	nd	nd	nd	nd	−	nd	nd	nd	nd	nd	nd	nd
Enzymic activities (API test):[h]																												
N-Acetyl-β-glucosaminidase	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	+	nd	nd	+	+	−	+	−	+	+	nd	+
Acid phosphatase	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	+	nd	nd	+	+	+	+	+	+	+	nd	+
Alkaline phosphatase	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	+	nd	nd	+	+	−	+	+	(+)	+	nd	+
Arginine dihydrolase	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	−	nd	nd	−	+	+	nd	+	nd	nd	nd	nd
Chymotrypsin	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	+	nd	nd	−	+	+	nd	−	(+)	−	nd	nd
Citrate utilization	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	−	nd	nd	−	+	+	nd	−	nd	nd	nd	nd
Cystine arylamidase	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	−	nd	nd	−	+	−	nd	−	+	+	nd	nd
Esterase (C4)	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	+	nd	nd	+	+	−	nd	+	−	−	nd	nd
Esterase lipase (C8)	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	+	nd	nd	+	+	−	nd	+	(+)	(+)	nd	nd
α-Fucosidase	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	−	nd	nd	−	−	−	nd	−	+	+	nd	nd
α-Galactosidase	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	+	nd	nd	+	+	−	nd	−	−	−	nd	nd
β-Galactosidase	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	+	nd	nd	v	+	v	+	−	+	+	nd	+
α-Glucosidase	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	+	nd	nd	+	+	+	nd	−	−	−	nd	nd
β-Glucosidase	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	+	nd	nd	+	+	+	nd	−	+	+	nd	nd
β-Glucuronidase	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	+	nd	nd	−	−	−	nd	−	−	−	nd	nd
Indole production	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	−	nd	nd	−	−	−	nd	−	nd	nd	nd	nd
Leucine arylamidase	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	−	nd	nd	+	+	−	nd	−	−	−	nd	nd
Lipase (C14)	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	−	nd	nd	−	−	−	nd	−	−	−	nd	nd
Lysine decarboxylase	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	−	nd	nd	−	+	+	nd	+	nd	nd	nd	nd
α-Mannosidase	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	+	nd	nd	−	+	−	nd	−	−	+	nd	nd
Naphthol-AS-BI-phosphohydrolase	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	+	nd	nd	−	+	−	nd	+	(+)	+	nd	nd
Ornithine decarboxylase	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	−	nd	nd	−	−	+	nd	+	nd	nd	nd	nd
Trypsin	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	+	nd	nd	−	+	−	nd	−	−	−	nd	nd
Tryptophan deaminase	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	−	nd	nd	−	−	−	nd	−	nd	nd	nd	nd
Urease	+	−[b], +[j]	nd	+	nd	−	nd	nd	nd	d	−[b], +[j]	−	+	nd	d	+	+	−	+	−	+	+[h,j], −[b]	nd	+	nd	+	+	nd
Valine arylamidase	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	−	nd	nd	−	−	−	nd	−	−	−	nd	nd
Biochemical tests:																												
H_2S production	+	+	−	−	nd	d	nd	nd	−	d	−	−	−	−	d	+[e], −[b]	+[b], −[h,e]	nd	−	−	−	−	−	+[e], −[h]	nd	nd	+	+
Melanin formation	−	+	−	−	−	nd	nd	nd	+	nd	+	+	+	−	nd	+	−	−	−	nd	+	−	nd	+	nd	nd	+	+
Nitrate reduction	+	−	+	+	+	−	nd	nd	+	d	+	d	+	−	+	−[b], +[e,j]	−	+	+	d	−	+	+	+	+	−	+	+
Coagulation of milk	−	nd	−	+	d	nd	nd	nd	−	nd	+	nd	nd	−	nd	−	−	+	−	nd	+	+	nd	−	nd	nd	−	nd
Peptonization of milk	−	nd	−	+	d	nd	nd	nd	−	nd	+	nd	nd	−	nd	+	−	+	−	nd	+	−	nd	+	nd	nd	−	nd
Other physiological properties:																												
Growth at:																												
pH 4.0	−[b], +[k]	−	nd	−	nd	−	nd	nd	nd	−	−	−	+	nd	−	−[b], +[j]	nd	−	−	−	+	−	nd	nd	−	nd	−[b], +[j]	nd
pH 6.0	+	+	nd	+	nd	+	nd	nd	nd	d[b]	+	d[b]	+	nd	+	+	nd	+	+	−	+	+	−	nd	+	+	−[b], +[j]	−
pH 8.0	+	+	nd	+	nd	+	nd	nd	nd	d[b]	+	d[b]	+	nd	+	+	nd	+	+	+	+	+	+	nd	+	+	+	+
pH 10.0	−[b]	−[b], +[j]	nd	−[b], +[j]	nd	−	nd	nd	nd	−	−[b], +[j]	−	+	nd	−	−[b], +[j]	nd	−	−	−	+	−[b], +[j]	+	nd	−	+	−[b], +[j]	−

(continued)

TABLE 195. (continued)

Characteristic	1. *A. philippinensis*	2. *A. auranticolor*	3. *A. brasiliensis*	4. *A. campanulatus*	5. *A. capillaceus*	6. *A. consettensis*	7. *A. couchii*	8. *A. cyaneus*	9. *A. deccanensis*	10. *A. derwentensis*	11. *A. digitatis*	12. *A. durhamensis*	13. *A. ferrugineus*	14. *A. globisporus*	15. *A. humidus*	16. *A. italicus*	17. *A. liguriensis*	18. *A. lobatus*	19. *A. missouriensis*	20. *A. palleronii*	21. *A. rectilineatus*	22. *A. regularis*	23. *A. sichuanensis*	24. *A. teichomyceticus*	25. *A. tereljensis*	26. *A. toevensis*	27. *A. utahensis*	28. *A. xinjiangensis*
Tolerance to (%, w/v):																												
Sodium chloride (1.0)	+	−	nd	+	nd	+	nd	nd	nd	d	+	+	nd	nd	+	+	nd	−	+	+	nd	−	+	nd	+	+	−	+
Sodium chloride (2.0)	+	−	nd	+	nd	d	nd	nd	nd	−	+	+	nd	nd	−	−	nd	−	+	+	nd	−	+	nd	+	+	−	+
Sodium chloride (3.0)	−	−	nd	+	−	−	nd	nd	nd	−	−	−	nd	nd	−	−	nd	−	−	d	nd	−	+	nd	+	−	−	+

[a]Symbols: +, >85% positive; d, different strains give different reactions (16–84% positive); −, 0–15% positive; (+), weakly positive; w, weak reaction; nd, not determined.

[b]Data from Goodfellow et al. (1990a).

[c]Kämpfer et al. (2007).

[d]Matsumoto et al. (2000).

[e]Palleroni (1989).

[f]Thiemann (1967).

[g]Vobis and Kothe (1989).

[h]Wink et al. (2006).

[i]Schäfer (1973).

[j]Kothe (1987); deviations are indicated by superscript letters.

[k]For isomers of inositol see[b,c,d,e].

[l]pNA, *p*-nitroanilide.

[m]pNP, *p*-nitrophenyl.

[n]oNP, *o*-nitrophenyl.

The zoospores of *Actinoplanes* exhibit chemotactic properties. In *Actinoplanes brasiliensis*, Palleroni (1976) found bromide and chloride ions acting as attractants at a relatively high concentration (0.1 M). Addition of methionine stimulated this chemotactic effect, suggesting that protein methylation may be involved (Palleroni, 1983). Spores of *Actinoplanes missouriensis* were attracted by fungal conidia and sclerotia and to their respective exudates (Arora, 1986). Phototactic effects have not been observed, but an apparent microaerophilic behavior was seen in *Actinoplanes brasiliensis* (Palleroni, 1976).

Sporangiospores of *Actinoplanes brasiliensis* are able to germinate in a minimal medium with glucose as sole carbon source. The germination rate is nearly 100% (Palleroni, 1989). In contrast, *Actinoplanes rectilineatus* gave a much lower proportion of spores able to germinate under minimal conditions and normal levels of germination depended on the addition of amino acids. Germination could be inhibited by chloramphenicol and actinomycin D and, even though it was stimulated by 6-azauracil, this compound inhibited further growth (Higgins, 1967).

Ecology. While studying the distribution and abundance of *Actinoplanes* in soils of Japan, Nonomura and Takagi (1977) introduced the convenient term "actinoplanetes", which was accepted for further ecological investigations (Makkar and Cross, 1982). In a more extended sense, the name "Actinoplanetes" was used for a long time for a suprageneric group within actinomycete systematics (Goodfellow, 1989) until the genera belonging to it could be harbored in the well-defined taxon *Micromonosporaceae* (Goodfellow et al., 1990b). Now, the term actinoplanetes can be used again in its original meaning, characterizing an ecological group of actinomycetes.

Actinoplanetes are widely distributed in soil throughout the world (Couch, 1963; Parenti and Coronelli, 1979; Schäfer, 1973). Strains of *Actinoplanes* occur in all types of soil, in arid desert areas (Couch, 1957; Garrity et al., 1996; Makkar and Cross, 1982), in sand dune systems close to seashores (Palleroni, 1976), and in subtropical and tropical regions. In a large-scale investigation of the distribution of the actinoplanetes in soil in Japan, Nonomura and Takagi (1977) demonstrated a correlation between their abundance, the type of soil, its pH value, and the content of organic matter. Relatively few actinoplanetes occurred in soils with pH 4.0–5.0 and abundant organic matter content. Their number increased with lower humus content and a pH value between 6.4 and 7.2. Soils with a permanent high content of water (e.g. paddy rice fields) have no advantage compared with cultivated fields, which are dry for longer periods.

Strains of *Actinoplanes* can also colonize plant or animal debris (Cross, 1981b; Makkar and Cross, 1982). A frequent drying and wetting of the substrates increases their occurrence. Favored habitats are edges of ponds, drainage ditches, and barnyards (Shearer, 1987). Sediments of rivers are also a good source for the isolation of *Actinoplanes* strains (Goodfellow et al., 1990b). They occur frequently on twigs submerged in streams (Willoughby, 1971), muddy dead leaves that are caught and dried on branches of overhanging trees (Cross, 1981b), and on allochthonous leaf litter cast up on the shores of lakes (Willoughby, 1969b). In general, the sporangiate actinoplanetes can be considered as normal inhabitants of soil and leaf litter (Cross, 1981b), although they can also be isolated directly from lake or river water (Willoughby, 1969a, 1971).

After 20 years of experience, Couch (1963) concluded that about 66% of the soil samples collected from all over the world contain sporangiate actinomycetes. This is in accordance with the observations of Schäfer (1973), who isolated *Actinoplanes* strains from 56% of soil samples investigated. Similar results were also obtained from geographically limited regions like Japan (strains isolated from 75% samples; Nonomura and Takagi, 1977) or Argentina (strains isolated from 65% samples; Vobis, 1987).

The function of actinoplanetes in soil ecosystems is poorly known. With a behavior of typical saprophytic micro-organisms, abilities to degrade any kind of biological material may be possible. Chitin has been used as a carbon source for the isolation of strains by Makkar and Cross (1982). However, chitin degradation seems to be extremely slow and the greatest advantage using chitin media is the inhibition/decrease in growth of other micro-organisms (Willoughby, 1968). Degradation tests using chitin from insects and fungi gave negative results (Schäfer, 1973), which is in contrast to the positive results of Goodfellow et al. (1990b), using the basal medium of Gordon (1967) supplemented with 0.5% (w/v) chitin.

Because *Actinoplanes* strains exhibit good growth on xylose and arabinose, it is conceivable that they play a role in decomposing pentosans of plant origin (Parenti and Coronelli, 1979). It was assumed that strains of *Actinoplanes* cannot decompose cellulose, with the exception of *Actinoplanes brasiliensis* (Palleroni, 1989; Parenti and Coronelli, 1979), but recently, a study of saprophytic actinomycete strains associated with the root system of the actinorhizal plant *Ochetophila* (*Discaria*) *trinervis*, revealed that all 27 isolated *Actinoplanes* strains can degrade starch, cellulose, and pectin. Furthermore, a third of them were also able to decompose hemicellulose and/or colonized preferably thin sections of dead wood (Solans and Vobis, 2003). It could be demonstrated in plant growth assays that one of the most active *Actinoplanes* strains, BCRU-ME 3, promotes *Frankia* symbiosis of *Ochetophila trinervis* as well as *Sinorhizobium meliloti*/*Medicago sativa* symbiosis (Solans, 2007; Solans et al., 2009). This helper effect could be increased by coinoculations with strains of *Streptomyces* and *Micromonospora*, respectively, which were isolated from the rhizosphere of the same host plant (Solans, 2007). The actinomycetes involved produce phytohormones, which seem to play an important role in those interactions with higher plants (Solans, 2008). Presumably, single strains of *Actinoplanes* have a multiple function in terrestrial ecosystems, as shown in this example.

Enrichment and isolation procedures

All isolation methods stated below, with exception of the incubation technique, are based on the knowledge of morphological or physiological properties of the organisms. Three main aspects of their life cycle are utilized: the sporangia are dry-resistant survival units that can release motile spores when sufficiently wet and the spores are attracted by organic or inorganic chemical substances. It is recommended that freshly collected samples are dried at about 35°C. Well enclosed in a glass vessel or even a plastic bag, the air-dried probes are suitable for later investigations and can be stored for a long time, preferably at 4°C (Makkar and Cross, 1982; Nonomura and Takagi, 1977).

Baiting technique. The baiting technique is the traditional isolation method, which was originally designed to isolate water

molds and chytrids, but led to the discovery of actinomycetes with motile spores (Couch, 1949, 1950).

About 1 g soil is placed in a small sterilized Petri dish (3 or 4 cm in diameter), which is then covered with sterile water. After cautiously stirring, the particles settle to the bottom. Natural baits are placed singly or in combination on the surface of the water: e.g. pollen of *Pinus*, *Liquidambar*, or *Sparganium*, boiled *Paspalum* grass leaves or other material of biological origin (Bland and Couch, 1981; Couch, 1949, 1954; Makkar and Cross, 1982; Schäfer, 1973). A ring of Parafilm can be used to ensure the baits do not stick to the wall of the Petri dish (Hayakawa, 2003). Pollen grains of *Pinus* are the most recommended baits (Hayakawa, 2003; Palleroni, 1989). The baits must be presterilized, depending on their consistency, either chemically with ethanol or propylene dioxide or by autoclaving (Gaertner, 1955; Makkar and Cross, 1982; Schäfer, 1973). The enrichment cultures with floating baits are stored undisturbed at room temperature for several days or weeks. The water level can be regulated by addition of sterile distilled water. The examination for actinoplanetes is carried out with a dissecting microscope, preferably with ×100 magnification and horizontal lighting (Bland and Couch, 1981). Typical sporangia of *Actinoplanes* are recognizable as glistening beads on the air-exposed sides of the baits. Such baits are then removed carefully from the water and transferred to a 3% agar plate (Bland and Couch, 1981). Individual sporangia can be separated from the bait and rolled several centimeters over the surface of agar, using a micromanipulator or a thin-pointed tungsten needle, which has a tip curved like a hockey stick (Vobis, 1992). In this way, contaminants are removed from the sporangial surface. Cleaned sporangia are transferred onto a Petri dish with suitable agar media such as Czapek sucrose or peptone Czapek agars (Bland and Couch, 1981), half-concentrated Casamino acids-peptone Czapek agar (Schäfer, 1973), or Emerson's yeast extract-starch agar (Vobis, 1992). The colonies originating from the individual sporangia are visible to the naked eye after 1–4 weeks.

Makkar and Cross (1982) designed a special apparatus to apply an additional enrichment step. The colonized baits are transferred onto a Nucleopore polycarbonate membrane filter, washed with distilled water, and immediately dried at 28°C for 7 d. If this sporangia-containing material is resuspended and incubated in water, the fluid is highly enriched with motile spores, and is used for spreading onto agar plates.

Hayakawa et al. (1991c) enhanced the effect of releasing the spores by desiccating the sporangia-bearing baits for 2 h at 30°C in a mixture of fine soil particles and silica gel. These specially treated baits were then immersed in water and portions of the liquid, now enriched with zoospores, were plated on humic acid-vitamin agar.

Another convenient variation consists of making a wet mount with the colonized pollen grains in a drop of water on a slide and following the spore liberation microscopically. When this occurs, the liquid, enriched with swimming spores, can be used as an inoculum for isolation (Palleroni, 1989).

Dehydration-rehydration technique. This technique utilizes the ability of the sporangia to withstand desiccation and to release motile spores when they are subsequently in contact with water. Besides soil samples, it is also applicable to leaf litter, decaying plant material from aquatic habitats, organic debris, etc. (Makkar and Cross, 1982).

Samples are dried at 28–30°C for 7 d. For rehydration, 0.5 g soil or corresponding substrate is mixed with 50 ml sterile tap water in a 150 ml beaker or Erlenmeyer flask, which is covered with sterile aluminum foil (Shearer, 1987; Vettermann and Prauser, 1979). The suspension is incubated at 20–30°C for about 1 h. During the first 30 min, the vessel can be shaken at irregular intervals. After that, the particles should be permitted to settle. From the supernatant, 0.5–1.0 ml is removed with a sterile Pasteur pipette and spread onto agar plates (Shearer, 1987). If necessary, dilutions can be prepared from the inoculation suspension (Makkar and Cross, 1982). Soil extract agar or colloidal chitin agar containing cycloheximide and nystatin (Makkar and Cross, 1982), oatmeal-soil extract agar, or starch-casein-sulfate agar (Shearer, 1987) have been used in combination with this technique. Plates are incubated at 28°C for 2–4 weeks.

Rehydration and centrifugation method. Hayakawa et al. (2000) described an enrichment method incorporating differential centrifugation. Samples are rehydrated with 10 mM phosphate buffer containing 10% soil extract at 30°C for 90 min. The liquid enriched with zoospores is centrifuged at 1500 × *g* for 20 min. Portions of the supernatant are then plated on humic acid-vitamin agar supplemented with nalidixic acid and trimethoprim. The centrifugation procedure specifically eliminates strains of *Streptomyces* and other nonmotile actinomycetes (Hayakawa, 2003).

Chemotactic method. Investigations of the chemotactic behavior of the sporangiospores of *Actinoplanes brasiliensis* demonstrated, among other things, that they are attracted by chloride ions (Palleroni, 1976). This fact induced the same author to develop a new, very effective, and time-saving isolation method (Palleroni, 1980).

The essential tool of this technique is the isolation chamber, a sterilizable plastic block (80 × 40 × 12 mm) with two cylindrical wells (9 mm deep and 24 mm in diameter) with centers 32 mm apart. The wells are connected by a channel that is 2 mm wide and 3 mm deep (Palleroni, 1980).

The soil sample (1 g) is divided equally between the two compartments. Sterile water is added nearly to the rim. After incubation for approximately 1 h at 30°C, spores are released from the sporangia and move freely in the water. A sterile 1 µl glass capillary about 32 mm long, filled with 0.01 M phosphate buffer (pH 7.0), containing 0.01 M KCl as chemoattractant (Palleroni, 1980), is placed in the channel. Buffer and attractants may vary: phosphate buffer (5–10 mM, pH 6.8) and KCI (2 mM) (Palleroni, 1989), or colloidine (100 mM) (Hayakawa, 2003). The capillary must be submerged about 1 mm below the surface of the liquid. One hour after immersion of the capillary, sufficient spores are attracted and trapped in the lumen of the capillary, which is then removed and washed from the outside with sterile water. The contents of the capillary are blown into 1 ml sterile water or buffer. Portions of the spore suspension are taken with a sterile pipette and spread onto agar plates. The plates are then incubated at 28°C. Starch casein-sulfate agar is recommended as the isolation medium by Palleroni (1980) and humic acid-vitamin agar is recommended by Hayakawa (2003). Although colonies can be selected after 4 d, slowly growing actinoplanetes may only be detectable after 3 weeks.

Moist incubation procedure. This method is suitable for the direct detection of actinoplanetes on natural substrates.

Although the ability to produce motile spores obviously plays no role, *Actinoplanes* strains can be readily enriched. Willoughby (1968) emphasized the importance of selecting materials, such as plant residues found on the shores of lakes and streams, that are alternatively wetted and dried as the level of water rises and falls.

Portions of decaying leaves or other biological substrates, freshly collected from the field, are washed with sterile water to remove adhering detritus. They are placed in Petri dishes, the bottoms of which have been covered with moist filter paper or layers of cellulose before autoclaving. The Petri dishes, acting as humid chambers, are sealed and incubated for about 4 weeks at 25°C. Examination by both dissecting and light microscopy is necessary to identify the sporangia of the actinoplanetes (Willoughby, 1969b). The isolation media recommended by Willoughby (1968) include starch-casein agar and chitin agar.

Maintenance procedures

The traditional method for maintaining *Actinoplanes* strains is lyophilization. The experiments of Miller and Couch (1959) show that both the mycelium and the sporangia can survive the freeze-drying process. As pointed out by Palleroni (1989), actinoplanetes do not differ from other actinomycetes in their conservation requirements. Good results are also obtained by freezing concentrated spore suspensions in liquid media or in dilute phosphate buffer with 5% (v/v) glycerol and storing them at −20°C or in liquid nitrogen.

Differentiation of the genus *Actinoplanes* from other related genera and taxonomic comments

With the discovery of *Actinoplanes* strains, Couch (1949) demonstrated for the first time the existence of actinomycetes having a motile phase during their life cycle, represented by flagellated spores produced within sporangia. In addition, he confirmed the close affinity to the genus *Micromonospora*, correlated by colonial characters like production of substrate mycelium and absence of aerial mycelium (Couch, 1950). During the following decades, newly described or formerly known sporangia-forming genera with or without motility were classified either closer or more distantly related to *Actinoplanes* (Bland and Couch, 1981; Goodfellow and Cross, 1984; Palleroni, 1989; Vobis, 1989b). Considering chemotaxonomic properties, DNA pairing experiments, rRNA cistron similarity, and 16S rRNA oligonucleotide cataloging, the genera *Ampullariella* and *Amorphosporangium* were combined in a redefined genus *Actinoplanes* (Stackebrandt and Kroppenstedt, 1987). Supported by chemotaxonomic and numerical taxonomic studies, as well as 16S rRNA gene sequence-based phylogenetic clustering, other sporangiate genera were separated from the "*Micromonospora–Actinoplanes*–complex" and classified within other families (Goodfellow et al., 1990b; Miyadoh et al., 1997, 2001; Stackebrandt et al., 1997). This was the case for the genera *Acrocarpospora*, *Cryptosporangium*, *Kutzneria*, *Planobispora*, *Planomonospora*, *Planotetraspora*, *Sphaerisporangium*, *Spirillospora*, *Streptoalloteichus*, and *Streptosporangium*, and the genera with multilocular sporangia, *Dermatophilus*, *Frankia*, and *Geodermatophilus*.

The genera *Actinoplanes* and *Micromonospora* form the central part of the well-established family *Micromonosporaceae* (Goodfellow et al., 1990b), which can be distinguished from other suprageneric groups by 16S rRNA gene sequence similarity (Koch et al., 1996a). Each genus of the family *Micromonosporaceae* is characterized by distinctive morphological and/or chemotaxonomic features (Ara et al., 2008b). The morphological heterogeneity of spore-producing structures allows differentiation of the genus *Actinoplanes* from the other genera. Members of the *Micromonospora*, *Salinospora*, and *Verrucosispora* can be clearly separated morphologically by forming single, nonmotile spores on substrate hyphae. Species of *Actinocatenispora*, *Asanoa*, *Catellatospora*, *Catenuloplanes*, *Longispora*, *Polymorphospora*, and *Spirilliplanes* produce spores in chains on the surface of the substrate mycelium. The spores of *Catenuloplanes* and *Spirilliplanes* are motile, a feature in common with *Actinoplanes*, but they are not developed within sporangia. Morphologically more related may be the group comprising the genera *Couchioplanes*, *Krasilnikovia*, and *Pseudosporangium*, which form spore chains within pseudosporangium-like structures or pseudosporangia on substrate mycelium; spores are motile in the case of *Couchioplanes*, but not in *Pseudosporangium* or *Krasilnikovia*.

The genera *Dactylosporangium*, *Luedemannella*, *Pilimelia*, *Planosporangium*, and *Virgisporangium* appear morphologically much more related to *Actinoplanes* because they all develop real sporangia on the surface of the substrate mycelium. *Luedemanella* can be distinguished by the production of nonmotile sporangiospores. The genera *Dactylosporangium*, *Planosporangium*, and *Virgisporangium* form finger-like or rod-shaped, oligospore sporangia, with 2–6 or more motile spores per sporangium in a single row. *Pilimelia* is a genus characterized by multispored sporangia and motile sporangiospores, features in common with *Actinoplanes*. The spores of most *Actinoplanes* species are spherical, but in the case of the former "*Ampullariella*" species, they are also rod-like as in *Pilimelia* species. The distinguishing properties are the size of spores and the type of flagellation: the rod-like spores of *Actinoplanes* are 0.5–1.0 × 2.0–4.0 µm and have a polarly inserted tuft of flagella; and the rod-like spores of *Pilimelia* are 0.3–0.7 × 0.7–1.5 µm with a lateral tuft of flagella. Based on the type of flagellation, the mode of movement is completely different. The rod-like spores of *Actinoplanes* rotate around their longitudinal axis, whereas those of *Pilimelia* rotate around the lateral axis (Vobis, 1992).

Differentiation of species of the genus *Actinoplanes*

Morphological characters are easy to recognize, but the strains have to be cultured onto suitable agar media and investigated microscopically at the right time, e.g. on M3 agar of Rowbotham and Cross (1977), supplemented with 0.1% (w/v) fructose, after incubation for 14 d at 25°C (Goodfellow et al., 1990b). The artificial soil agar of Henssen and Schäfer (1971) is also recommended (Vobis, 1992). Sporangia are the joint characteristic structures, which can be used to limit groups of species or even species. The spores produced within spherical sporangia have in general a globose shape and are arranged in coiled chains. The observation "irregular arrangement" depends possibly on an advanced process of maturity. All species with globose spores belong to *Actinoplanes* in the original concept of Couch (1963). Sporangia shapes other than spherical are characterized by spore chains orientated in parallel and rod-like spores, which are characteristic for the former "*Ampullariella*" species (Vobis and Kothe, 1989). Within this group, *Actinoplanes capillaceus* stands out, with a hairy ornamentation on the surface of the sporangia, and *Actinoplanes rectilineatus*, with globose spores.

Another useful morphological marker is the presence of aerial mycelium, which is well developed in two species, *Actinoplanes rectilineatus* and *Actinoplanes regularis*, and observed in a rudimentary form in *Actinoplanes couchii*, *Actinoplanes ferrugineus*, and *Actinoplanes liguriensis* (Table 194). None of the other species produce aerial mycelium. Fragmentation of substrate mycelium is described only in *Actinoplanes couchii* (Kämpfer et al., 2007) and *Actinoplanes philippinensis* (Couch and Bland, 1974b).

In many cases, the different colors of the substrate mycelium maybe useful for the separation of species. Physiological conditions like the type of agar medium or the age of cultures are factors that can influence exact determinations. Additionally, zonal divisions of the colonies like radial sectors or central and marginal areas may cause differences. Using modified Bennett's agar, Goodfellow et al. (1990b) assigned a total of 163 isolates to eight color groups. In Table 194, the spectrum of colors of substrate mycelium is restricted to six main colors. Their combinations permit specification of a characteristic color for each species. The color white also comprises the variations ivory, creamish, or pale; yellow also represents buff, tawny, amber, and ochre. Orange is the most frequent color, with all shades like apricot or orange-red. Orange may lead to the variations of red, brick-like, cherry, coral, pink, scarlet, cinnabar, purple, and mahogany. The color brown includes cinnamon, chestnut, rufous or rusty. Black may be interpreted as a very dense concentration of many colors.

The soluble pigments are considered by Goodfellow et al. (1990b) as a character of possible diagnostic value to define *Actinoplanes* clusters at the 83% similarity level (S_{SM}). Because the production and/or the color variations depend on the culture media, RNA agar was recommended. The soluble pigments may influence the basic orange color of the substrate mycelium and then, in the case of overproduction, are diffused into the substrate (Parenti and Coronelli, 1979). In Table 194, the most common pigments are compiled for each species; e.g. the rare blue soluble pigment allows a rapid diagnosis for the species *Actinoplanes cyaneus*. Brown pigments are formed by many strains; however, it is not known if these pigments are melanoid. The production of melanin, regarded as a constant physiological feature for *Streptomyces* strains (Shirling and Gottlieb, 1966), is also used as a taxonomic character for *Actinoplanes* species. Nevertheless, Goodfellow et al. (1990b) could not obtain reproducible results. Additionally, Kothe (1987) demonstrated by absorption spectra that the type strains of *Actinoplanes philippinensis* and *Actinoplanes brasiliensis* characterized as melanin-negative organisms show the same peaks at 275 nm and 345 nm like in *Actinoplanes utahensis*, a typically melanin-positive species (Table 195). The feature of melanin production seems to have no diagnostic value.

The assimilation of different organic compounds as sole sources of carbon is used as a diagnostic feature to recognize species. A total of 86 compounds is listed in Table 195, some of which have a differentiating value (Goodfellow et al., 1990b; Kämpfer et al., 2007; Palleroni, 1989; Wink et al., 2006). However, more than 70% of the tests have not been determined. Enzymic activities, including degradation and hydrolysis of organic compounds, can be used to differentiate *Actinoplanes* species, but the list is also incomplete for many species (Table 195). Other physiological properties like pH and sodium chloride tolerances (Table 195), growth in the presence of organic and inorganic substances, and resistance to various antibiotics (Table 194) supply further diagnostic data. Tests for antimicrobial activities against micro-organisms may also be integrated into the large list of diagnostic characters (Table 194).

Among the chemotaxonomic features, the fatty acid profiles, menaquinone components, and polar lipid patterns show sufficient quantitative differences, which could be used in particular cases for separating species of *Actinoplanes* (Table 194).

Many species are described only on the basis of their corresponding type strain. In this case, the species description is identical with the characterization of a single strain in all phylogenetic and phenotypic aspects. The studies of Goodfellow et al. (1990b), Kothe (1987), Matsumoto et al. (2000), and Schäfer (1973) have shown that not all features that are characteristic for one strain have diagnostic value to differentiate between species, but allow intraspecific variations. In this sense, the concept of many species of the genus *Actinoplanes* seems to be still ill-defined (Palleroni, 1989).

List of species of the genus *Actinoplanes*

The following list is based upon J.P. Euzéby's "List of Prokaryotic names with Standing in Nomenclature – Genus *Actinoplanes*" (http://www.bacterio.cict.fr/a/actinoplanes.html).

1. **Actinoplanes philippinensis** Couch 1950, 89[AL]

phi.lip.pi.nen′sis. N.L. masc. adj. *philippinensis* of or pertaining to the Philippines.

Sporangia are spherical to subspherical, 8.8–25.0 µm in diameter (most are about 16 µm thick), produced on the surface of substrate mycelium by palisade hyphae. Spores are arranged in coils within the sporangia, globose, 1.0–1.2 µm in diameter, motile with a tuft of flagella. Substrate hyphae are 0.5–1.5 µm wide, branched, and sparingly septate. Conidia may be developed under certain circumstances.

Growth on Czapek agar is moderate; colonies are flat, or slightly elevated, light buff to tawny, occasionally changing to purplish brown with age. Hyphae in the upper layer are arranged in palisades with abundant production of sporangia. On peptone Czapek agar, growth is very good, surface of the colonies with concentric rings and radial grooves, formation of sporangia is very rare. Hyphae may fragment into spheres and rods. Color is apricot-orange to orange-chrome. Vigorous growth is observed on potato glucose agar, with the color at first near apricot-orange, then mahogany red, chestnut, or cinnamon-rufous. On nutrient agar, colonies are flat, slightly elevated in the center, color orange to cinnamon-rufous. A dark brown soluble pigment is produced on potato glucose and glycerine Czapek agars.

Produces macrocyclic lactone antibiotics.

Source: the type strain was isolated from rice paddy soil (the Philippines).

DNA G+C content (mol%): 72.1 (Bd).

Type strain: ATCC 12427, CBS 107.58, DSM 43019, NBRC 13878, HAMBI 1927, JCM 3001, NRRL 2506, RIA 468, UNCC P-15, VKM Ac-842.

Sequence accession no. (16S rRNA gene): D85474.

Additional comments: the type strain shows the highest sequence similarity to the following *Actinoplanes* species (based on ~1470 nt): *Actinoplanes sichuanensis* 03-723^{T}, 99.0%; *Actinoplanes capillaceus* NBRC 16408^{T}, 98.9%; *Actinoplanes campanulatus* NBRC 12511^{T}, 98.9%.

2. **Actinoplanes auranticolor** (Couch 1963) Stackebrandt and Kroppenstedt 1988, 328^{VP} (Effective publication: Stackebrandt and Kroppenstedt 1987, 113.) (*Amorphosporangium auranticolor* Couch 1963, 65)

au.ran′ti.co.lor. N.L. n. *aurantium* a bitter orange; L. n. *color* tint, hue; N.L. adj. *auranticolor* orange colored.

Sporangia are very irregular in shape, multilobed, 6.0–25.0 μm wide and 8.0–15.0 μm long. Spore chains are irregularly arranged within the sporangium. Sporangiophores may be branched. Spores are short rod-shaped, 0.5–0.7 × 1.0–1.5 μm. The original description by Couch (1963) considers the spores to be nonmotile, but Kane Hanton (1968) later reported motility by means of polar flagella. Nonmotile microconidia may be produced on tyrosine medium.

Growth on Czapek agar is very good and colonies are frequently elevated and convoluted with slippery surfaces and apricot-orange in color; colonies are scarlet on peptone Czapek agar. Good growth is also seen on oatmeal agar and on casein media, but poor growth is observed on tyrosine media. In all cases, colony colors are various shades of orange. A diffusible dark pigment is formed in tyrosine media whereas, in other media, a yellow diffusible pigment may be observed.

Source: the type strain and a further strain were isolated from soil collected from meadows (Nevada, USA).

DNA G+C content (mol%): 72 (T_m).

Type strain: ATCC 15330, DSM 43031, NBRC 12245, HAMBI 1975, JCM 3038, NRRL B-3343, UNCC 253, VKM Ac-648.

Sequence accession no. (16S rRNA gene): D85471.

Additional comments: the type strain shows highest sequence similarity to the following *Actinoplanes* species (based on ~1470 nt): *Actinoplanes missouriensis* NBRC 13243^{T}, 99.8%; *Actinoplanes cosettensis* NBRC 14913^{T}, 98.7%; *Actinoplanes digitatis* NBRC 12512^{T}, 98.7%; *Actinoplanes humidus* NBRC 14915^{T}, 98.7%.

3. **Actinoplanes brasiliensis** Thiemann, Beretta, Coronelli and Pagani 1969, 119^{AL}

bra.si.li.en′sis. N.L. masc. adj. *brasiliensis* of or pertaining to Brazil.

Sporangia are produced abundantly on soil extract agar, calcium malate agar, and starch-casein agar. They are umbraculiformis (umbrella shaped) to irregular or very occasionally globose, with very wrinkled surfaces, measuring at their widest from 3.5 to 11.5 μm. Spores vary from subspherical (1.2 μm) to rod-shaped (1.2 μm in width and 1.7–2.3 μm in length).

Good growth occurs on skim milk, glucose asparagine, and potato agars. Growth varies from very good to moderate on different ISP media (Shirling and Gottlieb, 1966). Colonies have smooth to wrinkled surfaces and vary in color from light pink, orange rose, and orange to deep orange. No aerial mycelium is produced.

Produces the acidic antibiotic A/672.

Source: the type strain was isolated from a soil sample (State Bahia, Brazil).

DNA G+C content (mol%): 70.5 (HPLC).

Type strain: ATCC 25844, DSM 43805, NBRC 13938, JCM 3196, NRRL B-16714, VKM Ac-1320.

Sequence accession no. (16S rRNA gene): D85470.

Additional comments: the type strain shows the highest sequence similarity to the following *Actinoplanes* species (based on ~1470 nt): *Actinoplanes deccanensis* NBRC 13994^{T}, 98.2%; *Actinoplanes digitatis* NBRC 12512^{T}, 98.0%.

4. **Actinoplanes campanulatus** (Couch 1963) Stackebrandt and Kroppenstedt 1988, 328^{VP} (Effective publication: Stackebrandt and Kroppenstedt 1987, 113.) ["*Ampullaria campanulata*" Couch 1963, 59; *Ampullariella campanulata* (Couch 1963) Couch 1964, 29]

cam.pa.nu.la′tus. N.L. dim. n. *campanella* small bell; L. masc. suff. *-atus* suffix denoting provided with; N.L. masc. adj. *campanulatus* bell-shaped.

Sporangia are characteristically bell shaped, frequently lobed or irregular, sometimes pyriform or cylindrical, and 5.0–15.0 × 6.0–12.0 μm. They often have a papillate appearance at the proximal end because the spore chains are of unequal length. Spore chains are arranged in parallel rows within the sporangia. The spores are rod-shaped (0.5–1.0 × 2.0–4.0 μm) and motile by a polarly inserted tuft of flagella.

Good growth occurs on Czapek, peptone Czapek, and casein agars and moderate growth occurs on tyrosine agar. Colonies are elevated and convoluted with ridged areas at the margin. The color of the substrate mycelium is coral-red, coral-pink, orange, brown, or black. Soluble yellowish, greenish, and brownish pigments may be produced on various media. Formation of sporangia occurs on Czapek agar. D-Mannitol is utilized by the type strain; inositol and raffinose are not utilized. Gelatin is not liquefied.

The iso- and anteiso- fatty acids are unsaturated.

Source: the type strain was isolated from soil (Douglas, Kansas, USA). Three further isolates were from Chapel Hill, North Carolina, USA, and four were from soils collected in Tahiti (Couch, 1963). Additionally, 17 strains were isolated from soil by D. Schäfer (Marburg, Germany) originating from: Kenya (2), Ceylon (4), Corsica (1), Mexico (2), Austria (1), Taiwan (1), Germany (1), South Africa (1), Italy (1), USA (2), and Turkey (1) (Kothe, 1987).

DNA G+C content (mol%): 71 (T_m).

Type strain: ATCC 15348, CBS 190.64, DSM 43148, NBRC 12511, JCM 3059, NRRL B-3344, UNCC 65, VKM Ac-1319.

Sequence accession no. (16S rRNA gene): AB036995.

Additional comments: the type strain shows the highest sequence similarity to the following *Actinoplanes* species (based on ~1470 nt): *Actinoplanes capillaceus* K95-5561^{T}, 99.9%; *Actinoplanes lobatus* NBRC 12513^{T}, 99.3%.

5. **Actinoplanes capillaceus** Matsumoto, Takahashi, Kudo, Seino, Iwai and Ōmura 2001, 793[VP] (Effective publication: Matsumoto, Takahashi, Kudo, Seino, Iwai and Ōmura 2000, 114.)

ca.pil.la′ce.us. L. masc. adj. *capillaceus* capillary, hairlike, referring to the hairy surface of the sporangium.

Sporangia are produced by substrate hyphae on short sporangiophores and are bell-shaped (5.0–10.0 × 5.0–15.0 μm). The surface of the sporangia is covered with short hair-like elements. Spores are oblong in shape (0.7–0.8 × 2.0–3.0 μm) and motile by means of a polar tuft of flagella.

Good growth occurs on inorganic salts-starch agar; colonies are brick-red in color. Colonies on yeast extract-starch agar are pink to yellow colored. No soluble or melanoid pigments are produced. No growth occurs in the presence of 3% (w/v) NaCl. Sensitive to streptomycin (50 μg/ml), but resistant to novobiocin (20 μg/ml).

Source: two strains, including the type strain, were isolated from a soil sample collected in Sayama City, Saitama Prefecture, Japan. Regarding the subjective synonym species, further three strains were isolated from soil (China) (Jiang and Ruan, 1982; Juan and Zhang, 1974).

DNA G+C content (mol%): 71.4 (HPLC).

Type strain: DSM 44859, NBRC 16408, JCM 10268, K95-5561, NBRC 16408.

Sequence accession no. (16S rRNA gene): AB013495.

Additional comments: the type strain shows the highest sequence similarity to the following *Actinoplanes* species (based on ~1470 nt): *Actinoplanes campanulatus*, NBRC 12511[T], 99.9%; *Actinoplanes lobatus*, NBRC 12513[T], 99.2%. Subjective synonyms (*sensu* Matsumoto et al., 2000) are: "*Ampullariella cylindrica*" Jiang and Ruan 1982 (type strains: JCM 3329, NBRC 14264); "*Ampullariella pekinesis*" Juan (Ruan) and Zhang 1974 (type strains: JCM 3174, NBRC 13662, DSM 46148); and "*Ampullariella pilifera*" Jiang and Ruan 1982 (type strains: JCM 3330, NBRC 14265).

6. **Actinoplanes consettensis** Goodfellow, Stanton, Simpson and Minnikin 1990a, 320[VP] (Effective publication: Goodfellow, Stanton, Simpson and Minnikin 1990b, 33.)

con.set.ten′sis. N.L. masc. adj. *consettensis* of or belonging to Consett, UK, a town near the site where the organism was isolated.

Sporangia are globose; spores are motile and arranged irregularly within sporangia.

Abundant growth occurs on modified Bennett's agar, developing light to yellow-brown substrate mycelium. Degrades arbutin, casein, DNA, elastin, lecithin, starch, and RNA. Adonitol, L-arabinose, dextrin, D-fructose, D-galactose, D-glucose, D-lactose, maltose, D-mannitol, D-mannose, methyl β-D-glucoside, raffinose, L-rhamnose, and D-xylose are utilized as sole carbon sources, but not cellobiose, erythritol, *myo*-inositol, methyl α-D-glucoside, D-ribose, catechol, *m*-, *o*- or *p*-hydroxybenzoic acid, syringaldehyde, or vanillin. Nitrate is not reduced and urea is not hydrolyzed. Tolerant to a number of antibiotics and chemical inhibitors. Grows between 4 and 30°C.

The peptidoglycan of the cell wall contains *meso*- and hydroxy-diaminopimelic acid. The principal wall sugars are arabinose, galactose, glucose, mannose, and xylose. The organism contains complex mixtures of straight- and branched-chain fatty acids, with hexadecanoic and 12-methyltetradecanoic acids predominating. Tetrahydrogenated menaquinones with nine isoprene units are the predominant isoprenologue, and major amounts of phosphatidylglycerol, phosphatidylethanolamine, phosphatidylglycerol, and phosphatidylinositol are present.

Source: the type strain and five further strains were isolated from sediments of the River Derwent at Allensford, County Durham, UK.

DNA G+C content (mol%): not known.

Type strain: ATCC 49799, DSM 43942, NBRC 14913, LA 97, NCIB (now NCIMB) 20027, NRRL B-16688.

Sequence accession no. (16S rRNA gene): AB036996.

Additional comments: the type strain shows the highest sequence similarity to the following *Actinoplanes* species (based on ~1470 nt): *Actinoplanes humidus* NBRC 14915[T], 100%; *Actinoplanes digitatis* NBRC 12512[T], 98.8%.

7. **Actinoplanes couchii** Kämpfer, Huber, Thummes, Grün-Wollny and Busse 2007, 722[VP]

cou′chi.i. N.L. gen. masc. n. *couchii* of Couch, named after J.N. Couch (1896–1986), who proposed the genus name *Actinoplanes* in 1950.

Sporangia are globose to oval. A rudimentary sterile aerial mycelium is formed. Substrate hyphae fragment easily in irregular rod-shaped cells.

Good growth occurs on nutrient agar and DSMZ medium 65 at 25–30°C. Color of the substrate mycelium is yellow-orange to orange-red. A red to brown soluble pigment develops on DSMZ medium 65. Starch, xylan, tyrosine, casein, hypoxanthine, adenine, and xanthine are degraded.

The quinone system consists of MK-9(H_4) (75%) as the predominant compound, with moderate amounts of MK-9(H_6) (11%), minor amounts of MK-9(H_2) (4%), MK-9(H_8) (2%), and MK-10(H_2) (2%), and traces of MK-10(H_6) (<0.1%). The polar lipid profile contains the major compounds diphosphatidylglycerol and phosphatidylethanolamine, but lacks phosphatidylcholine and aminoglycolipids. Additionally, moderate amounts of phosphatidylinositol, two unknown phospholipids, a highly hydrophilic glycolipid, and a polar lipid are detectable and traces of a single phosphatidylinositol mannoside are present.

Source: the type strain was isolated from soil close to the Marmore waterfalls, Terni, Italy.

DNA G+C content (mol%): not known.

Type strain: CIP 109316, DSM 45050, GW8-1761.

Sequence accession no. (16S rRNA gene): AM400230.

Additional comments: the type strain shows the highest sequence similarity to the following *Actinoplanes* species (based on ~1470 nt): *Actinoplanes italicus* NBRC 13911[T], 99.2%; *Actinoplanes rectilineatus* NBRC 13941[T], 98.9%.

8. **Actinoplanes cyaneus** Terekhova, Sadikova and Preobrazhenskaya 1987, 179[VP] (Effective publication: Terekhova, Sadikova and Preobrazhenskaya 1977, 1059.)

cy.a.ne′us. L. masc. adj. *cyaneus* dark blue.

Spherical sporangia with diameters of 30–60 μm are formed by substrate mycelium, sporangiospores are globose

(1.2–1.4 µm) and motile. Aerial mycelium is not developed. On synthetic media, a soluble blue pigment is produced, belonging to the chemical group of celicomycin-actinorodine.

Source: the type strain was isolated from soil (Siberia, Russia).

DNA G+C content (mol%): not known.

Type strain: DSM 46137, NBRC 14990, INA 1569, JCM 9082, VKM Ac-1095.

Sequence accession no. (16S rRNA gene): AB036997.

Additional comments: the type strain shows the highest sequence similarity to the following *Actinoplanes* species (based on ~1470 nt): *Actinoplanes rectilineatus* NBRC 13941^{T}, 98.4%; *Actinoplanes italicus* NBRC 13911^{T}, 98.3%.

9. **Actinoplanes deccanensis** Parenti, Pagani and Beretta 1975, 248^{AL}

dec.ca.nen′sis. N.L. masc. adj. *deccanensis* of or pertaining to the Indian locality of Decca.

Sporangia are globose, small with irregular surfaces (4.0–7.0 µm in diameter), produced on hyphae of substrate mycelium, especially when grown on soil extract agar. Spores are subspherical, 1.0 × 1.5 µm in diameter, and motile. Soil extract agar promotes formation of sporangia. Hyphae of substrate mycelium are branched and about 1.0 µm in diameter. Aerial mycelium is not formed.

Growth is abundant on ISP agar media 2, 4, and 7 (Shirling and Gottlieb, 1966), and on other media like oatmeal, potato, Hickey–Tresner, Bennett's, or skim milk agars. Surfaces of the colonies are wrinkled, crusty, or rough. Color varies from light amber, light orange, light orange pinkish to orange. No growth on calcium malate agar. On tyrosine agar, a brown diffusible pigment is produced. Thermotolerant, grows between 26 and 42°C.

Produces the antibiotic lipiarmycin.

Source: the type strain was isolated from soil (Decca, India).

DNA G+C content (mol%): 72.0 (HPLC).

Type strain: A/10655, ATCC 21983, DSM 43806, NBRC 13994, JCM 3247, NRRL B-16715.

Sequence accession no. (16S rRNA gene): AB036998.

Additional comments: the type strain shows the highest sequence similarity to the following *Actinoplanes* species (based on ~1470 nt): *Actinoplanes missouriensis* NBRC 13243^{T}, 98.5%; *Actinoplanes digitatis* NBRC 12512^{T}, 98.3%.

10. **Actinoplanes derwentensis** Goodfellow, Stanton, Simpson and Minnikin 1990a, 320^{VP} (Effective publication: Goodfellow, Stanton, Simpson and Minnikin 1990b, 33.)

der.wen.ten′sis. N.L. masc. adj. *derwentensis* of or pertaining to The Derwent, a river in County Durham, England, from which the organism was isolated.

Globose sporangia are readily produced; spores are motile and arranged irregularly within sporangia.

Color of substrate mycelium is orange to dark orange on modified Bennett's agar. Degrades arbutin, casein, chitin, DNA, elastin, lecithin, RNA, and starch; utilizes adonitol, L-arabinose, dextrin, D-fructose, D-galactose, D-glucose, *myo*-inositol, D-lactose, maltose, D-mannitol, D-mannose, methyl β-D-glucoside, D-xylose, and sodium fumarate as sole carbon sources but not methyl α-D-glucoside, D-ribose, catechol, coumarin, *m*-, *o*- or *p*-hydroxybenzoic acid, syringaldehyde, syringic acid, or vanillin. Grows between 4 and 30°C.

The peptidoglycan of the cell walls contains *meso*- and hydroxy-diaminopimelic acid. The principal wall sugars are arabinose, galactose, glucose, mannose, and xylose. The organism contains complex mixtures of straight- and branched-chain fatty acids, with hexadecanoic, 14-methylpentadecanoic, and 14-methylhexadecanoic acids predominating. Tetrahydrogenated menaquinones with nine isoprene units are the predominant isoprenologue, and major amounts of diphosphatidylglycerol, phosphatidylethanolamine, phosphatidylglycerol, and phosphatidylinositol are present.

Source: the type strain and seven further strains were isolated from sediments of the River Derwent, Allensford, Durham, UK.

DNA G+C content (mol%): not known.

Type strain: ATCC 49798, DSM 43941, NBRC 14935, JCM 7556, LA 107, NCIB (now NCIMB) 12875, NRRL B-16692.

Sequence accession no. (16S rRNA gene): AB036999.

Additional comments: the type strain shows the highest sequence similarity to the following *Actinoplanes* species (based on ~1470 nt): *Actinoplanes couchii* GW8-1761^{T}, 98.6%; *Actinoplanes rectilineatus* NBRC 13941^{T}, 98.5%; *Actinoplanes utahensis* NBRC 13244^{T}, 98.5%.

11. **Actinoplanes digitatis** (Couch 1963) Stackebrandt and Kroppenstedt 1988, 328^{VP} (Effective publication: Stackebrandt and Kroppenstedt 1987, 113.) ["*Ampullaria digitata*" Couch 1963, 61; *Ampullariella digitata* (Couch 1963) Couch 1964, 29]

di.gi.ta′tis. L. masc. adj. *digitatis* (*sic*) having fingers.

Sporangia are usually digitate, sometimes subcylindrical or lobed, rarely bottle shaped, and 3.0–9.0 × 6.0–14.0 µm. Spore chains are arranged in parallel rows within the sporangia. Formation of sporangia occurs on Czapek agar. The motile spores are rod-shaped (0.5–1.0 × 2.0–4.0 µm).

Good growth occurs on Czapek, peptone Czapek, and tyrosine agars. Colonies are flat, wrinkled, or convoluted, and frequently ridged. The margin is sometimes fimbriate. The color of substrate mycelium is pinkish cinnamon or red-cinnamon to blackish brown, sometimes red with black sectors or very dark with red sectors, flesh-colored, or dirty buff. The marginal areas are usually lighter in color. Soluble yellowish, greenish, and brownish pigments may be produced on various media.

Inositol and raffinose are utilized, but D-mannitol is not utilized. Melanoid pigments are produced.

Source: the type strain was isolated from soil (Sheboygan, Michigan, USA); two further strains from soil were collected in Parfrey's Glen, Wisconsin, USA, and from garden soil (Highfield Cresent, Hindhead, UK). D. Schäfer (Marburg, Germany) isolated a total of 29 strains from soil samples originating from: Germany (11), Corsica (1), Portugal (3), Austria (3), Uruguay (1), England (2), Switzerland (3), Italy (3), Sardinia (1), and the Azores (1) (Kothe, 1987).

DNA G+C content (mol%): 73.0 (T_m).

Type strain: ATCC 15349, DSM 43149, NBRC 12512, JCM 3060, NRRL B-3345, VKM Ac-649.

Sequence accession no. (16S rRNA gene): AB037000.

Additional comments: the type strain shows the highest sequence similarity to the following *Actinoplanes* species (based on ~1470 nt): *Actinoplanes cosettensis* NBRC 14913^T, 98.8%; *Actinoplanes humidus* NBRC 14915^T, 98.8%; *Actinoplanes auranticolor* NBRC 12245^T, 98.7%.

12. **Actinoplanes durhamensis** Goodfellow, Stanton, Simpson and Minnikin 1990a, 320^{VP} (Effective publication: Goodfellow, Stanton, Simpson and Minnikin 1990b, 33.)

dur.ham.en′sis. N.L. masc. adj. *durhamensis* of or belonging to Durham, a city in the north-east of England.

Sporangia are globose; sporogenous hyphae are arranged irregularly within sporangia. Spores are motile.

Light to dark orange substrate mycelium is formed on modified Bennett's agar. Melanin is produced on ISP 7 medium.

Arbutin, casein, lecithin, and starch are degraded; L-arabinose, dextrin, D-fructose, D-galactose, D-glucose, *myo*-inositol, D-lactose, maltose, D-mannitol, D-mannose, melezitose, methyl β-D-glucoside, raffinose, L-rhamnose, D-sorbitol, D-xylose, and *p*-hydroxybenzoic acid are used as sole carbon sources, but not cellobiose, erythritol, catechol, coumarin, *o*-hydroxybenzoic acid, sodium fumarate, sodium succinate, syringaldehyde, syringic acid, or vanillin. Hydrogen sulfide and urease are not formed. Sensitive to a number of antibiotics and chemical inhibitors. Shows activity against *Staphylococcus aureus*. Grows between 4 and 30°C, but not at 37°C.

The peptidoglycan contains *meso*- and hydroxy-diaminopimelic acid. The principal cell-wall sugars are arabinose, galactose, glucose, mannose, and xylose. The organism contains a mixture of straight- and branched-chain fatty acids, with octadecanoic, 14-methylpentadecanoic, and 14-methylhexadecanoic acids predominating. Tetrahydrogenated menaquinones with nine isoprene units are the predominant isoprenologue, and major amounts of diphosphatidylglycerol, phosphatidylethanolamine, phosphatidylglycerol, and phosphatidylinositol are present.

Source: the type strain and four further strains were isolated from sediments of the River Derwent, Allensford, Durham, UK.

DNA G+C content (mol%): 70.8 (HPLC).

Type strain: ATCC 49800, DSM 43939, NBRC 14914, JCM 7625, LA 139, NCIB (NCIMB) 20041, NRRL B-16689.

Sequence accession no. (16S rRNA gene): AB037001.

Additional comments: the type strain shows the highest sequence similarity to the following *Actinoplanes* species (based on ~1470 nt): *Actinoplanes ferrugineus* NBRC 15555^T, 98.3%; *Actinoplanes digitatis* NBRC 12512^T, 97.7%.

13. **Actinoplanes ferrugineus** Palleroni 1979, 55^{AL}

fer.ru.gi′ne.us. L. masc. adj. *ferrugineus* of the color of iron rust.

Sporangia are globose to irregular, 4.0–12.0 µm in diameter, and develop on the surface of substrate mycelium. Abundant production of sporangia is observed on minimal media supplemented with single carbon sources like L-rhamnose, D-galactose, or D-fructose. Spores are spherical (0.9–1.0 µm), motile by means of a tuft of flagella. Rudimentary aerial mycelium may be formed on ISP medium 7.

Abundant growth occurs on various ISP agar media, Czapek-glucose, and potato-glucose agars. Colonies have flat to wrinkled surface, elevated in the center, and edges penetrating into the agar. Colors vary from amber to deep brown. On ISP 7 agar medium, a reddish-brown soluble pigment is produced.

Produces the proline analog L-azetidine-2-carboxylic acid.

Source: the type strain was isolated from a red soil sample collected at Dorrigo Mountain, Australia.

DNA G+C content (mol%): 70.5 (HPLC).

Type strain: ATCC 29868, DSM 43807, NBRC 15555, JCM 3277, NRRL B-16718, X-14695.

Sequence accession no. (16S rRNA gene): AB037002.

Additional comments: the type strain shows the highest sequence similarity to the following *Actinoplanes* species (based on ~1470 nt): *Actinoplanes tereljensis* MN07-A0371^T, 98.6; *Actinoplanes toevensis* MN07-A0368^T, 98.6%.

14. **Actinoplanes globisporus** (Thiemann 1967) Stackebrandt and Kroppenstedt 1988, 328^{VP} (Effectively published: Stackebrandt and Kroppenstedt 1987, 113.) (*Amorphosporangium globisporus* Thiemann 1967, 239)

glo.bi.spo′rus. L. n. *globus* a ball, sphere; Gr. n. *spora* a seed, and in biology a spore; N.L. masc. adj. *globisporus* round spored.

Sporangia (4.0–7.0 µm wide and 3.0–5.0 µm long) develop on short sporangiophores directly from the substrate mycelium, highly irregular in shape, resembling masses of spores not surrounded by a sporangial envelope. Spores are spherical, 0.8–1.0 µm, and motile (*fide* Palleroni, 1989). Production of chlamydospore-like structures is quite frequent.

Colors of the colonies vary from ivory, creamish, pale orange to orange.

Good growth occurs on Bennett's and Hickey–Tresner agars; moderate growth occurs on glucose asparagine, glycerol asparagine, oatmeal, starch, tyrosine, skim milk, and calcium malate agars. No growth is observed on Czapek, peptone iron, or cellulose agars. Grows on synthetic media only if supplemented with vitamin solution.

Source: the type strain was isolated from soil (Appiano Gentile, Como, Italy).

DNA G+C content (mol%): 70.2 (HPLC).

Type strain: ATCC 23056, DSM 43857, NBRC 13912, JCM 3186.

Sequence accession no. (16S rRNA gene): AB037003.

Additional comments: the type strain shows the highest sequence similarity to the following *Actinoplanes* species (based on ~1470 nt): *Actinoplanes deccanensis* NBRC 13994^T, 96.9%; *Actinoplanes ferrugineus* NBRC 15555^T, 96.5%.

15. **Actinoplanes humidus** Goodfellow, Stanton, Simpson and Minnikin 1990a, 320^{VP} (Effective publication: Goodfellow, Stanton, Simpson and Minnikin 1990b, 34.)

hu′mi.dus. L. masc. adj. *humidus* moist, damp, wet.

Abundant production of spherical sporangia; sporogenous hyphae are arranged irregularly within sporangia. Spores are motile.

Light to yellow-orange brown substrate mycelium is formed on modified Bennett's agar and a dark diffusible

pigment is produced on Bacto Tryptic soy agar supplemented with RNA.

Degrades arbutin, casein, DNA, elastin, lecithin, pectin, RNA, starch, and tyrosine. Utilizes D- and L-arabinose, dextrin, D-fructose, D-galactose, D-glucose, D-lactose, maltose, D-mannitol, D-mannose, methyl β-D-glucoside, D-sorbitol, D-xylose, *p*-hydroxybenzoic acid, sodium fumarate, and sodium succinate as sole carbon sources, but not cellobiose, erythritol, *myo*-inositol, catechol, coumarin, *m*- and *o*-hydroxybenzoic acid, syringaldehyde, syringic acid, or vanillin. Nitrate is reduced. Tolerant to a range of antibiotics and chemical inhibitors. Grows at 4 and 30°C, but not at 37°C.

Peptidoglycan contains *meso*- and hydroxy-diaminopimelic acid. The main cell-wall sugars are arabinose, galactose, mannose, and xylose. Contains complex mixtures of straight- and branched-chain fatty acids, with straight-chain components predominating. Tetrahydrogenated menaquinones with nine isoprene units are the predominant isoprenologue, and major amounts of diphosphatidylglycerol, phosphatidylethanolamine, phosphatidylglycerol, phosphatidylinositol, and two uncharacterized glycolipids are present.

Source: the type strain and eight further strains were isolated from sediments of the River Derwent (Allensford, Durham, UK).

DNA G+C content (mol%): not known.

Type strain: ATCC 49801, DSM 43938, NBRC 14915, JCM 7555, LA 6, NCIB (now NCIMB) 20000, NRRL B-16690.

Sequence accession no. (16S rRNA gene): AB037004.

Additional comments: the type strain shows the highest sequence similarity to following *Actinoplanes* species (based on ~1470 nt): *Actinoplanes cosettensis* NBRC 14913^T, 100.0%; *Actinoplanes digitatis* NBRC 12512^T, 98.8%.

16. **Actinoplanes italicus** Beretta 1973, 42^{AL}

i.ta′li.cus. L. masc. adj. *italicus* of or pertaining to Italy.

Sporangia are produced abundantly on starch (ISP 4) and skim milk agars, supported by straight sporangiophores arising from substrate mycelium. They are irregular in shape, varying from globose to oval and pyriform, 6.0–11.0 μm in diameter. Sporangiospores are spherical to oval, with a diameter of 1.0–2.0 μm, and highly motile.

Colonies on starch agar have a smooth surface and a dome-shaped center. Cherry-red substrate mycelium is produced on most media, but on ISP 2 and ISP 7 media (Shirling and Gottlieb, 1966), the color is orange; on Hickey–Tresner, Bennett's and nutrient agars, colonies are amber-brown to brown.

Production of a cherry-red soluble pigment is seen on oatmeal and starch agars; colors vary on other media from yellowish pink, rose, and amber to deep orange yellow. No pigment is produced on Bennett's, nutrient, or calcium-malate agar media.

Source: the type strain was isolated from a soil sample collected in an orchard at Pontelongo, Italy.

DNA G+C content (mol%): not known.

Type strain: A 5221, ATCC 27366, DSM 43146, NBRC 13911, JCM 3165, NRRL B-16722.

Sequence accession no. (16S rRNA gene): AB037005.

Additional comments: the type strain shows the highest sequence similarity to the following *Actinoplanes* species (based on ~1470 nt): *Actinoplanes couchii* GW8-1761^T, 99.2%; *Actinoplanes rectilineatus* NBRC 13941^T, 98.6%.

17. **Actinoplanes liguriensis** Wink, Kroppenstedt, Schuhmann, Seibert and Stackebrandt 2006, 2128^{VP} ("*Actinoplanes liguriae*" Parenti, Pagani and Beretta 1976, 505)

li.gu.ri.en′sis. N.L. masc. adj. *liguriensis* of or pertaining to the Italian region of Liguria.

Sporangia are globose to oval, 15.0–25.0 μm in diameter. Spore chains are coiled within the sporangia. Spherical motile spores (1.5–2.0 μm) with smooth surfaces are found. A rudimentary aerial mycelium is formed on ISP 6 medium. Abundant growth occurs on various agar media. Color of substrate mycelium is yellow-orange on ISP media 2, 3, 4, 5, 6, and 7. A yellow soluble pigment is produced on ISP media 4 and 5, and a red one on ISP 7.

Peptidoglycan contains *meso*-diaminopimelic acid; diagnostic sugars are xylose and arabinose. MK-9(H_4) and MK-10(H_4) are the principal menaquinones. The major phospholipid is phosphatidylethanolamine.

Produces the antibiotics gardimycin (Parenti and Coronelli, 1979) and Ala (*O*)-actagardine (Vértesy et al., 1999).

Source: the type strain was isolated from garden soil, Liguria region, Italy (Parenti et al., 1976).

DNA G+C content (mol%): not known.

Type strain: A/6353, ATCC 31048, BCRC 12121, CBS 355.75, DSM 43865, FH 2244, JCM 3250, KCC A-0250, NRRL B-16723.

Sequence accession no. (16S rRNA gene): AB047497.

Additional comments: the type strain shows the highest sequence similarity to the following *Actinoplanes* species (based on ~1470 nt): *Actinoplanes rectilineatus* NBRC 13941^T, 98.7%; *Actinoplanes regularis* NBRC 12514^T, 98.7%; *Actinoplanes palleronii* NBRC 14916^T, 98.5%.

18. **Actinoplanes lobatus** (Couch 1963) Stackebrandt and Kroppenstedt 1988, 328^{VP} (Effective publication: Stackebrandt and Kroppenstedt 1987, 113.) ["*Ampullaria lobata*" Couch 1963, 59; *Ampullariella lobata* (Couch 1963) Couch 1964, 29]

lo′ba.tus. N.L. masc. adj. *lobatus* lobed.

Strains have sporangia that vary in size and shape, are typically lobed and/or irregular, sometimes cylindrical, and are 4.0–20.0 × 12.0–23.0 μm. They often have a papillate appearance at the proximal end because of the varying length of the spore chains, which are arranged in parallel rows within the sporangia. The lobed sporangia sometimes are divided in several parts, giving the appearance of fused sporangia. Formation of sporangia occurs only on Czapek agar. The motile spores are rod-shaped (0.5–1.0 × 2.0–4.0 μm). Oval moniliform conidia may also be produced.

Colonies are up to 25 mm in diameter on Czapek agar or peptone Czapek agar and up to 10 mm on casein or tyrosine agars after 6 weeks' incubation. They are flattish or convoluted, and frequently ridged. The color of the substrate mycelium varies from coral red to dragon's blood red, jasper red, coral red, and brick red. Albino cultures may occur. On casein agar, the color is light coral red to

ferruginous. Soluble pale yellowish green pigments are produced on Czapek agar. Casein and tyrosine agars are slightly darkened. D-Mannitol and raffinose are utilized, but not inositol. Gelatin is liquefied.

Source: the type strain and a further strain were isolated from soil samples collected at Madison, Wisconsin, USA. Another isolate originated from pasture near Charleston, West Virginia, USA.

DNA G+C content (mol%): not known.

Type strain: ATCC 15350, DSM 43150, NBRC 12513, JCM 3061, NRRL B-3346, VKM Ac-676.

Sequence accession no. (16S rRNA gene): AB037006.

Additional comments: the type strain shows the highest sequence similarity to the following *Actinoplanes* species (based on ~1470 nt): *Actinoplanes sichuanensis* 03-723^{T}, 99.5%; *Actinoplanes campanulatus* NBRC 12511^{T}, 99.3%.

19. **Actinoplanes missouriensis** Couch 1963, 69^{AL}

mis.sou.ri.en′sis. N.L. masc. adj. *missouriensis* of or pertaining to the state of Missouri.

Sporangia are subglobose to globose, sometimes irregular, 6.0–14.0 µm in diameter. Spores are arranged in irregular coils within the sporangia, subspherical (1.0–1.2 µm), weakly motile by a tuft of polarly inserted flagella. Hyphae are less than 1.0 µm in diameter and branched. Production of palisade hyphae is rare.

Growth on Czapek agar is very good; colonies have an elevated center and flat border. Color is mostly ochraceous salmon, with whitish areas on the surface, where sporangia are abundant. Also, very good growth is observed on peptone Czapek agar (color zinc-orange to ochraceous orange). Production of sporangia is restricted to limited areas.

Produces the antibiotic 5-azacytidine.

Source: the type strain was isolated from soil collected near Hamilton, Missouri, USA. Members of the species also appeared in ten soil collections from the Mississippi Valley to the West Coast, USA.

DNA G+C content (mol%): not known.

Type strain: ATCC 14538, DSM 43046, NBRC 13243, JCM 3121, NBRC 102363, NRRL B-3342, UNCC 431.

Sequence accession no. (16S rRNA gene): AB037008.

Additional comments: the type strain shows the highest sequence similarity to the following *Actinoplanes* species (based on ~1470 nt): *Actinoplanes auranticolor* NBRC 12245^{T}, 99.2%; *Actinoplanes cosettensis* NBRC 14913^{T}, 98.6%; *Actinoplanes digitatis* NBRC 12512^{T}, 98.6%; *Actinoplanes humidus* NBRC 14915^{T}, 98.6%.

20. **Actinoplanes palleronii** Goodfellow, Stanton, Simpson and Minnikin 1990a, 320^{VP} (Effective publication: Goodfellow, Stanton, Simpson and Minnikin 1990b, 34.)

pal.le.ro′ni.i. N.L. gen. masc. n. *palleronii* of Palleroni, named after Noberto Palleroni, a distinguished taxonomist.

Sporangia are spherical; sporogenous hyphae are arranged irregularly within the sporangia. Spores are motile.

Light to yellow-brown substrate mycelium is formed on modified Bennett's agar. Melanoid pigments are produced on ISP 7 medium. Degrades arbutin, casein, lecithin, pectin, and starch; utilizes D-fructose, D-glucose, maltose, D-mannose, and D-xylose as sole carbon sources, but not D-arabinose, cellobiose, dextrin, erythritol, D-galactose, methyl β-D-glucoside, D-ribose, D-sorbitol, catechol, coumarin, *m*-, *o*- or *p*-hydroxybenzoic acid, sodium acetate, sodium fumarate, sodium succinate, syringaldehyde, syringic acid, or vanillin. Hydrogen sulfide and urease are not formed. Sensitive to potassium tellurite (0.005%, w/v), gentamicin sulfate (0.5%, w/v), and methacycline hypochloride (0.25%, w/v). Grows between 4 and 30°C, but not at 37°C.

The peptidoglycan contains *meso*- and hydroxy-diaminopimelic acid. The principal wall sugars are arabinose, galactose, glucose, rhamnose, ribose, and xylose. Contains complex mixtures of straight- and branched-chain fatty acids, with 12-methyltetradecanoic acid predominating. Tetrahydrogenated menaquinones with nine isoprene units are the predominant isoprenologue, and major amounts of diphosphatidylglycerol, phosphatidylethanolamine, phosphatidylglycerol, and phosphatidylinositol are present.

Source: the type strain and five further strains were isolated from sediments of the River Derwent at Allensford, Durham, UK.

DNA G+C content (mol%): not known.

Type strain: ATCC 49797, DSM 43940, NBRC 14916, JCM 7626, LA 83, NCIB (now NCIMB) 20021, NRRL B-16691.

Sequence accession no. (16S rRNA gene): AB037009.

Additional comments: the type strain shows the highest sequence similarity to the following *Actinoplanes* species (based on ~1470 nt): *Actinoplanes regularis* NBRC 12514^{T}, 99.4%; *Actinoplanes rectilineatus* NBRC 13941^{T}, 99.0%.

21. **Actinoplanes rectilineatus** Lechevalier and Lechevalier 1975, 371^{AL}

rec.ti.li.ne.a′tus. L. adj. *rectus* straight, upright; L. adj. *lineatus* striped, marked by fine parallel lines; N.L. masc. adj. *rectilineatus* marked with straight lines.

Sporangia are cylindrical, 8.0–15.0 µm, containing straight, longitudinally arranged rows of spores. Sporangiospores are globose, 1.5–2.0 µm in diameter, motile, and equipped with a polarly inserted tuft of about 30 flagella.

Color of the colonies vary from white, grayish tan, yellow-tan to yellow-brown (Lechevalier and Lechevalier, 1975), or pale, ochre, light-orange to orange (Kothe, 1987), depending on the agar medium used. White aerial mycelium is formed on some media. Grows between 10 and 37°C; abundant growth is observed at 23–28°C.

Source: the type strain was isolated from garden soil collected in Somerset, New Jersey, USA. The effective description (not validly published) of the species "*Actinoplanes penicillatus*" (Schäfer, 1973) was based on 15 strains, isolated from soil and characterized as having the same morphological features as *Actinoplanes rectilineatus.* The strains originated from Kenya (12), Australia (1, type strain), Corsica (1), and Spain (1).

DNA G+C content (mol%): 69 (T_m).

Type strain: ATCC 29234, DSM 43808, NBRC 13941, IMRU 3919, JCM 3194, NRRL B-16090, LL 7-10.

Sequence accession no. (16S rRNA gene): AB037010.

Additional comments: the type strain shows the highest sequence similarity to the following *Actinoplanes* species (based on ~1470 nt): *Actinoplanes couchii* GW8-1761^{T}, 98.9%; *Actinoplanes liguriensis* NBRC 13997^{T}, 98.7%.

22. **Actinoplanes regularis** (Couch 1963) Stackebrandt and Kroppenstedt 1988, 328[VP] (Effective publication: Stackebrandt and Kroppenstedt 1987, 113.) ["*Ampullaria regularis*" Couch 1963, 57; *Ampullariella regularis* (Couch 1963) Couch 1964, 29]

re.gu.la′ris. L. masc. adj. *regularis* regular.

Sporangia are mostly cylindrical, measuring 5.0–14.0 × 8.0–30.0 µm. The base of the sporangia is frequently mound shaped and resembles a corked bottle. Formation of sporangia occurs on Czapek and casein agars. Spores are arranged in parallel rows inside the sporangia, and are rod-shaped, 0.5–1.0 µm wide and 2.0–4.0 µm long, and motile by a polarly inserted tuft of flagella. Brushlike conidiophores may be produced on Czapek agar.

Good growth occurs on Czapek, peptone Czapek, casein, and tyrosine agars. Colonies are flat, frequently convoluted in the center with radial ridges on the margin. The color of the substrate mycelium is orange, red, brownish, ochre, or salmon to coral-pink, sometimes with white or gray areas on the surface, where the sporangia are produced. Soluble yellowish, greenish, or brownish (perhaps melanoid) pigments may be produced on various media.

Inositol, D-mannitol, and raffinose are not utilized by the type strain as sole carbon sources. Gelatin is liquefied.

Three strain variants are distinguishable on casein and tyrosine agars (Couch, 1963). The first variant utilizes casein and tyrosine and darkens both agars. The second variant utilizes casein and tyrosine, but darkens only the tyrosine agar. The third variant utilizes casein and tyrosine, but darkens only casein agar. The type strain of *Actinoplanes regularis* is from the first variant.

Source: the type strain was isolated from soil collected from a white pine grove, University of Wisconsin Arboretum, Madison, Wisconsin, USA. A further 28 strains were isolated, most from forest soils from North and South Carolina, Virginia, Mississippi, Wisconsin and Canada. D. Schäfer (Marburg, Germany) isolated a total of 26 strains from soil samples, originating from Germany (1), Kenya (3), Ceylon (1), Corsica (2), Tenerife (1), Austria (1), USA (3), Argentina (2), the Philippines (2), Taiwan (1), Uruguay (1), Cameroon (1), Mexico (1), France (2), the Azores (1), South Africa (1), and Yugoslavia (1) (Kothe, 1987).

DNA G+C content (mol%): 72.3 (Bd).

Type strain: CBS 193.64, DSM 43151, NBRC 12514, JCM 3062, NRRL B-3347, UNCC 65, VKM Ac-650.

Sequence accession no. (16S rRNA gene): AB037011.

Additional comments: the type strain shows the highest sequence similarity to the following *Actinoplanes* species (based on ~1470 nt): *Actinoplanes palleronii* NBRC 14916[T], 99.4%; *Actinoplanes rectilineatus* NBRC 13941[T], 98.9%.

23. **Actinoplanes sichuanensis** Sun, Dong, Zhang, Wei, Li, Yu, Klenk and Zhang 2009, 2766[VP]

si.chu.a.nen′sis. N.L. masc. adj. *sichuanensis* of or pertaining to Sichuan Province, southwest of China, soil of which was the source of the type strain.

Spherical sporangia are formed. The sporangiospores are motile and the surface is smooth. Aerial mycelium is absent. Color of substrate mycelium is cream to reddish orange on ISP 2, 3, 4, 5, 6, 7, yeast extract-starch agar, nutrient agar, Czapek solution agar, and modified Bennett's agar. Grows in the presence of 0–4% NaCl, but not in 5–10% NaCl. Growth occurs at initial pH of 6.5–10.5 and between 10 and 37°C, but not at pH 4.5–6.0 or at 40°C. Soluble pigments and melanin are not formed.

Esculin is hydrolyzed and nitrate is reduced to nitrite. Amylase and gelatinase are produced. H_2S is not produced. MK-9(H_4) (94.4%) is the predominant menaquinone, with MK-9(H_6) (3.7%) and MK-10(H_6) (1.9%) present as minor components. The major phospholipids are phosphatidylethanolamine and phosphatidylinositol. The predominant cellular fatty acids (>10%) are $C_{15:0}$ iso (22.2%), $C_{16:0}$ iso (15.3%) and *cis*-9-$C_{17:1}$ (11.1%); other fatty acids occurring in relatively small amounts (>5%) are $C_{14:0}$ (6.2%), $C_{16:0}$ (7.7%) and *cis*-9-$C_{18:1}$ (7.5%).

The organism is sensitive to filter-paper disks soaked in 1 ml of (µg/ml) erythromycin (15), gentamicin (10), and kanamycin (30), whereas it is resistant to ciprofloxacin (5), novobiocin (5), oxacillin (1), streptomycin (10), and tobramycin (10).

Source: the type strain was isolated from soil (Sichuan Province, China).

DNA G+C content (mol%): 70.4 (T_m).

Type strain: 03-723, CCM 7526, KCTC 19460.

Sequence accession no. (16S rRNA gene): EU531458.

Additional comments: the type strain shows the highest sequence similarity to the following *Actinoplanes* species (based on ~1470 nt): *Actinoplanes lobatus* NBRC 12513[T], 99.5%; *Actinoplanes philippinensis* NBRC 13878[T], 99.0%.

24. **Actinoplanes teichomyceticus** Wink, Kroppenstedt, Schuhmann, Seibert and Stackebrandt 2006, 2129[VP]

tei.cho.my.ce′ti.cus. Gr. n. *teichos* wall; Gr. n. *mukês -êtos* fungus; L. adj. suff. *-icus* suffix used with the sense of belonging to; N.L. masc. adj. *teichomyceticus* literally belonging to a fungus cell wall (referring to inhibition of cell-wall synthesis by teichomycin, produced by the type strain).

Spherical to oval sporangia (15.0–25.0 µm) are produced abundantly on many agar media, mainly on the dome of the colonies. Spores are highly motile, globose to oval, and 1.5–2.0 µm in diameter.

Abundant growth occurs on various agar media. Colonies may have a central protuberance or dome, and a smooth to wrinkled surface. Color of substrate mycelium may vary from beige on ISP media 5 and 6, pastel yellow on ISP media 2, 3, 4, and 7, and light orange on Bennett's and Czapek agars to deep orange on peptone glucose agar. On Hickey–Tresner, potato, and skim milk agars, it is light brownish to amber.

White aerial mycelium is produced on ISP 3 medium and Hickey–Tresner agar, rudimentary aerial mycelium with light pink or rose tinge is formed on Bennett's, Czapek glucose, and glycerol asparagine agars. A red-colored soluble pigment is formed on ISP medium 5, a yellow pigment on ISP media 4 and 5, and brown (melanoid) on ISP media 3 and 6.

Peptidoglycan contains *meso*-diaminopimelic acid; diagnostic sugars are xylose and arabinose; MK-9(H_4) and MK-10(H_4) are the principal menaquinones. Major phospholipid is phosphatidylethanolamine.

Produces the antibiotic teicoplanin RS-1 to RS-4, originally described as teichomycin A_1 and A_2 (Parenti et al., 1978).

Source: the type strain was isolated from a soil sample collected at Nimodi Village, Indore, India (Parenti et al., 1978).

DNA G+C content (mol%): not known.

Type strain: AB8327, ATCC 31121, BCRC 12106, DSM 43866, FH 2149, NBRC 13999, JCM 3252, KCC A-0252, NCIMB 12640, NRRL B-16726.

Sequence accession no. (16S rRNA gene): AB047513.

Additional comments: the type strain shows the highest sequence similarity to the following *Actinoplanes* species (based on ~1470 nt): *Actinoplanes liguriensis* NBRC 13997^T, 98.4%; *Actinoplanes palleronii* NBRC 14916^T, 98.3%. An earlier description of "*Actinoplanes teichomyceticus*" (Parenti, Beretta, Berti and Arioli 1978, 277) was not included in the Approved Lists of Bacterial Names (Skerman et al., 1980) and therefore treated in *Bergey's Manual of Systematic Bacteriology* (1st edition) under "*Species incertae sedis*" (Palleroni, 1989). Both species descriptions are based on the same type strain.

25. **Actinoplanes tereljensis** Ara, Yamamura, Tsetseg, Daram and Ando 2010, 925^{VP}

te.rel.jen′sis. N.L. masc. adj. *tereljensis* of or pertaining to the Mongolian region of Terelj, where the type strain was isolated.

Numerous irregular sporangia are formed on the surface of water agar, ISP 3 and 4, and Bennett's agar media; spores are motile. Cells grow well on ISP 2, 3, and 7, Bennett's, and yeast extract-starch agars, forming well-developed, extensively branched, and non-fragmented substrate hyphae. Optimal growth is at 20–28°C; no growth occurs at 37°C. Growth pH is 6–9. Tolerates up to 3% NaCl. Color of substrate mycelium is moderate grayish brown to dark brownish red on different agar media. A pale pinkish brown soluble pigment produced on ISP 7 and aerial mycelium is not observed.

The menaquinone system consists of MK-9(H_6) (85%) with a minor amount of MK-9(H_8) (15%). The polar lipid profile contains diphosphatidylglycerol, phosphatidylethanolamine, phosphatidyl-*N*-methylethylethanolamine, and phosphatidylinositol; phosphatidylcholine and phosphatidylglycerol have not been detected (phospholipid type PII). Major fatty acid methyl esters are $C_{16:0}$ iso (19%), $C_{17:0}$ anteiso (15%), $C_{15:0}$ anteiso (12%), and $C_{15:0}$ iso (11%).

Source: the type strain was isolated from soil (Terelj, Mongolia).

DNA G+C content (mol%): 70.6 (HPLC).

Type strain: MN07-A0371, NBRC 105297, VTCC D9-010.

Sequence accession no. (16S rRNA gene): AB468944.

Additional comments: the type strain shows the highest sequence similarity to the following *Actinoplanes* species (based on ~1470 nt): *Actinoplanes toevensis* MN07-A0368^T, 99.7%; *Actinoplanes ferrugineus* NBRC 15555^T (AB037002), 98.6%.

26. **Actinoplanes toevensis** Ara, Yamamura, Tsetseg, Daram and Ando 2010, 924^{VP}

to.e.ven′sis. N.L. masc. adj. *toevensis* of or pertaining to the Mongolian region of Töv Province, where the type strain was isolated.

Numerous globose to oval sporangia are formed on the surface of water agar, ISP 3 and 4, and Bennett's agar media. Spores formed in the sporangium are motile. Cells grow well on ISP 2, 3, and 4, Bennett's, and yeast extract-starch agar, forming well-developed, extensively branched, and non-fragmented substrate hyphae. Aerial mycelium is not observed. Optimal growth is at 25–30°C; no growth occurs at 40°C. Growth pH is 6–11. Tolerates up to 2% NaCl. Color of substrate mycelium is moderate yellow brown to orange brown on different agar media. A pale pinkish brown soluble pigment is produced on ISP 7 agar.

The menaquinone system consists of MK-9(H_6) (97%) with a minor amount of MK-9(H_8) (3%). The polar lipid profile contains diphosphatidylglycerol, phosphatidylethanolamine, phosphatidyl-*N*-methylethylethanolamine, and phosphatidylinositol; phosphatidylcholine and phosphatidylglycerol have not been detected (phospholipid type PII). Major fatty acid methyl esters are $C_{17:0}$ anteiso (25%), $C_{16:0}$ iso (22%), $C_{15:0}$ iso (13%), and $C_{15:0}$ anteiso (12%).

Source: the type strain was isolated from soil (Töv Province, Mongolia).

DNA G+C content (mol%): 70.6 (HPLC).

Type strain: MN07-A0368, NBRC 105298, VTCC D9-011.

Sequence accession no. (16S rRNA gene): AB468943.

Additional comments: the type strain shows the highest sequence similarity to the following *Actinoplanes* species (based on ~1470 nt): *Actinoplanes tereljensis* MN07-A0371^T, 99.7%; *Actinoplanes ferrugineus* NBRC 15555^T, 98.6%.

27. **Actinoplanes utahensis** Couch 1963, 67^{AL}

u.tah.en′sis. N.L. masc. adj. *utahensis* of or pertaining to the state of Utah.

Sporangia are very irregular in size and shape, lobed, pyriform, club-shaped, or digitate, 5.0–18.0 µm in diameter. Spores are arranged in irregular coils within the sporangia, subglobose, 1.0–2.0 µm in diameter, motile by polarly inserted polytrichous flagella. Microspores may be produced on peptone Czapek agar.

Good growth occurs on Czapek, peptone Czapek, casein, and tyrosine agars. Colonies are convoluted or flat; surfaces are slippery moist with minute or conspicuous bumps. Sporangial development is sparse and only on Czapek, oatmeal, and starch-casein agars. Color of the substrate mycelium is apricot-orange to salmon-orange. On peptone Czapek agar, the color may become ferrugineus toward the center. Substrate mycelium on oatmeal agar is amber colored and on peptone Czapek agar is slightly darkened.

Produces cyclic peptide antibiotics.

Source: the type strain was isolated from soil taken from Liberty Park, Salt Lake City, Utah, USA, together with two isolates from distinct samples from the same site. A further isolate originated from a soil sample collected between Carlin and Dunphy, Nevada, USA.

DNA G+C content (mol%): 70 (T_m).

Type strain: ATCC 14539, DSM 43147, NBRC 13244, JCM 3122, NRRL B-16727, UNCC 260, VKM Ac-674.

Sequence accession no. (16S rRNA gene): AB037012.

Additional comments: the type strain shows the highest sequence similarity to the following *Actinoplanes* species (based on ~1470 nt): *Actinoplanes regularis* NBRC 12514^T, 98.7%; *Actinoplanes palleronii* NBRC 14916^T, 98.6%.

28. **Actinoplanes xinjiangensis** Sun, Dong, Zhang, Wei, Li, Yu, Klenk and Zhang 2009, 2767^{VP}

xin.ji.an.gen′sis. N.L. masc. adj. *xinjiangensis* of or pertaining to Xinjiang Uyghur Autonomous Region, north-west China, soil of which was the source of the type strain.

Spherical to oval sporangia are formed. The sporangiospores are motile and the surface is almost smooth. Aerial mycelium is absent.

Grows well on ISP 2, 3, 4, 5, and 7, yeast extract-starch agar, nutrient agar, Czapek solution agar, and modified Bennett's agar; grows moderately on ISP 6 agar. Grows in the presence of 0–7% NaCl. Growth occurs at an initial pH value of 6.5–8.5 and between 10 and 37°C.

Esculin is hydrolyzed and nitrate is reduced to nitrite. Amylase is not produced but gelatinase is produced. H_2S production is positive. Melanin is produced on ISP 6 agar, but soluble pigment is not formed.

MK-9(H_4) (96.1%) is the predominant menaquinone and MK-10(H_6) (3.9%) is present as a minor component. The major phospholipid is phosphatidylethanolamine. The predominant cellular fatty acids (>10%) are $C_{15:0}$ iso (10.8%), $C_{16:0}$ iso (16.0%) and *cis*-9-$C_{17:1}$ (21.5%); other fatty acids occurring in relatively small amounts (>5%) are $C_{15:0}$ (6.7%), $C_{16:0}$ (6.5%) and *cis*-9-$C_{16:1}$ (5.9%).

The organism is sensitive to filter-paper disks soaked in 1 ml of (µg/ml) kanamycin (30), but resistant to (1 ml; µg/ml) ciprofloxacin (5), erythromycin (15), gentamicin (10), novobiocin (5), oxacillin (1), streptomycin (10), and tobramycin (10).

Source: the type strain was isolated from soil from Xinjiang Uyghur Autonomous Region, north-west China.

DNA G+C content (mol%): 71.0 (T_m).

Type strain: 03-8772, CCM 7527, KCTC 19461.

Sequence accession no. (16S rRNA gene): EU531457.

Additional comments: the type strain shows the highest sequence similarity to the following *Actinoplanes* species (based on ~1470 nt): *Actinoplanes sichuanensis* 03-723^T, 99.0%; *Actinoplanes lobatus* NBRC 12513^T, 98.9%.

Species and subspecies *incertae sedis*

The following list contains species and subspecies of *Actinoplanes*, *Ampullariella*, and *Amorphosporangium* that are effectively, but not validly published.

1. **"Actinoplanes arizonaensis"** Karwowski, Jackson, Theriault, Prokop, Maus, Hansen and Hensey 1988, 1210

   Produces arizonins.

   Type strain: AB660-122, ATCC 49796, NBRC 14837, JCM 9648.

2. **"Actinoplanes awaijnensis"** Torikata and Enokita 1978 *in* Torikata, Enokita, Imai, Itoh, Nakajima, Haneishi and Arai (1978)

   Produces 5-azacytidine.

   Type strain: ATCC 33917, JCM 9334, SANK 90277.

3. **"Actinoplanes awaijnensis subsp. mycoplanecinus"** Torikata, Enokita, Okazaki, Nakajima, Iwado, Haneishi and Arai 1983, 959

   Produces mycoplanecins.

   Type strain: ATCC 33919, NBRC 14279, JCM 6112.

4. **"Actinoplanes coloradoensis"** Jackson, Karwowski, Theriault, Fernandes, Semon and Kohl 1987, 1381

   Produces coloradocin.

   Type strain: AB 921J-26.

5. **"Actinoplanes deccanensis subsp. azaserinus"** Torikata, Enokita, Imai, Itoh, Nakajima, Haneishi and Arai (1978)

   Produces azaserine.

   Type strain: ATCC 33916, JCM 9333.

6. **"Actinoplanes friuliensis"** Aretz, Meiwes, Seibert, Vobis and Wink 2000, 813

   Produces friulimycins.

   Type strain: DSM 7358, HAG 010964.

   Additional comment: strain HAG 010964 (= DSM 7358) is a patent strain (EP 0 629 636, Hoechst AG, 1994). This strain can be obtained from the DSMZ for scientific research by a special agreement. According to Rules 27(3) and 30, "*Actinoplanes friuliensis*" is not validly published because, at the time of publication, the type strain was not deposited in two publicly accessible service collections in different countries.

7. **"Actinoplanes garbadinensis"** Parenti, Pagani and Beretta 1976, 502

   Produces gardimycin.

   Type strain: ATCC 31049, NBRC 13995, Lepetit A/10889.

8. **"Actinoplanes ianthinogenes"** Coronelli, Pagani, Bardone and Lancini 1974, 161

   Produces purpuromycin.

   Type strain: ATCC 21884, NBRC 13996, Lepetit A/1668.

9. **"Actinoplanes ianthinogenes subsp. octamycini"** Gause and Sveshnikova 1979 *in* Gause, Sveshnikova, Maksimova and Olkhovatova 1979

   Produces purpuromycin and octamycin.

   Type strain: ATCC 43632, NBRC 14524, JCM 9649.

10. **"Actinoplanes nipponensis"** Routien 1977 *in* Celmer, Moppett, Cullen, Routien, Jefferson, Shibakawa and Tone 1977a

    Produces antibiotic 41012.

    Type strain: ATCC 31145, DSM 43867, NBRC 14063, JCM 3264.

11. **"Actinoplanes nirasakinensis"** Torikata, Enokita, Imai, Itoh, Nakajima, Haneishi and Arai 1978, 92

    Produces actinomycins.

    Type strain: ATCC 33918, JCM 9335.

12. **"Actinoplanes pallidoaurantiacus"** Ruan, Zhang and Jiang 1976, 297

    Type strain: DSM 46145, NBRC 13968, JCM 3242.

13. **"Actinoplanes penicillatus"** Schäfer 1973, 185

    Type strain: CBS 558.75, DSM 46142, SE 2.

    Additional comments: the species "*Actinoplanes penicillatus*" (type strain: SE 2^T), was described by Schäfer (1973), describing

the same morphological features as those described for *Actinoplanes rectilineatus* (type strain: 7-10). A second investigation of both type strains (7-10 and SE 2) concluded in the description of a novel subspecies "*Actinoplanes rectilineatus* subsp. *penicillatus*" (*ex* Schäfer) nov. (sic!) rev. comb. nov. (Kothe, 1987).

14. **"Actinoplanes purpeobrunneus"** Ruan and Jiang 1979, 236
Type strain: NBRC 14020, JCM 3253, A58.

15. **"Actinoplanes pyriformis"** Ruan and Jiang 1979, 236
Type strain: NBRC 14030, JCM 3262, A68.

16. **"Actinoplanes rectilineatus subsp. penicillatus"** Kothe 1987, 70
See: "*Actinoplanes penicillatus*".

17. **"Actinoplanes roseosporangius"** Ruan, Zhang and Jiang 1976, 292
Type strain: DSM 46143, NBRC 13969, JCM 3243, 71-C29.

18. **"Actinoplanes rutilosporangius"** Ruan, Zhang and Jiang 1976, 298
Type strain: DSM 46151, NBRC 13970, JCM 3244, 71-C6.

19. **"Actinoplanes sarveparensis"** Japanese Patent (Kokai) 53-2402, 1978
Produces antibiotic L 13365.
Type strain: Lepetit A/13826, DSM 43901, NBRC 13993.

20. **"Actinoplanes tuftoflagellus"** Ruan and Jiang 1979, 235
Type strain: NBRC 14021, JCM 3254, A5.

21. **"Actinoplanes violaceus"** Jiang, Xu and Ruan 1983a
Type strain: ATCC 43537, NBRC 14458, JCM 3353, Y80-610.

22. **"Actinoplanes yunnanensis"** Jiang, Xu and Ruan 1983b, 212
Type strain: ATCC 43538, NBRC 14459, JCM 3354, Y79-21.

23. **"Ampullariella kinshanensis"** Ruan and Zhang 1974, 35
Type strain: DSM 46147, NBRC 13661, JCM 3173, 71-C11.

24. **"Ampullariella kunmingensis"** Jiang, Xu and Ruan 1983b, 214
Type strain: ATCC 43539, JCM 3355, Y79-15.

25. **"Ampullariella regularis subsp. intermedia"** Nonomura, Iino and Hayakawa 1979, 84
Type strain: DSM 43898, NBRC 14065, JCM 3235.

26. **"Ampullariella regularis subsp. mannitophila"** Itoh, Enokita, Okazaki, Iwado, Torikata, Haneishi and Arai 1981, 930
Produces candiplanecin.
Type strain: ATCC 33986, JCM 9336.

27. **"Ampullariella violaceochromogenes"** Nonomura, Iino and Hayakawa 1979, 84
Type strain: DSM 43899, NBRC 14066, JCM 3236.

28. **"Amorphosporangium castaneum"** Jiang and Yan 1984
Type strain: ATCC 43631, DSM 43914, NBRC 14428, JCM 3341, B-133.

Genus IV. **Asanoa** Lee and Hah 2002, 970[VP]

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As.a.n′a. N.L. fem. n. *Asanoa* named after Kozo Asano, the Japanese microbiologist who made the original description of the genus *Catellatospora*.

Weakly sporulating branched vegetative hyphae (0.3–0.4 µm in diameter). **Gram-stain-positive**. Nonmotile. **Aerobic**. Mesophilic. Cell wall contains diamino acids *meso*-diaminopimelic acid and 3-hydroxydiaminopimelic acid. Orange colony mass. Sporulation only occurs on tap-water agar and glycerol/calcium malate agar. Aerial mycelium and globose bodies are not observed. Possess a glycolylated peptidoglycan and whole-cell sugars of arabinose, rhamnose, ribose, xylose, galactose, mannose and glucose. Catalase-positive. Urease-negative. Nitrate is not reduced to nitrite. H_2S is not produced. Mycolic acids are not present. MK-10(H_6, H_8) are the major menaquinones. Polar lipid profile comprises phosphatidylethanolamine (a phospholipid type PII pattern). The fatty acid pattern is 2d type.

DNA G+C content (mol%): 69–71.5.

Type species: **Asanoa ferruginea** Lee and Hah 2002, 970[VP].

Further descriptive information

Phylogenetic analysis of the 16S rRNA gene positions the genus within the family *Micromonosporaceae*. The closest phylogenetic neighbor is *Micromonospora auratinigra* strain TT1-11 (97.8% sequence similarity, accession no. NR028659), which was isolated from a peat swamp forest in Thailand (Thawai et al., 2004b). An environmental clone with high 16S rRNA gene sequence similarity has been detected in cellulosic waste from a simulated low-level-radioactive-waste site (98.4 %, accession no. GQ263486) (Field et al., 2010).

Enrichment and isolation procedures

Asanoa ferruginea strain 6257-C[T] was isolated by a dilution method from woodland soil samples collected in Yamanashi, Japan (Asano and Kawamoto, 1986).

Maintenance procedures

Strains are cultivated on oatmeal agar (International *Streptomyces* Project, ISP, medium 3), inorganic salts/starch agar (ISP medium 4) and tap-water agar at 28°C for 21 d or on yeast extract/glucose broth for 3 d at 30°C. Stock cultures are maintained on yeast extract/malt extract agar (ISP medium 2) at 4°C or as a suspension in 20% (v/v) glycerol at −20°C (Asano and Kawamoto, 1986).

Differentiation of the genus *Asanoa* from closely related genera

Production of chains of nonmotile spores only from the vegetative mycelium differentiates *Asanoa* from phylogenetically related genera: *Micromonospora* produce single nonmotile spores on the vegetative hyphae (Luedemann, 1974); *Actinoplanes*, *Ampullariella*, *Amorphosporangiurn*, and *Pilimelia* form unique globose or cylindrical sporangia containing numerous motile spores (Couch, 1950, 1963, 1964); *Dactylosporangiurn* produce finger-shaped sporangia containing three to four motile spores arranged in a single row; and *Glycomyces* form chains of nonmotile spores on the aerial mycelium (Asano and Kawamoto, 1986). *Asanoa* possess major menaquinones MK-10(H_6, H_8), while members of the related genus *Catellatospora* have the major menaquinones MK-9(H_4, H_6) or MK-10(H_4).

List of species of the genus *Asanoa*

1. **Asanoa ferruginea** (Asano and Kawamoto 1986) Lee and Hah 2002, 970[VP] (Basonym: *Catellatospora ferruginea* Asano and Kawamoto 1986, 516.)

fer.ru.gi′ne.a. L. fem. adj. *ferruginea* rust-colored.

Growth occurs at mesophilic temperature. Does not grow on 3% NaCl or 0.01% lysozyme. Grows on 0.001% brilliant green and 0.0001% crystal violet. Antibiotic susceptibility (μg/ml): 50, gentamicin; 5, neomycin; and 100, streptomycin. Antibiotic resistance (μg/ml): 50, novobiocin; 20, vancomycin; and 10, tetracycline. Utilizes D-arabinose, L-arabinose, dextran, D-cellobiose, D-fructose, D-galactose, D-glucose, D-lactose, maltose, D-mannose, melibiose, methyl α-D-glucoside, D-raffinose, L-rhamnose, D-ribose, salicin, starch, sucrose, D-trehalose, D-xylose, adonitol, and D-mannitol. Does not utilize gluconate, inulin, D-melezitose, L-sorbose, dulcitol, butanol, *meso*-erythritol, ethanol, glycerol, *meso*-inositol, 2-propanol, 1-propanol, D-sorbitol, or D-xylitol. Hydrolyzes elastin and starch, but not casein, DNA, or gelatin. Does not decompose adenine, hippurate, hypoxanthine, DL-tyrosine, or xanthine. The phospholipid profile comprises diphosphatidylglycerol, phosphatidylinositol, phosphatidylinositol mannoside, phosphatidylethanolamine, phosphatidylglycerol, and an unknown phospholipid. The major fatty acids are $C_{15:0}$ anteiso (21%), $C_{17:0}$ (20%), $C_{16:0}$ iso (18%), $C_{17:1}$ (15%), and $C_{15:0}$ iso (10%) acids. Minor fatty acids are $C_{15:0}$ (5%), $C_{18:0}$ (4%), $C_{16:0}$ (3%), $C_{14:0}$ iso (2%), and $C_{17:0}$ iso (1%).

For all other characteristics, refer to the genus description.

DNA G+C content (mol%): 71.5 (HPLC).

Type strain: IMSNU 22009, NBRC 14496, DSM 44099.

Sequence accession no. (16S rRNA gene): AF152108.

2. **Asanoa iriomotensis** Tamura and Sakane 2005, 726[VP]

i.ri.o.mo.ten′sis. N.L. fem. adj. *iriomotensis* of or belonging to Iriomote Island, Okinawa, Japan, the origin of the soil sample from which the type strain was isolated.

Spore chains borne on the tip of short sporophores arising directly from the agar surface form on water agar and HV agar. Growth occurs optimally between 20 and 30°C; no growth at 15 or 37°C. Does not grow on 4% NaCl. Hydrolyzes starch. Does not reduce nitrate. Utilizes D-mannitol, D-melibiose, D-maltose, L-rhamnose, methyl α-D-glucoside, D-raffinose, D-galactose, D-mannose, and glucose. Does not utilize L-erythritol, adonitol, D-lactose, L-inositol, D-sorbitol, or dulcitol. Phosphatidylethanolamine is present as the diagnostic phospholipid. Unsaturated fatty acids and 10-methylated fatty acids are not detected. Major fatty acids are $C_{15:0}$ anteiso (22%), $C_{17:0}$ anteiso (20), $C_{15:0}$ iso (18%), $C_{17:0}$ (14%), and $C_{16:0}$ iso (12%). Minor fatty acids are $C_{17:0}$ iso, $C_{16:0}$, $C_{18:0}$, $C_{15:0}$, and $C_{14:0}$ iso.

For all other characteristics, refer to the genus description.

DNA G+C content (mol%): 69.0 (HPLC).

Type strain: TT 97-02, NBRC 100142, DSM 44745.

3. **Asanoa ishikariensis** Lee and Hah 2002, 971[VP] (Basonym: '*Catellatospora ishikariense*'.)

ish.i.ka.ri.en′sis. N.L. fem. adj. *ishikariensis* of or belonging to Ishikari-gun, Hokkaido, Japan, the origin of the soil sample from which the type stain was isolated.

Growth occurs at mesophilic temperatures below 37°C. Grows on 0.001% Brilliant green, but not on 0.0001% crystal violet, 3% NaCl or 0.01% lysozyme. Antibiotic susceptiblity (μg/ml): 50, gentamicin; 5, neomycin; 100, streptomycin; and 10, tetracycline. Antibiotic resistance (μg/ml): 50, novobiocin and 20, vancomycin. Utilizes D-arabinose, L-arabinose, dextran, D-cellobiose, D-fructose, D-galactose, gluconate, D-glucose, D-lactose, maltose, D-mannose, melibiose, methyl α-D-glucoside, D-raffinose, L-rhamnose, D-ribose, starch, sucrose, D-trehalose, D-xylose, adonitol, dulcitol, and D-mannitol. Does not utilize inulin, D-melezitose, salicin, L-sorbose, butanol, *meso*-erythritol, ethanol, glycerol, *meso*-inositol, 2-propanol, 1-propanol, D-sorbitol, or D-xylitol. Hydrolyzes casein, gelatin and starch but not DNA or elastin. Does not decompose adenine, hippurate, hypoxanthine, DL-tyrosine, or xanthine. The phospholipid profile consists of diphosphatidylglycerol, phosphatidylinositol, phosphatidylinositol mannoside, phosphatidylethanolamine, and an unknown phospholipid. A trace amount of phosphatidylglycerol is also detected. The major fatty acids are $C_{15:0}$ anteiso (25%), $C_{17:1}$ (24%), $C_{15:0}$ iso (16%) and $C_{17:0}$ (12%) acids. Minor fatty acids are $C_{17:0}$ iso (6%), $C_{16:0}$ iso (4%), $C_{15:0}$ (3%), $C_{18:0}$ (2%), $C_{16:0}$ (1%), and $C_{19:0}$ (1%).

For all other characteristics, refer to the genus description.

DNA G+C content (mol%): 71.1 (HPLC).

Type strain: IMSNU 22004, NBRC 14551.

Sequence accession no. (16S rRNA gene): AJ294715.

Genus V. **Catellatospora** Asano and Kawamoto 1986, 516[VP] emend. Lee and Hah 2002, 971[VP] emend. Ara, Bakir and Kudo 2008a, 1958[VP]

Fred A. Rainey

Ca.tel.la.to.spo′ra. L. n. *catella* small chain; Gr. n. *spora* a seed and in biology a spore; N.L. fem. n. *Catellatospora* (organism forming) small chain of spores.

Gram-stain-positive, forming chains of nonmotile spores that arise singly or in tufts from vegetative hyphae. Vegetative hyphae are branched but not fragmented. Substrate mycelia are light yellow to bright yellow. Aerial mycelia are absent. **Aerobic** and **chemo-organotrophic**. **Mesophilic**. **Catalase-positive**. The diagnostic amino acids of the peptidoglycan are ***meso*-diaminopimelic acid** and 3-hydroxydiaminopimelic acids. The muramic acid *N*-acyl type is glycolyl. The cell-wall hydrolysates contain arabinose, galactose, ribose, mannose, glucose and xylose. Rhamnose is present in some species. Mycolic acids are not present. The major fatty acids are $C_{17:0}$, $C_{17:1}$ ω8*c*, $C_{15:0}$ iso and $C_{16:0}$ iso. The polar lipids can include phosphatidylethanolamine, diphosphatidylglycerol, phosphatidylinositol and phosphatidylinositol mannosides. **Menaquinone MK-9(H_4)** is the major menaquinone. Species of the genus have been isolated from sandy and woodland soils from Japan and Bangladesh. Based on 16S rRNA gene sequence phylogeny the species of the genus form a distinct lineage within the family *Micromonosporaceae*.

DNA G+C content (mol%): 70.7–71.4.

Type species: **Catellatospora citrea** Asano and Kawamoto 1986, 516[VP].

List of species of the genus *Catellatospora*

1. **Catellatospora citrea** Asano and Kawamoto 1986, 516[VP]

cit′re.a. L. fem. adj. *citrea* of or pertaining to the citrus-tree, intended to mean lemon yellow.

Aerobic, Gram-stain-positive staining, short chains of nonmotile spores arise singly or in tufts from vegetative hyphae. Sporulation observed on Tyrosine agar, oatmeal agar, 1/5 yeast extract-starch agar, oatmeal-nitrate agar, tap-water agar and sucrose-nitrate agar. Yellow vegetative hyphae on oatmeal and Hickey–Tresner agar medium. Soluble or melanin-like pigments are not produced.

The cell-walls contain *meso*-diaminopimelic and 3-hydroxydiaminopimelic acids. The muramic acid *N*-acyl type is glycolyl. The cell-wall sugars are arabinose, galactose, rhamnose, ribose and xylose. Whole-cell fatty acids when grown at 30°C on yeast extract-starch broth include: $C_{15:0}$ (3.9%), $C_{16:0}$ (1.5%), $C_{17:0}$ (8.1%), $C_{18:0}$ (2.2%), $C_{16:1}$ 2-OH (5.5%), $C_{17:1}$ ω8*c* (7.8%), $C_{18:1}$ ω9*c* (2.2%), $C_{14:0}$ iso (2.4%), $C_{15:0}$ iso (28.4%), $C_{15:0}$ anteiso (5.3%), $C_{15:1}$ iso (2.6%), $C_{16:1}$ iso(1.8%), $C_{16:0}$ iso (10.5%), $C_{17:1}$ iso ω9*c* (1.4%), $C_{17:0}$ iso (3.3%), $C_{17:0}$ anteiso (4.3%), 10-methyl $C_{18:0}$ (1.0%) and summed feature 6 (1.5%) (Ara and Kudo, 2006). The polar lipid profile consists of phosphatidylethanolamine, phosphatidylglycerol, phosphatidylinositol, phosphatidylinositol mannosides. MK-9(H_4) is the major menaquinone with minor amounts of MK-9(H_6). Growth occurs between 20 and 37°C, with optimal growth at 31°C. Growth occurs between pH 6 and 9. Growth does not occur in the presence of 1 % (w/v) NaCl. Negative for milk coagulation and peptonization. Gelatin is not liquefied. Substrates used as sole carbon sources include: L-arabinose, glycerol, lactose, D-galactose, D-glucose, maltose, D-mannose, α-D(+)-melibiose, methyl α-D-glucoside, L-rhamnose, salicin, starch, sucrose, trehalose, and D-xylose. Adonitol, erythritol, D-(+)-raffinose, are not used as sole carbon sources. Utilization of D- fructose, *myo*-inositol, D-mannitol and D- ribose has been reported as both positive and negative for the type strain. Sensitive to the following antibiotics: novobiocin (20 μg/ml), vancomycin (50 μg/ml), gentamicin (50 μg/ml), demethylchlortetracycline (500 μg/ml) and streptomycin (100 μg/ml). Growth occurs in the presence of crystal violet at 0.0001% (w/v) but not at 0.001% (w/v).

Isolated from woodland soil collected in Itsukaichi-shi Tokyo, Japan.

DNA G+C content (mol%): 71.5 (T_m).

Type strain: 6183-E, ATCC 49964, CIP 107011, DSM 44097, NBRC 14495, JCM 7542, NRRL B-16429, VKM Ac-1421.

Sequence accession no. (16S rRNA gene): X93197.

2. **Catellatospora bangladeshensis** Ara and Kudo 2006, 399[VP]

ban.gla.desh.en′sis. N.L. fem. adj. *bangladeshensis* of or pertaining to Bangladesh, the origin of the soil from which the type strain was isolated.

Aerobic, Gram-stain-positive staining, short chains of nonmotile spores arise singly or in tufts from vegetative hyphae. Spores are spherical to cylindrical; spore surface is smooth. Forms well developed branched substrate mycelium. Sporulation observed on sucrose-nitrate agar and oatmeal-nitrate agar. Light yellow to bright yellow substrate mycelium form on yeast extract starch agar. Soluble or melanin-like pigments are not produced. The cell-walls contain *meso*-diaminopimelic and 3-hydroxydiaminopimelic acids. The muramic acid *N*-acyl type is glycolyl. The cell-wall hydrolysates contain arabinose, galactose, rhamnose, ribose, mannose, glucose and xylose. Mycolic acids are not present. Whole-cell fatty acids when grown at 30°C on yeast extract-starch broth include: $C_{15:0}$ (1.7%), $C_{17:0}$ (3.1%), $C_{17:1}$ ω8*c* (8.8%), $C_{18:1}$ ω9*c* (3.0%), $C_{14:0}$ iso (5.2%), $C_{15:0}$ iso (19.8%), $C_{15:0}$ anteiso (2.5%), $C_{16:1}$ iso (3.7%), $C_{16:0}$ iso (35.4%), $C_{17:1}$ iso ω9*c* (1.8%), $C_{17:0}$ iso (2.8%), $C_{17:0}$ anteiso (2.0%), $C_{18:0}$ iso (1.2%), 10-methyl $C_{18:0}$ (2.3%), and summed feature 3 (1.2%). The polar lipid profile consists of phosphatidylethanolamine, diphosphatidylglycerol, phosphatidylinositol, phosphatidylinositol mannosides. Glucosamine containing phospholipids and phosphatidylcholine are absent. MK-9(H_4) is the major menaquinone with minor amounts of MK-9(H_6) and MK-9(H_2). Growth occurs between 25 and 30°C

and between pH 6.8 and 7.2. Growth does not occur in the presence of 1 % (w/v) NaCl. Substrates used as sole carbon sources include: L-arabinose, D-glucose, D-galactose, lactose, D-mannose, maltose, α-D(+)-melibiose, L-rhamnose, salicin, sucrose, trehalose, and D-xylose. Weakly positive utilization of adonitol, glycerol, and D-mannitol. Erythritol, D- fructose, *myo*-inositol, methyl α-D-glucoside, D-(+)-raffinose, and D- ribose are not used as sole carbon sources.

Isolated from sandy soil collected at Chokoria, Bandladesh.

DNA G+C content (mol%): 71.0 (HPLC).

Type strain: 2-70(23), JCM 12949, DSM 44899.

Sequence accession no. (16S rRNA gene): AB200233.

3. **Catellatospora chokoriensis** Ara and Kudo 2006, 397[VP]

cho.kor.i.en′sis. N.L. fem. adj. *chokoriensis* of or pertaining to Chokoria, Bangladesh, the origin of the soil from which the type strain was isolated.

Aerobic, Gram-stain-positive staining, short chains of spores are borne directly on substrate mycelium. Spores are spherical to cylindrical and nonmotile. Forms well developed branched substrate mycelium. Aerial mycelium is absent. Sporulation observed on glycerol-asparagine agar, tyrosine agar, yeast-malt extract agar, oatmeal agar, Bennett agar, water agar, sucrose-nitrate agar, 1/5 yeast-starch agar and oatmeal-nitrate agar. Light yellow to bright yellow substrate mycelium form on yeast extract starch agar. Soluble or melanin-like pigments are not produced. The cell-walls contain *meso*-diaminopimelic and 3-hydroxydiaminopimelic acids. The muramic acid *N*-acyl type is glycolyl. The cell-wall hydrolysates contain arabinose, galactose, rhamnose, ribose, mannose, glucose and xylose. Mycolic acids are not present. Whole-cell fatty acids when grown at 30°C on yeast extract-starch broth include: $C_{15:0}$ (2.2%), $C_{17:0}$ (10.9%), $C_{18:0}$ (4.3%), $C_{17:1}$ ω8*c* (3.5%), $C_{14:0}$ iso (3.6%), $C_{15:0}$ iso (30.3%), $C_{15:0}$ anteiso (6.3%), $C_{16:1}$ iso (2.5%), $C_{16:0}$ iso (22.9%), $C_{17:0}$ iso (5.5%), and $C_{17:0}$ anteiso (7.9%). The polar lipid profile consists of phosphatidylethanolamine, diphosphatidylglycerol, phosphatidylinositol, phosphatidylinositol mannosides. Glucosamine containing phospholipids and phosphatidylcholine are absent. MK-9(H_4) is the major menaquinone with minor amounts of MK-9(H_6) and MK-9(H_2). Growth occurs between 15 and 30°C, between pH 6.0 and 9.0, and in the presence of 1 % (w/v) NaCl. Substrates used as sole carbon sources include: L-arabinose, D-glucose, D-galactose, lactose, D-mannose, maltose, α-D(+)-melibiose, salicin, sucrose, trehalose, and D-xylose. Weakly positive utilization of adonitol. Erythritol, D- fructose, glycerol, *myo*-inositol, D-mannitol, methyl α- D-glucoside, D-(+)-raffinose, L-rhamnose, and D-ribose are not used as sole carbon sources.

Isolated from sandy soil collected at a forest-side waterfall, Chokoria, Bangladesh.

DNA G+C content (mol%): 71.0 (HPLC).

Type strain: 2-25(1), JCM 12950, DSM 44900.

Sequence accession no. (16S rRNA gene): AB200231.

4. **Catellatospora coxensis** Ara and Kudo 2006, 398[VP]

cox.en′sis. N.L. fem. adj. *coxensis* of or pertaining to Cox's Bazar, Bangladesh, the origin of the soil from which the type strain was isolated.

Aerobic, Gram-stain-positive staining, short chains of nonmotile spores arise singly or in tufts from vegetative hyphae. Spores are spherical to cylindrical and nonmotile. Forms well developed branched substrate mycelium. Aerial mycelium is absent. Sporulation observed on oatmeal agar, water agar, 1/5 yeast-starch agar and oatmeal-nitrate agar. Light yellow to bright yellow substrate mycelium form on yeast extract starch agar. Soluble or melanin-like pigments are not produced. The cell-walls contain *meso*-diaminopimelic and 3-hydroxydiaminopimelic acids. The muramic acid *N*-acyl type is glycolyl. The cell-wall hydrolysates contain arabinose, galactose, rhamnose, ribose, mannose, glucose and xylose. Mycolic acids are not present. Whole-cell fatty acids when grown at 30°C on yeast extract-starch broth include: $C_{15:0}$ (4.8%), $C_{16:0}$ (1.6%), $C_{17:0}$ (14.4%), $C_{18:0}$ (1.8%), $C_{19:0}$ (1.2%), $C_{17:1}$ ω8*c* (7.9%), %), $C_{18:1}$ ω9*c* (1.6%), $C_{14:0}$ iso (4.8%), $C_{15:0}$ iso (22.2%), $C_{15:0}$ anteiso (7.4%), $C_{16:0}$ iso (18.5%), $C_{17:0}$ iso (2.2%), and $C_{17:0}$ anteiso (4.5%). The polar lipid profile consists of phosphatidylethanolamine, diphosphatidylglycerol, phosphatidylinositol, phosphatidylinositol mannosides. Glucosamine containing phospholipids and phosphatidylcholine are absent. MK-9(H_4) is the major menaquinone with minor amounts of MK-9(H_6) and MK-9(H_2). Growth occurs between 20 and 30°C and between pH 6.0 and 9.0. Growth does not occur in the presence of 1 % (w/v) NaCl. Substrates used as sole carbon sources include: L-arabinose, D-glucose, D-galactose, glycerol, lactose, D-mannose, maltose, α-D(+)-melibiose, L-rhamnose, D-ribose, sucrose, trehalose, and D-xylose. Weakly positive utilization of D- fructose, and methyl α- D-glucoside. Adonitol, erythritol, *myo*-inositol, D-mannitol, D-(+)-raffinose, salicin, and are not used as sole carbon sources.

Isolated from sandy soil collected at a forest-side waterfall, Chokoria, Cox's Bazar, Bangladesh.

DNA G+C content (mol%): 71.0 (HPLC).

Type strain: 2-29(17), JCM 12951, DSM 44901.

Sequence accession no. (16S rRNA gene): AB200232.

5. **Catellatospora methionotrophica** (*ex* Asano and Kawamoto 1988) Ara and Kudo 2006, 399[VP] (*Catellatospora citrea* subsp. *methionotrophica* Asano and Kawamoto 1988)

me.thi.o.no.tro′phi.ca. N.L. n. *methioninum* methionine; Gr. adj. *trophikos* nursing, tending or feeding; N.L. fem. adj. *methionotrophica*, methionine auxotroph.

Aerobic, Gram-stain-positive staining, straight chains of smooth surfaced spores. Light yellow to bright yellow vegetative hyphae formed. Soluble or melanin-like pigments are not produced. Forms well developed, branched substrate mycelium. Aerial mycelium is absent. The cell-walls contain *meso*-diaminopimelic and 3-hydroxydiaminopimelic acids. The muramic acid *N*-acyl type is glycolyl. The cell-wall hydrolysates contain arabinose, xylose, galactose, ribose, mannose, and glucose. Mycolic acids are absent. Whole-cell fatty acids when grown at 30°C on yeast extract-starch broth include: $C_{15:0}$ (2.9%), $C_{16:0}$ (1.3%), $C_{17:0}$ (8.9%), $C_{18:0}$ (1.7%), $C_{16:1}$ 2-OH (1.4%), $C_{17:1}$ ω8*c* (9.3%), $C_{18:1}$ ω9*c* (2.9%), $C_{14:0}$ iso (1.4%), $C_{15:0}$ iso (37.6%), $C_{15:0}$ anteiso (7.5%), $C_{16:0}$ iso (5.7%), $C_{17:1}$ iso ω9*c* (3.2%), $C_{17:0}$ iso (4.5%), $C_{17:0}$ anteiso (5.0%) and summed feature 6 (1.4%). The polar lipid profile consists of phosphatidylethanolamine, diphosphatidylglycerol, phosphatidylglycerol, phosphatidylinositol,

phosphatidylinositol mannosides. Glucosamine containing phospholipids and phosphatidylcholine are absent. MK-9(H_4) is the major menaquinone with minor amounts of MK-9(H_6) and MK-9(H_2). Growth occurs between 20 and 34°C and between pH 6.8 and 7.2. Growth does not occur in the presence of 1 % (w/v) NaCl. Negative for milk coagulation and peptonization. Gelatin is not liquefied. Requires methionine which cannot be replaced by cysteine or homoserine. Substrates used as sole carbon sources include: D-arabinose, L-arabinose, cellobiose, lactose, D-galactose, D-glucose, maltose, D-mannose, L-rhamnose, salicin, starch, sucrose, trehalose, and D-xylose. Weakly positive utilization of erythritol, glycerol, D-fructose, methyl α-D-glucoside and D-(+)-raffinose. Adonitol, ethanol, gluconate, D-mannitol, melezitose, melibiose, α-D(+)-melibiose, methanol, methyl α-D-glucoside, D- ribose and sorbitol are not used as sole carbon sources. Sensitive to the following antibiotics: novobiocin (50 μg/ml), vancomycin (50 μg/ml), gentamicin (50 μg/ml), demethylchlortetracycline (500 μg/ml) and streptomycin (100 μg/ml). Growth occurs in the presence of crystal violet at 0.0001% (w/v) but not at 0.001% (w/v).

Isolated from a woodland soil sample collected in Yamanashi, Japan.

DNA G+C content (mol%): 71.0 (HPLC).

Type strain: 6257-B, ATCC 49965, CIP 107012, NBRC 14553, NRRL B-16431, VKM Ac-2008, IMSNU 22006, JCM 7543, DSM 44098.

Sequence accession no. (16S rRNA gene): AF152107.

Genus VI. **Catenuloplanes** Yokota, Tamura, Hasegawa and Huang 1993, 809[VP]

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Ca.te.nul.o.plan′es. L. fem. n. *catenula* short chain; Gr. masc. n. *planes* a wanderer; N.L. masc. n. *Catenuloplanes* a short chain wanderer; intended to signify a motile short chain.

Gram-stain-positive. Not acid-fast. **Strictly aerobic**. **Forms branching and non-fragmenting vegetative hyphae**. Aerial mycelium is rudimentarily developed or absent. If produced, spores are arranged in chains, which arise from the vegetative hyphae or are formed on the rudimentary aerial hyphae. The spore chains are aggregated into clusters and may be enveloped by outer sheaths. The configuration of spore chains is curly or spiral with one or two turns and sometimes branched, The spores are rod-shaped, straight or curved (0.6–0.8 × 2–4 μm) with smooth surfaces, and motile by means of peritrichous flagella. **Cell-wall type is VI and the peptidoglycan contains D-glutamate**, **D-serine**, **L-serine, glycine**, **D-alanine, and L-lysine**. **Cell-wall sugars are mannose, xylose, ribose and glucose**. The muramic acid in the glycan moiety is N-glycolated. The Mycolic acids are absent. Major menaquinones are MK-10(H_4) and MK-11(H_4); some strains may also possess small amounts of MK-10, MK-10(H_2), MK-10(H_8), MK-10(H_6), MK-11(H_2) and MK-11(H_6). The major cellular fatty acids are $C_{18:1}$, $C_{16:0}$, and $C_{17:0}$ anteiso. Small amounts of $C_{17:0}$, $C_{16:1}$, $C_{16:0}$ iso, and $C_{18:0}$ may also be present. The diagnostic phospholipid is phosphatidylcholine.

DNA G+C content (mol%): 70.0–72.0.

Type species: **Catenuloplanes japonicas** Yokota, Tamura, Hasegawa and Huang 1993, 810[VP].

Further descriptive information

Phylogenetic analysis of the 16S rRNA gene classifies the genus within family *Micromonosporaceae.* The closest phylogenetic neighbor is *Asanoa iriomotensis* (97.3%, accession no. AB112081).

Enrichment and isolation procedures

Isolated from soil or leaf litter in Japan, India, and Nepal. Many strains were isolated on a medium containing 1.0% soluble starch, 0.1% casein, 0.05% K_2HPO_4, and 1.5% agar (pH 7.0–7.5) supplemented with (per ml) 25 μg nalidixic acid, 12.5 μg kanamycin, 5.0 μg cefsulodin, and 6.25 μg kabicidin (Yokota et al., 1993).

Maintenance procedures

Strains are cultivated on yeast extract/starch agar containing 2 g yeast extract (Difco), 10 g soluble starch and 15 g agar per l distilled water (pH 7.3) at 28°C for 14 d and maintained at 8°C.

Differentiation of the genus *Catenuloplanes* from closely related genera

Spore motility differentiates *Catenuloplanes* from closely related genera with nonmotile spores: *Actinocatenispora*, *Asanoa*, *Catellatospora*, *Krasilinkovia*, *Longispora*, *Luedemannella*, *Micromonospora*, *Polymorphospora*, *Salinispora*, and *Verrucosispora*. Sporangia or spore vesicles are absent in *Catenuloplanes*, but are present in *Actinoplanes*, *Dactylosporangium*, *Luedemannella*, *Pilimelia*, *Virgisporangium*, and *Planosporangium*. Within the family, only *Catenuloplanes* and *Couchioplanes* contain the diamino acid L-Lys in their cell walls. All other genera contain *m*-DAP. *Catenuloplanes* is differentiated from *Couchioplanes* by phospholipid type (III and II, respectively) and major menaquinones [MK-10(H_4) and MK-11(H_4) versus MK-9(H_4), respectively].

Taxonomic comments

Planopolyspora crispa was described nearly simultaneously with *Catenuloplanes japonicas* (Petrolini et al., 1993; Yokota et al., 1993). Because the name *Catenuloplanes japonicas* was validated first, it has priority, and *Planopolyspora crispa* was transferred to the genus *Catenuloplanes* as *Catenuloplanes crispus* by Kudo et al. (1999).

List of species of the genus *Catenuloplanes*

1. **Catenuloplanes japonicus** Yokota, Tamura, Hasegawa and Huang 1993, 810[VP]

ja.pon'i.cus. N.L. masc. adj. *japonicus* of or pertaining to Japan.

Vegetative mycelia of the strains are pale orange to orange yellow, and the aerial mycelia are white to pale yellow. They produce a pale yellowish soluble pigment on Czapek-sucrose agar, glucose-asparagine agar, and calcium malate agar. H_2S is produced. Nitrate is not reduced to nitrite. Gelatin liquefaction is positive. Hydrolyzes starch, but not hippurate. Decomposes calcium malate, tyrosine, esculin, and urea, but not adenine, xanthine, hypoxanthine, or cellulose. Susceptible to lysozyme. Milk is coagulated and peptonized. Utilizes acetate, lactate, malate, pyruvate, succinate, L-arabinose, cellobiose, fructose, galactose, glucose, glycerol, inositol, lactose, maltose, mannitol, melezitose, melibiose, α-methyl-D-glucoside, raffinose, L-ribose, salicin, starch, sucrose, trehalose, and D-xylose. Does not utilize benzoate, citrate, mucate, oxalate, ribitol, galactitol, L-erythritol, sorbitol, or L-sorbose. Growth occurs optimally at 21–28°C. Major fatty acids are $C_{18:1}$, $C_{16:0}$ and $C_{17:0}$ anteiso.

For all other characteristics, refer to the genus description.

DNA G+C content (mol%): 71.0 (HPLC).

Type strain: N381-16, NBRC 14176, ATCC 31637, DSM 44102, JCM 9106, VKM Ac-875.

Sequence accession no. (16S rRNA gene): X93201.

2. **Catenuloplanes crispus** Kudo, Nakajima and Suzuki 1999, 1858[VP]

cris'pus. L. masc. adj. *crispus* curly.

Yellow to brown vegetative mycelia on most media. Yellowish diffusible pigment is produced in glycerol/asparagine agar. Decomposes casein, elastin, esculin, testosterone, and urea, but not adenine, DNA, hypoxanthine, tyrosine, or xanthine. Utilizes L-arabinose, D-cellobiose, D-fructose, D-galactose, D-glucose, glycerol, *myo*-inositol, D-lactose, maltose, D-mannitol, D-mannose, L-rhamnose, salicin, starch, sucrose, D-trehalose, D-xylose, fumarate, L-malate, and succinate. Does not utilize adonitol, dulcitol, iso-erythritol, D-melezitose, methyl-α-D-glucoside, D-raffinose, citrate, mucate, benzoate, oxalate, or L-tartrate. Acids produced from L-arabinose, D-fructose, D-galactose, D-glucose, *myo*-inositol, L-rhamnose, sucrose and D-xylose, but not from D-melezitose, melibiose, methyl α-D-glucoside, D-raffinose, D-ribose or L-sorbose. No growth in the presence of 2% NaCl. Antibiotic susceptibility (20 μg/ml): ampicillin, benzylpenicillin, cephalexin, novobiocin, and kanamycin.

For all other characteristics, refer to the genus description.

DNA G+C content (mol%): 70.0 (HPLC).

Type strain: JCM 9312, ATCC 51431, DSM 44128, NBRC 15622, IPV 2867, NCB 1173, VKM Ac-1992.

Sequence accession no. (16S rRNA gene): AB024701.

3. **Catenuloplanes niger** Tamura, Yokota, Huang, Hasegawa and Hatano 1995, 860[VP]

ni'ger. L. masc. adj. *niger* black, referring to the production of a black soluble pigment.

The morphological, physiological, and chemotaxonomic characteristics are the same as those given previously for *Catenuloplanes japonicus*. Additionally, *Catenuloplanes niger* grows at 37°C and produces a black soluble pigment in peptone-yeast extract-iron agar (ISP medium 6) and nutrient agar, but does not produce any pigment in inorganic salts-starch agar (ISP medium 4). Resistant to penicillin, cephaloridine, cephalexin, and benzylpenicillin.

For all other characteristics, refer to the genus description.

DNA G+C content (mol%): 72.2 (HPLC).

Type strain: N406-14, NBRC 14177, ATCC 31638, DSM 44711, JCM 9533, VKM Ac-1964.

Sequence accession no. (16S rRNA gene): AB523881.

4. **Catenuloplanes indicus** Tamura, Yokota, Huang, Hasegawa and Hatano 1995, 860[VP]

in'di.cus. L. masc. adj. *indicus*, of or pertaining to India, where the organism was isolated.

The morphological, physiological, and chemotaxonomic characteristics are the same as those given previously for *Catenuloplanes japonicus*. Additionally, *Catenuloplanes indicus* grows at 37°C and does not produce any soluble pigment in inorganic salts-starch agar (ISP medium 4), tyrosine agar (ISP medium 7), Bennett agar, and Bennett agar containing maltose. Susceptible to penicillin, cephaloridine, cephalexin, and benzylpenicillin.

For all other characteristics, refer to the genus description.

DNA G+C content (mol%): 71.3–71.9 (HPLC).

Type strain: RA328^T, NBRC 15575, IMSNU 22099, ATCC 700014, DSM 44709, JCM 9534, VKM Ac-1999.

Sequence accession no. (16S rRNA gene): AJ294717.

5. **Catenuloplanes atrovinosus** Tamura, Yokota, Huang, Hasegawa and Hatano 1995, 860[VP]

at.ro.vi.no'sus. L. adj. *ater -tra -trum* dark; L. masc. adj. *vinosus* full of wine; N.L. masc. adj. *atrovinosus*, full of dark wine, dark wine color (red).

The morphological, physiological, and chemotaxonomic characteristics are the same as those given previously for *Catenuloplanes japonicus*. Additionly, *Catenuloplanes atrovinosus* grows at 37°C and produces a black soluble pigment in peptone-yeast extract-iron agar (ISP medium 6) and a pale brown soluble pigment in nutrient agar, but does not produce any soluble pigment in Bennett agar and Bennett agar containing maltose. It forms reddish colonies on inorganic salts-starch agar (ISP medium 4) and glycerol-asparagine agar (ISP medium 5). Galactose, mannose, xylose, and glucose are present as whole-cell sugars. Resistant to penicillin, cephaloridine, cephalexin, and benzylpenicillin.

For all other characteristics, refer to the genus description.

DNA G+C content (mol%): 72.2–72.7 (HPLC).

Type strain: RA332^T, NBRC 15579, IMSNU 22012, ATCC 700015, DSM 44707, JCM 9535, VKM Ac-1972.

Sequence accession no. (16S rRNA gene): AJ294716.

6. **Catenuloplanes castaneus** Tamura, Yokota, Huang, Hasegawa and Hatano 1995, 860[VP]

cas.ta'ne.us. L. masc. adj. *castaneus* chestnut-colored.

The morphological, physiological, and chemotaxonomic characteristics are the same as those given previously for *Catenuloplanes japonicus*. Additionally, *Catenuloplanes castaneus*

grows at 37°C and produces a scarlet soluble pigment in glycerol-asparagine agar (ISP medium 5), but does not produce any soluble pigments in inorganic salts-starch agar (ISP medium 4), tyrosine agar (ISP medium 7), and Bennett agar. Resistant to penicillin and benzylpenicillin, but susceptible to cephaloridine and cephalexin.

For all other characteristics, refer to the genus description.

DNA G+C content (mol%): 72.0–72.4 (HPLC).

Type strain: RA344, NBRC 15584, ATCC 700016, DSM 44708, JCM 9537, VKM Ac-1973.

Sequence accession no. (16S rRNA gene): AB523883.

7. **Catenuloplanes nepalensis** Tamura, Yokota, Huang, Hasegawa and Hatano 1995, 860[VP]

ne.pal.en′sis. N.L. masc. adj. *nepalensis* of or pertaining to Nepal, where the organisms were isolated.

The morphological, physiological, and chemotaxonomic characteristics are the same as those given previously for *Catenuloplanes japonicus*. In addition, *Catenuloplanes nepalensis* does not produce a soluble pigment in inorganic salts-starch agar (ISP medium 4), tyrosine agar (ISP medium 7), Bennett agar, and Bennett agar containing maltose. Resistant to penicillin, cephaloridine, and benzylpenicillin, but susceptible to cephalexin.

For all other characteristics, refer to the genus description.

DNA G+C content (mol%): 71.1 (HPLC).

Type strain: RA343, NBRC 15583, ATCC 700017, DSM 44710, JCM 9536, VKM Ac-1996.

Sequence accession no. (16S rRNA gene): AB523882.

Genus VII. **Couchioplanes** Tamura, Nakagaito, Nishii, Hasegawa, Stackebrandt and Yokota 1994, 199[VP]

Tomohiko Tamura

Couch.i.o.pla′nes. N.L. masc. n. *Couchius* a personal name, referring to J.N. Couch (1896–1986), a mycologist who contributed to the taxonomy of the family *Actinoplanaceae*; Gr. masc. n. *planes* a wanderer; N.L. masc. n. *Couchioplanes* a wanderer organism of the family *Actinoplanaceae* named after J.N. Couch.

Gram-stain-positive bacterium producing fine, nonfragmenting, branching mycelia. Not acid-fast. Strictly aerobic. The spore chains and aerial mycelia often **aggregate into clusters resembling sporangia**, but true sporangia are not observed (Figure 214, Figure 215, Figure 216 and Figure 217). Aerial mycelia with short spore chains are **arranged in spirals** that have one to five turns and are hooked or rarely flexuous (Figure 214). Several spores are present per spore chain, and the spores are **oval to short rods** (0.5–0.9 × 1.0–1.5 µm) and smooth. Upon immersion in water or phosphate buffer including soil extract, **motile spores** are released from the spore chain, but in many instances motility begins over 30–60 min after spore release. **Polar flagella** are present in motile spores (Figure 217). Cell wall contains D-**glutamic acid**, D- **and** L-**serine, glycine**, L-**alanine, and** L-**lysine** (molar ratio approx. 1:1:1:1:1). Xylose, arabinose, and galactose are present in whole-cell hydrolysates. Phosphatidylglycerol and phosphatidylethanolamine are present, but phosphatidylcholine is absent. $C_{16:0}$ iso and $C_{17:0}$ anteiso are the major cellular fatty acids. The major menaquinone is MK-9(H_4); in addition, small amounts of MK-9(H_6), MK-9(H_8), and MK-9(H_2) are also present. The acyl type of the cell-wall polysaccharides is glycolyl. Mycolic acid is absent.

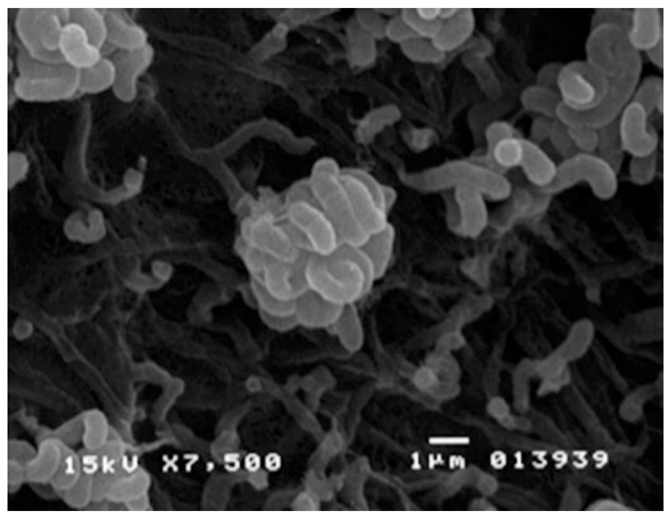

FIGURE 215. Scanning electron micrograph of *Couchioplanes caeruleus* subsp. *caeruleus* showing spore-chain clusters. Bar = 1 µm.

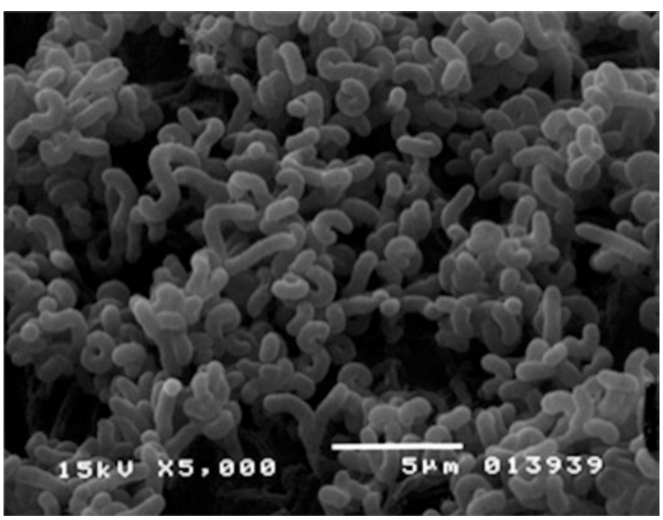

FIGURE 214. Scanning electron micrograph of *Couchioplanes caeruleus* subsp. *caeruleus* showing spiral, hook and/or rarely flexuous spore-chain forms. Bar = 5 µm.

DNA G+C content (mol%): 70–72 (HPLC).

Type species: **Couchioplanes caeruleus** (Horan and Brodsky 1986a) Tamura, Nakagaito, Nishii, Hasegawa, Stackebrandt and Yokota 1994, 200[VP] (*Actinoplanes caeruleus* Horan and Brodsky 1986a, 189).

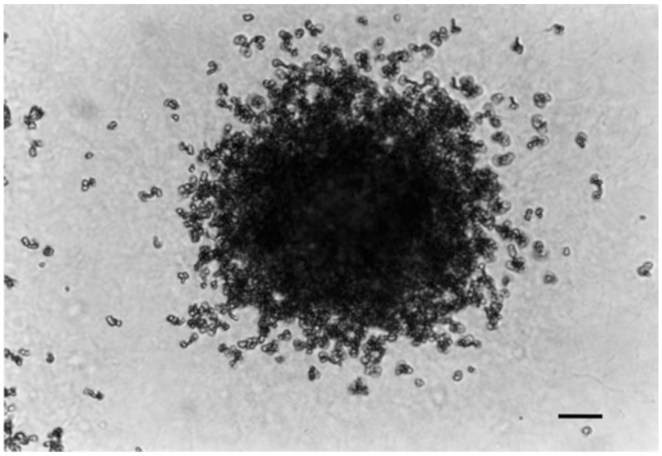

FIGURE 216. Light micrograph of the colony of *Couchioplanes caeruleus* subsp. *caeruleus*. Spore-chain cluster and aerial mycelium look like a sporangium. Bar = 10 µm.

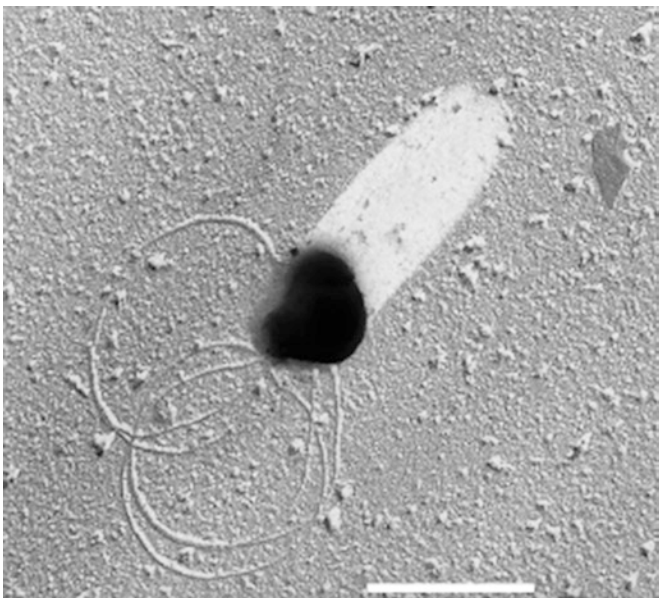

FIGURE 217. Transmission electron micrograph of a shadowing motile spore of *Couchioplanes caeruleus* subsp. *caeruleus* showing the polar flagellum. Bar = 1.0 µm.

Further descriptive information

In general, the vegetative mycelia of the strains are pale to yellowish orange in young cultures and change to a dark blue color in mature cultures. The aerial mycelia are white to gray. The zoospore exhibits active motility after incubation at 28°C for 1 h in 0.01 M phosphate buffer (pH 7.0) containing 10% soil extract. Using light microscopy (Figure 216), the physical appearance of spore chains and aerial mycelia may look like the sporangia of the genus *Actinoplanes*.

Good growth occurs at temperatures between 22 and 28°C. Grows well on yeast extract-malt extract agar, inorganic salts-starch agar, glycerol-asparagine agar, and Bennett's agar. Cultures grow well in yeast extract-glucose broth, consisting of yeast extract (1%) and D-glucose (1%) (pH 7.0), at 28°C on a rotary shaker for 4 d.

The wall chemotype is type VI according to the classification of Lechevalier and Lechevalier (1970a), and the peptidoglycan type is type A (most probably type A3α) according to the classification of Schleifer and Kandler (1972).

Enrichment and isolation procedures

The currently known strains of *Couchioplanes* have been isolated from soil. Generally, the enrichment and isolation methods for actinomycetes producing motile spores, such as centrifuge and capillary methods, may be applicable for strains of the genus *Couchioplanes*.

Maintenance procedures

Strains of the genus *Couchioplanes* are maintained by freezing in water containing 10–30% glycerol at −70°C. Lyophilization of suspensions in 10% skim milk +1% monosodium glutamate and L-drying in 0.01 M potassium phosphate buffer (pH 7.0) containing 3% monosodium glutamate (Sakane and Kuroshima, 1997) are also recommended for long-term preservation.

Differentiation of the genus *Couchioplanes* from other genera

The genera *Couchioplanes* and *Catenuloplanes* differ from other genera of the family *Micromonosporaceae* because their cell-wall peptidoglycan contains L-lysine instead of *meso*-diaminopimelate. These genera are also similar in the formation of motile arthrospores. However, the arthrospores of the genus *Couchioplanes* are oval to short rods with polar flagella, whereas the *Catenuloplanes* arthrospores are rods with peritrichous flagella. The genera *Couchioplanes* and *Catenuloplanes* also differ in their menaquinone systems [MK-9(H_4) vs MK-9(H_8), MK-10(H_8)], phospholipid types (PII vs PIII), and cellular fatty acids ($C_{16:0}$ iso and $C_{17:0}$ anteiso vs $C_{18:1}$, $C_{16:0}$ and $C_{17:0}$ anteiso) (Yokota et al., 1993).

Taxonomic comments

Although *Couchioplanes caeruleus* differs from other species of the genus *Actinoplanes* by forming a deep blue pigment in the vegetative mycelia, by the absence of diaminopimelic acid in its cell wall, by its ability to hydrolyze adenine and hypoxanthine, by its resistance to lysozyme, and by its inability to utilize L-arabinose, D-xylose, and succinate as sole carbon sources, Horan and Brodsky (1986a) included this organism in the genus *Actinoplanes* for the following reasons. It formed irregular to globose sporangia, which upon wetting released spherical to oval motile spores that were partially flagellated, and arabinose and xylose were present as diagnostic whole-cell sugars. However, Stackebrandt and Kroppenstedt (1987) reported that this organism should not be included in the genus *Actinoplanes* because of its peptidoglycan type. The results of numerical taxonomy studies of the genus *Actinoplanes* performed by Goodfellow et al. (1990b) also supported this conclusion.

Based on 16S rRNA gene sequence analysis, the closest neighbor of the genus *Couchioplanes* is the genus *Actinoplanes*, both of which belong to the family *Micromonosporaceae* of the order *Micromonosporales*.

List of species of the genus *Couchioplanes*

1. **Couchioplanes caeruleus** (Horan and Brodsky 1986a) Tamura, Nakagaito, Nishii, Hasegawa, Stackebrandt and Yokota 1994, 200[VP] (*Actinoplanes caeruleus* Horan and Brodsky 1986a, 189)

ca.e.ru′le.us. L. masc. adj. *caeruleus* dark blue, referring to the blue vegetative mycelial pigment.

A yellow to pale brownish soluble pigment is produced on peptone-yeast extract-iron agar. Hydrogen sulfide is produced. Reduces nitrate to nitrite. Gelatin liquefaction is positive. Hydrolyzes starch. Does not decompose calcium malate. Does not coagulate milk. Fructose, glucose, inositol, and sucrose are utilized as carbon sources, but arabinose, raffinose, and xylose are not.

Source: soil.

DNA G+C content (mol%): 70–72 (HPLC).

Type strain: SCC 1014, ATCC 33937, DSM 43634, NBRC 13939, JCM 3195, NRRL 5325, VKM Ac-1257.

Sequence accession no. (16S rRNA gene): D85479.

The species has subsequently been divided into subspecies.

1a. **Couchioplanes caeruleus subsp. caeruleus** (Horan and Brodsky 1986a) Tamura, Nakagaito, Nishii, Hasegawa, Stackebrandt and Yokota 1994, 201[VP] (*Actinoplanes caeruleus* Horan and Brodsky 1986a, 189)

A yellow to pale brownish soluble pigment is produced on glycerol-asparagine agar and glucose-asparagine agar, but this pigment is not produced on yeast extract-malt extract agar. Rhamnose and mannitol are utilized as carbon sources. A novel heptaene antifungal antibiotic is produced (Wagman et al., 1975), which has a broad spectrum of activity against pathogenic fungi (Wright et al., 1977). No growth occurs in the presence of 2% NaCl.

Source: soil.

DNA G+C content (mol%): 70 (HPLC).

Type strain: SCC 1014, ATCC 33937, DSM 43634, NBRC 13939, JCM 3195, NRRL 5325, VKM Ac-1257.

Sequence accession no. (16S rRNA gene): D85479.

1b. **Couchioplanes caeruleus subsp. azureus** Tamura, Nakagaito, Nishii, Hasegawa, Stackebrandt and Yokota 1994, 201[VP]

a.zu′re.us. N.L. masc. adj. *azureus* azure blue, referring to the blue vegetative mycelial pigment.

A yellow to pale brownish soluble pigment is produced on yeast extract-malt extract agar, but this pigment is not produced on glycerol-asparagine and glucose-asparagine agar. Growth occurs in the presence of 2% NaCl. Rhamnose is not utilized as a carbon source, and mannitol is weakly utilized. A mixture of antibiotics, which includes a number of macrocyclic lactones and depsipeptides, is produced (Celmer et al., 1977b). The individual compounds exhibit significant antibiotic activity. The crude antibiotic mixture or combinations of a pure macrocyclic lactone and a pure depsipeptide demonstrate marked synergistic antibiotic activity. These antibiotics act as growth promotants in chicks and swine and are effective in the treatment of swine dysentery. "*Actinoplanes azureus*" (Celmer et al., 1977b) is the basonym of this subspecies.

Source: soil.

DNA G+C content (mol%): 72 (HPLC).

Type strain: ATCC 31157, DSM 43900, NBRC 13993, JCM 3246, VKM Ac-2019.

Sequence accession no. (16S rRNA gene): D85478, X93202.

Genus VIII. **Dactylosporangium** Thiemann, Pagani and Beretta 1967, 43[AL]

Gernot Vobis

Dac.ty.lo.spo.ran′gi.um. Gr. n. *daktylos* finger; Gr. n. *spora* a seed, and in biology a spore; Gr. neut. n. *angeion* (L. translit. *angium*) vessel; N.L. neut. n. *Dactylosporangium* an organism with finger-shaped, spore-containing vessels (sporangia).

Finger-shaped to claviform sporangia (0.6–1.4 × 2.5–6.0 µm) are **formed on short sporangiophores on the substrate mycelium**. They develop singly or in clusters above the surface of the substrate. **Each sporangium contains a single row of normally three to four spores**. **The spores are oblong, ellipsoidal, ovoid, or slightly pyriform** (0.4–1.3 × 0.5–1.8 µm) and **motile** by means of a polarly inserted tuft of flagella. True aerial mycelium is not formed. Hyphae of the substrate mycelium are 0.5–1.0 µm in diameter, branched, and rarely septate. Large single spores, **globose bodies** (1.7–2.8 µm in diameter) are formed on short branches on substrate mycelium. Organisms are Gram-stain-positive and not acid-fast. The peptidoglycan of the cell walls contains ***meso*-diaminopimelic acid** (***meso*-DAP) and glycine, with xylose and arabinose** as characteristic sugars of whole-cell hydrolysates. Colonies grow on various agar media. They are compact, somewhat tough and leathery, and mostly flat or sometimes elevated with a smooth to slightly wrinkled surface. **The color of the substrate mycelium is pale orange to deep orange, rose or wine-colored to brown**. Aerobic, chemo-organotrophic, with optimum growth between 25 and 37°C and at pH 6.0–7.0.

DNA G+C content (mol%): 71–73 (T_m).

Type species: **Dactylosporangium aurantiacum** Thiemann, Pagani and Beretta 1967, 43[AL].

Further descriptive information

Phylogeny. The genus belongs to the family *Micromonosporaceae*. Based on 16S rRNA gene sequence analysis, it comprises six species with validly published names: *Dactylosporangium aurantiacum*, *Dactylosporangium fulvum*, *Dactylosporangium matsuzakiense*, *Dactylosporangium roseum*, *Dactylosporangium thailandense*,

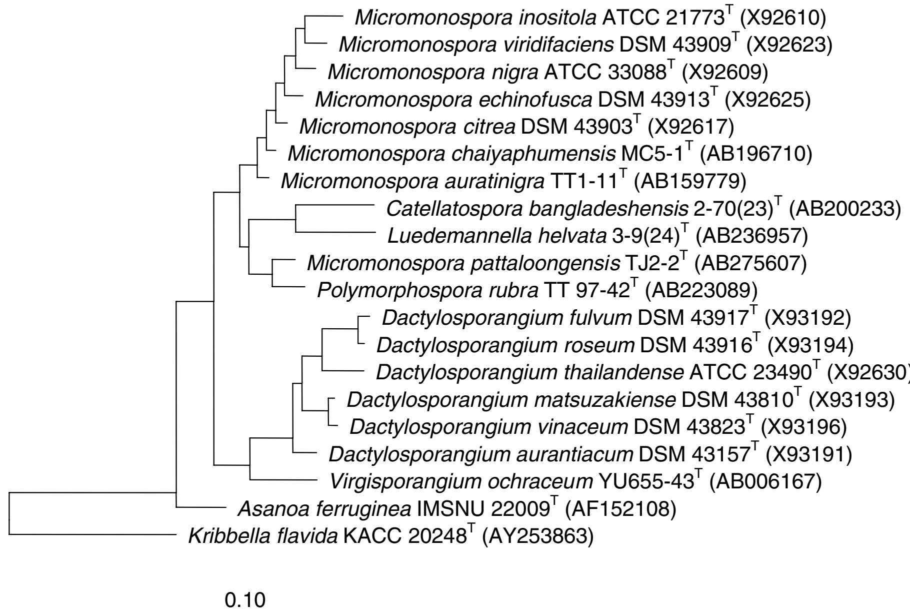

FIGURE 218. Phylogenetic tree showing the relationship of type strains of the genus *Dactylosporangium* and type strains of related genera of the family *Micromonosporaceae*. The tree was reconstructed with the maximum-likelihood method using the software environment ARB (Ludwig et al., 2004) and the corresponding SILVA SSURef 95 database (release July 2008; Pruesse et al., 2007). Bar = 0.10 substitutions per site.

and *Dactylosporangium vinaceum*. Sequence similarities between species range from 99.7 to 97.8%. The nearest genera of the family *Micromonosporaceae* are *Asanoa*, *Catellatospora*, *Luedemannella*, *Micromonospora*, *Polymorphospora*, and *Virgisporangium*, with sequence similarities between type strains of the genus *Dactylosporangium* and type strains of related genera ranging from 97.2 to 96.6% (genus *Polymorphospora*), 97.1 to 94.5% (genus *Micromonospora*), 97.1 to 95.7% (genus *Virgisporangium*), 96.8 to 96.1% (genus *Asanoa*), 96.5 to 94.4% (genus *Catellatospora*), and 96.5 to 95.4% (genus *Luedemannella*). A phylogenetic tree is shown in Figure 218.

Cell morphology and fine structure. The substrate hyphae are 0.5–1.0 µm in diameter and irregularly branched. They are rarely septate and do not separate into fragments either in agar or in liquid cultures. A true aerial mycelium is not formed; however, short hyphae in contact with the air are observed occasionally (Shomura et al., 1983b; Thiemann et al., 1967). *Dactylosporangium fulvum* develops rudimentary aerial mycelium (Shomura et al., 1986). The substrate hyphae may form coremia, also bearing sporangia and globose bodies (Shomura et al., 1986). The cell walls of the hyphae, the globose bodies, and the zoospores each consist of a single layer (Miyadoh et al., 1997; Vobis, 1987). The mesosomes are tubular-vesicular (Williams et al., 1973). Crystalline phage particles have been detected in the cytoplasm of the substrate hyphae of a strain of *Dactylosporangium thailandense* (Higgins and Lechevalier, 1969).

The oligosporous sporangia of the genus *Dactylosporangium* are formed on the surface of the colonies, singly or, more frequently, in tufts (Figure 219A). They are club- or finger-shaped, 0.6–1.4 µm in diameter, and 2.5–6.0 µm in length. The short sporangiophores are 0.5–1.5 µm long and usually branched (Figure 219B). Scanning and transmission electron micrographs have revealed a collar-like structure (Figure 219A, B) at the sporangiophore–sporangium juncture (Ensign, 1978; Sharples et al., 1974; Shomura et al., 1980; Vobis and Kothe, 1985). Each sporangium contains a single straight chain of three to four spores. A minimum of two spores and a maximum of five are produced (Shomura et al., 1980, 1983b, 1985; Thiemann, 1974; Thiemann et al., 1967). The formation of the sporangiospores corresponds to the scheme of spore formation as proposed for the sporangiate actinomycetes by Lechevalier and Holbert (1965). Inside a thin, expanding envelope, the unbranched sporogenous hypha grows up to the final length of the sporangium (Figure 219B). It is then divided into spore-shaped sections, which round off immediately (Miyadoh et al., 1997). The separating cross walls are double-layered (Vobis, 1989c; Vobis and Kothe, 1985). New sporangia are formed by lateral branches of the sporangiophore in a subterminal position at the base of an older sporangium (Figure 219B) (Ensign, 1978; Vobis and Kothe, 1985). Strains with abnormally long and branched sporangia are described by Thiemann (1970a).

The sporangiospores (zoospores) produced inside the sporangia have a smooth surface and are variable in shape, from oblong, ellipsoidal, and ovoid to slightly pyriform (Figure 219A, B, C). They measure 0.4–1.3 µm in diameter and 0.5–1.8 µm in length (Shomura et al., 1985; Shomura et al., 1980; Shomura et al., 1983b; Thiemann et al., 1967). Young spores are thin-walled and include various reserve substances in their cytoplasm (Vobis, 1987). The zoospores are motile by means of a polar tuft of flagella (Figure 219C) (Higgins et al., 1967; Lechevalier and Lechevalier, 1970; Shomura et al., 1985; Thiemann, 1974).

In addition to the zoospores, large globose bodies or aleuriospores of 1.7–2.8 µm in diameter are formed singly on substrate

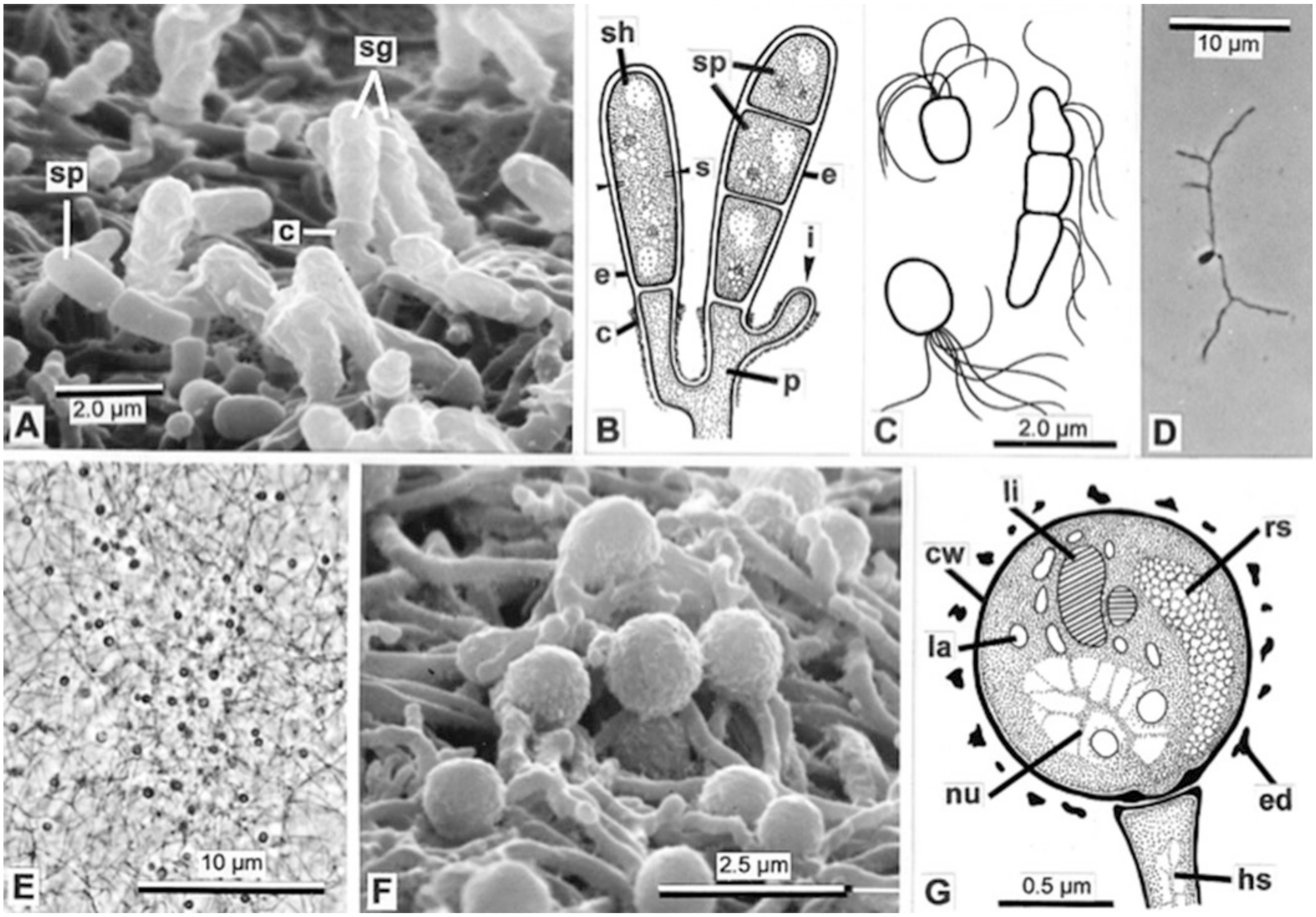

FIGURE 219. Morphological aspects of *Dactylosporangium*. A, Oligosporous sporangia (sg) with collars (c) and sporangiospores (sp) on the surface of the colony of *Dactylosporangium thailandense* (strain ATCC 23490[T]; SEM); B, scheme of sporangial development (c, collar; e, envelope; i, initium; p, sporangiophore; s, septum; sh, sporogenous hypha; sp, spore); C, flagellated sporangiospores (zoospores); D, germinated zoospore with hyphae developed after 36 h in distilled water (strain MB-VS 704; PHACO); E, globose bodies produced by substrate mycelium (strain MB-VS 699; PHACO); F, substrate hyphae and globose bodies, partially covered with granular deposits (strain A 1486; SEM); G, scheme of the ultrastructure of a globose body (cw, cell wall; ed, extracellular deposit; hs, hyphal stalk; la, light area; li, lamellate inclusion; nu, area of DNA; rs, reserve substance). Abbreviations: PHACO, phase contrast microscopy; SEM, scanning electron microscopy.

hyphae. They can be embedded in the agar or freely exposed on the surface (Figure 219E, F), but are also produced in liquid cultures (Thiemann et al., 1967). When observed by light microscopy using the phase-contrast technique (Figure 219E), they appear as refractile spores (Ensign, 1978; Vobis, 1992). The globose bodies contain nuclear material, large diffuse electron-transparent areas, smaller defined light areas, lamellated, protein-containing paracrystalline inclusions, and possible phage particles (Sharples and Williams, 1974). The deposit of electron-dense material, irregularly distributed and closely attached to the wall surface (Figure 219F, G), is conspicuous (Miyadoh et al., 1997).

Cell-wall composition. The peptidoglycan of the cell wall contains 3-hydroxy-DAP and/or *meso*-DAP and glycine, with xylose and arabinose as diagnostic sugars in whole-cell hydrolysates (Hasegawa et al., 1983; Lechevalier and Lechevalier, 1970b; Shomura et al., 1980, 1983b, 1985, 1986). The chemical composition of cell walls therefore conforms to chemotype II and sugar pattern D in the classification scheme of Lechevalier and Lechevalier (1970). *Dactylosporangium aurantiacum*, *Dactylosporangium thailandense*, and "*Dactylosporangium salmoneum*" possess cell-wall peptidoglycans of the *N*-glycolylmuramic acid type, exhibiting amounts of 74.6, 60.0, and 92.0 nmol glycolyl residues per mg dried bacterial cells, respectively (Uchida and Seino, 1997). An unknown sugar other than 3-*O*-methylrhamnose, which can be related to that found in species of *Catellatospora*, was detected in the type strains of *Dactylosporangium matsuzakiense* and *Dactylosporangium thailandense* (Asano et al., 1989b). This unknown sugar is probably identical to the deoxyhexose reported by Szaniszlo and Gooder (1967).

The polar lipid profile is characterized by the presence of diphosphatidylglycerol, phosphatidylethanolamine (PE), phosphatidylglycerol (PG), phosphatidylinositol, and phosphatidylinositol mannosides (Goodfellow et al., 1990b; Lechevalier et al., 1977). The presence of PG can be variable (Lechevalier et al., 1977). Further uncharacterized glycolipids may be present

and the type strain of *Dactylosporangium thailandense* is additionally characterized by two ninhydrin-positive, phosphate-positive lipids (Goodfellow et al., 1990b). Based upon the presence of PE and the absence of phosphatidylcholine and amino-containing phosphoglycolipid (GluNu) as diagnostic phospholipids, *Dactylosporangium* can be classified as having type II phospholipids of Lechevalier et al. (1981).

Fatty acids. Analysis of the fatty acids of the cytoplasmic membranes show that the principal components are branched iso- and anteiso-fatty acids (Kroppenstedt and Kutzner, 1978; Lechevalier et al., 1977). Cyclopropane and 10-methyl-branched fatty acids are not present (Kroppenstedt, 1979). In some strains, small amounts of unknown unsaturated fatty acids are found, which are assumed to have branched chains (Lechevalier et al., 1977). The type strains of *Dactylosporangium aurantiacum* and *Dactylosporangium thailandense* exhibit relatively high proportions of branched chain fatty acids, mainly iso-15 (4.1–15.8%), anteiso-15 (2.0–29.1%), iso-16 (6.2–18.3%), and anteiso-17 (4.4–14.2%), and smaller amounts of straight-chain constituents (13.7–32.9%) (Goodfellow et al., 1990b). The pattern of fatty acids corresponds to type 2d of the classification system of Kroppenstedt (1985).

The composition of the isoprenoid quinones is characterized by the possession of menaquinones with nine isoprene units (MK-9), whereas isoprenologues with ten units (MK-10) are absent. MK-9(H_2) and MK-9(H_4) are present in minor amounts, and MK-9(H_6) and MK-9(H_8) occupy predominant positions (Collins et al., 1984; Goodfellow et al., 1990b; Ruan et al., 1988). The presence of MK-9(H_4), MK-9(H_6), and MK-9(H_8) indicates that *Dactylosporangium* belongs to the menaquinone type 4b of the classification scheme of Kroppenstedt (1985).

Colonial characteristics. Colonies of *Dactylosporangium aurantiacum* are mostly flat with a smooth surface. The color of the substrate mycelium varies from whitish to pale orange to deep orange. The surface of the colonies of *Dactylosporangium thailandense* is usually smooth, but can be wrinkled on certain media. The substrate mycelium is light orange, amber, or brownish with a rose tinge (Thiemann et al., 1967). Colonies or *Dactylosporangium vinaceum* are compact, tough, and somewhat leathery; the mycelia are wine-colored to brown, depending on the medium (Shomura et al., 1983b). The colonial characteristics of *Dactylosporangium matsuzakiense* are similar to those of *Dactylosporangium vinaceum*; the substrate mycelium is orange (Shomura et al., 1983b). *Dactylosporangium roseum* has a rose-colored substrate mycelium on certain agar media (Shomura et al., 1985). Colonies of *Dactylosporangium fulvum* are yellowish brown (Shomura et al., 1986). Soluble pigments are produced by *Dactylosporangium vinaceum* (wine-colored to deep red), *Dactylosporangium thailandense* (amber to brown), and *Dactylosporangium matsuzakiense* (light brownish pink) (Shomura et al., 1985). Melanoid pigments are not produced (Table 196).

Antibiotic metabolites. The variety of the chemical structures of antibiotics isolated from *Dactylosporangium* strains indicates the great biosynthetic capability of this genus (Lancini and Lorenzetti, 1993). The aminoglycoside antibiotic dactimicin is produced by the type strains of *Dactylosporangium matsuzakiense* (SF-2052^T = ATCC 31570^T) and *Dactylosporangium vinaceum* (SF-2127^T = ATCC 35207^T) (Shomura et al., 1980; Shomura et al., 1983b). Dactimicin, a member of the pseudosaccharide group of antibiotics, is active against wide variety of Gram-stain-positive and Gram-stain-negative bacteria, including resistant strains with aminoglycoside-modifying enzymes (Omoto et al., 1987). *Dactylosporangium thailandense* strain G-367 produces the aminoglycoside antibiotics G-367-1 (2′-*N*-formylsisomicin) and G-367-2, a steric isomer of sisomicin, as well as sisomicin and gentamicin C1a and C2 (Fujii et al., 1982). Further aminoglycosides of the fortimicin antibiotic group are produced by *Dactylosporangium matsuzakiense* ATCC 31570^T (Dairi et al., 1992), and by the unidentified *Dactylosporangium* strain G 308. The antibiotic complex SF-2107 A-1, A_2, B and C, a member of the orthosomycin group, is produced by *Dactylosporangium roseum* strains SF-2107 (= ATCC 31744) and SF-2186^T (= NBRC 14352^T). The SF-2107 series substance is effective against Gram-stain-positive and Gram-stain-negative bacteria (Shomura et al., 1985).

Tiacumicins, a complex of 18-membered macrolide antibiotics, are isolated from strain AB718C-41 (= NRRL 18085), described as *Dactylosporangium aurantiacum* subsp. "*hamendensis*" (Theriault et al., 1987). The diarrhea-associated bacterium *Clostridium difficile* can be combated successfully *in vitro* and *in vivo* with tiacumicins B and C (Swanson et al., 1991).

The three strains FD 25647 (= ATCC 31222), FD 25712 (= ATCC 31223), and FD 25718 (= ATCC 718), determined as "*Dactylosporangium salmoneum*", produce the polycyclic ether antibiotic 44161, which is active against Gram-stain-positive bacteria, fungi, and protozoa, and promotes growth of animals. It exhibits potent anticoccidial activity (Celmer et al., 1978). The compound 44161 is identical to nigerimicin, which is also known as a herbicidal agent (Heisey and Putnam, 1990).

The tetracyclic antibiotic dactylocyclinone Sch 34164 (4α-hydroxy-8-methoxychlortetracycline) has been isolated from a culture of strain SCC 1695 (= ATCC 39499), determined as "*Dactylosporangium vescum*" (Patel et al., 1987). Sch 34164 (dactylocycline A) and dactylocycline B, isolated from strain SC 14051 (= ATCC 53693) have tested positive against tetracycline-resistant bacteria (Tymiak et al., 1993). Another polyketide-derived antibiotic, DK-7814-A (hydroxypurpuromycin), has been isolated from "*Dactylosporangium purpureum*" (Lancini and Lorenzetti, 1993). DK-7814-A, DK-7814-B, and DK-7814-CO are active against Gram-stain-positive bacteria and show antitumor activity.

The polypeptide compound capreomycin can also be obtained from strain D-409-5 (= ATCC 31203), originally described as "*Dactylosporangium variesporium*" (Tomita et al., 1977). Capreomycin is of primary interest for its use as an antituberculosis agent. Strain SF-2253 produces L-threo-β-hydroxyaspartic acid, an antibiotic useful against a wide spectrum of micro-organisms. This amino acid is also an inhibitor of glutamate uptake, frequently used in neurological studies (Alexander et al., 1997).

The type strain of *Dactylosporangium fulvum* (SF 2113^T = ATCC 43301^T) produces pyridomycin (Shomura et al., 1986), which is known as an antimycobacterial antibiotic (Maeda et al., 1953). The acidic substance SF 2185 is an antibiotic against plant pathogens, particularly the causal organisms of cucumber downy mildew and rice blast. The producing strain is SF-2185, determined as *Dactylosporangium aurantiacum* subsp. "*gifuense*" (Matsumoto et al., 1985). *Dactylosporangium aurantiacum* strain SANK 61299 produces the plant growth inhibitors streptol, A-79197-2 (disaccharide of streptol) and A-79197-3 (trisaccharide

TABLE 196. Diagnostic and physiological characteristics of the species of the genus *Dactylosporangium*[a]

Characteristic	*D. aurantiacum*	*D. fulvum*	*D. matsuzakiense*	*D. roseum*	*D. thailandense*	*D. vinaceum*
Morphological and colonial:						
Formation of globose bodies	+	+	–[c], v[g]	–[d], p[g]	+	+[b], v[g]
Formation of coremia	–	+	–	–	–	–
Color of substrate mycelium:						
Yellowish brown	–	+	–	–	–	–
Orange	+	–	+	–	–	–
Orange to brown	–	–	–	–	+	–
Wine to brown	–	–	–	–	–	+
Rose	–	–	–	+	–	–
Formation of diffusible pigment:						
Light brownish pink	–	v	v	–	–	–
Amber or brown	–	–	–	–	v	–
Wine to deep red	–	–	–	–	–	+
None	+	–	–	+	–	–
Production of melanoid pigment	–	–	–	–	–	–
Utilization of carbon sources:						
L-Arabinose	+	+	+	v	(+)	+
Dextrin	(+)	nd	nd	nd	(+)	nd
D-Dulcitol	–	nd	nd	nd	–	nd
D-Fructose	+	+	+	+	+	+
D-Galactose	+	nd	nd	nd	(+)	nd
D-Glucose	+	+	+	+	(+)	+
Glycerol	–	–	–	nd	–	–
i-Inositol	–	–	–	–	–	–
Inulin	(+)	nd	nd	nd	(+)	nd
Lactose	(+)	nd	nd	nd	(+)	nd
Maltose	(+)	nd	nd	nd	+	nd
D-Mannitol*	+	+	+	–	(+)	+
D-Mannose	+	nd	nd	nd	(+)	nd
Melibiose*	+	nd	–	nd	–	nd
Raffinose	(+)[e], –[b,c]	–	–	–	+[e], –[b,c]	–
L-Rhamnose*	(+)	–	+	–	(+)	+
D-Ribose*	–	nd	nd	nd	+	nd
D-Sorbitol	–	nd	nd	nd	–	nd
Sorbose	–	nd	nd	nd	nd	nd
Sucrose	(+)	+	+	+	+	+
D-Xylose	+	+	+	v	+	+
Degradation of:						
Calcium malate	–	nd	nd	nd	–	nd
Casein	+	+	nd	nd	+	nd
Cellulose	–	nd	nd	nd	–	nd
Esculin	nd	+	nd	nd	nd	nd
Gelatin	–[e], +[b,g]	–	–	+	+[e], –[b,g]	+
Hypoxanthine	nd	–	nd	nd	nd	nd
Starch*	+	+	+	–	+	+
Tyrosine	–	nd	nd	nd	+	nd
Xanthine	nd	–	nd	nd	nd	nd
Peptonization of milk	+[f], –[g]	–	–	–	+[f], –[g]	+
Coagulation of milk*	–	–	–	–	–	+
Reduction of nitrate*	+	+	–	+	–	–
Production of H_2S	+	nd	nd	nd	+	nd
Other physiological properties:						
Optimum pH for growth	6.0–7.0	nd	nd	nd	6.0–7.0	nd
Optimum temperature for growth, °C	28–37	26–34	25–37	28–37	28–37	25–37
Tolerance to NaCl, % (w/v)	3.0	3.0	<1.5	1.5	1.5	3.0

[a]v, Strain instability; p, poor; nd, not determined; (+), moderate. Characters of diagnostic value are marked with an asterisk. Deviations or incoherent data are indicated by superscript letters:

[b]Shomura et al. (1983b).

[c]Shomura et al. (1980).

[d]Shomura et al. (1985).

[e]Thiemann et al. (1967).

[f]Thiemann (1974).

[g]Shomura et al. (1986).

of streptol), which inhibit the germination of *Brassica rapa*. The streptol moiety seems to be important for herbicidal activity (Kizuka et al., 2002).

Life cycle. Members of the genus *Dactylosporangium* produce two kinds of spores. The zoospores, which do not seem to be dormant, may function to ensure dissemination of the organisms. The nonmotile globose bodies or aleuriospores, which are constitutively dormant, may function to provide insurance for surviving during long periods (Ensign, 1978).

The few-spored sporangia are developed by substrate mycelium in contact with the air, standing out permanently from the substrate. The short spore chains inside the sporangia are held together by the thin sporangial envelope. They may function as an "aerophilic" survival unit, easily distributed in the soil environment or by the wind. If the sporangia are immersed in water, the zoospores are released after a period of 10–60 min (Thiemann et al., 1967). A solution of soil extract promotes this process (Hayakawa et al., 2000). Spore release is probably initiated by the swelling of an intrasporangial matrix (Thiemann et al., 1967), or by swelling of the substance forming the collar at the base of the sporangium (Figure 219A, B). The short chain of sporangiospores is pressed upward and pushed through the apex of the sporangial envelope. High motility starts after a time lag of about 30 min when the spores first separate from one another (Shomura et al., 1980, 1983b; Thiemann et al., 1967). The cytoplasm of the spores includes reserve material (Vobis, 1987, 1992). Spores are able to swim for up to 30 h. Germination was reported after 24 h on agar medium (Thiemann et al., 1967). It occurs also in distilled water (Figure 219D). By a consequent production of new mycelium and sporangia, the typical "aero-aquatic" live cycle of an actinoplanete is formed (Cross, 1986; Vobis, 1987, 1992).

The globose bodies, which are formed by substrate hyphae, are in general submersed in the substrate, but can also develop very closely attached to the surface (Figure 219E, F). Like the zoospores, they contain reserve material (Figure 219G) (Vobis, 1992). Ensign (1978) demonstrated that the globose bodies can germinate in a 10% (w/v) yeast extract solution and concluded that they have the same function as true, nonmotile spores. Compared with the flagellated sporangiospores, the globose bodies have a different physiological behavior when treated with the chemical germicide benzethonium chloride (BC). After being exposed for 30 min at 30°C in 0.01% solution of BC, 51% of the globose bodies survived, whereas the zoospores of *Dactylosporangium* and *Actinoplanes* did not (Hayakawa, 2003). This is compatible with the ecological observation of Johnston and Cross (1976), namely that *Dactylosporangium* species can survive in lake sediments that contain no viable *Actinoplanes*. Cross (1986) concluded that *Dactylosporangium* has two different types of survival stages.

Cultural characteristics and physiology. *Dactylosporangium* species are aerobic and mesophilic. The optimum temperature for growth is between 25 and 37°C (Table 196). The species *Dactylosporangium aurantiacum*, *Dactylosporangium matsuzakiense*, and *Dactylosporangium roseum* tolerate temperatures up to 42°C; no growth occurs at 45°C (Shomura et al., 1986; Thiemann et al., 1967). Good vegetative growth occurs on various media, e.g. oatmeal agar, Bennett agar, Hickey–Tresner agar, nutrient agar, glucose-asparagine agar, sucrose-nitrate agar, sucrose-yeast extract agar, yeast extract-malt extract agar, and inorganic salt-starch agar. However, individual species grow well on only a small spectrum of these media (Shomura et al., 1980, 1983b, 1985, 1986; Thiemann et al., 1967).

For carbon utilization tests, a different basal media was employed because individual strains do not grow on a common basal medium. A modified M/40 medium of Magni and von Borstel was used for *Dactylosporangium aurantiacum*, which had to be additionally supplemented with vitamins for *Dactylosporangium thailandense* (Thiemann et al., 1967). For other species, carbohydrate utilization was determined on Luedemann–Brodsky basal medium (Shomura et al., 1986). The results of the carbon utilization tests are given in Table 196. *Dactylosporangium* can be characterized as a neutrophilic micro-organism, growing well at pH 6.0–7.0 (Thiemann et al., 1967). Species can grow on Luedemann agar medium containing concentrations of NaCl up to 3%, but do not tolerate a concentration of 4% (Shomura et al., 1986). The ability to degrade polymers of biological origin and other physiological characters are not sufficiently studied. The physiological characteristics are summarized in Table 196.

The development of sporangia depends on the agar media used. They can be formed after 2–3 d under favorable conditions (Ensign, 1978), although normally they are only evident after 5–15 d of incubation (Shomura et al., 1983b). Sporangial formation can be promoted by soil agar, calcium malate agar, and inorganic salts-starch agar (Shomura et al., 1986; Thiemann et al., 1967). Globose bodies appear to be produced mainly on complex agar media that promote the growth of substrate mycelium, but not the formation of sporangia (Ensign, 1978). *Dactylosporangium fulvum* develops globose bodies abundantly on glucose-asparagine agar and tyrosine agar (Shomura et al., 1986).

The physiological behavior of the flagellated sporangiospores has been demonstrated by their chemotactic responses towards chemoattractants (Hayakawa et al., 1991d). The studies were carried out by microcapillary assays and included the type strains of *Dactylosporangium thailandense*, *Dactylosporangium aurantiacum*, *Dactylosporangium vinaceum*, *Dactylosporangium matsuzakiense*, *Dactylosporangium roseum*, and *Dactylosporangium fulcrum*. γ-Collidine at a concentration of 100 mM was the most effective chemical attractant, followed by vanillin (10 or 100 mM), xylose (10 mM), and KCl (10 mM) (Hayakawa, 2003).

Phages. A poorly lytic bacteriophage was discovered in a strain of *Dactylosporangium thailandense* that did not infect other actinomycetes (Higgins and Lechevalier, 1969). Cross-infections with phages of various actinomycetes were not successful (Prauser, 1984; Willoughby et al., 1972).

Ecology. Members of the genus *Dactylosporangium* are distributed worldwide. Thiemann (1970a) reported that out of 454 soil samples from various parts of the world, 140 isolates of *Dactylosporangium* were obtained. They were present in sandy as well as in loamy soils. No correlation could be established between the type of soil, its pH (4.0–9.0), and the incidence of *Dactylosporangium*. A total of 33 strains could be isolated from different soil samples from Thailand, Brazil, and Argentina (Thiemann et al., 1967). Further strains could be isolated from soil of an uncultivated field of grass in Colombia, South America (Shearer, 1987), and from soil samples collected in tropical and subtropical regions in Yunnan, China (Xu et al., 1996).

The dactylocycline producing strain SCC 1695 (=ATCC 39449) was isolated from a soil sample collected in Zambia, Africa. We isolated strains MB-VS 699 und MB-VS 704, presented in Figure 219, from soil samples collected at Lüneburger Heide (Germany). Strain A 1486, together with two further undetermined *Dactylosporangium* strains, were isolated from a soil sample from the Nationalpark Taman Negawa, Malaysia (G. Vobis and J.M. Wink, unpublished results).

The species *Dactylosporangium fulvum*, *Dactylosporangium matsuzakiense*, *Dactylosporangium roseum*, and *Dactylosporangium vinaceum* all originated from soil samples collected at various localities in Japan (see "List of Species"). Hayakawa and Nonomura (1987a) obtained isolates from soil samples from vegetable and corn fields with pH ranging from 5.4 to 6.1, collected in different Prefectures (Nagano, Mie, Gunma, and Iwate) in Japan. Field soils seem to be the most fruitful sources for isolating *Dactylosporangium* strains, together with other diverse rare actinomycete taxa, but they were also isolated frequently from mountainous forest soils. Field, mountain grass-land, and rice paddy with soil of pH 6.0–7.0, organic matter content <5%, and immature brown humic acid <0.8 (Δlog K) are the characteristic soil habitats of *Dactylosporangium* (Hayakawa, 2003).

Only a few sources other than soil were successfully tested as natural substrates inhabited by *Dactylosporangium* or simply utilized as intermediate locations by its resistant structures like the globose bodies. Johnston and Cross (1976) isolated strains from the surface muds of two lakes of the English Lake District in Great Britain. *Dactylosporangium* strains were also found on plant debris (Lechevalier, 1981). Leaf litter in marsh water in New Jersey (USA) was used as substrate to isolate the antibiotic dactylocycline-producing strain SC 14051 (= ATCC 53693). More recently, Okazaki (2003) reported an antibiotic-producing strain of *Dactylosporangium aurantiacum* isolated from fresh plant leaves of *Cucubalus* sp.

Enrichment and isolation procedures

The details of the very effective isolation technique used by Thiemann et al. (1967) were never published. Strains of *Dactylosporangium* can be detected by isolation methods used for other genera of actinoplanetes, including *Micromonospora* (Vobis, 1992). They are characterized as slow-growing organisms, recognizable on poor nutritive agar media (Shomura et al., 1980). In general, the soil samples are pretreated, e.g. with dry heat, and dilutions are spread onto specific selective isolation media, which can be supplemented with antibiotic agents (Hayakawa and Nonomura, 1987a; Nonomura, 1984; Shearer, 1987; Xu et al., 1996). It seems that all these above-mentioned methods, including also the traditional chemotactic technique of Palleroni (1980), have only incidentally recovered *Dactylosporangium* species in soil.

More recently, Hayakawa (2003) summarized his own valuable experiences of isolating rare actinomycetes. To isolate selectively high numbers of strains of *Dactylosporangium* from soil, combinations of several techniques are recommended. At first, the soil samples are dried slowly at room temperature for a week, sieved, and ground slightly in a mortar (Nonomura and Ohara, 1969). After that, the samples can be pretreated physically with dry heat (120°C) for 1 h, followed by treatment with the chemical germicide BC (0.01 or 0.03%), exposed for 30 min at 30°C (Hayakawa et al., 1991a). Especially in the latter case, the globose bodies (aleuriospores) function as the surviving units.

The enrichment procedures profit by the release of zoospores from the sporangia in an aqueous environment. One possibility is an improved chemotactic method employing the capillary technique of Palleroni (1980), but using γ-collidine or vanillin (100 mM) as chemoattractant instead of the traditional 0.01 M KCl (Hayakawa et al., 1991d). A further enrichment method is named "rehydration and centrifugation" (RC) (Hayakawa et al., 2000). The samples are flooded with 10 mM phosphate buffer containing 1% soil extract at 30°C for 90 min. The fluid is centrifuged at 1500 × ***g*** for 20 min and the supernatant, containing actively swimming zoospores, is used for plating.

The preferable selective isolation medium is humic acid-vitamin (HV) agar, containing (per liter) 1.0 g humic acid, 0.02 g $CaCO_3$, 0.5 g NaH_2PO_4, 1.7 g KCl, 0.5 g $MgSO_4 \cdot 7H_2O$, 0.01 g $FeSO_4 \cdot 7H_2O$, 18.0 g agar, and B vitamins (0.5 mg each of thiamine·HCl, riboflavin, niacin, pyridoxin·HCl, inositol, Ca-pentothenate, *p*-aminobenzoic acid, and 0.25 mg biotin); pH 7.2 (Hayakawa and Nonomura, 1987b, 1987a). The HV agar is supplemented with nalidixic acid (20 mg/l) (Hayakawa et al., 1995; Hayakawa et al., 1991d; Nonomura and Hayakawa, 1988), and additionally with leucomycin or tunicamycin (20 mg/l or 30 mg/l) in the case of dry heat and BC pretreatment (Hayakawa et al., 1991a), or trimethoprim (20 mg/l) for the RC enrichment method (Hayakawa et al., 2000). Cycloheximide (50 mg/l) is also generally added as an antifungal compound. The plates are incubated at 30°C for 4–6 weeks.

An alternative source for isolation of *Dactylosporangium* was demonstrated by Okazaki (2003). Instead of soil, leaf samples can be investigated as a habitat for actinomycetes. Freshly picked leaves are cut into several pieces, rinsed with sterile water, and soaked in 70% ethanol for 1 min. They are then washed once more with sterile water, soaked in 1% NaClO for 3 min, and rinsed again with sterile water. After these treatments, the leaf pieces are incubated on 0.8% water agar for several weeks. Although the percentage of *Dactylosporangium* species recovered seems to be very low, a strain of particular interest based on its secondary metabolite production was isolated (Kizuka et al., 2002).

Maintenance procedures

Slant agar cultures can be stored for several weeks at room temperature or at 6°C. For long-term preservation, the strains can be lyophilized or maintained by other techniques recommended for the aerobic actinomycetes.

Differentiation of the genus *Dactylosporangium* from other related genera

The morphological characteristics of *Dactylosporangium*, namely the oligosporous sporangia with 2–5 flagellated sporangiospores and the typical globose bodies, both produced by substrate mycelium, are sufficient to differentiate the genus from other genera of the family *Micromonosporaceae* (Vobis, 1992). The newly described genera *Virgisporangium* (Tamura et al., 2001) and *Planosporangium* (Wiese et al., 2008) have similar sporangia, but small differences are observed (Table 197). Thiemann (1970a) observed *Dactylosporangium*-like strains with deviating morphologies of sporangia, resembling *Planosporangium* or *Virgisporangium* species, and also strains having short chains of nonmotile spores like *Catellatospora* species. He announced the description of new genera, which was never realized. The closely related genera *Dactylosporangium*, *Planosporangium*, *Virgisporangium*, *Catellatospora*,

TABLE 197. Characteristics differentiating *Dactylosporangium* from closely related genera forming either oligosporous sporangia with motile sporangiospores or single, nonmotile spores/globose bodies on substrate mycelium[a,b]

Characteristic	*Dactylosporangium*	*Planosporangium*	*Virgisporangium*	*Catellatospora*	*Asanoa*
Production of sporangia	+	+	+	–	–
Number of sporangiospores	2–5	3 or more	6 or more	–	–
Type of motility of sporangiospores	Tuft of flagella	Single polar flagella	nd	–	–
Production of single spores (0.7–1.5 µm in diameter)	–	+	–	–	–
Production of globose bodies (1.7–2.8 µm in diameter)	+[c]	–	–	+[b]	–
Production of short chains of nonmotile spores	–	–	–	+	+
Fatty acid type[d]	2d	3b	2d	nd	2d
Major menaquinones	MK-9(H_4, H_6, H_8)	MK-9(H_4), MK-10(H_4)	MK-10(H_4, H_6, H_8)	MK-9(H_4)	MK-10(H_6, H_8)

[a]Symbols: +, >85% positive; –, 0–15% positive; nd, not determined.

[b]Data compiled from Ara et al. (2008a), Asano and Kawamoto (1986), Lee and Hah (2002), Lee et al. (2000b), Tamura et al. (2001) and Wiese et al. (2008).

[c]Not confirmed with certainty for all species.

[d]According to the classification of Kroppenstedt (1985).

and *Asanoa* can be differentiated by a combination of morphological and chemical characteristics (Table 197).

Taxonomic comments

When the first two species of *Dactylosporangium* were described by Thiemann et al. (1967), the genus was classified in the family *Actinoplanaceae* (Couch, 1955a, 1955b), which comprised all sporangia-forming actinomycetes. Within this morphologically defined family, the three genera *Dactylosporangium*, *Planomonospora*, and *Planobispora* were characterized by having oligosporous sporangia and flagellated sporangiospores (Thiemann, 1970a). At the same time, it could be shown that the *Actinoplanaceae* could be divided into two groups, one characterized by cell-wall chemotype II and the other by chemotype III (Lechevalier and Lechevalier, 1970). By having cell-wall chemotype II, *Dactylosporangium* was closely related to the genus *Actinoplanes*, but could be plainly distinguished from other genera by morphological and chemotaxonomic characteristics (Vobis, 1989b, 1989c). The genus *Dactylosporangium* constantly kept its taxonomic position as the "good little brother" of *Actinoplanes* throughout all changes of classification concepts of generic and suprageneric ranks (Bland and Couch, 1981; Goodfellow, 1989; Goodfellow and Cross, 1984; Goodfellow et al., 1990b; Koch et al., 1996a; Miyadoh et al., 1997, 2001; Stackebrandt and Kroppenstedt, 1987; Stackebrandt et al., 1997; Zhi et al., 2009).

Differentiation of species of the genus *Dactylosporangium*

The species of *Dactylosporangium* have essentially the same morphological characteristics. They cannot be differentiated based on sporangia and zoospores, although globose bodies may or may not be present (Table 196). The formation of coremia is a special morphological characteristic for *Dactylosporangium fulvum* (Shomura et al., 1986).

Species differentiation is mainly based on the color of the substrate mycelium and on the production of soluble pigments. *Dactylosporangium aurantiacum* has orange substrate mycelium and does not produce pigment. *Dactylosporangium thailandense* has orange to brown mycelium and produces pigments on some media that are amber to brown with a reddish tinge (Thiemann, 1974; Thiemann et al., 1967). A wine-colored to deep red diffusible pigment characterizes *Dactylosporangium vinaceum* and the mycelium of this species is similarly colored. *Dactylosporangium matsuzakiense* has orange colonies and produces a light brownish-pink pigment on tyrosine agar (Shomura et al., 1983b). *Dactylosporangium roseum* is characterized by the rose color of its substrate mycelium (Shomura et al., 1985), and *Dactylosporangium fulvum* by a yellowish brown substrate mycelium and light brownish pink diffusible pigment (Shomura et al., 1986). *Dactylosporangium* species differ slightly in their patterns of carbon utilization, e.g. D-mannitol or L-rhamnose, and some physiological properties like degradation of starch and reduction of nitrate (Table 196).

List of species of the genus *Dactylosporangium*

1. **Dactylosporangium aurantiacum** Thiemann, Pagani and Beretta 1967, 43[AL]

au.ran.ti.a′cum. N.L. neut. adj. *aurantiacum* orange colored.

Sporangia (4.0–6.0 µm in length) develop on the surface of soil agar and on calcium malate agar. The zoospores, released 10–15 min after placing the sporangia in water, are oval, oblong, and slightly pyriform (1.5 µm long by 1.0 µm wide). They are extremely vigorous swimmers. Globose bodies are formed by substrate mycelium.

Colonies on agar media are mostly flat with a smooth surface. Abundant to good growth occurs on oatmeal agar and nutrient agar. The color of the substrate mycelium is pale

orange to orange. On Hickey–Tresner agar, growth is moderate with very pale mycelium. On tyrosine, nutrient, and skim milk agars, the colonies are orange to deep orange. Whitish colonies occur on glucose-asparagine agar and on starch agar. On glycerol-asparagine agar and on peptone-iron agar, the colonies are hyaline.

D-Mannitol, L-rhamnose, and melibose are utilized for growth; D-ribose is not. NaCl is tolerated up to 3% (w/v). Nitrate is reduced to nitrite. Litmus milk is not coagulated. No soluble pigments are produced on any media. The type strain shows the highest sequence similarity to *Dactylosporangium matsuzakiense* (98.9%) and *Dactylosporangium vinaceum* (98.6%). All other species show sequence similarities below 98.5%.

Source: the type strain was isolated from soil; no further information is given regarding its origin.

DNA G+C content (mol%): 73 (T_m).

Type strain: ATCC 23491, D-748, DSM 43157, NBRC 12592, JCM 3083, NRRL B-8111.

Sequence accession no. (16S rRNA gene): X93191.

2. **Dactylosporangium fulvum** Shomura, Amano, Yoshida and Kojima 1986, 169^{VP}

ful′vum. L. neut. adj. *fulvum* yellowish brown, referring to the color of the vegetative mycelium.

Sporangia (0.7–1.0 × 2.5–5.0 µm in size) with three to four spores in a single row are produced abundantly on sodium succinate agar, and moderately on calcium malate agar and inorganic salts-starch agar. Sporangiospores are motile. Globose bodies are formed abundantly on glucose-asparagine agar and tyrosine agar (ISP medium 5), and moderately on oatmeal agar, glycerol-asparagine agar and calcium malate agar. Coremia are formed abundantly on inorganic salts-starch agar and oatmeal agar, and moderately on yeast extract-malt extract agar and sucrose-yeast extract agar. Sporangia and globose bodies also develop directly from coremia.

The color of the substrate mycelium is yellowish brown and does not change when treated with alkaline or acid solutions. Except for a light brownish pink color on tyrosine agar, neither melanoid nor distinct diffusible pigments are not produced. Good growth occurs on sucrose-yeast extract agar, yeast extract-malt extract agar, and inorganic salts-starch (ISP medium 4) with light brown to cinnamon color. It grows moderately on Bennett agar, nutrient agar, tyrosine agar (ISP medium 7), oatmeal agar (ISP medium 3), and sucrose nitrate agar.

Decomposes casein and esculin; hydrolyzes starch. Nitrate is reduced to nitrite. Decomposition of xanthine and hypoxanthine, liquefaction of gelatin, and peptonization and coagulation of milk are negative. Optimum growth is between 26 and 34°C. Tolerates 3% NaCl, but no growth occurs on media containing more than 4% NaCl.

Produces pyridomycin. The type strain shows the highest sequence similarity to that of *Dactylosporangium roseum* (99.6%). The type strains of all other *Dactylosporangium* species show sequence similarities below 98.5%.

Source: the type strain was isolated from a soil sample collected in Chiba, Japan.

DNA G+C content (mol%): not determined.

Type strain: ATCC 43301, DSM 43917, NBRC 14381, JCM 5631, NRRL B-16292, SF-2113.

Sequence accession no. (16S rRNA gene): X93192.

3. **Dactylosporangium matsuzakiense** Shomura and Niida *in* Shomura, Kojima, Yoshida, Ito, Amano, Totsugawa, Niwa, Inouye, Ito and Niida 1983a, 672^{VP} (Effective publication: Shomura, Kojima, Yoshida, Ito, Amano, Totsugawa, Niwa, Inouye, Ito and Niida 1980, 928.)

mat.su.za.ki.en′se. N.L. neut. adj. *matsuzakiense* of or pertaining to Matsuzaki-cho, Izu Peninsula, Japan.

Finger-shaped sporangia are 4.0–6.0 × 0.9–1.4 µm in size, formed on short sporangiophores (0.5–1.0 µm), and occurring singly or in clusters. Each sporangium contains usually three spores, arranged in a single row. Sporangiospores are cylindrical to oblong (1.1–1.6 × 0.8–1.3 µm), motile. Abundant production of sporangia occurs on inorganic salts-starch agar, but is rare on Czapek, oatmeal, and tyrosine agars. Formation of globose bodies is not observed.

Colonies grow well on inorganic salts-starch agar, with a russet-orange substrate mycelium. Moderate growth occurs on Czapek agar and on yeast extract-malt extract agar, with an amber to light brown mycelium. Colonies grow moderately on glucose-asparagine agar and on oatmeal agar; the substrate mycelium is russet to orange. Poor growth occurs on glycerol-asparagine, nutrient, and calcium malate agars, the color of substrate mycelium varies from light yellow to light orange. Moderate growth occurs on tyrosine agar; the color of colonies is dusty orange to light brown and a light brownish-pink soluble pigment is produced.

D-Mannitol and L-rhamnose are utilized as sole carbon sources; melibiose is not. Growth occurs between 15 and 42°C. NaCl tolerance is lower than 1.5% (w/v). Gelatin is not liquefied; nitrate is not reduced. Skim milk is neither peptonized nor coagulated.

Produces the aminoglycoside antibiotic dactimicin. The type strain shows the highest sequence similarity to the type strains of *Dactylosporangium vinaceum* (99.7%) and *Dactylosporangium aurantiacum* (98.8%). The type strains of all other *Dactylosporangium* species show sequence similarities below 98.5%.

Source: the type strain was isolated from a soil sample collected at Matsuzaki-cho, Izu Peninsula, Japan.

DNA G+C content (mol%): not determined.

Type strain: ATCC 31570, DSM 43810, FERM-P 4670, NBRC 14259, JCM 3311, NRRL B-16293, SF-2052.

Sequence accession no. (16S rRNA gene): X93193.

4. **Dactylosporangium roseum** Shomura, Amano, Tohyama, Yoshida, Ito and Niida 1985, 4^{VP}

ro′se.um. L. neut. adj. *roseum* rose colored, pink.

Pod-shaped sporangia (2.5–5.5 × 0.8–1.1 µm), containing a single row of spores, are abundantly formed on chemically defined media such as sucrose-nitrate, glucose-asparagine, glycerol-asparagine, inorganic salts-starch, calcium malate, and tyrosine agars, but rarely on yeast extract-malt extract, nutrient, and Bennett agars. Sporangiospores are ellipsoidal (0.8–1.1 × 0.6–1.5 µm) and motile by means of polarly inserted flagella. The formation of globose bodies has not been observed.

Colonies grow well on sucrose-yeast extract agar, and moderately on yeast extract-malt extract, inorganic salts-starch, oatmeal, Bennett, and tyrosine agars. The typical rose color

of the substrate mycelium occurs on sucrose-yeast extract, inorganic salts-starch, and yeast extract-malt extract agars. On Bennett agar, the color of mycelium is pastel orange and on tyrosine agar it is yellowish. Colonies on sucrose-nitrate, glucose-asparagaine, glycerol-asparagine, calcium malate, oatmeal, and nutrient agars are colorless. Soluble pigments are not produced.

D-Mannitol and L-rhamnose are not utilized as sole carbon sources. NaCl is tolerated up to 1.5% (w/v). Growth occurs between 20 and 40°C; the temperature optimum ranges from 28 to 37°C. Nitrate is reduced to nitrite and gelatin is liquefied. Starch is not hydrolyzed; skim milk is neither peptonized nor coagulated. Melanoid pigments are not produced.

Produces antibiotic complex SF-2107, a member of orthosomycin group. The type strain shows the highest sequence similarity to the type strain of *Dactylosporangium fulvum* (99.6%). The type strains of all other *Dactylosporangium* species show sequence similarities below 98.5%.

Source: the type strain was isolated from a sample collected at Shizuoka, Japan; a further strain (SF-2107) was from soil collected at Yokohama, Japan.

DNA G+C content (mol%): not determined.

Type strain: DSM 43916, NBRC 14352, JCM 3364, NRRL B-16295, SF-2186.

Sequence accession no. (16S rRNA gene): X93194.

5. **Dactylosporangium thailandense** Thiemann, Pagani and Beretta 1967, 49[AL]

thai.lan.den′se. N.L. neut. adj. *thailandense* of or pertaining to Thailand. The correct Latin epithet of the species was proposed by Thiemann (1970b).

Abundant sporangial formation occurs on soil agar, calcium malate agar, and starch agar. Colonies grow on various agar media with a wrinkled or smooth surface. Good growth occurs on oatmeal agar with light orange-brown substrate mycelium, producing a light amber diffusible pigment. On Hickey–Tresner agar, growth is also good; the mycelium is brown with a rose tinge and the pigment is brown to reddish pink. Growth on glycerol-asparagine, glucose-asparagine, and starch agars is moderate, with a light orange to orange substrate mycelium. On nutrient agar, growth is good and the mycelium is pale orange.

D-Ribose, D-mannitol, and L-rhamnose are utilized for growth; melibiose is not. Tolerates up to 1.5% (w/v) NaCl. Tyrosine is hydrolyzed. Nitrate is not reduced to nitrite. Milk is not coagulated. No growth occurs at 42°C. Production of brown soluble pigments occurs on some media. Sequence similarities between the type strain and those of all other species of the genus are below 98.5%.

Source: the type strain was isolated from a soil sample collected in Thailand.

DNA G+C content (mol%): 71 (T_m).

Type strain: ATCC 23490, D-449, DSM 43158, NBRC 12593, JCM 3084.

Sequence accession no. (16S rRNA gene): X92630.

6. **Dactylosporangium vinaceum** Shomura, Yoshida, Miyadoh, Ito and Niida 1983b, 312[VP]

vi.na′ce.um. L. neut. adj. *vinaceum* of or belonging to wine, intended to mean wine-colored.

Finger-shaped sporangia (3.0–5.5 × 0.8–1.1 μm), produced singly or in tufts. Sporangia are occasionally apparent on Czapek and oatmeal agars, but less apparent on inorganic salts-starch and on calcium malate agar. Each sporangium contains three sporangiospores arranged in a single row. Spores are cylindrical to oblong (0.9–1.8 × 0.6–0.9 μm) and motile. Globose bodies are produced on glucose-asparagine and nutrient agars.

Colonies grow well on Czapek, glucose-asparagine, inorganic salts-starch, oatmeal, Bennett, and Hickey–Tresner agars. The color of substrate mycelium or reverse color ranges from light to dark wine-colored or occasionally ebony brown. Poor growth occurs on glycerol-asparagine agar with apricot substrate mycelium and no or very light wine-colored pigment production. Production of a wine red-colored diffusible pigment is conspicuous on various agar media, shaded from rose-wine to dark red or cherry. The pigments of the substrate mycelium and the soluble pigment are stable with changes in pH.

D-Mannitol and L-rhamnose are utilized as carbon source for growth. Gelatin and casein are hydrolyzed; milk is coagulated. Nitrate is not reduced to nitrite. NaCl concentrations of up to 3% (w/v) are tolerated.

Produces the pseudodisaccharide antibiotic dactimicin. The type strain shows the highest sequence similarity to the type strains of *Dactylosporangium matsuzakiense* (99.7%) and *Dactylosporangium aurantiacum* (98.6%). The type strains of all other *Dactylosporangium* species show sequence similarities below 98.5%.

Source: the type strain was isolated from a soil sample collected at Sekigahara, Gifu Prefecture, Japan.

DNA G+C content (mol%): not determined.

Type strain: ATCC 35207, DSM 43823, NBRC 14181, JCM 3307, NRRL B-16297, SF-2127.

Sequence accession no. (16S rRNA gene): X93196.

Species *incertae sedis*

1. **"Dactylosporangium salmoneum"** Routien *in* Celmer, Cullen, Moppett, Routien, Jefferson, Shibakawa and Tone 1978, 3 (U.S. Patent 4081532)

sal.mon′e.um. L. n. *salmon -onis* salmon; L. adj. suff. *-eus -a -um* suffix used with various meanings; N.L. neut. adj. *salmoneum* salmon-colored.

The species is not included in the Approved Lists of Bacterial Names and the name is not validly published in the *International Journal of Systematic Bacteriology*.

Finger-like sporangia (5.5–8.0 × 1.6 μm) containing 3–4 ellipsoidal to tear-drop shaped sporangiospores, develop abundantly on calcium malate agar. The color of the substrate mycelium is salmon, pale pink, orange, pink orange, pinkish, or creamish. Soluble pigments are not produced. No growth occurs on starch agar. Three polycyclic ether antibiotic-producing strains have been described as "*Dactylosporangium salmoneum*".

Source: the type strain was isolated from a soil sample collected in Japan.

Type strain: ATCC 31222, JCM 3272, Pfizer FD 25647.

2. **"Dactylosporangium variesporum"** Tomita, Kobaru, Hanada and Tsukiara 1977, 3 (U.S. Patent 4026766)

The species is not included in the Approved Lists of Bacterial Names and the name is not validly published in the *International Journal of Systematic Bacteriology*.

Finger shaped sporangia containing spores of various shapes. The substrate mycelium is orange to light reddish brown, creamy to light yellowish orange. A yellow pale brownish-to reddish-orange diffusible pigment is produced on various agar media. Glycerol, inositol, and D-ribose are utilized as sole carbon sources; L-rhamnose is not utilized. Nitrate is reduced to nitrite.

"*Dactylosporangium variesporum*" was established for a strain producing the antibiotic capreomycin.

Source: the type strain was isolated from a soil sample collected in India.

Type strain: ATCC 31203, Bristol Labs. D409-5, JCM 3273, NRRL B-16296, DSM 43911, NBRC 14104, KCC A-0273, NBRC 14104.

Further comment: "*Dactylosporangium variesporum*" differs in *N*-acyl type of muramyl residues of peptidoglycan from all other *Dactylosporangium* species in having acetyl. The taxonomic position is furthermore doubtful because of the lack of sporangiospore formation (Uchida and Seino, 1997).

Genus IX. **Krasilnikovia** Ara and Kudo 2007, 2449[VP] (Effective publication: Ara and Kudo 2007a, 6.)

Susmitha Seshadri

Kra.sil.ni.kov′i.a. N.L. fem. n. *Krasilnikovia* referring to N.A. Krasil'nikov, a Russian actinomycetologist who contributed to the taxonomy of the family *Micromonosporaceae*.

Aerobic with **branching hyphae**. **Gram-type positive**. Non-acid-fast. Pseudosporangia on short sporangiophores contain **oval to reniform, nonmotile spores** with a smooth surface. Growth occurs between **20–37°C** and **pH 5–9**. Did not grow on 3% NaCl. Cell wall contains ***meso*-diaminopimelic acid**. Major whole-cell sugars are galactose, mannose, xylose, arabinose, ribose, and glucose. The major menaquinone is **MK-9(H_6), and** the predominant fatty acids are **$C_{16:0}$ iso, $C_{14:0}$ iso** and **$C_{18:1}$ (ω9*c*)**. Phosphatidylethanolamine is the diagnostic phospholipid.

DNA G+C content (mol%): 71.

Type species: **Krasilnikovia cinnamomea** Ara and Kudo 2007, 2449[VP] (Effective publication: Ara and Kudo 2007a, 8.).

Further descriptive information

Isolated from sandy soils of a forest-side waterfall at Chokoria, Cox's Bazar, Bangladesh. Light microscopy revealed large, single or clustered spherical to irregularly shaped structures (~2.0–5.0 μm) on the substrate mycelium. These structures are pseudosporangia, i.e. lacking a sporangial wall, containing highly aggregated and hooked spore chains and substrate mycelium. The non-fragmenting substrate mycelium is light yellow to cinnamon in color. The acyl type of the cell-wall peptidoglycan is glycolyl. Most 16S rRNA gene nucleotide signatures of the family *Micromonosporaceae* are present except that a C is at position 222 instead of a U, a C–G pair is at positions 445:489 and 446:488 instead of a G–C pair, a U–A is at 1011:1018 instead of a C–G and G–U is at position 1263:1272 instead of A–U or C–G.

Enrichment and isolation procedures

Isolation was performed after 21 d of growth at 30°C on humic acid-vitamin (HV) agar supplemented with nalidixic acid (20 mg/l), nystatin and cycloheximide (each 50 mg/l). Strain 3-54(41), the type strain, was isolated by standard dilution plating and was further purified on yeast extract-malt extract agar.

Maintenance procedures

Routine subculturing is done on yeast-starch agar containing soluble starch (15.0 g); yeast extract (4.0 g); K_2HPO_4 (0.5 g); $MgSO_4 \cdot 7H_2O$ (0.5 g); and agar (15.0 g) in 1 liter of distilled water (pH 7.2).

Differentiation of the genus *Krasilnikovia* from closely related genera

16S rRNA gene sequence analysis showed that *Krasilnikovia* was 94.2–97.5% similar to the other genera in the family *Micromonosporaceae*. Phylogenetically, the closest relatives are *Couchioplanes* and *Actinoplanes globisporus*. *Krasilnikovia* lacks a true sporangium like *Couchioplanes*. However, its spores are nonmotile, unlike the motile spores of *Couchioplanes* and *Actinoplanes globisporus*. Also, *Krasilnikovia* differs from *Actinoplanes globisporus* in its menaquinone and whole cell sugar hydrolysate content. 16S rRNA gene signature sequences also differentiate this genus from phylogenetically closely related genera. Hence, on the basis of phylogenetic, phenotypic and chemotaxonomic studies, *Krasilnikovia* is classified as a novel genus in the family *Micromonosporaceae*.

List of species of the genus *Krasilnikovia*

1. **Krasilnikovia cinnamomea** Ara and Kudo 2007, 2449[VP] (Effective publication: Ara and Kudo 2007a, 8.)

cin.na.mo′mea. L. n. *cinnamomum* cinnamon; L. fem. suff. *-ea* suffix used with various meanings; N.L. fem. adj. *cinnamomea* cinnamon-colored.

The characteristics are as described for the genus with the following additional information. Spores are oval to short-rods (0.2–0.4 × 0.8–1.0 μm) and nonmotile. Growth is exhibited on glucose-asparagine agar, inorganic salts-starch agar, oatmeal-nitrate agar, sucrose-nitrate agar, yeast extract-starch agar, oatmeal agar, ISP medium 2, Bennett agar, Hickey–Tresner agar and glucose-yeast extract agar. Good sporulation is observed on glucose-asparagine agar, sucrose-nitrate agar and HV agar media. D-glucose, glycerol, D-xylose, D-galactose, D-fructose,

D-mannose, salicin and maltose are utilized, while adonitol, L-rhamnose, D-mannitol, α-D(+)melibiose, D-raffinose and trehalose are poorly utilized, and erythritol, L-arabinose, D-ribose, *myo*-inositol, α-methyl-D-glucoside and lactose are not utilized. In addition to the MK-9(H_6), small quantities of MK-9(H_4) and MK-9(H_8) are present. Saturated fatty acid $C_{18:0}$, $C_{17:0}$ 10 methyl, $C_{15:0}$ iso, $C_{15:0}$ anteiso, $C_{17:0}$ anteiso, and saturated $C_{16:0}$ are cellular fatty acids present in low amounts. Phospholipids present include phosphatidylethanolamine, diphosphatidylglycerol, phosphatidylglycerol, and phosphatidylinositol and phosphatidylinositol mannosides.

DNA G+C content (mol%): 71 (HPLC).

Type strain: 3-54(41), JCM 13252, MTCC 8094.

Sequence accession no. (16S rRNA gene): AB236956.

Genus X. **Longispora** Matsumoto, Takahashi, Shinose, Seino, Iwai and Ōmura 2003, 1558[VP]

Yōko Takahashi and Atsuko Matsumoto

Lon.gi.spo′ra. L. adj. *longus* long; Gr. fem. n. *spora* seed and in biology a spore; N.L. fem. n. *Longispora* long spore.

Leathery colonies. **Aerial hyphae bear long spore-chains of more than 20 spores on the tips of short sporophores** that branch from the vegetative mycelia. No fragmentation of vegetative mycelia. **No sporangia, synnemata, or sclerotia formed**. Cells are Gram-stain-positive, aerobic, non-acid-fast and **non-motile**. Cell-wall peptidoglycans contain ***meso*-diaminopimelic acid (DAP)**, glycine, and alanine. Arabinose, galactose, and xylose are detected in whole-cell hydrolysates. The acyl form of muramic acid in the peptidoglycans is **glycolyl**. Predominant menaquinones are **MK-10(H_4) and MK-10(H_6)**; MK-10(H_8) is a minor component. Mycolic acids are not detected. Diagnostic phospholipid is phosphatidylethanolamine (phospholipid type II). Mesophilic.

DNA G+C content (mol%): 70 (HPLC).

Type species: **Longispora albida** Matsumoto, Takahashi, Shinose, Seino, Iwai and Ōmura 2003, 1558[VP].

Further descriptive information

The almost-complete 16S rRNA gene sequence (1496 nt) [positions 10–1506, according to the *Escherichia coli* numbering system of Brosius et al. (1978)] of the type strain of *Longispora albida* has been determined. The phylogenetic tree based on 16S rRNA gene sequences revealed that the genus *Longispora* fell within the cluster of the family *Micromonosporaceae* and belonged to no other genera in the family (Figure 220). The pattern of the 16S rRNA gene signature nucleotides (Stackebrandt et al.,

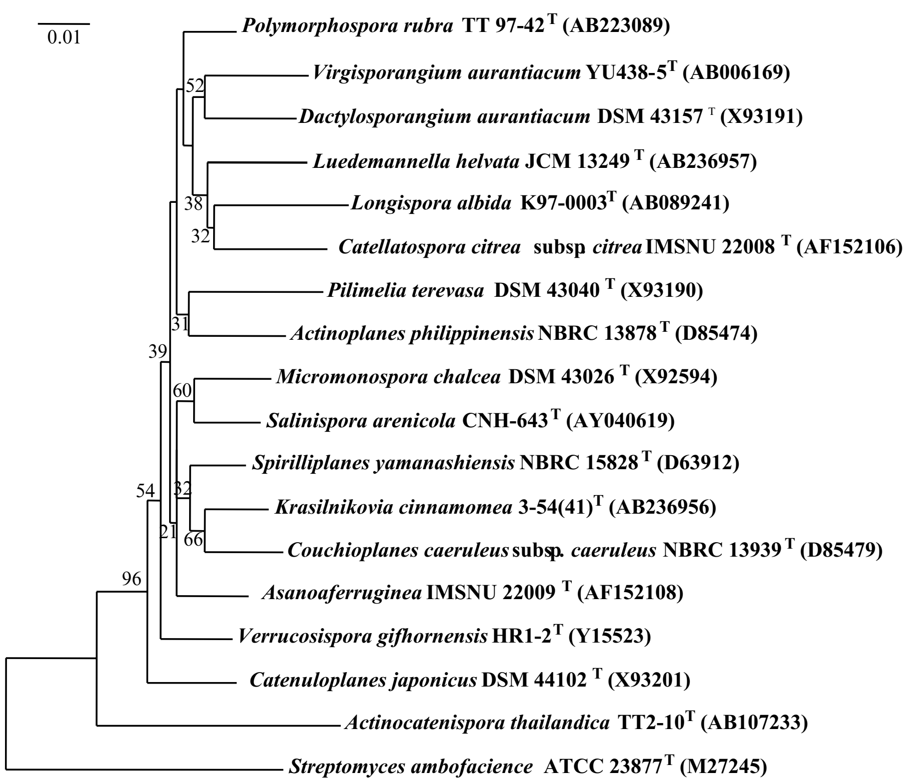

FIGURE 220. Phylogenetic tree based on 16S rRNA gene sequences of 17 genera in the family *Micromonosporaceae. Streptomyces ambofaciens* was used as an outgroup. Numbers at nodes indicate the level (%) of bootstrap support based on neighbor-joining analysis of 1000 resampled datasets. Only values >20% are shown. Bar, 1 nucleotide substitution per 100 nucleotides.

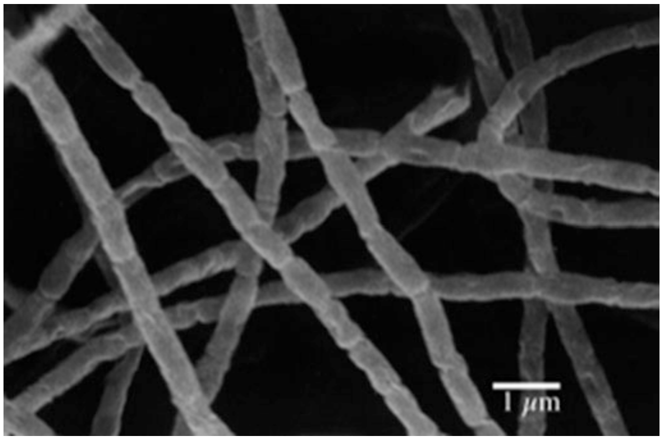

FIGURE 221. Scanning electron micrograph of spore-chains of *Longispora albida* K97-0003^{T} grown on 1/10 V8+$CaCl_2$ gellan gum medium for 20 d at 27°C. Bar = 1 μm.

TABLE 198. Aerial mycelium formation of *Longispora albida* on various media

	Solidifying agent			
	Agar		Gellan gum	
Medium	Growth	Aerial mycelium[a]	Growth	Aerial mycelium[a]
Yeast extract-malt extract (ISP 2)	+	$+^{t}$	++	$+^{t}$
Oatmeal (ISP 3)	+	−	+	−
Tyrosine (ISP 7)	+	−	+	+
Glucose-peptone	+	−	++	−
Nutrient	+	−	+	−
Water/proline	+	−	++	−
1/10 V8	+	−	+	+
1/10 V8 + $CaCl_2$	+	$+^{t}$	+	++

[a]Symbols: $+^{t}$, Trace aerial mycelium observed with a light microscope; +, poor aerial mycelium; ++, aerial mycelium produced (better than +).

1997) only differs from that of the family *Micromonosporaceae* (Koch et al., 1996a) at position 502 (A). The similarity values of the 16S rRNA gene with strains of other members of the family *Micromonosporaceae*, i.e. *Catellatospora citrea* subsp. *citrea*, *Virgisporangium aurantiacum*, *Asanoa ferruginea*, *Dactylosporangium aurantiacum*, *Pilimelia terevasa*, *Catenuloplanes japonicus*, *Verrucosispora gifhornensis*, *Spirilliplanes yamanashiensis*, *Micromonospora chalcea*, *Couchioplanes caeruleus* subsp. *caeruleus*, and *Actinoplanes philippinensis*, were 91.8–93.0%.

Short sporophores branch from the vegetative mycelia. Spore-chains from the sporophores have more than 20 spores per chain. Spores are cylindrical (Figure 221). Whirls, sclerotic granules, sporangia, and flagellated spores are not produced.

Longispora albida grows well on yeast extract/malt extract agar (ISP 2, Shirling and Gottlieb, 1966)*, oatmeal agar (ISP 3)†, peptone/yeast extract/iron agar (ISP 6)‡, and nutrient agar§, but aerial mycelia are not produced. When gellan gum is used as a solidifying agent, aerial mycelia grow slightly on some media. Spores are produced on 1/10 V8 + $CaCl_2$ gellan gum medium consisting of 2% V8 juice (Campbell's soup), 0.03% $CaCO_3$, 0.06% $CaCl_2 \cdot 2H_2O$ and 1.0% gellan gum in tap water (Table 198).

Enrichment and isolation procedures

Longispora albida was isolated from soil sample using water/proline/gellan gum medium consisting of 1% proline and 1% gellan gum in tap water. A feature of this medium is that gellan gum is used instead of agar as a solidifying agent. Samples (100 μl) from soil suspensions diluted with sterilized water were mixed with the water/proline/gellan gum medium kept at 55°C after sterilization at 121°C for 15 min in Petri dishes. The Petri dishes were incubated for 10 d at 27°C (Matsumoto et al., 2003). Although the recommended medium is water/proline/gellan gum medium, agar medium may be attempted for isolation of *Longispora* strains as *Longispora albida* grows on agar media.

Maintenance procedures

The organism can be maintained in the laboratory by transfer to the same media used for isolation. Viability of isolates during long-term storage is improved by addition of stabilizers. Recommended conditions are storage at −80°C or lyophilization in the presence of stabilizers such as 10% skim milk.

Differentiation of the genus *Longispora* from other genera

At the time of writing, the family *Micromonosporaceae* comprised 17 genera. Table 199 lists the characteristics that are useful for distinguishing *Longispora* from other genera in the family *Micromonosporaceae*. The phylogenetic tree revealed that *Longispora* branches within the family *Micromonosporaceae* (Figure 220). Although these genera have similar cultural, morphological, and chemotaxonomic characteristics, there are some differences. Genera that have spore-chains similar to those of *Longispora* are *Actinocatenispora* (Thawai et al., 2006a), *Asanoa* (Lee and Hah, 2002), *Catellatospora* (Asano and Kawamoto, 1986), *Polymorphospora* (Tamura et al., 2006), *Catenuloplanes* (Yokota et al., 1993), *Couchioplanes* (Tamura et al., 1994), and *Spirilliplanes* (Tamura et al., 1997). The latter three genera possess motile spores. The four genera that do not possess motile spores, *Actinocatenispora*, *Asanoa*, *Catellatospora*, and *Polymorphospora*, share this trait with *Longispora*. However, the genera *Asanoa* and *Catellatospora* form distinctive spore-chains that are borne directly from the vegetative hyphae growing on the surface of agar media and do not produce true aerial mycelia. *Actinocatenispora* and *Polymorphospora* contain MK-9 as major menaquinone.

*Yeast extract/malt extract agar: 0.4% yeast extract, 1.0% malt extract, 0.4% glucose, and 2.0% agar in distilled water, pH 7.3.

†Oatmeal agar. Cook 20 g oatmeal in 1.0 l distilled water for 20 min. Filter through gauze. Add distilled water to restore filtrate to 1.0 l, 1.0 ml of trace salts solution (consisting of 0.1 g $FeSO_4 \cdot 7H_2O$, 0.1 g $MnCl_2 \cdot 4H_2O$, 0.1 g, and $ZnSO_4 \cdot 7H_2O$ in 100 ml distilled water), and agar 18 g, pH 7.2.

‡Peptone/yeast extract/iron agar: 3.6% peptone iron agar, dehydrated (Difco), 0.1% yeast extract in distilled water, pH 7.0.

§Nutrient agar: 0.3% beef extract, 0.5% peptone, and 1.5% agar, pH 7.0.

TABLE 199. Differential characteristics of the genus *Longispora* and other genera in the family *Micromonosporaceae*[a,b]

Characteristic	*Longispora*	*Micromonospora*	*Actinocatenispora*	*Actinoplanes*	*Asanoa*	*Catellatospora*	*Catenuloplanes*	*Couchioplanes*	*Dactylosporangium*	*Krasilnikovia*	*Luedemannella*	*Pilimelia*	*Polymorphospora*	*Salinispora*	*Spirilliplanes*	*Verrucosispora*	*Virgisporangium*
Spore chain	+	−	+	−	+	+	+	+	−	−	−	−	+	−	+	−	−
Motile spore	−	−	−	+	−	−	+	+	+	−	−	−	−	−	+	−	+
Diamino acid	*meso*-DAP	*meso*-DAP	*meso*-DAP	*meso*-DAP	*meso*-DAP	*meso*-DAP	L-Lys	L-Lys	*meso*-DAP	*meso*-DAP	*meso*-DAP	*meso*-DAP	*meso*-DAP	*meso*-DAP	*meso*-DAP	*meso*-DAP	*meso*-DAP
Major menaquinones	MK-10($H_{4,6}$)	MK-10($H_{4,6}$), 9($H_{4,6}$)	9($H_{4,6}$)	MK-9(H_4)	MK-10($H_{6,8}$)	MK-9 ($H_{4,6}$) or 10(H_4)	MK-10(H_4), 11(H_4)	MK-9(H_4)	MK-9($H_{6,8}$)	MK-9 ($H_{6,4,8}$)	MK-9 ($H_{6,4,2,8}$)	MK-9($H_{4,2}$)	MK-10 ($H_{6,4}$), 9($H_{6,4}$)	MK-9(H_4)	MK-10(H_4)	MK-9(H_4)	MK-10($H_{4,6}$)
Phospholipid type[c]	PII	PII	PII	PII	PII	PII	PIII	PII	PII	PII	PII	PII	PII	PII	PII	PII	PII
Characteristic whole-cell sugars	Ara, Gal, Xyl	Ara, Xyl	Ara, Gal, Xyl	Ara, Xyl	Ara, Gal, Xyl	Ara, Gal, Xyl, or Xyl	Xyl	Ara, Gal, Xyl	Ara, Xyl	Gal, Man, Xyl, Ara, Rib,	Xyl, Gal, Man, Rham, Rib, Ara	Ara, Xyl	Xyl	Ara, Gal, Xyl	Ara, Xyl	Man, Xyl	Ara, Gal, Xyl
DNA G+C content (mol%)	70	71–72	72	72–73	71–72	71–73	70–72	69–73	71–73	71	71	nd	71	70–73	69	70	71

[a]Symbols: +, >85% positive; −, 0–15% positive; nd, not determined.

[b]Data from Matsumoto et al. (2003), Goodfellow et al. (1990b), Stackebrandt and Kroppenstedt (1987), Yokota et al. (1993), Vobis (1989b), Lee et al. (2000b), Lee and Hah (2002), Kudo et al. (1999), Tamura et al. (1994, 1997, 2001, 2006). Maldonado et al. (2005), Thawai et al. (2006a), Ara and Kudo (2007a, 2007d), and Rheims et al. (1998).

[c]According to the classification of Lechevalier et al. (1977).

List of species of the genus *Longispora*

1. **Longispora albida** Matsumoto, Takahashi, Shinose, Seino, Iwai and Ōmura 2003, 1558[VP]

al.bi′da. L. fem. adj. *albida* somewhat white.

Longispora albida grows on yeast extract/malt extract agar, oatmeal agar, peptone/yeast extract/iron agar, and nutrient agar, and the color of vegetative mycelium is light ivory to cream. Growth is better on gellan gum media than on agar media. Although aerial mycelia are not produced on these agar media, when gellan gum is used as a solidifying agent instead of agar, aerial mycelia grow on some media. Spores are well-produced on 1/10 V8 gellan gum medium containing $CaCl_2$ (Table 198). Spores are cylindrical (0.4–0.5 × 1.0–1.4 µm) and have a smooth surface (Figure 221). The temperature range for growth is 12–37°C (optimum range is 21–33°C). Growth occurs at pH 6–9. Melanoid pigment is not produced. Positive for nitrate reduction, gelatin liquefaction, milk coagulation, and milk peptonization. D-Glucose is utilized, but L-arabinose, D-fructose, *myo*-inositol, D-mannitol, melibiose, raffinose, L-rhamnose, sucrose, and D-xylose are not utilized. Cellulose is not decomposed. No growth occurs in the presence of 2% NaCl. The predominant components of the cellular fatty acids are $C_{16:0}$ iso, $C_{17:1}$, and $C_{18:1}$. Produces actinohivin, a novel antibiotic active against human immunodeficiency virus (Chiba et al., 2001).

Source: soil.

DNA G+C content (mol%): 70 (HPLC).

Type strain: K97-0003, NRRL B-24201, JCM 11711.

Sequence accession no. (16S rRNA gene): AB089241.

Acknowledgements

We thank Satoshi Ōmura, Akio Seino, and Yuzuru Iwai for their kind suggestions, and Ismet Ara for help with making phylogenetic tree.

Further reading

Suzuki, S., K. Takahashi, T. Okuda and S. Komatsubara. 1998. Selective isolation of *Actinobispora* on gellan gum plate. Can. J. Microbiol. *44*: 1–5.

Genus XI. **Luedemannella** Ara and Kudo 2007c, 1372[VP]

The Editorial Board

Lu.e.de.man.nel′la. N.L. fem. dim. n. *Luedemannella* here refering to George M. Luedemann, a Russian actinomycetologist who contributed to the taxonomy of family *Micromonosporaceae*.

Single or clustered spherical to irregular sporangia (variable in size, ~3.0–5.0 µm) with branched hyphae, forms a non-fragmenting substrate mycelium. **Gram-stain-positive**. Nonmotile. **Aerobic**. Forms nonmotile spherical to oval shaped spores (0.2–0.4 µm) with a smooth surface and loosely arranged in sporogenous hyphae. Temperature range for growth 20–37°C, pH range 5–9, NaCl tolerance <3%. Cell wall contains the diamino acid *meso*-diaminopimelic acid. The *N*-acyl group of muramic acid is glycolyl. Mycolic acids are not present. MK-9(H_6) and MK-9(H_4) are the major menaquinones, and small amounts of MK-9(H_2) and MK-9(H_8) are present. Polar lipid profile comprises phosphatidylethanolamine, diphosphatidylglycerol, phosphatidylglycerol, phosphatidylinositol and phosphatidylinositol mannosides.

DNA G+C content (mol%): 71.

Type species: **Luedemannella helvata** Ara and Kudo 2007c, 1372[VP].

Further descriptive information

Diagnostic whole-cell sugars are galactose, mannose, xylose and rhamnose as well as small amounts of ribose and arabinose (sugar pattern is D). Major fatty acids are $C_{17:0}$ anteiso (30.0–38.0%), $C_{15:0}$ anteiso (12.5–14.0%), $C_{16:0}$ iso (10.0–15.0%) and $C_{15:0}$ iso (10.1–12.0%) (fatty acid type 2d).

Phylogenetic analysis of the 16S rRNA gene positions the genus within the family *Micromonosporaceae*. The closest phylogenetic neighbor is *Micromonospora pattaloongensis* (96.7% sequence similarity) (Thawai et al., 2008). An environmental clone with high 16S rRNA gene sequence similarity has been detected in undisturbed tallgrass prairie soil in central Oklahoma (97.3%, accession no. FJ479324; Youssef et al., 2009).

Enrichment and isolation procedures

Strains 3-9(24)[T], 3-21(27) and 7-40(26)[T] were isolated from sandy soil collected in Chokoria and Cox's Bazar, Bangladesh. Serial diluents of the soil were transferred onto humic acid-vitamin agar (HV) (Hayakawa and Nonomura, 1987a) supplemented (per liter) with 50 mg cycloheximide, 50 mg nystatin and 20 mg nalidixic acid. Plates were incubated for 21 d at 30°C, then strains were transferred and purified on ISP medium 2 (yeast extract-malt extract agar, medium 2 of the International *Streptomyces* Project) (Shirling and Gottlieb, 1966).

Maintenance procedures

Working cultures are maintained on JCM medium 61 (per liter: 15.0 g soluble starch, 4.0 g yeast extract, 0.5 g K_2HPO_4, 0.5 g $MgSO_4 \cdot 7H_2O$, and 15.0 g agar; pH 7.2).

Differentiation of the genus *Luedemannella* from closely related genera

Possession of major menaquinones MK-9(H_6) and MK-9(H_4) as well small amounts of MK-9(H_2) and MK-9(H_8) distinguishes *Luedemannella* is from other phylogenetically related genera, which possess a variety of different menaquinone profiles: *Longispora*, MK-10(H_4) and MK-10(H_6); *Micromonospora*, MK-10(H_4), MK-10(H_6), MK-9(H_4) and MK-9(H_6); *Salinispora*, MK-9(H_4); *Actinocatenispora*, MK-9(H_4) and MK-9(H_6); *Polymorphospora*, MK-10(H_6), MK-10(H_4), MK-9(H_6) and MK-9(H_4); *Actinoplanes*,

MK-9(H_4) and MK-10(H_4); *Asanoa*, MK-10(H_6) and MK-10(H_8); *Catellatospora*, MK-9(H_4) and MK-9(H_6) or MK-10(H_4) and MK-10(H_6); *Catenuloplanes*, MK-9(H_8) and MK-10(H_8); *Couchioplanes*, MK-9(H_4); *Dactylosporangium*, MK-9(H_4), MK-9(H_6), and MK-9(H_8); *Pilimelia*, MK-9(H_4) and MK-9(H_2); *Spirilliplanes*, MK-10(H_4); *Verrucosispora*, MK-9(H_4); *Virgisporangium*, MK-10(H_4), MK-10(H_6), and MK-10(H_8); *Plantactinospora*, MK-10(H_6), MK-10(H_8), and MK-10(H_4); *Planosporangium*, MK-9(H_4) and MK-10(H_4); *Pseudosporangium*, MK-9(H_6). *Luedemannella* is also differentiated from other genera within the family by the presence of diagnostic whole-cell sugars including galactose, mannose, xylose and rhamnose along with small amounts of ribose and arabinose. *Luedemannella* possess all family-specific nucleotide signatures of 16S rRNA gene, except for the "U–A" pair at position 139:224, "A" at position 381, the "A/G–C" pair at position 656:750 and the C–U pair at position 999:1041.

List of species of the genus *Luedemannella*

1. **Luedemannella helvata** Ara and Kudo 2007c, 1372[VP]

hel.va′ta. N.L. fem. adj. *helvata* honey yellow, referring to the color of the substrate mycelium.

Vegetative mycelia are shell to melon yellow in color and aerial mycelia are not present. D-glucose, L-arabinose, maltose, and trehalose are utilized. D-Xylose, lactose, sucrose and D-raffinose are moderately utilized; erythritol, D-galactose and salicin are poorly utilized. Growth is not affected by glycerol, adonitol, D-ribose, D-fructose, D-mannose, L-rhamnose, *myo*-inositol, D-mannitol, α-methyl-D-glucoside, and α-D-melibiose. Temperature range for growth is 20–37°C, pH range is 5–9, NaCl tolerance is <3%. Grows well on yeast-extract-malt extract agar, Bennett agar, and glucose yeast extract agar. Moderate growth on ISP media 4 and 3, Hickey–Tresner agar, sucrose-nitrate agar, yeast extract-starch agar (JCM medium 61), oatmeal-nitrate agar, 1/5 yeast extract-starch agar (JCM medium 202), sucrose-beef extract agar, ISP medium 1, and 1/20 V8 juice agar. Melanin pigment production on ISP medium 7 is negative. Abundant sporulation occurs on nutrient agar, sucrose-nitrate agar, yeast extract-starch agar, 1/5 yeast extract-starch agar, and 1/20 V8 juice agar, and moderate sporulation occurs on glucose-asparagine agar, glycerol-asparagine agar, tyrosine agar, yeast extract-malt extract agar, oatmeal agar, Bennett agar, glucose-yeast extract agar, Hickey–Tresner agar, tap water agar, and oatmeal-nitrate agar.

For all other characteristics, refer to the genus description.

DNA G+C content (mol%): 71 (HPLC).

Type strain: 3-9(24), JCM 13249, MTCC 8091.

Sequence accession no. (16S rRNA gene): AB236957.

2. **Luedemannella flava** Ara and Kudo 2007c, 1372[VP]

fla′va. L. fem. adj. *flava* yellow, referring to the color of the substrate mycelium.

Vegetative mycelia are cream yellow to wheat yellow in color and aerial mycelia are not present. D-glucose, L-arabinose, maltose, trehalose, D-xylose, D-galactose, D-mannose, L-rhamnose, salicin, lactose, α-D-melibiose, and sucrose are utilized. Glycerol, D-ribose, D-fructose, *myo*-inositol, D-mannitol, and α-methyl-D-glucoside are poorly utilized. Growth is not affected by erythritol, adonitol, and D-raffinose. Temperature range for growth 20–30°C, pH range 6–9, NaCl tolerance <2%. Grows well on oatmeal agar (ISP medium 3), Bennett agar, glucose-yeast extract agar, Hickey–Tresner agar, yeast extract-starch agar (JCM medium 61) and 1/5 yeast extract-starch agar (JCM medium 202). Moderate growth on yeast extract-malt extract agar and oatmeal-nitrate agar. Melanin pigment production on ISP medium 7 is negative. Abundant sporulation occurs on glucose-asparagine agar, ISP media 5 and 4, tap water agar, and 1/5 yeast extract-starch agar; moderate sporulation on ISP medium 7, glucose-yeast extract agar, Hickey–Tresner agar, sucrose-nitrate agar, and oatmeal-nitrate agar; and no sporulation on nutrient agar, yeast extract-malt extract agar, ISP medium 3, Bennett agar, or yeast extract-starch agar.

For all other characteristics, refer to the genus description.

DNA G+C content (mol%): 71 (HPLC).

Type strain: 7-40(26), JCM 13250, MTCC 8095.

Sequence accession no. (16S rRNA gene): AB236959.

Genus XII. **Pilimelia** Kane 1966, 225[AL]

Gernot Vobis and Peter Kämpfer

Pi.li.mel′i.a. L. n. *pilus* a hair; Gr. fem. n. *melia* Melia, a nymph loved by the river god Inachus; N.L. fem. n. *Pilimelia* an aquatic organism growing on hair substrate.

Members of the genus produce substrate mycelium. Hyphae are Gram-stain-positive, 0.2–0.8 µm in diameter, branched, and septate. True aerial mycelium is not developed. **Sporangia** are produced **on the surface of the substrate** on sporangiophores. The shape of sporangia is **spherical, ovoid, pyriform, campanulate, or cylindrical**, approximately 10–15 µm in size. Sporangia contain **numerous spores in chains** that are arranged in parallel or irregularly in swirl-like rows. **Spores (zoospores)** are **rod-shaped** (0.4 × 1.2 µm) and **motile** by means of a **laterally inserted tuft of flagella**. Nonmotile spores develop in free chains arranged similarly to the zoospores. Colonies grow only on complex media and are small, compact, soft, pasty, or

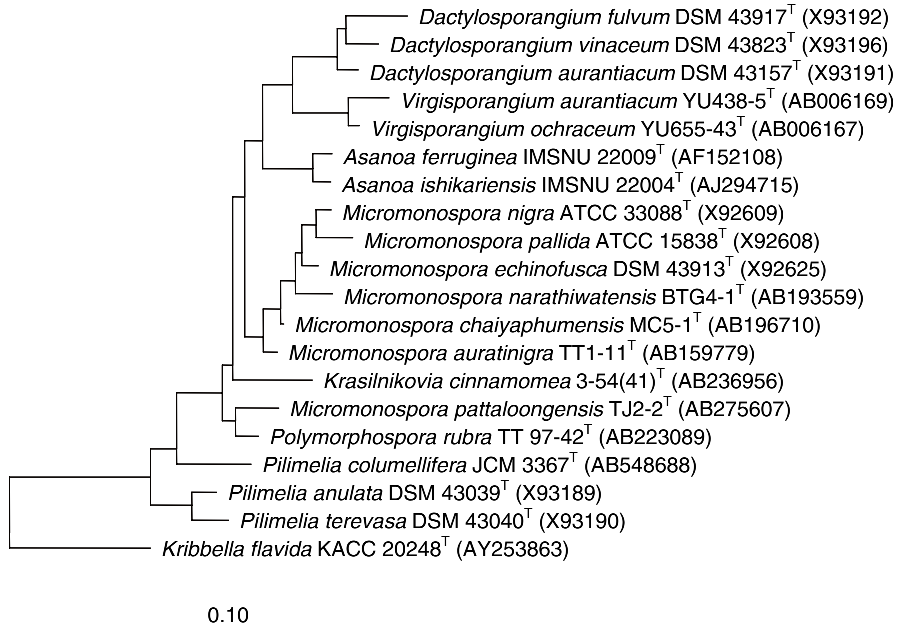

FIGURE 222. Phylogenetic tree showing the relationship of type strains of the genus *Pilimelia* and the related genus *Dactylosporangium*. The tree was reconstructed using the maximum-likelihood method using the software environment ARB (Ludwig et al., 2004) and the corresponding SILVA SSURef 95 database (release July 2008; Pruesse et al., 2007). Bar = 0.10 substitutions per site.

solid. **Color of substrate mycelium is pale lemon-yellow, golden yellow, orange, or pale**, turning brown to dark with age. Aerobic and chemo-organotrophic. Optimal growth is at pH 6.5–7.5 and 20–30°C (minimum 10°C, maximum 38°C). **Strains decompose keratinic substances** (e.g. hair of mammals). The peptidoglycan of the cell walls contains ***meso*-diaminopimelic acid** (*meso*-**DAP)** and **glycine**, with **xylose** and **arabinose** as characteristic sugars of whole-cell hydrolysates.

DNA G+C content (mol%): 71 (Miyadoh et al., 2001).

Type species: **Pilimelia terevesa** Kane 1966, 225^{AL}.

Further descriptive information

Phylogeny. On the basis of 16S rRNA gene sequence analysis, the genus *Pilimelia* belongs to the family *Micromonosporaceae* and contains three species and two subspecies with validly published names. *Pilimelia* species form a distinct phylogenetic cluster within the family *Micromonosporaceae* although the genus does not appear to be monophyletic. The 16S rRNA gene sequence similarity between the three species is between 96.1 and 98.6%. Similarity values of *Pilimelia columellifera* subsp. *columellifera*, *Pilimelia anulata*, and *Pilimelia terevasa* and type strains of the closely related genera are 94.1–96.85% for *Micromonospora*, 96.2–96.7% for *Polymorphospora*, 95.5–96.0% for *Krasilnikovia*, and 95.5–96.0% for *Asanoa*. Interestingly, *Pilimelia columellifera* subsp. *columellifera* JCM 3367^{T} shares a similarity value of 97.8% with *Micromonospora pattaloongensis* TJ2-2^{T}; however, chemotaxonomic and other phenotypic data indicate that this strain does not belong to the genus *Micromonospora*. A phylogenetic tree is shown in Figure 222. At present, no sequence is available for the type strain of *Pilimelia columellifera* subsp. *pallida*.

Cell morphology. The fine structure of the walls of hyphae and spores of *Pilimelia* is typical for those of Gram-stain-positive cells. A single compact layer surrounds the cytoplasm, which contains irregularly elongated nucleoid regions, ribosomes, and vacuole-like structures. Mesosomes are connected to the nuclear material or involved in the formation of cross-walls (Bland, 1968; Vobis, 1984). The cross-walls of substrate hyphae have the typical solid structure of Gram-stain-positive bacteria (cross-wall "type 1" of Williams et al., 1973). Typical interspace or "split" septa are observable in sporangial development (Vobis, 1984; Vobis et al., 1986b). Hyphae in contact with the air are additionally covered either by a thin sheath or by a thick layer of perhaps fibrous and mucilaginous material. The sporangial envelopes originate from these outer layers (Bland, 1968; Vobis, 1984).

The sporangia have different shapes and sizes and each species can be characterized by a special morphological type of sporangia (Figure 223; Table 200). Typical cylindrical sporangia are produced by *Pilimelia anulata*, reaching lengths of 10–35 µm (Kane, 1966); *Pilimelia columellifera* has spherical or oval to pyriform sporangia with diameters of 7–15 µm (Vobis et al., 1986b). Globose sporangia are also developed by *Pilimelia terevasa*, their size ranging between 5 and 23 µm (Kane Hanton, 1974). Other strains of *Pilimelia* have campanulate, inverse conical, heart-shaped, or flabelliform sporangia (Figure 223E) (Gaertner, 1955; Karling, 1954; Rothwell, 1957; Vobis et al., 1986b). Presumably, they belong to *Pilimelia terevasa*. After repeated subculturing, the strains may lose the ability to produce sporangia (Kane Hanton, 1974). The polysporous sporangia are developed at the tip of thickened hyphae that penetrate the surface of the substrate. These hyphae may be called palisade hyphae (Bland, 1968) or sporangiophores (Vobis, 1997). When sporangia are mature, the cytoplasm of the sporangiophores is

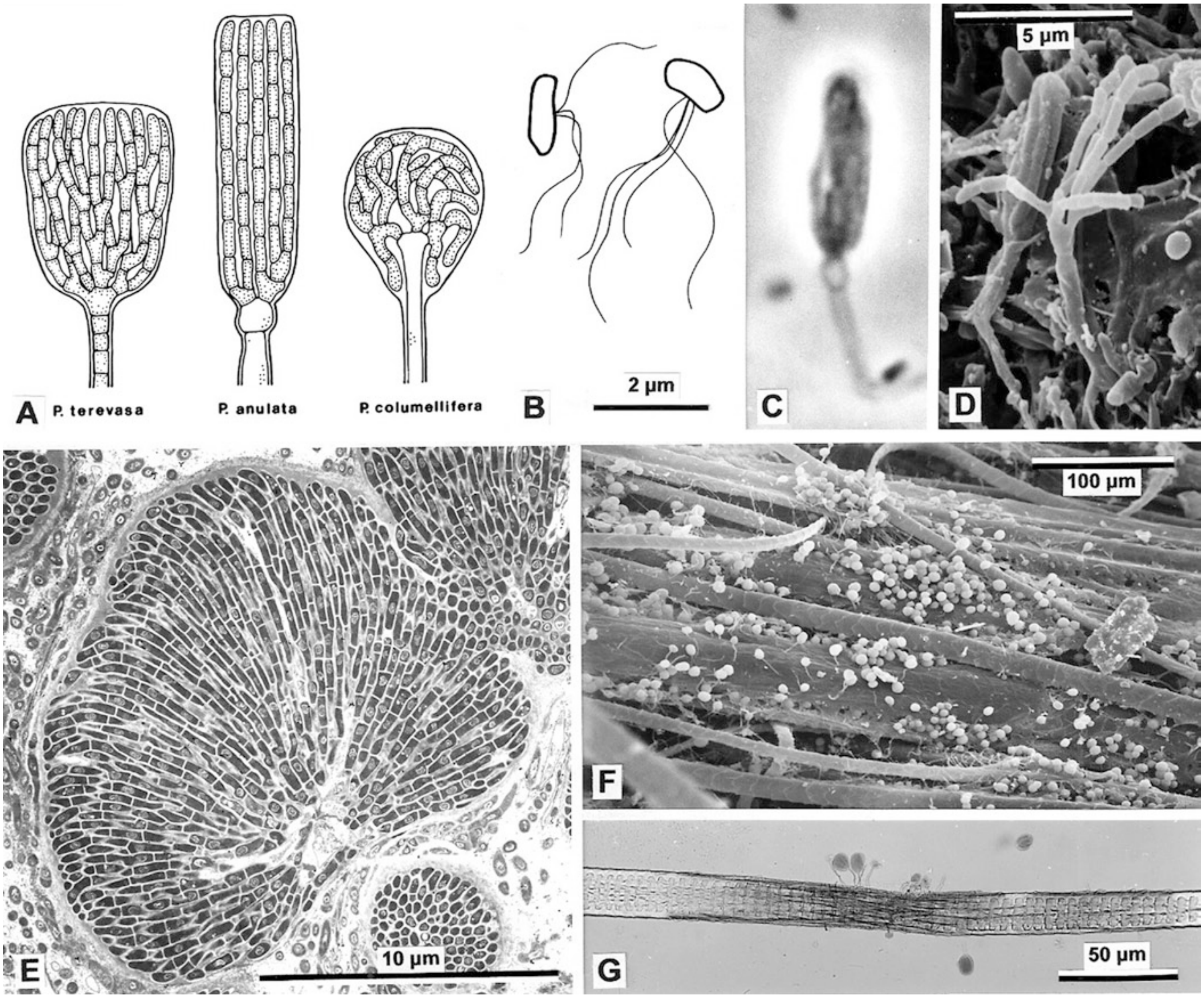

FIGURE 223. Morphological aspects of *Pilimelia*. A, Scheme of the different types of sporangia; B, rod-like sporangiosphores with laterally inserted tuft of flagella; C, cylindrical sporangium of *Pilimelia anulata* ATCC 25604^{T} with a basal ring-like structure (PHACO, magnification as in D); D, conidiophore of *Pilimelia anulata* ATCC 25604^{T} bearing chains of conidia (SEM); E, transverse section of a flabellate sporangium of strain MB-VK 122 with branched parallel rows of spores (TEM); F, globose sporangia on the surface of a colony of *Pilimelia columellifera* strain MB-SK6 cultivated on artificial soil agar and hair of white mice (SEM); G, hair colonized by a strain of *Pilimelia*, attacked section is darkly stained with cotton blue (LM). Abbreviations: LM, light microscopy; PHACO, phase contrast microscopy; SEM, scanning electron microscopy; TEM, transmission electron microscopy.

generally autolyzed. The different morphological characters of the sporangiophores are of diagnostic value (Table 200). In *Pilimelia terevasa* and *Pilimelia anulata*, they are fragmented by double-layered cross-walls (Vobis et al., 1986b). The uppermost fragment can be swollen to a ring-like structure or annulus, the distinctive structure of *Pilimelia anulata* (Figure 223C). In *Pilimelia columellifera*, the sporangiophores are not septate, but extend into the lumen of the sporangia to form clearly visible, nail-shaped columellae (Schäfer, 1973). The sporangiophores are arranged in either branched parallel (*Pilimelia terevasa* and *Pilimelia anulata*) or swirl-like (*Pilimelia columellifera*) rows (Figure 223A). It is estimated that one sporangium can contain from hundreds to several thousand spores (Vobis, 1984). The ultrastructure of sporangial development was described by Vobis (1984), confirming the scheme of sporangium formation as proposed by Lechevalier and Holbert (1965) for sporangiate actinomycetes.

The shape of the zoospores released from sporangia is rod-like. Occasional variations of slightly curved or reniform spores originate from branching segments of the sporogenous hyphae (Figure 223A, E). The spores can vary from 0.3 to 0.7 µm in diameter and 0.7 to 1.5 µm in length, and bear a laterally inserted tuft of flagella (Figure 223B) (Vobis et al., 1986b). A single flagellum might reach 5 µm in length with a diameter of about 11 nm. The flagella are frequently bundled and may function as a unit (Schäfer, 1973; Vobis, 1984).

TABLE 200. Diagnostic characters of the species of the genus *Pilimelia*[a]

Characteristic	*P. terevasa*	*P. anulata*	*P. columellifera*
Shape of sporangia:			
Spherical	+	−	+
Pyriform	−	−	+
Campanulate	+	−	−
Flabelliform	+	−	−
Cylindrical	−	+	−
Arrangement of spore chains:			
Parallel rows	+	+	−
Swirl-like	−	−	+
Sporangiophore:			
Septate	+	+	−
Annulate	−	+	−
Extended as columella	−	−	+
Consistency of colonies:			
Solid	−	−	+
Soft	+	+	−
Color of colonies:			
Lemon-yellow, yellow-gray	+	+	−
Golden-yellow, orange	−	−	+
Pale	−	−	+

[a]Symbols: +, >85% positive; −, 0–15% positive.

Nonmotile spores (conidia) may be produced (Figure 223D), mainly in the aqueous milieu of the substrate (Kane, 1966). The conidia develop in chains that are arranged in a pattern similar to the spore chains inside the corresponding sporangia (Kane, 1966; Schäfer, 1973; Vobis and Kothe, 1985). The formation of these unflagellated spores may be a variation of sporangial development in which the sporangial envelope is not formed (Gaertner, 1955).

Cell-wall composition. The peptidoglycan of the cell wall contains *meso*-DAP and glycine according to chemotype II of Lechevalier and Lechevalier (1970a), with xylose and arabinose as characteristic sugars in whole-cell hydrolysates (sugar pattern D) (Szaniszlo and Gooder, 1967; Vobis et al., 1986b). The acyl groups of the muramyl residues of the cell-wall peptidoglycans are of the glycolyl type, exhibiting high amounts of glycolic acid with 149 nmol/mg of cells for *Pilimelia anulata*, 141 nmol/mg for *Pilimelia terevasa*, and 74.0 nmol/mg for *Pilimelia columellifera* subsp. *columellifera* (Uchida and Seino, 1997).

The polar lipid profile is characterized by the presence of phosphatidylethanolamine and phosphatidylcholine and the absence of the unknown glucosamine-containing phospholipid (Vobis et al., 1986b). The phospholipid type of the cell membrane corresponds to type II of Lechevalier et al. (1981).

Fatty acids. *Pilimelia* strains have a high content (>50%) of C_{15} iso, $C_{15:1}$ iso, and $C_{17:1}$ fatty acids, and lack substantial amounts of C_{18} fatty acids (Stackebrandt and Kroppenstedt, 1987). The fatty acid pattern corresponds to type 2b of Kroppenstedt (1985).

The menaquinone composition in *Pilimelia* is predominantly MK-9(H_2) and MK-9(H_4), thus fitting into type 4a of the classification scheme of Kroppenstedt (1985).

Colonial characteristics. *Pilimelia* strains grow very slowly. Small colonies of about 5 mm in diameter develop after 4–6 weeks of incubation. These are compact and grow only with substrate mycelium, aerial mycelium is not produced. *Pilimelia terevasa* and *Pilimelia anulata* have soft and pasty colonies, colored bright lemon-yellow to yellow-gray. In both species, intra-mycelial pigments have been reported. The properties of absorption spectra are associated with carotenoids, with peaks at 479, 451, and 425 nm with an absorption maximum at 451 nm (Szaniszlo, 1968). In comparison with the spectral properties of extracts of *Actinoplanes* strains, the absorption peaks are shifted about 22 nm toward the UV region, indicating that the conjugated double bond system of the carotenoids of *Pilimelia terevasa* and *Pilimelia anulata* has one double bond less than that of *Actinoplanes* species (Parenti and Coronelli, 1979). The colonies of *Pilimelia columellifera* are solid and hard, either golden yellow to orange (subsp. *columellifera*) or pale (subsp. *pallida*) (Table 200). Growth occurs only on complex media such as 50% diluted skim milk agar (Gordon and Smith, 1955), Casamino acids-peptone Czapek agar (Henssen and Schäfer, 1971), nutrient-sugar agar (Henssen and Schäfer, 1971), peptone-yeast extract-iron agar (Shirling and Gottlieb, 1966), oatmeal-yeast extract agar (Vobis et al., 1986b), and yeast extract-starch agar (Emerson, 1958). On these nutrient-rich media, the colonies do not penetrate deeply into the agar but grow upward. Sporangial development rarely occurs. Nutrient-poor media with addition of natural keratinic substances promote the production of sporangia. Good examples are the artificial soil extract agar of Henssen and Schäfer (1971) combined with hair of white mice (Figure 223F), or highly diluted skim milk-mineral agar with the addition of cattle horn meal (Schäfer, 1973; Vobis, 1984, 1992). On these media, the colonies are more flat and the substrate hyphae penetrate deeply into the agar. The skim milk-cattle horn meal agar is highly recommended for starting a new culture (Vobis, 1992).

Life cycle. Sporangia with thick envelopes may function as a resistant form surviving in soil or disseminated as diaspores, possibly attached to soil particles and distributed by actively moving members of the edaphon like nematodes and arthropods. Transport over long distances by the wind is also easily imaginable. If sporangia are dipped into water, numerous flagellated spores are released, leaving behind the sporangial envelope (Vobis, 1984). The zoospores are also adapted to disseminate in water. If sporangia of *Pilimelia columellifera* are flooded with distilled water, motile spores are released after 45–60 min (Vobis et al., 1986b). The spores seem to swim randomly (Vobis, 1984). The laterally inserted tuft of flagella functions as a propelling flagellum and pushes the rod-shaped spore forwards. In microscopic water preparations, this type of movement in one direction, perpendicularly orientated to the longitudinal axis of the spore, is only hardly noticeable. Additionally, the spore moves like a slowly rotating screw. In addition to single spores, short chains of two or three spores can also be observed swimming actively in water (Vobis, 1984). On reaching natural keratinic substances, e.g. hair of mammals, they colonize the new substrate to produce mycelium and sporangia within 14 d (Vobis, 1989a). The life cycle of *Pilimelia* can be considered as "aero-aquatic" (Vobis, 1987), typical for all ecologically defined "actinoplanetes" (Makkar and Cross, 1982; Nonomura and Takagi, 1977).

TABLE 201. Other characteristics of species and subspecies of the genus *Pilimelia*[a,b]

Characteristic	*P. terevasa*	*P. anulata*	*P. columellifera* subsp. *columellifera*	*P. columellifera* subsp. *pallida*
Hydrolysis of starch	–	–	–	–
Degradation of tyrosine	+	+	–	–
Production of melanoid pigments[c]	v	v	v	+
Liquefaction of gelatin	–	–	+	+
Peptonization of casein	+	+	+	+
Reduction of nitrate	–	–	+	–
pH growth range	6.5–7.6	6.5–7.8	6.5–7.6	5.0–7.5
Temperature growth range (°C)	10–35	15–35	15–35	10–30

[a]Symbols: +, >85% positive; –, 0–15% positive; v, strain instability.

[b]Data from studies of type strains (Vobis et al., 1986b).

[c]Data compiled from Kane Hanton (1974), Schäfer (1973), and Vobis et al. (1986b).

Metabolism and physiology. Members of the genus *Pilimelia* are aerobic and mesophilic with growth optima between 20 and 30°C, although exceptional growth has been observed at 42°C (Schäfer, 1973). The pH range of growth is restricted to between 5.0 and 7.8. Colonies grow well at about pH 7.0. For more details and other physiological properties see Table 201. *Pilimelia* strains utilize neither the various carbon sources recommended by Shirling and Gottlieb (1966) (Vobis et al., 1986b) nor individual or combinations of purified amino acids as a sole source of nutrition (Kane Hanton, 1974). Four *Pilimelia* strains isolated from rhizosphere of the actinorhizal plant *Ochetophila* (= *Discaria*) *trinervis* did not show any enzymic activity to decompose substrates of vegetable origin like starch, cellulose, hemicellulose, pectin, or lignin (Solans and Vobis, 2003). The capacity to degrade natural keratinic substances, which can be observed easily by microscopic preparations of infected hairs, is highly conspicuous (Figure 223G). Further physiological investigations are needed to understand the high specialized enzymic activity of *Pilimelia*.

Ecology. Strains of *Pilimelia* must have a worldwide distribution (see also species descriptions). The discoverer of these rare actinomycetes, Karling (1954), first detected them in baited soil cultures from New York City, USA, in 1938 and later in soil samples from various parts of the Amazon Valley in Brazil, as well as from New Jersey, Virginia, Louisiana, Indiana, and Iowa (USA). Schäfer (1973) registered a total of 96 out of 427 soil samples, collected from different geographical regions of the world (22%), that produced of *Pilimelia* sporangia in enrichment cultures. Gaertner (1955) confirmed that 16% of his soil samples from the African continent were positive for the presence of *Pilimelia*. Strains presumptively identified as *Pilimelia terevasa* and *Pilimelia columellifera* have been reported to be widely distributed in diverse soils from England (Tribe and Abu El-Souod, 1979).

The investigations of Garrity et al. (1996) demonstrated that in the arid environment of the Mojave Desert along the California–Nevada border, 34 *Pilimelia*-like isolates could be recovered from eight out of 32 soil samples using the baiting technique. The total number of isolates was estimated as low, but the overall diversity was high. Accordingly, organisms belonging to *Pilimelia* do not occur so rarely in nature, but they are very difficult to isolate and cultivate (Schäfer, 1973; Vobis et al., 1986b).

Pilimelia strains are remarkable "nutrition specialists". In baiting experiments, they colonize and decompose natural keratinic material like hair of cows, horses, mice, rats, dogs, and deer (Gaertner, 1955; Makkar and Cross, 1982; Schäfer, 1973), and human hair (Kane, 1966; Rothwell, 1957). Infected parts of the hair lose their stability and external structure (Vobis, 1984). In addition to the degradation of mammalian hair, feathers of birds or snake skin can also be colonized and attacked by *Pilimelia* strains (Karling, 1954; Schäfer, 1973). These unusual keratinophilic micro-organisms may occupy an important ecological role in soil by decomposing the recalcitrant scleroproteins of vertebrates, especially hair of mammals (Gaertner, 1955). They have not yet been found to be dermatophytes.

Enrichment and isolation procedures

Strains of *Pilimelia* can be baited with natural keratinic substrates (Bland and Couch, 1981; Kane, 1966; Karling, 1954; Schäfer, 1973). Soil samples are placed in Petri dishes and stirred with double-distilled water. Sterilized hairs or bits of snake skin are laid upon the surface. After 3–4 weeks of incubation at room temperature, the hairs are examined microscopically. When they are covered with sporangia, they are transferred onto agar. With a thin, fine-pointed tungsten needle, slightly hooked at the tip, individual sporangia are removed and rolled in zig-zag curves over the surface of agar to remove bacteria, before transferring them to a suitable growth medium, such as 5% diluted skim milk-mineral agar containing cattle horn meal (Schäfer, 1973; Vobis, 1992) or Emerson's yeast extract-starch agar (Kane, 1966). Colonies of 1 mm in diameter can be transferred onto slants after about 3 weeks. Garrity et al. (1996) transferred the infected baits to sterile Whatman filter discs and washed them under vacuum on a mini-sieve. After a period of drying of 7–10 d, the filter discs with baits are suspended in 10 ml in a small Petri dish and incubated for 45–60 min at 22°C. The solutions are diluted (10^{-1} to 10^{-3}) and plated onto suitable or selective agar medium.

Maintenance procedures

Colonies growing on agar media must be subcultured after 4–5 weeks because autolysis of vegetative mycelium may occur after 2 weeks of incubation. For long-term preservation, *Pilimelia* strains must be lyophilized.

Procedures for testing special characters

The ability to decompose keratinic substances can be tested with hair of white mice. Sterilized hairs are incubated together with mycelium and checked microscopically after 2 weeks. Damaged hair segments are stainable with cotton blue (aniline blue) (Gams et al., 1980). Usually, the hairs are also deformed and splintered in the longitudinal axis (Figure 223G).

Differentiation of the genus *Pilimelia* from other related genera

The genus *Pilimelia* has a unique taxonomic position among the sporangiate genera belonging to the family *Micromonosporaceae*. It can be differentiated by morphological, physiological, and chemotaxonomic criteria. The genera *Dactylosporangium*, *Planosporangium*, and *Virgisporangium* can be separated from *Pilimelia* because they have oligospore sporangia with 2–6 or more spores per sporangium, organized in a single row. Among the polysporous sporangia producing genera of *Micromonosporaceae*, *Luedemanella* can be distinguished by the formation of nonmotile sporangiophores. *Actinoplanes*, which also forms polysporous sporangia with motile sporangiophores, produces globose or rod-like spores; *Pilimelia* spores are exclusively rod-like. The distinguishing properties are the size of the spores and the type of flagellation: the spores of *Pilimelia* are 0.3–0.7 × 0.7–1.5 µm with a laterally tuft of flagella, whereas the rod-like spores of *Actinoplanes*, characterizing the formerly "*Ampullariella*" species, are 0.5–1.0 × 2.0–4.0 µm and equipped with a polarly inserted tuft of flagella (Vobis, 1989b). In comparison to other members of *Micromonosporaceae*, the growth of *Pilimelia* strains is very slow; they need natural keratinic substances to produce well developed colonies (Vobis, 1989a, 1992). The menaquinone composition of MK-9(H_2) and MK-9(H_4), characteristic of type 4a of Kroppenstedt (1985), differentiates *Pilimelia* from other genera of the *Micromonosporaceae*, including *Micromonospora*, *Actinoplanes*, *Catellatospora*, *Catenuloplanes*, *Couchioplanes*, *Dactyloporangium*, *Spirilliplanes*, and *Verrucosispora* (Kudo, 2001). The pattern of fatty acids is also significantly different from those of other genera with high amounts of C15 and C17, and a lack of C18 fatty acids, corresponding to type 2b of Kroppenstedt (1985).

Taxonomic comments

Members of the genus *Pilimelia* were discovered by Karling (1954), Gaertner (1955), and Rothwell (1957), who were studying hair of mammals attacked by unusual, keratinophilic micro-organisms. Only preliminary diagnoses could be made, since pure cultures were not obtainable at this time. After successful culturing of single strains, Kane (1966) described the genus *Pilimelia* based on two species, *Pilimelia terevasa* and *Pilimelia anulata*. According to the original descriptions, the type strains appeared to be keratinophilic members of the genus *Ampullariella* (Cross and Goodfellow, 1973). This questionable taxonomic position could be clarified by re-examination of the type strains in connection with the description of a third species, *Pilimelia columellifera* (Schäfer, 1973; Vobis et al., 1986b). Finally, the recognition of the genus *Pilimelia* was supported by chemotaxonomic and phylogenetic investigations (Koch et al., 1996a; Kroppenstedt, 1985).

The proposals to transfer *Pilimelia terevasa* and *Pilimelia anulata* to "*Ampullariella*" (Juan and Zhang, 1974), and *Pilimelia columellifera* (Schäfer, 1973) to *Spirillospora* (Tribe and Abu El-Souod, 1979) were not considered in the Approved Lists of Bacterial Names (Skerman et al., 1980).

Differentiation of species of the genus *Pilimelia*

The species of *Pilimelia* are distinguishable by morphological and colonial criteria. Differential characteristics are listed in Table 200. Other physiological characters of the species and subspecies are indicated in Table 201.

List of species of the genus *Pilimelia*

1. **Pilimelia terevasa** Kane 1966, 225[AL]

ter.e.vas′a. L. adj. *teres* rounded (i.e. circular in transverse sections, tapering or narrow cylindric); L. pl. n. *vasa* vessels; N.L. n. *terevasa* rounded vessels, indicating "rounded", spherical sporangia.

Sporangia develop on hair of mammals floating on soil–water and on artificial soil extract agar. The shape of sporangia is spherical (type strain) or campanulate (Figure 223). The sporangia can reach diameters of up to 24 µm. The rod-shaped spores (0.3–0.6 × 0.7–1.5 µm) are motile by means of a tuft of laterally inserted flagella. Spores are released after 15–20 min from sporangia when flooded with water. Sporangiophores are septate, approximately 1 µm in diameter.

Colonies on agar media are bright lemon-yellow with rough lobed borders. The surface is tuberculate and warty with curled protrusions. The consistency is soft and pasty.

Source: the type strain was isolated from a soil sample collected in the area of St. Joseph Co., Indiana (USA).

DNA G+C content (mol%): unknown.

Type strain: ATCC 25603, CBS 570.75, DSM 43040, NBRC 15964, JCM 3091, KCC A-0091.

Sequence accession no. (16S rRNA gene): X93190.

2. **Pilimelia anulata** Kane 1966, 225[AL]

an.u.la′ta. L. fem adj. *anulata* having a ring.

Sporangia develop on mammalian hair and on agar media, supported by septate sporangiophores, where the uppermost fragment is swollen to a ring-like structure (Figure 223A, C). The sporangia are cylindrical, 2.8–11.2 µm wide and up to 35 µm long. Inside the sporangium, the spores are arranged in parallel chains. The spores are rod-shaped (0.3–0.7 × 0.8–1.3 µm) and equipped with a laterally inserted tuft of flagella. Conidiophores with conidia in chains (Figure 223D) are occasionally produced.

Colonies on agar media are bright lemon-yellow to yellow-gray and with lobed margins. The surface of the colonies is tuberculate with narrow and often branched, curled protrusions. The consistency is soft and pasty.

Source: the type strain was isolated from a soil sample collected in the area of St. Joseph Co., Indiana (USA).

DNA G+C content (mol%): unknown.

Type strain: ATCC 25604, DSM 43039, NBRC 16051, NBRC 15533, JCM 3090, KCC A-0090, NCIMB 12892.

Sequence accession no. (16S rRNA gene): X93189.

3. **Pilimelia columellifera** (*ex* Schäfer 1973) Vobis, Schäfer, Kothe and Renner 1986a, 573[VP] (Effective publication: Vobis, Schäfer, Kothe and Renner 1986b, 72.) (Schäfer 1973, 190.)

co.lu.mel.li′fe.ra. L. n. *columella* small column; L. suff. *-fer -fera -ferum* carrying; N.L. fem. adj. *columellifera* bearing a small column.

Sporangia are produced on the surface of hair and on agar media (Figure 223F). The shape of sporangia is spherical, ovoid, or pyriform and 7–16 µm in diameter. Sporangiophores develop in chains that are arranged like swirls. The nonseptate sporangiophore is extended into the lumen of the sporangium, thus forming a columella (Figure 223A). If sporangia are dipped into the water, spores start to swarm after 45 min. Occasionally, swarming occurs inside the sporangia until finally the envelope tears and the spores escape. The rod-shaped zoospores (0.35–0.45 µm wide and 0.8–1.5 µm long) are motile by means of two to four laterally inserted flagella.

Colonies on agar media are small, about 5 mm in diameter after 4 weeks of incubation. They are compact, irregular, and pulvinate. The surface is warty and squamous, and the consistency is solid. The color of mycelium is golden yellow to orange or colorless to pale brownish. The type strain shows the highest sequence similarity to *Micromonospora pattaloongensis* TJ2-2^{T} (97.3%), *Micromonospora auratinigra* TT1-11^{T} (97.2%), and *Polymorphospora rubra* (97%).

Source: Pilimelia columellifera was preliminarily described as "organism I" by Gaertner (1955), and could be registered by enrichment culture in 45 soil samples collected in Africa and in one sample from Germany. Tribe and Abu El-Souod (1979) reported the frequent presence of the species in soil samples collected in the Cambridge region (England).

DNA G+C content (mol%): unknown.

Type strain: ATCC 43728, CBS 569.75, DSM 43797, NBRC 16052, JCM 3367, MB-SK6.

Sequence accession no. (16S rRNA gene): AB548688.

3a. **Pilimelia columellifera subsp. columellifera** (*ex* Schäfer 1973) Vobis, Schäfer, Kothe and Renner 1986a, 573[VP] (Effective publication: Vobis, Schäfer, Kothe and Renner 1986b, 72) (Schäfer 1973, 190.)

The description is as for the species. It differs from the subspecies *Pilimelia columellifera* subsp. *pallida* by its golden yellow to orange substrate mycelium and by its ability to reduce nitrate.

Source: the type strain originated from a soil sample from Peru; a further 19 strains were isolated from soil samples collected in Germany, British Isles, Ceylon, Spain, Greece, Yugoslavia, Israel, Persia, and Austria (Schäfer, 1973).

DNA G+C content (mol%): unknown.

Type strain: ATCC 43728, CBS 569.75, DSM 43797, NBRC 16052, JCM 3367, MB-SK6.

Sequence accession no. (16S rRNA gene): AB548688.

3b. **Pilimelia columellifera subsp. pallida** (*ex* Schäfer 1973) Vobis, Schäfer, Kothe and Renner 1986a, 573[VP] (Effective publication: Vobis, Schäfer, Kothe and Renner 1986b, 72.) (Schäfer 1973, 192.)

pal′li.da. L. fem. adj. *pallida* pale.

The subspecies *pallida* is distinguishable from *Pilimelia columellifera* subsp. *columellifera* by a colorless to pale brownish substrate mycelium. The center of the colonies occasionally becomes blackish with increasing age. Colonies are flat or convex to slightly raised and umbonate. Sporangial development occurs occasionally on oatmeal-yeast extract agar or on artificial soil extract agar. Melanoid pigments are produced on peptone-yeast extract-iron agar and on tyrosine agar. Nitrate is not reduced to nitrite.

Source: the type strain was isolated from a soil sample collected in Germany; in addition, three further strains were isolated from soil in Germany and Portugal (Schäfer, 1973).

DNA G+C content (mol%): unknown.

Type strain: DSM 43799, MB-SK8.

Sequence accession no. (16S rRNA gene): not available.

Genus XIII. **Polymorphospora** Tamura, Hatano and Suzuki 2006, 1961[VP]

Tomohiko Tamura

Po.ly.mor.pho.spo′ra. Gr. adj. *polumorphos* multiform; Gr. fem. n. *spora* seed, and in biology, a spore; N.L. fem. n. *Polymorphospora* polymorphic spore.

Gram-stain-positive, not acid-fast, producing a fine, nonfragmenting, branching mycelium. Strictly aerobic. **Short spore chains** develop on short sporophores on the substrate mycelium. Immature spores are **oval or of various shapes**, and **short rods** are formed (0.6–0.9 × 0.8–1.5 µm wide) on maturation. Spores are **nonmotile**. Optimum temperature for growth generally ranges between 20 and 30°C. Cell wall contains D-glutamate, glycine, D-alanine, and *meso*-diaminopimelate. Mannose, 3-*O*-methylmannose, glucose, and galactose are detected as whole-cell sugars. The predominant cellular fatty acid is $C_{16:0}$ iso, followed by $C_{17:1}$ and $C_{17:0}$ anteiso. The major menaquinones are MK-10(H_6), MK-10(H_4), MK-9(H_6), and MK-9(H_4). Phosphatidylethanolamine is present as the diagnostic phospholipid, whereas phosphatidylcholine is absent (phospholipid pattern type PII). The acyl type of the cell wall is glycolyl. Mycolic acid is absent.

DNA G+C content (mol%): 70–71 (HPLC).

Type species: **Polymorphospora rubra** Tamura, Hatano and Suzuki 2006, 1963[VP].

Further descriptive information

The organism shows good growth on oatmeal agar, inorganic salts-starch agar, and peptone-yeast extract-iron agar. Cultures also grow well in yeast extract-glucose broth consisting of yeast

extract (1%) and D-glucose (1%), pH adjusted to 7.0, upon incubation at 28°C on a rotary shaker for 4 d. The chemotaxonomy of the genus *Polymorphospora* is chemotype II according to Lechevalier and Lechevalier (1970a), and the peptidoglycan type is presumed to be A1γ according to Schleifer and Kandler (1972). A unique nucleotide signature includes a U at position 1244 of the 16S rRNA gene.

Enrichment and isolation procedures

Samples are inoculated on humic acid-vitamin (HV) agar (Hayakawa and Nonomura, 1987a) using the yeast extract–SDS method (Hayakawa and Nonomura, 1989). Incubation is at 28°C for 2 weeks. The described strains were isolated from soil samples collected near the roots of *Bruguiera gymnorrhiza* and *Sonneratia alba* at the mouth of the River Shiira on Iriomote Island, Okinawa, Japan (Tamura et al., 2006).

Maintenance procedures

Strains of the genus *Polymorphospora* are maintained by freezing in water containing 10–30% glycerol at −70°C. Lyophilization of suspensions in 10% skim milk +1% monosodium glutamate and liquid-drying (Sakane and Kuroshima, 1997) in 0.01 M potassium phosphate buffer (pH 7.0) containing 3% monosodium glutamate are also recommended for long-term preservation.

Differentiation of the genus *Polymorphospora* from other genera

The genus *Polymorphospora* contains *meso*-diaminopimelic acid and glycine in its peptidoglycan. Unlike members of the genera *Catenuloplanes* and *Spirilliplanes*, arabinose is not detected in whole cells (Tamura et al., 1997; Yokota et al., 1993). 3-*O*-Methylmannose is contained as a whole-cell sugar, as in the genera *Spirilliplanes* and *Virgisporangium* (Tamura et al., 2001). The genus *Polymorphospora* forms spore chains and differs in this respect from the genera *Dactylosporangium* and *Micromonospora*, which are the closest phylogenetic neighbors.

Taxonomic comments

Based on 16S rRNA gene sequence analysis, the genus *Polymorphospora* forms an independent monophyletic clade within the family *Micromonosporaceae* of the order *Micromonosporales*. In addition, the two strains isolated in the original study showed DNA relatedness higher than 70% and are accommodated in the same species (Tamura et al., 2006).

List of species of the genus *Polymorphospora*

1. **Polymorphospora rubra** Tamura, Hatano and Suzuki 2006, 1963[VP]

   ru′bra. L. fem. adj. *rubra* red.

   Colonies that develop on ISP media 2, 3, and 4 are red to reddish-orange in color. Utilizes D-mannitol, melibiose, maltose, L-rhamnose, methyl α-D-glucoside, D-galactose, D-mannose, and D-glucose. Positive in tests for starch hydrolysis and urea decomposition. No growth in the presence of 4% NaCl. Esculin is not hydrolyzed. The major cellular fatty acids are $C_{16:0}$ iso and $C_{16:1}$.

   Source: the type strain was isolated from rhizosphere soil of mangrove.

   DNA G+C content (mol%): 70–71 (HPLC).

   Type strain: TT 97-42, DSM 44947, NBRC 101157.

   Sequence accession no. (16S rRNA gene): AB223089.

Genus XIV. **Salinispora** Maldonado, Fenical, Jensen, Kauffman, Mincer, Ward, Bull and Goodfellow 2005, 1763[VP]

Paul R. Jensen, Luis A. Maldonado and Michael Goodfellow

Sa.li.ni.spo′ra. L. adj. *salinus* saline; Gr. fem. n. *spora* a seed and, in bacteriology, a spore; N.L. fem. n. *Salinispora* a spore-forming bacterium originating from a saline habitat, indicating the marine habitat of the organism.

Obligately aerobic, Gram-stain-positive, non-acid-fast, nonmotile actinomycetes that form extensively branched substrate hyphae carrying single or clusters of smooth-surfaced spores, which may be sessile or borne on short sporophores. Vegetative hyphae are finely branched but do not show any fragmentation. Strains grow well at 10–30°C and pH 7–12. Colonies range from bright to pale orange and dark brown changing to black as they sporulate with no aerial mycelia observed. Brown diffusible pigments that darken agar media are normally produced. **Whole-organism hydrolysates are rich in *meso*-diaminopimelic acid and contain major amounts of arabinose, galactose, and xylose**. **The muramic acid moiety of the peptidoglycan is glycolated**. **Cells contain: diphosphatidylglycerol, phosphatidylethanolamine, phosphatidylglycerol, and phosphatidylinositol as major polar lipids; tetrahydrogenated menaquinones with nine isoprene units as the predominant isoprenologue; and complex mixtures of saturated iso- and anteiso-fatty acids but lack mycolic acids. Do not grow when seawater is replaced with deionized water in standard complex growth media**. The phylogenetic position of *Salinispora*, as determined by 16S rRNA gene sequence analysis, is in the family *Micromonosporaceae*.

Source: isolated from marine sediments and benthic organisms.

DNA G+C content (mol%): 69.4–69.5 (from genome sequences).

Type species: **Salinispora arenicola** Maldonado, Fenical, Jensen, Kaufman, Mincer, Ward, Bull and Goodfellow 2005, 1764[VP].

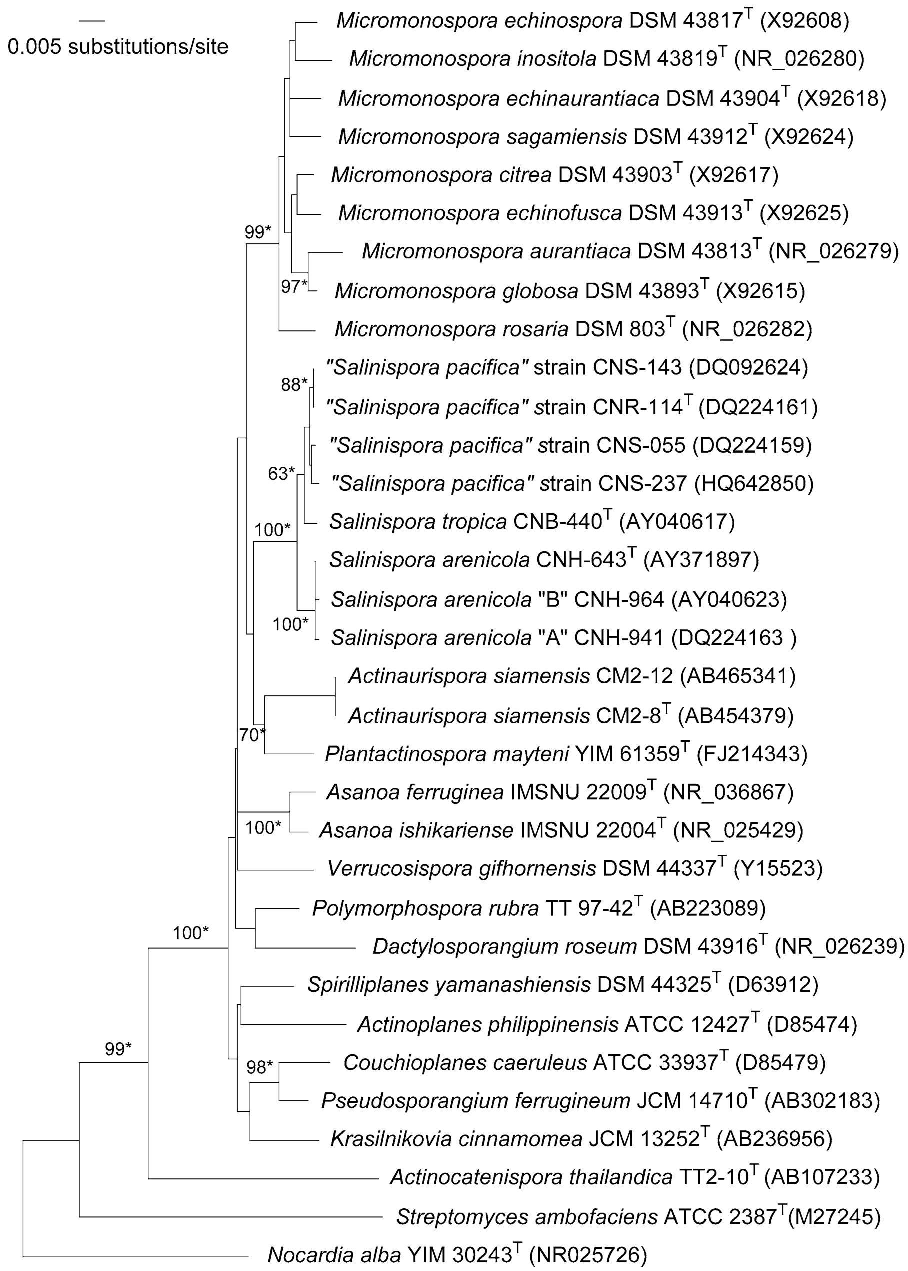

FIGURE 224. Neighbor-joining tree based on nearly complete 16S rRNA gene sequences showing relationships between *Salinispora* species and representatives of other genera classified in the family *Micromonosporaceae*. The numbers at the nodes indicate levels of bootstrap support based on an analysis of 1000 re-sampled datasets, only values above 60% are given. Asterisks indicate nodes that are also supported using the maximum-likelihood and maximum-parsimony methods. Bar = 0.005 substitutions per nucleotide position.

Further descriptive information

Phylogeny. The genus *Salinispora* forms a tight subclade in the 16S rRNA *Micromonosporaceae* gene tree (Figure 224). It encompasses two species with validly published names, *Salinispora arenicola* and *Salinispora tropica*, and a putative third species, "*Salinispora pacifica*" (Jensen and Mafnas, 2006). All *Salinispora* strains examined to date share 16S rRNA gene sequence identities greater than or equal to 99% and thereby constitute what has been described as a multidiverse ribotype cluster (Acinas et al., 2004). Initially, the genus was considered to be most closely related to the genus *Micromonospora* (Maldonado et al., 2005) but it is now known that its nearest phylogenetic neighbors are the genera *Actinaurispora* (Thawai et al., 2010) and *Plantactinospora* (Qin et al., 2009). Micromonosporae and salinisporae can also be distinguished by analysis of 16S–23S intergenic spacer regions (Maldonado et al., 2005) and by *gyrB* nucleotide sequence data (Jensen and Mafnas, 2006).

Cell morphology. *Salinispora* strains present extensively branched substrate hyphae which range from 0.25 to 0.5 µm in diameter. The substrate hyphae carry single or clusters of

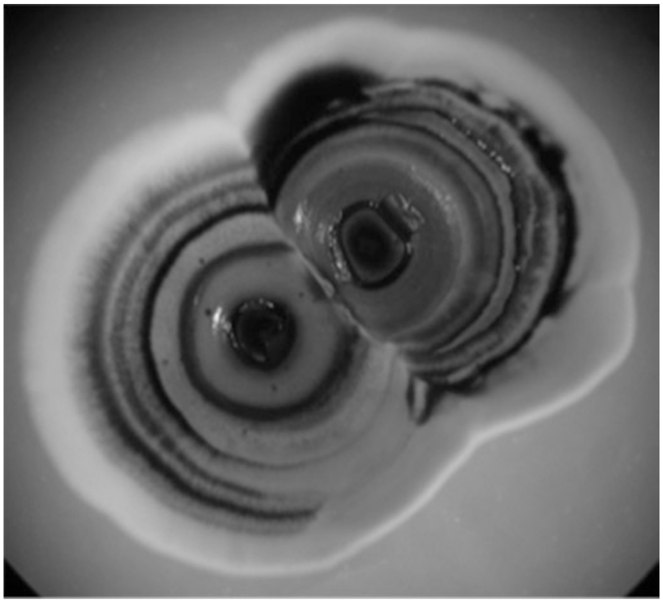

FIGURE 225. Colony of *Salinispora tropica* growing on seawater agar. Concentric rings of spores are visible on the colony surface. Photo credit: Erin Gontang.

smooth-surfaced spores. Spores range from 0.8 to 3.8 μm in diameter. Aerial hyphae have not been observed and spores may be sessile or borne on short sporophores.

Colony morphology. *Salinispora* strains grow slowly taking from 1 to 3 weeks to form small colonies on primary isolation plates (Jensen et al., 2005). Colonies are tough, leathery, and adhere to agar surfaces. On nutrient-rich media they exhibit a range of orange to brown pigments with spores blackening the colony surface (Figure 225), although on occasion poorly pigmented strains are encountered. In liquid culture, most strains are dispersed while some form dense clumps. The orange pigmentation produced in liquid culture generally fades to black over time.

Chemotaxonomy. Salinisporae contain *meso*-diaminopimelic acid as the diagnostic diamino acid of the cell-wall peptidoglycan, muramic acid residues that are *N*-glycolated, and produce whole-organism hydrolysates rich in arabinose, galactose, and xylose. They also contain diphosphatidylglycerol, phosphatidylethanolamine, phosphatidylglycerol, and phosphatidylinositol as diagnostic polar lipids, tetrahydrogenated menaquinones with nine isoprene units as the major isoprenologue, and complex mixtures of saturated iso- and anteiso-fatty acids, but lack mycolic acids (Maldonado et al., 2005). The predominant fatty acids (as percentages of total fatty acid content) are 13-methyltetradecanoic acid ($C_{15:0}$ iso; 3.7–13.3%), 14-methylpentadecanoic acid (C6:0; 36.4–53.5%), 16-methylheptadecanoic acid ($C_{18:0}$ iso; 3.5–10.2%), heptadecanoic acid ($C_{17:0}$; 4.1–7.8%) and 10-methyloctadecanoic acid ($C_{18:0}$ 10-methyl; 0.9–6.8%).

Nutrition and growth conditions. *Salinispora* strains were first recognized as being distinct from other actinomycetes based on their inability to grow when seawater was replaced with deionized water in the complex growth medium M4 (Jensen et al., 1991). Subsequently, it was reported that they failed to grow when sodium salts were replaced with equimolar concentrations of potassium salts suggesting a specific sodium ion requirement (Mincer et al., 2002). However, it is now clear that growth is possible at sodium concentrations as low as 5 mM (approx. 1% that found in seawater) if the medium is supplemented with sufficient concentrations of the appropriate non-sodium salts (Kim et al., 2005; Tsueng and Lam, 2008a). *Salinispora* cells are reported to lyze in the absence of a sufficiently high osmotic strength growth medium (Tsueng and Lam, 2010) and will only grow if the appropriate salts and high osmotic strength environment are provided (Tsueng and Lam, 2008b). *Salinispora* strains form well-developed colonies on media commonly employed to grow *Micromonospora* [e.g. yeast-malt extract agar (ISP medium 2); Shirling and Gottlieb, (1966)] when deionized water is replaced with seawater or an appropriate salts solution. Strains grow well on nutrient-rich agar media prepared with either natural or artificial seawater (Mincer et al., 2002).

Metabolism and metabolic pathways. *Salinispora* strains are heterotrophic and obligately aerobic. *Salinispora arenicola* and *Salinispora tropica* strains degrade arbutin, casein, elastin, gelatin, and starch, but not cellulose, chitin, tributyrin, or xylan (Maldonado et al., 2005). Similarly, cellobiose, α-lactose, melezitose, and starch are used as sole carbon sources, but not fructose, mannose, ribose, sorbose, or xylose. Salinisporae are best known metabolically for the production of secondary metabolites including the highly selective proteasome inhibitor salinosporamide A (Feling et al., 2003), which is undergoing clinical trials for the treatment of cancer (Fenical et al., 2009). Based on the analysis of two genome sequences, secondary metabolism is the major functionally annotated class of metabolic genes that differentiates the two species (Penn et al., 2009). This is supported by the observation that secondary metabolite production occurs in species-specific patterns with *Salinispora arenicola* strains producing rifamycins and staurosporines while *Salinispora tropica* strains produce salinosporamides and sporolides (Jensen et al., 2007). To date, at least 16 distinct structural types have been characterized from this genus including the cyanosporosides from "*Salinispora pacifica*" (Oh et al., 2006) and the sporolides from *Salinispora tropica* (Buchanan et al., 2005), both of which are proposed to be derived from enediyne intermediates. Other new structures from *Salinispora arenicola* include the polyketide-derived arenicolides (Williams et al., 2007) and the depsipeptide arenamides (Asolkar et al., 2009), while the antitumor antibiotic lomaivitacin (He et al., 2001) was isolated from what is now proposed to be "*Salinispora pacifica*".

Genetics. Representatives of all *Salinispora* 16S rRNA gene sequence types were originally aligned to *Escherichia coli* (accession no. J01695) and 71 type strains within the family *Micromonosporaceae*. This alignment revealed five genus-specific signature nucleotides consisting of A, C, T, T, and G (Mincer et al., 2002). This was subsequently revised to A, C, T, and G (Maldonado et al., 2005), while a new analysis including sequence data from the genera *Actinaurispora* (Thawai et al., 2010) and *Plantactinospora* (Qin et al., 2009) reveals three genus-specific nucleotides (C, T, T). Mean DNA:DNA hybridization values for *Salinispora tropica* CNB-440^T and *Salinispora arenicola* CNH-643^T are 68%. A PCR-targeted mutagenesis protocol has been developed to generate gene knock-outs in *Salinispora* species (Eustáquio et al., 2008) and used to characterize the function of enzymes involved in secondary metabolite biosynthesis in this taxon (Eustáquio et al., 2009).

Genomics. The complete genome sequences of *Salinispora tropica* strain CNB-440^T (accession no. NC_009380) and *Salinispora arenicola* strain CNS-205 (accession no. NC_009953) have been determined and analyzed (Penn et al., 2009; Udwary et al., 2007). Both chromosomes are circular with the *Salinispora arenicola* genome (5.8 Mb) being 600 kb larger in size. The *Salinispora arenicola* strain was chosen for sequencing because, unlike the type strain for this species (CNH-643^T), it produces the unusual cyclic peptide cyclomarin A (Renner et al., 1999). The two strains devote considerable proportions of their genomes (9–11%) to secondary metabolite production, which occurs in species-specific patterns (Jensen et al., 2007). Most genetic differences between the two strains are located in 21 major genomic islands, which house all of the species-specific secondary metabolic pathways. The mean nucleotide identity calculated for the two strains is 87.2%, far below the 94% value that has been suggested to delineate bacterial species (Konstantinidis and Tiedje, 2005). The genome sequences also provide evidence of marine adaptation in the form of a highly duplicated family of polymorphic membrane proteins (Penn et al., 2009). Intragenomic variability was not observed among the three copies of the 16S rRNA gene operon present in each genome.

Antibiotic sensitivity. Good growth is observed in the presence of (µg/ml) gentamicin sulfate (5 and 25), neomycin sulfate (5), streptomycin sulfate (5), novobiocin (5 and 25) penicillin G (5) and rifampin (5). *Salinispora arenicola* strains that produce compounds in the rifamycin class are also resistant to 25 µg/ml rifampin, whereas other *Salinispora* species are not.

Ecology. To date, *Salinispora* strains have only been cultured from tropical and subtropical marine environments. Most observations have been from sediments, where they have been shown to increase in abundance with depth (Jensen et al., 1991) and to occur at concentrations of $2–5 \times 10^3$ c.f.u./ml wet sediment (Mincer et al., 2005). Although the maximum depth limits are not known, strains have been successfully cultured from marine sediments collected at a depth of 1100 m (Mincer et al., 2005). Salinisporae have also been reported from a marine sponge (Kim et al., 2005) and are readily cultured from a variety of benthic marine plants and invertebrates (unpublished data, Scripps Institution of Oceanography), though there is not any evidence of specificity in these associations. The genus is broadly distributed in tropical and subtropical sediments (Jensen and Mafnas, 2006) but has yet to be cultured from more temperate environments despite culture-independent evidence that they occur in these regions (unpublished data, Scripps Institution of Oceanography). *Salinispora* species show distinct biogeographical patterns, with the only reports to date for *Salinispora tropica* coming from the Caribbean. *Salinispora arenicola* has the broadest distribution and has been recovered from all sites in which the genus has been reported, while "*Salinispora pacifica*" has an intermediate distribution, having yet to be cultured from the Caribbean (Jensen and Mafnas, 2006). Culture-independent studies have not revealed any new species-level diversity, while DNA extraction experiments have shown that most strains recovered from sediments were present as spores (Mincer et al., 2005).

Enrichment and isolation procedures

Salinispora strains are readily cultured from marine sediments using either of two approaches. The first involves drying approximately 1 g sediment at room temperature overnight in a laminar flow hood. A sterile foam plug is first stamped onto the dried sediment and then repeatedly stamped onto an appropriate agar growth medium creating a serial dilution effect. After 2–3 weeks of incubation at room temperature, *Salinispora* colonies can be seen on the agar surface. The second method involves serially diluting (1:10) 1 ml wet sediment in sterile seawater. The dilutions are then heated to 50°C for 6 min and 50 µl is spread-plated onto an appropriate growth medium using a sterile glass rod. Effective media formulations include seawater agar (1 liter natural or artificial seawater, 16 g agar) and A1 agar (10 g soluble starch, 4 g yeast extract, 2 g peptone, 16 g agar, 1 liter natural or artificial seawater). Cycloheximide (100 µg/ml final concentration) is used to supplement isolation media to reduce fungal growth, while rifampin (20 µg/ml) can be added to select for *Salinispora arenicola*, which produces related compounds in the rifamycin class and demonstrates a higher level of resistance to this antibiotic than the other taxa.

Maintenance procedures

Salinispora strains can be maintained as glycerol suspensions (20%, v/v) at −20 and −80°C for long-term preservation as suggested by Wellington and Williams (1978). The glycerol suspensions are prepared using 4–5 loopfuls of fresh biomass scraped using sterile disposable loops or sterile toothpicks from GYM or YEME agar plates that had been streaked for single colonies and incubated at 28–30°C for 10–14 d. Short-term storage can be in GYM or YEME agar slants which are inoculated from purified colonies grown at 28–30°C for 7–10 d.

Differentiation of the genus *Salinispora* from other genera

The most appropriate way of distinguishing salinisporae from other sporoactinomycetes is by PCR followed by sequencing. Suitable targets are 16S rRNA, *gyrB*, and 16S–23S rRNA intergenic spacer loci. Primers suitable for 16S rRNA gene amplification include FC27 (5′-AGAGTTTGATCCTGGCTCAG) and RC 1492 (5′-TACGGCTACCTTGTTACGACTT), as discussed by Mincer et al. (2005). *Salinispora* strains can be distinguished from well established genera classified in the family *Micromonosporaceae* using a combination of chemotaxonomic and morphological properties (see Table 190 in the treatment of the family *Micromonosporaceae*); they can also be separated from the genera *Actinaurispora* and *Plantactinospora* on this basis (Qin et al., 2009; Thawai et al., 2010). A quick and simple method is available for distinguishing between salinisporae and micromonosporae as only the latter grow on complex growth medium 4 prepared using deionized water (Jensen et al., 1991).

Taxonomic comments

The genus *Salinispora* was proposed by Maldonado et al. (2005) to accommodate representatives of large numbers of strains isolated from geographically diverse tropical and/or

subtropical locations and designated MAR1 (Mincer et al., 2002). The MAR1 strains were distinguished by morphological characteristics, 16S rRNA gene signature nucleotides, and failure to grow when seawater was replaced with deionized water in the growth medium. Comparative 16S rRNA gene sequence analysis showed that seven of the isolates formed a monophyletic clade in the family *Micromonosporaceae*, which suggested novelty at the genus level. The MAR1 isolates were provisionally assigned to a taxon that was informally designated "*Salinospora*" (Feling et al., 2003; Mincer et al., 2002).

The single most distinguishing feature of the genus is that, to date, it has only been reported from marine environments. It also appears to be unique among actinomycete genera in that strains fail to grow when seawater is replaced with deionized water in a complex growth medium or in defined media that lack specific salt combinations and a sufficiently high osmolarity (Tsueng and Lam, 2008b). Evidence that strains clading with "*Salinispora pacifica*" (M101 and SW02; Kim et al., 2005) have distinct spore arrangements relative to *Salinispora arenicola* warrants further investigation into the possibility that this is a species-defining morphological trait.

Differentiation of species of *Salinispora*

Members of the two species with validly published names can be distinguished using analysis of 16S rRNA gene sequence, DNA:DNA relatedness and phenotypic data (Maldonado et al., 2005). *Salinispora arenicola* strains, unlike those of *Salinispora tropica*, use L-proline, salicin, L-threonine, and L-tyrosine, but not galactose or inulin as sole carbon sources, and grow in the presence of 20 μg/ml rifampin. In addition, members of these taxa synthesize core-sets of secondary metabolites, *Salinispora arenicola* strains produce compounds that belong to the rifamycin and staurosporine classes whereas representatives of *Salinispora tropica* produce salinosporamides and sporalides (Jensen et al., 2007). In contrast, some "*Salinispora pacifica*" strains synthesize the structurally novel metabolites cyanosporasides A and B, salinipyrones A and B, and the polyketides pacificanones A and B (Oh et al., 2008).

List of species of the genus *Salinispora*

1. **Salinispora arenicola** Maldonado, Fenical, Jensen, Kaufman, Mincer, Ward, Bull and Goodfellow 2005, 1764[VP]

a.re.ni′co.la. L. n. *arena* sand; L. suff. -*cola* (from L. n. *incola*) inhabitant, dweller; N.L. n. *arenicola* sand-dweller, indicating isolation from marine sediments.

Aerobic, Gram-stain-positive actinomycetes, which form an extensively branched substrate mycelium that carries smooth-surfaced spores either singly or in clusters. Grows from 10–30°C; optimal growth occurs between 20 and 28°C.

Additional phenotypic properties are cited either in the genus description or in the text.

Source: isolated from coarse sand off the Bahamas.

DNA G+C content (mol%): 69.5 (from whole genome sequence).

Type strain: ATCC BAA-917, CNH-643, DSM 44817.

Sequence accession nos: AY040619 (16S rRNA gene); AY371897 (16S–23S rRNA intergenic spacer region); DQ228681 (*gyrB*); NC_009953 (genome of strain CNS-205).

2. **Salinispora tropica** Maldonado, Fenical, Jensen, Kaufman, Mincer, Ward, Bull and Goodfellow 2005, 1764[VP]

tro′pi.ca. L. fem. adj. *tropica* tropical, pertaining to the tropics, the source of the isolates.

Aerobic, Gram-stain-positive actinomycetes that form an extensively branched substrate mycelium that carries smooth-surfaced spores either singly or in clusters. Grows from 10–30°C; optimal growth occurs between 15–28°C.

Additional phenotypic properties are cited either in the genus description or in the text.

Source: isolated from sediment samples collected in the Caribbean. The type strain (CNB-440^{T}) was isolated from a sediment sample collected at a depth of 20 m off the coast of the Bahamas.

DNA G+C content (mol%): 69.4 (from whole genome sequence).

Type strain: ATCC BAA-916, CNB-440, DSM 44818.

Sequence accession nos: AY040617 (16S rRNA gene); AY371895 (16S–23S rRNA spacer region); DQ2288684 (*gyrB*); NC_009380 (whole genome of strain CNB-440^{T}).

Species *incertae sedis*

1. **"Salinispora pacifica"**

This interesting actinomycete was initially mentioned by Jensen and Mafnas (2006) but has yet to be deposited in culture collections and the name has not been validly published. Nonetheless, it provides insight into the phenotypic and phylogenetic diversity of the genus *Salinispora*. A representative of the taxon forms a distinct lineage in the 16S rRNA *Salinispora* gene tree (Figure 224) and has a range of phenotypic properties and DNA:DNA relatedness values that distinguish it from the type strains of *Salinispora arenicola* and *Salinispora tropica*. Members of this taxon have been isolated from marine sediments collected from around the islands of Guam and Palau in the Pacific Ocean (Gontang et al., 2007).

Genus XV. **Spirilliplanes** Tamura, Hayakawa and Hatano 1997, 101[VP]

Tomohiko Tamura

Spi.ril.li.plan′es. N.L. dim. neut. n. *spirillum* a small spiral; Gr. masc. n. *planes* a wanderer; N.L. fem. (*sic*) n. *Spirilliplanes* an organism with wandering cells, in spirals.

Gram-stain-positive, not acid-fast, producing a fine, nonfragmenting, branching mycelium. Strictly aerobic. The aerial hyphae **aggregate into clusters resembling coils** (Figure 226), but true sporangia are not observed; 14-d-old cultures grown on inorganic salts-starch agar have hyphae arranged in spirals of 5–10 turns with several spores per chain. Spores are ovals or short rods (0.5–0.7 × 0.7–1.0 μm) with smooth surfaces. Upon immersion in water or phosphate buffer containing soil extract, **motile spores** are released from spore chains, but in many instances motility begins over 30–60 min after spore release. **Polar flagella** are present in motile spores. In general, the vegetative mycelia are yellow to orange and aerial hyphae are white. Cell wall contains D-glutamate, glycine, D-alanine, and *meso*-diaminopimelate. Mannose, 3-*O*-methylmannose, glucose, xylose, and galactose are detected in the whole-cell sugars. $C_{17:1}$, $C_{17:0}$, $C_{15:0}$ anteiso, $C_{15:0}$ iso, and $C_{16:0}$ iso are present as major cellular fatty acids. The major menaquinone is MK-10(H_4); small amounts of MK-10(H_6) and MK-10(H_8) are also present. Phosphatidylethanolamine and phosphatidylinositol are diagnostic phospholipids (phospholipid pattern type PII). The acyl type of the cell-wall polysaccharides is glycolyl. Mycolic acid is absent.

DNA G+C content (mol%): 69 (HPLC).

Type species: **Spirilliplanes yamanashiensis** Tamura, Hayakawa and Hatano 1997, 102[VP].

Further descriptive information

The chains of very narrow and coiled sporogenous hyphae with zoospores often appear to be sporangium-like structures under a light microscope (Figure 227). Under a scanning electron microscope, however, these structures are found to be aggregated hyphae and not true sporangia (Figure 226). After incubation at 28°C for 1 h in either water or 0.01 M phosphate buffer (pH 7.0) containing 10% (v/v) soil extract, spores showed active motility.

Good growth occurs at temperatures between 25 and 30°C and on oatmeal agar, inorganic salts-starch agar, and peptone-yeast extract-iron agar.

Cultures also grow well in yeast extract-glucose broth consisting of yeast extract (1%) and D-glucose (1%), pH adjusted to 7.0, and incubated at 28°C on a rotary shaker for 4 d.

The wall chemotype is type II according to the scheme of Lechevalier and Lechevalier (1970a) and the peptidoglycan type is presumed to be type A1γ according to the classification of Schleifer and Kandler (1972).

Enrichment and isolation procedures

The type strain was isolated from a soil sample from Kofu, Yamanashi, Japan. The sample was spread on humic acid-vitamin (HV) agar (Hayakawa and Nonomura, 1987a) for isolation after dry heating (120°C, 1 h) (Nonomura and Ohara, 1969). Incubation was at 28°C for 2 weeks.

Maintenance procedures

Strains of the genus *Spirilliplanes* are maintained by freezing in water containing 10–30% glycerol at −70°C. Lyophilization of suspensions in 10% skim milk + 1% monosodium glutamate and L-drying in 0.01 M potassium phosphate buffer (pH 7.0) containing 3% monosodium glutamate (Sakane and Kuroshima, 1997) are also recommended for long-term preservation.

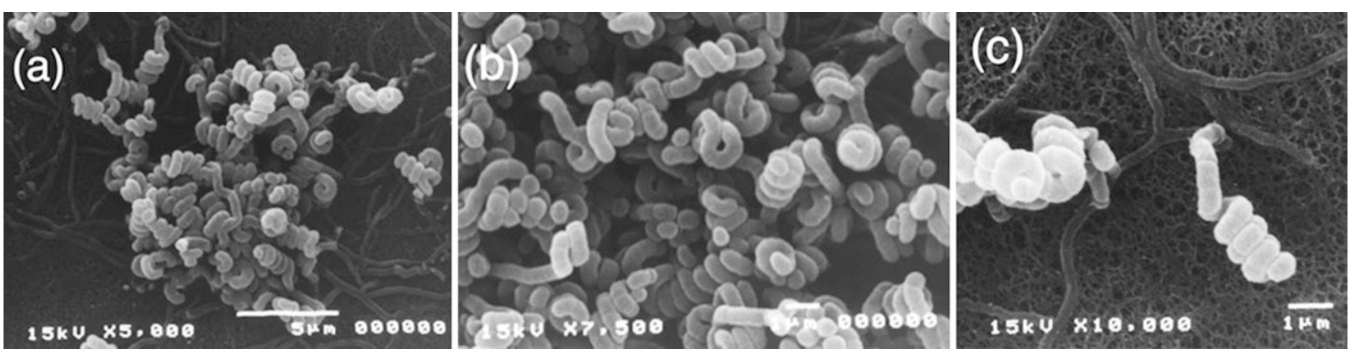

FIGURE 226. Scanning electron micrograph of a 14-d-old culture of *Spirilliplanes yamanashiensis* grown on HV agar revealed the presence of short hyphae arranged in spirals, which could arise from the substrate mycelia. The aerial mycelium at maturity formed short chains of spores (a–c), but no true sporangia were observed because the hyphae were not covered with a sheath.

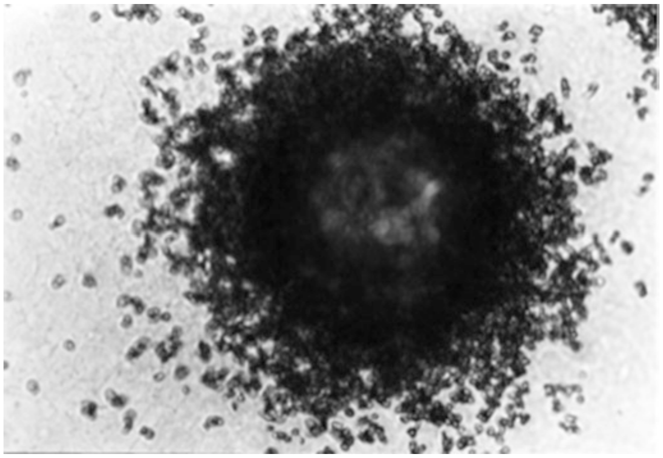

FIGURE 227. Light micrograph of a colony of a 14-d-old culture of *Spirilliplanes yamanashiensis* grown on HV agar. The sporogenous hyphae aggregate into clusters resembling sporangia under the light microscope, but are not true sporangia (see legend to Figure 226).

Differentiation of the genus from other genera

The genus *Spirilliplanes* possesses a chemotype II cell wall and chains of very narrow, coiled sporogenous hyphae with zoospores. Members of the order *Micromonosporales* with chemotype II cell walls are the genera *Actinoplanes*, *Dactylosporangium*, and *Pilimelia*, and they also have motile elements or spores. The genus *Spirilliplanes* can be distinguished from these genera because it does not form sporangia, lacks arabinose in whole cells, and possesses a different menaquinone pattern.

Taxonomic comments

Based on 16S rRNA gene sequence analysis, the genus *Spirilliplanes* clearly forms an independent lineage in the phylogenetic tree of the family *Micromonosporaceae* of the order *Micromonosporales*. The morphological and chemotaxonomic characteristics also clearly separate the genus *Spirilliplanes* from the other genera.

List of species of the genus *Spirilliplanes*

1. **Spirilliplanes yamanashiensis** Tamura, Hayakawa and Hatano 1997, 102[VP]

ya.ma.na.shi.en′sis. N.L. fem. adj. *yamanashiensis* of or pertaining to Yamanashi Prefecture, Japan, the source of soil from which the organism was isolated.

Brownish soluble pigment is produced on tyrosine agar (ISP medium 7). Liquefies gelatin. Hydrolyzes starch. Does not decompose calcium malate. Does not coagulate milk. Xylose, glucose, inositol, raffinose, rhamnose, mannitol, and sucrose are utilized, but fructose and inositol are not.

Source: soil sample from Kofu, Yamanashi, Japan.

DNA G+C content (mol%): 69 (HPLC).

Type strain: YU127-1, DSM 44325, NBRC 15828, JCM 10032, VKM Ac-1993.

Sequence accession no. (16S rRNA gene): D63912.

Genus XVI. **Verrucosispora** Rheims, Schumann, Rohde and Stackebrandt 1998, 1125[VP]

Erko Stackebrandt

Ver.ru.co.si.spo′ra. L. adj. *verrucosus -a -um* warty; Gr. fem. n. *spora* a seed and in biology a spore; N.L. fem. n. *Verrucosispora* an organism with warty spores.

Gram-stain-positive, non-acid-fast, **aerobic organism with branching hyphae**. **Well developed septate mycelium** averaging 0.4 µm in diameter. **Nonmotile spores are borne singly, sessile, or on short or long sporophores**. Warty spore surface changes to a hairy appearance with increased age. **Aerial mycelium is absent**. Strictly aerobic. Chemo-organotroph. Good growth occurs at temperatures between 30 and 40°C. The peptide side-chain of the peptidoglycan contains *meso*-diaminopimelic acid (*meso*-A_2pm), glycine, alanine, and glutamic acid. The peptide side-chains are directly cross-linked (type A1γ). The acyl type of the cell-wall polysaccharides is glycolyl. Mannose and xylose are present in whole cell hydrolysates; arabinose is absent. Characteristic phospholipids are phosphatidylethanolamine, diphosphatidylglycerol, phosphatidylinositol mannoside, and phosphatidylserine. Major menaquinone is MK-(H_4); minor amounts of MK-9(H_6), MK-10(H_4), and MK-9(H_2) are found. Major fatty acids (>15% of total) are $C_{15:0}$ iso, $C_{16:0}$ iso, and $C_{17:0}$ anteiso; $C_{17:0}$ iso, $C_{17:1}$ iso, and $C_{18:0}$ iso are found in minor amounts (>4% to <15%). Phylogenetically, a member of the *Micromonosporaceae*.

DNA G+C content (mol%): 70 (HPLC).

Type species: **Verrucosispora gifhornensis** Rheims, Schumann, Rohde and Stackebrandt 1998, 1126[VP].

Further descriptive information

The family *Micromonosporaceae* was phylogenetically placed in the suborder *Micromonosporineae* as one of several suborders of the order *Actinomycetales*, subclass *Actinobacteridae*, class *Actinobacteria* (Stackebrandt et al., 1997), but in the present volume the suborder has been elevated to order *Micromonosporales*, class *Actinobacteria*. The type strains of members of the most closely related genera are indicated in the phylogenetic tree

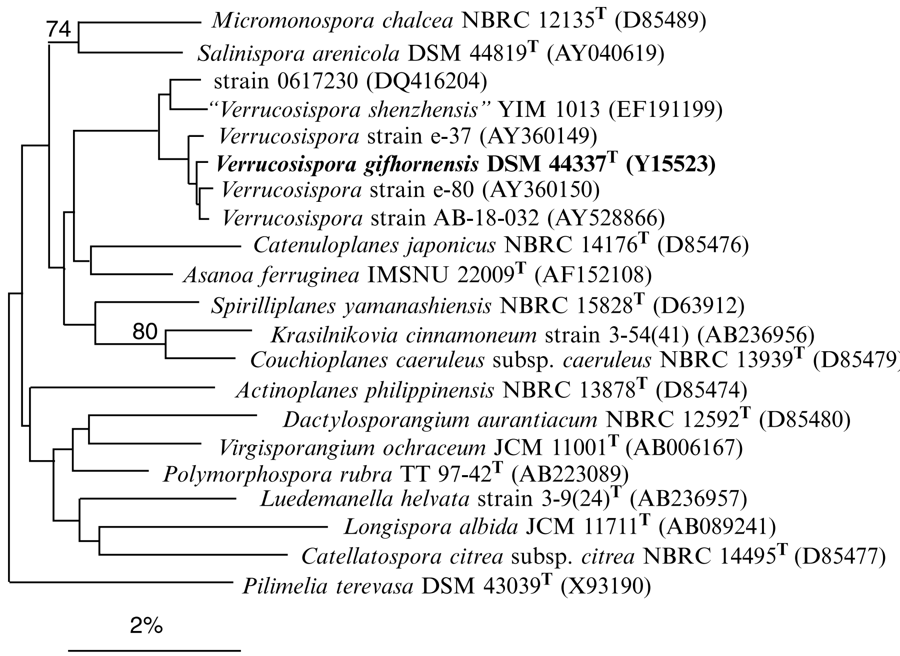

FIGURE 228. Phylogenetic tree showing the position of *Verrucosispora gifhornensis* and related strains among type strains of the family *Micromonosporaceae*, based upon 16S rRNA gene sequence analysis. The sequence of *Streptomyces ambofaciens* served as an outgroup sequence. Evolutionary distances were calculated by the method of Jukes and Cantor (1969). Phylogenetic dendrograms (DeSoete, 1983) were constructed using sequences from different sets of reference strains. Bootstrap analysis (>70% are shown) was used to evaluate the tree topology of the neighbor-joining data by performing 500 resamplings (Felsenstein, 1985). Bar = 2 nucleotide substitutions per 100 nucleotides.

based on 16S rRNA gene sequence similarities (Figure 228). All genera are closely related phylogenetically (Koch et al., 1996a; Maldonado et al., 2005; Matsumoto et al., 2003; Tamura et al., 1997, 2001, 2006; Thawai et al., 2006a), sharing >91% similarity among each other. *Verrucosispora gifhornensis* appears equally closely related (96.0–97.4%) to the majority of genera, sharing a slightly higher degree of relatedness with members of the *Catenuloplanes*, *Asanoa*, and *Spirilloplanes*. However, bootstrap values are low for most branching points indicating the low statistical significance of the branching order [see also dendrograms shown by Tamura et al. (2006) and Matsumoto et al. (2003)]. The close relatedness among type strains is also supported by DNA–DNA reassociation values. The experiments, carried between strain *Verrucosispora gifhornensis* and two close relatives, revealed 41.4% and 40.7% hybridization with the type strains of *Micromonospora olivasterospora* and *Spirilliplanes yamanashiensis*, respectively. The similarity value for the latter pair of organisms was 30.8%. A recently published 16S rRNA gene tree on the phylogenetic position of *Polymorphosporangium rubra* (Tamura et al., 2006) differs from other trees published for newly described type strains of novel genera of the *Micromonosporaceae* in that *Verrucosispora gifhornensis* represents the most deeply branching organism of the family.

Verrucosispora gifhornensis DSM 44337^T produces substrate mycelium on all media that promote growth, but does not produce aerial mycelium. The substrate mycelium is well developed. Hyphae are approximately 0.4 µm in diameter. Sporangia are not formed. On older parts, granular structures are observed on the mycelial surface. Spores are borne singly from the substrate mycelium and are 0.8 µm in diameter. The spore surface appears warty in younger states and changes to a hairy appearance with increased age (Figure 229). Spores are nonmotile.

Members of the genus are strictly aerobic. On all media tested, *Verrucosispora gifhornensis* develops orange colonies which became brownish with increased age. Best growth is observed on agar plates with Difco tryptic soy broth. Growth on the media recommended by the International *Streptomyces* Project (ISP) (Shirling and Gottlieb, 1966) is good on medium 1 (tryptone-yeast extract broth), medium 4 (inorganic salts-starch agar), and medium 5 (glycerol-asparagine agar); moderate growth is observed on medium 2 (yeast extract-malt extract agar), medium 6 (peptone-yeast extract iron agar), and medium 7 (tyrosine agar); no growth is observed on medium 3. Growth on HV-agar (Hayakawa and Nonomura, 1987a; Tamura et al., 1997) is slow. The type strain, DSM 44337^T, grows well in 0–2% NaCl, moderately in 2–4%, but fails to grow at concentrations above 4% NaCl.

No growth is observed below 20°C or above 40°C. Good growth is seen between 30 and 40°C, with optimum growth at 35°C. The pH range for growth is 6.5–8.2, with an optimum at 7.5.

Growth of *Verrucosispora gifhornensis* DSM 44337^T is inhibited by the following antibiotics. Strong inhibition (diameter of the inhibition zone: 31–50 mm): amikacin (concentration: 30 µg per disc), bacitracin (10 µg), cephalozin (30 µg), doxycycline (30 µg), gentamicin (10 µg), imipenem (10 µg), kanamycin (30 µg), neomycin (30 µg), polymyxin B (300 µg), and tetracycline

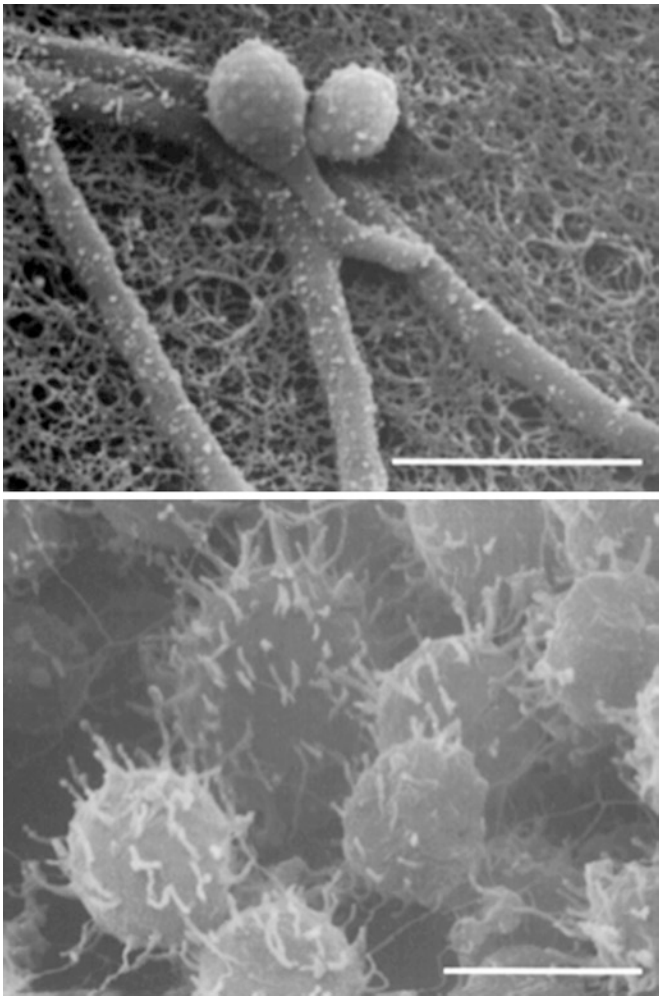

FIGURE 229. Scanning electron microscopic images of *Verrucosispora gifhornensis* DSM 44337^T. (Top) Young spores in detail; bar = 2 µm. (Bottom) Old spores in detail; the spore surface has become hairy; bar = 1 µm. (Reproduced with permission from Rheims et al., 1998. Int. J. Syst. Bacteriol. *48*: 1119–1127.)

(30 µg). Good inhibition (21–30 mm): aztreonam (30 µg), cefotaxime (30 µg), cephalothin (30 µg), norfloxacin (10 µg), penicillin G (6 µg), and ticarcillin (75 µg). Poor inhibition (10–20 mm): ampicillin (10 µg), azlocillin (30 µg), chloramphenicol (30 µg), colistin sulfate (10 µg), erythromycin (15 µg), mezlocillin (30 µg), and oflaxacin (5 µg). No inhibition is observed with lincomycin (15 µg), nitrofurantoin (100 µg), oxacillin (5 µg), or pipemidic acid (20 µg).

Strain AB-18-032, which is affiliated to the genus *Verrucosispora*, is the producer of the polycyclic polyketide Abyssomicin C. This antibiotic is an inhibitor of *para*-iminobenzoic acid biosynthesis and therefore inhibits folic acid biosynthesis at an early stage (Bister et al., 2004). Gram-stain-positive bacteria, including multiresistant and vancomycin-resistant *Staphylococcus aureus* strains, are strongly inhibited.

Enrichment and isolation procedures

Verrucosispora gifhornensis was isolated from a sample taken from a peat bog near Gifhorn, Lower Saxony, Germany (10°33′E, 52°30′N). Samples were taken from a depth of 20–40 cm after removal of the top peat layer. A dilution series of 10 g of an aged peat sample, stored at 4°C for 12 months, was set up in sterile tap water to a dilution of 10^{-6}. Aliquots of 100 µl were plated on Actinomycete isolation agar, pH 8.2 (Difco). After 11 d of aerobic incubation at 30°C, growth of strain DSM 44337^T was indicated by an expanding clear circular zone in the medium. After further incubation, an orange-red colony became visible which was transferred onto fresh Actinomycete isolation agar.

Several *Verrucosispora* strains have been isolated recently in studies exploring microbial diversity [Wang et al., (1999) (i.e. AF131629); Riedlinger et al., 2004; Fiedler et al., 2005; Rifaat et al., 2002; H Muramatsu and others, unpublished (i.e. AB123463)]. Several of these strains were isolated from the marine environment (L.A. Maldonado and others, unpublished, e.g. AY371894 and AY360149), mangrove (L.H. Liu and K. Hong, unpublished; DQ416204) and sponges (S. Jiang and others, unpublished, e.g. DQ994712). The name "*Verrucosispora shenzhensis*" has been proposed for one strain, the sequence of which is available under EF191199 (Z.L. Liao and others, unpublished). The phylogenetic position of a few strains, for which the almost complete 16S rRNA gene sequence has been determined, is shown in Figure 228. Their inter-strain relationship is >99.1%.

Maintenance procedures

Strains can be stored for some weeks as slants at 4°C and as 20% (w/v) glycerol suspensions at −20°C and −80°C. Long-term preservation methods include freeze-drying in skim milk and in liquid nitrogen at −196°C.

Differentiation of the genus *Verrucosispora* from other genera

Members of the genera can be distinguished mainly by their phylogenetic position, morphological features, e.g. presence or absence of sporangia, and motility of spores, and a combination of chemotaxonomic properties (see Table 191). The fatty acid profile of isolate DSM 44337^T is characterized by the predominance of $C_{15:0}$ iso, followed by $C_{16:0}$ iso and $C_{17:0}$ anteiso The predominance of $C_{15:0}$ iso and $C_{17:0}$ iso fatty acids over $C_{15:0}$ anteiso and $C_{17:0}$ anteiso fatty acids, the occurrence of significant amounts of unsaturated fatty acids, and the absence of 10-methyl- and 2-OH-fatty acids matches the diagnostic fatty acid type 2d (Kroppenstedt, 1985). This type has also been reported in members of the genera *Actinoplanes*, *Longispora*, *Virgisporangium*, *Actinocatenispora*, *Pilimelia*, and *Spirilliplanes*. The *meso*-A_2pm-containing, directly cross-linked peptidoglycan of the A1γ variation (cell-wall type II) is represented in all members of the family, except for members of the *Couchioplanes* and *Catenuloplanes* (type IV). The major isoprenoid quinone component, MK-9(H_4), is present in several members of the family, though often in combination with other menaquinones. The polar lipid pattern is PII (Lechevalier and Lechevalier, 1970a), consisting of the diagnostic phosphatidylethanolamine (phospholipid type PII), diphosphatidylglycerol, phosphatidylinositol mannoside, and phosphatidylserine. This combination is common among species of the family. *Verrucosispora gifhornensis* lacks arabinose among its whole cell sugars, a feature shared with members of the genera *Polymorphospora* (Tamura et al., 2006), *Catenuloplanes* (Yokota et al., 1993), and *Spirilliplanes* (Tamura et al., 1997).

List of species of the genus *Verrucosispora*

1. **Verrucosispora gifhornensis** Rheims, Schumann, Rohde and Stackebrandt 1998, 1126[VP]

gif.horn.en′sis. N.L. fem. n. *gifhornensis* of or belonging to the city of Gifhorn, adjacent to the peat bog from which the organism was isolated.

Morphological, chemotaxonomic, and general characteristics are as described for the genus. An orange pigment is produced on all ISP media tested. Gelatin liquefaction and peptonization of milk is positive. Hydrolyzes starch. Nitrite is not produced from nitrate. Cellulose decomposition is negative. D-Xylose, D-glucose, D-galactose, maltose, sucrose, D-arabinose, and α-trehalose are utilized; D-ribose, D-fructose, L-rhamnose, L-sorbose, lactose, α-melibiose, melezitose, raffinose, glycerol, dulcitol, *myo*-inositol, and salicin are not utilized. L-Serine, L-aspartic acid, L-glutamic acid, L-histidine, L-arginine, and L-phenylalanine are used as nitrogen source. Major fatty acids are (%) $C_{15:0}$ iso (31.1), $C_{16:0}$ iso (18.6), $C_{17:0}$ anteiso (16.0); minor components (>1.5% to <10%) are $C_{17:0}$ iso (8.3), $C_{17:1}$ iso (4.0), $C_{18:0}$ iso (4.4), $C_{15:0}$ anteiso (2.2), $C_{17:0}$ (2.9), and $C_{18:1}$ (1.8). The other chemotaxonomic markers are indicated in the genus description. Highly sensitive against a wide range of antibiotics.

Source: isolated from a peat bog near Gifhorn, Lower Saxony, Germany.

DNA G+C content (mol%): 70 (HPLC).

Type strain: HR1-2, DSM 44337.

Sequence accession no. (16S rRNA gene): Y15523.

Genus XVII. **Virgisporangium** corrig. Tamura, Hayakawa and Hatano 2001, 1814[VP]

Tomohiko Tamura

Vir.gi.spo.ran′gi.um. L. n. *virga* a slender green branch, rod; N.L. neut. n. *sporangium* (from Gr. n. *spora* a seed and, in biology, a spore; Gr. n. *angeion* vessel) sporangium (spore-containing vessel); N.L. neut. n. *Virgisporangium* an organism with rod-shaped sporangia (spore-containing vessels).

Gram-stain-positive, not acid-fast, produces a fine, nonfragmenting, branching mycelium. Strictly aerobic. **Slender sporangia** are formed on short sporangiophores on the substrate mycelium (Figure 230 and Figure 231). Each sporangium typically contains a single row of **six or more spores**. Spores are oval or short rods (0.6–0.9 × 0.8–1.5 µm) and exhibit motility. Cell wall contains D-glutamate, glycine, alanine, and 3-OH-diaminopimelate. Mannose, 3-*O*-methylmannose, rhamnose, glucose, arabinose, xylose, and galactose are present in whole-cell hydrolysates. The major cellular fatty acid is $C_{17:0}$ anteiso. The major menaquinones are MK-10(H_4) and MK-10(H_6). Phosphatidylethanolamine is present as the diagnostic phospholipid (phospholipid pattern type PII). The acyl type of the cell wall is glycolyl. Mycolic acid is not detected.

DNA G+C content (mol%): 71 (HPLC).

Type species: **Virgisporangium ochraceum** Tamura, Hayakawa and Hatano 2001, 1815[VP].

Further descriptive information

Good growth occurs between 20 and 30°C. In general, the vegetative mycelia are yellow to orange.

Cultures grow well in yeast extract-glucose broth, consisting of yeast extract (1%) and D-glucose (1%), pH adjusted to 7.0, and incubated at 28°C on a rotary shaker for 4 d.

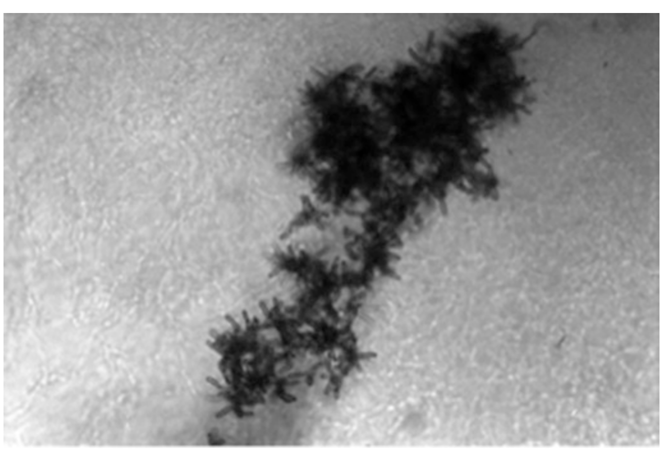

FIGURE 230. Light micrograph of 14-d-old cultures of *Virgisporangium ochraceum* isolates grown on HV agar showing rod-shaped sporangia on the substrate mycelium, which developed singly or in clusters above the surface of the substrate.

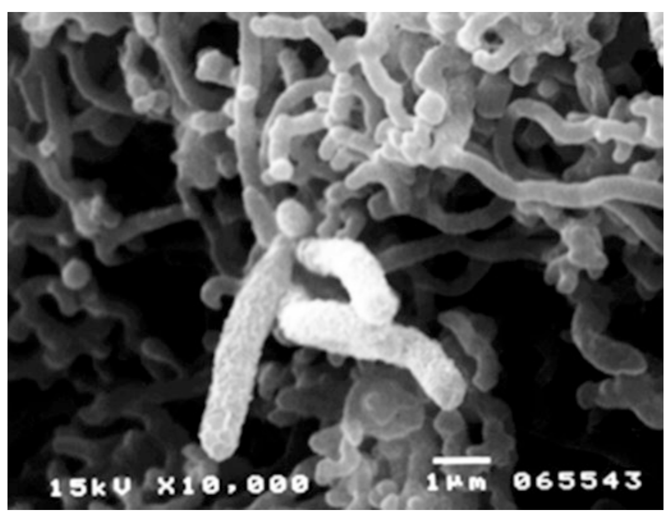

FIGURE 231. Scanning electron micrograph of 14-d-old cultures of *Virgisporangium ochraceum* isolates grown on HV agar showing that each sporangium typically contains a single row of six or more spores. Globose bodies were not observed. After incubation at 28°C for 1 h in distilled water, many spores exhibited active motility.

The cell-wall chemotype is II according to Lechevalier and Lechevalier (1970a) and the peptidoglycan type is presumed to be A1γ according to Schleifer and Kandler (1972). The cellular fatty acids are predominantly $C_{17:0}$ anteiso, with lesser amounts of $C_{18:1}$, $C_{16:0}$ iso, and $C_{15:0}$ iso, depending upon the species.

Enrichment and isolation procedures

For isolation of the strains of the genus *Virgisporangium*, samples are inoculated on humic acid-vitamin (HV) agar (Hayakawa and Nonomura, 1987a) using the capillary method (Hayakawa et al., 1991d; Palleroni, 1980) with vanillin as an attractant. Incubation is at 28°C for 2 weeks. The described species were isolated from a vegetable field soil in Yamanashi, from a mulberry field soil in Okinawa, and from a potato field soil in Okinawa, Japan (Tamura et al., 2001).

Maintenance procedures

Strains of the genus *Virgisporangium* are maintained by freezing in water containing 10–30% glycerol at −70°C. Lyophilization of suspensions in 10% skim milk + 1% monosodium glutamate and L-drying in 0.01 M potassium phosphate buffer (pH 7.0) containing 3% monosodium glutamate (Sakane and Kuroshima, 1997) are also recommended for long-term preservation.

Differentiation of the genus *Virgisporangium* from other genera

Strains of the genus *Virgisporangium* form rod-shaped sporangia on short sporangiophores, which resemble the extended sporangia of the genus *Dactylosporangium*. However, the genus *Virgisporangium* can be distinguished from the genus *Dactylosporangium* in the development of rod-shaped sporangia with six or more spores, the absence of 10-methylated fatty acids, and the presence of MK-10(H_4, H_6, H_8). Cell walls of *Virgisporangium* contain exclusively 3-OH diaminopimelic acid as the diamino acid. Some members of the genera *Micromonospora* and *Dactylosporangium* are known to have 3-OH-diaminopimelic acid as well as *meso*-diaminopimelic acid in their cell walls.

Taxonomic comments

The original spelling *Virgisporangium* (*sic*) proposed by Tamura et al. (2001), has been corrected by the List Editor, IJSEM (2001).

Based on 16S rRNA gene sequence analysis, this genus represents an independent lineage in the phylogenetic tree of the family *Micromonosporaceae* of the order *Micromonosporales*. *Virgisporangium aurantiacum* was proposed based upon a single strain. In contrast, the description of the type species *Virgisporangium ochraceum* was based upon three strains with relatively low values of DNA relatedness (40–60%), but high phenotypic and 16S rRNA gene sequence similarity (Tamura et al., 2001).

List of species of the genus *Virgisporangium*

1. **Virgisporangium ochraceum** corrig. Tamura, Hayakawa and Hatano 2001, 1815[VP]

och.ra′ce.um. L. n. *ochra* ochre, yellow ochre; N.L. neut. adj. *ochraceum* of the color of ochre, rust-colored.

Morphological, chemotaxonomic, and general characteristics are as given above for the genus. Brownish soluble pigment is produced on tyrosine agar (ISP medium 7). Gelatin liquefaction is negative. Hydrolyzes starch. Does not decompose calcium malate. Coagulation and peptonization of milk are positive. Optimum temperature for growth is 15–30°C. Grows at 37°C. Does not grow on 7% NaCl. Glucose, D-fructose, D-xylose, L-arabinose, glycerol, and mannose are utilized, but inulin is not. The major cellular fatty acids are $C_{17:0}$ anteiso, $C_{18:1}$, and $C_{15:0}$ iso, with $C_{16:0}$ iso, $C_{17:0}$, and $C_{19:0}$ in smaller amounts.

Source: soil.

DNA G+C content (mol%): 71 (HPLC).

Type strain: YU655-43, CIP 107213, NBRC 16418, JCM 11001.

Sequence accession no. (16S rRNA gene): AB006167.

2. **Virgisporangium aurantiacum** corrig. Tamura, Hayakawa and Hatano 2001, 1815[VP]

au.ran.ti.a′cum. N.L. neut. adj. *aurantiacum* orange-colored.

Morphological, chemotaxonomic, and general characteristics are as given above for the genus. Brownish soluble pigment is produced on tyrosine agar (ISP medium 7). Gelatin liquefaction is negative. Hydrolyzes starch. Does not decompose calcium malate. Coagulation and clearing of milk are positive. Optimum temperature for growth is 15–30°C. Does not grow at 37°C. Does not grow on 4% NaCl. Glucose, D-fructose, D-xylose, L-arabinose, glycerol, maltose, and mannose are utilized, but inositol, D-sorbitol, and inulin are not. The major cellular fatty acids are $C_{17:0}$ anteiso and $C_{16:0}$ iso.

Source: the type strain was isolated from soil.

DNA G+C content (mol%): 71 (HPLC).

Type strain: YU438-5, CIP 107212, NBRC 16421, JCM 11002.

Sequence accession no. (16S rRNA gene): AB006169.

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Order XII. **Propionibacteriales** ord. nov.

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Pro.pio.ni.bac.te.ri.a′les. N.L. neut. n. *Propionibacterium* type genus of the order; suff. *-ales* ending to denote an order; N.L. fem. pl. n. *Propionibacteriales* the *Propionibacterium* order.

This order is formed by elevation of the suborder *Propionibacterineae* Zhi et al. 2009. The pattern of 16S rRNA signatures defining this order consists of nucleotides at positions 127:234 (A–U), 598:640 (U–A), 657:749 (G–C), 828 (U), 829:851 (A–C), 832:854 (U–C), 833:853 (G–U), 952:1229 (C–G), and 986:1219 (U–A).

The order contains the families *Propionibacteriaceae* and *Nocardioidaceae*.

Type genus: **Propionibacterium** Orla-Jensen 1909, 337[AL] emend. Charfreitag, Collins and Stackebrandt 1988, 356.

References

Charfreitag, O., M.D. Collins and E. Stackebrandt. 1988. Reclassification of *Arachnia propionica* as *Propionibacterium propionicus* comb. nov. Int. J. Syst. Bacteriol. *38*: 354–357.
Orla-Jensen, S. 1909. Die Hauptlinien des natürlichen Bakteriensystems. Zentralbl. Bakteriol. Parasitenkd. Infektionskr. Hyg. Abt. 2 *22*: 305–346.
Zhi, X.-Y., W.-J. Li and E. Stackebrandt. 2009. An update of the structure and 16S rRNA gene sequence-based definition of higher ranks of the class *Actinobacteria*, with the proposal of two new suborders and four new families and emended descriptions of the existing higher taxa. Int. J. Syst. Evol. Microbiol. *59*: 589–608.

Family I. **Propionibacteriaceae** Delwiche 1957[AL] emend. Rainey, Ward-Rainey and Stackebrandt 1997, 484 emend. Zhi, Li and Stackebrandt 2009, 599

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Pro.pio.ni.bac.te.ri.a.ce′a.e. N.L. neut. n. *Propionibacterium* type genus of the family; suff. *-aceae* ending denoting family; N.L. fem. pl. n. *Propionibacteriaceae* the *Propionibacterium* family.

The pattern of 16S rRNA signatures consists of nucleotides at positions 328 (U), 407:435 (C–G), 451 (A), 453 (G), 819 (G), 825:875 (A–U), 827 (C), 828 (U), 832:854 (U–C), 833:853 (G–U), and 844 (U).

Type genus: **Propionibacterium** Orla-Jensen 1909, 337[AL] emend. Charfreitag, Collins and Stackebrandt 1988, 356.

Other genera of the family *Propionibacteriaceae* are: *Aestuariimicrobium* Jung et al. 2007; *Brooklawnia* Rainey et al. 2006 (*in* Bae et al. 2006b); *Friedmanniella* Schumann et al. 1997; *Granulicoccus* Maszenan et al. 2007; *Luteococcus* Tamura et al. 1994; *Microlunatus* Nakamura et al. 1995; *Micropruina* Shintani et al. 2000; *Propionicicella* Bae et al. 2006a; *Propionicimonas* Akasaka et al. 2003b; *Propioniferax* Yokota et al. 1994; *Propionimicrobium* Stackebrandt et al. 2002; and *Tessaracoccus* Maszenan et al. 1999b.

Genus I. **Propionibacterium** Orla-Jensen 1909, 337[AL] emend. Charfreitag, Collins and Stackebrandt 1988, 356

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Pro.pio.ni.bac.te′ri.um. N.L. n. *acidum propionicum* propionic aid; L. neut. n. *bacterium* a small rod; N.L. neut. n. *Propionibacterium* propionic (acid) bacterium.

Pleomorphic rods, 0.2–1.5 µm ×, 1–5 µm, often diphtheroid or club-shaped with one end rounded and the other tapered or pointed; **however, cells may be coccoid, bifid, branched, or filamentous with lengths up to 20 µm**. Cells may occur singly, in pairs or short chains, in V or Y configurations, or in clumps with "Chinese character" arrangement. Swollen spherical cells resembling sphaeroplasts, up to 5–20 µm in diameter, are formed by some strains. Highly filamentous microcolonies of long, branched, septate or non-septate filaments may be formed. **Gram-stain-positive, nonmotile**, non-acid-fast, nonsporeforming. **Anaerobic-to-aerotolerant-to-microaerophilic, although some strains can grow aerobically, most grow better anaerobically**. **Chemo-organotrophic mostly with complex nutritional requirements, grow in standard complex media:** fermentation of sugars, **polyhydroxy alcohols**, or lactate produced by the fermentative activities of other bacteria (secondary fermentation) and **produce large amounts of propionic and acetic acids** which is a distinguishing feature of the genus. Lesser amounts of iso-valeric, formic, succinic, or lactic acids and carbon dioxide are also formed. **Generally catalase-positive**. **Optimum growth temperature 30–37°C**. Colonies on solid media are smooth, convex, or rough. Floccular or granular masses of variable size may be observed in liquid media. White, gray, pink, red, yellow, or orange in color. Cell-wall peptidoglycan contains either *meso-* or LL-diaminopimelic acid (DAP) as the dibasic amino acid. Tetrahydrogenated menaquinones with nine isoprene units [**MK-9(H_4)**] are the major respiratory quinones. The long-chain **cellular fatty acids are of the straight-chain saturated**, **iso- and anteiso-methyl branched types**, **principally 12- and 13-methyltetradecanoic acids ($C_{15:0}$ iso; $C_{15:0}$ anteiso);** monounsaturated acids may also be present in small amounts.

DNA G+C content (mol%): 57–70.

Type species: **Propionibacterium freudenreichii** van Niel 1928, 162[AL].

Further descriptive information

Two principal groups of organisms are traditionally described in the genus *Propionibacterium*; namely the "classical" or "dairy" (Table 202) and the "cutaneous" (Table 203) propionibacteria (Table 204). Although the phylogenetic relationship between *Propionibacterium* species based on 16S rRNA gene sequences does not mirror exactly their classification based on habitat (Figure 232), the latter is still useful for categorizing some species.

1. The "classical" or "dairy" propionibacteria: typified by strains from dairy products and used, for example, in the manufacture of hard rennet Swiss-type cheese. The genus was first described as a result of the study of propionic acid-producing bacteria isolated from cheese by a number of authors in the early 1900s (Orla-Jensen, 1909; Von Freudenreich and Orla-Jensen, 1906). They are used as starter cultures in the dairy industry and have been studied in relation to the commercial production of propionic acid. They have also been isolated from fermenting food and plant materials such as silage and fermenting olives (Cancho et al., 1980; Cancho et al., 1970; Plastourgos and Vaughn, 1957), and from soil (van Niel, 1928), including soil from rice paddy fields (Hayashi and Furusaka, 1979). Related bacteria have been found associated with nematodes in the Zebra gut (Krecek et al., 1992) and anaerobic digestors (Riedel and Britz, 1993; Sarada and Joseph, 1994). The described species *Propionibacterium microaerophilum* and *Propionibacterium cyclohexanicum*, isolated from olive mill wastewater (Koussémon et al., 2001) and spoiled off-flavor orange juice (Kusano et al., 1997) respectively, do not fit the "classical" or "dairy" propionibacteria groups based on habitat, but they are more closely related to these species based on 16S rRNA gene sequence (97.5%

*This section is an updated version of the text written by Cecil S. Cummins and John L. Johnson in the previous edition of this *Manual*, 1986.

identity between *Propionibacterium microaerophilum* and *Propionibacterium acidipropionici*; 97.1% identity between *Propionibacterium cyclohexanicum* and *Propionibacterium freudenreichii*) compared with the "cutaneous" species. Similarly, based on 16S rRNA gene sequence, *Propionibacterium australiense*, isolated from granulomatous bovine lesions (Bernard et al., 2002b) clusters with *Propionibacterium cyclohexanicum* and is more closely related to *Propionibacterium freudenreichii* than "cutaneous" species. The recently described novel species *Propionibacterium acidifaciens*, isolated from human carious dentine, clusters with *Propionibacterium australiense* on the basis of 16S rRNA gene analysis (97.7% identity; Downes and Wade, 2009).

2. The "cutaneous" propionibacteria: strains isolated from human skin, although they also colonize the mouth, genitourinary tract, and the large intestine. *Propionibacterium propionicum*, which also causes human infection, clusters with "cutaneous" species based on 16S rRNA gene sequence.

On the basis of morphology, the human "cutaneous" propionibacteria were originally assigned to the genus *Corynebacterium* (up to seventh edition of the *Manual of Determinative Bacteriology*). They were transferred from that genus to *Propionibacterium* in the eighth edition because (a) they were anaerobic, (b) propionic acid is the main metabolic product, (c) LL-DAP is present in the peptidoglycan of most species, (d) they produce C_{15} iso- and anteiso- acids as the principal fatty acids of cell lipids, and (e) they lack mycolic acids and the arabinogalactan characteristic of *Corynebacterium* sensuo stricto. Prévot (1976), however, proposed that this group of organisms, because of their pathogenic and immunomodulatory properties, should not be transferred to *Propionibacterium* but should instead be accommodated in a separate subgenus *Coryneformis* in the family *Corynebacteriaceae*. Analysis of 16S rRNA gene sequence data confirms the distinction from the *Corynebacteriaceae* and relatedness to the other propionibacteria (Charfreitag and

TABLE 202. Characteristics differentiating the "classical" and "dairy" species of the genus *Propionibacterium*[a,b,c]

Characteristic	*P. freudenreichii*	*P. acidipropionici*	*P. jensenii*	*P. thoenii*
Acid from:				
Maltose	–	+	d+	d+
Sorbitol	–	+	–	d+
Sucrose	–	+	+	d+
Nitrate reduction	d	+	–	–
β-Hemolysis	–	–[d]	–	+[e]
Color/pigment	Cream	Cream-orange-yellow	Cream	Red-brown
DAP isomer	*meso*-	LL-	LL-	LL-

[a]Symbols: +, positive reaction in 90–100% of isolates; –, negative reaction in 90–100% of isolates; d, positive reaction in 11–89% of isolates; d+, reaction positive in 40–90% of isolates.

[b]Reactions determined by procedures given in Holdeman et al., (1977).

[c]DAP, diaminopimelic acid.

[d]May show slight β-hemolysis under area of confluent growth.

[e]Hemolytic on blood agar containing human, bovine, equine, sheep, rabbit, and pig blood (Vedamuthu et al., 1971).

TABLE 203. Principal characteristics of "cutaneous" species of the genus *Propionibacterium*[a]

Characteristic	*P. acnes* phylotype			*P. avidum* biovar		*P. granulosum*
	I	II	III[b]	I	II	
Fermentation of:						
L-Arabinose	–	–	–	d	d	–
Glycerol	d	d	+	+	+	+
Maltose	–	–	–	+	+	d+
Sorbitol	d+	–	–	–	–	–
Sucrose	–	–	nd	+	+	+
Esculin hydrolysis	–	–	nd	+	+	–
Biochemical tests:						
Gelatin liquefaction	+	+	nd	+	+	–
β-Hemolysis (rabbit blood) (5 d/37°C)	d+	–	–	+	+	–[c]
Indole production	+	+	+	–	–	–
Nitrate reduction	+	+	+	–	–	–
CAMP reaction (sheep blood) (2 d/37°C)	+	+	nd	nd	nd	–
Cell-wall composition:						
A_2pm isomer	LL-	LL- (*meso*-)	nd	LL-	*meso*-(LL-)	LL-
Amino acids	Ala, Gly, Glu	Ala, Gly[d], Glu	nd	Ala, Gly, Glu	Ala, Gly[d], Glu	Ala, Gly, Glu
Sugars	Galactose, glucose, mannose	Glucose, mannose	nd	Galactose, glucose, mannose	Glucose, mannose	Galactose, mannose

[a]Symbols: +, positive reaction in 90% or more of isolates; –, 90% or more of isolates are negative; d, 11–89% of isolates are positive; d+, 40–90% of isolates are positive; parentheses, present in some isolates.

[b]Data based on the study of five isolates (McDowell et al., 2008).

[c]Generally nonhemolytic on sheep, horse, or rabbit blood agar, but reported to be β-hemolytic on rabbit blood by Hoeffler, (1977).

[d]Gly not present in isolates with *meso*-DAP.

TABLE 204. Characteristics differentiating the species of the genus *Propionibacterium*[a]

Characteristic	"Classical" propionibacteria						"Cutaneous" propionibacteria					
	P. freudenreichii	*P. acidipropionici*	*P. jensenii*	*P. thoenii*	*P. cyclohexanicum*[b]	*P. microaerophilum*[c]	*P. acnes*	*P. avidum*	*P. granulosum*	*P. australiense*	*P. acidifaciens*[d]	*P. propionicum*
Acid from:												
Maltose	–	+	d+	d+	+	nd	–	+	d+	–	+	+
Sucrose	–	+	+	d+	nd	nd	–	+	+	–	+	+
Esculin hydrolysis	+	+	+	+	+	–	–	+	–	–	–	–
Indole production	d	–	–	–	–	nd	d+	–	–	–	–	–
Nitrate reduction	–	+	–	–	–	+[e]	d+	–	–	+	–	+
Gelatin hydrolysis	–	–	–	–	–	nd	+	+	d–	–	–	d
β-Hemolysis	–	–[f]	–	+[g]	nd	nd	d+	d+	–[h]	–	nd	+[i]
DAP isomer	*meso*-	LL-	LL-	LL-	*meso*-	nd	LL- (*meso*)	LL- (*meso*)	LL-	*meso*-	nd	LL-
DNA G+C content (mol%)	64–67	66–68	65–68	66–67	66.8	67.7	57–60	62–63	61–63	nd	70	63–65

[a]Symbols: +, positive reaction in 90% or more of isolates; –, 90% or more of isolates are negative; d, 11–89% of isolates are positive; d+, 40–90% of isolates are positive; d–, 10–40% isolates are positive; parentheses, present in some isolates.

[b]Data taken from Kusano et al. (1997).

[c]Data taken from Koussémon et al. (2001).

[d]Data taken from Downes and Wade (2009).

[e]Nitrate reduced to nitrogen rather than nitrite.

[f]May show slight β-hemolysis under area of confluent growth.

[g]Hemolytic on blood agar containing human, bovine, equine, sheep, rabbit, and pig blood (Vedamuthu et al., 1971).

[h]Generally nonhemolytic on sheep, horse, or rabbit blood agar, but reported to be β-hemolytic on rabbit blood by Hoeffler, (1977).

[i]+, for β-hemolysis of human blood; –, for sheep blood; d, for horse blood.

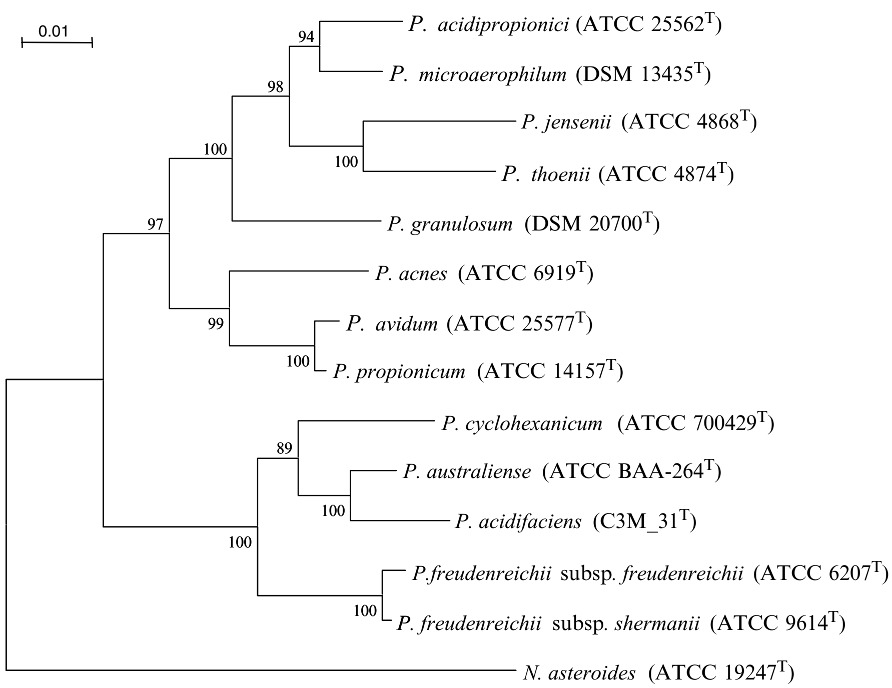

FIGURE 232. Phylogenetic tree of 16S rRNA gene sequences for the genus *Propionibacterium*. The neighbor joining tree was constructed using the Jukes–Cantor-based algorithm. The sequence input order was randomized, and bootstrapping resampling statistics were performed using 500 data sets. The 16S rRNA gene sequence from *Nocardia asteroides* was used as a distant outgroup to root the tree as it is also belongs to the class *Actinobacteria*. Bootstrap values are shown at each node of the tree. Bar = 1 substitution per 100 nucleotide positions.

Stackebrandt, 1989), and there is now general acceptance that *Propionibacterium* is a more fitting genus than *Corynebacterium* (Greenman, 1995) (see below for further discussion). This group has also been referred to as "anaerobic coryneforms", "anaerobic diphtheroids", "acnes group strains", or "cutaneous propionbacters".

In the early literature, anaerobic coryneform bacteria associated with infection were referred to as either *Corynebacterium acnes* (Douglas and Gunter, 1946) or *Corynebacterium parvum* (Cummins and Johnson, 1974). Douglas and Gunter (1946) proposed that these anaerobic coryneforms should be placed in the genus *Propionibacterium* due to the production of propionic acid, catabolic processes, and preference for anaerobic conditions, while others favored the retention of *Corynebacterium* (Puhvel, 1968). Furthermore, it is now clear that strains identified as *Corynebacterium acnes* included the two species *Propionibacterium acnes* and *Propionibacterium granulosum* (Cummins and Johnson, 1974). Cummins and Johnson (1974) also demonstrated that of 59 strains labeled *Corynebacterium parvum*, 90% were in fact *Propionibacterium acnes* by serological and physiological tests as well as DNA similarity, and almost all of the remainder were *Propionibacterium avidum*. The name *Corynebacterium parvum* has been used in immunological and oncological literature to describe the organism which has been extensively used as a reticulostimulant or immunomodulator but should be considered a synonym for *Propionibacterium acnes*. It should also be noted that the National Collection of Type Cultures (NCTC) strain 10387, still designated by the NCTC as *Corynebacterium parvum*, is identical with *Propionibacterium granulosum* ATCC 11829 (Cummins, 1980) and that NCTC 10390 (ATCC 12930), deposited as *Corynebacterium parvum*, is a strain of *Propionibacterium acnes* (phylotype II; see Table 203). We have kept much of the genus and species descriptions of Johnson and Cummins as given in the previous edition of the *Manual*, but have added to them in the light of recent molecular phylogenetic analyses. It should be noted that *Propionibacterium lymphophilum* (Torrey, 1916) has now been reclassified as *Propionimicrobium lymphophilum* (Stackebrandt et al., 2002) and *Propionibacterium innocuum* (Pitcher and Collins, 1991) as *Propioniferax innocua* (Yokota et al., 1994).

Morphologically, the "classical" propionibacteria tend to be shorter and rather thicker than the "cutaneous" species, although all strains may be very variable in morphology, especially in early exponential phase cultures. Strains of *Propionibacterium acnes* in particular give longer more slender irregular rods in young cultures, much resembling the "classical" description of *Corynebacterium diptheriae mitis*, making it easy to understand why the organism was called *Corynebacterium acnes*. In stationary phase cultures, all strains tend to be more coccal. The species *Propionibacterium propionicum* (Buchanan and Pine, 1962) and strains of *Propionibacterium acnes* phylotype III (McDowell et al., 2008) can form filaments up to at least 20 µm in length. Some strains of propionibacteria (e.g., *Propionibacterium avidum* and *Propionibacterium thoenii*) can be observed as capsulated (Skogen et al., 1974; Stackebrandt et al., 2006), and it seems that a considerable number of strains of all species may produce extracellular slime not organized in the form of clear-cut individual capsules. The extracellular material is carbohydrate in nature (Skogen et al., 1974). Purple/red polyphosphate granules may be observed by light microscopy with methylene blue stain or as electron-dense inclusions by electron microscopy with osmium staining, particularly in older cultures (Vorobjeva, 1999). Variations in the peptidoglycan composition and other sugar moieties of the cell envelopes of *Propionibacterium* species are detailed in Table 205. It has been suggested that peptidoglycan containing *meso*-DAP is related to a more coccoid morphology and LL-DAP with a coryneform morphology (Vorobjeva, 1999).

The cell lipids of both "classical" and "cutaneous" strains are characterized by the presence of large amounts of C_{15} branched-chain fatty acids (Moss et al., 1969). Mannose-containing phospholipids have been described in "*Propionibacterium shermanii*" strains (Prottey and Ballou, 1968). The mycolic acids, characteristic of the *Corynebacterium–Mycobacterium–Nocardia* group, are not found. Spermidine and spermine are the predominant compounds within the polyamine profile of the propionibacteria (Hamana, 1995).

With respect to nutritional requirements, the two groups of propionibacteria seem similar. Early studies indicated a requirement for pantothenate in all strains examined and many also require biotin, with growth generally much improved by thiamine and nicotinamide. The addition of oleate may stimulate growth and some strains require *p*-aminobenzoic acid (Delwiche, 1949; Ferguson and Cummins, 1978; Holland et al., 1979). In contrast to the auxotrophic behavior of most propionibacteria species, *Propionibacterium microaerophilum* does not require such growth factors and is, therefore, prototrophic. Amino acid requirements are complex for the "cutaneous" group (Ferguson and Cummins, 1978), but at least some of the "classical" propionibacteria can grow with ammonium sulfate as a nitrogen source (Wood et al., 1938). Most strains of propionibacteria produce vitamin B_{12}, and some, especially strains of *Propionibacterium freudenreichii*, produce large amounts (Hettinga and Reinbold, 1972; Janicka et al., 1976; Vorobjeva, 1999). Fermentation of glucose, pyruvate, dioxyacetate, and glycerol all result in propionic acid, acetic acid, and carbon dioxide production.

Bacteriophages have been reported for *Propionibacterium freundenreichii* subsp. *shermanii*; isometric and filamentous bacteriophage has been described and lysogeny demonstrated (Herve et al., 2001; Vorobjeva, 1999). Bacteriophages specific to *Propionibacterium acnes* have been used to distinguish types I and II (Webster and Cummins, 1978). The complete genome sequence of *Propionibacterium acnes* KPA171202 contains a putative pro-phage sequence (Brüggemann et al., 2004). The *Propionibacterium acnes* specific bacteriophage PA6, which has an icosahedral head and non-contractile tail characteristic of the *Siphoviridae*, has a genome sequence organization similar to the temperate mycobacteriophages, but does not appear to contain genes related to lysogeny (Farrar et al., 2007).

Isolation and maintenance procedures

Most methods for the primary isolation of propionibacteria from dairy products have relied on yeast extract-sodium lactate media (Malik et al., 1968). More complex media such as brain heart infusion broth or agar, however, have often been used for the "cutaneous" strains. Growth of all propionibacteria can be obtained in a trypticase-yeast extract-glucose medium containing 0.05% (v/v) Tween 80 (Cummins and Johnson, 1981). Defined media for the growth of propionibacteria have been devised (Ferguson and Cummins, 1978; Holland et al., 1979; Kurman, 1960; Reddy et al., 1973). A medium containing

TABLE 205. Summary of metabolic reactions and other biochemical characteristics of species of the genus *Propionibacterium*[a,b]

	"Classical" propionibacteria						"Cutaneous" propionibacteria					
Reaction	*P. freudenreichii*	*P. acidipropionici*	*P. jensenii*	*P. thoenii*	*P. cyclohexanicum*	*P. microaerophilium*	*P. acnes*	*P. avidum*	*P. granulosum*	*P. acidifaciens*	*P. australiense*	*P. propionicum*
Acid from:												
Adonitol	d+	+	d+	d+	–	nd	d+	d+	–	nd	+	d
Amygdalin	–	–	d+	d+	+	–	–	–	d–	nd	–	d
D-Arabinose	+	+	–	–	–	–	–	d+	–	nd	–	–
Cellobiose	–	+	d–	–	+	nd	–	–	–	–	–	–
Dulcitol	–	–	–	–	–	nd	–	–	–	nd	nd	–
Erythritol	+	+	+	d+	–	nd	d+	+	–	nd	–	d
Esculin	–	d+	–	+	+	nd	–	–	–	nd	–	nd
D-Fructose	+	+	+	+	+	nd	d+	+	+	+	+	+
Galactose	+	+	+	+	+	nd	d+	+	d–	nd	d–	d
D-Glucose	+	+	+	+	+	nd	d+	+	+	+	+	+
Glycerol	+	+	+	+	+	nd	d+	+	+	nd	nd	d
Glycogen	–	–	–	d+	–	+	–	–	–	nd	–	–
Inositol	d+	+	d+	d+	–	+	d–	d+	–	nd	+	d
Inulin	–	–	–	–	–	nd	–	–	–	nd	nd	–
Lactose	d–	+	d+	d–	+	–	–	d+	–	+	d–	d
Maltose	–	+	d+	d+	+	nd	–	+	d+	+	–	+
Mannitol	–	+	+	–	–	+	d–	d–	d+	+	d+	+
D-Mannose	+	+	+	+	+	nd	d+	+	+	+	d+	d
Melezitose	–	+	d+	d+	+	+	–	d+	d–	–	–	–
Melibiose	d–	d+	+	d+	nd	–	–	d+	d–	+	nd	d
D-Raffinose	–	d–	+	d+	–	–	–	d+	d+	+	d+	+
Rhamnose	–	+	–	–	–	+	–	–	–	+	–	–
Ribose	d+	+	+	+	–	nd	d+	d+	d–	+	d+	d
Salicin	–	+	+	d+	+	nd	–	d+	–	–	d+	d
Sorbitol	–	+	–	d+	–	+	d+	–	–	nd	–	d
L-Sorbose	–	d+	–	–	–	+	–	–	–	nd	nd	–
Starch	–	+	–	+	–	+	–	–	–	nd	–	d
Sucrose	–	+	+	d+	nd	nd	–	+	+	+	–	+
Trehalose	–	+	+	+	+	nd	–	+	d+	nd	–	d
D-Xylose	–	d+	d+	d+	–	–	–	d–	–	nd	–	–
Esculin hydrolysis	+	+	+	+	+	–	–	+	–	–	+	–
Gelatin hydrolysis	–	–	–	–	–	nd	+	+	d–	–	–	d
Starch hydrolysis	–	–	–	d+	–	nd	–	–	–	nd	–	d
Milk:												
Curd	d+	d–	d–	d–	nd	nd	d+	+	+	nd	d–	d
Digestion	–	–	d–	–	nd	nd	d+	+	–	nd	d–	d

(continued)

TABLE 205. (continued)

Reaction	"Classical" propionibacteria						"Cutaneous" propionibacteria					
	P. freudenreichii	*P. acidipropionici*	*P. jensenii*	*P. thoenii*	*P. cyclohexanicum*	*P. microaerophilium*	*P. acnes*	*P. avidum*	*P. granulosum*	*P. acidifaciens*	*P. australiense*	*P. propionicum*
Indole-produced	–	–	–	–	–	nd	d+	–	–	–	–	–
Nitrate-reduced	–	+	–	–	–	+[c]	d+	–	–	–	+	+
Catalase	+	d+	d+	+	–	–	d+	+	+	–	–	–
Gas[d]	–, 1	1, 2	–, 1	–, 1	nd	nd	–, 2	–, 1	–, 1	nd	–	–
Acetoin	–	d+	–	–	nd	nd	–	–	–	nd	nd	–
Growth in 20% bile[e]	+	+	+	–	nd	nd	+	+	–	–	–	–
Optimum temp for growth (°C)	30–32	30–32	30–32	30–32	35	30	36–37	36–37	36–37	37	35–37	35–37
Major respiratory quinone	MK-9(H_4)	MK-9(H_4)	MK-9(H_4)	MK-9(H_4)	MK-9(H_4)	nd	MK-9(H_4)	MK-9(H_4)	MK-9(H_4)	nd	nd	MK-9(H_4)
G+C content (mol%)	64–67	66–68	65–68	66–67	66.8	67.7	57–60	62–63	61–63	70	nd	63–65
Major fatty acid	Branched	Branched	nd	nd	ω-Cyclohexane	nd	Branched	Branched	Branched	nd	Branched	Straight
A_2pm isomer	*meso*-	LL-	LL-	LL-	*meso*-	nd	LL-(*meso*)	LL-(*meso*)	LL-	nd	*meso*-	LL-
Amino acids in peptidoglycan	Ala, Glu	Ala, Glu, Gly	Ala, Glu, Gly	Ala, Glu, Gly	Ala, Glu	nd	Ala, Glu, (Gly)	Ala, Glu, (Gly)	Ala, Glu, Gly	nd	nd	Ala, Gly, Glu
Sugars	Galactose, mannose, rhamnose	Glucose, (galactose), (mannose)	Glucose, galactose, mannose	Glucose, galactose,	Glucose, galactose, mannose, rhamnose, ribose	nd	Glucose, mannose, (galactose)	Glucose, mannose, (galactose)	Galactose, mannose	nd	nd	(Glucose), galactose, (mannose)

[a]Symbols: +, positive reaction in 90–100% of isolates, or pH below 5.7 in 90–100% of isolates; –, negative reaction in 90–100% of isolates, or pH 5.7 or above in 90–100% of isolates; d–, reaction positive in 10–40% of isolates; d+ reaction positive in 40–90% of isolates; d, reaction positive in 11–89% of isolates; parentheses, present in some isolates; nd, not determined.

[b]Reactions determined by procedures given in Holdeman et al. (1977).

[c]Nitrate reduced to nitrogen rather than nitrite.

[d]Numbers refer to amount of gas produced on a 1–4 scale.

[e]Data taken from Bernard et al., 2002b; –, unable to grow or grow poorly in 20% bile; +, able to grow well or luxuriantly in bile.

sodium oleinate for the growth of propionibacteria and inhibition of *Actinomyces* strains from human skin swabs has been described (Kishishita et al., 1980). The addition of bromocresol purple to the medium permits identification of *Propionibacterium acnes* and other cutaneous propionibacteria which appear as yellow or slightly yellow opaque colonies with yellow zones of lysis due to the fermentation of glycerol and the production of acid. *Staphylococcus epidermidis* appears as white translucent colonies which therefore enables visual differentiation from propionibacteria. Propionibacteria can also be recovered from skin swabs using TYEG agar (2% w/v tryptone, 1% w/v yeast extract, 0.5% w/v glucose) containing furazolidone (6 μg/ml) to inhibit staphylococcal growth (Ross et al., 2003). Although some strains of propionibacteria may be aerotolerant, a culture methodology used for the primary isolation for strict anaerobes should still be adopted, such as is detailed in the Wadsworth KTL Anaerobic Bacteriology Manual (Jousimies-Somer et al., 2002). Growth media and diluents should be pre-reduced and pre-equilibrated under anaerobic conditions. The incorporation of L-cysteine hydrochloride (0.05% w/v) into liquid media and diluents provides a suitable reducing agent. An anaerobic gas atmosphere within an anaerobic jar or cabinet, from which oxygen has been scavenged by palladium catalists, should be used for incubation. A lack of adherence to strict anaerobiosis when handling and processing clinical specimens will result in reduced detection of pathogenic *Propionibacterium* species (Martin-Rabadan et al., 2008; Tunney et al., 1998). The Hungate roll tube technique (and modifications thereof), where pre-reduced anaerobically sterilized (PRAS) medium is inoculated under N_2 gas (Holdeman et al., 1977), has also been used successfully (Sarada and Joseph, 1994). The "classical" propionibacteria grow best at 30–32°C, and the "cutaneous" group strains at 36–37°C. In general, maximum growth in complex media of pure cultures is attained in 48 h; primary isolation from clinical samples may, however, require incubation for 7 d or more for the "cutaneous" group and up to 14 d for the "classical" group.

For maintenance, chopped meat medium in stoppered tubes under N_2 or another anaerobic gas atmosphere is excellent. After 48 h at the optimum temperature for growth, cultures are kept at room temperature and will maintain viability better than at +4°C (Stackebrandt et al., 2006). For maintenance it is better to omit glucose from the medium to avoid formation of excess acid. The bacterium can also be maintained successfully at −70°C on commercially available microbeads stored in a cryopreservative solution.

Differentiation of the genus *Propionibacterium* from other genera

If molecular identification methods are not used, members of the genus *Propionibacterium* may need to be distinguished from other Gram-stain-positive, nonsporeforming, nonmotile, mainly anaerobic organisms with a rather irregular morphology. Although the production of large amounts of propionic and acetic acids is generally distinctive, the following may cause confusion in some cases.

Some members of the genus *Clostridium* produce considerable amounts of propionic acid, and not all will spore freely. This refers especially to strains of *Clostridium haemolyticum*, *Clostridium novyi*, *Clostridium botulinum* types C and D, *Clostridium propionicum*, *Clostridium quericolum*, and "*Clostridium arcticum*". However, all of these, except "*Clostridium arcticum*", produce large amounts of hydrogen during growth, unlike propionibacteria which produce largely CO_2.

The other genus whose members are most likely to cause confusion is *Corynebacterium*. Here the range of G+C contents (53–67 mol%) is very similar to that in the propionibacteria, and propionic acid may be an end product of metabolism. The corynebacteria generally grow much better aerobically than anaerobically and are characterized by the presence of arabinogalactan and mycolic acids in the cell wall, neither of which is found in propionibacteria. Some corynebacteria species, however, are lacking mycolic acids; these include *Corynebacterium amycolatum* (Collins et al., 1988), *Corynebacterium atypicum* (Hall et al., 2003), *Corynebacterium caspium* (Collins et al., 2004), *Corynebacterium ciconiae* (Fernández-Garayzabal et al., 2004), and *Corynebacterium kroppenstedtii* (Collins et al., 1988). On the other hand, corynebacteria do not have the large amount of C_{15} branched-chain fatty acids in the membrane lipids which are found in propionibacteria. Three types of menaquinones have been recognized in the genus; MK-8(H_4), MK-9, and MK-9(H_4) (Yamada et al., 1976). Corynebacteria have relatively low polyamine contents, with spermidine normally the major compound (Altenburger et al., 1997).

Taxonomic comments

In relation to the "classical" propionibacteria, the species "*Propionibacterium coccoides*" shows low reassociation values in DNA–DNA hybridizations with the propionibacteria, and 16S rRNA gene sequencing indicates that it is related to *Luteococcus* (Tamura et al., 1994; Vorobjeva, 1999).

List of species of the genus *Propionibacterium*

1. **Propionibacterium freudenreichii** van Neil 1928, 162[AL] ("*Bacterium acidi propionici a*", "*Bacterium acidi propionici d*" Von Freudenreich and Orla-Jensen 1906)

freu.den.reich′i.i. N.L. gen. masc. n. *freudenreichii* of Freudenreich; named for Edouard von Freudenreich, the Swiss bacteriologist who first isolated this species.

Description based on literature descriptions including those of Sakaguchi et al. (1941), Janoschek (1944), and Werkman and Brown (1933) and on a study of two strains of *Propionibacterium freudenreichii* (Williams E1.51 and ATCC 6207) and six strains of "*Propionibacterium shermanii*" (van Niel 1.11. IAM 1714 and ATCC 8262, 9615, 9617, 13673).

Surface colonies on horse blood agar (2 d) are 0.2–0.5 μm, circular, entire, convex, semi-opaque, glistening, gray-to-white (may become cream, tan, or pink). Colonies in deep agar are lenticular-to-4 mm, white, tan, or pink. Glucose broth cultures are turbid with a smooth or granular sediment, or clear with a granular sediment, and terminal pH of 4.5–4.9. Nonhemolytic based on Vedamuthu et al., (1971).

Rarely grows on the surface of agar incubated aerobically; grows in deep broth incubated aerobically, but more slowly than anaerobically. Strongly catalase-positive.

Strains require pantothenic acid and some also require biotin and thiamine. For others, thiamine is not essential but is stimulatory, while for all strains, *p*-aminobenzoic acid is not required (Delwiche, 1949).

The major long-chain fatty acids produced in thioglycollate cultures (Moss et al., 1969) are 12-methyltetradecanoic (~43%) and a 17-carbon branched-chain acid (~12%). Will produce large amounts of free proline in peptide-containing media (Langsrud et al., 1977, 1978). Peptidoglycan contains alanine, glutamic acid, and *meso*-DAP; cell-wall sugar components are galactose and moderate amounts of mannose and rhamnose but no glucose.

Source: raw milk, Swiss cheese and other dairy products; particularly associated with the ripening, flavor and aroma of Swiss cheese.

DNA G+C content (mol%): 64–67 (T_m).

Type strain: ATCC 6207, CCUG 7433, CIP 103026, DSM 20271, HAMBI 274, LMG 16412, NBRC 12424, NCTC 10470, NRRL B-3523.

Sequence accession no. (16S rRNA gene): X53217.

Further comments: member of the "classical" or "dairy" group of propionibacteria. On the basis of 16S rRNA gene sequence analysis, *Propionibacterium freudenreichii* clusters with *Propionibacterium australiense* (isolated from bovine lesions) and *Propionibacterium cycohexanicum*, (isolated from spoiled pasteurized orange juice (Figure 232). Lipolysis generated by esterase activity ripens the cheese and the free fatty acids that are produced contribute to flavor. Carbon dioxide produced from fermentation generates the characteristic holes in, for example, Emmental cheese (Dherbecourt et al., 2008). Used commercially as a starter culture in cheese production (Benjelloun et al., 2007). Strains in this species differ broadly from the other "classical" propionibacteria in the following ways:

1. They are generally very short rods, often almost coccal in shape.
2. They are more heat resistant (Malik et al., 1968).
3. They ferment a restricted range of carbohydrates: in particular the inability to ferment sucrose and maltose seems a reliable distinction from other species.
4. Their peptidoglycan contains *meso*-DAP instead of the LL-isomer and the cell wall contains rhamnose.

1a. **Propionibacterium freudenreichii subsp. freundereichii** van Niel 1928, 162[AL] emend. Moore and Holdeman 1970

freu.den.reich′i.i. N.L. gen. masc. n. *freudenreichii* of Freudenreich; named for Edouard von Freudenreich, the Swiss bacteriologist who first isolated this species.

Description as for *Propionibacterium freudenreichii*; distinguished from subsp. *shermanii* by nitrate reduction and by a lack of lactose fermentation.

Source: raw milk, Swiss cheese and other dairy products; particularly associated with the ripening, flavor and aroma of Swiss cheese.

DNA G+C content (mol%): 64–67 (T_m).

Type strain: ATCC 6207, CCUG 7433, CIP 103026, DSM 20271, HAMBI 274, LMG 16412, NBRC 12424, NCTC 10470, NRRL B-3523.

Sequence accession no. (16S rRNA gene): X53217.

1b. **Propionibacterium freudenreichii subsp. shermanii** van Niel 1928, 162[AL] emend. Moore and Holdeman 1970 ("*Propionibacterium shermanii*" van Niel 1928, 162)

sher.man′i.i. N.L. gen. masc. n. *shermanii* of Sherman; named for James M. Sherman, an American bacteriologist.

Description as for *Propionibacterium freudenreichii*; distinguished from subsp. *freudenreichii* by lactose fermentation and a lack of nitrate reduction.

DNA G+C content (mol%): 67 (T_m).

Type strain: ATCC 9614, CCUG 36819, CIP 103027, DSM 4902, LMG 16424.

Sequence accession no. (16S rRNA gene): Y10819.

Further comments: member of the "classical" or "dairy" group of propionibacteria. Strains of *Propionibacterium freudenreichii* subsp. *freudenreichii* and *Propionibacterium freudenreichii* subsp. *shermanii* have high DNA similarity with each other (Johnson and Cummins, 1972), and there seems to be little justification for separation at the species level. Of the species described by Sakaguchi et al. (1941), it is possible that *"Propionibacterium globosum", "Propionibacterium orientum"*, and *"Propionibacterium coloratum"* were in fact other variants of *Propionibacterium freudenreichii*, but no labeled strains appear to be extant and available for testing. Complete genome sequencing of the *Propionibacterium freudenreichii* subsp. *shermanii* strain ATCC9614 (~2.6 Mb; G+C content 67%) has been undertaken (Meurice et al., 2004).

2. **Propionibacterium acidifaciens** Downes and Wade 2009, 2780[VP]

a.ci.di.fa′ci.ens. N.L. n. *acidum* an acid; L. v. *facio* to produce; N.L. part. adj. *acidifaciens* acid-producing.

Description based on three strains isolated from the human oral cavity (Downes and Wade, 2009).

Microscopically, cultures are pleomorphic rods; straight, slightly curved, or club-shaped cells are arranged singly, in pairs, or short chains with some branched diphtheroids (0.7–0.8 × 1.2–4 µm). Surface colonies are 0.7–1.1 mm in diameter, circular, entire, high-convex-to-dome-shaped, white-to-pale cream, solid, and non-translucent after 7 d growth at 37°C on fastidious anaerobe agar (LabM) supplemented with 5% (v/v) horse blood under an atmosphere of 80% N_2, 10% H_2, and 10% CO_2.

Growth is evident in peptone yeast extract broth, with moderate turbidity that is enhanced by fermentable carbohydrates (1%). In peptone-yeast extract-glucose broth, large amounts of acetic and propionic acid are produced and, in moderate amounts, succinic acid is produced.

Growth was not detectable in air enriched with 5% CO_2. Catalase-negative.

Bile tolerant, although colonies are smaller on tryptone soy agar containing 20% bile compared to tryptone soy agar only.

Source: carious lesions in the human mouth.

DNA G+C content (mol%): 70 (T_m).

Type strain: C3M_31, CCUG 57100, DSM 21887, JCM 16571.

Sequence accession no. (16S rRNA gene): AB565481, EU979537.

Further comments: based on 16S rRNA gene sequences (1442 bp), the three strains differed between one and two bases. *Propionibacterium australiense* is the most closely related species based on 16S rRNA gene sequence (97.7% identity), although the species only share 8% DNA–DNA relatedness. On the basis of RpoB amino acid sequence, *Propionibacterium acnes* is the most closely related species. The strains examined represent a homogeneous group and are representative of the dominant *Propionibacterium* taxon associated with dentinal caries (Downes and Wade, 2009). Sensitive to vancomycin and kanamycin, but resistant to colistin.

3. **Propionibacterium acidipropionici** corrig. Orla-Jensen 1909, 337[AL] ("*Bacillus acidi propionici*" von Freudenreich and Orla-Jensen 1906; "*Propionibacterium pentosaceum*" van Niel 1928; "*Propionibacterium arabinosum*" Hitchner 1932)

a.ci.di.pro.pio′ni.ci. N.L. n. *acidum propionicum* propionic acid; N.L. gen. n. *acidipropionici* of propionic acid.

Description based on the study of the type strain ATCC 25562, ATCC 4875, *Propionibacterium pentosaceum*" (van Niel 4), ATCC 4965 ("*Propionibacterium arabinosum*", Hitchner), and IAM 1725. Phenotypic characteristics of these strains are similar to original descriptions.

Surface colonies on horse blood agar after 2 d anaerobic incubation are punctiform–1 mm, circular to slightly irregular, convex, entire or slightly scalloped, gray or white, and semiopaque. Colonies in deep agar are white, becoming pink with continued incubation.

Glucose broth cultures are turbid with smooth cream-colored sediment. Terminal pH 4.1–4.9.

Usually nonhemolytic, but may show slight β-hemolysis under area of confluent growth. May grow under both aerobic and anaerobic conditions. Weak or negative for catalase (van Niel, 1957; Werkman and Brown, 1933).

Requires pantothenate and biotin for growth. Thiamine is not essential but is stimulatory (Delwiche, 1949).

The major long-chain fatty acids produced in thioglycolate cultures are 13-methyltetradecanoic acid (17–40%) and 12-methyltetradecanoic acid (12–23%) (Moss et al., 1969). Peptidoglycan contains alanine, glutamic acid, glycine, and LL-DAP as diamino acid; cell-wall sugars are glucose with galactose and/or mannose (some strain variation: see Table 5 in Johnson and Cummins, 1972).

Source: dairy products.

DNA G+C content (mol%): 66–68 (T_m).

Type strain: ATCC 25562, CIP 103025, DSM 4900, VPI 399.

Sequence accession no. (16S rRNA gene): AJ704569.

Further comments: member of the "classical" or "dairy" group of propionibacteria. On the basis of 16*S* rRNA gene sequence analysis, *Propionibacterium acidipropionici* clusters with *Propionibacterium microaerophilum* (Figure 232). The strains originally described by van Niel (1928) and Hitchner (1932) as "*Propionibacterium pentosaceum*" and "*Propionibacterium arabinosum*", respectively, show high DNA similarity with each other and are also characterized by a weak or negative catalase reaction (Cummins and Johnson, 1986). Strains of "*Propionibacterium arabinosum*" were described as being unable to ferment xylose and rhamnose, while those of "*Propionibacterium pentsaceum*" could ferment these sugars. However, as in the case of *Propionibacterium freudenreichii* and "*Propionibacterium shermanii*" with lactose fermentation, this is not considered sufficient to warrant speciation. *Propionibacterium acidipropionici* produces a high yield of propionic acid, with low acetic acid production, from glycerol fermentation and has been studied as an alternative to petrochemicals as a commercial source of propionic acid (Himmi et al., 2000).

4. **Propionibacterium acnes** (Gilchrist 1900) Douglas and Gunter 1946, 22[AL] ("*Bacillus acnes*" Gilchrist 1900; "*Corynebacterium acnes*" Eberson 1918)

ac′nes. Gr. n. *acme* a point; incorrectly transliterated as N.L. n. *acne* acne; N.L. gen. n. *acnes* of acne.

Description based on a study of several hundred strains by Holdeman et al. (1977), Cummins and Johnson (1986), including the type strain ATCC 6919, and on the results of Kishishita et al. (1979), McGinley et al. (1978), and McDowell et al. (2008, 2005).

Four distinct genetic groups have been described within *Propionibacterium acnes*. These clades, known as types IA, IB, II, and III, share over 99.9% identity based on the analysis of 16S rRNA gene sequences (1484 bp section) but can be differentiated based on polymorphisms in *recA* and a putative hemolysin/cytotoxin gene (*tly*) (McDowell et al., 2005, 2008) (Figure 233).

Colonies in deep agar appear lenticular, 4 mm or less; colonies of some strains become tan, pink or orange in 3 weeks. Surface colonies on blood (horse or rabbit) agar (2–3 d) are punctiform-to-0.5 mm, circular, entire-to-pulvinate, translucent-to-opaque, white-to-gray, and glistening. On brain heart infusion (BHI) agar, type III strains may form very small, flat, dry colonies, which are difficult to recover from the plate, in contrast to types I and II which form circular, convex, glistening, opaque colonies. Strains within the type III clade have the capacity to form long slender filaments in addition to the "classical" coryneform shape (Figure 234 and Figure 235), a characteristic not been previously described for *Propionibacterium acnes*, but known for other propionibacteria such as *Propionibacterium propionicum* (Figure 236 and Figure 237) (Pine and Georg, 1969) and *Propionibacterium australiense* (Bernard et al., 2002b). Strains of phylotype II may appear more coccoid compared to those of phylotype I and are most similar to previous descriptions of "*Clostridium parvum*" and "*Clostridium adamsoni*" which are synonyms of *Propionibacterium acnes*.

In defined medium broth culture, *Propionibacterium acnes* type IA will form a turbid suspension, while in proteose peptone yeast (PPY) or BHI broth, the organism forms a settled granular sediment (due to aggregation) with a clear solution. With increasing concentrations of glucose greater than 4%, however, aggregation is significantly reduced and the broth becomes more turbid (Patrick and Glenn unpublished). Studies with types IB and II have shown the formation of a slight fine sediment upon culture in BHI broth and a turbid solution that contains suspended cells (Cohen et al., 2005). In suitable media with good growth, the final pH is 4.5–5.0. The auto-aggregating properties of type IA strains in broth culture may reflect, in part, a hydrophobic cell surface (Cohen et al., 2005).

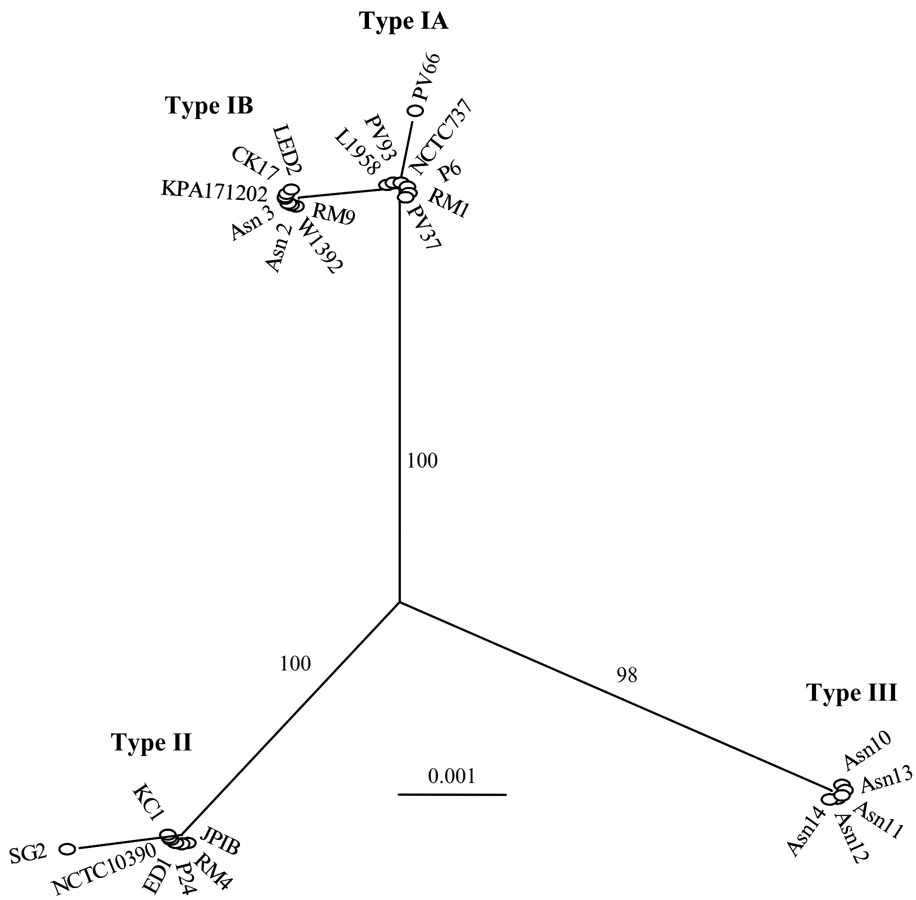

FIGURE 233. Unrooted phylogenetic tree of *Propionibacterium acnes* based on the complete *recA* gene sequence. The neighbor-joining tree was constructed using the Jukes–Cantor-based algorithm. The sequence input order was randomized, and bootstrapping resampling statistics were performed using 100 data sets. Bootstrap values are shown on the arms of the tree. The phylotype status for the different strains analyzed is shown. (Printed with permission from McDowell et al., 2008. J. Med. Microbiol. *57*: 218–224.)

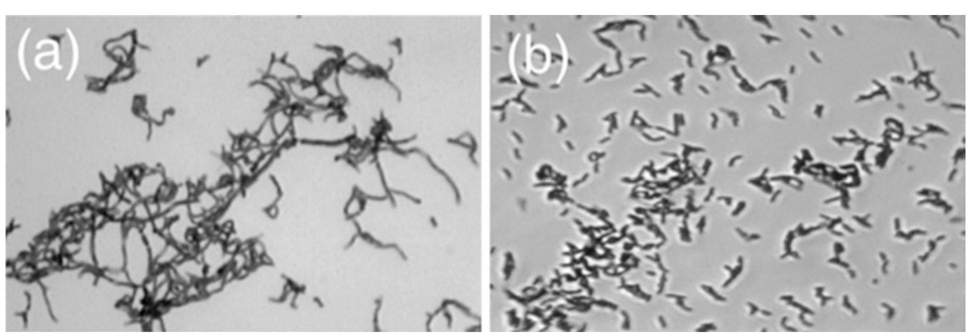

FIGURE 234. Gram stain of *Propionibacterium acnes* prepared from a mature colony (anaerobic blood agar; 7 d; 37°C); micrograph (obj. ×100). (a) Type III (Asn 10); (b) type IA (NCTC 737).

Approximately 65% of type I isolates (n=85) show β-hemolysis on horse blood, but this has not been observed with type II isolates (n=31) or any type III isolates examined to date (n=5). A gene family consisting of five homologs of the *Streptococcus agalactiae* co-hemolysin or CAMP (Christie–Atkins–Munch–Peterson)-factor has been identified within the genome of all *Propionibacterium acnes* phylotypes, and the co-hemolysin reaction similar to that originally described by Christie et al. (1944).has also been demonstrated among 50 isolates representing types IA, IB, and II (Valanne et al., 2005). Differential production of the different CAMP factor proteins has been observed among the phylotypes. In partic-

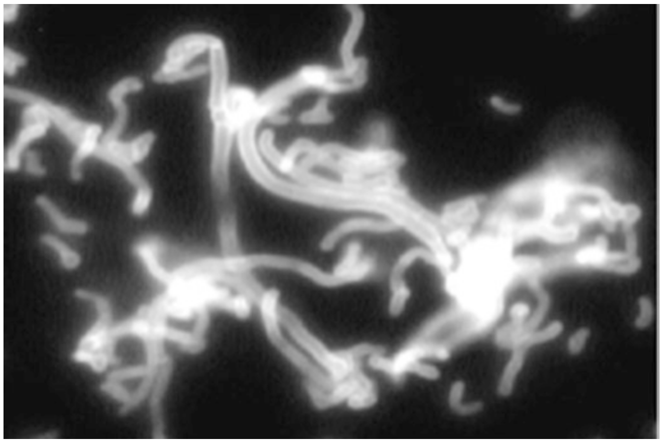

FIGURE 235. Immunolabelling of *Propionibacterium acnes* type III prepared from a mature colony (anaerobic blood agar; 7 d; 37°C); micrograph (obj × 100). Bacterium was labeled with a mouse IgG monoclonal antibody QUBPa3 (reacts with all *Propionibacterium acnes* strains) and a fluorescein isothiocyanate-conjugated goat anti-mouse IgG antibody.

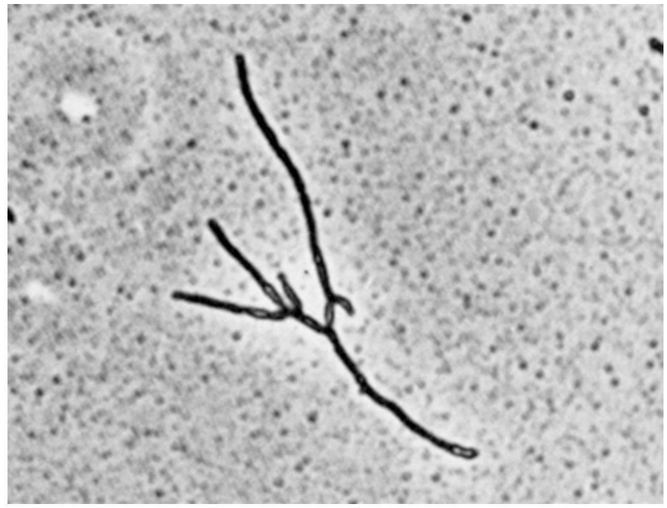

FIGURE 237. *Propionibacterium propionicum*, serovar 1. Young (20-h) microcolony on CC medium (slide culture); phase-contrast micrograph in situ (1600×).

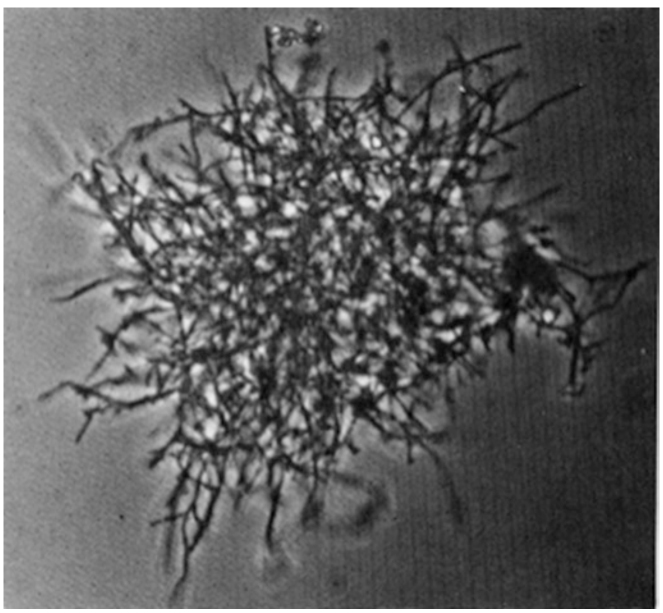

FIGURE 236. *Propionibacterium propionicum*, serovar 1. Wet mount in lactophenol cotton blue mounting fluid prepared from a 24 h culture in Tarozzi broth; phase contrast micrograph (640×).

ular, strains of type II and a commonly isolated type IB strain (ST-10 by MLST) produce large quantities of CAMP factor 1 compared to type IA organisms (Valanne et al., 2005).

The presence of oxygen is not lethal to *Propionibacterium acnes*, but anaerobiosis is usually required for isolation and growth (Cove et al., 1983). A small number of strains grow on the surface of blood agar plates incubated aerobically, while others (~30%) display variable growth on the surface of blood agar plates incubated in a candle jar for microaerophilic growth. Many strains grow in deep glucose broth without special provision for anaerobiosis. Strains are catalase-positive, although cultures need to be exposed to air for a time before testing. McGinley et al. (1978), who exposed colonies on BHI agar to air for 1 h before testing, found all 231 strains to be-positive for catalase. A study with one strain of *Propionibacterium acnes* has shown that increases in oxygen tension will alter the production of extracellular enzymes due to a decrease in growth rate (Cove et al., 1983).

The ability to ferment sorbitol is a characteristic of some type I strains, but not type II or type III strains and can, therefore, be used as a simple method of identification. On the collective basis of sorbitol, ribose, and erythritol fermentation, Kishishita et al. (1979) identified five fermentation biotypes (B1–B5) among 128 isolates of *Propionibacterium acnes* recovered from healthy facial skin. Biotypes B1-B5 were all identified among type I strains, but only B2 (ribose and erythritol fermenters) was characteristic of the type II clade. All type III strains examined to date (n=5) belong to biotype 4 (ribose fermenters) (McDowell et al., 2008).

Studies have shown that different strains produce many enzymes responsible for the degradation of host derived molecules. These include ribonuclease (Smith, 1969), neuraminidase (von Nicolai et al., 1980), hyaluronidase (Hoeffler, 1980; Ingham et al., 1980), acid phosphatase (Ingham et al., 1980) lecithinase (Werner, 1967), lipase (Ingham et al., 1981; Smith and Willett, 1968), and proteinase (Ingram et al., 1983). Analysis of the *Propionibacterium acnes* genome sequence has now identified the specific gene loci encoding these various extracellular products (Brüggemann et al., 2004). Free fatty acids released from skin lipids (e.g., sebum) by the action of lipase may (a) act as tissue irritants and (b) promote the growth of *Propionibacterium acnes* (e.g., oleate, see below). They may also assist bacterial adherence and colonization of the sebaceous follicle (Brüggemann, 2005).

Some strains also produce bacteriocin-like substances inhibitory for Gram-stain-positive and -negative anaerobes

(Fujimura and Nakamura, 1978; Ko et al., 1978; Paul and Booth, 1988).

All strains tested to date have an absolute requirement for pantothenate, while thiamine, biotin, and nicotinamide are stimulatory. Some strains of phylotype II require heme and vitamin K to grow (Ferguson and Cummins, 1978). The organic acids lactate, pyruvate, and 2-oxoglutarate also stimulate growth, as does oleate (usually used in the form of Tween 80). Amino acid requirements are complex (Ferguson and Cummins, 1978; Holland et al., 1979; Nielsen, 1983).

The major long-chain fatty acid produced in thioglycolate cultures (Moss et al., 1969) is 13-methyltetradecanoic acid (32–62%).

Peptidoglycan contains alanine, glutamic acid, glycine, LL-DAP and, in certain strains of type II, occasionally *meso*-DAP. Cell-wall sugars are glucose, mannose, and galactose. In contrast to *Propionibacterium acnes* type I, galactose is not found in the cell wall of type II strains. On the basis of differences in cell-wall composition, types I and II can be identified by serology and monoclonal antibody labeling methods (Johnson and Cummins, 1972; McDowell et al., 2005). Cell-wall sugar content of type III strains has not been determined.

Source: the bacterium primarily colonizes the sebaceous gland-rich areas of human skin, but the organism is also found in the mouth, as well as the genito-urinary tract and large intestine. In relation to infections, it has been recovered from comedones of acne vulgaris, wounds, blood, pus, soft tissue abscesses, the surface of indwelling medical devices, and eye infections. Levels of *Propionibacterium acnes* colonization on the skin vary from person-to-person and from the area of the body sampled (McLorinan et al., 2005). Levels can be as high as 10^6 organisms/cm^2, with the neck, forehead, and shoulder showing some of the highest concentrations of the bacterium compared to other sites such as the abdomen, hip, knee, and chest where levels are lower (Patel et al., 2009).

DNA G+C content (mol%): 57–60 (T_m).

Type strain: ATCC 6919, CCUG 1794, CIP 53.117, DSM 1897, JCM 6425, LMG 16711, NCTC 737, NRRL B-4224, VKM Ac-1450.

Sequence accession no. (16S rRNA gene): AB042288.

Further comments: member of the “cutaneous” group of propionibacteria. On the basis of 16S rRNA gene sequence analysis, *Propionibacterium acnes* clusters with *Propionibacterium avidum* and *Propionibacterium propionicum*. Multilocus Sequence Typing (MLST) of *Propionibacterium acnes* isolates based on the analysis of 7 core housekeeping genes (*aroE*, *atpD*, *gmk*, *guaA*, *lepA*, *recA*, and *sodA*) supports the IA, IB, II and III phylogenetic divisions (http://pubmlst.org/pacnes/; McDowell, Gao, Dowson and Patrick, unpublished). Strains of phylotypes I and II share DNA–DNA hybridization values of 88–99%. DNA–DNA hybridization values for phylotype III versus type I and II have not been determined. The *Propionibacterium acnes* genome sequence (type IB strain KPA171202; ST-10 by MLST) has revealed the presence of genes for oxidative phosphorylation, the Embden-Meyerhof and pentose phosphate pathways, and the tricarboxylic acid cycle (Brüggemann et al., 2004). Systems for anaerobic respiration, such as nitrate reductase, are also present. Reconstruction of the metabolic pathways in the bacterium demonstrates its capacity to deal with changing oxygen tensions and confirms laboratory observations (Cove et al., 1983).

Although the role of *Propionibacterium acnes* in the inflammatory condition acne has been debated, its involvement is generally accepted since acne which is refractory to antibiotic treatment is associated with strains resistant to the same antibiotic (Eady et al., 1989). Other conditions associated with *Propionibacterium acnes* include opportunistic biofilm infections of medical implants such as prosthetic joints (Tunney et al., 1998), native and prosthetic heart valves (Clayton et al., 2006), intravascular catheters (Martin-Rabadan et al., 2008), central nervous system shunts (Brook and Frazier, 1991), and also endophthalmitis post-eye surgery (Aldave et al., 1999). It has been also linked to synovitis-acne-pustulosis-hyperostosis-osteitis (SAPHO) syndrome (Kotilainen et al., 1996), sarcoidosis (Eishi et al., 2002), and chronic infection of the prostate gland, potentially leading to prostate cancer (Cohen et al., 2005). The possibility of wide variation in the potential for virulence among different *Propionibacterium acnes* isolates should not be discounted. The heterogeneity of *Propionibacterium acnes* in relation to putative virulence determinants is well documented (Lodes et al., 2006; McDowell et al., 2008; Valanne et al., 2005). Variation in expression of immune-reactive surface and secreted proteins has been described. For example, variation of proteins that bind to dermatan sulfate with carboxy-terminal repeats and the amino acid motif LPXTG, characteristic of MSCRAMMs (microbial surface components recognizing adhesive molecules), is generated by a variable number of C residues in a C_nTC_n motif upstream of the putative signal peptide for the proteins. In addition, there are variable numbers of repeats towards the mid-region and the carboxy-terminus that alter molecular mass and expression of the proteins (Lodes et al., 2006). This variation is probably generated by slipped strand mispairing during replication leading to phenotypic variation.

The strong pro-inflammatory nature of *Propionibacterium acnes* has been known for some time and homologs of GroEL, DnaK, and several other heat-shock proteins (Graham et al., 2004), which are major targets for the immune system, are present in the genome (Brüggemann et al., 2004). Three gene clusters putatively involved in extracellular polysaccharide biosynthesis have been identified in the genome sequence and may also be important in modulating immunogenicity. The production of porphyrins may lead to inflammation and generate toxic reduced oxygen species that cause cell damage. The immunostimulatory activity and adjuvant properties of isolates previously, and currently by some authors, referred to as “*Corynebacterium parvum*” are exploited in studies of the inflammatory response (Fink et al., 2008). *Propionibacterium acnes* has also been used as a non-specific immune stimulant in the equine industry (EqStim; Flaminio et al., 1998).

Propionibacterium acnes is often dismissed as a skin contaminant when present in clinical samples, especially when

present in low numbers. This has undoubtedly led to an under-reporting and, consequently, under-recognition of its association with different infections and conditions in the literature. Furthermore, many samples are not routinely cultured under anaerobic conditions. The use of protocols for anaerobic handling and isolation from clinical material increases the isolation of *Propionibacterium acnes* from clinical samples (Tunney et al., 1998). It has been demonstrated that aerobic incubation of agar plates inoculated with clinical specimens for up to 96 h prior to anaerobic reincubation will result in reduced *Propionibacterium acnes* colony numbers (Martin-Rabadan et al., 2008). This may have ramifications for the detection of *Propionibacterium acnes* associated with clinical samples that contain lower levels of the bacterium. In addition, the use of prophylactic antibiotics may also hinder growth of the organism from tissue samples. For phylotype III strains, the filamentous-like cellular morphology observed upon routine Gram-staining (Figure 234) and colony appearance (see above) may also confuse identification. When anaerobic growth from samples is performed, agar plates should be incubated long enough to observe growth (7–14 d). Infection of indwelling medical devices due to *Propionibacterium acnes* may also be under-described due to inappropriate sampling of the device and failure to detect the bacterium growing as an adherent biofilm (Tunney et al., 1998, 1999, 2007).

Analysis of isolates causing surgical, foreign body, and septicemia infections in Europe found that 3% were resistant to tetracycline, 15% to clindamycin, and 17% to erythromycin, while none were resistant to linezolid, benzylpenicillin, or vancomycin (Oprica and Nord, 2005). A study of acne patients in Europe found that the widespread use of topical formulations of erythromycin and clindamycin to treat this skin condition has resulted in a significant dissemination of cross-resistant propionibacteria strains. With the exception of Sweden and the UK, resistance rates to the orally administered tetracycline group of antibiotics are low (Ross et al., 2003). Similar results have also been described among acne patients being treated in Asia (Ishida et al., 2008; Tan et al., 2001). Resistance to erythromycin was most commonly encountered, and erythromycin-resistant strains were frequently cross-resistant to clindamycin. Among the tetracycline group of drugs, the mean MICs were higher than that for doxycycline and minocycline (Tan et al., 2001). Strains of *Propionibacterium acnes* are resistant to metronidazole (Chow et al., 1975), sulfonamides (Pochi and Strauss, 1961), and aminoglycosides (Wang et al., 1977). Studies have also shown that susceptibility of *Propionibacterium acnes* to common antimicrobials decreases as the bacterium forms a biofilm (Coenye et al., 2007; Ramage et al., 2003).

5. **Propionibacterium australiense** Bernard, Shuttleworth, Munro, Forbes-Faulkner, Pitt, Norton and Thomas 2002a, 1915[VP] (Effective publication: Bernard, Shuttleworth, Munro, Forbes-Faulkner, Pitt, Norton and Thomas 2002b, 45.)

aus.tra.li.en′se. N.L neut. adj. *australiense* of or belonging to Australia, the country where the bacterium was first isolated.

Description based on the literature description of the type strain 98A072 and the study of six strains in total (Bernard et al., 2002b; Forbes-Faulkner et al., 2000).

Microscopically, cultures are pleomorphic rods with filamentous, branching, and curved forms sometimes evident. Surface colonies grown on pre-reduced brain heart infusion agar supplemented with 5% sheep blood (BHI) under anaerobic conditions at 35°C are off-white or cream, rounded, convex, and do not adhere to the agar surface. Growth is evident in peptone-yeast broth with and without glucose. Nonhemolytic on sheep blood agar and negative for the CAMP and reverse CAMP reactions.

Growth is optimal under strict anaerobiosis and poor or non-existent in air enriched with 5% CO_2. Primary isolation from material derived from bovine granulomatous lesions required 5 d incubation on pre-reduced BHI agar. Good growth is, however, obtained within 24–48 h with repeated subculture. Catalase-negative. Peptidoglycan contains *meso*-DAP.

Source: granulomatous bovine lesions located throughout the animals (0.5–15 cm in diameter), from cattle in Queensland, Australia.

DNA G+C content (mol%): not available.

Type strain: 98A072, NML 98A072, ATCC BAA-264, CCUG 46075.

Sequence accession no. (16S rRNA gene): AF225962.

Further comments: all six strains examined had identical 16S rRNA gene sequences (>1460 bp) and cluster with *Propionibacterium acidifaciens* and *Propionibacterium cyclohexanicum* (Figure 232). These granulomatous lesions were distinguishable from those caused by *Mycobacterium bovis*, *Rhodococcus equi*, *Actinomyces bovis*, and fungi. The lesions consisted of a characteristic fibrous outer capsule surrounding thick yellow viscous pus with caseating granulomas. The lesions were present in large numbers throughout the animal, most commonly on the external surface of the rumen and reticulum, tongue, peritoneal surface of the gastrointestinal tract, and, less commonly, on the omemtum, flank, and flank muscles. Lesions were also more rarely present on the skin over the ribs, the cheek, or under the jaw. Affected carcasses were condemned. Cases were observed in cattle from different geographical sites in northern and central Queensland, Australia, on seven occasions over a period of more than two years. In one case, up to 30% of a group of 324 cows, between 10–12 yrs old, had lesions. Cases were also reported in three-year-old steers, bulls, and breeding cattle that were in calf. Initially observed on abattoir post-mortem inspection, multiple external lesions were subsequently found on live cattle at breeding properties. Post-mortem, these animals also had multiple internal lesions. The source of the *Propionibacterium australiense* has yet to be determined, although animal-to-animal transmission is thought to be most likely (Forbes-Faulkner et al., 2000).

6. **Propionibacterium avidum** (Eggerth 1935) Moore and Holdeman 1969, 7[AL] ("*Bacteroides avidus*" Eggerth 1935; "*Corynebacterium avidum*" Prévot 1938; "*Mycobacterium avidum*" Krasil'nikov 1949)

a′vi.dum. L. neut. adj. *avidum* greedy, voracious.

Description based on a study of 20 strains including ATCC 25577 by Holdeman et al. (1977) and 23 strains by Cummins and Johnson (1986).

Surface colonies on suitable media are 0.5–1.0 mm at 2–3 d, smooth, entire, circular, and white to light cream color. In general, grows better and gives larger colonies than either *Propionibacterium acnes* or *Propionibacterium granulosum*.

Glucose broth is generally turbid with a smooth abundant sediment which resuspends readily. Generally β-hemolytic on sheep, horse, or rabbit blood agar (19/23 strains; Cummins and Johnson, 1986) which agrees well with the results of Hoeffler (1977) on sheep, human, and rabbit blood agar. A thiol-activated extracellular hemolysin has been partially isolated (Fujimura et al., 1982).

Grows anaerobically or as a microaerophile when freshly isolated, but will often grow well aerobically after a few transfers. Appears to be the best adapted species of the "cutaneous" group for growth in aerobic environments (Cove et al., 1983). Catalase-positive.

Nutritional requirements are less exacting than either *Propionibacterium acnes* or *Propionibacterium granulosum* and, after several transfers, will grow in simple medium consisting of salts, glucose, and vitamins (Ferguson and Cummins, 1978). Pantothenic acid is an absolute requirement for growth.

Peptidoglycan contains alanine, glutamic acid, and glycine amino acid residues. Type I peptidoglycan contains LL-DAP, whereas type II contains *meso*-DAP, but a few strains have LL-DAP and no glycine (thus resembling similar strains of *Propionibacterium acnes* type II). Type I strains contain glucose, galactose, and mannose sugars in their cell wall, while type II contains glucose and mannose only, again resembling type II *Propionibacterium acnes* (Johnson and Cummins, 1972).

Strains are normally positive for gelatinase and deoxyribonuclease; most are negative for lecithinase, hyaluronidase, and chondroitin sulfatase (Hoeffler, 1977).

Source: principally, the moister areas of the skin: e.g., vestibule of the nose, axilla, perineum, and from chronically infected areas such as sinuses and occasionally from abscesses.

DNA G+C content (mol%): 62–63 (T_m).

Type strain: ATCC 25577, CCUG 36754, CIP 103261, DSM 4901, NBRC 15671, NCTC 11864, VPI 179.

Sequence accession no. (16S rRNA gene): AJ003055.

Further comments: member of the "cutaneous" group of propionibacteria. *Propionibacterium avidum* can be divided into two serological groups designated type I and II (Johnson and Cummins, 1972). By DNA–DNA hybridization, sequences within each group were at least 90% similar, but between the two groups the mean similarity was 80% (Goodsell et al., 1991). On the basis of 16S rRNA gene sequence analysis, *Propionibacterium avidum* is most closely related to *Propionibacterium propionicus* but also clusters with *Propionibacterium acnes* (Figure 232). Only two out of 22 type I strains (including the type strain) fermented inositol, in contrast to type II strains which all ferment inositol. *Propionibacterium avidum* has been rarely associated with severe infection after invasive procedures. These include skeletal infection such as sacroiliitis and septic arthritis of the hip (Million et al., 2008) and soft tissue infection such as splenic (Vohra et al., 1998) and breast abscesses (Panagea et al., 2005). The immunostimulatory properties of *Propionibacterium avidum* strain KP-40 have been studied and anti-metastatic activity demonstrated in mice (Isenberg et al., 1994; Isenberg et al., 1995). Previous studies have shown sensitivity to benzylpenicillin, ampicillin, cephalothin, rifampin, clindamycin, erythromycin, and minocycline (Eady et al., 1989). Little information is available on the current susceptibility of *Propionibacterium avidum* strains, but analysis of an isolate responsible for a post-surgical breast abscess revealed sensitivity to penicillin G, ampicillin, erythromycin, tetracycline, and vancomycin and resistance to metronidazole (Panagea et al., 2005). Erythromycin-resistant strains have been isolated from acne patients treated with oral erythromycin and topical clindamycin (Eady et al., 1989).

7. **Propionibacterium cyclohexanicum** Kusano, Yamada, Niwa, Yamasato 1997, 830[VP]

cy.clo.hex.a′ni.cum. Gr. n. *kuklos* circle; Gr. n. *hex* six; L. neut. suff. *-icum* suffix used with the sense of pertaining to; N.L. neut. adj. *cyclohexanicum* relating to ω-cyclohexyl fatty acid, the characteristic cellular fatty acid of the organism.

Description based on the literature description of the type strain TA-12[T] (Kusano et al., 1997; Walker and Phillips, 2007).

After 3 d growth, surface colonies on peptone-yeast extract-glucose (PYG) agar at 35°C in a gas atmosphere of 20% CO_2: less than 0.1% O_2 are circular, white-to-creamy, translucent, and 0.2–0.5 mm in diameter. Optimal growth temperature of 35°C.

Lactic acid is a major product of fermentation in addition to propionic and acetic acids.

Colony formation on plates incubated aerobically. Catalase-negative.

Growth at pH 3.2–7.5; optimum pH 5.5–6.5; can survive up to 90°C in PYG broth and 95°C in orange juice at pH 3.9 for 10 min.

ω-Cyclohexyl undecanoic acid accounts for 52.7% of the total fatty acids. Other fatty acids determined include the straight-chain and anteiso-branched fatty acids n-C_{15} (16.8%), C_{15}, (6.4%), n-C_{16} (2.8%), and n-C_{17} (5.3%).

Peptidoglycan contains alanine, glutamic acid, and *meso*-DAP; whole-cell sugars are galactose, glucose, mannose, ribose, and rhamnose.

Source: spoiled off-flavor pasteurized orange juice.

DNA G+C content (mol%): 66.8 (T_m).

Type strain: TA-12, ATCC 700429, CCUG 48885, CIP 105414, IAM 14535, JCM 21245, NBRC 103082, NRIC 0247.

Sequence accession no. (16S rRNA gene): D82046.

Further comments: on the basis of 16S rRNA gene sequence analysis, *Propionibacterium cyclohexanicum* is most closely related to *Propionibacterium australiense* but also clusters with *Propionibacterium freundenreichii*, the closest of the "classical" propionibacteria (Figure 232). The ω-cyclohexane fatty acids are also found in the cell membranes of *Alicyclobacillus* which is acidophilic and thermotolerant but

does not belong to the *Actinobacteria*, and *Curtobacterium pusillum*. It is the most thermotolerant of the propionibacteria as it can grow and survive in a number of fruit juices; it may therefore survive pasteurization processes used in the fruit juice industry (Kusano et al., 1997; Walker and Phillips, 2007).

8. **Propionibacterium granulosum** (Prévot 1938) Moore and Holdeman 1970, 15[AL] ("*Corynebacterium granulosum*" Prévot 1938)

gra.nu.lo′sum. L. n. *granulum* a small grain; L. neut. suff. -*osum* suffix meaning full of; N.L. neut. adj. *granulosum* full of granules.

Description based largely on a study of the reference strains and 36 other strains by Holdeman et al. and of 30 additional strains originally isolated by Charles Evans, University of Washington, Seattle, and examined by Cummins and Johnson (1986). Surface colonies on suitable media up to 1 mm at 2–3 d, generally white or grayish, smooth, circular, entire, usually larger and more whitish than colonies of *Propionibacterium acnes*. Glucose broth cultures are generally turbid with a rather coarsely granular deposit; often rather viscid and difficult to centrifuge cleanly. Generally nonhemolytic on sheep, horse, or rabbit blood, although reported β-hemolytic on rabbit blood by Hoeffler (1977). Very poor or no growth under aerobic conditions. Catalase-positive. Peptidoglycan amino acid residues are alanine, glutamic acid, glycine, and LL-DAP. Cell-wall sugar components are galactose, mannose, and glucosamine (Johnson and Cummins, 1972). Nutritional requirements are generally the same as *Propionibacterium acnes* requiring pantothenate, although some strains appear to require additional unidentified factors (Ferguson and Cummins, 1978).

Most strains had an active deoxyribonuclease andlecithinase; chondroitin sulfatase, hyaluronidase, and phosphates activity was detected in a few strains, but none produced gelatinase (Hoeffler, 1977). *Propionibacterium granulosum* has a lipase which is considerably more active than that of *Propionibacterium acnes* (Greenman et al., 1981).

Source: sebum-rich oily areas of the skin but in significantly smaller numbers than *Propionibacterium acnes* (McGinley et al., 1978). *Propionibacterium granulosum* is found along with *Propionibacterium acnes* in acne comedones and may play some part in the pathogenesis of acne. It is especially common in the region of the alae nasi (McGinley et al., 1978). The type strains was isolated as a contaminant from a culture labeled *Staphylococcus aerogenes*.

DNA G+C content (mol%): 63 (T_m).

Type strain: ATCC 25564, CCUG 32987, CIP 103262, DSM 20700, LMG 16726, NCTC 11865, VPI 507.

Sequence accession no. (16S rRNA gene): AJ003057.

Further comments: member of the "cutaneous" group of propionibacteria. *Propionibacterium granulosum* seems to have been first separated from *Propionibacterium acnes* (although those names were not used) by Brzin (1964) who recognized a group of strains that did not produce indole or reduce nitrate but could ferment sucrose and maltose. This distinction was later confirmed by Voss (1970) who showed that the indole-negative, nitrate-negative strains were serologically distinct from typical "*acnes*" strains. On the basis of acid extracts containing cell-wall polysaccharide, all strains belong to a single type, with little or no cross-reaction against polysaccharides from *Propionibacterium acnes* or *Propionibacterium avidum* (Cummins, 1975). However, Hoeffler et al. (1977) have found at least three types by tube or slide agglutination of intact suspensions which points to the existence of several surface antigens. Strains of *Propionibacterium granulosum* show low DNA similarity (~12–15%) to both *Propionibacterium acnes* and *Propionibacterium avidum* which is similar to the level of relationship that is found between *Propionibacterium acnes* and the "classical" propionibacteria (Johnson and Cummins, 1972). This is further illustrated by 16S rRNA gene sequence analysis which shows *Propionibacterium granulosum* more closely related to the "classical" propionibacteria than to *Propionibacterium acnes* or *Propionibacterium avidum* (Figure 232). Adjuvent therapy using *Propionibacterium granulosum* strain KP-45 resulted in a significant improvement in the survival of patients with stage I and stage II colorectal cancer (Isenberg et al., 1994). Antibiotic susceptibility studies with a septicemia isolate demonstrated sensitivity to penicillin G, ampicillin, erythromycin, cefotaxime, clindamycin, rifampin, and imipenem, but resistance to metronidazole, sulfonamide, fosfomycin, and tobramycin (Branger et al., 1987). Studies with an isolate responsible for endocarditis revealed susceptibility to penicillin, clindamycin, augmentin, piperacillin, chloramphenicol, cefotaxime, and cefazolin, but resistance to metronidazole (Chaudhry et al., 2000). As with other "cutaneous" propionibacteria, erythromycin-resistant strains of *Propionibacterium granulosum* have been isolated from the skin of acne patients treated with oral erythromycin and topical clindamycin (Eady et al., 1989).

9. **Propionibacterium jensenii** van Niel 1928, 163[AL] ("*Bacterium acidi propionici b*" von Freudenreich and Orla-Jensen 1906; "*Propionibacterium jensenii* var. *raffinosaceum*" van Neil 1928; "*Propionibacterium peterssonii*" van Niel 1928; "*Propionibacterium technicum*" van Niel 1928; "*Propionibacterium raffinosaceum*" Werkman and Kendall 1931; "*Propionibacterium zeae*" Hitchner 1932)

jen.se′ni.i. N.L. gen. masc. n. *jensenii* of Jensen; named for Sigurd Orla-Jensen (1870–1949), the Danish bacteriologist who first isolated this organism.

Description based on literature descriptions, including those of Werkman and Brown (1933), Sakaguchi et al. (1941), and Janoschek (1944) and on the study of 13 strains including 3 of *Propionibacterium jensenii* [ATCC 4867 (van Neil 24), ATCC 4868 (van Neil 29), and ATCC 4869 (van Neil 1)]; 2 of "*Propionibacterium technicum*" [ATCC 14073 (van Neil E.6.1) and ISL 106 (van Neil 22)]; 1 of "*Propionibacterium raffinosaceu*" [ISL 103 (van Neil 29)]; 1 of "*Propionibacterium peterssoni*" [ATCC 4870 (van Neil 20)]; 1 of "*Propionibacterium zeae*" [(ATCC 4964 (Hitchner)]; and ATCC 4871. Phenotypic characteristics of these strains are similar to original descriptions of *Propionibacterium jensenii*, "*Propionibacterium raffinosaceum*", "*Propionibacterium technicum*", "*Propionibacterium peterssonii*", or "*Propionibacterium zeae*".

Surface colonies on horse blood agar (2 d) are punctiform, circular, entire, convex, glistening, semiopaque, and white, cream, or pink. Colonies in deep agar are 4 mm or less, lenticular and white, pink or red-brown.

Glucose broth cultures are turbid or clear with smooth, granular or ropy sediment and terminal pH of 4.4–4.9.

Only strains producing the red polyene pigment granadaene have been shown to be hemolytic (Vedamuthu et al., 1971). Twenty pigmented strains produced β-hemolytic activity on horse blood agar. The hemolytic system of *Propionibacterium jensii* appears similar to that of *Streptococcus agalactiae* (Vanberg et al., 2007).

Some strains grow as well aerobically as anaerobically. Variable catalase activity.

Requires pantothenate and biotin for growth. Some require *p*-aminobenzoic acid while for others it is stimulatory. Thiamine is also stimulatory but not essential (Delwiche, 1949).

Peptidoglycan contains alanine, glutamic acid, glycine, and LL-DAP as diamino acid; cell-wall sugars are glucose and galactose, usually with small amounts of mannose.

Source: dairy products and silage.

DNA G+C content (mol%): 65–68 (T_m).

Type strain: ATCC 4868, CCUG 48883, CIP 103028, DSM 20535.

Sequence accession no. (16S rRNA gene): AJ704571, X53219.

Further comments: member of the "classical" or "dairy" group of propionibacteria. On the basis of 16S rRNA gene sequence analysis, *Propionibacterium jensenii* clusters with *Propionibacterium thoenii* (Figure 232). Some preliminary data suggest that the *Propionibacterium jensenii* strain PJ702 may have application as a living vaccine vector for tuberculosis and other mucosally transmitted diseases (Adams et al., 2005).

10. **Propionibacterium microaerophilum** Koussémon, Combet-Blanc, Patel, Cayol, Thomas, Garcia and Ollivier 2001, 1380^VP

mi.cro.a.e.ro′phi.lum. Gr. adj. *mikros* small, Gr. n. *aer* air; Gr. adj. *philos -ê -on* loving; N.L. neut. adj. *microaerophilum* slightly air-loving.

Description based on the literature description of the type strain M5^T (Koussémon et al., 2001).

Surface colonies on yeast extract minimal salts basal medium (YEM; prepared and stored using oxygen-free nitrogen) agar prepared under microaerophilic conditions (95:5% N_2/O_2) at 30°C are white after 1 week and lens-shaped with smooth edges and 2–3 mm in diameter.

Growth pH range 4.5–9.5; optimum 7.0. Mesophilic, with an optimum growth temperature of 30°C; range 20–45°C. Killing was observed at 80°C.

Growth occurs under anaerobic and aerobic conditions with an optimum O_2 concentration of 5% in the gas phase of the culture. Catalase-negative. Nitrate is reduced to nitrogen rather than nitrite.

The type strain M5 does not require growth factors such as pantothenate, biotin, or thiamin and is, therefore, prototrophic. This is in contrast to other propionibacteria which display auxotrophic behavior.

Source: decantation reservoir of olive mill wastewater.

DNA G+C content (mol%): 67.7 (T_m).

Type strain: M5, CNCM I-2360, DSM13435.

Sequence accession no. (16S rRNA gene): AJ234623.

Further comments: on the basis of 16S rRNA gene sequence analysis, *Propionibacterium microaerophilum* clusters with the "classical" propionibacteria and is most closely related to *Propionibacterium acidipropionici* (97.5% similarity) (Figure 232). Despite this, DNA–DNA hybridization experiments with *Propionibacterium acidipropionici* DSM 4900 only showed a reassociation value of 56.2%. Originally isolated from a decantation reservoir of olive mill wastewater that is stored to enable natural bioremediation prior to river disposal. Isolated by enrichment in deep minimal salts glucose basal medium agar (0.6 g KH_2PO_4, 0.2 g $MgCl_2 \cdot 6H_2O$, 1 g NH_2%Cl, 1 g NaCl, and 10 ml trace-element solution supplemented with 0.1 g yeast extract L <MIN>1 and 10 mM glucose) prepared under anaerobic conditions (Koussémon et al., 2001). This species is unlike other members of the genus as growth is sustained on a minimal salts glucose medium with ammonium salts as the only nitrogen source, although yeast extract enhances growth.

11. **Propionibacterium propionicum** corrig. (Buchanan and Pine 1962) Charfreitag, Collins and Stackebrandt 1988, 357^VP ("*Actinomyces propionicus*" Buchanan and Pine 1962)

pro.pio′ni.cum. N.L. n. *acidum propionicum* propionic acid; N.L. neut. adj. *propionicum* pertaining to propionic acid.

Previously *Actinomyces propionicus* (Buchanan and Pine, 1962) and *Arachnia propionica* (Pine and Georg, 1969; Schaal, 1986). Original spelling of the specific epithet, "propionicus" was corrected by Moore and Moore (1992).

Short irregular rods, 0.2–0.3 µm in diameter and 3–5 µm long, which may or may not be branched, as well as slender branching filaments 5–20 µm in length or longer (Figure 236). These filaments are especially evident in young liquid media cultures but commonly break up in older cultures into short rods (Figure 237). Rods are of variable length, often with clubbed ends and are commonly arranged in pairs, in Y or V configurations, or in parallel rows forming palisades. Gram stain may be uneven, with a beaded appearance. Swollen spherical cells resembling sphaeroplasts, up to 5–20 µm in diameter, are formed by some strains.

Cellular morphology varies with sugar substrate and growth conditions. Colony appearance may be variable; off-white to buff, breadcrumb, gritty, pitting or smooth, convex, entire edged. Young microcolonies are commonly composed of tangled filaments; older colonies may be heaped or convoluted with an undulant edge. Both types (rough and smooth) may occur together. Microscopically, colonies are mycelial with long hyphal branched elements but with no aerial filaments and can resemble *Actinomyces israelii.* Red fluorescence observed under ultraviolet light (365 nm) on blood agar.

Hemolytic on human blood agar, variable hemolysis on horse blood agar, and non-hemolytic on sheep blood agar.

Grows in pure culture both aerobically and anaerobically. Increased CO_2 is reported as a requirement for growth

anaerobically and to enhance growth aerobically by Schofield and Schaal (1981) whereas Buchanan and Pine (1962) report that CO_2 does not stimulate growth in broth culture. Obligately anaerobic and slow-growing on primary isolation from clinical samples, requiring up to 14 d incubation (Hall, 2006). Catalase-negative.

Peptidoglycan contains LL-DAP as the dibasic amino acid. Two distinct cell-wall sugar patterns have been described. Strains contain essentially only galactose or galactose and glucose with trace amounts of mannose, but not rhamnose or 6-deoxylatose.

Little or no proteloytic activity. API tests for hyaluronidase, esterase (C_4), esterase lipase (C_8), leucine arylamidase, and α-glucosidase are positive for all or the majority of isolates. Alkaline and acid phophatases, lipase (C_{14}), valine and cystine arylamidase, phosphoamidase, β-glucouronidase, β-glucosidase, α-mannosidase, and α-fucosidase are negative. Chymotrypsin, α-galactosidase, β-galactosidase, and *N*-acetyl-β-glucosamine are variable with ~10–50% isolates positive (Kilian, 1978; Schofield and Schaal, 1981).

Source: isolated from the oral cavity of humans including the mucosa, tonsils, and dental plaque, occasionally isolated from the gastrointestinal tract and female genital tract. It seems unlikely to represent part of the indigeous microbiotia. It may also be present in the oral cavity of other animals (Bowden, 1991). Isolated from lacrimal canaliculitis (inflammation of the short passage which drains tears form the lacrimal lake to the lacrimal sac).

DNA G+C content (mol%): 63–65 mol% (T_m).

Type strain: ATCC 14157, CCUG 4939, CIP 101941, DSM 43307, JCM 5830, NBRC 14587, NCTC 12967, VKM Ac-1449.

Sequence accession no. (16S rRNA gene): AJ003058, AJ315953, X53216.

Further comments: description based largely on the reference strain and eight other strains (Buchanan and Pine, 1962; Charfreitag et al., 1988; Cummins and Moss, 1990; Johnson and Cummins, 1972; Schofield and Schaal, 1981) and numerous clinical isolates (Hall, 2006). Two distinct serovars, based on fluorescent antibody labeling, have been described: serovar 1 (type strain ATCC 14157) and 2 (ATCC 29326, WVU† 346, F. Lentze strain "Fleischmann": VPI5067, CDCW904; isolated from a typical case of human actinomycosis in Germany), (Gerencser and Slack, 1967; Holmberg and Forsum, 1973). These serotypes also form separate clusters on numerical phenetic analysis (Schofield and Schaal, 1981). To date, there is no published information relating to any phylogenetic differences.

On the basis of 16S rRNA gene sequence analysis, *Propionibacterium propionicum* clusters with *Propionibacterium avidum* (Figure 232). Microscopically, the bacterium can be mistaken for *Streptococcus* species in clinical material as it can form chains of cells with coccoid appearance. Recognized cause of actinomycosis, a chronic granulomatous disease which commonly affects the cervicofacial area but may also cause infection of, for example, the thorax, abdomen, or pelvis. The organism is sometimes associated with the use of intra-uterine contraceptive devices. (Hall, 2006). Most commonly associated with infections of the lacrimal apparatus such as lacrimal canaliculitis (Brazier and Hall, 1993). It is not possible to clinically distinguish between *Actinomyces* species and *Propionibacterium propionicum* as the underlying cause of these diseases. *Propionibacterium propionicum* is morphologically similar to *Actinomyces israelii* but a distinguishing feature is propionic acid production from glucose metabolism. Long-term, high dose antibiotic therapy is frequently necessary; late diagnosis and insufficient treatment can lead to fatality (Hall, 2006). *Propionibacterium propionicum* is also associated with primary and persistent ondodontic infection (Siqueira and Rocas, 2003). Johnson and Cummins (1972) describe VPI 5077 as an unidentifiable isolate with 1% DNA similarity to ATCC 14157 (Type 1 *Propionibacterium propionicum*) and no antigenic cross-reactivity, received in their laboratory as "*Arachnia propoionica*". In the results, this isolate is listed as VPI 5067, a synonym for ATCC 29326: WVU 346 (F Lentz strain "Fleishman") reported by Gerencser and Slack (1967) as Type 2 "*Actinomyces propionicus*". Subsequent publications have quoted the results obtained by Johnson and Cummins (1972) as relating to VPI 5067. It is not known if the tabular designation VPI5067 in Johnson and Cummins (1972) is an error. Most isolates are sensitive to a wide selection of antibiotics including penicillins, erythromycin, clindamycin, imipenem cephalosporins, chloramphenicol, and tetracyclines. Resistant to metronidazole, fluoroquinolones, aztreonam, and aminoglycosides.

12. **Propionibacterium thoenii** van Niel 1928, 164[AL] ("*Bacterium acidi propionici* var. *rubrum*" Thöni and Allemann 1910; "*Propionibacterium rubrum*" van Niel 1928)

tho.e′ni.i. N.L. gen. masc. n. *thoenii* of Thöni; named for J. Thöni, the Swedish bacteriologist who first isolated this organism.

Description based on a study of the type strain and ATCC 4871 ("*Propionibacterium rubrum*" van Niel 23) and ATCC 4872 ("*Propionibacterium rubrum*" van Niel 19).

Surface colonies at 4 d are circular, entire, smooth, and generally orange or red-brown.

Broth cultures show generalized turbidity with brownish-red or orange-red deposit; terminal pH in glucose broth is 4.7–4.9.

Shows β-hemolysis on blood agar containing human, bovine, equine, sheep, rabbit or pig blood.

Less strictly anaerobic than the type species (van Niel, 1957). Catalase-positive.

Pantothenate and biotin are essential for growth. Thiamine is required by some strains and is stimulatory to others, while *p*-aminobenzoic acid is not required (Delwiche, 1949).

Peptidoglycan contains alanine, glutamic acid, glycine, and LL-DAP as diamino acid, and cell-wall sugars are glucose and galactose.

Source: originally isolated from red spots in Emmentaler cheese (Thöni and Alleman, 1910), also found in other dairy products.

DNA G+C content (mol%): 66–67 (T_m).

Type strain: ATCC 4874, CCUG 28149, CIP 103029, DSM 20276, HAMBI 247, JCM 6437, LMG 16455.

Sequence accession no. (16S rRNA gene): AJ704572, X53220.

Further comments: member of the "classical" or "dairy" group of propionibacteria. On the basis of 16S rRNA gene sequence analysis, *Propionibacterium thoenii* clusters with *Propionibacterium jensenii* (Figure 232). It has been recognized for some time that the strains described under the names *Propionibacterium thoenii* and "*Propionibacterium rubrum*" have many features in common, not least the production of an intense red or reddish brown pigment. The nature of the pigment is not known. Classically, (van Niel, 1957) "*Propionibacterium rubrum*" ferments raffinose and mannitol but not sorbitol, while *Propionibacterium thoenii* ferments sorbitol, but not raffinose or mannitol. However, strains of *Propionibacterium thoenii* and "*Propionibacterium rubrum*" show high DNA similarity (Johnson and Cummins, 1972) and, therefore, it was recommended that they be combined in a single species. Sequence comparison of the complete 16S rRNA genes revealed only 12 bp differences thus confirming the similarity (de Carvalho et al., 1995). Some strains of *Propionibacterium thoenii* have been shown to produce bacteriocins under different growth conditions (Ben-Shushan et al., 2003; Van der Merwe et al., 2004).

Genus II. **Aestuariimicrobium** Jung, Kim, Song, Lee, Oh and Yoon 2007, 2117[VP]

Susmitha Seshadri

A.es.tu.a.ri.i.mi.cro′bi.u.m. L. n. *aesturarium* part of the sea coast which, during the flood-tide, is overflowed, but at the ebb-tide is left covered with mud or slime, a tidal flat; N.L. neut. n. *microbium* microbe; N.L. neut. n. *Aestuariimicrobium* a microbe isolated from a tidal flat.

Short to coccoid nonsporeforming rods. Cells are nonflagellated and stain **Gram-positive**. **Aerobic**. Growth occurs between 4–40°C with **30°C as the optimal temperature**. The optimal pH range for growth is 7.5–8.5. Colonies are pigmented yellow. Nitrate is reduced to nitrite. **Catalase-positive**. The major **menaquinone is MK-9(H_4)** and the **predominant fatty acid is $C_{15:0}$ antesio**.

DNA G+C content (mol%): 68.8–69.2.

Type species: **Aestuariimicrobium kwangyangense** Jung, Kim, Song, Lee, Oh and Yoon 2007, 2117[VP].

Further descriptive information

The only species in this genus was isolated from an oil contaminated tidal flat sediment by enrichment with diesel oil and exhibits diesel oil degradation activity. Phylogenetic studies based upon the 16S rRNA indicate that *Aestuariimicrobium* is closely affiliated to the family *Propionibacteriaceae*. On the basis of phylogenetic, phenotypic, chemotaxonomic and genetic studies, *Aestuariimicrobium* is classified as a novel genus. Cell wall contains LL-diaminopimelic acid.

Enrichment and isolation procedures. Isolation was performed by inoculating 0.5 mg of the oil-contaminated sediment samples in 100 ml of Bushnell–Haas broth with 2% (w/v) diesel oil. Cultures were incubated at 30°C on a horizontal shaker at 150 r.p.m. Four strains R27^T, R44, R45, and R47 with diesel oil degradation activity were isolated by dilution plating on R2A agar.

Maintenance procedures

Routine subculturing is done on R2A agar at 30°C. Can be cultivated in R2A broth without the agar, supplemented with 2% (v/v) Hutner's mineral base and 0.1% (v/v) trace element solution.

Differentiation of the genus *Aestuariimicrobium* from closely related genera

The almost-complete 1472 nucleotide long 16S rRNA gene sequence of the four isolated strains showed low sequence similarity to other genera in the family *Propionibacteriaceae* and form a distinct lineage. *Aestuarimicrobium* is aerobic in comparison to phylogenetically closely related genera such as *Propionibacterium*, *Propioniferax*, *Tessaracoccus* and *Luteococcus*, which are all facultative anaerobes. The four above-mentioned genera differ from *Aestuariimicrobium* in one or more of phenotypic properties such as their shape, temperature and pH requirements for growth, catalase production, and nitrate reduction. *Aestuariimicrobium* and *Tessaracoccus* both contain only $C_{15:0}$ antesio as the major fatty acid while the other genera contain more than one major fatty acid. *Tessaracoccus* contains both MK-9(H_4) and MK-7(H_4) while *Aestuariimicrobium* contains only MK-9(H_4) as the predominant menaquinone.

List of species of the genus *Aestuariimicrobium*

1. **Aestuariimicrobium kwangyangense** Jung, Kim, Song, Lee, Oh and Yoon 2007, 2117[VP]

kwang.yang.en′se. N.L. neut. adj. *kwangyangense* of or belonging to Kwangyang, Korea, the source of isolation.

Cells are short rods or cocci (0.6–1.2 × 1.2–2.0 μm). Colonies measure 0.8–1.0 mm after 3 d of growth at 30°C on R2A agar. Colonies are circular, convex, smooth and yellow in color. No growth occurs anaerobically with or without nitrate supplementation on R2A agar. Esculin, casein and Tweens 20, 40, 60, and 80 are hydrolyzed, while starch, hypoxanthine, xanthine and tyrosine are not. D-Glucose, D-fructose, D-galactose, D-cellobiose, D-mannose, D-xylose, sucrose, maltose and salicin, are utilized as sole carbon and energy sources, but L-arabinose, acetate, citrate, succinate, L-malate, formate and L-glutamate are not utilized. Utilization of trehalose, benzoate, and pyruvate are variable. Susceptible to penicillin G, chloramphenicol, ampicillin, cephalothin, novobiocin, tetracycline and carbenicillin, but not to polymyxin B, streptomycin, gentamicin, kanamycin, lincomycin, oleandomycin or neomycin. The API ZYM system detects the presence of esterase (C4), esterase lipase

(C8), leucine, arylamidase, acid phosphatase, α-galactosidase, β-galactosidase, α-glucosidase and β-glucosidase but not alkaline phosphatase, lipase (C14), valine arylamidase, cystine arylamidase, trypsin, α-chymotrypsin, *N*-acetyl-β-glucosaminidase, α-mannosidase and α-fucosidase. Naphthol-AS-BI-phosphohydrolase and β -glucuronidase activities are negative for the type strain, but other strains are positive.

DNA G+C content (mol%): 68.8–69.2 (HPLC).

Type strain: R27, KCTC 19182, JCM 14204.

Sequence accession no. (16S rRNA gene): DQ830982.1.

Genus III. **Brooklawnia** Rainey, da Costa and Moe 2006, 1981[VP] (*in* Bae, Moe, Yan, Tiago, da Costa and Rainey 2006b)

Milton S. da Costa, Fred A. Rainey and William M. Moe

Brook.law′ni.a N.L. fem. n. *Brooklawnia* named after Brooklawn, the contaminated site from which members of the genus were first isolated.

Pleomorphic rods. Colonies are white. Nonsporeforming. Gram-stain-positive. Strains are **nonmotile**. **Mesophilic** with a temperature optimum for growth of about 37°C; neutrophilic with an optimum pH for growth of about 6.5. **Menaquinone 9(H_4)** is the predominant respiratory quinone. Fatty acids are primarily **iso-** and **anteiso-branched**; straight-chain fatty acids are also present. Cell-wall peptidoglycan contains *meso*-DAP (A1γ). Cells are catalase-positive and oxidase-negative, and nitrate is not reduced. **Facultatively anaerobic**. **Chemoheterotrophic**. Propionate and acetate are the predominant products of glucose fermentation. Strains of this genus were isolated from groundwater contaminated with chlorosolvents.

DNA G+C content (mol%): 67.5–67.9.

Type species: **Brooklawnia cerclae** Rainey, da Costa and Moe 2006, 1981[VP] (*in* Bae, Moe, Yan, Tiago, da Costa and Rainey 2006b).

Further descriptive information

One species, *Brooklawnia cerclae*, is currently classified in this genus (Bae et al., 2006b). The isolates of this species form colorless colonies that are about 1–3 mm in diameter under anaerobic growth conditions. The organisms are able to grow under aerobic and anaerobic conditions, but growth is better under an atmosphere of H_2 (10%), CO_2 (10%), and N_2 (80%) or under CO_2 (5%) and N_2 (95%).

Strains BL-34[T] and BL-35 are chemoheterotrophic, growing on a variety of complex media, namely Plate count Agar, Colombia Anaerobic Sheep Blood, and Peptone/Yeast Extract/Glucose (PYG) medium. Several carbon sources are assimilated, among them carbohydrates, organic acids, and methanol. The major fermentation products of glucose fermentation are propionate and acetate.

Iso-and anteiso-branched fatty acids are the major fatty acids; $C_{15:0}$ anteiso, and C_{15} iso account for about 84–86% of the total, which is much higher than in other closely related species classified in other genera (Bae et al., 2006b). The major respiratory quinone is MK-9(H_4) like those of other closely related organisms. Strains BL-34[T] and BL-35 are not able to assimilate or transform 1,2-dichloroethane (1,2-DCA) or 1,1,2-trichloroethane (1,1,2-TCA) despite being isolated from water containing high levels of these chlorosolvents. However, they are extremely resistant to these solvents and able to grow in media with solvent concentrations of up to 9.8 mM. The resistance to chlorosolvents may explain their ability to colonize and their subsequent isolation from the Brooklawn site where petrochemicals were disposed and where 1,1,2,2-tetrachloroethane, 1,1,2-trichloroethane, 1,2-dichloroethane, 1,2-dichloropropane, hexachloro-1,3-butadiene, hexachlorobenzene, vinyl chloride, and polycyclic aromatic hydrocarbons (PAHs) are present in ground water in large quantities (Clement et al., 2002; US EPA, 2005). Another organism isolated from this site, *Propionicicella superfundia* (Bae et al., 2006c), also tolerated high levels of chlorosolvents, but was not able to transform them.

Enrichment, isolation, and growth conditions. The isolates of *Brooklawnia cerclae* were recovered from ground water at the Brooklawn Site (Petro-Processors of Louisiana, Inc. Superfund Site) located near Baton Rouge, LA (USA). Strain BL-34[T] was isolated on Nutrient Agar (Difco) supplemented with 0.5 g/l, L-cysteine, 1.0 mg/l, and resazurin, adjusted to pH 7.0 prior to autoclaving. Incubation was at 30°C in an anaerobic chamber containing a gas mixture of 10% H_2, 10% CO_2, and 80% N_2 (by vol.). Strain BL-35 was isolated on Plate Count Agar (Difco) supplemented with the same amendments and incubated under the same conditions. The strains are maintained on Colombia Anaerobic Sheep Blood agar plates (BBL) or PYG agar plates (Akasaka et al., 2003b).

Maintenance procedures

The strains are maintained on Colombia Anaerobic Sheep Blood agar plates (BBL) or PYG agar plates (Akasaka et al., 2003b). Strains have been successfully maintained long-term in nutrient broth containing 15% (v/v) glycerol at −80°C.

Taxonomic comments

The genus *Brooklawnia* belongs to the family *Propionibacteriaceae* (Delwiche 1957; Stackebrandt et al. 1997) within the order *Propionibacteriales* (formerly suborder *Propionibacterineae*; Stackebrandt et al., 1997), sharing several characteristics with the species of the most closely related genera. The genera that fall within the radiation of the family *Propionibacteriaceae* include *Propionibacterium* Orla-Jensen 1909, *Aestuariimicrobium* Jung et al. 2007, *Friedmanniella* Schumann et al. 1997, *Granulicoccus* Maszenan et al. 2007, *Luteococcus* Tamura et al. 1994, *Microlunatus* Nakamura et al. 1995, *Micropruina* Shintani et al. 2000, *Propioniferax* Yokota et al. 1994, *Propionicicella* Bae et al. 2006c, *Propionicimonas* Akasaka et al. 2003b, *Propionimicrobium* Stackebrandt et al. 2002, and *Tessaracoccus* Maszenan et al. 1999b. The characteristics that can be used to differentiate *Brooklawnia* from the other genera of the family *Propionibacteriaceae* are shown in Table 206. *Brooklawnia* can be differentiated from *Propionimicrobium* (its closest phylogenetic relative based on branching – see

TABLE 206. Characteristics differentiating the genus *Brooklawnia* from other genera of the family *Propionibacteriaceae*[a]

Characteristic	*Brooklawnia*[b]	*Aestuariimicobium*[c]	*Friedmanniella*[d]	*Granulicoccus*[e]	*Luteococcus*[f]	*Microlunatus*[g]	*Micropuina*[h]	*Propionibacterium*[i]	*Propionicicella*[j]	*Propionicimonas*[k]	*Propioniferax*[l]	*Propionimicrobium*[m]	*Tessaracoccus*[n]
Origin	Chlorosolvent contaminated groundwater	Tidal flat sediment	Antarctic sandstone, activated sludge	Phenol degrading aerobic granules	Soil and water, human blood, human peritoneum	Activated sludge, soil	Activated sludge reactor	Dairy products, human sources, bovine lesions, wastewater, spolied orange juice	Chlorosolvent contaminated groundwater	Plant residue in rice field soil	Human epidermis	Human lymph nodes	Activated sludge, marine sediment
Cell morphology	Pleomorphic rods	Short rods, cocci	Cocci, in packets	Cocci	Coccoi, arranged in pairs and tetrads	Cocci	Cocci	Pleomorphic rods, cocci	Rods	Pleomorphic rods	Pleomorphic rods	Pleomor-phic rods	Coccoid, arranged in tetrads
O_2 metabolism	Facultatively anaerobic	Aerobic	Aerobic	Facultatively anaerobic	Facultatively anaerobic	Aerobic	Aerobic	Anaerobic, facultative anaero-bic, aerotolerant	Facultatively anaerobic	Facultatively anaerobic	Facultatively anaerobic	Anaerobic	Facultatively anaerobic
Catalase/ oxidase	+/−	+/−	+/v	−/+	+/+	+/v	+/+	v/v	−/−	−/−	+/+	v/−	+/−
Nitrate reduction	−	+	−	−	−	v	+	v	−	−	+	v	+
DNA G+C content (mol%)	67.5	68.8–69.2	69–74	69	66–68	67.9–70.9	70.5	57–68	69.9	68.7	59–63	53–56	74
Diamino acid in peptidoglycan	*meso*-DAP	LL-DAP	LL-DAP	LL-DAP	LL-DAP	LL-DAP	*meso*-DAP	*meso*-DAP; LL-DAP; *meso*- and LL-DAP	*meso*-DAP	*meso*-DAP	LL-DAP	Lys–Asp	LL-DAP
Murein type	A1γ	nd	A3γ′	A3γ	A3γ′	A3γ′	A1γ	A1γ; A3γ, A3γ′; A1γ and A3γ	nd	nd	A3γ′	A4-α	A3γ′
Major quinone	MK-9(H_4)	MK-9(H_4)	MK-9(H_4) or MK-9(H_2), MK-9(H_2) and MK-7(H_2) or MK-9(H_2) and MK-9(H_2)	MK-9(H_4)	MK-9(H_4)	MK-9(H_4)	MK-9(H_4)	MK-9(H_4)	MK-9	MK-9(H_4); MK-10(H_4)	MK-9(H_4)	MK-9(H_4)	MK-9(H_4), MK-7(H_4)

[a]Symbols: +, >85% positive; −, 0–15% positive; v, variable; nd, not determined.

[b]Data from Bae et al., (2006b).

[c]Data from Jung et al., (2007).

[d]Data from Schumann et al. (1997); Maszenan et al. (1999a); Lawson et al. (2000c).

[e]Data from Maszenan et al. (2007).

[f]Data from Tamura et al. (1994); Collins et al. (2000); Collins et al. (2003).

[g]Data from Nakamura et al. (1995); Cui et al. (2007); Wang et al. (2008).

[h]Data from Shintani et al. (2000).

[i]Data from Stackebrandt and Schaal (2006a).

[j]Data from Bae et al. (2006c).

[k]Data from Akasaka et al. (2003b).

[l]Data from Yokota et al. (1994).

[m]Data from Stackebrandt et al. (2002).

[n]Data from Maszenan et al. (1999b); Lee et al. (2008).

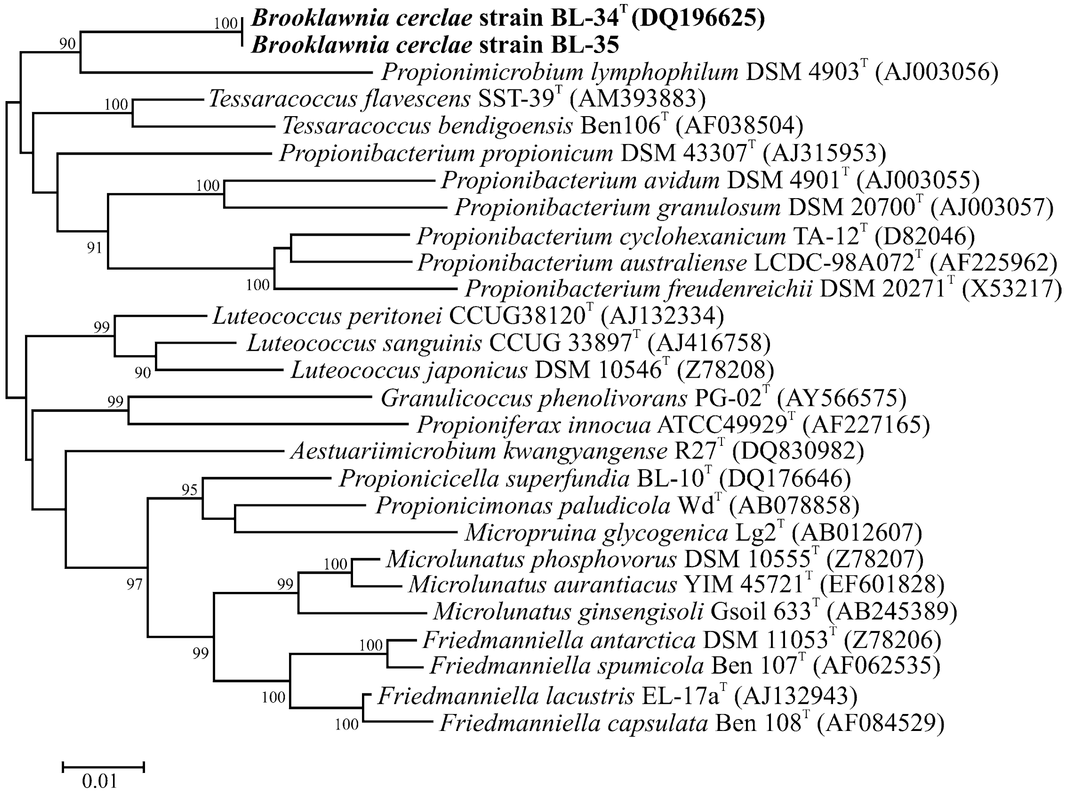

FIGURE 238. 16S rRNA gene sequence based phylogeny indicating the relationship of the genus *Brooklawnia* to other taxa of the family *Propionibacteriaceae*. The scale bar = 1 inferred nucleotide substitution per 100 nucleotides.

Figure 238) on the basis of its cell-wall type and G+C content of the genomic DNA. Differentiation from other genera is, in many cases, based on a combination of chemotaxonomic, physiological, and genomic characteristics (Table 206).

The species *Brooklawnia cerclae* is shown by 16S rRNA gene sequence comparisons to fall within the radiation of the species of the genera of the family *Propionibacteriaceae* (Figure 238). Pairwise sequence similarity values between the 16S rRNA gene sequence of *Brooklawnia cerclae* (DQ196625) and species within the family *Propionibacteriaceae* are in the range 93.5–95.6%. The highest pairwise similarities are to species of the genera *Tessaracoccus* and *Luteococcus* which have short branches as shown in Figure 238. *Brooklawnia cerclae* actually branches with *Propionimicrobium lymphophilum* although the branching is only supported by a bootstrap value of 90%.

List of species of the genus *Brooklawnia*

1. **Brooklawnia cerclae** Rainey, da Costa and Moe 2006, 1981[VP] (*in* Bae, Moe, Yan, Tiago, da Costa and Rainey 2006b)

cer′cla.e. N.L. gen. fem. n. *cerclae* of CERCLA, arbitrary name formed from CERCLA, acronym for Comprehensive Environmental Response, Compensation, and Liability Act, which has mandated cleanup of many hazardous waste sites in the United States.

Cells are Gram-stain-positive, nonmotile, nonspore-forming, pleomorphic rods. Growth occurs at 10°C–40°C; the optimum growth temperature is about 37°C. Growth occurs at pH 4.5–8.0; the optimum pH for growth is 6.5. Growth is not stimulated by addition of NaCl, but is sustained in the presence of up to 3% NaCl (v/v). Facultative anaerobic growth is supported by fermentation. Propionate and acetate are the main products of glucose fermentation. Chemotaxonomic features are the same as the genus description. Growth occurs on arabinose, fructose, glucose, galactose, maltose, rhamnose, xylose, ribose, mannose, starch, glycogen, glycerol, mannitol, lactate, and pyruvate, but not on acetate, ribitol, cellobiose, cellulose, galactitol, erythritol, ethanol, fucose, fumarate, inositol, lactose, malate, methanol, raffinose, sorbitol, succinate, sucrose, or xylan. Strains of this species have been isolated from chlorosolvent-contaminated ground water. Strain BL-35 (LMG 23249, NRRL B-41419) also belongs to this species.

DNA G+C content (mol%) of the type strain: 67.5 (HPLC).

Type strain: BL-34, JCM 14918, LMG 23248, NRRL B-41418.

Sequence accession no. (16S rRNA gene): DQ196625.

Genus IV. **Friedmanniella** Schumann, Prauser, Rainey, Stackebrandt and Hirsch 1997, 282[VP]

Peter Schumann and Rüdiger Pukall

Fri.ed.man.ni.el′la. N.L. fem. dim. n. *Friedmanniella* named after E. Imre Friedmann (1921–2007), an American microbiologist, in recognition of his contributions to Antarctic microbiology.

Spherical to ellipsoidal cells that occur mostly in more or less regular packets which develop through cell division in three perpendicular planes. The packets aggregate, forming clusters. **Gram-stain-positive**. **Nonmotile**. Nonsporeforming. Non-acid-fast. **Strictly aerobic**. **Oxidase-negative**. **Catalase-positive**. Chemo-organotrophic. Only a few carbohydrates, organic acids, and other carbon sources are metabolized. The **peptidoglycan type** is **A3γ** containing **LL-diaminopimelic acid**, glycine in position 1 of the peptide subunit, and a single glycine residue as interpeptide bridge. The major **menaquinone** is **MK-9(H_4)**, and the main **fatty acids** are 12- and 13-methyltetradecanoic acid (**$C_{15:0}$ anteiso and $C_{15:0}$ iso**). Mycolic acids are absent. The **phospholipid pattern** includes **phosphatidylglycerol, diphosphatidylglycerol, phosphatidylinositol**, and one unknown phospholipid.

Phylogenetically, this genus is affiliated to the family *Propionibacteriaceae* Delwiche 1957, emend. Rainey, Ward-Rainey and Stackebrandt 1997 of the order *Propionibacteriales* Zhi et al. 2009.

DNA G+C content (mol%): 69–74.

Type species: **Friedmanniella antarctica** Schumann, Prauser, Rainey, Stackebrandt and Hirsch 1997, 282[VP].

Further descriptive information

The four species of the genus *Friedmanniella*, *Friedmanniella antarctica* (Schumann et al., 1997), *Friedmanniella capsulata* (Maszenan et al., 1999a), *Friedmanniella lacustris* (Lawson et al., 2000a, 2000b) and *Friedmanniella spumicola* (Maszenan et al., 1999a), form a distinct cluster together with the members of the genera *Microlunatus* (Nakamura et al., 1995), *Micropruina* (Shintani et al., 2000), *Propionicicella* (Bae et al., 2006c), and *Propionicimonas* (Akasaka et al., 2003b) within the phylogenetic tree based on 16S rRNA gene sequences of the suborder *Propionibacterinae* (Figure 239). However, the affiliation of these genera to a family is the subject of conflicting taxonomic opinions (Garrity et al., 2007; Stackebrandt and Schaal, 2006b). For

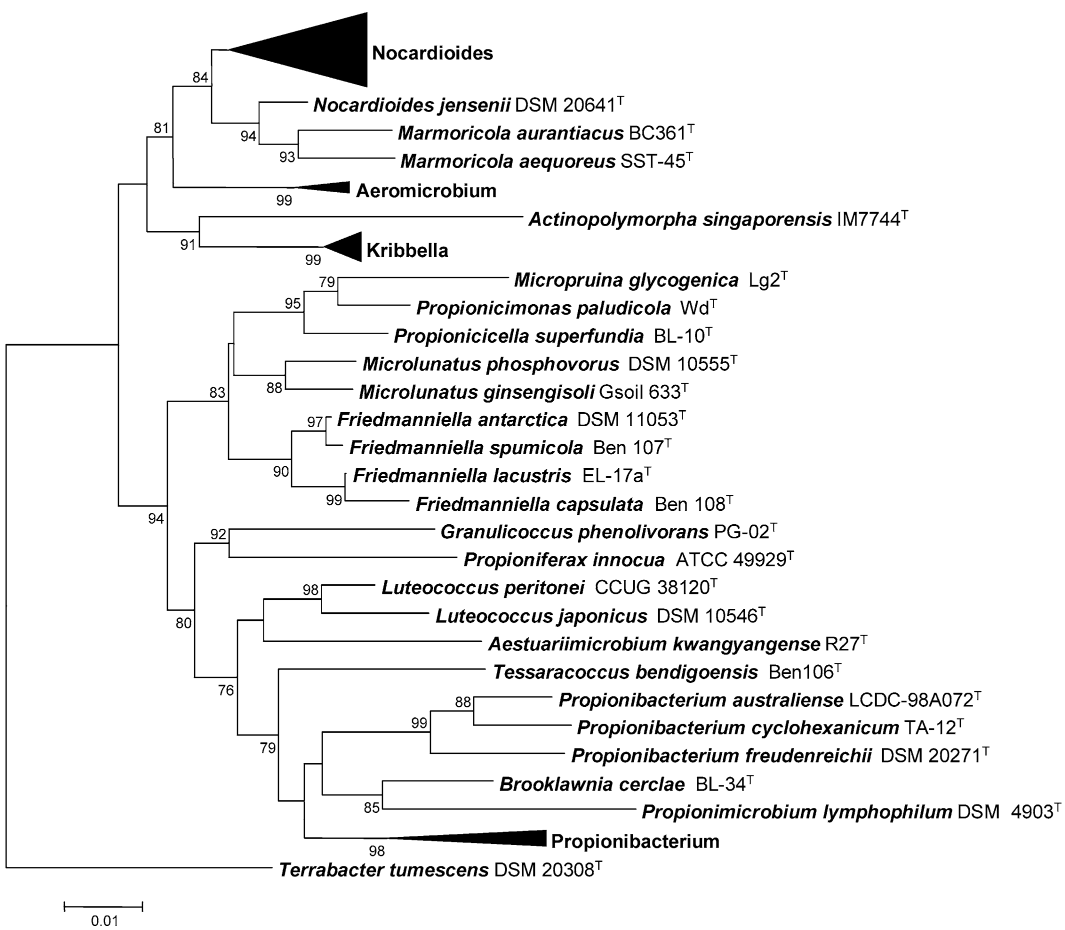

FIGURE 239. Neighbor joining analysis of almost complete 16S rRNA gene sequences from type strains of the genus *Friedmanniella* and other representatives of the *Actinobacteria*. The phylogenetic tree was conducted using Mega 3.1 (Kumar et al., 2004). The Kimura-2-parameter method was used for correction (Kimura, 1980). Bootstrap values were calculated from 1000 resamplings, but values >70% are indicated at branching points only. Bar = 1 subsitution per 100 nucleotides.

reasons given in *Taxonomic comments*, the genus *Friedmanniella* is considered a member of the family *Propionibacteriaceae* in this chapter. The type species with the highest 16S rRNA gene sequence similarity to *Friedmanniella antarctica* are *Microlunatus phosphovorus* (Nakamura et al., 1995), *Propionicicella superfundia* (Bae et al., 2006c), *Propionicimonas paludicola* (Akasaka et al., 2003b), *Aestuariimicrobium kwangyangense* (Jung et al., 2007), *Micropruina glycogenica* (Shintani et al., 2000), and *Brooklawnia cerclae* (Bae et al., 2006b) with values of 94.6%, 94.1%, 94.1%, 93.9%, 93.5%, and 93.6%, respectively. The type species *Friedmanniella antarctica* shows 16S rRNA gene sequence similarity values of 98.8%, 97.3%, and 96.5% to *Friedmanniella spumicola*, *Friedmanniella lacustris*, and *Friedmanniella capsulata*, respectively (all binary 16S rRNA gene sequence similarity values were calculated by the EzTaxon server, Chun et al., 2007). The DNA–DNA similarity values of type strains of *Friedmanniella spumicola* and *Friedmanniella capsulata* to *Friedmanniella antarctica* are 50% and 27%, respectively. *Friedmanniella spumicola* and *Friedmanniella capsulata* are related by more than 97% 16S rRNA gene sequence similarity but show only 29% DNA–DNA similarity (Maszenan et al., 1999a).

Members of the genus *Friedmanniella* are Gram-stain-positive, nonmotile cocci (usually 1.2–1.5 µm in diameter) or cells of nearly spherical shape which show a pronounced tendency to aggregate in clusters of packets which result from cell divisions in three perpendicular planes. The clusters are surrounded and internally subdivided by an extracellular capsular polymer (Maszenan et al., 1999a). Well-developed colonies are pigmented in shades of orange which may become more intense under the influence of light (Schumann et al., 1997). Ageing colonies change in color from orange to faint yellow.

The development of colonies of *Friedmanniella* strains on all tested agar media is usually slow and takes several days or up to 3 weeks. Growth in submerged shaking and standing cultures is often poor. *Friedmanniella lacustris* requires the vitamins biotin, thiamine, and nicotinic acid and grows optimally with 4% (w/v) NaCl. Medium PYGV (DSMZ medium 621; Staley, 1968) supplemented with artificial sea water and vitamins is recommended for cultivation of *Friedmanniella lacustris*. The details for preparation of this medium are given by Stackebrandt and Schaal (2006b). R-Medium (Yamada and Komagata, 1972) or modified organic medium 79 [containing per liter distilled water 10 g glucose, 10 g bacto peptone (Difco), 2 g Casamino acids (Difco), 2 g yeast extract (Serva), 6 g NaCl, and 15 g agar, pH 7.5] can be recommended for the cultivation of *Friedmanniella antarctica*. R2A agar [DSMZ medium 830, containing per liter distilled water 0.50 g yeast extract, 0.50 g proteose peptone (Difco no. 3), 0.50 g Casamino acids (Difco), 0.50 g glucose, 0.50 g soluble starch, 0.30 g Na pyruvate, 0.30 g K_2HPO_4, 0.05 g $MgSO_4 \cdot 7H_2O$, 15.00 g agar – adjust to pH 7.2 with crystalline K_2HPO_4 or KH_2PO_4 before adding agar] is suited for the cultivation of *Friedmanniella capsulata* and *Friedmanniella spumicola*.

Biochemical and physiological characteristics are given in the species descriptions and in Table 207. *Friedmanniella* strains are capable of polyphosphate accumulation (*Friedmanniella lacustris* has not been tested). *Friedmanniella lacustris*, *Friedmanniella capsulata*, and *Friedmanniella spumicola* are sensitive to penicillin; the latter two species were found also to be susceptible to chloramphenicol and vancomycin (Lawson et al., 2000a; Maszenan et al., 1999a). No data are available on the antibiotic sensitivity of *Friedmanniella antarctica*.

All members of the genus *Friedmanniella* exhibit the peptidoglycan type A3γ′ (A42.1, http://www.peptidoglycan-types.info) based on LL-diaminopimelic acid (LL-A_2pm), in which one glycine residue is included in the interpeptide bridge and another one is found at position 1 of the peptide subunit. The same peptidoglycan structure is found in the following members of the family *Propionibacteriaceae*: *Microlunatus* (Schumann et al., 1997), *Tessaracoccus* (Maszenan et al., 1999b), and *Propionibacterium propionicus* (Weiss et al., 1981). The predominating menaquinone is MK-9(H_4), while MK-7(H_4), MK-8(H_4), MK-7(H_2), and MK-9(H_2) may occur as minor components. $C_{15:0}$ anteiso exceeds the value of 50% in the cellular fatty acid profiles of all *Friedmanniella* strains, followed by $C_{15:0}$ iso (13.0–35.8%) and $C_{14:0}$ iso as minor component (1.8–3.9%). The polar lipids are phosphatidylglycerol, diphosphatidylglycerol, phosphatidylinositol, and one unidentified phospholipid. An unknown glycolipid additionally occurs in *Friedmanniella capsulata* and *Friedmanniella spumicola*. *Friedmanniella antartica* contains spermidine and spermine and corresponds in this polyamine pattern to *Microlunatus*, *Luteococcus*, and *Propioniferax* as members of the family *Propionibacteriacea* but differs from all tested species of the genera *Nocardioides* and *Aeromicrobium* of the family *Nocardioidaceae* which contains mainly cadaverine (Busse and Schumann, 1999).

Friedmanniella antarctica and *Friedmanniella lacustris* originate from Antarctica. *Friedmanniella antarctica* was isolated from a sandstone sample containing a cryptoendolithic microbial community from the Linnaeus Terrace (1600 m above ocean level), McMurdo Dry Valleys, Asgard Range of the Transarctic Mountains. *Friedmanniella lacustris* was isolated from a water sample taken at 1 m depth from the hypersaline and meromictic Ekho Lake in the ice-free area of the Vestfold Hills in East Antarctica. *Friedmanniella spumicola* was isolated from a stable surface foam of the aerobic reactor in an activated sludge plant treating mainly wastewater from an orange-juice-processing plant in Mildura, Victoria, Australia. The type strain of *Friedmanniella capsulata* was obtained from an activated sludge biomass sample from Haman Island, Queensland, Australia. Only a small number of additional strains related to *Friedmanniella spumicola* or *Friedmanniella antarctica* has been isolated from environmental habitats. *Friedmanniella* sp. Ellin 163 was isolated from pasture soil by using the liquid serial dilution culture method as described by Schoenborn et al. (2004). The 16S rRNA gene sequence of the isolate shows 99% similarity with *Friedmanniella spumicola*, but is also related to not yet cultivatable bacteria whose sequences have been submitted to EMBL. For instance, *Friedmanniella* sp. Ellin 163 shares 98–99% 16S rRNA gene sequence similarity with uncultured bacteria detected by analysis of the bacterial diversity in indoor dust (Rintala et al. not yet published; e.g., accession number AM697104) and also to sequences derived from analysis of clone libraries originating from metagenomes of endophytes and symbionts enriched from stem bark of the spurge *Trewia pudiflora*. Sequences related to *Friedmanniella spumicola* were also found in metagenomic analyses from soil as reported by Fierer et al. (2007). A *Friedmanniella spumicola*-like strain was isolated from pasture soil of perennial ryegrass (*Lolium perene*) and white clover (*Trifolium repens*) (Joseph et al., 2003), but also from 12,000 years old glacial ice, Sojana, Bolivia (Christner, 2002). Partial sequence analysis from airborne bacterial populations (Fierer

et al., 2008) led to the detection of a *Friedmanniella antarctica*-related strain (97% sequence similarity). Further 16S rRNA gene sequences from uncultured *Friedmanniella*-like bacteria were detected in soil under eucalyptus trees (Silveira et al., 2006) and bronchoalveolar lavage fluid from children with cystic fibrosis (Harris et al., 2007) showing similarity values of 98% to *Friedmanniella capsulata* and 97% to *Friedmanniella antarctica*.

Isolation and maintenance procedures

Friedmanniella antartica was isolated by sprinkling of loosened sandstone material onto PYGV agar (pH 6.9). Colonies developed around sand grains after incubation for 5 months at 9°C in dim light were picked and streaked onto the same medium. Resulting single colonies were subcultured after

TABLE 207. Characteristics differentiating *Friedmanniella* and *Microlunatus* species[a]

Characteristic	*F. antarctica*[b]	*F. capsulata*[c]	*F. lacustris*[d]	*F. spumicola*[c]	*M. phosphovorus*[e]	*M. ginsengisoli*[f]
Storage products	Polyphosphate	Polyphosphate	No intracellular granules	Polyphosphate	Polyphosphate	nt
NaCl tolerance range (%)	0–2	Inhibition	0–6	Inhibition	0–6	0–5
H_2S production	+	+	−	+	nt	−
Hydrolysis of gelatin	−	−	+	−	nt	+
Nitrate reduction to NO_2^-	−	−	+	−	+ (anaerobically)	+
Carbon source:						
Acetate	−	−	+	−	+	−
N-Acetyl-L-glutamate	nt	−	+	−	nt	nt
Alaninamide	−	−	+	+	nt	nt
Alanine	−	+	−	−	−	+
L-Alanylglycine, 2,3-butanediol, inosine, glucose-6-phosphate	−	+	−	−		
Asparagine,	−	+	−	−	+	+
β-Cyclodextrin	−	+	−	−	nt	nt
Dextrin	−	+	−	+	nt	nt
L-Fructose	−	+	+	−	nt	nt
Galactose	−	−	+	+	+	+
Gentiobiose	−	−	+	−	nt	nt
Glucose, mannose, raffinose	−	−	+	−	+	+
Glycogen	−	+	−	+	−	+
γ-Hydroxybutyrate	nt	−	+	−	nt	−
Inositol	−	−	+	−	+	−
Lactamide/succinamate	−	+	−	−	nt	nt
D-Lactate methylester	nt	+	−	+	nt	nt
Lactose	−	−	+	−	−	+
Malate	−	+	+	−	−	+
Maltose	−	+	+	−/+[g]	+	+
Maltotriose	nt	+	+	−	nt	nt
D-Mannitol	−	+	+	+	+	−
D-Melibiose	nt	+	+	−	+	+
Melicitose	nt	−	+	−	nt	nt
α-Methyl-D-galactoside, β-methyl-D-glucoside, palatinose, D-psicose	−	+	+	+	nt	nt
β-Methyl-D-galactoside	nt	−	+	−	nt	nt
3-Methyl-glucose	+	+	+	−	nt	nt
α-Methyl-D-mannoside	nt	−	+	−	nt	nt
Methylpyruvate	−	−	−	+	nt	nt
2-Oxoglutarate	nt	+	−	−	nt	nt
Propionate	−	+	+	−	−	+
Pyruvate	−	+	−	+	+	+
L-Rhamnose	−	+	+	−	nt	+
Succinate	−	+	−	+	−	−
Sucrose	−	+	+	−/+[g]	+	+
Thymidine	−	−	+	+	nt	nt
Thymidine-5′-monophosphate	−	+	−	+	nt	nt
Trehalose	−	−	+	−/+[g]	+	+
Turanose	−	−	+	+	nt	nt

(continued)

TABLE 207. (continued)

Characteristic	*F. antarctica*[b]	*F. capsulata*[c]	*F. lacustris*[d]	*F. spumicola*[c]	*M. phosphovorus*[e]	*M. ginsengisoli*[f]
Menaquinones (molar ratio)	MK-9(H_4), MK-9(H_2) (63:8)	MK-9(H_4), MK-9(H_2), MK-7(H_4) (72:10:8)	MK-9(H_4), MK-8(H_4) (83:4)	MK-9(H_4), MK-7(H_2), MK-8(H_2), MK-9(H_2), MK-8(H_4) (62:11:7:6:4)	MK-9(H_4)	MK-9(H_4)
Origin	Sandstone, Antarctica	Activated sludge, Australia	Lake water, Antarctica	Activated sludge, Australia	Activated sludge, Japan	Soil of a ginseng field, South Korea

[a]Symbols: +, positive reaction; –, negative reaction; nt, not tested.

[b]Data from Schumann et al. (1997); Maszenan et al. (1999a).

[c]Data from Maszenan et al. (1999a).

[d]Data from Lawson et al. (2000a).

[e]Data from Nakamura et al. (1995); Maszenan et al. (1999a).

[f]Data from Cui et al. (2007).

[g]Reactions variable in different test systems.

5 months on PYGV agar slants at 4–6°C. Serial transfers at 2-week intervals on R-agar at 22°C under diffuse daylight kept the type strain viable. Strain AA-1042[T] could be recultivated successfully from lyophilized conserves stored at 4°C for 27 months (Schumann et al., 1997). *Friedmanniella antarctica* DSM 11053[T] and *Friedmanniella capsulata* DSM 12936[T] could also be recovered successfully from freeze-dried cultures after a 10 year period of storage. Viability testing revealed 10^3–10^5 c.f.u./ml depending on the growth behavior of the strain (strength of aggregating packets and cluster formation).

The type strains of *Friedmanniella spumicola* and *Friedmanniella capsulata* were isolated from a foam sample and activated sludge biomass, respectively, by micromanipulation (Skerman, 1968). Colonies of both strains developed on standard methods agar (SMA, Difco) supplemented with 1% sterile horse serum at 25°C after 7–10 d. Subsequent purification was done on R2A agar. Short-term storage is possible in R2A or trypticase soy yeast extract medium containing 20% glycerol at −80°C. At the DSMZ, cultures are freeze-dried for long-term conservation according to described procedures which have been summarized in the Cabri guidelines (www.Cabri.org). In addition, preservation by freezing and low temperature storage in glass capillary tubes can be applied. *Friedmanniella lacustris* was isolated from a 20 ml water sample after enrichment by inoculation into 100 ml autoclaved PYGV medium (pH 8.0) prepared with filtered Ekho Lake water for 12 d at 15°C. Single colonies of strain EL17A[T] were obtained after streaking the enrichment culture onto PYGV agar. Cultures of the strain were freeze-dried and stored at 8°C in the dark.

Procedures for testing special characteristics

Because the peptidoglycan type A3γ′, variation A42.1, is a significant feature of members of the genera *Friedmanniella*, *Microlunatus*, *Tessaracoccus*, and of *Propionibacterium propionicus*, refer to the chapter on the genus *Terracoccus* (this volume) where the elucidation of the peptidoglycan structure is described. The structural variation A42.1 of the peptidoglycan based on LL-A_2pm can be concluded from the occurrence of the peptide Gly→D-Glu instead of the dipeptide L-Ala→D-Glu which is commonly found in A-type peptidoglycans (Schleifer and Kandler, 1972). The structure is confirmed by the molar ratio of the amino acids of ca. 1 LL-A_2pm:1 Glu:2 Gly:1 Ala, determined by gas chromatography as described by MacKenzie (1987).

Differentiation of the genus *Friedmanniella* from closely related genera

The genera with the highest phylogenetic relationship to the genus *Friedmanniella* are *Microlunatus* (Nakamura et al., 1995), *Micropruina* (Shintani et al., 2000), *Propionicicella* (Bae et al., 2006c), and *Propionicimonas* (Akasaka et al., 2003b). The genera *Micropruina*, *Propionicicella*, and *Propionicimonas* differ from the genus *Friedmanniella* in displaying the diagnostic diamino acid *meso*-A_2pm (Table 208). The latter two species can also be differentiated by their morphology. The unsaturated menaquinone MK-9 was reported for the genus *Propionicicella* (Bae et al., 2006c) which is unique within the families *Propionibacteriaceae* and *Nocardioidaceae*. The menaquinone profile of *Propionicimonas* differs from that of the genus *Friedmanniella* by the additional occurrence of MK-10(H_4) (Akasaka et al., 2003b). The genus *Microlunatus* shares not only the highest 16S rRNA gene sequence similarity with *Friedmanniella antarctica* but also peptidoglycan structure, major cellular fatty acids, menaquinone, and polar lipids (Table 208). As distinguishing chemotaxonomic characteristics are lacking, the differentiation of the genera *Friedmanniella* and *Microlunatus* is based on the lower tendency to aggregate and to form capsular polymers, better growth in submerged cultures, lack of orange pigments, and metabolism of a broader range of organic compounds of strains of the lat-

TABLE 208. Characteristics differentiating the genus *Friedmanniella* from the phylogenetically related genera *Microlunatus*, *Micropruina*, *Propionicicella*, and *Propionicimonas*[a]

Characteristic	*Friedmanniella*[b]	*Microlunatus*[c]	*Micropruina*[d]	*Propionicicella*[e]	*Propionicimonas*[f]
Morphology	Cocci in packets	Cocci	Coccoid	Rods	Irregular rods
Diamino acid of the peptidoglycan (peptidoglycan type)	LL-A_2pm (A3γ′, A42.1)	LL-A_2pm (A3γ′, A42.1)	*meso*-A_2pm (A1γ, A31)	*meso*-A_2pm (A1γ, A31)	*meso*-A_2pm (A1γ, A31)
Major menaquinones	MK-9(H_4)	MK-9(H_4)	MK-9(H_4)	MK-9	MK-9(H_4), MK-10(H_4)
Major fatty acids	$C_{15:0}$ anteiso, $C_{15:0}$ iso, $C_{14:0}$ iso	$C_{15:0}$ anteiso, $C_{15:0}$ iso, $C_{16:0}$ iso	$C_{15:0}$ anteiso, $C_{14:0}$ iso $C_{16:0}$, $C_{16:0}$ iso	$C_{15:0}$ anteiso, $C_{15:0}$, $C_{16:0}$ iso	$C_{15:0}$, $C_{15:0}$ anteiso, $C_{14:0}$ iso
Polar lipids	PI, PG, DPG, PL, (GL)	PI, PG, DPG, PL	nd	nd	nd
DNA G+C content (mol%)	69–74	67.9–69.8	70.5	69.9	67.4–68.7

[a]Abbreviations: A_2pm, diaminopimelic acid; peptidoglycan types according to http://www.peptidoglycan-types.info; MK-9(H_4), partially saturated menaquinone with two of 9 isoprene units hydrogenated; MK-10(H_4), partially saturated menaquinone with two of 10 isoprene units hydrogenated; MK-9, unsaturated menaquinone with 9 isoprene units; DPG, diphosphatidylglycerol; PG, phosphatidylglycerol; PI, phosphatidylinositol; PL, unidentified phospholipid; GL, unidentified glycolipid; nd, no data available.

[b]Data from Schumann et al. (1997); Maszenan et al. (1999a); Lawson et al. (2000a).

[c]Data from Nakamura et al. (1995); Maszenan et al. (1999a); Cui et al. (2007).

[d]Data from Shintani et al. (2000).

[e]Data from Bae et al. (2006c).

[f]Data from Akasaka et al. (2003b); Bae et al. (2006c).

ter genus (Schumann et al., 1997). The differentiation of both genera by the oxidase test (Maszenan et al., 1999a; Schumann et al., 1997) lost its importance as Lawson et al. (2000a) reports a weak oxidase reaction also for *Friedmanniella lacustris* and as the second *Microlunatus* species, *Microlunatus ginsengisoli* (Cui et al., 2007), differs from the type species of *Microlunatus* in its negative oxidase reaction. Members of the genus *Mirolunatus* differ from *Friedmanniella* species (except for *Friedmanniella lacustris*) in their capability to reduce nitrate. Several physiological and biochemical traits contribute to the differentiation of *Friedmanniella* and *Microlunatus* species (see Table 207).

Taxonomic comments

Stackebrandt and Schaal (2006b) considered the genus *Friedmanniella* as well as the genus *Micropruina* members of the family *Propionibacteriaceae* Delwiche (1957), emend. Rainey, Ward-Rainey and Stackebrandt (1997), while both genera were listed as members of the family *Nocardioidaceae* Nesterenko et al., (1985), emend. Rainey, Ward-Rainey and Stackebrandt (1997) in the Taxonomic Outline of the *Bacteria* and *Archaea* (Garrity et al., 2007). Because members of the genus *Friedmanniella* represent a phylogenetic subcluster within the clade of the family *Propionibacteriaceae* as shown in Figure 239, and because the peptidoglycan type A3γ′ in combination with the major menaquinone MK-9(H_4) and the polyamines spermidine and spermine can only be found in members of the family *Propionibacteriaceae*, the authors of this chapter take the view of Stackebrandt and Schaal (2006b) that the genus *Friedmanniella* shares the membership in the family *Propionibacteriaceae* with the genera *Propionibacterium*, *Luteococcus*, *Microlunatus*, *Micropruina*, *Propioniferax*, *Propionimicrobium*, and *Tessaracoccus* but also with *Propionicimonas* (Akasaka et al., 2003b), *Propionicicella* (Bae et al., 2006c), *Aestuariimicrobium* (Jung et al., 2007), *Granulicoccus* (Maszenan et al., 2007), and *Brooklawnia* (Bae et al., 2006b). The species *Jiangella gansuensis* YIM 002[T] (Song et al., 2005) was excluded from the phylogenetic analysis because all sequences available at present from EMBL and NCBI database are assigned to the family *Pseudonocardiaceae.* The EzTazon (Chun et al., 2007) also does not place the strain into the family *Nocardioidaceae* as stated by Song et al. (2005).

The species of the genus *Friedmanniella* are clearly differentiated from one another by their metabolic properties (Table 207). Both *Microlunatus* species were included in Table 207, as biochemical and physiological properties are useful for the differentiation of *Friedmanniella* and *Microlunatus* species. *Friedmanniella lacustris* and *Friedmanniella capsulata* are the metabolically most versatile organisms of the genus while *Friedmanniella antarctica* shows the lowest amount of positive reactions. *Friedmanniella lacustris* differs from the other members of the genus by lacking H_2S production, tolerance of up to 6% NaCl, growth below 9°C, and its ability to hydrolyze gelatin and reduce nitrate. The growth of *Friedmanniella capsulata* and *Friedmanniella spumicola* is inhibited by addition of NaCl to the media.

Acknowledgements

This chapter is dedicated to Dr. Helmut Prauser on the occasion of his 80th birthday in honor of his contribution to pioneering work on the taxonomy of actinomycetes and of the genera *Nocardioides* and *Friedmanniella* in particular.

List of species of the genus *Friedmanniella*

1. **Friedmanniella antarctica** Schumann, Prauser, Rainey, Stackebrandt and Hirsch 1997, 282[VP]

an.tarc′ti.ca. L. fem. adj. *antarctica* southern, isolated from Antarctica.

The spherical cells are 0.5–2.2 mm in diameter. The cells are arranged in more or less regular packets which adhere to one another to form clusters. Growth in submerged standing and shaking cultures is slow and poor. Colonies on R agar are up to 2 mm in diameter and raised and have irregular edges and shapes. The colony surface is crumbly and dull to smooth and shiny depending on the growth medium. The orange color of colonies may become more intense when the organism is cultured in diffuse daylight. The optimum growth temperature is 22°C. The temperature range for growth is approximately 9–25°C; no growth occurs at 6°C and 28°C. The optimal pH range for growth is 6.0–7.2. Catalase-positive. Oxidase-negative. Urease-positive. Does not reduce nitrate to nitrite. Acid is produced from D-ribose, is produced weakly from L-arabinose, and is produced very weakly from D-xylose. No acid is produced from L-rhamnose, D-glucose, D-fructose, D-mannose, D-galactose, maltose, lactose, sucrose, D-cellobiose, trehalose, D-raffinose, glycerol, D-mannitol, and *myo*-inositol. Starch and esculin are hydrolyzed, and Tween 80 and DNA are only weakly hydrolyzed. Casein and gelatin are not hydrolyzed. Sodium formate is utilized as a carbon source. Sodium acetate, sodium aconitate, sodium benzoate, sodium citrate, disodium succinate, and potassium hydrogentartrate are not utilized. Hypoxanthine, xanthine, adenine, DL-tyrosine, and sodium hippurate are not decomposed. H_2S is produced.

Source: a cryptoendolithic microbial community in sandstone on Linnaeus Terrace, McMurdo Dry Valleys, Antarctica.

DNA G+C content (mol%): 73 (HPLC).

Type strain: AA-1042, DSM 11053, JCM 11651, NBRC 16127.

Sequence accession no. (16S rRNA gene): Z78206.

2. **Friedmanniella capsulata** Maszenan, Seviour, Patel, Schumann, Burghardt, Webb, Soddell and Rees 1999a, 1678[VP]

cap.su.la′ta. L. n. *capsula* a small box or chest; L. fem. suff. *-ata* suffix denoting provided with; N.L. fem. adj. *capsulata* with a chest, capsuled.

This species is characterized by a bright orange color when grown in both solid and liquid media. Cell diameter is 0.6–1.2 µm. Can store polyphosphate aerobically. The optimum growth temperature is 20–25°C. The pH range for growth is 5.5–7.5, with an optimal growth pH of 6.5–7.0. Utilizes L-rhamnose, maltose, sucrose, β-cyclodextrin, L-fucose, 2-aminoethanol, 2,3-butanediol, glucose 6-phosphate, maltotriose, sedoheptulosan, stachyose, D-tagatose, lactamide, 2′-deoxyadenosine, α-ketoglutaric acid, propionic acid, succinamic acid, 3-methylglucose, bromosuccinic acid, glucuronamide, D-alanine, L-alanyl glycine, L-asparagine, L-aspartic acid, glycyl-L-aspartic acid, L-histidine, hydroxy-L-proline, L-threonine, DL-carnitine, γ-aminobutyric acid, urocanic acid, inosine, D-melezitose, malate, and trypsin. The following substrates are not utilized: *i*-erythritol, D-saccharic acid, formic acid, adonitol, D-galactose, D-psicose, turanose, methyl-pyruvate, α-ketobutyric acid, alaninamide, and thymidine. Cellular fatty acid profile is characterized by the presence of $C_{16:0}$ iso, $C_{17:0}$ iso, and $C_{17:0}$ anteiso. Contains the menaquinones MK-9(H_4) and MK-9(H_2) and characterized by the presence of MK-7(H_4). Mycolic acids are absent.

Source: activated sludge.

DNA G+C content (mol%): 74 (HPLC).

Type strain: Ben 108, ACM 5120, CCUG 43143, DSM 12936, JCM 13522.

Sequence accession no. (16S rRNA gene): AF084529.

3. **Friedmanniella lacustris** Lawson, Collins, Schumann, Tindall, Hirsch and Labrenz 2000b, 1953[VP] (Effective publication: Lawson, Collins, Schumann, Tindall, Hirsch and Labrenz 2000a, 226.)

la.cus′tris. N.L. fem. adj. *lacustris* (from L. n. *lacus*, a lake) belonging to the lake.

Cocci with some extracellular polymer, in older cultures also short rods. Cells nonmotile, 0.9–1.3 µm, nonsporeforming, aggregating in short chains, tetrads, or even packets. Grow well on medium PYGV + ASW containing vitamins. Agar colonies flat, watery, and slimy, with a brownish to pink color; older colonies more orange. Aerobic heterotrophs; carbon sources utilized for growth are acetate (weakly), pyruvate, α-D-glucose, glutamate, and citrate, but does not grow on succinate, malate, butyrate, or methanol. A large number of sugar compounds and several organic acids offered by the Biolog GP test system are metabolized. Polymers hydrolyzed: gelatin, starch, and DNA (weakly); does not hydrolyze alginate, casein, or Tween 80. Requires biotin, thiamine, and nicotinic acid for growth. Nitrate is weakly reduced aerobically. NH_3 is formed from peptone; produces acids from glucose. Voges–Proskauer, indole formation, and H_2S production-negative. Temperature optimum is 26°C; the range for growth is 3–3.5°C. The pH tolerance range for growth is 5.5–9.5, with an optimum at pH 7.5. Tolerates up to 6% (w/v) of NaCl with an optimum at 4%. Sensitive to chloramphenicol, penicillin G, and vancomycin. Peptidoglycan type is A3γ′ based on LL-diaminopimelic acid, a single glycine residue as interpeptide bridge, and a glycine residue at position 1 of the peptide subunit. Cell-wall sugars: glucose, mannose, ribose, rhamnose, and galactose. The major respiratory lipoquinone is MK-9(H_4) (83%), with MK-8(H_4) (4%) as minor component. Major fatty acids: $C_{15:0}$ anteiso and $C_{15:0}$ iso. Polar lipids include phosphatidylinositol, phosphatidylglycerol, diphosphatidylglycerol, and an unidentified phospholipid. Mycolic acids absent.

Source: a 1 m water sample from hypersaline Ekho Lake, East Antarctica.

DNA G+C content (mol%): 73 (HPLC).

Type strain: EL-17A, ATCC BAA-165, CIP 106992, DSM 11465, JCM 11951, NCFB 3066.

Sequence accession no. (16S rRNA gene): AJ132943.

4. **Friedmanniella spumicola** Maszenan, Seviour, Patel, Schumann, Burghardt, Webb, Soddell and Rees 1999a, 1678[VP]

spu.mi′co.la. L. fem. n. *spuma* foam; L. suffix *-cola* inhabitant; N.L. masc./fem. n. *spumicola* inhabitant of foam.

Cells adhere to one another and form aggregates of four and eight. The cell diameter is 0.5–1.4 µm. Growth in liquid and solid media is slow and poor requiring up to 2 weeks. Colony color when grown on solid and liquid media is dark yellow to pale orange. The temperature range for growth is 15–37°C with an optimum temperature of 25°C. The pH range for growth is 5.5–8.0, with an optimum pH of 7.0–7.5. Cells store PolyP granules aerobically. Can utilize the following substrates: i-erythritol, D-saccharic acid, formic acid, D-galactose, turanose, methylpyruvate, α-ketobutyric acid, alaninamide, and thymidine. The following substrates were not utilized: L-rhamnose, maltose, sucrose, β-cyclodextrin, L-fucose, 2-aminoethanol, 2,3-butanediol, glucose 6-phosphate, maltotriose, sedoheptulosan, stachyose, D-tagatose, lactamide, 2′-deoxyadenosine, α-ketoglutaric acid, propionic acid, succinamic acid, glucuronamide, D-alanine, L-alanyl-glycine, L-asparagine, L-aspartic acid, glycyl-L-aspartic acid, L-histidine, hydroxy-L-proline, L-threonine, DL-carnitine, γ-aminobutyric acid, uroconic acid, inosine, D-melezitose, malate, 3-methylglucose, bromosuccinic acid, and trypsin. Characterized by the presence of $C_{18:1}$ and $C_{15:0}$ iso. Unbranched fatty acids with 14, 15, and 16 carbons were found in trace amounts. Its major menaquinones are MK-9(H_4) and MK-7(H_2), with MK-9(H_2), MK-8(H_2), and MK-8(H_4) present in trace amounts. Mycolic acids are absent.

Source: activated sludge.

DNA G+C content (mol%): 69 (HPLC).

Type strain: Ben 107, ACM 5121.

Sequence accession no. (16S rRNA gene): AF062535.

Genus V. **Granulicoccus** Maszenan, Jiang, Tay, Schumann, Kroppenstedt and Tay 2007, 733[VP]

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Gra.nu.li.coc′cus. L. neut. n. *granulum* a small grain; N.L. masc. n. *coccus* (from Gr. masc. n. *kokkos* grain, seed) coccus; N.L. masc. n. *Granulicoccus* a coccus from (sludge) granules, here referring to the isolation source.

Non-spore-forming coccus (0.3–1.4 µm diameter). **Gram-stain-positive**. Nonmotile. **Facultative anaerobe**. Temperature range for growth 15–37°C, pH range 5–8.5. Cell wall contains type A3γ peptidoglycan (LL-A_2pm←Gly with alanine at position 1 of the peptide subunit). MK-9(H_4) is the major menaquinone, MK-8(H_4) is present as well in a 42:1 compositional ratio. Polar lipid profile comprises diphosphatidylglycerol and phosphatidylglycerol.

DNA G+C content (mol%): 69.

Type species: **Granuliococcus phenolivorans** Maszenan, Jiang, Tay, Schumann, Kroppenstedt and Tay 2007, 733[VP].

Further descriptive information

Major fatty acids are 13-methyltetradecanoic acid ($C_{15:0}$ iso, 50.5%) and 1,1-dimethoxy-iso-pentadecane ($C_{15:0}$ iso DMA, 37.4%). Stains contain polyphosphate granules but not poly-β-hydroxyalkanoates. Produces capsular material and can autoaggregate.

Phylogenetic analysis of the 16S rRNA gene affiliates the genus with the family *Propionibacteriaceae*. The closest phylogenetic neighbors are *Luteococcus peritonei* (93.9% sequence similarity) (Collins et al., 2000), *Microlunatus panaciterrae* (93.6%) (An et al., 2008), and *Propioniferax innocua* (93.6%) (Pitcher and Collins, 1991; Yokota et al., 1994).

Enrichment and isolation procedures

Strain PG-02[T] was isolated from phenol-degrading aerobic granules cultivated in a laboratory-scale sequencing batch reactor fed with synthetic wastewater containing phenol as the sole carbon source (Jiang et al., 2004). Activated sludge was the initial seed for the reactor. Granules (2.5 g) were added to 15 ml MP medium, and serial diluents of the supernatant were spread onto agar plates containing MP medium with 1.2% Bacto agar (Difco). Plates were incubated for 28 d at 25°C (2007). Colony morphology is visible after 10 d of incubation on MP agar plates.

Maintenance procedures

Strain PG-02[T] can be grown on synthetic wastewater medium with phenol as the sole carbon source with the following composition (per liter): phenol, 0.5 g; NH_4Cl, 0.20; $MgSO_4·7H_2O$, 0.13 g; K_2HPO_4, 1.65 g; KH_2PO_4, 1.35 g; and 1 ml of micronutrient solution. Stock cultures are maintained as a 20% glycerol suspension at −80°C.

Differentiation of the genus *Granuliococcus* from closely related genera

Presence of $C_{15:0}$ iso DMA differentiates *Granulicoccus* from the related genera *Luteococcus*, *Friedmanniella*, *Tessaracoccus*, *Propioniferax*, *Micropruina* and *Microlunatus*. *Granulicoccus* differs from *Microlunatus* in the possession of MK-8(H_4) and lack of phosphatidylinositol. The pleomorphic rod morphology and the polar lipid phosphatidylethanolamine of *Propioniferax* distinguish it from *Granulicoccus*.

List of species of the genus *Granuliococcus*

1. **Granulicoccus phenolivorans** Maszenan, Jiang, Tay, Schumann, Kroppenstedt and Tay 2007, 733^{VP}

phe.no.li.vo′rans. N.L. neut. n. *phenolum* phenol; L. part. adj. *vorans* devouring, consuming; N.L. part. adj. *phenolivorans* consuming phenol.

Growth occurs at mesophilic temperature and pH 5–8.5, with optima at 30°C and pH 7. Arginine dihydrolase, lysine decarboxylase, ornithine decarboxylase and tryptophan deaminase tests are negative. H_2S and indole are not produced. Voges–Proskauer-negative and does not produce acetoin or reduce nitrate to nitrite. Catalase-positive and oxidase-negative. Utilizes phenol, Tweens 40 and 80, L-arabinose, α-D-glucose, α-D-lactose, lactulose, maltose, maltotriose, D-mannose, D-melezitose, D-melibiose, methyl α-D-galactoside, methyl β-D-galactoside, 3-methyl glucose, methyl α-D-glucoside, methyl β-D-glucoside, psicose, D-raffinose, L-rhamnose, D-ribose, salicin, sedoheptulosan, stachyose, sucrose, D-tagatose, D-trehalose, turanose, D-xylose, *myo*-inositol, D-mannitol, D-sorbitol, xylitol, 2,3-butanediol, glycerol, DL-α-glycerol phosphate, glucose 1-phosphate, glucose 6-phosphate, adenosine, AMP, TMP, UMP and fructose 6-phosphate (Biolog GN and GP systems and API 20E). Acids and their derivatives utilized include methyl pyruvate, monomethyl succinate, acetic acid, citric acid, D-galactonic acid lactone, D-gluconic acid, D-glucuronic acid, α-, β- and γ-hydroxybutyric acids, *p*-hydroxyphenylacetic acid, itaconic acid, α-ketobutyric acid, α-ketoglutaric acid, α-ketovaleric acid, lactamide, D-lactic acid methyl ester, L- and DL-lactic acid, D- and L-malic acid, propionic acid, pyruvic acid, quinic acid, D-saccharic acid, sebacic acid, succinic acid, bromosuccinic acid, succinamic acid, *N*-acetylglutamic acid, L-glutamic acid, glycyl L-glutamic acid and L-pyroglutamic acid. Amino acid compounds utilized glucuronamide, alaninamide, D-alanine, L-alanine, L-alanyl glycine, L-asparagine, L-phenylalanine, L-proline, L-serine, inosine, uridine, thymidine and putrescine. Gentiobiose is weakly utilized. Growth is not affected by α-cyclodextrin, β-cyclodextrin, dextrin, glycogen, inulin, mannan, amygdalin, adonitol, D-arabitol, arbutin, cellobiose, i-erythritol, D-fructose, L-fucose, D-galactose, 2-aminoethanol, *N*-acetyl-D-galactosamine, *N*-acetyl-D-glucosamine, *N*-acetylmannosamine, phenyl ethylamine, deoxyadenosine, L-histidine, hydroxy-L-proline, L-leucine, L-ornithine, D-serine, L-threonine, DL-carnitine, D-galacturonic acid, formic acid, D-glucosaminic acid, malonic acid, L-aspartic acid, γ-aminobutyric acid and urocanic acid. Both the API ZYM and API 20E detect alkaline phosphatase, esterase, lipase, leucine arylamidase, valine arylamidase, naphthol-AS-BI-phosphohydrolase, α-galactosidase, β-galactosidase, β-glucuronidase, α-glucosidase and β-glucosidase. API 20E also detects β-galactosidase, urease and gelatinase. API ZYM did not detect acid phosphatase, esterase lipase, cystine arylamidase, trypsin, chymotrypsin, *N*-acetyl-β-glucosaminidase, α-mannosidase and α-fucosidase.

For all other characteristics, refer to the genus description.

DNA G+C content (mol%): 69 (HPLC).

Type strain: PG-02^T ATCC BAA-1292, DSM 17626.

Sequence accession no. (16S rRNA gene): AY566575.

Genus VI. **Luteococcus** Tamura, Takeuchi and Yokota 1994, 355^{VP} emend. Collins, Lawson, Nikolaitchouk and Falsen 2000, 181

TOMOHIKO TAMURA

Lu.te.o.coc′cus. L. adj. *luteus* yellow; N.L. masc. n. *coccus* (from Gr. masc. n. *kokkos* grain, seed) coccus; N.L. masc. n. *Luteococcus* yellow coccus.

Gram-stain-positive coccus or pleomorphic rod. Spherical cells are 0.7–1.0 μm in diameter and occur **singly, in pairs, or in tetrads**. Nonsporeforming. Colonies are circular and smooth and may be cream colored to yellow. **Facultatively anaerobic**. Catalase- and oxidase-positive. Urease-negative. The cells may or may not reduce nitrate to nitrite. Starch is hydrolyzed. Tween 20, 40, 60, and 80 are not hydrolyzed. Acid is produced from glucose and some other sugars. Propionic acid is the major product formed from glucose. Optimum growth temperature is 26–28°C.

Cell-wall peptidoglycan contains **LL-diaminopimelic acid**, alanine, glycine, and glutamate (approximately 1:2:1:1). The major menaquinone is MK-9(H_4). Mycolic acid is not present. The major cellular fatty acid is $C_{16:1}$, and among the minor components a small amount of $C_{18:0}$ iso 2OH is also present. Arabinose is present as a diagnostic sugar in the cell wall. The polar lipids phosphatidylinositol, diphosphatidyiglycerol, and phosphatidyiglycerol, are present.

DNA G+C content (mol%): 64–67.

Type species: **Luteococcus japonicus** Tamura, Takeuchi and Yokota 1994, 355^{VP}.

Further descriptive information

The description of the genus *Luteococcus* has been emended by Collins et al. (2000) to include pleomorphic rods as well as cocci. The type species grows well at temperatures of 25–30°C on oatmeal agar, inorganic salts-starch agar, and peptone-yeast extract-iron agar. For chemotaxonomic studies, these strains were grown in shake cultures (nutrient broth; Difco) at 28°C, and cells were harvested in the stationary phase, washed twice with water, and then, if necessary, freeze-dried.

Cellular fatty acids of the genus *Luteococcus* comprise predominantly $C_{15:1}$, $C_{17:1}$, and $C_{18:1}$ in addition to $C_{16:1}$. The composition differs among species. *Luteococcus peritonei* and *Luteococcus sanguinis* contains predominantly $C_{17:1}$ and followed by $C_{16:1}$ and $C_{15:1}$.

Other properties of the species of *Luteococcus* are presented in Table 209.

Enrichment and isolation procedures

The two strains of *Luteococcus japonicus* were isolated from soil on Tokara Island, Japan, and from spring water for brewing

TABLE 209. Phenotypic characteristics of *Luteococcus* species[a]

Characteristic	*L. japonicus*[b]	*L. peritonei*[c]	*L. sanguinis*[d]
Cell morphology	Cocci	Rods	Cocci
Source	Soil, water	Human peritoneum	Human blood
Acid from:			
L-Arabinose	+	−	nd
D-Glucose	+	+	+
Glycogen	nd	−	+
Mannitol	+	+	+
Maltose	+	v	+
Raffinose	+	−	nd
Ribose	+	−	−
Sucrose	+	+	+
Trehalose	+	−	nd
D-Xylose	−	−	−
Hydrolysis of:			
Esculin	+	+	+
Gelatin	v	−	+
Hippurate	+	−	nd
Acid phosphatase	nd	v	w
Alkaline phosphatase	nd	v	+
Chymotrypsin	nd	−	+
β-Glucuronidase	nd	+	−
Trypsin	nd	−	+
Urease	−	−	−
Reduction of nitrate to nitrite	−	+	+

[a]Symbols: +, >85% positive; −, 0–15% positive; nd, no data; v, variable. The data of each species are those of the type strains.

[b]Tamura et al. (1994).

[c]Collins et al. (2000).

[d]Collins et al. (2003).

named "miyamizu" in Hyogo prefecture, Japan, respectively (Oda, 1935). *Luteococcus peritonei* was isolated from human peritoneum during a fetal autopsy. *Luteococcus sanguinis* was isolated from a blood sample (one out of four bottles) of a 32-year-old man. Although the latter two species were isolated from human samples, the pathogenicity is not known.

Maintenance procedures

The strains of the genus *Luteococcus* are maintained by freezing in water containing 10–30% glycerol at −70°C. Lyophilization of suspensions in 10% skim milk + 1% monosodium glutamate and l-drying in 0.01 M potassium phosphate buffer (pH 7.0) containing 3% monosodium glutamate are also recommended for long-term preservation.

Differentiation of the genus *Luteococcus* from other genera

Luteococcus species show the same chemotaxonomic characteristics as most other genera of the family *Propionibacteriaceae* in possessing a cell-wall peptidoglycan containing LL-diaminopimelic acid (LL-A_2pm) and menaquinone MK-9(H_4). However, the cellular fatty acid composition containing predominantly straight monounsaturated acids (approximately 90% of total acids) is unusual and characteristic for the genus *Luteococcus* (Collins et al., 2000, 2003; Tamura et al., 1994). In contrast, the other members of the family *Propionibacteriaceae* possess mostly iso- and anteiso-branched fatty acids.

Taxonomic comments

Based on 16S rRNA gene sequence analysis, the species of the genus *Luteococcus* form an independent clade in the family *Propionibacteriaceae* of the order *Propionibacteriales*. Within the genus, phylogenetic distances based on 16S rRNA gene sequences between *Luteococcus peritonei* and the other two species are both 94%, indicating the distinctness of this species. In contrast, the sequence difference between *Luteococcus japonicus* and *Luteococcus sanguinis* is 96.9%, indicative of a closer relationship (Collins et al., 2003). However, species status was confirmed by the low DNA relatedness of 49% between these species (Collins et al., 2003). These relationships are confirmed by a comparison of the other phenotypic properties of these species, where *Luteococcus japonicus* and *Luteococcus sanguinis* share similarities in morphologies and carbon sources not seen in *Luteococcus peritonei* (Table 209).

Differentiation of species of the genus *Lutoecoccus*

Luteococcus peritonei can be distinguished from *Luteococcus japonicus* and *Luteococcus sanguinis* by its pleomorphic rod-shaped morphology. Nitrate is reduced to nitrite by *Luteococcus peritonei* and *Luteococcus sanguinis* but not by *Luteococcus japonicus*.

List of species of the genus *Luteococcus*

1. **Luteococcus japonicus** Tamura, Takeuchi and Yokota 1994, 355[VP]

ja.po′ni.cus. N.L. masc. adj. *japonicus* of or pertaining to Japan, where the organisms were isolated.

Cells are spherical and 0.7–1.0 µm in diameter and occur singly, in pairs, or in tetrads. Nonsporeforming. Gram-stain-positive. Facultatively anaerobic. Colonies are circular and smooth and may be cream colored to yellow. Catalase- and oxidase-positive. Urease-negative. Nitrate is not reduced to nitrite. Oxidation-fermentation is fermentative. Acid is produced from D-glucose, D-ribose, D-galactose, D-mannose, D-fructose, sucrose, maltose, trehalose, raffinose, glycerol, mannitol, inositol, and L-arabinose but not from D-xylose, D-arabinose, or L-rhamnose. Propionic acid is produced from glucose. Starch is hydrolyzed. Gelatin is weakly or not hydrolyzed. Optimum growth temperature is 26–28°C.

Source: soil of Tokara Islands, Japan.

DNA G+C content (mol%): 67 (HPLC).

Type strain: ATCC 51526, CCUG 38731, CIP 104067, DSM 10546, JCM 9415, NBRC 12422, VKM Ac-1951.

Sequence accession no. (16S rRNA gene): D21245, D85487, Z78208.

2. **Luteococcus peritonei** Collins, Lawson, Nikolaitchouk and Falsen 2000, 181[VP]

pe.ri.to.ne′i. L. n. *peritoneum* peritoneum; L. gen. neut. n. *peritonei* of the peritoneum.

Cells consist of pleomorphic rods that are Gram-stain-positive. Pigment is not produced. Facultatively anaerobic and catalase-positive. Acid is produced from glucose, lactose, sucrose, mannitol, and methyl β-D-glucopyranoside. Acid may or may not be produced from maltose. Acid is not produced from L-arabinose, D-arabitol, cyclodextrin, glycogen, melibiose, melezitose, pullulan, ribose, raffinose, sorbitol, trehalose, tagatose, or D-xylose. Esculin is hydrolyzed but gelatin and hippurate are not. α-Galactosidase, β-galactosidase, β-galacturonidase, α-glucosidase, β-glucosidase, β-glucuronidase, leucine arylamidase, and pyrazinamidase are produced. Arginine dihydrolase, lipase C14, chymotrypsin, α-fucosidase, glycyl-tryptophan arylamidase, *N*-acetylglucosaminidase, α-mannosidase, β-mannosidase, pyroglutamic acid arylamidase, trypsin, valine arylamidase, and urease are not produced. Activity for alkaline phosphatase and acid phosphatase may or may not be detected. Voges–Proskauer reaction is negative. Nitrate is reduced to nitrite.

Source: human peritoneum.

DNA G+C content (mol%): 65 (method of determination not reported).

Type strain: ATCC BAA-60 = CCUG 38120 = CIP 106441 = JCM 11685.

Sequence accession no. (16S rRNA gene): AJ132334.

3. **Luteococcus sanguinis** Collins, Hutson, Nikolaitchouk, Nyberg and Falsen 2003, 1891[VP]

san′gui.nis. L. gen. n. *sanguinis* of blood.

Nonmotile coccus; Gram-stain-positive. Facultatively anaerobic and catalase-positive.

Acid is produced from glucose, glycogen, mannitol, maltose, lactose, and sucrose, but not from ribose or D-xylose. Esculin and gelatin are hydrolyzed. When tested by using commercial API Coryne and API ZYM systems, acid phosphatase (weak reaction), alkaline phosphatase, chymotrypsin, ester lipase C8 (weak), cystine arylamidase (weak), α-galactosidase, β-galactosidase, α-glucosidase, β-glucosidase, leucine arylamidase, phosphoamidase (weak), pyrrolidonyl arylamidase, pyrazinamidase, valine arylamidase (weak), and trypsin are detected. Esterase C4, lipase C14, α-fucosidase, β-glucuronidase, *N*-acetyl-β-glucosaminidase, α-mannosidase, pyroglutamic acid arylamidase, and urease are not detected. Voges–Proskauer reaction is negative. Nitrate is reduced to nitrite.

Source: human blood.

DNA G+C content (mol%): 64 (method of determination not reported).

Type strain: CCUG 33897 = CIP 107216 = JCM 12371.

Sequence accession no. (16S rRNA gene): AJ416758.

Genus VII. **Microlunatus** Nakamura, Hiraishi, Yoshimi, Kawaharasaki, Masuda and Kamagata 1995, 21[VP]

Satoshi Hanada and Kazunori Nakamura

Mi.cro.lu.na′tus. Gr. adj. *mikros* small; L. masc. adj. *lunatus* half moon-shaped; N.L. masc. n. *Microlunatus* small moon-like microorganism.

Coccoid or spherical cells, 0.3–2.0 µm in diameter. Occurs singly and in pairs. Occasionally forms clusters. Some species show rod-shaped morphology. **Gram-stain-positive**. Nonmotile. Nonsporeforming. Catalase-positive. **Mesophilic**. Good growth occurs at 20–30°C. **Aerobic and chemo-organotrophic**. The following sugars and sugar alcohols can support good growth: glucose, arabinose, mannose, maltose, melibiose, rhamnose, and sorbitol. Some species reduce nitrate under anaerobic conditions and accumulate phosphate inside cells. The major quinone is MK-9(H_4). The cell-wall peptidoglycan contains LL-diaminopimeric acid. The major fatty acids are $C_{15:0}$ anteiso, $C_{15:0}$ iso, and $C_{16:0}$ iso. Isolated from activated sludge in wastewater treatment system, soil, spawn of a mushroom, and an indoor wall.

DNA G+C content (mol%): 65.1–70.9.

Type species: **Microlunatus phosphovorus** Nakamura, Hiraishi, Yoshimi, Kawaharasaki, Masuda and Kamagata 1995, 21[VP].

Further descriptive information

Microlunatus phosphovorus, the type species of the genus, was isolated from activated sludge in the wastewater treatment process (Nakamura et al., 1995). The process, consisting of alternating anaerobic and aerobic conditions, was designed to exhibit high phosphate removal activity. Because *Microlunatus phosphovorus* can accumulate a large amount of phosphate inside cells as a polyphosphate, it is considered to significantly contribute to phosphate removal from wastewater. The uptake and accumulation of phosphate are observed under aerobic conditions. Conversely, the accumulated polyphosphate is degraded and released outside under anaerobic conditions. Such phosphate uptake under aerobic conditions is also found in *Microlunatus aurantiacus*, but the amount of accumulation is obviously smaller than that of *Microlunatus phosphovorus* (Wang et al., 2008).

The cells of *Microlunatus phosphovorus* are 0.8–2.0 µm in diameter and occurred singly or in pairs (Figure 240). Small irregular clusters of cells are occasionally formed. A cell-wall structure (segmentation) is frequently observed in the middle of the spherical cells (Figure 241).

The species of the genus *Microlunatus* are mesophilic and neutrophilic and show good growth at 25–30°C and around pH 7.0. Nitrate reduction under anaerobic condition was included in the description of the genus based on the characteristic of *Microlunatus phosphovorus*. However, *Microlunatus ginsengsoli* and *Microlunatus panaciterrae* are negative for nitrate reduction. Although some species can reduce nitrate to nitrite under anaerobic conditions, the genus *Microlunatus* is basically a chemo-organotroph which grows by oxygen respiration using sugars and sugar alcohols as substrates.

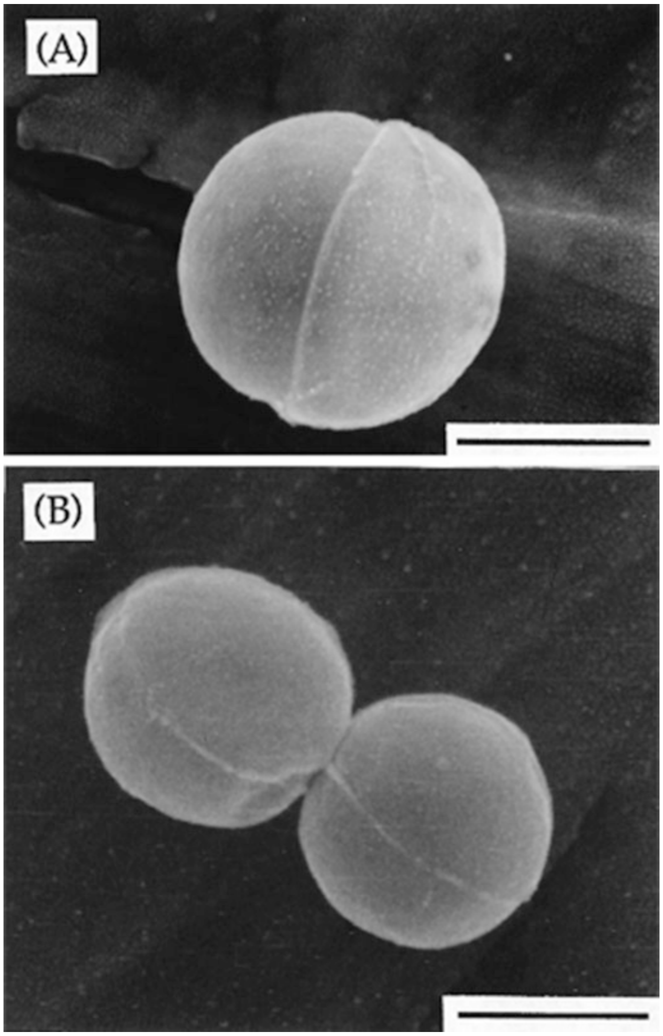

FIGURE 240. Scanning electron micrographs the type strain of *Microlunatus phosphovorus*. (A) Single cell. (B) Cells in pair. Bars = 1 μm. (Reproduced with permission from Nakamura et al., 1995. Int. J. Syst. Bacteriol. *45*: 17–22.)

In addition to *Microlunatus phosphovorus* as the type species, the genus *Microlunatus* contains five species. *Microlunatus ginsengisoli*, *Microlunatus aurantiacus*, and *Microlunatus panaciterrae* were all isolated from soil (An et al., 2008; Cui et al., 2007; Wang et al., 2008). While *Microlunatus phosphovorus* is slow growing with a doubling time of 13 h, two species of these isolates from soil, i.e., *Microlunatus ginsengisoli* and *Microlunatus panaciterrae*, can grow quickly on R2A medium. *Microlunatus soli* and *Microlunatus parietis* were isolated from the spawn of the mushroom *Agaricus brasiliensis* and an indoor wall, respectively. They are both oxidase-positive species like *Microlunatus panaciterrae*. *Microlunatus soli* contains unsaturated fatty acids, e.g., $C_{18:1}$ ω7*c*, that are rarely found in any other *Microlunatus* species.

The peptidoglycan structure of the genus *Microlunatus* was determined for *Microlunatus phosphovorus* (Schumann et al., 1997) and *Microlunatus soli* (Kämpfer et al., 2010b) and reveals that the interpeptide bridge is a single glycine and that position 1 of the peptide subunit is substituted by glycine. The structure is designated A3γ′ according to the classification of Schleifer and Kandler (1972).

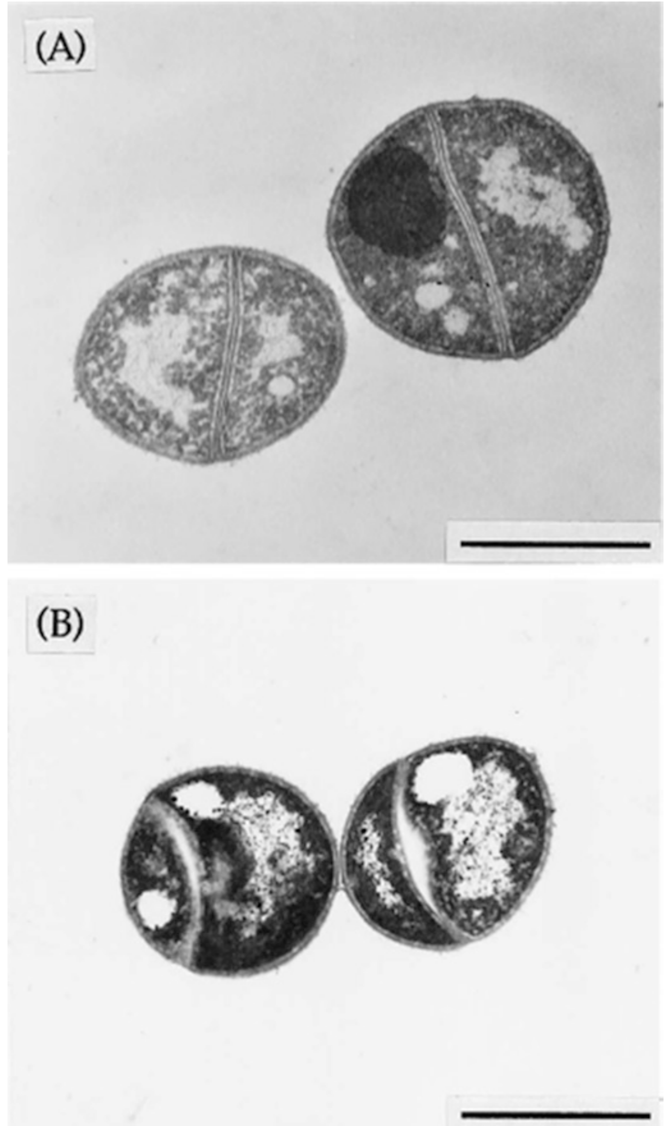

FIGURE 241. Transmission electron micrographs of thin sections of *Microlunatus phosphovorus* showing cell-wall structure in the middle of spherical cells and accumulation of polyphoisphate. (A) Cells harvested at the exponential phase. (B) Cells harvested at the stationary phase showing intracellular storage of polyphosphate (clear areas). Bars = 1 μm. (Reproduced with permission from Nakamura et al., 1995. Int. J. Syst. Bacteriol. *45*: 17–22.)

Although these six species of the genus *Microlunatus* have almost similar phenotypic characteristics as mentioned in the genus description above, they can be differentiated by comparing oxidase activity, nitrate reduction under anaerobic conditions, phosphate accumulation, and/or nutritional profiles (Table 210).

Enrichment and isolation procedures

For isolation of *Microlunatus phosphovorus*, a slow-growing bacterium inhabiting activated sludge, a relatively oligotrophic medium is used. Such an oligotrophic medium inhibits fast-growing bacteria from predominating. The isolation medium contains the following ingredients (per liter): 0.5 g of glucose, 0.5 g of peptone, 0.5 g of monosodium glutamate, 0.5 g of yeast

TABLE 210. Characteristics differentiating species of the genus *Microlunatus*[a,b]

Characteristics	*M. phosphovorus*	*M. aurantiacus*	*M. ginsengisoli*	*M. panaciterrae*	*M. parietis*	*M. soli*
Morphology	Cocci, occasionally. clusters	Cocci	Cocci	Cocci	Cocci to rods	Cocci
Cell diameter (μm)	0.8–2.0	0.9–1.3	0.5–0.8	0.3–0.7	nd	1.0–1.5
Habitat	Activated sludge	Soil	Soil	Soil	Indoor wall	Spawn of a mushroom
DNA G+C content (mol%)	67.9	70.9	69.8	65.1	nt	nt
Oxidase	w	−	−	+	+	+
Nitrate reduction under anaerobic conditions	+	−	−	nt	nt	nt
Phosphate uptake	+	w	−	−	nt	nt
Utilization of:						
Acetate	w	w	w	−	−	+
N-Acetyl-D-glucosamine	+	+	+	−	w	+
Adonitol	+	−	+	−	+	+
p-Arbutin	+	−	+	−	−	+
D-Cellobiose	+	+	+	−	+	+
D-Fructose	+	+	+	−	+	+
D-Galactose	+	w	+	−	+	+
myo-Inositol	+	+	+	−	+	+
L-Malate	+	+	w	−	−	w
D-Mannitol	+	+	+	−	+	+
Propionate	−	−	−	−	−	+
Sucrose	−	+	+	+	+	+
Salicin	+	−	+	+	−	+
D-Trehalose	+	+	+	−	+	+
D-Xylose	+	+	+	−	+	+

[a]Symbols: +, positive; −, negative; w, weakly positive, nt, not tested.

[b]Data from An et al. (2008) and Kämpfer et al. (2010a, 2010b).

extract, 0.44 g of KH_2PO_4, 0.1 g of $(NH_4)_2SO_4$, and 0.1 g of $MgSO_4 \cdot 7H_2O$ (pH adjusted to 7.0 with NaOH). Activated sludge (obtained from the wastewater treatment process with alternating anaerobic and aerobic conditions that exhibits high phosphate removal activity) is gently dispersed with an ultrasonicator and streaked on the isolation medium solidified with 1.5% agar. Circular, smooth, convex, and buff colonies of *Microlunatus phosphovorus* emerge on an agar plate after a few weeks of incubation at 25°C. The same liquid medium is used to enrich *Microlunatus phosphovorus* and is incubated at 25°C with gentle shaking.

The isolation procedure of *Microlunatus aurantiacus* from a soil sample includes an additional pre-incubation. Prior to spreading on agar plates, a soil sample dried at room temperature is suspended in a phosphate buffer solution (pH 7.0) containing 0.1% sodium cholate and incubated for 1 h at 45°C with vigorous shaking in order to eliminate fast-growing bacteria and disperse soil aggregates. The glycerol-asparagine based agar medium (ISP5 medium; Shirling and Gottlieb, 1966) is used as the isolation medium, and inoculated agar plates are incubated at 28°C for 21–30 d. To enrich this species, the modified ISP2 agar medium containing the following ingredients (per liter) is used: 4 g of glucose, 4 g of yeast extract, 5 g of malt extract, and a vitamin/amino acid mixture (1 mg of vitamin B_1, 1 mg of vitamin B_2, 1 mg of vitamin B_6, 1 mg of biotin, 1 mg of nicotinic acid, 1 mg of phenylalanine, 0.3 g of alanine); pH 7.2.

To isolate *Microlunatus ginsengisoli* and *Microlunatus panaciterrae*, MR2A agar medium is used. The MR2A agar medium is composed of (per liter): 0.25 g of tryptone, 0.25 g of peptone, 0.25 g of yeast extract, 0.125 g of malt extract, 0.125 g of beef extract, 0.25 g of Casamino acids, 0.25 g of soytone, 0.5 g of glucose, 0.3 g of soluble starch, 0.2 g of xylan, 0.3 g of sodium pyruvate, 0.3 g of K_2HPO_4, 0.05 g of $MgSO_4$, 0.05 g of $CaCl_2$, and 15 g of agar. These species can be maintained using standard R2A agar and nutrient agar as well as the modified R2A agar at 30°C.

Microlunatus soli and *Microlunatus parietis* can be cultured on R2A agar and nutrient agar at 30°C.

Maintenance procedures

A liquid culture of the type species, *Microlunatus phosphovorus*, retains viability for several months at room temperature in the dark. Long-term preservation at −80°C is possible in the presence of 10% (w/v) glycerol. Freeze-drying is also available with a suitable protective matrix.

Microlunatus ginsengisoli, *Microlunatus aurantiacus*, and *Microlunatus panaciterrae* can be preserved at −70°C in a 20% (w/v) glycerol suspension. Long-term preservation of *Microlunatus soli* and *Microlunatus parietis* is feasible by storage in 20% (v/v) glycerol stock and by lyophilization.

Differentiation of the genus *Microlunatus* from other genera

The genus *Microlunatus* can be differentiated clearly from other genera within the family *Propionibacteriaceae* by 16S rRNA gene sequence analysis (Figure 242). All species belonging to the genus *Microlunatus* are closely related to each other (the

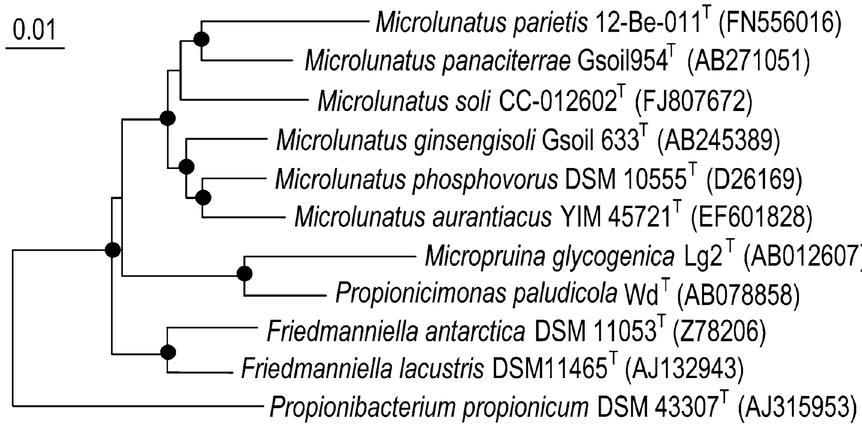

FIGURE 242. Phylogenetic relationships of the species of the genus *Microlunatus* based on 16S rRNA gene sequences constructed using the neighbor joining method. The EMBL/GenBank accession number of each sequence is indicated in parentheses. A bootstrap value (1000 replications) greater than 60% is shown as a closed circle at each branch point. The scale bar shows substitutions per 100 nucleotide positions.

sequence similarities among them are more than ~96%) and form a coherent cluster in the phylogenetic tree.

The related genera are *Micropruina*, *Propionicimonas*, and *Friedmanniella*, but all the members in the genus *Microlunatus* are phylogenetically distant from any species belonging to these related genera. All species in the genus *Microlunatus* and the related genera share the following common features in their phenotype: all are Gram-stain-positive, nonsporeforming, nonmotile cocci; contain $C_{15:0}$ anteiso as a main fatty acid component; and have menaquinone-9(H_4) as the major quinone. However, they can be differentiated from each other by comprehensive comparison of phenotypic characteristics such as their peptidoglycan types, nutritional profiles, and genomic G+C content.

Taxonomic comments

The type species of the genus, *Microlunatus phosphovorus*, was first described in 1995 with a proposal of the genus *Microlunatus* (Nakamura et al., 1995). The species was found in activated sludge in the wastewater treatment process. Since the first description, no species was newly proposed in this genus for more than ten years. From 2007 to 2008, three new bacteria belonging to the genus *Microlunatus* were found and proposed as new species. These new species, *Microlunatus ginsengisoli, Microlunatus aurantiacus*, and *Microlunatus panaciterrae*, were all isolated from soil, revealing that *Microlunatus* species inhabit not only the hydrosphere but also the lithosphere (An et al., 2008; Cui et al., 2007; Wang et al., 2008). In addition to these species, *Microlunatus soli* and *Microlunatus parietis* were newly isolated from the spawn of an edible mushroom, *Agaricus brasiliensis*, and an indoor wall, respectively. They were classified in the genus *Microlunatus* in 2010 (Kämpfer et al., 2010a, 2010b). At present, the genus *Microlunatus* consists of the following six species: *Microlunatus phosphovorus*, *Microlunatus aurantiacus*, *Microlunatus ginsengisoli*, *Microlunatus panaciterrae*, *Microlunatus parietis*, and *Microlunatus soli.*

List of species of the genus *Microlunatus*

1. **Microlunatus phosphovorus** Nakamura, Hiraishi, Yoshimi, Kawaharazaki, Masuda and Kamagata 1995, 21[VP]

phos.pho′vo.rus. L. n. *phosphorus* (from Gr. n. *phōsphoros* the light-bringer), the morning-star and, in chemistry, phosphorus; N.L. adj. *vorus* devouring; N.L. masc. adj. *phosphovorus* intended to mean phosphorus-accumulating microorganism.

The cells have a diameter of 0.8–2.0 µm and occur singly, in pairs, and occasionally in clusters. Often have a cell-wall structure in the middle of the cell and are hemispherical, especially at the stationary growth phase. The doubling time is about 13 h in a liquid medium. The colonies are circular (diameters, 0.5–1 mm), smooth, convex, and cream colored at the early stage of growth. After 10–14 d of incubation, the colonies are 1–2 mm in diameter and yellowish. Oxidase is positive but weak. The cells reduce nitrate to nitrite but do not reduce nitrite to nitrogen. The species has high phosphate-accumulating activity in the absence of any carbon substrate in the medium when it is exposed to exogenous phosphate under aerobic conditions (an intracellular phosphorus content sometimes increases more than 10% on a dry cell weight). The cells utilize glucose, mannose, galactose, xylose, arabinose, sucrose, maltose, cellobiose, trehalose, and melibiose, but not lactose. Starch is utilized, but glycogen is not utilized. Sugar alcohols such as inositol, dulcitol, and mannitol are utilized. Alcohols like methanol, ethanol, propanol, and glycerol are not utilized. Pyruvate is utilized, and acetate is utilized slowly. Growth occurs at 5–35°C with an optimum growth temperature of 25°C. The optimum pH is 7.0.

Source: sludge operating under alternating anaerobic and aerobic conditions.

DNA G+C content (mol%): 67.9 (HPLC).

Type strain: NM-1, ATCC 700054, CIP 104466, DSM 10555, HAMBI 2303, JCM 9379, NBRC 101784, VKM Ac-1990.

Sequence accession no. (16S rRNA gene): D26169, Z78207.

2. **Microlunatus aurantiacus** Wang, Cai, Zhi, Zhang, Tang, Xu, Cui and Li 2008, 1875[VP]

au.ran.ti.a′cus. N.L. masc. adj. *aurantiacus* orange-colored, referring to the orange color of the colonies.

Cells are cocci, 0.9–1.3 μm in diameter. Colonies are very small (~0.5–2.0 mm in diameter after incubation for 4 d on ISP2 agar medium at 28°C), smooth, circular, convex, and orange–yellow. Growth occurs at 15–37°C and pH 7.0–7.5. Growth occurs in the absence of NaCl. Oxidase-negative. Nitrate is reduced under anaerobic conditions. Cells show a weak phosphate-accumulating activity. Acid is produced from amygdalin, fructose, D-glucose, lactose, maltose, mannose, melibiose, raffinose, sucrose, trehalose, xylitol, and L-xylose. Utilizes arbutin, fructose, D-adonitol, esculin, alanine, amygdalin, fumarate, inulin, D-mannose, methyl α-D-galactoside, methyl β-D-galactoside, methyl α-D-glucoside, raffinose, D-ribose, salicin, D-sorbitol, starch, sucrose, trehalose, Tween 20, urea, xylitol, DL-xylose, adenine, asparagine, arginine, glutamate, histidine, hypoxanthine, threonine, tyrosine, and xanthine as sole carbon sources. The phospholipid pattern consists of diphosphatidylglycerol, phosphatidylglycerol, and phosphatidylinositol.

Source: a soil sample collected from the rhizosphere of *Taxus chinensis* in Yunnan Province, China.

DNA G+C content (mol%): 70.9 (HPLC).

Type strain: YIM 45721, CCTCC AB 206067, DSM 18424.

Sequence accession no. (16S rRNA gene): EF601828.

3. **Microlunatus ginsengisoli** Cui, Im, Yin, Yang and Lee 2007, 715[VP]

gin.seng.i.so′li. N.L. n. *ginsengum* ginseng; L. n. *solum* soil; N.L. gen. n. *ginsengisoli* of soil of a ginseng field, the source of the type strain.

Cells are 0.5–0.8 μm in diameter. Colonies are very small, smooth, circular, non-glossy, yellowish, and convex. Grows well at 20–30°C and at pH 5.5–8.5. Grows on nutrient agar but not MacConkey agar. Growth on MR2A agar occurs both in the absence of NaCl and in the presence of 4.0% (w/v) NaCl. No growth at 6% NaCl and higher. Oxidase-negative. Nitrate, as a nitrogen source, is reduced under aerobic conditions, but not under anaerobic conditions. β-Galactosidase and gelatinase activities and the Voges–Proskauer reaction are positive (API 20E). The following compounds are utilized as sole carbon sources: D-glucose, L-rhamnose, D-fructose, D-lyxose, D-ribose, L-xylose, propionate, valerate, fumarate, salicin, lactate, malate, tartrate, sucrose, D-trehalose, D-raffinose, gluconate, D-adonitol, D-sorbitol, xylitol, amygdalin, inulin, dextran, alanine, asparagine, aspartate, histidine, phenylalanine, praline, and tyrosine.

Source: soil from a ginseng field in Pocheon province, South Korea.

DNA G+C content (mol%): 69.8 (HPLC).

Type strain: Gsoil 633, DSM 17942, JCM15306, KCTC 13940.

Sequence accession no. (16S rRNA gene): AB245389.

4. **Microlunatus panaciterrae** An, Im and Yoon 2008, 2736[VP]

pa.na.ci.ter′ra.e. N.L. n. *Panax -acis* scientific name for ginseng; L. n. *terra* soil; N.L. gen. n. *panaciterrae* of soil of a ginseng field.

Cells are cocci, 0.3–0.7 μm in diameter. Colonies grown on R2A agar for 5 d are smooth, circular, non-glossy, yellowish, convex, and 1–2 mm in diameter. Grows well at 20–30°C and at pH 5.0–9.0. Grows on nutrient agar, but not on MacConkey agar or trypticase soy agar. Growth on R2A agar occurs both in the absence of NaCl and in the presence of 5.0% (w/v) NaCl but not at 6.0% NaCl. Oxidase-positive. Anaerobic growth does not occur. Nitrate is not reduced under anaerobic condition. Acid is produced from amygdalin, L-arabinose, D-glucose, melibiose, and L-rhamnose. β-Galactosidase, β-glucosidase, gelatinase, and the Voges–Proskauer reaction are positive (API20E). The following compounds are utilized as sole carbon sources: D-arabinose, citrate, D-fucose, L-fucose, D-glucose, lactate, maltose, D-mannose, melibiose, L-rhamnose, D-ribose, salicin, D-sorbitol, and sucrose. Starch is degraded. The polar lipids detected are phosphatidylethanolamine, diphosphatidylglycerol, and phosphatidylglycerol.

Source: soil from a ginseng field in Pocheon Province, South Korea.

DNA G+C content (mol%): 65.1 (HPLC).

Type strain: Gsoil 954, DSM 18662, KCTC 13058.

Sequence accession no. (16S rRNA gene): AB271051.

5. **Microlunatus parietis** Kämpfer, Schäfer, Lodders and Martin 2010a, 2422[VP]

pa.ri′e.tis. L. gen. n. *parietis* of the wall of a house.

Cells are rod-shaped and cocci. Good growth occurs on R2A agar, tryptone soy agar, M79 (modified Letheen Broth) agar, and nutrient agar at 25–30°C. Utilizes arbutin, cellobiose, D-fructose, D-galactose, sucrose, salicin, trehalose, D-xylose, adonitol, *myo*-inositol, maltitol, and D-mannitol. Weakly utilizes *N*-acetyl-D-glucosamine. The polar lipid profile mainly consists of diphosphatidylglycerol, phosphatidylglycerol, phosphatidylinositol, two unknown phospholipids, and one unknown glycolipid.

Source: an indoor wall in Berlin, Germany.

DNA G+C content (mol%): not determined.

Type strain: 12-Be-011, CCM 7636, DSM 22083.

Sequence accession no. (16S rRNA gene): FN556016.

6. **Microlunatus soli** Kämpfer, Young, Busse, Chu, Schumann, Arun, Shen and Rekha 2010b, 827[VP]

so′li. L. gen. n. *soli* of soil, the source of the type strain.

Cocci, 1.0–1.5 μm in diameter. Good growth occurs on R2A agar and nutrient agar at 25–30°C. Oxidase-positive. Utilizes *N*-acetyl-D-glucosamine, arbutin, cellobiose, D-fructose, D-galactose, sucrose, salicin, trehalose, D-xylose, adonitol, *myo*-inositol, maltitol, D-mannitol, acetate, and propionate. Weakly utilizes L-malate, L-histidine, L-proline, and L-serine. Cell-wall peptidoglycan is A3γ′ containing LL-diaminopimelic acid and glycine. The polyamine pattern is composed of spermidine and spermine as major compounds. Major polar lipids are phosphatidylglycerol and an unknown phospholipid followed by diphosphatidylglycerol, some unknown phospholipids, and an unknown glycolipid. A significant amount of an unsaturated fatty acid, $C_{18:1}$ ω7*c*, is the major cellular fatty acid. A small amount of $C_{14:0}$ 2-OH is also found.

Source: spawn of the edible mushroom *Agaricus brasiliensis*.

DNA G+C content (mol%): not determined.

Type strain: CC-12602, CCM7685, DSM21800.

Sequence accession no. (16S rRNA gene): FJ807672.

Genus VIII. **Micropruina** Shintani, Liu, Hanada, Kamagata, Miyaoka, Suzuki and Nakamura, 2000, 205[VP]

Lyudmila I. Evtushenko

Mi.cro.prui′na. Gr. adj. *mikros* small, fine; L. fem. n. *pruina* hoarfrost; N.L. fem. n. *Micropruina* fine hoarfrost.

Spherical cells, 0.5–2.2 µm in diameter (mostly about 1.0 µm) that are arranged in pairs, short chains, or clusters. Nonmotile. Nonsporeforming. **Gram-stain-positive**. Capsules are produced. **Chemo-organotroph, with a respiratory type of metabolism. Catalase and oxidase activities are positive**. Nitrate is used as an electron acceptor under anaerobic conditions; no production of nitrogen gas. The only known species, *Micropruina glycogenica*, is a slowly growing organism. Carbohydrates, sugar alcohols, carbonic acids, and amino acids are utilized as carbon sources; oxidative acid production occurs. **Capable of accumulating cellular glycogen**. Mesophilic and neutrophilic; growth occurs at 20–35°C and at pH 6–8. The optimum temperature and pH are 30°C and 7.0.

The cell-wall peptidoglycan contains *meso*-diaminopimelic acid (*meso*-A_2pm). Menaquinones are the only detected respiratory quinines; the tetrahydrogenated menaquinone with nine isoprene units, **MK-9(H_4), is the predominant** component. The major cellular fatty acids are $C_{15:0}$ anteiso, $C_{14:0}$ iso, $C_{16:0}$ iso, and $C_{16:0}$. No tuberculostearic or other 10-methyl branched acids are detected. Mycolic acids are absent.

The type species was isolated from an anaerobic-aerobic sequential batch biofilter reactor. No evidence of pathogenic properties.

DNA G+C content (mol%): 70.5.

Type species: **Micropruina glycogenica** Shintani, Liu, Hanada, Kamagata, Miyaoka, Suzuki and Nakamura 2000, 206[VP].

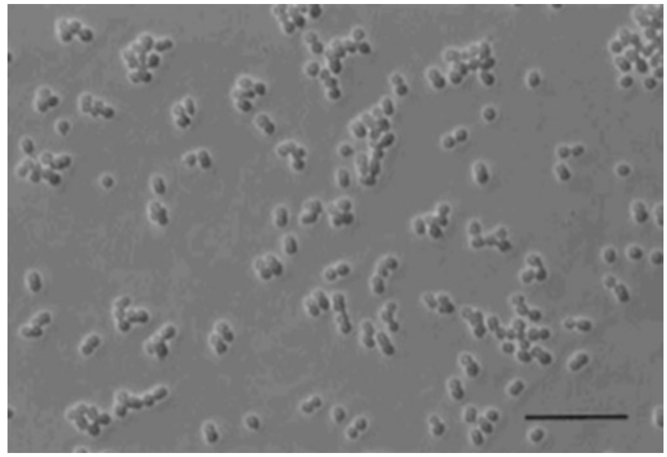

FIGURE 243. Phase-contrast photomicrograph of *Micropruina glycogenica* cells grown in NM-1 medium at 30°C. Bar = 10 µm. (Reproduced with permission from Shintani et al., 2000; Int. J. Syst. Evol. Microbiol. *50*: 201–207).

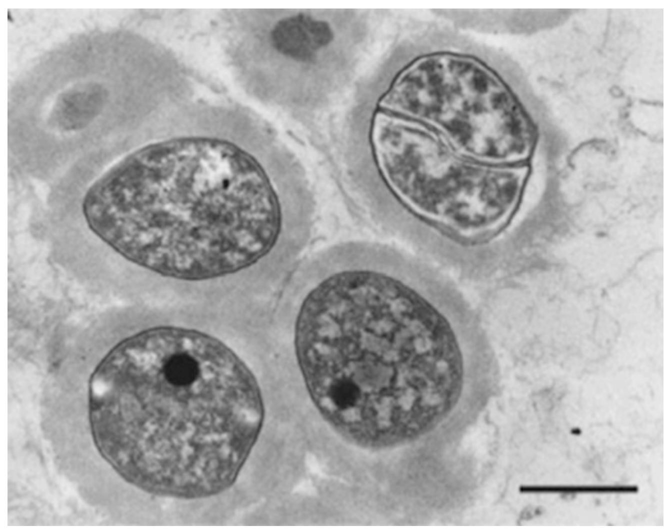

FIGURE 244. Transmission electron micrograph of *Micropruina glycogenica* cells with surrounding capsules. Cells were grown in NM-1 medium with a gentle shaking at 30°C. Bar = 1 µm. (Reproduced with permission from Shintani et al., 2000; Int. J. Syst. Evol. Microbiol. *50*: 201–207).

Further descriptive information

The growing cells divide at the middle by a septum to form pairs of cells which may be arranged in short chains, packets, or clusters (Figure 243 and Figure 244). Cells are individually surrounded by a capsule. The cell-wall structure is typical of Gram-stain-positive bacteria; Gram-stain-positive.

Little information is available on the habitats and ecology of organisms of the genus *Micropruina*. The type strain of *Micropruina glycogenica* was isolated from an anaerobic-aerobic sequential batch biofilter reactor exhibiting a biological phosphorus removal activity. However, neither orthophosphate uptake activity nor accumulation of intracellular polyphosphate has been detected in this bacterium by Neisser staining. Two organisms closely related to *Micropruina glycogenica* (>98% 16S rRNA gene sequence similarity) have been revealed in activated sludge and in sea water (GenBank accession numbers EU104262 and FJ545597, respectively).

Enrichment and isolation procedures

The *Microlunatus* agar medium (NM-1) containing glucose (0.5 g), peptone (0.5 g), monosodium glutamate (0.5 g), yeast extract (0.5 g), KH_2PO_4 (0.44 g), $(NH_4)_2SO_4$ (0.1 g), $MgSO_4 \cdot 7H_2O$ (0.1 g), agar (15 g), and 1 liter of distilled water (final pH, 7.0) (Nakamura et al., 1995) was used to isolate the type strain as described by Liu et al. (1997).

Maintenance procedures

Long-term conservation is achieved by freeze-drying and in liquid nitrogen.

Differentiation of the genus *Micropruina* from related genera

Tables 206, 208, and 212 lists characteristics differentiating *Micropruina* from the other genera of the family *Propionibacteriaceae*. Coccoid cells, along with the positive catalase and oxidase reactions and the presence of the predominant menaquinone MK-9(H_4), are the most salient characteristics that differentiate *Micropruina* from the phylogenetically closest genera *Propionicicella* and *Propionicimonas*. The extent of growth with (or without)

oxygen may also be used for the purposes of delineating *Micropruina* from the facultativelly anaerobic *Propionicicella* and *Propionicimonas*. The *meso*-A_2pm isomer in the cell-wall peptidoglycan readily differentiates *Micropruina* from the phylogenetically close genera of coccoid bacteria which have LL-A_2pm as the diagnostic diamino acid.

Taxonomic comments

On the basis of 16S rRNA gene analysis, *Micropruina glycogenica* was originally shown to form a separate phylogenetic cluster with the genera *Microlunatus* and *Friedmanniella*, which was loosely associated with a cluster encompassing *Nocardioides* and closely related genera of the family *Nocardioidaceae* (Shintani et al., 2000). Further phylogenetic analysis with the extended selection of related sequences led Stackebrandt and Schaal (2006a) to the conclusion that the genus *Micropruina*, along with *Microlunatus* and *Friedmanniella*, should be placed in the family *Propionibacteriaceae*.

Acknowledgements

The author was supported by the program MCB of the Russian Academy of Sciences.

List of species of the genus *Micropruina*

1. **Micropruina glycogenica** Shintani, Liu, Hanada, Kamagata, Miyaoka, Suzuki and Nakamura 2000, 206[VP]

gly.co.ge.ni′ca. N.L. n. *glycogenum* glycogen; L. fem. suff. *-ica* suffix used with the sense of pertaining to; N.L. fem. adj. *glycogenica* referring to the ability to accumulate glycogen.

Morphological and general phenotypic characteristics of the species are the same as described for the genus. Punctuated nonpigmented colonies are formed after 2–3 weeks on NM-1 agar medium. Large amount (up to 8.4% dry wt) of intracellular glycogen is accumulated as a storage material under aerobic and anaerobic conditions.

Aerobic growth in the liquid NM-1 medium is very slow. The doubling time is approximately 12.6 h at 30°C (the optimum growth temperature). The temperature range for growth is 20–35°C; growth is not observed below 16°C or above 37°C. Tolerates NaCl up to 3.0% (w/v). The pH range for growth was 6–8, with no growth at pH 5 and 9.

Reduces nitrate to nitrite under anaerobic conditions, but formation of nitrogen gas is not observable. The following carbon sources are utilized aerobically as determined using the Biolog system: glucose, arabinose, cellobiose, galactose, lactose, maltose, mannose, melibiose, sucrose, xylose, inositol, mannitol, acetate, propionate, pyruvate, succinate, alanine, arginine, and histidine. Trehalose, dulcitol, ethanol, glycerol, methanol, propanol, malate, asparagine, glutamate, and glutamine are not utilized. Starch and glycogen are hydrolyzed.

The cellular fatty acids of the type strain include $C_{15:0}$ anteiso (37.1%), $C_{16:0}$ (12.9%), $C_{14:0}$ iso (14.1%), $C_{16:0}$ iso (10.8%), $C_{15:0}$ iso (5.9%), $C_{18:0}$ (4.6%), $C_{14:0}$ (3.7%), and $C_{15:0}$ iso (2.2%).

Source: an anaerobic-aerobic sequential batch biofilter reactor.

DNA G+C content (mol%): 70.5 (HPLC).

Type strain: strain Lg2, DSM 15918, JCM 10248.

Sequence accession no. (16S rRNA gene): AB012607.

Genus IX. **Propionicicella** Bae, Moe, Yan, Tiago, da Costa and Rainey 2006a, 2026[VP]

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Pro.pi.o.ni.ci.cel′la. N.L. n. *acidum propionicum* propionic acid; L. fem. n. *cella* a storeroom, chamber, and in biology, a cell; N.L. fem. n. *Propionicicella* propionic-acid-producing cells.

Nonsporeforming rods, (0.5 × 1.0–2.5 µm). **Gram-stain-positive**. Nonmotile. **Facultative anaerobe**. Temperature range for growth 15–37°C, pH range 4.5–8.5, NaCl tolerance 0–4%. The peptidoglycan type is A1γ with *meso*-diaminopimelic (*meso*-DAP) in the cell wall. MK-9 is the major menaquinone. Propionate and acetate are produced when grown anaerobically with glucose.

DNA G+C content (mol%): 69.9.

Type species: **Propionicicella superfundia** Bae, Moe, Yan, Tiago, da Costa and Rainey 2006a, 2026[VP].

Further descriptive information

Major fatty acids are $C_{15:0}$ (54.3%), $C_{15:0}$ (16.3%), $C_{16:0}$ iso (12.8%), $C_{17:0}$ anteiso (5.4%), $C_{14:0}$ iso (5.3%), $C_{17:0}$ (2.9%) and $C_{16:0}$ (1.1%).

Phylogenetic analysis of the 16S rRNA gene suggests that the genus is affilitiated with the family *Propionibacteriaceae*. The closest phylogenetic neighbor is *Propionicimonas* sp. F6 (97.3% sequence similarity, accession AY570689), which was isolated from production waters of a low-temperature, biodegraded oil reservoir (Grabowski et al., 2005). Nearest type strains are *Propionicimonas paludicola* (accession no. AB078858; Akasaka et al., 2003b) and *Micropruina glycogenica* (accession no. AB012607; Shintani et al., 2000), 97.5% and 95.4%, respectively. An environmental clone with high 16S rRNA gene sequence similarity have been detected colonizing the gut epithelium (97.5%, accession no. AB198497) (Nakajima et al., 2005).

Enrichment and isolation procedures

Strain BL-10[T] was isolated from groundwater samples collected from well W-1024-1 located in the dense non-aqueous-phase liquid (DNAPL) source zone at the Brooklawn portion of the Petro-Processors of Louisiana, Inc. (PPI) EPA Superfund Site, located approximately 10 miles north of Baton Rouge, LA (USA). Sterile, 1-l glass sample collection bottles were filled with groundwater, leaving little or no headspace, and placed on ice during transport to the laboratory (approx. 1 h). Serial diluents (anaerobically in 100 mM potassium phosphate buffer, pH 7.0) were transferred onto Columbia Anaerobic Sheep Blood agar plates (CASB, BBL) and incubated anaerobically under an atmosphere of 90% N_2, 5% CO_2, and 5% H_2 at 30°C for up to 5 weeks.

Maintenance procedures

Strains are maintained anaerobically on CASB or PYG media (Akasaka et al., 2003b). Stock cultures are maintained as a 15% (v/v) glycerol and 5% (v/v) dimethylsulfoxide suspension at −80°C.

Differentiation of the genus *Propionicicella* from closely related genera

Facultative anaerobic growth, rod-shaped morphology, and predominant menaquinone MK-9 differentiate *Propionicicella* from phylogenetically related genera *Ponticoccus*, *Friedmanniella*, *Microlunatus*, and *Micropruina*, which are all aerobic cocci with MK-9(H_4) as the predominant menaquinone. Predominant menaquinones MK-9(H_4) and MK-10(H_4) distinguishes *Propionicimonas* from *Propionicicella*. *Ponticoccus*, *Friedmanniella*, *Microlunatus*, and *Micropruina* are catalase-positive, and *Propionicicella* is catalase-negative. *Ponticoccus* and *Micropruina* reduce nitrate and *Propionicicella* does not.

List of species of the genus *Propionicicella*

1. **Propionicicella superfundia** Bae, Moe, Yan, Tiago, da Costa and Rainey 2006a, 2026[VP]

   su.per.fun′di.a. L. prep. *super* above/on top; L. masc. n. *fundus* land owned by someone; L. adjectival ending *-ius -ia -ium* indicating the meaning of "belonging to"; N.L. fem. adj. *superfundia* referring to land designated as a US Environmental Protection Agency Superfund Site.

   Colonies are white, circular, convex, smooth and 2–3 mm in diameter on PYG agar. Growth occurs at mesophilic temperatures and pH 4.5 and 8.5, with optima at 30°C and pH 6.5. NaCl tolerance ≤ 4.0% (w/v). Catalase- and oxidase-negative. Nitrate is not reduced. Glucose fermentation products are formic acid, acetic acid, propionic acid, and succinic. Utilizes adonitol, erythritol, fructose, glucose, glycerin, lactate, maltose, mannitol, mannose, pyruvate, sorbitol, sucrose, and xylose. Does not utilize acetate, arabinose crystalline cellulose, dulcitol, ethanol, fucose, galactose, lactose, malate, meliobiose, methanol, raffinose, rhamnose, starch, succinate, and fumarate. Rapid ID 32A kits (bioMérieux) detects α-glucosidase, β-glucosidase, glycine arylamidase, histidine arylamidase, proline arylamidase, leucyl glycine arylamidase, phenylalanine arylamidase, leucine arylamidase, and alanine arylamidase, but not glutamyl glutamic acid arylamidase, alkaline phosphatase, α-fucosidase, glutaminc acid decarboxylase, arginine dehydrogenase, urease, α-galactosidase, and β-galactosidase. Fermentation occurs in the presence of 0–9.8 mM and 0–5.9 mM 1,2-dichloroethane and 1,1,2-trichloroethane, respectively.

   For all other characteristics, refer to the genus description.

   DNA G+C content (mol%): 69.9 (HPLC).

   Type strain: BL-10, ATCC BAA-1218, LMG 23096.

   Sequence accession no. (16S rRNA gene): DQ176646.

Genus X. **Propionicimonas** Akasaka, Ueki, Hanada, Kamagata and Ueki 2003b, 1996[VP]

Atsuko Ueki, Hiroshi Akasaka and Katuji Ueki

Pro.pio.ni.ci.mo′nas. N.L. n. *acidum propionicum* propionic acid; L. fem. n. *monas* a unit, monad; N.L. fem. n. *Propionicimonas* propionic acid-producing monad.

Irregular, often slightly curved rods. **Gram-stain-positive**. Endospores are not produced. Nonmotile. **Facultatively anaerobic**. **Chemo-organotrophic**. Negative for oxidase, catalase, and nitrate reduction. Mesophilic. **In the presence of an excess amount of cobalamin, acetate and propionate are produced anaerobically as major fermentation products from glucose**. Cell-wall peptidoglycan contains ***meso*-diaminopimelic acid (DAP)**, and major cellular fatty acids are **$C_{13:0}$, $C_{15:0}$ anteiso, and $C_{15:0}$**. The major respiratory quinones are **MK-9(H_4) and MK-10(H_4)**. Based on 16S rRNA gene sequences, species in the genera *Micropruina*, *Microlunatus*, and *Friedmanniella* in the *Nocardioidaceae* are the most closely related to those of *Propionicimonas*. Isolated from rice plant residue (straw and roots) in irrigated rice field soil in Japan.

DNA G+C content (mol%): 68.7 (HPLC).

Type species: **Propionicimonas paludicola** Akasaka, Ueki, Hanada, Kamagata and Ueki 2003b, 1996[VP].

Further descriptive information

The following information is based on the description of strains of *Propionicimonas paludicola*. Grows weakly under aerobic conditions; much better growth occurs anaerobically. Colonies are white and 2–3 mm in diameter after 2–3 d of anaerobic cultivation. Growth rates in PYG medium (Holdeman et al., 1977) are very slow for most strains, but growth, as well as propionate production, is stimulated significantly by the addition of cobalamin to the medium. Some strains do grow well in PYG medium without cobalamin and produce substantial amounts of acetate and propionate; however, their other phenotypic and phylogenetic characteristics are almost identical to those of strains that do require cobalamin. The cell-wall structure is typical of Gram-stain-positive bacteria. Intracellular storage compounds in the cells are shown as electron-translucent regions by electron microscopy.

Isolation procedures

The plant residue samples collected from irrigated rice field soil are washed with anoxic diluent, cut to pieces, and homogenized using a Waring blender under N_2 gas. The homogenized samples are diluted consecutively with anoxic diluent and inoculated into the medium by the anaerobic roll-tube method (Holdeman et al., 1977). Colonies formed in the roll tubes during incubation for 2 weeks are picked up and strains can be purified by repeating the roll-tube method (Akasaka et al., 2003a). Strains can also often be isolated from roots of living rice plants of the same rice field.

TABLE 211. Differential characteristics of the genus *Propionicimonas* and closely related genera[a,b]

Characteristic	*Propionicimonas*	*Micropruina*	*Microlunatus*	*Friedmanniella*
Source	Plant residue in paddy soil	Activated sludge reactor	Activated sludge reactor	Antarctic sandstone
Cell shape	Irregular rods	Cocci (single, pair, or packet)	Cocci (single or pair)	Cocci (arranged in packet)
Cell size (μm)	0.4–0.5 × 1.4–2.2	0.5–2.2	0.8–2.0	0.5–2.2
Color of colony	White	White	Cream	Orange
Optimum growth temperature (°C)	35	30	25–30	20–26
O_2 requirement	Facultative anaerobe	Aerobe	Aerobe	Aerobe
Oxidase	−	+	+	−
Catalase	−	+	+	+
Nitrate reduction	−	+	+	−
Acid production from glucose	+	+	+	−
DNA G+C content (mol%)	67.4–68.7	70.5	67.9	69–74
Major quinone	MK-9(H_4), MK-10(H_4)	MK-9(H_4)	MK-9(H_4)	MK-9(H_4)
Major cellular fatty acids	$C_{13:0}$, $C_{15:0}$ anteiso, $C_{15:0}$	$C_{14:0}$ iso, $C_{15:0}$ anteiso, $C_{16:0}$ iso, $C_{16:0}$	$C_{15:0}$ iso, $C_{15:0}$ anteiso	$C_{15:0}$ iso, $C_{15:0}$ anteiso
Peptidoglycan	*meso*-DAP	*meso*-DAP	LL-DAP	LL-DAP

[a]Symbols: +, >85% positive; -, 0–15% positive.

[b]Data from Shintani et al. (2000); Nakamura et al. (1995); Schumann et al. (1997); Maszenan et al. (1999a); Lawson et al. (2000c).

Maintenance procedures

The strains are cultivated anaerobically at 30°C using PY medium (Holdeman et al., 1977) as a basal medium with oxygen-free N_2/CO_2 (95:5) mixed gas as headspace closed with butyl-rubber stoppers. PY medium supplemented with (per litre) 0.25 g each of glucose, cellobiose, maltose, and soluble starch, as well as 50 ml plant residue extract (RE) and 15 g agar (pH 7.3) is used for maintenance in agar slants. Rice plant residue collected from irrigated rice field soil during the flooding period is autoclaved (120°C for 30 min) with a fivefold amount (wet weight basis) of deionized H_2O, and the supernatant obtained after centrifugation is used as RE for the provision of growth factors (Akasaka et al., 2003a). RE prepared as above contains 3–5 μg/l cobalamin (Akasaka et al., 2004). Cobalamin itself is not usually added to the medium for slant cultures to avoid excess growth. Anaerobic slant cultures kept at 4°C can remain viable for at least several months, or the slant cultures can be maintained at −70°C without transfer for more than 5 years.

Taxonomic comments

Based on 16S rRNA gene sequences, the species forms a cluster close to the genera *Micropruina* (Shintani et al., 2000), *Microlunatus* (Nakamura et al., 1995), and *Friedmanniella* (Schumann et al., 1997) in the *Actinobacteria*. The closest relative is *Micropruina glycogenica* (Shintani et al., 2000), with sequence similarity of 95.8%. The closest relative to the type strain of *Propionicimonas paludicola* is an environmental clone, SJA-181, which is derived from an anaerobic microbial consortium in a trichlorobenzene-transforming bioreactor (16S rRNA gene sequence similarity of 98.3%). *Propionicimonas paludicola* is only distantly related to the propionate-producing species *Propionibacterium propionicus* and *Propioniferax innocua* in the *Propionibacteriaceae*, with sequence similarities of 91.5–92.0% and 90.0%, respectively.

Differentiation of the genus *Propionicimonas* from other genera

Unlike *Propionicimonas paludicola*, all species in the closely related genera *Micropruina*, *Microlunatus*, and *Friedmanniella* have spherical cells, often arranged in packets. Related species are strictly aerobic and catalase-positive. Both *Micropruina* and *Microlunatus* species have oxidase and nitrate-reducing activities. Major cellular fatty acids of these relatives are $C_{14:0}$ iso, $C_{15:0}$ iso, $C_{16:0}$ iso, and $C_{16:0}$ in addition to $C_{15:0}$ anteiso. $C_{13:0}$ and $C_{15:0}$ fatty acids are absent or only present as minor components. All relatives also have MK-9(H_4) as a major respiratory quinone, but do not contain MK-10(H_4), which is found in *Propionicimonas paludicola* (Lawson et al., 2000c; Maszenan et al., 1999a; Nakamura et al., 1995; Schumann et al., 1997; Shintani et al., 2000). Among the close relatives, only *Micropruina* species has *meso*-DAP in the peptidoglycan of the cell wall (Shintani et al., 2000); species in other two genera have LL-DAP (Table 211).

List of species of the genus *Propionicimonas*

1. **Propionicimonas paludicola** Akasaka, Ueki, Hanada, Kamagata and Ueki 2003b, 1996

pa.lu.di′co.la. L. n. *palus -udis* a swamp, marsh; L. suff. *-cola* (from L. n. *incola*) inhabitant, dweller; N.L. masc. n. *paludicola* an inhabitant of swamps.

Cells are pleomorphic rods, frequently arranged in irregular V- or crescent-shapes, 1.8–2.0 × 0.4–0.5 μm. In the presence of excess amounts of cobalamin in the medium, all strains produce acetate and propionate in the molar ratio of 2:1, with small amounts of lactate and succinate, from

glucose; however, lactate production is predominant under cobalamin limitation for cobalamin-requiring strains. Optimal growth occurs at 35°C and pH 6.5. The species grows in the presence of up to 2.0% (w/v) NaCl. Acids are produced from arabinose, xylose, fructose, galactose, glucose, mannose, cellobiose, maltose, sucrose, trehalose, glycerol, and mannitol. Ribose and lactose are poorly utilized. Grows on pyruvate and lactate, and very weakly on malate, fumarate, and succinate. Does not utilize fucose, rhamnose, sorbose, melibiose, melezitose, raffinose, cellulose, glycogen, soluble starch, xylan, adonitol, dulcitol, erythritol, inositol, sorbitol, ethanol, methanol, or propanol. Other characteristics are as described for the genus.

Source: isolated from rice plant residue (straw and roots) in irrigated rice field soil in Japan.

DNA G+C content (mol%): 68.7 (HPLC).

Type strain: JCM 11033, DSM 15597.

Sequence accession no. (16S rRNA gene): AB078858.

Genus XI. **Propioniferax** Yokota, Tamura, Takeuchi, Weiss and Stackebrandt 1994, 581[VP]

Akira Yokota

Pro.pio.ni.fe′rax. N.L. n. *acidum propionicum* propionic acid; L. adj. *ferax* fertile; N.L. fem. n. *propioniferax* propionic acid-producing.

Cells are **Gram-stain-positive**, non-acid-fast, **nonmotile**, nonsporing, **pleomorphic rods**, appearing in clusters and V forms. **Facultatively anaerobic** but luxuriant growth occurs aerobically. Oxidase- and catalase-positive. Cell wall contains **LL-diaminopimelic acid**, arabinose, and mannose, but not galactose. Glycine forms the interpeptide bridge of peptidoglycan. Mycolic acids are not present. Major fatty acids are $C_{15:0}$ anteiso and $C_{15:0}$ iso. The major respiratory quinone is **MK-9(H_4)**.

DNA G+C content (mol%): 59–63 (T_m).

Type species: **Propioniferax innocua** Yokota, Tamura, Takeuchi, Weiss and Stackebrandt 1994, 581[VP].

Further descriptive information

Propionibacterium innocuum was proposed by Pitcher and Collins (1991) for a coryneform bacterium species containing LL-diaminopimelic acid (LL-A_2pm) in the cell wall. However, in contrast to other species of the genus *Propionibacterium* (Charfreitag et al., 1988; Cummins and Johnson, 1986), strains of *Propionibacterium innocuum* showed aerobic growth, did not require blood, serum, or Tween 80 for growth, and contained arabinose in cell-wall hydrolyzates. In addition, 16S rRNA analysis indicated that *Propionibacterium innocuum* was only remotely related to other members of the genus *Propionibacterium.* On the basis of physiological and chemotaxonomic characteristics, and a 16S rRNA gene sequence comparison, the species *Propionibacterium innocuum* was transferred from the genus *Propionibacterium* to a new genus, *Propioniferax*, as *Propioniferax innocua* by Yokota et al. (1994). The genus *Propioniferax* is a single species genus. Some characteristics in the original description of the genus by Yokota et al. (1994) were transferred to the species description. The genus *Propioniferax* is a member of the family *Propionibacteriaceae* within the order *Propionibacteriales* represented by the genus *Propionibacterium* Orla-Jensen 1909 (Figure 245).

Isolation and maintenance procedures

Strains of *Propioniferax innocua* originated from human skin, isolated from laboratories in Philadelphia (USA), Leiden (The Netherlands), and London (UK). Strains were routinely cultured aerobically on nutrient agar (Oxoid) at 37°C. Anaerobic growth on nutrient agar under an atmosphere of 95% H_2 and 5% CO_2 was much reduced. Cells grow well in CPY broth (casein peptone, 10 g; yeast extract, 5 g; and H_2O, 1 liter; pH 7.2) and tryptone soy broth (Difco) supplemented with 1% yeast extract.

Strains of the genus *Propioniferax* can be preserved by lyophilization suspended in 10% skim milk containing 1% monosodium glutamate, as well as by deep-freezing suspended in 10–20% glycerol solution at temperatures below −80°C.

Differentiation of the genus *Propioniferax* from other genera

The genus *Propioniferax* can be differentiated from closely related genera in the family *Propionibacteriaceae* based on chemotaxonomic and phylogenetic data, as shown in Table 212.

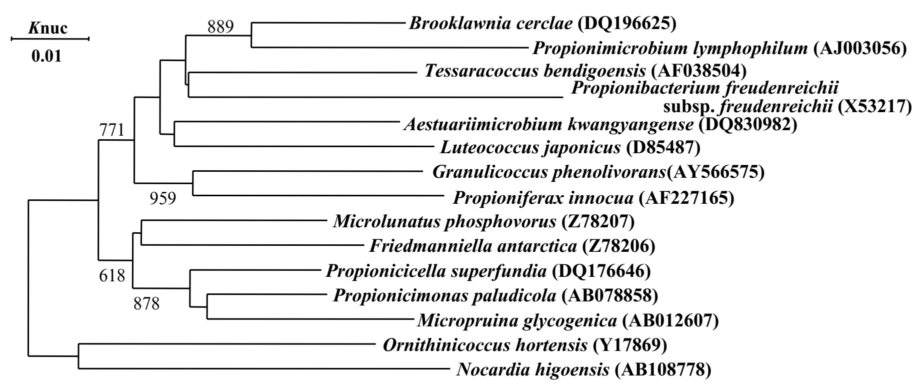

FIGURE 245. Phylogenetic relationships of the type strains of *Propioniferax* and named genera of the family *Propionibacteriaceae.* The neighbor-joining method was used for tree construction, 1000 bootstrap trees were generated and bootstrap values (>600; out of 1000 reiterations) are indicated at branch points.

TABLE 212. Differential characteristics of the genus *Propioniferax* and other genera of the family *Propionibacteriaceae*[a]

Characteristic	*Propioniferax*	*Propionibacterium*	*Aestuariimicrobium*	*Brooklawnia*	*Friedmanniella*	*Granulicoccus*	*Luteococcus*	*Microlunatus*	*Micropruina*	*Propionicicella*	*Propionicimonas*	*Propionimicrobium*	*Tessaracoccus*
O_2 requirement	Facultative anaerobes	Facultative anaerobes	Aerobes	Facultative anaerobes	Aerobes	Facultative anaerobes	Facultative anaerobes	Aerobes	Aerobes	Facultative anaerobes	Facultative anaerobes	Anaerobes	Facultative anaerobes
Cell morphology	Pleomorphic rods	Pleomorphic rods	Short rods or cocci	Pleomorphic rods	Cocci in packets	Cocci, singly and in pairs	Cocci, singly and in pairs	Cocci, singly and in pairs	Cocci	Rods	Irregular rods	Pleomorphic rods	Cocci, in tetrads
Cell size, μm	0.5–1.2	0.2–0.8	nk	nk	0.5–2.2	0.3–1.4	0.7–1.0	0.8–2.0	0.5–2.2	0.5–1.7	0.4–0.5 × 1.8–2.0	1.0–2.5	0.8–2.0
Isolation source(s)	Human epidermal surface	Human oral cavity, cervicovaginal secretion	Tidal flat	Chlorosolvent-contaminated groundwater	Sandstone of Antarctica	Phenol-degrading aerobic granules	Soil, water	Sewage treatment plant	Activated sludge reactor	Groundwater	Plant residue in paddy soil	Lymph nodes of patients	Sewage treatment plant
Growth temperature (°C):													
Optimum	37	35–37	30	37	9–25	30	26–28	25–30	nd	30	35	36–37	25
Range	10–40	30–37	4–40	10–40	22	15–37	12–38	5–35	20–30	15–37	10–40	nd	20–37
pH for growth:													
Optimum	7.0	nd	7.5–8.5	6.5	6.0–7.2	7.0	nd	7.0	nd	6.5	6.5	nd	7.5
Range	nd	nd	nd	4.5–8.0	nd	5.0–8.5	nd	5.0–9.0	6–8	4.5–8.5	4.5–7.5	nd	5.5–9.3
Presence of metachromatic granules	+, NK	nd	nd	nd	nd	+, Poly P	nd	+, Poly P	–	nd	nd	nd	+, Poly P
Oxidase	+	nd	–	–	–	–	+	+w	+	–	–	–	–
Catalase	+	–	+	+	+	+	+	+	+	–	–	v	+
Production of indole	–	–	nd	nd	nd	–	–	+	nd	nd	nd	–	–
Production of H_2S	–	+	nd	nd	+	–	–	nd	nd	nd	nd	nd	–
Major menaquinone(s)	MK-9(H_4)	MK-9(H_4)	MK-9(H_4)	MK-9(H_4)	MK-9(H_4)	MK-9(H_4), MK-8(H_4)	MK-9(H_4)	MK-9(H_4)	MK-9(H_4)	MK-9	MK-9(H_4), MK-10(H_4)	MK-9(H_4)	MK-9(H_4), MK-7(H_4)
A_2pm/murein type	LL-A_2pm/A3-γ	LL-A_2pm/A3-γ′	LL-A_2pm	*meso*-A_2pm/A1-γ	LL-A_2pm/A3-γ′	LL-A_2pm/A3-γ	LL-A_2pm/A3-γ	LL-A_2pm/A3-γ′	*meso*-A_2pm	*meso*-A_2pm	*meso*-A_2pm	Lysine/A4-α	LL-A_2pm/A3-γ′
Polar lipids[b]	PE, PG, PL, GL	nd	nd	nd	PG, DPG, PI, PL	PG, DPG	PG, DPG, PI, GL	PG, DPG, PI, GL	nd	nd	nd	nd	PG, DPG, PI, GL
Urease	+	–	–	nd	nd	+	–	+	nd	nd	nd	nd	–
Nitrate reduction	+	+	+	–	nd	–	–	+	+	–	–	v	+
DNA G+C content (mol%)	59–63	63–65	69	68	73	69	66–68	68	71	70	69	56	74

[a]Data for the various genera are from the following sources: *Aestuariimicrobium*, Jung et al. (2007); *Brooklawnia*, Bae et al. (2006b); *Friedmanniella*, Schumann et al. (1997); *Granulicoccus*, Maszenan et al. (2007); *Luteococcus*, Tamura et al. (1994); *Microlunatus*, Nakamura et al. (1995); *Micropruina*, Shintani et al. (2000); *Propionicicella*, Bae et al. (2006c); *Propionicimonas*, Akasaka et al. (2003b); *Propionimicrobium*, Stackebrandt et al. (2002); *Tessaracoccus*, Maszenan et al. (1999b). Symbols: +, positive; –, negative; +w, weak positive; v, variable; nd, not determined; NK, not known.

[b]DPG, Diphosphatidylglycerol; GL, unknown glycolipid; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; PI, phosphatidylinositol; PL, unknown phospholipid.

List of species of the genus *Propioniferax*

1. **Propioniferax innocua** (Pitcher and Collins 1992) Yokota, Tamura, Takeuchi, Weiss and Stackebrandt 1994, 581^VP (*Propionibacterium innocuum* Pitcher and Collins 1992, 327)

in.noc′u.a. L. fem. adj. *innocua* harmless.

Cells are Gram-stain-positive, non-acid-fast, nonmotile, nonsporing, pleomorphic rods appearing in clusters and V forms. Colonies are white, shining, and convex to domed. The optimum growth temperature is approximately 37°C. Grows at 10 and 40°C. Facultatively anaerobic, but substantial growth occurs aerobically (colonies are approximately 0.5–3 mm in diameter after growth on nutrient or horse blood agar at 37°C for 2 d). Catalase- and oxidase-positive. The principal carboxylic acid produced from glucose is propionic acid. Glucose, sucrose, maltose, trehalose, fructose, mannose, and glycerol are fermented. Inositol, arabinose, adonitol, salicin, erythritol, mannitol, cellobiose, dulcitol, raffinose, lactose, sorbose, sorbitol, and rhamnose are not utilized, and galactose utilization is variable. Gelatin is hydrolyzed. Growth occurs in the presence of 7.5% NaCl, but not in 10% NaC1. Reduces nitrate. Hydrolyzes urea and starch. Growth is not stimulated by Tween 80, but Tween 80 is hydrolyzed. The following tests are negative: arginine dihydrolase, esculin hydrolysis, glutamate oxidation, arginine, lysine and ornithine decarboxylase, phenylalanine deaminase, citrate and malonate utilization, indole, H_2S, and decomposition of tyrosine and xanthine.

The cell wall contains LL-A_2pm, arabinose, and mannose, but not galactose. Major fatty acids are $C_{15:0}$ anteiso and $C_{15:0}$ iso. The major respiratory quinone is MK-9(H_4).

Source: human epidermal surface.

DNA G+C content (mol%): 59 to 63 (T_m).

Type strain: strain L60, ATCC 49929, CCUG 33480, DSM 8251, JCM 13395, LMG 16732, NCTC 11082.

Sequence accession no. (16S rRNA gene): AF227165.

Genus XII. **Propionimicrobium** Stackebrandt, Schumann, Schaal and Weiss 2002, 1926^VP

Erko Stackebrandt

Pro.pio.ni.mi.cro′bi.um. N.L. *acidum propionicum* propionic acid; N.L. neut. n. *microbium* (from Gr. adj. *mikros* small and Gr. n. *bios* life) a microbe; N.L. neut. n. *Propionimicrobium* propionic acid-producing microbe.

Pleomorphic rods, 0.5–0.8 × 1–2.5 µ, often diphtheroid or club-shaped. **Cells may be coccoid**. They may occur singly, in pairs or short chains, in V or Y configurations, or in clumps. Gram-stain-positive, nonmotile, nonsporeforming chemo-organotroph. **Anaerobic**. **Fermentation end products include propionic acid, acetic acid, succinic acid, iso-valeric acid, and lesser amounts of formic acid**. One out of two strains is catalase-positive. The major menaquinone is MK-9(H_4). **The peptidoglycan contains lysine and aspartic acid (Lys–Asp type)**. Major fatty acids are $C_{18:1}$ ω9*c*, $C_{15:0}$ anteiso, and $C_{16:0}$. The genus is a member of the family *Propionibacteriaceae* Delwiche (1957), emend. Stackebrandt, Rainey and Ward-Rainey (1997) *in* Rainey, Ward-Rainey and Stackebrandt (1997).

DNA G+C content (mol%): 53–56.

Type species: **Propionimicrobium lymphophilum** (Torrey 1916) Stackebrandt, Schumann, Schaal and Weiss 2002, 1926^VP ("*Bacillus lymphophilus*" Torrey 1916; *Propionibacterium lymphophilum* Johnson and Cummins 1972, 1057).

Further descriptive information

The exclusion of *Propionimicrobium lymphophilum* species from the genus *Propionibacterium* (Stackebrandt et al., 2002) was based upon phylogenetic evidence (Dasen et al., 1998) and information given by Cummins and Johnson (1986) based on the characterization of four strains (Holdeman et al., 1977; Johnson and Cummins, 1972). The description of the species is based on two strains, VIP 0202 and VIP 0383, which show 75% DNA similarity to one another. *Propionimicrobium lymphophilum* DSM 4309^T shares less than 91.8% sequence similarity with the *Propionibacterium* species (Dasen et al., 1998) which themselves show more than 93% similarity among each other. Depending upon the algorithm used, analysis of the 16S rRNA gene indicates that *Propionimicrobium lymphophilum* forms either the deepest branch of the genus *Propionibacterium* (Koussémon et al., 2001) (neighbor-joining program; Felsenstein, 1993), or branches among the other genera of the family *Propionibacteriaceae* (maximum-likelihood; Felsenstein, 1993) and distance matrix analyses (De Soete, 1983).

Enrichment and isolation procedures

The organism was first isolated by Torrey (1916) from lymph glands of a patient with Hodgkin's disease. Later, strains were isolated from urinary tract infections and from a mesenteric ganglion of a monkey inoculated with an unidentified "actinobacterium" isolate. Strains of the species have been reported to thrive in soil of rice paddy fields (Hayashi and Furusaka, 1980) and have been found in preparations of green olives (Cancho et al., 1980), but the identification of these strains did not include verification of the distinct cell-wall composition or the base composition of DNA (Cummins and Johnson, 1986).

Maintenance procedures

The organism can be maintained on modified Peptone-Yeast-Glucose agar (DSMZ medium 104; DSMZ (2001) or Columbia agar supplemented with 5% defibrinated sheep blood (Becton and Dickinson). Plates are sealed in plastic bags containing an Anerocult A (Merck) bag. Storage is in 20% (v/v) glycerol at −80°C or as lyophilized cultures.

Taxonomic comments

Strain VIP 0202 was originally described as "*Bacillus lymphophilus*" Torrey 1916, then as "*Corynebacterium lymphophilum*" (Torrey 1916) Eberson 1918 and as "*Mycobacterium lymphophilum*" (Torrey 1916) Krasil'nikov 1949, before it was included in a taxonomic study on coryneforms and propionibacteria (Johnson and Cummins, 1972). While membership of strain VIP 0202 in

Corynebacterium was excluded because of low DNA reassociation with members of this genus, it was tentatively classified as *Propionibacterium lymphophilum* based on its anaerobic growth and its ability to form propionic acid. *Propionibacterium lymphophilum* was included in the *Approved Lists of Bacterial Names* (Skerman et al., 1980), with reference to the publication of Johnson and Cummins (1972) and to the non-formal description of physiological and morphological properties by Holdeman et al. (1977) in the *Anaerobe Laboratory Manual of the Virginia Polytechnic Institute and State University*, Blacksburg, VA (VIP). The formal description of the species was made by Cummins and Johnson (1986) in their coverage of the genus *Propionibacterium* in *Bergey's Manual of Systematic Bacteriology*. Interestingly, both Holdeman et al. (1977) and Skerman et al. (1980) indicated strain VIP 7625B^T (=ATCC 27520^T) as the type strain, but neither Johnson and Cummins (1972), Holdeman et al. (1977), nor Cummins and Johnson (1986) refer to strain VIP 7625B^T but to strain VIP 0202. According to information obtained from the Technical Services of the ATCC in August 2001, strain ATCC 27520^T had originally been received as strain VIP 7625B^T.

In order to determine, whether strain VIP 7625B^T (=ATCC 27520^T = DSM 4309^T) was a subculture of strain VIP 0202, Stackebrandt et al. (2002) reinvestigated the base composition of DNA and the composition of peptidoglycan of strain DSM 4309^T, originally obtained for strain VIP 0202. The presence of lysine and the A4α (Lys–Asp) peptidoglycan type were confirmed as was the rather low base composition of DNA of 56 mol% G+C, which was slightly higher than the 53–54 mol% reported for strain VIP 0202.

Differentiation of the genus *Propionibacterium* from other genera

16S rRNA gene sequence analysis reveals the moderate 16S rRNA gene sequence relationship of strain DSM 4903^T to members of the genus *Propionibacterium* (>92%). The position of *Propionibacterium lymphophilum* outside the *Propionibacterium* cluster is not supported by high bootstrap values and its position changes with the algorithm applied. While it branches with *Tessarococcus bendigoensis* ACM 5119^T and *Propionibacterium propionicum* DSM 43307^T in the tree of Stackebrandt et al. (2002), it branches with *Luteococcus japonicus* IFO 12422^T in the tree of Dasen et al. (1998).

Physiologically the species can be differentiated from members of the genus *Propionibacterium* by a combination of results from esculin hydrolysis, indole production, and nitrate reduction, as well as by a combination of acid production from adonitol, erythritol, maltose, ribose, and L-sorbose, (Koussémon et al., 2001; Kusano et al., 1997). The composition of major fatty acids consisting of $C_{18:1}$ ω9*c*, $C_{15:0}$ anteiso, and $C_{16:0}$ differs significantly from those reported for *Propionibacterium* strains in which branched fatty acids (Moss et al., 1969) or ω-cyclohexane (Kusano et al., 1997) dominate.

List of species in the genus *Propionimicrobium*

1. **Propionimicrobium lymphophilum** (Torrey 1916) Stackebrandt, Schumann, Schaal and Weiss 2002, 1926^{VP} ("*Bacillus lymphophilus*" Torrey 1916; *Propionibacterium lymphophilum* Johnson and Cummins 1972, 1057)

lym.pho′phi.lum L. fem. n. *lympha* clear water and, in biology, lymph; N.L. neut. adj. *philum* (from Gr. neut. adj. *philon*), friend, loving; N.L. neut. adj. *lymphophilum*, lymph-loving.

Surface colonies on horse blood in 4 d are punctiform to 0.5 mm, circular, entire, convex to pulvinate, white, glistening, and smooth. Glucose cultures (24 h) are turbid, becoming clear, with a ropy sediment and a terminal pH of 5.4–5.7. Anaerobic, producing no growth on the agar surface when incubated aerobically, but growth develops in deep broth incubated aerobically. Acid production from adonitol, erythritol, fructose, glucose, maltose, and ribose, as well as from starch and inositol (reaction positive in 40–90% of strains). Acid is not formed from amygdalin, arabinose, cellobiose, dulcitol, esculin, galactose, glycerol, glycogen, inulin, lactose, mannitol, mannose, melezitose, raffinose, rhamnose, salicin, sorbitol, or sorbose. Nitrate reduction is positive for some strains. Esculin and gelatin are not hydrolyzed; indole, and acetoin are not produced. Hemolysis-negative. Weak production of gas and weak growth in 20% bile.

Cell walls contain alanine, glutamic acid, lysine, and aspartic acid. Glucose, galactose, and mannose are the principal cell-wall sugars. Major fatty acids are $C_{18:1}$ ω9*c*, $C_{15:0}$ anteiso, and $C_{16:0}$; smaller amounts (>2 < 5%) of $C_{14:0}$, $C_{15:0}$ iso, $C_{17:0}$ anteiso, and $C_{18:1}$ ω7*c* are present.

Source: urinary tract infections and a mesenteric ganglion of a monkey. Strains originally described by Torrey (1916) were from lymph nodes of patients suffering from Hodgkin's disease.

DNA G+C content (mol%): 53–54 mol% (T_m), 56 (HPLC).

Type strain: ATCC 27520, CCUG 27816, CIP 103263, DSM 4903, JCM 5829, LMG 16728, NCTC 11866, VPI 7625B.

Sequence accession no. (16S rRNA gene): AJ003056.

Genus XIII. **Tessaracoccus** Maszenan, Seviour, Patel, Schumann and Rees 1999b, 466^{VP}

Robert J. Seviour and Abdul M. Maszenan

Tes.sa.ra.coc′cus. Gr. adj. num. *tessares* four; N.L. masc. n. *coccus* (from Gr. masc. n. *kokkos* grain, seed) coccus; N.L. masc. n. *Tessaracoccus*, four round cells.

Gram-stain-positive, nonsporeforming, facultatively anaerobic nonmotile cocci 0.5–1.1 μm in diameter, arranged in regular tetrads. **Contains polyphosphate granules**. **Oxidase-negative, catalase-positive, and nitrate reduced**. Cell wall contains LL-diaminopimelic acid with an **A3γ′ peptidoglycan type and a glycine residue at position 1 of the tetrapeptide**. **Major menaquinones are MK-9(H_4) and MK-7(H_4), while the polar lipids are phosphatidylinositol, phosphatidylglycerol, and diphosphatidylglycerol,**

together with three unidentified glycolipids. The major fatty acid is anteiso hexadecanoic acid. Growth at 20–37°C, with the optimal temperature at 25°C. Growth at pH 6.0–9.0 with optimum pH at 7.5.

DNA G+C content (mol%): 74.

Type species: **Tessaracoccus bendigoensis** Maszenan, Seviour, Patel, Schumann and Rees 1999b, 466[VP].

Further descriptive information

Tessaracoccus is currently placed as a member of the family *Propionibacteriaceae* emend. Rainey, Ward-Rainey and Stackebrandt despite different signature nucleotides in some positions (e.g. G–T instead of A–T and A instead of G at positions 602:636 and 686, respectively) from those proposed by Stackebrandt et al. (1997) to circumscribe members of this family (Maszenan et al., 1999b). Only one species, *Tessaracoccus bendigoensis*, is currently recognized. It is related to members of the genus *Propionibacterium* with *Propionibacterium cyclohexanicum*, *Propionibacterium freudenreichii*, *Propionimicrobium lymphophilum*, and *Propionibacterium propionicum* as its closest relatives (Figure 246). It can utilize a wide range of sugars and sugar derivatives, as well as organic acids, although it does not utilize most amino acids and amines (Maszenan et al., 1999b). *Tessaracoccus* was isolated from activated sludge and synthesizes polyphosphate under aerobic conditions, thereby raising the possibility that it might play some role in enhanced biological phosphate removal (EBPR). However, there are not any studies which show this organism occurs in high numbers in such systems, and 16S rRNA targeted probes have not been generated for its *in situ* identification. There is not any evidence that the organism can accumulate poly β-hydroxyalkanoates (Maszenan et al., 1999b), a feature generally considered necessary in any putative polyphosphate accumulating organism in such systems (Seviour et al., 2003). It has also been detected in, but not cultured from, a Cr (IV) degrading community by denaturing gradient gel electrophoresis profiling of its 16S rRNA gene fragments (Arias and Tebo, 2003).

Enrichment and isolation procedures

Tessaracoccus bendigoensis was isolated with difficulty by micromanipulation (Skerman, 1968) from activated sludge biomass taken from an anaerobic:aerobic laboratory scale EBPR sequencing batch reactor seeded from a full scale EBPR activated sludge plant (Maszenan et al., 1999b). The only medium successful for its isolation and subsequent growth was the GS medium described by Williams and Unz (1985).

Maintenance procedures

Tessaracoccus bendigoensis was cultivated on GS medium and incubated at 25°C. After purity was confirmed by microscopic examination, cultures were stored successfully on GS medium in 20% glycerol (v/v) at −80°C.

Taxonomic comments

Only a single strain of a single species of *Tessaracoccus* has been characterized and described so far, hence it seems likely that the genus description may require emending as more species are isolated and characterized. Based collectively on its G+C mol% composition, 16S rRNA gene sequence, menaquinone and fatty acid composition, and its peptidoglycan type (A3γ′), with LL-diaminopimelic acid (LL-A_2pm), and only a single glycine residue in its interpeptide bridge, and glycine at position 1 in the tetrapeptide, *Tessaracoccus* seems to differ markedly from all other genera in the family *Propionibacteriaceae* (Table 213). *Propionimicrobium lymphophilum*, for example, has aspartate as the interpeptide bridge and an A4α cell-wall type (Stackebrandt et al., 2002). In addition to the MK-9(H_4) common to all members of the family *Propionibacteriaceae*, it contains MK-7(H_4), while its predominant fatty acid is $C_{15:0}$ anteiso.

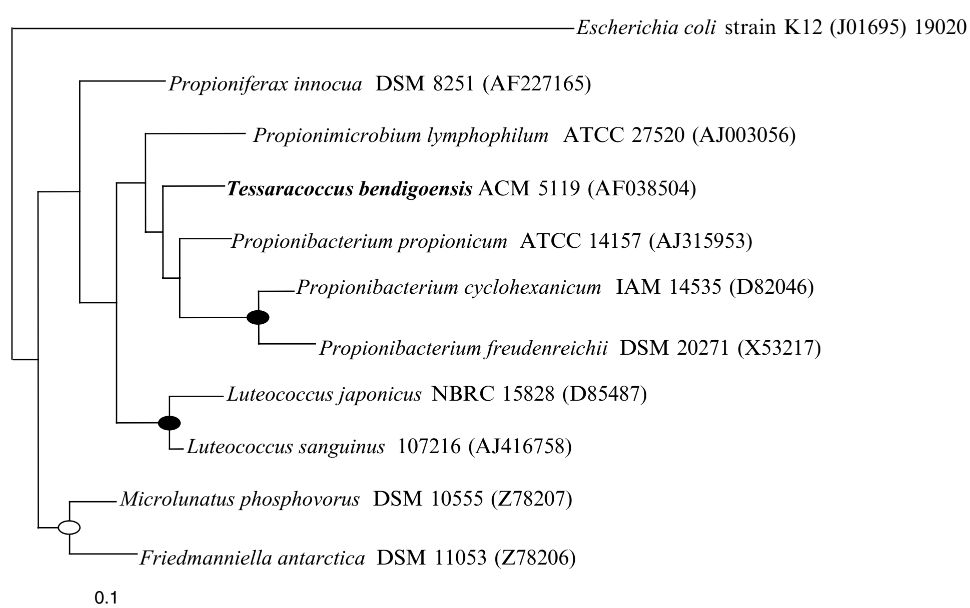

FIGURE 246. Phylogenetic tree based on 16S rRNA sequence data indicating the placement of *Tessaracoccus bendigoensis* within the family *Propionibacteriaceae*. Nodes receiving ≥75% and <97% bootstrap support (1000 replicates) with parsimony and neighbor-joining algorithms (○). Those receiving ≥97% are indicated (●). Scale bar = 0.1 nucleotide substitutions per site.

TABLE 213. Characteristics differentiating *Tessaracoccus bendigoensis* and related taxa[a]

Characteristics	*Tessaracoccus bendigoniensis*	*Luteococcus japonicus*	*Propionibacterium freudenreichii*	*Propionibacterium propionicum*	*Propioniferax innocua*	*Propionimicrobium lymphophilum*
Origin	Activated sludge	Soil and water	Cheese and dairy produce	Human oral cavity and cervicovaginal secretion	Human skin	Lymph nodes of patients
Cell shape and arrangement	Coccoid cells in tetrads	Coccoid cells; arranged in pairs and tetrads	Pleomorphic rods	Pleomorphic rods	Pleomorphic rods	Pleomorphic rods
Optimal temperature for growth	25	26–28	30–32	35–37	37	36–37
Optimal pH for growth (optimum)	6.0–9.0 (7.5)	nd	4.5–8.5	nd	7.0	nd
O_2 requirement	Facultatively anaerobic	Facultatively anaerobic	Anaerobic, aerotolerant	Facultatively anaerobic	Facultatively anaerobic	Anaerobic
Catalase	+	+	+	−	+	v
Oxidase	−	+	nd	nd	+	−
Nitrate reduction	+	−	v	+	+	v
DNA G+C content (mol%)	74	66–68	64–67	63–65	59–63	53–56
Major diamino acid	LL-A_2pm	LL-A_2pm	*meso*-A_2pm	LL-A_2pm	LL-A_2pm	Lysine
Peptidoglycan type	A3γ′	A3γ	nd	A3γ′	A3γ′	A4-α
Major menaquinone	MK-9(H_4), MK-7(H_4)	MK-9(H_4)	MK-9(H_4)	MK-9(H_4)	MK-9(H_4)	MK-9(H_4)

[a]Symbols: +, positive; −, negative; v, variable; nd, not determined.

[b]References: *Tessaracoccus bendigoniensis* (Maszenan et al., 1999b); *Luteococcus japonicus* (Maszenan et al., 1999b; Tamura et al., 1994); *Propionibacterium freudenreichii* (Bae et al., 2006b; Cummins and Johnson, 1986; Kusano et al., 1997); *Propionibacterium propionicum* (Cummins and Moss, 1990; Maszenan et al., 1999b); *Propioniferax innocua* (Maszenan et al., 1999b; Pitcher and Collins, 1991; Schumann et al., 1997; Yokota et al., 1994); *Propionimicrobium lymphophilum* (Stackebrandt et al., 2002).

List of species of the genus *Tessaracoccus*

1. **Tessaracoccus bendigoensis** Maszenan, Seviour, Patel, Schumann and Rees 1999b, 466[VP]

ben.di.go′en.sis. N.L. masc. adj. *bendigoensis* of or belonging to Bendigo, Australia, the place of origin of the isolate.

The following enzymes are detected by APIzyme: *N*-acetyl-β-glucosaminidase, acid phosphatase, alkaline phosphatase, cystine arylamidase, esterase, esterase lipase, α-galactosidase, β-galactosidase, α-glucosidase, β-glucosidase, gelatinase, leucine aryl amidase, lipase, α-mannosidase, naphthol-AS-B1-phosphohydrolase, and valine aryl amidase. Produces acetoin, but not H_2S or indole. It can liquefy gelatin, and grows at 20–37°C and pH 6.0–9.0. Optimal growth is at 25°C and optimal pH is 7.5.

The following substrates are utilized as detected with Biolog™: acetate, *N*-acetylglucosamine, amygdalin, L-arabinose, arbutin, α-cyclodextrin, β-cyclodextrin, dextrin, D-fructose, D-galactose, gentiobiose, D-gluconic acid, α-D-glucose, glucose 1-phosphate, glucose 6-phosphate, glycerol, DL-glycerol phosphate, glycogen, α-hydroxybutyrate, DL-lactate, L-lactate, α-D-lactose, lactulose, maltose, maltotriose, D-mannitol, D-mannose, D-melibiose, methyl-α-D-glucoside, methyl-β-D-glucoside, methyl pyruvate, palatinose, pyruvate, D-raffinose, D-ribose, salicin, stachyose, sucrose, turanose, D-xylose, uridine, and UMP.

Source: activated sludge.

DNA G+C content (mol%): 74 (T_m).

Type strain: Ben 106, ACM 5119, DSM 12906, JCM 13525.

Sequence accession no. (16S rRNA gene): AF038504.

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Family II. **Nocardioidaceae** Nesterenko, Kvasnikov and Nogina 1990, 320[VP] (Effective publication: Nesterenko, Kvasnikov and Nogina 1985a, 9.) emend. Rainey, Ward-Rainey and Stackebrandt 1997, 484 emend. Zhi, Li and Stackebrandt 2009, 599

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No.car.di.o.i.da.ce.a.e. N.L. masc. n. *Nocardioides* type genus of the family; suff. *-aceae* ending to denote a family; N.L. fem. pl. n. *Nocardioidaceae* the *Nocardioides* family.

Young cultures exhibit different morphologies, ranging from extensively branching vegetative hyphae, growing on and penetrating the surface of agar media, **to irregular rods** and **spherical cells. Aerial hyphae are produced** by organisms of many taxa and may be abundant or scant, at times discernible only microscopically**. The vegetative and aerial hyphae**, depending on organisms and growth conditions, **undergo varying degrees of fragmentation and differentiation eventually resulting in rod-like and coccoid cells (arthrospores)** often arranged in chains or small aggregates. **Cell division and hyphal elongation by pronounced budding** are characteristic of some taxa. **Clusters of tightly packed irregularly shaped cells** can be observed. The non-mycelial bacteria of the genus *Aeromicrobium* and the majority of *Nocardioides* species usually exhibit **a rod–coccoid morphogenetic cycle** and **can be motile. Spherical cells during all growth phases** are characteristic mostly of the genus *Marmoricola*. **Gram-stain-positive type of cell wall.** Non-acid-fast. **Colonies are non-pigmented** or of different intensity and shades of **cream, yellow, or orange.** Aerial mycelium, if any, is typically white, but may be cream or light yellow in color.

Chemo-organotrophs, having a respiratory type of metabolism, with a potential for metabolic flexibility. **Grow under aerobic conditions on standard laboratory media**, including chemically defined (synthetic) media. Some organisms are nutritionally fastidious. Some show weak growth in media rich in organics under anaerobic conditions. Mostly catalase-positive. Oxidase activity varies with the species. Utilize a wide range of carbon and nitrogen sources, including unusual organic compounds and toxic environmental pollutants, and possess a wide spectrum of enzymatic activities. **Mostly mesophiles, non-halophiles. Prefer a neutral to mildly alkaline pH.** However, thermophilic, salt-requiring, and alkaliphilic species occur.

The cell-wall peptidoglycan contains LL-diaminopimelic acid and glycine as the diagnostic amino acid **(the peptidoglycan type A3γ)** The muramic acid residue of the peptidoglycan is *N*-acetylated. **Anionic (acidic) polysaccharides are usually present** in the cell wall, which can be either phosphorous-containing or phosphorous-free (**teichoic, teichuronic, teichulosonic acids** or some not yet identified polymers). Menaquinones are the sole respiratory quinines detected; **the predominant component is menaquinone with partially saturated side chain consisting of either 8, 9, or 10 isoprene units – MK-8(H_4), MK-9(H_4), MK-9(H_6), or MK-10(H_4).** Cellular fatty acids are **different combinations of branched-chain saturated (iso-, anteiso-, 10-methyl- and 9-methyl-branched), straight-chain saturated and monounsaturated, and hydroxylated components.** Mycolic acids are absent. Phospholipids of the three types are present: **type I (with no nitrogenous phospholipids), type III (phosphatidylcholine as diagnostic component), and rarely type II (with diagnostic phosphatidylethanolamine).** Occur in various terrestrial and aquatic environments, including polluted sites, and can be found in microbial communities associated with plants, algae, lichens, animals, or humans. No medically relevant strains or species have been described within the family so far.

DNA G+C content (mol%): 65.5–74.8 (T_m, HPLC).

Type genus: **Nocardioides** Prauser 1976, 61[AL].

Further descriptive information

The family *Nocardioidaceae* (Nesterenko et al., 1985a, 1990; Zhi et al., 2009) belongs to the order *Propionibacteriales*, class *Actinobacteria* (Stackebrandt et al., 1997). Genera currently assigned to this family form a 16S rRNA-based phylogenetic group which includes two main clusters (Figure 247). The first one comprises the genera *Nocardioides* (type genus of the family), *Marmoricola* and *Aeromicrobium*. The second cluster includes the genera *Actinopolymorpha*, *Flindersiella**, *Kribbella*, and *Thermasporomyces**. Delineation of genera within the *Nocardioidaceae*, like in many other actinomycete families, is primarily based on 16S rRNA gene sequence phylogenetic clustering and on morphological and chemotaxonomic characteristics of species included (Table 214).

Morphology. Three main morphological groups can be distinguished among organisms of this family: (1) organisms producing rod-shaped cells and typically displaying a rod-to-coccus morphogenetic cycle, (2) organisms with mostly spherical cells during all growth phases, and (3) organisms forming a mycelium during the morphogenetic cycle. The first morphological group consists of the majority of *Nocardioides* species and all members of the genus *Aeromicrobium*. Cells display irregular rods in young cultures and divide by transverse septum formation, often showing V-forms. While growth proceeds, the cells typically become shorter, and a significant proportion of cells can be coccoid in older cultures. The distinct rod–coccoid growth cycle resembling that of *Arthrobacter* (Keddie et al., 1986) is often observed, especially in synchronized cultures on rich agar media. The second morphological group is mostly represented by coccoid organisms of the genus *Marmoricola*. To this group might also be assigned some *Nocardioides* species producing very short rods and cocci, and showing outward morphological resemblance with *Marmoricola*, e.g. *Nocardioides aquaticus* (Lawson et al., 2000a). The third morphological group encompasses organisms (genera *Actinopolymorpha*, *Kribbella*, *Flindersiella*, *Thermasporomyces*, and two *Nocardioides* species, *Nocardioides albus* and *Nocardioides luteus*) with a common feature of forming branched hyphae but varying significantly in developmental events and cell differentiation (Table 214). Both vegetative (substrate) and, if any, aerial hyphae vary in length and branching intensity, depending on the genus or species and the composition and consistency of the growth medium. The substrate hyphae

*After this chapter was accepted for publication, the descriptions of genera *Flindersiella* (Kaewkla and Franco, 2010a) and *Thermasporomyces* (Yabe et al., 2011) were published. The main characteristics of these genera are outlined only in this chapter and mentioned in the chapters "Genus *Actinopolymorpha*" and "Genus *Nocardioides*" (for further details, see the original descriptions).

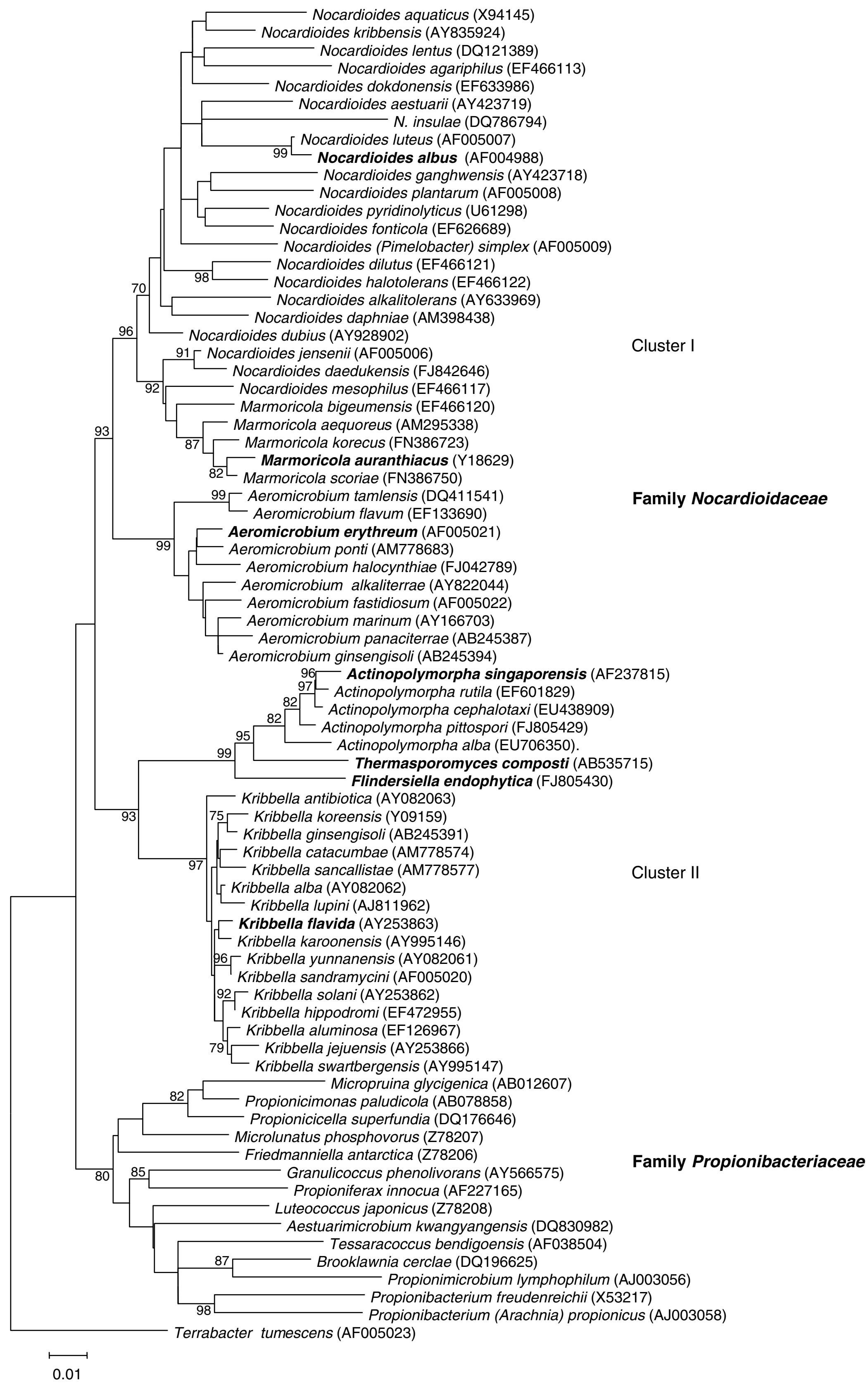

FIGURE 247. Phylogenetic dendogram based on 16S rRNA comparison of type strains of the species comprising the family *Nocardioidaceae* (genera *Aeromicrobium*, *Actinopolymorpha*, *Flindersiella*, *Kribbella*, *Marmoricola*, and *Thermasporomyces*, and representative species of the genus *Nocardioides*). The type species of the genera are given in bold. For the extended tree of the genus *Nocardioides*, see Figure 248. Bar = 0.01 inferred substitutions per nucleotide. Values at nodes indicate bootstrap values for 1000 replicates.

can be of uneven thickness and/or have local swellings, often located at the top of hyphae. The hyphae usually break up into elongated, Y-shaped, or rod-like fragments which can undergo further division by transverse septum formation. The fragments may become thicker and rounder, becoming spore-like cells (arthrospores) after maturation, which can be strung together into short, straight, or zig-zag shaped chains. In some cultures (e.g. in the genus *Kribbella*), the substrate and aerial hyphae or significant hyphal area may remain stable, and no distinct regular fragmentation into rod-like elements can be observed *in situ*. In addition, short chains of poorly to well-differentiated cells (conidia) may occasionally occur on terminal parts of aerial hyphae (*Flindersiella*, *Kribbella*). A characteristic feature of some representatives of the family, especially of the genus *Actinopolymorpha*, is the production of novel cells through marked apical and lateral budding and hyphal elongation by budding (similar to that in *Pseudonocardia*; Henssen, 1989; Henssen et al., 1983), along with the hyphal growth in an ordinary way. Cells produced by budding, and also by septum formation, can form aggregates or compact clusters (most pronounced in *Actinopolymorpha* and some *Kribbella*). The cells in clusters, as recently revealed in kribbellae, are usually tightly packed and irregular in size and shape (often angular) and are produced via transverse and/or differently directed septa, like that reported, e.g. for *Geodermatophilus* (Eppard et al., 1996; Ishiguro and Wolfe, 1970). Both the hyphal fragments and products of other modes of cell fission usually give rise to new hyphae (or buds) when transferred to a fresh medium. Notably, all organisms of the first and the second morphological groups with rod-shaped and coccoid cells fall into the phylogenetic cluster I (Figure 247). The third morphological group, except two nocardioform *Nocardioides* species (*Nocardioides albus* and *Nocardioides luteus*), corresponds to the phylogenetic cluster II.

Chemotaxonomy. All species comprising the genera of the family *Nocardioidaceae* are characterized by the cell-wall peptidoglycan containing LL-diaminopimelic (LL-A_2pm) acid (cell-wall chemotype I *sensu* Lechevalier and Lechevalier, 1970). The peptidoglycan type is A3γ *sensu* Schleifer and Kandler (1972) (type A41.1 according to DSMZ Catalog of Strains, 2001), which was determined for several species (Fiedler et al., 1970; Lawson et al., 2000a; Prauser, 1986; Schippers et al., 2005; Schleifer and Kandler, 1972; Schumann et al., 1997; Trujillo et al., 2006; Urzì et al., 2000) or inferred from available data on the peptidoglycan amino acid composition (e.g. Lee et al., 2000; Miller et al., 1991; Park et al., 1999; Tamura and Yokota, 1994). The subunit of this polymer is characterized by the tetrapeptide L-Ala–D-Glu–LL-A_2pm–D-Ala and a glycine residue as an interpeptide bridge linking the amino group located on the D-carbon of LL-A_2pm and the C-terminal D-alanine of an adjacent subunit. It should be emphasized that this polymer structure differs from those reported for some other LL-A_2pm-containing actinobacteria, belonging, e.g. to the phylogenetically neighboring family *Propionibacteriaceae* or to more distant *Intrasporangiaceae*. Peptidoglycans of organisms of these families may have additional glycine molecules in the interpeptide bridge, along with glycine or glycine amide linked to the α-carboxyl group of D-glutamic acid at position 2 of the peptide subunit (type A3γ; A41.2) or contain glycine instead of alanine in position 1 of the peptide subunit (type A3γ′; A42.1) (DSMZ, 2001; Schleifer and Kandler, 1972; Schumann et al., 1997, 2009; Stackebrandt and Schaal, 2006; Stackebrandt and Schumann, 2006; Weon et al., 2007). Thus, the peptidoglycans of members of the family *Nocardioidaceae* analyzed so far contain one glycine in the peptide subunit, in contrast to that of some *Propionibacteriaceae* or *Intrasporangiaceae*, which may contain up to 4 glycine molecules per peptide subunit. An exception might be the genus *Thermasporomyces* reported to possess peptidoglycan with glycine, glutamic acid, alanine, and LL-A_2pm in a molar ratio of 3.9:1.0:0.6:0.5, although the peptidoglycan structure was not yet studied (Yabe et al., 2011). For a few organisms of the family *Nocardioidaceae*, the acyl type of muramyl residues of the peptidoglycans was determined and found to be acetyl type (Kaewkla and Franco, 2010a; Kubota et al., 2005a; Lee et al., 2000; Uchida and Seino, 1997; Urzì et al., 2000).

The cell-wall monosugars of genera of the family *Nocardioidaceae* routinely recorded during chemotaxonomic studies lack coherence. The sugars revealed in cell walls or whole cells of different species include various combinations of glucose, galactose, mannose, rhamnose, and less frequently 2-*O*-methyl-D-galactose (madurose), 2,3-*O*-dimethyl-D-galactose, ribose, xylose, as well as some unidentified sugars. On the other hand, the neutral cell-wall monosugars may be absent (*Nocardioides plantarum*), while aminosugars, polyols, carbonic acids, and some unusual compounds originating from different types of peptidoglycan-attached polysaccharides may be present (see the chapters on *Aeromicrobium*, *Kribbella*, and *Nocardioides* in this volume for more details). The data available suggest that the monosugar patterns, individual sugars, and/or other components originating from the cell-wall polysaccharides linked to the peptidoglycan are indicative of individual species or species group within a genus and may also predict membership of a bacterium to a certain genus.

The peptidoglycan-linked polysaccharides from representatives of this family were found to possess mostly anionic (acidic) polymers of different types (Table 214). One such acidic polymer type is represented by teichoic acids which are poly(polyol phosphate) polymers often including glycosyl moieties in the basic chain and bearing lateral branches (substituents) (Baddiley, 1970; Ward, 1981). The teichoic acids were found in almost all organisms of the genera *Nocardioides* and *Aeromicrobium* so far analyzed (Naumova et al., 2001; Shashkov et al., 2000b; Shashkov et al., 1999; Takeuchi and Yokota, 1989; Tul'skaya, 2009). The exception is *Nocardioides plantarum* (which lacks teichoic acids and other phosphate-containing polysaccharides, and most likely has a different kind of the peptidoglycan-linked polymer). *Aeromicrobium fastidiosum*, in addition to a ribitol teichoic acid, has another type of phosphate-containing polymer, presumably a phosphorhamnan. Two types of phosphorous-free acidic polymers (along with minor amounts of a neutral glycopolymer, mannan) have been found in the cell walls of 15 *Kribbella* strains representing different phylogenetic groups within the genus (Shashkov et al., 2009; A.S. Shashkov, E.M. Tulskaya, and L.I. Evtushenko, unpublished). One type is represented by teichuronic acids with a rare diaminosugar, 2,3-diacetamido-2,3-dideoxyglucose, in the basic chain. The second type comprises unusual glycopolymers with the backbone containing a nine-carbon sialic-acid-like keto sugar, a nonulosonic (mostly pseudaminic) acid, and is characterized by an unusual linkage in the polymeric chain (Figure 275). By analogy with teichuronic acids, the name for nonulosonic acid-containing polymers was

TABLE 214. Differential characteristics of the genera in the family *Nocardioidaceae*[a]

Characteristic	*Nocardioides*	*Aeromicrobium*	*Marmoricola*	*Kribbella*	*Actinopolymorpha*	*Flindersiella*	*Thermasporomyces*
Phylogenetic group	I	I	I	II	II	II	II
Morphology	Hyphae[b], rods, coccoid cells[c,d]	Rods, coccoid cells[d]	Coccoid cells	Hyphae, rods, coccoid cells[c], conidia, hyphal swellings, cell clusters[e]	Hyphae, pleomorphism, marked budding, cell aggregates (clusters)[e]	Hyphae, rods[c], conidia	Hyphae, rods, coccoid cells[c]
Aerial mycelium	D +	–	–	+	D + (w)	+	–
Motility	D–	D–	D–	–	–	–	–
Optimal temperature (°C)	20–30	25–35	28–30	25–30	27–28	27–35	50–55
Major menaquinones[f]	8/4[g]	9/4	8/4[h]	9/4[i]	9/4 or 9/6 (9/4–8, 10/4–8)	10/6 (10/8,4,2)	9/4, 10/4, 11/4
Predominant fatty acids (>10%)[j]	$C_{16:0}$ iso, ($C_{18:1}$ ω9*c*, $C_{17:1}$ ω6/8c, 10-Me-$C_{18:0}$, 10-Me-$C_{17:0}$, $C_{17:0}$ anteiso, $C_{16:0}$, $C_{16:1}$ iso, $C_{17:0}$, $C_{17:0}$ iso, $C_{15:0}$ iso)	$C_{18:1}$ ω9*c*, 10-Me-$C_{18:0}$, $C_{16:0}$, ($C_{16:0}$ 2-OH, $C_{18:0}$, 10-Me-$C_{16:0}$)	$C_{16:0}$, $C_{18:1}$ ω9*c*, (10-Me $C_{18:0}$, $C_{17:1}$ ω8*c*, $C_{16:1}$) or $C_{16:0}$ iso[k]	$C_{15:0}$ anteiso, $C_{16:0}$ iso, ($C_{15:0}$ iso, $C_{17:1}$ iso, $C_{14:0}$ iso, $C_{17:0}$ anteiso, 9-Me-$C_{16:0}$)	$C_{16:0}$ iso, $C_{15:0}$ iso, ($C_{17:0}$ iso, $C_{17:0}$ anteiso, $C_{16:1}$ iso, $C_{15:0}$ anteiso)	$C_{16:0}$ iso, $C_{17:0}$ anteiso, $C_{15:0}$ anteiso	$C_{17:0}$ anteiso, $C_{15:0}$ anteiso, $C_{17:0}$ iso, $C_{15:0}$ iso
Polar lipids[l]	DPG, PG, PL (PG-OH, PI, PI-OH, PIM, acyl-PG, PC[m], PE[m])	DPG, PG (PE, PI, PL)	PI, DPG, PG, (PC, PL)	PC, PI, DPG, PG, (PI-OH, PL, GL)	PG, PIM, (DPG, PI)	DPG, PG	PG, DPG, PGL, GL
Major polyamines[n] (>20%)	CAD (PUT, SPM)	CAD (SPD, SPM)	nd	nd	nd	nd	nd
Major murein-linked glycopolymers[o]	Teichoic acids[p]	Teichoic acids[q]	nd	Teichuronic or teichulosonic acids	nd	nd	nd
DNA G+C content (mol%)	67.5–74.8	65.5–74.0	71.0–72.9	66.3–71.3	66.6–69.6	68.8	69.2

Symbols and abbreviations: +, present; –, absent; D, different between species within a genus (character for the type species is indicated); anteiso, iso-, 9-Me-, and 10-Me indicate anteiso-, iso-, 9-, and 10-methyl-branched acids, respectively; 2-OH, 2-hydroxylated acids; DPG, diphosphatidylglycerol; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; PG-OH, phosphatidylglycerol containing 2-hydroxy fatty acids; PGL, ninhydrin-positive phosphoglycolipid; PI, phosphatidylinositol; PI-OH, phosphatidylinositol containing 2-hydroxy fatty acids; PIM, phosphatidylinositol mannosides; PL, unidentified phospholipid(s); GL, glycolipid(s); CAD, cadaverine; PUT, putrescine; SPD spermidine; SPM, spermine.

[a]Data compiled from the original descriptions of the genera, the species composing the genera and publications cited in the respective genus chapters.

[b]Vegetative and aerial hyphae develop in cultures of *Nocardioides albus* and *Nocardioides luteus* only.

[c]Rod-shaped and coccoid cells are usually produced owing to fragmentation of vegetative and/or aerial hyphae, with different genera and species showing varied degrees of hyphal fragmentation and additional cell differentiation.

[d]Coccoid cells are typically produced in later stages of the morphogenetic cycle.

[e]Cells in clusters are often tightly packed and irregular in shape and size (can be produced internally by differently oriented septa, as shown for kribbellae and supposed for *Actinopolymorpha*).

[f]Numerals indicate the numbers of isoprene units and the number of hydrogen atoms in the partially saturated side chain, e.g. 8/4 is a menaquinone with 8 isoprene units and 4 hydrogen atoms in the side chain. Components irregularly detectable among species or reported to occur in lesser amounts are given in parentheses.

[g]Menaquinones 6/4, 7/4, 8/0, 8/2, 8/6, and 9/4 may be produced as minor components or in trace amounts (see the chapter on *Nocardioides* for more details and references).

[h]Menaquinone system including 8/4, 7/4, 8/2, and 6/4 (peak area ratio, 73:4:1:1) has been reported for *Marmoricola aurantiacus* (Urzì et al., 2000).

[i]Menaquinone 9/4 constitutes 93% (Carlsohn et al., 2007) or more of the total.

[j]Numbers before and after colons represent chain lengths and numbers of double bonds of fatty acids; ω indicates the double bond position. Compounds are listed in the order of decreasing amounts and frequency among species within a genus. Compounds found to irregularly contribute more than 10% among species of multi-species genera or in different experiments are given in parenthesis (see the respective genus chapters in this volume for details). Relative amounts of the predominant components in the fatty acid profiles may vary depending on growth conditions and growth phase.

[k]Found only in *Marmoricola bigeumensis*.

[l]PL usually stands for principal unidentified or incompletely identified compounds which may be the same or different. Parenthesis indicates that a compound was present in only some species within a genus. Minor or trace amounts of other unidentified polar lipids may also be present (see respective genus chapters in this volume).

[m]The key components of the phospholipid types II and III (according to Lechevalier et al., 1977) were reported for *Nocardioides daedukensis* and *Nocardioides dubius* (phosphatidylethanolamine) and *Nocardioides furvisabuli* (phosphatidylcholine).

[n]Polyamines found in some representatives of a genus are given in parentheses.

[o]See the text and relevant genus chapters for the composition of polymers indicated. Minor or trace amounts of neutral polysaccharides may occur in organisms of some taxa so far tested.

[p]An exception is *Nocardioides plantarum*, which most likely contains a peptidylglycan-linked polymer of a different type.

[q]Available for the only species, *Aeromicrobium fastidiosum*; this species also contains another polysaccharide, presumably phosphorhamnan.

suggested to be teichulosonic acids (Knirel, 2009). Notably, each *Kribbella* strain contained as the major polysaccharide either teichuronic or teichulosonic acid, or, occasionally both, while a neutral polymer (mannan) was present in varying amounts in all 15 strains investigated. The teichulosonic acids of a different type were found previously (along with some other acidic polymers) in the cell wall of some streptomycetes, including plant pathogenic strains (Shashkov et al., 2002a; Shashkov et al., 2000a; Shashkov et al., 2002b; Tul'skaya et al., 2007; Tul'skaya, 2009). Pseudaminic acid (or its derivatives) are a rather common component of polysaccharides of Gram-negative bacteria (Knirel et al., 2003; Schoenhofen et al., 2006; Vimr et al., 2004); in the cell walls of Gram-positives, the pseudaminic acid and pseudaminic acid-containing polymers have been found so far solely in kribbellae. Lipoteichoic acids (which, in contrast to the cell-wall teichoic acids, are membrane-anchored) were found in abundance in all representatives of the family investigated so far, i.e. in several mycelium-forming *Nocardioides* strains (E.M. Tulskaya and L.I. Evtushenko, unpublished).

Members of the *Nocardioidaceae* are characterized by different types and the predominant components of cellular fatty acids (Table 214). The complex fatty acid type (Suzuki and Komagata, 1983a; Suzuki et al., 1993), which includes both straight- and branched-chain fatty acids (iso-, anteiso, and 10-methyl-branched) and also hydroxylated acids is characteristic of the genus *Nocardioides*. The fatty acid profile of the majority *Nocardioides* species are dominated by $C_{16:0}$ iso which may contribute up to 65–70% (Choi et al., 2007; Kim et al., 2008a; Schumann et al., 1997). Species of the genera *Aeromicrobium* and *Marmoricola*, except *Marmoricola bigeumensis*, contain large proportions of straight-chain fatty acids as well as 10-methyl-branched and hydroxylated acids, whereas iso- and anteiso-branched components are usually present in minor amounts. In contrast, the four remaining (mycelium-forming) genera of the family are characterized by the predominance of iso- and/or anteiso-branched acids (Table 214). The fatty acid profiles in members of the family may vary considerably with culture age, growth conditions and analytical procedure. Nevertheless, the fatty acid type and, in general, the patterns of major fatty acids, together with some rarely encountered components, e.g. 9-methyl branched chain acids (*Kribbella*) appear to be indicative of certain genera (Table 214).

Three phospholipid types *sensu* Lechevalier et al. (1977) can be identified: type I (no nitrogenous phospholipids), type II (phosphatidylethanolamine as diagnostic phospholipid), and type III (phosphatidylcholine as diagnostic phospholipid). Other polar lipids include phosphatidylglycerol, diphosphatidylglycerol, phosphatidylinositol, phosphatidylinositol mannosides, hydroxy-phosphatidylglycerol, as well as unidentified phospholipids and glycolipids (Table 214). The type of phospolipids and the pattern of principal polar lipids are uniform in some genera (e.g. *Kribbella*, all species of which contain phosphatidylcholine) and heterogenous particularly in the genera *Nocardioides, Aeromicrobium*, and *Marmoricola*. As with the fatty acids, the polar lipid patterns may be affected by growth conditions and analytical procedure, resulting in conflicting data reported by different authors (see, e.g. Collins et al., 1989, 1983; Komura et al., 1975b; Lechevalier et al., 1981, 1977; Lee and Kim, 2007; O'Donnell et al., 1982; Prauser, 1989; Tamura and Yokota, 1994). Besides, many publications report "unknown" polar lipid components, which might be the same as or different from those identified by other authors. Detailed analysis of the polar lipids in members of the family will indeed increase their discriminative power.

Polyamines have so far been detected in only a few representatives of the genus *Nocardioides* and two strains of *Aeromicrobium* (Busse and Schumann, 1999). In most species, cadaverine (which distinguishes these two genera from the other LL-A_2pm-containing actinobacteria analyzed) was present as the primary polyamine with either putrescine, spermine, or spermidine as a secondary one. Others such as 1,3-diaminopropane, tyramine, and sym-homospermidine were detected in some strains in minor or trace amounts (Busse and Schumann, 1999).

Physiology. Bacteria of the *Nocardioidaceae* are considered to be heterotrophic aerobes growing in media containing peptone and/or yeast extract as well as in chemically defined (synthetic) media with glucose and other sugars or organic acids as sole carbon sources; some require vitamins and other growth factors. Representatives of the family, especially *Nocardioides* strains, also exhibit the capacity to degrade and metabolize recalcitrant molecules, including diverse hydrocarbons and numerous substituted aromatic compounds, many of which are toxic (see respective genera chapters in this volume for references and details). Some species of the genus *Kribbella* show weak or moderate anaerobic growth on agar media rich in organics in an atmosphere of $H_2/CO_2/N_2$ (5:10:85), but not on ISP 9 with glucose as the sole carbon source (Kirby et al., 2006). The ability of some kribbellae to grow aerobically on tap-water agar (Lee et al., 2000; L.M. Baryshnikova and L.I. Evtushenko, recent observations) suggests that they may possess an oligotrophic or even autotrophic lifestyle under certain conditions, as reported earlier for representatives of the genus *Pseudonocardia* (Goodfellow and Lechevalier, 1986; Lechevalier et al., 1986; Mahendra and Alvarez-Cohen, 2005; Parales et al., 1994; Takamiya and Tubaki, 1956). There is some indirect evidence of possible chemolithotrophic growth with CO and hydrogen (King and Weber, 2007; Mattes et al., 2005; Osborne et al., 2010). Bacteria of the family are mostly mesophiles, exhibiting optimal growth within the temperature range 25–37°C; some grow best at 16–26°C (Lawson et al., 2000a). So far, only one thermophilic species, *Thermasporomyces composti*, growing at 35–62°C (optimum growth at 50–55°C) has been reported (Yabe et al., 2011). Some mesophilic species can grow at temperatures up to 45°C (Cao et al., 2009; Kaewkla and Franco, 2010a; Kirby et al., 2006; Song et al., 2011). Members of the family usually prefer a neutral to mildly alkaline pH, but some (representatives of *Marmoricola*, *Kribbella*, and *Nocardioides*) resist alkaline conditions and can grow at initial pH values up to pH 12 (e.g. Dastager et al., 2008b; Lee, 2007a; Lee and Lee, 2010; Lee et al., 2010; Yoon et al., 2005a). Moreover, some species appear to be alkaliphilic, in particular, *Marmoricola scoriae* (optimal growth at initial pH 8–11; Lee et al., 2010). Some species show weak growth at pH 4.5 or slightly below (Everest and Meyers, 2008). Bacteria of the family are largely non-halophiles, but some species of *Actinopolymorpha*, *Aeromicrobium*, and *Nocardioides* require salt, grow best in the presence of NaCl up to 6–8% (w/v), and/or tolerate NaCl concentrations up to 15% (w/v) (e.g. Bruns et al., 2003; Cao et al., 2009; Choi et al., 2007; Kim et al., 2008a; Lawson et al., 2000a; Lee and Lee, 2008; Wang et al., 2001).

Genomic characteristics. The G+C content of the DNA are largely about 70 mol%, varying between 65.5 mol% (HPLC) determined for *Aeromicrobium panaciterrae* (Cui et al., 2007a) and 74.8 mol% (HPLC) reported for *Nocardioides lentus* (Yoon et al., 2006a). The sequences of the internal transcribed spacer (ITS) region of the 16S–23S rRNA gene (Yoon and Park, 2000) and the ribonuclease P (RNase P) RNA gene (Yoon et al., 1998a) were analyzed in representatives of *Nocardioides*, *Aeromicrobium*, and *Kribbella*. It is worth noting that the 16S–23S ITS and RNase P RNA genes do not necessarily show higher sequence differences between genera than between some *Nocardioides* species. In particular, differences were greater between *Nocardioides albus* and some rod-shaped *Nocardioides* species than between representatives of this and the other genera of the family included in the study. The RNase P RNA gene transfer between organisms of this group can probably take place, as follows from the nearly identical (>99% similarity) sequences of this gene in *Nocardioides jensenii* and *Luteococcus japonicus* (*Propionibacteriaceae*) (Yoon and Park, 2000, 2006). The whole genome has been sequenced for only two representatives of the family, a rod-shaped *Nocardioides* sp. JS614 (Copeland et al., 2006; GenBank accession no. NC_008699) capable of assimilating vinyl chloride and ethene as carbon and energy sources (Coleman et al., 2002; Mattes et al., 2005) and the type strain of *Kribbella flavida*, DSM 17836 (Pukall et al., 2010); GenBank accession no. NC_013729). The genome of *Nocardioides* sp. JS614 is represented by a 4.99-Mb circular chromosome, 91% of which possesses a protein-coding capacity (4645 protein coding genes), and harbors 55 predicted pseudogenes. The strain also contains a circular plasmid with DNA G+C content of 68 mol%, which is lower than that calculated for the genome (71 mol%) of this strain. The *Kribbella flavida* genome, a circular chromosome of 7.58 Mb, has 7086 protein-coding genes, 60 RNA genes, 2 rRNA operons, and 143 predicted pseudogenes.

Ecology and habitats. Bacteria of the *Nocardioidaceae* are widespread in soil and also in other terrestrial and aquatic environments, including sub-zero-temperature, deep subsurface, and nutrient-limited ecosystems, as well as sites polluted by toxic organic compounds (for references and details, see respective generic chapters in this volume). They have also been detected in uranium and nuclear waste-contaminated sites (Desantis et al., 2006; Fredrickson et al., 2004). It has been reported that representatives of the *Nocardioidaceae* from desert top soils, along with other bacteria and fungi, survive long-distance transport through the atmosphere by dust storms (Griffin, 2007; Polymenakou et al., 2008) and occur in urban aerosols (Brodie et al., 2007). It is worth mentioning that the dust flux only from the Saharan-Sahel region to the atmosphere approximates 1 billion tons per year (Moulin et al., 1997) and, accordingly, huge bacterial and fungal mass are transferred through the atmosphere. Cells or spores of some *Kribbella* remained alive in a soil sample after exposure at 120°C (dry heating) for 1 h (Kirby et al., 2006). Thus, presumably members of this family, like many other actinobacteria, can survive environmental hazards, including desiccation, low and high temperatures, oxygen radicals, UV damage, toxic compounds, etc.

Bacteria of the family, like other actinomycetes, are considered to be consumers of organic material in ecosystems. Many organisms, especially of the genus *Nocardioides*, show biodegradative activities, secreting a range of extracellular enzymes and exhibiting the capacity to metabolize recalcitrant and toxic environmental pollutants. They may also use traces of organics or engage in chemolithotrophic metabolism with input from some atmospheric gases and minerals. Organisms of the genus *Kribbella*, along with some other soil bacteria, are suggested to utilize hydrogen at low concentrations (in the plant rhizosphere) and might contribute to the function of soil as a sink in the global hydrogen cycle (Osborne et al., 2010). Members of the *Nocardioidaceae* can be associated with plants and may exist as mutualistic plant endophytes (e.g. Collins et al., 1994; Coombs and Franco, 2003; Coombs et al., 2003; Kaewkla and Franco, 2010b, 2010a; Song et al., 2004; Trujillo et al., 2006). They can also occur in association with algae, lichens, fungi, and other eukaryotic organisms, including warm-blooded animals (e.g. Fall et al., 2007; Gill et al., 2006; Harris et al., 2007; Lauer et al., 2007, Lee and Kim, 2007; Li et al., 2007b; Sfanos et al., 2005; Tóth et al., 2008). They have also been found in the human microbiome (Grice et al., 2008, 2009). All members of the family are considered nonpathogenic to humans, vertebrate animals, and plants, although some representatives can be occasionally detected among members of bacterial populations found in diseased humans and plants (e.g. Filion et al., 2004; Harris et al., 2007; Song et al., 2004).

Taxonomic comments

The family *Nocardioidaceae* (Nesterenko et al., 1985a, 1990), as initially described on the basis of data accumulated by that time, included the genus *Nocardioides* (Prauser, 1976) with the species *Nocardioides albus* (Prauser, 1976), *Nocardioides luteus* (Prauser, 1984b, 1985), *Nocardioides simplex* (O'Donnell et al., 1982, 1983), and related organisms containing LL-A_2pm in the cell wall, i.e. *Pimelobacter jensenii* (Suzuki and Komagata, 1983b, 1983c) and *Arthrobacter tumescens* (Conn and Dimmick, 1947; Jensen, 1934). In the same publication, the authors also proposed to reclassify *Pimelobacter jensenii* Suzuki and Komagata (1983b, 1983c) as *Nocardioides jensenii*, but the name was not validated, and treated *Arthrobacter tumescens* (*Pimelobacter tumescens*, according to Suzuki and Komagata, 1983b, 1983c) as a separate taxon outside the genus *Nocardioides*. In this context, the genus *Pimelobacter* with the species *Pimelobacter simplex*, *Pimelobacter jensenii*, and *Pimelobacter tumescens* was validly described by Suzuki and Komagata (1983b, 1983c) almost simultaneously with the proposal of O'Donnell et al. (1982, 1983) to reclassify *Arthrobacter simplex* (Lochhead, 1957) as *Nocardioides simplex*, and shortly before the publication of Nesterenko and colleagues (Nesterenko et al., 1985a) (see the *Nocardioides* chapter, below, for more details on the taxonomic and nomenclatural history of the organisms).

Subsequent taxonomic re-evaluation of this group, involving the use of 16S rRNA analysis, showed that *Nocardioides albus*, *Nocardioides luteus*, *Pimelobacter jensenii*, and *Pimelobacter simplex* formed a common phylogenetic group, whereas *Pimelobacter tumescens* represented a separate line of descent (Collins et al., 1989). Based on the sequence data which were mainly in accordance with the data on polar lipid and fatty acid composition (Collins et al., 1983; O'Donnell et al., 1982), and the results of phage typing (Prauser, 1976), the authors transferred *Pimelobacter jensenii* to the genus *Nocardioides* and reclassified *Pimelobacter tumescens* in a newly established genus *Terrabacter* (currently within the family *Intrasporangiaceae*; Stackebrandt

et al., 1997). Simultaneously, Collins and Stackebrandt (1989a, 1989b) proposed the species *Nocardioides fastidiosus* which was later transferred (Tamura and Yokota, 1994) to the genus *Aeromicrobium* (Miller et al., 1991). In 1994, another species, *Nocardioides plantarum*, was added to the genus *Nocardioides* (Collins et al., 1994). Thus, the four *Nocardioides* species (*Nocardioides albus*, *Nocardioides luteus*, *Nocardioides simplex*, *Nocardioides jensenii*, and *Nocardioides plantarum*) and two species of *Aeromicrobium* (*Aeromicrobium erythromycini* and *Aeromicrobium fastidiosum*) had been recognized by 1997, when E. Stackebrandt and colleagues (1997) proposed the emended description of the family *Nocardioidaceae* in a paper introducing a novel hierarchic classification scheme of actinobacteria, in which delineation of higher taxa was based on the 16S rRNA gene sequence-based phylogenetic clustering and distribution of signature nucleotides.

The remaining genera, i.e. *Actinopolymorpha*, *Flindersiella*, *Kribbella*, *Hongia*, *Marmoricola*, and *Thermasporomyces* were added to this family during the years 1999–2011 as a result of using a taxonomic strategy based on the polyphasic approach (Collwell, 1970; Stackebrandt, 2006; Vandamme et al., 1996) which integrates genomic and phenotypic, including chemotaxonomic, characteristics and assumes a certain level of their consensus while establishing or revising the genera and delineating their boundaries. Among them, the genera *Kribbella* (Park et al., 1999) and *Hongia* (Lee et al., 2000) were independently published by different research teams to accommodate the phenotypically and phylogenetically very similar mycelial organisms. Later, Sohn et al. (2003) reclassified the only species of the genus *Hongia*, *Hongia koreensis*, as *Kribbella koreensis*, providing strong evidence that the name *Hongia* Lee et al. (2000) is a junior heterotypic synonym of *Kribbella* Park et al. (1999).

The genus *Jiangella* was also assigned at its original description to the family *Nocardioidaceae* (Song et al., 2005). The family affiliation of this genus was accepted by Zhi et al. (2009) who suggested an updated structure and 16S rRNA gene sequence-based definition of higher ranks of the class *Actinobacteria*. The authors, along with other proposals concerned with establishment and emendation of higher taxa, provided the emended description of the family *Nocardioidaceae* (comprising the genera *Nocardioides*, *Actinopolymorpha*, *Aeromicrobium*, *Jiangella*, *Kribbella*, and *Marmoricola*). However, the genus *Jiangella* has recently been transferred from the *Nocardioidaceae* to the newly proposed family *Jiangellaceae*, suborder *Jiangellineae* (Tang et al., 2010), which has been elevated to order *Jiangellales* in the present volume. In addition, two novel genera, *Flindersiella* and *Thermasporomyces*, have been added to the *Nocardioidaceae*. All the above data suggest that the current family *Nocardioidaceae* Nesterenko et al. (1990) emend. Zhi et al. (2009) is in need of re-evaluation and further emendation.

Our recent analyses of 16S rRNA gene sequences using different clustering algorithms and different selections of strains, including type strains of *Flindersiella*, *Thermasporomyces*, and representatives of the family *Propionibacteriaceae*, showed, as already mentioned, that the genera comprising the current family *Nocardioidaceae* form at least two separate phylogenetic clusters (Figure 247) which can be equated with families. The two clusters are approximately equidistant from each other and from the cluster of the family *Propionibacteriaceae*; the grouping appears to be consistent with morphological and chemotaxonomic features of the genera encompassed (Table 214). The first group, the *bona fide Nocardioidaceae* (in general, corresponding to the *Nocardioidaceae* Nesterenko et al. (1990) emend. Rainey et. al. (1997), includes the genera *Nocardioides*, *Aeromicrobium*, and *Marmoricola*. The second group, here provisionally named "*Kribellaceae*" comprises the genera *Kribbella*, *Actinopolymorpha*, *Flindersiella*, and *Thermasporomyces*. Properties shared primarily or exclusively by organisms of the bona fide *Nocardioidaceae* include: (a) relatively simple morphology and developmental cycles (mostly irregular rods to coccoid cells, or occasionally fragmenting substrate hyphae giving rise to scant aerial hyphae); (b) the presence of motile cells; (c) significant proportions of straight-chain saturated and unsaturated fatty acids (and their 10-methyl-branched derivatives); (d) one predominating menaquinone (containing a tetra-hydrogenated side chain with 8 or 9 isoprene units) in the respiratory chain; (e) teichoic acids or other phosphorous-containing cell-wall polymers (*Nocardioides*, *Aeromicrobium*).

In contrast to the *bona fide Nocardioidaceae*, the "*Kribellaceae*" comprises organisms which are characterized by: (a) generally more complex cell morphology reflecting more complex developmental events, manifested in the course of their reproductive cycles; (b) the absence of motile cells; (c) fatty acid profile dominated by iso- and anteiso-branched acids with minor quantity of straight-chain components; (d) more complex menaquinone system (and, therefore the respiratory system as a whole) with several major isoprenologues tending to have longer and more saturated side chains; (e) phosphorous-free acidic polysaccharides in the cell wall, i.e. teichulosonic and teichuronic acids (found in *Kribbella*). In addition, representatives of these two groups have different-sized genomes, i.e. 4.99 Mb (*Nocardioides* sp. JS614) and 7.58 Mb (*Kribbella flavida* DSM 17836), with different numbers of protein-coding genes, 4645 and 7086, respectively (Copeland et al., 2006; Pukall et al., 2010). Thus, it appears reasonable to divide the current family *Nocardioidaceae* into at least two families, to reflect more robust phylogenetic grouping of the genera encompassed and to achieve more focused and practicable family definitions. In this way, the bona fide *Nocardioidaceae* would be restricted to the genera *Nocardioides*, *Aeromicrobium*, and *Marmoricola*, while the family provisionally named "*Kribellaceae*" is suggested to include *Kribbella*, *Actinopolymorpha*, *Flindersiella*, and *Thermasporomyces*. Along with the 16S rRNA gene sequence data used to define actinobacterial families, the data on this and the other actinobacterial families tend to suggest the feasibility of the phenotypic circumscription (at least of selected families at the beginning) within the framework of contemporary taxonomic structure of the class *Actinobacteria* and the taxonomic methods presently available.

In general, the current multi-species genera of the group under consideration are clearly defined by 16S rRNA gene sequence clustering (Figure 247), and the allocation of a novel strain to a certain genus is achievable by determination of full or partial 16S rRNA gene sequences. Exceptions are the genera *Marmoricola* and *Nocardioides*, members of which form a common clade at a periphery of the *Nocardioides* radiation. Additionally, *Nocardioides dubius* and some other species rather occupy a phylogenetic position intermediate between this group and the remaining *Nocardioides* species. Correspondingly, phylogenetic delineation of organisms of the genera *Marmoricola* and *Nocardioides* might be problematic owing to this situation and also to uncertainty with the chemotaxonomic differentiation between these two genera (Table 214). Allocation of novel strains to the genus *Marmoricola* or related *Nocardioides* species

can be achieved via the determination of both the phylogenetic position and chemotaxonomic characteristics (mostly fatty acid composition), as well as by comparison with individual species of these two genera.

It is quite likely that the taxonomic structure of the genus *Marmoricola* will be revised to reflect more focused definition of the inter-generic boundaries between *Marmoricola* and *Nocardioides* with removal of *Marmoricola bigeumensis* from the genus *Marmoricola*. Notably, the establishment of the genus *Marmoricola* was based on the priority of differences in morphology, lipid composition, and several secondary-structure-forming nucleotides between the novel strain and the phylogenetically closest *Nocardioides* species (Urzì et al., 2000). The proposal of *Marmoricola bigeumensis*, in contrast, relied mostly on the result of the 16S rRNA gene-clustering (Dastager et al., 2008b), although the organism has a higher (97%) binary 16S rRNA gene sequence similarity to *Nocardioides jensenii* than to the type species *Marmoricola aurantiacus* and markedly differs from the two *Marmoricola* species described by that time (Lee and Kim, 2007; Urzì et al., 2000) at least in fatty acid type (which is commonly considered to be a chemotaxonomic marker differentiating actinobacterial taxa above species level; Kroppenstedt, 1985; Suzuki and Komagata, 1983a). Recent descriptions of two additional *Marmoricola* species (Lee and Lee, 2010; Lee et al., 2010) and a few related *Nocardioides* species provided additional evidence for the *insertae sedis* status of the species *Marmoricola bigeumensis*.

The data accumulated since the original description of the genus *Nocardioides* (Prauser, 1976) tend to suggest that the taxonomic structure of this genus will be re-evaluated to achieve a more coherent phylogenetic and phenotypic circumscription. The original description of the genus *Nocardioides* (created for mycelium-producing actinomycetes with the peptidoglycan type similar to that of the genus *Streptomyces*, and showing susceptibility to specific actinophages) does not reflect characteristics of organisms subsequently described under the generic name *Nocardioides*. At present the genus comprises phylogenetically distant and phenotypically dissimilar species, including both the mycelium-forming and rod-shaped organisms. The most unrelated species show 16S rRNA gene sequence similarity of ~92–93%, which is equal to or lower than cut-off values separating many well-defined genera in this and other actinomycete families. In addition, the differences between some *Nocardioides* species in the 16S–23S ITS and RNase P gene sequences exceed those found between representatives of different genera (Yoon et al., 1998a; Yoon and Park, 2000). As for chemotaxonomic heterogeneity, some *Nocardioides* species differ from the type species in the phospholipid types (*sensu* Lechevalier et al., 1977) which is usually believed to differentiate genera (Kämpfer, 2006; Kroppenstedt and Evtushenko, 2006; Lechevalier et al., 1977, 1981), and in the polar lipid patterns as a whole (Table 214). Strains of several *Nocardioides* species so far tested differ in the second major polyamine (Busse and Schumann, 1999) and in the nature of the peptidoglycan-bound cell-wall polysaccharides (Shashkov et al., 1999, 2000b; Tul'skaya, 2009), although the taxonomic value of such characteristics in the family *Nocardioidaceae* is in need of further evaluation. On the other hand, the cell-wall peptidoglycan structures, which are well recognized taxonomic markers differentiating actinomycete genera, have not yet been determined for the majority of *Nocardioides* species, and might turn out to be dissimilar in some organisms.

It can be expected that further comprehensive study of phenotypic and genotypic characteristics of organisms currently encompassed by the genus *Nocardioides*, including new relevant isolates, and involving the genomic and proteomic data for focused circumscription, will provide stronger grounds for dividing the current genus *Nocardioides* into several genera. Dissection of the taxonomic structure of this genus is consistent with the recent way that classification schemes have been improved for other phylogenetically heterogeneous actinomycete genera. Phenotypic (chemotaxonomic) characteristics in particular were used to discriminate between incoherent species and establish separate genera (Behrendt et al., 2011; Tamura et al., 2009, and other studies published in IJSEM during the last decade). Dissection is also in line with the recent tendencies to describe species with more homogenous sets of strains and very high 16S rRNA gene sequence similarity (99% and higher) to recognized species. The tendencies, in turn, reflect genome variation among strains within current bacterial species evolving in different ecological settings (Konstantinidis et al., 2006; Konstantinidis and Tiedje, 2005, 2007).

Acknowledgements

This work was supported by the program MCB of the Russian Academy of Sciences.

Genus I. **Nocardioides** Prauser 1976, 61[AL]

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No.car.di.o.i′des. N.L. fem. n. *Nocardia* name of a genus; L. suff. *-oides* (from Gr. suff. *-eides* from Gr. n. *eidos*, that which is seen, form, shape, figure) ressembling, similar; N.L. masc. n. *Nocardioides Nocardia*-like, referring to the similarity of life cycles of the type species of this genus and *Nocardia*.

Abundantly branched vegetative hyphae or irregular rods may be formed in young cultures. The morphogenetic cycle is usually observable, with **different organisms showing more or less complex succession of morphological stages**. The morphogenetic cycle usually starts with the coccoid cells or short rods which may simply germinate into rods or longer filaments, show elementary branching or form extensively branched hyphae on and below the surface of agar media, and may give rise to **aerial mycelium**. The latter consists of irregular, sparsely branching or unbranched hyphae and may totally or partially cover the primary mycelium, or be discernible only microscopically. Both the **vegetative and aerial hyphae and the rod-shaped cells undergo various degrees of fragmentation** (division via septa formation). Fragmentation finally results

in the next generation of short rod-like and coccoid cells. No endospores are formed. **Rod-shaped bacteria may be motile. Gram-stain-positive** type of cell wall. Non-acid-fast. The colony color is mainly **whitish, creamy, or yellow** of different tint and intensity and rarely **orange**. Diffusible pigments are not usually produced. Colonies not covered by aerial mycelium are **mostly pasty**, with **smooth to wrinkled surface**. Chemo-organotrophic, with an oxidative type of metabolism. Predominantly catalase-positive. The level of oxidase activity varies among species. Grows under aerobic conditions on standard laboratory media, including chemically defined (synthetic) media or media with low nutrient concentrations. Certain vitamins or other growth factors may be required. Utilizes a wide range of carbon and nitrogen sources, including unusual organic compounds and toxic environmental pollutants. Mostly mesophilic and neutrophilic; some grow at initial pH values of 5–5.5 and/or 11–12. Mostly non-halophilic, but salt-requiring organisms occasionally occur.

The cell-wall peptidoglycan type is A3γ, with LL-diaminopimelic acid and glycine as the diagnostic amino acids. **Muramic acid is of the acetyl type**. The **cell-wall teichoic acids are present** in most organisms examined. **Menaquinones** are the sole respiratory quinones, the predominant component is **MK-8(H_4)** containing a tetra-hydrogenated side chain with eight isoprene units. **Cellular fatty acids are complex mixtures** of saturated and monounsaturated, straight-chain and iso-, anteiso- and 10-methyl-branched components, including 10-methyl octadecanoic acid (tuberculostearic acid, TBSA), among which **14-methyl pentadecanoic acid ($C_{16:0}$ iso) usually predominates.** Mycolic acids are not present. **The principal phospholipids are typically composed of non-nitrogenous components**, but phosphatidylethanolamine and phosphatidylcholine may occasionally occur. The polyamine patterns usually include **cadaverine as the predominant component**, with putrescine, spermine, or spermidine representing the second major polyamine.

Natural habitats include various terrestrial and aquatic environments. Can be associated with plants, animals, and humans. Some are occasionally found among bacteria associated with human diseases, but considered to be of no relevance to the disease agents.

DNA G+C content (mol%): 67.5 (T_m)–74.8 (HPLC).

Type species: **Nocardioides albus** Prauser 1976, 61[AL].

Further descriptive information

The genus *Nocardioides* belongs to the family *Nocardioidaceae*, order *Propionibacteriales*. Based on the 16S rRNA gene sequence analysis, the species currently comprising this genus form a phylogenetic radiation which is clearly separated from the other genera of the family *Nocardioidaceae*, except for *Marmoricola* (Figure 247 and Figure 248). Several *Nocardioides* species rather occupy intermediate phylogenetic position between the majority of *Nocardioides* species and the genus *Marmoricola* or are intermixed with members of the genus *Marmoricola*. The 16S rRNA gene sequence similarity levels between *Nocardioides* species are quite dissimilar and range from ~92–93 to 99.6%.

Morphology and colony appearance. Bacteria of the genus *Nocardioides* usually display life cycles that most closely resemble those of *Nocardia* or *Arthrobacter*. Information on the mycelium-forming organisms presented in this section is based to a large extent on the works of H. Prauser (Prauser, 1976; Prauser, 1984a; Prauser, 1989).

Visible growth of *Nocardioides albus* and *Nocardioides luteus* and related mycelium-forming organisms is observed within 1–2 d on standard nutrient media. On media rich in organic nitrogen, and in submerged shaken culture, the extent of mycelium development and its persistence are reduced. The hyphae of the primary mycelium are 0.5–0.8 μm in diameter and irregularly septate. Preceding fragmentation, additional septa are formed. Fragmentation of the vegetative hyphae begins in the older parts of the colonies (Figure 249). Depending on the growth media, the elements resulting from fragmentation may be irregular, rod-like, or coccoid (Figure 249 and Figure 250; Prauser, 1989; Suzuki and Komagata, 1983c). The fragments may give rise to new mycelia by extruding one, two, or more hyphae. The irregularly shaped and branched hyphae of the aerial mycelium (Figure 251 and Figure 252) are slightly thicker (0.6–1.0 μm). The aerial hyphae become septate and break up more regularly than those of the primary mycelium. They usually fragment completely into rod- or coccus-like elements that resemble at maturity the surface-smooth conidia (arthrospores) of some streptomycetes (Figure 253 and Figure 254). The spore-like elements germinate by producing one or two germ tubes when transferred to a fresh medium. Motility does not occur at any stage of the life cycle of *Nocardioides albus* and *Nocardioides luteus*. Indeed, the spore-like elements may be unrecognizable *in situ* but observable after mechanical disruption of the mycelium threads. Notably strains may occasionally lose the ability to form aerial mycelium on continued subculture. Both hyphae and fragments show the ultrastructure typical for Gram-stain-positive bacteria (Figure 250 and Figure 254).

The colony appearance of *Nocardioides albus*, *Nocardioides luteus*, and related filamentous organisms is influenced to a large extent by the culture conditions. The colony surface on agar media may be smooth, rough or wrinkled, or covered by aerial mycelium. Depending on the growth medium, individual strain, and the culture age, the aerial mycelium may totally cover the primary mycelium, may be formed only in patches or at the margins of the colonies, may be visible only microscopically or absent. Colonies not covered by aerial mycelium may be dull to bright, but usually are faintly glistening. The colony consistency (mass of the primary mycelium) is mostly pasty, particularly in the center of colonies where hyphal fragmentation begins and in older agar cultures on rich media.

The majority of species currently attributed to the genus *Nocardioides* produce neither primary nor aerial mycelium. Cells of such species are mostly slender irregular rods in young cultures (Table 215; Figure 255, Figure 256, Figure 257, Figure 258, Figure 259A, Figure 261, and Figure 262) that may occur singly, in short chains, in palisades or other side-by-side formations (Figure 258) probably due to adhesive properties. V-forms may be produced. Older cultures of the majority of species predominantly or exclusively consist of coco-bacillary or coccoid cells (Figure 259B). A marked rod-to-coccus morphogenetic cycle resembling that of *Arthrobacter* was reported or suggested for nearly all rod-shaped *Nocardioides* species. Larger club-shaped or spherical forms significantly exceeding the remaining cells in diameter (Figure 257) may be occasionally observable both in young and older cultures (Dastager et al., 2009d; Dastager et al., 2008f, c, 2009e; Jensen, 1934). The cell division proceeds by means of irregular or binary

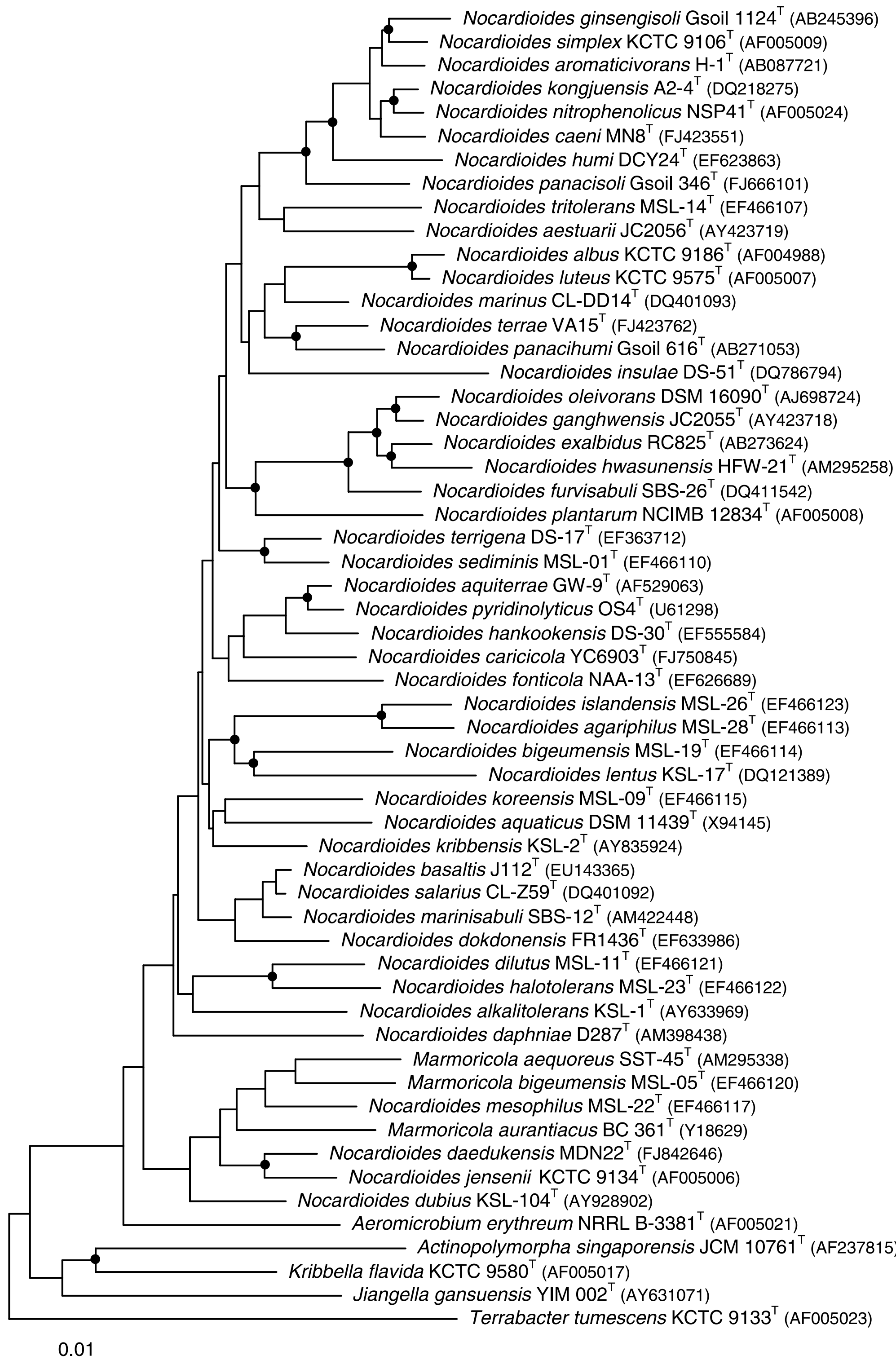

FIGURE 248. Neighbor-joining phylogenetic tree based on 16S rRNA gene sequences showing the positions of *Nocardioides* species and some related taxa. Filled circles indicate that the corresponding nodes were also recovered in the trees generated with the maximum-likelihood and maximum-parsimony algorithms. The bar = 0.01 substitutions per nucleotide position.

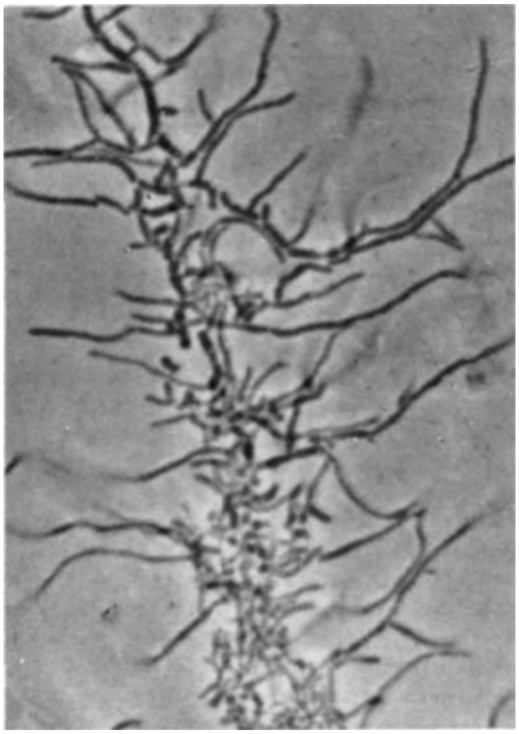

FIGURE 249. *Nocardioides albus* IMET 7807. Fragmentation of hyphae of the primary mycelium *in situ*; 7-d-old culture on glycerol asparagine agar. Phase-contrast micrograph (1600×). (Reprinted from Prauser, 1986. *Bergey's Manual of Systematic Bacteriology*, vol. 2, Williams & Wilkins, Baltimore, pp. 1481–1485.)

FIGURE 250. *Nocardioides albus* IMET 7807. Part of a hypha of the primary mycelium with branches originating from one segment. Beginning of fragmentation of the hypha at the upper right angle (arrow). Electron micrograph (~40,000×). (Reprinted from Prauser, 1986). *Bergey's Manual of Systematic Bacteriology*, vol. 2, Williams & Wilkins, Baltimore, pp. 1481–1485.)

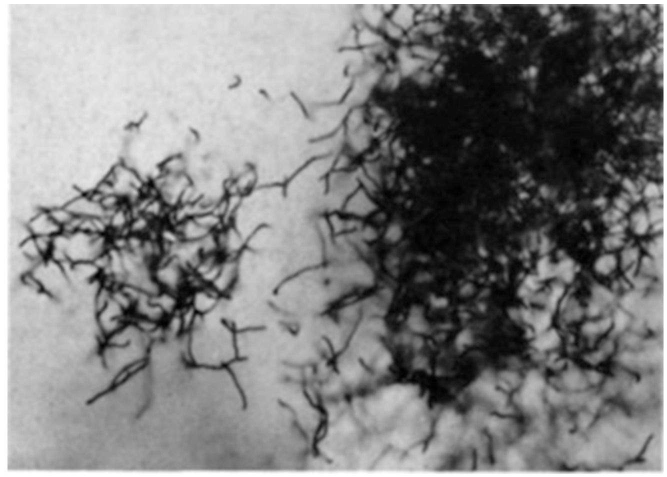

FIGURE 251. *Nocardioides albus* IMET 7807. Aerial mycelium of 11-d-old culture on chitin agar. Phase-contrast micrograph (~400×). (Reprinted from Prauser, 1986. *Bergey's Manual of Systematic Bacteriology*, vol. 2, Williams & Wilkins, Baltimore, pp. 1481–1485.)

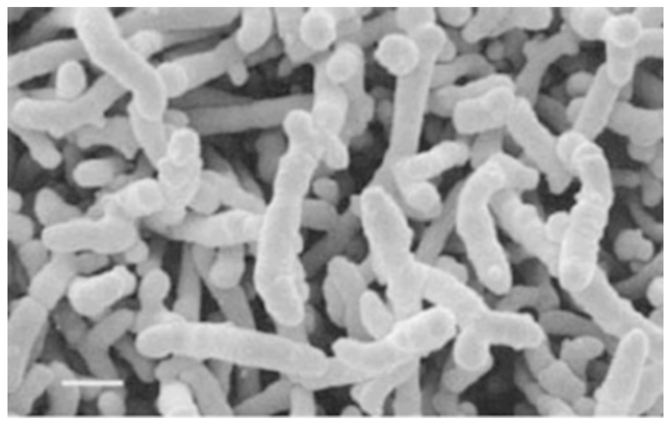

FIGURE 252. Aerial mycelium of *Nocardioides albus* KCTC 9186 on glucose-asparagine agar. Scanning electron micrograph. Bar = 1 µm. (Reprinted from Yoon and Park, 2006. *The Prokaryotes*, 3rd edn, vol. 3, Springer, New York, pp. 1099–1113.)

fission via septa production, so as very short rods or coccoid cells are eventually formed. At the same time, a bud-like mode of cell division may occur. The cell reproduction through constricting was also reported (Kvasnikov et al., 1974). Interestingly, the rod-shaped strain *Nocardioides* sp. JS614, which is the only member of the genus whose complete genome is sequenced, contains a single *ssg* gene that is most likely functionally related to *ssgB* (hypothesized to be essential for septa formation in actinomycetes) (Traag and van Wezel, 2008). The cells may be nonmotile or motile (Table 215) either with single polar, subpolar, or peritrichous flagella (Figure 260, Figure 261, and Figure 262). Colonies of rod-shaped organisms are usually small, circular, convex, or rarely flat, smooth and glistening, with entire margins. They typically are indistinct in color (whitish, light creamy, or yellowish white), with some organisms producing distinct yellow pigments of different tint and intensity; orange-pigmented colonies may occur on some media (Table 215).

Chemotaxonomy. The cell-wall peptidoglycan contains 2,6-LL-diaminopimelic acid (LL-A_2pm), along with alanine, glutamic acid, and glycine. As available for several species (Lawson et al., 2000a; Prauser, 1976; Schippers et al., 2005; Schleifer and Kandler, 1972), the peptidoglycan type is A3γ type *sensu* Schleifer and Kandler (1972); variation A41.1 (http://www.peptidoglycan-types.info). It contains LL-A_2pm in position 3 and L-alanine in position 1 of the tetrapeptide subunit, with a glycine residue forming the interpeptide bridge (like that in the majority of LL-A_2pm-containing actinomycetes). This polymer differs from the

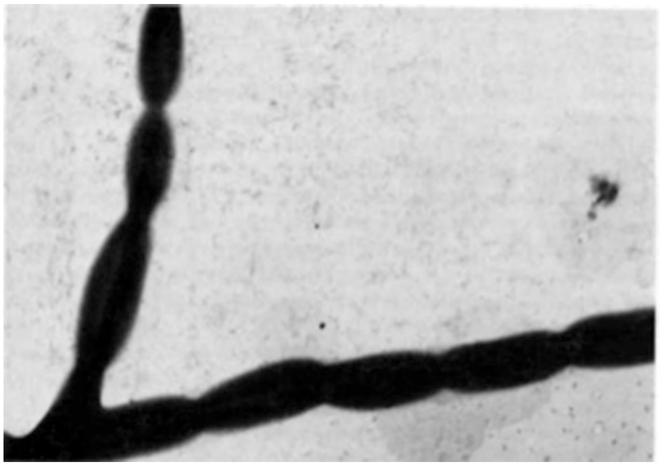

FIGURE 253. *Nocardioides albus* IMET 7807. Aerial hyphae fragmented into spore-like elements; 14-d-old culture on yeast extract-malt extract agar. Electron micrograph (~17,000×). (Reprinted from Prauser, 1986. *Bergey's Manual of Systematic Bacteriology*, vol. 2, Williams & Wilkins, Baltimore, pp. 1481–1485.)

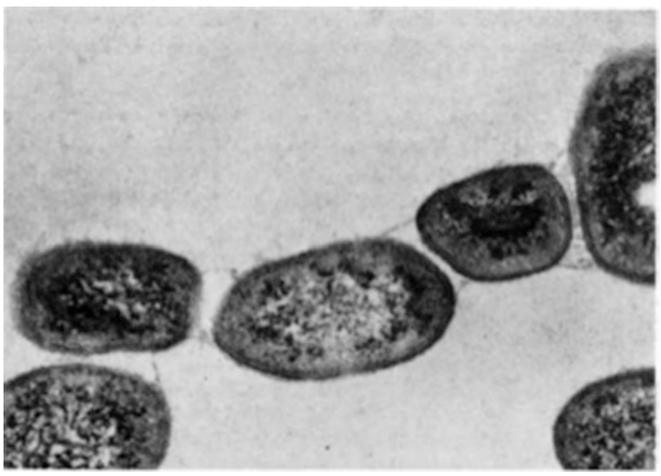

FIGURE 254. *Nocardioides albus*. Developing spore-like elements (arthrospores) still connected by the surface sheath of the aerial hypha. Electron micrograph (~50,000×). (Reprinted from Prauser, 1986. *Bergey's Manual of Systematic Bacteriology*, vol. 2, Williams & Wilkins, Baltimore, pp. 1481–1485.)

LL-A_2pm-based peptidoglycans of some other representatives of the suborder *Propionibacterineae* or the family *Intrasporangiaceae*, which may contain 3 glycine molecules in the interpeptide bridge and glycine or glycine amide at D-glutamic acid of the peptide subunit (type A3γ; variation A41.2), or contain glycine instead of alanine in position 1 of the peptide subunit (type A3γ′; variation A42.1) (Schleifer and Kandler, 1972; Schumann et al., 2009; Stackebrandt and Schaal, 2006; Weon et al., 2007; and the respective chapters in this volume). No isomer of A_2pm was reported for *Nocardioides dilutus*. The acetyl type of the muramyl residues in the cell-wall peptidoglycans was reported for a few *Nocardioides* species so far studied with respect to this characteristic (Kubota et al., 2005a; Uchida and Seino, 1997). The data on cell-wall sugars routinely examined in actinomycetes during taxonomic studies are also available for some species only, namely, *Nocardioides albus*, *Nocardioides luteus*, *Nocardioides simplex*, *Nocardioides jensenii*, and *Nocardioides aquaticus*. These typically include galactose and different combinations of glucose, mannose, and rhamnose (Cummins and Harris, 1959; Keddie and Cure, 1977; Lawson et al., 2000a; Prauser, 1989; Sadikov et al., 1983; Shashkov et al., 2000b; Shashkov et al., 1999; Takeuchi and Yokota, 1989; Tul'skaya, 2009). On the other hand, no monosugars have been revealed in the cell wall of the type strain of *Nocardioides plantarum* (Tul'skaya, 2009).

The cell-wall teichoic acids of different structure were found in strains of *Nocardioides albus*, *Nocardioides luteus*, *Nocardioides jensenii*, *Nocardioides simplex*, and some mycelium-producing organisms of this genus (Evtushenko et al., 1984; Sadikov et al., 1983; Shashkov et al., 2000b; Shashkov et al., 1999; Takeuchi and Yokota, 1989; Tul'skaya, 2009). No phosphorous-containing polymers was revealed in the cell wall of *Nocardioides plantarum* (Tul'skaya, 2009), which, along with the absence of monosugars, is rather indicative of a different kind of the peptidoglycan-linked polymer in this species. The type strain of *Nocardioides albus* possesses a galactosylglycerol phosphate polymer, with repeating units joined by phosphodiester links involving the glycerol C3 and the β-D-galactopyranose C3 atoms. The β-D-galactopyranosyl residues are substituted at C2 with acetate groups and at C4 with β-D-glucopyranose carrying a 4,6 pyruvate ketal group (Shashkov et al., 1999). The type strain of *Nocardioides simplex* was reported to contain a poly(glycerol phosphate) teichoic acid (Sadikov et al., 1983; Takeuchi and Yokota, 1989). Additional sugars (galactose, mannose, glucose, and/or rhamnose) detected by these authors might be involved in the side chains of the polymer(s). The teichoic acid of the type strain of *Nocardioides jensenii* was not identified, but reported to include glycerol, galactose, *N*-acetylglycosamine, and pyruvic acid (Tul'skaya, 2009). Takeuchi and Yokota (1989) revealed galactose and glycerol as predominant components, together with lesser amounts of *N*-acetylglycosamine, glucose, and rhamnose, in a phosphorus-containing polysaccharide fraction of the cell wall of *Nocardioides jensenii*. The type strain of *Nocardioides luteus* and several very closely related strains, including *Nocardioides luteus* ("*Nocardioides flavus*") VKM Ac-2525 (= DSM 46114 = IMET 7844 = J.-S. Ruan, 71-N54) and *Nocardioides* sp. ("*Nocardioides fulvus*") VKM Ac-2526 (= DSM 46115 = IMET 7846 = J.-S. Ruan, 71-N86) have identical 1,5-poly(ribitol phosphate) teichoic acids (Shashkov et al., 1999; Tul'skaya, 2009). All ribitol molecules of these strains are substituted at C4 with α-D-galactopyranosyl residues carrying a 4,6 pyruvate ketal group (Shashkov et al., 2000b; Tul'skaya, 2009). It is noteworthy that regardless of the polymer structure, galactose and pyruvic acid are present in the teichoic acids of all *Nocardioides* strains so far characterized (no precise data are available for *Nocardioides simplex* from early work; Sadikov et al., 1983). High abundance of lipoteichoic acids that, in contrast to the cell-wall teichoic acids, are the membrane-anchored molecules in the cell envelopes, were found in all mycelium-forming *Nocardioides* strains investigated to date (E.M. Tul'skaya and L.I. Evtushenko, unpublished).

The predominant menaquinone in *Nocardioides* species is MK-8(H_4). The data available for some species show that other menaquinones, i.e. MK-6(H_4), MK-7(H_4), MK-8, MK-8(H_2), MK-8(H_6), and MK-9(H_4) may be produced as minor components or in trace amounts (Collins et al., 1994; Collins et al., 1979; Collins et al., 1983; Lawson et al., 2000a; Schippers et al., 2005; Suzuki and Komagata, 1983c; Yamada et al., 1976; Yoon et al., 1997b).

TABLE 215. Descriptive and differential phenotypic characteristics of *Nocardioides* species[a,b]

Characteristic	1. *N. albus*	2. *N. aestuarii*	3. *N. agariphilus*	4. *N. alkalitolerans*	5. *N. aquaticus*	6. *N. aquiterrae*	7. *N. aromaticivorans*	8. *N. basaltis*	9. *N. bigeumensis*	10. *N. caeni*	11. *N. caricicola*	12. *N. daedukensis*	13. *N. daphniae*	14. *N. dilutus*	15. *N. dokdonensis*	16. *N. dubius*
Colony color[c]	White	Ivory	White to cream	White	Cream to dull orange	Cream	White	Cream	Cream	Grayish yellow	White	Yellowish	Yellowish	White to cream	Cream	Yellowish white
Mycelium (M) or rods (R)	M	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
Motility	–	–	+	–	–	+	–	–	+	–	–	–	–	+	–	+
Cell size (μm)[d]	0.5–0.8 (width)	0.3–0.4 × 0.9–2.1	0.4–0.5 × 1.3–3.0	0.8–1.0 × 1.5–2.0	0.9–1.0 × 0.9–1.4	0.8–1.0 × 1.7–2.0	0.5–0.7 × 1.0–2.0	0.7–1.0 × 1.2–2.0	0.3–0.8 × 0.8–4.0	0.3–0.7 × 0.7–2.5	0.4–0.6 × 2.0–5.0	0.4–0.8 × 0.8–3.0	0.8–1.0 × 1.2–2.2	0.4–0.8 × 1.9–4.0	0.6–0.9 × 1.2–1.8	0.8–1.0 × 1.5–2.5
Temperature optimum (°C)	28	30	28	25–30	16–26	30	30	25–30	28	30	30	30	28	26–28	25	30
Temperature range (°C)[e]	15–42	20–35	25–37	4–34	16–26	15–42	22–40	10–37	20–35	10–35	10–45	4–37	4–38	nd	4–30	10–37
NaCl requirement, optimum (%)[f]	–	– (0–2)	–	–	– (1–6)	–	–	+ (1–2)	–	– (0–0.5)	–	– (0–0.5)	–	–	– (0–3)	– (0)
NaCl maximum (%)[g]	8	8	<2	5	15	nd	nd	10	<1	1.0	0.5	9	5	<1	7	5
pH optimum	~7	7	7.5	7–9	7–8	6–7	7	6–7	7.5–9	6.5–7.5	8	7–8	7.5–8.5	7–8	7	7–8
pH range[h]	6–9	6–10	nd	5.0–12	5.5–9.5	nd	5–8	5.5–8	nd	6.0–9.5	7–9	6–10	5.5–10.5	nd	5–10	6–10.5
Catalase	+	+	–	+	+	+	+	+	–	+	+	+	+	nd	+	+
Oxidase	–	–	–	+	–	+	–	–	–	+	–	+	–	nd	–	+
Nitrate reduction	–	–	+	+	+	+	–	–	–	–	+	+	+	+	+	–
Decomposition of:																
Esculin	w	w	–	–	–	v	+	–	–	–	+	+	+	–	–	+
Casein	+	+	nd	+	+	+	+	nd	–	+	+	+	w	+	–	+
DNA	w	+	–	nd	+	+	nd	nd	–	nd	nd	nd	nd	–	–	nd
Gelatin	+	+	–	v	+	+	+	+	–	+	–	+	+	–	–	v
Starch	+	–	–	–	v	v	–	–	–	+	–	–	+	–	–	–
Tween 80	+	+	+	+	+	v	v	–	+	+	+	–	v	+	+	–
Tyrosine	+	–	–	+	+	–	–	–	+	–	–	–	+	+	–	–
Urea	–	–	nd	–	–	–	+	–	–	–	–	–	–	–	+	–
Hypoxanthine	+	–	nd	–	–	–	+	–	nd	–	nd	–	–	–	nd	–
Xanthine	+	–	nd	–	–	v	–	–	nd	–	nd	–	–	–	–	–
Enzymes (API ZYM):																
N-Acetyl-glucosaminidase	–	–	–	.–	.–	–	.–	–	–	–	–	–	.–	–	–	–
Acid phosphatase	–	w	nd	+	+	+	v	–	nd	+	+	–	w	+	+	+
Alkaline phosphatase	+	w	+	+	v	–	+	+	+	+	+	–	w	–	+	+
α-Chymotrypsin	–	+	nd	–	–	–	–	–	nd	–	–	–	–	–	+	–
Cystine arylamidase	–	–	+	–	v	w	v	w	–	w	–	–	–	–	–	–
Esterase (C4)	+	+	+	+	w	–	v	+	+	–	+	–	+	+	+	+
Lipase (C14)	–	–	nd	v	–	–	–	–	nd	–	–	–	w	+	–	–
α-Fucosidase	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
α-Galactosidase	–	–	nd	–	–	–	–	–	nd	–	–	–	–	–	–	–
β-Galactosidase	v	+	–	–	–	v	–	–	–	–	+	–	–	v	–	–
α-Glucosidase	+	+	nd	v	+	+	+	+	nd	+	+	–	–	v	+	+
β-Glucosidase	w	–	+	–	–	w	v	–	+	–	+	–	–	+	–	+

Leucine arylamidase	+	+	nd	v	+	+	+	+	nd	+	+	+	+	*v*	+	+
α-Mannosidase	v	–	nd	–	–	–	–	–	nd	–	–	–	–	–	–	–
Naphthol-AS-BI-phosphohydrolase	w	w	nd	w	–	+	v	+	nd	w	–	w	w	+	+	+
Trypsin	+	+	–	–	w	v	v	w	–	–	+	–	–	–	+	–
Valine arylamidase	–	+	–	–	w	–	v	–	–	w	–	–	w	nd	w	–
Utilization of carbon source:[i]	*ISP9, B-YC*	*B-YC*	*MS1+ GF1*	*ISP9*[j]	*API CH50, B-YC*	*ISP9, B-YC*	*API CH50, MS2+VE*	*API 50 CH*	*MS1+GF1*	*API 50 CH*	*API 20NE*	*ISP9*	*ISP9*[j]	*ISP9*	*YNB+CA*	*ISP9*[j]
L-Arabinose	+	–	nd	v	–	–	+	–	nd	+	–	–	–	–	–	–
Cellobiose	+	+	nd	+	–	+	+	+	nd	+	nd	–	+	+	–	v
D-Fructose	+	+	–	–	+	+	+	–	–	–	nd	–	+	–	+	v
D-Galactose	+	+	–	–	+	+	–	–	–	–	nd	–	–	+	–	–
D-Glucose	+	+	nd	–	+	+	+	+	nd	–	–	–	+	+	–	+
Lactose	v	w	–	–	–	v	–	–	+	–	nd	nd	+	+	–	–
Maltose	+	nd	–	v	nd	+	+	–	–	–	–	–	–	–	–	–
D-Mannitol	+	+	–	–	+	+	+	+	–	–	–	nd	–	+	+	+
D-Mannose	+	–	–	–	–	v	v	–	+	–	–	–	+	+	+	–
Melezitose	–	nd	nd	+	nd	–	–	+	nd	–	nd	nd	nd	nd	+	nd
Melibiose	–	nd	+	–	nd	–	v	–	+	–	nd	nd	–	+	+	–
D-Raffinose	–	w	+	+	–	–	–	–	–	–	nd	nd	–	+	–	–
L-Rhamnose	+	–	–	–	nd	v	+	–	–	+	nd	nd	–	+	–	–
D-Ribose	v	–	–	–	–	v	+	–	–	–	nd	nd	–	–	+	–
Sucrose	+	+	nd	v	+	+	+	+	nd	+	nd	–	+	+	–	v
D-Trehalose	–	nd	nd	+	nd	+	+	+	nd	+	nd	+	nd	nd	+	–
D-Xylose	+	+	nd	v	+	+	+	–	nd	–	nd	–	+	+	w	v
Glycerol	+	w	nd	nd	+	–	v	–	nd	–	nd	nd	nd	nd	+	nd
Inositol	–	–	–	–	–	–	–	–	–	–	nd	nd	+	+	–	–
Inulin	–	–	nd	nd	–	–	–	–	nd	–	nd	nd	nd	nd	–	nd
Salicin	w	+	nd	–	–	–	–	+	nd	–	nd	–	nd	nd	–	w
N-Acetylglucosamine	w	–	nd	–	–	–	–	–	nd	–	–	nd	nd	nd	–	nd
Principal phospholipids[k]	PG, DPG, PI, APG, [PIMs, PI-OH][l,m,n]	nd	DPG, PG	nd	PI, PG, DPG, PL	nd	nd	nd	DPG, PG	nd	nd	DPG, PL, L [PG, PE, PL][n]	DPG, PG PL	DPG, PG	nd	DPG, PG, PE, PI
DNA G+C content (mol%)[p]	68.6 (T_m)	70	69.4	72.4–73.6	69	73	72.0–72.4	68 (T_m)	69.3	71.5	71.7	68.7	69.9	71.8	69.1	70.6
Isolation source	Soil	Tidal flat sediment	Soil	Alkaline soil	Saline lake, Antarctica	Ground water	Polluted environments	Black sand	Soil	Sludge of wastewater	Halophilic plant, *Carex* sp.	Soil	Water flea *Daphnia cucullata*	Soil	Beach sand sediment	Alkaline soil

(continued)

TABLE 215. (continued)

Characteristic	17. *N. exalbidus*	18. *N. fonticola*	19. *N. furvisabuli*	20. *N. ganghwensis*	21. *N. ginsengisoli*	22. *N. halotolerans*	23. *N. hankookensis*	24. *N. humi*	25. *N. hwasunensis*	26. *N. insulae*	27. *N. islandensis*	28. *N. jensenii*	29. *N. kongjuensis*	30. *N. koreensis*	31. *N. kribbensis*	32. *N. lentus*
Colony color[c]	White	Yellowish	Yellow	Ivory	Yellow-white	Cream-white	White	Pale yellow	Yellowish	Ivory	White to cream	Yellowish white	Yellowish white	Cream white	Cream	Yellow
Mycelium (M) or rods (R)	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
Motility	–	–	+	–	–	nd	–	+	–	–	–	–	–	+	–	–
Cell size (μm)[d]	~ 0.5 × 2.0	~ 0.8 × 2.0–9.0	0.4–0.5 × 0.6–1.2	0.4–0.5 × 0.9–4.5	0.2–0.4 × 0.8–1.2	~ 0.6 × 1.2–3.4	0.4–0.8 × 1.5–10.0	0.3–0.5 × 0.8–1.0	0.4–0.7 × 1.0–1.7	0.6–1.0 × 1.3–6.0	0.5–0.6 × 1.0–3.7	0.6–0.8 × 3.0–7.0	0.4–0.7 × 0.8–3.0	0.4–0.6 × 0.8–2.0	0.8–1.0 × 1.5–2.0	0.4–0.7 × 1.0–4.5
Temperature optimum (°C)	30	30	30	30	30	28	25–28	30–37	30	30	28	28	30–37	30	30	28
Temperature range (°C)[e]	15–35	25–37	4–37	10–40	15–37	nd	10–34	25–42	4–37	10–34	nd	18–37	10–40	27–37	4–35	4–34
NaCl requirement, optimum (%)[f]	–	+ (0.5–1)	–	– (0–1)	–	– (3)	– (0–0.5)	nd	–	– (0)	nd	–	– (0)	–	–	– (0.5)
NaCl maximum (%)[g]	nd	1	6	8	5	10	2	nd	4	3	7	7	5	5	3	5
pH optimum	~7	7–8	~7	7	7	7–8	6–7	~7	~7	8	7	7–9	7–8	7–8	9	8
pH range[h]	~6–9	5–9	5.1–10.1	6–10	5–8.5	nd	5.5–8.0	5–11	5–9	6.5 – >8	5–12	nd	5.5– >8	nd	6–11	6.5–9.5
Catalase	+	+	+	+	+	–	+	–	+	+	–	+	+	–	+	+
Oxidase	–	–	–	–	–	–	+	+	–	+	–	–	+	–	+	W
Nitrate reduction	–	–	+	+	–	+	–	–	–	+	–	+	–	–	+	+
Decomposition of:																
Esculin	–	+	–	w	nd	–	+	+	–	–	–	–	–	–	+	–
Casein	nd	+	+	+	nd	–	+	nd	–	+	c	+	+	nd	+	+
DNA	nd	+	+	+	+	nd	nd	nd	nd	nd	nd	+	nd	–	nd	nd
Gelatin	nd	+	–	+	+	nd	+	–	v	+	–	+	+	–	+	+
Starch	–	+	+	+	nd	v	+	nd	+	–	+	–	–	+	–	–
Tween 80	nd	+	–	+	nd	v	w	+	nd	+	+	+	+	+	+	+
Tyrosine	nd	nd	–	+	nd	+	v	nd	–	–	–	+	–	+	+	v
Urea	–	–	–	–	v	+	–	–	–	–	–	+	–	nd	–	–
Hypoxanthine	nd	nd	–	–	nd	nd	–	nd	–	–	–	+	–	nd	–	–
Xanthine	nd	nd	–	w	nd	nd	–	nd	–	–	–	+	–	nd	–	–
Enzymes (API ZYM):																
N-Acetyl-glucosaminidase	nd	–	–	–	nd	–	–	–	–	–	–	–	–	–	–	–
Acid phosphatase	w	+	–	w	nd	+	+	+	w	+	+	v	+	nd	+	+
Alkaline phosphatase	+	+	+	+	nd	+	+	+	+	+	+	w	+	+	+	+
α-Chymotrypsin	nd	–	–	–	nd	–	–	+	–	–	–	–	–	nd	+	–
Cystine arylamidase	w	–	–	w	nd	c	v	+	–	–	–	–	–	–	–	–
Esterase (C4)	w	+	–	v	nd	+	+	+	w	+	–	w	v	+	+	+
Lipase (C14)	–	–	–	–	nd	–	–	–	–	–	–	–	–	nd	–	–
α-Fucosidase	–	–	–	–	nd	–	–	–	–	–	–	–	–	–	–	–
α-Galactosidase	+	–	w	+	nd	–	–	–	nd	–	–	–	–	nd	–	–
β-Galactosidase	+	+	+	+	–	+	v	+	–	–	–	–	–	–	w	–
α-Glucosidase	+	+	–	+	nd	+	–	+	w	–	v	v	+	nd	+	–

β-Glucosidase	w	+	−	−	+	+	−	+	nd	−	v	−	v	+	−	+
Leucine arylamidase	+	+	+	+	nd	nd	+	+	+	−	+	+	+	nd	+	+
α-Mannosidase	−	−	−	−	nd	−	−	−	−	−	−	−	−	nd	−	−
Naphthol-AS-BI-phosphohydrolase	+	+	−	−	nd	+	+	+	−	−	+	v	+	nd	+	+
Trypsin	−	−	−	−	nd	−	v	+	−	−	−	v	v	−	−	−
Valine arylamidase	+	−	w	+	nd	−	−	+	+	−	−	v	v	−	−	−
Utilization of carbon source[i]*:*	*API CH50*	*API CH50*	*ISP9*	*B-YC, ISP9*	*MS2+VE*	*ISP9*	*API CH50*	*API 20NE, API 32GN*	*ISP9*	*API 20E*	*ISP9*	*ISP9, B-YC*[j]	*ISP9, API 50 CH, MS2+VE*	*MS1+ GF1*	*ISP9*	*ISP9*
L-Arabinose	−	+	+	+	c	−	+	−	v	−	−	−	v	nd	−	w
Cellobiose	−	+	+	+	nd	−	+	nd	+	+	v	−	−	nd	+	+
D-Fructose	+	+	+	+	+	−	+	nd	+	−	−	v	v	−	−	−
D-Galactose	−	+	+	+	nd	+	+	nd	+	+	−	−	−	−	+	+
D-Glucose	+	+	+	+	+	+	+	+	+	+	+	+	+	nd	+	+
Lactose	−	nd	+	+	nd	+	−	nd	−	nd	+	−	−	+	−	v
Maltose	−	−	+	+	+	−	+	−	+	w	−	v	−	−	+	+
D-Mannitol	+	nd	+	+	+	+	+	+	+	nd	−	−	−	−	+	+
D-Mannose	−	+	+	+	−	+	−	−	+	w	v	−	v	+	−	−
Melezitose	−	nd	−	nd	nd	nd	−	nd	−	nd	nd	nd	−	nd	−	+
Melibiose	+	nd	−	+	−	nd	+	+	−	nd	+	−	−	+	−	−
D-Raffinose	−	nd	+	+	nd	+	−	nd	−	nd	−	−	−	−	+	+
L-Rhamnose	+	nd	−	v	−	+	+	+	+	nd	−	+	v	−	+	+
D-Ribose	−	nd	−	−	−	−	−	+	−	nd	−	−	−	−	+	−
Sucrose	+	−	−	+	+	nd	+	+	+	−	+	+	+	nd	+	+
D-Trehalose	−	−	+	+	nd	+	+	nd	+	+	+	+	+	nd	+	+
D-Xylose	−	+	+	+	+	+	+	nd	+	w	+	v	−	nd	−	v
Glycerol	−	nd	+	+	nd	nd	−	nd	−	nd	nd	w	−	nd	nd	nd
Inositol	−	nd	w	−	−	+	−	−	−	−	−	−	−	−	−	−
Inulin	−	nd	+	−	−	nd	−	nd	+	nd	nd	−	−	nd	nd	nd
Salicin	−	nd	−	+	−	nd	−	−	+	w	nd	−	−	nd	nd	nd
N-Acetylglucosamine	−	nd	nd	+	v	nd	−	−	nd	nd	nd	−	−	nd	nd	nd
Principal phospholipids[k]	DPG, PI	nd	PG, PC, PI, PL	nd	nd	DPG, PG, PL	nd	DPG, PG, PL	DPG, PI PG, PL	nd	DPG, PG, PL	DPG, PG, PI, OH-PG	nd	DPG, PG, PL	nd	nd
DNA G+C content (mol%)[p]	74	71.8	69.1	72	70.2	69.7	71.3	71.0	71.1–72.2	71.1	71.4	68.8 (T_m)	72.1	69.9	73–74	74.6–74.8
Isolation source	Lichen	Freshwater from spring	Beach sand	Tidal flat	Soil	Agricultural soil	Soil	Soil	Sea water	Soil	Agricultural soil	Soil	Soil	Soil	Alkaline soil	Alkaline soil

(continued)

TABLE 215. (continued)

Characteristic	33. *N. luteus*	34. *N. marinisabuli*	2.515 pt	36. *N. mesophilus*	37. *N. nitrophenolicus*	38. *N. oleivorans*	39. *N. panacihumi*	40. *N. panacisoli*	41. *N. plantarum*	42. *N. pyridinolyticus*	43. *N. salarius*	44. *N. sediminis*	45. *N. simplex*	46. *N. terrae*	47. *N. terrigena*	48. *N. tritolerans*
Colony color[c]	Yellow to orange	Pale yellow	Creamy white	Cream whitish	Yellowish white	Orange	None or grayish	Yellowish white	White	Cream	Creamy white	Cream whitish	Yellowish white	Cream	Ivory	Cream
Mycelium (M) or rods (R)	M	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
Motility	–	–	–	+	+	–	–	–	–	+	–	+	+	–	–	+
Cell size (μm)[d]	0.5–1.0, width	0.6–0.8 × 1.4–2.1	0.4–0.6 × 1.0–1.8	0.3–0.8 × 0.9–1.4	0.5–0.8 × 1.0–3.0	~ 0.3 × 1.1	0.3–0.5 × 0.7–1.2	0.2–0.4 × 0.8–1.2	0.4–0.7 × 0.8–2.4	0.5–0.6 × 1.2–1.6	0.3–0.6 × 0.6–1.6	0.3–0.4 × 0.9–1.4	0.5–0.7 × 1.5–3.0	0.2–0.5 × ~ 1 or more	0.4–0.7 × 0.7–2.0	0.3–0.5 × 0.8–4.0
Temperature optimum (°C)	28	30	25–30	28	30	30	30	30	25	35	25–30	28	26–30	29	30	28–30
Temperature range (°C)[e]	20–45	4–40	10–40	20–37	15–40	25–30	15–30	10–42	<37	20–40	10–35	20–37	10–37	16–34	4–35	20–40
NaCl requirement, optimum (%)[f]	–	–	+ (1–3)	nd	–	–	–	+(2)	–	–	+(3)	nd	–	0–0.25	–(0.5–1)	–
NaCl maximum (%)[g]	8	8	8	nd	nd	>2	1	<3	<5	nd	10	7–7.5	4–5	1	3	7
pH optimum	~7	7–8	7–8	7–7.5	8	7	7	7	~7	8	6–7	nd	~7	6.2–6.5	8	7–7.5
pH range[h]	5–9	5–12	6–9	nd	6–10	nd	5–8	5.5–8.5	nd	5–9	6–10	nd	nd	5.5–8.5	nd	6–12
Catalase	+	+	w	+	+	+	nd	+	+	+	+	+	+	nd	+	–
Oxidase	v	–	–	–	v	–	nd	+	–	–	–	–	+	–	+	–
Nitrate reduction	–	–	–	–	–	–	w	+	–	+	–	+	–	+	+	c
Decomposition of:																
Esculin	+	+	+	–	+	+	+	nd	w	+	+	–	+	+	–	–
Casein	+	+	+	+	+	+	+	–	v	+	+	+	+	+	+	+
DNA	+	nd	nd	nd	+	+	+	nd	–	+	nd	nd	+	nd	nd	–
Gelatin	+	–	–	–	+	–	+	+	+	+	+	+	+	–	+	–
Starch	+	w	–	+	v	–	–	–	–	+	w	+	v	–	w	+
Tween 80	+	–	+	–	+	–	nd	nd	+	–	+	+	+	–	w	+
Tyrosine	+	–	–	+	+	–	nd	nd	–	+	+	+	+	nd	–	+
Urea	–	–	–	–	v	–	–	–	–	–	–	–	–	–	–	–
Hypoxanthine	+	–	–	–	–	–	nd	nd	–	–	–	–	–	nd	–	–
Xanthine	v	–	–	–	–	–	nd	nd	–	+	–	–	–	nd	–	+
Enzymes (API ZYM):																
N-Acetyl-glucosaminidase	–	–	–	–	–	–	+	–	–	–	–	–	–	+	–	–
Acid phosphatase	–	–	w	+	+	–	+	+	w	+	+	–	w	+	–	+
Alkaline phosphatase	v	+	+	+	+	+	+	+	–	+	+	+	+	+	+	+
α-Chymotrypsin	–	–	–	–	–	–	–	+	–	–	+	–	–	–	–	–
Cystine arylamidase	–	–	–	–	w	w	w	–	+	–	+	–	w	–	–	+
Esterase (C4)	+	–	w	+	–	w	+	–	+	–	+	+	–	–	+	+
Lipase (C14)	–	–	–	–	–	–	–	–	w	–	–	–	–	–	–	–
α-Fucosidase	–	–	–	–	–	–	+	–	–	–	–	–	–	–	–	–
α-Galactosidase	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–

β-Galactosidase	v	–	–	–	–	w	–	–	–	–	–	–	–	+	–	–
α-Glucosidase	v	+	+	–	+	+	+	+	+	+	+	–	+	+	+	+
β-Glucosidase	–	–	–	–	v	–	+	–	+	–	–	–	v	–	–	–
Leucine arylamidase	+	+	+	+	+	+	+	–	+	+	+	+	+	+	+	+
α-Mannosidase	+	–	–	–	–	–	+	–	–	–	–	–	–	–	–	–
Naphthol-AS-BI-phosphohydrolase	v	–	–	+	+	v	–	+	+	+	+	–	v	+	+	nd
Trypsin	+	–	+	–	+	–	+	+	–	+	+	–	v	+	–	–
Valine arylamidase	–	–	w	–	+	+	w	–	w	w	+	–	w	–	–	+
Utilization of carbon source:[j]	*ISP9, B-YC*	*ISP9*	*API 20 NE*	*ISP9*	*ISP9, B-YC, API 50 CH, MS2+VE*	*MS1+ GF2*	*API 20NE*	*MS2+VE*	*MS4+GF3, B-YC*	*YNB+CA, B-YC*	*MS3+YE*	*ISP9*	*ISP9, B-YC, API 50 CH, MS2+VE*	*API 20 NE*	*API CH 50*	*LBM*
L-Arabinose	+	–	–	–	v	–	–	–	–	–	–	–	–	–	–	–
Cellobiose	+	–	nd	+	–	+	–	–	+	+	+	+	–	nd	+	+
D-Fructose	v	+	+	–	+	+	+	+	+	+	–	+	–	nd	+	–
D-Galactose	–	+	+	–	–	+	–	nd	–	v	–	+	–	nd	+	+
D-Glucose	+	–	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lactose	–	–	–	–	–	+	nd	nd	–	v	–	+	–	nd	+	+
Maltose	+	v	nd	–	v	+	+	+	+	+	nd	+	v	+	+	–
D-Mannitol	+	–	+	+	v	+	–	–	–	v	+	+	v	+	+	+
D-Mannose	+	–	–	–	v	+	–	–	–	v	–	v	v	–	–	+
Melezitose	nd	nd	nd	nd	–	nd	nd	nd	+	+	nd	–	–	nd	–	nd
Melibiose	nd	nd	nd	–	v	+	–	–	nd	–	nd	+	–	nd	+	+
D-Raffinose	–	–	–	+	–	nd	+	nd	–	–	–	–	v	nd	–	+
L-Rhamnose	v	–	+	–	+	+	+	–	+	+	–	+	–	nd	+	+
D-Ribose	–	–	–	–	+	–	–	–	–	+	+	–	v	nd	–	–
Sucrose	w	–	+	+	+	+	+	+	+	+	+	+	+	nd	+	+
D-Trehalose	nd	+	+	nd	+	+	–	nd	+	+	+	+	+	nd	+	nd
D-Xylose	+	+	+	+	+	–	+	–	+	+	–	+	–	nd	+	+
Glycerol	w	–	–	nd	–	–	nd	–	+	–	–	–	–	nd	–	nd
Inositol	–	–	–	+	–	–	nd	–	–	+	–	+	–	nd	–	+
Inulin	–	+	nd	nd	–	–	nd	nd	–	–	nd	–	–	nd	–	nd
Salicin	–	–	nd	nd	v	–	+	nd	+	–	–	–	–	nd	–	nd
N-Acetylglucosamine	+	–	nd	nd	–	+	nd	–	–	–	nd	nd	–	–	nd	nd
Principal phospholipids[k]	PG, DPG, PI, APG, PL [PIMs][m,n,o]	PG, PI	nd	nd	nd	nd	nd	PG, PI	nd	nd	nd	nd	DPG, PG, PI, PL, [OH-PG][n]	nd	nd	PG, DPG
DNA G+C content (mol%)[p]	68 (T_m)	73.1	72.9	68.7	71.4	nd	73	73	69(T_m)	72.5	73.3	71.5	71.7–73.5	71.6 (T_m)	71.5	67.6
Isolation source	Soil	Beach sand	Sea water	Soil	Industrial wastewater	Crude oil	Soil	Soil	Herbage	Oil shale column	Zooplankton enriched sea water	Sediment	Soil	Soil	Soil	Soil

(continued)

TABLE 215. (continued)

[a]Symbols and abbreviations: +, positive; −, negative; w, weakly positive; v, variable results (between strains, different experiments or the test methods), or conflicting data; nd, not determined. DPG, diphosphatidylglycerol; PG, phosphatidylglycerol; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PI, phosphatidylinositol; PI-OH, phosphatidylinositol apparently containing hydroxylated fatty acids; OH–PG, hydroxy phosphatidylglycerol; PL, unidentified phospholipids; L, unidentified lipids; APG, acylphosphatidylglycerol; PIMs, phosphatidylinositol mannosides. Data are from An et al. (2007), Cho et al. (2010), Choi et al. (2007), Chou et al. (2008), Cui et al. (2009), Collins et al. (1994, 1989, 1979, 1983), Dastager et al. (2009a, 2008d, 2008a, 2008e, 2008f, 2008c, 2009e, 2010), Jones and Collins (1986), Kim et al. (2008a), Kim et al. (2009a, 2009b), Kubota et al. (2005a), Lawson et al. (2000), Lee (2007b), Lee et al. (2007), Lee et al. (2008), Li et al. (2007b), O'Donnell et al. (1982), Park et al. (1999), Park et al. (2008), Prauser (1976, 1984a, 1989), Schippers et al. (2005), Song et al. (2011), Suzuki and Komagata (1983c), Tóth et al. (2008), Yano et al. (1970, 1971), Yi and Chun (2004b, 2004a), Yoon et al. (2008, 2007a, 1999, 2007b, 2009, 2004, 2005a, 2005b, 2005d, 2006a, 2006b, 2010, 1997b), Zhang et al. (2009a) and recent observations.

[b]See also the tree (Figure 248) for phylogenetic position of species and Table 216, Table 217, Table 218, and Table 221 for characteristics of some species groups.

[c]Differences in the color intensity and shade may be influenced by growth conditions and culture age (see also the species description section for more details). For *Nocardioides albus* and *Nocardioides luteus*, the color of vegetative mycelium is indicated.

[d]Cell morphology and size as usually observable in young cultures; some differences in the cell size may be caused by different growth conditions. In older cultures, cells as a rule become shorter and often consist predominantly or exclusively of coccoid or coccobacillary forms (see the species descriptions for details).

[e]The temperature range in which the growth was registered; the actual growth temperature range may be broader for some species (see the species descriptions for further details).

[f]Optimal NaCl concentrations (%, w/v) where available are indicated in parentheses. Zero indicates that addition of NaCl to culture medium is not required for growth, but minor or trace amounts of NaCl may be present. The salinity level may slightly differ depending on the test conditions (see the species descriptions for further details).

[g]Maximal NaCl concentration for growth as registered in the test media used (see the species descriptions for further details); the values may be influenced by the culture medium composition.

[h]Initial pH of test media, with exception of data for *Nocardioides fonticola*; the pH range values may slightly vary depending on the test conditions.

[i]Data on utilization of carbon sources were obtained using different methods, and, therefore, the results presented may not be comparable in some cases. Basal media used in conventional tests to monitor the growth on carbon sources are as follows: *ISP9* (Shirling and Gottlieb, 1966); *B–YC*, basal mineral salts medium (Baumann et al., 1972), supplemented with 2% (v/v) Hutner's mineral base (Cohen-Bazire et al., 1957) and modified by reducing the concentration of sea salts to half-strength (Yi and Chun, 2004a); *LBM*, Leifson's basal medium (Leifson, 1963); *MS1+GF1*, mineral salts medium supplemented with the following growth factors (GF): yeast extract, Oxoid (0.02 g/l), bio-Lactysat, bioMérieux (0.02 g/l), a vitamin solution and a trace element solution (Kämpfer et al., 1990); *MS1+GF2*, the same test medium, but bio-Lactysat is replaced by peptone (Merck) (0.02 g/l) (Kämpfer et al., 1991; Schippers et al., 2005); *MS2+VE*, mineral salts medium supplemented with vitamins and element solution (Cui et al., 2009); *MS3+YE*, mineral salts medium with 0.05 (g/l) of yeast extract (Kim et al., 2008a); *MS4+GF3*, basal test medium containing (per liter) mineral base E (Owens and Keddie, 1969), 0.2 g of Bacto Yeast Extract (Difco), 12 g of agar, 2 µg of vitamin B_{12}, 10 mg of sodium glutamate, and 10 mg of methionine (Collins et al., 1994); *YNB+CA*, Bacto Yeast Nitrogen Base without amino acids (Difco), modified by the addition of 10 mg/l Casamino acids (Difco), and agar (Stevenson, 1967).

[j]Data obtained with the Biolog GP2 test system are displayed in Table 217.

[k]Some differences in quantitative and qualitative composition of phospholipids may be influenced by growth conditions, the culture age and analytical procedure (see the *Further descriptive information* section for more details).

[l]Data from Collins at al. (1983), Lechevalier at al. (1981, 1977), and O'Donnell et al. (1982). Lechevalier et al. (1981, 1977) identified PG, PI, APG, and traces of PIMs (see also the *Further descriptive information* section and Figure 263).

[m]Glycolipids may be detected among principal polar lipids (see the *Further descriptive information* section and Figure 263).

[n]Polar lipids detected in minor amounts are given in square brackets.

[o]*Nocardioides luteus* is generally similar to *Nocardioides albus* in the principal polar lipids (see the *Further descriptive information* section and Figure 263).

[p]DNA base composition as determined by direct quantification of nucleosides by the HPLC method, unless indicated; data for *Nocardioides simplex* were estimated by the thermal denaturation method, with the HPLC-based method for the type strain.

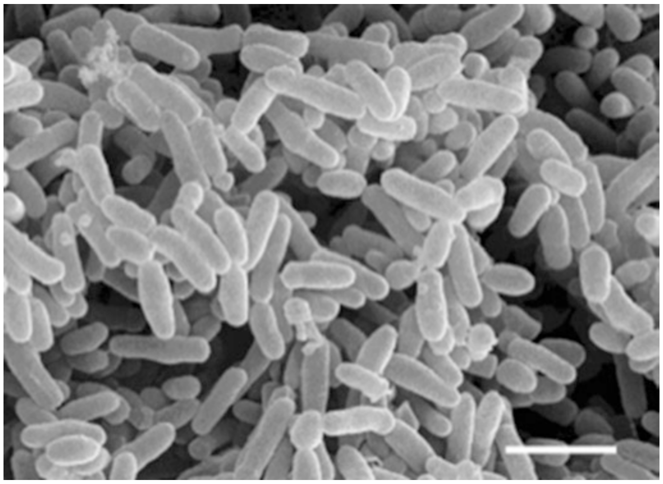

FIGURE 255. *Nocardioides koreensis* MSL-09, 7-d-old culture on R2A agar (28°C). Scanning electron micrograph. Bar = 2 μm. (Reproduced with permission from Dastager et al., 2008d. Int. J. Syst. Evol. Microbiol. *58*: 2292–2296.)

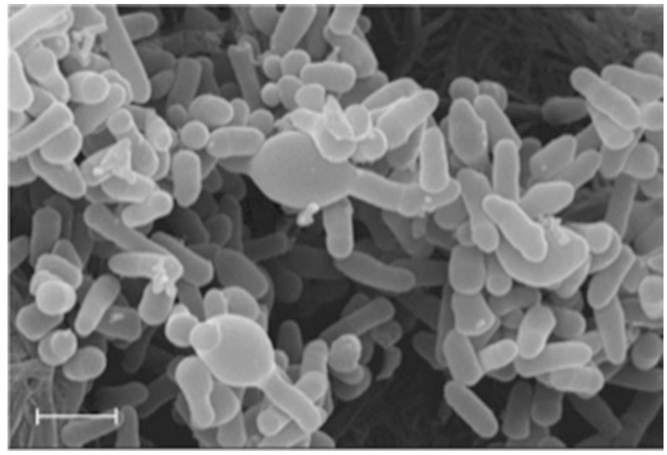

FIGURE 257. *Nocardioides tritolerans* MSL-14; 7-d-old culture grown on R2A agar at 28°C. Scanning electron micrograph. Bar = 1.0 μm. (Reproduced with permission from Dastager et al., 2008c. J. Microbiol. Biotechnol. *18*: 1203–1206.)

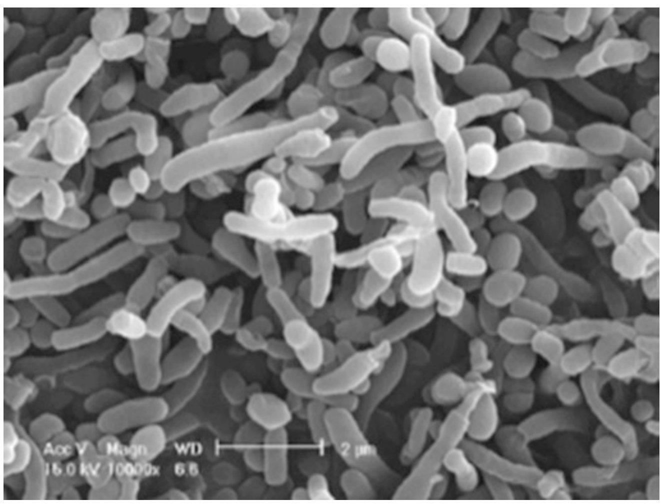

FIGURE 256. *Nocardioides caricicola* YC6903; 5-d-old culture grown in half-strength R2A broth at 30°C. Scanning electron micrograph. Bar = 2 μm. (Reproduced with permission from Song et al., 2011. Int. J. Syst. Evol. Microbiol. *61*: 105–109.)

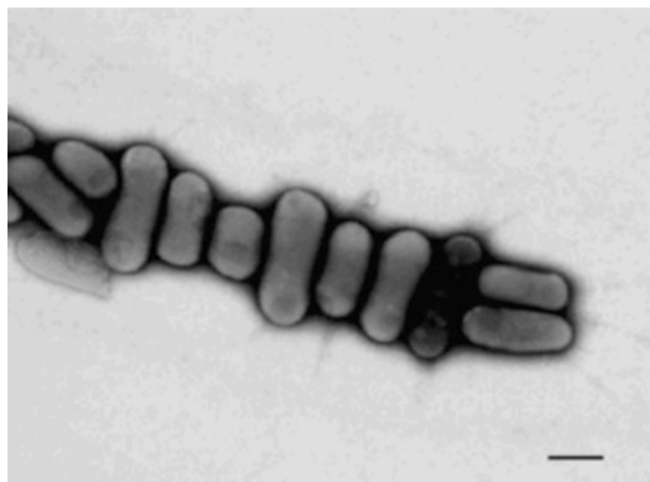

FIGURE 258. Palisade-arranged cells of *Nocardioides furvisabuli* SBS-26; 3-d-old culture grown at 30°C on ISP 2 medium prepared with 60% (v/v) natural seawater. Negative stain; transmission electron microscopy. Bar = 0.5 μm. (Reproduced with permission from Lee, 2007b. Int. J. Syst. Evol. Microbiol. *57*: 35–39.)

The cellular fatty acid profiles of *Nocardioides* species contain complex mixtures of saturated and monounsaturated, straight-chain and iso-, anteiso- and 10-methyl-branched acids, including 10-methyl-octadecanoic acid (tuberculostearic acid, TBSA), as well as 2-OH and 3-OH hydroxylated acids. In addition, minor quantities of ω-alicyclic fatty acids were reported (Song et al., 2010). The growth (developmental) phase, growth conditions, e.g. temperature, pH, oxygen supply, and growth medium composition (carbon sources, an excessive supply of primer sources) and consistency (solid, liquid), along with analytical procedure, may considerably influence the proportions of principal components or the components considered to be diagnostic for this genus (see Kaneda, 1991; Kroppenstedt, 1985; Suzuki and Komagata, 1983a; Suzuki et al., 1993; Yano et al., 1970, 1971); and also the species descriptions in this chapter). The majority of the data on fatty acids for *Nocardioides* species were obtained using the procedure of Sasser (1990) to extract methyl esters and then gas chromatography analysis. Many recent data were obtained using an automated MIDI system consisting of a Hewlett Packard model 5890 capillary gas chromatograph and a computer with specific software (Microbial ID, Inc., Newark, DE) to automatically identify and quantify the fatty acids.

Comparison of data on fatty acids determined in some *Nocardioides* cultures of different age grown on Nutrient agar (Difco) at 30°C for 4 and 7 d (Yoon et al., 1997a; Yoon et al., 2010) and in some corynebacteria (Suzuki and Komagata, 1983a) demonstrated that the content of TBSA and its homologs increases as the cells aged. On the other hand, the content of 10-methyl acids plus their biosynthetic precursors (respective monoenoic acids) in some analyzed bacteria is rather constant, at least throughout 120 h of cultivation (Suzuki and Komagata, 1983a). Correspondingly, the total contents of these fatty acids

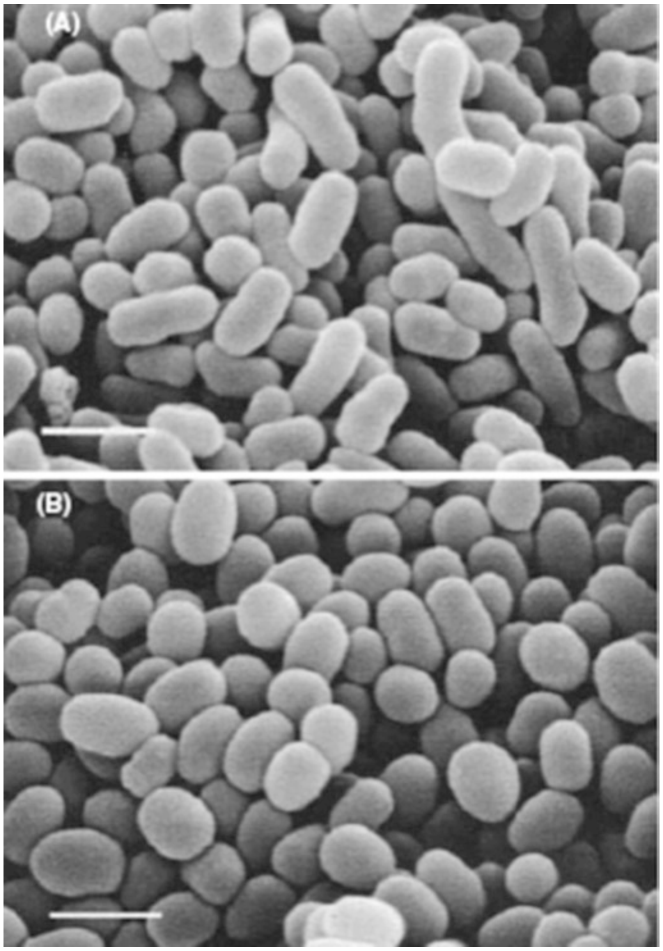

FIGURE 259. *Nocardioides pyridinolyticus* KCTC 0074BP; 3-d-old (A) and 7-d-old (B) cultures on nutrient agar. Scanning electron micrographs. Bar = 1 μm. (Reprinted from Yoon and Park, 2006. *The Prokaryotes*, 3rd edn, vol. 3, Springer, New York, pp. 1099–1113.)

(and other biosynthetically related acids) should be taken into account when comparing the cellular fatty acid compositions of organisms and drawing conclusions about the fatty acid type. In addition, some *Nocardioides* strains grown at higher temperatures usually exhibit a larger proportion of saturated acids (Song et al., 2011; Suzuki and Komagata, 1983a; Yoon et al., 2008). The content of saturated and unsaturated fatty acids may be influenced by the consistency of the medium (liquid, solid) and the extent of aeration. Nevertheless, irrespective of the culture conditions and analytical procedure, the 14-methylpentadecanoic acid ($C_{16:0}$ iso) predominate in most cases and may reach up to 65–82% (Choi et al., 2007; Kim et al., 2008a; Kim et al., 2009a; Schumann et al., 1997; Yoon et al., 2006a). A substantially larger proportion of $C_{16:0}$ iso (65–70%) might be a chemotaxonomic marker of some species, as demonstrated by the data on the phylogenetically closest relatives *Nocardioides salarius* (Kim et al., 2008a) and *Nocardioides basaltis* (Kim et al., 2009b). Similar profiles were obtained independently by different research teams for cultures of different age (1 and 3 d) grown on marine agar (MA; Difco) at 30°C. Very high content of $C_{16:0}$ iso (71.5%) was also reported for *Nocardioides marinus* (MA agar, 30°C, 1 d), also isolated from sea water but phylogenetically distant from the above species (Choi et al., 2007).

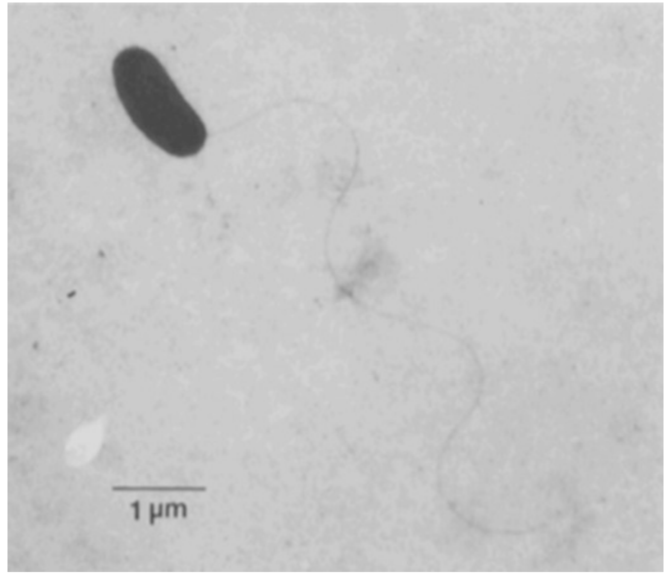

FIGURE 260. *Nocardioides pyridinolyticus* KCTC 0074BP. Flagellated cell from 3-d-old culture on nutrient agar. Negative stain; transmission electron microscopy. Bar = 1 μm. (Reprinted from Yoon. and Park, 2006. *The Prokaryotes*, 3rd edn, vol. 3, Springer, New York, pp. 1099–1113.)

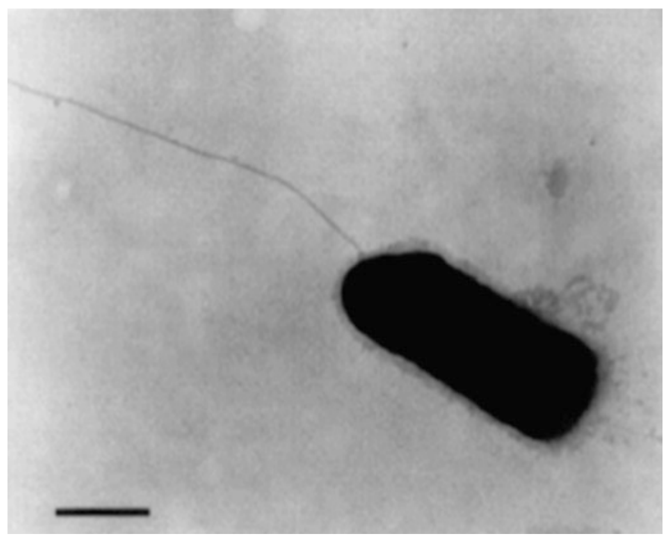

FIGURE 261. Flagellated cell of *Nocardioides nitrophenolicus* KCTC 0457BP; 2-d-old culture grown on nutrient agar at 30°C. Negative stain; transmission electron microscopy. Bar = 0.5 μm. (Reprinted from Yoon et al., 1999. Int. J. Syst. Bacteriol. *49*: 675–680.)

Different predominant fatty acids were also reported, which may be characteristic of certain species or species groups, or reflect the particular culture conditions, or both. For example, a straight-chain unsaturated acid $C_{18:1}$ ω9*c* (27.5%), and $C_{16:0}$ iso (18.6%) were the main components for *Nocardioides oleivorans* (Schippers et al., 2005). In one report (Dastager et al., 2009e), $C_{18:1}$ ω7*c* (50.3%) predominates with a substantially smaller content of $C_{16:0}$ iso (4.2%) for *Nocardioides islandensis* cultured on R2A agar (Difco) for 10 d at 28°C. *Nocardioides aquaticus* grown at 16–20°C also contained an abundance of straight-chain unsaturated acid, $C_{18:1}$ (30.6%), as well as $C_{16:0}$ (14.1%), and $C_{17:0}$ anteiso (10.6%) (Lawson et al., 2000a). *Nocardioides fonticola* had

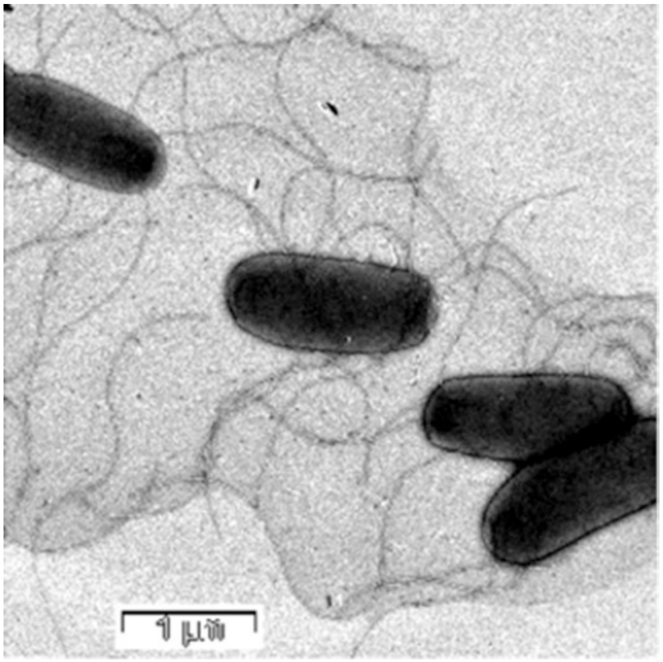

FIGURE 262. Cells of *Nocardioides humi* DCY24 with peritrichous flagella at different developmental stages; cells grown on Luria–Bertani agar at 30°C for 18 h. Transmission electron micrographs of negatively stained cells. Bar = 1 µm. (Reproduced with permission from Kim et al., 2009b. Int. J. Syst. Evol. Microbiol. *59*: 2724–2728.)

$C_{16:0}$ iso and $C_{17:0}$ in equal proportions, nearly 20% when grown on R2A for 3 d at 25°C (Chou et al., 2008). *Nocardioides hankookensis*, on the other hand, contained nearly similar proportions of three acids, $C_{14:0}$, $C_{16:0}$ iso, and $C_{18:1}$ ω5*c* (9–11%) when grown on twofold-diluted R2A agar for 3 d at 30°C (Song et al., 2011), while $C_{16:0}$ iso (42.7%) was found to be the major component in cells mass-harvested from the standard R2A agar after 7 d incubation at 25°C (Yoon et al., 2008). Cells of *Nocardioides furvisabuli*, harvested from MA after incubation at 30°C contained almost equal proportions of $C_{18:0}$ and $C_{16:0}$ iso (19.5%), whereas only $C_{16:0}$ iso (34.1%) was predominating in cells of the same species grown on ISP 2 medium prepared with natural sea water (Lee, 2007b). Strains of *Nocardioides simplex* may exhibit (13–18%) $C_{17:0}$ anteiso larger proportions of $C_{17:0}$ anteiso (13–18%) and $C_{18:1}$ (13–28%) in some experiments (Suzuki and Komagata, 1983c) or have $C_{16:0}$ iso as predominating (reaching up to 41.8%) (Schumann et al., 1997). However, when analyses are performed under identical experimental conditions, strains of this and some other species typically exhibit very similar fatty acid profiles (see, e.g. Lee et al., 2008; Yoon et al., 2005a, 2005b, 2009) and this characteristic may be useful to discriminate *Nocardioides* species, including closely related ones (Cui et al., 2009; Song et al., 2011; Yi and Chun, 2004a; Yoon et al., 1997a, 2010). On the other hand, species (type strains) with rather undistinguishable profiles also occur.

With regard to polar lipids, phosphatidylglycerol (PG), diphosphatidylglycerol (DPG) as well as several incompletely characterized or "unknown" phosphorus-containing or other lipids are typically reported among principal components in *Nocardioides* species (Table 215). The majority of species do not contain nitrogenous phospholipids (i.e. possess phospholipids type I *sensu* Lechevalier et al., 1981, 1977). On rare occasions, nitrogenous phospholipids, such as phosphatidylethanolamine (PE) (Yoon et al., 2005d, 2010) and phosphatidylcholine (PC) (Lee, 2007b) were reported, that are diagnostic of phospholipid types II and III (Lechevalier et al., 1977, 1981). The other phospholipids revealed for some organisms of this genus included phosphatidylinositol (PI), hydroxy-phosphatidylglycerol (PG-OH), acylphosphatidylglycerol (APG), and phosphatidylinositol mannosides (PIMs) (Collins at al., 1983, 1989; Lechevalier et al., 1981, 1977; O'Donnell et al., 1982; see Table 215). It should be emphasized that certain "unknown" phospholipids reported by different authors for some *Nocardioides* species may correspond to those identified (named) in other studies, or only slightly differ from such phospholipids in R_f value owing to some differences in chemical structure of the components involved, mostly fatty acids. Indeed, different patterns of principal phospholipids were reported for some strains in this bacterial group (e.g. Collins et al., 1989, 1983; Komura et al., 1975a; Lechevalier et al., 1977, 1981; O'Donnell et al., 1982; Tamura and Yokota, 1994; Yano et al., 1970, 1971). Glycolipids are usually not mentioned among major polar lipids for *Nocardioides* species. However, several distinct glycolipids can be detected in *Nocardioides albus*, *Nocardioides luteus* (Figure 263), and some other *Nocardioides* strains.

There is now substantial evidence for the effect of culture conditions on the quantitative and qualitative composition of polar lipids in *Nocardioides* strains (which is reminiscent of the phenomenon known for the cellular fatty acids). A prominent example is the polar lipid profile of *Nocardioides albus*. Lechevalier et al. (1981, 1977) reported the presence of PG, PI, APG, and traces of PIMs for *Nocardioides albus*, emphasizing the lack of DPG. According to O'Donnell et al. (1982) and Collins et al. (1983), DPG still occurs among the major phospholipids of this species, along with PG and several incompletely characterized phospholipids, two of which supposedly contain a 2-hydroxy acid. In our recent experiments (N.G. Vinokurova and L.I. Evtushenko, unpublished), both young (8 h, mycelial stage) and older (24 h, fragmentation stage) cultures contained PG, DPG, PL_1 (supposedly APG, run above the DPG spot, just after neutral lipids) and two (or three) characteristic very closely related phospholipids. One of them is most likely PI (contained inositol) and the second was tentatively identified as hydroxy-acid-containing phosphatidylinositol (PI-OH). The older cultures *Nocardioides albus* and *Nocardioides luteus* differed from young cultures mainly in that they contained additional glycolipids (varying amounts) and had an unknown phosphorous-involving yellowish-pigmented compound PL_x in some experiments (Figure 263C, D). The latter, which stained well with Mo-blue (Dittmer and Lester, 1964; Komagata and Suzuki, 1987), shows very weak coloration when sprayed with 5% molybdophosphoric acid in ethanol, but has no distinct specific staining either with ninhydrin, α-naphthol (Jacin and Mishkin, 1965; Komagata and Suzuki, 1987), periodate Schiff reagent (Komagata and Suzuki, 1987; Minnikin et al., 1977) or 5% $AgNO_3$ in aqueous ammonia solution. Certain differences between the polar lipid patterns of *Nocardioides albus* and *Nocardioides luteus* also occur, especially in older cultures (Figure 263C, D). Notably, PL_1 (supposedly APG and/or another closely related compound) characteristic of *Nocardioides albus* and *Nocardioides luteus*, was not revealed in *Nocardioides simplex*. Thus, the polar lipid pattern, even with some components incompletely identified and varying to some extent between experiments, may serve to differentiate species

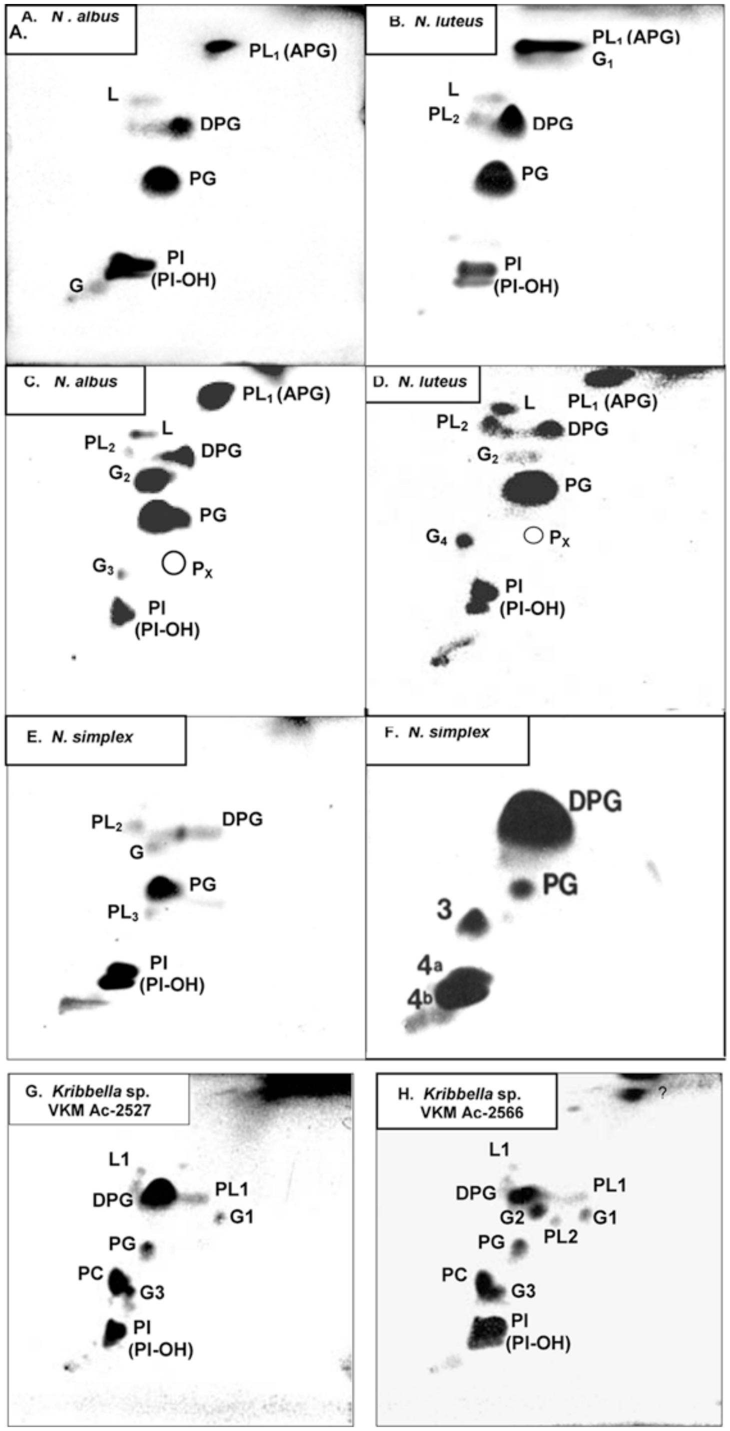

FIGURE 263. Two-dimensional thin-layer chromatograms of polar lipids from the type strains of *Nocardioides albus*, *Nocardioides luteus*, *Nocardioides simplex*, and representatives of the genus *Kribbella* (A–D) Polar lipids of *Nocardioides albus* VKM Ac-805 and *Nocardioides luteus* VKM Ac-1246 in young (nonfragmenting mycelium) (A, C) and older (fragmentation stage) (B, D) cultures grown in complex liquid medium, PYGP (peptone, 0.5%; yeast extract, 0.3%; glucose, 0.5%; K_2HPO_4, 0.02%; pH 7.2). Chloroform-methanol-water (65:25:4, by vol.) was used in the first direction and chloroform-acetic acid-methanol-water (80:15:12:5, v/v) in the second direction (modified from Minnikin et al., 1977). (E and F) Polar lipids patterns from subcultures of the type strain of *Nocardioides simplex*, VKM Ac-1118 (E) and *Nocardioides simplex* NCIB 8929 (F) grown under different conditions. (G and H) Examples of PC-containing polar lipids patterns from nocardioform strains of *Kribbella* (PYGP, 16 h, 28°C; mycelial stage). Abbreviations: DPG, diphosphatidylglycerol; PG, phosphatidylglycerol; PI, phosphatidylinositol; PI-OH, a compound (supposedly phosphatidylinositol) that contains hydroxylated fatty acid(s); PL, unknown phospholipids; PL_1, supposedly acylphosphatidylglycerol (APG); G, glycolipids; L, unknown non-phosphorylated polar lipid; P_x, unknown phosphorus-containing pigment (see the text for details). (A–E, G, and H are courtesy of Natalia G. Vinikurova; F is reprinted from O'Donnell et al., 1982. Arch. Microbiol. *133*: 323–329.)

within this genus and from members of other genera. For differential purposes, the polar lipid patterns have to be analyzed under standardized and controlled experimental conditions and in the same laboratory. Further detailed studies of polar lipids in members of the genus *Nocardioides* will elucidate the spectra of the polar lipid components characteristic of particular species (species groups) and hopefully increase the discriminative power of this characteristic.

Polyamine patterns have been analyzed for 10 strains comprising five *Nocardioides* species (Busse and Schumann, 1999). The majority of strains grown in liquid R medium (Yamada and Komagata, 1972) contained cadaverine in maximum proportion (34–77%). The second major component (nearly 20% or more) for most strains was putrescine (*Nocardioides albus*, *Nocardioides luteus*, and *Nocardioides jensenii*), spermidine (the type strain of *Nocardioides simplex*), or spermine (*Nocardioides simplex* IMET 10283). For *Nocardioides plantarum*, spermine was found to predominate (42%), followed by cadaverine. This strain was also distinguished by a significantly lower concentration of total polyamines. Other compounds, such as 1,3-diaminopropane, tyramine, and sym-homospermidine, were revealed in lesser or trace quantities, with larger proportions (>10%) of 1,3-diaminopropane and tyramine found in a few strains.

In combination with cadaverine, putrescine (in high concentration and proportion in the majority of *Nocardioides* strains) appears to be useful in distinguishing many species of this genus from *Aeromicrobium*. Both *Nocardioides* and *Aeromicrobium* differed from all other analyzed LL-A,pm-containing coryneform and nocardioform bacteria used in the comparative study (families *Propionibacteriaceae*, *Intrasporangiaceae*, and *Sporichthyaceae*) by the presence of cadaverine among major components (Busse and Schumann, 1999; Yoon et al., 2007b). The distinguishing polyamine patterns, along with the overall polyamine concentrations, indicates the potential of polyamines to differentiate individual species or species groups within the genus, as demonstrated by the data on major and additional polyamines for the type strains: *Nocardioides albus* DSM 43109 (cadaverine, 49%; putrescine, 50%); *Nocardioides luteus* DSM 43366 (cadaverine, 57%; putrescine, 28%; spermidine, 11%); *Nocardioides jensenii* DSM 2064I (cadaverine, 54%; putrescine, 22%; spermidine, 12%; spermine, 9.3%); *Nocardioides plantarum* DSM 11054 (spermine, 42%; cadaverine, 34%; tyramine, 12%; 1,3-diaminopropane, 6.8%; spermidine, 5.1%); *Nocardioides simplex* DSM 20130 (cadaverine, 57%; spermidine, 19%; putrescine, 15%; spermine, 5.6%; 1,3-diaminopropane, 4.2%). Notably, the polyamine profiles of strains within the species *Nocardioides albus* and *Nocardioides simplex* (for which more than one strain were studied) were not uniform. A prominent example is the pair *Nocardioides albus* DSM 43874 (53% cadaverine, 30% putrescine, 15% 1,3-diaminopropane, with the total polyamine concentration of 1.5 μmol/g, dry wt) and the type strain *Nocardioides albus* DSM 43109 (49% cadaverine and 50% putrescine, and the total polyamine content of 10.5 μmol/g). The heterogeneity of the polyamine patterns, at least for strains referred to as *Nocardioides albus*, is most likely evidence for the presence of different species within this group (see *Taxonomic comments* for more information). Further studies of polyamines in *Nocardioides* strains strictly proven to be members of one species will elucidate the polyamine set of diagnostic importance for individual species or species groups within the genus.

Nutrition and growth conditions. Strains of *Nocardioides* species usually grow well in standard nutrient media with glucose, peptone, and yeast extract under aerobic conditions. Some organisms of this genus prefer the nutrient-poor media, including a tenfold-diluted nutrient agar (NA; Difco) (Yoon et al., 2005a, 2005b, 2005d), tenfold-diluted R2A agar (Difco) (Dastager et al., 2008f), and some other diluted complex media. Knowledge of minimal nutritional requirements of *Nocardioides* species is incomplete. Most species are nutritionally non-exacting and grow well on a suitable minimal salts medium, e.g. ISP 9 (Shirling and Gottlieb, 1966)* with glucose or other compounds as a sole carbon and energy source and an ammonium salt as a sole nitrogen source (Table 215).

Some organisms, including *Nocardioides albus*, *Nocardioides luteus*, *Nocardioides dokdonensis*, *Nocardioides exalbidus*, *Nocardioides jensenii*, and *Nocardioides simplex* (Collins et al., 1989; Keddie et al., 1986; Li et al., 2007b; Park et al., 2008) and probably many others can use nitrate as a sole nitrogen source. Traces of complex organic substrates (e.g. yeast extract or casein peptone) in minimal salts media commonly enhance growth rates and biomass production of *Nocardioides* species. The good growth of *Nocardioides plantarum* was reported to require thiamine (Collins et al., 1994), while that of *Nocardioides aquaticus* needed both thiamine and biotin (Lawson et al., 2000a). However, both these organisms can grow in the absence of thiamine and biotin (Yi and Chun, 2004a), e.g. in a mineral salts medium consisting of the basal medium (BM; Baumann et al., 1972, 1971) supplemented with 2% (v/v) Hutner's mineral base (Cohen-Bazire et al., 1957), and modified by reducing the concentration of sea salts to half-strength. The BM medium (Baumann et al., 1971) contained 50 mM tris(hydroxymethyl) aminomethane [(Tris)-hydrochloride pH 7.5], 190 mM NH_4C1, 0.33 mM $K_2HPO_4 \cdot H_2O$, 0.1 mM $FeSO_4 \cdot 7H_2O$, and half-strength artificial sea water (MacLeod, 1968).

Some mycelium-forming non-pigmented *Nocardioides* strains (capable of growing on glucose or sucrose with ISP 9 as a basal medium) can utilize other sugars (L-rhamnose, D-xylose, and D-mannitol) as carbon and energy sources only in the presence of thiamine or a vitamin solution (thiamine, riboflavin, nicotinic acid, pyridoxine, and *p*-aminobenzoic acid) (Suzuki and Komagata, 1983c). A similar situation probably takes place with some other species, carbon sources, and growth factors. Some components (or their concentrations) of the above vitamin solution probably inhibit growth or growth rate (Suzuki and Komagata, 1983c). The ability to grow in mineral salts medium with certain carbon sources may also depend on other specific test conditions (e.g. composition of salts in the test medium, carbon source concentrations, the growth medium used to obtain cells for testing, amount of cell mass used as inoculate, etc.).

Bacteria of this genus are largely non-halophilic and neutrophilic mesophiles. A few species, particularly isolated from marine and marine-related environments are salt-requiring and/or grow better in the presence of 0.5–6% NaCl and show resistance to NaCl concentration up to 10–15% (w/v) (Table 215). Some organisms (*Nocardioides bigeumensis* and *Nocardioides caricicola*), in contrast, were reported to be highly sensitive to salt and did not grow even in the presence of 1% (w/v) NaCl (Dastager et al., 2008d; Song et al., 2011). A number of species

*See Shirling and Gottlieb, (1966) (Int. J. Syst. Bacteriol. *16*: 313–340) for the composition of ISP media cited here and in other sections of this chapter.

may start growth at pH 5–5.5 and/or pH 11–12, while others grow in a narrow pH range (pH 7–9) (Table 215). The reported optimal growth temperatures vary insignificantly among species (most organisms grow well between 25 and 30°C; some show best growth at lower temperatures, 16–26°C. Among the recognized *Nocardioides* species, *Nocardioides caricicola* (Song et al., 2011) had the highest growth temperature (45°C) and weak growth was observable for *Nocardioides luteus* (recent experiments). Representatives of several species, i.e. *Nocardioides albus*, *Nocardioides aquiterrae*, *Nocardioides humi*, and *Nocardioides panacisoli* exhibit growth at 42°C (Cho et al., 2010; Kim et al., 2009b; Prauser, 1984b; Yoon, 2004). However, not all organisms have been tested for growth at 42 and/or 45°C.

Metabolism, enzymic and degradative activities. Bacteria of this genus are commonly considered to be chemo-organotrophic aerobes that are able to utilize and degrade a wide range of carbon and energy sources, including various unusual organic compounds. The ability to grow in nutrient-poor media, as well as the finding of representatives of *Nocardioides* in environments of nutrient scarcity, e.g. in cavities (Barton et al., 2004; Groth and Saiz-Jimenez, 1999; Groth et al., 2001), suggests that they may be sufficiently adapted to the oligotrophic lifestyle, be involved in a complex metabolic network of oligotrophic microbial communities, or even engage in chemolithotrophic metabolism with possible input from some atmospheric gases and minerals. In particular, strain *Nocardioides* sp. JS614 is probably able to grow chemolithotrophically on CO, as it might be suggested both from the finding of form I *Cox* genes in its genome (Mattes et al., 2005) and the experiments demonstrating CO-oxidizing activity and chemolithotrophic growth on CO by some actinomycetes containing this gene (King and Weber, 2007). The ability of strains of the recognized species to grow anaerobically on heterotrophic media under different gas atmospheres was not revealed (An et al., 2007; Chou et al., 2008; Cui et al., 2009; Dastager et al., 2008c, 2009e, 2010; Kim et al., 2008a; Lawson et al., 2000a; Schippers et al., 2005; Tóth et al., 2008; Yi and Chun, 2004a, 2004b).

All or the majority of *Nocardioides* strains studied taxonomically possessed activity for esterase lipase (C 8) (conflicting results were reported for a few species by Cho et al., 2010), but were negative in tests for β-glucuronidase, α-fucosidase, and α-mannosidase (API ZYM, bioMérieux). A limited number of strains tested to date (mostly by the API 20NE system; bioMérieux) were negative in the tests for arginine dihydrolase, lysine decarboxylase, ornithine decarboxylase, and tryptophan deaminase. Negative results were also typically registered in the tests for production of H_2S from peptone or cysteine. The only species, *Nocardioides panacisoli*, was reported to produce H_2S from thiosulfate (Cho et al., 2010). For all strains studied so far, tests showed no utilization of thiamine, L-ascorbate, salicylate, 2-propanol, dulcitol, erythritol, sorbitol, xylitol), and polyethylene glycol as carbon sources (Kubota et al., 2005a; Yi and Chun, 2004a, 2004b; Yoon et al., 2009). The results of Voges–Proskauer test vary with species and experiments (Cui et al., 2009; Lee, 2007b; Yoon et al., 2009). The ability to degrade adenine is uncommon (Prauser, 1989; Yi and Chun, 2004a, 2004b), although many species degrade DNA (Table 215) and some utilize thymine (Collins et al., 1994). Although conventional tests have not shown pronounced cellulolytic activity or utilization of cellulose (carboxymethylcellulose) as a sole or principal carbon source in recognized *Nocardioides* species, some unnamed strains of this genus were found to degrade rice straw pieces in minimal salts medium (Abdulla and El-Shatoury, 2007) and many species possess some relevant enzymatic activities. A few organisms can degrade chitin (Prauser, 1976; Tóth et al., 2008) or xylan (Park et al., 2008). Several bacteria were unable to decompose alginate (Lawson et al., 2000a; Yi and Chun, 2004b, a). Varying or conflicting test results (Table 215) for some characteristics of some species (type strains) were reported by different or the same authors. In most cases (disregarding obvious technical mistakes), such data indicate that a particular activity or ability exists, but it is weak or indistinct under the experimental conditions employed.

A prominent feature of members of the genus *Nocardioides* is their capability of performing transformation and degradation of complex and unusual compounds, including very common persistent and toxic environmental pollutants. Examples of such compounds and pollutants include alkanes of various lengths, petroleum chemicals, crude oil and its derivatives (Hamamura and Arp, 2000; Hamamura et al., 2001; Iizuka and Komagata, 1964; Jung et al., 2002; Purswani et al., 2008; Schippers et al., 2005), including carbazole, a recalcitrant *N*-heterocyclic aromatic compound derived from crude oil, creosote, and shale oil (Inoue et al., 2007; Inoue et al., 2006), as well as a sulfur heterocyclic compound dibenzothiophene (Sandhya et al., 1997; Sandhya et al., 1995). There are reports on the ability of members of this genus to degrade and utilize phenols and nitrophenols (Collins et al., 1994; Cui et al., 2009; Gundersen and Jensen, 1956; Keddie et al., 1986; Yoon et al., 2009), including 2,4-dinitrophenol and 2,4,6-trinitrophenol (picric acid) (Behrendt and Heesche-Wagner, 1999; Cho et al., 1998, 2000; Ebert et al., 1999, 2001; Rajan et al., 1996; Yoon et al., 1999), as well as pyridine (Lee et al., 1994; Lee et al., 1991; Rhee et al., 1997; Yoon et al., 1997b). They are capable of degrading a three aromatic ring compound, phenanthrene (Iwabuchi and Harayama, 1997, 1998a, 1998b; Saito et al., 1999, 2000), heterocyclic compounds such as dibenzofurans and chloroaromatics, including dibenzo-*p*-dioxins (Futamata et al., 2004; Inoue et al., 2007; Inoue et al., 2006; Kubota et al., 2005a; Sukda et al., 2009) and 2,4,5-trichlorophenoxyacetic acid which is one of the most persistent herbicides (Ebert et al., 1999, 2001; Golovleva et al., 1990; Männisto et al., 2001; Männisto et al., 1999; Traag and van Wezel, 2008; Travkin et al., 1999). There are reports on the ability to degrade different other herbicides, including *s*-triazine herbicides (Mulbry et al., 2002; Topp et al., 2000; Vibber et al., 2007; Yamazaki et al., 2008). *Nocardioides* sp. PD653 is the first naturally occurring aerobic bacterium reported to be capable of mineralizing hexachlorobenzene, a recalcitrant environmental pollutant, and degrading pentachloronitrobenzene (Takagi et al., 2009).

Nocardioides sp. JS614 is one of the most studied representatives of *Nocardioides* with respect to degradative activities, genetics, and metabolism (Chuang and Mattes, 2007; Mattes et al., 2003, 2005; Owens et al., 2009) and the only *Nocardioides* strain for which the complete genome (CP000509) has been sequenced so far. It is capable of aerobic growth with ethene (a plant hormone and greenhouse gas) and vinyl chloride (a known human carcinogen and common groundwater contaminant), which

is often generated in groundwater by the incomplete reduction of chlorinated solvents (Coleman et al., 2002). This strain also transforms other short-chain alkenes, including propene, 1-butene, and *trans*-2-butene to their corresponding epoxyalkanes, and produces highly enantio-enriched epoxyalkanes via stereoselective monooxygenase-mediated alkene epoxidation (Owens et al., 2009).

Nocardioides simplex and other *Nocardioides* strains possess various enzymes transforming sterols (Arima et al., 1969; Fokina et al., 2003a, 2003b; Nagasawa et al., 1969; Yu et al., 2007). An extracellular adenosine deaminase was described for *Nocardioides* sp. J-326TK (Jun et al., 1994). Histamine dehydrogenase, catalyzing the oxidative deamination of histamine to produce imidazole acetaldehyde and an ammonium ion, and also oxidizing agmatine and putrescine had been reported for *Nocardioides simplex*; the gene encoding this enzyme had been characterized and overexpressed in *Escherichia coli* (Fujieda et al., 2004, 2005; Limburg et al., 2005; Reed et al., 2008; Tsutsumi et al., 2008). Two mycelium-forming strains, *Nocardioides albus* SC13912 (ATCC 55426) and *Nocardioides luteus* SC13911, produce enzymes facilitating the production of a 10-deacetylbaccatin III, a precursor used for semisynthesis of paclitaxel and analogs. An extracellular enzyme from *Nocardioides albus* is a polypeptide (47 kDa) that specifically removes the C-13 side chain from paclitaxel, cephalomannine, and other analogs. An intracellular 10-deacetylase (40 kDa) from *Nocardioides luteus* SC13912 removes the 10-acetate from baccatin III and paclitaxel (Hanson et al., 1994, 2004). *Nocardioides kongjuensis* is able to degrade *N*-hexanoyl-L-homoserine lactone, a signaling molecule in quorum-sensing system of many bacteria (Yoon et al., 2006b). Extremely thermostable deoxycytidine deaminase (52 kDa) with the activity maximum at 99°C was revealed in *Nocardioides* sp., strain CT16 (Sakai et al., 2002). A chitosanase catalyzing the hydrolysis of glycosidic bonds in chitosan and its encoding gene were described for a soil isolate NlO6 identified as *Nocardioides* sp. on chemotaxonomic grounds (Masson et al., 1995). A number of other medically and industrially useful enzymes and degradation activities of *Nocardioides* strains were reported (e.g. Hanson et al., 1994; Jun et al., 1994; Masson et al., 1995; Nishimoto et al., 1996; Nobile and Belleville, 1958; Patel et al., 2000; Siddiqui et al., 2000). Numerous cloning, sequencing, and three-dimensional structure studies of enzymes and relevant genes have been published and the literature and sequences are available from public databases.

Antibiotic sensitivity and antibiotic production. The antibiotic sensitivity and production were determined for some *Nocardioides* species (*Nocardioides aquiterrae*, *Nocardioides aquaticus*, *Nocardioides caricicola*, *Nocardioides daedukensis*, *Nocardioides dokdonensis*, *Nocardioides fonticola*, *Nocardioides hankookensis*, *Nocardioides insulae*, *Nocardioides pyridinolyticus*, and *Nocardioides terrigena*). All strains, except for *Nocardioides pyridinolyticus* (Song et al., 2011) were sensitive to chloramphenicol and streptomycin in different concentrations. Various other antibiotics (ampicillin, carbenicillin, cephalothin, gentamicin, kanamycin, lincomycin, neomycin, novobiocin, oleandomycin, penicillin G, polymyxin B, and tetracycline) suppress growth of individual strains (species) as given in the species descriptions in this chapter. The type strain of *Nocardioides albus* was reported to be highly susceptible to antifungal drugs, such as bifonazole, econazole, miconazole, and clotrimazole, but not to the triazoles fluconazole and voriconazole (100 μg/ml) (Dabbs et al., 2003). No production of β-lactamases associated with resistance to β-lactam antibiotics was revealed for type strains of *Nocardioides albus*, *Nocardioides luteus*, *Nocardioides plantarum*, *Nocardioides pyridinolyticus*, and *Nocardioides simplex* (Ogawara et al., 1999).

The data about antibiotic production of *Nocardioides* strains are relatively sparse as compared with members of some other actinomycete genera. Representatives of *Nocardioides* inhabiting marine shellfish were reported to exhibit wide-spectrum antimicrobial activities and also show antitumor activities (El-Shatoury et al., 2009). Mycelium-forming strains of this genus from wheat roots were capable of suppressing *in vitro* some fungal pathogens of wheat, including *Rhizoctonia solani*, *Pythium* spp., and *Gaeumannomyces graminis* var. *tritici* (Coombs et al., 2004). A *Nocardioides* strain phylogenetically close to *Nocardioides oleivorans* inhibited growth of filamentous fungi (representatives of *Aspergillus* and *Fusarium*) but was not active towards the yeasts or bacteria tested (Romanenko et al., 2008). A strain, identified as *Nocardioides* sp., produces along with known piericidins, a new biologically active 4-pyridinol compound that inhibits NADH dehydrogenase and electron transfer; the new compound inhibited cell division of fertilized starfish (*Asterina pectinifera*) eggs at the minimum inhibitory concentration of 0.09 mg/ml (Kubota et al., 2003). Strains DSM 3176 and DSM 3177 identified as *Nocardioides albus* on morphological and chemotaxonomic grounds have been reported to synthesize leucylblasticidin S, a precursor in the biosynthesis of blasticidin S, as well as to produce a peptidylnucleoside (Dellweg et al., 1988), named rodaplutin (Gullo et al., 1988). They show insecticidal and acaricidal activities, along with weak antimycotic and antibacterial ones (Dellweg et al., 1988). Some strains described under the generic name *Nocardioides* produce macrolide antibiotics, such as luminamicin with activity against anaerobic bacteria (Ōmura et al., 1985), or an antibiotic with antifungal activities against *Candida albicans* and *Candida tropicalis* (Loppinet et al., 1997). Indeed, data on phylogenetic position of some antibiotic-producing strains described in early works under the name *Nocardioides* are not available and some of them (e.g. *Nocardioides* ATCC 39419, a producer of antitumor antibiotic, sandramycin) might not belong to this genus (Matson. and Bush, 1989). This strain had been reclassified as a member of the genus *Kribbella* (Park et al., 1999).

Bacteriophages. A large collection of bacteriophages that multiply in *Nocardioides albus*, *Nocardioides luteus*, *Nocardioides simplex*, and *Nocardioides jensenii* strains but not in strains of other actinomycete genera, including *Aeromicrobium*, have been described (Miller et al., 1991; Prauser, 1976, 1984b; Prauser, 1984a; Prauser, 1989; Prauser and Falta, 1968; Wellington and Williams, 1981; Williams et al., 1980). Some *Streptomyces* phages were reported to cause clearing effects, i.e. phage-dependent lysis without phage propagation, on strains of the species *Nocardioides albus*, *Nocardioides luteus*, and related mycelium-forming organisms of this genus (Prauser, 1976, 1984b, 1989). The sensitivity to specific *Nocardioides* bacteriophages was used to differentiate the *Nocardioides* species from other mycelium-forming actinomycetes (Kurtboke and Williams, 1991; Prauser, 1976, 1984b) and was an additional criterion that justified the transfer of *Arthrobacter simplex* and *Arthrobacter jensenii* to the genus *Nocardioides* (Collins et al., 1989; O'Donnell et al., 1982). The phage susceptibility is considered to rely to a certain degree on

adsorption to specific bacterial cell-wall receptors present in a limited number of closely related bacterial strains (Kurtboke and Williams, 1991; Young, 1967). In the case of the aforementioned *Nocardioides* species and the specific *Nocardioides* phages (Prauser, 1976, 1984b; Prauser, 1984a; Prauser and Falta, 1968), the critical determinants in the initial phage binding site are most likely located on teichoic acids, as reported for representatives of *Bacillus*, *Listeria* and other bacteria (Archibald, 1976; Monteville et al., 1994; Schleifer and Steber, 1974; Wendlinger et al., 1996). Such specific binding sites in strains of *Nocardioides albus*, *Nocardioides luteus*, and related mycelium-forming *Nocardioides* strains may include D-galactose and/or D-glucose linked to pyruvate, which are common structural elements of teichoic acids in these organisms (Shashkov et al., 2000b; Shashkov et al., 1999; Tul'skaya, 2009).

Genomic characteristics and plasmids. The mol% G+C of the DNA of members of this genus is around 70 (Table 215), with a lower value of 66.5 (T_m) reported for strain *Nocardioides albus* IMET 7801 (Prauser, 1976) and a higher value of 74.8 (HPLC) for a strain of *Nocardioides lentus* (Yoon et al., 2006a). Analysis of the 16S–23S rRNA gene's internally transcribed spacer (ITS) region found no tRNA sequences in more than 30 strains of the genus *Nocardioides* and related organisms (Yoon et al., 1998a) in contrast to some other actinomycetes (Baylis and Bibb, 1988; Kim et al., 1993; Normand et al., 1992; Pernodet et al., 1989; Yoon et al., 1997b). Moreover, two types of 16S–23S ITS sequences (differing in size from 1 to 12 bp) were found in a few strains of *Nocardioides albus* (DSM 43874, JCM 5851, and JCM 5862) and the strain *Nocardioides simplex* NCIMB 12919, which suggests that at least two rRNA operons might occur in genomes of these organisms. The 16S–23S ITSs in *Nocardioides* strains vary in size from 328 (*Nocardioides nitrophenolicus*) to 539 bp (the *Nocardioides albus* group). The levels of nucleotide similarity between the type strains ranged from 48.4% (for the pair *Nocardioides jensenii – Nocardioides nitrophenolicus*) or 51.3% (for the pair *Nocardioides jensenii – Nocardioides simplex*) to 84.8% (for the type strains of *Nocardioides albus* and *Nocardioides luteus*). The sequences of *Nocardioides simplex* strains used in the study were identical or nearly identical (386–388 bp) and exhibited a high nucleotide sequence similarity (97.7–100%). The 16S–23S ITSs of the type strain of *Nocardioides luteus* and "*Nocardioides fulvus*" 71-N86 (= IMET 7846 = DSM 46115 = JCM 3335), "*Nocardioides flavus*" 71-N54 (= IMET 7844 = DSM 46114 = NBRC 14396), and 71-N82 (= KCTC 9579 = NBRC 14397) were also identical in size (473 bp) and invariant or almost similar (1 bp difference) in the sequences. The data indicate that these three strains (isolated from soils from different parts of worldwide) are *Nocardioides luteus* (Prauser, 1984a; Ruan and Zhang, 1979; Tille et al., 1978). In contrast, the 18 strains referred to as *Nocardioides albus* substantially differed in the 16S–23S ITS nucleotide sequences, sharing 84.2–100% sequence similarity (the former figure is lower than the value for type strains of this species and *Nocardioides luteus*). They also differed in size of 16S–23S ITS sequences (468–539 bp), with the type strain containing 514 bp and some strains containing 473 bp (identical to that in *Nocardioides luteus*).

The sequences of RNase P RNA genes were analyzed for seven *Nocardioides* species and representatives of other genera (Yoon and Park, 2000). The sequence similarity between the type strains of *Nocardioides* species was 77.6–94.7 (approx. 64–89% if gaps are included). Like its 16S–23S ITS sequences, the RNase P RNA gene sequence of the type strain of *Nocardioides luteus* is identical or almost identical (differing by 1 bp) to the sequences in "*Nocardioides flavus*" 71-N54 and "*Nocardioides fulvus*" 71-N86. Phylogenetic analysis based on RNase P RNA gene sequences showed the clustering of the type strain of *Nocardioides albus* with *Nocardioides luteus* (94% sequence similarity), *Nocardioides simplex* with *Nocardioides nitrophenolicus* (94.7% similarity), and the formation of distinct lineages by other species (*Nocardioides jensenii*, *Nocardioides plantarum* and *Nocardioides pyridinolyticus*). Importantly, the sequence similarity values (77.6–78.6%) between more distant *Nocardioides* species were lower than, or close to, the values between *Nocardioides* species and strains of *Kribbella* (79.3–83.3%) or *Aeromicrobium* (77.4–82.0%). Accordingly, the *Nocardioides* species did not form a coherent cluster in the extended tree including members of these and other genera used in the study. Furthermore, *Luteococcus japonicus* (*Propionibacteriaceae*) possessed a gene with a nucleotide sequence that was nearly identical (>99% similarity) to that of *Nocardioides jensenii* (Yoon and Park, 2000), which suggests that the RNase P RNA gene transfer can take place between these groups.

As mentioned before, *Nocardioides* sp. JS614 is the only completely sequenced member of the genus capable of assimilating vinyl chloride and ethene, and degrading a number of short-chain alkenes (GenBank accession no. NC_008699; Copeland et al., 2006). The genome is a circular molecule (4.99 Mb), which harbors 4546 protein-coding genes and 55 predicted pseudogenes. The circular plasmid pNOCA01 (307,8 bp) of this strain (GenBank accession number CP000508) has the DNA G+C content of 68 mol%, which is lower than that calculated for the genome (71 mol%) and carries genes encoding key enzymes participating in alkene oxidation and assimilation (Chuang and Mattes, 2007; Mattes et al., 2007; Mattes et al., 2003; Mattes et al., 2005). A few other plasmids were also found in *Nocardioides* strains possessing degradative activities. A plasmid with the *dfdBC* gene cluster encoding a ring-cleavage dioxygenase that degrades dibenzofuran (an oxygen-containing heterocyclic compound) was reported for strain *Nocardioides* sp. DF412 (Miyauchi et al., 2008). A plasmid (34.2 kb) bearing genes encoding the sulfur oxidizing enzyme system involved in degradation of dibenzothiophene (an environmentally persistent heterocyclic sulfur compound) was found in *Nocardioides* sp. PKSP 12 (Sandhya et al., 1995, 1997).

Habitat and ecology. Bacteria of the recognized species of the genus *Nocardioides* have been isolated from various environments (Table 215). Numerous ecological studies indicate the presence of these organisms in various terrestrial and aquatic ecosystems worldwide, including low-temperature and deep subsurface ecosystems as well as nutrient-limited environments (e.g. Barton et al., 2007; Boivin-Jahns, et al., 1995; Gontang et al., 2007; Groth and Saiz-Jimenez, 1999; Groth et al., 1999; Katayama et al., 2006; Rintala et al., 2008; Vishnivetskaya et al., 2006; Ward and Bora, 2006; Zhang et al., 2009a). Mycelial *Nocardioides* strains were reported to predominate in kaolin prepared for the ceramic industry (Prauser, 1976, 1986). There is now substantial evidence that representatives of the genus *Nocardioides* also occur in soils and industrial wastewater polluted by different organic and inorganic compounds, including toxic polyaromatics, heavy metals, and nuclear waste (e.g. Coleman et al., 2002; Desantis et al., 2006; Fredrickson et al., 2004;

Futamata et al., 2004; Golovleva et al., 1990; Hamamura and Arp, 2000; Iizuka and Komagata, 1964; Kubota et al., 2005a; Lee et al., 1994, 1991); Maltseva and Oriel, 1997; Männisto et al., 1999; Mattes et al., 2003; Miyauchi et al., 2008; Rajan et al., 1996; Sandhya et al., 1995; Schippers et al., 2005; Suzuki and Komagata, 1983c; Yoon and Park, 2006; Yoon et al., 1999).

Members of the genus *Nocardioides*, like many other actinobacteria, are most likely involved in the turnover of organic matter in ecosystems because they can degrade and metabolize a wide range of natural organic compounds. The ability to degrade aromatic compounds, including polyaromatics and other chemicals, implies a significant role in the natural degradation of such compounds. In organics-limited environments, organisms of this genus, as mentioned before, may use traces of organics dissolved in water or derived from the decomposition of other micro-organisms, or interact mutualistically with other micro-organisms in such environments (Barton et al., 2004; Barton et al., 2007; Groth and Saiz-Jimenez, 1999). Bacteria of the genus *Nocardioides* can also be detected in the water phase of tropospheric clouds (Amato et al., 2006) where they may be active and take part (as described for other bacteria) in the biodegradation of organic compounds dissolved in cloud water, reaching up to 20 mg per liter (Vaitilington et al., 2010). In desert dust clouds, they can migrate great distances through the atmosphere during dust storms (Griffin, 2007; Polymenakou et al., 2008).

All the above data indicate that the resting and/or vegetative cells of organisms of the genus *Nocardioides* can survive and maintain a suitable metabolic activity under extreme conditions such as increased UV and nuclear radiation, and heavy metal or other pollutant contaminations. The data also indicate the high adaptive potential of organisms of this genus. At the genomic level, this potential is often associated with increased numbers of genes in the hypervariable regions, often called "gene islands", and the ability to move horizontally (Dobrindt et al., 2004; Wilmes et al., 2009). In the latter context, it might be relevant to note that thin channel-like structures connecting cells of *Nocardioides* are occasionally seen on electron micrographs.

Bacteria of this genus occur in the rhizosphere, roots, and the above-ground parts of plants; some have been reported to be mutualistic plant endophytes (Coombs and Franco, 2003; Coombs et al., 2004; Qin et al., 2009; Song et al., 2011; Tian, 2007; Ulrich et al., 2008). Similar to other endophytic bacteria, they may obtain nutrients from plants, obtain protection from abiotic stress like desiccation or freezing, promote plant growth via different mechanisms, and prevent plant disease caused by plant-pathogenic bacteria, fungi, and insects by secreting secondary metabolites (Coombs and Franco, 2003; Dimock et al., 1988; Firakova et al., 2007; Hallmann et al., 1997; Kloepper et al., 1991; Reiter et al., 2002; Schrey et al., 2005; Tian et al., 2007; Tian et al., 2004). Members of this genus can also be found in association with lichens, daphnia, termites and other eukaryotic organisms, including vertebrates and humans (e.g. Delbes et al., 2007; El-Shatoury et al., 2009; Fall et al., 2007; Grice et al., 2009; Li et al., 2007b; Tóth et al., 2008). Although bacteria of the genus *Nocardioides* have been occasionally found in bacterial populations associated with human diseases (Cox et al., 2003; Frank et al., 2007; Harris et al., 2007), they are considered non-pathogenic either to humans or other warm-blooded animals.

Enrichment and isolation procedures

Most *Nocardioides* strains have been isolated at random from terrestrial or marine environments during studies of bacterial diversity in certain biotopes or during screening for strains possessing biodegradative activities. Organic media with low nutrient concentrations are generally advantageous for recovering diverse *Nocardioides* species from natural habitats. *Nocardioides* strains are successfully isolated by the standard dilution plating technique on suitable nonselective ("total count") agar media including the standard R2A agar (Difco), twofold or tenfold-diluted R2A, tenfold-diluted nutrient agar (NA, Difco), oligotrophic medium PYGV (Staley, 1968) and other media (see, e.g. Prauser, 1976; Yoon and Park, 2006), and the descriptions of *Nocardioides* species in this chapter). Oligotrophic medium PYGV (Staley, 1968) with sea water(Lawson et al., 2000a), marine agar (Difco), and a number of other nutrient media supplemented with sea water or salts in a proper concentration (Choi et al., 2007; Lee, 2007a; Park et al., 2008; Yi and Chun, 2004b, a) can be used for isolation of *Nocardioides* strains from marine ecosystems or other hypersaline environments. For isolation of *Nocardioides* strains from alkaline soil, a diluted nutrient agar (NA; Difco) with the pH adjusted to 9.0–10.0 using Na_2CO_3 can be applied (Yoon et al., 2005a; Yoon et al., 2005b; Yoon et al., 2005d, 2006a). *Nocardioides* can also be isolated using media and procedures recommended for arthrobacters (Keddie et al., 1986) and some rhodococci (Goodfellow and Maldonado, 2006).

Since the cells or mycelium fragments are usually closely associated with the mineral and organic particles of the soil, a special procedure for their suspending may be useful, e.g. vigorous shaking of the sample with diluent or mechanical or chemical desorption methods [see Futamata et al. (2004), Herron and Wellington (1990), and Kämpfer (2006), for details and references]. Soil samples pretreated with electric pulses (at a field intensity of 16 kV/cm^2) can result in a highly selective outgrowth of filamentous *Nocardioides*-like strains (Bulina et al., 1998). A subsequent treatment of sample suspension and plating procedures are generally similar to those reported for other mycelial and coryneform actinomycetes [see, e.g. Kämpfer (2006), and Keddie et al. (1986), for details and references]. Isolation of endophytic representatives of *Nocardioides* is carried out from the thoroughly washed and surface-sterilized roots or other parts of plants as described (e.g. Coombs and Franco, 2003; Chung et al., 2008; Song et al., 2011; Tian et al., 2004; Zinniel et al., 2002; or in other works dealing with isolation of plant endophytes). Nutrient-poor media such as humic acid-vitamin B agar (HV; Hayakawa and Nonomura, 1987), tap water-yeast extract agar (TWYE, containing 0.25 g of yeast extract, 0.5 g of K_2HPO_4, and 18 g of agar per liter of tap water) (Crawford et al., 1993), yeast extract-casein hydrolysate agar (YECD, containing 0.3 g of yeast extract, 0.3 g of D-glucose, 2 g of K_2HPO_4, and 18 g of agar per liter of distilled water) (Coombs and Franco, 2003) were successfully applied to isolate endophytic *Nocardioides* from wheat roots. To control fungal growth, the above media can be supplemented with 50 µg/ml benomyl (DuPont). Other antibacterial and antifungal agents might be helpful, but some, including antifungal agents (Dabbs et al., 2003) may inhibit the growth of *Nocardioides* organisms.

Selective enrichment procedures and special media containing certain synthetic organic chemicals (pollutants) or their derivatives are advantageous for isolation of *Nocardioides* strains from polluted environments. Such procedures and media were reported while isolating strains of *Nocardioides pyridinolyticus* (Yoon et al., 1997b), *Nocardioides nitrophenolicus* (Yoon et al., 1999), *Nocardioides aromaticivorans* (Kubota et al., 2005a), *Nocardioides simplex* ("*Brevibacterium lipolyticum*") (Iizuka and Komagata, 1964), *Nocardioides oleivorans* (Schippers et al., 2005), and a number of other not yet named representatives of the genus (e.g. Maltseva and Oriel, 1997; Männisto et al., 1999; Mattes et al., 2003; Miyauchi et al., 2008; Rajan et al., 1996). Because organisms of this genus may be sufficiently adapted to the environmental conditions, isolation conditions may be employed that mimic natural environmental conditions. While intended for isolation of strains belonging to or closely related to the described *Nocardioides* species, the selective (semi-selective) media may be prepared taking into account isolation sources and the specific characteristics listed in Table 215 and mentioned in the species description.

Incubation of isolation plates or enrichment cultures is usually carried out at 25–30°C for an appropriate time period, but may also be carried out at a lower (10–15°C) or higher (40°C) temperature (Iwabuchi et al., 1998; Kim et al., 2008a; Lawson et al., 2000a). Colonies of mycelium-producing *Nocardioides* strains formed on many isolation media can be distinguished from those of other actinomycetes (mostly streptomycetes that are abundantly present in the same soil sample) based on the absence of mycelium or scant aerial mycelium, pasty or sand-pasty consistency of colonies, and whitish, light creamy-white, yellowish-white, or yellow colony color. These features, however, are also characteristic of representatives of some other nocardioform actinomycetes, including *Agromyces*, *Kribbella*, or *Promicromonospora* often present in the same soil sample. The *Nocardioides* species with rod-shaped cell morphologies usually occur among coryneform soil isolates that have slender cells and white, yellowish, or indistinctly pigmented colonies.

Maintenance procedures

Cultures may be maintained as 20% glycerol suspensions at −20 and −80°C. Long-term conservation is achieved by freeze-drying or in liquid nitrogen by standard procedures.

Differentiation of the genus *Nocardioides* from other genera

The characteristics essential for phenotypic delineation of the currently recognized genus *Nocardioides* are listed in Table 214 and Table 216. The major menaquinone MK-8(H_4) is the most prominent chemotaxonomic marker that differentiates members of the genus *Nocardioides* from all other genera of the family, except for *Marmoricola*. The presence of well-developed branching hyphae or relatively long rods (in young cultures), along with a marked rod-to-coccoid morphogenetic life cycle, allows primary separation of the majority of *Nocardioides* species from members of the genus *Marmoricola*, which typically produce spherical cells. Genus level identification of novel strains that form short rods to coccoid cells in young cultures or exhibit to a greater or lesser extent phylogenetic relatedness to the genus *Marmoricola* and related *Nocardioides* species is achieved (in the current classification system) via comparison of such strains with respective individual species of these genera (Table 216). The data on cellular fatty acids are of a practical importance, along with other phenotypic characteristics (Table 214 and Table 216).

Taxonomic comments

The genus *Nocardioides*, with the type species *Nocardioides albus*, originally encompassed the actinomycetes that produced branched fragmenting hyphae, possessed the peptidoglycan type similar to that of the genus *Streptomyces*, and showed susceptibility to specific actinophages (Prauser, 1976). The second mycelium-forming species, *Nocardioides luteus*, was added to the genus in 1984 (Prauser, 1984b, 1985). Shortly before, O'Donnell et al. (1982, 1983) reclassified *Arthrobacter simplex*, which was first described as *Corynebacterium simplex* (Jensen, 1934) and then affiliated to the genus *Arthrobacter* (Lochhead, 1957), as *Nocardioides simplex*. There was general agreement by 1982, mainly on chemotaxonomic grounds, that atypical LL-A_2pm-containing arthrobacters should be removed from this genus (for further details and references, see, e.g. Keddie and Jones, 1981; Keddie et al., 1986; Suzuki and Komagata, 1983c). The distant relatedness of *Arthrobacter simplex* to *Arthrobacter globiformis* was also shown by the rRNA cataloguing studies (Stackebrandt et al., 1980). However, the LL-A_2pm-containing arthrobacters remained species "in search of a genus" at that time.

The proposal of O'Donnell et al. (1982, 1983) to affiliate *Arthrobacter simplex* to *Nocardioides* was based on similarity of the type strain of this species to the mycelium-forming *Nocardioides* in lipid composition (fatty acids, phospholipids, and menaquinones), as well as on other supportive data including, in particular, data on the presence the peptidoglycan type A3γ (Prauser, 1978; Schleifer and Kandler, 1972), the sensitivity to some of specific *Nocardioides* actinophages (Prauser, 1976, 1981; Wellington and Williams, 1981), the DNA G+C content (Tille et al., 1978; Yamada and Komagata, 1970), and the DNA–DNA similarity level of 15–20% (Prauser, 1981). While discussing the generic affiliation of coryneform *Arthrobacter simplex* to *Nocardioides*, O'Donnell et al. (1982) also referred to some actinomycete genera that accommodate morphologically diverse strains and to the evidence from early 16S rRNA sequencing studies (Stackebrandt et al., 1980; Stackebrandt and Woese, 1981) that morphological properties are not always reliable indicators of natural relationships.

Owing to the inclusion of *Arthrobacter simplex* into *Nocardioides* (the transfer was validated in 1983), the genus became heterogeneous with respect to morphology. The authors proposed the emended description of the genus *Nocardioides* with inclusion of the extended range of morphological and chemotaxonomic characters (O'Donnell et al., 1982). This proposal, however, was not validated. There were conflicting opinions with regard to the assignment of organisms with markedly different morphology to one genus, mainly for practical reasons prevailing at that time. The aggregation of a typical nocardioform organism with abundant primary and aerial mycelium (*Nocardioides albus*) with a motile, single-celled bacterium (*Arthrobacter simplex*) was questioned, in particular, by the author of the genus *Nocardioides*, H. Prauser (Prauser, 1986). In line with this view, Suzuki and Komagata (1983b, 1983c) validly published the new genus *Pimelobacter* to accommodate some LL-A_2pm-containing

TABLE 216. Selected characteristics for preliminary differentiation of species of the *Nocardioides jensenii* assemblage, some phylogenetically related *Nocardioides* species, and species of the genus *Marmoricola*[a,b,c]

Characteristic	*N. jensenii*	*N. daedukensis*	*N. dubius*	*N. mesophilus*	*N. daphnia*	*N. alkalitolerans*	*N. dilutus*	*N. halotolerans*	*M. aurantiacus*	*M. aequoreus*	*M. bigeumensis*	*M. korecus*	*M. scoriae*
Colony color[d]	Yellowish white	Yellowish	Yellowish white	Cream-white	Yellowish	Yellowish	Cream-white	Cream-white	Orange	Yellow	Lemon yellow	Yellow	Vivid yellow
Cell appearanced[e]	Irregular rods to cocci	Irregular rods to cocci	Irregular rods to cocci	Short rods to cocci	Irregular rods to cocci	Irregular rods to cocci	Irregular rods to cocci	Irregular rods to cocci	Cocci	Cocci	Cocci	Cocci	Cocci
Cell diameter (μm)	0.6–0.8	0.4–0.8	0.8–1.0	0.3–0.8	0.8–1.0	0.8–1.0	0.4–0.8	0.6–0.9	0.5–0.7	0.5–0.7	0.3–0.5	1.1–1.2	0.6–1.0
Motility	–	–	+	+	–	–	+	nd	–	–	+	–	–
Growth at 37°C	+	+	+	+	+	–	nd	nd	–	+	+	+	+
Catalase activity	+	+	+	+	+	+	nd	–	+	+	–	+	+
Oxidase activity	–	+	+	–	–	+	nd	–	–	–	–	–	–
Nitrate reduction	+	+	–	–	+	+	+	+	–	+	+	–	–
Decomposition of:													
Esculin	–	+	+	–	+	–	–	–	+	+	+	+	+
Starch	–	–	–	+	+	–	–	v	–	–	+	–	–
Xanthine	+	–	–	–	–	–	–	–	–	–	+	–	–
Maximal NaCl (%)[f]	>5	9	5	nd	5	5	<1	10	2	5 (7 w)	7	2.0	3.0
Optimum and (max.) pH[f]	7	7–8 (10)	7–8 (10.5)	7–7.5	7.5–8.5 (10.5)	7–9 (12)	7–8	7–8	5.1–8.7	7.1 (12)	7.2 (12)	6.1–10.1 (5.1–12.1)	8.1–11.1 (6.1–12.1)
Major fatty acids (>20%) (*growth conditions*)[g]	$C_{16:0}$ iso (*NA, 30°C, 7d*)	$C_{16:0}$ iso (*NA, 30°C, 7d*)	$C_{16:0}$ iso (*NA, 30°C, 7d*)	$C_{16:0}$ iso (*R2A, 28°C, 7d*)	$C_{16:0}$ iso (*TSA, 28°C*)	$C_{16:0}$ iso, 10-Me-$C_{18:0}$ (*NA, 30°C, 7d*)	$C_{16:0}$ iso (*1/2 R2A, 28°C, 7d*)	$C_{16:0}$ iso (*1/2 R2A, 28°C, 7d*)	$C_{16:0}$, $C_{18:1}$ (*R2A, 30°C, 5 d; TSB, 28°C*)	$C_{18:1}$, $C_{16:0}$ (*R2A, 30°C, 5 d; TSB, 30°C, 3d*)	$C_{16:0}$ iso (*R2A, 30°C, 5 d; TSA*)	$C_{16:0}$, $C_{17:1}$ ω8*c* (*R2A, 30°C, 5 d*)	$C_{16:0}$, $C_{18:1}$ ω9 (*R2A, 30°C, 5 d*)
Phospholipids	DPG, PG, PI, OH-PG, PL	DPG, PL, UL [PG, PE, PL]	DPG, PG, PE, PI	nd	DPG, PG, PL	nd	DPG, PG	nd	DPG, PG, PI, PL	DPG, PG, PI, UL	DPG, PG, PI, UL	DPG, PC, PG, PI, PL	DPG, PC, PG, PI, PL

(continued)

TABLE 216. (continued)

Characteristic	*N. jensenii*	*N. daedukensis*	*N. dubius*	*N. mesophilus*	*N. daphnia*	*N. alkalitolerans*	*N. dilutus*	*N. halotolerans*	*M. aurantiacus*	*M. aequoreus*	*M. bigeumensis*	*M. korecus*	*M. scoriae*
DNA G+C content (mol%)[h]	68.8 (T_m)	68.7	70.6	68.7	69.9	69.9	71.8	67.9	72.0	72.4	72.9	71.0	72.0
Source of isolation	Soil	Soil	Alkaline soil	Soil	Water flea	Alkaline soil	Soil	Agricultural soil	Marble statue	Beach sand	Soil	Volcanic ash	Volcanic ash

[a]Data are for the type strains. Symbols and abbreviations: +, positive; −, negative; w, weak; v, variable or conflicting data; nd, no data available; UL, unknown polar lipid(s); PL, unidentified phospholipids(s); other abbreviations, see also Table 215.

[b]Data are from Suzuki and Komagata (1983c), Yi and Chun (2004a), Dastager et al. (2008d, 2008f, 2010), Lee (2007a), Urzì et al. (2000), Yoon et al. (2005a, 2005d, 2010), Tóth et al. (2008), Lee and Lee (2010), and Lee et al. (2010).

[c]See Figure 248 and Figure 247 for phylogenetic position of the organisms.

[d]Differences in the color intensity and shade and the cell appearance may be influenced by the growth conditions and culture age.

[e]Older cultures of *Nocardioides* species as a rule consist of short rods, coco-bacillary and/or coccoid forms.

[f]Tolerance to NaCl and pH and optimal pH values for growth may vary depending on the test medium composition (mineral, rich in organics, liquid and/or agar-containing).

[g]*NA*, nutrient agar (Difco); *R2A*, R2A agar (Difco); *1/2 R2A*, half-strength R2A agar (1.6 g of R2A broth powder supplemented with 1.5% agar in 1 l of distilled water); *TSA*, trypticase soy agar (Difco); *TSB*, trypticase soy broth (Difco).

[h]DNA base composition as determined by the HPLC method; data for *Nocardioides jensenii* were estimated by the thermal denaturation method.

arthrobacters. Pointing out a rather close relatedness of the studied atypical arthrobacters to *Nocardioides albus* in chemotaxonomic characteristics along with a dissimilarity in the cell morphology and colony appearance, the authors noted some difference between these organisms in the fatty acid and menaquinone composition, a higher DNA G+C content in strains assigned to *Pimelobacter*, and low DNA–DNA similarity values clearly showing distant relationship of *Nocardioides albus* and *Pimelobacter* (Suzuki and Komagata, 1983c). The following species were included in the genus: the type species *Pimelobacter simplex* (*Arthrobacter simplex*), *Pimelobacter jensenii* (created for a single strain NCMB 9770 = JCM 1364, formerly a strain of *Arthrobacter simplex*; Jensen and Gundersen, 1956), and *Pimelobacter tumescens* (the former *Arthrobacter tumescens*; Jensen, 1934; Conn and Dimmick, 1947) currently belonging to the genus *Terrabacter* (see below).

Thus, the valid descriptions of *Arthrobacter simplex* (Lochhead, 1957), *Nocardioides simplex* (O'Donnell et al., 1982, 1983), and *Pimelobacter simplex* (Suzuki and Komagata, 1983a, 1983c) are based on the same type strain and their names are considered to be objective synonyms, with *Arthrobacter simplex* being basonym. In the first edition of *Bergey's Manual of Systematic Bacteriology*, this species was treated under *Arthrobacter*, in Addendum II to "The list of species of the genus *Arthrobacter*", mentioning *Nocardioides simplex* and *Pimelobacter simplex* in the list of synonyms and emphasizing that "the taxonomic position of *Arthrobacter simplex* (and *Arthrobacter tumescens*) remains unresolved" (Keddie et al., 1986). The species *Pimelobacter jensenii* was briefly discussed in the *Further comments* to the description of *Arthrobacter simplex* (Keddie et al., 1986).

Subsequent taxonomic study of the above group using the 16S rRNA-based phylogenetic analysis showed that *Nocardioides albus*, *Nocardioides luteus*, *Nocardioides* (*Pimelobacter*) *simplex*, and *Pimelobacter jensenii* formed a coherent phylogenetic cluster among the strains included in the study, whereas *Pimelobacter tumescens* was phylogenetically distinct (Collins et al., 1989). Accordingly, *Pimelobacter jensenii* was transferred to the genus *Nocardioides*, while *Pimelobacter tumescens* was reclassified as a representative of the newly established genus *Terrabacter* (in the family *Intrasporangiaceae*). Shortly thereafter, Collins and Stackebrandt (1989a, 1989b) proposed the new species *Nocardioides fastidiosus* that seemed at that time to be phylogenetically affiliated to the genus *Nocardioides*. This species was later transferred by Tamura and Yokota (1994) to the genus *Aeromicrobium* (Miller et al., 1991).

Proposals of all other *Nocardioides* species published within the period 1994–2010 and described in this volume rely on the 16S RNA-based phylogenetic analysis, key chemotaxonomic characteristics as well as differences from related species in phenotypic traits and DNA–DNA hybridization values (currently accepted for species delineation) (Stackebrandt et al., 2002; Wayne et al., 1987). The DNA–DNA hybridization experiments (mostly by the method of Ezaki et al., 1989) were performed mainly when particular strains and closely related organisms had high 16S rRNA sequence similarities. However, DNA–DNA hybridization values or other data justifying delineation at the genomic level from the closest related species are absent in a few cases. These mostly include species proposed independently and almost at the same time by different research groups.

At present the genus *Nocardioides* displays an assemblage of phylogenetically rather distant and phenotypically dissimilar species whose features are not reflected by the original genus description created for mycelium-forming actinomycetes (Prauser, 1976). The most unrelated species share 92–93% sequence similarities which are equal to or even less than values separating the well-defined genera in the family *Nocardioidaceae* and other families of the suborder *Actinomycetales*. In addition, as stated before, the differences in both 16S–23S ITS and RNase P gene sequence similarities between some *Nocardioides* species are less than those between some *Nocardioides* species and representatives of other actinomycete genera used in the study (Yoon et al., 1998a; Yoon and Park, 2000).

At the chemotaxonomic level, there are only two characteristics identical for all *Nocardioides* species, i.e. the presence of LL-A_2pm in the cell-wall peptidoglycan and the predominance of the menaquinone MK-8(H_4). Both these characteristics are also typical of some other actinomycete genera (e.g. in the family *Intrasporangiaceae*). In general, *Nocardioides* species seem to display a common fatty acid type, the complex fatty acid type *sensu* Suzuki et al. (1993) and type 3 according to Kroppenstedt (1985). However, it appears that several different subtypes may be distinguished. The data on other chemotaxonomic features are absent for most *Nocardioides* species; if reported, do not appear to be uniform. In particular, the phospholipid types, I, II, and III (with no nitrogenous phospholipids or with phosphatidylethanolamine and phosphatidylcholine as diagnostic components) are usually considered to represent chemotaxonomic markers useful in distinguishing actinomycete genera (Kämpfer, 2006; Kroppenstedt and Evtushenko, 2006; Kroppenstedt and Goodfellow, 2006; Lechevalier et al., 1977, 1981). On the other hand, some specific component(s), e.g. an acylphosphatidylglycerol-like phospholipid (PL_1 in Figure 263) are variable among *Nocardioides* species with the phospholipid type I. Some representatives of this genus also differ in the composition of peptidoglycan-bound cell-polymers (Shashkov et al., 1999, 2000b; Tul'skaya, 2009), and in polyamine patterns (Busse and Schumann, 1999). Furthermore, the cell-wall peptidoglycan structure (undetermined for the majority of *Nocardioides* species) might turn out to be dissimilar in some phylogenetically distant organisms of this genus. This has been shown, in particular, for some LL-A_2pm-containing organisms of the family *Propionibacteriaceae* (the sister family of the *Nocardioidaceae*) and for members of the family *Intrasporangiaceae* (see Schumann et al., 1997, 2009; Stackebrandt and Schaal, 2006; and the respective chapters in this volume). The acyl type of muramic acid, a characteristic feature of the cell envelope differentiating some genera within a family or a suborder (Uchida and Seino, 1997), might be dissimilar for distantly related *Nocardioides* species as well. Organisms of the genus *Nocardioides*, as mentioned before, also differ in cell morphology, catalase activity (a possible indication that different factors are utilized to prevent oxygen damage or cope with certain environmental conditions), optimal and maximal salinity for growth, pH growth range, and other properties affected by ecological niche and in turn affecting speciation.

The data available suggest that further taxonomic study of *Nocardioides*, involving the data on genomics and proteomics, will result in the dissection of this genus into several genera and the establishment of phenotypically and phylogenetically coherent taxa. Dissection of the genus *Nocardioides* is consistent with the current trend toward improving the classification

schemes of heterogeneous actinomycete genera, e.g. by finding of additional phenotypic (chemotaxonomic) differentiating characteristics and establishing novel genera (see recent publications in IJSEM). Dissection is also in line with a tendency to describe novel species (often originating from the same or similar ecological niches) that show very high 16S rRNA gene sequence (99% and higher) and include strains exhibiting greater genomic and phenotypic homogeneity. Finally, the classification system must take into account ecological studies revealing diverse novel organisms associated with, or belonging to, the recent genus *Nocardioides* and showing high 16S rRNA gene sequence similarity to species of this genus.

At the moment, some recognized *Nocardioides* species need to be revised taking into account their more focused circumscriptions and current taxonomic concepts. Prominent examples of such species requiring further revision and emendation of the species description are *Nocardioides albus* Prauser 1976 and *Nocardioides luteus* Prauser 1984b. According to the original description of *Nocardioides albus*, based on the study of 17 strains from a variety of soils and related sources from different sites of the world (Prauser, 1976), the species encompassed strains showing a certain phenotypic similarity, mostly in formation of indistinctly colored (whitish to faintly yellowish or faintly brownish) primary mycelium and white aerial mycelium. The species *Nocardioides luteus* was proposed to accommodate strains distinguished by distinct yellow to orange-yellow primary mycelium and cream aerial mycelium (when well developed) (Prauser, 1984b). The separate species status of these organisms was justified by DNA–DNA hybridization experiments with the type strains of the two above species. However, relatedness to *Nocardioides albus* of the remaining strains studied in the original work (Prauser, 1976) and also many other mycelial organisms later described in the literature under the species names *Nocardioides albus* (Collins et al., 1994; Evtushenko and Zelenkova, 1989; Prauser, 1976; Suzuki and Komagata, 1983c; Yoon et al., 1998a; Yoon et al., 1998b) has not been verified in by DNA–DNA hybridization experiments or other adequate genomic analysis. An exception is *Nocardioides albus* IMET 7832 with a high DNA–DNA relatedness to the type strain (Prauser, 1984b) and probably some strains that harbor 16S rRNA gene and 16S–23S ITS sequences identical or almost identical to those of the type strain (Yoon et al., 1998a; Yoon et al., 1998b).

Meanwhile, much evidence has accumulated showing that strains referred to as *Nocardioides albus* are heterogeneous group. As mentioned before, some strains of this assemblage are affiliated with *Nocardioides luteus* or distinct from *Nocardioides albus* or *Nocardioides luteus* on the basis of 16S rRNA gene and 16S–23S rDNA ITS sequence analyses (Yoon et al., 1998a, 1998b). Some were found to contain teichoic acids identical to those of the type strain of *Nocardioides luteus* or differ from those of both the *Nocardioides luteus* and *Nocardioides albus* type strains (Tul'skaya, 2009). Differences in susceptibility to individual actinophages (Prauser, 1976) might indicate differences in cell-wall chemistry between strains of the *Nocardioides albus* complex. Representatives of this group tend also to differ from the *Nocardioides albus* type strain in the proportions of the predominant polyamines (Busse and Schumann, 1999). There is also a report of one *Nocardioides albus* strain (IMET 7819) with a fatty acid composition anomalous to that of the type and other strains studied under the same experimental conditions (O'Donnell et al., 1982). In addition, there are some differences in physiological characteristics, including the range of pH and NaCl concentration for growth (Prauser, 1976; Suzuki and Komagata, 1983c; L. Dorofeeva and L. Evtushenko, unpublished). Some strains of the *Nocardioides albus* assemblage might be eventually be proven to be *Nocardioides luteus* strains and described as non-pigmented subspecies (biovars), while other strains most likely will be assigned to novel species.

Another group of *Nocardioides* species in need of further attention includes a few recent species proposed shortly after the description of their phylogenetic relatives. For such strains, the DNA–DNA hybridization or other relevant experiments justifying their separation into species at the genomic level were not performed. As an example, *Nocardioides basaltis* (Kim et al., 2009a) is closely related to *Nocardioides salarius* (Kim et al., 2008a) (Figure 248). Both these species have very similar fatty acid profiles and other phenotypic traits (Table 215) and originate from marine-related environments in the same geographic region. In this context it is worth noting that many bacteria including actinomycetes have more than one rRNA operon and multiple 16S rRNA gene variants (Acinas et al., 2004). The difference between 16S rRNA genes in one genome may range from 0 to several percent (Conville and Witebsky, 2007; Wang et al., 1997; Yap et al., 1999). In the case of *Nocardioides* species, at least two rRNA operons with two types of 16S–23S ITS sequences (differing in size by up to 12 bp) may occur in some strains (Yoon et al., 1998a). Analogously, other organisms of this genus may contain more than one rRNA operon, which may include 16S rRNA genes with substantially different sequences. Accordingly, the species status of organisms, which is mostly based on direct 16S rRNA sequencing of a single isolate, might be ambiguous (particularly taking into account the limitations of measuring DNA–DNA relatedness by the hybridization technique and of delineating phenotypic features with certainty). The *Nocardioides* strains in assemblages in need of further attention include those very similar in phenotypic traits and isolated from the same or similar ecological niches located at the same or nearby geographical sites. Further comparative taxonomic studies of such species and newly isolated strains, utilizing 16S rRNA gene sequence analysis and reanalysis, with cloning and evaluation for the presence of multiple copies of this gene as well as multilocus sequence analysis (MLSA; Gevers et al., 2005; Rong and Huang, 2010) would help clarify the taxonomy of this genus.

Differentiation of the species of the genus *Nocardioides*

The phenotypic properties differentiating species of the genus *Nocardioides* are listed in Table 215, Table 216, Table 217, Table 218, Table 219, and Table 220. Additional details are given in the *List of species of the genus Nocardioides*.

Assignment of a previously unknown or a dubious isolate to a certain species normally is a two-stage process. In the first stage, the strain in question is assigned to a certain genus; in the second stage, the species level is identified. During stage one the data from phylogenetic analyses are matched to a spectrum

TABLE 217. Oxidization of carbon sources by the type strains *Nocardioides jensemi* and some related species tested by the Biolog GP2[a,b]

Carbon source	*N. akalitolerans*	*N. daphniae*	*N. dubius*	*N. jensemi*
D-Cellobiose	+	−	−	−
D-Fructose	+	+	−	−
D-Glucose	+	+	−	−
Maltose	−	−	+	−
Maltotriose	+	−	+	−
D-Mannose	+	+	−	−
Palatinose	+	+	−	−
D-Psicose	+	+	−	−
D-Ribose	+	+	−	−
Sucrose	+	−	−	−
Trehalose	+	+	−	−
3-Methyl glucose	+	−	−	−
Mannan	+	−	−	−
L-Lactic acid	+	+	−	+
Succinic acid monomethyl ester	+	−	+	−
Propionic acid	+	−	−	+
Pyruvic acid methyl ester	−	+	+	+
L-Alaninamide	−	−	+	+
L-Alanyl glycine	−	−	+	−
Inosine	−	+	+	+
Tween 40	+	−	+	+
Tween 80	−	−	+	+

[a]Symbols: +, positive; −, negative.

[b]Data from Tóth et al. (2008).

TABLE 218. Differential characteristics of organisms of the *Nocardioides simplex* assemblage[a,b]

Characteristic	*N. aromaticivorans*	*N. caeni*	*N. ginsengisoli*	*N. kongjuensis*	*N. nitrophenolicus*	*N. simplex*
Colony color[c]	MW	GY	YW	YW	YW	W, YW
Motility	−	−	−	−	+	+
Growth at 37°C	+	−	+	+	+	+
Growth at 4% (w/v) NaCl	nd	−	+	+	nd	+
Oxidase test	−	+	−	+	+[d]	+
Carbon source utilization:[e]						
L-Arabinose	+	+	−	−	−	−
D-Cellobiose	+	+	nd	−	−	−
D-Fructose	+	−	+	+	+	−
D-Glucose	+	−	+	+	+	+
D-Maltose, D-mannitol	+	−	+	−	−[f]	−[f]
L-Rhamnose	+	+	−	−[f]	+	−
D-Ribose	+	−	−	−	+	−[f]
D-Xylose	+	−	+	−	+	−
Utilization of aromatic chemicals:[g]						
Dibenzofuran (100 p.p.m.)	+	nd	+	+	+	−
Dibenzofuran (200 p.p.m.)	+	nd	−	−	(+)	−
p-Nitrophenol (50 p.p.m.)	+	nd	(+)	+	+	−
p-Nitrophenol (100 p.p.m.)	(+)	nd	−	−	+	−
Decomposition of:						
Esculin	+	−	nd	−	+	+
Starch	−	+	nd	−	+	−[h]
Tyrosine	−	−	nd	−	+	+
Hypoxanthine	+	−	nd	−	−	−
DNA G+C content (mol%) (HPLC)	72.1	71.5	70.2	72.1	71.4	71.7

[a]Data presented are for the type strains. Symbols and abbreviations: +, positive; −, negative; (+), weakly positive; nd, no data available; MW, milky white; GY, grayish yellow; YW, yellowish white; W, white.

[b]Data from Yoon et al. (2006b, 2009), Kubota et al. (2005a), Suzuki and Komagata (1983c), Yi and Chun (2004a), Cui et al. (2009) and unpublished data of the authors of this chapter.

[c]Some variation in the color shade and intensity may be influenced by the growth conditions and culture age.

[d]Negative reaction was reported by Yi and Chun (2004a).

[e]Data on carbon source utilization for all species, except for *Nocardioides ginsengisoli* according to the API 50 CH test system (bioMérieux), with the AUX (bioMérieux) used as the inoculation medium (Yoon et al., 2009). Data for *Nocardioides ginsengisoli* and some data for other species (except for *Nocardioides caeni*) were also obtained using a mineral salts medium supplemented with vitamins and trace element solution as the basal medium (Cui et al., 2009).

[f]Positive results were reported by Cui et al. (2009).

[g]Data from Cui et al. (2009).

[h]Weak activity was reported by Yi and Chun (2004b).

TABLE 219. Percentage cellular fatty acid compositions of the type strains of the *Nocardioides simplex* assemblage[a]

Fatty acid	*N. aromaticiborans*[b]	*N. ginsengisoli*[b]	*N. kongjuensis*[b]	*N. nitrophenolicus*[b]	*N. simplex*[b]	*N. caeni*[c]
Saturated, straight-chain:						
$C_{16:0}$	2.5	1.5	6.4	6.4	1.4	4.6
$C_{17:0}$	2.4		2.9	2.8		1.3
$C_{18:0}$					5.1	1.2
Saturated, iso-branched:						
$C_{14:0}$ iso	2.2	2.9	1.7		1.4	1.0
$C_{15:0}$ iso	2.1	1.9	3.2	4.6	1.1	2.9
$C_{16:0}$ iso	52.0	57.5	52.9	39.8	35.8	40.3
$C_{17:0}$ iso	2.8	2.4	5.4	9.3	3.5	6.3
$C_{18:0}$ iso	5.5	2.3	3.7	8.2		1.9
Saturated anteiso-branched:						
$C_{17:0}$ anteiso			1.7	3.4		0.8
$C_{19:0}$ anteiso	1.8					
Unsaturated straight-chain:						
$C_{17:1}$ ω6*c*	7.5	8.1	2.3	5.6	13.3	8.0
$C_{17:1}$ ω8*c*	5.4	9.0	2.4	1.2	1.0	8.6
$C_{18:1}$ ω7*c*						1.7
$C_{18:1}$ ω9*c*	8.4	5.1	6.7	8.0	8.1	16.4
Unsaturated, iso-branched:						
$C_{16:1}$ iso		1.1			2.1	
10-Methyl-branched:						
$C_{16:0}$ 10-methyl					4.6	
$C_{17:0}$ 10-methyl	3.7	3.2	3.1	1.3	6.2	0.6
$C_{18:0}$ 10-methyl (TBSA)	1.1	1.7	2.0	4.3	11.0	
Hydroxylated acids:						
$C_{15:0}$ 3-OH	3.2		4.2			
$C_{15:0}$ iso 2-OH and/or $C_{16:1}$ ω7*c*	1.3	3.3	1.5		2.2	2.2

[a]Fatty acids that represented <1% in all strains are omitted (except for $C_{17:0}$ anteiso.and $C_{17:0}$ 10-methyl for *Nocardioides caeni*).

[b]Data from Cui et al. (2009). Fatty acids as determined in cell mass harvested from trypticase soy agar (TSA; Difco) after incubation at 30°C for 2 d.

[c]Data from Yoon et al. (2009). Fatty acids as determined in cell mass harvested from nutrient agar (Difco) after incubation at 30°C for 7 d (different proportions of fatty acids will be obtained for this strain if grown on TSA for 2 d).

of phenotypic characters identified with a supposedly coherent group bearing the genus name. During stage two, characters said to be associated with the genus taxon but in a less consistent way may be the focus of attention. Sometimes the results of such a preliminary screening provides useful hints to help further the species level differentiation. In particular, the morphological features (extensively branched hyphae or rod-shaped cells) and chemotaxonomic traits (fatty acid composition, polar lipids, cell-wall polysaccharides, and polyamine patterns) are helpful. Strains phylogenetically related to the *Marmoricola* species require special attention (Figure 247 and Figure 248). The morphological, chemotaxonomic, and other phenotypic characteristics distinguishing *Nocardioides* and *Marmoricola*-related organisms is of practical significance and some key characteristics are given in Table 216.

Strains can be preliminarily assigned to *Nocardioides* species or species group using the multiplex PCR assay and specific primers generated by aligning 16S rRNA gene sequences of *Nocardioides* and one universal reverse primer (Park et al., 1998). Most *Nocardioides* species were described on the basis of a single isolate designated the type strain. Therefore, the other members of such species might differ in some growth and physiological features from the type strains. In addition, some enzymes are inducible, so the test results for enzymic activities (and some other phenotypic characteristics) may be influenced by the medium used to obtain the cells used in a particular experiment. Moreover, the type and reference strains maintained for many years in culture collections might have characteristics altered to some extent since their isolation. A prominent example is the strain IMET 7801 that lost the ability to produce aerial mycelium (Prauser, 1976; Vandamme et al., 1996). In light of very close 16S rRNA gene relatedness and phenotypic similarity of some species, the precise affiliation of a novel strain with a recognized *Nocardioides* species or its classification as a representative of a novel species requires thorough comparison of phenotypic characteristics and DNA–DNA hybridization results or analyses of other adequate genomic characteristics capable of taxonomic resolution at the strain-species level (Gevers et al., 2005; Rong and Huang, 2010; Rosselló-Mora and Amann, 2001; Schumann et al., 2009). Analysis of the 16S–23S rRNA gene internal transcribed spacer region (Yoon et al., 1998a), RNase P RNA gene (Yoon and Park, 2000), or *gyrB* gene (encoding the β-subunit of DNA gyrase, a type II DNA topoisomerase) for novel strains and related species may facilitate the affiliation strains to species. Correlation between the DNA–DNA similarity level and the *gyrB*-based genetic distance was demonstrated for species of the related genus *Kribbella* (Kirby et al., 2010).

Acknowledgements

This work was supported by the program MCB of the Russian Academy of Sciences. The author is grateful to Drs L.V. Dorofeeva, N.G. Vinokurova, E.M. Tul'skaya, and A.M. Shashkov for helpful cooperation.

TABLE 220. Differential characteristics of species of the *Nocardioides ganghwensis* assemblage[a,b]

Characteristic	*N. exalbidus*	*N. furvisabuli*	*N. ganghwensis*	*N. hwasunensis*	*N. oleivorans*
Colony color[c]	W	Y	I	YW	O
Maximal NaCl for growth (%)	nd	6	8	4	(2.34)[d]
Motility	−	+	−	−	−
Nitrate reduction	−	+	+	−	−
Utilization of carbon sources:[e]					
Adonitol	−	+	−	−	−
Glycerol, D-raffinose	−	+	+	−	−
L-Arabinose, D-xylose	−	+	+	+	−
Cellobiose, D-galactose, D-mannose	−	+	+	+	+
D-Salicin	−	−	+	+	−
Sucrose	+	−	+	+	+
Melibiose	+	−	+	−	+
L-Rhamnose	+	−	−[f]	+	+
Inulin	−	+	−	+	−
Enzymes (API ZYM):					
α-Glucosidase	+	−	+	+	+
β-Galactosidase	+	+	+	−	+[g]
Naphthol-AS-BI-phosphohydrolase	+	−	−	−	−[h]
Degradation of:					
Esculin	−	−	(+)	−	+
Casein	nd	+	+	−	+
Starch	−	+	+	+	−
Tyrosine	nd	−	+	−	−
DNA G+C content (mol%) (HPLC)	74	69.1	72.0	71.1	nd

[a]Data are for the type strains. Symbols and abbreviations: +, positive; −, negative; (+), weakly positive; nd, no data available; I, ivory; O, orange; YW, yellowish white; W, white.

[b]Data from Yi and Chun (2004a), Schippers et al. (2005), Li et al. (2007b), Lee (2007b), and Lee et al. (2008).

[c]Some variation in the color shade and intensity may be influenced by the growth conditions and culture age.

[d]No data are available on the ability to grow at a higher concentration of NaCl.

[e]Data on utilization of carbon sources for *Nocardioides exalbidus* are according to the *API CH50* test system (Li et al., 2007a). Data for the remaining species are according to conventional methods (see Table 215 and the original species description for the basal media employed, as well as for the other methods).

[f]Positive result was reported by Lee (2007b).

[g]Activity may be weak (Lee et al., 2008).

[h]Weak activity may be observed (Li et al., 2007b).

List of species of the genus *Nocardioides*

1. **Nocardioides albus** Prauser 1976, 61[AL]

al′bus. L. masc. adj. *albus* white, referring to the white aerial mycelium.

Characteristics are as described for the genus and listed in Table 215. Additional information given below is taken from the works of Prauser (1976, 1989) and Yi and Chun (2004a), unless indicated.

Primary mycelium is white or may be whitish to faintly cream-colored in aged cultures on some ISP media, including glycerol-nitrate agar. White aerial mycelium is typically well formed on yeast extract-malt extract agar, oatmeal agar, chitin agar, and glucose-asparagine agar (Figure 251 and Figure 252); usually fairly well formed on inorganic salts-starch agar and glycerol-asparagine agar, and absent on glycerol-nitrate agar and many complex media rich in organics. No distinct soluble pigments are usually produced. Colonies lacking aerial mycelium are mostly pasty, with smooth to wrinkled and dull to bright surfaces. Growth is optimum at about 28°C, good at 10°C and 37°C, but not at 50°C; weak growth may occur at 42°C. Grows in up to 8% (w/v) NaCl and at initial pH 6–9; weak growth may be observable at initial pH 5 and 10, as assessed in PYGP medium containing 0.5% peptone, 0.3% yeast extract, 0.5% glucose, and 0.02% K_2HPO_4 (L. Dorofeeva and L. Evtushenko, unpublished data). Nutritionally non-exacting; grows well on a suitable mineral salts medium with glucose as sole carbon-plus-energy source and an ammonium salt or nitrate as sole nitrogen source. Growth and biomass production are enhanced in the presence of vitamins (Lawson et al., 2000a) and traces of organics. Utilization of some sugars may depend on the presence of vitamins; thiamine supports growth with D-mannitol, while a solution of vitamins (thiamine, riboflavin, nicotinic acid, pyridoxine and *p*-aminobenzoic acid) supports growth on L-rhamnose or D-xylose (Suzuki and Komagata, 1983c). The type strain utilizes succinate, L-asparagine (weak), L-ornithine (weak), and *N*-acetylglucosamine (weak), but not acetamide, L-arginine, L-lysine, and D-sorbitol as sole carbon sources. The ability to use azelate, malonate, suberate, L-proline, histamine, and

tetradecane as sole carbon and energy sources was reported (Collins et al., 1994). Acetate, citrate, and benzoate are utilized, but the test results may vary between test methods (Park et al., 1999; Yi and Chun, 2004a). Acid is produced by oxidation of D-glucose, L-arabinose, sucrose, D-xylose, D-mannitol, D-fructose and L-rhamnose in conventional tests, but not from inositol and raffinose. However, acid was not produced from glucose using the API 20NE test system. No distinct reaction is usually observable in the test for oxidase activity (assessed with tetramethyl-*p*-phenylenediamine). The type strain degrades chitin and grows on chitin agar (Prauser, 1976) as a sole source of carbon and nitrogen. The type strain is highly susceptible to antifungal drugs such as the imidazoles (bifonazole, econazole, miconazole, and clotrimazole), while resistant to triazoles fluconazole and voriconazole (100 μg/ml) (Dabbs et al., 2003).

Peptidoglycan type is A3γ (LL-A_2pm-glycine). The cell-wall sugars are galactose and glucose. The cell wall of the type strain contains poly(galactosylglycerol phosphate) teichoic acid, each monomeric unit containing glycerol, galactose, glucose, pyruvate, and phosphate (Shashkov et al., 1999). The presence of glycerol in the cell wall is the most salient characteristic differentiating this species from *Nocardioides luteus* with cell wall containing ribitol-based teichoic acid (Shashkov et al., 1999, 2000b). The polyamine pattern of the type strain of *Nocardioides albus* grown in rich medium (Yamada and Komagata, 1972) contains almost equal amounts of diamines cadaverine and putrescine (49.0 and 49.6%, respectively), with a minor quantities (<1%) of 1,3-diaminopropane and tyramine, and traces of spermidine and spermine (Busse and Schumann, 1999). The predominant isoprenoid quinone is MK-8(H_4); minor amounts of MK-8(H_2) and MK-8 are detected (Collins et al., 1983; O'Donnell et al., 1982; Suzuki and Komagata, 1983c). The cellular fatty acid profile to a large extent depends on the growth conditions and analytical procedures, but $C_{16:0}$ iso is typically predominating (25–71%) and hydroxylated acids are not detected (Collins et al., 1983; Lee et al., 2000; Miller et al., 1991; Schumann et al., 1997; Suzuki and Komagata, 1983c; Yoon et al., 1997a). The major fatty acids of cells harvested from Nutrient agar (Difco) after 4 d incubation at 30°C were $C_{16:0}$ iso (53.6%), $C_{17:1}$ ω6*c* (15.6%), and $C_{17:0}$ 10-methyl (12.2%); other acids included tuberculostearic acid (TBSA; 2.3%) and homologous components, as well as saturated, unsaturated, iso-, and anteiso- branched acids (Yoon et al., 1997b). The fatty acids of the type strain grown in trypticase soy broth at 28°C for 24–48 h contained mainly $C_{16:0}$ iso (70.8%), along with small proportions of other fatty acids, including TBSA (2.6%) (Schumann et al., 1997). A larger proportion of TBSA (18.3%) was detected in cells from modified Sauton's medium (30°C; Mordarska et al., 1972); $C_{16:0}$ iso contributed 26.6% (O'Donnell et al., 1982). A higher level of TBSA, 34%, was reported by Lee et al. (2000). The qualitative and quantitative composition of polar lipids is also influenced by culture conditions, but phosphatidyl glycerol (PG), diphosphatidyl glycerol (DPG), phosphatidyl inositol (PI) as well as phospholipid (PL_1) [corresponding to acyl phosphatidyl glycerol (APG) in chromatographic behavior] and PI-OH (supposedly a hydroxylated PI) are usually among the principal phosphorous-containing components. Glycolipids may be detected in significant amounts (see the section *Further descriptive information* and Figure 263). Susceptible to specific *Nocardioides* actinophages. The DNA–DNA similarity of the type strain of *Nocardioides albus* to the type strain of *Nocardioides luteus* was 49% and 38% in different experiments (Prauser, 1984b, 1989). Typically occur in soil. The type strain of *Nocardioides albus* was isolated on asparagine agar, containing asparagine 1.0 g, glucose 10.0 g, K_2HPO_4 (anhyd.) 1.0 g, $MgSO_4$ (anhyd.) 0.5 g, NaCl 0.5 g, agar 20.0 g and distilled water 1 liter; pH 7.0–7.2 (Prauser, 1976).

Source (type strain): soil of a lavender field, Tihany peninsula, Balaton region, Hungary.

DNA G+C content (mol%): 68.6 (T_m) (for the type strain; Suzuki and Komagata, 1983c).

Type strain: IMET 7807, ATCC 27980, DSM 43109, KCTC 9186, CCUG 37987, CIP 103451, IFO (now NBRC) 13917, JCM 3185, LMG 16326, NRRL B-5389, VKM Ac-805.

Sequence accession no. (16S rRNA gene): AF004988, X53211.

Additional remarks: the mycelium-forming strains mentioned in the literature under the species name *Nocardioides albus* compose a rather phylogenetically and chemotaxonomically heterogeneous group and some strains most likely will be described as novel species in future (see Further descriptive information for more information).

2. **Nocardioides aestuarii** Yi and Chun 2004b, 2152[VP]

a.es.tu.a'ri.i. L. gen. n. *aestuarii* of the tidal flat.

Characteristics are as described for the genus and listed in Table 215. Information presented below is taken from the original species description (Yi and Chun, 2004b).

Cells are nonmotile rods (ca. 0.3–0.4 × 0.9–2.1 μm) in 3-d-old culture on marine agar 2216 (MA; Difco). Colonies on MA are ivory, approximately 0.5–1 mm in the diameter after 3 d at 30°C, and reach the maximum diameter of 1–2 mm after 5 d. Substrate or aerial mycelium is not observed. Grows at 20–35°C, 0–8% (w/v) NaCl, and at initial pH 6–10; optimal growth was observed at 30°C, 0–2% (w/v) NaCl, and pH 7 as tested on ZoBell medium (Yi and Chun, 2004a; ZoBell, 1941) containing Bacto agar (Difco) 15 g, Bacto peptone (Difco) 5 g, yeast extract (Difco) 1 g, ferric citrate 0.1 g, sea salts 40 g, and 1 liter of distilled water (sea salts were not added when the relation to NaCl was tested). No growth occurs at 15 or 40°°C, at 9% (w/v) NaCl, and at pH 5 and 11 under the same experimental conditions. Utilizes acetate, L-lysine (weak), succinate, and other substrates (Table 215) in basal medium described by Baumann et al. (1972) supplemented with 2% (v/v) Hutner's mineral base (Cohen-Bazire et al., 1957) and modified by reducing the concentration of sea salts to half-strength. L-Arginine, L-asparagine, L-ornithine, D-sorbitol, and tartrate are not used as sole carbon sources on the same test medium. Acid was not produced from glucose using the API 20NE test system. Major fatty acids determined for the cells grown on MA at 30°C for 3 d were $C_{16:0}$ iso (52.0%), $C_{16:1}$ iso H (14.5%), $C_{17:1}$ ω8*c* (7.0%), $C_{17:0}$ 10-methyl (3.1%), and a small quantity (0.3%) of TBSA. The type strain (and the only strain described) was isolated using R2A agar (Difco) supplemented with artificial sea salts (Sigma) by the dilution plating method.

Source (type strain): sediment of tidal flat in Ganghwa Island, Korea (37°36′22.3 N, 126°22′59.4″ E).

DNA G+C content (mol%): 70 (HPLC).

Type strain: JC2056, IMSNU 14029, KCTC 9921, JCM 12125.

Sequence accession no. (16S rRNA gene): AY423719.

3. **Nocardioides agariphilus** Dastager, Lee, Ju, Park and Kim 2008f, 2295[VP]

a.ga.ri.phi′lus. N.L. n. *agarum* agar; N.L. adj. *philus -a -um* (from Gr. adj. *philos -ê -on*) friend, loving; N.L. masc. adj. *agariphilus* agar-loving.

Characteristics are as described for the genus and listed in Table 215. Information presented below is taken from the original species description (Dastager et al., 2008f).

Cells are irregular rods (0.4–0.5 × 1.3–3.0 µm) or cocci. Club-shaped and larger spherical forms reaching up to 2 µm in diameter occur in 7-d-old culture (28°C) on R2A agar. No aerial or substrate mycelium is formed. Motility is reported. Colonies on R2A agar are white to cream in color, and 1.5–2.7 mm in diameter after 7 d of incubation at 28°C. Will grow at 25–35°C, with optimum at 28°C; no growth occurs below 20°C or above 37°C. Optimum pH for growth is 7.5. Prefers a low salinity level; prefers not to grow in twofold-diluted R2A medium (Difco) in the presence of 2% (w/v) NaCl. The carbon sources for growth (according to Kämpfer, 1991) using mineral salts medium supplemented with yeast extract, Oxoid (0.02 g/l), bio-Lactysat, bioMérieux (0.02 g/l), a vitamin solution, and trace element solution (Kämpfer et al., 1990) are listed in Table 215. The organism was also reported to show growth on agar medium without any addition of carbon and nitrogen sources. Major fatty acids of cells harvested from twofold-diluted R2A broth (pH 7.5) after incubation for 10 d at 28°C were $C_{16:0}$ iso (37.6%), $C_{17:1}$ ω8*c* (9.8%), $C_{17:0}$ (7.1%), $C_{17:0}$ iso (5.8%), $C_{17:1}$ iso ω9*c* (5.1%), $C_{15:0}$ iso (4.9%), $C_{18:1}$ ω9c (4.3%), $C_{17:0}$ anteiso (3.9%), and $C_{17:0}$ 10-methyl (3.0%). Neither TBSA nor hydroxylated fatty acids were reported among components exceeding 2.4% of the total fatty acids. The type strain (and the only strain described) was isolated using a tenfold-diluted R2A medium (Difco).

Source (type strain): soil, Bigeum Island, Korea.

DNA G+C content (mol%): 69.4 (HPLC).

Type strain: MSL-28, DSM 19323, JCM 16020, KCTC 19276.

Sequence accession no. (16S rRNA gene): EF466113.

4. **Nocardioides alkalitolerans** Yoon, Kim, Lee, Lee and Oh 2005a, 813[VP]

al.ka.li.to′le.rans. Arabic article *al* the; Arabic n. *qaliy* ashes of saltwort; L. part. adj. *tolerans* tolerating; N.L. masc. part. adj. *alkalitolerans* referring to the ability to tolerate high pH.

Characteristics are as described for the genus and listed in Table 215, Table 216, and Table 217. Information presented below based on the original species description Yoon et al. (2005a), unless indicated.

Cells are rods (0.8–1.0 × 1.5–2.0 µm) in the exponential phase of growth and show rod-to-coccus morphogenesis from the early exponential phase to the stationary phase. Neither substrate nor aerial mycelium is formed. Gram-stain-positive, but Gram staining is variable in old cultures. Colonies on twofold-diluted nutrient agar (NA; Difco) adjusted to pH 9.0 are milky-white in color and 0.7–1.0 mm in diameter after 7 d incubation at 30°C. Growth occurs at 4 and 34°C, but not at or above 35°C. Optimal pH for growth is 7.0–9.0, with the pH growth range of 5.5–12.0 [assessed in two-fold diluted nutrient broth, adjusted to alkaline pH with Na_2CO_3 (below pH 10.5) or KOH (above pH 10.5)]. Growth at pH 5.0 is variable, with no growth for type strain. Growth occurs at in up to 5% (w/v) NaCl (in trypticase soy broth). Nutritionally non-exacting; grows on a mineral salts medium (ISP 9) with glucose as carbon-plus-energy source and an ammonium salt as a sole nitrogen source. Adonitol and D-sorbitol are not utilized on the basal medium ISP 9; utilization of maltose varies among strains (the type strain is negative). Tweens 20, 40, and 60 are hydrolyzed; some strains, including the type strain, are able to hydrolyze gelatin. Some test results may vary between experiments (Dastager et al., 2010; Yoon et al., 2005a). The ability to oxidize some carbon substrates (Tóth et al., 2008) in the Biolog GP2 system are shown in Table 216. The major fatty acids determined for 4 strains grown on two-fold diluted NA agar (pH 9.0) at 30°C for 7 d, included TBSA (21.1–23.2%), $C_{16:0}$ iso (19.9–22.9%), $C_{18:1}$ ω9*c* (10.2–13.4%), $C_{17:1}$ ω6*c* (8.2–10.4%), $C_{16:0}$ (5.9–8.1%), $C_{18:0}$ (4.8–5.9%), and $C_{18:0}$ iso (4.0–5.2%). The DNA–DNA similarity levels between four strains of this species, including the type strain and strains KSL-9, KSL-10, and KSL-12) are 85–91%. The four characterized strains of this species were isolated using the dilution plating technique at 30°C on tenfold-diluted nutrient agar (NA; Difco) adjusted to pH 10.0 with Na_2CO_3.

Source (type strain): alkaline serpentinite soil, Korea.

DNA G+C content (mol%): 72.4–73.6 (HPLC); 71.7 for the type strain.

Type strain: KSL-1, KCTC 19037, DSM 16699, JCM 13365.

Sequence accession no. (16S rRNA gene): AY633969 for the type strain, and AY633970–AY633972 for strains KSL-9, KSL-10, and KSL-12.

5. **Nocardioides aquaticus** Lawson, Collins, Schumann, Tindall, Hirsch and Labrenz 2000b, 1953[VP] (Effective publication: Lawson, Collins, Schumann, Tindall, Hirsch and Labrenz 2000a, 226.)

a.qua′ti.cus. L. masc. adj. *aquaticus* living in water.

Characteristics are as described for the genus and listed in Table 215. Information presented below is based on the original species description (Lawson et al., 2000a), unless indicated.

Cells are predominantly coccoid (around 1.0 µm or more in diameter) or very short rods (0.9 × 1.0 µm), arranged in pairs or small clusters, as observable in 10–14 d cultures grown at 26°C on oligotrophic medium PYGV (DSM No. 621) (DSMZ, 2001; Staley, 1968) prepared with artificial sea water. Neither substrate nor aerial mycelium is formed. Colonies are mostly creamy or dull orange, and older cultures may be orange-colored. Grows at 16–26°C, in the presence of 1–6% NaCl (w/v) and at pH 7–8. The ranges of salinity and pH growth are 0–15% (w/v) NaCl and pH 5.5–9.5 (the maximal pH value examined); prefers not to grow at

3°C or 33.5°C. Growth occurs on marine agar (Difco) (Yi and Chun, 2004a) and is enhanced in the presence of thiamine and biotin (Lawson et al., 2000a). Acetate, pyruvate, glutamate, succinate, malate, and butyrate are utilized as carbon sources for growth in mineral salts medium supplemented with vitamins and traces of yeast extract (Labrenz et al., 1999). No growth with methanol as a carbon source. According to Yi and Chun (2004a), the type strain is able to grow with glucose, acetate, succinate, and other substrates as sole carbon sources on mineral salts medium (Baumann et al., 1971) supplemented with 2% (v/v) Hutner's mineral base (Cohen-Bazire et al., 1957) and modified by reducing the concentration of sea salts; does not utilize benzoate, tartrate, L-arginine L-asparagine, L-lysine, L-ornithine, sorbitol, and acetamide as sole carbon sources for growth. Weak (Lawson et al., 2000a) or no growth (Yi and Chun, 2004a) occurs with citrate. Tween 40 is hydrolyzed. NH_3 is produced from peptone. Methyl red test is negative. No acid is produced from glucose using the API 20NE strip (Yi and Chun, 2004a). More than 20 substrates are oxidized using the Biolog GP test system, including D-glucose, D-fructose, maltose, D-melibiose, D-psicose, L-rhamnose, stachyose, sucrose, D-trehalose, turanose, D-xylose, adonitol, glycerol, inositol, D-mannitol, acetate, succinamate, thymidine, and Tweens 40 and 80. Sensitive to chloramphenicol, penicillin G, and streptomycin as determined with bioDiscs (bioMérieux) after 4 d of growth. Peptidoglycan type is A3γ (LL-A_2pm-glycine). The cell-wall sugars are galactose and glucose. The major respiratory menaquinone is MK-8(H_4) (94% of the total), with MK-6(H_4) and MK-7(H_4) as minor components. The fatty acids analyzed according to Groth et al. (1996) in relatively young cells grown in trypticase soy broth (Difco) at 20°C included the following components: $C_{18:1}$ (30.6%), $C_{16:0}$ (14.1%), $C_{17:0}$ anteiso (10.6%), $C_{16:0}$ iso (8.1%), $C_{18:0}$ (6.9%), $C_{17:0}$ (5.9%), as well as TBSA (3.6%). The type strain (and the only strain characterized) was isolated using PYGV medium prepared with the natural water from Antarctic Ekho Lake, both for enrichment (15°C, 12 d) and subsequent isolation.

Source (type strain): water sample from Antarctic Ekho Lake (depth 1 m, salinity of 9.5%, temperature of 2.8°C, and pH of 8.04) located in the Vestfold Hills in East Antarctica.

DNA G+C content (mol%): 69 (HPLC).

Type strain: EL-17K, ATCC BAA-164, CIP 106993, DSM 11439, JCM 11266, NBRC 100371, NCIMB 703076 (formerly NCFB 3076).

Sequence accession no. (16S rRNA gene): X94145

6. **Nocardioides aquiterrae** Yoon, Kim, Kang, Oh and Park 2004, 74[VP]

a.qui.ter′ra.e. L. n. *aqua* water; L. gen. fem. n. *terrae* of earth or ground; N.L. gen. fem. n. *aquiterrae* pertaining to groundwater.

Characteristics are as described for the genus and listed in Table 215. Information presented below is based on the publications of Yoon et al. (2008, 2007a, 2004), unless indicated.

Cells are irregular rods (0.8–1.0 × 1.7–2.0 µm) in 7 d culture on nutrient agar (NA; Difco) at 30°C, forming small (0.5–1.0 mm in diameter) cream-colored colonies and show rod-to-coccus morphogenesis from the early exponential phase to the stationary phase. Motile, a single lateral flagellum was observed. Neither substrate nor aerial mycelium is formed. Stained Gram-stain-positive or may be Gram-stain-variable in old cultures. Grows at 15 and 42°C (optimum, 30°C), but not at 10°C or temperatures above 43°C. Optimal pH for growth is 6.0–7.0; no growth occurs at pH 5.0. Nutritionally non-exacting; grows on a suitable mineral salts medium (ISP 9 medium) with glucose as the carbon-plus-energy source and an ammonium salt as the sole nitrogen source. Utilizes gentiobiose, turanose, gluconate, and malate as sole carbon and energy sources but not D-arabinose, D- and L-fucose, D-lyxose, D-tagatose, sorbose, stachyose, L-xylose, adonitol, L-arabitol, D-sorbitol, xylitol, glycogen, methyl β-D-xyloside, methyl α-D-mannoside, methyl α-D-glucoside, amygdalin, adipate, 2- and 5-ketogluconate, caprate, and phenylacetate. According to Yi and Chun (2004a), the type strain also uses acetate, succinate, tartrate (weak), acetamide, L-asparagine, and L-lysine as carbon sources for growth and energy, but not benzoate, L-arginine, and L-ornithine. Tributylin is hydrolyzed (Song et al., 2011). Utilization of citrate, lactose, L-rhamnose, D-ribose, and esculin, hydrolysis of Tween 80 and starch, activities for β-galactosidase and trypsin may vary with experiments or the test method (Song et al., 2011; Yi and Chun, 2004a; Yoon et al., 2008; Yoon et al., 2004). Acid not produced from glucose using the API 20NE test system (Yi and Chun, 2004a). Sensitive to chloramphenicol (100 µg), streptomycin (50 µg) and ampicillin (10 µg), but tolerates tetracycline (30 µg) and rifampin (30 µg), as assessed using filter-paper discs (Song et al., 2011).

The fatty acids of cells harvested from NA after 6 d incubation at 30°C included (>4%): $C_{16:0}$ iso (57.6%), $C_{17:0}$ anteiso (9.0%), $C_{16:1}$ iso H (6.8%), $C_{15:0}$ iso (4.9%), and $C_{17:0}$ 10-methyl (4.1%) as well as TBSA (0.8%). The fatty acids determined for cells grown on half-strength R2A agar plates for 3 d at 30°C (Song et al., 2011) were a smaller proportion of $C_{16:0}$ iso (29.9%) and larger proportions of straight-chain unsaturated (>15%) and saturated (7.7%) acids. Song et al. (2011) compared the fatty acid profiles of *Nocardioides aquiterrae* and phylogenetically related species (*Nocardioides pyridinolyticus*, *Nocardioides hankookensis*, and *Nocardioides caricicola*). The levels of DNA–DNA hybridization between the type strains of *Nocardioides aquiterrae* and the phylogenetically closest species *Nocardioides pyridinolyticus* (99.2% 16S rRNA gene similarity) were 32.5 and 28.7% in reciprocal experiments using the procedure of Ezaki et al. (1989). A lower DNA similarity value (19%) was determined with respect to *Nocardioides hankookensis* (98.1% 16S rRNA gene sequence similarity) (Yoon et al., 2008). The following characteristics are helpful in distinguishing *Nocardioides aquiterrae* from the above-mentioned close species: the cell size and motility, optimal and maximal growth temperatures, activity for cytochrome oxidase, nitrate reduction, alkaline phosphatase, esterase (C4) and α-glucosidase, utilization of D-melezitose, D-melibiose, gentiobiose, D-ribose, D-arabitol, and inositol, as well as sensitivity to antibiotics (ampicillin, chloramphenicol, streptomycin, rifampin, and tetracycline; Song et al., 2011; Yoon et al., 2004, 2008). The type strain (and the only strain described) was isolated by the dilution plating technique using NA at 30°C.

Source (type strain): groundwater, Korea.
DNA G+C content (mol%): 73 (HPLC).
Type strain: GW-9, KCCM 41647, JCM 11813.
Sequence accession no. (16S rRNA gene): AF529063.

7. **Nocardioides aromaticivorans** Kubota, Kawahara, Sekiya, Uchida, Hattori, Futamata and Hiraishi 2005b, 984[VP] (Effective publication: Kubota, Kawahara, Sekiya, Uchida, Hattori, Futamata and Hiraishi 2005a, 172.)

a.ro.ma.ti.ci.vo′rans. L. adj. *aromaticus* aromatic, fragrant; L. part. adj. *vorans* devouring; N.L. part. adj. *aromaticivorans* devouring aromatic (compounds).

Characteristics are as described for the genus and listed in Table 215, Table 218, Table 219, and Table 221. Information presented below is based on the original species description (Kubota et al., 2005a), unless indicated.

Cells are nonmotile rods (0.5–0.7 × 1.0–2.0 µm). Transmission electron microscopy with negatively stained cells demonstrated the presence of septa in the middle of some rods, which suggests that coccoid cells may occur in old cultures on some media. Neither substrate nor aerial mycelium is formed. Colonies are milky-white on complex agar media, e.g. on a peptone-beef extract–yeast extract agar medium (Futamata et al., 2004). Growth occurs in the temperature range of 22–40°C (optimum, 30°C) and pH range 5–8 (optimum, pH 7). Dibenzofuran, biphenyl, and dibenzo-*p*-dioxin (weak) are utilized as sole carbon and energy sources, with formation of yellow-orange pigments. Cui et al. (2009) reported the ability of the type strain to utilize *p*-nitrophenol. Cellobiose, maltose, D-mannose, sucrose, trehalose, and D-mannitol are utilized as carbon and energy sources (API CH50), but growth may be weak with these sugars. The carbon sources not utilized include D-arabinose, L-sorbose, L-xylose, fucose, D-tagatose, adonitol, arabitol, dulcitol, erythritol, sorbitol, xylitol, gluconate, 2- and 5-ketogluconate, glycogen, amygdalin, α-methyl-D-glucoside, and α-methyl-D-mannoside (API CH50). Yoon et al. (2009) additionally reported positive reaction of the type strain in the tests for utilization of D- and L-fucose, D- and L-arabitol, adipate and malate, as well as negative results in the tests for utilization of gentiobiose, arbutin, esculin, starch, caprate, and phenylacetate (API 50 CH, API 20NE). Weak or no growth is observable with D-lyxose (Kubota et al., 2005a; Yoon et al., 2009). The type strain grows with L-alanine, L-histidine, L-proline, L-serine, acetate, caprate, 2-ketogluconate, malic acid, propionic acid, suberic acid, and valeric acid as sole carbon and energy sources (conventional tests with mineral salts medium supplemented with vitamins and element solution) but not with adipate, citrate, itaconate, 3- and 4-hydroxybenzoic acids, 3-hydroxybutyrare, 5-ketogluconate, lactate, malonate under the same test conditions (Cui et al., 2009). Production of acetoin was reported (Cui et al., 2009). The major cellular fatty acids determined using the method of Suzuki and Komagata (1983a, 1983c) for seven strains grown at 25°C in a complex liquid medium included $C_{16:0}$ iso (32.8–49.0%), $C_{18:1}$ (11.8–22.1%), $C_{17:0}$ iso (11.9–13.5%), $C_{18:0}$ iso (4.9–7.0%), $C_{17:1}$ (3.0–5.6%), $C_{19:0}$ iso (2.6–3.7%), $C_{17:0}$ (2.0–5.0%), and $C_{17:0}$ anteiso (2.4–4.2%). Neither 10-methyl-branched nor hydroxylated fatty acids were detected. In contrast, the cells harvested from trypticase soy agar (Difco) after incubation at 30°C for 2 d produced $C_{17:0}$ 10-methyl and TBSA, as well as $C_{15:0}$ 3-OH acid (Cui et al., 2009) as shown in Table 219 (also contains data on fatty acids of the type strains of the phylogenetically closest species). The levels of DNA–DNA hybridization between seven strains (H-1, H-2, A-1, A-2, A-3, NSA1-1, and NSA1-2) assigned to *Nocardioides aromaticivorans* exceed 78%. The two strains (H-1 and NSA1-2) affiliated with this species showed DNA–DNA hybridization levels of 17–55% with the type strains of the phylogenetically closest *Nocardioides* species Table 221. The DNA–DNA hybridization values for type strains of more distant species obtained by the method of Ezaki et al. (1989) were 25–29% (*Nocardioides plantarum*) to 32–40% (*Nocardioides albus*, *Nocardioides luteus*, and *Nocardioides jensenii*). Selected phenotypic characteristics differentiating *Nocardioides aromaticivorans* from the phylogenetically closest species are listed in Table 218. Occur in various environments (river water, river sediments, and soil) polluted with polychlorinated dibenzo-*p*-dioxins and dibenzofurans. A dibenzofuran-containing mineral salts medium supplemented with a vitamin solution can be used for enrichment and subsequent isolation. Colonies showing soluble yellow pigment production on the dibenzofuran-containing agar are picked up and subjected to the standard purification procedure, culture on a suitable medium, and strain identification. For details on the enrichment and

TABLE 221. Mean levels of DNA–DNA hybridization (%) between the type strains of *Nocardioides simplex* and closely related species[a,b]

Species	*N. simplex*	*N. aromaticivorans*	*N. nitrophenolicus*	*N. kongjuensis*
1. *N. aromaticivorans*	48–55			
2. *N. nitrophenolicus*	41	48–49		
3. *N. kongjuensis*	21–28	22–36	38–46	
4. *N. ginsengisoli*[c]	33	28	25	22
5. *N. caeni*	13	17	22	31

[a]Data from Yoon et al. (1999, 2006b, 2009), Kubota et al. (2005a), Kim et al. (2009b) and Cui et al. (2009) obtained by the method of Ezaki et al. (1989).

[b]There are no data on the DNA–DNA hybridization level between *Nocardioides ginsengisoli* and *Nocardioides caeni*. These species were described independently by different research groups almost simultaneously (Cui et al., 2009; Yoon et al., 2009).

[c]Close binding values (18–36%) in the reciprocal experiments were obtained for the four species (*Nocardioides aromaticivorans*, *Nocardioides kongjuensis*, *Nocardioides nitrophenolicus*, and *Nocardioides simplex*).

the isolation procedure, see Futamata et al. (2004), Hiraishi and Kitamura (1984), and Kubota et al. (2005b).

Source (type strain): water from the Hikichi river polluted with polychlorinated dibenzo-*p*-dioxins and dibenzofurans, Kanagawa, Japan.

DNA G+C content (mol%): 72.0–72.4 (HPLC).

Type strain: H-1, CIP 108782, DSM 15131, IAM 14992, JCM 11674.

Sequence accession no. (16S rRNA gene; type strain): AB087721.

8. **Nocardioides basaltis** Kim, Roh, Chang, Nam, Yoon, Jeon, Oh and Bae 2009a, 46[VP]

ba.sal′tis. L. gen. n. *basaltis* of basalt, pertaining to the composition of sand, a source of isolation.

Characteristics are as described for the genus and listed in Table 215. Characteristics presented below are taken from the original species description (Kim et al., 2009a).

Cells are nonmotile rods (0.7–1.0 × 1.2–2.0 µm), forming creamy-colored colonies (0.5–1.5 mm in diameter) after 3 d growth on marine agar (MA; Difco) at 30°C. Grows at 10–37°C (optimum about 25–30°C) but not at 4 or 41°C. Grows in marine broth at initial pH 5.5–8.0 (optimally at pH 6–7). Salt-dependent; grows in marine broth containing 1–10% (w/v) NaCl (optimally at 1–2% NaCl), but not in the absence of NaCl. No growth also occurs on the standard R2A agar (Difco) or tripticase soy agar (TSA; Difco). Positive for assimilation of D-arabitol, gluconate and turanose according to the API 50 CH test system examined using inoculation medium AUX supplemented with 1.5% (w/v) NaCl. Negative for assimilation of D-adonitol, amygdalin, D-arabinose, L-arabitol, arbutin, dulcitol, erythritol, D-fucose, L-fucose, gentiobiose, glycogen, inulin, 2- and 5-ketogluconate, D-lyxose, methyl α-D-glucopyranoside, methyl α-D-mannopyranoside, methyl D-xylose, D-sorbitol, L-sorbose, D-tagatose, L-xylose, and xylitol under the same test conditions. No acid is produced from glucose using the API 20NE test system. Fatty acids (>2%) determined for cells grown on MA at 30°C for 3 d included $C_{16:0}$ iso as the predominant component (70.3%), along with $C_{17:1}$ ω8*c* (4.3%), $C_{16:1}$ iso H (3.7%), $C_{14:0}$ iso (3.5%), $C_{17:0}$ 10-methyl (3.2%), $C_{18:1}$ ω9*c* (2.8%), and $C_{18:0}$ iso (2.7%). No TBSA was revealed among fatty acids exceeding 1%. Generally similar profile, with $C_{16:0}$ iso predominating (65.3%), was reported for the phylogenetically closest species, *Nocardioides salarius*, obtained for a younger (1 d) culture grown under the same conditions (Kim et al., 2008a). The fatty acid profile of another phylogenetically close species, *Nocardioides marinisabuli*, grown for 3 d and analyzed under the same experimental conditions as *Nocardioides basaltis*, differed from the latter in that it contained a smaller proportion of $C_{16:0}$ iso (35.9%), and larger proportions of $C_{18:1}$ ω9*c* (15.4%) and $C_{17:0}$ iso (12.3%). The DNA–DNA relatedness between the type strains of this species and phylogenetically very close species *Nocardioides marinisabuli* (99.2% 16S rRNA similarity) was 15.8±1.5%. Mean DNA–DNA similarities with the type strains of more distant species, *Nocardioides terrigena* and *Nocardioides kribbensis*, were 7.0 and 28.7%, respectively. There are no available data on DNA–DNA relatedness to the phylogenetically closest species, *Nocardioides salarius* (Kim et al., 2008a). Sharing many phenotypic features in common with *Nocardioides salarius*, including the fatty acid profile and physiological and biochemical traits (Table 215), the type strain of *Nocardioides basaltis* still appears to differ from that of *Nocardioides salarius* by absence of acid phosphatase, α-chymotrypsin, and valine arylamidase (API ZYM), inability to decompose esculin, Tween 80, and tyrosine, ability to use salicin as a carbon source, having larger-sized cells, and a lower DNA G+C content. The type strain (and the only strain described) was isolated by the dilution plating technique using NA at 30°C.

Source (type strain): black sand, beach, Soesoggak, Jeju Island, Korea.

DNA G+C content (mol%): 68 (T_m).

Type strain: J112, JCM 14945, KCTC 19365.

Sequence accession no. (16S rRNA gene): EU143365.

Additional remarks: the species *Nocardioides basaltis* Kim et al. 2009a described shortly after the establishment of the species *Nocardioides salarius* Kim et al. 2008a has very similar 16S rRNA gene sequence similarity (99.6%) to *Nocardioides salarius*, while the data on the DNA–DNA relatedness between these species are absent. Further comparative taxonomic studies of these two species seem to be needed to justify the separate species status of *Nocardioides basaltis*.

9. **Nocardioides bigeumensis** Dastager, Lee, Ju, Park and Kim 2008f, 2295[VP]

bi.ge.um.en′sis. N.L. masc. adj. *bigeumensis* of or pertaining to Bigeum Island, Korea, the geographical origin of the type strain.

Characteristics are as described for the genus and listed in Table 215. Information presented below based on the original species description (Dastager et al., 2008f).

Cells are cocci or irregular rods (0.3–0.8 × 0.8–4.0 µm). Larger spherical forms, up to 1.5 µm in diameter are reported. Motile. Colonies are cream in color, flat and 1.0–2.5 mm in diameter after 4–5 d incubation on R2A at 28°C. No aerial or substrate mycelium is produced. Grows at 20–35°C (optimum 28°C) and at pH 7.5–9.0. Prefers low salinity; no growth is observed in twofold-diluted R2A in the presence of 1% (w/v) NaCl or more. The carbon sources for growth (Kämpfer, 1991) using mineral salts medium supplemented with yeast extract, Oxoid (0.02 g/l), bio-Lactysat, bioMérieux (0.02 g/l), a vitamin solution, and trace element solution (Kämpfer et al., 1990) are listed in Table 215. The major fatty acids determined in cell mass harvested from twofold-diluted R2A broth (pH 7.5) after incubation for 10 d at 28°C were reported to include $C_{16:0}$ iso (38.3), $C_{15:0}$ iso (13.1), $C_{14:0}$ iso (9.0), $C_{18:1}$ ω9*c* (6.3), $C_{16:0}$ 10-methyl (4.8), $C_{16:0}$ (4.4), and $C_{17:1}$ ω8*c* (3.9). Neither TBSA nor hydroxylated fatty acids exceeded 1%. The type strain (and the only strain described) was isolated using a tenfold-diluted R2A medium by the standard dilution plating method.

Source (type strain): soil, Bigeum Island, Korea.

DNA G+C content (mol%): 69.3 (HPLC).

Type strain: MSL-19, DSM 19320, JCM 16021, KCTC 19290.

Sequence accession no. (16S rRNA gene): EF466114.

10. **Nocardioides caeni** Yoon, Kang, Park, Kim and Oh 2009, 2796[VP]

ca.e′ni. L. gen. n. *caeni* of sludge, isolated from wastewater.

Characteristics are as described for the genus and listed in Table 215, Table 218, Table 219, and Table 221. Information presented below is taken from the original species description (Yoon et al., 2009), unless indicated.

Cells are nonmotile rods or cocci (0.3–0.7 × 0.7–2.5 µm) and can undergo a rod-to-coccus morphogenetic cycle from the early exponential phase to the stationary phase. Gram-stain-positive but results of Gram-staining vary in old cultures. Colonies on nutrient agar (NA; Difco) are grayish yellow and 1.5–2.5 mm in diameter after incubation for 7 d at 30°C. Neither substrate nor aerial mycelium is formed. Optimal temperature for growth is 30°C. The organism grows at 10 and 35°C, but not at 4 or 37°C. Growth occurs in the presence of 0–1.0% (w/v) NaCl, with optimum growth at 0–0.5% (w/v) NaCl and no growth at 2% (w/v) NaCl (assessed in trypticase soy broth prepared according to the formula of the Difco medium without NaCl). Optimal pH for growth is 6.5–7.5; growth occurs at pH 6.0 and 9.5, but not at pH 5.5 or 10.0 [initial pH of the test medium, nutrient broth (NB; Difco) adjusted to various pH by adding HCl or Na_2CO_3]. Positive for assimilation of gluconate, adipate, and malate, but negative for assimilation of 2- and 5-ketogluconate, caprate, and phenylacetate (API 20NE), as well as D-arabinose, sorbose, adonitol, D- and L-arabitol, dulcitol, erythritol, sorbitol, xylitol, methyl α-D-mannoside, methyl α-D-glucoside, amygdalin, arbutin, glycogen, gentiobiose, D-tagatose, D-fucose, and L-fucose (API 50 CH, AUX as suspending medium). Tweens 20, 40, and 60 are hydrolyzed. Susceptible to the following antibiotics (amounts per disc): carbenicillin (100 µg), cephalothin (30 µg), chloramphenicol (100 µg), gentamicin (30 µg), kanamycin (30 µg), neomycin (30 µg), novobiocin (5 µg), oleandomycin (15 µg), penicillin G (20 U), polymyxin B (100 U), streptomycin (50 µg), and tetracycline (30 µg). Resistant to the following antibiotics (amounts per disc): ampicillin (10 µg) and lincomycin (15 µg). Major fatty acids (>5.0% of the total) determined in cell mass harvested from NA plates after incubation for 7 d at 30°C included $C_{16:0}$ iso, $C_{18:1}$ ω9*c*, $C_{17:1}$ ω8*c*, $C_{17:1}$ ω6*c*, and $C_{17:0}$ iso (Table 219); no TBSA was not detected among fatty acids exceeding 0.5%. The type strain exhibited the DNA–DNA similarity values of 13–31% to type strains of the most closely related species (Table 221). Selected phenotypic characteristics differentiating *Nocardioides caeni* from phylogenetically closely related species are listed in Table 218. The type strain (and the only strain described) was isolated by means of the standard dilution plating technique on NA at 30°C.

Source (type strain): sludge of domestic wastewater, Korea.

DNA G+C content (mol%): 71.5 (HPLC).

Type strain: MN8, KCTC 19600, CCUG 57506.

Sequence accession no. (16S rRNA gene): FJ423551.

11. **Nocardioides caricicola** Song, Yasir, Bibi, Chung, Jeon and Chung 2011, 108[VP]

ca.ri.ci′co.la. L. n. *carex -icis* reed-grass, rush or sedge, and also a botanical genus name (*Carex*); L. suff. *-cola* (from L. n. *incola*), inhabitant, dweller; N.L. n. *caricicola*, *Carex*-dweller, isolated from a halophytic plant *Carex scabrifolia* Steud.

Characteristics are as described for the genus and listed in Table 215. Information presented below is taken from the original species description (Song et al., 2011).

Cells are irregular rods (0.4–0.6 × 2.0–5.0 µm) to cocci (Figure 256) in 5-d-old culture grown in half-strength R2A broth (Difco). No flagella were observed for cells grown in the same medium for 2 d at 30°C. Colonies are convex, glistening, white in color and 0.5–0.7 mm in diameter after 5 d of incubation at 30°C on half-strength R2A agar (1.6 g R2A broth powder supplemented with 1.5% agar in 1 L of distilled water). The organism grows in half-strength R2A broth with 0.5% (w/v) NaCl and on marine agar 2216 (MA, Difco). No growth is observable in half-strength R2A broth supplemented with 1% (w/v) NaCl. The pH range for growth reported is also narrow, 7.0–9.0 (optimum pH 8.0); no growth at pH 6.5 and 9.5 (assessed on half-strength R2A agar). Grows at 10 and 45°C, where the latter is the maximal growth temperature reported for the recognized *Nocardioides* species, and not at 50°C (half-strength R2A agar plates). Acid is produced from D-glucose (API 20E). No assimilation of any carbon source was observed using API 20NE test system (no inoculation medium is reported). Tween 20 is decomposed. Tributylin or xylan is not decomposed. Nitrate is reduced to nitrogen gas. Susceptible to the following antibiotics (µg per filter-paper disc): chloramphenicol (100), gentamicin (10), kanamycin (30), penicillin (10), rifampin (30), streptomycin (50), and vancomycin (30); resistant to ampicillin (10) and tetracycline (30). The major cellular fatty acids (>5.0%) determined for cells harvested from half-strength R2A agar plates after incubation for 3 d at 30°C comprised $C_{16:0}$ iso (28.9%), $C_{18:1}$ ω5*c* (7.0%), and $C_{14:0}$ (6.6%), as well as $C_{18:2}$ ω6*c*, $C_{18:2}$ ω9*c*, and/or $C_{18:0}$ anteiso (8.1%). Minor contents of TBSA (1.7%) and $C_{17:0}$ 10-methyl (3.3%) were also detected. Song et al. (2011) compared the fatty acid profiles of the type strains of *Nocardioides caricicola* and related species (*Nocardioides aquiterrae*, *Nocardioides hankookensis*, and *Nocardioides pyridinolyticus*) obtained under the same experimental conditions. DNA–DNA relatedness between the type strains of this species and *Nocardioides pyridinolyticus* was determined to be 53.5 ± 5.5%. The type strain (and the only strain described) was isolated from the surface-sterilized plant roots. Following sterilization (70% ethanol, 1.0% NaOCl and again 70% ethanol), washing and checking for the surface sterility, a root sample was dried, grounded in sterile sea water and plated on 1/10-strength R2A agar, followed by incubation at 25°C for 1–2 weeks (for details on the isolation procedure see Chung et al., 2008; Song et al., 2011).

Source (type strain): roots of a halophytic plant, *Carex scabrifolia* Steud, growing on sand dunes, Namhae Island, Korea.

DNA G+C content (mol%): 71.7 (HPLC).

Type strain: YC6903, KACC 13778, DSM 22177.

Sequence accession no. (16S rRNA gene): FJ750845.

12. **Nocardioides daedukensis** Yoon, Park, Kang, Lee, Lee and Oh 2010, 1337[VP]

da.e.duk.en′sis. N.L. masc. adj. *daedukensis* of or pertaining to Daeduk Science Park, where the Korea Research Institute of Bioscience and Biotechnology is located.

Characteristics are as described for the genus and listed in Table 215 and Table 216. Information presented below is taken from the original species description (Yoon et al., 2010).

Cells are nonmotile irregular rods or cocci (0.4–0.8 × 0.8–3.0 μm) and show rod-to-coccus morphogenesis from the early exponential phase to the stationary phase. Gram-stain-positive but Gram-staining is variable in old cultures. Colonies on nutrient agar (NA; Difco) are pale yellow in color, glistening, raised, and 1.0–1.5 mm in diameter after incubation for 7 d at 30°C. Neither substrate nor aerial mycelium is formed. Grows at 4 and 37°C (optimally at 30°C), but not at 40°C. Growth is observable at pH 6.0 and 10.0 (optimum at pH 7.0–8.0), but not at pH 5.5 and 10.5, as assessed in nutrient broth (NB; Difco) adjusted to different pH values with HCl or Na_2CO_3. Tolerates salinity up to 9.0% (w/v) NaCl (tested in trypticase soy broth prepared according to the Difco formula, with a definite NaCl concentration), however prefers low NaCl concentrations for growth, 0–0.5% (w/v). Nutritionally non-exacting; grows on a mineral salts medium (ISP 9 medium) with glucose as carbon-plus-energy source and an ammonium salt as sole nitrogen source. Glutamate, acetate, L-malate and pyruvate are utilized as a sole carbon and energy sources on the same basal medium, while negative test results are observable for benzoate, citrate, formate, and succinate. Tweens 20, 40, and 60 are hydrolyzed. Susceptible to the following antibiotics (amounts per disc): carbenicillin (100 μg), cephalothin (30 μg), chloramphenicol (100 μg), gentamicin (30 μg), kanamycin (30 μg), lincomycin (15 μg), neomycin (30 μg), oleandomycin (15 μg), penicillin G (20 U), polymyxin B (100 U), streptomycin (50 μg), and tetracycline (30 μg). Resistant to ampicillin (10 μg) and novobiocin (5 μg).The major fatty acids determined for cells harvested from NA plates after cultivation for 7 d at 30°C were $C_{16:0}$ iso (40.9%), $C_{17:1}$ (15.5%), $C_{17:0}$ 10-methyl (12.2%), $C_{18:1}$ (7.6%), TBSA (6.3%), and $C_{16:1}$ iso (3.5%), as well as minor amounts of other acids, including $C_{17:0}$ 3-OH (1.2%). The fatty acid profile differed from that of the type strain of phylogenetically close *Nocardioides jensenii* and *Nocardioides dubius* (analyzed under the same experimental conditions). In particular, *Nocardioides jensenii* contained a greater proportion of $C_{16:1}$ iso (10.9%), whereas *Nocardioides dubius* had a smaller proportion of $C_{17:1}$ (1.4%) and a significantly larger total iso-branched acids. The major polar lipids of cells grown in NB for 7 d at 30°C are diphosphatidylglycerol, an unidentified phospholipid, and two unidentified lipids. Minor components detected included phosphatidylglycerol, phosphatidylethanolamine, and an unidentified phospholipid. The organism did not contain a compound identical to phosphatidylinositol that was reported for *Nocardioides jensenii* (Collins et al., 1989), *Nocardioides dubius* (Yoon et al., 2005d), and *Marmoricola aurantiacus* (Urzì et al., 2000).

The type strain exhibited mean DNA–DNA relatedness values of 19% to the type strain of the phylogenetically closest species, *Nocardioides jensenii* (98.3% 16S rRNA similarity) and of 10% to *Nocardioides dubius.* The type strain (and the only strain described) was isolated by the standard dilution plating technique on ten-diluted NA at 30°C.

Source (type strain): soil at Taejon, South Korea.

DNA G+C content (mol%): 68.7 (HPLC).

Type strain: MDN22, KCTC 19601, CCUG 57505.

Sequence accession no. (16S rRNA gene): FJ842646.

13. **Nocardioides daphniae** Tóth, Kéki, Homonnay, Borsodi, Márialigeti and Schumann 2008, 81[VP]

daph.ni′a.e. N.L. gen. n. *daphniae* of *Daphnia*, generic name of water flea (*Daphnia cucullata*) from which the type strain was isolated.

Characteristics are as described for the genus and listed in Table 215, Table 216, and Table 217. Information presented below is based on the original description (Tóth et al., 2008), unless indicated.

Cells are short rods or coccoids (0.8–1.0 × 1.2–2.2 μm). Nonmotile. Colonies on King B agar medium (King et al., 1954) are of yellowish color. Neither substrate nor aerial mycelium is produced.

The organism grows at 4–38°C, at initial pH values of 5.5–10.5, and at NaCl concentrations up to 5% (w/v), with optimum growth at 28°C and pH 7–9; no growth occurs at 45°C, at pH 11, and in the presence of 10% (w/v) NaCl (King B agar or broth as basal media). Good growth also occurs on standard nutrient agar (NA, Difco) and trypticase soy agar. The organism grows on mineral salts medium (ISP 9) with glucose and other substrates (Table 215) as carbon-plus-energy sources and an ammonium salt as a sole nitrogen source (Dastager et al., 2010). Produces chitinase (tested using a modified version of the method of Holding and Collee, 1971). No acid produced from glucose in the O/F test (Hugh and Leifson, 1953). Hemolysin is not detected. Data on the oxidation of carbon substrates assessed with the Biolog GP2 test system are listed in Table 216. In addition, the type strain oxidizes methyl-β-D-glucoside, sorbitol, stachyose, acetic acid, β-hydroxybutyric acid, γ-hydroxybutyric acid, α-ketovaleric acid, L-lactic acid, D-malic acid, methyl-pyruvate, glycyl L-glutamic acid, pyruvic acid, 2,3-butanediol, adenosine, 2-deoxyadenosine, thymidine, uridine, adenosine 5′-monophosphate, and uridine 5′-monophosphate but not the remaining Biolog GP2 substrates (about 50 substrates). The major fatty acids determined for cells grown on trypticase soy agar (Difco) at 28°C included $C_{16:0}$ iso (42.7%), $C_{18:1}$ ω9*c* (9.9%), $C_{17:0}$ 10-methyl (9.7%), $C_{17:0}$ iso (8.2%), and $C_{17:1}$ iso ω9*c* (8.1%). Components detected in smaller proportions were $C_{17:1}$ ω8*c* (3.6), $C_{16:1}$ iso (2.6), $C_{17:0}$ anteiso (2.4), $C_{18:0}$ iso (2.4), $C_{16:1}$ ω7*c* (2.2), and some others typical of the genus. The type strain (and the only strain described) was isolated using King B agar medium (King et al., 1954).

Source (type strain): whole-body homogenate sample of *Daphnia cucullata* (Crustacea: Cladocera) adult individuals, Lake Balaton at Tihany (46°55′20″N; 17°55′39″E), Hungary.

DNA G+C content (mol%): 69.9 (HPLC).

Type strain: D287, DSM 18664, CCM 7403, JCM 16608.

Sequence accession no. (16S rRNA gene): AM398438.

14. **Nocardioides dilutus** Dastager, Lee, Ju, Park and Kim 2009b, 1555[VP] (Effective publication: Dastager, Lee, Ju, Park and Kim 2008d, 572.)

di.lu′tus. L. masc. adj. *dilutus* weak, diluted, intended to mean that the organism is able to grow in 100 times diluted R2A medium.

Characteristics are as described for the genus and listed in Table 215 and Table 216. Information presented below is taken from Dastager et al. (2008d, 2010).

Cells are irregular rods (0.4–0.8 µm in diameter; up to 4.0 µm in length) or cocci. The 5-d-old culture grown on R2A agar (Difco) at 28°C is composed of shorter cells (mostly 0.4–0.6 × 0.8–1.6 µm) with a small proportion of coccoid cells. Motile. Colonies on R2A are irregular, smooth, flat, and white to cream in color; 1.5–2.7 mm in diameter after 5 d incubation at 28°C. Neither substrate nor aerial mycelium is formed. Nutritionally non-exacting; grows on a mineral salts medium (e.g. ISP 9) with glucose and other substrates as carbon-plus-energy sources and an ammonium salt as a sole nitrogen source. Growth occurs on solid media with low nutrient concentrations, including 100-fold diluted R2A. Prefers not to grow in R2A broth supplemented with 1% (w/v) NaCl or more. Cell-wall peptidoglycan contains A_2pm as the diagnostic diamino acid (no A_2pm isomer is reported). Fatty acids determined in cell mass harvested from R2A broth after incubation for 7 d at 28°C included (% of the total): $C_{16:0}$ iso (46.7%), $C_{18:1}$ ω9*c* (12.4%), $C_{17:1}$ ω8*c* (5.5%), $C_{14:0}$ iso (4.3%), $C_{17:0}$ anteiso (4.2%), $C_{16:0}$ (3.8%), $C_{17:0}$ (2.9%), $C_{18:0}$ (2.7%), $C_{15:0}$ anteiso (2.5%), $C_{15:0}$ iso (2.4%), $C_{16:1}$ iso (2.0%), and $C_{17:0}$ (1.2%). The type strain (and the only strain described) was isolated by serial dilution plating on tenfold-diluted R2A agar at 30°C after 7 d incubation.

Source (type strain): soil, Bigeum Island, Korea.

DNA G+C content (mol%): 71.8 (HPLC).

Type strain: MSL-11, KCTC 19288, DSM 19318.

Sequence accession no. (16S rRNA gene): EF466121.

15. **Nocardioides dokdonensis** Park, Baik, Kim, Chun and Seong 2008, 2622[VP]

dok.do.nen′sis. N.L. masc. adj. *dokdonensis* of or pertaining to Dokdo, the Korean island from where the type strain was isolated.

Characteristics are as described for the genus and listed in Table 215. Information presented below based on the original description (Park et al., 2008).

Cells are relatively short rods (0.6–0.9 × 1.2–1.8 µm) in the exponential phase of growth. Nonmotile. Colonies on trypticase soy agar (TSA; Difco) are cream in color and approximately 1.0–2.0 mm in diameter after 3 d at 25°C, reaching a maximum diameter of 3 mm after 7 d. Substrate and aerial mycelia are not observed. Grows at 4–30°C (optimum, 25°C), at pH 5–10 (optimum, pH 7), and in up to 7% (w/v) NaCl, with the optimum growth in 0–3% (w/v) NaCl, as assessed on TSA. The following substrates are utilized as sole carbon and energy sources for growth: adonitol, sodium acetate, sodium citrate, sodium propionate, sodium pyruvate, and other carbon sources (Table 215) as tested on Stevenson's basal medium (Stevenson, 1967).* Acetate, adipate, caprate, phenyl acetate, malate, gentiobiose, dextran, DL-xylitol are not utilized on the same basal medium. The following substrates are utilized as sole nitrogen sources: L-cysteine, L-hydroxyproline, L-phenylalanine, L-threonine, L-valine, and nitrate, but not DL-α-amino-*n*-butyric acid or L-histidine (examined using Tsukamura's medium; Tsukamura, 1975). Allantoin, guanine, Tween 20, and xylan are hydrolyzed, but arbutin, elastin, and hippurate are not. Acids are not produced from D-glucose and maltose using the API 20 NE system. In the Biolog GP2 system, the type strain oxidizes 29 of 97 substrates including acetate, adenosine, adenosine 5′-monophosphate, β-cyclodextrin, 2′-deoxyadenosine, D-fructose, glycerol, β-hydroxybutyric acid, γ-hydroxybutyric acid, inosine, α-ketovaleric acid, maltotriose, D-mannitol, D-mannose, melezitose, melibiose, propionic acid, D-psicose, pyruvic acid, D-ribose, sedoheptulosan, D-sorbitol, succinic acid, thymidine, trehalose, Tween 40, Tween 80, uridine, and D-xylose. The type strain is sensitive to the following antibiotics (µg per disc, unless indicated): amikacin (30), ampicillin (10), chloramphenicol (30), erythromycin (15), gentamicin (10), kanamycin (30), nalidixic acid (30), streptomycin (10), tetracycline (30), vancomycin (30), penicillin (10 U), and polymyxin B (300 U). The major cellular fatty acids determined for the type strain grown on TSA for 2 d at 25°C were $C_{16:0}$ iso (40.4%), $C_{18:1}$ ω9*c* (11.2%), $C_{16:0}$ (7.7%), $C_{18:0}$ (7.2%), $C_{17:0}$ iso (4.8%), and $C_{17:1}$ ω8*c* (4.6%). TBSA was not detected; the only 10-methyl-branched acid, i.e. $C_{17:0}$ 10-methyl contributed 1.4%. The DNA–DNA similarity between the type strains of *Nocardioides dokdonensis* and *Nocardioides marinisabuli* was 23.1±5.6%. The type strain (and the only strain described) was isolated by the standard dilution plating technique using R2A agar (Difco) supplemented with 3.5% artificial sea salts (Sigma).

Source (type strain): sand sediment, a beach on Dokdo Island, Korea (37°05′N; 131°13′E).

DNA G+C content (mol%): 69.1 (HPLC).

Type strain: FR1436, KCTC 19309, JCM 14815.

Sequence accession no. (16S rRNA gene): EF633986.

16. **Nocardioides dubius** Yoon, Lee and Oh 2005d, 2211[VP]

du′bi.us. L. masc. adj. *dubius* doubtful of the taxonomic position.

Characteristics are as described for the genus and listed in Table 215, Table 216, and Table 217. Information presented below is based on the original description (Yoon et al., 2005d; Yoon et al., 2010), unless indicated.

Cells are irregular rods (0.8–1.0 × 1.5–2.5 µm) in the exponential phase of growth and show rod-to-coccus morphogenesis from the early exponential phase to the stationary phase. Motile; a single flagellum was observed. Neither substrate nor aerial mycelia is formed. Colonies are irregular, raised with erose margins, yellowish-white in color and 2.0–3.0 mm in diameter after 3 d incubation on nutrient agar (NA; Difco) at 30°C. Grows at 10 and 37°C, but not at 4 or 38°C. Grows in up to 5% (w/v) NaCl, but better without addition of NaCl in trypticase soy broth (TSB; Difco). Optimal pH for growth is 7.0–8.0; grows at pH 6.0 and 10.5, but not at pH 5.5 (initial pH values; examined on NA as a basal medium after 10 d incubation at 30°C). Nutritionally non-exacting; grows on a mineral salts medium (ISP 9) with glucose and some other substrates (Table 215) as carbon-plus-energy source and an ammonium salt as sole

*Bacto Yeast Nitrogen Base medium without amino acids (Difco) supplemented with Casamino acids (10 mg per liter) and agar (15 g per liter), and neutralized by K_2HPO_4.

nitrogen source. Does not utilize D-cellobiose, D-fructose, sucrose, and D-xylose (Yoon et al., 2010); conflicting data were reported by Dastager et al. (2010) using the same basal medium (ISP 9). Utilizes glutamate and pyruvate as a sole carbon and energy sources in the same basal medium, but not benzoate, citrate, formate, and L-malate. Table 216 shows the carbon sources in the Biolog GP2 test system that are oxidized. Tweens 20, 40, and 60 are not hydrolyzed. The composition of the major polar lipids is unique: phosphatidylethanolamine, along with diphosphatidylglycerol, phosphatidylglycerol, and phosphatidylinositol [cells for analysis were grown in NB (Difco) at 30°C]. The major fatty acids (more than 3% of the total in at least one experiment) determined in cells harvested from NA after 6 and 7 d incubation at 30°C were $C_{16:0}$ iso (58.5 and 57.8%), $C_{17:0}$ 10-methyl (7.0 and 8.7%), $C_{16:0}$ 10-methyl (4.2 and 5.3%), $C_{18:0}$ iso (4.3 and 3.0%), $C_{16:1}$ iso (3.7 and 5.1%), $C_{17:0}$ iso (3.2 and 2.4%), and $C_{14:0}$ iso (2.5 and 3.0%). TBSA (2.7%) was detected only in the 6-d-old culture (among fatty acids exceeding 0.5%). The fatty acid profile of 7-d-old culture of *Nocardioides dubius* differed from those of *Nocardioides jensenii* and *Nocardioides daedukensis* (analyzed under the same experimental conditions) in the proportion of iso-branched acids, which was larger contributing nearly 75%, and in the proportions of some individual components (for details, see the descriptions of *Nocardioides jensenii* and *Nocardioides daedukensis* in this chapter and the paper of Yoon et al., 2010). The mean DNA–DNA relatedness of the type strain to the type strain of *Nocardioides daedukensis* was 19% (Yoon et al., 2010). The type strain (and the only strain described) was isolated by the standard dilution plating technique on a tenfold-diluted NA with the pH 10.0 adjusted using Na_2CO_3 (30°C).

Source (type strain): alkaline soil, Kwangchun, Korea.

DNA G+C content (mol%): 70.6 (HPLC).

Type strain: KSL-104, KCTC 9992, JCM 13008.

Sequence accession no. (16S rRNA gene): AY928902.

17. **Nocardioides exalbidus** Li, Xie and Yokota 2007a, 2449[VP] (Effective publication: Li, Xie and Yokota 2007b, 24.)

ex.al.bi′dus. L. masc. adj. *exalbidus* whitish, referring to the color of the colonies.

Characteristics are as described for the genus and listed in Table 215 and Table 220. Additional information presented below is based on the original description (Li et al., 2007b).

Cells are nonmotile, coccoid- to rod-shaped (~ 0.5 µm wide, up to 2.0 µm long), predominantly coccoid or very short rods (~0.6–0.8 µm in diameter) when grown 2 d on ISP 2 agar at 30°C, and show rod-to-coccus morphogenesis from the early exponential phase to the stationary phase. Colony color is white, as observed on trypticase soy agar (TSA; BBL) and on IAM-A1 agar (that is a modified Detmer's medium; see IAM Catalog of Strains, 2004). Grows at 15–35°C, optimally at 30°C, and over a pH range of 6.0–9.0, with the optimal pH around 7.0. The organism grows in a mineral salts medium (IAM-A1 agar) with glucose as carbon-plus-energy source and nitrate as sole nitrogen source. The type strain utilizes amygdalin, 2-keto-gluconate, and 5-keto-gluconate as sole carbon and energy sources but not gluconate, gentiobiose, D-lyxose, sorbose, D-tagatose, D-turanose, adonitol, D- and L-arabitol, dulcitol, erythritol, sorbitol, xylitol, methyl β-D-xyloside, methyl α-D-mannoside, methyl α-D-glucoside, arbutin, starch, and glycogen.

Diphosphatidylglycerol and phosphatidylinositol were reported as major polar lipids; no phosphatidylcholine was detected. The main fatty acids determined in culture grown on TSA for 3 d at 27°C, included $C_{16:0}$ iso (28.0%), TBSA (18.2%), $C_{17:0}$ 10-methyl (7.7%), $C_{17:0}$ iso (9.1%), $C_{17:0}$ anteiso (6.9%), and $C_{18:0}$ (5.8%), and $C_{17:1}$ ω6*c* (4.7%). The DNA–DNA relatedness of the type strain of *Nocardioides exalbidus* to the type strains of phylogenetically neighboring species was 44% (*Nocardioides oleivorans*) and 39% (*Nocardioides ganghwensis*). Selected phenotypic characteristics useful in distinguishing *Nocardioides exalbidus* from the above and other phylogenetically closely related species are listed in Table 220. The type strain (the only strain characterized) was isolated from a lichen sample that was washed and crushed in sterilized water, followed by streaking the effluent on IAM-A1 agar medium, after incubation for 2 weeks and purification on TSA.

Source (type strain): lichen, Izu-Oshima Island, Japan.

DNA G+C content (mol%): 74 (HPLC).

Type strain: RC825, IAM 15416, JCM 23199, CCTCC AA206016.

Sequence accession no. (16S rRNA gene): AB273624.

18. **Nocardioides fonticola** Chou, Cho, Arun, Young and Chen 2008, 1866[VP]

fon.ti′co.la. L. masc. n. *fons*, *fontis* a spring, fountain; L. suff. *-cola* (from L. masc. or fem. n. *incola*) an inhabitant of a place; N.L. n. *fonticola* an inhabitant of a fountain or spring.

Characteristics are as described for the genus and listed in Table 215. Information presented below is based on the original species description (Chou et al., 2008).

Cells are nonmotile rods (~ 0.8 × 2.0–9.0 µm). Colonies on R2A (Difco) are pale yellowish, approximately 0.9–1.0 mm in diameter after 3 d incubation at 25°C, and reach a maximum of 2 mm after 5 d of incubation. Grows at 25–37°C, with optimum at 30°C. Grows at low salinity (0.5–1.0%, w/v, NaCl; optimum at 0.5%), but not in the absence of NaCl or at 2% (w/v) NaCl as assessed in nutrient broth prepared according to the Difco formula without NaCl. The pH range for growth is 5–9 (optimum pH 7–8), as examined in nutrient broth (Difco) with appropriate buffers; no growth is observed at pH 4.5 or 9.5 under the same experimental conditions. No acid is produced by oxidation of D-glucose, D-xylose, D-mannitol, maltose, D-lactose, sucrose, D-ribose, and glycogen (API Coryne test system). Negative for fermentation of glucose, and for assimilation of gluconate, malate, caprate, adipate, citrate, and phenylacetate (API 20NE). Positive for pyrazinamidase and pyrrolidonyl arylamidase. Tweens 20, 40, and 60 are hydrolyzed. Corn oil is not degraded. Sensitive to the following antibiotics (µg per disk): ampicillin (10), chloramphenicol (30), gentamicin (10), kanamycin (30), novobiocin (30), rifampin (5), penicillin G (10), streptomycin (10), and tetracycline (30). Resistant to nalidixic acid (30), ceftizoxime (30), or sulfamethoxazole (23.75) plus trimethoprim (1.25). Major fatty acids detected for cells harvested from

R2A after 3 d incubation at 25°C included $C_{16:0}$ iso (19.9%), $C_{17:0}$ (19.9%), $C_{17:1}$ ω8*c* (13.3%), $C_{18:1}$ ω9*c* (11.7%), $C_{18:0}$ (4.8%), $C_{18:0}$ iso (4.5%), as well as $C_{19:1}$ ω11*c* and/or $C_{19:1}$ ω9*c* (8.3%), 10-methyl-branched fatty acids [10-Me-$C_{17:0}$ (2.6%) and TBSA (1.7%)], and other acids [$C_{16:0}$, $C_{19:0}$, and $C_{17:0}$ iso (each 2.6–3.6%)]. The type strain (and the only strain described) was isolated by plating a water sample on R2A agar medium and incubation at 25°C for 3 d.

Source (type strain): freshwater of spring, Kaoshiung County, Taiwan.

DNA G+C content (mol%): 71.8 (HPLC).

Type strain: NAA-13, BCRC 16874, JCM 16703, LMG 24213.

Sequence accession no. (16S rRNA gene): EF626689.

19. **Nocardioides furvisabuli** Lee 2007b, 37[VP]

fur.vi.sa′bu.li. L. neut. adj. *furvum* black-colored; L. neut. n. *sabulum* gravel, sand; N.L. gen. n. *furvisabuli* of black-colored sand, the source of isolation of the type strain.

Characteristics are as described for the genus and listed in Table 215 and Table 220. Information presented below is based on the original publication (Lee, 2007b).

Cells are short motile rods (0.4–0.5 × 0.6–1.2 μm) after 3 d in culture at 30°C on YE/SW agar (ISP 2 medium prepared in 60%, v/v, natural sea water), and tend to adhere and form palisades (Figure 258). Colonies are yellow and 0.6–0.8 mm in diameter after 5 d incubation under the same conditions. The organism shows good growth on ISP 2 medium, marine agar (MA; Difco), and nutrient agar (Difco) with or without the addition of natural sea water. Prefers not to grow on standard trypticase soy agar (Difco). Grows at 4–37°C, with optimum at 30°C. The reported pH range for growth is pH 5.1–10.1 (initial pH of media), and the optimum is about 7.0. Grows in up to 6% (w/v) NaCl, as determined in ISP 2 as the basal medium. Nutritionally non-exacting; grows in a suitable mineral salts medium with glucose as carbon-plus-energy source and an ammonium salt as sole nitrogen source (e.g. ISP 9 medium). Citrate, formate, malate, succinate, tartrate, adonitol, D-dulcitol, and methyl α-D-mannoside but not benzoate, D-arabinose, L-sorbose, dextran, methyl α-D-glucoside, *meso*-erythritol, D-xylitol, and 2,3-butanediol are utilized as sole carbon and energy sources. Weakly positive for utilization of acetate, D-sorbitol, and 1,2-propanediol. Negative for acid production (API 20NE) from glucose. Elastin is degraded. The fatty acid profiles were reported for cells grown for 5 d at 30°C on YE/SW agar and MA agar. In the YE/SW culture, $C_{16:0}$ iso (34.1%), $C_{18:1}$ ω9*c* (27.2%), $C_{18:0}$ (7.9%), and $C_{16:0}$ (7.5%) were predominating; the straight-chain saturated acids comprised 17.1% of the total, and straight-chain unsaturated acids comprised almost 33%. Cells grown on MA agar had a significantly larger proportion (44%) of straight-chain saturated components, including $C_{18:0}$ (19.3%) and $C_{16:0}$ (14.6%) and a smaller proportion of straight-chain unsaturated acids (10.5%). The other major acids were $C_{16:0}$ iso (19.6%), $C_{18:1}$ ω9*c* (6.5%), $C_{16:1}$ iso (7.2%), and $C_{15:0}$ anteiso (7.5%). Notably, cells grown on MA agar had anteiso-branched acids ($C_{15:0}$ and $C_{17:0}$; 9.6% in sum), whereas cells grown on YE/SW had one 10-methyl-branched acid (10-Me-$C_{17:0}$; 1.7%). The polar lipid pattern (determined for cells harvested from YE/SW broth after 3 d incubation at 30°C) for *Nocardioides* is unique: phosphatidylcholine, phosphatidylglycerol, phosphatidylinositol, and an unidentified phospholipid. Selected phenotypic characteristics differentiating *Nocardioides furvisabuli* from the phylogenetically closely related species are listed in Table 220. The type strain (the only strain described) was isolated on ISP 4 medium prepared with 60% (v/v) natural sea water.

Source (type strain): black sand, Samyang Beach on Jeju Island, Korea.

DNA G+C content (mol%): 69.1 (HPLC).

Type strain: SBS-26, JCM 13813, NRRL B-24465.

Sequence accession no. (16S rRNA gene): DQ411542.

20. **Nocardioides ganghwensis** Yi and Chun 2004, 1298[VP]

gang.hwen′sis. N.L. masc. adj. *ganghwensis* of or belonging to Ganghwa island in Korea, the geographical origin of the type strain.

Characteristics are as described for the genus and listed in Table 215 and Table 220. Information presented below is based on the original species description (Yi and Chun, 2004a), unless indicated.

Cells are nonmotile irregular rods (~0.4–0.5 × 0.9–4.5 μm) in 3-d-old culture on marine agar (Difco). Colonies on marine agar (MA; Difco) are ivory, butyraceous, and approximately 1–2 mm in diameter after 3 d at 30°C and reach a maximum diameter of 3–4 mm after 5 d. Substrate or aerial mycelium is not observed. Grows at 10–40°C (with no growth at 5 and 50°C), at initial pH 6–10 (no growth at pH 5 and 11), and in the presence of NaCl up to 8% (w/v), as assayed on synthetic ZoBell medium (ZoBell, 1941).* The best growth is observed at 30°C, pH 7, and at a low NaCl concentration (1% or less). Nutritionally non-exacting; grows on a suitable mineral salts medium (e.g. ISP 9) with glucose as sole carbon-plus-energy source and an ammonium salt as sole nitrogen source (Lee, 2007b). The type strain utilizes acetate, citrate, succinate, L-asparagine, L-ornithine, and *N*-acetylglucosamine and other substrates (Table 215) as sole carbon and energy sources in mineral salts medium (Baumann et al., 1971) supplemented with 2% (v/v) Hutner's mineral base (Cohen-Bazire et al., 1957) and modified by reducing the concentration of sea salts to half-strength. Benzoate, tartrate, L-arginine, L-lysine, acetamide, and D-sorbitol are not used under the same test conditions. According to Lee (2007b), the organism can also utilize maltose, melibiose, and trehalose. L-Rhamnose is utilized as a carbon source for growth and energy (Schippers et al., 2005; Yi and Chun, 2004a); a conflicting result was reported by Lee (2007b) with ISP 9 as the basal medium. Crude oil is not degraded (Schippers et al., 2005). No acid production from glucose (API 20NE kit). Major fatty acids determined for cells grown at 30°C on MA for 3 d included $C_{16:0}$ iso (30.4%), $C_{17:1}$ ω8*c* (25.7%), $C_{18:1}$ ω9*c* (5.7%), $C_{16:1}$ iso H (4.4%), and $C_{17:0}$ 10-methyl (3.9%). Other fatty acids (various saturated, unsaturated, iso- and anteiso-branched and hydroxylated fatty acids) were present in smaller quantities.

*ZoBell medium: Bacto agar (Difco) 15 g; Bacto peptone (Difco), 5 g; yeast extract (Difco), 1 g; ferric citrate, 0.1 g; sea salts, 40 g; distilled water, 1 liter; sea salts were not added when the relation to NaCl was tested.

TBSA was not detected under the test conditions employed. The DNA–DNA similarity to the phylogenetically closest species, *Nocardioides oleivorans* and *Nocardioides exalbidus*, was respectively 32% (Schippers et al., 2005) and 39% (Li et al., 2007b). Selected phenotypic characteristics differentiating *Nocardioides ganghwensis* from these and other phylogenetically closely related species are listed in Table 220. The type strain (the only strain described) was isolated using R2A (Difco) supplemented with artificial sea salts (Sigma).

Source (type strain): sediment sample of getbol (tidal flat), Ganghwa Island, South Korea (37°35′31.9″N; 126°27′ 24.5″E).

DNA G+C content (mol%): 72 (HPLC).

Type strain: JC2055, IMSNU 14028, KCTC 9920, JCM 12124.

Sequence accession no. (16S rRNA gene): AY423718.

21. **Nocardioides ginsengisoli** Cui, Lee and Im 2009, 3048[VP]

gin.sen.gi.so′li. N.L. n. *ginsengum* ginseng; L. n. *solum* soil; N.L. gen. n. *ginsengisoli* of soil of a ginseng field, the source of the organism.

Characteristics are as described for the genus and listed in Table 215, Table 218, Table 219, and Table 221. Information presented below is taken from the original species description (Cui et al., 2009).

Cells are nonmotile, slender, short rods (~ 0.2–0.4 × 0.8–1.2 μm) after culture on R2A agar (Difco) for 3 d at 30°C. Colonies developed under the same growth conditions are light yellow–white and convex. Grows at 15–37°C (optimum, 30°C), but not at 4 or 42°C after 5 d incubation. Growth occurs at pH 5.0–8.5, with optimum at pH 7.0, and in up to 5.0% (w/v) NaCl (assessed on R2A agar as basal medium after culture for 5 d). The organism grows on nutrient agar (Difco) and mineral salt medium* supplemented with glucose as a sole carbon source for growth-plus-energy, vitamins (Widdel and Bak, 1992), trace element solution SL-10 (Widdel et al., 1983), and selenite/tungstate solution (Tschech and Pfennig, 1984), but not on MacConkey agar. Utilizes n-nitrophenol (50 p.p.m.) and dibenzofuran (50–150 p.p.m.) as well as the following compounds as sole carbon and energy sources in the above basal medium: D-lyxose, pyruvic acid, acetate, propionate, 3-hydroxybutyrate, valerate, phenylacetate, benzoic acid, 3- and 4-hydroxybenzoate, malate, oxalate, gluconate, L-alanine, arginine, asparagine, aspartic acid, glutamic acid, glutamine, glycine, histidine, isoleucine, leucine, lysine, phenylalanine, L-proline, L-serine, threonine, tryptophan, tyrosine, and valine. The organism is unable to use the following substrates as sole carbon sources under the same test conditions: L-fucose, L-sorbose, citrate, formate, malonate, glutaric acid, tartaric acid, adipate, caprate, itaconate, maleinate, suberate, ethanol, methanol, dulcitol, D-sorbitol, xylitol, amygdalin, glycogen, dextran, cysteine, and methionine. Voges–Proskauer reaction is observable. Tests for degradation of chitin and xylan are negative. No acid or gas is produced from glucose. Nitrate is not used as a terminal electron acceptor in R2A broth supplemented with KNO_3 (10 mM) and thioglycolate with incubation under a nitrogen gas atmosphere. The fatty acid profiles determined for the type strain of this and closely related species are displayed in Table 219. Other selected phenotypic characteristics useful in preliminary discriminating *Nocardioides ginsengisoli* from the closely related species are listed in Table 218. DNA–DNA relatedness of the *Nocardioides ginsengisoli* type strain to the type strains of the phylogenetically closest species were 22–33% (Table 221). The type strain (the only strain described) was isolated from a soil sample suspended in 50 mM phosphate buffer (pH 7.0). After serial dilution with the same phosphate buffer, the suspension was spread on plates with one-fifth strength modified-R2A agar†, and the plates were incubated at 30°C for 1 month.

Source (type strain): soil of a ginseng field, Pocheon province, South Korea.

DNA G+C content (mol%): 70.2 (HPLC).

Type strain: Gsoil 1124, KCTC 19135, CCUG 52478, DSM 17921, JCM 16930.

Sequence accession no. (16S rRNA gene): AB245396.

22. **Nocardioides halotolerans** Dastager, Lee, Ju, Park and Kim 2009c, 1555[VP] (Effective publication: Dastager, Lee, Ju, Park and Kim 2008a, 26.)

ha.lo.to′le.rans. Gr. n. *hals halos* salt; L. part. adj. *tolerans* tolerating; N.L. part. adj. *halotolerans*, salt-tolerating, referring to the ability of the organism to tolerate high salt concentrations.

Characteristics are as described for the genus and listed in Table 215 and Table 216. Information presented below is taken from the original species description (Dastager et al., 2008a).

Cells are irregular rods (normally about 0.6 μm but may be 0.9 μm in diameter, up to 3.4 μm in length) on twofold diluted R2A agar plates at 28°C. A 7-d-old culture is mostly composed of coccoid cells and very short rods, up to 1 μm long. Data on motility are conflicting. Colonies are cream-white and 1–2 mm in diameter after 3 d under the same growth conditions. Nutritionally non-exacting; grows in a suitable mineral salts medium (e.g. ISP 9) with glucose as a sole carbon-plus-energy source and an ammonium salt as a sole nitrogen source. Also grows on twofold dilute R2A medium with up to 10% (w/v), optimally 3%, NaCl. Acid production from carbohydrates is rather uncommon or weak; no acid formed from any of the carbon sources tested. The major fatty acids determined for cells harvested from twofold-diluted R2A after incubation at 28°C for 7 d included $C_{16:0}$ iso (59.29%), $C_{17:0}$ anteiso (8.2%), $C_{18:1}$ ω9*c* (6.8%) and 10 methyl-$C_{16:0}$ (5.6%). The type strain (and the only strain described) was isolated by diluting plating method on tenfold-diluted R2A.

Source (type strain): farming soil, Bigeum Island, South Korea.

DNA G+C content (mol%): 69.7 (HPLC).

Type strain: MSL-23, KCTC 19274, DSM 19273.

Sequence accession no. (16S rRNA gene): EF466122.

*Mineral salt medium: 1.8 g of K_2HPO_4, 1.08 g of KH_2PO_4, 0.5 g of $NaNO_3$, 0.5 g of NH_4Cl, 0.1 g of KCl, 0.1 g of $MgSO_4$, and 0.05 g of $CaCl_2$ per 1 liter.

† One-fifth strength modified-R2A agar (per liter of distilled water): 0.25 g of tryptone, 0.25 g of peptone, 0.25 g of yeast extract, 0.125 g of malt extract, 0.125 g of beef extract, 0.25 g of Casamino acids, 0.25 g of Soytone, 0.5 g of glucose, 0.3 g of soluble starch, 0.2 g of xylan, 0.3 g of sodium pyruvate, 0.3 g of K_2HPO_4, 0.05 g of $MgSO_4$, 0.05 g of $CaCl_2$, and 15 g of agar.

23. **Nocardioides hankookensis** Yoon, Kang, Lee and Oh 2008, 437[VP]

han.ko.o.ken′sis. N.L. masc. adj. *hankookensis* of or belonging to Hankook, the Korean name of South Korea from where the type strain was isolated.

Characteristics are as described for the genus and listed in Table 215. Information presented below is based on the original species description (Yoon et al., 2008), unless indicated.

Cells are nonmotile irregular rods (0.4–0.8 µm in width, 1.5–10 µm in length). No coccoid cells are observable if grown on R2A (Difco) or trypticase soy agar (Difco) at 25°C. Neither substrate nor aerial mycelium is formed. Gram-stain-positive, but Gram-stain variable in old cultures. Colonies on R2A agar are white in color and 0.7–1.0 mm in diameter after 7 d incubation at 25°C. Grow at 10 and 34°C (optimum, 25°C), but not at 4 or 35°C, at pH 5.5–8.0, but not at pH 5.0 or 8.5 [in trypticase soy broth (Difco) with the pH adjusted by HC1 or Na_2CO_3], and in the presence of 0–2% (w/v) NaCl (optimum 0–0.5%), but not 3% NaCl (in a complex medium prepared according to the Difco trypticase soy broth formula, except that NaCl was omitted). Utilizes gentiobiose (weak), turanose, adipate, gluconate (weak), and malate as carbon sources, but not D-arabinose, D-lyxose, D- and L-fucose, sorbose, D-tagatose, L-xylose, adonitol, D-arabitol, dulcitol, erythritol, sorbitol, xylitol, caprate, citrate, 2- and 5-ketogluconate, phenylacetate, methyl β-D-xyloside, methyl α-D-mannoside, methyl α-D-glucoside, glycogen, and amygdalin (API 20NE and API 50 CH; AUX suspending medium). Tweens 20, 40, and 60 are decomposed. Tributylin is hydrolyzed (Song et al., 2011). Weak activity for cystine arylamidase, β-galactosidase, and trypsin, as well as tyrosine hydrolysis, but the test results vary between experiments or test methods (Song et al., 2011; Yoon et al., 2008). The type strain is susceptible to the following antibiotics (µg per disc, unless indicated otherwise): carbenicillin (100), chloramphenicol (100), gentamicin (30), kanamycin (30), lincomycin (15), neomycin (30), oleandomycin (15), polymyxin B (100 U), and streptomycin (50), but resistant to ampicillin (10), cephalothin (30), novobiocin (5), and penicillin G (20 U). The type strain is also sensitive to rifampin (30 µg) but reports on its tetracycline (30 µg) sensitivity conflict (Song et al., 2011). Major fatty acids (>5% of the total) of cells grown on R2A agar for 7 d at 25°C were $C_{16:0}$ iso (42.7%), $C_{17:1}$ ω8*c* (14.8%), $C_{18:1}$ ω9*c* (13.1%), $C_{17:0}$ 10-methyl (5.5%), and TBSA (2.3%). A different fatty acid profile was reported for cells grown on double-strength R2A agar plates for 3 d at 30°C (Song et al., 2011). The most pronounced differences were in a smaller proportion of $C_{16:0}$ iso (9.1%) and a larger proportion of total straight-chain saturated acids (26.3%) with $C_{14:0}$ predominating (10.2%). Song et al. (2011) reported the fatty acid profiles for the type strains of *Nocardioides hankookensis* and phylogenetically related species (*Nocardioides aquiterrae*, *Nocardioides pyridinolyticus*, and *Nocardioides caricicola*) obtained under the same experimental conditions.

The type strain exhibited DNA–DNA hybridization levels of 19 and 22% with the type strains of phylogenetically closest species, *Nocardioides aquiterrae* and *Nocardioides pyridinolyticus*, respectively. Phenotypic characteristics useful in distinguishing *Nocardioides hankookensis* from both above species include the cell appearance, size and non-motility, a lower growth temperature optimum and maximum, a lower DNA G+C content, the ability to produce esterase (C4), growth on L-arabinose and D-melibiose, sensitivity to rifampin (30 µg per disc), as well inability to reduce nitrate and produce α-glucosidase (Song et al., 2011; Yoon et al., 2004, 2008; Table 215). The type strain (and the only strain described) was isolated by the standard dilution plating technique at 25°C with tenfold-diluted nutrient agar (Difco).

Source (type strain): soil, Dokdo Island, Korea (37°14′12″N, 131°52′07″E).

DNA G+C content (mol%): 71.3 (HPLC).

Type strain: DS-30, JCM 15302, KCTC 19246, CCUG 54522.

Sequence accession no. (16S rRNA gene): EF555584.

24. **Nocardioides humi** Kim, Srinivasan, Park, Sathiyaraj, Kim and Yang 2009b, 2725[VP]

hu′mi. L. gen. n. *humi* of/from soil, pertaining to the source of isolation of the type strain.

Characteristics are as described for the genus and listed in Table 215. Information presented below is taken from the original species description (Kim et al., 2009b).

Cells incubated on Luria–Bertani (LB) agar at 30°C for 18 h are small rods (0.3–0.5 × 0.8–1.0 µm) and motile with peritrichous flagella (Figure 262). Colonies developed on R2A agar (Difco) after 3 d at 30°C are irregular, flat, and pale yellow in color. Grows at 25 and 42°C (the maximal temperature tested) optimally at 30–37°C, but not at 4°C. Optimal pH for growth is 6.5–7.5, the pH growth range is 5–11. L-Alanine, L-proline, L-serine, glycogen, 2-ketogluconate, 3-hydroxybutyrate, acetate, gluconate, itaconate, malate, propionate, and valerate (API 20NE, API 32GN), but not D-sorbitol, L-histidine, 3-hydroxybenzoate, 4-hydroxybenzoate, 5-ketogluconate, adipate, caprate, citrate, lactate, malonate, phenylacetate, and suberate are utilized as carbon sources. No acid produced from glucose (API 20NE). The major fatty acids (>5%) determined in cell mass harvested from TSA after 2 d incubation at 30°C included $C_{16:0}$ iso (23.1%), $C_{17:0}$ iso (16.2%), $C_{18:1}$ ω9*c* (11.8%), TBSA (9.7%), $C_{17:1}$ ω6*c* (7.7%), $C_{18:0}$ (6.0%), and $C_{16:0}$ (5.2%). The type strain (the only strain described) was isolated using the standard dilution-plating method on a tenfold-diluted R2A agar.

Source (type strain): surface soil of a ginseng field, South Korea.

DNA G+C content (mol%): 71.0 (HPLC).

Type strain: DCY24, JCM 14942, JCM 17026, KCTC 19265, LMG 24128

Sequence accession no. (16S rRNA gene): EF623863.

25. **Nocardioides hwasunensis** Lee, Lee and Kim 2008, 280[VP]

hwa.sun.en′sis. N.L. masc. adj. *hwasunensis* of or belonging to Hwasun, the place where the type strain was isolated.

Characteristics are as described for the genus and listed in Table 215 and Table 220. Information presented below is based on the original species description (Lee et al., 2008).

Nonmotile short rods (0.4–0.7 × 1.0–1.7 μm), occurring singly, in pairs, or in aggregates, are observed in young cultures grown in marine broth (MB; Difco) or in trypticase soy broth at 30°C. Shorter rods, and oval or coccoid cells are observed in older cultures. Colonies are yellowish cream in color on Marine agar (MA; Difco) and light yellow on ISP 2 agar or trypticase soy agar (TSA; Difco). Grows at 4–37°C (optimum, 30°C) but not at 42°C. Grows at pH 5.1 and 9.1, but not at pH 10.1 (on MA with pH adjusted using HC1 or NaOH) and in 4% (w/v) NaCl, but not in 5% (w/v) NaCl (on ISP 2 as the basal medium). Nutritionally non-exacting; grows on a suitable mineral salts medium with glucose as sole carbon-plus-energy source and an ammonium salt as sole nitrogen source (e.g. ISP 9 medium). Acid is produced from glucose by oxidation (API 20NE system), but glucose is not fermented. Acetate, citrate, formate (weak), malate, succinate, tartrate, D-sorbitol, and dextran are used as sole carbon and energy sources, but not D-arabinose, L-sorbose, adonitol, D-dulcitol, *meso*-erythritol, D-xylitol, 2,3-butanediol, 1,2-propanediol, benzoate, methyl-β-D-glucoside, methyl-β-D-mannoside. Utilization of L-arabinose is variable between strains, with the type strain showing positive reaction. The ability to hydrolyze gelatin varies among strains. The fatty acid profiles determined for cells grown on TSA at 30°C for 3 d were almost identical for two strains studied. The major fatty acids (>5%) for the type strains were as follows: $C_{16:0}$ iso (27.8%), $C_{16:0}$ (10.8%), $C_{18:0}$ (8.8%), $C_{18:1}$ ω9*c* (8.2%), $C_{15:0}$ iso (7.5%). 10-Methyl-branched acids were represented by TBSA (4.5%), 10-Me-$C_{17:0}$ (3.5%), and 10-Me-$C_{16:0}$ (2.9%). Selected characteristics differentiating *Nocardioides hwasunensis* from the phylogenetically closely related species are listed in Table 220. The two described strains (HFW-18 and HFW-21) were isolated from a water sample using ISP 2 medium prepared with 60% (v/v) natural sea water.

Source (type strain): water, the area, where running water from a valley merges into sea water on Hwasun beach, Jeju Island, Korea.

DNA G+C content (mol%): 71.1–72.2 (HPLC); 71.1 for the type strain.

Type strain: HFW-21, DSM 18584, JCM 15307, KCTC 19197.

Sequence accession no. (16S rRNA gene): AM295258.

26. **Nocardioides insulae** Yoon, Kang, Lee and Oh 2007b, 138[VP]

in.su′la.e. L. fem. gen. n. *insulae* of an island, referring to the source of isolation of the type strain.

Characteristics are as described for the genus and listed in Table 215. Information presented below is based on the original species description (Yoon et al., 2007b).

Cells are irregular rods (0.6–1.0 × 1.3–6.0 μm) or cocci in the exponential phase of growth and show rod-to-coccus morphogenesis from the early exponential phase to the stationary phase. Neither substrate nor aerial mycelium is formed. Colonies are ivory in color and 1.0–1.5 mm in diameter after 7 d incubation on nutrient agar (NA; Difco) at 30°C.

Grows at 10 and 34°C (optimally at 30°C), but not at 4 or 35°C, and at low NaCl concentration [up to 3%, w/v, in trypticase soy broth (Difco formula without NaCl)]. Media without the addition of NaCl are preferable for growth. Growth is optimal at pH 8.0, observed at pH 6.5, but not observed at pH 6.0 [tested in Nutrient broth (Difco) adjusted to various pH values by the addition of HCl or Na_2CO_3]. In the API 20E test, acetate, L-malate, pyruvate, L-glutamate, and salicin (weakly), but not citrate, succinate, benzoate or formate are utilized as sole carbon and energy sources. Tweens 20, 40, and 60 are hydrolyzed. The type strain is susceptible to the following antibiotics (μg per disk, unless indicated): carbenicillin (100), cephalothin (30), chloramphenicol (100), gentamicin (30), kanamycin (30), lincomycin (15), neomycin (30), oleandomycin (15), polymyxin B (100 U), streptomycin (50), tetracycline (30), but not ampicillin (10), novobiocin (5), or penicillin G (20 U). The predominant fatty acid, as determined in cells harvested from NA plates after incubation at 30°C for 7 d, was $C_{16:0}$ iso (49.7%). The amounts of TBSA (8.3%), $C_{17:0}$ 10-methyl (2.9%), $C_{18:1}$ ω9*c* (7.4%), $C_{17:1}$ ω6*c* (5.4%), $C_{17:0}$ anteiso (4.9%), and other acids typical of the genus were smaller. The type strain (and the only strain described) was isolated on tenfold-diluted NA at 30°C using the standard dilution plating technique.

Source (type strain): soil, Dokdo Island, Korea.

DNA G+C content (mol%): 71.1 (HPLC).

Type strain: DS-51, JCM 15308, KCTC 19180, DSM 17944.

Sequence accession no. (16S rRNA gene): DQ786794.

27. **Nocardioides islandensis** Dastager, Lee, Ju, Park and Kim 2009d, 1555[VP] (Effective publication: Dastager, Lee, Ju, Park and Kim 2008e, 405.)

is.lan.den′sis. N.L. masc. adj. *islandensis* from or pertaining to Bigeum Island in Korea, from where the type strain was isolated.

Characteristics are as described for the genus and listed in Table 215. Information presented below is taken from the original species description (Dastager et al., 2008e).

Cells (cultured 10 d on R2A agar or twofold-diluted R2A at 28°C) are mostly slender irregular rods (ca. 0.5–0.6 μm in diameter, up to 3.7 μm in length) with nearly flat (just after division) and rounded ends. Short chains and branching forms may occur. Coccoid cells and larger spherical or club-shaped forms may be up to 1.6 μm in diameter. Nonmotile. Colonies are white to cream in color, moist and approximately 1–2 mm in diameter after 3 d incubation R2A agar at 28°C. Aerial mycelium is not formed. Nutritionally non-exacting; grows on ISP 9 with glucose as the carbon-plus-energy source and an ammonium salt as the sole nitrogen source. Acid production from carbohydrates is rather uncommon or weak; no acid formed from any of the carbon sources examined (the test method, not reported). Acetoin not produced. The major fatty acids (cells from R2A agar plates after incubation for 10 d at 28°C) included (% of the total): $C_{18:1}$ ω7*c* (50.3), $C_{16:0}$ (11.5), $C_{14:0}$ (7.5), $C_{16:0}$ iso (4.2), and $C_{15:0}$ anteiso (4.2); other acids ($C_{15:0}$ iso, $C_{17:1}$ ω8*c*, $C_{18:0}$, $C_{18:1}$ ω5*c*, $C_{17:0}$ iso, $C_{17:0}$ anteiso, and $C_{17:0}$) each contributed 1–1.9%. No TBSA was detected (limit, 1%).

The type strain (and the only strain described) was isolated by the standard dilution plating on tenfold-diluted R2A agar at 28°C.

Source (type strain): farming soil, Bigeum Island, Korea.

DNA G+C content (mol%): 71.4 (HPLC).

Type strain: MSL-26, KCTC 19275, DSM 19321.
Sequence accession no. (16S rRNA gene): EF466123.

28. **Nocardioides jensenii** (Suzuki and Komagata 1983b) Collins, Dorsch and Stackebrandt 1989, 3[VP] (*Pimelobacter jensenii* Suzuki and Komagata 1983b, 673[VP]; effective publication: Suzuki and Komagata 1983c, 69.)

jen.se′ni.i. N.L. gen. masc. n. *jensenii* of Jensen; named after H.L. Jensen, the Danish bacteriologist who contributed to the taxonomy of coryneform bacteria.

Characteristics are as described for the genus and listed in Table 215, Table 216, and Table 217. Information presented below based on the descriptions of Suzuki and Komagata (1983b), Collins et al. (1989), Yi and Chun (2004a, 2004b), and Yoon et al. (2010) unless otherwise indicated.

Cells are nonmotile irregular rods [mostly 0.6–0.8 × 2.0–4.0 μm or longer, up to 7.0 μm, after culture 1 d on YM agar (0.5% Bacto-peptone, 0.3% yeast extract, 0.3% malt extract, 1% glucose, and 2% agar)] can undergo a distinct rod–coccus morphogenetic cycle. Branching cells and V-forms occasionally occur, but neither substrate nor aerial mycelium is formed. Week-old cultures on YM agar are mostly coccoid cells and very short rods (0.6–1.0 × 0.8–1.0 μm) arranged singly, in pairs, short chains, or small clusters. Colonies are white, yellowish white, and may be light yellowish gray in older cultures on some media. A yellowish soluble pigment may be occasionally produced in older cultures, e.g. on Chapek's agar with glucose instead of sucrose. Grows at 18 and 37°C, but not at 42°C; in 5% (w/v) NaCl, but not in 10% NaCl. The ability to grow in 7% (w/v) NaCl was reported (Nesterenko et al., 1985b). Nutritionally non-exacting; grows in a suitable mineral salts medium with glucose as sole carbon-plus-energy source and an ammonium salt or nitrogen as sole nitrogen source. The type strain of *Nocardioides jensenii* can degrade dinitro-*o*-cresol herbicides (Gundersen and Jensen, 1956). Assimilates acetate, but shows no alkaline reaction with citrate and succinate (in tests described by Yamada and Komagata, 1972). The type strain utilizes acetate, pyruvate, L-asparagine, L-lysine (weak), but not succinate, acetamide, L-arginine, L-ornithine, and D-sorbitol as sole carbon and energy sources for growth (Yi and Chun, 2004a). Uses azelate, suberate, thymine, and uracil, but not tetradecane as sole carbon and energy sources (Collins et al., 1994). Some test results displayed in Table 215, including citrate and benzoate utilization, may vary among experiments or test methods (Dastager et al., 2010; Yi and Chun, 2004a; Yoon et al., 2010). No acid produced during glucose oxidation in the API 20NE test (Yi and Chun, 2004a) or in conventional tests in peptone-based media. The test results for oxidation of carbon substrates (Tóth et al., 2008) obtained with the Biolog GP2 system are shown in Table 217.

The cell-wall peptidoglycan contains LL-A_2pm, glycine, alanine, and glutamic acid. The peptidoglycan type is A3γ (A41.1; http://www.peptidoglycan-types.info), as reported by Schleifer and Kandler (1972) for the type strain *Nocardioides jensenii* (*Arthrobacter simplex* NCIB 9770). Conflicting data on the composition of peptidoglycan (with three-glycine interpeptide bridge) was reported by Collins et al. (1989). Re-examination of the data for the type strain of *Nocardioides jensenii* (L. Evtushenko and L. Dorofeeva, unpublished) showed the molar ratio of alanine, glutamate, LL-A_2pm, and glycine to be 2.1:1.0:1.0:1.2, which is similar to the peptidoglycan composition reported by Schleifer and Kandler (1972). The sugars detectable in the cell wall include galactose, glucose, and rhamnose, along with *N*-acetylglucosamine and glycerol (Takeuchi and Yokota, 1989; Tul'skaya, 2009) and minor amounts of mannose (Tul'skaya, 2009). The cell wall contains teichoic acid of unidentified structure, which involves glycerol, galactose, *N*-acetylglycosamine, and pyruvate as components, along with phosphate (Takeuchi and Yokota, 1989; Tul'skaya, 2009). The polyamine pattern of the type strain grown in rich (R) medium (Yamada and Komagata, 1972) includes cadaverine as the predominant component (54%), followed by putrescine (22%), spermidine (12%), spermine (9%), and 1,3-diaminopropane (2.5%) (Busse and Schumann, 1999). The major isoprenoid quinone is MK-8(H_4), with isoprenyl units saturated at sites II and III.

Fatty acid composition depends on the culture conditions and analytical procedures (Miller et al., 1991; Schumann et al., 1997; Suzuki and Komagata, 1983a, 1983c; Yoon et al., 2010; Yoon et al., 1997b). The fatty acids [for cells harvested from nutrient agar (NA, Difco) after 4 and 7 d cultivation at 30°C] included $C_{16:0}$ iso (45.5 and 33.4%), $C_{16:1}$ iso (14.0 and 10.9%), $C_{17:1}$ (8.7 and 10.1%), $C_{18:1}$ (3.2 and 4.6%), $C_{15:0}$ iso (3.7 and 4.0%), $C_{17:0}$ iso (1.7 and 2.1%), $C_{18:0}$ iso (2.7 and 1.2%), $C_{18:0}$ (2.0 and 2.9%), $C_{17:0}$ 10-methyl (5.4 and 5.5%), $C_{16:0}$ 10-methyl (2.5 and 6.4%), TBSA (2.4 and 9.5%), and other fatty acids characteristic of the genus, each contributing 0.3–1.4% (Yoon et al., 2007b; Yoon et al., 2010). The major fatty acids (for cells grown in TSB medium for 24–48 h at 28°C) included $C_{16:0}$ iso (28.6%), $C_{15:0}$ iso (16.7%), $C_{18:1}$ (10.3%), $C_{17:0}$ iso (9.6%) and $C_{17:1}$ (6.5%), with TBSA constituting 2.4% (Schumann et al., 1997). A larger proportion of TBSA (19.1%), along with similar amounts of $C_{17:0}$ 10-methyl (5.3%), $C_{16:0}$ 10-methyl (3.6), and $C_{16:0}$ iso (34%), was reported by Miller et al. (1991). In some experiments, 2-hydroxy fatty acids may be detected (O'Donnell et al., 1982; Suzuki and Komagata, 1983c; Suzuki and Komagata, 1983a). Diphosphatidylglycerol, phosphatidylglycerol, phosphatidylinositol and supposedly hydroxy-phosphatidylglycerol were the principal polar lipids (Collins et al., 1989). Susceptible to some actinophages (Collins et al., 1989) that multiply in mycelium-forming *Nocardioides* strains (Prauser, 1976; Prauser, 1984a; Prauser, 1989). The mean DNA–DNA relatedness was 16% to the type strains of *Nocardioides albus* and *Nocardioides simplex*, 13% to the type strain of *Terrabacter tumescens* (membrane filter method with tritium-labeled DNA; Suzuki and Komagata, 1983c), and 10% to *Nocardioides daedukensis* (Yoon et al., 2010; procedure of Ezaki et al., 1989). The type strain (the only strain described) was isolated as a herbicide-decomposing bacterium (Jensen and Gundersen, 1956).

Source (type strain): soil.

DNA G+C content (mol%): 68.8 (T_m).

Type strain: ATCC 49810, CCUG 37988, CIP 102404, CNF 091, DSM 20641, IAM 12581, IFO (now NBRC) 14755, KCTC 9134, NBRC 14755, JCM 1364, LMG 16325, NCIB (now NCIMB) 9770, VKM Ac-1878.

Sequence accession no. (16S rRNA gene): AF005006, X53214.

Additional remarks: the type strain of this species was originally identified as a strain of *Arthrobacter simplex* (Jensen and Gundersen, 1956). Suzuki and Komagata (1983c) proposed to reclassify it as *Pimelobacter jensenii.* Collins et al. (1989) transferred this species to *Nocardioides* as *Nocardioides jensenii* (see *Further descriptive information* for more details).

29. **Nocardioides kongjuensis** Yoon, Lee, Jung, Kim, Kim and Oh 2006b, 1786[VP]

kong.ju.en′sis. N.L. masc. adj. *kongjuensis* of or belonging to Kongju, Korea, pertaining to the geographical origin of the type strain.

Characteristics are as described for the genus and listed in Table 215, Table 218, Table 219, and Table 221. Information presented below is from Yoon et al. (2009, 2006b), unless indicated.

Cells are irregular rods (0.4–0.7 × 0.8–3.0 µm) in the exponential phase of growth and exhibit rod-to-coccus morphogenesis from the early exponential phase to the stationary phase. Neither substrate nor aerial mycelium is formed. Colonies are yellowish white in color, reaching 3.0–5.0 mm diameter after 3 d incubation on nutrient agar (NA; Difco) at 30°C. Grows at 10 and 40°C, but not at 4 or 41°C, at pH 5.5, but not at pH 5.0 [optimum, 7.0–8.0; nutrient broth (NB; Difco) with the pH adjusted with HCl or Na_2CO_3 prior to sterilization], and in up to 5% (w/v) NaCl, with optimal growth in the absence of NaCl (trypticase soy broth prepared according to the Difco formula without NaCl).

Nutritionally non-exacting; grows on a suitable mineral salts medium (e.g. ISP 9 medium) with glucose as sole carbon-plus-energy source and an ammonium salt as sole nitrogen source. The organism is capable of degrading *N*-hexanoyl-L-homoserine lactone and using this compound as a sole source of carbon and energy for growth. L-Arabinose, D-mannose (weak), malate, succinate, and pyruvate are utilized as carbon sources, but not D-fructose, acetate, benzoate, citrate, and formate (basal medium ISP 9). In the API 20NE or API 50 CH (AUX as suspending medium) test, D-fructose, adipate, citrate (weak), gluconate, and malate were used, but not D- and L-arabinose, D- and L-fucose, gentiobiose, D-lyxose, D-mannose, D-melibiose, L-rhamnose, L-sorbose, D-tagatose, D-turanose, L-xylose, D-adonitol, D-and L-arabitol, dulcitol, erythritol, D-sorbitol, xylitol, methyl α-D-mannoside, methyl α-D-glucoside, methyl β-D-xyloside, *N*-acetylglucosamine, amygdalin, arbutin, esculin, starch, glycogen, 2- and 5-ketogluconate, caprate, and phenylacetate. In conventional tests with mineral salts medium supplemented with vitamins and element solution, this organism used L-alanine, L-histidine, L-proline, L-serine, acetate, 3- and 4-hydroxybenzoic acids, 3-hydroxybutyrate, malic acid, propionic acid, suberic acid, and valeric acid as sole carbon sources for growth, but not lactate, itaconate, and malonate (Cui et al., 2009). Tweens 20, 40, and 60 are hydrolyzed. Acetoin is produced (Cui et al., 2009). For cells harvested from NB agar after incubation at 30°C for 6 d, the fatty acids (5% or more of the total) included $C_{16:0}$ iso (27.1%), TBSA (14.4%), $C_{17:0}$ 10-methyl (6.0%), $C_{17:1}$ ω6*c* (11.2%), $C_{18:1}$ ω9*c* (5.0%), and $C_{17:0}$ iso (6.3%). A different fatty acid profile was reported for cells grown on trypticase soy agar (Difco) at 30°C for 2 d (Table 219). The fatty acids composition of the type strain of *Nocardioides kongjuensis* and type strains of related species are compared in Table 219. A 21–46% DNA–DNA similarity was found between the type strain of *Nocardioides kongjuensis* and the type strains of phylogenetically closest species (Table 221). Selected phenotypic characteristics differentiating *Nocardioides ginsengisoli*, *Nocardioides caeni*, and other phylogenetically related species are listed in Table 218. The type strain (the only strain described) was isolated using minimal medium (Leadbetter and Greenberg, 2000) supplemented with *N*-hexanoyl-L-homoserine lactone as the carbon source and NH_4Cl as the nitrogen source; pH 6.5 (for further details on the isolation procedure see Yoon et al., 2006b).

Source (type strain): soil, Kongju, South Korea.

DNA G+C content (mol%): 72.1 (HPLC).

Type strain: A2-4, KCTC 19054, JCM 12609.

Sequence accession no. (16S rRNA gene): DQ218275.

30. **Nocardioides koreensis** Dastager, Lee, Ju, Park and Kim 2008f, 2294[VP]

ko.re.en′sis. N.L. masc. adj. *koreensis* of or pertaining to Korea, the geographical origin of the type strain.

Characteristics are as described for the genus and listed in Table 215. Information presented below is based on the original species description (Dastager et al., 2008f).

Cells are typically irregular rods (mostly 0.4–0.7 µm wide, up to 3.2 µm long). The irregular rods do not exceed 2 µm in length and coccoid cells occasionally occur after 7 d of incubation in R2A medium (Difco) at 28°C (Figure 255). Branching cells may be observed. Motility is reported. Colonies are cream to whitish in color, flat, and 0.9–1.4 mm in diameter after 4–5 d incubation in R2A medium at 30°C. No aerial or substrate mycelium is formed. Optimum growth is at pH 7.0–8.0 and 30°C. Grows at 27–37°C, but not at 25°C or above 37°C, and in up to 5% (w/v) NaCl (in twofold-diluted R2A medium). The carbon sources for growth tested according to Kämpfer (1991) by using mineral salts medium supplemented with yeast extract, Oxoid (0.02 g/l), bio-Lactysat, bioMérieux (0.02 g/l), a vitamin solution, and trace element solution (Kämpfer et al., 1990) are listed in Table 215. The major fatty acids reported for cells harvested from twofold-diluted R2A broth (pH 7.5) after incubation for 10 d at 28°C included (% of the total): $C_{16:0}$ iso (62.9%), $C_{18:1}$ ω9*c* (5.9%), $C_{14:0}$ iso (4.0%), $C_{17:0}$ 10-methyl (3.4%), $C_{16:0}$ (3.3%), $C_{16:1}$ iso (3.1%). Neither TBSA nor hydroxylated fatty acids were reported among components equal or exceeding 1% of the total fatty acids. The type strain (and the only strain described) was isolated using a tenfold-diluted R2A.

Source (type strain): soil, Bigeum Island, South Korea.

DNA G+C content (mol%): 69.9 (HPLC).

Type strain: MSL-09, DSM 19266, JCM 16022, KCTC 19272.

Sequence accession no. (16S rRNA gene): EF466115.

31. **Nocardioides kribbensis** Yoon, Kim, Lee and Oh 2005b, 1614[VP]

krib.ben′sis. N.L. masc. adj. *kribbensis* pertaining to KRIBB, arbitrary adjective formed from the acronym of the Korea Research Institute of Bioscience and Biotechnology, KRIBB, where taxonomic studies on this species were performed.

Characteristics are as described for the genus and listed in Table 215. Information presented below from the original publication (Yoon et al., 2005b).

Cells are irregular rods (0.8–1.0 × 1.5–2.0 µm) in the exponential phase of growth and can undergo a rod-to-coccus morphogenetic cycle from the early exponential phase to the stationary phase. Neither substrate nor aerial mycelium is formed. Gram-stain-positive; Gram-staining is variable in old cultures. Colonies are cream in color and 1.0–1.5 mm in diameter after 6 d incubation on twofold diluted NA (pH 9.0). Grows at 4 and 35°C but not at 36°C, and in up to 3% (w/v) NaCl. Growth occurs at pH 6.0 and 11.0, with optimum growth at pH 9, but not at pH 5.5 or pH 12 [in twofold diluted NA adjusted to alkaline pH with Na_2CO_3 (below pH 10.5) or KOH (above pH 10.5)]. Nutritionally non-exacting; grows on a suitable mineral salts medium (e.g. on ISP 9) with glucose as sole carbon-plus-energy source and an ammonium salt as sole nitrogen source. D-Sorbitol but not adonitol is used as a carbon source. Tweens 20, 40, and 60 are hydrolyzed. The predominant fatty acids of three strains of this species [harvested from twofold-diluted NA (pH 9.0) after incubation for 5 d at 30°C] were almost identical and that of the type strain (% of the total) were: $C_{16:0}$ iso (42.5), $C_{15:0}$ iso (9.7), $C_{18:1}$ ω9*c* (7.2), $C_{17:0}$ iso (5.7), $C_{14:0}$ iso (5.4), $C_{17:0}$ anteiso (4.2), $C_{16:0}$ (3.6), and TBSA (1.2%). DNA–DNA relatedness between the three strains of this species were 88–93%. Mean DNA–DNA hybridization values of 28.7% and 18% were reported between the type strain of *Nocardioides kribbensis* and type strains of *Nocardioides basaltis* and *Nocardioides salarius*, respectively (Kim et al., 2008a; Kim et al., 2009a). DNA–DNA relatedness of strains of *Nocardioides kribbensis* to the type strain of *Nocardioides aquiterrae* was 8–15% (Yoon et al., 2005b). The three described strains of this species were isolated on tenfold-diluted NA with pH adjusted to 10.0 using Na_2CO_3.

Source (type strain): alkaline soil, Korea.

DNA G+C content (mol%): 73–74 (HPLC); 74 for type strain.

Type strain: KSL-2, DSM 16314, JCM 13594, KCTC 19038.

Sequence accession no. (16S rRNA gene): AY835924.

32. **Nocardioides lentus** Yoon, Lee and Oh 2006a, 274[VP]

len′tus. L. masc. adj. *lentus* slow, delayed, referring to slow growth.

Characteristics are as described for the genus and listed in Table 215. Characteristics presented below taken from the original description (Yoon et al., 2006a).

Cells are irregular rods (0.4–0.7 × 1.0–4.5 µm) in the exponential phase of growth and can undergo rod-to-coccus morphogenesis from the early exponential phase to the stationary phase. Neither substrate nor aerial mycelium is formed. Gram-stain-positive; staining is variable in old cultures. Colonies are yellow in color and 0.5–1.0 mm in diameter after 10 d incubation on twofold diluted nutrient agar (NA; Difco) at 28°C. Grows at 4 and 34°C, but not at 35°C, at pH 6.5 and 9.5, but not at pH 6.0 or 10.0 [initial pH of the test medium, twofold diluted nutrient broth (Difco) adjusted prior to sterilization with HCl or Na_2CO_3], and optimally at pH 8.0. Growth occurs in up to 5% (w/v) NaCl, and is optimal in 0.5% [w/v; as assessed using trypticase soy broth (Difco)]. Nutritionally non-exacting; grows on a suitable mineral salts medium (e.g. ISP 9 medium) with glucose as sole carbon-plus-energy source and an ammonium salt as sole nitrogen source. Lactose and D-xylose are also used as carbon sources by some strains, including the type strain. Utilizes D-sorbitol but not adonitol as a carbon source. Positive for hydrolysis of Tweens 20, 40, and 60; hydrolysis of tyrosine is variable among strains and positive for the type strain. The fatty acids of the type strain (cells harvested from twofold diluted NA [pH 8.0] after incubation for 10 d at 28°C) included $C_{16:0}$ iso (60.9%), $C_{17:1}$ ω8*c* (6.6%), and $C_{17:0}$ 10-methyl (5.8%) as well as TBSA (1.6%), a hydroxylated fatty acid ($C_{17:0}$ iso 3-OH; 2.7%) and minor amounts of other fatty acids characteristic of *Nocardioides*. One strain of this species, KSL-18, was found to contain $C_{16:0}$ iso in the highest proportion (81.9%) reported for the genus *Nocardioides* so far. Strains KSL-17, KSL-18, and KSL-19 are identical in their 16S rRNA gene sequences and exhibit 85–90% DNA–DNA hybridization to each other. The type strain and both reference strains described were isolated by the standard dilution plating technique on tenfold-diluted NA with the pH adjusted to 9.0 using Na_2CO_3 at 30°C.

Source (type strain): alkaline soil, Kwangchun, Korea.

DNA G+C content (mol%): 74.6–74.8 (HPLC).

Type strain: KSL-17, DSM 16315, JCM 14046, KCTC 19039.

Sequence accession no. (16S rRNA gene): DQ121389 for the type strain and DQ121390 and DQ121391 for the reference strains KSL-18 and KSL-19, respectively.

33. **Nocardioides luteus** Prauser 1985, 223[VP] (Effective publication: Prauser 1984b, 647.) ("*Nocardioides flavus*" Ruan and Zhang 1979, 347; "*Nocardioides fulvus*" Ruan and Zhang 1979, 350.)

lu′te.us. L. masc. adj. *luteus* yellow, referring to the yellow primary mycelium of the type strain.

Characteristics are as described for the genus and listed in Table 215. Information presented below is based on the author's description (Prauser, 1984b, 1989) and recent observations, unless indicated.

The branching hyphae of the primary mycelium are usually yellow on most ISP media to orange-yellow (oatmeal agar, glycerol-nitrate agar) or cream to faintly yellowish-brown on some peptone-containing media, particularly in aged cultures. The aerial mycelium is white or cream-colored if well developed. No pronounced soluble pigments are usually produced, but a light reddish-brown or light yellowish-brown pigment may be observable in older cultures on some media. Colonies lacking aerial mycelium are usually pasty on most media, with smooth to wrinkled and dull to bright surfaces. Growth is optimum at about 28°C, observable at 10°C and 42°C, and may occur at 45°C but not at 50°C. The organism grows well in up to 8% (w/v) NaCl (weak growth at 9%) and at initial pH values of 5–9, but not at pH 10, as assessed using a complex medium, PYGP (peptone, 0.5%; yeast extract, 0.3%; glucose, 0.5%; K_2HPO_4, 0.02%). Nutritionally non-exacting; grows aerobically on a suitable mineral salts medium (e.g. ISP 9) with glucose or sucrose as carbon-plus-energy source and an ammonium salt or nitrate as sole nitrogen source. Growth

and biomass production is enhanced in the presence of vitamins (Lawson et al., 2000a) and traces of yeast extract or peptone. Acids are produced from D-glucose, L-arabinose, D-xylose, D-mannitol, and D-fructose under aerobic conditions in conventional tests; some strains may produce acids from L-rhamnose and sucrose. However, no acid production from glucose was observed with the API 20NE system (Yi and Chun, 2004a). Citrate, succinate, and benzoate but not tartrate are used as carbon sources. The type strain also utilizes acetate but not acetamide, L-arginine, L-asparagine, L-lysine, L-ornithine, and D-sorbitol as a sole carbon sources (Yi and Chun, 2004a), uses azelate, malonate, suberate, L-proline, tetradecane, and phenol as sole carbon and energy sources (Collins et al., 1994). Uses D-fructose, L-rhamnose and sucrose as carbon sources, and can hydrolyze xanthine, but the test results may vary between experiments or test conditions (Prauser, 1984b; Yi and Chun, 2004a). Relatively weak but distinct oxidase activity is observable (assessed with tetramethyl-*p*-phenylenediamine in young culture). Production of α-mannosidase was reported (Yi and Chun, 2004a).

Galactose is the only cell-wall monosugar which is clearly detectable in routine chemotaxonomic studies; traces of glucose and/or rhamnose may also be found (Shashkov et al., 2000b; Tul'skaya, 2009). The cell wall contains a 1,5-poly(ribitol phosphate) teichoic acid (Shashkov et al., 2000b), which is the most essential chemotaxonomic marker differentiating *Nocardioides luteus* from *Nocardioides albus* that possesses a poly (galactosylglycerol phosphate) polymer (see Further descriptive information for details). The major isoprenoid quinone is MK-8(H_4); minor amounts of MK-8(H_2) and MK-8 may be detected (O'Donnell et al., 1982). The type strain of *Nocardioides luteus* harvested from rich (R) medium (Yamada and Komagata, 1972) had nearly 6 μmol/g (dry wt) of polyamines, with the predominant compounds cadaverine (53.7%) and putrescine (28.2%), and lesser amounts of spermidine (11.3%), 1,3-diaminopropane (2.6%), spermine (2.4%), and tyramine (1.7%) (Busse and Schumann, 1999). The cellular fatty acid profile of the type strain of *Nocardioides luteus* is generally similar to that obtained for *Nocardioides albus* under the same experimental conditions (Miller et al., 1991; O'Donnell et al., 1982; Yoon et al., 1997b). In some experiments, a small quantity of a 3-hydroxy fatty acid may additionally occur (Yoon et al., 1997b) and a significantly larger proportion of TBSA may be detected (Miller et al., 1991). The qualitative polar lipid composition of the type strain of *Nocardioides luteus* is also generally reminiscent of that detected in *Nocardioides albus* (Figure 263). Susceptible to taxon-specific *Nocardioides* actinophages. The DNA–DNA hybridization levels between the type strains of *Nocardioides luteus* and *Nocardioides albus* were 49% and 38% in different experiments (Prauser. 1984a, 1989). The DNA–DNA relatedness of *Nocardioides albus* type strain to "*Nocardioides flavus*" 71-N54 (= DSM 46114) and "*Nocardioides fulvus*" 71-N86 (= DSM 46115) was 33% and 41%, respectively (Prauser, 1989).

Source (type strain): soli.

DNA G+C content (mol%): 67.5 (T_m).

Type strain: KCTC 9575, ATCC 43052, CCUG 37986, CIP 103450, DSM 43366, IMET 7830, JCM 3358, LMG 16209, IFO (now NBRC) 14491, VKM Ac-1246.

Sequence accession no. (16S rRNA gene): AF005007, X53212.

Additional remarks: some strains without distinct yellow pigmentation cited in the literature under the species name *Nocardioides albus* might also belong to *Nocardioides luteus* (see the section *Further descriptive information* for more details).

34. **Nocardioides marinisabuli** Lee, Hyun and Lee 2007, 2961[VP]

ma.ri.ni.sa'bu.li. L. adj. *marinus -a -um* of the sea, marine; L. neut. n. *sabulum* gravel, sand; N.L. gen. n. *marinisabuli* of sea sand, referring to the sand sample from which the type strain was isolated.

Characteristics are as described for the genus and listed in Table 215. Data presented below based on information published by Lee et al. (2007), unless indicated.

Cells are irregular rods (0.6–0.8 × 1.4–2.1 μm). Colonies are pale yellow in color approximately 1.0–1.5 mm in diameter after growth on trypticase soy agar (TSA; Difco) for 7 d.

Grows at pH 5.1–12.1, in up to 8% NaCl, as determined in the basal medium ISP 2, at 4–40°C but not at 42°C. Nutritionally non-exacting; grows on a suitable mineral salts medium with glucose as the carbon-plus-energy source and an ammonium salt as the sole nitrogen source (e.g. ISP 9 medium). Citrate and gluconate are assimilated, while D-arabinose, D-sorbitol, acetate, adipate, caproate, citrate, malate, and phenylacetate are not sole carbon and energy sources. Glucose is not fermented (API 20NE). The major fatty acids (in cells harvested from TSA and MA after 3 d incubation at 30°C) were mainly the same components but had slightly different proportions (% of the total): $C_{16:0}$ iso (48.7 and 35.9), $C_{17:0}$ iso (10.8 and 12.3), $C_{18:1}$ ω9*c* (5.6 and 15.4), $C_{17:1}$ ω8*c* (2.8 and 5.6), $C_{17:0}$ anteiso (4.3 and 4.1). Less than 1% TBSA was found in both experiments; the other 10-methyl-branched acid were $C_{17:0}$ 10-methyl (1.7%) and $C_{16:0}$ 10-methyl (<1%) for the MA culture and $C_{17:0}$ 10-methyl (3.2%) for the TSA culture. The fatty acid profile of the type strain of a phylogenetically close species, *Nocardioides basaltis* (grown on MA at 30°C for 3 d and analyzed using the same procedure) differed from that of *Nocardioides marinisabuli* mainly by having a larger proportion of $C_{16:0}$ iso (70%), smaller proportions of $C_{18:1}$ ω9*c* (2.8%), and traces of $C_{17:0}$ iso and $C_{17:0}$ anteiso (<1%). A larger proportion of $C_{16:0}$ iso (65.3%) was also detected in a younger culture of *Nocardioides salarius* grown on MA at 30°C (Kim et al., 2008a). The type strain of *Nocardioides marinisabuli* had DNA–DNA hybridization values of 33±9% and 15.8±1.5%, respectively, with type strains of the most closely related species (99.2% 16S rRNA gene sequence similarity) *Nocardioides salarius* and *Nocardioides basaltis* (Kim et al., 2008a, 2009a) and 23.1±5.6% (Park et al., 2008) and 18 ± 6% (Lee et al., 2007) with type strains of more distant species, *Nocardioides dokdonensis* and *Nocardioides kribbensis* (<97.7% sequence similarity). The type strain (the only strain described) was isolated using ISP 4 medium (Shirling and Gottlieb, 1966) prepared with 60% (v/v) natural sea water.

Source (type strain): sand, Samyang Beach, Jeju Island, Korea.

DNA G+C content (mol%): 73.1 (HPLC).

Type strain: SBS-12, DSM 18965, JBRI 2003, KCCM 42681

Sequence accession no. (16S rRNA gene): AM422448.

35. **Nocardioides marinus** Choi, Kim, Noh and Cho 2007, 778[VP]

ma.ri′nus. L. masc. adj. *marinus* of or belonging to the sea, from where the type strain was isolated.

Characteristics are as described for the genus and listed in Table 215. The data presented below are from Choi et al. (2007), unless indicated.

Cells are nonmotile small rods (0.4–0.6 × 1.0–1.8 µm) in the exponential phase and coccoid forms in the stationary phase. Colonies on marine agar (MA; Difco) are creamy white.

Grows at 10–40°C (optimum, 25–30°C) but not at 5 or 45°C, and in 0.5–8% (w/v) NaCl (optimum, 1–3%), but not 0% NaCl (test medium, ZoBell broth*; ZoBell, 1941). Utilizes L-asparagine, but not benzoate, citrate, succinate, tartrate, acetamide, L-arginine, L-lysine, and L-ornithine as a sole or principal carbon source for growth and energy. Glucose is not fermented (API 20NE). The major fatty acids in cells grown on MA agar plates at 30°C for 1 d included $C_{16:0}$ iso (71.5%), $C_{17:0}$ 10-methyl (5.1%), $C_{18:1}$ ω9*c* (4.4%), and $C_{17:1}$ ω8*c* (3.8%). Other components of the fatty acid profile were various saturated, monounsaturated, iso-, anteiso- and 10-methyl-branched fatty acids (0.3–2.1%), including TBSA (1.7%) and $C_{15:0}$ iso 2-OH and/or $C_{16:1}$ ω7*c* (1.3%). Level of DNA–DNA similarity (thermal denaturation and renaturation method) between the type strain of this species and the type strain of *Nocardioides terrae* was 16% (Zhang et al., 2009a). The type strain (the only strain described) was isolated using a selective S medium† (Fialho et al., 1999).

Source (type strain): sea water around Dokdo Island, the East Sea, Korea.

DNA G+C content (mol%): 72.9 (HPLC).

Type strain: CL-DD14, JCM 15615, KCCM 42321, DSM 18248.

Sequence accession no. (16S rRNA gene): DQ401093.

36. **Nocardioides mesophilus** Dastager, Lee, Pandey and Kim 2010, 2291[VP]

me.so.phi′lus. Gr. adj. *mesos* middle; Gr. adj. *philos* loving; N.L. masc. adj. *mesophilus* middle (temperature)-loving, i.e. mesophilic.

Characteristics are as described for the genus and listed in Table 215 and Table 216. Information presented below is taken from the original species description (Dastager et al., 2010).

Cells are slender short rods, 0.3–0.8 × 0.9–1.4 µm on R2A agar. Motility but no flagella were reported. Colonies are translucent, cream-whitish, and slightly raised after 2 d incubation at 28°C on twofold-diluted R2A agar. Optimum growth temperature and pH are 28°C and 7.0–7.5, respectively. Grows at 20–37°C. Acid is not produced from any of the carbon sources tested. Nutritionally non-exacting; grows on a mineral salts medium (ISP 9) with glucose as the carbon-plus-energy source and an ammonium salt as the sole nitrogen source. The major cellular fatty acids in cells harvested from R2A plates after incubation at 28°C for 7 d included $C_{16:0}$ iso (44.6%), $C_{16:1}$ (12.1%), $C_{14:0}$ iso (11.0%), $C_{16:0}$ (6.5%), $C_{18:1}$ ω9*c* (3.1%), $C_{16:0}$ 10-methyl (2.8%), $C_{15:0}$ iso (2.3%), and $C_{15:0}$ (2.2%). No TBSA was reported. DNA–DNA relatedness between the type strains of *Nocardioides mesophilus* and *Nocardioides jensenii* was 34±2.0% (Dastager et al., 2010). The type strain (and the only strain described) was isolated by serial dilution plating on tenfold-diluted R2A agar after 7 d incubation at 28°C.

Source (type strain): soil, Bigeum Island, Jeollanam-do Province, Korea.

DNA G+C content (mol%): 68.7 (HPLC).

Type strain: MSL-22, DSM 19432, KCTC 19310.

Sequence accession no. (16S rRNA gene): EF466117.

37. **Nocardioides nitrophenolicus** Yoon, Cho, Lee, Suzuki, Nakase and Park 1999, 679[VP]

ni.tro.phe.no′li.cus. N.L. n. *nitrophenol* nitrophenol; L. masc. suff. *-icus* suffix used with the sense of pertaining to; N.L. masc. adj. *nitrophenolicus* relating to nitrophenols.

Characteristics are as described for the genus and listed in Table 215, Table 218, Table 219, and Table 221. Information presented below is from Yoon et al. (1999, 2009, 2006b), unless indicated.

Cells are irregular rods (0.5–0.8 × 1.0–3.0 µm) in 2 d culture on nutrient agar and can undergo rod-to-coccus morphogenesis from the early exponential phase to the stationary phase. Motile, a single flagellum was observed (Figure 261). Neither substrate nor aerial mycelium is produced. Gram-stain-positive but the Gram staining test result is variable in old cultures. Colonies are yellowish white on nutrient agar (NA; Difco). Grows at 15 and 40°C (optimum, 30°C) but not at 45 or 10°C, as assessed after one-month incubation on nutrient agar (NA, Difco), and at initial pH 6 and 10 (optimum, pH 8; tested on NA).

Nutritionally non-exacting; grows on a suitable mineral salts medium (e.g. ISP 9) with glucose and an ammonium salt as sole carbon and nitrogen sources. The organism is capable of utilizing phenol, *p*-nitrophenol, and dibenzofuran (at suitable concentrations in the test medium) as sole carbon sources for growth (Cui et al., 2009; Kubota et al., 2005a; Yoon et al., 1999; Table 218). D-Mannose (weak), acetate, fumarate, succinate are used as sole carbon and energy sources for growth with ISP9 as the basal medium, but not L-arabinose, lactose, maltose, D-melibiose, adonitol, D-mannitol, D-sorbitol, citrate, and benzoate. The organism can also grow on a mineral salts medium with L-arginine, but not with tartrate, L-asparagine, L-lysine, L-ornithine, acetamide, D-salicin, and *N*-acetylglucosamine as sole carbon sources (Yi and Chun, 2004a). In API 20NE or API 50 CH tests with AUX as suspending medium, adipate, gluconate, and malate were used as sole carbon sources but not D- and L-arabinose, D- and L-fucose, gentiobiose, D-lactose, D-melibiose, L-xylose, D-sorbose, D-tagatose, adonitol, D- and L-arabitol, dulcitol, erythritol, sorbitol, xylitol, methyl α-D-mannoside, methyl

*Zobell Broth: 5 g of Bacto peptone, 1 g of yeast extract, 0.1 g of ferric citrate, and 1 liter of distilled water.

†S medium: 10 g of Na_2HPO_4, 3 g of KH_2PO_4, 1 g of K_2SO_4, 30 g of NaCl, 0.2 g of $MgSO_4{\cdot}7H_2O$, 0.01 g of $CaCl_2$, 0.001 g of $FeSO_4{\cdot}7H_2O$, 1 g of Casamino acids, 1 g of yeast extract, 20 g of glucose, 20 g of Bacto agar, and 1 liter of distilled water.

α-D-glucoside, *N*-acetylglucosamine, amygdalin, arbutin, esculin, starch, glycogen, citrate, 2-and 5-ketogluconate, caprate, and phenylacetate. According to Cui et al. (2009), the type strain grows with L-alanine, L-histidine, L-proline, L-serine, lactate, caprate, 3- and 4-hydroxybenzoic acids, 3-hydroxybutyrate, malic acid, propionate, suberate, valerate, as well as with maltose, D-mannose, D-mannitol, melibiose, and salicin as carbon and energy sources but not with adipate, itaconate, and malonate (tested on mineral salts medium supplemented with vitamins and an element solution). Acetoin is produced (Cui et al., 2009). According to Yi and Chun (2004a), the type strain cannot produce acid from glucose (API 20NE kit). Arbutin is weakly hydrolyzed. Elastin is not degraded.

Major fatty acids in cells harvested from NA after 4 d incubation at 30°C were $C_{16:0}$ iso (28.1%), TBSA (13.9%), $C_{17:1}$ ω6*c* (10.8%), $C_{17:0}$ iso (12.3%), $C_{17:0}$ anteiso (5.8%), $C_{17:0}$ 10-methyl (5.1%), $C_{16:0}$ 10-methyl (3.6%), $C_{18:1}$ ω9*c* (4.5%), and $C_{15:0}$ iso (3.1%). The major fatty acids for cells grown on trypticase soy agar (Difco) at 30°C for 2 d (Table 219) differed in having larger proportions of saturated iso-branched acids (61.7% in total, with $C_{16:0}$ iso contributing 39.8%) and saturated straight-chain acids (14.4%), smaller proportions of total 10-methyl-branched acids (5.7%) and the absence of 2-hydroxylated acids. *Nocardioides nitrophenolicus* is substantially distinguished from *Nocardioides simplex* and other species of this genus by the length of the 16S–23S ITS region (328 bp, the shortest among the analyzed strains) and the nucleotide sequences (Yoon et al., 1998a). Distinct differences from other species, including *Nocardioides simplex*, was also revealed in the RNase P RNA gene sequences (Yoon and Park, 2000). The DNA–DNA similarity values (Table 221) between the type strain of *Nocardioides nitrophenolicus* and the type strains of the closest species ranged from 22 to 49% (Cui et al., 2009; Kubota et al., 2005a; Yoon et al., 1999; Yoon et al., 2009; Yoon et al., 2006b). Selected phenotypic characteristics useful in distinguishing *Nocardioides nitrophenolicus* from phylogenetically close species are listed in Table 218. The type strain (the only strain described) was isolated using minimal salts medium supplemented with a trace element solution and *p*-nitrophenol for enrichment and subsequent isolation (for further details on the isolation procedure see Yoon et al., 1999).

Source (type strain): industrial wastewater, Cheong-ju, South Korea.

DNA G+C content (mol%): 71.4 (HPLC).

Type strain: NSP41, CIP 107017, DSM 15529, KCTC 0457BP, JCM 10703.

Sequence accession no. (16S rRNA gene): AF005024.

38. **Nocardioides oleivorans** Schippers, Schumann and Spröer 2005, 1502[VP]

o.le.i.vo′rans. L. n. *oleum* oil; L. v. *vorare* to devour; N.L. part. adj. *oleivorans* capable of utilizing oil (hydrocarbons).

Characteristics are as described for the genus and listed in Table 215 and Table 220. Information presented below is from the original description (Schippers et al., 2005), unless indicated.

Nonmotile, irregular rods (about 0.3 × 1.1 μm). Colonies (maximum diameter after 2 weeks, 2 mm) are translucent and orange-pigmented. Grows at 30°C and in 2% NaCl. According to the original species description, *N*-acetyl-D-glucosamine, acetate, propionate, fumarate, DL-3-hydroxybutyrate, DL-lactate, L-malate, pyruvate, L-aspartate, L-histidine, L-proline, putrescine, phenylacetate, L-ornithine, and other substrates but not aconitate, adipate, citrate, α-D-galacturonate, 3-hydroxybenzoate, 4-hydroxybenzoate, suberate, adonitol, sorbitol, L-alanine, L-hydroxyproline, L-serine, glycogen, and *N*-acetyl-D-galactosamine are utilized as sole carbon and energy sources (tested using a basal medium containing vitamins and trace elements (Kämpfer et al., 1991). Crude oil is also used as a growth substrate. Acid is not produced from D-glucose, rhamnose, sucrose, adonitol, inositol, xylose, or sorbitol under aerobic conditions in conventional tests (Kämpfer et al., 1991). Glucose is not fermented (API 20NE; Lee, 2007b). The following substrates are hydrolyzed: *p*-nitrophenyl (pNP) *N*-acetyl-β-D-glucosaminide, pNP β-D-galactopyranoside, pNP α-D-glucopyranoside, pNP β-D-glucopyranoside, pNP α-D-maltoside, bis-pNP phosphate, benzolphosphonacid pNP-ester, pNP phosphocholine, 2-deoxythymidine 5′-pNP phosphate, L-alanine *p*-nitroanilide (pNA), L-glutamate pNA, L-glutamate-γ-3-carboxy pNA, L-leucine pNA, and L-lysine pNA, whereas pNP *N*-acetyl-β-D-galactosaminide, pNP α-L-arabinopyranoside, pNP β-D-cellobioside, pNP β-D-glucuronide, pNP β-D-lactoside, pNP α-D-mannoside, pNP β-D-xyloside, glycine pNA, L-proline pNA, and L-valine pNA are not hydrolyzed. Some additional characteristics of this organism were reported by Lee (2007b). The cell-wall peptidoglycan is of A3γ-type (LL-A_2pm-glycine). MK-8(H_4) is the predominant menaquinone and MK-8(H_2) is a minor menaquinone component. The major fatty acids determined for cells grown in basal medium* included $C_{18:1}$ ω9*c* (27.5%), $C_{16:0}$ iso (18.6%), TBSA (9.0%), $C_{18:0}$ (7.5%), $C_{17:0}$ iso (6.6%), and $C_{17:1}$ ω6*c* (5.7%).

The DNA–DNA relatedness (using the spectrophotometric method) of the type strain of *Nocardioides oleivorans* to the type strain of the phylogenetically closest species, *Nocardioides ganghwensis* (99% 16S rRNA gene sequence similarity) was 32% whereas its relatedness to *Nocardioides exalbidus* (using the method of Ezaki et al., 1989) was 44% (Li et al., 2007b). Selected phenotypic characteristics useful in distinguishing *Nocardioides oleivorans* from phylogenetically closely related species are listed in Table 220. The type strain (the only strain described) was isolated by enrichment in mineral salts medium (Fedorak and Westlake, 1981) supplemented with crude oil (30°C; several weeks) and subsequent isolation on the complex oil-free medium (Bosecker et al., 1991; Schippers et al., 2005).

Source (type strain): crude oil sample, the oilfield Oerrel of the Gifhorn Trough, Germany.

DNA G+C content (mol%): not determined.

Type strain: BAS3, DSM 16090, JCM 14342, NCIMB 14004.

Sequence accession no. (16S rRNA gene): AJ698724.

*Basal medium: 23.4 g of NaCl, 0.75 g of KCl, 7.0 g of $MgSO_4 \cdot 7H_2O$, 0.5 g of peptone from meat, 0.5 g of peptone from casein, 1.0 g of yeast extract, and 18 g of agar per 1 liter of water; pH 7.3.

39. **Nocardioides panacihumi** An, Im, Lee and Yoon 2007, 2145[VP]

pa.na.ci.hu′mi. N.L. n. *Panax* scientific name of ginseng; N.L. gen. n. *panacis* of ginseng; L. n. *humus* soil; N.L. gen. n. *panacihumi* of soil of a ginseng field.

Characteristics are as described for the genus and listed in Table 215. Other characteristics presented below are from the original publication (An et al., 2007), unless indicated.

Cells are nonmotile, small irregular rods (0.3–0.5 × 0.7–1.2 μm), forming white colonies, after 3 d incubation on R2A agar at 30°C. Undergo rod-to-coccus morphogenesis from the early exponential phase to the stationary phase. No growth occurs on MacConkey agar.

Grows well at 30°C and at pH 7.0; growth occurs at 15 and 30°C and at pH 5.0–8.0, but not at 4 and 37°C and at pH 8.5. Prefers low salinity, growing on R2A agar in 1% (w/v) NaCl, but not in 2%. Acetate, gluconate, glycogen, 3-hydroxybenzoate, 4-hydroxybenzoate, 3-hydroxybutyrate, propionate, valerate, phenylacetate, L-alanine, and L-proline but not L-fucose, D-sorbitol, adipate, caprate, citrate, itaconate, 2- and 5-ketogluconate, L-lactate, malate, malonate, suberate, L-histidine and L-serine are utilized as sole carbon sources. Acids are produced from D-glucose, D-fructose, D-lyxose, D-raffinose, L-rhamnose, sucrose, D-turanose, and *N*-acetylglucosamine (API 50 CH), but not from D- and L-fucose, gentiobiose, D-lactose, D-lyxose, D-mannose, melezitose, melibiose, L-sorbose, D-tagatose, L-xylose, adonitol, arabitol, dulcitol, erythritol, glycerol, inositol, D-mannitol, D-sorbitol, xylitol, gluconate, 2- or 5-ketogluconate, starch, glycogen, arbutin, inulin, salicin, methyl α-glucopyranoside, methyl α-D-mannoside, or methyl β-D-xyloside. In API 50 CH tests, acids are also not produced from cellobiose, D-galactose, maltose, D-ribose, and trehalose (characteristics that differentiate this species from the phylogenetically close species *Nocardioides terrae*; Zhang et al., 2009a). Glucose is not fermented (API 20NE). Nitrate reduction is weak under aerobic conditions. Catalase-positive (Cho et al., 2010), oxidase negative (Zhang et al., 2009a). Xylan and chitin are not degraded. The major fatty acids of cells harvested from R2A agar after incubation for 6 d at 30°C were $C_{16:0}$ iso (45.5%), $C_{17:1}$ ω6*c* (7.5%), $C_{16:0}$ (6.4%), and $C_{17:0}$ (5.9%); the minor fatty acids were TBSA and its homologs, as well as $C_{15:0}$ iso 3-OH. The DNA–DNA relatedness (thermal denaturation and renaturation method) of the type strains of *Nocardioides panacihumi* and *Nocardioides terrae* was 21% (Zhang et al., 2009a). To isolate the type strain (the only strain described), the sample was suspended in 50 mM phosphate buffer (pH 7.0) and seeded on one-fifth-strength R2A agar plates after serial dilution in the same buffer.

Source (type strain): soil of a ginseng field, Pocheon Province, South Korea.

DNA G+C content (mol%): 73 (HPLC).

Type strain: Gsoil 616, DSM 18660, JCM 15309, KCTC 19187.

Sequence accession no. (16S rRNA gene): AB271053.

40. **Nocardioides panacisoli** Cho, Lee, An, Whon and Kim 2010, 390[VP]

pa.na.ci.so′li. N.L. n. *Panax -acis* scientific name of ginseng; L. n. *solum* soil; N.L. gen. n *panacisoli* of soil of a ginseng field, the source of isolation of the organism.

Characteristics are as described for the genus and listed in Table 215. Information presented below is taken from the original species description (Cho et al., 2010).

Cells are slender short rods (0.2–0.4 × 0.8–1.2 μm) after 3 d in culture on R2A agar (Difco) at 30°C, forming light yellowish and convex colonies. Will grow within the temperature range of 10–42°C but not at 4 or 45°C, with the best growth at 18–37°C. Grows at pH 5.5–8.5 (on R2A agar prepared with suitable buffers). Grows on R2A agar with 2.0% (w/v) but not 3.0% NaCl. Grows in mineral salts medium (1.8 g of K_2HPO_4, 1.08 g of KH_2PO_4, 0.5 g of $NaNO_3$, 0.5 g of NH_4Cl, 0.1 g of KCl, 0.1 g of $MgSO_4$, and 0.05 g of $CaCl_2$ per 1 liter), supplemented with glucose, vitamins (Widdel and Bak, 1992), trace element solution SL-10 (Widdel et al., 1983), and selenite/tungstate solution (Tschech and Pfennig, 1984). Acetate, adipate, citrate, 3-hydroxybutyrate, gluconate, L-malate, L-proline, propionate, and valerate are also used as a sole carbon source for growth-plus-energy as tested on the above basal medium, but not the following compounds: L-alanine, caprate, L-fucose, glycogen, L-histidine, 3-hydroxybenzoic acid, itaconate, 5-ketogluconate, L-lactic acid, malonate, phenylacetate, L-serine, D-sorbitol, and suberate. The organism produces H_2S from sodium thiosulfate. Xylan or chitin is not degraded. Neither acid nor gas is produced from glucose (API 20E). Xylan or chitin is not degraded. Major fatty acids (>5% of the total) determined in cell mass scraped from trypticase soy agar Difco) after incubation at 30°C for 2 d included $C_{16:0}$ iso (28.0%), $C_{18:1}$ ω9*c* (10.7%), $C_{17:1}$ ω8*c* (9.0%), $C_{17:1}$ ω6*c* (5.8%), TBSA (6.7%), and $C_{16:0}$ (6.1%). The type strain (and the only strain described) was isolated according to the procedure and medium described by Cui et al. (2007b) for isolation of *Nocardioides ginsengisoli*; the plates were incubated at 30°C for 1 month under aerobic conditions.

Source (type strain): soil of a ginseng field in Pocheon Province, South Korea (37°58′N; 127°15′E).

DNA G+C content (mol%): 73.0 (HPLC).

Type strain: Gsoil 346, JCM 16953, KCTC 19470, DSM 21348.

Sequence accession no. (16S rRNA gene): FJ666101.

41. **Nocardioides plantarum** Collins, Cockcroft and Wallbanks 1994, 525[VP]

plan.ta′rum. N.L. gen. pl. n. *plantarum* of plants.

Characteristics are as described for the genus and listed in Table 215. Data presented below based on information published by Collins et al. (1994), unless indicated.

Cells are irregular rods (mean 0.4–0.7 × 0.8–2.4 μm or longer) in young culture and may produce V-forms. In older cultures, coccoid cells usually predominate. Nonmotile. Colonies are typically non-pigmented, but may be light brownish-gray in older cultures on some media. Growth occurs at 5 and 30°C, but not at 37°C (see Table 215) or in the presence of 5% NaCl. The organism grows well on nutrient agar (Difco) (Yi and Chun (2004a) and some other organic media based on yeast extract and peptone. Growth occurs with glucose and other sources (including fumarate, propionate, succinate, and isobutyrate) as sole or principal carbon sources on the basal mineral salts medium containing (per liter) mineral base E (Owens and Keddie, 1969), 0.2 g of Bacto Yeast Extract (Difco), 12 g of agar, 2 μg of

vitamin B_{12}, 10 mg of sodium glutamate, and 10 mg of methionine (Collins et al., 1994). Some strains utilize L-proline and tetradecane. Histidine, histamine, malonate, phenol, azelate, suberate, crotonate, pentoate, thymine, and uracil are not utilized. Tween 40 and Tween 60 are hydrolyzed. Hippurate is not decomposed. According to Lawson et al. (2000a), thiamine is requited as the only growth factor in a glucose-mineral salts medium (Grainger, 1963). However, the type strain can also grow with glucose or other substrates as sole carbon sources in minimal media without thiamine (Yi and Chun, 2004a), for instance on mineral salts medium (Baumann et al., 1971) supplemented with 2% (v/v) Hutner's mineral base (Cohen-Bazire et al., 1957) and modified by reducing the concentration of sea salts to half-strength. On the above basal medium, according to Yi and Chun (2004a), the type strain is able to grow with acetate, citrate, L-arginine, and L-ornithine as sole carbon sources, but not with benzoate, tartrate, D-sorbitol, L- and D-asparagine, L-lysine, and acetamide. No acid production from glucose (API 20NE kit) (Yi and Chun (2004a). The main component of the quinone system is MK-8(H_4); a minor amount of MK-7(H_4) is detectable. Neither cell-wall neutral monosugars nor cell-wall teichoic acids were revealed (Tul'skaya, 2009).

Polyamine pattern of the type strain grown in rich (R) medium (Yamada and Komagata, 1972) included cadaverine (33.9%) and spermine (42.4%) as the predominant components, with minor amounts of tyramine (11.9%), 1,3-diaminopropane (6.8%), and spermidine (5.1%) (Busse and Schumann, 1999). The major fatty acids reported for cells harvested after 1-d incubation on marine agar (Difco) at 30°C included $C_{16:0}$ (10.5%), TBSA (8.4%), $C_{18:0}$ (6.8%), $C_{16:0}$ iso (6.2%), $C_{17:0}$ 10-methyl (6.0%), $C_{10:0}$ (5.7%), and $C_{18:1}$ ω9*c* (5.0%) (Yi and Chun, 2004a). The main cellular fatty acids determined for the type strain grown in TSB medium at 28°C for 24–48 h included $C_{16:0}$ iso (26.0%), $C_{17:0}$ anteiso (16.8%), $C_{18:1}$ (15.7%), $C_{17:0}$ (6.1%), and $C_{17:0}$ iso (5.4%) (Schumann et al., 1997). The two strains described (Grainger J70 and Grainger J6) were isolated from herbage as reported by Grainger (1963).

Source (type strain): herbage.

DNA G+C content (mol%): 69 (T_m).

Type strain: Grainger J70, ATCC 51889, CIP 104157, DSM 11054, JCM 9626, LMG 16210, NCIMB 12834, VKM Ac-1998.

Sequence accession no. (16S rRNA gene): AF005008, X69973.

42. **Nocardioides pyridinolyticus** Yoon, Rhee, Lee, Park and Lee 1997b, 935[VP]

py.ri.di.no.ly'ti.cus. N.L. n. *pyridinum* pyridine; N.L. masc. adj. *lyticus* (from Gr. masc. adj. *lutikos*) able to loosen, able to dissolve; N.L. masc. adj. *pyridinolyticus* pyridine-dissolving.

Characteristics are as described for the genus and listed in Table 215. Data presented below based on information published by Yoon et al. (2008, 2004, 1997b), unless indicated.

Cells are small rods (0.5–0.6 × 1.2–1.6 μm) in young culture on minimal salts medium supplemented with 0.05% (w/v) yeast extract. Show rod-to-coccus morphogenesis from the early exponential phase to the stationary phase (Figure 259). Gram-stain-positive, but Gram straining is indistinct in old cultures. Motile, a single flagellum was observed (Figure 260). Neither substrate nor aerial mycelium is produced. Colonies are cream colored on nutrient agar (NA; Difco) and are approximately 1.0–1.5 mm in diameter after 5 d at 35°C. The organism grows well on minimal salts media supplemented with additional growth factors, yeast extract and tryptone, at pH 5 and 9 (optimum, pH 8) and at 20 and 40°C (optimum, 35°C). Utilizes pyridine and phenol as carbon and energy sources for growth, and also acetate, adipate, L-arabitol, turanose and other carbon sources (Table 215) as tested on basal Stevenson's medium* (Stevenson, 1967). The following substrates are not utilized on the same basal medium: D- and L-fucose, gentiobiose, D-lyxose, D-tagatose, sorbose, stachyose, adonitol, dulcitol, erythritol, D-sorbitol, xylitol, caprate, 2-ketogluconate, 5-ketogluconate, malate, phenylacetate, methyl α-D-glucoside, methyl α-D-mannoside, methyl β-D-xyloside, amygdalin, glycogen, esculin, and arbutin. According to Yi and Chun (2004b, 2004a), L-asparagine and succinate, but not benzoate, tartrate, L-arginine, L-lysine, L-ornithine, and acetamide are utilized as carbon and energy sources. Tributylin is hydrolyzed (Song et al., 2011). Data on utilization of D-galactose, lactose, D-mannose and D-mannitol as sole sources of carbon and energy vary between experiments or the test methods (Yi and Chun, 2004a, 2004b; Yoon et al. 1997b, 2004, 2008). Similarly, hydrolysis of tyrosine and production of cysteine arylamidase vary between experiments or test methods (Song et al., 2011; Yoon et al., 2008). Acid production from glucose is not observable with the API 20NE test system (Yi and Chun, 2004a, 2004b). Sensitive to tetracycline (30 μg) and shows resistance to ampicillin (10 μg), chloramphenicol (100 μg), streptomycin (50 μg), and rifampin (30 μg), as assessed using filter-paper discs (Song et al., 2011). The predominant menaquinone is MK-8(H_4); minor or trace amounts of MK-7(H_4) and MK-8(H_2) may occur. The major cellular fatty acids determined for cells harvested after 4 d incubation on nutrient agar (NA; Difco) at 30°C included $C_{16:0}$ iso (47.3%), $C_{17:0}$ anteiso (14.2%), $C_{15:0}$ iso (5.1%), $C_{17:0}$ 10-methyl (5.5%), $C_{16:0}$ 10-methyl (5.0%), TBSA (3.4%), and $C_{16:1}$ iso H (4.2%). A different fatty acid profile was recently reported for cells grown on 1/2-strength R2A agar (1/2-R2A) for 3 d at 30°C (Song et al., 2011). The most remarkable differences were in $C_{14:0}$ iso 3-OH (8.8%), $C_{18:1}$ ω5*c* (8.8%) and minor amounts of cyclic fatty acids, which were not mentioned among fatty acids exceeding 0.5% in cells grown on NA (Yoon et al. 1997a, 2004), as well as a smaller proportion of $C_{16:0}$ iso (22%). The fatty acid profiles of *Nocardioides pyridinolyticus* and phylogenetically close species (*Nocardioides aquiterrae*, *Nocardioides hankookensis*, and *Nocardioides caricicola*) grown on 1/S-R2A agar are compared by Song et al. (2011). The DNA–DNA relatedness of the type strains of *Nocardioides pyridinolyticus* to it closest phylogenetic relative, *Nocardioides aquaterrae* (99.2% 16S rRNA gene sequence similarity) were

*Stevenson's Medium: Bacto yeast nitrogen base medium without amino acids (Difco) supplemented with Casamino acids (10 mg per liter) and agar (15 g per

found to be 28.7 and 32.5% in reciprocal experiments using the procedure of Ezaki et al. (1989). Mean similarity to *Nocardioides hankookensis* using the same method was 22%. A higher DNA–DNA relatedness (53.5±5.5%) determined by the same method was reported for the pair *Nocardioides pyridinolyticus*–*Nocardioides caricicola* (Song et al., 2011). The characteristics useful in distinguishing *Nocardioides pyridinolyticus* from the phylogenetically closest species, *Nocardioides aquiterrae* and *Nocardioides hankookensis*, include the growth temperature range (higher optimal temperature for growth), cell motility, negative reaction in the test for cytochrome oxidase activity, the ability to grow with inositol, D-melezitose and D-ribose as sole carbon sources, and resistance to chloramphenicol (100 µg per disc), streptomycin (50 µg per disc), and probably the ability to utilize pyridine. The type strain (and the only strain taxonomically characterized) was isolated as described by Lee et al. (1991).

Source (type strain): oxic zone of an oil shale column.

DNA G+C content (mol%): 72.5 (HPLC).

Type strain: OS4, CIP 106800, DSM 15530, KCTC 0074BP, JCM 10369.

Sequence accession no. (16S rRNA gene): U61298.

43. **Nocardioides salarius** Kim, Choi, Hwang and Cho 2008a, 2062[VP]

sa.la′ri.us. L. masc. adj. *salarius* of salt, referring to the salt resistance of this micro-organism.

Characteristics are as described for the genus and listed in Table 215. Additional data presented below are based on information published by Kim et al. (2008a).

Cells are nonmotile rods (0.3–0.6 × 0.6–1.6 µm), forming creamy white colonies on marine agar (MA; Difco) MA at 30°C. Grows at 10–35°C, optimally at 25–30°C; no growth occurs at pH 5 or at temperatures 5 or 40°C. Salt-requiring; grows at NaCl concentrations of 1–10% (w/v), with optimum growth at 3% NaCl, as tested in ZoBell broth (ZoBell, 1941), containing 5 g of Bacto peptone, 1 g of yeast extract, 0.1 g of ferric citrate, and 1 liter of distilled water; no growth is observable at 0.5% (v/w) or without NaCl in the same test medium. Growth occurs in marine broth at initial pH values of 6 and 10 (the highest value examined); the optimum pH for growth is 6–7. A number of carbon sources (Table 215), as well as L-arginine and L-ornithine enhance growth in a basal mineral salts medium* supplemented with 0.05 g of yeast extract. Citrate, succinate, L-asparagine, and L-lysine are not used as sole or principal carbon sources. Glucose is not fermented (API 20NE). The predominant fatty acid detected in cells harvested from MA after 1 d incubation at 30°C was $C_{16:0}$ iso (65.3%), followed by $C_{16:0}$ 10-methyl (5.2%) and other iso-branched acids, such as $C_{16:1}$ iso H (4.6%), $C_{15:0}$ iso (3.4%), $C_{14:0}$ iso (3.2%), and $C_{15:0}$ iso2-OH (and/or $C_{16:1}$ ω7*c*) (3.2%). Components detected in lesser amounts (up to 2.5%) included mostly straight-chain monounsaturated and iso- and anteiso-branched fatty acids, as well as TBSA (0.5%) and $C_{17:0}$ 2-OH (0.7%). A mean DNA–DNA hybridization value of 33±9% was obtained to the type strain of *Nocardioides marinisabuli* (99.2% 16S rRNA gene sequence identity), with a lower DNA–DNA similarity (18 ± 6%) to more distant *Nocardioides kribbensis*. There are no data on the DNA–DNA similarity between *Nocardioides salarius* and the phylogenetically closest (99.6% 16S rRNA gene sequence similarity) species *Nocardioides basaltis* described by Kim et al. (2009a) shortly after the establishment of the species *Nocardioides salarius*). The type strains of these two species show many phenotypic features in common, including the fatty acid composition and physiological and biochemical traits. See the description of the species *Nocardioides basaltis* in this section, Table 215 and the original publications (Kim et al., 2008a, 2009a) for characteristics that might be helpful in distinguishing these two species. The type strain (and the only strain described) was isolated by enrichment culture (sea water supplemented with zooplankton incubated at 10–15°C for about 1 year) on low-nutrient heterotrophic medium (for details on the isolation procedure and the medium composition see Kim et al., 2008a; Cho and Giovannoni, 2004).

Source (type strain): sea water enriched with zooplankton, the South Sea, Korea.

DNA G+C content (mol%): 73.3 (HPLC).

Type strain: CL-Z59, KCCM 42320, DSM 18239.

Sequence accession no. (16S rRNA gene): DQ401092.

Additional remarks: the absence of data on the DNA–DNA similarity between *Nocardioides salarius* and the phylogenetically closest species *Nocardioides basaltis*, along with their very similar phenotypic traits suggest that further comparative taxonomic studies of these two species, are needed to support distinctions between these two species.

44. **Nocardioides sediminis** Dastager, Lee, Ju, Park and Kim 2009e, 281[VP]

se.di.mi′nis. L. gen. n. *sediminis* of a sediment, referring to the source from which the type strain was isolated.

Characteristics are as described for the genus and listed in Table 215. Information presented below is taken from the original species description (Dastager et al., 2009e).

Cells are mostly slender irregular rods (0.3–0.4 × 0.9–1.4 µm) after 3–5 d incubation on R2A agar (Difco) at 28°C. Non-separated coccoid cells and larger club-shaped and spherical forms up to 0.8 µm in diameter may occur. The cells from two-fold diluted R2A agar appeared to be motile under light microscopy but no flagella were detected. Colonies are translucent, cream–whitish, and slightly raised on R2A agar after 2 d growth at 28°C. Nutritionally non-exacting; grows on a mineral salts medium (ISP 9) with glucose as the carbon-plus-energy source and an ammonium salt as the sole nitrogen source. Growth also occurs with turanose but not with adonitol, adipate, D-arabitol, esculin, arbutin, dulcitol, erythritol, D-fucose, gentiobiose, gluconate, glycogen, D-lyxose, malate, sorbose, sorbitol, xylitol, or L-xylose tested under the same experimental conditions. Acid production from carbohydrates is rather uncommon or weak; no acid was formed from any of the tested carbon sources. The predominant fatty acids determined for cell mass harvested from R2A plates after incubation at 28°C for 7 d included $C_{16:0}$ iso (48.3%), $C_{17:1}$ ω8*c* (17.1%), $C_{17:0}$ iso (7%), $C_{17:0}$ 10-methyl (4.8%), and $C_{18:1}$ ω9*c* (4.3%). DNA–DNA

liter), and neutralized by K_2HPO_4.

similarity of the two type strains of the species *Nocardioides sedimentis* and *Nocardioides terrigena* was reported to be 34%. The type strain (the only strain described) was isolated using standard dilution techniques on tenfold-diluted R2A at 28°C.

Source (type strain): sediment sample taken at a depth of 60–70 m, Bigeum Island, Jeollanam-do Province, South Korea.

DNA G+C content (mol%): 71.5 (HPLC).

Type strain: MSL-01, DSM 19263, KCTC 19271.

Sequence accession no. (16S rRNA gene): EF466110.

45. **Nocardioides simplex** (Jensen 1934) O'Donnell, Goodfellow and Minnikin 1983, 896[VP] (Effective publication: O'Donnell, Goodfellow and Minnikin 1982, 327.) [*Pimelobacter simplex* (Jensen 1934) Suzuki and Komagata 1983b, 673[VP]; effective publication: Suzuki and Komagata 1983c, 69; *Arthrobacter simplex* (Jensen) Lochhead 1957, 608[AL]; "*Corynebacterium simplex*" Jensen, 1934, 43.]

sim′plex. L. masc. adj. *simplex* simple.

Characteristics are as described for the genus and listed in Table 215, Table 218, Table 219, and Table 221. Additional data are from O'Donnell et al. (1982), Suzuki and Komagata (1983c), and Keddie et al. (1986), unless indicated.

Cells are typically slender irregular rods, exhibiting a rod-to-coccus morphogenesis during the growth cycle. Irregular rods (~0.4–0.5 × 1.0–3.0 µm or longer) in late lag and exponential phase cultures and coccoid cells or very short rods (~0.4–0.5 µm in diameter) in older cultures were reported for the type strain on complex media by O'Donnell et al. (1982). Slightly wider cells (~0.5–0.9 µm in diameter), both in young and older cultures, were reported for this species by Keddie et al. (1986). Rods are typically motile with single- or peritrichous flagella. Colonies on yeast-peptone agar and most ISP media usually show no distinctive pigmentation. On YM agar and some other rich media, they may be slightly yellowish white or ivory in color, particularly in older cultures. A light brownish soluble pigment may occur in older cultures on some peptone-based media. Optimum growth temperature is ~26–30°C; grows at 10°C, and may or may not grow at 37°C. The type strain grows well in 5% NaCl but not in 7% NaCl (Nesterenko et al., 1985b).

Nutritionally non-exacting; grows in a suitable mineral salts medium with glucose as sole carbon-plus-energy source and an ammonium salt or nitrate as sole nitrogen source (Keddie et al., 1966; Owens and Keddie, 1969). The organism utilized about 60 out of the 180 compounds tested as sole or principal sources of carbon and energy. These include a very narrow range of carbohydrates and sugar derivatives, a wide range of fatty acids, simple alcohols, and amino acids, including some hydroxy-acids, oxo-acids, amines, pyrimidines, and other compounds (Keddie, 1974). The type strain utilizes acetate, citrate, succinate, fumarate, acetamide, L-asparagine, L-arginine, L-ornithine (Yi and Chun, 2004b, a), azelate, malonate, L-proline, histamine, thymine, uracil, and tetradecane (Collins et al., 1994) as sole carbon and energy sources, and tartrate in conventional tests. Negative results were recorded for utilization of L-lysine, benzoate, and hippurate(Yi and Chun, 2004b, a). Among carbohydrates tested by Yi and Chun (2004a), only D-glucose and sucrose supported growth. In the API 20NE or API 50 CH test system (AUX as suspending medium), the type strain utilized a few carbon sources for growth and energy, such as D-glucose, sucrose, trehalose, gluconate, adipate, and malate but not many other substrates, including D- and L-arabinose, D-cellobiose, D-fructose, D- and L-fucose, D-galactose, gentiobiose, D-lactose, D-melibiose, D-lyxose, D-maltose, D-mannose, D-melezitose, D-raffinose, L-rhamnose, D-ribose, D-tagatose, D-turanose, D- and L-xylose, D-sorbose, D- and L-adonitol, D- and L-arabitol, dulcitol, erythritol, glycerol, inositol, D-mannitol, D-sorbitol, xylitol, methyl α-D-mannoside, methyl α-D-glucoside, methyl β-D-xyloside, *N*-acetylglucosamine, amygdalin, arbutin, esculin, D-salicin, inulin, starch, glycogen, 2- and 5-ketogluconate, caprate, and phenylacetate (Kubota et al., 2005b; Yoon et al., 2009). Cui et al. (2009) reported the ability of the type strain to grow with L-alanine, L-histidine, L-serine, lactate, caprate, 3- and 4-hydroxybenzoic acids, 3-hydroxybutyrate, malic acid, propionate, suberate, and valerate (on mineral salts medium supplemented with vitamins and element solution); the strain in addition was reported to grow with maltose, D-mannose, melibiose, D-mannitol, salicin, *N*-acetylglucosamine as carbon and energy sources under the same experimental condition.

No acid is produced from glucose or other sugars by oxidation in peptone-based media in conventional tests (Keddie et al., 1986). No acid is produced from glucose and other sugars by the type strain using the API 20NE and API 50 CH test systems (Kubota et al., 2005a; Yi and Chun, 2004a). As mentioned before, the *Nocardioides simplex* strains transform sterols and their derivates (Arima et al., 1969; Nagasawa et al., 1969). They also were reported to utilize phenol as a sole carbon and energy source (Collins et al., 1994; Keddie et al., 1986), but the type strain could not grow in the presence of phenol at a concentration of 125 p.p.m. (Cui et al., 2009). The authors also found that this strain utilized dibenzofuran at 50 p.p.m. in the test medium (higher concentrations were growth-inhibiting) (Cui et al., 2009). According to Kubota et al. (2005a), the type strain *Nocardioides simplex* JCM 1363 did not use biphenyl and dibenzofuran as a carbon and energy source, in contrast to *Nocardioides aromaticivorans*. Five tested strains of *Nocardioides simplex*, in contrast to closely related species, were also unable to utilize *p*-nitrophenol as the sole carbon and energy source (Cui et al., 2009; Yoon et al., 1999) (Table 218).

The cell-wall peptidoglycan is of the A3γ type (LL-A_2pm-glycine) (Schleifer and Kandler, 1972). The well-documented cell-wall sugar is galactose (Keddie and Cure, 1977; Sadikov et al., 1983; Takeuchi and Yokota, 1989). In addition, the presence of glucose (Takeuchi and Yokota, 1989) or rhamnose (Sadikov et al., 1983) was reported for the type strain, and mannose for strain NBRC (IFO) 12679 = JCM 1366 (Takeuchi and Yokota, 1989). (Relationship to *Nocardioides simplex* was supported by DNA–DNA hybridization experiments; Suzuki and Komagata, 1983c; Yoon et al., 1999.) The wall polysaccharides of both the above strains were also found to contain a small or trace amount of glucosamine (Takeuchi and Yokota, 1989). Both strains had cell walls containing glycerol teichoic acids (Sadikov et al., 1983; Takeuchi and Yokota, 1989). The polyamine composition of the type strain, DSM 43109, harvested from rich (R) medium (Yamada and Komagata, 1972) was cadaverine

(56.6%), spermidine (18.6%), putrescine (15.0%), spermine (5.6%), and 1,3-diaminopropane (4.2%), with a trace amount of *sym*-homospermidine (Busse and Schumann, 1999). The predominant isoprenoid quinone is MK-8(H_4); minor quantities of MK-7(H_4), MK-8, MK-8(H_6), and a trace amount of MK-9(H_4) may be detected (Collins et al., 1979; Collins et al., 1983; O'Donnell et al., 1982; Suzuki and Komagata, 1983c; Yamada et al., 1976).

The fatty acids of *Nocardioides simplex* to a large extent depends on the culture conditions and analytical procedures (Collins et al., 1983; Cui et al., 2009; Miller et al., 1991; O'Donnell et al., 1982; Schumann et al., 1997; Suzuki and Komagata, 1983c; Yoon et al., 1999; Yoon et al., 1997a). When strains of this and other species of this genus are analyzed under similar experimental conditions, the fatty acid profiles of *Nocardioides simplex* are very similar (Yoon et al., 1999) and differ from those of other species (Cui et al., 2009; Yoon et al., 1999), including closely related ones (Table 219). The cellular fatty acids (4% or more), determined for 5 strains grown on nutrient agar (NA; Difco) for 4 d at 30°C were $C_{16:0}$ iso (29.0–36.6%), $C_{18:1}$ ω9*c* (7.3–12.0%), $C_{17:1}$ ω6*c* (11.9–14.8%), TBSA (9.3–15.3%), $C_{17:0}$ 10-methyl (2.4–6.3%), $C_{16:0}$ 10-methyl (1.1–4.2%), $C_{16:1}$ iso H (2.8–6.6%), and $C_{17:0}$ iso (2.2–6.4%). The cells of the type strain grown in trypticase soy broth (Difco) for 24–48 h at 28°C contained different proportions of predominant fatty acids: $C_{16:0}$ iso (41.8%), $C_{18:1}$ (13.1%), $C_{17:1}$ (5.2%), TBSA (5.2%), $C_{16:1}$ (5.0%), $C_{14:0}$ iso (4.6%), and also had a lower level of 10-methyl-branched components (Schumann et al., 1997). The presence of 2-hydroxylated acids was also detected (Collins et al., 1983; Suzuki and Komagata, 1983c; Yoon et al., 1999), which may contribute up to 10% in some experiments (Suzuki and Komagata, 1983c). The phospholipids also varied between different studies and included diphosphatidylglycerol, phosphatidylglycerol, and a few incompletely characterized components (Collins et al., 1989; Collins et al., 1983; O'Donnell et al., 1982). The presence of phosphatidylglycerol with a significant amount of 2-hydroxy fatty acids at the 2-position was reported by Yano et al. (1970, 1971). There is also a report about tentative identification of substantial proportions of phosphatidylinositol mannosides and phosphatidylethanolamine in *Arthrobacter simplex* ("*Corynebacterium*" *simplex*) grown on *n*-alkanes (Yanagawa et al., 1972). In our recent experiment, only three clearly dominating phospholipids were detected for the type strain of *Nocardioides simplex*, namely, phosphatidylglycerol and two similar unidentified phospholipids, one of which was found to include inositol and is most likely phosphatidylinositol, while the second is supposedly phosphatidylinositol containing a hydroxy acid (Figure 263).

Nocardioides simplex is susceptible to some actinophages among those multiplying on mycelium-forming *Nocardioides* strains (Prauser, 1976, 1981, 1989).

Strains affiliated with this species had 60–100% DNA–DNA similarity (using the membrane filter method with tritium-labeled DNA; Suzuki and Komagata, 1983c) and more than 85% (Yoon et al., 1999, using the method of Ezaki et al., 1989). The levels of DNA–DNA hybridization between the type strain of *Nocardioides simplex* and type strains of the species composing the tight *Nocardioides simplex* phylogenetic group were reported to be 13–55% (Cui et al., 2009; Kubota et al., 2005a; Yoon et al., 1999, 2006b, 2009; Table 221). The DNA–DNA hybridization values of the type strain of *Nocardioides simplex* with those of the type strains of more distant species (*Nocardioides plantarum*, *Nocardioides albus*, *Nocardioides luteus*, and *Nocardioides jensenii*) obtained by the method of Ezaki et al. (1989) were reported to be 28–33% (Kubota et al., 2005a). A lower DNA–DNA binding level (16–22%) was found between members of *Nocardioides simplex* and the type strains of *Nocardioides albus* and *Nocardioides jensenii* (using the membrane filter method; Suzuki and Komagata, 1983c). Similar values (15–20%) were reported for *Nocardioides simplex* and mycelium-forming strains of *Nocardioides* by Prauser (1981). Strains of *Nocardioides simplex* are very similar in the length and nucleotide sequences of the 16S–23S ITS region and clearly distinguished from *Nocardioides nitrophenolicus* and other members of this genus by these characteristics (Yoon et al., 1998a). The type strains of *Nocardioides simplex* and other species included in the study are also different in the RNase P RNA gene sequences (Yoon and Park, 2000).

Selected phenotypic characteristics useful in distinguishing *Nocardioides simplex* from phylogenetically closely related species are listed in Table 218. Occur mostly in soil. The type strain was isolated as described by Jensen (1934).

Source (type strain): rice soli.

DNA G+C content (mol%): 71.7–73.5 (HPLC, T_m); 71.7 (HPLC) for the type strain.

Type strain: AJ 1420, ATCC 6946, CCM 1652, CCUG 23611, CIP 82.106, DSM 20130, HAMBI 90, HAMBI 1861, IAM 1660, IFO (now NBRC) 12069, JCM 1363, KCTC 9106, LMG 16261, NRRL B-14051, NRRL B-3157, VKM Ac-1118.

Sequence accession no. (16S rRNA gene): X53213, AF005009.

Additional remarks: first described as "*Corynebacterium simplex*" (Jensen, 1934), this species was then affiliated to the genus *Arthrobacter* as *Arthrobacter simplex* (Lochhead, 1957) and later reclassified, mainly on chemotaxonomic grounds, as *Nocardioides simplex* (O'Donnell et al., 1982, 1983). Independently, Suzuki and Komagata (1983b, 1983c) established the genus *Pimelobacter* to accommodate *Arthrobacter simplex* and some related organisms. Those, in particular, included two strains (IAM 1398 = IFO 1366 and IAM 1413 = IFO 1367) isolated from soils of an oilfield in Japan and originally described as "*Brevibacterium lipolyticum*" (Iizuka and Komagata, 1964). Strain NCIB 9770, originally identified as *Arthrobacter simplex* (Jensen and Gundersen, 1956) was reclassified as a representative of a novel species of the genus *Pimelobacter*, *Pimelobacter jensenii* (Suzuki and Komagata, 1983b, 1983c), and subsequently transferred to *Nocardioides* as *Nocardioides jensenii* (Collins et al., 1989). Some other strains (ATCC 13260, 19565, and 19566) originally described under the name *Nocardioides simplex* were reidentified as members of the genus *Rhodococcus* (Yoon et al., 1997a). Notably, other strains cited in the literature under the species name *Nocardioides simplex* might also not belong to this species, particularly in light of recent proposals regarding some species phylogenetically closely related to *Nocardioides simplex* (Table 218). Although some of these strains (e.g. *Nocardioides simplex* VKM Ac-2033D involved in transformation of sterols (Fokina et al., 2003a, 2003b) or *Nocardioides simplex* 3E involved in degradation of 2,4,5-trichlorophenoxyacetic acid (Golovleva et al., 1990)

exhibit a very high 16S rRNA gene sequence similarity to the type strain of *Nocardioides simplex*, their relationship to this species was not firmly supported by DNA–DNA hybridization or other relevant genotypic studies.

46. **Nocardioides terrae** Zhang, Liu and Liu 2009a, 2447[VP]

ter′ra.e. L. gen. n. *terrae* of or from the earth.

Characteristics are as described for the genus and listed in Table 215. Information presented below is based on the original species description (Zhang et al., 2009a).

Cells are slender irregular rods or coccoid. Data on the cell size are conflicting. In the species description, the cells are 0.2–0.3 µm in diameter and 0.3–1.0 µm long. As seen from the micrograph provided, most cells after 3 d in culture on R2A agar (30°C) appear as short rods, mostly about 0.3–0.5 µm in diameter and up to 1.0 µm long; longer cells, up to 2–2.3 µm may also occur. Coccoid cells of the same diameter, slightly smaller or increased are observable. The general appearance of the cells suggests the occurrence of a rod-to-coccoid growth cycle. Nonmotile, no flagella are observed. Colonies are cream-colored on R2A agar. Grows at 16–34°C (optimum, 29°C), but prefers not to grow at 14 or 36°C. Grows at pH 5.5–8.5 (optimum pH 6.2–6.5) and in 0–1% (w/v) NaCl (optimum 0–0.25%), as assessed in R2A broth. Along with substrates listed in Table 215, the organism assimilates gluconate as a carbon source, but not adipate, caprate, and citrate (API 20 NE). Acids are produced from D-glucose, L-rhamnose, and weakly from starch (API 50 CH). Acids are also formed from D-cellobiose, D-galactose, maltose, D-ribose, and trehalose (characteristics differentiating this species from the phylogenetically close species *Nocardioides panacihumi*). The type strain contains *N*-acetyl-β-glucosaminidase. Tween 20 is not hydrolyzed. Glucose fermentation (API 20NE) occurs. The major cellular fatty acids (>5% of the total) determined for cell mass scraped from R2A after incubation at 30°C for 3 d were $C_{16:0}$ iso (18.1%), TBSA (11.8%), $C_{17:0}$ 10-methyl (10.7%), $C_{17:1}$ ω8*c* (7.0%), $C_{15:0}$ iso (6.1%), $C_{18:1}$ ω9*c* (5.3%), and $C_{16:0}$ 10-methyl (5.1%). Levels of DNA–DNA similarity between the type strain of this species and the type strains of *Nocardioides panacihumi* and *Nocardioides marinus* were reported to be 21 and 16%, respectively (thermal denaturation method). The type strain (the only strain described) was isolated on VL55 medium (Sait et al., 2002) modified by adding 10 ml per liter of an amino acid mixture (Davis et al., 2005) and using gellan as a solidifying agent. The strain was isolated from a plate inoculated with the 10^{-7} dilution after 1 week of incubation at 30°C.

Source (type strain): forest soil, the Changbai Mountains, Heilongjiang Province, China.

DNA G+C content (mol%): 71.6 (T_m).

Type strain: VA15,CGMCC 1.7056, JCM 16799, NBRC 104259.

Sequence accession no. (16S rRNA gene): FJ423762.

47. **Nocardioides terrigena** Yoon, Kang, Lee and Oh 2007a, 2474[VP]

ter.ri.ge′na. L. masc. or fem. n. *terrigena* child of the earth, referring to the isolation of the type strain from soil.

Characteristics are as described for the genus and listed in Table 215. Additional information is from the original species description (Yoon et al., 2007a).

Cells are nonmotile rods or cocci (0.4–0.7 × 0.7–2.0 µm) in the exponential phase of growth. A rod–coccus morphogenetic growth cycle occurs during growth on complex media. Neither substrate nor aerial mycelium is formed. Gram-staining is variable in old cultures. Colonies are ivory in color and 1.0–1.2 mm in diameter after 7 d incubation on R2A agar at 30°C. Grows at 4 and 35°C, but prefers not to grow at 36°C. Grows in 0–3% but not at 4% (w/v) NaCl, with optimum growth at 0.5–1.0% (w/v) NaCl (assessed in R2A broth prepared according to the Difco formula without agar). Optimal pH for growth is around 8.0. Gentiobiose, D-lyxose (weak), D-turanose, adipate, gluconate and L-malate, but not D-arabinose, D- and L-fucose, sorbose, D-tagatose, L-xylose, D- and L-arabitol, erythritol, dulcitol, sorbitol, xylitol, caprate, 2- and 5-ketogluconate, phenylacetate, methyl β-D-xyloside, methyl α-D-mannoside, methyl α-D-glucoside, glycogen, esculin, amygdalin, and arbutin are utilized as sole carbon and energy sources. Tweens 20, 40, and 60 are hydrolyzed. The type strain is susceptible to the following antibiotics (µg per disk, unless indicated): ampicillin (10), carbenicillin (100), cephalothin (30), chloramphenicol (100), kanamycin (30), lincomycin (15), neomycin (30), novobiocin (5), oleandomycin (15), penicillin G (20 U), and streptomycin (50) and weakly susceptible to tetracycline (30); resistant to gentamicin (30) or polymyxin B (100 U). The main fatty acids included $C_{16:0}$ iso (35.5%), $C_{17:1}$ ω8*c* (30.2%), and $C_{17:0}$ (11.5%). Minor compounds reported were represented by various saturated, monounsaturated, iso-, anteiso- and 10-methyl-branched fatty acids characteristic of the genus *Nicardioides*, each contributing 0.8–2.7%. TBSA was not detected among fatty acids that exceeded 0.5%. The DNA–DNA hybridization value was 34% for the type strain *Nocardioides terrigena* and type strain of the most closely related species, *Nocardioides sediminis* (Dastager et al., 2009e), and 7% for the type strains of *Nocardioides terrigena* and *Nocardioides basaltis* (Kim et al., 2009a). The type strain (the only strain described) was isolated using the standard dilution plating technique on tenfold-diluted nutrient agar (Difco) at 25°C.

Source (type strain): soil, Dokdo, Korea (37°14′12″N; 131°52′07″E).

DNA G+C content (mol%): 71.5 (HPLC).

Type strain: DS-17, KCTC 19217, JCM 14582.

Sequence accession no. (16S rRNA gene): EF363712

48. **Nocardioides tritolerans** Dastager, Lee, Ju, Park and Kim 2009a, 1555[VP] (Effective publication: Dastager, Lee, Ju, Park and Kim 2008c, 1205.)

tri.to′le.rans L. pref. *tri-* (from L. num. adj. *tris*), three; L. part. adj. *tolerans* tolerating; N.L. part. adj. *tritolerans* referring to the ability of the organism referring to the ability of the organism to tolerate relatively high salinity, alkalinity and temperature.

Characteristics are as described for the genus and listed in Table 215. Information presented below is taken from the original work on species description (Dastager et al., 2008c).

Cells are typically motile irregular rods, mostly about 0.3–0.5 µm wide and up to 4.0 µm long on R2A medium. A week-old culture on the same medium is mostly composed of short rods, not exceeding 1 µm in length; a small fraction

of coccoid cells is present. Larger club-shaped or spherical forms, up to 1.4 µm in diameter may occur (Figure 257). Colonies are translucent, cream in color, slightly raised, and 0.9–1.3 mm in diameter after 4–5 d incubation on R2A medium at 30°C. Grows at 20–40°C, but not at 19°C or 41°C. Grows well on R2A medium at initial pH 6.0–11.0 (optimum, pH 7.0–7.5), with weak growth at pH 12, and on R2A medium containing up to 7.0% (w/v) NaCl.

Acids are produced from many sugars listed in Table 215, as tested by conventional methods. The fatty acids of cells harvested from R2A after incubation for 5 d at 28°C included $C_{16:0}$ iso (40.6%), $C_{17:1}$ ω8*c* (7.3%), $C_{18:1}$ ω9*c* (6.1%), $C_{17:1}$ (4.5%), $C_{15:0}$ iso (4.5%), $C_{17:0}$ anteiso (4.4%), $C_{17:0}$ iso (4.1%), $C_{17:0}$ 10-methyl (2.9%), $C_{17:1}$ ω6*c* (2.9%), $C_{17:1}$ iso ω9*c* (2.8%), $C_{16:1}$ iso (2.6%), and $C_{16:0}$ (2.4%); other acids reported were $C_{14:0}$ iso, $C_{18:0}$ iso, $C_{18:0}$, and $C_{15:1}$ anteiso, each contributing 1.4–1.8%. The type strain (and the only strain described) was isolated by serial dilution plating on tenfold-diluted R2A agar at 30°C after 7 d incubation.

Source (type strain): soil, Bigeum Island, Korea.

DNA G+C content (mol%): 67.6 (HPLC).

Type strain: MSL-14, KCTC 19289, DSM 19319.

Sequence accession no. (16S rRNA gene): EF466107.

Genus II. **Actinopolymorpha** Wang, Zhang, Xu, Ruan and Wang 2001, 472[VP]

Lyudmila I. Evtushenko

Ac.ti.no.po.ly.mor'pha. Gr.n. *actis*, *actinos* a ray; Gr. adj. *polumorphos* multiform, manifold; N.L. fem. n. (N.L. fem. adj. used as a substantive) *Actinopolymorpha* actinomycete of many shapes.

Branched fragmenting vegetative hyphae of uneven thickness to highly pleomorphic cells of different sizes, both usually **growing on the agar surface**. Marked **apical and lateral budding** is often observable. Cells may remain attached after division, forming **short chains and small aggregates**. Colonies are white, yellow, or orange in color, mostly of a pasty consistency, smooth, or wrinkled. Scant or moderate aerial mycelium occasionally occurs in older cultures on some media. Endospores are not formed. Nonmotile. Gram-stain-positive. Non-acid-fast. **Chemo-organotrophic with a respiratory type of metabolism.** Catalase- and oxidase positive. Grow aerobically on standard laboratory media, including the chemically defined (synthetic) media. Oxidative acid production from glucose and some other carbohydrates. Mesophilic (optimum temperature ~28°C), non-salt-requiring. Diagnostic diamino acid of the cell wall is **LL-diaminopimelic acid**. Menaquinones are the sole respiratory quinones detected; the major component is tetrahydrogenated or hexahydrogenated menaquinone with nine isoprene units, **MK-9(H_4) or MK-9(H_6)**. The fatty acid profile dominated by **iso-branched acids ($C_{16:0}$ iso, $C_{15:0}$ iso, $C_{17:0}$ iso, and $C_{16:1}$)**; anteiso-branched, hydroxylated, and 10-methyl branched fatty acids usually contribute lesser or minor amounts. Polar lipid pattern includes **phosphatidylinositol mannosides, phosphatidylinositol, diphosphatidylglycerol, and phosphatidylglycerol.** The described *Actinopolymorpha* species have been isolated from soil or plant tissues.

DNA G+C content (mol%): 66.6–69.6.

Type species: **Actinopolymorpha singaporensis** Wang, Zhang, Xu, Ruan and Wang 2001, 472[VP].

Further descriptive information

Based on the 16S rRNA gene sequence analysis, the genus *Actinopolymorpha* represents, together with the recently described genera *Thermasporomyces* (Yabe et al., 2011) and *Flindersiella* (Kaewkla and Franco, 2010a), a separate subcluster within the family *Nocardioidaceae*, order *Propionibacteriales* (formerly suborder *Propionibacterineae* Stackebrandt et al. 1997) (see Figure 247). The genus currently includes five species: the type species *Actinopolymorpha singaporensis* (Wang et al., 2001), *Actinopolymorpha rutila* (Wang et al., 2008b), *Actinopolymorpha alba* (Cao et al., 2009), *Actinopolymorpha cephalotaxi* (Yuan et al., 2010), and *Actinopolymorpha pittospori* (Kaewkla and Franco, 2010a). The information given below is from the original species descriptions, unless indicated.

All organisms, when cultured on agar media, grow on the agar surface, and occasionally weakly penetrate it (reported for *Actinopolymorpha singaporensis*). The colonies are pasty, usually smooth at first and later wrinkled. A marked morphological differentiation within colonies may be observable (Figure 264). The colony color varies depending on species and growth medium, and usually is white or yellow to orange of different intensity and shade (see Table 222 and the species description for more details). Aerial mycelia white in color may develop in older cultures on certain agar media (Table 222), which is mostly scant but may be moderate to good, e.g. mycelia of *Actinopolymorpha*

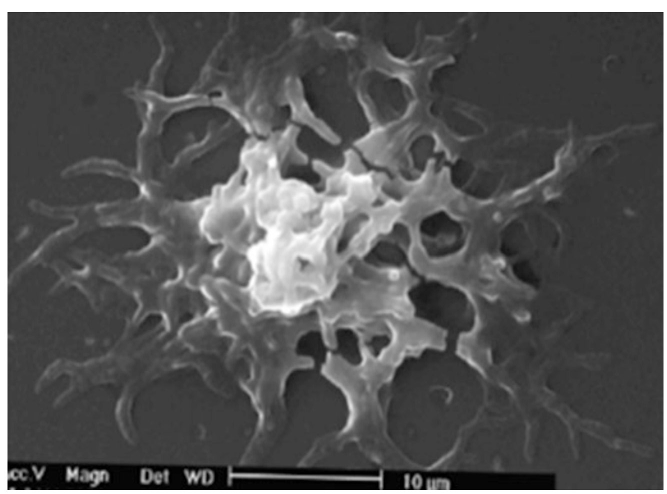

FIGURE 264. Scanning electron micrograph of a microcolony of *Actinopolymorpha rutila* YIM 45725 grown on ISP 2 agar at 28°C for 12 d. (Reproduced with permission from Wang et al., 2008b. Int. J. Syst. Evol. Microbiol. *58*: 2443–2446.)

TABLE 222. Descriptive and differentiating characteristics of *Actinopolymorpha* species[a]

Characteristic	*A. singaporensis*	*A. alba*	*A. cephalotaxi*	*A. pittospori*	*A. rutila*
Colony color on ISP 2	Brilliant orange	Milk white	Brilliant orange	Pale yellow	Deep orange-yellow
Colony color on ISP 3	Yellow	Light grey-white	Yellow or buff	Yellowish white	Brilliant orange-yellow
Aerial hyphae (media)	w (ISP 3)	w (ISP 2)	−	+ (HPDA), w (BA, ISP 2)	w (ISP 2)
Diffusible pigment (media)	−	+ (ISP 7)	+ (ISP, NA)	−	−
Growth at:[b]					
15°C	−	+[c]	+	+	+
37°C	+	+[c]	− or w	−	+
45°C	− or w	+[c]	−	−	−
Maximal % of NaCl (medium)[d]	8, 15 w (TSB)	7 (ISP 2)	5 (TSB)	3 w (ISP 2)	5 (ISP 2)
Nitrate reduction	+	−	+	nd	−
Utilization of C-sources:[e]					
D-Glucose	+	+	+	+	+
D-Arabinose	−	+	+	w	+
Fructose	+	−	+	+	+
D-Galactose	+	−	+	+	+
myo-Inositol	+	−	+	−	+
D-Mannitol	+	−	+	−	+
Raffinose	−	+	+	w	+
L-Rhamnose	+	v	+	nd	+
Sorbitol	+	−	+	+	v
Sucrose	+	−	+	+	+
D-Xylose	+	v	+	nd	+
Acid production from:[f]					
D-Glucose	+	+	+	+	+
Fructose	+	+	+	−	+
Dulcitol	w	−	−	−	w
D-Galactose	+	+	+	−	v
Maltose	v	+	+	−	+
D-Mannose	+	+	+	w	+
myo-Inositol	+	w	+	−	− or w
D-Mannitol	+	w	+	−	+
Salicin	+	+	+	w	+
Sucrose	+	+	+	−	+
Trehalose	+	+	+	+	+
D-Xylose	+	+	+	w	+
Adonitol	+	+	−	−	+
Sorbitol	+	−	+	−	v
Cell-wall diamino acid	LL-A_2pm	LL-A_2pm	LL-A_2pm	LL-A_2pm	LL-A_2pm
Whole-cell sugars	Glc, Rha, Rib	Glc, Rha, Rib	Glc	Glc, Rib, Gal	Glc, Rib, Gal
Menaquinone pattern[g]	9/6 (9/4, 9/8, 10/4)	9/6 (9/8, 10/6, 10/8)	9/4 (9/6, 9/8, 10/4)	9/4 (9/6, 10/4, 10/8)	9/4 (9/6, 9/8, 10/6)
Phospholipids	DPG, PG, PIM, PI	DPG, PG, PIM, PI	PI, PG, PIM	nd	DPG, PG, PIM, PI
Major acids (>5%):[h]	$C_{16:0}$ iso (**25.3**; 19.4), $C_{15:0}$ iso (**33.7**; 33.3), $C_{17:0}$ iso (**12.1**; 7.0), $C_{17:0}$ anteiso (**11.1**; 7.9), $C_{15:0}$ anteiso (**<0.5;** 4.0), $C_{16:1}$ iso (**<0.5**; 21.6)	$C_{16:0}$ iso (**25.3**; 7.1), $C_{15:0}$ iso (**33.7**; 28.1), $C_{17:0}$ iso (**12.1**; n.d), $C_{17:0}$ anteiso (**11.1**; n.d.), $C_{15:0}$ anteiso (**<0.5;** 14.5), $C_{16:1}$ iso (**<0.5**; 6.6)	$C_{16:0}$ iso (**40.5**; 21.4), $C_{15:0}$ iso (**17.5**; 12.0), $C_{17:0}$ iso (**10.6**; 3.0), $C_{17:0}$ anteiso (**9.4**; 6.0), $C_{15:0}$ anteiso (**1.2;** 1.5), $C_{16:1}$ iso (**1.6**; 32.9)	$C_{16:0}$ iso (**29.2**), $C_{15:0}$ iso (**17.0**), $C_{17:0}$ iso (**16.4**), $C_{17:0}$ anteiso (**20.7**), $C_{15:0}$ anteiso (**7.5**), $C_{16:1}$ iso (**<0.5**)	$C_{16:0}$ iso (**35.8**; 22.4), $C_{15:0}$ iso (**15.3**; 22.1), $C_{17:0}$ iso (**10.0**; 8.9), $C_{17:0}$ anteiso (**9.8;** 8.3), $C_{15:0}$ anteiso (**3.5;** 3.7), $C_{16:1}$ iso (**1.9**; 21.6)
10-Methyl-branched[i]	$C_{16:0}$, $C_{17:0}$	$C_{16:0}$, $C_{17:0}$	$C_{16:0}$, $C_{17:0}$	$C_{16:0}$, $C_{17:0}$	$C_{17:0}$
Hydroxylated[i]	$C_{9:0}$ 3-OH, $C_{15:0}$ 3-OH, $C_{14:0}$ iso 3-OH	$C_{14:0}$ iso 3-OH (11.8), $C_{9:0}$ 3-OH, $C_{15:0}$ 3-OH	$C_{9:0}$ 3-OH, $C_{14:0}$ 3-OH, $C_{15:0}$ 3-OH, $C_{16:1}$ 2-OH	$C_{9:0}$ 3-OH	$C_{14:0}$ 3-OH, $C_{15:0}$ 3-OH, $C_{18:0}$ 2-OH
DNA G+C content (mol%)	69.5 (HPLC)	66.6 (HPLC)	69.3 (T_m)	69.6 (HPLC)	67.7 (HPLC)

(continued)

TABLE 222. (continued)

Characteristic	*A. singaporensis*	*A. alba*	*A. cephalotaxi*	*A. pittospori*	*A. rutila*
Source of type strain	Soil, primary rain-forest	Soil, plant rhizo-sphere	Soil, rhizosphere of Chinese cowtail pine (*Cephalotaxus fortunei*)	Leaves of apricot tree (*Pittosporum phylliaeoides*)	Soil, forest

[a]Based on characteristics of type strains. Data from Wang et al. (2008b, 2001), Cao et al. (2009), Yuan et al. (2010), and Kaewkla and Franco (2010b, 2010a). Symbols and abbreviations: +, positive; –, negative; v, variable between experiments or test methods; w, weak; LL-A_2pm, 2,6-diaminopimelic acid; Glc, glucose; Gal, galactose; Rib, ribose; Rha, rhamnose; HPDA, half-strength potato dextrose agar (Atlas, 1993); BA, Bennett's agar (Atlas, 1993); NA, nutrient agar (Atlas, 1993); TSB, tryptic soy broth (Difco); nd, not determined.

[b]Tested on ISP 2 agar, except *Actinopolymorpha alba* examined on TSA [3% (w/v) trypticase soy broth (BBL); 1.5% (w/v) Bacto agar (Difco)] (data from the original species descriptions and Kaewkla and Franco, (2010a, 2010b).

[c]Conflicting test results were reported by Kaewkla and Franco (2010b, 2010a).

[d]Lower NaCl concentrations allowing growth of *Actinopolymorpha singaporensis* (3%), *Actinopolymorpha alba* (1%), and *Actinopolymorpha rutila* (3%) were obtained using basal medium ISP 2 at 27°C (Kaewkla and Franco, 2010a, 2010b).

[e]Tested on basal medium ISP 9 (data from the original species descriptions, and from Kaewkla and Franco, 2010b).

[f]According to the method of Gordon et al. (1974).

[g]Predominant menaquinone and components detected in lesser amounts (in parenthesis); numerals indicate the numbers of isoprene units and the number of hydrogen atoms on the side chain (e.g. 9/4 is partially saturated chain with 4 hydrogen atoms on the side chain containing 9 isoprene units).

[h]Obtained using the standard protocol of the MIDI System in cells cultured under different conditions. Data for cells grown in liquid Tryptic Soya Broth medium, TSB (Oxoid) at 27°C for 10 d (Kaewkla and Franco, 2010b) are given in bold; data for cells grown at 28°C for 6 d in liquid TSB (Difco) (Yuan et al., 2010) are underlined; data for *Actinopolymorpha rutila* (Wang et al., 2008b) and *Actinopolymorpha alba* (Cao et al., 2009) cells cultured for 5 d at 28°C on TSA agar [3% trypticase soy broth (BBL) and 1.5% Bacto agar (Difco)] are presented neither in bold nor underlined.

[i]Other diagnostic fatty acids detectable irregularly in minor (< 5%) or trace amounts, except $C_{14:0}$ iso 3-OH (which is reported to constitute 11.8% in 5-d cultures of *Actinopolymorpha alba* grown at 28°C on TSA agar (Cao et al., 2009).

pittospori grown on half-strength potato dextrose agar (HPDA; Atlas, 1993).

Various morphological forms, extending from well developed branched hyphae with swollen hyphal segments to highly pleomorphic cells (irregularly-sized globular, coccoid, rod-shaped, and angular), which are often produced by apical and lateral budding, is a characteristic feature of organisms of this genus (Figure 265, Figure 266, and Figure 267). The observable morphologies depend on species, growth medium, the culture age, and location in colonies. The cells produced by budding often remain attached after division, forming aggregates and short chains (pseudomycelium). The cells in chains resemble at maturation exospores (arthrospores) of spore-forming actinomycetes (Figure 265c). The cells may give rise to new buds which further develop into cells of different shapes or hyphae as growth proceeds or when a culture is transferred to fresh medium. The marked hyphal elongation by budding was emphasized for *Actinopolymorpha singaporensis* (which has been described in more morphological detail) at a later growth stage (Figure 265c), but probably takes place in some other species of this genus. No data are available on the fine structure of thickened hyphae, hyphal swellings or globular forms (presumably cell clusters) examined by electron microscopy of thin sections. There are certain analogues of images of such morphological structures with those observed, e.g. in strains of *Kribbella* (chapter in this volume), *Friedmanniella* (Schumann et al., 1997), and, especially, *Pseudonocardia* which, in addition, are characterized by the growth of hyphae by budding (Agre et al., 1984; Evtushenko et al., 1989; Henssen et al., 1983; Kuimova and Malishkaite, 1984). Likewise with these genera, it might be suggested that the cells composing clusters or thickened hyphal masses may divide internally by differently oriented septa. Members of *Actinopolymorpha* were reported not to produce "classical" sporangia typical of sporangial actinomycetes (e.g. *Actinoplanes*). However, it cannot be excluded that the aforementioned cell clusters (subclusters) may have a common envelope (to be designated as "sporangia"?).

Gram-stain-positive. Non-acid and non-alcohol-fast (examined for *Actinopolymorpha pittospori*). The cell wall contains LL-diaminopimelic acid (LL-A_2pm), as reported for all species; no data on the other peptidoglycan amino acids are available. The whole-cell sugars detected include glucose, galactose, rhamnose, and ribose in different combinations (Table 222). The menaquinone systems contain MK-9(H_6) as the predominant compound for *Actinopolymorpha singaporensis* and *Actinopolymorpha alba*, or MK-9(H_4) for the remaining species. Additional menaquinones, i.e. MK-9(H_8), MK-10(H_4), MK-10(H_6), and MK-10(H_8) in different combinations have been found in lesser or minor amounts (Table 222). Some shifts in the predominant menaquinone and the menaquinone patterns in this genus may be influenced by the medium composition and the age (developmental stage) of cultures, as suggested from some examples with other actinomycetes (Li et al., 2003; Saddler et al., 1986; Tanaka et al., 1996). All species contain similar patterns of principal polar lipids, i.e. phosphatidylinositol mannosides, phosphatidylinositol, diphosphatidylglycerol, and phosphatidylglycerol. An exception is *Actinopolymorpha cephalotaxi* which has been reported to lack diphosphatidylglycerol; no data are available for *Actinopolymorpha pittospori.* The fatty acid profiles are dominated by iso-branched components, mostly $C_{16:0}$ iso, $C_{15:0}$ iso, $C_{17:0}$ iso, and $C_{16:1}$ iso (Table 222). In addition, anteiso-branched and hydroxylated acids may be detected in significant proportions for certain species in some experiments. 10-Methyl-branched acids ($C_{16:0}$ 10-methyl and $C_{17:0}$ 10-methyl) are usually

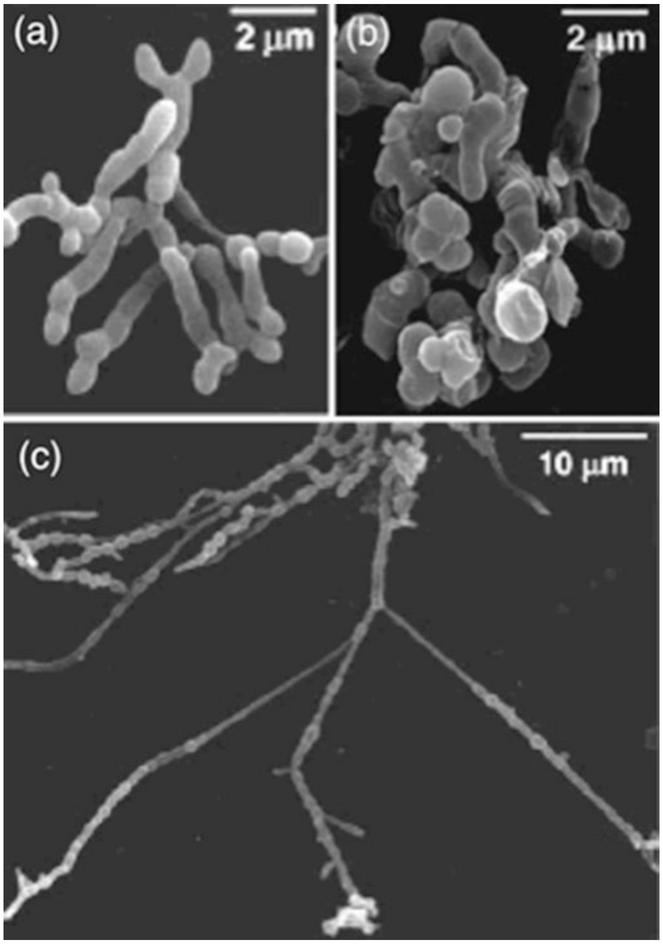

FIGURE 265. Cellular morphology of *Actinopolymorpha singaporensis*. Scanning electron micrographs of cells of strain *Actinopolymorpha singaporensis* IM 7744 grown on ISP 4 (a) and ISP 3 (b) media for 8 d; hyphae at the periphery of a colony on ISP 3 medium in 35-d-old culture (c). (Reproduced with permission from Wang et al., 2001. Int. J. Syst. Evol. Microbiol. *51*: 467–473.)

present but not in excess of 5%. The patterns of predominant fatty acids (and the fatty acid profiles as a whole) vary with species and the culture age (developmental stage). The most common change for cultures grown on trypticase soy broth (agar) is that the proportions of saturated iso-branched acids increase in older (10 d) cultures compared with younger (5–6 d) cultures, while that of unsaturated ($C_{16:1}$ iso) decreases (Table 222).

All species grow aerobically on complex agar media at nearly neutral pH at ~28°C and also are capable of growing on chemically defined ISP* media, including minimal salts medium ISP 9 supplemented with glucose or some other carbon sources. Some species were reported to grow on inorganic salt-starch agar (ISP 4) and Czapek's agar (Pridham and Lyons, 1980). The species differ in the rate and abundance of growth, but all form colonies within 8–10 d at 27–28°C on yeast extract-malt extract agar (ISP 2) and some other media.

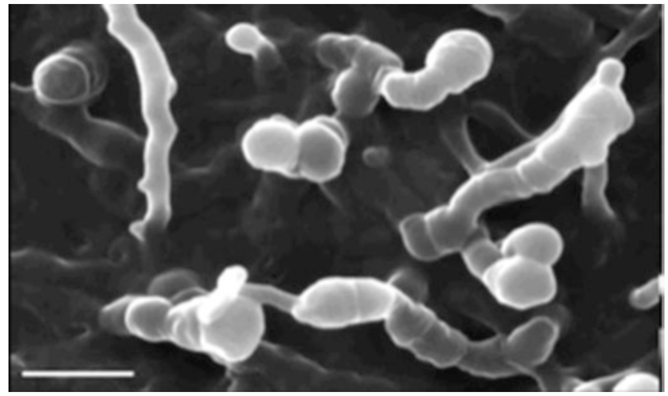

FIGURE 266. Cellular morphology of *Actinopolymorpha alba*. Scanning electron micrograph of cells of strain *Actinopolymorpha alba* YIM 48868 grown on ISP 2 agar for 12 d at 28°C. Bar = 2 µm. (Reproduced with permission from Cao et al., 2009. Int. J. Syst. Evol. Microbiol. *59*: 2200–2203.)

All species produce acid during aerobic growth from glucose and other carbohydrates (Table 222). All tested strains exhibit positive reactions for catalase and oxidase (no data on oxidase for *Actinopolymorpha pittospori*). Nitrate reduction varies with species (Table 222). All tested strains hydrolyze gelatin, but show negative results in the classical tests for hydrolysis of cellulose or starch (no data on *Actinopolymorpha pittospori*). *Actinopolymorpha singaporensis* and *Actinopolymorpha rutila* so far examined test positive for hydrolysis of casein, Tween 20, but negative for decomposition of Tween 80, and adenine. The four species (*Actinopolymorpha alba*, *Actinopolymorpha cephalotaxi*, *Actinopolymorpha rutila*, and *Actinopolymorpha singaporensis*) so far tested with the API ZYM system exhibit various enzymatic activities (see the species descriptions for details). The optimal growth temperature is ~28°C, some species can grow at 37°C or somewhat higher temperatures in appropriate conditions (Table 222). The recognized *Actinopolymorpha* species are non-salt-requiring, but some may tolerate up to 7% NaCl (e.g. *Actinopolymorpha alba* when tested on ISP 2 medium at 28°C; Cao et al., 2009), or even 15% (weak growth was reported for *Actinopolymorpha singaporensis* in liquid TSB as basal medium; Wang et al., 2001). A lower salt resistance of the above species and also *Actinopolymorpha rutila* was observed by Kaewkla and Franco (2010a, 2010b) on ISP 2 as basal medium at a slightly lower temperature (27°C). Organisms of this genus are considered to be neutrophilic, grow best at pH 6–8. They also may show growth at initial pH 6 and 10 on ISP 2 medium; some can grow at pH 5 (Kaewkla and Franco, 2010a, 2010b).

The DNA base ratio is 66.6–69.6 mol% (Table 222). The 16S rRNA gene sequence similarity ranges from 97.5% (the pair *Actinopolymorpha alba*–*Actinopolymorpha pittospori*) to 99.5% (*Actinopolymorpha cephalotaxi*–*Actinopolymorpha rutila*). The DNA–DNA similarity between the phylogenetically closest species obtained by the thermal denaturation method was 33.8% (Yuan et al., 2010). The highest value of DNA–DNA similarity (53.1%) was reported (Cao et al., 2009) for the pair *Actinopolymorpha alba*–*Actinopolymorpha rutila*, obtained by using the modified (Christensen et al., 2000) fluorometric micro-well method of Ezaki et al. (1989).

* Mineral salts medium: 11.8 g of NaCl, 0.32 g of KC1, 2.26 g of MgCl2·6H_2O, 2.97 g of $MgSO_4$·7H_2O, 0.65 g of $CaCl_2$·2H_2O, 0.2 g of $NaNO_3$, 0.2 g of NH_4C1 in 1 liter

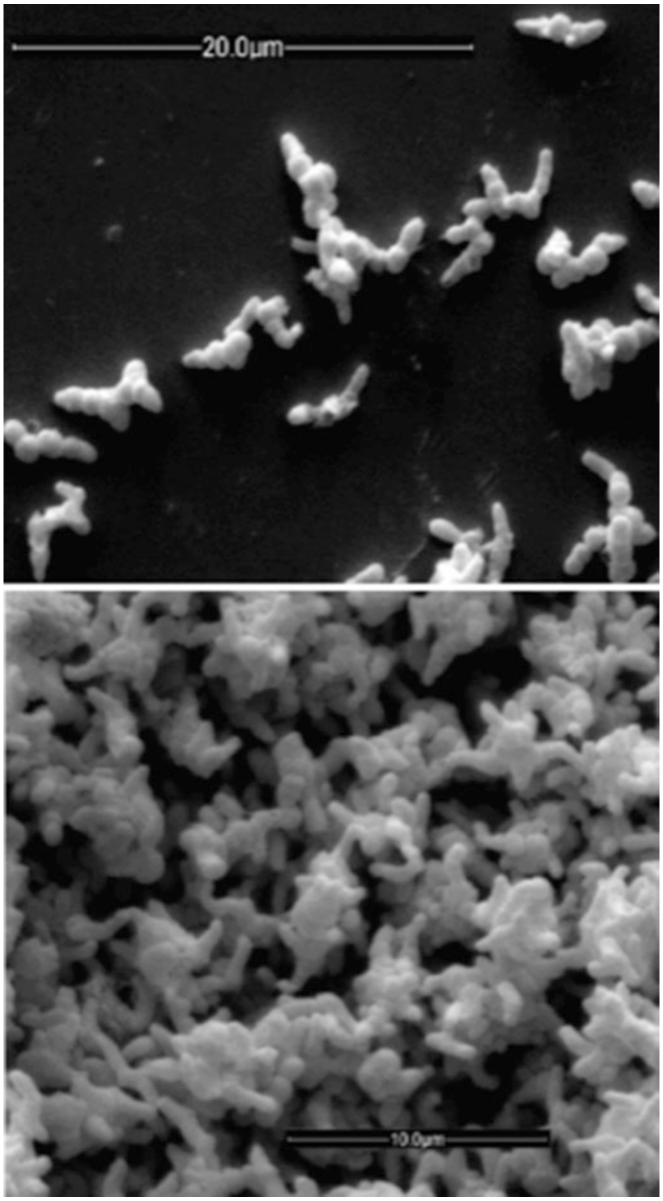

FIGURE 267. Cellular morphology of *Actinopolymorpha cephalotaxi*. Scanning electron micrographs of strain *Actinopolymorpha cephalotaxi* I06-2230 grown on ISP 2 agar for 14 d at 28°C. (Reproduced with permission from Yuan et al., 2010. Int. J. Syst. Evol. Microbiol. *60:* 51–54.)

All species are sensitive to 0.1% phenol. The sensitivity to antibiotics has not been tested directly, but it may be assumed from the composition of the isolation media that *Actinopolymorpha cephalotaxi* resists aztreonam (25 mg/l), while *Actinopolymorpha pittospori* tolerates nalidixic acid (20 µg/ml). *Actinopolymorpha singaporensis* may be resistant to penicillin or streptomycin, or both (the isolation procedure included pre-incubation of a soil sample in LB medium containing both these antibiotics, each 10 µg/ml). *Actinopolymorpha rutilus* YIM 45725 produces an estrogenic ligand, actinopolymorphol A, that preferentially induces heterodimerization of estrogen receptors ERα and ERβ and modulate estrogen receptor function (Huang et al., 2010; Powell et al., 2010).

Knowledge of the natural habitat of *Actinopolymorpha* species is fragmentary. A few available strains of this genus were isolated from forest soils or plant rhizosphere. An endophytic species, *Actinopolymorpha pittospori*, inhabited leaves of the Australian native apricot tree, *Pittosporum phylliraeoides*. Notably, members of *Actinopolymorpha* have not been reported so far in numerous ecological studies performed by direct non-culture (metagenomic) methods, in contrast to vast diversity of other organisms (clones) of the family *Nocardioidaceae*. No data have been reported on the possible pathogenic properties.

Enrichment and isolation procedures

Several strains of this genus have been isolated by plating on suitable agar media and incubation at 27–30°C for 3–11 weeks. The isolation media were yeast extract–malt extract agar (ISP 2), oatmeal agar (ISP 3) used for isolation of *Actinopolymorpha singaporensis*, inorganic salt-starch agar (Gauze 1; Gauze et al., 1983) for *Actinopolymorpha alba*, and starch-glycerol-proline agar with aztreonam (25 mg/l) for *Actinopolymorpha cephalotaxi*. A complex medium VL70 containing a mixture of 17 amino acids and solidified with 0.8% gellan gum (Hudson et al., 1989; Schoenborn et al., 2004) and supplemented with nalidixic acid and nystatin (each 20 µg/ml) was used to isolate *Actinopolymorpha pittospori* from the surface-sterilized and crushed leaves of *Pittosporum phylliraeoides*. The sterilization procedure included treatment with 70% ethanol and 6% hypochlorite (for 5 min each), followed by repeated rinsing with sterile water, treatment with 10% $NaHCO_3$ (10 min), and final rinsing with sterile water. Seeded plates were kept for 11 weeks until isolation in plastic sealed boxes, which contained wet paper towels to maintain humidity. To isolate *Actinopolymorpha singaporensis*, a soil sample was pre-incubated in Luria–Bertani (LB) medium containing penicillin and streptomycin (each 10 µg/ml), which eliminated many fast-growing bacteria. The soil sample was first dried in a chemical fume hood for 3 d and ground in a mortar. One milligram of the sample was suspended in 10 ml of LB medium containing antibiotics and incubated for a 3 h at 37°C with vigorous shaking. Then, the culture was centrifuged, washed three times with 10 ml of sterile water, resuspended in 1 ml of sterile water, spread onto an appropriate agar media, and incubated at 28–30°C for a month.

Maintenance procedures

Cultures may be maintained as 20% glycerol suspensions at −20 and −80°C. Long-term conservation is achieved by freeze-drying or in liquid nitrogen by standard procedures.

Differentiation of the genus *Actinopolymorpha* from other genera

Characteristics useful for phenotypic delineation of *Actinopolymorpha* from the other genera of the family *Nocardioidaceae* are listed in Table 222. The menaquinone systems dominated by MK-9(H_6) or MK-9(H_4), and the growth temperature range and optima are the most notable phenotypic characteristics that, along with morphological features, differentiate species of *Actinopolymorpha* from the phylogenetically neighboring genera *Flindersiella* and *Thermasporomyces*. The phosphatidylcholine-lacking polar lipid profile sharply differentiates this genus from *Kribbella*. *Actinopolymorpha* is readily distinguished by the morphological features

from the phylogenetically more distant non-mycelium-forming *Aeromicrobium*, *Marmoricola* and the majority of *Nocardioides* species. In addition, *Actinopolymorpha* can by separated by the menaquinone pattern both from the rod-shaped and mycelium-forming *Nocardioides* species and *Marmoricola*, which possess the predominant menaquinone MK-8(H_4).

Taxonomic comments

After the establishment of the genus *Actinopolymorpha* based on the study of a single strain (Wang et al., 2001), additional four species have been isolated and described on the basis of polyphasic taxonomic approach within 2008–2011. The data accumulated since 2001 show that some characteristics in the original genus description do not reflect features of all species currently composing the genus, or the detailed data provided conflict with those of individual species. The genus description therefore needs harmonization while preparing the emended description of this genus.

Acknowledgements

The author was supported by the program MCB of the Russian Academy of Sciences.

Differentiation of species of the genus *Actinopolymorpha*

Primary differentiation of the currently recognized species of this genus is achievable taking into consideration the colony color and morphological features, differences in salt tolerance, the composition of whole-cell sugars, fatty acids, menaquinones, and some other characteristics listed in Table 222 and outlined in the species descriptions below. When using the chemotaxonomic characteristics for differentiation purposes, the possible age- and medium-dependent shifts in the fatty acid compositions and probably in the menaquinone and whole cell sugar patterns, should be taken into account. In comparative taxonomic studies, cells must be grown and analyzed (tested) under similar conditions.

List of species of the genus *Actinopolymorpha*

1. **Actinopolymorpha singaporensis** Wang, Zhang, Xu, Ruan and Wang 2001, 472[VP]

sin.ga.po.ren′sis. N.L. fem. adj. *singaporensis* of or belonging to Singapore, signifying the country where the type strain was isolated.

Characteristics are as described for the genus and listed in Table 222. Information given below is based on the data and microphotographs provided in the original paper (Wang et al., 2001), unless indicated.

Grows usually on the agar surface; poor penetration of vegetative hyphae into the agar medium, e.g. ISP 3, may occur after prolonged cultivation. The colony color is brilliant orange on ISP 2 medium, and yellow on ISP 3, ISP 4, TSA (Difco), and Bennett's agar (Atlas, 1993). Aerial mycelium is absent or scarcely formed in old cultures on some solid media. No diffusible pigment is produced. The cells of varied size and shape (irregular rods, squarish, and coccoid) are usually observed. The cells can be arranged in small aggregates and short chains. Budding cell division and hyphal elongation by budding take place. Grows at 25–37°C; weak growth may be observable at 45°C on ISP 2 medium (Kaewkla and Franco, 2010a). Grows well in liquid TSB medium containing NaCl up to 8% and slowly with 10–15% NaCl. A lower salt resistance may be observed on other growth media (Kaewkla and Franco, 2010b, a). Utilizes maltose, dextrin, glycerol, and salicin, as sole carbon sources, but not cellobiose or lactose (Wang et al., 2008b; Wang et al., 2001). An alkaline color of the phenol red in the tests (Gordon et al., 1974) for utilization of citrate or malonate, but not succinate (Wang et al., 2008b). Hydrolyzes hypoxanthine and xanthine. Positive in the API ZYM test for esterase lipase (C8), but negative for esterase (C4) and urease (Yuan et al., 2010).

Source (type strain): soil of the primary rainforest of the Bukit Timah nature reserve, Singapore.

DNA G+C content (mol%): 69.5 (HPLC).

Type strain: IM 7744, JCM 10761, NBRC 100040, NRRL B-24113.

Sequence accession no. (16S rRNA gene): AF237815.

2. **Actinopolymorpha alba** Cao, Jiang, Wu, Xu and Jiang 2009, 2202[VP]

al′ba. L. fem. adj. *alba* white, referring to the white substrate mycelium.

Characteristics are as described for the genus and listed in Table 222. Information presented below is taken from the original paper (Cao et al., 2009), unless indicated.

Good growth occurs at 28°C on ISP 2, ISP 2, ISP 5, Czapek's agar, and potato agar, but not nutrient agar and ISP 4. The colony color is usually milk–white to gray-white on the all media tested. Sparse aerial hyphae develop on ISP 2 medium but not on the other test media. No diffusible pigments are observable, except brown melanoid pigment on tyrosine agar, ISP 7 (Kaewkla and Franco, 2010b). Branched vegetative hyphae of uneven thickness, with marked budding and swellings (Figure 266). The ranges of temperatures and salinity allowing growth are 0–7% NaCl and 10–45°C (on ISP 2) according to the original description; lower threshold values were reported (Kaewkla and Franco, 2010b, a). Utilizes dextrin, fucose, maltose, mannose, and ribose as carbon sources, but not cellobiose, lactose, glycerol, or xylitol. Negative for milk coagulation and peptonization. Hydrolyzes L-tyrosine, but not hypoxanthine, xanthine, or urea. Positive in the API ZYM tests for α-chymotrypsin, esterase (C4), β-galactosidase, α-glucosidase, *N*-acetyl-β-glucosaminidase, leucine arylamidase, α-mannosidase, naphthol-AS-BI-phosphohydrolase, but negative for acid phosphatase, alkaline phosphatase, cystine arylamidase, esterase lipase (C8), α-fucosidase, α-galactosidase, β-glucosidase, β-glucuronidase, lipase (C14), trypsin, and valine arylamidase.

Source (type strain): rhizosphere soil, Yunnan Province, China.

DNA G+C content (mol%): 66.6 (HPLC).

Type strain: YIM 48868, CCTCC AA 208030, DSM 45243, JCM 16897.

Sequence accession no. (16S rRNA gene): EF601829.

3. **Actinopolymorpha cephalotaxi** Yuan, Zhang,Yu, Sun, Wei, Liu, Li and Zhang 2010, 53[VP]

ce.pha.lo.ta′xi. N.L. gen. n. cephalotaxi of *Cephalotaxus*, of *Cephalotaxus fortunei*, a plant from which the rhizosphere soil sample was collected for isolation.

Characteristics are as described for the genus and listed in Table 222. Information presented below is taken from the original paper (Yuan et al., 2010), unless indicated.

Grows at 28°C, forming brilliant orange colonies on ISP 2, and buff or yellow on ISP 3, ISP 4, ISP 5, Czapek's agar, nutrient agar (Difco), and tomato-paste-oatmeal agar (Waksman, 1961). Aerial mycelium is absent. Buff diffusible pigment is produced on ISP 3 agar, nutrient agar and tomato-paste-oatmeal agar. Cells usually of irregular shapes, divide through apical and lateral budding, and remain attached after division, forming small aggregates and short chains (Figure 267). Grows well in the temperature range of 20–28°C, will grow at 15°C (Kaewkla and Franco, 2010b) but not at 4°C or above 37°C. Tolerates up to 5% NaCl both on ISP 2 agar (Kaewkla and Franco, 2010b) and in liquid TSB medium. Utilizes glucosamine as sole carbon sources, but not acetate, citrate, gluconate, or tartrate. Positive for milk coagulation and peptonization. H_2S is not produced. Positive in the API ZYM tests for acid and alkaline phosphatases, esterase, esterase lipase, urease, trypsin, chymotrypsin, naphthol-AS-BI-phosphohydrolase, α- and β-galactosidases, α- and β-glucosidases, *N*-acetyl-β-glucosaminidase, and α-mannosidase.

Source (type strain): rhizosphere soil of *Cephalotaxus fortunei*; Yunnan Province, China.

DNA G+C content (mol%): 69.3 (T_m).

Type strain: I06-2230, CCM 7466T, DSM 45117, KCTC 19293.

Sequence accession no. (16S rRNA gene): EU438909.

4. **Actinopolymorpha pittospori** Kaewkla and Franco 2010b, 000[VP]

pit.to.spo′ri. N.L. gen. n. *pittospori* of *Pittosporum*, isolated from *Pittosporum phylliraeoides*

Characteristics are as described for the genus and listed in Table 222. Information presented below is taken from the original paper (Kaewkla and Franco, 2010b).

Good growth on ISP 2, Bennett's agar, and half-strength potato dextrose agar (HPDA; Atlas, 1993); poor growth is observed on ISP 3, ISP 4, ISP 5, ISP 7, and nutrient agar (Atlas, 1993). Colony color is white on ISP 4, and pale yellow or yellowish white on the all other above media. Aerial mycelium is moderate to good on HPDA, scant on ISP 2 and Bennett's agar, and absent on the other test media. Diffusible pigments are not produced. Branched substrate mycelium of irregular thickness is well developed on most media. In the later stage of growth, hyphae fragment to rod-like elements or V-shaped forms. Short chains and aggregates of cells occur. Will grow between 15–27°C, but not at 4 or 37°C. Good growth is observed at a low (1%) NaCl concentration, with weak growth at 3% NaCl, as assessed on ISP 2 agar.

Source (type strain): surface-sterilized leaves of *Pittosporum phylliraeoides*; the campus of Flinders University, Adelaide, South Australia.

DNA G+C content (mol%): 69.6 (HPLC).

Type strain: PIP 143, DSM 45354, ACM 5288.

Sequence accession no. (16S rRNA gene): FJ805429.

5. **Actinopolymorpha rutila** Wang, Zhang, Xu and Li 2008b, 2445[VP]

ru.ti′la. L. fem. adj. *rutila* red inclining to golden-yellow, referring to the color of colonies.

Characteristics are as described for the genus and listed in Table 222. Information presented below is taken from the original paper (Wang et al., 2008b), unless indicated.

Colonies develop well within 3–4 d at 28°C on suitable agar media. Good growth occurs on ISP 2, ISP 5, and potato agar; moderate growth on ISP 3, ISP 4, Czapek's agar, and nutrient agar. The color of colonies is deep orange-yellow on ISP 2 and potato agar; brilliant orange-yellow on ISP 3, ISP 5, and Czapek's agar; pink orange-yellow on ISP 4 and nutrient agar. Scant aerial hyphae are formed on ISP 2 in the later stages of growth. Diffusible pigments are not produced. Branched substrate mycelia of uneven thickness are formed, and fragment in the early stages of growth. Hyphae exhibit varied degrees of fragmentation and differentiation as growth proceeds, with short to elongated rod-like or V-shaped elements typically observable in older cultures. Will grow at 15–37°C, and in the presence of 3% NaCl on ISP 2 medium; data on higher values of the above growth parameters are conflicting (Kaewkla and Franco, 2010b, a; Wang et al., 2008b). Positive oxidase reaction with 1% *p*-aminodimethylalanine oxalate. Assimilates cellobiose, lactose, maltose, mannose, melibiose, melezitose, ribose, dextrin, salicin, adonitol, glycerol, and xylitol as sole carbon sources but not arabitol or dulcitol. Acid is produced from fucose, raffinose, D-xylose, and erythritol. Utilizes malonate or succinate, but not acetate, citrate, or oxalate (tested according to Gordon et al., 1974). Negative test results for hydrolysis of DNA, Tween 60, or L-tyrosine, and for H_2S production. Hydrolyzes hypoxanthine and xanthine and decomposes urea in conventional tests (but negative for urease in the API ZYM test system; Yuan et al., 2010). Positive in the API ZYM for alkaline phosphatase, α-glucosidase, β-glucosidase, α-galactosidase, β-galactosidase, and α-mannosidase, but negative for *N*-acetyl-β-glucosaminidase, lipase (C14), β-glucuronidase, acid phosphatase, naphthol-AS-BI-phosphohydrolase, leucine arylamidase, cystine arylamidase, valine arylamidase, α-fucosidase, chymotrypsin, and trypsin. Variable results in the API ZYM for esterase (C4) and esterase lipase (C8) (Wang et al., 2008b; Yuan et al., 2010).

Source (type strain): forest soil collected in Yunnan Province, south-west China.

DNA G+C content (mol%): 67.7 (HPLC).

Type strain: YIM 45725, CCTCC AA 206004, DSM 18448, JCM 16537.

Sequence accession no. (16S rRNA gene): EF601829.

Genus III. **Aeromicrobium** Miller, Woese and Brenner 1991, 367[VP] emend. Yoon, Lee and Oh 2005c, 2174

Lyudmila I. Evtushenko and Valentina I. Krausova

A.e.ro.mi.cro′bi.um. Gr. n. *aer, aeros* air; N.L. neut. n. *microbium* microbe; N.L. neut. n. *aeromicrobium* aerobic microbe.

Small irregular rods (mostly 0.3–0.6 µm in diameter) **to coccoid forms.** Elongated rods, branched filamentous elements, and V-forms rarely occur. A marked rod-to-coccoid growth cycle can be observed. Endospores are not formed. Nonmotile or motile. Extracellular diffuse haloes or capsules can be produced. **Gram-positive** cell-wall chemotype, non-acid-fast. Colonies are non-pigmented, yellow or beige to amber-beige in color. **Chemo-organotrophs, metabolism primarily respiratory** with oxygen as the terminal electron acceptor. **Mostly catalase-positive;** negative catalase reaction may occasionally occur. Nitrate can be reduced by some species. Acids are produced oxidatively from some carbohydrates. Generally nutritionally exacting; grows aerobically on complex media based on peptone, yeast extract, and similar sources of nutrients. Some species can grow in chemically defined media. Mesophilic, optimal growth at 25–37°C; growth range is ~ 4–42°C. Mostly non-halophilic or slightly halophilic; some species are salt-requiring. Prefer a neutral to mildly alkaline pH. **The cell-wall peptidoglycan contains LL-diaminopimelic acid, along with alanine, glutamic acid, and glycine.** Menaquinones are the sole respiratory quinones detected; the tetrahydrogenated menaquinone with nine isoprene units **[MK-9(H_4)] is predominant**. **The major cellular fatty acids are octadecenoic ($C_{18:1}$ ω9*c*), hexadecanoic ($C_{16:0}$), tuberculostearic ($C_{18:0}$ 10-methyl), and 2-hydroxy hexadecanoic ($C_{16:0}$ 2-OH) acids.** Mycolates are absent. Principal polar lipids reported are diphosphatidylglycerol, phosphatidylglycerol, and phosphatidylethanolamine, or diphosphatidylglycerol, phosphatidylglycerol, and phosphatidylinositol. Occur in terrestrial and aquatic environments and can be associated with plants, animals, and humans.

DNA G+C content (mol%): 65.5–75.9.

Type species: **Aeromicrobium erythreum** Miller, Woese and Brenner 1991, 367[VP].

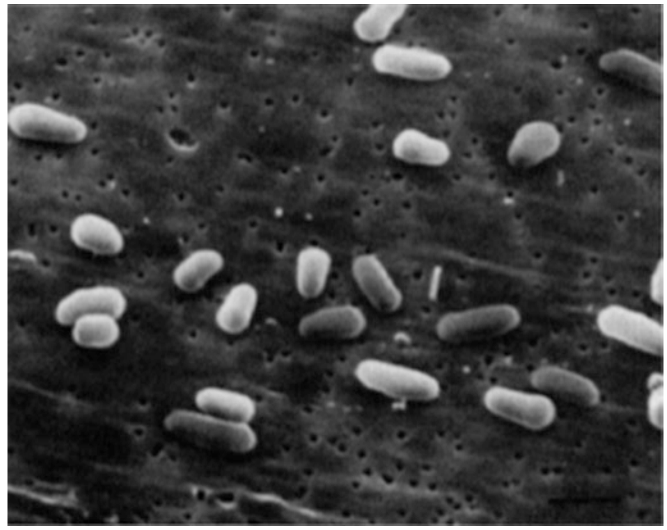

FIGURE 268. Cells of *Aeromicrobium erythreum*, 36-h culture grown in Marine broth (Difco). Scanning electron micrograph. Bar = 1 µm. (Reproduced with permission from Miller et al., 1991. Int. J. Syst. Bacteriol. *41*: 363–368.)

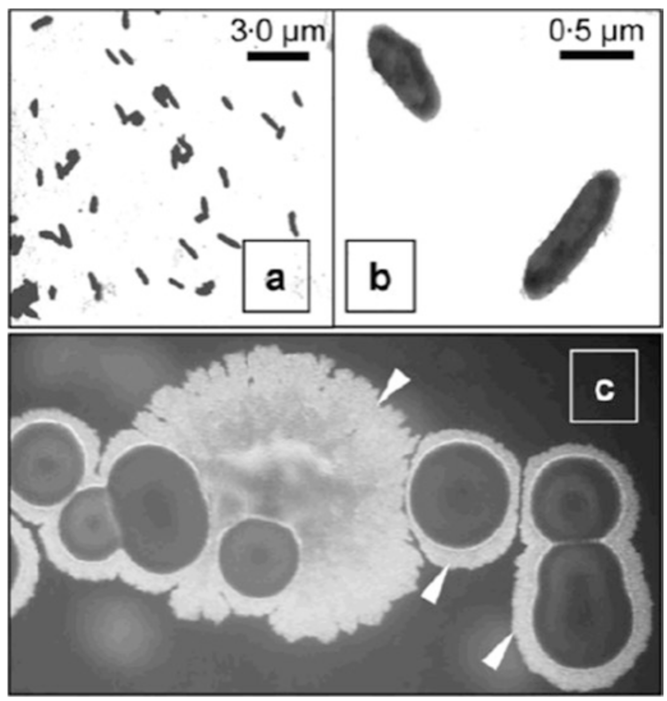

FIGURE 269. Morphological features of *Aeromicrobium marinum.* (a, b) Transmission electron micrographs of negatively stained cells from a 10-d-old culture in Marine broth; (c) colony morphology on Marine agar observed by light and epifluorescence microscopy; haloes are indicated by arrowheads. (Reproduced with permission from Bruns et al., 2003. Int. J. Syst. Evol. Microbiol. *53*: 1917–1923.)

Further descriptive information

The genus *Aeromicrobium* belongs to the family *Nocardioidaceae* and the order *Propionibacteriales* (formerly suborder *Propionibacterineae* Stackebrandt et al. 1997). The current *Aeromicrobium* species form a coherent 16S rRNA-based phylogenetic cluster which is clearly separated from other genera and species comprising the family *Nocardioidaceae* (Figure 247).

Morphology and colony appearance. The cells of *Aeromicrobium* species are typically small irregular rods (mostly 0.3–0.6 µm in width and up to 2.4 µm in length) and can be coccoid (Figure 268 and Figure 269a, b; Table 223). Longer cells can be produced by *Aeromicrobium tamlense* (up to 4.8 µm; Lee and Kim, 2007) and *Aeromicrobium halocynthiae* (up to 6.0 µm or more; Kim et al., 2010). Cells of *Aeromicrobium halocynthiae* can also form straight or curved filaments with rudimentary branching (in A1+C liquid medium containing 10 g of starch, 4 g of peptone, 2 g of yeast extract, and 1 g of calcium carbonate in 1 liter of filtered seawater; pH 7.0; Kim et al., 2010). Where reported, the cells of *Aeromicrobium* species stain Gram-positive.

TABLE 223. Descriptive and differential characteristics of *Aeromicrobium* species[a,b,c]

Characteristic	1. *A. erythreum*	2. *A. alkaliterrae*	3. *A. fastidiosum*	4. *A. flavum*	5. *A. ginsengisoli*	6. *A. halocynthiae*	7. *A. marinum*	8. *A. panaciterrae*	9. *A. ponti*	10. *A. tamlense*
Colony color	Beige, yellow	Cream	Whitish	Yellow	White	Light yellowish	Ivory	Light yellow	Yellow	Yellow
Cell width (µm)[d]	0.5	0.3–0.5	0.4–0.5	0.2–0.4	0.3–0.4	0.4–0.5	0.3–0.5	0.2–0.4	0.7	0.4–0.6
Cell length (µm)[d]	0.5–1.2	0.8–1.4	1.5–2.2	0.6–1.2	0.5–1.2	4.1–6.0	0.7–1.3	1.0–1.5	2.4	0.8–4.8
Motility	–	–	+	–	–	–	–	–	–	–
Optimal temperature (°C)	35	25	25	30	30	25	25	~30	30–37	30
Temperature range (°C)[e]	21–40	4–35	5–30	25–37	4–30	10–42	4–35	15–30	4–42	10–42
NaCl growth range (%, w/v)	0–4	0–8	0–4	0–4	0–4(w)	0–6	0.1–10.7	0–3	0–10	0–5
Optimal pH[f]	~7.0	7.0–7.5	7.0–7.4	~7.0	~7.0	~7.0	7.0–8.5	5.0–8.5	5.1–7.1	~7.0
pH growth range[f]	5.0–9.0	6.0–11.0	5.0–8.0	5.0–10.0	5.0–8.5	5.0–10.0	5.5–9.5	5.0–8.5	4.1–12.1	5.1–10.1
Catalase activity	+	+	+	+	–	+	+	+	+	+
Oxidase activity	+	–	+	+	+	–	–	–	–	–
Nitrate reduction[g]	–	–[h]	–[h]	+	+		–[h]	–[h]	–	–
Growth on ISP 9 with glucose[i]	–	+	–				–		+	+
Utilization of carbon sources:[j]										
L-Arabinose	+	v	+	–	+	+	–	–	+	–
D-Cellobiose	–	+	+	–	+	–	+	+	+	+
D-Fructose	+	–	+	+	+	+	–	–	+	+
D-Galactose	+	+	+		+	–	+	+	+	+
D-Glucose	+	+	+	+	+	+	v	+	+	+
Lactose	–	–	–		+	–	–	–	–	+
Maltose	–	+	v	+	+	+	v	+	+	+
D-Mannose	–	–	+	–	+	+	v	+	+	+
Melibiose	–				–	–		+		
D-Ribose	+	–		+	–	+		–		
L-Rhamnose	–	v	v	–	+	–	–	–	–	v
L-Sorbose	–	–	–	+	–	–	–	–	–	–
Sucrose	+	+	+	+	+	+	–	v	+	+
D-Trehalose	+	+	+	+	+	+	+	+	+	+
D-Xylose	+	–	+	–	+	+	–	–	+	–
Dextran	+	+	–	+	–		w		+	+
Glycerol	+	+	+	+	+	+	v	+	+	+
Inositol	+	+	+		+	+	–	–	–	–
D-Mannitol	–	v	v		–	+	+	v	+	–
Inulin	–			–	+	–			–	–
Salicin	–	+		–	–	–	v	+	–/w	–
Alanine	+	–	+		+		–	+		
Acetate	+	v	+	+	+	+	+	v	+	+
Adipate		+	+		–		+	+		
Citrate	v	v	v		–	–	v	v	v	–
Caprate		+	+		–		+	+		
Gluconate		+	+		+	–	+	+		
3-Hydroxybutyrate		+	+	–	+		+	–		
DL-Lactate		–	+		–		–	–		
Malate	+	+	+		+		+	+	+	+
Malonate		+	–		–		–	–		
Phenyl aceatate		+	+		–		+	+		
Propionate		+	+		+		–	–		
Pyruvate		–	+	+	+		+			–
Suberate		+	+		–		–	–		
Succinate	+	+	–		+		+	+	+	+
Valerate		+	+		+		–	–		
Hydrolysis/decomposition:[k]										
Esculin	+	–		+		–				+
Casein	+	+	+	–			–	–	+	–
Cellulose	+	–	–		–		–	–	+	
DNA	w	+	+	–	–		w	–	+	
Elastin	+	+	–				–	–	+	
Gelatin	+	+	v	+	+		–	+	+	+

(continued)

TABLE 223. (continued)

Characteristic	1. *A. erythreum*	2. *A. alkaliterrae*	3. *A. fastidiosum*	4. *A. flavum*	5. *A. ginsengisoli*	6. *A. halocynthiae*	7. *A. marinum*	8. *A. panaciterrae*	9. *A. ponti*	10. *A. tamlense*
Hypoxanthine	–	–	–				–	–	–	+
Starch	+	–	+			–	–	–	–	
Tween 80		+		–						+
Tyrosine	w	–	–		+		–	–	+	–
Enzyme activities (*API*, bioMérieux)*:*										
Acid phosphatase	+	+	+	–	+	–	–	+	+	+
Alkaline phosphatase	v	–	+	–	–	–	–	+	+	+
Arginine dihydrolase	–	–	–	–	–	–	–	v	–	–
Esterase (C 4)	+	+	+	+	+	+	+	w	+	–
Esterase lipase (C8)	+	+	+	+	+	+	+	+	+	w
Lipase (C14)	–	–	–	w	–	–	–	–	–	–
β-Galactosidase	–	v	v	–	+	–	v	v	–	–
α-Glucosidase	+	+	+	+	v	w	–	–	v	+
β-Glucosidase	v	v	v	+	+	–	v	v	–	v
Leucine arylamidase	+	+	+	+		+	+	+	–	+
Naphthol-AS-BI-phosphohydrolase	–/w	+	w	–	+	w	w	+	–	w
N-Acetyl-β-glucosaminidase	–	–/w	–	–	+	–	–/w	–/w	–	–
Valine arylamidase	–	–	–	–		w	–	–	w	–
Urease	–	–	–	w	–	–	–	–	–	–
Trypsin	–	–	–	–	–	–	–	–	–	–/w
Voges–Proskauer reaction	+	–	–	w		+	+	–	+	w
Major menaquinone	9(H_4)	9(H_4)	9(H_4)	9(H_4), 8(H_4)	9(H_4)	9(H_4)	9(H_4)	9(H_4)	9(H_4)	
Principal phospholipids	DPG, PG, PE		DPG, PG, PE						DPG, PG, PI, PL	DPG, PG, PI
DNA G+C content (mol%)	71–73	71.5	71–72	73.3	66.8	75.9	70.6	65.5	74.0	72.7
Isolation source	Soil	Soil	Herbage	Air	Soil	Ascidian	Sea water	Soil	Sea water	Seaweed

[a]Based on characteristics of type strains. Symbols and abbreviations: –, negative; +, positive; v, variable between different experiments or test methods; w, weak or slow; –/w, either negative, or weak and slow; 9(H_4) and 8(H_4), menaquinone having nine and eight isoprene units, two of which are saturated; PE, phosphatidylethanolamine, PG, phosphatidylglycerol; DPG, diphosphatidylglycerol; PI, phosphatidylinositol, PL, unidentified phospholipids.

[b]Data compiled from: Miller et al. (1991), Tamura and Yokota (1994), Collins and Stackebrandt (1989b), Bruns et al. (2003), Yoon et al. (2005c), Cui et al. (2007a), Lee and Kim (2007), Kim et al. (2008b), Lee and Lee (2008), Tang et al. (2008), and Kim et al. (2010).

[c]The following characteristics were reported to be negative for all species (type strains) tested: Activities for lysine decarboxylase, ornithine decarboxylase, tryptophan deaminase; decomposition of chitin or chitinase activity (no data for *Aeromicrobium halocynthiae*); H_2S production and activities for cystine arylamidase, α-chymotrypsin, α-fucosidase, α-galactosidase, β-glucuronidase and α-mannosidase (no data for *Aeromicrobium ginsengisoli*); decomposition of xanthine (no data for *Aeromicrobium flavum*, *Aeromicrobium ginsengisoli* and *Aeromicrobium halocynthiae*).

The following characteristics were tested only for a few species (type strains) and were reported to be negative: Utilization of raffinose, dulcitol, and D-sorbitol (*Aeromicrobium ginsengisoli*, *Aeromicrobium halocynthiae*, *Aeromicrobium ponti*, and *Aeromicrobium tamlense*); utilization of tartrate (*Aeromicrobium ginsengisoli*, *Aeromicrobium ponti*, and *Aeromicrobium tamlense*); utilization of adonitol (*Aeromicrobium erythreum*, *Aeromicrobium ginsengisoli*, *Aeromicrobium halocynthiae*, and *Aeromicrobium tamlense*); utilization of benzoate (*Aeromicrobium alkaliterrae*, *Aeromicrobium ginsengisoli*, *Aeromicrobium ponti*, and *Aeromicrobium tamlense*); utilization of formate (*Aeromicrobium alkaliterrae*, *Aeromicrobium ginsengisoli*, *Aeromicrobium panaciterrae*, *Aeromicrobium ponti*, and *Aeromicrobium tamlense*); utilization of methyl-α-D-mannoside (*Aeromicrobium flavum*, *Aeromicrobium ponti*, and *Aeromicrobium tamlense*); indole production (*Aeromicrobium alkaliterrae*, *Aeromicrobium flavum*, *Aeromicrobium halocynthiae*, and *Aeromicrobium tamlense*), and xylanase activity/xylan degradation (*Aeromicrobium ginsengisoli* and *Aeromicrobium panaciterrae*).

[d]Cell size as usually observable in young cultures. Some differences in the cell size and morphology can be caused by different growth conditions; *Aeromicrobium halocynthiae* can produce filamentous elements with primary branching. In older (synchronized) cultures of some species, cells may become shorter and may consist predominantly or exclusively of shorter rods, coco-bacillary or coccoid forms (see the species descriptions for details).

[e]The lower and higher growth temperatures reported in the original species descriptions are indicated; actual growth temperature range can be slightly broader for some species (for more information, see the species description).

[f]Initial pH values of media.

[g]According to the data reported in the original species descriptions.

[h]Conflicting results obtained with the API 20NE (bioMérieux) test have been reported (Kim et al., 2008b).

[i]Basal mineral medium (Shirling and Gottlieb, 1966) without vitamins.

[j]Determined using different conventional methods, the API 20E, API 20NE, API ID32 GN (bioMérieux), and Biolog GP2 test systems, therefore the data for some species can be incomparable. See Table 224 and the original species descriptions for methods.

[k]See Lee and Lee (2008), Cui et al. (2007a), Kim et al. (2008b), and Kim et al. (2010) for methods.

Although cocci and irregular rods are characteristic of many species, little information is available on the rod–coccus growth cycle, which is characteristic of arthrobacters and many other actinobacteria. In the cycle, irregular rods in young cultures are replaced by coccoid forms in older cultures, and these coccoid forms, when transferred to fresh medium, produce outgrowths to give irregular rods again (Jones and Keddie, 2006). No correlation was observed between the age of the culture and prevalence of rod-shaped or coccoid cells for *Aeromicrobium erythreum* in TYE broth (1.6% tryptone, 1% yeast extract, 0.5% NaC1, and 0.4% D-glucose; Miller at al., 1991). The cell morphology (irregular rod-shaped cells) of *Aeromicrobium marinum* in Marine broth (MB; Difco) was also reported to remain constant irrespective of the culture age (Bruns et al., 2003). On the other hand, irregular spherical forms were reported in a 5-d-old culture of *Aeromicrobium ginsengsoli* on R2A agar (Difco; Kim et al., 2008b). Our recent observations showed that cultures of *Aeromicrobium fastidiosum* can exhibit a distinct rod–coccoid growth cycle on some organic media, e.g. on modified Corynebacterium agar (DSMZ medium No. 53) containing glucose (5 g), yeast extract (5 g), Casamino acids (10 g), NaCl (5 g), agar (15 g), and distilled water (1 liter); pH 7.0–7.2. When resting cells of old (2–3 weeks) or lyophilized cultures of this species are transferred to fresh agar medium, the cells grow as rods (mean, 0.3–0.5 × 1.8–2.2 μm) in young cultures (1–2 d) and become shorter (~3–4 fold) rods or coccoid cells in 5-d-old cultures. Most likely the irregular rods of *Aeromicrobium flavum* and elongated cells or branched filamentous elements of *Aeromicrobium halocynthiae* are also replaced by short rods and/or coccoid cells in older cultures, as can be suggested from the data and micrographs provided in the original species descriptions (Kim et al., 2010; Tang et al., 2008). The marked change of morphology during the growth cycle can probably be shown by some other *Aeromicrobium* species in synchronized cultures on suitable agar media, at least those species reportedly having both irregular rods and coccoid forms. As in arthrobacters and many other coryneform bacteria (Cure and Keddie, 1973; Jones and Keddie, 2006), the composition of the nutrient medium probably influences the extent to which the morphology of *Aeromicrobium* species changes during the growth cycle. The replacement (if any) of the (irregular) rods in cultures by coccoid or coco-bacillary forms varies with individual species and culture conditions and can take up to 5–7 d or even more.

Colonies are usually 0.5–1.5 mm in diameter; larger colonies (up to 3 mm) are occasionally formed (reported for 5 d-old culture R2A agar; Cui et al., 2007a). Colonies are typically circular, smooth, convex, and may have thin, translucent edges, probably due to production of extracellular slime as demonstrated for *Aeromicrobium marinum* (Bruns et al., 2003) and *Aeromicrobium flavum* (Tang et al., 2008). After treatments aimed at detection of proteins or acidic polysaccharides, the diffuse haloes around the *Aeromicrobium marinum* colonies stain weakly with Alcian blue, indicating that the haloes may contain acidic polysaccharides (Bruns et al., 2003). The color of colonies is white or whitish to yellow and amber beige. The colony pigment color and intensity may vary depending on growth medium and culture age, as reported, e.g. for *Aeromicrobium erythreum* (Miller et al., 1991). Diffusible brown or purple pigments can be produced under certain conditions (Miller et al., 1991).

Chemotaxonomy. All species contain LL-diaminopimelic acid (LL-A_2pm) as the diamino acid in the cell-wall peptidoglycan. The amino acid composition (LL-A_2pm, alanine, glutamic acid, and glycine) reported for *Aeromicrobium erythreum* and *Aeromicrobium fastidiosum* (Busse and Schumann, 1999; Miller et al., 1991; Tamura and Yokota, 1994) is consistent with the peptidoglycan type A3γ *sensu* Schleifer and Kandler (1972). The cell wall of the type strain of *Aeromicrobium fastidiosum* was found to have glucose, rhamnose, ribitol, and amino sugars, which are derived from at least two cell-wall anionic polymers. One of these polymers is a ribitol teichoic acid that contains an amino sugar, and another is supposedly phosphorhamnan (Tul'skaya, 2009). The major component of the respiratory quinine system is MK-9(H_4); other menaquinones, MK-8(H_4), MK-7(H_4), and MK-9(H_6), may occur in minor or trace amounts (Tamura and Yokota, 1994). A larger proportion (25.4%) of MK-8(H_4) has been reported for *Aeromicrobium flavum* (Tang et al., 2008).

The fatty acids of *Aeromicrobium* species are of complex type (Suzuki and Komagata, 1983a). The predominant components are mostly $C_{18:1}$ ω9*c* (reaching up to 68%; Tang et al., 2008), $C_{16:0}$ (up to 47%; Schumann et al., 1997), $C_{18:0}$ 10-methyl (up to 42%; Tamura and Yokota, 1994), and $C_{16:0}$ 2-OH (up to 23%; and Lee, 2008). Their proportions (and the fatty acid profiles as a whole) vary with species, and usually are influenced by the composition of media, the growth temperature, the availability of oxygen, the culture age, and analytical procedure (Lee and Lee, 2008; Lee et al., 2000; Miller et al., 1991; Park et al., 1999; Tamura and Yokota, 1994; Yoon et al., 2005c). The saturated iso- and anteiso-branched fatty acids that dominate the fatty acid profiles of many other coryneform bacteria, including members of the family *Nocardioidaceae*, occur in minor or trace amounts. Principal polar lipids reported for *Aeromicrobium erythreum* and *Aeromicrobium fastidiosum* are phosphatidylethanolamine and phosphatidylglycerol (Tamura and Yokota, 1994); in addition, Lee and Kim (2007) reported the presence of diphosphatidylglycerol in these species. A different phospholipid pattern, including phosphatidylinositol, diphosphatidylglycerol, and phosphatidylglycerol, has been reported for *Aeromicrobium tamlense* (Lee and Kim, 2007) and *Aeromicrobium ponti* (Lee and Lee, 2008); additionally three unidentified phospholipids have been indicated for *Aeromicrobium ponti* (Lee and Lee, 2008).

Analysis of polyamines in the type strains of *Aeromicrobium erythreum* and *Aeromicrobium fastidiosum* (Busse and Schumann, 1999) revealed that both strains contained a large amount of cadaverine (48.6% and 24.8%, respectively, of the polyamine content). The principal polyamine in *Aeromicrobium fastidiosum* was spermine (58.4%), whereas the second most abundant polyamine in *Aeromicrobium erythreum* was spermidine (37.2%). Putrescine and 1,3-diaminopropane occurred in minor amounts in both strains. Notably, the total polyamine concentration in *Aeromicrobium fastidiosum* was rather low.

Nutrition and growth conditions. Bacteria of this genus will grow aerobically in nutritionally complex media containing peptone and yeast extract and utilize a wide range of carbohy-

drates and other compounds as carbon sources in appropriate growth media (Table 223). The data on minimal nutritional requirements of *Aeromicrobium* species are incomplete. *Aeromicrobium fastidiosum* requires thiamine and biotin (Tamura and Yokota, 1994). *Aeromicrobium erythreum* additionally needs nicotinic acid (Miller et al., 1991). Bruns et al. (2003) reported that *Aeromicrobium marinum* will grow on a minimal, artificial seawater medium with defined carbon sources (e.g. mannitol and amino acids), a 10-vitamin solution (Balch et al., 1979) and 1 ml of trace element solution (Widdel and Bak, 1992). *Aeromicrobium panaciterrae* grows in a minimal salts medium with glucose (or some other carbon sources), vitamins, and trace elements (Cui et al., 2007a). Vitamins (biotin, thiamine, and nicotinic acid) are not necessary for growth of *Aeromicrobium alkaliterrae* (Yoon et al., 2005c). Most likely *Aeromicrobium ponti* and *Aeromicrobium tamlense* do not require vitamins for growth on basal medium ISP 9 (Shirling and Gottlieb, 1966) containing defined carbon sources (Lee and Lee, 2008; Lee and Kim, 2007).

The optimal growth temperatures (25–37°C) and the temperature ranges (4–42°C or slightly higher) vary with species (Table 223). The recognized *Aeromicrobium* species are considered to be non-salt-requiring, except for *Aeromicrobium marinum*, which is reported to be obligately salt-dependent and exhibits optimum growth at salt concentrations similar to that of sea water (Bruns et al., 2003). Some other species have also been reported to prefer salt-containing media. *Aeromicrobium halocynthiae*, which grows on A1+C agar (prepared with seawater supplemented with 0–6% NaCl), shows robust growth in the presence of NaCl in this medium, but does not grow on standard TSA medium (Difco) (Kim et al., 2010). *Aeromicrobium flavum* displays the optimal growth rate in LB broth with 1–2% NaCl (Tang et al., 2008). Bacteria of this genus prefer a neutral to mildly alkaline pH. However, they may also grow in test media with acidic (up to 4.1) or alkaline (up to 11–12.1) initial pH values (Table 223).

Genomic characteristics. The DNA G+C content of most *Aeromicrobium* species are higher than 70 mol% (up to 75.9 mol%; Kim et al., 2010); lower values (65.5 and 66.8 mol%; HPLC) have been reported for *Aeromicrobium panaciterrae* (Cui et al., 2007a) and *Aeromicrobium ginsengisoli* (Kim et al., 2008b). The current *Aeromicrobium* species form a coherent phylogenetic group based on 16S rRNA gene sequence analysis (similarity, >96%; Figure 247). The similarity based on 16S–23S ITS sequence analysis for the type strains of *Aeromicrobium erythreum* and *Aeromicrobium fastidiosum* was found to be 71.2% (Yoon et al., 1998a). The authors emphasized that the 16S–23S ITS similarity of *Aeromicrobium erythreum* was higher to the type strain of *Nocardioides jensenii* (73.1%) than to *Aeromicrobium fastidiosum*, and higher to three *Streptomyces* species (62.9–70.0%) and one *Frankia* strain (68.8%) than to some organisms of the family *Nocardioidaceae*. Likewise, *Aeromicrobium fastidiosum* had high, but slightly lower, similarity to *Nocardioides jensenii* (70.1%) and higher similarity to representatives of *Streptomyces* and *Frankia* than to most members of the family *Nocardioidaceae* used in the analysis. Similarity of the ribonuclease P RNA gene sequences between the type strains of *Aeromicrobium erythreum* and *Aeromicrobium fastidiosum*, in contrast, was highest (88% or 78.6% when nucleotide gaps were included) than between *Aeromicrobium* strains and organisms of other genera used in the study (Yoon and Park, 2000).

Bacteriophages. Four bacteriophages infectious for *Nocardioides albus*, *Nocardioides simplex*, and/or *Terrabacter tumescens* were not infectious for *Aeromicrobium erythreum* NRRL B-3381; the complete absence of plaques suggests the existence of barriers to phage adsorption or phage replication (Miller et al., 1991).

Antibiotic sensitivity and antibiotic production. The growth of the type strains of two *Aeromicrobium* species (*Aeromicrobium erythreum* and *Aeromicrobium flavum*) are inhibited by tetracycline, chloramphenicol, kanamycin, and vancomycin, as well as other antibiotics as indicated in the species descriptions (Miller et al., 1991; Tang et al., 2008). *Aeromicrobium erythreum* JCM 8359 but not *Aeromicrobium fastidiosum* JCM 8088 produces β-lactamase (Ogawara et al., 1999). The type strain of *Aeromicrobium erythreum* produces the macrolide antibiotic erythromycin A (French et al., 1970; Miller et al., 1991). The erythromycin-biosynthetic (*ery*) gene cluster of *Aeromicrobium erythreum* was cloned and characterized (Brikun et al., 2004). The 55.4-kb cluster contained 25 *ery* genes, and homologs were found for each gene in the previously characterized *ery* gene cluster from *Saccharopolyspora erythraea*. In addition, four new predicted *ery* genes were revealed (Brikun et al., 2004). The characteristics of the erythromycin resistance gene *ermA* of *Aeromicrobium erythreum* were described by Roberts et al. (1985).

Habitats and ecology. The recognized *Aeromicrobium* species have been isolated from soils of different origin, plants, air, and aquatic environments (Table 223). *Aeromicrobium halocynthiae* was recovered from the siphon tissue of a marine ascidian, *Halocynthia roretzi* (Kim et al., 2010).

The species *Aeromicrobium tamlense* and *Aeromicrobium marinum* were or could be associated with algae. *Aeromicrobium marinum* was found to occur in large numbers in the German Wadden Sea and to assimilate mannitol, which is present in seaweed in large amount as well as in algal exudates (Bruns et al., 2003; Budavari, 1989; Spencer, 1990). This bacterium, in addition, produces abundant exopolysaccharides that might serve to attach to algal surfaces (Bruns et al., 2003). The ability of another seawater species, *Aeromicrobium ponti*, to use mannitol as a carbon source and to hydrolyze cellulose (Lee and Lee, 2008) might be indicative of its association with algae as well. Recent ecological studies show that bacteria of the genus *Aeromicrobium* are distributed in various other terrestrial and aquatic environments, including low-temperature and deep subseafloor ecosystems (Glöckner et al., 2000; Hansen et al., 2007; Imazaki and Kobori, 2010; Katayama et al., 2007; Kobayashi et al., 2008; Lesaulnier et al., 2008; Rintala et al., 2008; Stevens et al., 2007; Zhang et al., 2007; Zhang et al., 2009b). Some unnamed members of this genus have been found in polluted environments, including high-level nuclear waste-contaminated sediments (Fredrickson et al., 2004), hydrocarbon-contaminated soils (Militon et al., 2010), and copper-contaminated substrates (Sun et al., 2010). They can occur on the surface of copper coins (Santo et al., 2010) and

are associated with plant tissues (Sun et al., 2010; Wang et al., 2008a) and deep-water marine sponge (Sfanos et al., 2005). Representatives of the genus also can be isolated from the skin microbiota of salamanders (Lauer et al., 2008) and humans (e.g. GenBank numbers HM336148 and HQ616221) and are also among viable microorganisms in retropharyngeal lymph nodes (organs once believed to be largely amicrobic in the absence of overt disease) of healthy wild ungulates (mule deer, *Odocoileus hemionus*; Wittekindt et al., 2010). No species or strains pathogenic for humans, other warm-blooded animals, or plants have yet been identified.

Enrichment and isolation procedures

No selective medium for the isolation of strains of *Aeromicrobium* has been described. Dilution plating and different media, e.g. R2A agar (Difco), tenfold-diluted nutrient agar (NA, Difco), and other agar media containing peptone and yeast extract may be used for isolation. Incubation is usually carried out at 25–30°C from a week to a month. Adjustment of pH to 10.0 with Na_2CO_2, as reported for *Aeromicrobium alkaliterrae* (Yoon et al., 2005c), facilitates isolation of alkalitolerant (alkaliphilic) species. Marine agar (MA, Difco) and SC-SW medium (1% soluble starch, 0.03% casein, 0.2% KNO_3, 0.2% NaCl, 0.002% $CaCO_3$, 1.8% agar, 0.005% $MgSO_4 \cdot 7H_2O$, and 0.001% $FeSO_4 \cdot 7H_2O$ in a 60:40 mixture of natural seawater and distilled water; Lee and Kim, 2007) can be used to isolate *Aeromicrobium* species from marine environments. Pre-incubation of a water sample in Marine broth (Difco) as described for isolation of *Aeromicrobium marinum* (Bruns et al., 2003) can be useful. When isolating strains of *Aeromicrobium* species, selective (semi-selective) media may be developed on the basis of characteristics listed in Table 223, the minimal nutritional requirements of *Aeromicrobium* species, and resistance to certain antibiotics. Characteristics of environmental samples should be taken into account. Alkaline pre-treatment before serial dilution and plating, suggested for isolation of some bacteria (Påhlson et al., 1986; Wakisaka et al., 1982), facilitates reducing numerous Gram-negative bacteria. The filtration of (suspended) environmental samples through membrane filters with a pore size of 0.45 or 0.65 µm or other approaches may be useful to separate small cells of *Aeromicrobium* (which may be even smaller in environmental samples) from large-sized microorganisms occurring in the same sample.

Maintenance procedures

Cultures may be maintained as 20% glycerol suspensions at −20 and −70°C. Long-term conservation is achieved by freeze-drying by standard procedures.

Differentiation of the genus *Aeromicrobium* from other genera

Table 214 lists the phenotypic characteristics differentiating *Aeromicrobium* from other genera of the family *Nocardioidaceae*. *Aeromicrobium* can be easily distinguished from the genera and species comprising mycelium-forming organisms on the basis of cell morphology. The predominant menaquinone MK-9(H_4) is the most salient characteristic which differentiates *Aeromicrobium* from all members of the genera *Nocardioides* and *Marmoricola*. Other chemotaxonomic characteristics which may serve to distinguish *Aeromicrobium* from related genera with LL-A_2pm in the cell wall include the profiles of cellular fatty acids, polar lipids, polyamines, and probably the composition of cell-wall polysaccharides.

Taxonomic comments

The genus *Aeromicrobium* with the type species *Aeromicrobium erythreum* was described by Miller et al. (1991) to accommodate the strain "*Arthrobacter*" sp. NRRL B-3381, an unusual non-mycelium-forming producer of the macrolide antibiotic erythromycin A, that differed in many ways from *Arthrobacter sensu stricto*. The second species, *Aeromicrobium fastidiosum*, was added to the genus by Tamura and Yokota (1994) as a result of the reclassification of *Nocardioides fastidiosa* (Collins and Stackebrandt, 1989a, 1989b).

Eight remaining *Aeromicrobium* species included in this volume were described within the 2003–2010 period on the basis of taxonomic study of single environmental isolates. The descriptions relied on 16S rRNA gene sequence analysis, DNA-DNA hybridization studies (examined in cases of high 16S rRNA sequence similarity), and phenotypic traits. The DNA-DNA similarities between *Aeromicrobium* species obtained by different methods ranged from very low (3–11.5%; Cui et al., 2007a; Tang et al., 2008; Yoon et al., 2005c) to high (up to 63.5% for the *Aeromicrobium halocynthiae-Aeromicrobium ponti* pair; Kim et al., 2010). The original genus description (Miller et al., 1991), which was based on one strain and included many features specific for this strain, was emended by Yoon et al. (2005c) to take into account other relevant data accumulated by 2005.

Differentiation of species of the genus *Aeromicrobium*

Phenotypic characteristics useful in distinguishing the currently recognized ten species of the genus *Aeromicrobium* are listed in Table 224. Additional characteristics useful for differentiation are given in Table 223 and in the species descriptions below. Since many test results can be influenced by the test method, a comparative experimental study of phenotypic characteristics of a novel isolate and type strains of recognized species rather than comparisons with data presented is recommended (Schumann et al., 2009; Tindall et al., 2010).

Acknowledgements

The authors were supported by the program MCB RAS of the Russian Academy of Sciences.

TABLE 224. Differential characters useful for identification of *Aeromicrobium* species[a,b]

Characteristic	1. *A. erythreum*	2. *A. alkaliterrae*	3. *A. fastidiosum*	4. *A. flavum*	5. *A. ginsengisoli*	6. *A. halocynthiae*	7. *A. marinum*	8. *A. panaciterrae*	9. *A. ponti*	10. *A. tamlense*
Colony color[c]	Beige, yellow	Cream	White	Yellow	White	Light yellowish	Ivory	Light yellow	Yellow	Yellow
Motility	−	−	+	−	−	−	−	−	−	−
Growth at:										
10°C	−	+	+	−	+	+	+	−	+	+
42°C	−	−	−	−	−	+	−	−	+	+
6% NaCl	−	+	−	−	−	+	+	−	+	−
8% NaCl	−	+	−	−	−	−	+	−	+	−
Catalase activity	+	+	+	+	−	+	+	+	+	+
Oxidase activity	+	−	+	+	+	−	−	−	−	−
Utilization of carbon sources:[d]										
L-Arabinose	+	+[e]	+	−	+	+	−	−	+	−
D-Cellobiose	−	+	+	−	+	−	+	+	+	+
D-Fructose	+	−	+	+	+	+	−	−	+	+
D-Glucose	+	+	+	+	+	+	−[e]	+	+	+
D-Mannose	−	−	+	−	+	+	−[e]	+	+	+
D-Xylose	+	−	+	−	+	+	−	−	+	−
Succinate	+	+	−		+		+	+	+	+
Hydrolysis of casein	+	+	+	−			−	−	+	−
Hydrolysis of tyrosine	w	−	−		+		−	−	+	−
Acid phosphatase	+	+	+	−	−	−	−	+	+	+
Alkaline phosphatase	v	−	+	−	−	−	−	+	+	+
DNA G+C content (mol%)	71–73 (T_m)	71.5 (HPLC)	71–72 (T_m)	73.3 (T_m)	66.8 (HPLC)	75.9 (HPLC)	70.6 (HPLC)	65.5 (HPLC)	74.0 (HPLC)	72.7 (HPLC)

[a]Based on characteristics of type strains. Symbols: −, negative; +, positive; v, variable between experiments; w, weakly positive.

[b]See Table 223 for references.

[c]Color intensity and shade may vary depending on growth conditions; for details concerning *Aeromicrobium erythreum*, see the species description.

[d]Identical test results (unless indicated) were obtained by using both conventional methods and the API test systems (data for *Aeromicrobium erythreum*, *Aeromicrobium alkaliterrae*, *Aeromicrobium fastidiosum*, *Aeromicrobium marinum*, and *Aeromicrobium panaciterrae*). The results for *Aeromicrobium ponti* and *Aeromicrobium tamlense* were obtained using ISP 9 (Shirling and Gottlieb, 1966) as the basal medium (Lee and Lee, 2008; Lee and Kim, 2007). API test systems (bioMérieux) were used to obtain the data for *Aeromicrobium ginsengisoli* (Kim et al., 2008b) and *Aeromicrobium halocynthiae* (Kim et al., 2010), and the Biolog GP2 test system was used to obtain the data for *Aeromicrobium flavum* (Tang et al., 2008).

[e]The opposite result was obtained using the API test system (Kim et al., 2008b).

List of species of the genus *Aeromicrobium*

1. **Aeromicrobium erythreum** Miller, Woese and Brenner 1991, 367[VP]

e.ryth′re.um. N.L. neut. adj. *erythreum* intended to mean erythromycin-producing.

Characteristics are as described for the genus and listed in Table 223. Additional information given below is taken from the original paper (Miller et al., 1991) unless indicated. Predominantly irregular rods to coccoid forms (0.5 × 0.5–1.2 µm; Figure 268) in TEY broth (1.6% tryptone, 1% yeast extract, 0.5% NaC1, and 0.4% D-glucose) at all growth phases (24, 48, and 120 h). Cells are non-motile. Colonies are beige to amber beige and 1 mm in diameter after 3 d of cultivation on TYE agar medium (1% tryptone, 0.5% yeast extract, 0.8% NaCl, and 1.5% agar) at 32°C. Colonies sometimes have thin, translucent edges. The colony color changes to a shade of yellow on media allowing even slight acid production and to distinct yellow (e.g. on R2YE medium containing sucrose; Hopwood et al., 1985) or other media (Miller et al., 1991; Tamura and Yokota, 1994). Diffusible brown or purple pigments may be produced on R2YE plates (but are not observed on TYE agar) after extended incubation at 32°C. The optimum growth temperature is 35 ± 2°C; no growth occurs at 18°C or 43°C. Grows in nutritionally complex media. Biotin, nicotinic acid, and thiamine are required for growth in chemically defined media. A wide range of organic compounds are utilized as sole or principal carbon plus energy sources for growth (Table 223). Acid production from carbohydrates is generally very weak, as determined using the phenol red indicator medium (Difco), but growth on fructose gives readily detectable acidification. The type strain produces the macrolide antibiotic erythromycin A (which can be detected by standard growth inhibition assays with susceptible bacteria on various media). The strain tested using a Gram-positive minimal inhibitory concentration (MIC) panel (Baxter, Sacramento, CA) is sensitive to amikacin,

cefotaxime, ceftriaxone, chloramphenicol, ciprofloxacin, gentamycin, hygromycin, kanamycin, neomycin, oxacillin, streptomycin, sulfamethoxazole, tetracycline, thiostrepton, vancomycin, and viomycin. Resistant to amoxicillin, ampicillin, cefamandole, clindamycin, erythromycin, imipenem, naladixic acid, nitrofurantoin, norfloxacin, penicillin, rifampin, spiramycin, and tylosin. Ogawara et al. (1999) reported that benzylpenicillin potassium inhibited growth at a concentration of 50 μg/ml, but not at 10 μg/ml.

The major compounds of polyamine pattern include cadaverine (48.6%) and spermidine (37.2%); other compounds, i.e. spermine, 1,3-diaminopropane, and putrescine, occur in minor amounts (Busse and Schumann, 1999). The predominant menaquinone is MK-9(H_4), with minor amounts of MK-7(H_4) and MK-8(H_4) (Tamura and Yokota, 1994). The major phospholipids are phosphatidylethanolamine, phosphatidylglycerol, and diphosphatidylglycerol (Lee and Kim, 2007; Tamura and Yokota, 1994). The major (>5%) fatty acids, as determined in cells grown under various conditions and harvested at different growth phase were reported to be $C_{18:0}$ 10-methyl (16.2–39%), $C_{18:1}$ ω9*c* (15.1–29.1%), $C_{16:0}$ (16.2–25.2%), $C_{16:0}$ 2-OH (11.3–14.4%), $C_{17:0}$ 10-methyl (0.3–9.5%), and $C_{18:0}$ (4.3–10.9%) (Lee and Lee, 2008; Lee et al., 2000; Miller et al., 1991; Park et al., 1999; Tamura and Yokota, 1994). A higher content of $C_{16:0}$ (47%) and lower proportions of other acids were detected for cells harvested from trypticase soy broth (Difco) after incubation at 28°C for 24–48 h (Schumann et al., 1997).

Source (type strain): tropical soil in Puerto Rico.

DNA G+C content (mol%): 71–73 (T_m).

Type strain: NRRL B-3381, ATCC 51598, DSM 8599, JCM 8359, LMG 16472, NBRC 15406, NRRL B-3381.

Sequence accession no. (16S rRNA gene): AF005021

2. **Aeromicrobium alkaliterrae** Yoon, Lee and Oh 2005c, 2174[VP]

al.ka.li.ter′ra.e. N.L. n. *alkali* (from Arabic *al-qalyi*, the ashes of saltwort), soda ash; L. gen. n. *terrae* of the soil or earth; N.L. gen. n. *alkaliterrae* of alkaline soil.

Characteristics are as described for the genus and listed in Table 223. Additional information given below is taken from the original paper (Yoon et al., 2005c) unless indicated. Small rods (0.3–0.5 × 0.8–1.4 μm) or cocci. No motility by flagella. Colonies are yellow and 1.0–1.5 mm in diameter after 7 d of cultivation at 25°C on nutrient agar (Difco). Capable of growth on simple chemically defined media, including ISP 9 medium (Shirling and Gottlieb, 1966) supplemented with glucose or other carbon sources. No growth occurs under anaerobic conditions on trypticase soy agar (TSA; Difco) or on TSA supplemented with nitrate. Grows at 35°C, but not at 37°C. Tolerates NaCl up to 8% in trypticase soy broth (TSB; Difco), but optimal growth is observed when no salt is added. Growth occurs at pH 6.0 and pH 11.0, but not at pH 5.5 or 11.5 (initial pH values of medium, TSB). Displays the ability to hydrolyze Tweens 20, 40, and 60. The major fatty acids (5% or more), as determined in cultures grown on TSA and MA at 25°C for 6 d, are $C_{16:0}$ (34.1 and 33.8%), $C_{18:0}$ 10-methyl (16.3 and 20.9%), $C_{16:0}$ 2-OH (17.6 and 15.5%), $C_{18:1}$ ω9*c* (5.7 and 9.9%), and $C_{17:0}$ 10-methyl (5.0 and 1.0%), respectively. Generally similar proportions of the above acids, except for slightly decreased $C_{18:1}$ ω9*c* (2.3%) were reported by Lee and Lee (2008) for cells grown on TSA agar at 30°C for 5 d.

Source (type strain): an alkaline soil in Kwangchun, Korea.

DNA G+C content (mol%): 71.5 (HPLC).

Type strain: strain KSL-107, KCTC 19073, DSM 16824, JCM 13518.

Sequence accession no. (16S rRNA gene): AY822044.

3. **Aeromicrobium fastidiosum** (Collins and Stackebrandt 1989b) Tamura and Yokota 1994, 610[VP] (*Nocardioides fastidiosa* Collins and Stackebrandt 1989b, 293)

fas.ti.di.o′sum. L. neut. adj. *fastidiosum* fastidious, referring to the nutritionally fastidious nature of the organism when it is first isolated.

Characteristics are as described for the genus and listed in Table 223. Additional information given below is taken from the original descriptions (Collins and Stackebrandt, 1989b; Tamura and Yokota, 1994), unless indicated. Irregular rods (average, 0.4–0.5 × 1.5–2.2 μm) in young culture (1–2 d) on modified CB agar at 26°C (recent observation). The rods fragment and become shorter as growth proceeds (usually after 3 d) and can be short cell chains at this stage. After 5–6 d of culture, coco-bacillary to coccoid cells, usually single or in pairs (recent observation), predominate. In contrast to all other *Aeromicrobium* species, *Aeromicrobium fastidiosum* is motile. Colonies are white or grayish-white. Grows in nutritionally complex media. Biotin and thiamine are required for growth in a suitable minimal salts medium with glucose. Growth occurs at 30°C, but not at 40°C. A wide range of organic compounds are utilized as sole or principal carbon plus energy sources for growth (Table 223), including L-phenylalanine, L-threonine, tyrosine, butanol, crotonate, dodecane, hexadecane, oxalacetate, and pentoate. Acid is produced from glucose. The cell-wall polysaccharides contain glucose, rhamnose, an aminosugar, and ribitol, which originate from at least two anionic polymers, i.e. the ribitol-based teichoic acid and supposedly phosphorhamnan (Tul'skaya, 2009). *Aeromicrobium fastidiosum* is characterized by a rather low concentration of polyamines; the polyamime pattern includes spermine (58.4%), cadaverine (24.8%), and minor amounts of putrescine, spermidine, and 1,3-diaminopropane (Busse and Schumann, 1999). The major menaquinone is MK-9(H_4), with minor amounts of MK-9(H_6) and MK-8(H_4). Principal phospholipids are phosphatidylethanolamine, phosphatidylglycerol, and diphosphatidylglycerol (Lee and Kim, 2007; Tamura and Yokota, 1994). The major fatty acids (>5%) determined in cells grown under different conditions are $C_{18:1}$ ω9*c* (12.3–48.2%), $C_{18:0}$ 10-methyl (9.1–42%), $C_{16:0}$ 2-OH (8.9–23.2%), $C_{16:0}$ (10–18.3%), $C_{18:0}$ (6.1–16.1%), and $C_{17:0}$ 10-methyl (<1–8.7%) as reported by Lee and Lee (2008), Lee et al. (2000), Park et al. (1999), and Tamura and Yokota (1994).

Source (type strain): herbage.

DNA G+C content (mol%): 71–72 (T_m).

Type strain: strain J41, ATCC 49363, DSM 10552, IFO (now NBRC) 14897, JCM 8088, LMG 16205, NCIB (now NCIMB) 12713, VKM Ac-1324.

Sequence accession no. (16S rRNA gene): AF005022; X53189, X76862, Z78209.

4. **Aeromicrobium flavum** Tang, Zhou, Zhang, Mao, Luo, Wang and Fang 2008, 1862[VP]

fla′vum. L. neut. adj. *flavum* yellow, referring to the colony color.

Characteristics are as described for the genus and listed in Table 223. Additional information given below is taken from the original description (Tang et al., 2008). Cells are mostly irregular rods (ca. 0.2–0.4 in width and up to 1.2 μm in length) as observed after 16 h of culture on LB agar, and become shorter in a 2-d-old culture. The cells are surrounded by an amorphous polysaccharide-like layer and form conglomerates. No motility by flagella. Colonies are yellow pigmented. Grows readily in nutritionally complex media at 25–37°C but not at 10 and 42°C. The optimum NaCl concentration for growth is 1–2% (tested in LB broth). In addition to characteristics listed in Table 223, the type strain shows positive test reactions in the Biolog GP2 test system for D-psicose, turanose, α-ketovaleric acid, γ-hydroxybutyric acid, and D-fructose-6-phosphate. Negative responses have been recorded for D-arabitol, L-fucose, α-cyclodextrin, glycogen, mannan, *N*-acetyl-β-D-mannosamine, amygdalin, L-alanyl glycine, arbutin, L-asparagine, 2′-deoxyadenosine, putrescine, sedoheptulosan, glycyl-L-glutamic, α-hydroxybutyric, and L-lactic acids. Displays also negative responses in the Biolog GP2 plate for assimilation of dextrin, thymidine, and uridine, which along with other features listed in Table 223 and Table 224 differentiate this species from its phylogenetically closest relative *Aeromicrobium tamlense* (98.4% 16S rRNA gene sequence similarity). The DNA-DNA relatedness value between the type strains of these two species was reported to be 35%. Susceptible to tetracycline, chloramphenicol, kanamycin, vancomycin, erythromycin, rifampicin, and tobramycin; resistant to penicillin and nalidixic acid (determined by the agar-diffusion method using antibiotic-impregnated discs as described by Buczolits et al., 2002). The major menaquinones are MK-9(H_4) (74.6%) and MK-8(H_4) (25.4%). The fatty acids (1% or more) recorded for cells grown for 24 h at 30°C in trypticase soy broth (Difco) include $C_{18:1}$ ω9*c* (68.4%), $C_{18:0}$ (11.7%), $C_{16:0}$ (8.7%), $C_{16:0}$ 2-OH (5.3%), $C_{18:0}$ 10-methyl (2.3%), and $C_{16:1}$ ω6*c* (1.0%).

Source (type strain): air (on the campus of Wuhan University, China).

DNA G+C content (mol%): 73.3 (T_m).

Type strain: TYLN1, CCTCC AB 206046, DSM 19355.

Sequence accession no. (16S rRNA gene): EF133690.

5. **Aeromicrobium ginsengisoli** Kim, Park, Im and Yang 2008b, 2028[VP]

gin.sen.gi.so′li. N.L. n. *ginsengum* ginseng; L. n. *solum* soil; N.L. gen. n. *ginsengisoli* of/from ginseng soil.

Characteristics are as described for the genus and listed in Table 223. Additional information given below is taken from the original description (Kim et al., 2008b). Cells (typically 0.3–0.4 × 0.5–1.2 μm) display coccoid forms after 5 d in culture at 30°C on R2A agar (Difco). No motility was observed with a light microscope. Colonies are white on R2A after 5 d. Will grow in nutritionally complex media at temperatures up to 30°C or slightly higher, but not at 37°C. Grows on R2A agar with 3% NaCl and weakly with 4% NaCl. No growth on MacConkey agar. In contrast to all other recognized *Aeromicrobium* species, this species was reported to display negative catalase reaction. Oxidase activity (evaluated by using 1% *p*-aminodimethylaniline oxalate) is positive. Reduces nitrate to nitrite, but not to nitrogen gas. Positive for assimilation of D-fucose, D-lyxose, ethanol, fumarate, L-asparagine, glutamate, glutamine, L-leucine, L-phenylalanine, L-proline, L-serine, L-tryptophan, and L-tyrosine in the API 20E and API 20NE tests (bioMérieux). Negative for assimilation of L-fucose, melibiose, L-xylose, *myo*-inositol, methanol, glycogen, 3- and 4-hydroxybenzoate, glutarate, 2- and 5-ketogluconate, itaconate, maleic acid, oxalate, L-arginine, L-aspartate, L-cysteine, L-glycine, L-histidine, L-isoleucine, L-lysine, L-methionine, L-threonine, and L-valine. Fatty acids determined in cells grown on trypticase soy agar (Difco) at 30°C for 48 h include $C_{16:0}$ (32.7%), $C_{18:0}$ 10-methyl (20.6%), $C_{18:0}$ (12.9%), $C_{17:0}$ (3.2%), $C_{19:0}$ 10-methyl (3.1%), and $C_{17:0}$ 10-methyl (2.4%).

Source (type strain): soil of a ginseng field in Daejeon, South Korea.

DNA G+C content (mol%): 66.8 (HPLC).

Type strain: Gsoil 098, GBS 39, JCM 14732, KCTC 19207.

Sequence accession no. (16S rRNA gene): AB245394.

6. **Aeromicrobium halocynthiae** Kim, Yang, Sohn and Kwon 2010, 2797[VP]

ha.lo.cyn.thi′a.e. N.L. gen. n. *halocynthiae* of *Halocynthia roretzi*, the ascidian from which the type strain was isolated.

Characteristics are as described for the genus and listed in Table 223. Additional information presented below is taken from the original paper (Kim et al., 2010). Cells are typically elongated rods (approximately 0.4–0.5 × 4.1–6.0 μm); straight, curved and rudimentarily branched filaments can be produced (e.g. in culture growing in A1+C liquid medium at 25°C). Colonies are light yellowish and 0.6–1 mm in diameter after incubation on A1+C agar plates at 25°C for 5 d. Grows on marine agar (Difco) and some other complex media but not on standard TSA medium (Difco). Will grow on A1+C agar with 0–6% NaCl, but NaCl is required for robust growth. Grows at 10–42°C (but not at 7 and 45°C), with optimal growth at 25°C. Will grow in liquid A1+C medium over a pH range of 5.0–10.0. The following carbon sources are utilized (API 50 CHE system): turanose, D-tagatose and 5-ketogluconate. Erythritol, D-arabinose, L-xylose, adonitol, methyl-β-xyloside, dulcitol, sorbitol, methyl α-D-mannoside, methyl-α-D-glucoside, *N*-acetylglucosamine, amygdalin, arbutin, esculin, salicin, melezitose, raffinose, starch, glycogen, xylitol, β-gentiobiose, D-lyxose, D-fucose, L-fucose, D-arabitol, L-arabitol, and 2-ketogluconate are not utilized as sole carbon and energy sources. Oxidizes arabinose, does not oxidize glucose, mannitol, inositol, sorbitol, rhamnose, sucrose, melibiose or amygdalin. α-Galactosidase and β-glucuronidase activities are not detected. The type strain produces taurocholic acid, a bile acid, as a major secondary metabolite. Predominant fatty acids (>5%) are $C_{18:1}$ ω9*c* (35.2%), $C_{16:0}$ (31.5%), $C_{18:0}$ 10-methyl (21.1%), and $C_{18:0}$ (5.5%). The hydroxylated fatty acid ($C_{16:0}$ 2-OH), which typically occurs in significant amounts in all other recognized *Aeromicrobium* species, has been not detected in *Aeromicrobium halocynthiae*. This species

is phylogenetically closest to *Aeromicrobium ginsengisoli*, *Aeromicrobium erythreum* and *Aeromicrobium ponti* (97.7, 97.6, and 97.5% 16S rRNA gene sequence similarities, respectively). The DNA–DNA similarity values between the type strain of *Aeromicrobium halocynthiae* and the type strains of the aforementioned species are 49.6–63.5%.

Source (type strain): siphon tissue of a marine ascidian, *Halocynthia roretzi*, collected at a depth of 15 m near Kyung-Po beach, coast of Gangneung, Korea.

DNA G+C content (mol%): 75.9 (HPLC).

Type strain: KME 001, JCM 15749, KCCM 90079.

Sequence accession no. (16S rRNA gene): FJ042789.

7. **Aeromicrobium marinum** Bruns, Philipp, Cypionka and Brinkhoff 2003, 1922[VP]

ma.ri′num. L. neut. adj. *marinum* of the sea, marine.

Characteristics are as described for the genus and listed in Table 223. Additional information presented below is taken from the original description (Bruns et al., 2003) unless indicated. Cells are irregular rods (0.3–0.5 × 0.7–1.3 μm; Figure 269a,b). The morphology is reported to remain constant in Marine broth (Difco), irrespective of the culture age (up to 10 d). No flagella, pili, or other appendages are observed. Colonies are ivory-colored and 0.5–1.0 mm in diameter; diffuse haloes of different size (Figure 269c) are observed around the colonies. It is the sole species within the genus proven to be salt-requiring so far. Grows well in ASW/YPG medium (1 liter of artificial sea water, 0.3% yeast extract, 0.6% peptone, and 0.3% glucose) with salt concentrations in the range 0.63–10.7%. A maximal growth rate is observable at 5.35%; growth is weak at 0.08% salinity and absent when no salts are added. The type strain grows with carbon sources such as crotonic acid, fumarate, 2-oxoglutarate, arginine, and glutamic acid but not with pimelic acid, nicotinic acid, salicylic acid, ethanol, propanol, butanol, asparagine, aspartic acid, cysteine, glutamine, glycine, or histidine. Cannot grow with D-glucose as a carbon source in minimal salts media containing vitamins and microelements (Bruns et al., 2003; Cui et al., 2007a). Unable to hydrolyze vegetable oil. Bruns et al. (2003) and Lee and Lee (2008) reported the following major fatty acids (>5%) for the type strain: $C_{18:1}$ ω9*c* (28.5 and 21.7%), $C_{16:0}$ (15.0 and 16.6%), $C_{16:0}$ 2-OH (14.0 and 7.9%), and $C_{18:0}$ 10-methyl (12.5 and 8.2%). Lee and Lee (2008) reported additionally significant amounts of $C_{17:0}$ (9.9%) and $C_{18:0}$ (11.5%) for cells harvested from TSA agar (Difco) after incubation at 30°C for 5 d.

Source (type strain): surface waters of the German Wadden Sea.

DNA G+C content (mol%): 70.6 (HPLC).

Type strain: T2, DSM 15272, JCM 13314, LMG 21768.

Sequence accession no. (16S rRNA gene): AY166703.

8. **Aeromicrobium panaciterrae** Cui, Im, Yin, Lee, Lee and Lee 2007a, 690[VP]

pa.na.ci.ter′ra.e. N.L. n. *Panax, -acis* scientific name of ginseng; L. n. *terra* soil; N.L. gen. n. *panaciterrae* of soil of a ginseng field.

Characteristics are as described for the genus and listed in Table 223. Information presented below is taken from the original paper (Cui et al., 2007a). Cells are short rods (0.2–0.4 × 1.0–1.5 μm) after culture for 3 d in modified R2A broth. No motility was observed with a light microscope. Colonies grown on R2A agar (Difco) for 5 d are light yellowish white and reach 1–3 mm in diameter. Grows well at 15–30°C (but not at 4 or 35°C), in the pH range of 5.0–8.5, and in the presence of up to 3% NaCl on R2A agar. Will grow readily on nutrient agar (0.5% peptone, 0.3% beef extract, and 1.5% agar). Growth on TSA agar is slow and looks like very thin layer on the agar surface after 5 d at 30°C. No growth on MacConkey agar. Capable of growth in suitable minimal salts media with glucose, vitamins, and trace elements. Utilizes melibiose, L-lactate, D-glycogen, and glycine as carbon sources but not D-fucose, D-lyxose, L-lysine, and DL-serine. Does not produce acid or gas from glucose oxidatively or fermentatively as determined using O-F basal medium (Atlas, 1993) with bromothymol blue. The major components (>5%) of the fatty acid profile determined in cells grown in modified R2A broth for 6 d are $C_{18:0}$ 10-methyl (14.5%), $C_{16:0}$ (13.0%), $C_{16:0}$ 2-OH (12.8%), $C_{17:0}$ 10-methyl (11.9%), $C_{16:0}$ 10-methyl (11.7%), $C_{16:1}$ ω7*c*, $C_{15:0}$ iso 2-OH (6.6%), and $C_{18:1}$ ω9*c* (5.3%).

Source (type strain): soil of a ginseng field in Pocheon Province, South Korea.

DNA G+C content (mol%): 65.5 (HPLC).

Type strain: Gsoil 161, KCTC 19131, DSM 17939, CCUG 52476.

Sequence accession no. (16S rRNA gene): AB245387.

9. **Aeromicrobium ponti** Lee and Lee 2008, 990[VP]

pon′ti. L. gen. n. *ponti* of the sea, belonging to the sea, referring to the isolation of the type strain from seawater.

Characteristics are as described for the genus and listed in Table 223. Information presented below is taken from the original paper (Lee and Lee, 2008). Cells are irregular rods (0.7 × 2.4 μm) as observed in 24-h culture on TSB at 30°C. Colonies are yellow in color and reach 0.8–1.2 mm in diameter after 5 d on TSA (Difco). Neither motility nor flagella are observed. Will grow on simple chemically defined media, e.g. ISP 9 (Shirling and Gottlieb, 1966) with glucose or other carbohydrates, sugar alcohols, or organic acids (Table 223). Does not grow on ISP 9 medium with D-arabinose, melezitose, methyl-α-D-glucoside, or *meso*-erythritol. Grows well at 30–37°C; can grow at 42°C or even higher. Displays growth in the pH range of 4.1–12.1 (initial pH of medium, TSA), but the optimal pH for growth is 5.1–7.1. Tolerates 10% NaCl as tested on ISP 2 agar medium. Able to hydrolyze carboxymethylcellulose. The polar lipids are phosphatidylinositol, diphosphatidylglycerol, phosphatidylglycerol, and three unidentified phospholipids. Predominant fatty acids determined in cells grown on TSA agar at 30°C for 5 d are $C_{18:1}$ ω9*c* (34.7%), $C_{16:0}$ (19.7%), $C_{16:0}$ 2-OH (14.8%), and $C_{18:0}$ 10-methyl (11.0%).

Source (type strain): seawater from the coast of Jeju Island, Republic of Korea.

DNA G+C content (mol%): 74.0 (HPLC).

Type strain: HSW-1, DSM 19178, KACC 20565.

Sequence accession no. (16S rRNA gene): AM778683.

10. **Aeromicrobium tamlense** Lee and Kim 2007, 339[VP]

tam.len′se. N.L. neut. adj. *tamlense* of or belonging to Tamla, the old name of Jeju, Republic of Korea, the site of isolation of the type strain.

Characteristics are as described for the genus and listed in Table 223. Additional information presented below is taken from the original paper (Lee and Kim, 2007) unless indicated. Cells are irregular rods ranging in size from 0.4–0.6 × 0.8–1.2 µm to 0.5 × 3.8–4.8 µm, as observed in 3-d-old cultures on YE-SW agar at 30°C. Colonies are yellow and 0.6–0.8 mm in diameter after incubation for 5 d on YE-SW at 30°C. No growth occurs at 4 or 45°C. Growth is observed at pH 10.1 (initial pH of medium, TSA) and in the presence of up to 5% NaCl (tested on ISP 2 medium; Shirling and Gottlieb, 1966). Grows in simple chemically defined media, e.g. ISP 9 (Shirling and Gottlieb, 1966), with glucose or other carbohydrates, sugar alcohols, or organic acids (Table 223) and also with L-ribose, or methyl-α-glucoside. The following carbon sources are not utilized as sole carbon and energy sources: D-arabinose, D-melezitose, 2,3-butanediol, *meso*-erythritol, and 1,2-propanediol. The type strain displays positive responses in the Biolog GP2 plate for assimilation of dextrin, thymidine, and uridine (Tang et al., 2008), which, along with other characteristics listed in Table 223 and Table 224, may serve to differentiate *Aeromicrobium tamlense* from the phylogenetically closest species *Aeromicrobium flavum.* Principal phospholipids include phosphatidylinositol, diphosphatidylglycerol, and phosphatidylglycerol. The major fatty acids in cells harvested from TSA agar (Difco) after incubation at 30°C for 5 d are $C_{18:1}$ ω9*c* (47.2%), $C_{16:0}$ (13.8%), $C_{18:0}$ (13.6), $C_{18:0}$ 10-methyl (8.7%), and $C_{16:0}$ 2-OH (6.8%).

Source (type strain): dried seaweed collected from Samyang beach in Jeju Island, Korea.

DNA G+C content (mol%): 72.7 (HPLC).

Type strain: SSW1-57, JCM 13811, CIP 09549, DSM 19087, NRRL B-24466.

Sequence accession no. (16S rRNA gene): DQ411541.

Genus IV. **Kribbella** Park, Yoon, Shin, Suzuki, Kudo, Seino, Kim, Lee and Lee 1999, 750^VP emend. Sohn, Hong, Bae and Chun 2003, 1007

Lyudmila I. Evtushenko and Valentina I. Krausova

Krib.bel′la. N.L. fem. dim. n. *Kribbella* arbitrary name formed from the acronym of the Korea Research Institute of Bioscience and Biotechnology, KRIBB, where taxonomic studies of this taxon were performed.

Extensively branched vegetative (substrate) hyphae, mostly ~0.4–0.7 µm in diameter, growing on the surface of, and penetrating, agar media. **Aerial hyphae are typically produced** on suitable agar media. Both the substrate and aerial **hyphae usually undergo various degrees of fragmentation** eventually **resulting in irregular rod-shaped to coccoid, nonmotile, elements**. Short chains of well-to-poorly differentiated conidia may occur on aerial hyphae. Bud-like cells, apical swellings, and cell conglomerates may be produced by the substrate mycelium. No endospores are formed. **Colonies are pasty to soft-leathery**, often with lichenous shape and usually no distinct coloration. Diffusible pigments are usually not produced, except for melanoid pigments characteristic of some species. **Gram-stain-positive**, but may be variable. Non-acid-fast. **Chemoorganotrophs, having a respiratory type of metabolism**, with a potential for mixotrophy and metabolic flexibility. **Grow well under aerobic conditions** on standard laboratory media, including the chemically defined (synthetic) media. Some species show also weak growth under anaerobic conditions. Catalase-positive. Oxidase test reaction intensity varies with species. Utilize a wide range of carbon and nitrogen sources and possess a significant spectrum of hydrolytic activities. Mesophilic, optimal growth temperature is ~28–30°C; the lower and higher growth temperatures reported for different species are 8°C and 45°C. Usually non-halophilic and prefer a neutral to mildly alkaline pH; some species grow at initial pH values of 10 and slightly below 5.0. **The cell-wall peptidoglycan contains LL-diaminopimelic acid**, along with alanine, glutamic acid, and glycine. The muramic acid residue of the peptidoglycan is ***N*-acetylated.** The **main secondary cell-wall polymer is teichuronic or teichulosonic acid.** Menaquinones are the sole respiratory quinones detected; the tetrahydrogenated menaquinone with nine isoprene units **[MK-9(H_4)] is the major component**. **The saturated iso- and anteiso branched acids ($C_{15:0}$ anteiso, $C_{16:0}$ iso, and $C_{15:0}$ iso) are the main** cellular fatty acids, with **$C_{15:0}$ anteiso usually contributing a maximal proportion**. Monounsaturated iso-branched, saturated 9-methyl branched fatty acids typically occur and may constitute significant amounts. Other fatty acids, including 2-hydroxy and 10-methyl branched acids may occur in minor or trace quantities. Mycolates are absent. **The diagnostic component of the polar lipid patterns is phosphatidylcholine** (PC); additional principal polar lipids reported are diphosphatidylglycerol (DPG), phosphatidylglycerol (PG), and phosphatidylinositol (PI).

Natural habitats are various terrestrial environments; some organisms are associated with plants and fungi, and occur in the human skin microbiome.

DNA G+C content (mol%): 67–71.3 (T_m; HPLC).

Type species: **Kribbella flavida** Park, Yoon, Shin, Suzuki, Kudo, Seino, Kim, Lee and Lee 1999, 750^VP.*

Further descriptive information

The genus *Kribbella* belongs to the family *Nocardioidaceae*, order *Propionibacteriales.* Based on the 16S rRNA gene sequence analysis, the current 16 *Kribbella* species form a tight, clearly separated phylogenetic cluster (Figure 247) with a high 16S rRNA gene sequence similarity (>97.5% and up to 99.6%).

Morphology and colony appearance. Aerial mycelium varies in abundance, depending on growth media and the individual strain or species, and at times is discernible only microscopically or lacking, e.g. on some agar media rich in organics.

of distilled water; pH 6.7±0.5.

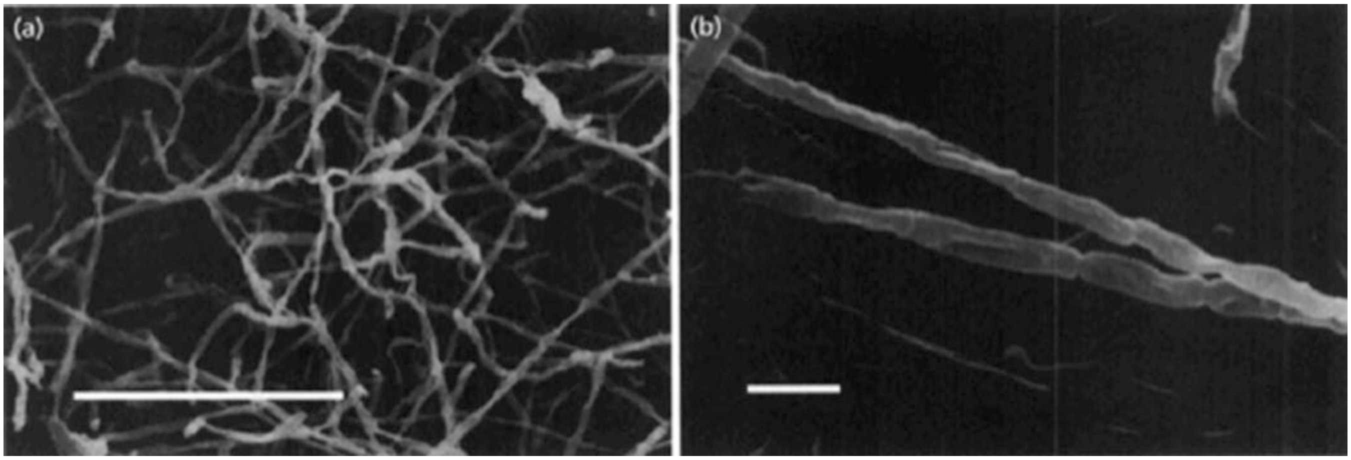

FIGURE 270. Aerial mycelium of *Kribbella flavida* on oatmeal agar (ISP 3 medium). Scanning electron micrographs. Bars = 10 µm (a) and 1 µm (b). (Reproduced with permission from Park et al., 1999. Int. J. Syst. Bacteriol. *49*: 743–752.)

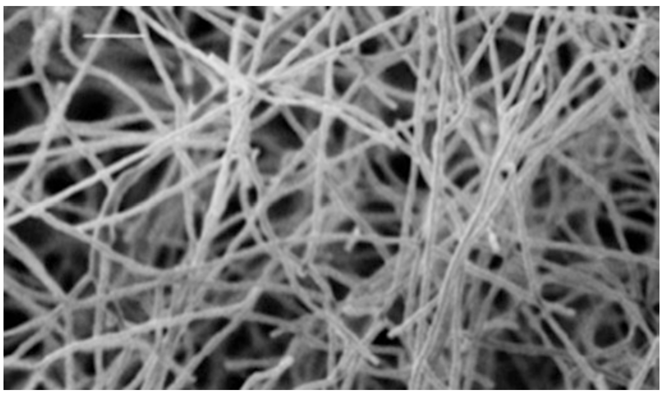

FIGURE 271. Aerial mycelium of *Kribbella swartbergensis*, 14-d-old culture on oatmeal agar (ISP 3). Scanning electron micrograph. Bar = 3 µm. (Reproduced with permission from Kirby et al., 2006. Int. J. Syst. Evol. Microbiol. *56*: 1097–1101.)

The aerial mycelium, if present, is mostly white or pale yellow and consists of straight or curved branched hyphae (Figure 270 and Figure 271). Well-developed and extensively branching substrate mycelium is typically white, off-white, pale yellow, cream, or rarely orange (Table 225). Both the aerial and substrate hyphae, depending on the individual organism and the consistency and composition of growth medium, undergo various degrees of fragmentation to yield nonmotile elongated, Y-shaped, and irregular rod-like elements with square ends (Figure 272, Figure 273, and Figure 274). Shorter fragments often become rounded-off and have the appearance of arthrospores at maturation. Hyphae in many areas may remain stable and not fragmented *in situ* on agar or in liquid media. Short chains of well-to-poorly differentiated conidia can be observed on aerial hyphae. Some organisms may produce bud-like cells on substrate hyphae (Figure 274). Hyphae of many organisms have a tendency to form apical swellings (Lee et al., 2000; recent observation of the authors of this chapter). Spherical or irregularly-shaped structures (up to 3–5 µm in diameter) can be produced on substrate hyphae of some organisms, most abundantly on agar media rich in organics when aerial mycelium is absent (recent observations). These consist of tightly packed, irregularly sized polymorphic cells remaining closely associated with each other after cell division and embedded in a common matrix of extracellular polysaccharide-like material. Both the hyphal fragments (arthrospores) and the cells produced by different division modes usually give rise to new vegetative hyphae when transferred to a fresh medium.

Growth may be pasty, sand-pasty, soft-leathery, or leathery, depending on species, growth media, and growth phase. Diffusible pigments are usually absent, except for melanoid pigments, which are produced on tyrosine agar (ISP 7 medium*) (Table 225) and occasionally on peptone-yeast extract agar (ISP 6 medium). The cell-wall architecture of *Kribbella* species is typical of Gram-positive bacteria (recent observation), but the Gram-staining test may show variable results (Park et al., 1999).

Chemotaxonomy. All *Kribbella* species are characterized by the presence of LL-diaminopimelic acid (LL-A_2pm) as the diamino acid in the cell wall. The peptidoglycan type is A3γ according to classification of Schleifer and Kandler (1972), as follows from the peptidoglycan amino acid composition [alanine, LL-2,6-diaminopimelic acid (LL-A_2pm), glutamic acid, and glycine] of the total cell-wall hydrolysate, and specific dipeptides (L-Ala–D-Glu, LL-A_2pm–D-Ala and LL-A_2pm–Gly) found in the partial cell-wall hydrolysate of *Kribbella lupini* (Trujillo et al., 2006). The monomeric unit of the A3γ polymer type contains L-alanine in the first position and LL-A_2pm in the third position of the tetrapeptide subunit, with a single glycine residue as an interpeptide bridge. The same peptidoglycan amino acids were reported for *Kribbella flavida*, *Kribbella sandramycini* (Park et al., 1999), *Kribbella koreensis* (Lee et al., 2000), *Kribbella solani*, *Kribbella jejuensis* (Song et al., 2004), and *Kribbella ginsengisoli* (Cui et al., 2010); Park et al. (1999) indicated the presence of both D- and L-alanine for *Kribbella flavida* and *Kribbella sandramycini*.

The sugar patterns determined in the cell walls or whole organisms of the recognized *Kribbella* species include combinations of galactose, glucose, mannose, ribose, xylose, and

*See Shirling and Gottlieb (1966) for the composition of ISP media cited here and in other sections of this chapter.

TABLE 225. Differential characters useful for preliminary identification of *Kribbella* species[a,b]

Characteristic	1. *K. flavida*	2. *K. alba*	3. *K. aluminosa*	4. *K. antibiotica*	5. *K. catacumbae*	6. *K. ginsengsoli*	7. *K. hippodromi*	8. *K. jejuensis*	9. *K. karoonensis*	10. *K. koreensis*	11. *K. lupini*	12. *K. sancticallisti*	13. *K. sandramycini*	14. *K. solani*	15. *K. swartbergensis*	16. *K. yunnanensis*
Aerial mycelium[c]	White	White to soft yellow	White	Yellow white	White	White	White	White	White, pale cream	White	White	White	White	White	White	White to moderate yellow
Substrate mycelium[c]	Pale yellow	Pale yellow to soft yellow	Beige to pale yellow	Yellow white to yellow	Yellow to yellow-orange[d]	Cream	Cream	Cream	Cream to yellow	Cream	Cream	Cream	Pale yellow	Cream	Cream	Yellow white to yellow
Melanin production	+	–	–	+	+	nr	–	–	–	+	nr	w	–	–	–	w
Anaerobic growth	w[e]	nr	nr	–[e]	–[f]	nr	–[e]	w[e]	–[e]	w[e]	nr	–[f]	w[e]	+[e,f]	–[e]	nr
Maximal NaCl (%)[g]	3[h]	nr	2	<2	3	4	6	1–2[i]	4	2	7	5	4	5	4	nr
Growth at 37°C	v	nr	+	–	–	+	+	+	+	v	+	+	v	–	+	–
Growth at 45°C	–	nr	–	–	–	–	–	–	–	–	–	–	–	–	+	–
Growth at initial pH 5[g]	v	nr	+	–	+	–	+	–	+	–	–	+	+	–	v	–
Growth at initial pH 9[g]	+	–	–	–	+	–	v	–	+	+	+	+	+	–	+	–
Oxidase	+	nr	–	+	+	+	nr	nr	nr	–	+	+	+	nr	nr	nr
Nitrate reduction	+	–	–	–	–	–	+	–	+	v	–	+	–	v	+	–
H_2S production	–	–	+	–	nr	–	+	nr	+	+	nr	nr	–	nr	+	–
Growth on sole carbon sources:[j]																
L-Arabinose	–	+	+	+	+	+	+	+	–	+	+	+	w	+	w	+
D-Galactose	–	+	nr	+	+	+	+	+	nr	+	+	+	+	–	nr	–
D-Xylose	–	+	+	+	+	+	–	+	+	+	nr	+	+	+	w	+
Inositol	+	+	+	+	–	+	+	–	+	+	nr	–	+	v	w	+
Mannitol	+	+	+	+	+	+	+	–	+	+	+	+	+	v	w	+
Degradation of:[k]																
Casein	+	–	+	+	+	+	+	v	+	+	+	+	+	v	+	–
Gelatin	–	+	nr	+	+	+	+	+	+	+	+	+	+	nr	+	–
Hypoxanthine	–	nr	+	+	w	nr	+	v	+	+	nr	+	–	v	+	nr
Starch	–	+	+	+	–	w	w	v	+	v	–	–	v	v	+	+
Tween 80	+	nr	+	nr	+	nr	+	–	w	–	nr	+	+	+	+	nr
Urea	+	nr	+	nr	–	–	–	+	+	+	–	–	+	–	–	nr
DNA G+C content (mol%)	70	67.9	nr	67	nr	66.3	nr	68	nr	71.3	68	nr	68.3	69	nr	68.6

Sugar composition[l]	Man, Glu, Gal	Glu, Gal, Rib	Glu, Man, Rib	Glu, Xyl, Rib	nr	Gal, Rib, Xyl	Glu, US1	Man, Glu, Gal Rib	Man, Rib	Man, Glu, Gal, Rib	Gal, US2	nr	Man, Glu, Gal	Man, Glu, Gal, Rib	Man, Rib	Man, Glu, Gal, Rib
Principal phospholipids	PC, DPG, PG, PI	PC, DPG, PG, PI	PC, DPG, PG, PI [m]	PC, DPG, PG, PI	PC, DPG, PG, PI[m]	PC, DPG, PG, PI	nr	PC, DPG, PI	nr	PC, DPG, PG, PI	PC, DPG, PG, PI	PC, DPG, PG, PI [m]	PC, PG, DPG, PI	PC, DPG, PI	nr	PC, PG, DPG
Source of isolation	Soil	Soil	Medieval alum slate mine	Soil	Catacomb, deteriorated surface	Soil	Soil	Soil	Soil	Gold-mine cave	Root of lupine	Catacomb, tufacean surface	Soil	Potato tuber	Soil	Soil

[a]Based on characteristics of the type strains, except for *Kribbella aluminosa*, *Kribbella catacumbae*, and *Kribbella sancticallisti*. Symbols and abbreviations: +, positive; −, negative; w, weakly positive or delayed; v, variable among experiments or methods used (sometimes because of weak growth or reaction); Glc, glucose; Gal, galactose; Man, mannose; Rib, ribose; Xyl, xylose, US1 and US2, unidentified sugars; PC, phosphatidylcholine; PG, phosphatidylglycerol; DPG, diphosphatidylglycerol; PI, phosphatidylinositol; nr, Not reported.

[b]Data from: Park et al. (1999), Lee et al. (2000), Sohn et al. (2003), Song et al. (2004), Li et al. (2004, 2006), Kirby et al. (2006), Trujillo et al. (2006), Carlsohn et al. (2007), Urzì et al. (2008), Everest and Meyers (2008), and Cui et al. (2010).

[c]According to the original descriptions; color may slightly change depending on the growth medium and the culture age.

[d]Dark orange in old colonies on Luedemann medium (Luedemann, 1968).

[e]Obtained on ATCC Medium No. 172 (Cote et al., 1984) according to Kirby et al. (2006) and Everest and Meyers (2008).

[f]Tested on VL agar (Tiecco, 1975) according to Urzì et al. (2008).

[g]Tested on solidified or/and in liquid media of different composition (see the text for details).

[h]Obtained on nutrient agar as basal medium (Trujillo et al., 2006); no growth on ISP 2 as basal medium (Kirby et al., 2006).

[i]Different results reported (Carlsohn et al., 2007; Kirby et al., 2006; Song et al., 2004) are likely influenced by different test conditions.

[j]ISP 9 (Shirling and Gottlieb, 1966) as basal medium, except for *Kribbella ginsengisoli* tested on a minimal salts medium with vitamins and microelements, or using the API API 20NE and ID 32GN test systems (Cui et al., 2010).

[k]See the original species description for test methods.

[l]Determined in whole cells or purified cell walls. Cui et al. (2010) reported glucosamine instead of glucose for *Kribbella flavida, Kribbella koreensis, Kribbella sandramycini* and *Kribbella yunnanensis*, and mannose instead of glucose for *Kribbella alba* and *Kribbella antibiotica*.

[m]Unidentified phospholipids and glycolipids reported in addition (Carlsohn et al., 2007; Urzì et al., 2008).

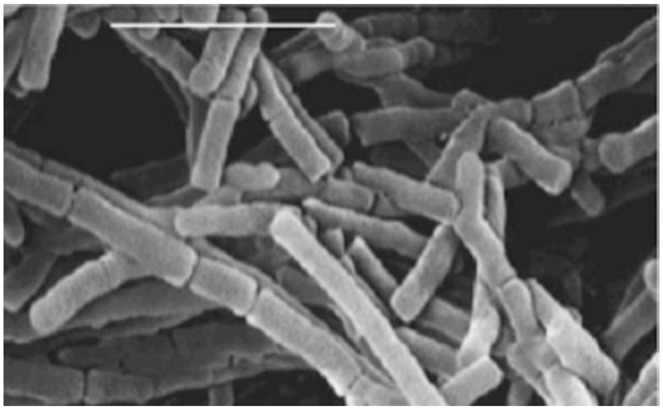

FIGURE 272. Fragmented aerial mycelium of *Kribbella koreensis*, 14-d-old culture on inorganic salts/starch agar (ISP medium 4). Scanning electron micrograph. Bar = 2 μm. (Reproduced with permission from Lee et al., 2000. Int. J. Syst. Evol. Microbiol. *50*: 191–199.)

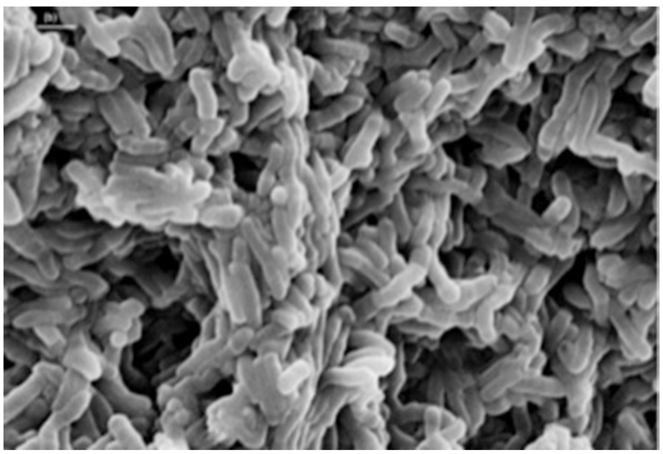

FIGURE 273. Fragmented substrate mycelium of *Kribbella karoonensis*, 14-d-old culture on oatmeal agar (ISP 3 medium). Samples were prepared by placing agar blocks containing substrate mycelium in liquid nitrogen and freezing prior to viewing by cryo-SEM. Bar = 3 μm. (Reproduced with permission from Kirby et al., 2006. Int. J. Syst. Evol. Microbiol. *56*: 1097–1101.)

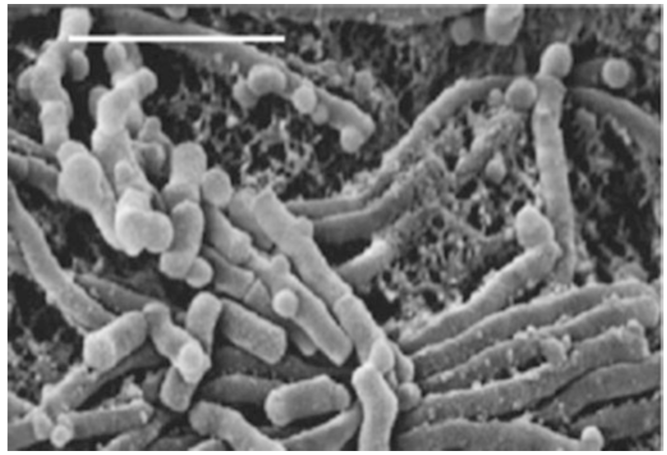

FIGURE 274. Irregularly fragmented substrate mycelium of *Kribbella koreensis* with bud-like cells, 14-d-old culture on inorganic salts/starch agar (ISP medium 4). Scanning electron micrograph. Bar = 2 μm. (Reproduced with permission from Lee et al., 2000. Int. J. Syst. Evol. Microbiol. *50*: 191–199.)

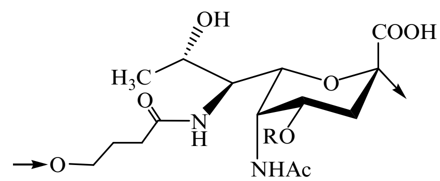

FIGURE 275. Repeating unit of teichulosonic acid of strain *Kribbella* sp. VKM Ac-2541, where R = H (45%), α-D-Gal*p*3OMe (37%) or α-D-Gal*p*2,3OMe (18%).

unidentified sugars (Table 225). In addition, 3-*O*-methyl-D-galactose (madurose), 2,3-di-*O*-methyl-D-galactose, and rhamnose were found in several recently analyzed *Kribbella* strains which supposedly (based on 16S rRNA gene sequence analysis and phenotypic traits) represent at least four novel species (Shashkov et al., 2009; E.M. Tul'skaya and L.I. Evtushenko, unpublished results). In traditional terms (Lechevalier and Lechevalier, 1970), neither individual cell-wall sugars nor sugar pattern are characteristic of all organisms of the genus *Kribbella*. However, the sugars, along with other compounds derived from the cell-wall polysaccharides linked to the peptidoglycan, can serve as chemical markers of individual species or species groups within the genus. Recent studies performed with six of the aforementioned novel *Kribbella* strains revealed that each strain contained two cell-wall polysaccharides, i.e. an acidic phosphate-less polymer as the predominant one and a neutral polysaccharide, a mannan, found in minor or trace amounts (Shashkov et al., 2009; Tul'skaya, 2009). Notably, the mannan (the basic chain built of 1–6-linked α-mannopyranose substituted at O-2 with α-mannopyranose) was common to all six strains studied. The predominant acidic polymers, in contrast, differed to a greater or lesser extent, and were represented either by different teichuronic acids (Ward, 1981) or unusual polysaccharides, the so-called teichulosonic acids (polymers with nonulosonic acids; Knirel, 2009; Knirel et al., 1987, 2003). The disaccharide subunits of the two identified teichuronic acids consisted of aminomannuronic acid and a rare sugar, 2,3-diacetamido-2,3-dideoxy-α-glucopyranose (strain VKM Ac-2539), or of the same sugar and diaminomannuronic acid (strains VKM Ac-2538 and VKM Ac-2540). The subunit of teichulosonic acid of strain VKM Ac-2541 contained in the basic chain a residue of oxybutyrate and pseudaminic acid, a sialic acid-like nine-carbon compound (5,7-diacylamido-3,5,7,9-tetradeoxy-L-glycero-β-L-manno-nonulosonic acid; Knirel et al., 2003; Vimr et al., 2004) with madurose and 2,3-di-*O*-methyl-D-galactose as irregular substituents at O-4 (Figure 275). The teichulosonic acids of the two other strains had the same structure as in strain VKM Ac-2541 plus rhamnose as an additional substituent, or the polymer subunit contained additional galactose (unknown position). Remarkably, neither teichoic acids nor other related phosphorous-containing polysaccharides have been revealed in the cell walls of more than 15 *Kribbella* strains examined up to now (Shashkov et al., 2009; E.M. Tul'skaya, A.S. Shashkov, and L.I. Evtushenko, unpublished), in contrast to organisms of the genera *Nocardioides* and *Aeromicrobium* (Naumova et al., 2001; Takeuchi and Yokota, 1989; Tul'skaya, 2009). Also, although

pseudaminic acid (or its derivatives) is common in capsular polysaccharides, lipopolysaccarides, pili, and flagella of many Gram-negative bacteria (see, e.g. Knirel, 2009; Knirel et al., 1987, 2003; Schoenhofen et al., 2006; Vimr et al., 2004), it has not been found until recently in Gram-positive bacteria except kribbellae.

The predominant menaquinone is MK-9(H_4) in all bacteria of the genus *Kribbella* (Table 226) and may represent up to 93% of the total quinone content as reported for *Kribella aluminosa* (Carlsohn et al., 2007), or even more. The cellular fatty acids of *Kribbella* species are dominated by the saturated iso- and anteiso branched acids, mainly $C_{15:0}$ anteiso, $C_{16:0}$ iso, and $C_{15:0}$ iso. Among these, $C_{15:0}$ anteiso was the predominant component in all species (usually contributing about 30%), except *Kribbella sancticallisti* and *Kribbella swartbergensis* which were reported to contain $C_{16:0}$ iso in maximal proportions (~24–27%) (Carlsohn et al., 2007; Urzì et al., 2008). Monounsaturated *iso*-branched (mostly $C_{17:1}$ iso) as well as saturated 9-methyl branched (mostly 9-methyl-$C_{16:0}$) fatty acids typically occur and may reach 10% or more of the total fatty acids. Other components, including 2-hydroxy (mostly $C_{16:0}$ iso 2-OH) and 10-methyl branched acids ($C_{18:0}$ 10-methyl and $C_{17:0}$ 10-methyl) were reported for some species in minor amounts. Notably, the qualitative and quantitative compositions of fatty acids in strains of this genus may depend on the growth conditions, the culture age, and analytical procedure employed, as demonstrated, e.g. for *Kribbella flavida* and *Kribbella sandramycini* (Park et al., 1999; Sohn et al., 2003; Song et al., 2004; Trujillo et al., 2006).

The principal phospholipids of most *Kribbella* species include PC, DPG, PG, and PI. PG was not reported in *Kribbella jejuensis* and *Kribbella solani* (Song et al., 2004), while PI was not mentioned among main polar lipids of *Kribbella yunnanensis* (Li et al., 2006). On the other hand, an additional phospholipid (tentatively identified as phosphatidylinositol containing 2-hydroxy fatty acids) and some unidentified minor phospholipids occur in all 15 yet-unnamed *Kribbella* strains analyzed (N.G. Vinokurova and L.I. Evtushenko, unpublished data). In addition, one to three glycolipids may present in lesser but significant amounts in *Kribbella* strains, together with a variety of minor or trace amounts of unidentified phospho-, glyco-, and other kinds of polar lipids (Figure 263 in the chapter Genus *Nocardioides*).The clearly detectable glycolipids have chromatographic mobility close to that of PC and DPG. Several unknown minor phospho- and glycolipids, along with PC, DPG, PG, and PI, were also reported for *Kribbella aluminosa* (Carlsohn et al., 2007), *Kribbella catacumbae*, and *Kribbella sancticallisti* (Urzì et al., 2008).

Nutrition and growth conditions. The *Kribbella* strains usually grow well under aerobic conditions at neutral pH or mildly alkaline pH on standard laboratory media, including the chemically defined media, e.g. ISP media used to culture streptomycetes. Kribbellae also utilize a wide range of other organic compounds as carbon sources in ISP9 or other minimal salts media, and possess various enzymatic activities towards complex macromolecules resulting in production of low molecular weight compounds which can be used as carbon (and/or nitrogen) sources (Table 225 and Table 226; see the section *List of species of the genus Kribbella*). *Kribbella ginsengsoli* was reported (Cui et al., 2010) to grow on a minimal medium containing basal salts, a vitamin solution (Widdel and Bak, 1992), trace elements (SL-10; Widdel et al., 1983), selenite/tungstate solution (Tschech and Pfennig, 1984), and a definite carbon source (0.1%). Acids are rather weakly produced by kribbellae under aerobic conditions from carbohydrates as shown for *Kribbella koreensis* (Lee et al., 2000) and some other species. Optimal temperatures for growth vary insignificantly among species, but all organisms of this genus grow well between 25 and 30°C. The higher and lower growth temperatures reported are 45°C (*Kribbella swartbergensis*; Everest and Meyers, 2008) and 8°C (*Kribbella ginsengisoli*; Cui et al., 2010). Members of the genus *Kribbella* usually prefer neutral or slightly alkaline pH for growth. The pH growth range varies with species (Table 225 and Table 226). A few species can grow at initial pH of 5; *Kribbella hippodromi* showed weak growth even at pH 4.3 (Everest and Meyers, 2008). Some species grow at initial pH 10 (*Kribbella catacumbae*, *Kribbella koreensis*, and *Kribbella sancticallisti*; Lee et al., 2000; Urzì et al., 2008). Bacteria of this genus are typically non-halophilic and tolerate low NaCl concentrations; *Kribbella antibiotica* and *Kribbella jejuensis* are the most sensitive to salts (cells grow in 2% NaCl; Carlsohn et al., 2007; Kirby et al., 2006; Song et al., 2004).

It should be noted that actinomycetes of the genus *Kribbella* were originally described as chemo-organotrophs with strictly aerobic metabolism (Lee et al., 2000; Park et al., 1999). However, some species were subsequently shown to grow moderately well (*Kribbella solani*) or weakly (*Kribbella flavida*, *Kribbella jejuensis*, *Kribbella koreensis*, and *Kribbella sandramycini*) on ATCC Medium No. 172* anaerobically in an atmosphere of $H_2/CO_2/N_2$ (5:10:85) (Kirby et al., 2006). According to Urzì et al. (2008), *Kribbella solani* also grew anaerobically on VL agar (Tiecco, 1975). Some yet-unnamed *Kribbella* strains exhibit weak but distinct growth in organic semi-solid medium (0.5% glucose, 0.5% peptone, 0.3% yeast extract, 0.02% K_2HPO_4, 0.3% agar; pH 7.2) under anaerobic and/or microaerophilic conditions; L.M. Baryshnikova and L.I Evtushenko, recent observation). None of the tested kribbellae grew anaerobically on ISP 9 agar with glucose (Kirby et al., 2006). On the other hand, weak aerobic growth was observed for some yet-unnamed *Kribbella* strains on ISP 9 medium without addition of a carbon source (recent observation). *Kribbella koreensis*, when seeded with a diluted soil suspension (during the isolation procedure), showed the ability to grow aerobically on a tap-water agar with antifungal antibiotics (Lee et al., 2000). The data suggest that members of the genus *Kribbella* can adapt to an oligotrophic life style and grow in the presence of traces of carbon and/or nitrogen sources, and probably may scavenge nutrient substances from air, e.g. ammonium. Perhaps, some organisms may even have an autotrophic life style under certain conditions, like that reported or assumed, e.g. for some *Pseudonocardia* and *Streptomyces* (Goodfellow and Lechevalier, 1986; O'Donnell et al., 1993; Selesi et al., 2005; Takamiya and Tubaki, 1956). Recent finding showing that kribbellae increase in numbers in a soil microcosm after hydrogen exposure is indicative of their possible ability to utilize hydrogen as a source of energy (Osborne et al., 2010). All the above data, along with data showing that *Kribbella* often occur in nutrient-limited environments, including acidic and heavy-metal-contaminated ones (see below the section Habitats and ecology), suggest that at least certain organisms of this genus are rather aerobic chemo-organotrophs with a potential for mixotrophy and metabolic flexibility.

* *Hongia* Lee, Kang and Hah 2000, 197[VP] is a later heterotypic synonym of *Kribbella* Park, Yoon, Shin, Suzuki, Kudo, Seino, Kim, Lee and Lee 1999, 750[VP]

TABLE 226. Additional differentiating and descriptive characteristics of *Kribbella* species[a]

Characteristic	1. *K. flavida*	2. *K. alba*	3. *K. aluminosa*	4. *K. antibiotica*	5. *K. catacumbae*	6. *K. ginsengsoli*	7. *K. hippodromi*	8. *K. jejuensis*	9. *K. karoonensis*	10. *K. koreensis*	11. *K. lupini*	12. *K. sancticallisti*	13. *K sandramycini*	14. *K. solani*	15. *K. swartbergensis*	16. *K. yunnanensis*
Predominant menaquinone[b]	$9(H_4)$	$9(H_4)$	$9(H_4)$	$9(H_4)$	$9(H_4)$	$9(H_4)$	nr	$9(H_4)$	nr	$9(H_4)$	$9(H_4)$	$9(H_4)$	$9(H_4)$	$9(H_4)$	nr	$9(H_4)$
pH growth range	(5.0)–9.0	5.5–8.5	5.0–9.0	nr	5.0–(10.0)	5.5–8.5	(4.3)–(9.0)	5.5–8.5	5.0–9.9	7.0–10.0	6.0–9.0	5.0–10.0	5.0–9.0	5.5–8.5	(5.0)–9.0	5.5–8.5
Catalase reaction	+	+	+	nr	+	+	nr	+	+	+	+	+	+	+	+	+
Growth on sole carbon sources:[c]																
D-Cellobiose	nr	nr	nr	nr	nr	+	+	+	nr	+	+	nr	nr	+	nr	nr
D-Fructose	v	+	+	+	+	+	+	nr	+	+	nr	+	+	nr	+	+
D-Glucose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lactose	v	+	nr	+	+	+	w/–	+	+	+	nr	+	v	+	w	+
Maltose	+	+	nr	+	+	+	+	nr	w/–	+	–	+	+	nr	+	+
D-Mannose	v	+	nr	+	+	+	+	–	+	+	+	+	v	+	w	+
D-Melezitose	+	nr	nr	nr	nr	nr	+	+	nr	+	–	nr	+	+	nr	nr
Melibiose	+	nr	nr	nr	nr	+	+	+	–	+	+	nr	+	+	+	nr
Raffinose	v	+	+	+	w	+	+	+	+	+	+	+	+	+	w	+
L-Rhamnose	v	+	+	+	+	nr	+	+	+	+	+	+	+	+	w	+
Sucrose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Trehalose	nr	nr	nr	nr	nr	+	+	+	nr	+	+	nr	nr	+	nr	nr
Adonitol	+	nr	nr	nr	nr	–	+	–	w/–	+	nr	nr	+	v	nr	nr
Glycerol	–	+	nr	+	+	+	+	nr	+	+	nr	+	+	+	w	+
Sorbitol	v	+	nr	+	w	–	nr	nr	w	v	+	–	v	nr	+	+
Xylitol	–	nr	nr	nr	nr	nr	–	–	nr	–	+	nr	+	–	nr	nr
Inulin	+	nr	nr	nr	nr	–	+	+	–	+	nr	nr	+	–	–	nr
N-Acetyl-β-glucosamine	nr	nr	nr	nr	+	+	nr	nr	nr	v	+	+	nr	nr	nr	nr
Utilization of organic acids:[d]																
Acetate	–	+	+	+	nr	+	w	nr	w/–	+[e]	nr	nr	–	nr	w	+
Citrate	v	–	+	+	–	+	w	nr	w/–	+[e]	–	–	–	nr	w	+
Lactate	nr	nr	nr	nr	nr	+	nr	nr	w/–	+[e]	nr	nr	nr	nr	w	nr
Malate	nr	nr	+	nr	nr	+	nr	nr	nr	+[e]	+	+	nr	nr	nr	nr
Oxalate	+	+	nr	+	nr	+	nr	nr	nr	+[e]	nr	nr	–	nr	w	+
Succinate	+	nr	+	nr	nr	+	w	nr	+	+[e]	nr	nr	+	nr	w	nr
Degradation of:																
Adenine	nr	nr	+	nr	nr	nr	+	–	+	–	nr	nr	nr	v	+	nr
Esculin	+	nr	+	nr	+	nr	+	+	+	+	+	+	+	+	+	nr
Arbutin	+	nr	nr	nr	+	nr	+	+	+	+	+	w	+	+	+	nr
Cellulose	–	–	–	–	–	–	–	nr	nr	–	nr	nr	–	nr	nr	–
Elastin	+	nr	nr	nr	w	nr	nr	–	nr	+	nr	+	+	–	nr	nr

Tyrosine	nr	nr	+	nr	+	nr	+	−	+	+	+	+	nr	v	+	nr
Xanthine	+	nr	+	nr	nr	nr	−	−	nr	+	nr	w	nr	−	−	nr
Xylan	nr	nr	nr	nr	nr	−	−	−	nr	nr	−	nr	nr	−	nr	nr
API ZYM (bioMérieux):																
Cystine arylamidase	+	nr	−	nr	nr	nr	nr	w	nr	+	w	nr	+	w	+	nr
Esterase (C4)	+	nr	+	nr	nr	nr	nr	nr	nr	+	−	nr	+	nr	nr	nr
α-Fucosidase	nr	nr	+	nr	nr	nr	nr	+	nr	nr	+	nr	nr	−	−	nr
β-Galactosidase	+	nr	+	nr	nr	nr	nr	nr	nr	+	w	nr	+	nr	nr	nr
Lipase (C14)	nr	nr	−	nr	nr	nr	nr	−	nr	nr	+	nr	nr	+	+	nr
Naphthol-AS-BI-phosphohydrolase	+	nr	+	nr	nr	nr	nr	nr	nr	−	−	nr	+	nr	nr	nr

[a]See Table 225 for symbols, for references and other details. Data in parentheses (for the pH growth range) indicate variable, weak or delayed growth; w/−, doubtful growth.

[b]9(H_4), menaquinone having nine isoprene units two of which are saturated.

[c]ISP 9 (Shirling and Gottlieb, 1966) as basal medium. See Table 225 for *Kribbella ginsengisoli*; positive results were also obtained for *Kribbella koreensis* in the same study (Cui et al., 2010) with D-glucose, lactose, maltose, D-mannose, D-melibiose, L-rhamnose, and sucrose.

[d]Growth with the listed organic acids as sole carbon sources on basal medium ISP 9 (Shirling and Gottlieb, 1966), unless other methods indicated.

[e]An alkaline reaction, according to the method of Gordon et al. (1974); growth also occurs with acetate, lactate, or malate, but not with citrate (API 20NE; Cui et al., 2010).

Genomic characteristics. The DNA base ratio of species of the genus *Kribbella* ranges from 66.3 mol% (Cui et al., 2010) to 71.3 mol% (Lee et al., 2000), with 70 mol% reported for the type strains of the type species *Kribbella flavida* (Park et al., 1999). As mentioned before, the current 16 *Kribbella* species form a coherent phylogenetic cluster, with some species showing more than 99% 16S rRNA gene sequence similarity. The DNA–DNA similarities between *Kribbella* species obtained by different methods ranged from 0% for the pair *Kribbella koreensis*–*Kribbella sandramycini* (Sohn et al., 2003), which looks rather ambiguous, to 61.2% for *Kribbella yunnanensis*–*Kribbella sandramycini* (Li et al., 2006). Recently, the sequences of *gyrB* gene (encoding the β-subunit of DNA gyrase, a type II DNA topoisomerase) have been analyzed for strains of the 15 recognized *Kribbella* species (Kirby et al., 2010). The type strains of *Kribbella* species had partial *gyrB* gene sequence (1108 nt, 56% of the full-length *gyrB*) similarity values in the range 89.91–94.95%, except for the pair *Kribbella solani*–*Kribbella hippodromi* (98.22%).

The 16S–23S rDNA internally transcribed spacer (ITS) sequences and the ribonuclease P RNA gene sequences were analyzed for *Kribbella flavida* and *Kribbella sandramycini* (Yoon et al., 1998a; Yoon and Park, 2000). The type strain of *Kribbella sandramycini* was reported to contain two types of 16S–23S ITS sequences (*rrn* 1, 429 bp; *rrn* 2, 439 bp) showing high sequence divergence, with the nucleotide similarity level of 80.8% (and 78.4% when gaps are included). The type strain of *Kribbella flavida* exhibits a higher degree of ITS sequence similarity to that of *Kribbella sandramycini* (75.4%, *rrn* 1; 73.6%, *rrn* 2) than to the ITS sequences of representatives of *Nocardioides* and *Aeromicrobium* used in the study. The same *Kribbella* strains shared 92±2% similarity of the ribonuclease P RNA gene sequences and formed a cluster distinct from the strains of other genera analyzed (Yoon and Park, 2000). Gao and Gupta (2005) revealed in *Kribbella sandramycini*, along with an insertion in the 23S rRNA gene, three conserved indels in three widely distributed proteins, i.e. a deletion in cytochrome *c* oxidase subunit 1, an insertion in CTP synthetase, and an insertion in glutamyl-tRNA synthetase that are distinctive characteristics of most *Actinobacteria* and not found in any other groups of bacteria. The phylogenetic analysis based on CTP synthetase sequences showed that the overall topology of the tree of actinobacteria studied was very similar to that seen in the 16S rRNA gene trees. One of the interesting exceptions relevant to this chapter is *Kribbella sandramycini*, which is grouped with *Saccharopolyspora erythrea* DSM 40517 (not with *Nocardioides simplex*) and has an almost identical CTP synthetase sequence to that of *Saccharopolyspora erythrea* DSM 40517 (Gao and Gupta, 2005). Recently, the sequence of the complete genome for *Kribbella flavida* DSM 17836 (a circular chromosome of 7,579,488 nt, 7086 genes with a protein-coding capacity, 60 RNA genes, and 143 predicted pseudogenes) was determined (Pukall et al. 2010, GenBank accession no. NC_013729). The majority of the protein-coding genes (70.7%) were assigned with a putative function while those remaining were annotated as hypothetical proteins).

Antibiotic sensitivity and antibiotic production. Strains of *Kribbella flavida*, *Kribbella antibiotica*, *Kribbella jejuensis*, *Kribbella karoonensis*, *Kribbella koreensis*, *Kribbella sandramycini*, *Kribbella solani*, and *Kribbella swartbergensis* were reported to be sensitive to ampicillin sodium salt (100 μg/ml) and also (except *Kribbella jejuensis*) to cefotaxime (10 μg/ml) (Kirby et al., 2006). Except for *Kribbella solani*, all the above strains, as well as *Kribbella catacumbae* and *Kribbella sancticallisti*, are inhibited by tobramycin (10 μg/ml) (Kirby et al., 2006; Urzì et al., 2008). All the above-mentioned species are resistant to carbenicillin (100 μg/ml) and, except *Kribbella sancticalisti* and *Kribbella ginsengisoli*, and to streptomycin (10 μg/ml) (Cui et al., 2010; Kirby et al., 2006; Urzì et al., 2008). The listed and other members of this genus are sensitive to a wide range of other antibiotics (see the *List of species of the genus Kribbella*). A few *Kribbella* strains were reported to produce biologically active compounds. *Kribbella sandramycini* ATCC 39419 produces an antitumor antibiotic, sandramycin (a cyclic decadepsipeptide with a two-fold axis of symmetry and 3-hydroxyquinaldic acid as an appended chromophore; Matson and Bush, 1989; Matson et al., 1993). *Kribbella koreensis*is produces novel neuropilin/growth factor complexes (Alitalo et al., 2003). *Kribbella antibiotica* possesses antifungal activity (Li et al., 2004) and *Kribbella jejuensis* inhibits growth of *Streptomyces scabiei* (Song et al., 2004).

Habitats and ecology. Kribbellae are widely distributed in terrestrial ecosystems worldwide, mostly in soils of different origin, including acidic ones (Ding et al., GenBank accession no. FJ938352; Zheng and Huang, GenBank accession no. EU697199) and polluted sites (Cho et al., 2006; Leigh et al., 2007). Representatives of *Kribbella* were found in the root zone of an Austrian pine (*Pinus nigra* L.) growing naturally in soil contaminated with polychlorinated biphenyls (PCBs) (Leigh et al., 2007). Furthermore, kribbellae along with *Pseudonocardia*, *Nocardiodes*, and *Sphingomonas* were the predominating representatives of 75 genera which utilized carbon from PCBs (Leigh et al., 2007). Organisms of this genus often occur in sub-subsurface and nutrient-limited environments, including gold-mine caves (Lee et al., 2000), an alum slate mine (Carlsohn et al., 2007), and catacombs (Urzì et al., 2008). Heterotrophic lifestyle and enzymatic activity towards complex macromolecules assume that kribbellae function as consumers of organic material in soil. In environments of nutrient scarcity, they probably use traces of organics dissolved in water or derived from the decomposition of other microorganisms, are involved in mutualistic interactions with other microorganisms, or engage in chemolithotrophic metabolism with input from some atmospheric gases and minerals, like those suggested for other actinomycetes from such environments (Barton et al., 2007; Groth and Saiz-Jimenez, 1999). Organisms of this genus and some other soil bacteria are assumed to be important utilizers of hydrogen at low concentrations in soil, and could be important contributors to the function of soil as a sink in the global hydrogen cycle (Osborne et al., 2010). Kribbellae also occur among microbial endophytes in plant tissues (Conn and Franco, 2004; Trujillo et al., 2006) and in bacterial communities associated with spores of the endomycorrhizal fungus *Gigaspora margarita* (L. Long, H. Zhu, and Q. Yao, GenBank accession no. EU589433). *Kribbella solani* was isolated from a potato tuber with scab lesions (Song et al., 2004), although there is no evidence of its plant pathogenic properties. Representatives of this genus were also found in the bacterial community of human skin (Kong et al., GenBank accession no. HM277981). To the authors' knowledge, kribbellae have not been discovered up to now in marine ecosystems or in clinical specimens. The spores or cells of *Kribbella* are thought to survive or maintain a low metabolic activity under extreme conditions in nature, since these remain alive in a soil sample (from which some species have been isolated) after exposure to temperature as high as 120°C for 1 h (dry heating) (Kirby et al., 2006).

Enrichment and isolation procedures

The available strains of the genus *Kribbella* are mostly random isolates obtained during studies on (actinobacterial) microbiota of certain biotopes or screenings for biologically active compounds. Strains of this genus are normally isolated by plating on suitable "total count" agar media, e.g. R2A agar (Difco), as well as those used for isolation of certain actinomycete groups. Incubation is usually carried out at 25–30°C for a week to a month. Generally, kribbellae relatively frequently occur among the mycelial soil isolates having sand-pasty or soft-leathery, agar-penetrating and non-pigmented, yellowish or light cream colonies developing on R2A agar. Other media, reported in the original species descriptions, e.g. glycerol-asparagine agar (ISP 5 medium), glucose-yeast extract-malt extract (ISP 2 medium), SM1 agar (proposed for isolation of *Amycolatopsis*; Tan et al., 2006), yeast extract-mannitol agar (used for isolation of *Rhizobia*; Vincent, 1970), tap-water agar, etc. were successfully employed for isolation of kribbellae. Various isolation strategies used to isolate mycelial actinomycetes, including air-drying and heating (up to 120°C for 1 h; Kirby et al., 2006) of soil samples, adding antifungal antibiotics, exposition in alkaline solutions, etc., may be used to reduce the numbers or inhibit the growth of accompanying microbes. When intending isolation of strains belonging or closely related to the described *Kribbella* species, some selective (semi-selective) media may be formulated on the basis of characteristics listed in Table 225 and Table 226, and a consideration of the resistance to certain antibiotics and heavy metal salts and the isolation sources.

Maintenance procedures

Cultures may be maintained as 20% glycerol suspensions at −20 and −80°C. Long-term conservation is achieved by freeze-drying or in liquid nitrogen by standard procedures.

Differentiation of the genus *Kribbella* from other genera

The phenotypic characteristics essential for delineation of *Kribbella* from other genera of the family are listed in Table 214. The formation of well-developed branching hyphae allows primary separation of *Kribbella* from the genera *Aeromicrobium*, *Marmoricola*, and the vast majority of *Nocardioides* species. The predominant menaquinone MK-9(H_4) and the presence of phosphatidylcholine among principal polar lipids clearly distinguish kribbellae from all species of the genus *Nocardioides*. The polar lipid pattern containing phosphatidylcholine is also the most distinctive feature that differentiates kribbellae both from the genera of the family *Nocardioidaceae* and other actinomycete genera comprising mycelium-forming organisms with LL-A_2pm in the cell wall. A rapid method based on single-digestion restriction analysis of the PCR-amplified 16S rRNA gene with endonucleases (*Mbo*I, *Vsp*I, *Sph*I, *Sna*BI, *Sal*I, and *Age*I), useful for identification of *Kribbella* strains, is described (Cook and Meyers, 2003).

Taxonomic comments

The genus *Kribbella* was established by Park et al. (1999) with *Kribbella flavida* (type species) and *Kribbella sandramycini* to accommodate two strains, which were previously ascribed to the genus *Nocardioides*, i.e. "*Nocardioides fulvus*" (Ruan and Zhang, 1979) and *Nocardioides* sp. ATCC 39419, a producer of the antibiotic sandramycin (Matson and Bush, 1989). Almost simultaneously, Lee et al. (2000) independently published the description of *Hongia koreensis* for an original isolate which was very close to *Kribbella* at the phylogenetic level and similar in many phenotypic characteristics. Subsequent taxonomic analysis of the above species resulted in the reclassification of *Hongia koreensis* as *Kribbella koreensis* (Sohn et al., 2003). The remaining 13 *Kribbella* species were described within the 2004–2010 period on the basis of taxonomic studies mostly of single environmental isolates. As mentioned before, some species of this genus are very close phylogenetically and share up to 99% or more 16S rRNA gene sequence similarity, with the highest value being between *Kribbella solani* and *Kribbella hippodromi* (99.64%) by local alignment (Everest and Meyers, 2008). The type strains of *Kribbella solani* and *Kribbella hippodromi* also display the closest identity (98.2%) by *gyrB* gene sequence similarity (Kirby et al., 2010). Nevertheless, these two species had a DNA–DNA similarity of approximately 40%, as revealed by hybridization experiments, and differed in a number of phenotypic properties (Everest and Meyers, 2008). Analogously, the DNA–DNA similarity values between the type strains of other phylogenetically very close species (see Cui et al., 2010, and Kirby et al., 2010, for details and references) are below the threshold value of 70% recommended for the definition of bacterial species (Wayne et al., 1987).

Differentiation of species of the genus *Kribbella*

The phenotypic properties useful in preliminarily distinguishing of *Kribbella* species are listed in Table 225. Additional differentiating and descriptive characteristics are given in Table 226 and outlined in the descriptions of species. It is worth noting that the available differential physiological and biochemical characteristics are based, in most cases, on the data obtained for a single representative of a species. Moreover, conflicting results have been reported for strains which give rather weak or doubtful growth on some carbon sources and showed very weak activities in degradation tests. Some differences in salt resistance and the pH growth range of a strain can be influenced by the test medium composition (Carlsohn et al., 2007; Cui et al., 2010; Kirby et al., 2006; Song et al., 2004; Trujillo et al., 2006). Chemotaxonomic features such as the composition of cell-wall sugars, polar lipids, and fatty acid profiles are important for differentiation purposes. It should be emphasized that whole cells but not cell walls were analyzed in most *Kribbella* species or the purified cell walls were obtained (and analyzed) by different methods, and this should be taken into account when comparing the sugar composition. Also the qualitative and quantitative compositions of fatty acids (and polar lipids) in strains of this genus may be influenced by growth conditions and analytical procedures. For the comparison of fatty acid profiles, the data should therefore be obtained solely by growing cells under standardized cultivation conditions and performing analyses in the same laboratory rather than from the literature. The distance dendrogram generated on the basis of fatty acid profiles by the software of the Sherlock Microbial Identification System may be useful to separate strains of phylogenetically related species (Urzì et al., 2008). Matrix Assisted Laser Desorption Ionization Time-of-Flight (MALDI/TOF) mass spectrometry of proteins and the RiboPrint analyses (with *Pvu*II as the restriction enzyme) are also quite helpful for clustering of phylogenetically close *Kribbella* strains at the strain-species level (Carlsohn et al., 2007; Urzì et al., 2008).

DNA–DNA hybridization studies may be necessary to support the species affiliation of a strain or strain group which is closely related to known species on the basis of 16S rRNA gene sequences or other characteristics. Analysis of *gyrB* gene sequences is advantageous to assess membership of a strain in question to an established species. According to Kirby et al. (2010), strains with *gyrB*-based genetic distance exceeding 0.04 to a known species (calculated from partial *gyrB* gene sequences, 1108 nt) most likely represent a distinct species. This value therefore is suggested as a cut-off point to determine whether DNA-DNA hybridization is required. A 390-nucleotide sequence of the *gyrB* gene (a variable region from 1010 to 1400 bp according to *Streptomyces avermitilis* MA-4680 *gyrB* numbering) is sufficient to preliminarily assess whether a strain is likely to represent a new species. The GenBank numbers for the *gyrB* gene sequences of *Kribbella* strains, PCR primers for amplification of the *Kribbella gyrB* gene, and the details of the method are available from Kirby et al. (2010).

Acknowledgements

The authors were supported by the program MCB of the Russian Academy of Sciences.

List of species of the genus *Kribbella*

1. **Kribbella flavida** Park, Yoon, Shin, Suzuki, Kudo, Seino, Kim, Lee and Lee 1999, 750[VP]

fla′vi.da. L. fem. adj. *flavida* yellowish, pale yellow.

Characteristics are as described for the genus and listed in Table 225 and Table 226. Additional information given below is taken from the original paper (Park et al., 1999), unless indicated.

Substrate mycelium is usually pale yellow and consists of extensively branched hyphae penetrating into agar media and often fragmenting into rod-shaped to coccoid elements. Aerial hyphae appear straight or curved and break up into short to elongated rod-like fragments (Figure 270). Colonies are usually pasty and have lichenous shapes. Melanin is produced on ISP 7 medium. Gram-staining reaction is variable. Grows at 12–30°C and at pH 9 (initial pH value of test media); growth at 37°C and at pH 5 varies with experiments (Kirby et al., 2006; Park et al., 1999; Trujillo et al., 2006). Grows at 3% NaCl on nutrient agar (Trujillo et al., 2006) but not on ISP 2 medium supplemented with the same salt concentration (Kirby et al., 2006). Produces pyrrolidonyl arylamidase (API ZYM; Trujillo et al., 2006).

Grows in the presence (µg/ml) of carbenicillin disodium salt (100), cephaloridine (10), chloramphenicol (50), cycloheximide (10), erythromycin (10), gentamycin sulfate (10, weak), and streptomycin sulfate (10). Growth is inhibited by ampicillin sodium salt (100 mg/ml), cefotaxime (10), neomycin sulfate (10), tobramycin sulfate (10), and vancomycin hydrochloride (10) (Kirby et al., 2006). The major fatty acids (>5% of the total registered at least in one experiment) reported for cultures grown under different conditions were $C_{15:0}$ anteiso (24.9–32), $C_{16:0}$ iso (14.0–21.3), $C_{16:1}$ iso (2–13.5), $C_{17:0}$ anteiso (3.2–10.0), $C_{14:0}$ iso (3–9.1), $C_{15:0}$ iso (5.0–5.4), $C_{17:1}$ iso ω9*c* (< 1–6.7), 9-methyl-$C_{16:0}$ (< 1–8) (Park et al., 1999; Sohn et al., 2003; Song et al., 2004), $C_{17:1}$ ω8*c* (10.6) (Song et al., 2004), and $C_{17:1}$ ω9*c* (7.0) (Sohn et al., 2003).

Source (type strain): soil near Beijing, China.

DNA G+C content (mol%): 70 (HPLC); 70.6% (calculated for the genome; Pukall et al., 2010).

Type strain: DSM 17836, KACC 20248, KCTC 9580, IFO (now NBRC) 14399, JCM 10339.

Sequence accession no. (16S rRNA gene): AY253863, AF005017.

Sequence accession no. (complete genome): NC_013729.

2. **Kribbella alba** Li, Wang, Zhang, Xu and Jiang 2006a, 1459[VP] (Effective publication: Li, Wang, Zhang, Xu and Jiang 2006b, 34.)

al′ba. L. fem. adj. *alba* white.

Characteristics are as described for the genus and listed in Table 225 and Table 226. Additional information given below is taken from the original paper (Li et al., 2006).

Substrate mycelium ranges from pale yellow on ISP 3, ISP 4, Czapek's agar, and potato agar to soft yellow on ISP 2 and nutrient agar. The hyphae are extensively branched and often fragment into irregular rod-shaped elements. Aerial mycelium is developed on all the media tested, and it is white (on ISP 3, 4, and 5 media and on potato agar), yellow-white (on ISP 2 and Czapek's agar) or pale yellow (on nutrient agar). Some areas of aerial hyphae fragment into short to elongated rod-like elements which can be arranged in chains. The major fatty acids (> 5% of the total) determined in cells grown on TSA at 28°C include $C_{15:0}$ anteiso (27.9), $C_{16:0}$ iso (15.3), $C_{15:0}$ iso (9.1), $C_{17:1}$ iso ω9*c* (10.4), and $C_{17:0}$ anteiso (6.1).

Source: soil in Yunnan Province, the west of China.

DNA G+C content (mol%): 67.9 (T_m).

Type strain: YIM 31075, CCTCC AA 001020, DSM 15500, JCM 14306, NBRC 103561.

Sequence accession no. (16S rRNA gene): AY082062.

3. **Kribbella aluminosa** Carlsohn, Groth, Spröer, Schütze, Saluz, Munder and Stackebrandt, 2007, 1946[VP]

a.lu.mi.no′sa. L. fem. adj. *aluminosa* aluminous, foil of alum, alum-containing, referring to the source of isolation of the first strains.

Characteristics are as described for the genus and listed in Table 225 and Table 226. Additional information given below is taken from the original paper (Carlsohn et al., 2007).

Extensively branched, beige to pale yellow substrate mycelium is produced on all media tested. White aerial mycelium varies in abundance and typically forms well on ISP 5 medium and less abundantly on ISP media 2, 3, and 4, but not on organic medium 79 (Prauser and Falta, 1968). Both vegetative and aerial hyphae fragment into irregular rod-shaped elements. Colonies on organic medium 79 are wrinkled and of a pasty consistency. Grows at 20 and 37°C; no growth occurs at 6 or 42°C. No growth is also observed

at pH 4.5 or 9.0 (initial pH values of liquid organic medium 79). Grows in the same medium with 2% NaCl but not with 4% NaCl. Growth occurs on minimal agar medium (Amoroso et al., 2000) in the presence of $NiCl_2$ (5.0 mM) or $CuSO_4$ (0.5 mM). Aconitate (delayed) is utilized as a sole carbon source, but benzoate and DL-tartrate are not. In API ZYM tests, positive reactions are observed for leucine arylamidase, valine arylamidase, α-galactosidase, α-glucosidase, β-glucosidase, esterase lipase (C8), α-mannosidase, *N*-acetyl-β-glucosaminidase, acid phosphatase, and alkaline phosphatase, but not β-glucuronidase or trypsin; weak or negative reaction for α-chymotrypsin. Hippurate is hydrolyzed. Indole is not produced. Susceptible to the following antibiotics (μg per disc): ampicillin (10), chloramphenicol (30), ciprofloxacin (5, weakly), imipenem (10), novobiocin (5, weakly), ofloxacin (10, weakly), oxytetracycline hydrochloride (30, weakly), as well as polymyxin B (300 IU per disc, weakly). Resistant to (μg per disc) lincomycin hydrochloride (2), methicillin (5), nalidixic acid (30), norfloxacin (10), and penicillin G (10 IU per disc). The major fatty acid (>5% of the total registered at least for one strain) as determined in three strains grown at 28°C in tryptic soy broth (TSB; Sigma-Aldrich) and harvested at exponential growth phase include $C_{15:0}$ anteiso (36.0–44.6), $C_{16:0}$ iso (7.5–17.6), $C_{15:0}$ iso (6.1–9.7), $C_{14:0}$ iso (2.7–6.1), $C_{17:0}$ anteiso (4.6–5.4), and 9-methyl-$C_{16:0}$ (7.0–8.7).

Source: acidic and heavy-metal-containing rock surfaces, a medieval alum slate mine in Thuringia, Germany.

DNA G+C content (mol%): not determined.

Type strain: HKI 0478, DSM 18824, JCM 14599.

Sequence accession no. (16S rRNA gene): EF126967.

4. **Kribbella antibiotica** Li, Wang, Zhang, Schumann, Stackebrandt, Xu and Jiang 2004a, 1425[VP] (Effective publication: Li, Wang, Zhang, Schumann, Stackebrandt, Xu and Jiang 2004b, 164.)

an.ti.bio′ti.ca. Gr. prep. *anti* against, in opposition to; Gr. n. *bios* life; L. suff. *-icus -a -um*, suffix used in adjectives with the sense of belonging to, related to; N.L. fem. adj. *antibiotica* related to antibiotic (the type strain of *Kribbella antibiotica* is an antifungal strain).

Characteristics are as described for the genus and listed in Table 225 and Table 226. Additional information given below is taken from the original paper (Li et al., 2004b), unless indicated.

Substrate mycelium is yellow white (on ISP 3, 4, and 5 media) or yellow (on potato agar), extensively branched, and often fragments into irregular rod-shaped elements. Aerial mycelium is developed on all the media tested, particularly on Czapek's agar and ISP 5 medium, and fragments into elongated to short rod-like elements. The color of aerial mycelium is yellow white on ISP 3, ISP 4, ISP 5, Czapek's agar, and nutrient agar, soft yellow on ISP 2, and gray yellow on potato agar. Melanin is produced on ISP 7 medium. Grows at 22–30°C. No growth occurs at 12 or 37°C (Trujillo et al., 2006). This species does not grow on ISP 2 medium supplemented with 2% NaCl (Kirby et al., 2006). Grows in the presence (μg/ml) of carbenicillin disodium salt (100), cephaloridine (10), chloramphenicol (20, weak), cycloheximide (10), streptomycin sulfate (10), and vancomycin hydrochloride (10) but not in the presence of ampicillin sodium salt (100), cefotaxime (10), chloramphenicol (50), erythromycin (10), gentamycin sulfate (10), neomycin sulfate (10), and tobramycin sulfate (10) (Kirby et al., 2006). Produces a substance with antifungal activity. The predominant fatty acids determined in cells grown in liquid ISP 2 medium at 28°C were $C_{15:0}$ anteiso and $C_{15:0}$ iso.

Source (type strain): soil in Yunnan Province, China.

DNA G+C content (mol%): 67 (T_m).

Type strain: YIM 31530, CCTCC AA 001021, DSM 15501, JCM 13523, NBRC 101882.

Sequence accession no. (16S rRNA gene): AY082063.

5. **Kribbella catacumbae** Urzì, De Leo and Schumann 2008, 2095[VP]

ca.ta.cum′ba.e. L. gen. n. *catacumbae* of a catacomb, isolated from a Roman catacomb.

Characteristics are as described for the genus and listed in Table 225 and Table 226. Additional information given below is taken from the original paper (Urzì et al., 2008).

Substrate hyphae (diameter 0.36 μm) are branched and tend to penetrate into the agar. They are pale yellow to orange-yellow on the test media, becoming dark orange in old cultures on Luedemann medium [0.5% yeast extract (Difco), 0.5% NZ Amine type A (Sheffield Chemical Co., Norwich, NY), 1% glucose, 2% soluble starch (Difco), 0.1% $CaCO_3$, and 1.5% agar (Luedemann, 1968)]. Colonies are irregular (diameter 5–10 mm), with lobate margin, crateriform profile and rough surface, that are covered with white aerial hyphae. No distinct formation of rod-shaped elements due to fragmentation has been observed in cultures on Luedemann medium, but morphological structures resembling short chains of non-separated arthrospores are observable by electron microscopy. Melanin is produced on ISP 7 medium. Grows at 15–30°C; weak growth is observed at 10°C. Grows in the presence of 2% NaCl and weakly at 3%, but not at 5% as determined on Luedeman agar without $CaCO_3$ addition. Grows at pH 5–9, with weak growth at pH 10 (initial pH values of the same medium). Weak growth on D-raffinose and sorbitol after 21 d. Tweens 20, 40, and 60 are hydrolyzed, and DNA is weakly hydrolyzed. Resistant to the following antibiotics (μg/ml): cephaloridine (10), neomycin sulfate (1.5), streptomycin sulfate (10), and vancomycin hydrochloride (10). Growth is inhibited by chloramphenicol (50), gentamycin sulfate (3), and tobramycin sulfate (1.5). Sensitive to 0.01% lysozyme. Major fatty acids (>10% of the total) as determined for 6 strains grown on TSA at 28°C for 24 h were $C_{15:0}$ anteiso (22.4–28.3), $C_{15:0}$ iso (20.5–28.7), and $C_{17:1}$ iso ω9*c* (9.9–10.6).

Source: deteriorated surfaces in the St. Callistus catacombs, Rome, Italy.

DNA G+C content (mol%): not determined.

Type strain: BC631, DSM 19601, JCM 14968.

Sequence accession no. (16S rRNA gene): AM778575.

6. **Kribbella ginsengisoli** Cui, Lee, Lee and Im 2010, 366[VP]

gin.sen.gi.so′li. N.L. n. *ginsengum* ginseng; L. n. *solum* soil; N.L. gen. n. *ginsengisoli* of soil of a ginseng field.

Characteristics are as described for the genus and listed in Table 225 and Table 226. Additional information given below is taken from the original paper (Cui et al., 2010).

Extensively branched substrate mycelium penetrating into the agar and aerial mycelium are observed on R2A agar plates (Difco) and most other media tested. Cells are not lyzed in the KOH test according to the method of Buck (1982). Grows at 8–37°C but not at 6 or 42°C. Will grow on R2A agar with up to 4.0% NaCl, and in the pH range of 5.5–8.5. Grows on nutrient agar (Difco) but not on MacConkey agar. Unable to reduce nitrate or nitrite under either aerobic or anaerobic conditions. Utilizes glucose as a sole carbon source but does not produce acid or gas from it (API 20NE and ID 32GN tests systems). In addition to compounds listed in Table 225 and Table 226, the following carbon sources are utilized: amygdalin, asparagine, aspartate, fumarate, gluconate, glutamate, glutamine, glycogen, L-histidine, oxalate, phenylalanine, pyruvate, threonine, and valerate; the following compounds are not utilized as sole carbon sources: adipate, L-alanine, arginine, benzoic acid, 3- and 4-hydroxybenzoates, caprate, cysteine, dextran, dulcitol, ethanol, formic acid, D-fucose, glutarate, glycine, isoleucine, itaconate, leucine, lysine, D-lyxose, maleic acid, malonate, methanol, methionine, phenyl acetate, proline, propionate, D-rhamnose, D-ribose, salicin, L-serine, L-sorbose, suberate, tartarate, tryptophan, tyrosine, valine, or xylitol (conventional method with minimal salts medium containing vitamins and microelements, and/or the API 20NE and ID 32GN test systems). Negative for production of arginine dihydrolase, lysine decarboxylase, ornithine decarboxylase, and tryptophan deaminase. Degrades chitin, but not DNA. Indole is not produced from tryptophan. Voges–Proskauer test is positive. Growth is inhibited by the following antibiotics (μg per disc): streptomycin sulfate (6), gentamycin sulfate (4), tetracycline hydrochloride (10), chloramphenicol (15), kanamycin (6), penicillin (18), or erythromycin (6). The major fatty acids (5% or more), as determined for cells grown in tryptic soy broth for 5 d at 30°C include $C_{15:0}$ anteiso (20.6), $C_{16:0}$ iso (11.5), $C_{16:0}$ (8.8), $C_{16:0}$ 2-OH (8.1), $C_{15:0}$ iso (6.4), $C_{17:0}$ iso (5.0), and $C_{18:0}$ (6.3).

Source (type strain): soil from of a ginseng field, Pocheon Province, South Korea.

DNA G+C content (mol%): 66.3 (HPLC).

Type strain: Gsoil 001, KCTC 19134, DSM 17941.

Sequence accession no. (16S rRNA gene): AB245391.

7. **Kribbella hippodromi** Everest and Meyers, 2008, 444[VP]

hip.po.dro′mi. Gr. masc. n. *hippodromos* horse racecourse, N.L. gen. masc. n. *hippodromi* of/from a horse racecourse, referring to the source of isolation of the type strain, Kenilworth Racecourse, Cape Town, Western Cape, South Africa.

Characteristics are as described for the genus and listed in Table 225 and Table 226. Additional information given below is taken from the original paper (Everest and Meyers, 2008).

Substrate mycelium is usually cream-colored, with extensively branched hyphae, which fragment in both liquid and agar cultures. Aerial mycelium is white on ISP 4 medium. Colonies appear convoluted with irregular edges on most media. Grows at 20–37°C, but not at 45°C. Growth occurs in the presence of 5% NaCl, with very weak growth at 6% NaCl, and no growth at 7% NaCl (tested on ISP 2 medium). Grows weakly at pH 4.3 (initial pH value of Bennett's agar). Utilizes salicin as a sole carbon source. Utilizes L-arginine, L-asparagine, L-histidine, L-threonine, and nitrate as sole nitrogen sources, with weak growth on L-cysteine and L-serine. Gives also weak growth on DL-α-amino-*n*-butyric acid, L-4-hydroxyproline, and L-valine as sole nitrogen sources and no growth on L-methionine and L-phenylalanine (characteristics distinguishing this species from *Kribbella solani* which shows clear growth on these amino acids). Allantoin, guanine, and pectin are not degraded or hydrolyzed. Resistant to the following antibiotics (μg/ml): lincomycin hydrochloride (100), neomycin sulfate (50), oleandomycin phosphate (100), rifampicin (50), streptomycin sulfate (100), and penicillin G (10 IU /ml). Sensitive to (μg/ ml) cephaloridine (100), tobramycin sulfate (50), and vancomycin hydrochloride (50). No antibacterial activity against *Mycobacterium aurum*.

Source (type strain): soil sample from a fynbos-rich site, Kenilworth Racecourse, Cape Town, South Africa.

DNA G+C content (mol%): not determined.

Type strain: S1.4, DSM 19227, JCM 15572, NRRL B-24553.

Sequence accession no. (16S rRNA gene): EF472955.

8. **Kribbella jejuensis** Song, Kim, Hong, Cho, Sohn, Chun, Sun, 2004, 1347[VP]

je.ju.en′sis. N.L. fem. adj. *jejuensis* of or belonging to Jeju, Korea.

Characteristics are as described for the genus and listed in Table 225 and Table 226. Additional information given below is taken from the original paper (Song et al., 2004), unless indicated.

Substrate mycelium is usually cream-colored and consists of broadly branched hyphae. Aerial hyphae are white and fragmenting into rod-shaped elements. Colonies are usually sand-pasty and have lichenous shapes. Grows at 28–30 and 42°C (Carlsohn et al., 2007; Song et al., 2004) and in the presence of 2% NaCl on ISP 2 medium (Kirby et al., 2006); no growth is observed (Carlsohn et al., 2007) in liquid organic medium 79 (Prauser and Falta, 1968) at the same salt concentration. No growth occurs at initial pH 5 or pH 9 as assessed both on Bennet's agar and in liquid organic medium 79 (Carlsohn et al., 2007; Kirby et al., 2006). Utilizes DL-arginine, L-histidine, DL-homoserine, DL-α-amino-*n*-butyric acid, *trans*-4-hydroxy-L-proline, ammonium, and nitrate as nitrogen sources. Does not utilize salicin as a sole carbon source. Hydrolyzes tributyrin. Does not decompose guanine, allantoin, and chitin. Produces trypsin (Carlsohn et al., 2007). Tolerant to 0.01% (w/v) lysozyme, 0.1% (w/v) phenyl ethanol, and 0.001% (w/v) potassium tellurite, but not to 0.01% (w/v) sodium azide, 0.01% (w/v) thallous acetate, or 0.1% (w/v) phenol. Grows in the presence (μg/ml) of carbenicillin disodium salt (100), cefotaxime (10), chloramphenicol (50), cycloheximide (10), erythromycin (10), gentamycin (0.4), neomycin sulfate (10, weak), rifampicin (4), streptomycin sulfate (10), tetracycline (1), tobramycin (4, weak), and vancomycin hydrochloride (10) but not in the presence of ampicillin sodium salt (100), cephaloridine (10), gentamycin sulfate (10), streptomycin (20), tobramycin sulfate (10), and troleandomycin (10) (Kirby et al., 2006; Song et al., 2004). Weak growth occurs

in the presence of penicillin G (10 U/ml). Carlsohn et al. (2007) reported that the type strain of this species resisted ampicillin (10 µg per disk) and imipenem (10 µg per disk). Shows weak antimicrobial activity towards *Streptomyces scabiei* but not against *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Micrococcus luteus*, *Candida albicans*, *Aspergillus niger*, *Trichoderma harzianum*, *Fusarium acuminatum*, and *Colletotrichum gloeosporioides*. The major fatty acids (>5% of the total) determined for the cultures grown on TSA (BBL) at 30°C (Song et al., 2004) and in TSB at 28°C and harvested at the logarithmic growth phase (Carlsohn et al., 2007) were similar: $C_{15:0}$ anteiso (31.2 and 27.1), $C_{16:0}$ iso (16.2 and 17.3), $C_{15:0}$ iso (10.8 and 11.3), $C_{14:0}$ iso (7.1 and 10.4), 9-methyl-$C_{16:0}$ (9.3 and 7.9), $C_{16:0}$ iso 2-OH (6.5 and 6.3).

Source (type strain): soil from Jeju, Korea.

DNA G+C content (mol%): 68 (T_m).

Type strain: HD9, JCM 12204, KACC 20266, NBRC 101070.

Sequence accession no. (16S rRNA gene): AY253866.

9. **Kribbella karoonensis** Kirby, Le Roes and Meyers, 2006, 1100[VP]

ka.ro.o.nen′sis. N.L. fem. adj. *karoonensis* of or pertaining to the Karoo Desert National Botanical Garden in Worcester, South Africa.

Characteristics are as described for the genus and listed in Table 225 and Table 226. Additional information given below is taken from the original paper (Kirby et al., 2006).

Substrate mycelium is cream/yellow on inorganic salts starch agar (ISP 4) and fragment into rod-shaped elements in broth and on agar media (Figure 273). Aerial mycelium is pale cream/white and fragments into rod-shaped elements. Grows at 20°C (minimal temperature tested) and 37°C, but not at 45°C. Grows well on ISP 2 medium supplemented with 2% NaCl, with weak growth in the presence of 3 or 4% NaCl. Survives in a soil sample heated (dry heating) at 120°C for 1 h. Grows in the presence of (µg/ml) carbenicillin disodium salt (100), chloramphenicol (20), cycloheximide (10), erythromycin (10), and streptomycin sulfate (10). Growth is inhibited by ampicillin sodium salt (100), cefotaxime (10), cephaloridine (10), chloramphenicol (50), gentamycin sulfate (10), neomycin sulfate (10), tobramycin sulfate (10), and vancomycin hydrochloride (10).

Source (type strain): soil collected from the base of a giant quiver tree, *Aloe pillansii*, in the Karoo Desert National Botanical Garden, Worcester, Western Cape Province, South Africa.

DNA G+C content (mol%): not determined.

Type strain: Q41, DSM 17344, JCM 14304, NBRC 101884, NRRL B-24425.

Sequence accession no. (16S rRNA gene): AY995146.

10. **Kribbella koreensis** (Lee, Kang and Hah 2000) Sohn, Ohn, Hong, Bae and Chun 2003, 1007[VP] (*Hongia koreensis* Lee, Kang and Hah, 2000, 197)

ko.re.en′sis. N.L. fem. adj. *koreensis* of or pertaining to Korea, the location of the soil sample from which the type strain was isolated.

Characteristics are as described for the genus and listed in Table 225 and Table 226. Additional information given below is taken from the papers of Lee et al. (2000) and Sohn et al. (2003), unless indicated.

Substrate mycelium is usually cream on the media tested and consists of irregularly branched hyphae tending to form apical swellings (observed, e.g. on ISP 3 medium). The hyphae fragment into elongated or short rod-shaped elements; bud-like cells are formed on some media, for instance, on ISP 3 medium (Figure 274). Aerial mycelium is white and fragmenting (Figure 272). Melanin is produced on ISP 7 medium. Grows at 10 and 30°C; growth at 37°C varies with experiments (Kirby et al., 2006; Lee et al., 2000; Trujillo et al., 2006). Grows well on ISP 2 medium in the presence of 2% NaCl (Kirby et al., 2006); no growth occurs at 3% NaCl (Kirby et al., 2006; Lee et al., 2000; Trujillo et al., 2006). Growth is observable at initial pH 7–10. Grows on D-arabinose, D-lyxose, methyl-α-D-glucoside, methyl-α-D-mannoside, and salicin as sole carbon sources; no growth is observed with dextran, D-glucosamine, L-sorbose, and dulcitol. Growth occurs on a tap-water agar (supplemented with cycloheximide and nystatin). Produces pyrrolidonyl arylamidase (API ZYM; Trujillo et al., 2006). Positive response in the API 20NE and ID 32GN systems for utilization of acetate, adipate, L-fucose, gluconate, 3-hydroxybutyrate, itaconate, malate, D-ribose, and suberate as sole carbon sources, and production of β-glucosidase; negative for utilization of caprate, citrate, glycogen, 3- and 4-hydroxybenzoate, 2- and 5-ketogluconate, L-alanine, malonate, phenyl acetate, L-proline, propionate, valerate, L-histidine, and L-serine, as well as reduction of nitrate to nitrogen, production of arginine dihydrolase and indole, and acidification of glucose (Cui et al., 2010). Acid is produced from D-raffinose in the Bacto OF basal medium (Difco); no acid production was found with all other compounds tested: D-glucose, D-arabinose, L-arabinose, D-cellobiose, D-fructose, D-galactose, inulin, D-lactose, maltose, D-mannose, D-melezitose, melibiose, methyl-α-D-glucoside, methyl-α-D-mannoside, L-rhamnose, L-ribose, salicin, sucrose, D-trehalose, D-xylose, adonitol, 2, 3-butanediol, *meso*-erythritol, glycerol, *meso*-inositol, D-mannitol, or 1, 2-propanediol. Gives an alkaline reaction in the tests for utilization of organic acids, such as α-ketoglutarate, malonate, propionate, pyruvate, and *trans*-aconitate with phenol red indicator; negative responses with *cis*-aconitate, benzoate, formate, maleate, salicylate, sebacate, sorbate, or tartarate (according to the method of Gordon et al. (1974). DNA and pectin are not hydrolyzed. Sodium hippurate is decomposed. Resistant to lysozyme, 0.1% (w/v) phenol, 0.01% (w/v) potassium tellurite, 0.00005% (w/v) crystal violet, and 0.001% (w/v) brilliant green, but not to 0.01% (w/v) sodium azide, 0.3% (w/v) phenylethanol, or 0.01% (w/v) thallous acetate. Grows in the presence of (µg/ml) carbenicillin disodium salt (100), cephaloridine (10), chloramphenicol (20, weak), cycloheximide (10), erythromycin (10), neomycin sulfate (10, weak), streptomycin sulfate (10), and vancomycin hydrochloride (10); growth is inhibited by (µg/ml) ampicillin sodium salt (100), cefotaxime (10), chloramphenicol (50), gentamycin sulfate (10), and tobramycin sulfate (10) (Kirby et al., 2006). No antimicrobial activity was observed against *Bacillus subtilis*, *Micrococcus luteus*, *Staphylococcus aureus*, *Streptomyces murinus*, *Escherichia coli*, *Enterobacter aerogenes*, *Saccharomyces cerevisiae*, *Candida albicans*, and *Aspergillus niger*. Produces neuropilin/growth factor complexes (Alitalo et al., 2003). The major

cellular fatty acids (representing >5% of the total recorded in at least one experiment) reported for cultures grown under different conditions were: $C_{15:0}$ anteiso (24.7–36.4), $C_{16:0}$ iso (8.4–24.0), $C_{15:0}$ iso (12.3–20.1), $C_{17:0}$ iso (4.5–9.0), $C_{17:0}$ anteiso (3.1–5.0), 9-methyl-$C_{16:0}$ (<1–12.7), $C_{17:1}$ ω9*c* (<1–5.0), $C_{14:0}$ iso (2.0–5.9), $C_{16:0}$ (<1–6.6), and 10-methy-$C_{16:0}$ (<1–5.1) (Lee et al., 2000; Sohn et al., 2003; Song et al., 2004).

Source (type strain): a gold-mine cave in Kongju, Republic of Korea.

DNA G+C content (mol%): 71.3 (T_m).

Type strain: LM 161, IMSNU 50530, JCM 10977, NBRC 101069.

Sequence accession no. (16S rRNA gene): Y09159.

11. **Kribbella lupini** Trujillo, Kroppenstedt, Schumann and Martinez-Molina 2006, 410[VP]

lu.pi′ni. L. gen. n. *lupini* of lupin, isolated from *Lupinus angustifolius.*

Characteristics are as described for the genus and listed in Table 225 and Table 226. Additional information given below is taken from the original paper (Trujillo et al., 2006) unless indicated.

Substrate mycelium is white to cream, extensively branching and fragmenting into rod-shaped and coccoid elements. Abundant white aerial mycelia are produced that break up into rod-shaped fragments. Grows at 12 and 37°C. Tolerates up to 7% NaCl, as tested on nutrient agar. Growth is observed at pH 6–9, but not at pH 5 (initial pH values of nutrient agar). Grows on L-sorbose, adipate, gluconate, L-alanine, L-arginine, L-histidine, L-lysine, L-proline, and DL-valine but not caprate and L-serine as sole carbon sources. Acid is produced from glucose. Produces arginine dihydrolase, esterase lipase (C8), acid and alkaline phosphatases, leucine arylamidase, valine arylamidase, α-chymotrypsin, trypsin, α-galactosidase, α-glucosidase, β-glucosidase, *N*-acetyl-β-glucosaminidase, and α-mannosidase but not β-glucuronidase or pyrrolidonyl arylamidase (API ZYM). Sensitive (μg per disk) to amoxicillin (30), gentamycin (10), neomycin (5), novobiocin (5), oxytetracycline (30), streptomycin (300), tobramycin (10), vancomycin (30), and tetracycline (30). Resistant to ampicillin (2 μg/disk), penicillin G (10 U/disk), and rifampicin (2 μg/disk). The purified cell wall contains galactose and an unidentified sugar which is neither arabinose, glucose, mannose, rhamnose, ribose nor xylose. The major fatty acids (>5% of the total), as determined for cells grown in tryptic soy broth, were $C_{15:0}$ anteiso (28.2), $C_{16:0}$ iso (21.3), $C_{17:1}$ iso (11.0), $C_{14:0}$ iso (9.1), $C_{15:0}$ iso (5.3), $C_{16:1}$ iso (8.1), and $C_{17:0}$ anteiso (5.4).

Source (type strain): root nodules of *Lupinus angustifolius.*

DNA G+C content (mol%): 68 (T_m).

Type strain: LU14, DSM 16683, JCM 14303, LMG 22957, NBRC 101883.

Sequence accession no. (16S rRNA gene): AJ811962.

12. **Kribbella sancticallisti** Urzì, De Leo and Schumann 2008, 2095[VP]

sanc.ti.cal.li′sti. L. n. *Sanctus Callistus* Saint Callistus; N.L. gen. n. *sancticallisti* of Saint Callistus, isolated from the Saint Callistus Roman catacombs.

Characteristics are as described for the genus and listed in Table 225 and Table 226. Additional information given below is taken from the original paper (Urzì et al., 2008).

Substrate hyphae (diameter, 0.51 μm) are cream on Luedemann medium (Luedemann, 1968), branched, and tend to penetrate into the agar. Colonies are irregular (diameter 5–10 mm), with lobate margin, crateriform profile, and rough surface that is covered with white aerial hyphae. Produces melanin (weakly) on ISP 7 medium. No distinct formation of rod-shaped elements due to fragmentation has been observed in cultures on Luedemann medium, but morphological structures resembling short chains of non-separated or separated arthrospores are observable by electron microscopy. Grows at 15 and 37°C with weak growth at 10°C. Grows in the presence of 4% NaCl and weakly in the presence of 5% on Luedeman agar (Luedemann, 1968) without $CaCO_3$ addition. Growth occurs at pH 5 and 10 (initial pH values of the above medium). Utilizes gluconate; hydrolyzes DNA and Tweens 20, 40, and 60. Resistant to the following antibiotics (μg/ml) cephaloridine (10), gentamycin sulfate (3), oleandomycin (15), rifampicin (4) tobramycin (1.5), and penicillin G (10 U /ml); growth is inhibited by chloramphenicol (50), streptomycin sulfate (l0), and vancomycin chloride (10). Sensitive to 0.01% lysozyme. The major fatty acids (>10% of the total) determined for three strains grown on TSA at 28°C for 24 h were $C_{16:0}$ iso (24.0–27.6), $C_{15:0}$ anteiso (19.7–20.8), and $C_{15:0}$ iso (9.1–10.8).

Source: deteriorated tufacean surfaces with grey-whitish alterations in the Roman catacombs of St. Callistus (Rome, Italy).

DNA G+C content (mol%): not determined.

Type strain: BC633, DSM 19602, JCM 14969.

Sequence accession no. (16S rRNA gene): AM778577.

13. **Kribbella sandramycini** Park, Yoon, Shin, Suzuki, Kudo, Seino, Kim, Lee and Lee 1999, 750[VP]

san.dra.my.ci′ni. N.L. n. *sandramycinum* sandramycin (an antibiotic); N.L. gen. n. *sandramycini* of sandramycin, intended to mean producing sandramycin.

Characteristics are as described for the genus and listed in Table 225 and Table 226. Additional information given below is taken from the original paper (Park et al., 1999), unless indicated.

Substrate mycelium consists of extensively branched hyphae which penetrate into agar media; they often fragment into rod- to coccoid-shaped and rod elements. Aerial hyphae appear straight or curved and break up into short to elongated rod-like elements. Colonies are pasty and have lichenous shapes with irregular edges. Gram-stain variable. Grows at 20 and 28–30°C; growth at 37°C varies with experiments (Kirby et al., 2006; Park et al., 1999; Trujillo et al., 2006). Weak growth may occur at 40°C. Grows well on ISP 2 medium in the presence of 2% NaCl, with moderate growth at 3% NaCl and weak growth in the presence of 4% NaCl (Kirby et al., 2006). Produces pyrrolidonyl arylamidase (Trujillo et al., 2006). Growth is observed at initial pH 5 and pH 9 (Kirby et al., 2006; Park et al., 1999; Trujillo et al., 2006). Grows in the presence of (μg/ml) carbenicillin disodium salt (100), chloramphenicol (50), cycloheximide (10), erythromycin (10), neomycin sulfate (10), streptomycin

sulfate (10), and vancomycin hydrochloride (10) but not in the presence of (µg/ml) cefotaxime (10), cephaloridine (10), gentamycin sulfate (10), and tobramycin sulfate (10) (Kirby et al., 2006). Produces an anti-tumor antibiotic, sandramycin (Matson et al., 1993). The major fatty acids (>5% of the total at least in one experiment) reported for cultures grown under different conditions were $C_{15:0}$ anteiso (32.2–42.6), $C_{14:0}$ iso (3.0–14.8), $C_{15:0}$ iso (11.2–15.0), $C_{16:0}$ iso (5.6–8.1), $C_{17:0}$ iso (1.6–6.0), $C_{17:1}$ iso ω8*c* (<1–7.5), $C_{17:1}$ ω8*c* (3.9–6.1), and 9-methyl-$C_{16:0}$ (<1–7.0) (Park et al., 1999; Sohn et al., 2003; Song et al., 2004; Trujillo et al., 2006).

Source (type strain): soil in Mexico.

DNA G+C content (mol%): 68.3 (HPLC).

Type strain: ATCC 39419, KCTC 9609, JCM 10340, NBRC 100341.

Sequence accession no. (16S rRNA gene): AF005020, AY253864.

14. **Kribbella solani** Song, Kim, Hong, Cho, Sohn, Chun and Sun 2004, 1347[VP]

so.la′ni. N.L. gen. n. *solani* of *Solanum*, the genus of the potato, *Solanum tuberosum*, from which the type strain was isolated.

Characteristics are as described for the genus and listed in Table 225 and Table 226. Additional information given below is taken from the original paper (Song et al., 2004), unless indicated. The species description is based on the type strain only.

Substrate mycelium is usually cream, the hyphae are broadly branched. Aerial mycelium is white and consists of hyphae fragmenting into rod-shaped elements. Colonies are typically sand-pasty, with irregular edges, and have lichenous shapes. Among the strains tested, the best (but moderate) anaerobic growth of the type strain of this species is observed on ATCC Medium No. 172 (Kirby et al., 2006). According to Urzì et al. (2008), the strain also grows anaerobically on VL agar (Tiecco, 1975). Grows well in liquid organic medium 79 (Prauser and Falta, 1968) and on ISP 2 agar supplemented with 4% NaCl under aerobic conditions; moderate or weak growth is observed in the presence of 5% NaCl on ISP 2 medium, with no growth at 6% (Carlsohn et al., 2007; Everest and Meyers, 2008; Kirby et al., 2006). No growth is observed at initial pH 5 or pH 9 as assessed in liquid organic medium 79 (Carlsohn et al., 2007) and on Bennett's agar (Kirby et al., 2006). Grows on L-histidine, DL-arginine, and nitrate as nitrogen sources. Gives also clear growth on DL-α-amino-*n*-butyric acid, L-4-hydroxyproline, L-valine, L-methionine, and L-phenylalanine as sole nitrogen sources, in contrast to the phylogenetically closest relative *Kribbella hippodromi*, which shows weak or doubtful growth on these amino acids (Everest and Meyers, 2008; Song et al., 2004). Utilizes salicin as a sole carbon source. Produces (weakly) trypsin (Carlsohn et al., 2007). DNA and tributyrin are hydrolyzed but guanine, allantoin, and chitin are not. Tolerant to 0.01% lysozyme, 0.1% phenyl ethanol, and 0.01% potassium tellurite but not to 0.01% sodium azide, 0.01% thallous acetate, and 0.1% phenol. Grows in the presence (µg/ml) of carbenicillin disodium salt (100), chloramphenicol (50), cycloheximide (10), erythromycin (10), neomycin (80), streptomycin (20), tetracycline (4), rifampicin (4), tobramycin sulfate (10, weak), and vancomycin hydrochloride (10), with weak growth in the presence of penicillin G (10 IU/ml); growth is inhibited (µg/ml) by ampicillin sodium salt (100), cefotaxime (10), cephaloridine (10), and treoleandomycin (10) (Kirby et al., 2006; Song et al., 2004). Resists ampicillin (10 µg per disk) and shows weak growth in the presence of imipenem (10 µg per disk) (Carlsohn et al., 2007). No antimicrobial activity against *Bacillus subtilis*, *Micrococcus luteus*, *Streptomyces scabiei*, *Pseudomonas aeruginosa*, *Candida albicans*, *Aspergillus niger*, *Trichoderma harzianum*, *Fusarium acuminatum*, and *Colletotrichum gloeosporioides*. The major fatty acids (>5%) as determined for cultures grown on TSA agar (BBL) (Song et al., 2004) and in tryptic soy broth (Carlsohn et al., 2007) were $C_{15:0}$ anteiso (21.6 and 31.2), $C_{16:0}$ iso (19.5 and 16.9), $C_{15:0}$ iso (5.8 and 10.0), $C_{14:0}$ iso (12.6 and 10.4), and $C_{16:1}$ iso (6.2 and 1.5).

Source (type strain): a potato tuber with scab lesions, Jeju, Korea.

DNA G+C content (mol%): 69 (T_m).

Type strain: DSA1, JCM 12205, KACC 20196, NBRC 101071.

Sequence accession no. (16S rRNA gene): AY253862.

15. **Kribbella swartbergensis** Kirby, Le Roes and Meyers 2006, 1100[VP]

swart.berg.en′sis. N.L. fem. adj. *swartbergensis* of or pertaining to the Groot Swartberg mountain range, South Africa.

Characteristics are as described for the genus and listed in Table 225 and Table 226. Additional information given below is taken from the original paper (Kirby et al., 2006), unless indicated.

Substrate hyphae are cream-colored on inorganic salts/starch agar (ISP 4 medium) and fragment into rod-shaped elements in broth and on agar media. Aerial hyphae appear straight (Figure 271) and white in color on ISP 4 agar. Optimal temperature for growth is 30°C; grows occurs at 20 and 45°C (minimal and maximal temperatures tested). The ability to grow at 45°C readily distinguishes this species from all other kribbellae. It also grows in the presence of 3% NaCl, with weak growth at 4% NaCl as tested on ISP 2 medium. Grows at initial pH 5 (weak) and pH 9 both on Bennett's agar (Kirby et al., 2006) and in liquid organic medium 79 (Carlsohn et al., 2007). Produces trypsin (Carlsohn et al., 2007). Grows in the presence (µg/ml) of carbenicillin disodium salt (100), cephaloridine (10), chloramphenicol (20), cycloheximide (10), erythromycin (10), gentamycin sulfate (10), neomycin sulfate (10, weak), and streptomycin sulfate (10) but not in the presence of ampicillin sodium salt (100), cefotaxime (10), chloramphenicol (50), tobramycin sulfate (10), and vancomycin hydrochloride (10). Carlsohn et al. (2007) reported that the type strain of this species resisted ampicillin (10 µg per disk) and imipenem (10 µg per disk). The major fatty acids (>5%) determined for the culture grown in TSB were $C_{16:0}$ iso (27.4), $C_{15:0}$ anteiso (16.4), 9-methyl $C_{16:0}$ (13.8), $C_{17:0}$ anteiso (11.7), and $C_{15:0}$ iso (5.7) (Carlsohn et al., 2007).

Source (type strain): soil from the banks of the River Gamka, Die Hel, Groot Swartberg mountain range, Western Cape Province, South Africa.

DNA G+C content (mol%): not determined.

Type strain: HMC25, DSM 17345, JCM 14305, NBRC 101885, NRRL B-24426.

Sequence accession no. (16S rRNA gene): AY995147.

16. **Kribbella yunnanensis** Li, Wang, Zhang, Xu and Jiang, 2006a, 1459[VP] (Effective publication: Li, Wang, Zhang, Xu and Jiang 2006b, 33.)

yun.nan.en′sis. N.L. fem. adj. *yunnanensis* of or pertaining to Yunnan, a province of south-west China in which the sample was collected.

Characteristics are as described for the genus and listed in Table 225 and Table 226. Additional information given below is taken from the original paper (Li et al., 2006) unless indicated.

Substrate mycelium is moderate yellow/soft yellow on ISP 2, potato agar, and nutrient agar, and pale yellow or yellow white on ISP 3, ISP 4, ISP 5, and Czapek's agar. The hyphae are extensively branched and often fragmenting into irregular rod-shaped elements. Aerial mycelia develop on all media tested, especially on Czapek's agar; it is typically white on ISP 3, ISP 4, and ISP 5 media and potato agar, yellow-white on ISP 2 medium and Czapek's agar, and pale yellow on nutrient agar. Aerial hyphae fragment into short to elongated rod-like elements which can be arranged in short chains and have an arthrospore appearance on some media, e.g. on ISP 2 agar. Melanin is weakly produced on ISP 7 medium (Urzì et al., 2008). The major fatty acids (>5% of the total) determined in cells grown on TSA at 28°C include $C_{15:0}$ anteiso (31.6), $C_{15:0}$ iso (14.8), $C_{16:0}$ iso (10.2), $C_{14:0}$ iso (7.8), $C_{17:1}$ iso ω9*c* (6.7), and $C_{17:0}$ iso (6.5).

Source (type strain): soil in Yunnan Province, the west of China.

DNA G+C content (mol%): 68.6 (T_m).

Type strain: YIM 30006, CCTCC AA 001019, DSM 15499, JCM 14307, NBRC 103562.

Sequence accession no. (16S rRNA gene): AY082061.

Genus V. **Marmoricola** Urzì, Salamone, Schumann and Stackebrandt 2000, 534[VP] emend. Lee and Lee 2010, 2138

Lyudmila I. Evtushenko

Mar.mo.ri′co.la. L. neutr. n. *marmor* marble; L. masc. suff. -*cola* inhabitant of; *Marmoricola* inhabitant of marble.

Spherical cells, mostly about 0.5–1.0 µm in diameter, that occur singly, in pairs, short chains, or small clusters. Nonmotile or motile**.** Endospores are not formed. **Gram-positive** cell-wall type. Non-acid-fast. **Chemo-organotrophic with a respiratory type of metabolism. Catalase-positive, test for oxidase is usually negative.** Grow under aerobic conditions on a range of standard nutrient media, including the chemically defined (synthetic) media, forming circular, pasty, and **yellow or orange colonies.** Acids are produced oxidatively rather weakly from carbohydrates. Nitrate reduction varies among species. **Mesophilic**, grow best at 28–30°C; no growth occurs at 42°C. Prefer a neutral to mildly alkaline pH; some species are alkalitolerant or display alkaliphilic properties. Usually non-halophilic. **The cell-wall peptidoglycan contains LL-diaminopimelic acid and glycine** as the diagnostic amino acid **(the peptidoglycan type A3γ).** The acyl type of muramic acid is acetyl. **Menaquinones are the only respiratory quinones; the major component is the tetrahydrogenated menaquinone with eight isoprene units, MK-8(H_4).** The cellular fatty acid pattern is dominated by **straight-chain saturated and unsaturated ($C_{16:0}$ and $C_{18:1}$) components. Tuberculostearic ($C_{18:0}$ 10-methyl) acid is usually detected.** Mycolic acids are absent. The principal identified polar lipids are **phosphatidylinositol, phosphatidylglycerol, and diphosphatidylglycerol; phosphatidylcholine may be present.** Occur in various terrestrial and marine environments and can be found in bacterial communities associated with plants and humans.

DNA G+C content (mol%): 72–73 (HPLC).

Type species: **Marmoricola aurantiacus** Urzì, Salamone, Schumann and Stackebrandt 2000, 534[VP].

Further descriptive information

The genus *Marmoricola* belongs to the family *Nocardioidaceae*, order *Propionibacteriales* (formerly suborder *Propionibacterineae* Stackebrandt et al. 1997), and currently harbors five species, with *Marmoricola aurantiacus* as the type species (Table 227). Based on the 16S rRNA gene analysis, the *Marmoricola* species together with *Nocardioides jensenii* and a few related species of this genus form a group at the periphery of the genus *Nocardioides* radiation (Figure 247). The 16S rRNA gene sequence similarities between the recognized *Marmoricola* species range from 95.3 to 98.7%.

The information given below originates from the papers on the descriptions of the genus *Marmoricola* (Urzì et al., 2000) and the species currently assigned to the genus (Dastager et al., 2008b; Lee and Lee, 2010; Lee, 2007a; Lee et al., 2010; Urzì et al., 2000), unless indicated.

Cells of most species are non-motile cocci, ranging from 0.5 to 1.0 µm in diameter (Figure 276) or a bit more, while the coccoid cells of *Marmoricola bigeumensis* are motile and smaller (~0.3–0.5 µm). A rod–coccus morphogenetic cycle does not occur (data for *Marmoricola aurantiacus* and *Marmoricola aequoreus*). Colonies are typically circular, entire, convex, shiny, pasty, and yellow- or orange-pigmented. They may become rough, crater-shaped, or irregularly shaped in old (1–1.5 months) cultures.

Marmoricola aurantiacus contains the cell-wall peptidoglycan A3γ type *sensu* Schleifer and Kandler (1972), with LL-diaminopimelic acid (LL-A_2pm) at the third position of the peptide chain and a single glycine as an interpeptide bridge. The same diamino acid, LL-A_2pm, has been reported for the remaining

TABLE 227. Descriptive and differential characteristics of *Marmoricola* species[a]

Characteristic	*M. aurantiacus*	*M. aequoreus*	*M. bigeumensis*	*M. korecus*	*M. scoriae*
Colony color[b]	Orange	Yellow	Lemon yellow	Yellow	Vivid yellow
Cell shape (size, μm)	Cocci (0.5–0.7)	Cocci (0.5–0.7)	Cocci (~0.3–0.5)	Cocci (1.1–1.2)	Cocci (0.6–1.0)
Motility	–	–	+	–	–
Temperature range (°C)[c]	18–28	4(w)–37	20–37	4–37	10–37
Growth at pH[d]	5.1–8.7	5.1–12.1	6.0–12.0	5.1–12.1	6.1–12.1
Optimal pH[d]	~7.0	~7.1	~7.2	6.1–10.1	8.1–11.1
Max. NaCl (%, w/v)	2.0	7.0 (w)	7.0	2.0	3.0
Catalase activity	+	+	+	+	+
Oxidase test	–	–	–	–	–
Nitrate reduction	–	+	+	–	–
Hydrolysis of:					
Casein	–	+	–	+	+
DNA	–		–	–	+
Gelatin	–	+	–	+	+
Starch	–	–	+	–	–
Tyrosine	–	–	+	–	–
Hypoxanthine	+	–	nd	–	–
Xanthine	–	–	+	–	–
Utilization of:[e]					
L-Arabinose	+	–	+	nd	+
D-Arabinose	+	–	–	–	+
D-Cellobiose	+	+	+	+	–
Dextran	+	+	+	–	–
D-Fructose	+	+	–	–	+
D-Galactose	+	+	–	–	+
D-Lactose	+	+	+	–	–
Maltose	+	+	+	–	+
D-Mannose	+	+	+[f]	+	v
D-Melezitose	+	+	–	–	+
Raffinose	+	–	–	–	+
L-Rhamnose	+	–	+	–	+
L-Ribose	+	+	–	–	+
Salicin	+	–	–	–	+
Sucrose	+	+	–	–	+
D-Trehalose	+	+	–	–	+
D-Xylose	+	+	+	+	+
Adonitol	+	–	–	+	–
Dulcitol	–	–	–	+	–
meso-Erythritol	–	–	–	+	–
Glycerol	+	+	–	–	–
D-Mannitol	+	+	+[f]	+	+
D-Sorbitol	+	+	+	–	–
D-Xylitol	+	–	+	+	–
Acetate	+	+	+	–	+
Citrate	+	+	–	–	+
DL-Malate	+	+	–	–	+
Succinate	+	+	–	+	–
DL-Tartrate	+	–	–	–	–
Enzymatic activity (API ZYM):					
Acid phosphatase	+	–	+	+	+
Cystine arylamidase	–	w	+	w	w
Valine arylamidase	w	+	–	+	+
Esterase (C4)	w	–	+	–	–
β-Galactosidase	w	+	v	–	+
β-Glucosidase	+	w	+	w	+
Trypsin	–	–	+	w	w
α-Chymotrypsin	–	–	+	–	–
Naphthol-AS-BI-phosphohydrolase	+	–	+	+	+
Cell-wall diamino acid	LL-A_2pm	LL-A_2pm	LL-A_2pm	LL-A_2pm	LL-A_2pm
Predominant fatty acid type	Straight-chain	Straight-chain	iso-Branched	Straight-chain	Straight-chain

(continued)

TABLE 227. (continued)

Characteristic	*M. aurantiacus*	*M. aequoreus*	*M. bigeumensis*	*M. korecus*	*M. scoriae*
Major fatty acids (>10% of the total)[g]	$C_{16:0}$, $C_{18:1}$ ω9*c*, ($C_{16:1}$)[h]	$C_{16:0}$, $C_{18:1}$ ω9*c*	$C_{16:0}$ iso	$C_{16:0}$, $C_{17:1}$ ω8*c*, $C_{18:1}$ ω9*c*	$C_{16:0}$, $C_{18:1}$ ω9*c*, $C_{18:0}$ 10-methyl
Phospholipids	DPG, PG, PI, PL	DPG, PG, PI, PL	DPG, PG, PI, PL	DPG, PC, PG, PI, PL	DPG, PC, PG, PI, PL
Major menaquinone	MK-8(H_4)	MK-8(H_4)	MK-8(H_4)	MK-8(H_4)	MK-8(H_4)
DNA G+C content (mol%)	72.0	72.4	72.9	71.0	72.0
Source of type strain	Marble statue	Beach sandy sediment	Agricultural soil	Volcanic ash	Volcanic ash

[a]Based on characteristics of type strains; see the text for other descriptive characteristics. Data from Urzì et al. (2000), Lee (2007a), Dastager et al. (2008b), Lee and Lee (2010), and Lee et al. (2010). Abbreviations: LL-A_2pm, LL-2,6-diaminopimelic acid; PI, phosphatidylinositol; PG, phosphatidylglycerol; DPG, diphosphatidylglycerol; PC, phosphatidylcholine; PL, unknown phospholipid(s); MK-8(H_4), a menaquinone with eight isoprenoic units in the side chain, two of which are saturated. Symbols: −, negative; +, positive; w, weak or slow; v, variable between experiments; nd, no data available; see the text for other symbols.

[b]The pigmentation intensity and shade may vary depending on the growth medium and the culture age.

[c]The recorded lower and higher growth temperatures are indicated; the actual growth temperature range can be slightly broader for some species (see the text).

[d]Initial pH values of media.

[e]Data from Lee and Lee (2010) and Lee et al. (2010) obtained using ISP 9 (Shirling and Gottlieb, 1966) as basal medium.

[f]The opposite result has been reported by Dastager et al. (2008b) obtained using different test medium.

[g]Either $C_{16:0}$ or $C_{18:1}$ ω9*c* may represent a larger proportion depending on culture conditions (see the text and original papers for the culture conditions and the methods).

[h]$C_{16:1}$ may be undetectable or comprising <1% of the total fatty acids (Lee and Lee, 2010).

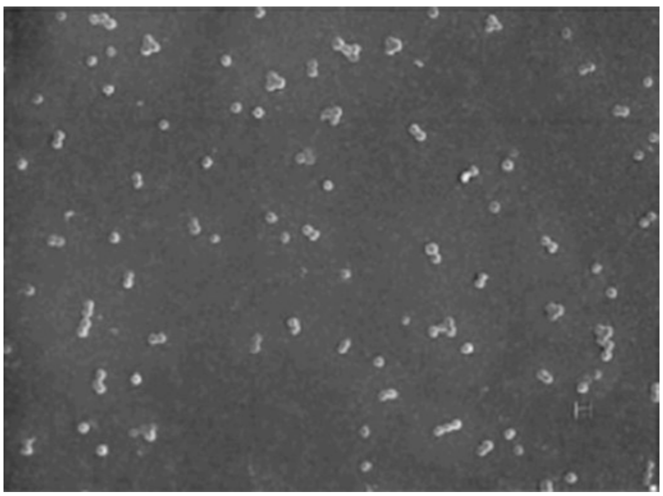

FIGURE 276. Cellular morphology of *Marmoricola aurantiacus*. Cells from a 14-d-old culture on Luedemann medium (Luedemann, 1968). Scanning electron micrograph. Bar = 1 µm. (Reproduced with permission Urzì et al., 2000. Int. J. Syst. Evol. Microbiol. *50*: 529–536.)

species. The acyl type of peptidoglycan is acetyl, as determined for *Marmoricola aurantiacus* (using the glycolate test of Uchida and Aida, 1984). The whole-cell sugars were also reported only for this species and included glucose and traces of ribose. The major component of the respiratory quinone system is MK-8(H_4); minor amounts of MK-7(H_4), MK-8(H_2), and MK-6(H_4) have been reported for *Marmoricola aurantiacus* (peak area ratio, 73:4:1:1). The cellular fatty acids of most species are dominated by straight-chain saturated and straight-chain unsaturated acids. A significant proportion (up to 10%) of tuberculostearic acid ($C_{18:0}$ 10-methyl) can be detected, along with other branched-chain and hydroxylated components occurring in minor or trace amounts (see Table 227 and *List of species of the genus Marmoricola* for more details). In *Marmoricola bigeumensis*, in contrast, branched-chain fatty acids predominate, with $C_{16:0}$ iso comprising 27.6 or almost 60% depending on experiment. All species are characterized by similar principal phospholipids in the polar lipid fraction, including phosphatidylinositol (PI), phosphatidylglycerol (PG), diphosphatidylglycerol (DPG), and an additional unidentified component (unidentified phospholipids in all cases except for *Marmoricola bigeumensis*). There is no evidence, however, that this unidentified component is the same for all species or different. Recently, phosphatidylcholine has been reported in *Marmoricola scoriae* and *Marmoricola korecus* along with PI, PG, DPG, and unidentified phospholipids (Lee and Lee, 2010; Lee et al., 2010).

Bacteria of this genus grow aerobically on a range of standard laboratory media based on peptone and yeast extract, and also display growth on mineral media, e.g. ISP 9* (contains essential salts, trace microelements, and ammonium as a source of nitrogen) supplemented with particular carbon sources, including various carbohydrates, sugar alcohols, organic acids, and other compounds. According to Lee and Lee (2010) and Lee et al. (2010), all species of this genus can grow on ISP 9 with D-glucose, D-mannose, D-mannitol, xylose, or α-methyl-D-glucoside, but not with formate, *meso*-inositol, α-methyl-D-mannoside, or L-sorbose, and display specificity with regard to a variety of other carbon sources (Table 227). Generally, species of this genus are rather non-exacting in their growth requirements, but some may be nutritionally fastidious. *Marmoricola aurantiacus* is unable to grow on certain media rich in organics, including *Corynebacterium* agar (DSMZ medium No. 53). *Marmoricola bigeumensis* has been reported (Dastager et al., 2008b) not to grow on yeast extract-malt extract agar (ISP 2), oatmeal agar (ISP 3), and inorganic salt-starch agar (ISP 4). Bacteria of this genus decompose and hydrolyze a range of

*See Shirling and Gottlieb (1966) for the composition of ISP media cited here and in other sections of this chapter.

*Contains 10 g of glucose, 20 g of soluble starch, 5 g of yeast extract, 5 g of N-Z amine type A, 1 g of $CaCO_3$, 15 g of agar, and 1 liter of distilled water (Cote et al., 1984).

*See Shirling and Gottlieb (1966) for the composition of ISP media cited here and in other sections of this chapter.

complex organic compounds and possess various enzymatic activities (Table 227). In addition, all species show activities for alkaline phosphatase, esterase lipase (C8), leucine arylamidase, α-glucosidase, β-glucosidase, but are negative in tests for lipase (C14), α-galactosidase, β-glucuronidase, *N*-acetyl-β-glucosaminidase, α-mannosidase, and α-fucosidase (the API ZYM assay). All give negative responses in the API 20NE tests for arginine dihydrolase, lysine decarboxylase, ornithine decarboxylase, urease, tryptophan deaminase, and indole production, but are positive in the Voges–Proskauer test. Only two species (*Marmoricola aequoreus* and *Marmoricola bigeumensis*) display positive reaction in the test for nitrate reduction. H_2S production is not observed (examined for *Marmoricola aequoreus* and *Marmoricola scoriae*). All species hydrolyze esculin. Do not decompose carboxymethyl cellulose (data for *Marmoricola korecus* and *Marmoricola scoriae*). *Marmoricola* species so far described are mesophilic and generally considered to be non-halophilic, but some have been reported to grow best at a low salinity (1.5–2% NaCl; *Marmoricola bigeumensis*) or tolerate up to 7% NaCl (*Marmoricola aequoreus* and *Marmoricola bigeumensis*). All prefer a neutral to mildly alkaline pH; some species can grow in test media at high initial pH values (up to pH 12); some exhibit alkaliphilic properties (*Marmoricola scoriae*).

Bacteria of this genus occur in various environments, including nutrient-limited ones. The recognized *Marmoricola* species have been isolated from agricultural soil, sandy beach sediments, volcanic ash, and a marble statue (Table 227). Ecological studies show that representatives of *Marmoricola* or phylogenetically very close organisms occur in soils of different origins, including pastures (Schoenborn et al., 2004) and the mound of a soil-feeding termite (*Cubitermes niokoloensis*; Fall et al., 2007). Bacteria of this genus have also been associated with dust particles transported from desert top soils by Saharan dust storms (Polymenakou et al., 2008) and with urban aerosols (Brodie et al., 2007), and found in indoor environments (Rintala et al., 2008; GenBank no. FM872516 and no. AM697095). They also occur in marine ecosystems (Gontang et al., 2007) and among bacteria cultured from granular activated carbon filters in water treatment installations (Magic-Knezev et al., 2009). Bacteria of the genus *Marmoricola* are detected in human skin microbiome (Grice et al., 2009, 2008; see, e.g. GenBank numbers HM267307, HM326534, etc.) and have been found in microbial populations of bronchoalveolar lavage fluid from children with cystic fibrosis (Harris et al., 2007). A bacterium remotely related to *Marmoricola aurantiacus* was also detected in the rhizosphere-associated bacterial and fungal communities of diseased seedlings (Filion et al., 2004). However, no species or strains pathogenic for humans or warm-blooded animals or plants have yet been identified within the genus *Marmoricola*.

Enrichment and isolation procedures

No selective media or enrichment procedures have been devised for isolating organisms of the genus *Marmoricola*. The available strains of this genus are isolates obtained during studies on microbiota of certain biotopes. *Marmoricola aurantiacus* was isolated from a marble statue on Bunt and Rovira medium (Bunt and Rovira, 1955), modified by the addition of 0.5% glucose, 0.5% NaCl, and 0.03% Na_2CO_3, pH 8.6 (BRII) and further cultivated in Luedemann medium (Luedemann, 1968). *Marmoricola korecus* and *Marmoricola scoriae* were isolated on starch–casein agar (1% soluble starch, 0.03% casein, 0.2% KNO_3, 0.2% NaCl, 0.2% KH_2PO_4, 0.002% $CaCO_3$, 0.005% $MgSO_4 \cdot 7H_2O$, 0.001% $FeSO_4 \cdot 7H_2O$, and 1.8% agar, pH 7.2). SC-SW agar (the same composition but without addition of KH_2PO_4 and prepared in a 60:40 mixture of natural sea water and distilled water; Lee, 2006) was used for isolation of *Marmoricola aequoreus*, and one-tenth-strength R2A agar (Difco) was used for isolation of *Marmoricola bigeumensis*. Isolations were carried out using the serial dilution plating method, followed by incubation at 28–30°C for ~2–4 weeks.

Maintenance procedures

Cultures may be maintained as 20% glycerol suspensions at −20 and −80°C. Long-term conservation is achieved by freeze-drying or in liquid nitrogen by standard procedures.

Differentiation of the genus *Marmoricola* from other genera

The phenotypic characteristics differentiating *Marmoricola* from other genera of the family are listed in Table 214 and Table 216. The genus *Marmoricola* is readily distinguished from most genera of the family by the presence of coccoid cells and predominant menaquinone MK-8(H_4). The same major menaquinone is also characteristic of the genus *Nocardioides*, but members of the latter have mycelial or rod-shaped cell morphologies in young cultures and usually form coccoid cells (along with short rods) at later growth stages. Further, the *Marmoricola* species readily differ from the genus *Nocardioides* in the fatty acid profile dominated by straight-chain compounds (exception is *Marmoricola bigeumensis*). It should be emphasized that delineation of the current genera *Marmoricola* and *Nocardioides*, as a whole, remains somewhat uncertain (See *Taxonomic comments*, below). Therefore, the side-by-side comparison of novel strains with individual species of the genus *Marmoricola* and closely related *Nocardioides* species is advisable during the identification process. Also, (short) rods in some organisms of this group, owing to their possible fast fragmentation into cocci on certain media, may be difficult to observe.

Taxonomic comments

The genus *Marmoricola* was described by Urzì et al. (2000) with one species, *Marmoricola aurantiacus*, represented by a single nonmotile coccoid strain having high (96.4%) 16S rRNA gene sequence similarity with the phylogenetically neighboring species, *Nocardioides jensenii*. The establishment of the genus *Marmoricola* was based on the assumed priority of unusual phenotypic, including chemotaxonomic, features of the strain and its difference from other members of the family *Nocardioidaceae* described by that time in several secondary-structure-forming nucleotides. The key chemotaxonomic marker separating the genus *Marmoricola* from *Nocardioides* included the cellular fatty acid profile (mostly straight-chain components with minor amounts of $C_{18:0}$ 10-methyl). The species *Marmoricola aequoreus*, *Marmoricola scoriae*, and *Marmoricola korecus*, described subsequently (Lee and Lee, 2010; Lee, 2007a; Lee et al., 2010) have generally similar morphological and chemotaxonomic properties to those of *Marmoricola aurantiacus*, except the presence of phosphatidylcholine (detected in *Marmoricola scoriae* and *Marmoricola korecus*). This finding rendered the genus heterogeneous with respect to principal polar lipids, and the genus

description has been emended (Lee and Lee, 2010) to include this characteristic and also reflect some features of *Marmoricola bigeumensis* described in 2008 (Dastager et al., 2008b). The latter was assigned to the genus mainly on the basis 16S rRNA-based phylogenetic clustering (Dastager et al., 2008b).

However, *Marmoricola bigeumensis*, as mentioned before, markedly differs from the other *Marmoricola* species by possessing a large proportion of saturated iso-branched acids and resembling *Nocardioides* by the fatty acid profile. Notably, iso- and anteiso-branched acids are synthesized in a different way than the straight-chain saturated acids (for details and references see Suzuki et al., 1993), and the respective difference in fatty acid composition is typically used as a criterion to differentiate the actinomycete genera (Kroppenstedt, 1985; Suzuki and Komagata, 1983a; Urzì et al., 2000). *Marmoricola bigeumensis* is also distinguished from the other *Marmoricola* species by motile and smaller cells, and exceeds those in NaCl resistance. In addition, it is not quite clear whether the cells of *Marmoricola bigeumensis* are cocci at all growth phases, or arise from (short) rods at later stages of the developmental cycle as in *Nocardioides* species. Furthermore, with the recent descriptions of additional *Marmoricola* species and closely related organisms of the genus *Nocardioides*, it becomes clear that *Marmoricola bigeumensis* is branching outside the group of the remaining *Marmoricola* species. This species is also closest to members of the genus *Nocardioides* (*Nocardioides jensenii* and *Nocardioides mesophilus*) in terms of 16S rRNA gene sequence similarity (97%; calculated from 1420 bp and 1396 bp, respectively), while the sequence similarity to the other *Marmoricola* species is 96.6–95.3% (1398–1435 bp compared). Taken together, all the above data indicate that the current taxonomic structure of the genus *Marmoricola* is in need of re-evaluation to improve coherence of its phylogenetic and phenotypic circumscription and delimitation from the genus *Nocardioides*. The available data suggest that further detailed taxonomic studies of *Marmoricola bigeumensis* at the genomic and phenotypic level, involving available and newly isolated relevant strains, may provide strong grounds for movement of *Marmoricola bigeumensis* to the genus *Nocardioides* or to a new genus.

Differentiation of species of the genus *Marmoricola*

Phenotypic characteristics useful in distinguishing the current *Marmoricola* species are discussed in the above sections and listed in Table 227 and in the *List of species of the genus Marmoricola*, below. When using the compositions of fatty acids and polar lipids for differentiation, note that these characteristics may depend on growth conditions, age of the culture, and analytical procedure. In comparative taxonomic studies, cells must be grown and analyzed under the same conditions.

Acknowledgements

The author was supported by the MCB program of the Russian Academy of Sciences.

List of species of the genus *Marmoricola*

1. **Marmoricola aurantiacus** Urzì, Salamone, Schumann and Stackebrandt 2000, 534[VP]

au.ran.ti′a.cus. N.L. masc. adj. *aurantiacus* orange-colored.

Characteristics are as described for the genus and listed in Table 227. Additional information presented below is taken from the original paper (Urzì et al., 2000), unless indicated. Spherical cells (0.5–0.7 µm in diameter) occurring singly, in pairs, as short chains, or small clusters (Figure 276). Nonmotile. No rod–coccus life cycle. Cells are Gram-stain-positive and not lysed in the KOH test. Non-acid-fast. Colonies are orange-pigmented and 2–5 mm diameter after 30-d incubation on Luedemann medium (Luedemann, 1968) at 28°C. Grows at 18°C; no growth occurs at 6 or 37°C. Growth is restricted on Luedemann medium supplemented with 2% NaCl and absent in the presence of 4% NaCl. The type strain will grow on brain heart infusion agar (BHIA; Oxoid), potato glucose agar (PDA; Oxoid), ISP 2 agar, and in Bacto tryptic soy broth (Difco). According to Lee and Lee (2010), growth is also supported by R2A agar (Difco). No growth is observed in several other tested complex media. Will grow on inorganic medium ISP 9 with various carbon sources (Lee and Lee, 2010) but not on Czapek–Dox modified agar (CZ; Oxoid) or on water agar. Acid is not produced from D-glucose, D-ribose, L-arabinose, D-galactose, D-cellobiose, lactose, maltose, D-mannose, D-raffinose, L-rhamnose, D-trehalose, D-xylose, glycerol, D-mannitol, or *myo*-inositol, as registered on the medium containing 0.5% tryptone (Oxoid), 0.4% Bacto Casamino acids (Difco), 0.07% $(NH_4)_2HPO_4$, 0.5% NaCl, 0.003% bromocresol purple, and the substrate at a concentration of 1%. Hydrolyzes Tween 80 (weak). The whole-cell sugars are glucose and traces of ribose. The major cellular fatty acids (about 5% or more at least in one experiment) determined in cells grown in Bacto tryptic soy broth at 28°C (Urzì et al., 2000) and in cells cultured on R2A agar at 30°C for 5 d (Lee and Lee, 2010) are $C_{16:0}$ (41.4 and 37%, respectively), $C_{18:1}/C_{18:1}$ ω9*c* (33.7 and 27), $C_{16:1}/C_{16:1}$ ω9*c* (14.9 and <1), $C_{18:0}$ 10-methyl (2.8 and 5.4), and $C_{16:0}$ 2-OH (1.4 and 4.7). In addition, Lee and Lee (2010) detected a significant amount of $C_{16:1}$ ω7*c* and/or $C_{15:0}$ iso 2-OH (9.9%) in cells from R2A agar.

Source (type strain): a Carrara marble statue (Wagmüller's monument) located in the Nordfriedhof Cemetery in Munich, Germany.

DNA G+C content (mol%): 72.1 (HPLC).

Type strain: BC 361, DSM 12652, CIP 106770, JCM 10917.

Sequence accession no. (16S rRNA gene): Y18629.

2. **Marmoricola aequoreus** Lee 2007a, 1392[VP]

a.e.qu.o.re′us. L. masc. adj. *aequreus* belonging to the sea, referring to the isolation site of the type strain.

Characteristics are as described for the genus and listed in Table 227. Additional information presented below is taken from the papers of Lee (2007a) and Lee and Lee (2010).

Spherical cells (0.5–0.7 μm in diameter) occurring singly, in pairs, as short chains, or small clusters. Nonmotile. No rod–coccus life cycle is observed. Colonies are bright yellow after incubation for 5 d at 30°C on YE-SW agar (ISP 2 agar prepared in a 60:40 mixture of natural sea water and distilled water). Growth is good at 10–37°C (with an optimum at 30°C), poor at 4°C, and absent at 42°C. Will grow on ISP medium 2 with 5% NaCl, but poorly at 6–7% NaCl. Grows at pH 5.1–12.1 (initial pH of medium, YE-SW agar) and optimally at pH 7.1. The type strain produces acid from dextran, maltose, and salicin but not from D-glucose and almost 30 other carbon sources tested using Bacto OF basal medium (Difco) supplemented with a filter-sterilized carbon source at a final concentration of 1%. The cellular fatty acids (>5% at least in one experiment), determined in cells grown on TSA for 3 d at 30°C and on R2A agar for 5 d at the same temperature, include $C_{18:1}$ ω9*c* (40.1 and 26.4), $C_{16:0}$ (35 and 30.2), $C_{18:0}$ (7.6 and 2.3%), $C_{16:1}$ ω9*c* (6.6 and <1). In cells harvested from R2A agar, 10-methyl-branched acids were detected (7.1% $C_{18:0}$ 10-methyl and 1.7% $C_{17:0}$ 10-methyl), as well as $C_{19:0}$ anteiso (6.3%), $C_{16:1}$ 2-OH (4.3%), and $C_{16:1}$ ω7*c* and/or $C_{15:0}$ iso 2-OH (8.2%).

Source (type strain): sandy sediment 1 m below the surface of Samyang Beach on Jeju Island, Republic of Korea.

DNA G+C content (mol%): 72.4 (HPLC).

Type strain: SST-45, JCM 13812, NRRL B-24464.

Sequence accession no. (16S rRNA gene): AM295338.

3. **Marmoricola bigeumensis** Dastager, Lee, Ju, Park and Kim 2008b, 1062[VP]

bi.ge.um.en′sis. N.L. masc. adj. *bigeumensis* of or pertaining to Bigeum Island, Korea, from where the type strain was isolated.

Characteristics are as described for the genus and listed in Table 227. Additional information presented below is taken from the original paper (Dastager et al., 2008b), unless indicated.

Coccoid and motile cells (0.3–0.5 μm in diameter). Colonies are lemon-yellow and 1–3 mm in diameter on R2A agar. Grows in the presence of up to 7% NaCl and at pH 6.0–12.0 (initial pH of medium), but best at 1.5–2% NaCl and pH ~7.2 (all tested on twofold diluted in R2A agar). Will grow in the temperature range of 20–37°C. No growth is observed on yeast extract-malt extract agar (ISP 2), oatmeal agar (ISP 3), and inorganic salt-starch agar (ISP 4). D-Glucose, L-arabinose, L-rhamnose, D-ribose, and D-xylose are utilized as carbon sources for growth and energy, as determined both in the original study (Dastager et al., 2008b) and using ISP 9 as basal medium (Lee and Lee, 2010); growth with D-mannose and D-mannitol is variable (Dastager et al., 2008b; Lee and Lee, 2010). No acid is produced (the Hugh–Leifson test; Hugh and Leifson, 1953) from the aforementioned carbohydrates and also from cellobiose, D-galactose, lactose, maltose, and raffinose. Tween 80 is hydrolyzed. The reported cellular fatty acids [determined in cells grown on Trypticase soy agar plates (BBL) using the procedures described by Miller (1982)] include $C_{16:0}$ iso (59.1%), $C_{17:0}$ anteiso (6.9%), $C_{16:0}$ (4.0%), and $C_{14:0}$ iso (3.3%). A wider spectrum of fatty acids was detected by Lee and Lee (2010) for cells grown on R2A agar at 30°C for 5 d, with $C_{16:0}$ iso predominating (27.6%). The other components (3% or more) were $C_{16:0}$ (6.8), $C_{17:0}$ (3.8), $C_{17:1}$ ω8*c* (6.9), $C_{18:1}$ ω9*c* (4.2), $C_{14:0}$ iso (4.3), $C_{15:0}$ iso (5.5), $C_{17:0}$ iso (3.5), $C_{15:0}$ anteiso (3.3), $C_{17:0}$ anteiso (5.9), $C_{16:0}$ iso 3-OH (3.0), and $C_{16:1}$ 2-OH (3.5); a 10-methyl-branched acid ($C_{17:0}$ 10-methyl) was found as a minor component (2.2%).

Source (type strain): a soil sample collected from an agricultural area of Bigeum Island, Korea.

DNA G+C content (mol%): 72.9 (HPLC).

Type strain: MSL-05, KCTC 19287, JCM 15624, DSM 19426.

Sequence accession no. (16S rRNA gene): EF466120.

4. **Marmoricola korecus** Lee, Lee and Ko 2010, 000[VP]

ko.re′cus. N.L. masc. adj. *korecus* pertaining to Korea, where the type strain was isolated.

Characteristics are as described for the genus and listed in Table 227. Additional information presented below is taken from the original paper (Lee et al., 2010).

Spherical (1.1–1.2 μm diameter) and nonmotile cells. Colonies are yellow-pigmented after incubation for 5 d on ISP 2 agar at 30°C (optimal temperature). Growth occurs at 4–37°C but not at 42°C. Grows on ISP 2 with 2% NaCl (but not with 3%), and in the pH range of pH 5.1–12.1; optimal pH is 6.0–10.1 (initial pH of the same test medium). The major fatty acids determined in cells grown on R2A agar at 30°C for 5 d are $C_{16:0}$, $C_{17:1}$ ω8*c*, $C_{18:1}$ ω9*c*, as well as $C_{16:1}$ ω7*c* and/or $C_{15:0}$ iso 2-OH.

Source (type strain): a red-colored layer of volcanic ash, Jeju, Republic of Korea.

DNA G+C content (mol%): 71.0 (HPLC).

Type strain: Sco-A36, KCTC 19596, DSM 22128.

Sequence accession no. (16S rRNA gene): FN386723.

5. **Marmoricola scoriae** Lee and Lee 2010, 2138[VP]

sco.ri′a.e. L. gen. n. *scoriae* of scoria (volcanic ash), referring to the site from which the type strain was isolated.

Characteristics are as described for the genus and listed in Table 227. Additional information presented below is taken from the original paper (Lee and Lee, 2010).

Spherical cells (0.6–1.0 μm in diameter), occur singly, in pairs or in clusters. Nonmotile. Colonies are vivid yellow, 0.1–0.2 mm in diameter after incubation on ISP 2 agar for 5 d at 30°C (optimal growth temperature). Will grow at 10–37°C but not at 4 or 42°C. Growth occurs at pH 6.1–12.1; optimal pH is 8.1–11.1 (initial pH values of test medium, ISP 2). Grows on the same medium at 1–3% NaCl but better in the absence of NaCl. The major fatty acids (>5% of the total) determined in cells grown on R2A agar at 30°C for 5 d are $C_{16:0}$ (27.7), $C_{18:1}$ ω9 (25.9), $C_{18:0}$ 10-methyl (10.2), $C_{16:0}$ 2-OH (8), as well as $C_{16:1}$ ω7*c* and/or $C_{15:0}$ iso 2-OH (6).

Source (type strain): volcanic ash of Oreum (a parasitic volcanic cone), Jeju, Republic of Korea.

DNA G+C content (mol%): 72.0 (HPLC).

Type strain: Sco-D01, KCTC 19597, DSM 22127.

Sequence accession no. (16S rRNA gene): FN386750.

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Order XIII. **Pseudonocardiales** ord. nov.

David P. Labeda and Michael Goodfellow

Pseu.do.no.car.di′a.les. N.L. fem. n. *Pseudonocardia* type genus of the order; suff. *-ales* ending to denote an order; N.L. fem. pl. n. *Pseudonocardiales* the *Pseudonocardia* order.

The order was formed by elevation of the suborder *Pseudonocardineae* Stackebrandt, Rainey, Ward-Rainey 1997, 486[VP] emend. Zhi, Li and Stackebrandt 2009, 599. Labeda et al. (2011) proposed the elimination of the family *Actinosynnemataceae* Labeda and Kroppenstedt 2000 and emended the description of the family *Pseudonocardiaceae* to include the genera formerly found within this family. As a result, the order *Pseudonocardiales* contains a single family and **the description and signature nucleotides of the 16S rRNA gene are that of the family *Pseudonocardiaceae*.**

Type genus: **Pseudonocardia** Henssen 1957, 408[AL].

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Family I. **Pseudonocardiaceae** Embley, Smida and Stackebrandt 1989, 205[VP] emend. Labeda, Goodfellow, Chun, Zhi and Li 2010a

David P. Labeda and Michael Goodfellow

Pseu.do.no.car.di.a.ce′a.e. N.L. fem. n. *Pseudonocardia* the type genus of the family; suff. *-aceae* ending to denote a family; N.L. fem. pl. n. *Pseudonocardiaceae* the *Pseudonocardia* family.

Aerobic, mesophilic, or thermophilic, Gram-stain-positive, non-acid-fast, catalase-positive, lysozyme resistant, actinomycetes comprising the type genus *Pseudonocardia* (Henssen 1957) emend. Park et al. 2008, as well as the genera *Actinoalloteichus* Tamura et al. 2000, *Actinokineospora* (Hasegawa 1988b) Labeda et al. 2010b, *Actinomycetospora* Jiang et al. 2008, *Actinosynnema* Hasegawa et al. 1978, *Allokutzneria* Labeda and Kroppenstedt 2008, *Amycolatopsis* (Lechevalier et al. 1986) Lee 2009, *Crossiella* Labeda 2001, *Goodfellowiella* Labeda et al. 2008 (previous illegitimate name *Goodfellowia* Labeda and Kroppenstedt 2006), *Kibdelosporangium* Shearer et al. 1986a, *Kutzneria* Stackebrandt et al. 1994, *Lechevalieria* Labeda et al. 2001, *Lentzea* (Yassin et al. 1995) Labeda et al. 2001, *Prauserella* (Kim and Goodfellow 1999) Li et al. 2003a, *Saccharomonospora* Nonomura and Ohara 1971, *Saccharopolyspora* (Lacey and Goodfellow 1975) Korn-Wendisch et al. 1989, *Saccharothrix* (Labeda et al. 1984) Labeda and Lechevalier 1989, *Sciscionella* Tian et al. 2009, *Streptoalloteichus* (Tomita et al. 1987) Tamura et al. 2008b, *Thermocrispum* Korn-Wendisch et al. 1995, and *Umezawaea* Labeda and Kroppenstedt 2007. Figure 277 shows a phylogenetic tree for the genera of the family *Pseudonocardiaceae* calculated from almost-complete 16S rRNA gene sequences.

Morphologically heterogeneous; single or short chains of spores may be present on aerial mycelium and the substrate mycelium. Vegetative mycelium branches, diameter approximately 0.5–0.7 µm; aerial mycelium is produced and fragments into single or chains of smooth-surfaced, rod-shaped elements in some genera. Other structures such as synnemata or dome-like bodies, sporangia or pseudosporangia may be produced by some genera. Some taxa may fail to produce aerial mycelia. Motile spores may be produced by some genera. Marked fragmentation of hyphae occurs in some taxa, but is absent in others. Most taxa are chemo-organotropic, although some are autotrophic. A few taxa are halophilic. Members of the family are found in a diversity of environments, including soils, plant material, manure, and clinical or veterinary samples.

All genera contain *meso*-diaminopimelic acid as the diamino acid in their peptidoglycan and galactose as one of many diagnostic whole-cell sugars. Mycolic acids are not present in any of the genera. Tetrahydrogenated menaquinones of nine isoprene units are characteristic components, although menaquinones containing eight isoprene units predominate in the genus *Pseudonocardia*. The phospholipid profile generally includes phosphatidylethanolamine, sometimes containing hydroxylated fatty acids, as a major constituent, although representatives of one or more genera may also contain phosphatidylcholine. Chemotaxonomic properties of genera of the family are shown in Table 228. The pattern of 16S rRNA signatures consists of nucleotides at positions 127:234 (G–C), 564 (U), 672:734 (U–G), 831:855 (U–G), 832:854 (G–Y), 833:853 (U–G), 952:1229 (U–A), and 986: 1219 (U–A).

DNA G+C content (mol%): 66–76.

Type genus: **Pseudonocardia** Henssen 1957, 408[AL] emend. Park, Park, Lee and Kim 2008, 2477.

Further descriptive information

The family *Pseudonocardiaceae* Embley, Smida and Stackebrandt 1989, 205[VP] emend. Zhi, Li and Stackebrandt 2009, 599 was proposed by Embley et al. (1988a, 1989) and Warwick et al. (1994) on the basis of 16S rRNA gene sequence phylogeny for the genera. The family was emended by Stackebrandt et al. (1997) and, subsequently, Labeda and Kroppenstedt (2000) proposed that the genera *Actinosynnema*, *Actinokineospora*, *Lentzea*, and *Saccharothrix* be placed in the new family *Actinosynnemataceae* based on phylogenetic analysis of 16S rRNA gene sequences for a subset of all taxa within the family. The description of the family *Actinosynnemataceae* was emended by Zhi et al. (2009) to include additional genera described since 2000, namely *Lechevalieria* Labeda et al. 2001 and *Umezawaea* Labeda and Kroppenstedt 2007. A recent study of the taxonomic status of the families *Actinosynnemataceae* and *Pseudonocardiaceae* (Labeda et al., 2010a), based on the 16S rRNA gene sequence data available for the 142 validly named taxa and the chemotaxonomic and morphological properties available from the literature, concluded that the retention of the family *Actinosynnemataceae* could no longer be supported, nor could adequate support be found for any other subdivision of the order *Pseudonocardiales* at this time. It was proposed that the genera within the family *Actinosynnemataceae* be included in the family *Pseudonocardiaceae* and the family description emended accordingly.

Key to the genera of the family *Pseudonocardiaceae*

I. Produces sporangia on the colony surface observable with light microscopy.

A. Polar lipid pattern includes phosphatidylethanolamine containing hydroxylated fatty acids and *lyso*-phosphatidylethanolamine.

→Genus *Allokutzneria*

B. Polar lipid pattern does not include phosphatidylethanolamine containing hydroxylated fatty acids or *lyso*-phosphatidylethanolamine; whole-cell sugar pattern includes arabinose and galactose.

→Genus IX. *Kibdelosporangium*

C. Polar lipid pattern does not include phosphatidylethanolamine containing hydroxylated fatty acids or *lyso*-phosphatidylethanolamine; whole-cell sugar pattern includes galactose and rhamnose.

→Genus X. *Kutzneria*

II. Produces pseudosporangia on the colony surface.

A. Polar lipid pattern includes phosphatidylethanolamine containing hydroxylated fatty acids.

→Genus XVIII. *Thermocrispum*

B. Polar lipid pattern does not include phosphatidylethanolamine containing hydroxylated fatty acids.

1. Polar lipid pattern includes phosphatidylinositol and phosphatidylinositol mannosides; whole-cell sugar pattern includes rhamnose.

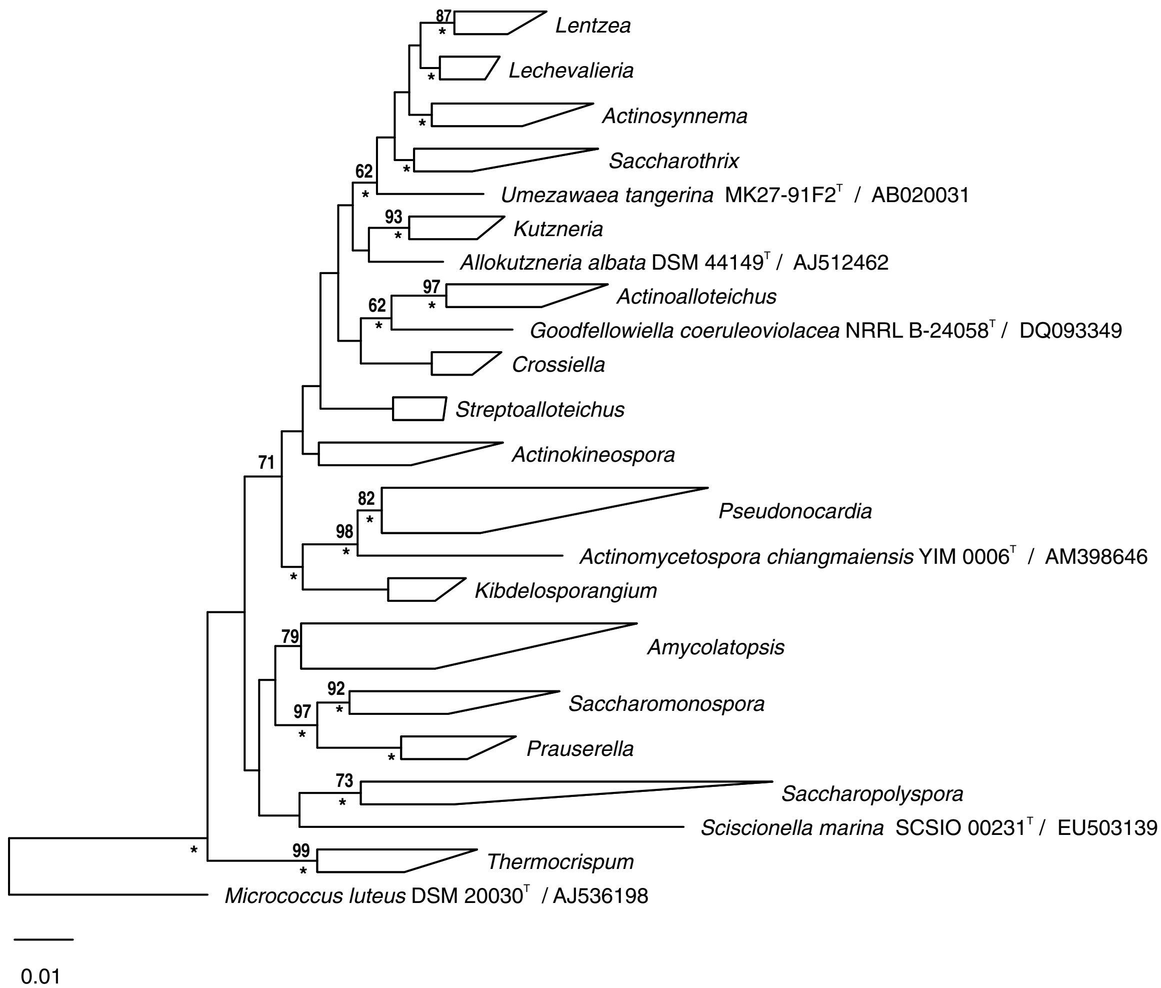

FIGURE 277. Phylogenetic tree for the genera of the family *Pseudonocardiaceae* calculated from almost-complete 16S rRNA gene sequences using the Kimura evolutionary distance method (Kimura, 1980) and the neighbor-joining algorithm of Saitou and Nei (1987). Percentages at the nodes represent levels of bootstrap support from 100 resampled datasets; values less than 60% are not shown. Branches also conserved in maximum-parsimony (Felsenstein, 1993) and maximum-likelihood (Stamatakis et al., 2002) trees are marked with an asterisk. Bar = 0.01 nucleotide substitutions per site.

→Genus VII. *Crossiella*

2. Polar lipid pattern does not include phosphatidylinositol or phosphatidylinositol mannosides; whole-cell sugar pattern does not include rhamnose.

→Genus XVII. *Streptoalloteichus*

III. Does not produce sporangia or pseudosporangia.

A. Produces motile spores.

1. Produces chains of motile spores borne on synnemata or raised dome-like structures on the vegetative mycelium.

→Genus V. *Actinosynnema*

2. Produces chains of motile spores on the aerial mycelia; synnemata not produced

→Genus III. *Actinokineospora*

B. Does not produce motile spores.

1. Does not contain arabinose as a diagnostic whole-cell sugar.

a. Polar lipid pattern includes phosphatidylethanolamine containing hydroxylated fatty acids and *lyso*-phosphatidylethanolamine.

→Genus XIX. *Umezawaea*

b. Polar lipid pattern includes phosphatidylethanolamine containing hydroxylated fatty acids but not *lyso*-phosphatidylethanolamine.

i. Diagnostic whole-cell sugars include mannose and rhamnose.

→Genus XVI. *Saccharothrix*

ii. Diagnostic whole-cell sugars do not include either mannose or rhamnose.

→Genus VIII. *Goodfellowiella*

c. Polar lipid pattern does not include phosphatidylethanolamine containing hydroxylated fatty acids.

i. 16S rRNA gene contains diagnostic nucleotide signature pattern of TT (844–845) and GGT (1107–1109).

→Genus XI. *Lechevalieria*

ii. 16S rRNA gene contains diagnostic nucleotide signature patterns of TCAA (617–620) and GCC (843–845).

→Genus XII. *Lentzea*

2. Contains arabinose as diagnostic whole-cell sugar.

a. Polar lipid pattern contains phosphatidylcholine and/or phosphatidylethanolamine.

TABLE 228. Comparison of chemotaxonomic profiles of genera within the family *Pseudonocardiaceae*

Character	*Pseudonocardia*	*Actinoalloteichus*	*Actinokineospora*	*Actinomycetospora*	*Actinosynnema*	*Allokutzneria*	*Amycolatopsis*	*Crossiella*	*Goodfellowiella*	*Kibdelosporangium*	*Kutzneria*	*Lechevalieria*	*Lentzea*	*Prauserella*	*Saccharomonospora*	*Saccharopolyspora*	*Saccharothrix*	*Scisionella*	*Streptoalloteichus*	*Thermocrispum*	*Umezawaea*
Sporangia produced	None	None	None	None	Synnemata	Yes, no spores	None	None	None	Yes	Yes	None	None	None	None	None	None	None	Pseudosporangia	Pseudosporangia	None
Motile spores	No	No	Variable	No	Yes	No	No	No	No	No	No	No	No	No	No	No	No	No	Variable	No	No
Whole-cell sugar pattern[b]	Ara, Gal	Glu, Gal, Man, Rib	Ara, Gal, Rha, Man	Ara, Gal	Gal, Man	Ara, Gal, Man	Ara, Gal	Gal, Man, Rha, Rib	Gal, Rib	Ara, Gal, Glu (v), Mad (v), Rha (v)	Gal, Rha	Gal, Man, Rha (trace)	Gal, Man, Rib	Ara, Gal	Ara, Gal	Ara, Gal	Gal, Rha, Man (trace)	Ara, Gal, Glu	Gal, Man, Rha,	Ara, Glu, Gal (trace), Man	Gal, Man, Rib, Rha (trace)
Phospholipids[a]	PC, PE, PME, PI, PIM, OH-PE	PE, PIM, PI, PG, DPG PME	PE, DPG, PI	PC, PI, PG	PE, OH-PE, DPG	PE, PME, OH-PE, PI, lyso-PME, DPG, PG, *lyso*-PE	PE, DPG, PG, PI	PE, PME, PI, PIM	PE, DPG, OH-PE, PME	PE, PI, PME, PG, DPG, PIM	PE, DPG, PI, PG, PME	PE, DPG, PG, PI	PE, DPG, DPG, PI	DPG, PE	PE, DPG, PG, PI	PC, PE, DPG, PG, PI	PE, OH-PE, DPG, PG, PI, PIM	DPG, PC, PE, PI, PL, PME	PE, DPG, PI, PIM, DPG, PME	PE, PI, OH-PE	PE, PI, OH-PE, *lyso*-PE
Predominant menaquinone(s)	MK-8 (H_4)	MK-9 (H_4)	MK-9 (H_4)	MK-9(H_4)	MK-9(H_4), some MK-9(H_6)	MK-9 (H_4)	MK-9(H_4)	MK-9 (H_4)	MK-9(H_4), MK-10(H_4)	MK-9(H_4), MK-9(H_6), MK-9 (H_{10})	MK-9(H_4)	MK-9(H_4)	MK-9 (H_4)	MK-9 (H_2), MK-9 (H_4)	MK-9(H_4)	MK-9 (H_4)	MK-9(H_4), MK-10(H_4)	MK-9 (H_4)	MK-9(H_6), MK-10(H_6)	MK-9 (H_4)	MK-9(H_4), MK-10(H_4)
DNA G+C content, mol%	68–69	72–72.5	72.0	69.0	73.0	71.6	66.0–69.0	74.1	69.2	66	70.3–70.7	68.0–71.4	71.4	67–68.9	66.0–70.0	66.0–74.0	71.4	69.0	71.6	69.0–73.0	74.0

[a]Ara, Arabinose; Gal, galactose; Glu, glucose; Mad, madurose; Man, mannose; Rha, rhamnose; Rib, ribose. v, Variable.

[b]DPG, Diphosphatidylglycerol; OH-PE, phosphatidylethanolamine with hydroxy fatty acids; *lyso*-PE, phosphatidylethanolamine where one fatty acid chain is missing from the glycerol backbone; *lyso*-PME, phosphatidylmethylethanolamine where one fatty acid chain is missing from the glycerol backbone; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; PI, phosphatidylinositol; PIM, phosphatidylinositol mannosides; PL, unknown phospholipids; PME, phosphatidylmethylethanolamine.

i. Polar lipid pattern includes phosphatidylethanolamine containing hydroxylated fatty acids; predominant menaquinone is MK-8(H_4); chains of spores not produced on aerial mycelia, but vegetative mycelium fragments.
→Genus I. *Pseudonocardia*

ii. Polar lipid pattern does not include phosphatidylethanolamine containing hydroxylated fatty acids; predominant menaquinone is MK-9(H_4); chains of spores not produced on aerial mycelia, but vegetative mycelium fragments.
→Genus *Sciscionella*

b. Polar lipid pattern contains phosphatidylcholine; predominant menaquinone is MK-9(H_4).

i. Short chains of spores, sometimes with spiny to hairy ornamentation, are produced on the aerial mycelia.
→Genus XV. *Saccharopolyspora*

ii. Short chains of smooth-surfaced spores are produced from the vegetative mycelium; phosphatidylinositol and phosphatidylglycerol are only other polar lipids.
→Genus III. *Actinomycetospora*

c. Polar lipid pattern contains phosphatidylethanolamine but not phosphatidylcholine.

i. Single spores, often densely packed, are borne along the aerial mycelium.
→Genus XIV. *Saccharomonospora*

ii. Substrate mycelium fragments into irregular rods; aerial mycelium may be produced which fragments into spore chains; nucleotide signature regions in the 16S rRNA gene sequence include 51 (A/C), 208 (U), 210 (G), 213 (A), 280 (C), 1004 (A), and 1257 (U).
→Genus VI. *Amycolatopsis*

iii. Substrate mycelium fragments into irregular rods; aerial mycelium may be produced which fragments into spore chains; nucleotide signature regions in the 16S rRNA gene sequence include 51 (A), 72 (A), 183:194 (G–C), 208 (G), 210 (A), 213 (C), 280 (U), 1004 (G), and 1257 (G).
→Genus XIII. *Prauserella*

Genus I. **Pseudonocardia** Henssen 1957, 408[AL] emend. Park, Park, Lee and Kim 2008, 2477

Ying Huang and Michael Goodfellow

Pseu.do.no.car′di.a. Gr. adj. *pseudês* false; N.L. fem. n. *Nocardia* a bacterial genus name; N.L. fem. n. *Pseudonocardia* the false *Nocardia*.

Aerobic, Gram-stain-positive, non-acid-fast, nonmotile, catalase-positive, actinomycetes which form branched substrate hyphae that may fragment into rod-shaped elements. Hyphae vary in thickness and in the degree of branching. Aerial hyphae, when formed, may be sterile, fragmented into chains of oval or squarish elements, or into chains of two or more spores. **Substrate and aerial hyphae may exhibit cell division in different directions with a tendency to form apical or intercalary swellings. Spores are usually smooth and are produced on the substrate and/or aerial mycelium by acropetal budding and/or by basipetal septation (fragmentation), or else are formed in longitudinal pairs on vegetative hyphae and singly or in longitudinal pairs on aerial hyphae. In some species, the mycelium is covered by an electron-dense outer layer.** Grows on a variety of organic and synthetic media. Some species are facultative autotrophs. **Cell wall contains *meso*-diaminopimelic acid (*meso*-A2pm), arabinose, and galactose. The major menaquinone is MK-8(H_4) and the predominant fatty acid is iso-branched hexadecanoic acid. Mycolic acids are absent.** The polar lipid profile, which varies between species, may include any of the following: phosphatidylcholine, phosphatidylethanolamine, phosphatidylglycerol, phosphatidylmethylethanolamine, and glucosamine-containing phospholipids. **The 16S rRNA gene sequence contains two unique regions which correspond to nucleotide positions 179–219 and 813–833 of the *Streptomyces ambofaciens* 16S rRNA gene** (Pernodet et al., 1989). The phylogenetic position of *Pseudonocardia*, as determined by 16S rRNA gene sequence analysis, is in the family *Pseudonocardiaceae.*

DNA G+C content (mol%): 68–79 (HPLC, T_m).

Type species: **Pseudonocardia thermophila** Henssen 1957, 408[AL].

Further descriptive information

Phylogeny. The genus *Pseudonocardia* forms a distinct clade in the *Pseudonocardiaceae* 16S rRNA gene tree (Bowen et al., 1989; Embley et al., 1988b, 1988c; Warwick et al., 1994). It encompasses 29 species with validly published names, half of which have been proposed in the past 10 years on the basis of polyphasic taxonomic studies (Chen et al., 2009; Huang et al., 2002; Kämpfer and Kroppenstedt, 2004; Lee et al., 2000a; Park et al., 2008). *Pseudonocardia* species can be phylogenetically assigned to four multimembered clades and one single-membered clade (Figure 278). The earliest described species in the three multi-membered subclades, which are supported by bootstrap values that range from 70 to 100%, are *Pseudonocardia halophobica*, *Pseudonocardia hydrocarbonoxydans*, and *Pseudonocardia nitrificans.* The earliest described species in the least well circumscribed multi-membered subclade is *Pseudonocardia thermophila. Pseudonocardia spinosispora* forms a single-membered clade that is loosely associated with the *Pseudonocardia nitrificans* phyletic line. The most distant phylogenetic relationship in the genus is between the type strains of *Pseudonocardia ammonioxydans* and *Pseudonocardia thermophila* and these organisms share a 16S rRNA gene sequence similarity of 93%. In contrast, the type strains of *Pseudonocardia chloroethenivorans* and *Pseudonocardia dioxanivorans* have identical 16S rRNA gene sequences.

Cell morphology. *Pseudonocardia* strains form extensive substrate and aerial hyphae that vary in the degree of branching and in diameter (0.3–2 µm). Some species, such as *Pseudonocardia asaccharolytica*, *Pseudonocardia halophobica*, and *Pseudonocardia sulfidoxydans*, form swollen hyphal segments up to 5 µm in diameter, zig-zag mycelial growth, and longitudinal and transverse septa in the mycelium (Akimov et al., 1989; Reichert et al., 1998). A characteristic feature of many *Pseudonocardia* species, such as *Pseudonocardia ammonioxydans*, *Pseudonocardia spinosa*, and *Pseudonocardia thermophila*, is acropetal budding (Figure 279, Figure 280, and Figure 281); a constriction is formed behind the tip of the terminal segment, the tip then enlarges to form a new segment, another constriction is formed near the tip, and the process continues (Figure 279, Figure 280, Figure 281, Figure 282, Figure 283, and Figure 284; Henssen and Schäfer,

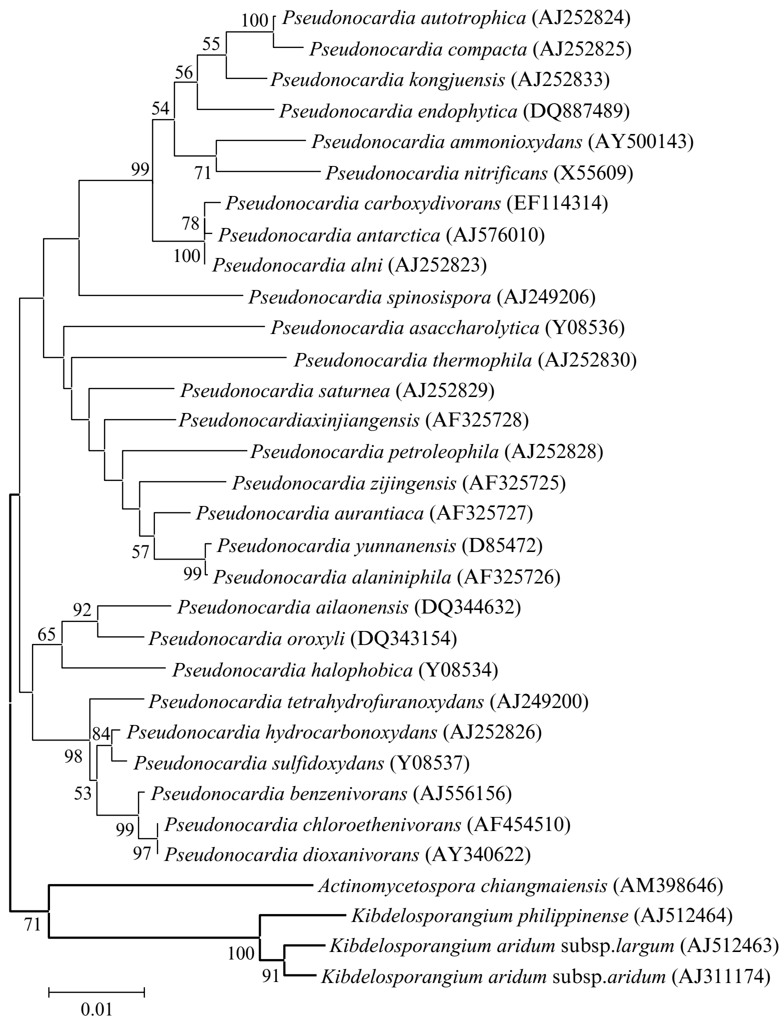

FIGURE 278. Neighbor-joining tree based on nearly complete 16S rRNA gene sequences showing relationships between type strains belonging to the genus *Pseudonocardia*. The numbers at the nodes indicate levels of bootstrap support based on a neighbor-joining analysis of 1000 resampled datasets; only values above 50% are given. Bar = 0.01 substitutions per nucleotide position.

1971). The constrictions may be secondarily separated by septa (Figure 280), which may be formed some distance behind the constriction, as in *Pseudonocardia compacta*. Side branches are usually formed below a septatum, more rarely from the center of a septation (Figure 281 and Figure 282). Aerial hyphae can be sterile, may fragment into chains of squarish to oval fragments, or differentiate into chains of spores (Lechevalier et al., 1986).

Spores may be formed on substrate or aerial hyphae by successive acropetal budding, by basipetal septation (fragmentation), or by irregular spore formation along senescing hyphae (Henssen, 1989; Henssen and Schnepf, 1967). *Pseudonocardia alaniniphila*, *Pseudonocardia aurantiaca*, *Pseudonocardia xinjiangensis*, and *Pseudonocardia yunnanensis* bear spores either in longitudinal pairs on substrate hyphae or singly on aerial hyphae (Xu et al., 1999). In *Pseudonocardia chloroethenivorans*, the aerial and substrate hyphae carry single spores at the ends of hyphae (Lee et al., 2004). Single spores are also formed at the ends of substrate hyphae in *Pseudonocardia tetrahydrofuranoxydans* (Kämpfer et al., 2006). Spores are smooth-walled or spiny and vary greatly in size, but are usually 0.5–1 µm wide by 1.5–4.5 µm long. Spore walls are of uniform thickness and intersporal pads are not formed. In *Pseudonocardia spinosa*, the ornamentation of the spiny spores is formed by folds in the fibrous sheath.

The fine structure of some *Pseudonocardia* species, notably *Pseudonocardia autotrophica*, *Pseudonocardia spinosa*, and *Pseudonocardia thermophila*, has been investigated extensively by Henssen and coworkers (Henssen and Schäfer, 1971; Henssen et al., 1983; Kothe et al., 1989; Kuimova and Malishkaite, 1984). The substrate and aerial mycelial wall of these organisms is composed of two layers: an inner electron-transparent, uniformly thick layer; and an outer electron-dense irregular layer to which the fibrous sheath is attached (Figure 281, Figure 282, and Figure 283). The cross walls are interspace septa (Henssen et al., 1981, 1983) of type 2 *sensu* Williams et al. (1973). Hyphal fragments may become subdivided by septa growing inwards at different angles. Spores of *Pseudonocardia thermophila* show resistance to dry and wet heat at 100°C (Fergus, 1967).

Colony morphology. *Pseudonocardia* strains form well developed colonies on most standard media used to cultivate filamentous actinomycetes (Shirling and Gottlieb, 1966). The substrate

FIGURE 279. Budding, zig-zag-shaped hyphae of *Pseudonocardia spinosa* strain MB SF-1 (light microscopy; bar = 5 µm). (Reproduced with permission from Henssen and Schäfer, 1971. Int. J. Syst. Bacteriol. *21*: 29–34.)

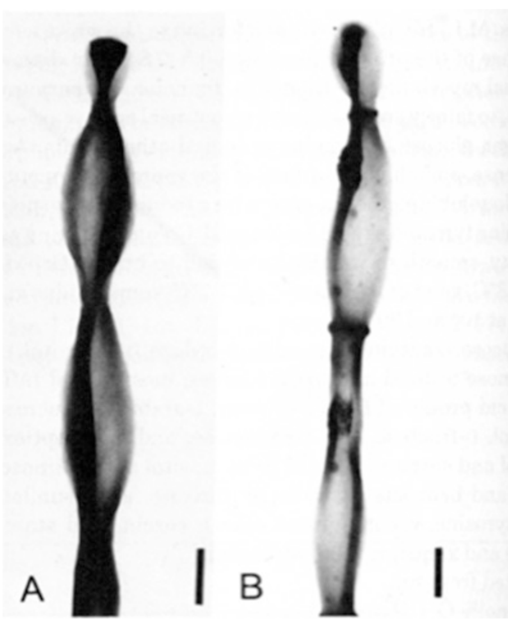

FIGURE 280. A and B. Budding aerial hyphae of *Pseudonocardia thermophila* strain MB-A18.B. Constrictions separated by septae (whole mount silhouettes, transmission electron microscopy; bar = 0.5 µm). (Reproduced with permission from Henssen and Schäfer, 1971. Int. J. Syst. Bacteriol. *21*: 29–34.)

mycelium is typically yellowish to brown and the aerial mycelium, if produced, is whitish. The colors of substrate and aerial mycelia, however, can vary between species and are influenced by the cultivation medium. *Pseudonocardia compacta* strains form

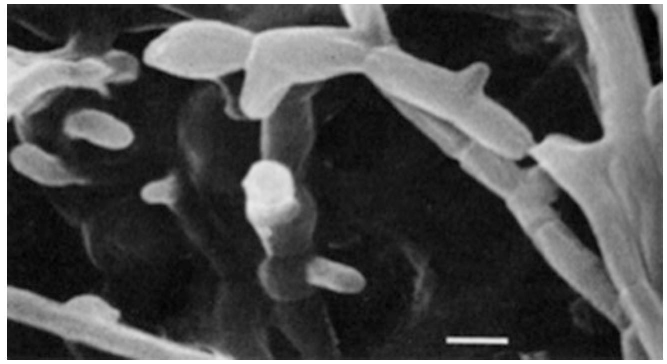

FIGURE 281. Budding substrate and aerial hyphae of *Pseudonocardia compacta* strain MB H-146 (scanning electron microscopy; bar = 1 µm). (Reproduced with permission from Henssen et al., 1983. Int. J. Syst. Bacteriol. *33*: 829–836.)

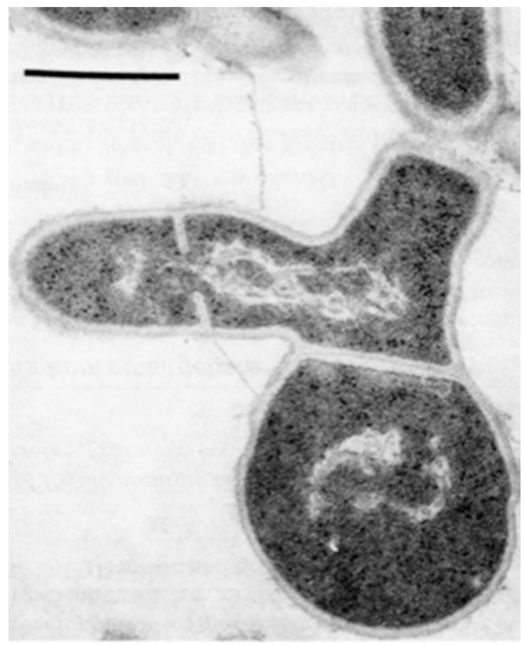

FIGURE 282. *Pseudonocardia thermophila* MB-A18: tip of aerial hyphae with apical swelling and side branch showing septate formation (TEM, glutaraldehyde/osmium tetroxide fixation; bar = 0.5 µm).

lumpy colonies composed of densely aggregated aerial hyphae, which frequently bear apical and intercalary swellings (Henssen et al., 1983). The straight or occasionally helically twisted hyphae of these organisms are more or less regularly segmented. The substrate mycelium of *Pseudonocardia spinosa* forms a compact mass composed of non-septate hyphae of various thicknesses constricted at intervals (Henssen and Schäfer, 1971).

Cell-wall composition. The peptidoglycan of *Pseudonocardia* strains contains *meso*-A_2pm, arabinose, and galactose; these components, which are observed in whole-organism hydrolysates (Embley, 1992; Huang et al., 2002; Reichert et al., 1998; Takeuchi et al., 1992; Warwick et al., 1994), are typical of wall chemotype IV *sensu* Lechevalier and Lechevalier (1970). They typically contain tetrahydrogenated menaquinones with eight isoprene units [MK-8(H_4)] as the predominant isoprenologue

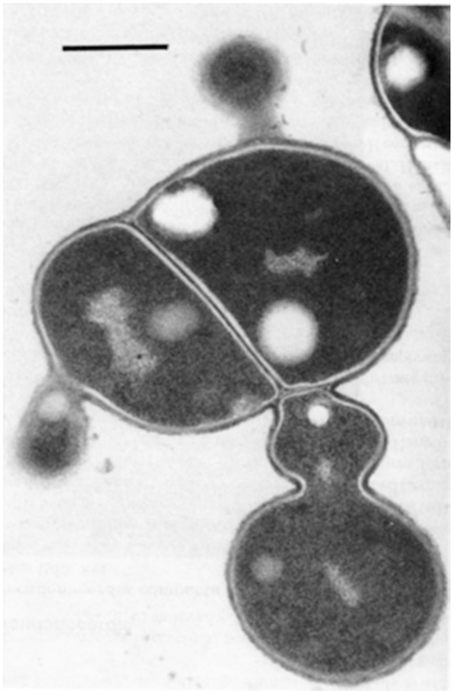

FIGURE 283. Budding cells of *Pseudonocardia spinosa* MB SF-1, the upper bud with constriction (TEM, glutaraldehyde/osmium tetroxide fixation; bar = 0.5 μm).

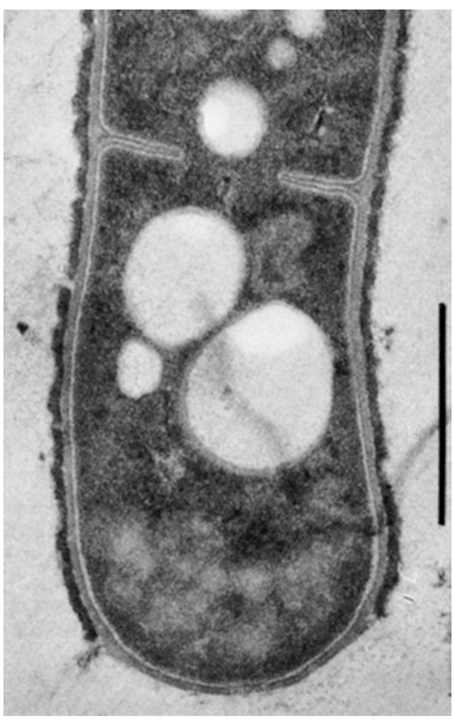

FIGURE 284. *Pseudonocardia compacta* MB H-146: tip of spore wall formed by inward growth of double annulus (TEM; bar = 0.5 μm). (Reproduced with permission from Henssen et al., 1983. Int. J. Syst. Bacteriol. *33*: 829–836.)

(Chen et al., 2009; Collins et al., 1985; Huang et al., 2002; Kroppenstedt, 1985; Liu et al., 2006; Qin et al., 2008b) and iso-branched hexadecanoic acid ($C_{16:0}$ iso) as the major fatty acid (Gu et al., 2006; Huang et al., 2002; Kroppenstedt, 1985; Park et al., 2008), but lack mycolic acids (Goodfellow and Minnikin, 1981; Lechevalier et al., 1986; Lee et al., 2004). *Pseudonocardia* species may exhibit either a phospholipid type II (phosphatidylethanolamine or its derivatives as major diagnostic components) or type III (phosphatidylcholine) pattern *sensu* Lechevalier et al. (1981, 1977a). Variations in fatty acid profiles and polar lipid patterns are cited in the species descriptions. *Pseudonocardia carboxydivorans* is unusual as it contains MK-9 as the predominant menaquinone (Park et al., 2008).

Nutrition and growth conditions. Many pseudonocardiae grow on standard media, such as Czapek (Waksman, 1967), glucose-asparagine (ISP medium 5, Shirling and Gottlieb, 1966), and yeast extract (Henssen, 1989) agars. Some species such as *Pseudonocardia petroleophila*, however, grow poorly on most media (Hirsch and Engel, 1956). *Pseudonocardia spinosispora* produces sparse, if any, substrate mycelium on most agar media, but does grow in stationary, but not shaken, broth cultures (Lee et al., 2002). *Pseudonocardia halophobica* does not grow in the presence of 3% NaCl (Akimov et al., 1989). Most pseudonocardiae grow well between 15 and 37°C, although *Pseudonocardia thermophila* grows optimally between 40 and 50°C (Henssen, 1957) and *Pseudonocardia antarctica* is psychrotolerant (Prabahar et al., 2004a).

Metabolism. *Pseudonocardia* strains have an oxidative metabolism and exhibit considerable physiological versatility. Some species, such as *Pseudonocardia ammonioxydans*, *Pseudonocardia autotrophica*, *Pseudonocardia dioxanivorans*, *Pseudonocardia petroleophila*, and *Pseudonocardia saturnea*, are facultative autotrophs, whereas most *Pseudonocardia* species are heterotrophs. *Pseudonocardia autotrophica*, *Pseudonocardia petroleophila*, and *Pseudonocardia saturnea* grow in the presence of CO_2, H_2, and O_2 (Hirsch and Engel, 1956; Lechevalier et al., 1986; Takamiya and Tubaki, 1956); *Pseudonocardia carboxydivorans* uses CO as a sole carbon source (Park et al., 2008); *Pseudonocardia ammonioxydans* oxidizes ammonia to nitrate as an energy source, and grows autotrophically on modified nitrifying medium and heterotrophically on Luria–Bertani medium (Liu et al., 2006).

Pseudonocardia (previously *Amycolata*) *autotrophica* strains can degrade lignin and related phenolic compounds (Ball et al., 1989; Haider et al., 1978; Malarczyk et al., 1987; McCarthy and Broda, 1984). When grown in a medium containing carbohydrates and supplemented with a radiolabeled dehydropolymer of coniferyl alcohol they show a wide variety of activities, releasing $^{14}CO_2$ from the methoxyl group, the 2-carbon of the side chain, and from the benzene ring (Haider et al., 1978). In contrast, only small amounts of $^{14}CO_2$ were released from the labeled lignin component of corn stalks and *Pseudonocardia autotrophica* strains also do not significantly degrade ^{14}C-lignin labeled wheat lignocellulose (McCarthy and Broda, 1984). Malarczyk et al. (1987) studied the metabolism of radiolabeled methoxyphenolic acids supplied to *Pseudonocardia autotrophica* strains as sole carbon sources and found that they could release $^{14}CO_2$ from vanillic acid irrespective of the position of the label, and from ring-labeled benzylovanillic, isovanillic and veratric acids. They proposed a pathway for the degradation of vanillic acid that proceeded via guaiacol, isovanillic acid, and protocatechuic acid.

Pseudonocardia hydrocarbonoxydans and *Pseudonocardia petroleophila* grow well in an atmosphere containing simple hydrocarbons (Hirsch and Engel, 1956; Nolof and Hirsch, 1962); *Pseudonocardia hydrocarbonoxydans* is able to partially oxidize gaseous

aldehydes, hydrocarbons, monocarboxylic acids (C_6–C_{14}), and two dicarboxylic acids, namely sebacic and succinic acids (Lacey, 1988; Nolof, 1962). *Pseudonocardia petroleophila* oxidizes the cyclohexane ring of methylcyclohexane (Tonge and Higgins, 1974) and *Pseudonocardia nitrificans* is unusual in its ability to convert ethyl-, ethyl-N-ethyl-, *n*-butyl-, and *n*-propyl-carbamates to nitrite and use these compounds as sole carbon, energy, and nitrogen sources (Isenberg et al., 1954). *Pseudonocardia* sp. strain TY-7 oxidizes propane to 1-propanol and 2-propanol through both terminal and subterminal oxidations (Kotani et al., 2006).

Pseudonocardiae have physiological properties which suggest that they may play a role in the degradation of hazardous compounds and in biogeochemical cycles: *Pseudonocardia asaccharolytica* and *Pseudonocardia sulfidoxydans* utilize dimethyl disulfide as an energy source (Reichert et al., 1998); *Pseudonocardia benzenivorans* utilizes 1,2,3,5-tetrachlorobenzene as a sole carbon source (Kämpfer and Kroppenstedt, 2004); *Pseudonocardia chloroethenivorans* degrades chloroethene, *cis*-1,2-dichloroethene, and trichloroethene, and metabolizes phenol as a source of carbon and energy by a *meta*-cleavage pathway (Lee et al., 2004); *Pseudonocardia dioxanivorans* degrades 1,4-dioxane (Mahendra and Alvarez-Cohen, 2005); and *Pseudonocardia hydrocarbonoxydans* oxidizes hydrocarbons (Lechevalier et al., 1986; Nolof and Hirsch, 1962). Similarly, *Pseudonocardia nitrificans* oxidizes urethane to nitrite as a sole source of carbon, nitrogen, and energy (Schatz et al., 1954). *Pseudonocardia* sp. strain ENV478 degrades 1,4-dioxane and other potentially important ether pollutants and, hence, may be a useful biocatalyst for *in situ* and *ex situ* systems designed for treating recalcitrant pollutants (Vainberg et al., 2006).

Pseudonocardia thermophila exhibits an inducible exo- and intracellular carboxymethylcellulase and β-D-glucosidase activity, although these attributes are suppressed in the presence of free glucose (Malfait et al., 1984). Isolates of *Pseudonocardia thermophila* from cattle compost metabolize different types of native and crystalline cellulose under laboratory conditions (Goddon and Penninckx, 1984). Ca^{2+}-alginate-immobilized cells of *Pseudonocardia thermophila* IFO (now NBRC) 12133 have been used to produce optically active α-hydroxy-esters (Ishihara et al., 1997).

It is evident from the phylogenetic tree calculated from 16S rRNA gene sequences that the type strains of *Pseudonocardia benzenivorans*, *Pseudonocardia hydrocarbonoxydans*, *Pseudonocardia sulfidoxydans*, and *Pseudonocardia tetrahydrofuranoxydans* form a distinct subclade with high bootstrap support (Figure 278). All of these organisms grow on 4-aminobutyrate, 4-hydroxybutyrate, serine, and succinate (each at 10 mM) although the growth yields differ (Kämpfer et al., 2006). Moreover, these species, as well as *Pseudonocardia dioxanivorans*, tolerate a high concentration of tetrahydrofuran (THF, 60 mM), but exhibit differences in their ability to form visible cell aggregations in liquid media containing this compound (Kämpfer et al., 2006; Mahendra and Alvarez-Cohen, 2005). *Pseudonocardia benzenivorans* and *Pseudonocardia sulfidoxydans* form cell aggregates at a low THF concentration (10 mM), whereas *Pseudonocardia hydrocarbonoxydans* and *Pseudonocardia tetrahydrofuranoxydans* exhibit dispersed cell suspensions at this concentration. THF degradation in *Pseudonocardia tetrahydrofuranoxydans* is initiated by a three-component binuclear iron-containing monooxygenase which contains an NADH-dependent reductase component with an unusual covalently bound flavin (Thiemer et al., 2001, 2003).

In general, *Pseudonocardia* species produce acid from a range of sugars and use diverse compounds as sole carbon and nitrogen sources, but tend to vary in their ability to degrade adenine, casein, gelatin, hypoxanthine, starch, tyrosine, and xanthine (Chen et al., 2009; Goodfellow, 1971; Lechevalier et al., 1986; Lee et al., 2004; Liu et al., 2006; Orchard and Goodfellow, 1980; Prabahar et al., 2004a). They are also a potentially rich source of useful enzymes, as exemplified by *Pseudonocardia thermophila*, which produces cobalt-containing nitrile hydratases (Miyanaga et al., 2001; Peplowski et al., 2007) and a constitutively expressed thermostable amidase (Egorova et al., 2004), as well as exhibiting cellulase and xylanase activity (Li et al., 1984; Malfait et al., 1984; Zimmermann et al., 1988). *Pseudonocardia autotrophica* strains can convert vitamin D_3 into hydroxylated active forms, such as 1α and 2α,25-dihydroxyvitamin D_3 (Kim et al., 2002b; Takeda et al., 2006). Kang et al. (2006) optimized the culture conditions for the bioconversion of vitamin D_3 to 1α,25-dihydroxyvitamin D_3 using *Pseudonocardia autotrophica* ID 9302. *Pseudonocardia* strains are also known to metabolize 4-ethylpyridine and 4-methylpyridine (Lee et al., 2006a), and polyethylene glycol (Yamashita et al., 2004).

Pseudonocardia strains have been reported to produce antimicrobial compounds such as the novel glycopeptides helvecardins A and B (Takeuchi et al., 1991), phenazostatin D (Maskey et al., 2003), and eight new quinolone compounds with selective and potent anti-*Helicobacter pylori* activity (Dekker et al., 1998). There has also been a report that *Pseudonocardia autotrophica* produces a broad spectrum antifungal compound (Antoun et al., 1978). *Pseudonocardia* strains isolated from coastal soils have also been found to produce metabolites inhibitory against *Micrococcus luteus* (Srivibool and Sukchotiratana, 2006).

Genetics. There have been relatively few studies on the genetics of *Pseudonocardia* strains, although a nitrile hydratase gene from *Pseudonocardia thermophila* has been cloned and sequenced (Yamaki et al., 1997), as has the gene for cytochrome P-450 hydroxylase in *Pseudonocardia autotrophica* (Kim et al., 2002b). There have been studies on gene clusters involved in fatty acid metabolism in *Pseudonocardia autotrophica* BCRC 12444 (Chen et al., 2005), THF degradation in *Pseudonocardia tetrahydrofuranoxydans* (Thiemer et al., 2003), and on the cloning and expression of a gene that encodes an ether-bond-cleaving enzyme, diglycolic acid dehydrogenase, in polyethylene glycol-utilizing *Pseudonocardia tetrahydrofuranoxydans* $K1^T$ (Yamashita et al., 2004). Two gene clusters encoding putative propane monooxygenases have been cloned from *Pseudonocardia* sp. strain TY-7 and shown to be induced by *n*-alkanes, suggesting that the products of these genes are involved in gaseous *n*-alkane oxidation (Kotani et al., 2006).

A cryptic polyene gene cluster has been isolated and partially characterized from a strain of *Pseudonocardia autotrophica*, an organism that does not exhibit antifungal activity (Lee et al., 2006b), and this strain was shown to contain a novel polyene-specific cytochrome P-450 hydroxylase (CYP) genes. Genomic DNA library screening using the probe for the polyene-specific CYP gene identified a positive cosmid clone containing a DNA fragment of approximately 34.5 kb. Complete sequencing of this DNA fragment showed the presence of seven complete and two incomplete open reading frames that were found to be unique, but comparatively similar to previously known polyene biosynthetic genes. The nine open reading frames were thought to be involved in the biosynthesis of a novel cryptic *Pseudonocardia* polyene metabolite. Polyene-specific CYP genes were not detected, however, in representatives of several other

Pseudonocardia species (Hwang et al., 2007). Two large cryptic plasmids, about 80 and 120 MDa, have also been detected in *Pseudonocardia alni* strains (Dobritsa, 1984).

Pathogenicity. There is no definitive evidence that *Pseudonocardia* strains have a role as clinical or veterinary pathogens. *Pseudonocardia autotrophica* has been occasionally isolated from clinical materials and may be an opportunistic pathogen of immunocompromised hosts (Mishra et al., 1980; Schaal and Beaman, 1984). In addition, unidentified *Pseudonocardia* strains have been associated with allergic diseases in Kuwait (Diab and Al-Gunaim, 1982).

Antibiotic sensitivity. Little is known about the antibiotic sensitivity patterns of *Pseudonocardia* species, although certain closely related species can be distinguished on the basis of their susceptibility to a range of antibiotics (Evtushenko et al., 1989; Prabahar et al., 2004a; Xu et al., 1999). The antibiotic sensitivity profiles of individual taxa, where known, are provided in the species descriptions.

Ecology. Pseudonocardiae have been primarily isolated from enrichment cultures, plant materials, and soils, but have also been recovered from various other habitats, including air, clinical materials, coastal and marine sediments, composts, and sludge, although population numbers, distribution, and roles of *Pseudonocardia* species in natural habitats is virtually unknown. Several species, including *Pseudonocardia ailaonensis*, *Pseudonocardia alaniniphila*, *Pseudonocardia aurantiaca*, *Pseudonocardia xinjiangensis*, *Pseudonocardia yunnanensis*, and *Pseudonocardia zijingensis*, were isolated from soils collected in Yunnan and Xinjiang Provinces in China (Huang et al., 2002; Jiang et al., 1991; Qin et al., 2008b; Xu et al., 1999). Similarly, *Pseudonocardia ammonioxydans* was isolated from a coastal sediment (Liu et al., 2006), *Pseudonocardia antarctica* from a moraine sample from the McMurdo Dry Valleys region of Antarctica (Prabahar et al., 2004a), *Pseudonocardia benzenivorans* from contaminated soil (Kämpfer and Kroppenstedt, 2004), and *Pseudonocardia kongjuensis* and *Pseudonocardia spinosispora* from gold mine cave soil (Lee, 1996; Lee et al., 2001, 2002).

Several (Aragno and Schlegel, 1981)species have been isolated from plant materials, including *Pseudonocardia alni*, which is thought to be associated with the nitrogen-fixing root nodules and rhizospheres of the alder species *Alnus glutinosa* and *Alnus incana* (Evtushenko et al., 1989; Sharaya et al., 1982) where it might utilize the excess hydrogen produced by nitrogen-fixing bacteria (Aragno and Schlegel, 1981). *Pseudonocardia asaccharolytica* and *Pseudonocardia sulfidoxydans* were isolated from bark compost biofilters in an animal rendering plant (Reichert et al., 1998), *Pseudonocardia endophytica* from the Chinese medicinal plant, *Lobelia clavata* (Chen et al., 2009), and *Pseudonocardia oroxyli* from the surface-sterilized elongation root zone of *Oroxylum indicum*, another traditional Chinese medicinal plant (Gu et al., 2006). Enrichment cultures from contaminated industrial sludge and waste water plants led to the isolation of strains of *Pseudonocardia benzenivorans* (Kämpfer and Kroppenstedt, 2004) and *Pseudonocardia tetrahydrofuranoxydans* (Kämpfer et al., 2006), respectively. *Pseudonocardia* strains have been isolated from marine sediments following their detection using culture-independent procedures (Maldonado et al., 2005).

It seems likely that *Pseudonocardia* species with an ability to oxidize hydrogen and fix carbon dioxide (Aragno and Schlegel, 1981; Hirsch, 1960) are widely distributed in hydrogen-rich habitats that are abundant in nature (Aragno and Schlegel, 1981). *Amycolata autotrophica* is able to colonize the surfaces of substrates and actively oxidizes hydrogen when continuously supplied with a mixture of H_2:O_2:CO_2 (7:2:1) in the presence of a small amount of mineral medium (Kriukov, 1981). This organism has been isolated from decomposing vegetable matter, marshland, and soil (Kuznetsov et al., 1978; Lechevalier et al., 1986; Okazaki et al., 1983), and from degraded polyester polyurethane (Pommer and Lorenz, 1986). *Pseudonocardia autotrophica* has been shown to have the capacity to degrade methoxyphenolic compounds (Haider et al., 1978; Malarczyk et al., 1987) and thus may have a role in the turnover of phenolic compounds derived from lignin degradation.

The relative biodiversity of toluene-degrading bacteria isolated from a compost biofilter treating toluene vapor was studied by Juteau et al. (1999). The toluene-degrading community was composed of *Pseudonocardia* and *Rhodococcus* strains that dominated usually faster-growing bacteria, such as members of the genera *Acinetobacter* and *Pseudomonas*. These authors considered that the actinomycetes might be *K*-strategists (adapted to a resource-restricted and crowded environment) and that the compost biofilter was a *K*-environment. These observations would explain why the actinomycetes were not outnumbered by the faster-growing acinetobacters and pseudomonads, which can be considered as *r*-strategists (adapted to a resource-abundant and undercrowded environment).

Pseudonocardia thermophila has been isolated from cattle manure and may play a part in the turnover of plant materials (Goddon and Penninckx, 1984; Henssen, 1989; Malfait et al., 1984). Goddon and Penninckx (1984) studied the succession of the microflora in composting cattle manure using a bench-scale reactor and observed that *Pseudonocardia thermophila* was one of the most abundant actinomycetes [approx. 10^6–10^7 colony forming units (c.f.u.)/g dry weight compost] when the temperature exceeded 40°C, although the numbers stabilized around 10^5–10^6 c.f.u./g after 15 d when the temperature fell to 10°C.

Morón et al. (1999) generated genus-specific primers for PCR identification of *Pseudonocardia* species. The primers were used to identify 106 strains presumptively assigned to the family *Pseudonocardiaceae* that had been isolated from geographically diverse soil and decomposing plant material samples. Nearly half of the isolates produced the genus-specific amplification product and partial 16S rRNA gene sequencing of representative isolates was used to validate their genus assignment. Fatty acid analyses of all of the isolates showed that they formed a heterogeneous group indicating that the genus *Pseudonocardia* is underspeciated.

Mutualistic associations. Attine leaf-cutting ants maintain two highly specialized, vertically transmitted, mutualistic symbionts, basidiomycete fungi (*Agaricales*: mostly *Lepiotaceae*: *Leucocopineae*) that are cultivated for food in underground gardens (Chapela et al., 1994; Martin, 1970; Weber, 1966, 1972), and pseudonocardiae, which produce antibiotics that specifically inhibit the growth of *Escovopsis* parasites (*Ascomycota*: anamorphic *Hypocreales*) of the fungal gardens (Cafaro and Currie, 2005; Currie et al., 1999a, 1999b, 2003a; Kost et al., 2007). The ants provide the fungus with fresh substrate and protection against competition and pathogens (Bass and Cherett, 1994; Mueller et al., 2004 ; North et al., 1997), and virgin ant queens carry their mother's symbiont when leaving their colony to mate. In return, the fungus produces nutrient-rich bodies (gongylidia), which workers harvest as a sole source of food for their larvae and the queen. The relationships between attine ants, their fungal cultivars, pseudonocardial mutualists, and cultivar antagonists is considered to be one that has evolved over 50 million years (Currie, 2001; Currie et al., 2003b, 2006; Mueller et al., 2001; Schultz and Brady, 2008).

The fungal gardens are propagated asexually as clonal monocultures and hence are susceptible to parasitism by micro-organisms that are competitively superior to the fungus cultivated by the ants (Weber, 1966), notably to a highly specialized parasite belonging to the genus *Escovopsis* (Currie et al., 1999a). Genetically diverse strains of the horizontally transmitted pathogen (Gerardo et al., 2006; Taerum et al., 2007) are capable of rapidly devastating fungal gardens and thereby threaten the survival of ant colonies (Currie et al., 1999a; Currie and Stuart, 2001). Bioassay and *in vivo* infection experiments indicate differences in the inhibitory capabilities of ant-associated *Pseudonocardia* strains, as well as variation in *Escovopsis* strain susceptibility to different antibiotics (Poulsen et al., 2007).

Pseudonocardial symbionts typically reside on the cuticle of ants in elaborate cuticular crypts linked to endocrine glands, which apparently produce nutrients to support bacterial growth (Currie et al., 2006). A degree of broad-scale co-evolution is considered to be apparent between *Pseudonocardia* and fungus-growing ants (Cafaro and Currie, 2005), though this relationship is not clear-cut due to the ability of *Pseudonocardia* strains to cross attine species boundaries, both within and between genera (Poulsen et al., 2005). These workers detected frequent *Pseudonocardia* exchanges between sympatric ant species within the genus *Acromyrineae*, thereby indicating that horizontal transmission had occurred. However, symbiotic recognition and behavioral choice may play a crucial role in the fungus-growing, ant-pseudonocardial mutualism (Bot et al., 2001; Mueller et al., 2004; Viana et al., 2001), possibly by recognizing clone-specific chemical signatures (Richard et al., 2007). The operation of such factors might allow ants to retain the ecological flexibility they need to defend their gardens from *Escovopsis* strains and, at the same time, resolve potential conflict that can arise from rearing competing symbiotic *Pseudonocardia* strains (Zhang et al., 2007b).

Symbiotic pairing assay experiments have revealed the presence and extent of antagonistic interactions between *Pseudonocardia* symbionts of fungus-growing ants (Poulsen et al., 2007). The widespread ability of ant-associated pseudonocardiae to inhibit one another suggests that competition will often arise when *Pseudonocardia* strains mix and that such interactions may have a direct impact on host/symbiont dynamics. Indeed, the detection of antagonistic interactions across the phylogenetic diversity of pseudonocardial symbionts indicates that such interactions may have shaped the ant/pseudonocardial symbioses from its inception. Antagonism between *Pseudonocardia* strains can be expected to prevent new strains from gaining a foothold in ant colonies, thereby enforcing single strain rearing within individual ant colonies. However, while ant recognition of *Pseudonocardia* strains may prevent conflict between them, it may also allow ants to replace their resident *Pseudonocardia* strain with an organism that produces novel antibiotics against the garden parasite, *Escovopsis*. The results of the experiments conducted by Poulsen and his colleagues support the view that the evolution of sociality required dramatic increases in anti-microbial defenses and that micro-organisms have been powerful selective agents (Stow and Beattie, 2008).

The coevolution of attine ants and pseudonocardiae has been reassessed by Mueller et al. (2008). They found that phylogenetic data from culture-dependent and -independent microbial surveys indicated close relationships between free-living and ant-associated pseudonocardiae, and a lack of topological correspondence between ant and *Pseudonocardia* phylogenies, indicating frequent pseudonocardial acquisition from environmental sources. Identity of ant-associated pseudonocardiae and isolates from plants and soil implicated them as sources from which attine ants acquire *Pseudonocardia* strains. These data are at variance with the prevailing views of specific coevolution between attine ants, pseudonocardiae, and garden diseases. Consequently, the effectiveness of pseudonocardial antibiotics may not be due to advantages in the coevolutionary arms race with specialized garden diseases, but from the frequent recruitment of effective *Pseudonocardia* strains from the environment. Current models of *Pseudonocardia*/disease coevolution need to be re-examined in light of these new findings.

Multiple lines of evidence shown that a black yeast, closely related to the genus *Phialophora* (Ascomycota), is the fifth symbiont to be recognized in the fungus-growing attine ant/microbe symbiosis (Little and Currie, 2007). The black yeasts, which form a monophyletic group, grow on the ants' cuticle and derive nutrients from the pseudonocardial symbiont (Little and Currie, 2008). Ants infected with black yeasts are significantly less effective at defending their fungal gardens from *Escovopsis*. It is possible that the reduction of mutualistic actinobacterial biomass in ants, probably caused by the black yeast symbionts, reduces the quantity of antibiotics available to inhibit the garden symbiont. However, the reduction in the ability of ants to inhibit *Escovopsis* indicates that it is an integral component of a complex symbiotic association which is not fully understood. Nevertheless, it seems clear that each member of the fungus-growing ant community is directly and/or indirectly influenced by the other partners in both positive and negative ways (Little and Currie, 2008; Poulsen and Currie, 2006).

Enrichment and isolation procedures

Most *Pseudonocardia* species have been isolated on nutrient-poor media under aerobic conditions at 20–30°C. Several species, including *Pseudonocardia xinjiangensis* and *Pseudonocardia yunnanensis*, have been recovered from soil samples collected from Xinjiang and Yunnan Provinces in China following inoculation of soil suspensions onto AV (Nonomura and Ohara, 1971) and HV (Hayakawa, 1990) agars and incubation at 28°C for 21–28 d (Xu et al., 1999). *Pseudonocardia ailaonensis* and *Pseudonocardia zijingensis* were also isolated from Yunnan soil samples, albeit on starch-casein and yeast extract-starch agars, respectively (Huang et al., 2002; Qin et al., 2008b). Similarly, *Pseudonocardia antarctica* was isolated on yeast extract-peptone agar (Prabahar et al., 2004a), and *Pseudonocardia kongjuensis* and *Pseudonocardia spinosispora* were isolated on tap water agar (Lee et al., 2001, 2002). *Pseudonocardia ammonioxydans* was isolated on a modified nitrifying medium inoculated with a coastal sediment suspension prior to incubation at 30°C for a month (Liu et al., 2006), and *Pseudonocardia oroxyli* was recovered from sterile root samples inoculated onto BL-2 agar plates supplemented with penicillin and incubated at 27°C for up to 4 weeks (Gu et al., 2006).

The remaining *Pseudonocardia* species that oxidize hydrocarbons or deleterious organic compounds can be selectively isolated either by enrichment culture or by plating suspensions onto media containing selected compounds as sole carbon sources. *Pseudonocardia asaccharolytica* and *Pseudonocardia sulfidoxydans* were isolated from samples enriched with methyl sulfide-containing gas (Reichert et al., 1998), *Pseudonocardia carboxydivorans* from soil enriched with low concentrations of CO (Park et al., 2008), and *Pseudonocardia chloroethenivorans* by using trichloroethene-contaminated air with phenol as a source

of carbon for energy and growth (Lee et al., 2004). Similarly, *Pseudonocardia benzenivorans* was isolated on a medium containing 1,2,3,5-tetrachlorobenzene as a sole carbon source (Kämpfer and Kroppenstedt, 2004), *Pseudonocardia dioxanivorans* on a basal medium supplemented with THF and yeast extract (Parales et al., 1994), *Pseudonocardia nitrificans* on tap-water agar supplemented with urethan and trace metal salts (Schatz et al., 1954), and *Pseudonocardia tetrahydrofuranoxydans* on a medium enriched with THF (Kohlweyer et al., 2000). *Pseudonocardia petroleophila* and *Pseudonocardia saturnea* strains were isolated using CO_2 enrichments of mineral salts solutions seeded with compost (Hirsch, 1960), and *Pseudonocardia carboxydivorans* was isolated from soil enriched with low concentrations of CO (Park et al., 2008). *Pseudonocardia hydrocarbonoxydans* was isolated as an aerial contaminant on a silica gel plate (Nolof and Hirsch, 1962).

The type strain of *Pseudonocardia autotrophica* was isolated as an airborne contaminant of phosphate buffer that had been left standing next to a coal gas leak (Hirsch, 1960; Takamiya and Tubaki, 1956). *Pseudonocardia autotrophica*, in common with other autotrophic *Pseudonocardia* species, grows faster under heterotrophic conditions. Strains of *Pseudonocardia autotrophica*, "*Amycolata* (i.e. *Pseudonocardia*) *autotrophica* subsp. *amethystina*" and "*Amycolata* (i.e. *Pseudonocardia*) *autotrophica* subsp. *canberrica*" were isolated from suspensions of eucalyptus forest soil, lake side sand, and lake bottom mud using heterotrophic media supplemented with selective agents (Okazaki et al., 1983).

Henssen (1957) isolated the type strain of *Pseudonocardia thermophila* from fresh and rotten manure using a nutrient medium supplemented with cellulose dextrin as a carbon source (Fuller and Norman, 1942). The exact composition of this nutrient medium is not entirely clear from the original paper, however, as a number of variations were used. Subsequently, large numbers of *Pseudonocardia thermophila* strains were isolated from the heating phase of composting cattle manure after 20 d at 45°C on a cellulose medium supplemented with streptomycin sulfate (Goddon and Penninckx, 1984). *Pseudonocardia composta* and *Pseudonocardia spinosa* have been isolated from soil using nutrient-poor media, such as soil extract agar, and incubated at 20–30°C (Henssen, 1989; Henssen and Schäfer, 1971).

Maintenance procedures

Working cultures of *Pseudonocardia* can be maintained at 4°C on appropriate standard media. Long-term preservation of strains is best achieved as frozen stocks in 20% (v/v) aqueous glycerol at −20°C, −80°C (mechanical freezer), or −172°C (liquid nitrogen vapor phase), or by traditional lyophilization procedures.

Procedures for testing special characters

Two pairs of genus specific oligonucleotide primers, AMP3 (5′-GCGGCACAGAGACCGTGGAAT-3′)/AMP2 (5′-GTGGAAA GTTTTTTCGGCTGGGG-3′) and AMP4 (5′-GCGGCACAGAAA CCGTGGAAT-3′)/AMP5 (5′-GTGGAAAGTTTTTTCGGTGGG GG-3′), can be used to identify members of the genus *Pseudonocardia* at annealing temperatures of 53 and 60°C respectively (Morón et al., 1999). The amplification conditions are: initial denaturation at 95°C for 4 min; 40 cycles of 30 s at 93°C, 30 s at 53°C for primer set AMP3/AMP2 or 30 s at 60°C for primer set AMP4/AMP5, and 2 min at 72°C; followed by 10 min at 72°C. Both primers sets yield a 640 bp genus-specific amplification product. The AMP3/AMP2 primer set is particularly effective for the detection and identification of *Pseudonocardia* strains at a lower annealing temperature.

Differentiation of the genus *Pseudonocardia* from other genera

The genus *Pseudonocardia* can be distinguished from other genera classified in the family *Pseudonocardiaceae* by using a combination of chemotaxonomic and morphological criteria (see Table 228 in the treatment of the family *Pseudonocardiaceae*). It also forms a distinct clade in the *Pseudonocardiaceae* 16S rRNA gene tree and can be distinguished from the phylogenetically close genera *Actinomycetospora* and *Kibdelosporangium* (Figure 278) as they contain MK-9(H_4) as the predominant isoprenologue and form pseudosporangia (*Kibdelosporangium*) or no aerial mycelium (*Actinomycetospora*). *Pseudonocardia* strains can also be recognized using genus-specific oligonucleotide primer sets (Morón et al., 1999) and can be distinguished from other genera classified in the family *Pseudonocardiaceae* based on partial amino acid sequencing of ribosomal AT-L30 proteins (Ochi, 1995).

Taxonomic comments

The genus *Pseudonocardia* was proposed to accommodate *Pseudonocardia thermophila*, a moderately thermophilic organism that showed distinctive morphological properties (Henssen, 1957). Morphologically similar strains were designated *Pseudonocardia compacta* (Henssen, 1957) and *Pseudonocardia spinosa* (Henssen and D. Schäfer, 1971; Schäfer, 1969). Another thermophilic organism, "*Pseudonocardia thermospinosa*", was isolated from soil samples from Hanoi, Vietnam (Lu and Yan, 1978), but the name was not validly published. The three species with validly published names could be distinguished from members of other genera of actinomycetes which formed aerial spores in chains by exhibiting acropetal budding of substrate and aerial hyphae (Henssen, 1970; Henssen and Schäfer, 1971; Henssen and Schnepf, 1967), and by forming a two-layered wall and interspace septa (Henssen et al., 1981). However, most of the 29 species with validly published names assigned to the genus have been circumscribed using a combination of genotypic and phenotypic properties. These taxa include species that were previously classified in the genera *Actinobispora* (Xu et al., 1999), *Amycolata* (Lechevalier et al., 1986), and *Pseudoamycolata* (Akimov et al., 1989).

The genus *Actinobispora* was proposed to encompass members of a single species, *Actinobispora yunnanensis*, which formed spores in longitudinal pairs on vegetative hyphae, in longitudinal pairs or singly on aerial hyphae, and which contained *meso*-A_2pm as the wall diamino acid, arabinose, galactose and xylose in whole-organism hydrolysates (an unusual wall chemotype IV), phosphatidylethanolamine and glucosamine-containing phospholipids (phospholipid type 1), and MK-7(H_2) and MK-9(H_2) as predominant isoprenologues, but lacked mycolic acids (Jiang et al., 1991). Subsequently, three additional species, *Actinobispora alaniniphila*, *Actinobispora aurantiaca*, and *Actinobispora xinjiangensis*, were described (Xu et al., 1999). Suzuki et al. (1998) selectively isolated *Actinobispora* strains on gellan gum plates. Huang et al. (2002) found that *Actinobispora* and *Pseudonocardia* species were intermixed in a distinct clade in the *Pseudonocardiaceae* 16S rRNA gene tree, shared key chemical markers, and gave a taxon-specific amplification product using PCR primers designed to differentiate the genus *Pseudonocardia* from related taxa. They also found that the type strains of the

original *Actinobispora* species contained MK-8(H_4) as the major isoprenologue. Huang and her colleagues proposed that the genus *Actinobispora* should become a junior synonym of the genus *Pseudonocardia* and that *Actinobispora alaniniphila*, *Actinobispora aurantiaca*, *Actinobispora xinjiangensis*, and *Actinobispora yunnanensis* be transferred to this genus.

The genus *Amycolata* was proposed by Lechevalier et al. (1986) for a group of actinomycetes that formed substrate hyphae that tended to fragment into squarish subunits, and aerial hyphae which, when formed, were either sterile or fragmented into chains of squarish to oval elements or spore-like structures. The organisms had a wall chemotype IV, a type III phospholipid pattern, and MK-8(H_2, H_4) as predominant isoprenologues, but lacked mycolic acids. The genus provided a taxonomic home for organisms that had previously been misclassified in the genera *Nocardia* and *Streptomyces*. The type species, *Amycolata autotrophica*, included strains previously classified as *Nocardia autotrophica* (Hirsch, 1960) or "*Streptomyces autotrophicus*" (Takamiya and Tubaki, 1956), and also encompassed some strains of *Nocardia coeliaca* (Gordon et al., 1974). *Amycolata autotrophica* strains isolated from root nodules and rhizospheres of *Alnus* species were reclassified as *Amycolata alni* following DNA–DNA pairing studies (Evtushenko et al., 1989). Similarly, *Amycolata hydrocarbonoxydans* and *Amycolata saturnea* were initially described as *Nocardia* species by Nolof and Hirsch (1962) and Hirsch (1960), respectively. "*Streptomyces nitrificans*" and "*Nocardia petroleophila*" were also considered to be species of *Amycolata* based on 16S rRNA gene sequence data (Embley, 1992). Two subspecies of *Amycolata autotrophica* have been described but their names have not been validly published, namely "*Amycolata autotrophica* subsp. *amethystine*" and "*Amycolata autotrophica* subsp. *canberrica*".

In an extensive phylogenetic study of the family *Pseudonocardiaceae*, Warwick et al. (1994) found that the 16S rRNA gene sequences of the type strains of *Amycolata* and *Pseudonocardia* species formed a mixed, but distinct, phyletic branch. These results were in line with those from previous studies which showed that members of these genera contained phosphatidylcholine as a diagnostic phospholipid (Embley et al., 1988b; Lechevalier et al., 1977a, 1986), MK-8(H_4) as the predominant isoprenologue (Embley et al., 1988b; Lechevalier et al., 1986), formed a characteristic electron-dense layer in the substrate and aerial mycelia (Kothe et al., 1989), and contained ribosomal AT-130 proteins that had very similar electrophoretic mobilities (Ochi and Yoshida, 1991) and partial amino acid sequences (Ochi, 1995). Consequently, they proposed that the genus *Amycolata* Lechevalier et al. (1986) be recognized as a junior synonym of the genus *Pseudonocardia* Henssen (1957); they also provided an emended description of the genus, and proposed that *Amycolata alni*, *Amycolata autotrophica*, *Amycolata hydrocarbonoxydans*, *Amycolata saturnea*, "*Nocardia petroleophila*" and "*Streptomyces nitrificans*" be transferred to the genus *Pseudonocardia*.

The monospecific genus *Pseudoamycolata* was proposed for two strains that had previously been classified as *Amycolata autotrophica* (Akimov et al., 1989). The strains resembled *Amycolata* species in overall morphology and phenotype, and contained MK-8(H_4) as the major isoprenologue, but lacked phosphatidylcholine, the absence of which was judged to be sufficient to justify the proposal for a new genus. McVeigh et al. (1994) found that apart from the absence of phosphatidylcholine, *Pseudoamycolata halophobica* resembled *Pseudonocardia* species with respect to cultural, morphological, and physiological properties. They also noted that bacteria synthesized phosphatidylcholine by sequential methylation of phosphatidylethanolamine and speculated that minor mutations might prevent this transformation. These considerations led them to propose that *Pseudoamycolata halophobica* be reclassified in the genus *Pseudonocardia* as *Pseudonocardia halophobica*.

Nearly all of the *Pseudonocardia* species with validly published names form distinct phyletic lines in the *Pseudonocardia* 16S rRNA gene tree (Figure 278). However, comparative taxonomic studies, including DNA–DNA relatedness experiments, are needed to determine whether *Pseudonocardia chloroethenivorans* and *Pseudonocardia dioxanivorans* are distinct species as the type strains of these taxa have identical 16S rRNA gene sequences. The position of *Pseudonocardia spinosa* in the *Pseudonocardia* 16S rRNA tree remains to be established. It has been reported that the type strain of this organism is no longer extant (Embley, 1992), but this is not so as the strain is held in the JCM and NBRC collections.

Improvements in the classification of wall chemotype IV actinomycetes that lack mycolic acids have helped to clarify the taxonomy of the genus *Pseudonocardia* (Embley et al., 1988a, 1988b; Embley, 1992; Goodfellow et al., 1989b; Warwick et al., 1994) and have thereby facilitated the recognition and description of novel species (Gu et al., 2006; Kämpfer et al., 2006; Liu et al., 2006). The improved taxonomy also led to the reclassification of some species previously assigned to the genus. Ōmura et al. (1983) proposed *Pseudonocardia azurea* for an organism that produced two water-soluble antibiotics, azureamycin A and B (Ōmura et al., 1979). This organism was considered to show acropetal budding, as was "*Pseudonocardia fastidiosa*", which produced a macrobicyclic peptide antibiotic (Celmer et al., 1977). Henssen et al. (1987) were unable to confirm that these organisms exhibited acropetal budding, but did find that they had chemotaxonomic and morphological properties consistent with their reclassification in the genus *Amycolatopsis*, as *Amycolatopsis azurea* and *Amycolatopsis fastidiosa*.

It is clear that additional *Pseudonocardia* species remain to be described as it is evident that the genus is underspeciated (Bredholdt et al., 2007; Morón et al., 1999; Schabereiter-Gurtner et al., 2001; Song et al., 2001; Zhang et al., 2008a). It seems likely that the isolation of additional novel pseudonocardiae will be facilitated by the application of innovative selective isolation procedures (Li et al., 2002).

Differentiation of the species of the genus *Pseudonocardia*

The chemotaxonomic, morphological, and physiological properties shown in Table 229 can be used to distinguish between species of *Pseudonocardia*, but members of these taxa have yet to be examined using a common set of tests. The type strains of all but two *Pseudonocardia* species can be separated based on their 16S rRNA gene sequences and phylogeny, as seen in Figure 278.

Acknowledgements

The authors thank Dr T. Kudo (JCM) for providing the type strain of *Pseudonocardia spinosa*, and Dr J.P. Euzéby for clarifying the etymology of *Pseudonocardia nitrificans*. We are particularly indebted to Dr D.P. Labeda (USDA, Peoria) for critically reading our initial manuscript and thereby making many helpful suggestions on how it could be improved.

TABLE 229. Diagnostic characteristics for *Pseudonocardia* species[a]

Characteristic	1. *P. thermophila*	2. *P. ailaonensis*	3. *P. alaniniphila*	4. *P. alni*	5. *P. ammonioxydans*	6. *P. antarctica*	7. *P. asaccharolytica*	8. *P. aurantiaca*	9. *P. autotrophica*	10. *P. benzenivorans*	11. *P. carboxydivorans*	12. *P. chloroethenivorans*	13. *P. compacta*	14. *P. dioxanivorans*	15. *P. endophytica*	16. *P. halophobica*	17. *P. hydrocarbonoxydans*	18. *P. kongjuensis*	19. *P. nitrificans*	20. *P. oroxyli*	21. *P. petroleophila*	22. *P. saturnea*	23. *P. spinosa*	24. *P. spinosispora*	25. *P. sulfidoxydans*	26. *P. tetrahydrofuranoxydans*	27. *P. xinjiangensis*	28. *P. yunnanensis*	29. *P. zijingensis*
Fragmentation of:																													
Substrate mycelium	+	+	−	+	+	nd	+	−	+	+	nd	−	+	+	+	+	+	nd	nd	+	+	+	+	nd	+	nd	−	−	+
Aerial mycelium	+	+	−	+	+	+	+	−	+	+	+	−	+	+	−	+	+	+	+	+	+	+	+	+	+	+	−	−	+
Single spores	−	−	+	−	−	−	−	+	−	−	−	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	+	−
Pairs of spores	−	−	+	−	−	−	−	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	+	−
Acid produced from:																													
Adonitol	nd	+	−	+	+	−	−	−	+	nd	nd	−	−	+	−	−	−	+	+	nd	nd	−	nd	+	−	−	+	+	+
L-Arabinose	+	+	−	+	+	−	−	(+)	+	−	nd	+	−	−	−	+	+	−	nd	−	nd	+	−	+	+	nd	+	−	(+)
Cellobiose	+	+	+	−	−	−	−	+	+	−	−	+	+	−	−	−	+	+	nd	−	nd	+	−	−	−	nd	+	+	+
Erythritol	+	+	−	+	nd	−	+	−	+	−	nd	−	−	−	+	+	−	−	nd	−	nd	−	−	+	+	nd	−	−	−
Fructose	+	+	−	+	+	−	−	+	+	+	−	+	+	+	(+)	−	−	(+)	nd	+	nd	+	−	+	+	nd	+	+	−
Glucose	+	+	(+)	+	+	nd	−	−	+	+	nd	+	+	+	+	+	+	(+)	nd	+	nd	+	−	+	+	nd	+	−	+
Lactose	+	−	−	−	−	nd	−	−	−	−	−	+	−	−	−	−	+	−	nd	−	nd	−	−	−	−	nd	+	−	+
Maltose	+	+	−	+	+	+	−	+	+	−	−	+	−	−	+	+	−	+	nd	+	nd	+	−	−	−	nd	(+)	−	(+)
Mannitol	+	+	+	−	nd	(+)	−	+	+	−	−	+	+	−	−	+	+	+	nd	+	nd	+	−	−	−	nd	+	+	(+)
Rhamnose	+	+	+	−	−	+	−	+	−	nd	nd	+	−		−	+	+	nd	−	nd	nd	−	−	+	−	nd	+	+	+
Salicin	+	+	−	+	nd	nd	−	(+)	−	−	nd	+	−	−	+	−	+	−	nd	−	nd	−	−	−	−	nd	(+)	(+)	−
Sorbitol	+	−	−	+	−	−	−	−	+	−	−	−	−	−	+	−	−	−	nd	−	nd	−	−	−	−	nd	−	−	+
Trehalose	+	+	−	+	+	−	−	−	+	−	−	+	−	+	+	+	+	+	nd	+	nd	+	−	−	+	nd	−	−	+
D-Xylose	+	+	+	+	(+)	+	−	−	+	+	−	+	−	(+)	−	(+)	+	+	nd	−	nd	+	−	+	+	nd	+	+	+
Decomposition of:																													
Adenine		−	−	+	nd	nd	−	−	+	−	−	−	−	−	nd	(+)	−	nd	nd	+	nd	+		−	−		−	−	−
Hypoxanthine	nd	+	+	+	−	+	−	+	−	+	nd	+	−	+	+	+	−	+	nd	+	nd	+	−	−	+	nd	+	+	+
L-Tyrosine	−	+	−	+	+	+	−	−	+	−	nd	+	−	−	+	−	−	+	nd	+	nd	+	−	−	+	nd	−	−	+
Xanthine	nd	+	−	+	−	−	−	−	−	−	nd	+	−	+	nd	+	−	−	nd	+	nd	+	−	−	+	nd	−	(+)	+
Growth in NaCl (w/v):																													
3%	+	+	−	+	+	+	−	−	+	+	+	(+)	−	+	+	+	−	+	nd	+	+	+	−	−	+	+	−	−	+
4%	nd	+	−	+	+	+	−	−	+	+	+	−	−	+	+	+	−	+	nd	+	+	nd	−	−	−	+	−	−	+
5%	nd	+	−	+	+	+	−	−	+	−	−	−	−	(+)	+	(+)	−	+	nd	+	+	−	−	−	−	−	−	−	(+)
7%	nd	−	−	+	+	+	−	−	+	−	−	−	−	−	−	(+)	−	+	nd	(−)	+	−	−	−	−	−	−	−	−
Urease production	+	+	−	+	+	+	−	+	+	nd	+	−	−	nd	−	−	−	+	nd	−	nd	+	−	+	−	nd	+	−	−

[a]+, Positive; −, negative; (+), weakly positive; nd, not determined.

List of species of the genus *Pseudonocardia*

1. **Pseudonocardia thermophila** Henssen 1957, 408[VP]

ther.mo′phi.la. Gr. n. *thermê* heat; Gr. adj. *philus* loving; N.L. fem. adj. *thermophila* heat loving.

Substrate hyphae are septate, often zig-zag shaped; swellings are usually present and become divided by transverse and longitudinal septa. Aerial hyphae are often zig-zag shaped, young stages with constrictions; later on they are septate throughout; swellings are rarely present. Spores on substrate and aerial mycelium are formed by budding or secondary septation of hyphal segments. The inner wall layer in hyphae and spores is uniformly thin and is not thickened in mature spores. Good growth occurs on nutrient and yeast agars. Colonies are yellow and covered by thick white aerial hyphae. Good growth also occurs on asparagine-glycerol and yeast-glucose agars; colonies are yellow and covered by sparse aerial hyphae. Does not grow on oatmeal agar. Grows between at 28 and 60°C (optimally between 40 and 50°C).

Casein and starch are degraded, but not gelatin. H_2S is produced. Acid is produced from galactose, inositol, maltose, melezitose, and 1,2-propanediol, but not from mannose, methyl α-D-glucoside, or sucrose. Additional phenotypic features are shown in Table 229.

Source: isolated from fresh and rotten manure.

DNA G+C content (mol%): not determined.

Type strain: strain MB A-18, ATCC 19285, CBS 277.66, DSM 43832, JCM 3095, NBRC 15559, VKM Ac-896.

Sequence accession no. (16S rRNA gene): X53195.

2. **Pseudonocardia ailaonensis** Qin, Su, Zhang, Wang, Jiang, Xu and Li 2008b, 2088[VP]

ai.la.o.nen′sis. N.L. fem. adj. *ailaonensis* pertaining to Ailao Mountain, Yunnan province, China, the source of the soil sample from which the type strain was isolated.

Forms substrate hyphae that fragment into rod-shaped elements and aerial hyphae that differentiate into long chains of smooth-walled rod-shaped spores. Cream-white aerial hyphae are formed on Czapek, glucose-asparagine, potato, and yeast extract-malt extract agars. The color of the substrate mycelium is orange-yellow on Czapek agar, yellow-brown on glucose-asparagine, and deep orange-yellow on yeast extract-malt extract and potato agars; does not form diffusible pigments on any of these media. Melanin pigments are produced on peptone-yeast extract-iron agar. Grows between 15 and 37°C (optimum at 28°C). The pH range for growth is 6.0–8.0.

Does not liquefy gelatin, produce H_2S, lecithinase, or degrade either cellulose or starch. Milk is coagulated and peptonized. Reduces nitrate. Acid is produced from dulcitol (weak), galactose, glycerol, mannose, and sucrose, but not from inositol, D-lactulose, melezitose, methyl-D-glucoside, or raffinose. Grows on cellobiose, inositol, lactose, mannitol, melezitose (weak), oxalate, ribose, sorbitol, and sucrose as sole carbon sources, but not on acetate, galactose, or xylose. L-Arginine, L-cysteine, L-ornithine, L-threonine, and L-tyrosine are used as sole carbon sources.

The predominant fatty acids are $C_{16:0}$ iso (35.5%), $C_{16:0}$ iso 2-OH (10.8%), and $C_{16:0}$ 10-methyl (9.0%). The cellular polar lipid pattern contains diphosphatidylglycerol, phosphatidylglycerol, and phosphatidylinositol.

Source: the type strain was isolated from a soil sample collected from Ailao Mountain in Yunnan Province, south-west China.

DNA G+C content (mol%): 74.1 (HPLC).

Type strain: strain YIM 45505, DSM 44979, KCTC 19315.

Sequence accession no. (16S rRNA gene): DQ344632.

3. **Pseudonocardia alaniniphila** (Xu, Jin, Mao, Lu, Cui and Jiang 1999) Huang, Wang, Lu, Hong, Liu, Tan and Goodfellow 2002, 981[VP] (Basonym: *Actinobispora alaniniphila* Xu, Jin, Mao, Lu, Cui and Jiang 1999, 885)

a.la.ni.ni′phi.la. N.L. n. *alaninum* alanine; Gr. adj. *philus* loving; N.L. fem. adj. *alaniniphila* alanine loving.

Forms substrate and aerial hyphae that are branched, but which do not fragment. Spores are borne in longitudinal pairs on vegetative hyphae and either singly or in longitudinal pairs on aerial hyphae at 45°C. Substrate hyphae are orange-yellowish and aerial hyphae are sparse and pink-white. Neither diffusible nor melanin pigments are produced. Does not grow at 45°C.

Pectin is degraded, but not cellulose or starch. Nitrate is reduced. Does not produce H_2S. Cells utilize D-fructose, L-rhamnose, D-xylose, D-mannitol, alanine, L-histidine, and proline, but not adonitol cellobiose, raffinose, or inulin. Acid is not produced from these carbon sources. Alanine, L-histidine, and proline are used as sources of nitrogen. Resistant to neomycin and rifampicin (each at 50 μg/ml). Weak antifungal activity is shown against *Aspergillus niger*. Additional phenotypic features are shown in Table 229.

The cellular polar lipid pattern contains phosphatidylcholine, phosphatidylethanolamine, phosphatidylglycerol, phosphatidylmethylethanolamine, and glucosamine-containing phospholipids.

Source: the type strain was isolated from a soil sample collected at Xichou, Yunnan Province, China.

DNA G+C content (mol%): 69.3 (T_m).

Type strain: strain Y-16303, CCTCC AA 97001, CGMCC 4.1536, DSM 44660, JCM 11837.

Sequence accession no. (16S rRNA gene): EU722519.

4. **Pseudonocardia alni** (Evtushenko, Akimov, Dobritsa and Taptykova 1989) Warwick, Bowen, McVeigh and Embley 1994, 298[VP] (Basonym: *Amycolata alni* Evtushenko, Akimov, Dobritsa and Taptykova 1989, 76)

al′ni. L. gen. n. *alni* of alder, referring to the isolation of the type strain and some other strains from alder associations.

Substrate and aerial mycelia fragment into rod-shaped and oval elements. Abundant chains of spores (up to 1.5 μm in diameter) are formed on aerial hyphae. Substrate hyphae are characterized by the presence of swellings, polygonal shaped cells, and their conglomerates. Swollen hyphal segments (up to 2.5 μm in diameter and 10 μm long) are present and become divided by transverse and longitudinal septa. The aerial mycelium is white to cream colored and the substrate mycelium is orange to brown. Moderate growth occurs on water agar.

Degrades gelatin, starch, and Tweens 40, 60 and 80, but not casein or cellulose. Esculin is hydrolyzed, but not allantoin. Acid is produced from D-arabinose, fructose, galactose,

glycerol, methyl α-D-glucoside, 1,2-propanediol, ribose, and sucrose, but not from dulcitol, galactose, inositol, inulin, mannose, melezitose, rhamnose, ribose, or sucrose. Acetate, aconitate, benzoate, citrate, formate, fumarate, 2-oxoglutarate, lactate, malate, maleate, propionate, pyruvate, sebacate, succinate, and *trans*-aconitate are assimilated. Arginine, asparagine, glutamine, histidine, leucine, ornithine, and proline are used as nitrogen sources. Grows in the presence of phenol (0.001%, w/v) and thymol (0.01%, w/v). Susceptible (μg/ml) to chloramphenicol (32), fucidin (10), gentamicin (4), kanamycin (16), lysozyme (50), monomycin (8), nalidixic acid (64), neomycin (4), novobiocin (0.25), rifampin (0.125), roseofungin (10), streptomycin (4), tetracycline (1), and vancomycin (0.5), but resistant to ampicillin (1–16), benzylpenicillin (0.5–10), carbenicillin (1–10), ristomycin (0.05), tobramycin (5), and vancomycin (0.25). Additional phenotypic features are shown in Table 229.

The major fatty acids are $C_{16:0}$ iso (36–40%) and $C_{17:0}$ anteiso acid (14–19%); also contains smaller amounts of $C_{16:1}$ iso (5.8–8.6%), $C_{16:0}$ (3.8–8.1%), $C_{17:0}$ iso (4.4–14.4%), $C_{17:0}$ (4.5–9.6%), $C_{18:0}$ methyl (3.5–6.9%), and $C_{18:0}$ anteiso (1.2–7.5). The cellular polar lipid pattern contains phosphatidycholine and phosphatidylethanolamine.

Source: isolated from root nodules and rhizospheres of alders [*Alnus glutionosa* (L.) Gaerth. and *Alnus incana* (L.) Moench.], and from marine sediments.

DNA G+C content (mol%): 72–74 (T_m).

Type strain: strain 3LS, DSM 44104, IFO (now NBRC) 14991, JCM 9103, NBRC 14991, VKM Ac-901.

Sequence accession no. (16S rRNA gene): AJ252823.

5. **Pseudonocardia ammonioxydans** Liu, Wu, Liu and Liu 2006, 556[VP]

am.mo.ni.o.xy′dans. N.L. n. *ammonia* ammonia; N.L. part. adj. *oxydans* oxidizing; N.L. part. adj. *ammonioxydans* oxidizing ammonia.

Forms aerial and substrate mycelia which fragment into rod-shaped elements. Short chains of smooth-surfaced spores are formed on the substrate mycelium by acropetal budding. A brown substrate mycelium and a white aerial mycelium are formed on Luria–Bertani and trypticase soy broth agars. Grows between 4 and 40°C.

Ammonia is oxidized to nitrate in a modified nitrifying medium (nitrification in MNM broth) and in blends of MNM and Luria–Bertani media (dissimilatory, heterotrophic ammonia oxidation). Degrades casein, gelatin (weak), and starch (weak). Produces H_2S. Acid is produced from *N*-acetyl-D-glucosamine, D-arabitol, D-galacturonic acid, gluconic acid, glycerol, 1,2-propanediol, and ribose, but not from *N*-acetyl-β-D-mannosamine, arbutin, L-fucose, D-tagatose, gentiobiose, inositol, α-D-lactose, lactulose, maltotriose, mannose, methyl α-D-galactoside, methyl β-D-galactoside, methyl α-D-glucoside, methyl α-D-mannoside, melezitose, palatinose, D-psicose, raffinose, sucrose, turanose, or xylitol.

Growth occurs in the presence of 0–8% NaCl, with optimal growth at 3.5% NaCl. Additional phenotypic features are shown in Table 229.

The major fatty acids are $C_{16:0}$ iso (41.1%), $C_{16:1}$ iso (15.7%), and $C_{17:1}$ ω8*c* (12.1%). The cellular polar lipid pattern contains diphosphatidylglycerol, phosphatidylcholine, phosphatidylglycerol, phosphatidylinositol mannosides, and phosphatidylmethylethanolamine, but not glucosamine-containing phospholipids.

Source: the type strain was isolated from coastal sediment collected from the Jiao-Dong peninsula, near Qingdao, Shandong Province, China.

DNA G+C content (mol%): 69.6 (T_m).

Type strain: strain H9, CGMCC 4.1877, JCM 12462.

Sequence accession no. (16S rRNA gene): AY500143.

Further comments: metabolizes diverse carbon compounds on Biolog GP2 microplates (Liu et al., 2006).

6. **Pseudonocardia antarctica** Prabahar, Dube, Reddy and Shivaji 2004b, 1005[VP] (Effective publication: Prabahar, Dube, Reddy and Shivaji 2004a, 69.)

an.tarc′ti.ca. L. fem. adj. *antarctica* southern, and by extension, pertaining to the continent Antarctica.

Filamentous brown substrate and aerial mycelia form a white conglomerate. Spores are formed by fragmentation of aerial hyphae. Grows at 7–38°C (optimum at 25°C) and pH 4–10. β-Galactosidase-, oxidase-, and phosphatase-positive, but negative for arginine dihydrolase, arginine decarboxylase, esculin, lipase, and lysine. Acid is produced from galactose, mannose, and ribose, but not from fructose, inositol, or melezitose.

Resistant to co-trimoxazole, nalidixic acid, and norfloxacin. Additional phenotypic features are shown in Table 229.

The major fatty acids are $C_{16:0}$ iso (30%) and $C_{16:0}$ (15%). The cellular polar lipid pattern contains diphosphatidylglycerol, phosphatidylcholine, phosphatidylethanolamine, phosphatidylglycerol, phosphatidylinositol, and phosphatidylmethylethanolamine.

Source: isolated from a moraine sample collected from the McMurdo Dry Valleys region of Antarctica.

DNA G+C content (mol%): 71 (T_m).

Type strain: strain DVS 5a1, DSM 44749, JCM 12172, MTCC 4297.

Sequence accession no. (16S rRNA gene): AJ576010.

Further comments: the specific epithet *antarctica* is a L. adj. not a N.L. adj. as cited in the paper by Prabahar et al. (2004a).

7. **Pseudonocardia asaccharolytica** Reichert, Lipski, Pradella, Stackebrandt and Altendorf 1998, 447[VP]

a.sac.cha.ro.ly′ti.ca. Gr. pref. *a* not; Gr. n. *sakchâr* sugar; Gr. fem. adj. *lutikê* able to dissolve, able to loosen; N.L. fem. adj. *asaccharolytica* referring to the failure to produce acid from carbohydrates.

Substrate hyphae are yellow on trypticase soy agar and fragment into coccoid and rod-shaped elements. Swollen hyphal segments up to 2 μm in diameter are formed, as are transverse hyphal septa. White, aerial hyphae carry long chains of oval spores. Spore surfaces are smooth. Dimethyl disulfide is oxidized. Acid is produced from 1,2-propanediol, but not from galactose, inositol, mannose, melezitose, methyl α-D-glucoside, sucrose, or xylitol. Additional phenotypic features are shown in Table 229.

The major fatty acids are $C_{16:0}$ iso (21.5–28.1%), $C_{16:0}$ (19.3–23.3%), $C_{17:0}$ iso (11.6–12.7%), $C_{15:0}$ iso (8.2–11.4%), and $C_{17:0}$ anteiso (4.8–6.1%); also contains small amounts of 10-methyl fatty acids. Mycolic acids are not formed. The cellular polar lipid pattern contains diphosphatidylglycerol, hydroxyphosphatidylethanolamine, phosphatidylglycerol, phosphatidylinositol, phosphatidylinositol mannosides, phosphatidylmethylethanolamine, and a ninhydrin-positive component.

Source: isolated from tree-bark compost biofilters from an animal-rendering plant.

DNA G+C content (mol%): not determined.

Type strain: strain 580, CIP 105685, DSM 44247, IFO (now NRBC) 16224, JCM 10410.

Sequence accession no. (16S rRNA gene): Y08536.

8. **Pseudonocardia aurantiaca** (Xu, Jin, Mao, Lu, Cui and Jiang 1999) Huang, Wang, Lu, Hong, Liu, Tan and Goodfellow 2002, 981[VP] (Basonym: *Actinobispora aurantiaca* Xu, Jin, Mao, Lu, Cui and Jiang 1999, 885.)

au.ran.ti′a.ca. N.L. fem. adj. *aurantiaca* orange-colored.

Forms substrate and aerial hyphae that are branched, but do not fragment. Spores are borne in longitudinal pairs on vegetative hyphae and either singly or in longitudinal pairs on aerial hyphae. The substrate mycelium is orange to orange-yellow and is covered by sparse, pink-white aerial hyphae. A brilliant yellow diffusible pigment is produced on oatmeal agar. Melanin pigments are formed. Does not grow at 45°C.

Pectin is degraded, but not cellulose or starch. Gelatin is liquefied. Milk is neither coagulated nor peptonized. Does not produce H_2S, lecithinase, or reduce nitrate. Cellulose, fructose, raffinose, and rhamnose are used as sole carbon sources, but not adonitol, inositol, mannitol, or xylose. Acid is not produced from these sugars. Alanine, L-histidine, and proline are used as sole nitrogen sources.

Shows weak antimicrobial activity against *Bacillus subtilis.* Resistant to neomycin, but not to rifampin (each at 50 µg/ml). Additional phenotypic features are shown in Table 229.

The cellular polar lipid pattern contains phosphatidylcholine, phosphatidylethanolamine, phosphatidylglycerol, and glucosamine-containing phospholipids.

Source: the type strain was isolated from a soil sample collected at Jianchuan, Yunnan Province, China.

DNA G+C content (mol%): 71.5 (T_m).

Type strain: strain Y-14860, CCTCC AA 97002, CGMCC 4.1537, JCM 11838.

Sequence accession no. (16S rRNA gene): AF056707.

9. **Pseudonocardia autotrophica** (Takamiya and Tubaki 1956) Warwick, Bowen, McVeigh and Embley 1994, 298[VP] [Basonym: *Nocardia autotrophica* Hirsch 1961, 362; "*Streptomyces autotrophicus*" Takamiya and Tubaki 1956, 59; *Amycolata autotrophica* (Takamiya and Tubaki 1956) Lechevalier, Prauser, Labeda and Ruan 1986, 34]

au.to.tro′phi.ca. Gr. pron. *autos* himself; N.L. fem. adj. *trophica* (from Gr. fem. adj. *trophikê*) nursing, tending or feeding; N.L. fem. adj. *autotrophica* self-nourishing, referring to the ability to grow at the expense of H_2 and CO_2.

Yellow to brown substrate hyphae fragment into squarish spores of unequal size, especially in liquid media. White to cream aerial hyphae differentiate into long chains of cylindrical to oval spores. Zig-zag mycelial growth and swollen hyphal segments are formed. Longitudinal and transverse septa are formed in the mycelium. Grows at 10–37°C, but not at 42°C. Autotrophic. Hydrogen is utilized in the presence of oxygen and carbon dioxide in a mineral salts medium.

Decarboxylates acetate, citrate, lactate, malate, propionate, pyruvate, and succinate, but not benzoate, mucate, oxalate, or tartrate. Produces phosphatase and H_2S. Does not hydrolyze esculin. Degrades testosterone, and Tweens 20, 40, 60 and 80, but not casein, gelatin, or starch. Acid is produced from galactose, inositol, mannose, melezitose, methyl α-D-glucoside, 1,2-propanediol, and sucrose.

Does not grow in lysozyme, or salicylate broth. Susceptible to (µg/ml) gentamicin (50), minocycline (50), rifampin (50), streptomycin (50), and vancomycin (50), but is resistant to erythromycin (50), fusidic acid (50), penicillin (10 IU). Additional phenotypic features are shown in Table 229.

The major fatty acids are $C_{16:0}$ iso (33.5%), $C_{17:0}$ iso (7.4%), and $C_{17:0}$ ω-methyl (10%). The cellular polar lipid pattern contains diphosphatidylglycerol, phosphatidylcholine, phosphatidylglycerol, phosphatidylinositol, phosphatidylglycerol, phosphatidylinositol, phosphatidylinositol mannoside, and phosphatidylmethylethanolamine, but not phosphatidylethanolamine.

Source: isolated from phosphate buffer solution, aluminum hydroxide gel, vegetable matter, soil, and clinical specimens.

DNA G+C content (mol%): 70 (T_m).

Type strain: ATCC 19727, CIP 107114, DSM 535, IFO (now NRBC) 12743, JCM 4348, NRRL B-11275, VKM Ac-941.

Sequence accession no. (16S rRNA gene): AJ252824.

10. **Pseudonocardia benzenivorans** Kämpfer and Kroppenstedt 2004, 751[VP]

ben.ze.ni.vo′rans. N.L. neut. n. *benzenum* benzene; L. part. adj. *vorans* devouring, digesting; N.L. fem. part. adj. *benzenivorans* digesting benzene.

Forms pale substrate hyphae that fragment into rod-shaped and coccoid elements. Aerial mycelium is white. Good growth on R2A and nutrient agars at 25–30°C after incubation for 3 d. Does not grow at 4–20 or 40–55°C. L-Alanine-*p*-nitroanilide (NA), *p*-nitrophenyl-(NP)-α-D-glucopyranoside, *p*NP-phenylphosphonate (weak), *bis*-*p*NP-phosphate, and L-proline-*p*NA are hydrolyzed, but not esculin, 2-deoxythymidine-5′-*p*NP-phosphate, *o*NP-β-D-galactopyranoside, *p*NP-β-D-glucopyranoside, L-glutamate-3-carboxy-*p*NA, *p*NP-phosphorylcholine, proline-*p*NA, or *p*NP-α-D-xylopyranoside. Acid is produced from galactose, glycerol, D-lactulose, and mannose, but not from dulcitol, inositol, melezitose, methyl α-D-glucoside, raffinose, sorbose, or sucrose. Grows on 4-aminobutyrate and 4-hydroxybutyrate (each at 10 mM). Additional phenotypic features are shown in Table 229.

The predominant fatty acids are $C_{16:0}$ iso (52.1%), $C_{16:1}$ iso *cis*9 (6.0%), $C_{17:0}$ iso (6.9%), $C_{17:1}$ *cis*9 (5.2%), and $C_{17:0}$

10-methyl (5.5%); small amounts of methyl-branched fatty acids are also present. The cellular polar lipid pattern contains diphosphatidylglycerol, phosphatidylethanolamine, and phosphatidylinositol, but not phosphatidylcholine.

Source: the type strain, which originated from a soil sample collected in Bitterfeld, Germany, was isolated from an enrichment culture containing 1,2,3,5-tetrachlorobenzene as the sole source of carbon.

DNA G+C content (mol%): not determined.

Type strain: strain B5, CCUG 49018, CIP 107928, DSM 44703, JCM 12694.

Sequence accession no. (16S rRNA gene): AJ556156.

Further comments: metabolizes a diverse range of carbon compounds on Biolog GP2 microplates (Kämpfer and Kroppenstedt, 2004)

11. **Pseudonocardia carboxydivorans** Park, Park, Lee and Kim 2008, 2477[VP]

car.bo.xy.di.vo′rans. N.L. neut. n. *carboxydum* carbon monoxide; L. part. adj. *vorans* devouring, digesting; N.L. part. adj. *carboxydivorans* digesting carbon monoxide.

Forms brown substrate mycelium and a white aerial mycelium. The aerial hyphae fragment into rod-shaped elements. Grows optimally at 25°C. Oxidizes carbon monoxide. Does not produce oxidase. Does not degrade gelatin or starch. Acid is produced from inulin, but not from cellobiose, inositol, maltose, mannitol, mannose, melezitose, ribose, trehalose, or xylose. *N*-Acetyl-D-glucosamine, inulin, and mannan are used as sole carbon sources, but not cellobiose, fructose, galactose, *myo*-inositol, maltose, mannitol, mannose, melezitose, raffinose, L-rhamnose, D-ribose, sorbitol, sucrose, trehalose, xylitol, or D-xylose. Additional phenotypic features are shown in Table 229.

The predominant fatty acids are $C_{16:0}$ iso (47.2%) and $C_{16:1}$ iso (22.8%); also contains smaller amounts of $C_{15:0}$ iso (5.0%), $C_{16:1}$ *cis*9 (5.9%), $C_{16:0}$ 10-methyl (4.6%), $C_{17:0}$ iso (3.0%), $C_{17:0}$ anteiso (2.6%), and $C_{17:1}$ *cis*9 (2.4%). The major menaquinone is MK-9.

Source: the type strain was isolated from soil collected at a roadside in Seoul, Korea.

DNA G+C content (mol%): 77 (HPLC).

Type strain: strain Y8, JCM 14827, KCCM 42678.

Sequence accession no. (16S rRNA gene): EF114314.

12. **Pseudonocardia chloroethenivorans** Lee, Strand, Stensel and Herwig 2004, 138[VP]

chlo.ro.e.the.ni.vo′rans. N.L. neut. n. *chloroethenum* chloroethene; L. part. adj. *vorans* devouring; N.L. fem. part. fem. adj. *chloroethenivorans* chloroethene-devouring.

Forms substrate and aerial hyphae that are branched, but do not fragment. Spores are formed at the ends of hyphae. Substrate hyphae are white. Diffusible pigments not formed. Grows on phenol as a source of carbon and energy. Chloroethane, *cis*1,2-dichloroethene, and trichloroethane are degraded. Additional phenotypic features are shown in Table 229.

The major fatty acids are $C_{16:0}$ iso (37.3%), $C_{15:0}$ iso (4.5%), $C_{16:0}$ (5.6%), $C_{16:0}$ 10-methyl (6.1%), $C_{17:0}$ iso (5.9%), $C_{17:0}$ anteiso (6.0%).

Source: the type strain was isolated from an aerobic laboratory enrichment in the Department of Civil and Environmental Engineering, University of Washington, Seattle, WA, USA.

DNA G+C content (mol%): not determined.

Type strain: strain SL-1, ATCC BAA-742, DSM 44698, JCM 12679.

Sequence accession no. (16S rRNA gene): AF454510.

Further comments: metabolizes a wide range of substrates on Biolog GP2 microplates (Lee et al., 2004).

13. **Pseudonocardia compacta** Henssen, Happach-Kasan, Renner and Vobis 1983, 834[VP]

com.pac′ta. L. fem. adj. *compacta* compact.

Substrate hyphae are septate and densely branched; swellings are common and, in part, multidivided. Aerial hyphae are compact, constricted or septate, and show apical and intercalary swellings. Spores of varying shapes and lengths are formed on substrate and aerial mycelia. The inner layer of hyphal walls vary in thickness. The inner layer of spore walls are thick in mature spores. Moderate to good growth occurs on artificial soil agar, with scanty yellow substrate hyphae and abundant white aerial hyphae. Optimum temperature for growth is 20–30°C.

Does not degrade casein, gelatin, or starch. Acid is not formed from galactose, mannose, melezitose, methyl α-D-glucoside, inositol, 1,2-propanediol, sucrose, or xylitol. Additional phenotypic features are shown in Table 229.

The cellular polar lipid pattern contains diphosphatidylglycerol, phosphatidylcholine, phosphatidylethanolamine, phosphatidylinositol, phosphatidylinositol mannosides, and phosphatidylmethylethanolamine. The major fatty acids are $C_{16:0}$ iso (34.5%), $C_{15:0}$ (10.0%), and $C_{17:0}$ (4.8%).

Source: isolated from garden soil collected in Wohra, near Marburg, Germany.

DNA G+C content (mol%): not determined.

Type strain: strain MB H-146, ATCC 35407, CBS 160.82, DSM 43592, IFO (now NRBC) 14343, JCM 7438, NRRL B-16170, VKM Ac-897.

Sequence accession no. (16S rRNA gene): AJ252825.

14. **Pseudonocardia dioxanivorans** Mahendra and Alvarez-Cohen 2005, 597[VP]

di.o.xa.ni.vo′rans. N.L. n. *dioxanum* dioxane; L. part. adj. *vorans* devouring; N.L. part. adj. *dioxanivorans* dioxane-devouring.

Forms white aerial hyphae and yellowish branched substrate hyphae which fragment into rod-shaped elements. Hyphal swellings and budding are sometimes observed. Does not form diffusible pigments. Optimal growth temperature is 30°C.

Aerobic autotrophic growth occurs on CO_2 and H_2. Fixes dinitrogen. Grows on 1,3- and 1,4-dioxane, butyl methyl ether, 2-methyl-1,3-dioxolane, THF, and tetrahydropyran. Acid is produced from glycerol, but not from dulcitol, inositol, lactulose, mannose, melezitose, or sucrose. Additional phenotypic features are shown in Table 229.

The major fatty acids are $C_{16:0}$ iso (27.5%), $C_{16:1}$ iso *cis*9 (9.3%), and $C_{17:1}$ iso *cis*9 (23.6%). The predominant menaquinone is MK-8(H_4); also contains a small amount of MK-7(H_4).

Source: isolated from a 1,4-dioxane-contaminated sludge sample collected at Darlington, South Carolina, USA (Parales et al., 1994).

DNA G+C content (mol%): 74 (HPLC).

Type strain: strain CB1190, ATCC 55486, DSM 44775, JCM 13855.

Sequence accession no. (16S rRNA gene): AY340622.

Further comments: metabolizes a diverse range of carbon compounds on Biolog GP2 and SEP2 microplates, and nitrogen sources on Biolog PM3 plates (Mahendra and Alvarez-Cohen, 2005).

15. **Pseudonocardia endophytica** Chen, Qin, Li, Zhang, Xu, Jiang, Kim and Li 2009, 559[VP]

en.do.phy′ti.ca. Gr. *endo* within; Gr. n. *phyton* plant; L. fem. suff. *-ica* adjectival suffix used with the sense of belonging to; N.L. fem. adj. *endophytica* within plant, endophytic, pertaining to the original isolation from plant tissues.

Forms yellowish-brown substrate mycelium and white aerial mycelium on tryptic soy and yeast extract-malt extract agars. The substrate mycelium fragments into rod-shaped elements. Spore chains form on aerial mycelium. Does not form diffusible pigments. Growth occurs at 15–37°C and pH 6.0–8.0.

Catalase-positive and oxidase-negative. Malonate and ornithine decarboxylase tests are positive, but negative for the gluconate test, H_2S production, milk coagulation and peptonization, and nitrate reduction. Does not degrade casein, cellulose, gelatin, or starch. Acid is produced from arbutin, esculin, galactose, glycerol, melezitose, D-ribose, sucrose, trehalose, and turanose, but not from amygdalin, arabitol, dulcitol, fucose, gentiobiose, gluconate, glycogen, inositol, inulin, 2-ketogluconate, lyxose, mannose, melibiose, methyl α-D-glucoside, methyl α-D-mannoside, methyl β-D-xyloside, *N*-acetylglucosamine, raffinose, sorbose, starch, tagatose, or xylitol. Utilizes L-asparagine, L-glutamic acid, *p*-hydroxyphenylacetic acid, α-ketoglutaric acid, α-ketovaleric acid, D-malic acid, *N*-acetyl-L-glutamic acid, propionic acid, putrescine, L-pyroglutamic acid, pyruvic acid, succinamic acid, and succinic acid, but not acetic acid, L-alaninamide, D- or L-alanine, L-alanyl glycine, glycyl L-glutamic acid, α-, β- or γ-hydroxybutyric acid, lactamide, L-lactic acid, L-malic acid, methyl pyruvate, monomethyl succinate, pyruvic acid, or L-serine. Additional phenotypic features are shown in Table 229.

The cellular polar lipid pattern contains diphosphatidylglycerol, phosphatidylcholine, phosphatidylethanolamine, and phosphatidylmethylethanolamine. The major fatty acids are $C_{16:0}$ iso (34.0%), $C_{17:1}$ *cis*9 (14.5%), and $C_{15:0}$ iso (9.6%). The predominant menaquinone is MK-8 (H_4); also contains traces of MK-7(H_6) and MK-9.

Source: isolated from a plant sample collected from Xishuangbanna, Yunnan Province, south-western China.

DNA G+C content (mol%): 70.3 (HPLC).

Type strain: strain YIM 56035, CCTCC AA 206026, DSM 44969, KCTC 19150.

Sequence accession no. (16S rRNA gene): DQ887489.

16. **Pseudonocardia halophobica** (Akimov, Evtushenko and Dobritsa 1989) McVeigh, Munro and Embley 1994, 302[VP] (Basonym: *Pseudoamycolata halophobica* Akimov, Evtushenko and Dobritsa 1989, 460)

ha.lo.pho′bi.ca. Gr. n. *hals* halos salt, the sea; Gr. n. *phobos* fear, dread; L. fem. suff. *-ica* suffix used with the sense of pertaining to; N.L. fem. adj. *halophobica* salt-fearing, referring to the inability to grow in the presence of 3% NaCl.

Forms substrate and aerial hyphae which tend to fragment into rod-shaped and oval elements. Shows zig-zag mycelial growth and longitudinal and transverse hyphal septa. Short chains of spores may be produced on aerial hyphae. Swollen hyphal segments (up to 3 μm long) are formed. The aerial mycelium is white to cream colored and the substrate mycelium ranges from yellowish orange to brown, depending on the medium. An orange substrate mycelium is formed on peptone-yeast extract agar.

Degrades Tweens 40 and 60. Arbutin and esculin are hydrolyzed, but not allantoin. Decarboxylates aconitate, formate, lactate, mannose, malate, propionate, pyruvate, succinate, sebacate, and *trans*-aconitate, but not maleate or salicylate. Gaseous aliphatic hydrocarbons (C6–C14) are used for growth. Grows on 4-aminobutyrate and 4-hydroxybutyrate (each at 10 mM). Acid is produced from inositol and sucrose, but not from dulcitol, glycerol, D-lactulose, mannose, melezitose, methyl α-D-glucoside, raffinose, or sorbose. Alanine, arginine, hydroxyproline, ornithine, phenylalanine, proline, threonine, tryptophan, and valine are used as sole nitrogen sources, but not glycine, histidine, leucine, lysine, or tyrosine.

Susceptible to (μg/ml) fucidin (10), lincomycin (1), methacycline (1), novobiocin (50), penicillin (10), polymyxin B (20), roseofungin (10), and tetracycline (10). Does not grow in the presence of crystal violet (0.000001, w/v); azide (0.001%, w/v) or sodium chloride (3%, w/v). Additional phenotypic features are shown in Table 229.

Major fatty acids are $C_{16:0}$ iso (19–26%), $C_{17:0}$ anteiso (17–21%), and $C_{15:0}$ iso (5.5–9%); also contains smaller amounts of $C_{15:0}$, $C_{16:0}$, $C_{16:1}$, $C_{17:0}$ 10-methyl, and $C_{18:0}$ 10-methyl. The cellular polar lipid pattern contains diphosphatidylglycerol, hydroxyphosphatidylethanolamine, phosphatidylethanolamine, phosphatidylglycerol, phosphatidylinositol, and phosphatidylinositol mannoside; also contains small amounts of phosphatidylmethylethanolamine and a ninhydrin-positive component, but does not contain either phosphatidylcholine or glucosamine-containing phospholipids.

Source: isolated from soil.

DNA G+C content (mol%): 72 (T_m).

Type strain: strain SS1/1, ATCC 51535, DSM 43089, IFO (now NRBC) 15408, IMRU 1300, JCM 9421, NRRL B-16514, VKM Ac-1069.

Sequence accession no. (16S rRNA gene): AJ252827.

17. **Pseudonocardia hydrocarbonoxydans** (Nolof and Hirsch 1962) Warwick, Bowen, McVeigh and Embley 1994, 298[VP] [Basonym: *Nocardia hydrocarbonoxydans* Nolof and Hirsch 1962, 267; *Amycolata hydrocarbonoxydans* (Nolof and Hirsch 1962) Lechevalier, Prauser, Labeda and Ruan 1986, 34]

hy.dro.car.bo.no′xy.dans. Gr. n. *hudôr* water; L. n. *carbo -onis* coal, charcoal; N.L. part. adj. *oxydans* oxidizing; N.L. pres. part. *hydrocarbonoxydans* oxidizing hydrocarbons.

Zig-zag mycelial growth and swollen hyphal segments are evident. Longitudinal and transverse hyphal septa are formed. Aerial and substrate hyphae may fragment into

squarish elements. Sparse to moderate white aerial hyphae are formed. Aerial hyphae are white and sparse to moderate; substrate hyphae are off-white, yellowish white, gold, or brown depending on the growth medium. Grows at 10–37°C, but not at 45°C.

Gaseous aliphatic hydrocarbons (C6–C14) are used for growth. Grows on 4-aminobutyrate and 4-hydroxybutyrate (each at 10 mM). Positive for catalase, phosphatase, and esculin. Decarboxylates lactate, propionate, and pyruvate, but not benzoate, mucate, or tartrate. Does not degrade casein, or grow in lysozyme or salicylate broths. Acid is produced from inositol and sucrose, but not from dulcitol, glycerol, D-lactulose, mannose, melezitose, methyl α-D-glucoside, raffinose, or sorbose. Additional phenotypic features are shown in Table 229.

L-Alanine-*p*NA, *o*NP-β-galactopyranoside (weak), *p*NP-α- and *p*NP-β-D-glucopyranoside, *p*NP-phenylphosphonate, *bis*-*p*NP-phosphate, L-proline, *p*NA-glucopyranoside, and *p*NP-β-D-xylopyranoside are hydrolyzed, but not 2-deoxythymidine-5′-*p*NP-phosphate, esculin, L-glutamate-γ-3-carboxy-*p*-NA, or *p*NP-phosphorylcholine.

The predominant fatty acids are $C_{15:0}$ iso (10.9%), $C_{16:0}$ iso (30.8%), and $C_{16:0}$ (25.8%). The cellular polar lipid pattern contains diphosphatidylglycerol, phosphatidycholine, phosphatidylglycerol, phosphatidylinositol, phosphatidylinositol mannosides, and phosphatidylmethylethanolamine, but not phosphatidylethanolamine. The predominant menaquinones are MK-8(H_2) and MK-8(H_4).

Source: air contaminant; isolated from a silica gel plate.

DNA G+C content (mol%): 69 (T_m).

Type strain: ATCC 15104, DSM 43281, IFO (now NRBC) 14498, JCM 3392, NRRL B-16171, VKM Ac-799.

Sequence accession no. (16S rRNA gene): AJ252826.

Further comments: metabolizes a diverse range of substrates on Biolog GP2 and SFP2 microplates (Mahendra and Alvarez-Cohen, 2005).

18. **Pseudonocardia kongjuensis** Lee, Kim, Min, Lee, Kang and Hah 2001, 1509[VP]

kong.ju.en′sis. N.L. fem. adj. *kongjuensis* of Kongju, Republic of Korea.

Forms an abundant yellowish brown substrate mycelium and a white aerial hyphae mycelium that fragments into rod-shaped spores. The spore surface is smooth. Growth occurs between 4 and 37°C.

Catalase-positive. Produces H_2S. Casein is degraded, but not gelatin or starch. Acid is produced from galactose, glycerol (weak), inositol, mannose, melezitose, and sucrose, but not from 2,3-butanediol, dulcitol, melibiose, methyl α-D-glucoside, methyl α-D-mannoside, 1,2-propanediol, raffinose, sorbose, or D-xylitol. Additional phenotypic features are shown in Table 229.

The major fatty acids are $C_{16:0}$ iso (31.4%), $C_{17:1}$ (13.8%), $C_{18:0}$ (9.6%), $C_{16:1}$ (7.4%), $C_{16:0}$ (6.0%), $C_{16:1}$ iso (6.7%), and $C_{17:1}$ iso (6.2%). The cellular polar lipid pattern contains diphosphatidylglycerol, phosphatidylcholine, phosphatidylethanolamine, phosphatidylglycerol, and phosphatidylinositol. The predominant menaquinone is MK-8(H_4).

Source: isolated from a gold mine cave in Kongju, Republic of Korea.

DNA G+C content (mol%): 71 (HPLC).

Type strain: strain LM 157, DSM 44525, IMSNU 50583, JCM 11896, KCTC 9990, NBRC 100380.

Sequence accession no. (16S rRNA gene): AJ252833.

19. **Pseudonocardia nitrificans** (*ex* Schatz, Isenberg, Angrist and Schatz 1954) Warwick, Bowen, McVeigh and Embley 1994, 298[VP] ("*Streptomyces nitrificans*" Schatz, Isenberg, Angrist and Schatz 1954, 2)

ni.tri.fi′cans. N.L. part. adj. *nitrificans* nitrifying.

Produces straight, branched, sporulating aerial hyphae. Grows on various simple and complex agar media. Grows better at 25–30°C than at 37°C. Develops a brick-red mycelium without hemolysis on blood agar, and a gray sporulated surface with a pink to buff reverse on other media; does not produces diffusible pigments. Forms a well sporulating pellicle in nutrient broth and other liquid media. Growth is gnarled and wrinkled on beet, carrot, and potato slants.

Urethane is used as a sole source of carbon, energy, and nitrogen. Produces nitrite from carbamate, but does not oxidize urethane nitrogen beyond nitrite. Slowly alkalinizes milk without curdling. Does not degrade cellulose or produce indole. Ethanol, fumarate, lactate, and *n*-propanol are used as sole carbon sources. Ammonia, guanidine, nitrate, nitrite, purines, urea, and several amino acids are used as sole nitrogen sources. Additional phenotypic features are shown in Table 229.

Source: isolated from soil enriched with urethane as a sole carbon, nitrogen, and energy source.

DNA G+C content (mol%): not determined.

Type strain: IFAM 379.

Sequence accession no. (16S rRNA gene): X55609.

20. **Pseudonocardia oroxyli** Gu, Luo, Zheng, Liu and Huang 2006, 2194[VP]

o.ro.xy′li. N.L. gen. n. *oroxyli* of the plant genus *Oroxylum*.

Forms aerial and substrate hyphae which fragment into smooth rod-shaped elements. Swellings are produced at hyphal tips. The cinnamon-buff substrate mycelium carries pinkish cinnamon aerial hyphae. Grows well on glucose-yeast extract-malt extract agar after 3 d at 25–30°C. Does not produce diffusible pigments.

Acid is produced from galactose, glycerol, mannose, melezitose, and sucrose, but not from dulcitol, inositol, D-lactulose, or D-sorbose. Acetate, L-alanine, L-arabinose, L-arginine, cellobiose, citrate, fructose, galactose, glucose, glutamic acid (weak), glycerol, glycogen, inulin, inositol, lactose, D-lactulose, L-leucine, malate, mannitol, maltose, mannose, melezitose, methyl α-D-glucoside, L-methionine, L-ornithine, oxalate (weak), L-phenylalanine, L-proline, raffinose, rhamnose, ribose, salicin (weak), sorbitol, succinate, sucrose, trehalose, L-valine, and D-xylose are used as sole carbon sources, but not L-arginine, L-cysteine, L-leucinamide, L-tyrosine, or malonate. L-Ornithine is used as a sole nitrogen source, but not L-cysteine, L-threonine, or L-tyrosine. Additional phenotypic features are shown in Table 229.

The predominant fatty acid is $C_{16:0}$ iso (45.1%); smaller amounts of $C_{16:0}$ 10-methyl (11.1%), $C_{17:1}$ ω6*c* (7.4%), iso-H-$C_{16:1}$ (7.1%), $C_{18:1}$ ω9*c* (5.1%), $C_{15:0}$ iso (4.4%), $C_{16:1}$ ω7*c*

(4.3%), $C_{14:0}$ iso (3.5%), and $C_{16:0}$ (3.0%) are also formed. The cellular polar lipid pattern contains phosphatidylethanolamine, phosphatidylinositol, phosphatidylmethylethanolamine, two glucosamine-containing phospholipids of unknown structure, and two glycolipids.

Source: the type strain was isolated from a surface-sterilized root of *Oroxylum indicum* collected in the rain forest around Liusha River, southwest of Jinghong City, Yunnan Province, China.

DNA G+C content (mol%): 70.6 (T_m).

Type strain: strain D10, CGMCC 4.3143, DSM 44984, JCM 13909.

Sequence accession no. (16S rRNA gene): DQ343154.

Further comments: metabolizes a diverse range of carbon compounds on Biolog GP2 and SEP2 microplates, and nitrogen sources on Biolog PM3 plates (Mahendra and Alvarez-Cohen, 2005).

21. **Pseudonocardia petroleophila** (Hirsch and Engel 1956) Warwick, Bowen, McVeigh and Embley 1994, 298[VP] (Basonym: *Nocardia petroleophila* Hirsch and Engel 1956, 445.)

pe.tro.le.oph′il.a. Gr. n. *petra* stone; L. n. *oleum* oil; Gr. adj. *philus* loving; N.L. fem. adj. *petroleophila* loving stone oil, petroleum.

Substrate mycelium is yellowish, citron, or ochre-yellow depending on the carbon or nitrogen source. It fragments into bacillary elements under autotrophic and heterotrophic growth conditions, but much more rapidly in the latter case. Abundant snow-white aerial hyphae, which fragment into spore-like fragments with age, are produced on mineral media. Optimal temperature for growth is 25–28°C; slight growth is evident at 37°C, but does not grow at 50°C. Grows at pH 6.8–8.3. Grows on certain organic media, but without aerial mycelium production and with rapid fragmentation of substrate mycelium. Grows slowly but abundantly on all mineral salts media. Growth rate increases in a petroleum atmosphere. Atmospheric carbon dioxide is used as a carbon and energy source. Does not coagulate or peptonize milk. Grows in the presence of 10% NaCl. Additional phenotypic features are shown in Table 229.

Source: isolated from soil collected in Germany.

DNA G+C content (mol%): not determined.

Type strain: ATCC 15777, CIP 104515, DSM 43193, IFAM 78, IFO (now NRBC) 14406, JCM 3378, JCM 3394, NRRL B-16301, VKM Ac-865.

Sequence accession no. (16S rRNA gene): AJ252828.

22. **Pseudonocardia saturnea** (Hirsch 1960) Warwick, Bowen, McVeigh and Embley 1994, 298[VP] [Basonym: *Nocardia saturnea* Hirsch 1960, 401; *Amycolata saturnea* (Hirsch 1960) Lechevalier, Prauser, Labeda and Ruan 1986, 34.]

sa.tur′ne.a. L. n. *Saturnus* Saturn, Roman god of seed sowing; N.L. fem. adj. *saturnea* pertaining to Saturn, referring to the colonies which have a Saturnian shape.

Yellowish white to bright butter yellow substrate hyphae fragment into rod-shaped elements, especially on organic media. Aerial hyphae are white to yellowish and fragment into long rectangular elements on nutritionally poor media. Grows at 10–37°C (optimum at 28–30°C), but does not grow at 42°C.

CO_2 is used as a sole source of carbon for energy and growth. Positive for catalase, esculin, and phosphatase. Does not degrade casein. Decarboxylates benzoate, lactate, propionate, and pyruvate, but not mucate or tartrate. Acid is produced from inositol, mannose, melezitose, methyl α-D-glucoside, 1,2-propanediol, and sucrose, but not from galactose or xylitol. Additional phenotypic features are shown in Table 229.

The cellular polar lipid pattern contains phosphatidycholine and phosphatidylmethylethanolamine, but not phosphatidylethanolamine. The predominant menaquinones are MK-8(H_2) and MK-8(H_4).

Source: isolated from air and compost in Germany.

DNA G+C content (mol%): 72 (T_m).

Type strain: ATCC 15809, DSM 43195, IFO (now NRBC) 14499, IMRU 1181, JCM 3187, NRRL B-16172, VKM Ac-781.

Sequence accession no. (16S rRNA gene): AJ252829.

23. **Pseudonocardia spinosa** Schäfer *in* Henssen and Schäfer 1971, 31[AL]

spi.no′sa. L. fem. adj. *spinosa* spiny.

Substrate hyphae are compact, irregularly branched, constricted, and septate; swellings are common. Aerial hyphae are constricted or septate. Spores are formed on substrate and aerial mycelia either by budding or secondary septation of hyphal segments. Inner wall layers of hyphae and spores vary in thickness. Grows extremely slowly. Moderate to good growth occurs on oatmeal and yeast-starch agars. Yellow colonies covered by abundant white aerial mycelium are formed on asparagine-glycerol agar. Optimal temperature for growth is between 20 and 30°C. Additional phenotypic features are shown in Table 229.

Source: isolated from soil collected in Turkey.

DNA G+C content (mol%): not determined.

Type strain: strain MB SH-1, ATCC 25924, CBS 818.70, IFO (now NRBC) 16002, JCM 3136.

Sequence accession no. (16S rRNA gene): not determined.

24. **Pseudonocardia spinosispora** Lee, Kim, Kang and Hah 2002, 1607[VP]

spi.no.si.spo′ra. L. adj. *spinosus* thorny; Gr. fem. n. *spora* seed; N.L. fem. n. *spora* spore; N.L. fem. n. *spinosispora* thorny spore.

Substrate hyphae are sparse or absent on most cultivation media; the reverse color is deep brown in older cultures. A well developed white aerial mycelium fragments into rod-shaped elements (approx. 0.54 × 2.25 μm), which are covered with numerous spines. Growth occurs in standing, but not in shaken, cultures. Grows at temperatures between 4 and 30°C.

Produces H_2S, but does not degrade casein, gelatin, or starch. Acid is produced from glycerol, inositol, mannose, and D-xylitol, but not from 2,3-butanediol, dulcitol, galactose, melezitose, melibiose, methyl α-D-glucoside, methyl α-D-mannoside, 1,2-propanediol, raffinose, sorbose, or sucrose. Additional phenotypic features are shown in Table 229.

The major fatty acids contain $C_{16:0}$ iso (33.8%), $C_{15:0}$ iso (13.8%), $C_{17:0}$ iso (10.0%), $C_{17:1}$ (9.5%), $C_{16:1}$ iso (7.4%), $C_{17:1}$ iso (7.2%), and $C_{16:1}$ iso (6.0%); also contains small amounts of 10-methyl-branched fatty acids, but not tuber-

culostearic acid or hydroxy fatty acids. The cellular polar lipid pattern contains diphosphatidylglycerol, phosphatidylcholine, phosphatidylethanolamine, phosphatidylglycerol, phosphatidylinositol, phosphatidylinositol mannosides, phosphatidylmethylethanolamine, hydroxyphosphatidylethanolamine, and an unknown phospholipid.

Source: isolated from soil from a gold mine cave near Kongju City, Korea.

DNA G+C content (mol%): 70.4 (HPLC).

Type strain: strain LM 141, IMSNU 50581, KCTC 9991, NRRL B-24156.

Sequence accession no. (16S rRNA gene): AJ249206.

25. **Pseudonocardia sulfidoxydans** Reichert, Lipski, Pradella, Stackebrandt and Altendorf 1998, 448[VP]

sul.fi.do′xy.dans. N.L. n. *sulfidum* sulfide; N.L. part. adj. *oxydans* oxidizing; N.L. part. adj. *sulfidoxydans* oxidizing sulfides.

Aerial and substrate mycelia fragment into rod-shaped elements. Hyphal segments are sometimes swollen up to 5 µm. Shows zig-zag mycelial growth and longitudinal and transverse hyphal septa. Yellow substrate hyphae and white aerial hyphae, which carry long chains of oval spores, are formed on trypticase soy agar.

Acid is produced from galactose, glycerol, mannose, and sucrose, but not from dulcitol, inositol, D-lactulose, melezitose, methyl α-D-glucoside, D-sorbose, or raffinose. L-Aniline-*p*NA, *o*NP-β-D-galactopyranoside, *p*NP-α-D-glucopyranoside, *bis*-*p*NP-phosphate, *p*NP-phenyl-phosphate, and L-proline-*p*NA are hydrolyzed, but not 2-deoxythymidine-5′-*p*NP-phosphate, esculin, *p*NP-β-D-glucopyranoside, L-glutamate-γ-3-carboxy-*p*NA, or *p*NP-phosphorylcholine. Dimethyl disulfide and dimethyl sulfide are oxidized. Grows on 4-hydroxybutyrate and 4-aminobutyrate (10 mM each). Additional phenotypic features are shown in Table 229.

The major fatty acids are $C_{16:0}$ iso (24.8–32.3%), $C_{16:0}$ (15.0–30.5%), and $C_{15:0}$ iso (11.2–16.4%). Mycolic acids are not formed. The cellular polar lipid pattern contains diphosphatidylglycerol, hydroxyphosphatidylethanolamine, phosphatidylethanolamine, phosphatidylglycerol, phosphatidylinositol, phosphatidylinositol mannosides, phosphatidylmethylethanolamine, and a ninhydrin-positive component.

Source: isolated from tree-bark compost biofilters from an animal-rendering plant.

DNA G+C content (mol%): not determined.

Type strain: strain 592, CIP 105686, DSM 44248, IFO (now NRBC) 16205, JCM 10411.

Sequence accession no. (16S rRNA gene): Y08537.

Further comments: metabolizes a diverse range of substrates on Biolog GP2 and SFP2 microplates and nitrogen sources on Biolog PM3 plates (Mahendra and Alvarez-Cohen, 2005).

26. **Pseudonocardia tetrahydrofuranoxydans** Kämpfer, Kohlweyer, Thiemer and Andreesen 2006, 1536[VP]

te.tra.hy.dro.fu.ra.no′xy.dans. N.L. n. *tetrahydrofuranum* tetrahydrofuran; N.L. v. *oxydare* to make acid, to oxidize; N.L. part. adj. *tetrahydrofuranoxydans* oxidizing tetrahydrofuran.

Forms branched filaments (e.g. 1.3 µm in width) that produce cell aggregates in THF-containing medium. Single spore-like bodies are formed at the end of cells. Aerial mycelium on agar is white, branched, and becomes fragmented. Good growth occurs on nutrient and R3A agars after 3 d at 25–30°C. Growth on THF occurs between 11 and 36°C.

L-Aniline-*p*NA and L-proline-*p*NA are hydrolyzed, but not 2-deoxythymidine-5′-*p*NP-phosphate, esculin, *o*NP-β-D-galactopyranoside, *p*NP-β-D-glucuronide, *p*NP-α-glucopyranoside, *p*NP-β-D-glucopyranoside, L-glutamate-γ-3-carboxyl-*p*NA, *bis-p*NP-phosphate, *p*NP-phenylphosphonate, *p*NP-phosphorylcholine, or *p*NP-α-D-xylopyranoside. Grows on 4-aminobutyrate, 1,4-butanediol, 2,4-diaminobutyrate, 4-hydroxybutyrate, DL-3-hydroxybutyrate, and some purines and ethers, but not on 4-aminobutyrate, chlorinated benzenes, dimethylsulfide, or hydrocarbons (C6 or petroleum). Additional phenotypic features are shown in Table 229.

Major fatty acids are $C_{16:0}$ iso (24.8–32.3%), $C_{15:0}$ iso (11.2–16.4%), and $C_{16:0}$ (15.0–30.5%); small to moderate amounts of methyl-branched fatty acids are also present.

Source: the type strain was isolated from an enrichment culture containing THF as the sole source of carbon, and originated from sludge of a wastewater treatment plant in Göttingen, Germany.

DNA G+C content (mol%): 71.3 (HPLC).

Type strain: strain K1, CIP 109050, CCUG 52126, DSM 44239, JCM 14745.

Sequence accession no. (16S rRNA gene): AJ249200.

Further comments: metabolizes a broad range of carbon compounds on Biolog GP2 plates (Kämpfer et al., 2006).

27. **Pseudonocardia xinjiangensis** (Xu, Jin, Mao, Lu, Cui and Jiang 1999) Huang, Wang, Lu, Hong, Liu, Tan and Goodfellow 2002, 981[VP] (Basonym: *Actinobispora xinjiangensis* Xu, Jin, Mao, Lu, Cui and Jiang 1999, 885.)

xin.ji.ang.en′sis. N.L. fem. adj. *xinjiangensis* pertaining to Xinjiang, an autonomous region in northwest China.

Forms substrate and aerial hyphae that are branched, but do not fragment. Spores are borne in longitudinal pairs on vegetative hyphae and either singly or in longitudinal pairs on aerial hyphae. An orange-yellowish substrate mycelium carries sparse, white aerial hyphae. Neither diffusible nor melanin pigments are produced. Does not grow at 45°C. Does not degrade cellulose, pectin, or starch, produce lecithinase or H_2S, or reduce nitrate. Adonitol, cellobiose, fructose, inositol, inulin, mannitol, raffinose, and xylose are used as sole carbon sources. Acid is not produced from these carbon sources. Alanine, L-histidine, and proline are used as nitrogen sources. Additional phenotypic features are shown in Table 229.

The cellular polar lipid pattern contains phosphatidycholine, phosphatidylethanolamine, phosphatidylglycerol, phosphatidylinositol, and glucosamine-containing phospholipids.

Source: the type strain was isolated from a soil sample collected from Tolufan, Xinjiang Province, China.

DNA G+C content (mol%): 72.1 (T_m).

Type strain: strain XJ-45, CCTCC AA 97020, CGMCC 4.1538, DSM 44661, JCM 11839.

Sequence accession no. (16S rRNA gene): EU722520.

28. **Pseudonocardia yunnanensis** (Jiang, Xu, Yang, Guo, Ma and Liu 1991) Huang, Wang, Lu, Hong, Liu, Tan and Goodfellow 2002, 981[VP] (Basonym: *Actinobispora yunnanensis* Jiang, Xu, Yang, Guo, Ma and Liu 1991, 527)

yun.nan.en′sis. N.L. fem. adj. *yunnanensis* pertaining to Yunnan, a province of south China.

Substrate and aerial hyphae are branched and do not fragment. A yellow substrate mycelium carries sparse, white aerial hyphae. Spores are borne in longitudinal pairs on vegetative hyphae and either singly or in longitudinal pairs on aerial hyphae. Neither diffusible nor melanin pigments are produced. Grows at 45°C. Does not degrade cellulose, gelatin, starch, or pectin. Milk is coagulated and peptonized. Nitrate is not reduced; lecithinase and H_2S are not produced. Adonitol, cellobiose, fructose, inositol, mannitol, raffinose, and xylose are used as sole carbon sources.

Susceptible to neomycin and rifampin (each at 50 µg/ml). Additional phenotypic features are shown in Table 229.

The cellular polar lipid pattern contains phosphatidylethanolamine, phosphatidylmethylethanolamine, and glucosamine-containing phospholipids

Source: isolated from a soil sample collected in Weixin, Yunnan Province, China.

DNA G+C content (mol%): 73.4 (T_m).

Type strain: strain Y-11981, CCTCC M 90959, CGMCC 4.1542, DSM 44253, NBRC 15681, JCM 9330, VKM Ac-1991.

Sequence accession no. (16S rRNA gene): AJ252822.

29. **Pseudonocardia zijingensis** Huang, Wang, Lu, Hong, Liu, Tan and Goodfellow 2002, 981[VP]

zi.jing.en′sis. N.L. fem. adj. *zijingensis* pertaining to Zijing, the source of the soil from which the organism was isolated.

The aerial and substrate mycelia fragment into rod-shaped elements. Chains of smooth spores are produced by acropetal budding on the substrate mycelium. Branched yellow substrate hyphae and white aerial hyphae are formed on trypticase soy broth agar. Does not produce diffusible pigments. Growth occurs at 15–45°C. Produces lipase. Additional phenotypic features are shown in Table 229.

The cellular polar lipid pattern contains diphosphatidylglycerol, phosphatidycholine, phosphatidylglycerol, phosphatidylinositolmannosides, and phosphatidylmethylethanolamine, but not glucosamine-containing phospholipids.

Source: isolated from a soil sample of Zijing Mountain, Yunnan Province, China.

DNA G+C content (mol%): 70.9 (T_m).

Type strain: strain 6330, CGMCC 4.1545, DSM 44774, JCM 11117.

Sequence accession no. (16S rRNA gene): AF325725.

Genus II. **Actinoalloteichus** Tamura, Zhiheng, Yamei and Hatano 2000, 1439[VP]

Tomohiko Tamura

Ac.ti.no.al.lo.tei′chus. Gr. n. *actis*, *actinos* ray (used to refer to actinomycetes); Gr. adj. *allos* another, the other; Gr. masc. n. *teichos* wall; N.L. masc. n. *Actinoalloteichus* actinomycete with a different wall.

Stains Gram-positive, not-acid-fast. A fine, non-fragmenting, branching mycelium is produced. Strictly aerobic. Non-fragmentary substrate mycelia are present. Aerial mycelia are formed with an **aggregate of straight spore chains**. The aerial and substrate mycelia tend to fragment. Good growth occurs at 20–37°C. The organism shows good growth on yeast extract-malt extract agar and oatmeal agar. Cell walls contain glutamate, glucosamine, alanine, and *meso*-diaminopimelate. $C_{17:0}$ anteiso, $C_{15:0}$ iso, $C_{16:0}$ iso, and $C_{15:0}$ anteiso are the major cellular fatty acids present. The major menaquinone is MK-9(H_4); small amounts of MK-8(H_4) and MK-9(H_2) are also present. Phosphatidylethanolamine and phosphatidylmonomethylethanolamine are present as diagnostic phospholipids. The acyl type of the cell wall is acetyl.

DNA G+C content (mol%): 72–73.

Type species: **Actinoalloteichus cyanogriseus** Tamura, Zhiheng, Yamei and Hatano 2000, 1439[VP].

Further descriptive information

Ribose, mannose, galactose, and glucose are detected in whole-cell sugars, but arabinose is not. The wall corresponds to chemotype III according to Lechevalier and Lechevalier (1970). The fatty acid and menaquinone compositions of the species of *Actinoalloteichus* are given in Table 230. *Actinoalloteichus hymeniacidonis* can be distinguished from the other species of the genus by its higher concentrations of $C_{17:1}$ ω8*c*, $C_{17:0}$, and $C_{15:0}$ anteiso. Species of *Actinoalloteichus* grow well on a variety of sugars. Other physiological characteristics are summarized in Table 231.

Enrichment and isolation procedures

The type strain of *Actinoalloteichus cyanogriseus* was isolated from a soil sample collected from Yunnan, China (Liu et al., 1984). The type strain of *Actinoalloteichus hymeniacidonis* was isolated

TABLE 230. Fatty acids and menaquinones of type strains of *Actinoalloteichus* species

Characteristic	*A. cyanogriseus*[a]	*A. hymeniacidonis*[b]	*A. spitiensis*[c]
Cellular fatty acids (%):			
$C_{14:0}$ iso	5	4	6
$C_{15:0}$ iso	15	6	17
$C_{15:0}$ anteiso	10	20	7
$C_{15:0}$		6	
$C_{16:1}$ iso	8		6
$C_{16:1}$	2		2
$C_{16:0}$ iso G		6	
$C_{16:0}$ iso	19	16	33
$C_{16:0}$	2		2
$C_{17:0}$ iso	3		3
$C_{17:0}$ anteiso	20	4	8
$C_{17:1}$ ω8*c*		19	
$C_{17:0}$		11	
Others	16	8	16
Menaquinones (%):			
MK-8(H_4)	10		5
MK-9(H_2)	9		
MK-9(H_4)	75	64	82
MK-9(H_6)	2	23	2
MK-9(H_8)		12	
MK-10(H_4)	3		9

[a]Tamura et al. (2000).

[b]Zhang et al. (2006).

[c]Singla et al. (2005).

TABLE 231. Physiological characteristics of *Actinoalloteichus* species[a]

Characteristic	*A. cyanogriseus*[b]	*A. hymeniacidonis*[c]	*A. spitiensis*[d]
Utilization of:			
Arabinose	w	−	−
Fructose	−	+	−
Glucose	+	+	−
Maltose	+	+	−
Mannitol	+	+	+
Mannose	+	+	−
Raffinose	−	−	+
Rhamnose	+	+	−
Sucrose	w	+	+
Sorbitol	+	+	−
Sodium citrate	−	+	+
Sodium succinate	−	−	+
Xylose	+	+	−
Decomposition of casein	−	+	+
Hydrolysis of starch	w	+	+
Resistance to methyl violet	−	+	−
Pigmentation on:			
ISP 1	Absent	Black	Absent
ISP 6	Black	Black	Absent

[a]Data are for the type strain of each species. +, Positive; w, weakly positive; −, negative.

[b]Tamura et al. (2000).

[c]Zhang et al. (2006).

[d]Singla et al. (2005).

from a marine sponge collected at the inter-tidal beach of Dalian, on the Chinese Yellow Sea, located in northern China. Freshly collected sponge specimens were rinsed five times in sterile seawater to remove transient and loosely attached bacteria and then thoroughly homogenized in a sterile mortar. A 10-fold dilution series of sponge homogenate was plated in triplicate on modified arginine-glycerol agar (ISP medium 5) plates, consisting of glycerol (0.6%, v/v), arginine (0.1%, w/v), K_2HPO_4 (0.1%, w/v), $MgSO_4$ (0.05%, w/v), and agar (1.8%, w/v) in natural seawater. The type strain of *Actinoalloteichus spitiensis* was isolated from a soil sample collected from the Lahaul–Spiti Valley, a cold desert of the Indian Himalayas, by using a dilution plating technique on actinomycetes isolation agar, containing (in w/v): sodium caseinate (0.2%), asparagine (0.01%), sodium propionate (0.4%), dipotassium phosphate (0.05%), magnesium sulfate (0.01%), ferrous sulfate (0.0001%), and agar (1.5%), pH 8.1. Incubation was at 28°C for 2 to 4 weeks.

Maintenance procedures

Strains of the genus *Actinoalloteichus* are maintained by freezing in water containing 10–30% glycerol at −70°C. Lyophilization of suspensions in 10% skim milk+1% monosodium glutamate and L-drying in 0.01 M potassium phosphate buffer (pH 7.0) containing 3% monosodium glutamate (Sakane and Kuroshima, 1997) are also recommended for long-term preservation.

Differentiation of the genus *Actinoalloteichus* from other genera

The genus *Actinoalloteichus* develops aerial mycelium with straight chains of spores, contains galactose as the characteristic whole-cell sugar, and *meso*-diaminopimelate, glutamate, and alanine as the cell-wall amino acids. Phylogenetic analysis indicates that the genus *Actinoalloteichus* is closely related to the genera *Goodfellowiella* (Labeda and Kroppenstedt, 2006) and *Streptoalloteichus* (Tomita et al., 1987). The genus *Goodfellowiella* has $C_{17:0}$ 10-methyl and anteiso-branched 2-hydroxy fatty acids. *Streptoalloteichus* develops motile spores in sporangia. On the other hand, the genus *Actinoalloteichus* lacks 10-methyl fatty acids and does not develop sporangia or motile cells. The genera *Saccharothrix* (Labeda et al., 1984), *Actinosynnema* (Hasegawa et al., 1978), and *Thermocrispum* (Korn-Wendisch et al., 1995) have *meso*-diaminopimelic acid in the cell wall (cell-wall type III) and long chains of spores. However, the genus *Actinoalloteichus* can be distinguished from these genera by its morphological characteristics, fatty acid components, and the absence of motility.

Taxonomic comments

The species *Streptomyces caeruleus* has been reclassified as *Actinoalloteichus cyanogriseus* based on 16S rRNA gene sequence analysis and DNA hybridization (Tamura et al., 2008a). The genus *Actinoalloteichus* belongs to the family *Pseudonocardiaceae* of the order *Pseudonocardiales*.

Differentiation of the species of the genus *Actinoalloteichus*

Characteristics of the three *Actinoalloteichus* species are given in Table 230 and Table 231.

List of species of the genus *Actinoalloteichus*

1. **Actinoalloteichus cyanogriseus** Tamura, Zhiheng, Yamei and Hatano 2000, 1439[VP]

cya.no.gri′se.us. L. adj. *cyaneus* deep blue; N.L. adj. *griseus* gray; N.L. masc. adj. *cyanogriseus* blue-gray.

The vegetative mycelia are brown to gray in color and the aerial mycelia are blue to gray. A black soluble pigment is produced on yeast extract-malt extract agar. Decomposition of urea, growth in Sabouraud glucose broth and MacConkey agar, hydrolysis of esculin and hippurate, utilization of sodium citrate, sodium succinate and calcium malate, gelatin liquefaction and reduction of nitrate are all negative. Glucose, maltose, xylose, mannitol, rhamnose, mannose, and sorbitol are utilized, but inositol, fructose, and raffinose are not. No growth at 15 or 45°C.

Source: the type strain was isolated from cultivated soil.

DNA G+C content (mol%): 73 (HPLC).

Type strain: AS 4.1159, CIP 106755, DSM 43889, NBRC 14455, JCM 6095, NRRL B-16252.

Sequence accession no. (16S rRNA gene): AB006178.

2. **Actinoalloteichus hymeniacidonis** Zhang, Zheng, Huang, Luo, Jin, Zhang, Liu and Huang 2006, 2311[VP]

hy.me.ni.a.ci′do.nis. N.L. gen. n. *hymeniacidonis* of *Hymeniacidon*, the generic name of the marine sponge *Hymeniacidon perleve*, the source of the type strain.

Aerial mycelia are produced with aggregates of straight chains of spores (0.6–0.8 µm). Grows well on yeast extract-malt extract agar and oatmeal agar at 20–37°C. A black soluble pigment is produced on yeast extract-malt extract agar and peptone-yeast extract-iron agar. Decomposition of urea, growth in Sabouraud glucose broth and MacConkey agar, hydrolysis of esculin and hippurate, utilization of calcium malate, sodium oxalate and sodium succinate, and reduction of nitrate are all negative. Fructose, glucose, maltose, mannose, mannitol, rhamnose, sucrose, sorbitol, and xylose are utilized as sole carbon sources, but arabinose, inositol, and raffinose are not. Grows weakly at 15°C and does not grow at 45°C. The cell-wall chemotype is III. The major menaquinone is MK-9(H_4); small amounts of MK-9(H_6) and MK-9(H_8) are also present. The phospholipid profile comprises mainly phosphatidylethanolamine, phosphatidylglycerol, phosphatidylinositol, and phosphatidylinositol mannoside. Major cellular fatty acids are $C_{15:0}$ anteiso (20%), $C_{17:1}$ ω8*c* (19%), $C_{16:0}$ iso (16%), and $C_{17:0}$ (11%).

Source: the type strain was isolated from a marine sponge, *Hymeniacidon perleve*, in Dalian, China (type strain).

DNA G+C content (mol%): not reported.

Type strain: HPA177, CGMCC 4.2500, JCM 13436.

Sequence accession no. (16S rRNA gene): DQ144222.

3. **Actinoalloteichus spitiensis** Singla, Mayilraj, Kudo, Krishnamurthi, Prasad and Vohra 2005, 2563[VP]

spi.ti.en′sis. N.L. masc. adj. *spitiensis* pertaining to Spiti Valley, located in the Indian Himalayas, where the type strain was isolated.

No aerial mycelium or spores are produced. Positive for utilization of D-mannitol, raffinose, sucrose, salicin, sodium citrate, and sodium succinate as sole carbon sources. Negative for utilization of D-glucose, maltose, *myo*-inositol, D-fructose, D-arabinose, D-xylose, L-rhamnose, D-mannose, and D-sorbitol as sole carbon sources. Positive for decomposition of casein and negative for decomposition of urea. No growth occurs on MacConkey agar or in Sabouraud glucose broth. Positive for hydrolysis of starch and negative for hydrolysis of hippurate and esculin. Tolerates up to 2% NaCl. Grows at temperatures between 20 and 37°C, with an optimum temperature of 25°C; it cannot grow at 15 or 42°C. Growth occurs at initial pH values between 6 and 11, the optimum being pH 8.0. Major fatty acids are $C_{16:0}$ iso (33.0%) and $C_{15:0}$ iso (17.0%), with lesser amounts of $C_{15:0}$ anteiso (7.0%) and $C_{17:0}$ anteiso (8.0%). The major menaquinone is MK-9(H_4) (82%). MK-9(H_2) is absent.

Source: the type strain was isolated from soil 3600 m above sea level, at Rangrik Village in Spiti Valley, Himachal Pradesh, India.

DNA G+C content (mol%): 72 (HPLC).

Type strain: RMV-1378, DSM 44848, JCM 12472, MTCC 6194.

Sequence accession no. (16S rRNA gene): AY426714.

Genus III. **Actinokineospora** Hasegawa 1988a, 449[VP] (Effective publication: Hasegawa 1988b, 33.)

David P. Labeda

Ac.ti.no.ki.ne.o.spo′ra. Gr. n. *aktis -inos* ray; Gr. v. *kineo* to set in motion; Gr. fem. n. *spora* seed and in biology a spore; N.L. fem. n. *Actinokineospora* actinomycete bearing zoospores.

Forms hyphae (approx. 0.5 µm in diameter) that differentiate into a vegetative mycelium that penetrates the agar medium and forms colonies on the surface; aerial mycelium arises from the colony. **Aerial hyphae bear chains of conidia capable of forming flagella in an aqueous environment.** Gram-stain-positive. Catalase-positive. Aerobic. **The cell wall contains *meso*-diaminopimelic acid as the diamino acid along with glycine, D-glutamic acid, and L-alanine, properties characteristic of type A1γ peptidoglycan. The characteristic whole-cell sugars are arabinose and galactose, but very little arabinose is found in purified cell walls. The phospholipid pattern consists of significant amounts of phosphatidylethanolamine along with diphosphatidylglycerol and phosphatidylinositol. The principal menaquinone is MK-9(H_4).** Phylogenetically, within the the order *Pseudonocardiales* based on 16S rRNA gene sequences.

DNA G+C content (mol%): 72.0 (T_m).

Type species: **Actinokineospora riparia** Hasegawa 1988a, 449[VP] (Effective publication: Hasegawa 1988b, 33.).

Further descriptive information

The genus *Actinokineospora* was described by Hasegawa (1988b, 1988a) for a novel actinomycete strain isolated from a soil sample collected on the side of the Ado River in Shiga Prefecture, Japan. This strain was unique in that it produced motile zoospores (Figure 285) from aerial hyphae (Figure 286) and was found to have both arabinose and galactose as diagnostic sugars in whole-cell hydrolysates, thereby distinguishing it from all described actinomycete genera. Subsequently, members of an additional six species of this genus that exhibited similar morphology and chemotaxonomy were isolated and described. Phylogenetic analysis of *Actinokineospora* species based on 16S rRNA gene sequences places the genus within the suborder *Pseudonocardineae* (Figure 287), elevated to the order *Pseudonocardiales* in this volume, along with other genera which produce motile zoospores, such as *Actinosynnema* and *Streptoalloteichus*, but members of these taxa produce structures such as synnemata and sporangia, respectively, which are not present in *Actinokineospora*. All *Actinokineospora* species described to date have been found associated with plant leaves and soil samples.

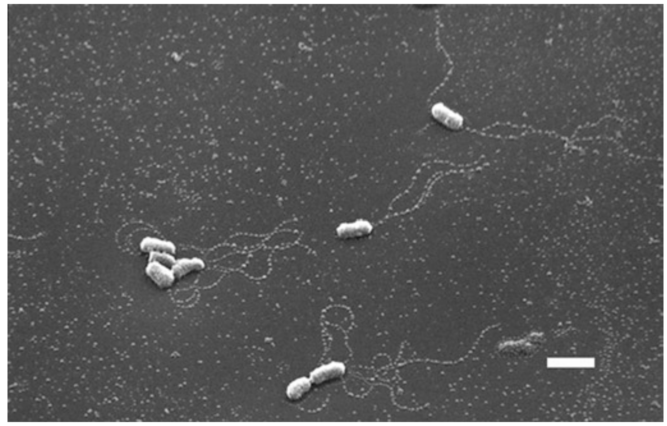

FIGURE 286. Electron micrograph of zoospores of *Actinokineospora enzanensis* NBRC 16517^T. Bar = 1.0 µm. (Electron micrograph courtesy of Misa Otogura, NBRC, Japan.)

Enrichment and isolation procedures

Strains of *Actinokineospora* have been routinely isolated on humic acid-vitamin agar (HV agar; Hayakawa and Nonomura, 1987), using a modification of the procedure of Makkar and Cross (1982). Soil or leaf litter samples are air-dried at 28°C for 7 d, 0.5 g sample is mixed with 50 ml sterile tap water, and the mixture is incubated at 20°C for 55 min with occasional shaking. Aliquots (0.1 ml) of dilutions of the supernatant are spread on the surface of plates and incubated at 28°C for 2–3 weeks.

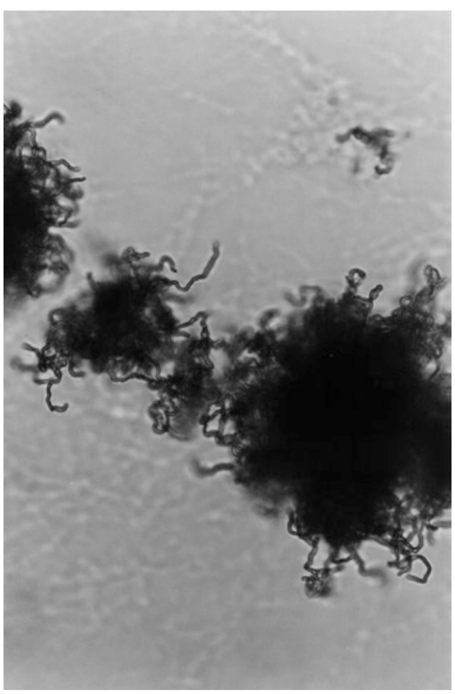

FIGURE 285. Light micrograph of the aerial mycelium on a culture of *Actinokineospora riparia* on sucrose-nitrate agar.

Hayakawa et al. (2000) described an isolation method for strains of *Actinokineospora* and other actinomycetes that have motile zoospores from soils and plant material. They placed a 0.5 g sample of air-dried soil or leaf litter in a beaker and gently flooded it with 50 ml sterile 10 mM phosphate buffer (pH 7.0) containing 10% soil extract. After incubating the sample for 90 min at 30°C, the motile zoospores that might be present are released. Following low speed (i.e. 1500 × *g*) centrifugation for 20 min and a subsequent 30 min incubation period, the samples were serially diluted and 200 µl aliquots plated onto an appropriate isolation medium, such as HV medium (Hayakawa and Nonomura, 1987) containing 50 µg/ml cycloheximide and with/without trimethoprim (20 µg/ml) and nalidixic acid (10 µg/ml). After a 2–3 week incubation period at 30°C, the plates are observed using a microscope fitted with a long working distance objective for the tentative identification of putative *Actinokineospora* strains based on morphological criteria.

Maintenance procedures

Working cultures of *Actinokineospora* can be maintained as refrigerated (4°C) agar slants on an appropriate medium such as NZamine medium (DSMZ medium 554; DSMZ, 2001). The slants are generally incubated for 5–7 d at 28–30°C prior to refrigeration and then should be subcultured at monthly intervals. Long-term preservation of strains is best accomplished as frozen stocks in 20% (v/v) aqueous glycerol at −80°C (mechanical freezer) to −172°C (liquid nitrogen vapor phase) or by using traditional lyophilization techniques.

Procedures for testing special characters

Strains are routinely cultivated on NZamine medium (DSMZ medium 554) at 28°C. Morphological observations are made on the media of Shirling and Gottlieb (1966) and NZamine medium.

Chemotaxonomic analysis of strains for fatty acids, menaquinones, and polar lipids are performed using methods described previously by Grund and Kroppenstedt (1989), Minnikin et al. (1984), Saddler et al. (1991), and Sasser (1990).

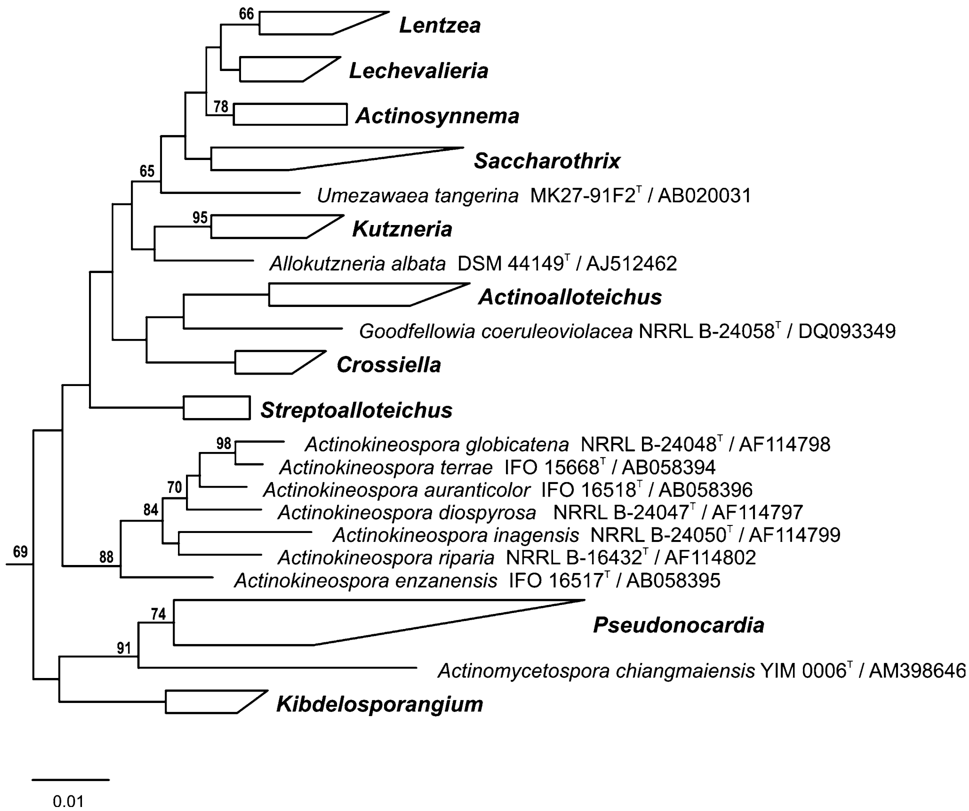

FIGURE 287. Phylogenetic tree for the genus *Actinokineospora* and related genera within the suborder *Pseudonocardineae* (now the order *Pseudonocardiales*) calculated from almost-complete 16S rRNA gene sequences using the Kimura's evolutionary distance method (Kimura, 1980) and the neighbor-joining algorithm of Saitou and Nei (1987). Numbers at the nodes represent levels of bootstrap support (%; Felsenstein, 1989) from 100 resampled datasets; values less than 60% are not shown. Bar = 0.01 nucleotide substitutions per site.

Physiological tests, including production of acid from carbohydrates, utilization of organic acids, and hydrolysis and decomposition of adenine, casein, esculin, guanine, hippurate, hypoxanthine, tyrosine, xanthine, and urea are typically determined using the media of Gordon et al. (1974). Phosphatase activity is evaluated by using the method of Kurup and Schmitt (1973) substituting NZamine agar as the basal medium. Temperature range for growth is determined on slants of NZamine medium and salt tolerance on slants of the same medium supplemented with 4 and 5% (w/v) NaCl.

Differentiation of the genus *Actinokineospora* from other genera

Actinokineospora strains can be easily differentiated from other genera morphologically based on their production of motile zoospores from aerial mycelia in the absence of elaborate structures such as sporangia or synnemata. They are chemotaxonomically distinct from other related actinomycete genera producing motile zoospores in the presence of arabinose in the whole-cell sugar hydrolysate pattern (Table 232) and are phylogenetically distinct based on 16S rRNA gene sequences (Figure 287).

Differentiation of the species of the genus *Actinokineospora*

The physiological characteristics of *Actinokineospora* species are summarized in Table 233 and can be used to differentiate between species, although a common set of physiological tests has not been used in the description of all species. Gross colonial morphology provides some additional information of the species based on the color of the substrate mycelium, production and color of aerial mycelium, and on the formation and color of soluble pigments when growing on agar media.

TABLE 232. Chemotaxonomic profiles of *Actinokineospora* and phylogenetically closely related genera

Character	*Actinokineospora*	*Actinoalloteichus*	*Actinosynnema*	*Allokutzneria*	*Crossiella*	*Goodfellowiella*	*Kibdelosporangium*	*Kutzneria*	*Streptoalloteichus*
Motile spores	+	–	+	–	–	–	–	–	+
Whole-cell sugar pattern	Galactose, arabinose, rhamnose	Glucose, galactose, mannose, ribose	Galactose, mannose	Arabinose, galactose, mannose	Galactose, mannose, rhamnose, ribose	Galactose, ribose	Arabinose, galactose, glucose, rhamnose	Galactose, rhamnose	Galactose, mannose, rhamnose, ribose
Phospholipids[a]	PE, OH-PE	PIM, PI, PG, DPG, PME	PE, OH-PE, PG	PE, PME, OH-PE, PI, *lyso*-PME, DPG,PG, *lyso*-PE	PE, DPG, PI, PIM, PME	PE, DPG, OH-PE, PME	PE, PME, PG, PI	PE, DPG, PI, PG, PME	PE, DPG, PI, PIM, DPG, PME
Predominant menaquinones	MK-9(H_4), MK-7(H_4), MK-8(H_4)	MK-9(H_4)	MK-9(H_4), MK-9(H_6)	MK-9(H_4)	MK-9(H_4)	MK-9(H_4), MK-10(H_4)	MK-9(H_4)	MK-9(H_4)	MK-9(H_6), MK-10(H_6)
DNA G+C content (mol%)	69.1–72.0	72–72.5	71	71.6	71.4	69.2	66	70.3–70.7	71.6

[a]Abbreviations: DPG, diphosphatidylglycerol; OH-PE, phosphatidylethanolamine with hydroxy fatty acids; *lyso*-PE, phosphatidylethanolamine where one fatty acid chain is missing from the glycerol backbone; *lyso*-PME, phosphatidylmethylethanolamine where one fatty acid chain is missing from the glycerol backbone; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; PI, phosphatidylinositol; PIM, phosphatidylinositol mannosides; PME, phosphatidylmethylethanolamine.

TABLE 233. Physiological properties of *Actinokineospora* species[a,b]

Character	*A. riparia*	*A. auranticolor*	*A. diospyrosa*	*A. enzanensis*	*A. globicatena*	*A. inagensis*	*A. terrae*
Colony reverse color	Yellow/brown	Orange	Yellow/brown	Gray	Yellow/brown	Yellow/brown	Yellow/brown
Hydrolysis of:							
Calcium malate	−	+	−	+	+	−	v
Elastin	−	+	+	+	+	+	+
Gelatin	−	+	+	+	+	−	+
Milk (peptonization)	−	nd	+	nd	−	−	+
Starch	−	nd	+	nd	+	−	+
Testosterone	+	+	+	+	v	−	+
Production of:							
Nitrate reductase	+	−	−	+	−	+	−
Hydrogen sulfide	−	+	+	+	+	+	+
Growth on sole carbon source (1.0%, w/v):							
Arabinose	−	−	−	−	−	−	w
D-Fructose	w	−	+	−	+	−	+
Galactose	−	nd	−	nd	−	−	−
Glucitol	−	nd	−	nd	−	−	−
Glycerol	+	+	+	+	+	−	v
Maltose	−	+	+	−	+	w	+
D-Mannose	w	−	w	−	+	−	+
Rhamnose	−	−	w	−	+	w	w
Sucrose	−	+	+	−	+	−	+
Trehalose	nd	+	nd	+	nd	nd	nd
Growth on sole carbon source (0.1%, w/v):							
D-Alanine	−	v	v	+	v	−	+
L-Proline	−	+	+	+	−	+	+
Sodium acetate	−	v	−	v	−	+	v
Growth in the presence of (w/v):							
Bismuth citrate, 0.001%	+	+	v	+	−	−	v
Brilliant green, 0.001%	−	−	v	−	−	−	+
Furazolidone, 0.004%	−	−	+	−	+	+	+
Potassium tellurite 0.01%	−	+	+	+	+	−	+
Sodium chloride, 2.0%	+	+	+	+	+	−	+
Sodium chloride, 3.0%	−	v	+	−	+	−	+
Vanillin, 0.05%	−	v	−	v	v	+	+
Resistance to antibiotics:							
Ampicillin, 10 µg/ml	−	+	−	+	−	−	−
Benzyl penicillin, 10 µg/ml	−	−	−	+	−	+	−
Cephaloridine, 10 µ/ml	−	−	−	+	+	+	v
Chloramphenicol, 10 µg/ml	−	+	v	−	−	−	v
Lincomycin, 20 µg/ml	−	+	v	−	v	−	v
Norfloxacin, 40 µg/ml	−	+	v	v	−	−	−
Oleandomycin, 5 µg/ml	−	+	+	−	v	−	v
Rifampin, 20 µg/ml	+	−	−	+	−	−	−
Growth at:							
10°C	−	+	+	−	+	−	+
37°C	+	+	v	−	−	−	v

[a]Symbols: v, variable reaction; w, weak positive reaction; nd, not determined.

[b]All species hydrolyzed hippurate but not xylan. None grew on erythritol, inositol, D-lactose, mannitol, raffinose, sorbitol, or xylose as sole carbon sources or in the presence of 0.2% *p*-hydroxybenzaldehyde or 0.1% phenylethanol. All species grew at 25°C and 30°C and were resistant to bekanamycin (20 and 40 µg/ml), kanamycin (40 and 80 µg/ml), oxytetracycline (20 µg/ml), and vancomycin (0.25 µg/ml).

List of species of the genus *Actinokineospora*

1. **Actinokineospora riparia** Hasegawa 1988a, 449[VP] (Effective publication: Hasegawa 1988b, 33.)

ri.pa′ri.a. L. fem. adj. *riparia* that frequents the banks of rivers, riverside, referring to the collection site of the soil sample from which the type strain was isolated, along the Ado River, Japan.

Vegetative mycelium varies in color from colorless on most media to yellowish-white on some media and tan to brown on yeast extract-malt extract agar. Aerial mycelium is white in color when produced. Tyrosine is decomposed, but not adenine, hypoxanthine, or xanthine. Growth is observed on D-glucose, soluble starch, and trehalose, with weak growth on cellulose, inulin, and melibiose; does not grow on adonitol, erythritol, ribose, salicin, L-sorbose, or dulcitol. Temperature for growth is 28°C.

Source: isolated from a soil sample collected on the side of the Ado River, Shiga Prefecture, Japan.

DNA G+C content (mol%): 72 (T_m).

Type strain: C-39162, ATCC 49499, DSM 44259, NBRC 14541, JCM 7471, NRRL B-16432, VKM Ac-1980.

Sequence accession no. (16S rRNA gene): AF114802.

2. **Actinokineospora auranticolor** Otoguro, Hayakawa, Yamazaki, Tamura, Hatano and Iimura 2003, 1[VP] (Effective publication: Otoguro, Hayakawa, Yamazaki, Tamura, Hatano and Iimura 2001, 38.)

au.ran.ti.co′lor. N.L. n. *Aurantium* generic name of the orange; L. n. *color* tint, hue; N.L. adj. *auranticolor* orange colored.

Yellowish-orange substrate mycelium. Aerial mycelium is white to gray. Temperature for growth is 28°C.

Source: isolated from leaves and soil.

DNA G+C content (mol%): 71.3 (HPLC).

Type strain: YU 961-1, DSM 44650, NBRC 16518, JCM 11646.

Sequence accession no. (16S rRNA gene): AB058396.

3. **Actinokineospora diospyrosa** Tamura, Hayakawa, Nonomura, Yokota and Hatano 1995, 378[VP]

di.o.spy′ro.sa. N.L. fem. adj. *diospyrosa* pertaining to the fruit tree *Diospyros kaki*.

Tan substrate mycelium. Aerial mycelia are white to gray. Temperature for growth is 28°C.

Source: isolated from fallen leaves of the persimmon tree, *Diospyros kaki*.

DNA G+C content (mol%): 69.3 (HPLC).

Type strain: YU8-1, DSM 44255, NBRC 15665, JCM 9921, NRRL B-24047, VKM Ac-1984.

Sequence accession no. (16S rRNA gene): AF114797.

4. **Actinokineospora enzanensis** Otoguro, Hayakawa, Yamazaki, Tamura, Hatano and Iimura 2003, 1[VP] (Effective publication: Otoguro, Hayakawa, Yamazaki, Tamura, Hatano and Iimura 2001, 38.)

en.za.nen′sis. N.L. fem. adj. *enzanensis* pertaining to Enzan City, Yamanashi, Japan, where the organism was isolated.

Vegetative mycelium is greenish-gray. Aerial mycelium is sparse and white, when formed. Temperature for growth is 28°C.

Source: isolated from soil.

DNA G+C content (mol%): 70.0 (HPLC).

Type strain: YU 924-101, DSM 44649, NBRC 16517, JCM 11647.

Sequence accession no. (16S rRNA gene): AB058395.

5. **Actinokineospora globicatena** Tamura, Hayakawa, Nonomura, Yokota and Hatano 1995, 377[VP]

glo.bi.ca.te′na. L. n. *globus* a ball, sphere; L. n. *catena* chain; N.L. n. *globicatena* ball of chains, referring to the mass of spore chains that aggregate into balls.

Vegetative mycelium is yellow to golden brown. Aerial mycelium is white to gray. Temperature for growth is 28°C.

Source: isolated from soil and fallen leaves.

DNA G+C content (mol%): 69.5–69.8 (HPLC).

Type strain: YU6-1, DSM 44256, NBRC 15664, JCM 9922, NRRL B-24048, VKM Ac-1981.

Sequence accession no. (16S rRNA gene): AF114798.

6. **Actinokineospora inagensis** Tamura, Hayakawa, Nonomura, Yokota and Hatano 1995, 377[VP]

in.ag.en′sis. N.L. fem. adj. *inagensis* pertaining to Lake Inaga, the lake from which the organism was first isolated.

Yellow to tan substrate mycelium is produced. Aerial mycelium is white to gray in color. Temperature for growth is 28°C.

Source: isolated from fallen leaves.

DNA G+C content (mol%): 69.1 (HPLC).

Type strain: YU4-1, DSM 44258, NBRC 15663, JCM 9923, NRRL B-24050, VKM Ac-1982.

Sequence accession no. (16S rRNA gene): AF114799.

7. **Actinokineospora terrae** Tamura, Hayakawa, Nonomura, Yokota and Hatano 1995, 377[VP]

ter′ra.e. L. gen. n. *terrae* of the earth.

Yellow to golden brown substrate mycelium is produced. Aerial mycelium is white to gray. Temperature for growth is 28°C.

Source: isolated from soil and fallen leaves.

DNA G+C content (mol%): 70.0 (HPLC).

Type strain: YU6-3, DSM 44260, NBRC 15668, JCM 9924, NRRL B-24049, VKM Ac-1983.

Sequence accession no. (16S rRNA gene): AB058394.

Genus IV. **Actinosynnema** Hasegawa, Lechevalier and Lechevalier 1978, 305[AL]

DAVID P. LABEDA

Ac.ti.no.syn′ne.ma. Gr. n. *actis*, *actinos* ray; Gr. adv. *syn* together; Gr. n. *nema*, *nematos* thread; N.Gr. n. *synnema*, threads wrapping together, synnema; N.L. neut. n. *Actinosynnema* indicates a synnema-forming actinomycete.

Fine hyphae (about 0.5 μm in diameter) are **differentiated into substrate mycelium**, with long branching hyphae that penetrate the agar and also grow into and **form synnemata, dome-like bodies, or flat colonies on the agar surface, and aerial hyphae** (0.5–1.0 μm in diameter) **that arise from synnemata, dome-like bodies, or flat colonies. The aerial hyphae bear chains of spores capable of forming flagella in an aqueous environment. The cell walls contain major amounts of *meso*-diaminopimelic acid** (***meso*-DAP**), glutamic acid, alanine, glucosamine, and muramic acid. **The whole-cell sugar pattern consists of galactose and mannose. Principal phospholipids include phosphatidylethanolamine, phosphatidylethanolamine containing 2-hydroxy fatty acids, and diphosphatidylglycerol. Menaquinones are predominantly MK-9(H_4), with some MK-9(H_6)**. Gram-stain-positive. Non acid-fast. Catalase-positive. Aerobic. Mesophilic. Most strains isolated directly from plant tissue. Phylogenetically nearest neighbors are the genera *Lechevalieria* and *Lentzea*.

DNA G+C content (mol%): 71–73 (T_m).

Type species: **Actinosynnema mirum** Hasegawa, Lechevalier and Lechevalier 1978, 305[AL].

Further descriptive information

The genus *Actinosynnema* was described by Hasegawa et al. (1978) to accommodate actinomycetes that produce unique morphological structures called synnemata (Figure 288) or dome-like bodies on most media. Aerial mycelia are produced on these synnemata or dome-like bodies and are initially whitish in color and become yellow to yellowish-orange in color. Regular septation occurs in mature aerial hyphae making it look bamboo-like when observed microscopically and then the hyphae become chains of spores. Suspension of the aerial mycelia in liquid media under a coverslip permits the observation of peritrichously motile zoospores (Figure 289) within 30 min to 1 h.

Phylogenetic analyses based on 16S rRNA gene sequencing demonstrates that the genus *Actinosynnema* is related to the genera *Lechevalieria*, *Lentzea*, and *Saccharothrix*, being intermediate between the first two genera and *Saccharothrix* (Figure 290) and is the type genus for the family *Actinosynnemataceae* Labeda and Kroppenstedt (2000), now part of the family *Pseudonocardiaceae*.

FIGURE 288. Scanning electron micrograph of a synnemata from *Actinosynnema mirum*. Bar = 10 μm.

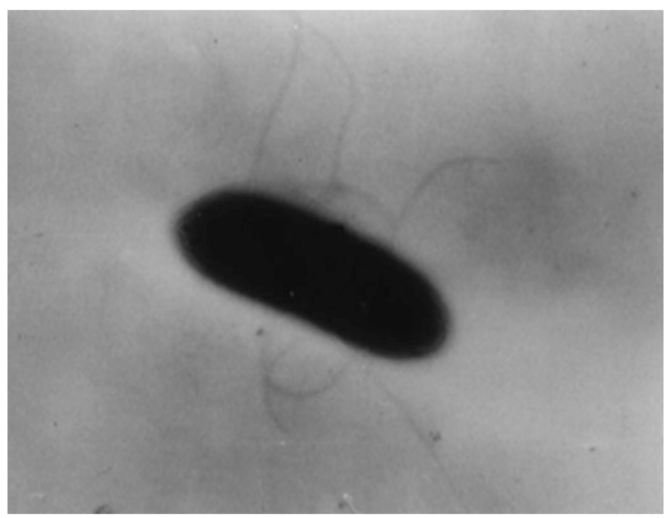

FIGURE 289. Electron micrograph of a zoospore of *Actinosynnema mirum*.

Enrichment and isolation procedures

Strains of the genus *Actinosynnema* can be isolated from grass blades by placing them on the surface of yeast extract agar plates (0.02% yeast extract and 1.5% agar in distilled water) and incubating for 3 weeks at 28°C. The agar surface will most likely be covered with various types of microbial growth, but small synnemata can be observed on the grass blade itself using a stereoscopic microscope. These synnemata can be carefully removed with a sterile loop and transferred to fresh growth media. *Actinosynnema mirum* has been reported not to be inhibited by the presence of 100 μg/ml nystatin or 50 μg/ml candicidin and *Actinosynnema pretiosum* has been found to be resistant to 100 μg/ml amphotericin B so it might be possible to use these antibiotics to suppress the growth of fungal competitors. Hayakawa et al. (2000) described an isolation method for strains of *Actinokineospora* and other actinomycetes that have motile zoospores which has also been reported to permit the isolation of *Actinosynnema* strains from soils and plant material. They placed a 0.5 g sample of air-dried soil or leaf litter in a beaker and gently flooded it with 50 ml sterile 10 mM phosphate buffer (pH 7.0) containing 10% soil extract. After incubating the sample for 90 min at 30°C, the motile zoospores that might be present are released. Following

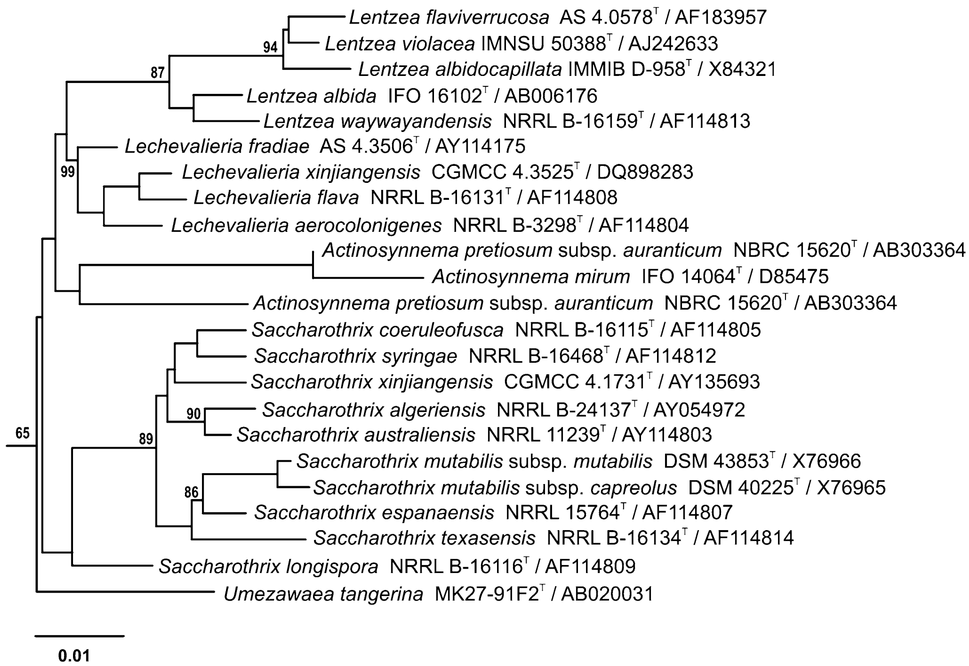

FIGURE 290. Phylogenetic tree for the genera *Actinosynnema*, *Lechevalieria*, *Lentzea*, *Saccharothrix*, and *Umezawaea* of the suborder *Pseudonocardineae* (now order *Pseudonocardiales*) calculated from almost-complete 16S rRNA gene sequences using the Kimura's evolutionary distance method (Kimura, 1980) and the neighbor-joining algorithm of Saitou and Nei (1987). Percentages at the nodes represent levels of bootstrap support (Felsenstein, 1989) from 100 resampled datasets; values less than 60% are not shown. Bar = 0.01 nucleotide substitutions per site.

low speed (i.e. 1,500 × *g*) centrifugation for 20 min and a subsequent 30 min incubation period, the samples are serially diluted and 200 µl aliquots plated onto an appropriate isolation medium such as HV medium (Hayakawa and Nonomura, 1987) containing 50 µg/ml cycloheximide with and without trimethoprim (20 µg/ml) and nalidixic acid (10 µg/ml). After a 2–3 week incubation period at 30°C, the plates are observed for the presence of synnemata using a microscope fitted with a long working distance objective and those observed can be transferred to fresh media.

Maintenance procedures

Working cultures of *Actinosynnema* can be maintained as plate or agar slant cultures on an appropriate medium such as NZamine agar (DSMZ medium 554; DSMZ, 2001) with bi-weekly subculturing. Survival of the cultures is often better at room temperature than at 4°C. Longer term preservation of strains is best accomplished as frozen stocks in 40% (v/v) aqueous glycerol at −80°C (mechanical freezer) to −172°C (liquid nitrogen vapor phase) or by using traditional lyophilization techniques.

Procedures for testing special characters

Strains are routinely cultivated on NZamine medium (DSMZ medium 554) at 28°C. Morphological observations are made on the media of Shirling and Gottlieb (1966) and NZamine medium, but for production of synnemata, strains should be grown on plates of 1.5% agar in tap water or Bennett's agar (DSMZ medium 548). Chemotaxonomic analysis of strains for fatty acids, polar lipids, and menaquinones are performed using methods described previously by Grund and Kroppenstedt (1989), Minnikin et al. (1984), Saddler et al. (1991), and Sasser (1990).

Physiological tests, including production of acid from carbohydrates, utilization of organic acids, and hydrolysis and decomposition of adenine, casein, esculin, guanine, hippurate, hypoxanthine, tyrosine, xanthine, and urea are typically determined using the media of Gordon et al. (1974). Phosphatase activity is evaluated by using the method of Kurup and Schmitt (1973) substituting NZamine agar as the basal medium. Temperature range for growth is determined on slants of NZamine medium and salt tolerance is evaluated on slants of the same medium supplemented with 2% to 5% NaCl.

Differentiation of the genus *Actinosynnema* from other genera

Strains of *Actinosynnema* can be differentiated easily from other actinomycetes by observation of the very characteristic synnemata produced on most growth media. Numerous other actinomycete genera produce motile zoospores, notably *Actinokineospora*, *Actinoplanes*, *Planobispora*, *Planomonospora*, *Spirillospora*, and several others, but none produce synnemata. The chemotaxonomic profile of *Actinosynnema* species is different from those of members of the other genera in the *Actinosynnemataceae*, particularly the whole-cell sugar pattern consisting of only galactose and mannose as the diagnostic sugars. The phospholipid pattern of *Actinosynnema* strains is quite similar to those of *Saccharothrix* species, but the lack of rhamnose in the whole-cell sugar pattern and the presence of MK-9(H_6) and lack of MK-10(H_4) menaquinones differentiates them from members of this genus. Characteristics of *Actinosynnema* and some related genera are given in Table 234.

Differentiation of the species of the genus *Actinosynnema*

The physiological characteristics of *Actinosynnema* species are summarized in Table 235 and can be used to differentiate between species, although a common set of physiological tests has not been used in the description of all species. Gross colonial morphology provides some additional information of the species based on the color of the substrate mycelium, production and color of aerial mycelium, and production and color of soluble pigments when growing on agar media.

TABLE 234. Chemotaxonomic and morphological characteristics of *Actinosynnema* and related genera[a,b]

Characteristic	*Actinosynnema*	*Lechevalieria*	*Lentzea*	*Saccharothrix*	*Umezawaea*
Production of synnemata and motile zoospores	+	–	–	–	–
Whole-cell sugar pattern	Galactose, mannose	Galactose, mannose, rhamnose	Galactose, mannose, ribose	Galactose, rhamnose, mannose (trace)	Galactose, rhamnose
Phospholipids	PE, OH-PE, PI, PIM, DPG	PE	PE, DPG, PG, PI	PE, OH-PE, PI, PIM, DPG, PG (v)	PE
Menaquinone(s)	MK-9(H_4), MK-9(H_6)	MK-9(H_4)	MK-9(H_4)	MK-10(H_4), MK-9(H_4)	MK-9(H_4), trace MK-10(H_4)

[a]Symbols: DPG, diphosphatidylglycerol; OH-PE, phosphatidylethanolamine containing hydroxylated fatty acids; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; PI, phosphatidylinositol; PIM, phosphatidylinositol mannosides; PME, phosphatidylmethylethanolamine.

[b]All genera have *meso*-DAP as the cell-wall diamino acid, are of cell-wall chemotype III, and contain straight-chain, mono-unsaturated, iso, and anteiso fatty acids.

TABLE 235. Physiological properties of *Actinosynnema* species[a]

Character	*A. mirum*	*A. pretiosum* subsp. *pretiosum*	*A. pretiosum* subsp. *auranticum*
Decomposition of:			
Adenine	–	nd	nd
Casein	+	+	+
Esculin	nd	+	+
Gelatin	+	+/–	+/–
Hypoxanthine	–	–	–
Starch	+	+	+
Tyrosine	+	+	+
Urea	–	–	–
Xanthine	–	–	–
Growth on:			
Arabinose	+/–	+	–
Galactose	+	+	+
Glucose	+	+	+
Glycerol	+	–	+
Inositol	–	–	–
Lactose	–	–	+/–
Mannitol	+	+	+
Maltose	+	+/–	+
Mannose	+	+	+
Melibiose	–	+	+/–
Raffinose	–	+/–	+/–
Rhamnose	+	+	+
Soluble starch	+/–	+	+
Sorbitol	–	–	–
Sucrose	+	+	+
Trehalose	+	+	+
Xylose	+	+	+
Production of:			
Catalase	+	+	+
Hydrogen sulfide	nd	–	–
Nitrate reductase	+	+	+
Phosphatase	+	+	+
Growth in the presence of:			
NaCl (2%, w/v)	nd	+	+
Lysozyme	+	+	+
Amphotericin B (100 μg/ml)	nd	+	+
Candicidin (50 μg/ml)	+	nd	nd
Chloramphenicol (20 μg/ml)	–	–	–
Dihydrostreptomycin (10 μg/ml)	–	nd	nd
Nystatin (100 μg/ml)	+	nd	nd
Streptomycin (20 μg/ml)	nd	–	–
Sulbenicillin (100 μg/ml)	nd	+	+
Tetracyline (1 μg/ml)	–	nd	nd
Tetracyline (20 μg/ml)	nd	–	–
Growth at:			
10°C	+	–	–
37°C	–	+	+

[a]Symbols: +, positive; –, negative; +/–, doubtful response; nd, not determined.

List of species of the genus *Actinosynnema*

1. **Actinosynnema mirum** Hasegawa, Lechevalier and Lechevalier 1978, 305[AL]

mi′rum. L. neut. adj. *mirum* marvellous.

Substrate mycelium is yellow to orange-yellow in color, producing whitish synnemata that become yellow to orange-yellow in color on some media, notably thin potato-carrot agar of Higgins et al. (1967). White to yellowish-white aerial mycelia are produced on most media. A pale yellow-brown soluble pigment is produced on tyrosine agar and a pale greenish pigment on oatmeal agar.

Lactate, malate, pyruvate, and tartrate are assimilated for growth, but not acetate, benzoate, citrate, or succinate. Acid is produced from adonitol, L-arabinose, cellobiose, dextrin, fructose, galactose, glucose, glycogen, lactose, maltose, D-mannitol, D-mannose, D-melibiose, raffinose, L-rhamnose, soluble starch, sucrose, trehalose, and xylose, but not from dulcitol, erythritol, *i*-inositol, inulin, glycerol, methyl α-D-glucoside, methyl α-D-mannoside, D-ribose, salicin, or sorbitol. Temperature for growth is 28–30°C.

Source: isolated from a sedge (grass) blade.

DNA G+C content (mol%): 73 ± 1 (T_m).

Type strain: ATCC 29888, DSM 43827, NBRC 14064, IMRU 3971, JCM 3225, NRRL B-12336, VKM Ac-843.

Sequence accession no. (16S rRNA gene): D85475.

2. **Actinosynnema pretiosum** Hasegawa, Tanida, Hatano, Higashide and Yoneda 1983b, 314[VP]

pre.ti.o′sum. L. neut. adj. *pretiosum* precious.

2a. **Actinosynnema pretiosum subsp. pretiosum** Hasegawa, Tanida, Hatano, Higashide and Yoneda 1983b, 317[VP]

pre.ti.o′sum. L. neut. adj. *pretiosum* precious.

Substrate mycelium is pale yellow to pale orange-yellow in color. Branched hyphae in liquid culture fragment into motile elements with peritrichous flagella. The aerial mycelium consists of long, straight, helical, or (rarely) branching hyphae that are white to pale yellow in color. The aerial hyphae form in tufts at the tips of synnemata or on the surface of dome-like or irregular structures on the surface of the colonies. The aerial hyphae have a bamboo-like appearance and transform into chains of peritrichously flagellated spores. Yellow soluble pigments are produced on glucose-asparagine and peptone-yeast extract agars. The type strain produces antibiotics of the ansamitocin complex and tomaymycin. Temperature for growth is 28–30°C.

Source: isolated from a blade of *Carex* grass.

DNA G+C content (mol%): 71 ± 1 (T_m).

Type strain: C-15003(N-1), ATCC 31281, DSM 44132, FERM-P 3992, NBRC 15621, JCM 7344, NRRL B-16060, VKM Ac-1963.

Sequence accession no. (16S rRNA gene): AF114800.

2b. **Actinosynnema pretiosum subsp. auranticum** Hasegawa, Tanida, Hatano, Higashide and Yoneda 1983b, 320[VP]

au.ran′ti.cum. N.L. neut. adj. *auranticum* orange.

Substrate mycelium is yellowish-orange or orange on various media. Aerial hyphae are white to yellowish white in color. Pale yellowish-brown soluble pigments are produced on several media. The type strain produces the antibiotics dnacins and ansamitocins. Temperature for growth is 28–30°C.

Source: isolated from a blade of *Carex* species grass.

DNA G+C content (mol%): 71 ± 1 (T_m).

Type strain: C-14482(N-1001), ATCC 31309, DSM 44131, FERM-P 4130, NBRC 15620, JCM 7343, NRRL B-16078, VKM Ac-1961.

Sequence accession no. (16S rRNA gene): AB303364.

Genus V. **Amycolatopsis** Lechevalier, Prauser, Labeda and Ruan 1986, 34[VP] emend. Lee 2009, 1403

Geok Yuan Annie Tan and Michael Goodfellow

A.my.co.la.top′sis. N.L. fem. n. *Amycolata* genus belonging to the order *Actinomycetales*; Gr. fem. n. *opsis* aspect, appearance; N.L. fem. n. *Amycolatopsis* that which appears similar to *Amycolata*.

Aerobic to facultatively anaerobic, Gram-stain-positive, non-acid-fast, nonmotile, catalase-positive actinomycetes that form branching substrate hyphae which fragment into squarish and rod-shaped elements. When formed, aerial hyphae may be sterile or differentiate into chains of smooth-walled, squarish to ellipsoidal spore-like structures. Chemo-organotrophic to facultatively autotrophic. Grows on a broad range of organic and synthetic media. Mesophilic or thermophilic. **Whole-organism hydrolysates are rich in *meso*-2,6-diaminopimelic acid, arabinose, and galactose.** The peptidoglycan is of the A1γ type. Muramic acid moieties are *N*-acetylated. **The diagnostic phospholipid is phosphatidylethanolamine (with or without phosphatidylmethylethanolamine) or phosphatidylmethylethanolamine** with variable occurrence of diphosphatidylglycerol, phosphatidylglycerol, phosphatidylinositol, and phosphatidylinositol mannosides; contains **complex mixtures of saturated and branched chain fatty acids**. Does not contain mycolic acids. The phylogenetic position of *Amycolatopsis,* as determined by 16S rRNA gene sequence analysis, is in the family *Pseudonocardiaceae.*

Common in arid soils but has also been isolated from activated sludge, equine placentas, and from clinical and plant material.

DNA G+C content (mol%): 66–75 (T_m; HPLC).

Type species: **Amycolatopsis orientalis** (Pittenger and Brigham 1956) Lechevalier, Prauser, Labeda and Ruan 1986, 35[VP].

Further descriptive information

Phylogeny. The genus *Amycolatopsis* forms a clade in the *Pseudonocardiaceae* 16S rRNA gene tree. It encompasses 39 species with validly published names, most of which have been proposed in the past five years using polyphasic taxonomic approaches (Carlsohn et al., 2007; Labeda et al., 2003; Lee, 2009; Tan et al., 2006a, 2007). These species can be assigned to five multimembered and two single-membered phyletic lines in the *Amycolatopsis* 16S rRNA gene tree (Figure 291). The taxonomic integrity of the multimembered taxa, the *Amycolatopsis methanolica*, *Amycolatopsis nigrescens*, *Amycolatopsis orientalis*, *Amycolatopsis palatopharyngis*, and *Amycolatopsis sulphurea* subclades, are supported by all of the tree-making algorithms and by high bootstrap values.

Amycolatopsis methanolica subclade strains grow well at temperatures up to 60°C (Chun et al., 1999; De Boer et al., 1990; Henssen et al., 1987; Kim et al., 2002a) and can thereby be considered to be thermophilic actinomycetes (Brock, 1986; Cross, 1968). Further comparative taxonomic studies between members of this and the other multimembered subclades are needed to determine whether the *Amycolatopsis methanolica* subclade merits generic status. The type strain of *Amycolatopsis fastidiosa*, now reclassified as *Actinokineospora fastidiosa*, which grows between 10 and 60°C, forms a distinct phyletic line at the foot of the *Amycolatopsis* 16S rRNA gene tree (Goodfellow et al., 2001; Huang et al., 2001, 2004; Labeda et al., 2010b).

Everest and Meyers (2009) found good congruence between 16S rRNA and partial *gyrB* phylogenies of representative *Amycolatopsis* strains. Differences were found between the overall topologies of the trees, though groups of closely related strains clustered together in both trees, a result consistent with corresponding studies on the genera *Gordonia* (Shen et al., 2006) and *Micromonospora* (Kasai et al., 2000). A concatenated tree based on *gyrB*/16S rRNA gene sequences closely resembled the *gyrB* tree and contained conserved phyletic lines evident in the separate trees. It was apparent that the number of phyletic lines supported by high bootstrap values was much greater in the concatenated gene tree than in the corresponding 16S rRNA and partial *gyrB* trees. The type strain of *Amycolatopsis fastidiosa* formed a distinct phyletic line outside the evolutionary radiation occupied by the other *Amycolatopsis* strains in both the partial *gyrB* and concatenated trees.

DNA–DNA relatedness studies show that the type strain of *Amycolatopsis fastidiosa* has little, if any, DNA in common with the other *Amycolatopsis* strains to which it has been compared (Table 236). This is also the case with the type strain of *Amycolatopsis sulphurea*, an organism which forms a distinct phyletic line with *Amycolatopsis jejuensis* NRRL B-24427[T] in the 16S rRNA/*gyrB* concatenated tree (Everest and Meyers, 2009) though its nearest neighbor in the 16S rRNA gene tree, *Amycolatopsis sulphurea*, was not included in this study. In general, good congruence exists between DNA–DNA pairing and corresponding 16S rRNA gene sequence data.

Cell morphology. *Amycolatopsis* strains show regular to occasional fragmentation of either the substrate mycelium or the aerial mycelium or both (Figure 292). They do not form sclerotia, spore vesicles, or synnemata. Kothe et al. (1989) examined the morphology and fine structure of members of the family *Pseudonocardiaceae* and found that *Amycolatopsis* strains produced long chains of smooth, squarish to ellipsoidal spore-like structures on substrate and aerial mycelia. They also noted that the fine structure of the hyphae varied as some *Amycolatopsis* strains produced an electron-dense layer that covered the hyphal wall but others did not. Some strains show an unusual degree of morphological variation, as exemplified by the formation of short chains of oval, smooth-surfaced spores on the substrate mycelium of *Amycolatopsis taiwanensis* KCTC 19116[T] (Figure 293) and the production of globose, smooth-surfaced pseudosporangia by *Amycolatopsis decaplanina* DSM 44594[T] (Figure 294). However, spores were not detected either inside or outside the pseudosporangiae (Wink et al., 2004).

Chemotaxonomy. *Amycolatopsis* strains have cell walls which contain *meso*-diaminopimelic acid (*meso*-A_2pm), arabinose, and galactose (Carlsohn et al., 2007; Lechevalier et al., 1986; Takeuchi et al., 1992), that is, they have a wall chemotype IV *sensu* Lechevalier and Lechevalier (1970); an A1γ type peptidoglycan (Schleifer and Kandler, 1972); muramic acid in the *N*-acetylated form (Lee and Hah, 2001; Tan et al., 2006a, 2007), and phosphatidylethanolamine (PE; taxonomically significant phospholipid) and phosphatidylglycerol (PG) as major polar lipids (phospholipid type II *sensu* Lechevalier et al., (1981, 1977a) with a variable occurrence of diphosphatidylglycerol (DPG), hydroxyphosphatidylethanolamine (HPE), phosphatidylinositol (PI), phosphatidylinositol mannosides (PIMs), phosphatidylserine (PS), and phosphatidylmethylethanolamine (PME). The type strains of *Amycolatopsis nigrescens* and *Amycolatopsis saalfeldensis*, for instance, contain DPG, PE, HPE, PG, PI, PS, and uncharacterized glycolipids (Carlsohn et al., 2007; Groth et al., 2007). Details of the polar lipid composition of individual species are given in the species descriptions.

In general, most *Amycolatopsis* species have di-, tetra-, or hexahydrogenated menaquinones with nine isoprene units [MK-9(H_2,H_4,H_6)] as the predominant isoprenologue (Alderson et al., 1981; Lechevalier et al., 1986; Tan et al., 2006a, 2006b, 2007; Wink et al., 2003b; Yassin et al., 1991). In contrast, *Amycolatopsis decaplanina* contains a mixture of tetrahydrogenated menaquinones with eight and nine isoprene units (Wink et al., 2004), whereas *Amycolatopsis nigrescens* is characterized by the presence of major amounts of tetrahydrogenated menaquinones with eleven isoprene units and corresponding components with nine, ten, and twelve isoprene units (Groth et al., 2007). Variations in the proportions of di-, tetra-, and hexahydrogenated components with nine isoprene units amongst several *Amycolatopsis* species were attributed to the collection of biomass from different stages of the growth cycle (Yassin et al., 1991).

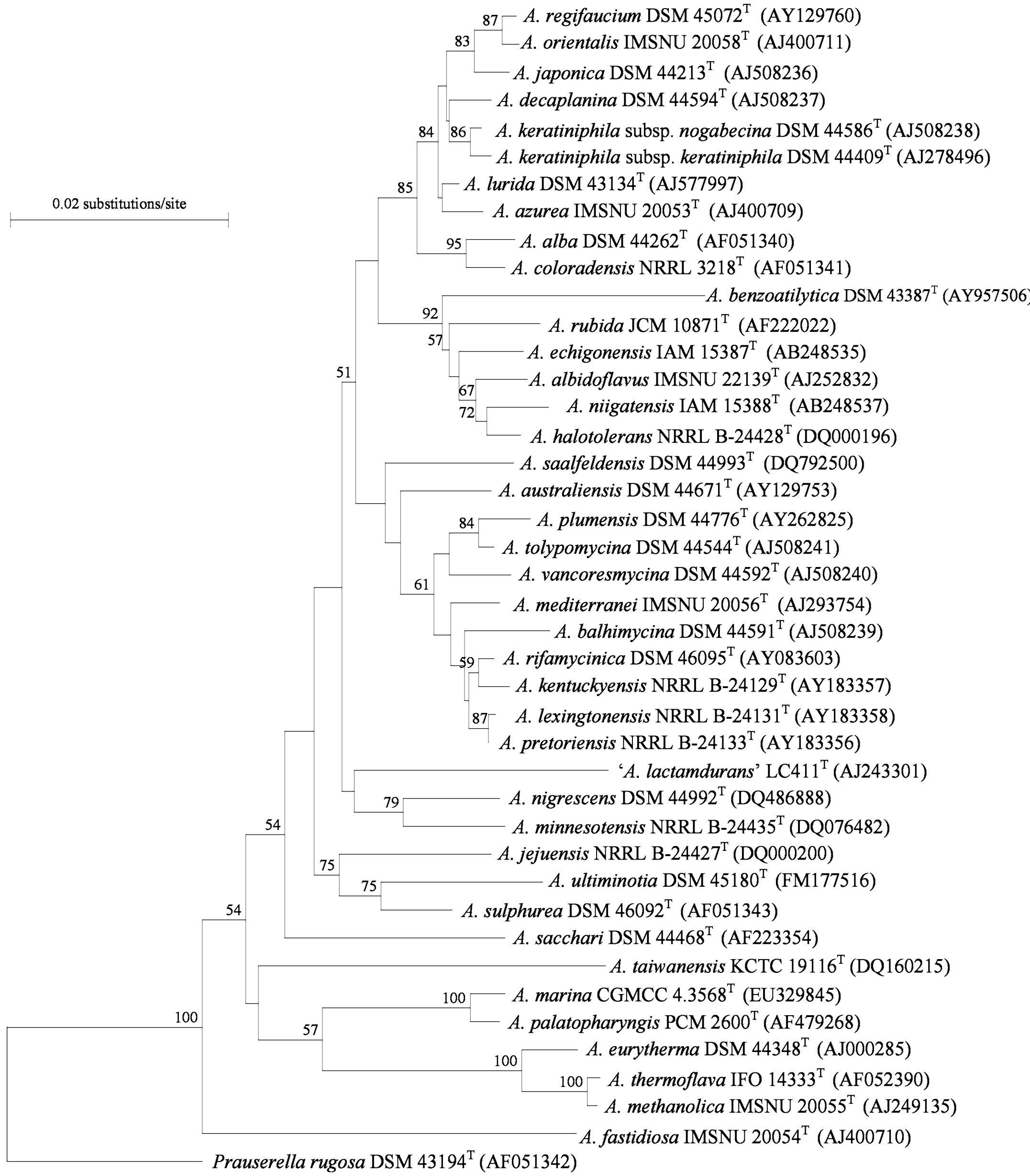

FIGURE 291. Neighbor-joining tree (Saitou and Nei, 1987) based on nearly complete 16S rRNA gene sequences showing relationships between the type strains of *Amycolatopsis* species. The numbers at the nodes indicate the levels of bootstrap support based on 1000 resampled datasets; only values above 50% are given. Bar = 0.02 substitutions per nucleotide position.

Amycolatopsis strains contain complex mixtures of iso-branched, straight chain saturated, and unsaturated components (Alderson et al., 1981; Kroppenstedt, 1985; Wink et al., 2003b; Yassin et al., 1993), but lack mycolic acids (Carlsohn et al., 2007; Huang et al., 2004; Lechevalier et al., 1986). Most strains have similar qualitative fatty acid profiles in which 14-methylpentadecanoic acid ($C_{16:0}$ iso) is the major component (Carlsohn et al., 2007; Kothe et al., 1989; Mertz and Yao, 1993; Wink et al., 2003b) though considerable amounts of hexadecanoic ($C_{16:0}$), 12-methyltridecanoic ($C_{14:0}$ iso), 13-methyltetradecanoic ($C_{15:0}$ iso), heptadecanoic ($C_{17:0}$), and octadecanoic ($C_{18:0}$) acids may be present (Huang et al., 2004; Lee, 2006; Lee and Hah, 2001). Quantitative differences in fatty acid profiles have been used to distinguish between *Amycolatopsis* species (Groth et al., 2007; Mertz and Yao, 1993).

Colony morphology. *Amycolatopsis* strains form well developed colonies on most standard media used to cultivate filamen-

TABLE 236. Percentage DNA–DNA relatedness values between *Amycolatopsis* species[a]

	A. orientalis	*A. alba*	*A. albidoflavus*	*A. azurea*	*A. balhimycina*	*A. benzoatilytica*	*A. coloradensis*	*A. decaplanina*	*A. echigonensis*	*A. eurytherma*	*A. fastidiosa*	*A. japonica*	*A. kentuckyensis*	*A. keratiniphila*	*A. lexingtonensis*	*A. lurida*	*A. marina*	*A. mediterranei*	*A. methanolica*	*A. niigatensis*	*A. regifaucium*	*A. rifamycinica*	*A. rubida*	*A. sulphurea*	*A. thermoflava*	*A. tolypomycina*	*A. tucumanensis*	*A. ultiminotia*	*A. vancoresmycina*
A. orientalis		24–30	18–19			24	0–8	51			0–7					48–59		0–7			43			0					
A. alba			56				27				0					24		25						0					
A. albidoflavus						46														33–34									
A. azurea		56					33	56	45–48		0					37		0						0					
A. balhimycina																		46								61			
A. benzoatilytica																													
A. coloradensis		27	33								0					37		0						0					53
A. decaplanina																32													
A. eurytherma																			60								40		
A. fastidiosa		0	0				0									0		11						7					
A. japonica														60															
A. kentuckyensis																		14				67							
A. lexingtonensis													43		42			22				47							
A. lurida	46	24–45	37				37	32			0							6						0					
A. marina																													
A. mediterranei		25	0				0				0											40		0		55			54
A. methanolica										58–60															21				
A. niigatensis								58–62																					
A. rubida								39–41												28–35									
A. palatopharyngis																	49												
A. pretoriensis													26		54			31				48							
A. sulphurea	0	0	0	–							7					0		0										29	
A. tolypomycina																													54
A. tucumanensis																													
A. ultiminotia																								31					

[a]Data taken from Al-Musallam et al. (2003), Bala et al. (2004), Chun et al. (1999), Kim et al. (2002a), Labeda (1995), Lechevalier et al. (1986), Everest and Meyers (2009), Stackebrandt et al. (2004), Tan et al. (2007), and Wink et al. (2004).

FIGURE 292. Scanning electron micrograph of *Amycolatopsis orientalis* KCTC 9412^T showing the formation of squarish fragments from the mycelium after growth on modified Bennett's agar at 28°C for 14 d. Bar = 1.0 µm.

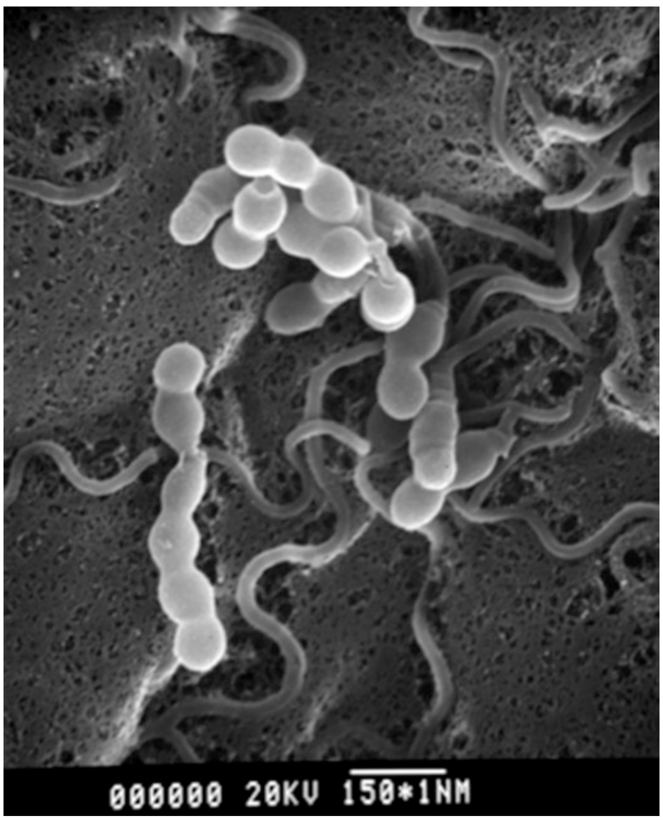

FIGURE 293. Scanning electron micrograph of *Amycolatopsis taiwanensis* KCTC 19116^T showing the formation of spore chains following growth on HV agar at 28°C for 14 d. Bar = 1.5 µm (Reproduced with permission of Tseng et al., 2006. Int. J. Syst. Evol. Microbiol. *56*: 1811–1815.)

tous actinomycetes and may carry abundant aerial hyphae (Mertz and Yao, 1993; Wink et al., 2003b, 2004). They grow particularly well on modified Bennett's agar supplemented with mannitol

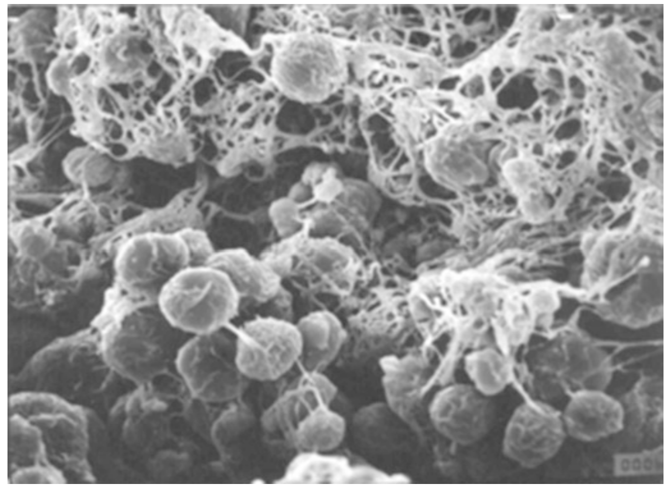

FIGURE 294. Scanning electron micrograph (×1000) of *Amycolatopsis decaplanina* DSM 44594^T showing the formation of pseudosporangiae following growth on oatmeal agar at 28°C for 14 d (Reproduced with permission from Wink et al., 2004. Int. J. Syst. Evol. Microbiol. *54*: 235–239.)

(0.5%, w/v) and soybean flour (0.5%, w/v) following incubation at 28°C for 14 d (Tan et al., 2006b). Tan and her colleagues were able to assign strains growing under these conditions to 35 color-groups based on aerial hyphal color, substrate mycelial pigmentation, and diffusible pigment color. Strains taken to represent many of the color groups formed distinct phyletic lines in the 16S rRNA *Amycolatopsis* gene tree indicating that color groupings can be predictive from a taxonomic perspective. Isolates assigned to two of these color groups were subsequently classified as novel *Amycolatopsis* species, namely *Amycolatopsis australiensis* and *Amycolatopsis regifaucium* (Tan et al., 2006a, 2007).

Nutrition and growth conditions. *Amycolatopsis* strains grow well on standard media such as glycerol-asparagine, inorganic-salts starch, oatmeal, peptone-yeast extract-iron, tyrosine, tryptone-yeast extract and yeast extract-malt extract agars (ISP media 2–7; Shirling and Gottlieb, 1966), and on modified Bennett's agar supplemented with mannitol and soybean flour (Tan et al., 2006b). *Amycolatopsis eurytherma*, *Amycolatopsis fastidiosa*, *Amycolatopsis methanolica*, and *Amycolatopsis thermoflava* strains grow well at 50°C or above (Chun et al., 1999; De Boer et al., 1990; Henssen et al., 1987; Kim et al., 2002a) and can be considered to be thermophilic actinomycetes (Brock, 1986; Cross, 1968). A fourth species, *Amycolatopsis sacchari*, contains organisms that are moderate thermophiles (Goodfellow et al., 2001). In contrast, organisms assigned to the remaining *Amycolatopsis* species are mesophiles (Lee and Hah, 2001; Tan et al., 2006a, 2007). *Amycolatopsis* strains grow well from pH 6.0 to 9.0.

Metabolism. *Amycolatopsis* strains are aerobic to facultatively anaerobic, chemo-organotrophic to facultatively autotrophic actinomycetes that degrade several organic substrates, produce acid from a range of sugars, and use diverse compounds as sole sources of carbon for energy and growth (Chun et al., 1999; De Boer et al., 1990; Gordon et al., 1978; Groth et al., 2007). The

TABLE 237. Bioactive compounds produced by members of the genus *Amycolatopsis*

Strain/taxon	Product	Feature	Reference
A. alba	Glycopeptide antibiotic	Inhibits peptidoglycan synthesis	Mertz & Yao (1993)
A. azurea	Azureomycins A and B	Glycopeptide antibiotics	Ōmura et al. (1979)
A azurea	Octacosamicins	Antifungal activity	Dobashi et al. (1988)
A. balhimycina	Balhimycin	Glycopeptide antibiotic	Nadkarni et al. (1994)
A. coloradensis	Avoparcin	Glycopeptide antibiotic	Kunstmann et al. (1968)
A. fastidiosa	Antibiotics 41034 and 41494	Macrobicyclic peptides	Celmer et al. (1977)
A. japonica	*(S,S)-N,N′*-ethylene-diaminedisuccinic acid	Inhibits phospholipase C	Nishikori et al. (1984)
	Dethymicin	Immunosuppressant	Ueno et al. (1992)
A. keratiniphila subsp. *nogabecina*	Nogabecin	Glycopeptide antibiotic	Shorin et al. (1957)
"*A. lactamdurans*"	Cephamycin C	β-Lactam antibiotic	Stapley et al. (1972)
	Efrotomycin	β-Isomer	Wax et al. (1976)
	3-Methylpseudouridine	Polyether	Nielsen and Arison (1989)
A. mediterranei	Rifamycins	Clinically useful ansamycins, active against *Mycobacterium* spp.	Margalith and Beretta (1960); Lancini et al. (1967); Birner et al. (1972); Lancini and Sartori (1976)
	31-Homorifamycin W		Wang et al. (1994)
	Kanglemycin A	Ansamycin-type antibiotic	Wang et al. (1988)
	Dethymicin	Immunosuppressant	Ueno et al. (1992)
	3-Hydroxyrifamycin S	Ansamycin antibiotic	Traxler et al. (1981)
	Protorifamycins	Ansamycin antibiotics	Ghisalba et al. (1978, 1979, 1980)
	Aromatic amino acids	Suitable for strain improvement	de Boer et al. (1990)
A. orientalis	Vancomycin	Glycopeptide antibiotic, active against severe bacterial infections	Pittenger and Brigham (1956)
	Glycopeptide compounds	Glycosyltransferase gene, *gtfA*	Baltz (2000)
	Muraceins	Muramyl peptide inhibitors	Bush et al. (1984)
	N-Demethylvancomycin	Vancomycin analog	Boeck et al. (1984)
	D-Amino acid specific peptidase		Sugie et al. (1988)
	MM47761, MM49721, MM55266, MM55268	Glycopeptide antibiotics	Box et al. (1991, 1990)
	Orienticin	Glycopeptide antibiotic	Tsuji et al. (1988)
	Quartromicin	Antiviral antibiotics	Tsunakawa et al. (1992)
	Antibiotic UK-69753	Efrotomycin-like antibiotic	Ruddock et al. (1987); Pacey et al. (1989)
A. lurida	Benzathrins	Quinone antibiotic with antitumor activity	Philip et al. (1957); Theriault et al. (1986); Rasmussen et al. (1986)
	Ristocetin	Glycopeptide antibiotic	Grundy et al. (1957)
A. regifaucium	Kigamicins	Antitumor antibiotics	Kunimoto et al. (2003)
A. sulphurea	Cetocycline	Tetracycline derivative	Proctor et al. (1978)
A. tolypomycina	Tolypomycin	Ansamycin-type antibiotic	Hasegawa et al. (1971); Kishi et al. (1972)
A. vancoresmycina	Homorifamycin; vancoresmycin	Ansamycin antibiotic; polyketide antibiotic	Hopmann et al. (2002).

type strain of *Amycolatopsis benzoatilytica* metabolizes *m*-hydroxybenzoate through a central protocatechuate intermediate, unlike other *Amycolatopsis* species which are unable to use this compound as growth substrate (Grund et al., 1990).

Members of the genus are a rich source of antibiotics and secondary metabolites (Table 237). Medically important antibiotics produced by *Amycolatopsis* strains include balhimycin (Nadkarni et al., 1994), dethymicin (Ueno et al., 1992), rifamycin (Sensi et al., 1959), and vancomycin (Barna and Williams, 1984). Balhimycin and vancomycin show antibiotic activity against methicillin-resistant *Stapylococcus aureus* strains, whereas dethymicin has a mode of action which distinguishes it from other immunosuppresants, such as cyclosporine, FK506, and rapamycin. Rifamycin is used to treat leprosy and tuberculosis, and to control infections in organ implants and AIDS patients.

Much of the emphasis in studies on the metabolism of *Amycolatopsis* strains has been focused on the application of biochemical and genetic approaches to the synthesis of antibiotics, as exemplified by studies on the biosynthesis of rifampin (Floss and Yu, 2005; Ghisalba and Nuesch, 1981; Hu et al., 1999; Schupp et al., 1998; Xu et al., 2005). The polyketide framework of rifamycin B is assembled from 3-amino-5-hydroxybenzoic acid (AHBA), a product of the aminoshikimate pathway (Kim et al., 1998; Yu et al., 2001), two molecules of acetate and eight of

propionate. Five multifunctional proteins (RifA–RifE) and an amide synthase (RifF) catalyze the synthesis of the core structure of rifampin (August et al., 1998; Schupp et al., 1998; Xu et al., 2005).

A number of non-antibiotic bioactive metabolites are formed by *Amycolatopsis* strains, including (*S,S*)-*N,N*′-ethylenediamine disuccinic acid from *Amycolatopsis japonica* (Nishikori et al., 1984), a D-amino acid-specific peptidase from *Amycolatopsis orientalis* (Sugie et al., 1988), and a polylactic acid depolymerase from *Amycolatopsis* sp. strain K104-1 (Nakamura et al., 2001). *Amycolatopsis orientalis* produces chitinases of potential value in the production of chitin hexamers (Tominaga and Tsujisaka, 1976; Usui et al., 1987); this organism converts tetra-*N*-acetylchitotelraose to a mixture of hexa-*N*-acetyl-chitohexaose and the corresponding dimer by a transglycosylation reaction (Usui et al., 1987). *Amycolatopsis* sp. strain HR167 is used to produce vanillic acid from ferulic acid (Rabenhorst and Hopp, 1997), and an *Amycolatopsis* isolate for the bioconversion of lovastatin to the novel compound wuxistatin (Zhuge et al., 2008).

Amycolatopsis methanolica NCIB 11946^T is a facultative methylotrophic actinomycete which was previously assigned to the genera *Nocardia* and *Streptomyces* (De Boer et al., 1990). The organism assimilates formaldehyde by the ribulose monophosphate shunt (Hazeu et al., 1983), a finding that made it a candidate for fermentative overproduction of aromatic amino acids (Dijkhuizen et al., 1985; Morinaga and Hirose, 1984). Regulation of aromatic amino acid biosynthesis in the strain has been studied in detail (Abou-Zeid et al., 1995; De Boer et al., 1989; Euverink et al., 1992, 1995) and stable mutants have been isolated and grown under diverse conditions in batch and continuous culture (De Boer et al., 1988; Hazeu et al., 1983). Prephenate dehydratase (PDT) is a key regulatory enzyme in L-phenylalanine biosynthesis, the PDT protein of which has been purified and characterized as a homotetrameric enzyme with 34 kDa subunits (Euverink et al., 1995). It has been shown that *Amycolatopsis methanolica* metabolizes glucose via the Embden–Meyerhof–Parnas pathway (Alves et al., 1994), and produces a PPi-dependent phosphofructokinase with biochemical characteristics similar to both ATP- and PPi-dependent enzymes during growth on glucose (Alves et al., 1996).

A copper-resistant *Amycolatopsis* strain was isolated from copper-polluted sediment by Albarracin et al. (2008). The organism accumulates high levels of copper and is considered to have potential as an agent of bioremediation. It was subsequently characterized and described as a novel *Amycolatopsis* species, *Amycolatopsis tucumanensis* (Albarracin et al., 2010). These workers also found that the type strain of *Amycolatopsis eurytherma*, a close relative of *Amycolatopsis tucumanensis*, has a moderate copper resistance profile.

Genetics. Genetic analyses show that the antibiotic potential of members of the genus *Amycolatopsis* is much greater than previously recognized (Banskota et al., 2006; Wood et al., 2007). Improved genetic tools, including new cloning, expression, and shuttle vectors are being developed to manipulate and regulate genes involved in secondary metabolite production and to gain an insight into the origin, evolution, and functional properties of extrachromosomal elements (Malhotra and Lal, 2007; te Poele et al., 2007, 2008).

Amycolatopsis methanolica NCIB 11946 contains a 13.3 kb conjugative element, pMEA300, which is maintained as an integrated element, but may also be present as a free circular plasmid (Malhotra and Lal, 2007; te Poele et al., 2007, 2008). Various cloning vectors based on these plasmids have been constructed (Madón and Hutter, 1991; Vrijbloed et al., 1995a), and used to clone genes involved in glucose and methanol metabolism and in aromatic amino acid biosynthesis into *Amycolatopsis methanolica* (Alves et al., 1996; Hektor, 1997; Vrijbloed et al., 1995c, 1995d). The conjugative element pMEA100 (23.7 kb) from *Amycolatopsis mediterranei* LBG A3136 also exists in an integrated form and in the free state, but in *Amycolatopsis mediterranei* ATCC 13685 it is only found in the free form (Moretti et al., 1985).

The integrated elements of *Amycolatopsis mediterranei* and *Amycolatopsis methanolica* have been sequenced (te Poele et al., 2006; te Poele et al., 2008; Vrijbloed, 1996), and on the basis of structural and functional similarities shown to belong to a class of integrative and conjugative elements (ICEs; Moretti et al., 1985; Burrus et al., 2002) that have been found in several other filamentous actinomycetes, such as pSE211 and pSE101 from *Saccharopolyspora erythraea* (Brown et al., 1988, 1994), pSAM2 from *Streptomyces ambofaciens* (Boccard et al., 1989; Pernodet et al., 1984; Smokvina et al., 1991), SLP1 from "*Streptomyces coelicolor*" A3(2) (Bibb et al., 1981) and pSG1 from *Streptomyces griseus* (Cohen et al., 1985). This class of elements integrate site-specifically in a tRNA gene of the host genome, but unlike other ICEs, replicate autonomously like a plasmid. Most are self-transmissible and several can mobilize chromosomal markers (Bibb et al., 1981; Brown et al., 1988; Hopwood et al., 1984; Moretti et al., 1985; Smokvina et al., 1988; Vrijbloed, 1996). The integrase (Int) directs site-specific DNA recombination between the attP site and a chromosomal attB site (Boccard et al., 1989).

Characterization of deletion derivatives of pMEA300 led to the identification of genes needed for replication, regulation, integration, and conjugation (Vrijbloed et al., 1994, 1995a, 1995b, 1995c, 1995d). The organization of genes involved in replication, excision, and integration (attp, int, repAM, xis) in pMEA300 is conserved (te Poele et al., 2007, 2008). It has been shown that most of these elements can mediate the pock-formation phenotype, reflecting growth retardation of the recipient which occurs during conjugation (Vrijbloed et al., 1995b). These workers found that the *traJ* gene was required for the transfer of pMEA300 into recipient strains lacking this element. TraJ of pMEA300 shows 33% identity to TraJ of pMEA100 (te Poele et al., 2008) and 27% identity to a cell division FtsK/SpoIII protein of *Frankia* strain EAN1 pec, and to TraB of *Streptomyces ghanaenis* plasmid pS65 (te Poele et al., 2007). This latter protein is a septal DNA translocator which mediates a unique conjugation mechanism that translocates unprocessed double-stranded DNA molecules to recipient strains (Reutler et al., 2006).

te Poele et al. (2007, 2008) determined the presence and distribution of a new class of replication initiator and transfer proteins, namely RepAM and TraJ. They screened a collection of over 100 *Amycolatopsis* strains that had been isolated from different geographical locations and found pMEA sequences to be widely distributed. They identified two geographically distinct pMEA-like elements. Phylogenetic analysis of their deduced RepAM and TraJ protein sequences revealed clusters with protein sequences of either pMEA100 or pMEA300. The sequences which clustered with pMEA100 were from European strains and

those from pMEA300 encompassed Australasian strains. The *repAM* and *traJ* genes were linked with the 16S rRNA genes of the host strains suggesting that the pMEA-elements co-evolved with their hosts rather than being dispersed by horizontal transfer of the free replicating form.

Amycolatopsis benzoatilytica (previously *Amycolatopsis orientalis*) DSM 43387^T (Majumdar et al., 2006) contains a 29.6 kb non-integrative, freely replicating plasmid, pA387 (Lal et al., 1991). This plasmid has been completely sequenced and used to construct cloning and conjugative shuttle vectors (Lal et al., 1991; Malhotra et al., 2008) and to generate *Escherichia coli–Amycolatopsis* shuttle vectors (Ding et al., 2003; Khanna et al., 1998; Kumar et al., 1994; Lal et al., 1998; Priefert et al., 2002; Tuteja et al., 2000). These vectors replicate and are maintained in members of several *Amycolatopsis* species, including *Amycolatopsis japonica* (Stegmann et al., 2001), "*Amycolatopsis lactamdurans*" (Kumar et al., 1994), *Amycolatopsis mediterranei* (Ding et al., 2003; Lal et al., 1991, 1998; Tuteja et al., 2000), *Amycolatopsis orientalis* (Lal et al., 1991), and *Amycolatopsis rifamycinica* (Khanna et al., 1998; Lal et al., 1998; Tuteja et al., 2000). The value of pA387-derived shuttle vectors has been demonstrated in several studies (Kumar et al., 1996; Xu et al., 2005), notably by increased production of cephamycin C by "*Amycolatopsis lactamdurans*" (Chary et al., 1997, 2000).

The direct mycelium transformation system developed for *Amycolatopsis mediterranei* by Madón and Hütter (1991) was applied and optimized to enhance the conversion of ferulic acid into vanillin using *Amycolatopsis* sp. strain HR 167 (Priefert et al., 2002). The density of the mycelial suspensions in the transformation mixture and the methylation state of the plasmid DNA used for the transformation were critical factors. The transformation rates achieved with plasmid DNA isolated from *Escherichia coli* ET 12567 was 3,500-fold higher than those obtained with DNA isolated from *Escherichia coli* XLI-Blue. Other interesting developments include the isolation of balhimycin genes from *Amycolatopsis mediterranei* using a reverse cloning procedure (Pelzer et al., 1999), the discovery that the *pfp* gene of *Amycolatopsis methanolica*, which encodes PPi-dependent phosphofructokinase, is located on a 2.3 kb *Pvu*II fragment (Alves et al., 1996), and the detection of genes involved in the modification of the polyketide backbone of rifamycin B by *Amycolatopsis mediterranei* S699 (Xu et al., 2005).

Antibiotic sensitivity. Comprehensive comparative studies on the antibiotic sensitivity patterns of all *Amycolatopsis* species are needed now that there is evidence that members of this genus may be considered as emerging pathogens. Preliminary studies show that closely related *Amycolatopsis* species can be distinguished using antibiotic sensitivity data (De Boer et al., 1990).

Pathogenicity. There is no conclusive evidence that *Amycolatopsis* strains can cause disease in humans, although the type strain of *Amycolatopsis benzoatilytica* has been implicated as an agent of submandibular mycetoma (Majumdar et al., 2006; Scharfen, 1971). In addition, members of the genus are occasionally encountered in clinical material (Mishra et al., 1980; Schaal and Beaman, 1984). *Amycolatopsis orientalis* has been isolated from cerebrospinal fluid (Gordon et al., 1978) and *Amycolatopsis palatopharyngis* has been isolated from infected palatopharyngeal mucosa of a 70-year-old male (Huang et al., 2004). There is evidence that actinomycetes can cause placentitis and abortion in horses (Donahue and Williams, 2000; Giles et al., 1993; Hong et al., 1993). The distinctive type of nocardiosis in horses, nocardioform placentitis, is recognized by the formation of lesions on the chorionic surface of the placenta and the isolation of Gram-stain-positive, branching bacteria. Three *Amycolatopsis* species, *Amycolatopsis kentuckyensis*, *Amycolatopsis lexingtonensis*, and *Amycolatopsis pretoriensis*, were proposed to accommodate strains isolated from equine placentas (Labeda et al., 2003).

Ecology. Most members of the genus *Amycolatopsis* have been isolated from soil and vegetable matter though their roles in these environments have not been studied. It seems likely that the primary habitat is soil, as exemplified by the isolation of *Amycolatopsis australiensis* from an arid composite soil (Tan et al., 2006a), *Amycolatopsis eurytherma* from scrubland soil (Kim et al., 2002a), *Amycolatopsis keratiniphila* from marsh soil (Al-Musallam et al., 2003), *Amycolatopsis plumensis* from a brown hypermagnesian ultramafic soil (Saintpierre-Bonaccio et al., 2005), *Amycolatopsis rubida* from a coniferous forest soil (Huang et al., 2001), and *Amycolatopsis ultiminotia* from rhizosphere soil (Lee, 2009). Additional *Amycolatopsis* species have been recovered from more unusual sources, such as the isolation of *Amycolatopsis echigonensis* from a filtrate substrate (Ding et al., 2007), *Amycolatopsis jejuensis* from a natural cave (Lee, 2006), *Amycolatopsis nigrescens* from a Roman catacomb (Groth et al., 2007), *Amycolatopsis saalfeldensis* from a medieval alum slate mine (Carlsohn et al., 2007), and *Amycolatopsis tucumanensis* from copper-polluted sediments (Albarracin et al., 2010). *Amycolatopsis* strains have also been isolated from hyper-arid soils of the Atacama Desert (Okoro et al., 2009).

Large numbers of *Amycolatopsis* strains have been recovered from Australian arid soils, and from an environmental sample collected from under a sycamore tree in Newcastle-upon-Tyne, UK, using a dilution plate procedure and three selective media (Tan et al., 2006b). Many of the isolates were related to either *Amycolatopsis mediterranei* or *Amycolatopsis orientalis* on the basis of 16S rRNA gene sequencing, but others formed new phyletic lines. Some of the novel isolates were classified as *Amycolatopsis australiensis* sp. nov. (Tan et al., 2006a) and *Amycolatopsis regifaucium* sp. nov. (Tan et al., 2007).

Enrichment and isolation procedures

Members of most *Amycolatopsis* species have been isolated by plating dilutions of soil suspensions onto nonselective media, supplemented with antifungal antibiotics, followed by colony selection and characterization. This hit and miss approach, for instance, led to the isolation of *Amycolatopsis halotolerans* and *Amycolatopsis jejuensis* on starch-casein agar (Lee, 2006), *A mediterranei* on Bennett's agar (Margalith and Beretta, 1960), *Amycolatopsis nigrescens* on nutrient agar (Groth et al., 2007), *A rubida* on glucose-asparagine agar (Huang et al., 2001), *Amycolatopsis saalfeldensis* on casein mineral agar (Carlsohn et al., 2007), and *Amycolatopsis taiwanensis* on HV agar (Tseng et al., 2006); in the main, isolation plates were incubated at either 28°C or 30°C for 14 d.

Several *Amycolatopsis* species have been isolated from environmental samples using less subjective isolation procedures. *Amycolatopsis keratiniphila* was isolated from soil enriched with

sterilized and defatted wool (Al-Musallam et al., 2003), *Amycolatopsis palatopharyngis* was isolated on brain-heart infusion agar at 37°C for 5 d under microaerophilic conditions (Huang et al., 2004), and *Amycolatopsis plumensis* was isolated on yeast extract-malt extract agar supplemented with streptomycin (Saintpierre-Bonaccio et al., 2005). In addition, *Amycolatopsis sacchari* strains were recovered from diverse environmental samples using a wind tunnel technique (Lacey, 1971; Lacey and Dutkiewicz, 1976a); the Andersen sampler was loaded with Petri dishes containing half-strength nutrient agar (Goodfellow et al., 2001).

Tan et al. (2006b) designed a number of agar media to isolate *Amycolatopsis* strains from soil using putative selective agents drawn from phenotypic databases generated in numerical taxonomic studies on members of the family *Pseudonocardiaceae.* Three of the resultant media formulations proved to be particularly effective in recovering *Amycolatopsis* strains from soil samples, namely media SM1 (Stevenson's basal medium supplemented with D-sorbitol and neomycin sulfate), SM2 (Stevenson's basal medium supplemented with D-melezitose and neomycin sulfate), and SM3 (Gauze's medium supplemented with nalidixic acid and novobiocin); all three media contained antifungal antibiotics. Inoculated plates were incubated at 28°C for up to 21 d; the appearance of crusty or leathery colonies covered with white or whitish-yellow aerial hyphae proved to be characteristic of *Amycolatopsis* strains. Representative isolates were found to have morphological and chemotaxonomic features consistent with their assignment to the genus *Amycolatopsis.* Tan and her colleagues generated a set of genus-specific oligonucleotides which were used for the rapid identification of putative *Amycolatopsis* strains recovered from the selective isolation plates.

Maintenance procedures

Amycolatopsis strains can be cultivated on glucose-yeast extract agar or modified Bennett's agar. After incubation at 28°C to allow abundant growth, cultures may be maintained at 4°C or room temperature for up to 6 months. Long-term preservation can be achieved by lyophilization or in 20% glycerol frozen at −20°C or −80°C.

Differentiation of the genus *Amycolatopsis* from other genera

The genus *Amycolatopsis* can be distinguished from other genera in the *Pseudonocardiaceae* family by using a combination of chemotaxonomic and morphological markers (see Table 228 in the treatment of the family *Pseudonocardiaceae*). *Amycolatopsis* strains can also be distinguished from related taxa by using the genus-specific primers AMY2 and ATOP (Tan et al., 2006b).

Taxonomic comments

The genus *Amycolatopsis* was proposed by Lechevalier et al. (1986) for actinomycetes that formed substrate hyphae that tended to fragment into squarish elements, and aerial hyphae which, when formed, were either sterile or fragmented into chains of squarish to oval bodies or spore-like structures. The organisms had a wall chemotype IV (*meso*-diaminopimelic acid, arabinose, and galactose), a type II phospholipid pattern (PE with or without PME), and MK-9(H_2,H_4) as predominant isoprenologues, but lacked mycolic acids.

The founder members of the genus, *Amycolatopsis orientalis* (comprising *Amycolatopsis orientalis* subsp. *orientalis* and *Amycolatopsis orientalis* subsp. *lurida*), *Amycolatopsis mediterranei*, *Amycolatopsis rugosa*, and *Amycolatopsis sulphurea* were formally members of other taxa, notably the genera *Nocardia* and *Streptomyces.* The type species, *Amycolatopsis orientalis*, had been classified as *Actinomyces orientalis* (Krasil'nikov, 1981), *Nocardia orientalis* (Pridham, 1970), *Proactinomyces orientalis* (Rautenstein et al., 1975), and *Streptomyces orientalis* (Pittenger and Brigham, 1956). Similarly, *Amycolatopsis mediterranei* had previously been known as *Nocardia mediterranei* (Thiemann et al., 1969) and "*Streptomyces mediterranei*" (Margalith and Beretta, 1960). "*Nocardia lurida"* (Grundy et al., 1957), *Nocardia rugosa* (di Marco and Spalla, 1957), and "*Nocardia sulphurea*" (Oliver and Sinclair, 1964) were transferred to the genus *Amycolatopsis* as *Amycolatopsis orientalis* subsp. *lurida*, *Amycolatopsis rugosa*, and *Amycolatopsis sulphurea*, respectively.

The valid publication of *Amycolatopsis orientalis* subsp. *lurida* (*ex* Grundy et al. (1957) Lechevalier et al. (1986) automatically created the name *Amycolatopsis orientalis* subsp. *orientalis* (Pittenger and Brigham, 1956) Lechevalier et al. (1986) according to Rule 46 of the Bacteriological Code (Howey et al., 1990). However, the elevation of *Amycolatopsis orientalis* subsp. *lurida* to full species status automatically meant that the name *Amycolatopsis orientalis* subsp. *orientalis* reverted to the earlier name, *Amycolatopsis orientalis* (Pittenger and Brigham, 1956) Lechevalier et al. (1986), according to Rule 40d (formerly Rule 46) of the Bacteriological Code.

The taxonomic positions of *Amycolatopsis mediterranei*, *Amycolatopsis orientalis*, and *Amycolatopsis sulphurea* were underpinned by molecular systematic data (Embley et al., 1988a; Warwick et al., 1994), but the remaining species, *Amycolatopsis rugosa*, was subsequently reclassified as *Prauserella rugosa* gen. nov., sp. nov. (Kim and Goodfellow, 1999). Similarly, *Amycolatopsis orientalis* subsp. *lurida* was found to merit species status as *Amycolatopsis lurida* (Stackebrandt et al., 2004). Several other taxa were soon assigned to the genus, notably *Amycolatopsis coloradensis* Labeda (1995), *Amycolatopsis azurea* Henssen et al. (1987), and *Amycolatopsis fastidiosa* Henssen et al. (1987); these taxa previously had been classified as *Streptomyces candidis* (Kunstmann et al., 1968), *Pseudonocardia azurea* (Ōmura et al., 1979), and *Amycolatopsis fastidiosa* (Henssen et al., 1987), respectively. Another organism, *Amycolatopsis methanolica* de Boer et al. (1990) had formally been a member of the genus *Nocardia* (Hazeu et al., 1983) and, prior to that, of the genus *Streptomyces* (Kato et al., 1977).

The species *Amycolatopsis fastidiosa* was proposed by Henssen et al. (1987), based on chemotaxonomic and morphological properties, for a strain initially described as "*Pseudonocardia fastidiosa*" by Celmer et al. (1977) in a US patent. However, it has become increasingly clear from 16S rRNA gene sequence and *gyrB* sequence analysis, and DNA–DNA relatedness data mentioned earlier, that the strain is misplaced in the genus *Amycolatopsis.* It has also been noted that the strain did not give an amplification product using *Amycolatopsis*-specific oligonucleotide primers (Tan et al., 2006b). The organism has recently been transferred to the genus *Actinokineospora* as *Actinokineospora fastidiosa* comb. nov. following a careful evaluation of its chemotaxonomic, morphological, and physiological properties

and of its position in the order *Pseudonocardiales* (formerly suborder *Pseudonocardineae*) 16S rRNA gene tree (Labeda et al., 2010b).

Differentiation of the species of the genus *Amycolatopsis*

Amycolatopsis species can be distinguished using a combination of 16S rRNA gene sequence (Figure 291), DNA: DNA relatedness (Table 236) and phenotypic (Table 237) data, although a common set of phenotypic tests has not been used in the description of all species. Some *Amycolatopsis* species have been differentiated using MALDI-TOF MS spectra (Groth et al., 2007) and on the basis of fatty acid (Ding et al., 2007; Lee, 2006; Mertz and Yao, 1993) and ribotype (Wink et al., 2003b, 2004) patterns.

List of species of the genus *Amycolatopsis*

1. **Amycolatopsis orientalis** (Pittenger and Brigham 1956) Lechevalier, Prauser, Labeda and Ruan 1986, 35[VP] (Basonym: *Nocardia orientalis* Pridham and Lyons 1969, 183; "*Streptomyces orientalis*" Pittenger and Brigham 1956, 642.)

or.i.en.tal′is. L. fem. adj. *orientalis* of the orient.

Forms an extensive branched substrate mycelium which appears slightly zig-zag in places. When produced, the aerial mycelium differentiates into cylindrical, occasionally ovoid spores which are carried in straight to flexuous chains. Blue colored colonies and a light brown diffusible pigment are formed on modified Bennett's agar. Grows at 10–42°C, but not at 45°C.

Positive for *N*-acetyl-β-glucosamidase, acid and alkaline phosphatases, α-chymotrypsin, esterase (C4), and esterase lipase (C8), but negative for cystine arylamidase, α-fucosidase, α- and β-galactosidases, α- and β-glucosidases, β-glucuronidase, α-mannosidase, trypsin, and valine arylamidase (API ZYM tests). Positive for β-galactosidase, but not for arginine dehydrolase or lysine and ornithine decarboxylases (API 20E tests). Acetoin-positive, but indole-negative. Arbutin is hydrolyzed, but does not produce H_2S.

Elastin, Tween 40, uric acid, and xylan are degraded, but not adenine. Acid is not produced from either dulcitol or methyl β-D-xyloside. Acetate, citrate, malate, propionate, pyruvate, and succinate are used as sole carbon sources, but not benzoate, mucate, oxalate, or tartrate.

Resistant (μg/ml) to gentamicin sulfate (5), neomycin sulfate (8), novobiocin (10), penicillin G (20), polymyxin B (50), rifampin (10), streptomycin sulfate (16), tobramycin sulfate (8), and vancomycin hydrochloride (0.25), but sensitive to lysozyme. Additional phenotypic properties are shown in Table 238.

Whole-organism hydrolysates are rich in arabinose and galactose. Cellular fatty acid profiles contain major proportions of $C_{15:0}$ iso (15.2%), $C_{16:0}$ iso (23.8%), $C_{17:0}$ anteiso (13.7%), and $C_{17:0}$ (12.6%), and smaller proportions (<10%) of $C_{14:0}$ iso (4.3%), $C_{15:0}$ anteiso (11%), $C_{15:1}$ (1.3%), $C_{15:0}$ (7.2%), $C_{16:1}$ (3.5%), $C_{16:0}$ (8.6%), $C_{17:0}$ iso (1.5%), $C_{17:1}$ (1.2%), $C_{18:1}$ iso (1.5%), and $C_{18:0}$ (3.0%). Mycolic acids are absent. The polar lipid pattern contains PE as the diagnostic component. The predominant menaquinones are MK-9(H_2,H_4).

Source: isolated from soil, vegetable matter, and clinical specimens.

DNA G+C content (mol%): 66 (T_m).

Type strain: ATCC 19795, CIP 107113, DSM 40040, IFO (now NRBC) 12806, IMSNU 20058, JCM 4235, JCM 4600, NRRL 2450, UNIQEM 181, VKM Ac-866.

Sequence accession nos: AJ400711 (16S rRNA gene); EU822906 (*gyrB*).

2. **Amycolatopsis alba** Mertz and Yao 1993, 719[VP]

al′ba. L. fem. adj. *alba* white, referring to the white aerial hyphae.

Forms a yellowish-brown substrate mycelium and a white aerial mycelium on Bennett's, glycerol-asparagine, tomato paste-oatmeal, and yeast extract-glucose agars. A light brown soluble pigment is produced on Emerson's and yeast extract-malt extract agars. Cylindrical, smooth spores are formed with a typical cobweb morphology. Grows between 15 and 37°C, but not at 10 or 45°C.

Arbutin is hydrolyzed and H_2S is produced. Elastin, Tween 40, uric acid, and xylan are degraded.

Positive for *N*-acetyl-β-glucosamidase, acid and alkaline phosphatases, α-chymotrypsin, cystine arylamidase, esterase (C4), esterase lipase (C8), β-galactosidase, α-glucosidase, β-glucuronidase, leucine arylamidase, α-mannosidase, naphthol-AS-BI-phosphohydrolase, trypsin, and valine phosphatase, but negative for α-fucosidase, α-galactosidase, and lipase C14 (API ZYM tests). Positive for arginine dihydrolase, β-galactosidase, and lysine and ornithine decarboxylases (API 20E tests).

Acid is produced from ribose, but not from cellulose, dulcitol, ethanol, glycogen, inulin, or xylitol. Acetate, butyrate, formate, lactate, malate, propionate, pyruvate, and succinate are used as sole carbon sources, but not oxalate, mucate, or tartrate.

Resistant (μg/ml, unless otherwise indicated) to bacitracin (10 U), kanamycin (30), nalidixic acid (30), neomycin (30), oleandomycin (15), rifampin (5), streptomycin (10), sulfonamide (200), and tetracycline (30), but sensitive to cephalothin (30), gentamicin (10), lincomycin (2), penicillin (10 U), streptomycin (10), tobramycin (10), and vancomycin (30). Grows in the presence of lysozyme. Additional phenotypic properties are shown in Table 238.

Whole-organism hydrolysates are rich in arabinose and galactose. The cellular fatty acid profile includes major proportions of $C_{15:0}$ iso (30.0%) and $C_{17:0}$ (18.6%), and smaller proportions (<10%) of $C_{14:0}$ iso (2.9%), $C_{15:0}$ anteiso (6.6%), $C_{15:0}$ (6.0%), $C_{16:0}$ iso (9.3%), $C_{16:0}$ iso (7.8%), $C_{17:0}$ iso (2.8%), $C_{17:0}$ anteiso (3.8%), and $C_{18:0}$ (1.5%). Mycolic acids are absent. PE is the diagnostic polar lipid. The predominant menaquinone is MK-9(H_4); also contains minor amounts of MK-8(H_4).

Source: isolated from soil.

TABLE 238. Characteristics differentiating the species of the genus *Amycolatopsis*[a]

Characteristic	1. *A. orientalis*	2. *A. alba*	3. *A. albidoflavus*	4. *A. australiensis*	5. *A. azurea*	6. *A. balhimycina*	7. *A. benzoatilytica*	8. *A. coloradensis*	9. *A. decaplanina*	10. *A. echigonensis*	11. *A. eurytherma*	12. *A. halotolerans*	13. *A. japonica*	14. *A. jejuensis*	15. *A. kentuckyensis*	16a. *A. keratiniphila* subsp. *keratiniphila*	16b. *A. keratiniphila* subsp. *nogabecina*	17. *A. lexingtonensis*	18. *A. lurida*	19. *A. marina*	20. *A. mediterranei*	21. *A. methanolica*	22. *A. minnesotensis*	23. *A. nigrescens*	24. *A. niigatensis*	25. *A. palatopharyngis*	26. *A. plumensis*	27. *A. pretoriensis*	28. *A. regifaucium*	29. *A. rifamycinica*	30. *A. rubida*	31. *A. saalfeldensis*	32. *A. sacchari*	33. *A. sulphurea*	34. *A. taiwanensis*	35. *A. thermoflava*	36. *A. tolypomycina*	37. *A. tucumanensis*	38. *A. ultiminotia*	39. *A. vancoresmycina*
Acid production from:																																								
Adonitol	+	+	+	−	+	nr	+	−	nr	$+^{w}$	+	+	+	+	+	+	nr	+	+	nr	−	+	+	nr	$+^{w}$	+	+	−	−	+	+	nr	+	−	nr	+	−	nr	−	−
Arabinose	+	+	+	+	+	+	+	−	+	nr	+	−	+	+	+	+	+	+	+	−	+	−	+	+	nr	+	+	+	+	+	+	+	+	−	+	+	+	nr	−	+
Cellobiose	+	+	+	+	+	nr	+	+	nr	nr	+	+	+	−	+	+	nr	+	+	+	+	+	+	nr	nr	−	+	+	+	+	+	nr	+	−	nr	+	+	nr	+	+
Dextrin	+	−	nr	v	nr	nr	+	+	nr	nr	$+^{w}$	nr	+	nr	+	nr	nr	+	+	nr	+	−	−	nr	nr	−	nr	+	−	nr	−	nr	+	+	nr	−	nr	nr	nr	nr
meso-Erythritol	+	+	+	nr	+	nr	+	−	nr	$+^{w}$	+	−	+	−	−	nr	nr	$+^{w}$	+	nr	−	+	+	nr	$+^{w}$	+	nr	+	−	nr	+	nr	+	−	nr	+	nr	nr	−	nr
Fructose	+	+	+	+	+	+	+	+	+	nr	+	+	+	+	+	+	+	+	+	+	+	+	+	+	nr	+	+	+	nr	+	+	+	+	nr	−	+	+	$+^{w}$	−	+
Galactose	+	+	+	+	+	nr	+	+	nr	+	+	+	+	+	+	+	nr	+	+	+	+	+	+	nr	+	+	−	+	+	+	+	nr	+	+	nr	+	+	nr	+	+
Glucose	+	+	−	+	nr	nr	+	nr	+	$+^{w}$	nr	−	nr	−	+	nr	nr	+	nr	+	+	nr	+	+	$+^{w}$	nr	nr	+	nr	nr	nr	+	nr	nr	+	nr	nr	nr	+	nr
Glycerol	+	+	nr	+	nr	nr	nr	nr	nr	nr	nr	+	nr	−	+	+	nr	+	+	nr	+	nr	+	nr	nr	nr	nr	+	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	+	nr
myo-Inositol	+	+	+	+	+	+	+	+	−	+	+	+	+	+	+	+	+	+	+	+	+	−	+	+	−	+	nr	+	+	+	+	+	−	+	nr	−	+	−	+	+
Lactose	+	+	+	+	+	nr	+	−	nr	+	$+^{w}$	−	+	−	+	+	nr	+	+	−	+	−	+	nr	−	−	−	+	−	−	−	nr	+	−	nr	+	−	nr	+	−
Maltose	+	+	−	v	nr	nr	−	+	nr	nr	−	−	+	−	+	nr	nr	+	+	+	+	nr	+	nr	nr	−	+	+	nr	−	−	nr	+	+	nr	−	nr	nr	+	nr
Mannitol	+	+	−	+	+	+	+	+	+	−	+	+	+	+	−	+	+	$+^{w}$	+	+	+	+	+	+	+	−	+	−	nr	−	+	+	+	+	nr	+	+	nr	+	+
Mannose	−	+	+	+	nr	nr	+	nr	nr	−	nr	+	nr	+	+	nr	nr	+	nr	nr	+	nr	+	nr	−	nr	nr	+	+	nr	nr	nr	nr	nr	nr	nr	nr	nr	+	nr
Melezitose	+	−	−	−	+	nr	nr	−	nr	nr	−	−	−	−	−	nr	nr	−	nr	nr	−	+	−	nr	nr	−	nr	−	−	−	−	−	−	nr	−	−	nr	nr	−	nr
Melibiose	+	+	−	−	nr	+	nr	−	nr	nr	−	+	+	−	+	+	nr	+	−	nr	+	−	−	nr	nr	−	−	+	−	nr	−	nr	−	−	nr	+	−	nr	−	−
Methyl α-D-glucoside	+	+	−	nr	nr	nr	nr	+	nr	nr	−	−	+	−	+	nr	nr	+	+	nr	+	−	−	nr	nr	−	nr	+	nr	nr	−	nr	+	−	nr	+	nr	nr	−	nr
Raffinose	−	+	−	v	+	+	nr	−	−	nr	−	−	+	−	+	+	−	+	−	−	+	−	−	+	nr	+	−	+	−	−	−	−	nr	−	nr	+	−	−	−	+
Rhamnose	+	−	nr	nr	nr	+	+	−	−	+	+	−	−	−	+	+	+	+	−	+	+	+	+	$+^{w}$	+	−	−	+	nr	−	+	$+^{w}$	+	−	nr	−	+	$+^{w}$	+	+
Salicin	+	+	−	nr	nr	nr	nr	+	nr	nr	−	−	+	−	+	nr	nr	+	+	nr	+	$+^{w}$	+	nr	nr	−	nr	+	nr	nr	+	nr	+	−	−	+	nr	nr	−	nr
D(−)-Sorbitol	−	−	−	−	nr	−	−	−	nr	+	+	−	−	−	+	nr	nr	−	−	−	$+^{w}$	+	+	nr	$+^{w}$	−	−	$+^{w}$	−	−	−	nr	−	−	nr	+	−	nr	−	−
Sucrose	+	−	+	v	nr	+	−	+	−	nr	−	+	+	+	+	+	+	+	+	−	+	+	+	−	nr	−	−	+	−	nr	+	+	+	+	+	−	+	−	−	+
Trehalose	+	+	+	v	nr	nr	+	+	nr	nr	+	−	+	−	+	nr	nr	−	+	+	+	+	+	nr	nr	+	+	+	nr	nr	+	nr	+	+	nr	+	nr	$+^{w}$	−	nr
Xylose	+	+	+	+	+	−	+	+	−	+	+	−	−	−	+	+	+	+	+	+	+	+	+	+	−	+	−	+	+	+	+	+	+	−	nr	+	−	$+^{w}$	+	−
Decomposition of:																																								
Allantoin	−	+	nr	v	−	nr	+	−	nr	nr	+	nr	+	nr	−	nr	nr	−	nr	nr	nr	−	nr	nr	nr	−	nr	−	−	−	−	nr	+	nr	nr	+	nr	nr	nr	nr
Casein	+	+	+	+	+	nr	−	+	nr	nr	+	+	+	+	+	nr	nr	+	+	−	+	+	+	+	nr	+	+	+	nr	+	+	+	+	+	+	+	+	+	+	−
Esculin	+	+	+	+	+	nr	+	+	nr	nr	−	nr	+	nr	+	nr	nr	+	+	nr	+	$+^{w}$	nr	+	nr	−	nr	+	+	+	+	+	+	+	+	+	nr	+	+	nr
Gelatin	+	+	+	+	+	nr	−	+	+	nr	+	+	+	+	+	nr	nr	+	+	+	+	+	+	nr	nr	+	+	+	nr	+	$+^{w}$	+	+	+	+	−	+	nr	+	−
Hypoxanthine	+	+	+	v	−	nr	−	+	nr	nr	$+^{w}$	+	+	+	+	nr	nr	+	+	+	+	+	+	+	nr	+	−	+	+	+	+	−	−	+	$+^{w}$	+	nr	nr	+	nr
Tyrosine	+	nr	+	nr	nr	nr	nr	+	nr	nr	nr	+	+	+	+	nr	nr	+	+	−	+	+	+	+	nr	+	+	$+^{w}$	+	nr	nr	+	+	+	nr	nr	nr	nr	+	nr
Xanthine	+	+	+	−	−	−	−	−	nr	nr	+	+	+	−	−	nr	nr	−	+	nr	−	−	+	+	nr	+	−	−	+	−	+	+	+	−	−	+	+	nr	+	−

Production of:																																								
Amylase	−	+	+	+	−	−	−	+	nr	nr	−	−	+	−	−	nr	nr	−	−	$+^{w}$	−	−	−	−	nr	−	+	−	+	−	−	−	−	−	−	−	−	nr	−	−
Nitrate reductase	+	−	+	+	+	−	nr	+	nr	+	+	+	−	+	−	+	−	+	+	−	+	+	+	+	−	+	nr	−	+	nr	+	−	+	+	+	+	+	−	−	−
Urease	+	+	$+^{w}$	−	+	+	+	−	+	+	+	+	−	$+^{w}$	+	+	+	+	+	−	+	−	+	+	+	$+^{w}$	+	$+^{w}$	−	+	+	+	+	−	−	+	+	+	−	+
Growth in/ at:																																								
5% NaCl	+	+	+	−	+	nr	nr	+	nr	nr	+	+	+	−	+	+	nr	+	−	+	−	nr	+	+	nr	+	−	+	+	$+^{w}$	+	−	+	+	−	+	+	nr	+	+
10°C	+	−	+	v	+	nr	nr	+	nr	+	−	+	+	+	−	+	nr	nr	+	+	+	−	+	−	+	+	−	nr	+	+	+	$+^{w}$	−	−	−	−	nr	nr	+	nr
45°C	−	−	−	+	−	−	nr	−	nr	+	+	−	+	−	+	−	nr	+	−	+	−	+	−	−	+	−	−	−	−	−	−	−	+	−	−	+	+	nr	−	−

[a]+, Positive; $+^{w}$, weakly positive; −, negative; v, variable; nr, not reported.

DNA G+C content (mol%): not determined.

Type strain: A83850, ATCC 51368, DSM 44262, NBRC 15602, JCM 10030, NRRL 18532.

Sequence accession nos: AF051340 (16S rRNA gene); EU822885 (*gyrB*).

3. **Amycolatopsis albidoflavus** Lee and Hah 2001, 649[VP]

al.bi.do.fla'vus. L. adj. *albidus* white; L. adj. *flavus* yellow; N.L. adj. *albidoflavus* whitish yellow.

Forms well developed aerial and vegetative mycelia that fragment into rod-shaped elements. The aerial mycelium is white and the substrate mycelium is orange yellow; a yellow soluble pigment is formed on modified Bennett's agar. Grows between 10 and 37°C, but not at 45°C.

Phosphatase-positive. Does not produce acid from L-sorbose or xylitol. Benzoate, citrate, lactate, oxalate, and propionate are used as sole carbon sources, but not mucate or tartrate. Grows in the presence of 7% (w/v) NaCl.

Resistant (μg/ml) to gentamicin sulfate (5), neomycin sulfate (8), novobiocin (10), penicillin G (20), polymyxin B (50), rifampin (10), streptomycin sulfate (16), tobramycin sulfate (8), and vancomycin hydrochloride (0.25). Additional phenotypic properties are shown in Table 238.

Whole-organism hydrolysates are rich in arabinose and galactose. Muramic acid is in the *N*-acetylated form. The cellular fatty acid profile includes major proportions of $C_{16:0}$ iso (26.1%), $C_{14:0}$ iso (9.8%), and $C_{16:0}$ (10.2%), and smaller proportions (<10%) of $C_{14:0}$ (1.1%), $C_{15:0}$ iso (8.7%), $C_{15:1}$ (2.3%), $C_{15:0}$ (5.8%), $C_{16:1}$ (4.0%), $C_{17:0}$ iso (1.6%), $C_{17:1}$ (4.4%), $C_{17:0}$ (7.8%), and $C_{18:0}$ (6.2%). The cellular polar lipid pattern includes PE and PI. The predominant menaquinone is MK-9(H_4).

Source: isolated from soil.

DNA G+C content (mol%): 68.5 (HPLC).

Type strain: ATCC 53205, DSM 44639, IMSNU 22139, JCM 11300, KCTC 9471, NBRC 100337, NRRL B-24149.

Sequence accession nos: AJ252832 (16S rRNA gene); EU822886 (*gyrB*).

4. **Amycolatopsis australiensis** Tan, Robinson, Lacey and Goodfellow 2006a, 2299[VP]

aus.tra.li.en'sis. N.L. fem. adj. *australiensis* pertaining to Australia, the source of the soil from which the first strains were isolated.

Forms an extensively branched substrate mycelium which fragments into squarish rod-shaped elements. Abundant, white aerial hyphae, a pale yellow substrate mycelium, and a medium yellow diffusible pigment are produced on modified Bennett's agar supplemented with mannitol and soybean flour. Melanin pigments are not formed on peptone-yeast extract-iron or tyrosine agars. Grows between 28 and 45°C, and from pH 5 to 7.

Tween 40 and uric acid are degraded. Acid is produced from arabitol, glycogen, and turanose, but not from dulcitol, D-ribose, or xylitol. Citrate, oxalate, and propionate are used as sole carbon sources, but not benzoate, lactate, or mucate (all at 0.1%, w/v).

Resistant (μg/ml) to gentamicin sulfate (5), neomycin sulfate (8), polymyxin B (50) and tobramycin sulfate (8), but sensitive to novobiocin (10), penicillin G (20), rifampin (10), and streptomycin sulfate (16). Additional phenotypic properties are shown in Table 238.

Whole-organism hydrolysates are rich in arabinose and galactose. Muramic acid is *N*-acetylated. Mycolic acids are not present. The polar lipid pattern includes DPG, PE, PG, PI, and PME. The predominant menaquinone is MK-9(H_4); also contains MK-8(H_2) (26%) and MK-9(H_8) (8%).

Source: isolated from arid soil collected from Western Australia.

DNA G+C content (mol%): not determined.

Type strain: GY048, DSM 44671, JCM 15587, NCIMB 14142.

Sequence accession nos: AY129753 (16S rRNA gene); EU822887 (*gyrB*).

5. **Amycolatopsis azurea** (Ōmura, Tanaka, Tanaka, Spiri-Nakagawa, Oiwa, Takahashi, Matsuyama and Iwai 1983) Henssen, Kothe and Kroppenstedt 1987, 294[VP] (Basonym: *Pseudonocardia azurea* Ōmura, Tanaka, Tanaka, Spiri-Nakagawa, Oiwa, Takahashi, Matsuyama and Iwai 1983, 673.)

a.zu're.a. N.L. fem. adj. *azurea* azure blue, referring to the color of aerial mycelium.

Forms irregularly branched substrate hyphae which tend to be zig-zag-shaped. The aerial mycelium is usually white, but is blue on sucrose-nitrate and tyrosine agars, and pink on glucose-peptone agar. The substrate mycelium is brownish black and the aerial mycelium is light gray on modified Bennett's agar. Blue soluble pigments are produced on glucose-nitrate and starch-yeast extract agars. Aerial spores are smooth and cylindrical (0.4–1.1 × 3.7 μm). Grows between 10 and 36°C, but not at 37 or 45°C.

Positive for *N*-acetyl-β-glucosamidase, α-chymotrypsin, esterase lipase (C8), β-galactosidase, α- and β-glucosidases, leucine arylamidase, naphthol-AS-BI-phosphohydrolase, and trypsin, but negative for alkaline phosphatase, cystine arylamidase, esterase (C4), α-fucosidase, α-galactosidase, β-glucuronidase, lipase (C14), α-mannosidase, and valine arylamidase (API ZYM tests). Positive for arginine dihydrolase, β-galactosidase, and lysine and ornithine decarboxylases (API 20E tests). Acetoin-positive, but indole-negative. Arbutin is hydrolyzed, but does not produce H_2S. Elastin, Tween 40, uric acid, and xylan are degraded. Citrate, oxalate, and propionate are used as sole carbon sources, but not benzoate, lactate, or mucate (all at 0.1%, w/v). Grows in the presence of 7% (w/v) NaCl.

Resistant (μg/ml) to gentamicin sulfate (5), kanamycin (30), nalidixic acid (30), neomycin sulfate (8), penicillin G (20), polymyxin B (50), rifampin (10), streptomycin sulfate (16), sulfonamide (200), tobramycin sulfate (8), and vancomycin hydrochloride (30). Additional phenotypic properties are shown in Table 238.

Whole-organism hydrolysates are rich in arabinose and galactose. Cellular fatty acid profiles contain major proportions of $C_{15:0}$ iso (30.0%), $C_{17:0}$ (11.8%), and $C_{17:0}$ (25.7%), and smaller proportions (>10) of $C_{15:0}$ anteiso (3.9%), $C_{15:0}$ (6.5%), $C_{16:0}$ iso (2.9%), $C_{16:1}$ (8.8%), $C_{16:0}$ (5.7%), $C_{17:0}$ iso (2.6%), and $C_{18:0}$ iso (2.1%). Mycolic acids are not present. The polar lipid pattern contains DPG, PE, PG, PI, and PIMs. The predominant menaquinone is MK-9(H_4); also contains MK-8(H_4) and MK-9(H_4).

Source: isolated from soil.

DNA G+C content (mol%): 66 (T_m).

Type strain: AM-3696, ATCC 51273, DSM 43854, FERM-P 4738, IFO (now NRBC) 14573, IMSNU 20053, JCM 3275, NRRL 11412, VKM Ac-1418.

Sequence accession nos: AJ400709 (16S rRNA gene); EU822888 (*gyrB*).

6. **Amycolatopsis balhimycina** Wink, Kroppenstedt, Ganguli, Nadkarni, Schumann, Seibert and Stackebrandt 2003a, 935[VP] (Effective publication: Wink, Kroppenstedt, Ganguli, Nadkarni, Schumann, Seibert and Stackebrandt 2003b, 44.)

bal.hi.my′ci.na. N.L. n. *balhimycinum* balhimycin (an antibiotic produced by the organism); L. fem. suff. *-ina* suffix used with the sense of belonging to; N.L. fem. adj. *balhimycina* pertaining to balhimycin.

Forms a chrome yellow substrate mycelium on glycerol-asparagine, inorganic salts-starch, oatmeal, peptone-yeast extract, tyrosine, and yeast-extract-malt extract agars (ISP media 2–7); soluble pigments are not formed on any of these media. Fragmentation of the substrate mycelium and white aerial hyphae into more or less irregular arthrospores is only seen on oatmeal agar.

Positive for alkaline phosphatase, *N*-acetyl-β-glucosamidase, α-chymotrypsin, cystine arylamidase, α-fucosidase, α- and β-galactosidases, α-glucosidase, leucine arylamidase, lipase (C14), naphthol-AS-BI-phosphohydrolase, trypsin, and valine arylamidase, but negative for β-glucuronidase and α-mannosidase (API ZYM tests). Produces H_2S. Additional phenotypic properties are shown in Table 238.

Whole-organism hydrolysates are rich in arabinose and galactose. Cellular fatty acid profiles contain major proportions of $C_{16:0}$ iso (36.0%), and smaller proportions (<10%) of $C_{15:0}$ (5.3%), $C_{16:0}$ (2.0%), $C_{17:0}$ (2.2%), $C_{15:1}$ (5.1%), $C_{14:0}$ iso (3.8%), $C_{15:0}$ iso (5.7%), C_{161} (7.3%), $C_{17:0}$ iso (1.2%), $C_{15:0}$ anteiso (1.5%), $C_{17:0}$ anteiso (4.1%), $C_{16:1}$ *cis*9 (4.0%), $C_{17:1}$ *cis*9 (7.6%), $C_{16:0}$ 10-methyl (2.4%), $C_{17:0}$ 10-methyl (3.2%), 2-hydroxy-$C_{16:0}$ (7.7%), and $C_{17:0}$ anteiso 2-OH (0.7%). Mycolic acids are absent. The polar lipid pattern contains DPG, HPE, PE, PG, and PI. The predominant menaquinone is MK-9(H_4).

Source: isolated from a soil sample collected in India.

DNA G+C content (mol%): not determined.

Type strain: FH 1894, DSM 44591, JCM 12668, NRRL B-24207.

Sequence accession nos: AJ508239 (16S rRNA gene); EU822889 (*gyrB*).

7. **Amycolatopsis benzoatilytica** Majumdar, Prabhagaran, Shivaji and Lal 2006, 202[VP]

ben.zo.a.ti.ly′ti.ca. N.L. n. *benzoas -atis* benzoate; N.L. adj. *lyticus-a-um* (from Gr. adj. *lutikos-ê-on*) dissolving; N.L. fem. adj. *benzoatilytica* benzoate-degrading.

Forms cream-colored, irregularly shaped, rough, flat colonies with undulating margins. The substrate mycelium tends to fragment in liquid medium. Grows between 10 and 37°C, and from pH 5 to 10.

Tween 80 is degraded and *m*-hydroxybenzoate is metabolized. Additional phenotypic properties are shown in Table 238.

Whole-organism hydrolysates are rich in arabinose and galactose. Cellular fatty acid profiles contain major proportions of $C_{14:0}$ iso (11.3%), $C_{15:0}$ (14.7%), $C_{15:1}$ ω6*c* (12.8%), $C_{16:0}$ iso (12.8%), $C_{17:0}$ (11.3%), and $C_{17:1}$ ω8*c* (11.3%), and smaller proportions (<10%) of $C_{15:0}$ iso (6.8%), $C_{15:0}$ anteiso (3%), $C_{16:0}$ (5.2%), and $C_{15:0}$ 2-OH (1%). The polar lipid pattern contains DPG, PE, PG, PI, and PME. The predominant menaquinone is MK-9(H_4).

Source: isolated from a patient with submandibular mycetoma.

DNA G+C content (mol%): not determined.

Type strain: AK 16/65, ATCC 55165, DSM 43387, IMRU 1389, JCM 13851.

Sequence accession no. (16S rRNA gene): AY957506.

Additional comments: the strain was initially identified as *Nocardia brasiliensis* (Scharfen, 1971) and reclassified as *Nocardia orientalis* by Gordon et al. (1978).

8. **Amycolatopsis coloradensis** Labeda 1995, 126[VP]

co.lo.rad.en′sis. N.L. fem. adj. *coloradensis* of Colorado, the source of the soil sample from which the type strain was isolated.

Forms a yellow to orange substrate mycelium. When formed, the aerial mycelium is sparse and white to olive buff. Aerial hyphae differentiate into chains of straight to flexuous cylindrical to ovoid spores. An orange yellow soluble pigment is produced on most growth media, including modified Bennett's agar. Grows at 10 to 37°C, but not at 45°C.

Arbutin is not hydrolyzed nor is H_2S produced. Elastin, Tween 40, and xylan are degraded, but not uric acid. Positive for *N*-acetyl-β-glucosamidase, acid and alkaline phosphatases, α-chymotrypsin, esterase (C8), leucine arylamidase, lipase (C14), and trypsin, but negative for cystine arylamidase, esterase (C4), α-fucosidase, α- and β-galactosidases, α- and β-glucosidases, β-glucuronidase, α-mannosidase, naphthol-AS-BI-phosphohydrolase, and valine arylamidase (API ZYM tests). Positive for arginine dihydrolase, β-galactosidase, and lysine and ornithine decarboxylases, but not for β-galactosidase (API 20E tests). Acetate, malate, propionate, pyruvate, and succinate are used as sole carbon sources, but not benzoate, citrate, mucate, oxalate, or tartrate.

Resistant (μg/ml) to gentamicin sulfate (5), neomycin sulfate (8), polymyxin B (50), streptomycin sulfate (16), tobramycin sulfate (8), and vancomycin hydrochloride (0.25). Additional phenotypic properties are shown in Table 238.

Whole-organism hydrolysates are rich in arabinose and galactose. Cellular fatty acid profiles contain major proportions of $C_{15:0}$ (14.6%), $C_{15:1}$ ω6*c* (12.8%), $C_{16:0}$ iso (25) and $C_{17:0}$ (11.3%), and smaller proportions of $C_{14:0}$ iso (0.5%), $C_{15:0}$ iso (6.8%), $C_{15:0}$ anteiso (3.0%), $C_{16:0}$ (5.2%), $C_{16:1}$ iso H (0.2%), $C_{17:0}$ iso (0.4%), $C_{17:0}$ anteiso (0.8%), and $C_{18:0}$ (0.4%). Mycolic acids are absent. The cellular polar lipid pattern contains PE as the diagnostic nitrogen-containing component. The predominant menaquinone is MK-9(H_2,H_4).

Source: isolated from a soil sample collected in Colorado.

DNA G+C content (mol%): 66 (T_m).

Type strain: ATCC 53629, DSM 44225, IFO (now NRBC) 15804, JCM 9869, NRRL 3218, VKM Ac-1732.

Sequence accession nos: AF051341 (16S rRNA gene); EU822890 (*gyrB*).

9. **Amycolatopsis decaplanina** Wink, Gandhi, Kroppenstedt, Seibert, Sträubler, Schumann and Stackebrandt 2004, 237[VP]

de.ca.pla'ni.na. N.L. neut. n. *decaplaninum* decaplanin; L. fem. suff. *-ina* related to; N.L. fem. adj. *decaplanina* related to decaplanin, an antibiotic produced by the organism.

Honey yellow substrate mycelium is formed on glycerol-asparagine, inorganic salts-starch, oatmeal, peptone-yeast extract-iron, tyrosine, tryptone-yeast extract, and yeast extract-malt extract agars (ISP media 1–7). Only produces an aerial mycelium on oatmeal agar and a soluble red pigment on tyrosine agar. Melanin pigments are not formed. Regular shaped to globose and smooth-surfaced pseudosporangia are produced, but spores are not seen either inside or outside these bodies.

Positive for *N*-acetyl-β-glucosamidase, acid and alkaline phosphatases, α-chymotrypsin, β-galactosidase, α- and β-glucosidases, leucine arylamidase, α-mannosidase, naphthol-AS-BI-phosphohydrolase, trypsin, and valine arylamidase, but negative for cystine arylamidase, esterase (4), esterase lipase (C8), α-fucosidase, α-galactosidase, β-glucuronidase, and lipase (C14) (API ZYM tests). Positive for β-galactosidase, but not for arginine dihydrolase or lysine and ornithine decarboxylases (API 20E tests). Produces acetone, but not indole. Arbutin is hydrolyzed, but H_2S is not produced. Elastin and Tween 40 are degraded, but not uric acid or xylan. Additional phenotypic properties are shown in Table 238.

Whole-organism hydrolysates are rich in arabinose and galactose. Cellular fatty acid profiles contain major proportions of $C_{15:0}$ iso (22.5%), $C_{17:0}$ (11.4%), and $C_{16:0}$ iso (10.3%), and smaller proportions (<10%) of $C_{14:0}$ iso (6.4%), $C_{15:0}$ anteiso (9.4%), $C_{15:0}$ iso 2-OH (8.7%), $C_{15:0}$ (7.6%), $C_{17:1}$ (5.8%), $C_{16:0}$ (3.3%), $C_{15:1}$ (2.1%), $C_{17:0}$ iso (1.7%), $C_{17:0}$ anteiso (3.4%), $C_{16:0}$ iso 2-OH (2.8%), $C_{15:0}$ anteiso 2-OH (1.9%), $C_{17:0}$ 2-OH (1.5%) and $C_{17:0}$ 10-methyl iso (1.2%). The polar lipid pattern contains DPG, HPE, PE, PG, PI, and PIMs. The predominant menaquinones are MK-8(H_4) and MK-9(H_4).

Source: isolated from a soil sample collected in India.

DNA G+C content (mol%): not determined.

Type strain: FH 1845, DSM 44594, JCM 12669, NRRL B-24209.

Sequence accession nos: AJ508237 (16S rRNA gene); EU822891 (*gyrB*).

Additional comments: the type strain of *Amycolatopsis decaplanina* has a ribotype pattern which distinguishes it from phylogenetically related *Amycolatopsis* strains (Wink et al., 2004).

10. **Amycolatopsis echigonensis** Ding, Hirose and Yokota 2007, 1750[VP]

e.chi.go.nen'sis. N.L. fem. adj. *echigonensis* referring to Echigo, the old name of Niigata Prefecture, Japan, the source of the type strain.

The substrate mycelium is light yellow and the aerial mycelium white. Grows between 5 and 45°C, and optimally at 30°C, and from pH 6 to 11, optimally at pH 9.0.

Positive for *N*-acetyl-β-glucosamidase, acid and alkaline phosphatases, α-chymotrypsin, cystine arylamidase, esterase (C4), esterase lipase (C8), α- and β-galactosidases, α-glucosidase, β-glucuronidase, leucine arylamidase, α-mannosidase, trypsin, and valine arylamidase, but negative for α-fucosidase, β-glucosidase, and lipase (C14) (API ZYM tests). Grows in the presence of 7% (w/v) NaCl. Additional phenotypic properties are shown in Table 238.

Whole-organism hydrolysates are rich in arabinose and galactose. Cellular fatty acid profiles contain major proportions of $C_{15:0}$ anteiso (16.7%) and $C_{16:0}$ iso (31.9%), and smaller proportions (<10%) of $C_{14:0}$ iso, (6.5%), $C_{14:0}$ (0.4%), $C_{15:0}$ iso (7.0%), $C_{15:0}$ (5.2%), $C_{15:1}$ ω6*c* (3.6%), $C_{16:0}$ (2.5%), $C_{16:1}$ iso OH (1.3%), $C_{17:0}$ iso (1.1%), $C_{17:}$ (6.9%), $C_{17:0}$ (3.4%), $C_{17:1}$ ω6*c* (5.6%), and $C_{17:1}$ ω8*c* (2.4%). Mycolic acids are absent. The predominant menaquinone is MK-9(H_4).

Source: isolated from filtration material made from volcanic soil, Niigata, Japan.

DNA G+C content (mol%): 72.4 (HPLC).

Type strain: LC2, CCTCC AB206019, IAM 15387, JCM 21831.

Sequence accession nos: AB248535 (16S rRNA gene); EU822892 (*gyrB*).

11. **Amycolatopsis eurytherma** Kim, Sahin, Tan, Zakrzewska-Czerwinska and Goodfellow 2002a, 893[VP]

eur.y.ther'ma. Gr. adj. *eurus* wide, broad; Gr. adj. *thermos* hot; N.L. fem. adj. *eurytherma* grows over a wide temperature range.

Extensively branched substrate hyphae fragment into squarish, rod-like elements (approx. 0.4–0.5 × 0.7–1.6 µm). Forms a yellow substrate mycelium on modified Bennett's and glucose-yeast extract agars, but distinct pigments are not evident on Czapek-Dox, glycerol-asparagine, or oatmeal agars; diffusible pigments are not formed on any of these media. Melanin pigments are not produced on peptone-yeast extract-iron or tyrosine agars. Aerial mycelium is white, sparse, and sterile. Grows from 25 to 55°C, but not at 10 or 60°C and from pH 6 to 9.

Negative for β-galactosidase and *N*-acetyl-β-glucosamidase. Elastin and xylan are decomposed, but not chitin or guanine. Grows on adonitol, L-arabinose, cellobiose, fructose, galactose, *myo*-inositol, lactose, mannitol, mannose, melezitose, melibiose, rhamnose, ribose, sorbitol, starch, sucrose, trehalose, turanose, xylitol, and xylose as sole carbon sources, but not on raffinose (all at 1%, w/v).

Resistant (µg/ml) to ampicillin (32), bacitracin (16), carbenicillin (12), cefoxitin (32), cephaloridine (128), cephradine (32), doxycyline hydrochloride (4), ethionamide (16), fusidic acid (4), gentamicin sulfate (32), gramicidin (8), isoniazid (16), lincomycin hydrochloride (128), lividomycin A (8), nalidixic acid (32), neomycin sulfate (8), novobiocin (4), oleandomycin phosphate (128), penicillin G (15 IU), polymyxin B (50), rifampin (64), spiramycin (10), streptomycin sulfate (64), tetracycline hydrochloride (32), tobramycin sulfate (8), vancomycin hydrochloride (64), and viomycin sulfate (20), but sensitive to amikacin (4), bacitracin (32), doxycyclin (6), fusidic acid (8), gentamicin (64), lividomycin A (16), neomycin sulfate (32),

novobiocin (16), streptomycin sulfate (64), ticarcillin (16), and tyrothricin (16). Additional phenotypic properties are shown in Table 238.

Whole-organism hydrolysates are rich in arabinose and galactose. Cellular fatty acid profiles contain major proportions of $C_{16:0}$ iso (46%) and $C_{16:0}$ (23%), and smaller proportions (<10%) of $C_{15:0}$ iso, (4%), $C_{17:0}$ iso (6%), $C_{17:0}$ anteiso (9%), $C_{17:0}$ (3%), and $C_{16:0}$ (3%). Mycolic acids are absent. The polar lipid pattern contains DPG, PE, PG, PI, PI dimannoside, and PME. The predominant isoprenologue is MK-9(H_4).

Source: isolated from arid and scrubland soil collected in Madurai, India, and Van, Turkey, respectively.

DNA G+C content (mol%): 72.2–74.0 (HPLC).

Type strain: NT202, DSM 44348, JCM 12071, NBRC 100338, NCIMB 13795.

Sequence accession nos: AJ000285 (16S rRNA gene); EU822893 (*gyrB*).

12. **Amycolatopsis halotolerans** Lee 2006, 552[VP]

ha.lo.to′le.rans. Gr. n. *hals halos* salt; L. part. adj. *tolerans* tolerating, enduring; N.L. part. adj. *halotolerans* salt-tolerating.

Forms well-developed, branched aerial and substrate mycelia that fragment into rod-shaped elements. The substrate mycelium is brown and the aerial mycelium is grayish white on yeast extract-malt extract agar (ISP medium 2). Grows between 10 and 37°C, but not at or above 45°C.

Does not produce acid from D-arabinose, dulcitol, methyl α-D-mannoside, sorbose, or xylitol. Growth occurs in the presence of 7%, but not 10% (w/v) NaCl. Additional phenotypic properties are shown in Table 238.

Whole-organism methanolyzates are rich in arabinose and galactose. Muramic acid moieties are *N*-acetylated. Cellular fatty acids contain major proportions of $C_{15:0}$ iso (13.0%), $C_{16:0}$ (16.2%), $C_{16:0}$ iso (17.6%), $C_{17:0}$ (14.5%), and $C_{18:0}$ (13.2%), and smaller proportions (<10%) of $C_{15:0}$ (3.7%), $C_{17:0}$ iso, (5.0%), $C_{15:0}$ anteiso (3.2%), $C_{17:0}$ anteiso, (3.1%), $C_{14:0}$ 2-OH (0.8%), and $C_{15:0}$ iso 3-OH (5.2%). Mycolic acids are absent. The polar lipid pattern contains DPG, PE, PG, and PI. The predominant menaquinone is MK-9(H_4); minor amounts of MK-9(H_6) and MK-9(H_8) are also present.

Source: isolated from soil collected inside a natural cave on Jeju Island, Republic of Korea.

DNA G+C content (mol%): 72.5 (HPLC).

Type strain: N4-6, DSM 45041, JCM 13279, NRRL B-24428.

Sequence accession nos: DQ000196 (16S rRNA gene); EU822895 (*gyrB*).

13. **Amycolatopsis japonica** corrig. Goodfellow, Brown, Cai, Chun and Collins 1997b, 915[VP] (Effective publication: Goodfellow, Brown, Cai, Chun, Collins 1997a, 81.)

ja.po′ni.ca. N.L. fem. adj. *japonica* pertaining to Japan.

Forms substrate and aerial mycelia which fragment into squarish elements. A yellow brown substrate mycelium, a white aerial mycelium and a dark olive brown diffusible pigment are formed on modified Bennett's agar. Grows between 10 and 45°C.

Positive for *N*-acetyl-β-glucosamidase, acid and alkaline phosphatases, α-chymotrypsin, cystine arylamidase, esterase (C4), esterase lipase (C8), α-fucosidase, α- and β-galactosidases, α- and β-glucosidases, leucine arylamidase, lipase (C14), α-mannosidase, naphthol-AS-BI-phosphohydrolase, trypsin, and valine arylamidase, but negative for β-glucuronidase (API ZYM tests). Positive for β-galactosidase, arginine dihydrolase, and lysine and ornithine decarboxylases (API 20E tests). Acetoin-positive, but indole- and lecithanase-negative. Produces lipolytic enzymes. Arbutin is hydrolyzed, but not hippurate. H_2S is not produced.

DNA, elastin, RNA, Tweens 20, 40, 60 and 80, uric acid, and xylan are degraded, but not adenine, chitin, or pectin. Adonitol, L-arabinose, fructose, galactose, *myo*-inositol, lactose, mannitol, melibiose, raffinose, rhamnose, salicin, sucrose, and xylose are used as sole carbon sources, but not dextran, melezitose, or xylitol (all at 0.1%, w/v). Similarly, citrate, propionate, and pyruvate are used as sole carbon sources (all at 1.0%, w/v). DL-α-Amino-*n*-butyric acid, L-cysteine, L-histidine, L-hydroxyproline, L-phenylalanine, potassium nitrate, and L-threonine are used as sole carbon and nitrogen sources, but not L-valine. Grows in the presence of crystal violet (0.0001%, w/v) and potassium tellurite (0.001%, w/v), but is inhibited by phenol (0.1%, w/v), sodium azide (0.01%, w/v), sodium chloride (7%, w/v), and thallous acetate (0.001%, w/v).

Resistant (μg/ml) to gentamicin sulfate (5), neomycin sulfate (8), novobiocin (10), penicillin G (20), polymyxin B (50), rifampin (10), streptomycin sulfate (16), tobramycin sulfate (8), and vancomycin hydrochloride (0.25), but sensitive to neomycin sulfate (50), triacyl oleandomycin (100) and rifampin (50). Additional phenotypic properties are shown in Table 238.

Whole-organism methanolyzates are rich in arabinose and galactose. Cellular fatty acid profiles contain major proportions of $C_{16:0}$ iso (41.4%), $C_{16:0}$ (13.2%), and $C_{15:0}$ iso (13.5%). Mycolic acids are absent. The polar lipid pattern contains DPG, PE, PG, PI, and PIMs. The predominant menaquinone is MK-9(H_4).

Source: isolated from soil.

DNA G+C content (mol%): 69.5 (T_m).

Type strain: MG417-CF17, CIP 106801, DSM 44213, JCM 10140, NRRL B-24138.

Sequence accession nos: AJ508236 (16S rRNA gene); EU822896 (*gyrB*).

Additional comments: the original spelling, *Amycolatopsis japonicum* (*sic*) was corrected at validation in line with Rule 61 of the Bacteriological Code (Associate Editor, 1997).

14. **Amycolatopsis jejuensis** Lee 2006, 552[VP]

je.ju.en′sis. N.L. fem. adj. *jejuensis* of Jeju Island, Republic of Korea.

Forms well-developed, branched aerial and substrate mycelia that fragment into rod-shaped elements. The substrate mycelium is yellowish brown and the aerial mycelium is white on yeast extract-malt extract agar (ISP medium 2); does not produce soluble pigments. Grows between 10 and 30°C.

Acid is produced from D-arabinose, but not from dextran, dulcitol, inulin, methyl α-D-mannoside, ribose, sorbose, L-xylose, or D-xylitol. Grows in the presence of 2%,

but not 3% (w/v) NaCl. Additional phenotypic properties are shown in Table 238.

Whole-organism hydrolysates are rich in arabinose and galactose. Muramic acid moieties are *N*-acetylated. Cellular fatty acids profiles contain major proportions of $C_{15:0}$ iso (14.3%), $C_{16:0}$ (20.2%), $C_{16:0}$ iso (14.6%), and $C_{18:0}$ (17.5%), and minor proportions (<10%) of $C_{14:0}$ (1.2%), $C_{16:1}$ (0.9%), $C_{14:0}$ iso (2.2%), $C_{15:0}$ (2.6%), $C_{17:0}$ (4.8%), $C_{17:0}$ iso (5.0%), $C_{15:0}$ anteiso (3.2%), $C_{17:0}$ anteiso (3.5%), $C_{20:0}$ (1.1%), $C_{14:0}$ 2-OH (0.6%), and $C_{15:0}$ iso 3-OH (7.6%). Mycolic acids are absent. The polar lipid pattern contains DPG, PE, PG, PI, and PIMs. The predominant menaquinone is MK-9(H_4).

Source: isolated from dried bat dung from a natural cave on Jeju Island, Republic of Korea.

DNA G+C content (mol%): 71.7 (HPLC).

Type strain: N7-3, DSM 45042, JCM 13280, NRRL B-24427.

Sequence accession nos: DQ000200 (16S rRNA gene); EU822897 (*gyrB*).

15. **Amycolatopsis kentuckyensis** Labeda, Donahue, Williams, Sells and Henton 2003, 1602[VP]

ken.tuc.ky.en′sis. N.L. fem. adj. *kentuckyensis* from Kentucky, named after the place of origin, the state of Kentucky, USA.

Forms a well-developed, yellow-orange to brownish-orange substrate mycelium and a light orangish-white to grayish orange-white aerial mycelium on most media. A faint brownish soluble pigment is produced on some media. Grows from 15 to 42°C.

Hippurate is hydrolyzed. Does not degrade adenine. Acid is produced from dulcitol, but not from methyl β-xyloside. Acetate and citrate are used as sole carbon sources; benzoate, lactate, malate, mucate, oxalate, propionate, succinate, and DL-tartrate are used weakly, if at all. Additional phenotypic properties are shown in Table 238.

Whole-organism hydrolysates are rich in arabinose and galactose. Cellular fatty acids profiles contain major proportions of $C_{15:0}$ iso (14.2%), $C_{16:0}$ iso (17.2%) and $C_{17:1}$ *cis*9 (10.1%), and smaller proportions (<10%) of $C_{14:0}$ iso (2.2%), $C_{15:0}$ anteiso (3.3%), $C_{15:1}$ (1.0%), $C_{15:0}$ (5.1%), $C_{16:1}$ *cis*9 (2.3%), $C_{16:0}$ (3.0%), $C_{16:0}$ 9-methyl (3.3%), $C_{17:0}$ iso (2.4%), $C_{17:0}$ anteiso (6.6%), $C_{16:0}$ 2-OH (2.9%), $C_{17:0}$ (6.2%), and $C_{17:0}$ 10-methyl (5.3%). The major phospholipid is PE; smaller amounts of PME are also present. The predominant menaquinones are MK-9(H_2) and MK-9(H_4).

Source: isolated from equine placentas.

DNA G+C content (mol%): not determined.

Type strain: LDDC 9447-99, DSM 44652, JCM 12570, NRRL B-24129.

Sequence accession no. (16S rRNA gene): AY183357.

16. **Amycolatopsis keratiniphila** Al-Musallam, Al-Zarban, Fasasi, Kroppenstedt and Stackebrandt 2003, 872[VP]

ke.ra.ti.ni′phi.la. N.L. n. *keratinum* keratin; N.L. adj. *philus -a -um* (from Gr. adj. *philos -ê -on*) friend, loving; N.L. fem. adj. *keratiniphila* keratin-loving, referring to the ability of the species to degrade keratin.

Additional comments: the type strain NRRL B-24117 was mistakenly cited as NRRL B24117 in Al-Musallam et al. (2003). The taxon was subsequently divided into two subspecies.

16a. **Amycolatopsis keratiniphila subsp. keratiniphila** Wink, Kroppenstedt, Ganguli, Nadkarni, Schumann, Seibert and Stackebrandt 2003a, 935[VP] (Effective publication: Wink, Kroppenstedt, Ganguli, Nadkarni, Schumann, Seibert and Stackebrandt 2003b, 44.) (*Amycolatopsis keratiniphila* Al-Musallam, Al-Zarban, Fasasi, Kroppenstedt and Stackebrandt 2003, 872)

Light gray aerial mycelium is formed; does not form a soluble pigment. Grows between 10 and 28°C.

Positive for *N*-acetyl-β-glucosamidase, acid and alkaline phosphatases, α-chymotrypsin, cystine arylamidase, esterase (C4), esterase lipase (C8), α- and β-galactosidases, α- and β-glucosidases, leucine arylamidase, lipase (C14), trypsin, and valine arylamidase, but negative for α-fucosidase, β-glucuronidase, α-mannosidase and naphthol-AS-BI-phosphohydrolase (API ZYM tests). Positive for arginine dehydrolase, β-galactosidase, and lysine and ornithine decarboxylases (API 20E tests). Acetoin-positive, but indole-negative. Arbutin is hydrolyzed, but does not produce H_2S. Elastin and Tween 40 are degraded, but not uric acid or xylan.

Resistant (filter paper discs soaked in μg/ml) to nalidixic acid (30), streptomycin (10), sulfonamide (200), and vancomycin (30), but sensitive to kanamycin (30). Additional phenotypic properties are shown in Table 238.

Whole-organism hydrolysates are rich in arabinose and galactose. The cellular fatty acid profile contains major proportions of $C_{16:0}$ iso (33.5%) and $C_{14:0}$ iso (13.9%), and smaller proportions (<10%) $C_{17:1}$ (9.9%), $C_{15:0}$ iso (8.0%), $C_{17:0}$ (7.6%), 2-hydroxy-$C_{16:0}$ iso (7.4%), $C_{15:0}$ (7.1%), $C_{16:1}$ (4.9%), $C_{16:0}$ (2.0%), $C_{15:0}$ anteiso, (1.9%), $C_{17:0}$ anteiso (1.3%), $C_{17:0}$ 10-methyl iso (1.0%), 2-hydroxy-$C_{15:0}$ anteiso (0.9%) and $C_{17:0}$ iso (0.8%). Mycolic acids are not formed. The polar lipid pattern contains DPG, HPE, PE, and PI. The predominant menaquinone is MK-9(H_4).

Source: isolated from agricultural soil in Kuwait using animal wool in bait.

DNA G+C content (mol%): not determined.

Type strain: D2, DSM 44409, JCM 12683, NRRL B-24117.

Sequence accession nos: AJ278496 (16S rRNA gene); EU822898 (*gyrB*).

16b. **Amycolatopsis keratiniphila subsp. nogabecina** Wink, Kroppenstedt, Ganguli, Nadkarni, Schumann, Seibert and Stackebrandt 2003a, 935[VP] (Effective publication: Wink, Kroppenstedt, Ganguli, Nadkarni Schumann, Seibert and Stackebrandt 2003b, 44.)

no.ga.be′ci.na. N.L. n. *nogabecinum* nogabecin (an antibiotic produced by the organism); L. fem. suff. *-ina* suffix used with sense of belonging to; N.L. fem. adj. *nogabecina* pertaining to nogabecin, an antibiotic produced by the organism.

The substrate mycelium is sand yellow on oatmeal, peptone-yeast extract-iron, and yeast extract-malt extract agars and beige on glycerol-asparagine, inorganic salts, and tyrosine agars; soluble pigments are not formed on any of these media. Aerial hyphae are not produced.

Positive for *N*-acetyl-β-glucosamidase, acid and alkaline phosphatases, α-chymotrypsin, cystine arylamidase, esterase

(C8), α- and β-galactosidases, α-glucosidase, leucine arylamidase, lipase (C14), α-mannosidase, naphthol-AS-BI-phosphohydrolase, trypsin, and valine arylamidase, but negative for esterase (C4), α-fucosidase, and β-glucuronidase (API ZYM tests). Positive for arginine dihydrolase, β-galactosidase, and lysine and ornithine decarboxylases (API 20E tests) Arbutin is hydrolyzed and H_2S produced. Tween 40 is degraded, but not elastin, uric acid, or xylan.

Resistant (filter paper discs soaked in µg/ml) to nalidixic acid (30), streptomycin (10) and vancomycin, but is susceptible to kanamycin (30) and sulfonamide (200). Additional phenotypic properties are shown in Table 238.

Whole-organism hydrolysates are rich in arabinose and galactose. The fatty acid profile contains major proportions of $C_{16:0}$ iso (31.9%), $C_{14:0}$ iso (12.3%), and $C_{17:0}$ (10.9%), and smaller proportions of $C_{15:0}$ (9.1%), $C_{16:0}$ (2.2%), $C_{17:1}$ *cis*9 (3.8%), $C_{15:0}$ iso (9.8%), $C_{17:0}$ iso (0.9%), $C_{15:0}$ anteiso (2.0%), $C_{17:0}$ anteiso (1.8%), $C_{18:0}$ iso (0.4%), $C_{17:0}$ 10-methyl iso (2.1%), $C_{17:0}$ 10-methyl (0.3%), 2-hydroxy-$C_{15:0}$ iso (3.9%), 2-hydroxy-$C_{15:0}$ iso (0.9%), 2-hydroxy-$C_{16:0}$ iso (7.7%), and 2-hydroxy-$C_{17:0}$ anteiso (0.9%). Mycolic acids are absent. The polar lipid pattern contains DPG, HPE, PE, and PI. The predominant menaquinone is MK-9(H_4).

Source: isolated from a soil sample collected in India.

DNA G+C content (mol%): not determined.

Type strain: FH 1893, DSM 44586, JCM 12671, NRRL B-24206.

Sequence accession nos: AJ508238 (16S rRNA gene); EU822899 (*gyrB*).

17. **Amycolatopsis lexingtonensis** Labeda, Donahue, Williams, Sells and Henton 2003, 1603[VP]

lex.ing.ton.en′sis. N.L. fem. adj. *lexingtonensis* from Lexington, named after the place of origin, Lexington, Kentucky, USA.

Forms an abundant dark orange-brown to dark reddish-brown substrate mycelium, a light yellow to purplish-tan aerial mycelium, and a dark red to reddish-brown soluble pigment on most media. Grows between 15 to 42°C.

Hippurate is hydrolyzed, but does not degrade adenine. Acid is produced from dulcitol (weak), but not from methyl α-D-glucoside. Acetate, citrate, oxalate, and propionate are used as sole carbon sources, but not benzoate, lactate, malate, mucate, succinate, or DL-tartrate. Additional phenotypic properties are shown in Table 238.

Whole-organism hydrolysates are rich in arabinose and galactose. Cellular fatty acid profiles contain major proportions of $C_{15:0}$ iso (10.2%) and $C_{16:0}$ iso (42.9%), and smaller proportions (<10%) of $C_{14:0}$ iso (4.1%), $C_{15:0}$ anteiso (1.2%), $C_{15:1}$ (1.7%), $C_{15:0}$ (4.2%), $C_{16:1}$ *cis*9 (4.0%), $C_{16:0}$ (2.2%), 9-methyl-$C_{16:0}$ (1.5%), $C_{17:0}$ iso (2.1%), $C_{17:0}$ anteiso (1.7%), $C_{17:1}$ *cis*9 (7.8%), $C_{16:0}$ 2-OH (7.8%), $C_{17:0}$ (3.3%), and $C_{17:0}$ 10-methyl (1.8%). The major phospholipid is PE. The predominant menaquinone is MK-9(H_4).

Source: isolated from an equine placenta.

DNA G+C content (mol%): not determined.

Type strain: LDDC 12275-99, DSM 44653, JCM 12672, NRRL B-24131.

Sequence accession no. (16S rRNA gene): AY183358.

18. **Amycolatopsis lurida** (Lechevalier, Prauser, Labeda and Ruan 1986) Stackebrandt, Kroppenstedt, Wink and Schumann 2004, 267[VP] [Basonym: *Amycolatopsis orientalis* subsp. *lurida* (*ex* Grundy, Sinclair, Theriault, Goldstein, Rickher, Warren, Oliver and Sylvester 1957) Lechevalier, Prauser, Labeda and Ruan 1986, 35; "*Nocardia lurida*" Grundy, Sinclair, Theriault, Goldstein, Rickher, Warren, Oliver and Sylvester 1957, 687.]

lu′ri.da. L. fem. adj. *lurida* pale yellow, sallow.

White aerial mycelium carries cylindrical, occasionally ovoid, smooth spores in straight to flexuous chains. Yellow to beige substrate mycelium branches frequently and appears to be slightly zig-zag. Grows at 10°C, but not at 45°C.

Positive for *N*-acetyl-β-glucosamidase, acid and alkaline phosphatases, α-chymotrypsin, esterase lipase, (C8), β-galactosidase, β-glucosidase, leucine arylamidase, lipase (C14), naphthol-AS-BI-phosphohydrolase, and trypsin, but negative for esterase (C4), cystine arylamidase, α-fucosidase, α-galactosidase, α-glucosidase, β-glucuronidase, α-mannosidase, and valine arylamidase (API ZYM tests). Positive for β-galactosidase, but not for arginine dihydrolase, or lysine and ornithine decarboxylases (API 20E tests). Acetoin positive, but indole-negative. Arbutin is hydrolyzed, but does not produce H_2S. Elastin and xylan are degraded, but not Tween 40 or uric acid.

Resistant (filter paper discs soaked in µg/ml) to nalidixic acid (30), streptomycin (10) and vancomycin (30), but sensitive to kanamycin (30) and sulfonamide (200). Grows in the presence of lysozyme. Additional phenotypic properties are shown in Table 238.

Whole-organism hydrolysates are rich in arabinose and galactose. Mycolic acids are present. The polar lipid pattern contains DPG as the diagnostic component. The predominant menaquinones are MK-9(H_2,H_4).

Source: isolated from soil.

DNA G+C content (mol%): 67 (T_m).

Type strain: ATCC 14930, DSM 43134, IFO (now NRBC) 14500, JCM 3141, LMG 4064, NRRL 2430, NRRL WC-3860, VKM Ac-1242.

Sequence accession nos: AJ577997 (16S rRNA gene); EU822900 (*gyrB*).

19. **Amycolatopsis marina** Bian, Li, Wang, Song, Liu, Dai, Ren, Gao, Hu, Liu, Li and Zhang 2009, 480[VP]

ma.ri′na. L. fem. adj. *marina* of the sea, marine.

Sparse, white aerial mycelium and branched yellow to yellow-brown substrate mycelium fragment into rod-like elements on yeast extract-malt extract agar medium (ISP medium 2). Diffusible pigments are not produced. Grows between 10 and 45°C (optimally at 28°C) and at pH 6–9 (optimally between pH 7 and 8).

H_2S is not produced. Tweens 20, 40, 60, and 80 are degraded, but not adenine or elastin. Cellobiose, fructose, galactose, maltose, mannitol, *myo*-inositol, rhamnose, ribose, trehalose, xylitol, and sodium acetate are used as sole carbon sources, but not DL-arabinose, methyl-D-lactose, raffinose, sorbitol, sucrose, or sodium citrate dehydrate (all at 1%, w/v). Grows in the presence of 12% (w/v) NaCl.

Resistant (filter paper discs soaked in 30 µg/ml antibiotic) to amikacin, carbenicillin, clarithromycin, kanamycin, penicillin G, rifampin, sulfamethoxazole and tobramycin, but sensitive to acetylspiramycin, carbenicillin, cephalothin, chloramphenicol, doxycycline, erythromycin, gentamicin, midecamycin, minocycline, novobiocin, and streptomycin. Additional phenotypic properties are shown in Table 238.

Whole-organism hydrolysates are rich in arabinose and galactose. Cellular fatty acid profiles contain major proportions of $C_{16:0}$ iso (40.4%) and $C_{16:0}$ iso 2-OH (11.4%), and smaller proportions (<10%) of $C_{17:1}$ *cis*9 (8.6%), $C_{16:1}$ *cis*9, (7.8%), $C_{16:0}$ (7.1%), $C_{17:0}$, (4.9%), $C_{17:0}$ iso (3.5%), $C_{15:0}$ (2.9%), $C_{16:1}$ iso H (2.7%), $C_{17:0}$ anteiso (2.3%), $C_{15:1}$ B (1.6%), $C_{15:0}$ iso (1.5%), $C_{16:0}$ 10-methyl (1.4%), $C_{18:0}$ (1.4%), $C_{18:1}$ *cis*9 (1%), $C_{14:0}$ iso (0.9%), and $C_{18:0}$ iso (0.8%). The polar lipid pattern contains DPG, PE, PG, PI, PIMs, and PME. The predominant menaquinone is MK-9(H_4) (79%); also contains minor amounts of MK-8(H_4).

Source: isolated from a sediment sample collected from the South China Sea.

DNA G+C content (mol%): 70.1 (HPLC).

Type strain: Ms392A, CGMCC 4.3568, NBRC 104263.

Sequence accession no. (16S rRNA gene): EU329845.

20. **Amycolatopsis mediterranei** (Margalith and Beretta 1960) Lechevalier, Prauser, Labeda and Ruan 1986, 35[VP] [Basonym: *Nocardia mediterranei* (Margalith and Beretta 1960) Thiemann, Zucco, Pelizza 1969, 106; "*Streptomyces mediterranei*" Margalith and Beretta 1960, 321.]

med.i.ter.ra′ne.i. L. neut. gen. n. *mediterranei* of the interior of the land, from the Mediterranean area.

Substrate hyphae may show a zig-zag appearance. Aerial hyphae, when formed, differentiate into long, straight to flexuous chains of ellipsoid to oblong spores. Yellow colored colonies are formed on modified Bennett's agar. Grows from 10 to 42°C, but not at 45°C.

Acid and alkaline phosphatase-positive, but is negative for *N*-acetyl-β-glucosamidase, α-chymotrypsin, cystine arylamidase, esterase (C4), esterase lipase C8), α-fucosidase, α- and β-galactosidases, α- and β-glucosidases, leucine arylamidase, lipase (C14), α-mannosidase, naphthol-AS-BI-phosphohydrolase, trypsin, and valine arylamidase. Acetate, citrate, lactate, malate, oxalate, propionate, pyruvate, and succinate are decarboxylated, but not benzoate, mucate, or tartrate.

Resistant (µg/ml) to gentamicin sulfate (5), neomycin sulfate (8), tobramycin sulfate (8), novobiocin (10), polymyxin B (50), rifampin (10) and vancomycin hydrochloride (0.25). Additional phenotypic properties are shown in Table 238.

Whole-organism hydrolysates are rich in arabinose and galactose. Muramic acid moieties are *N*-acetylated. The cellular fatty acid profile contains major proportions of $C_{16:0}$ iso (44.9%), and smaller proportions (<10%) of $C_{14:0}$ iso (1.2%), $C_{15:0}$ iso (9.3%), $C_{15:0}$ anteiso (1.4%), $C_{15:1}$ (2.0%), $C_{15:0}$ (3.1%), $C_{16:1}$ iso H (2.3%), $C_{16:0}$ (1.0%), $C_{16:1}$ *cis*9 (1.3%), $C_{17:1}$ iso G (1.5%), $C_{17:0}$ iso (2.0%), $C_{17:0}$ anteiso (4.0%), $C_{17:1}$B (6.1%), $C_{17:1}$ C (9.0%), $C_{17:0}$ C (2.9%), and $C_{18:1}$ (5.6%). Does not contain mycolic acids. The predominant menaquinones are MK-9(H_4) and MK-9(H_6).

Source: isolated from a soil sample collected in a *Pinus* arboretum near St Raphael in France.

DNA G+C content (mol%): 67–69 (T_m).

Type strain: ATCC 13685, CCUG 43144, CIP 107074, DSM 43304, NBRC 13415, IMET 7651, ISP 5501, JCM 4789, KCTC 1739, NRRL B-3240, VKM Ac-798.

Sequence accession nos: AJ293754 (16S rRNA gene); EU822901 (*gyrB*).

Additional comments: Amycolatopsis mediterranei DSM 46095 and DSM 40696 have been reclassified as *Amycolatopsis rifamycinica* (Bala et al., 2004).

21. **Amycolatopsis methanolica** de Boer, Dijkhuizen, Grobben, Goodfellow, Stackebrandt, Parlett, Whitehead and Witt 1990, 203[VP]

me.tha.no′li.ca. N.L. n. *methanol* methanol; L. fem. suff. -*ica* suffix used with the sense of pertaining to; N.L. fem. adj. *methanolica* relating to methanol.

Forms a yellow substrate mycelium which bears white aerial hyphae that differentiate into smooth, squarish to oval spores (0.4 × 0.6–0.8 µm) on long, straight to flexuous chains on Czapek Dox agar. Grows from 10 to 50°C.

Decomposes DNA and elastin, but not adenine, arbutin, or tributyrin. Acetate, benzoate, fumarate, 2-oxoglutarate, lactate, malate, propionate, pyruvate, and succinate are used as sole carbon sources, but not citrate, formate, oxalate or gluconate. Acid is produced from ribose, but not from dulcitol.

Resistant to (µg/ml) cephaloridine hydrochloride (10), gentamicin sulfate (5), lincomycin hydrochloride (10), neomycin sulfate (10), oleandomycin phosphate (2), penicillin G (20), novobiocin (10), polymyxin B (50), and vancomycin hydrochloride (0.25), but sensitive to lysozyme. Additional phenotypic properties are shown in Table 238.

Whole-organism hydrolysates are rich in arabinose and galactose. The cellular fatty acid profile contains major proportions of $C_{16:0}$ iso (32.0%), $C_{16:0}$ (24.3%), and $C_{17:0}$ anteiso (21.8%). Mycolic acids are absent. The polar lipid pattern contains DPG, PE, PME, and PIMs. The predominant menaquinones are MK-9(H_2,H_4).

Source: isolated from a soil sample collected in New Guinea.

DNA G+C content (mol%): not determined.

Type strain: 239, DSM 44096, NBRC 15065, IMSNU 20055, JCM 8087, LMD 80.32, NCIB (now NCIMB) 11946, NRRL B-24139.

Sequence accession nos: AJ249135 (16S rRNA gene); EU822902 (*gyrB*).

Additional comments: the type strain of *Amycolatopsis methanolica* cleaves a broad range of 7-amino-methylcoumarin and 4-methylumbelliferone conjugated substrates and grows in a mineral medium broth containing diverse sole carbon compounds (De Boer et al., 1990).

22. **Amycolatopsis minnesotensis** Lee, Kinkel and Samac 2006c, 268[VP]

min.ne.sot.en′sis. N.L. fem. adj. *minnesotensis* pertaining to Minnesota, the origin of the soil sample from which the type strain was isolated.

Forms a well-developed, white aerial mycelium and a yellow vegetative mycelium that fragment into rod-shaped elements. Grows from 10 and 30°C, but not at 37°C.

Positive for acid and alkaline phosphatase, esterase (C4), and naphthol-AS-BI-phosphohydrolase, but negative for α-chymotrypsin, cystine arylamidase, α- and β-galactosidases, α-glucosidase, lipase (C14), α-mannosidase, trypsin, and valine arylamidase. Acid is produced from D-xylitol, but not from 2,3-butanediol, dextran, dulcitol, or 1,2-propanediol. Additional phenotypic properties are shown in Table 238.

Resistant (filter paper discs soaked in µg/ml) to kanamycin (30), streptomycin (10) and vancomycin (30), but sensitive to nalidixic acid (30) and sulfonamide (200).

Predominant menaquinones are MK-9(H_4); major fatty acids are $C_{16:0}$ iso, $C_{16:0}$, $C_{15:0}$ iso, and $C_{17:0}$.

Source: isolated from a prairie soil.

DNA G+C content (mol%): 69.5.

Type strain: 32U-2, DSM 44988, JCM 14545, KCCM 42246, NRRL B-24435.

Sequence accession nos: DQ076482 (16S rRNA gene); EU822903 (*gyrB*).

23. **Amycolatopsis nigrescens** Groth, Tan, González, Laiz, Carlsohn, Schütze, Wink and Goodfellow 2007, 517[VP]

ni.gres′cens. L. part. adj. *nigrescens* becoming black.

Forms extensively branched hyphae (0.7–0.9 µm in diameter) that fragment into squarish rod-like elements. The color of the substrate mycelium changes from orange to black with the production of a dark reddish-black soluble pigment. The substrate mycelium carries sparse to moderate white or pale-orange aerial hyphae. Grows between 20 and 40°C, but not at 10 or 42°C, and between pH 5 and 9 but not at pH 4.5 or 10.

Tween 80 is degraded, but not adenine. Positive for *N*-acetyl-β-glucosamidase, acid and alkaline phosphatases, α-chymotrypsin, cystine arylamidase, esterase (C4), esterase lipase (C8), leucine arylamidase, lipase (C14), naphthol-AS-BI-phosphohydrolase, and valine arylamidase, but negative for α-galactosidase, α-glucosidase, α-mannosidase, and trypsin (API ZYM tests).

Hippurate is hydrolyzed. L-arabinose, fructose, glucose, mannitol, *myo*-inositol, raffinose, and xylose are used as sole carbon sources, but not cellulose, or sucrose. Similarly, acetate, aconitate, citrate, malate, and succinate are used as sole carbon sources, but not benzoate or DL-tartrate. Grows in the presence of 6% (w/v) NaCl.

Resistant (filter paper discs soaked in µg/ml) to lincomycin hydrochloride (2), kanamycin (30), meticillin (5), nalidixic acid (30), norfloxacin (10), streptomycin (10), sulfonamide (200), and vancomycin (30), but sensitive to chloramphenicol (30), imipenem (10), ofloxacin (10), oxytetracycline hydrochloride (30), and rifampin (30). Additional phenotypic properties are shown in Table 238.

Whole-organism hydrolysates are rich in arabinose and galactose. Muramic acid moieties are *N*-acetylated. The cellular fatty acid profile contains major proportions of $C_{16:0}$ iso (27–28%), and smaller proportions (<10%) of $C_{17:0}$ (7.3–9.3%), $C_{17:1}$ *cis*9 (8.4–9.8%), $C_{16:0}$ (7.2–7.4%), $C_{16:0}$ iso 2-OH (6.4–7.9%), $C_{15:0}$ iso (5.2–5.7%), $C_{18:1}$ *cis*9 (4.8–4.9%), $C_{16:1}$ iso (4.2–4.4%), $C_{17:0}$ anteiso (3.7–3.8%), $C_{16:1}$ *cis*9 (3.1–3.8%), $C_{18:0}$ (3.3%), and an unidentified component (3.0–3.7%). Mycolic acids are absent. The polar lipid pattern includes DPG, HPE, PG, PI, and PS. The predominant menaquinone is MK-11(H_4) (52–54%); also contains smaller proportions of MK-12(H_4) (18%), MK-9(H_4) (8–9%), and MK-10(H_4) (9%).

Source: isolated from the wall of St Callistus hypogean Roman catacomb.

DNA G+C content (mol%): not determined.

Type strain: CSC17Ta-84, DSM 44992, HKI 0330, JCM 14717, NRRL B-24473.

Sequence accession nos: DQ486888 (16S rRNA gene); EU822904 (*gyrB*).

24. **Amycolatopsis niigatensis** Ding, Hirose and Yokota 2007, 1750[VP]

ni.i.ga.ten′sis. N.L. fem. adj. *niigatensis* referring to Niigata Prefecture, Japan, the source of the type strain.

The substrate mycelium is purple brown and the aerial mycelium white to light yellow. Grows between 5 to 45°C, optimally at 30°C, and from pH 6 to 11, optimally at pH 9.

Positive for *N*-acetyl-β-glucosamidase, alkaline phosphatase, α-chymotrypsin, cystine arylamidase, esterase (4), esterase lipase (C8), α- and β-galactosidases, α-glucosidase, leucine arylamidase, α-mannosidase, naphthol-AS-BI-phosphohydrolase, and valine arylamidase, but negative for α-fucosidase, β-glucosidase, and lipase (C14). Grows in the presence of 7% (w/v) NaCl. Additional phenotypic properties are shown in Table 238.

Whole-organism hydrolysates are rich in arabinose and galactose. Cellular fatty acids contain a major proportion of $C_{16:0}$ iso (40%), and smaller proportions (<10%) of $C_{14:0}$ iso (7.1%), $C_{14:0}$ (0.4%), $C_{15:0}$ iso (9.3%), $C_{15:0}$ anteiso (7.7%), $C_{15:0}$ (4.5%), $C_{16:0}$ (2.1%), $C_{16:1}$ iso OH (2.6%), $C_{17:0}$ iso (1.6%), $C_{17:0}$ anteiso (6.9%), $C_{17:0}$ (2.6%), $C_{17:1}$ ω6*c* (6.6%), and $C_{17:1}$ ω8*c* (1.7%). Mycolic acids are absent. The predominant menaquinone is MK-9(H_4).

Source: isolated from filtration material made from volcanic soil, Niigata, Japan.

DNA G+C content (mol%): 72.4 (HPLC).

Type strain: LC11, CCTCC AB206020, IAM 15388, JCM 21832.

Sequence accession nos: AB248537 (16S rRNA gene); EU822905 (*gyrB*).

25. **Amycolatopsis palatopharyngis** Huang, Paściak, Liu, Xie and Gamian 2004, 361[VP]

pa.la.to.pha.ryn′gis. N.L. n. *palatopharynx* palatopharynx; N.L. gen. n. *palatopharyngis* of the palatopharynx.

Forms a branched yellow to yellow-brown substrate mycelium that fragments into rod-like elements. White aerial hyphae are produced sparsely on Bennett's, glucose-yeast extract-malt extract, and brain-heart infusion agars and moderately on inorganic salts-starch agars. Aerial hyphae differentiate into long chains of spore-like structures. Diffusible pigments are not produced. Grows between 10 and 40°C, but not at 45°C, and between pH 6 and 10. Grows in the presence of 10% (w/v) NaCl.

Resistant (µg/ml) to ampicillin (10), carbenicillin (100), and cephalothin (30), but sensitive to acetylspiramycin (15), chloramphenicol (30), clarithromycin (10), doxycycline (30), gentamicin (10), kanamycin (30), midecamycin (15),

minocycline (30), novobiocin (5), penicillin (30), rifampin (5), streptomycin (10), and tobramycin (10). Additional phenotypic properties are shown in Table 238.

Whole-organism hydrolysates are rich in arabinose and galactose. The cellular fatty acid profile contains major proportions (<10%) of $C_{16:0}$ iso (50.3%), $C_{16:0}$ (21.7%), and $C_{17:0}$ anteiso (12.4%), and smaller proportions of $C_{16:0}$ anteiso (3.6%), $C_{17:0}$ (8.5%), and $C_{18:0}$ (3.7%). Mycolic acids are absent. The polar lipid pattern includes PE, DPG, and PI. The predominant menaquinone is MK-9(H_4).

Source: isolated from an infected palatopharyngeal mucosa of an elderly human patient.

DNA G+C content (mol%): 65.8 (T_m).

Type strain: 1BDZ, AS 4.1729, DSM 44832, JCM 12460, PCM 2600.

Sequence accession nos: AF479268 (16S rRNA gene); EU822907 (*gyrB*).

26. **Amycolatopsis plumensis** Saintpierre-Bonaccio, Amir, Pineau, Tan and Goodfellow 2005, 2060[VP]

plum.en′sis. N.L. fem. adj. *plumensis* referring to the Plum region of the main island of New Caledonia, the source of the soil from which the type strain was isolated.

Forms extensively branched substrate hyphae that fragment into squarish, rod-like elements. A pale-orange substrate mycelium is formed on modified Bennett's agar, but diffusible pigments are absent. The substrate mycelium carries abundant pale-orange aerial hyphae. Grows between 20 and 37°C, but not at 10 or 45°C, and between pH 4 to12.

H_2S is formed. Elastin, Tween 80, and xylan are degraded.

Resistant (μg/ml) to erythromycin (4), gentamicin sulfate (10), rifampin (6), and streptomycin sulfate (5). Grows in the presence of crystal violet (0.0002%, w/v), phenol (0.01%, w/v), and potassium tellurite (0.005%, w/v). Additional phenotypic properties are shown in Table 238.

Whole-organism hydrolysates are rich in arabinose and galactose. The predominant menaquinone is MK-9(H_4).

Source: isolated from brown hypermagnesian ultramafic soil at the southern end of the main island of New Caledonia.

DNA G+C content (mol%): not determined.

Type strain: SBHS Strp1, DSM 44776, JCM 13852, NBRC 102106, NRRL B-24324.

Sequence accession nos: AY262825 (16S rRNA gene); EU822908 (*gyrB*).

27. **Amycolatopsis pretoriensis** Labeda, Donahue, Williams, Sells and Henton 2003, 1604[VP]

pre.tor.i.en′sis. N.L. fem. adj. *pretoriensis* from Pretoria, named after the place of origin, Pretoria, South Africa.

Forms a well-developed grayish-yellow to orange-brown substrate mycelium and an abundant white to orange-white aerial mycelium on most media. Faint soluble pigments are produced on some media, such as yeast extract-malt extract agar. Grows from 15 to 37°C.

Positive for cystine arylamidase and β-galactosidase, but negative for α-fucosidase, β-glucosidase, and valine arylamidase (API ZYM tests). Hippurate is hydrolyzed. Acid is produced from dulcitol, but not from methyl β-xyloside or melezitose. Acetate, benzoate (weak), citrate (weak), and lactate (weak) are used as a sole carbon sources, but not malate, mucate, or tartrate. Additional phenotypic properties are shown in Table 238.

Whole-organism hydrolysates are rich in arabinose and galactose. Cellular fatty acid profiles contain major proportions of $C_{16:0}$ iso (45.5%) and $C_{16:0}$ 2-OH (12.3%), and smaller proportions (<10%) of $C_{14:0}$ iso (3.4%), $C_{15:0}$ iso (7.9%), $C_{15:1}$ (3.3%), $C_{15:0}$ (3.1%), $C_{17:0}$ anteiso (1.3%), $C_{17:1}$ *cis*9 (8.9%), $C_{17:0}$ (2.7%), and $C_{17:0}$ 10-methyl (2.6%). The major polar lipids are PE and PME. The predominant menaquinones are MK-9(H_2) and MK-9(H_4).

Source: isolated from an equine placenta in Pretoria, South Africa.

DNA G+C content (mol%): not determined.

Type strain: ARC OVI 0181, DSM 44654, JCM 12673, NRRL B-24133.

Sequence accession no. (16S rRNA gene): AY183356.

28. **Amycolatopsis regifaucium** Tan, Robinson, Lacey, Brown, Kim and Goodfellow 2007, 2566[VP]

re.gi.fau′ci.um. L. n. *rex regis* king; L. gen. pl. n. *faucium* of a defile; N.L. gen. pl. n. *regifaucium* of King's Canyon, Australia, the source of the soil from which the first strains were isolated.

Forms an extensively branched substrate mycelium which fragments into squarish, rod-like elements. An abundant, light-gray aerial mycelium and a dark yellow–brown substrate mycelium with filamentous edges are formed on modified Bennett's agar supplemented with mannitol and soybean flour; a dark gray–brown diffusible pigment is also produced. Grows between 10 and 37°C, and between pH 5 and 10.

Arbutin is hydrolyzed. Elastin, Tween 40, uric acid, and xylan are degraded. L-Arabinose, arabitol, cellobiose, dextrin, galactose, glucose, glycerol, glycogen, *myo*-inositol, maltose, mannitol, methyl α-D-glucoside, ribose, sucrose, glycogen, and xylitol are used as sole carbon sources, but not adonitol, *meso*-erythritol, melezitose, melibiose, raffinose, or sorbitol.

Resistant (μg/ml) to gentamicin sulfate (5), neomycin sulfate (8), novobiocin (10), penicillin G (20), polymyxin B sulfate (50), rifampin (10), streptomycin sulfate (16), and tobramycin sulfate (8). Additional phenotypic properties are shown in Table 238.

Whole-organism hydrolysates are rich in arabinose and galactose. Does not contain mycolic acids. The polar lipid pattern includes DPG, PE, PG, PI, PIMs, and PME. The predominant menaquinone is MK-9(H_4) (71%); also contains MK-9(H_6) (29%).

Source: isolated from an arid soil collected from King's Canyon, Australia.

DNA G+C content (mol%): not determined.

Type strain: GY080, DSM 45072, JCM 15588, NCIMB 14277.

Sequence accession nos: AY129760 (16S rRNA gene); EU822909 (*gyrB*).

29. **Amycolatopsis rifamycinica** Bala, Khanna, Dadhwal, Prabagaran, Shivaji, Cullum and Lal 2004, 1148[VP]

rif.a.my.ci′na. N.L. n. *rifamycinum* rifamycin; L. suff. *-icus -a -um* related to; N.L. fem. adj. *rifamycinica* referring to the ability to produce rifamycin.

Orange-colored substrate mycelium is produced on yeast extract and glucose-asparagine agars and a white to very pale pink aerial mycelium is produced on oatmeal and yeast extract-molasses agars. A light pale to brown yellow pigment is formed on tyrosine, yeast extract-glucose, and oatmeal agars. Grows from 10 to 37°C, but not at 45°C.

Positive for cystine arylamidase, β-galactosidase, β-glucosidase, and valine arylamidase, but negative for α-fucosidase (API ZYM tests). Additional phenotypic properties are shown in Table 238.

The cellular fatty acid profile contains major proportions of $C_{16:0}$ iso (24%), $C_{17:0}$ anteiso (11%), and $C_{18:1}$ (25%), and smaller proportions (<10%) of $C_{15:0}$ iso (3%), $C_{16:0}$ (4%), iso $C_{17:0}$ (9%), $C_{17:1}$ (4%), and $C_{18:4}$ (4%). The polar lipid pattern includes cardiolipin, PE, PG, and PI.

Source: isolated from a soil sample in an arid region near Alice Springs, Northern Territory, Australia.

DNA G+C content (mol%): not determined.

Type strain: nt 19, ATCC 27643, DSM 46095, JCM 12674.

Sequence accession nos: AY083603 (16S rRNA gene); EU822910 (*gyrB*).

Additional comments: DNA restriction profiles of seven *Amycolatopsis mediterranei* strains provided further evidence that strain DSM 46095 was misclassified in this species (Bala et al., 2004), a result in line with the results of an earlier study (Kaur et al., 2001).

30. **Amycolatopsis rubida** Huang, Qi, Lu, Liu and Goodfellow 2001, 1096[VP]

ru.bi′da. L. fem. adj. *rubida* reddish.

Forms branching white to yellowish substrate mycelium which fragments into squarish elements (0.4–0.5 × 1.2–3.0 µm). An abundant white aerial mycelium which fragments into squarish elements is produced on modified Bennett's agar. A reddish diffusible pigment is formed on glucose-asparagine agar. Grows between 10 and 40°C. Grows in the presence of 7% (w/v) NaCl.

Resistant (µg/ml) to gentamicin sulfate (5), neomycin sulfate (8), novobiocin (10), penicillin G (20), polymyxin B (50), streptomycin sulfate (30), tobramycin sulfate (8), and vancomycin hydrochloride (0.25), but sensitive to chloramphenicol (30) and erythromycin (15). Also resistant to lysozyme (0.005%, w/v). Additional phenotypic properties are shown in Table 238.

Whole-organism hydrolysates are rich in arabinose and galactose. The cellular fatty acid profile contains major proportions of $C_{14:0}$ iso (11.3%), $C_{15:0}$ (14.6%), $C_{15:1}$ ω6*c* (12.8%), $C_{16:0}$ iso (12.8%) and $C_{17:0}$ (11.3%), and smaller proportions (<10%) of $C_{14:0}$ (0.5%), $C_{15:0}$ iso (6.8%), $C_{15:0}$ anteiso (3%), $C_{16:0}$ (5.2%), $C_{16:1}$ iso H (0.2%), $C_{17:0}$ iso (0.4%), $C_{17:0}$ anteiso (0.8%), and $C_{18:0}$ (0.4%). Mycolic acids are absent. The polar lipid pattern includes DPG, PE, PIMs, and PME. The predominant menaquinone is MK-9(H_4).

Source: isolated from coniferous forest soil collected in Guangxi Province, China.

DNA G+C content (mol%): 67.4 (T_m).

Type strain: 13.4, AS4. 1541, CIP 107102, DSM 44637, JCM 10871 NBRC 100041, NRRL B-24150.

Sequence accession nos: AF222022 (16S rRNA gene); EU822911 (*gyrB*).

31. **Amycolatopsis saalfeldensis** Carlsohn, Groth, Tan, Schütze, Saluz, Munder, Yang, Wink and Goodfellow 2007, 1644[VP]

sa.al.fel.den′sis. N.L. fem. adj. *saalfeldensis* from Saalfeld, named after the place of origin, a town in Thuringia, Germany.

Forms extensively branched substrate mycelium (hyphal diameter 0.5–0.6 µm) that fragments into squarish rod-like elements on oatmeal and yeast-extract-malt extract agars. The substrate mycelium carries moderate amounts of white aerial hyphae on oatmeal agar which fragment into squarish rod-like elements. Diffusible pigments are not produced. Grows between 20 and 35°C, and between pH 4.5 and 8, but not at pH 9.

Positive for *N*-acetyl-β-glucosamidase, acid and alkaline phosphatases, α-chymotrypsin, esterase (C4), esterase lipase (C8), α-glucosidase, naphthol-AS-BI-phosphohydrolase, and valine arylamidase, but negative for cystine arylamidase, α-fucosidase, α- and β-galactosidases, lipase (C14), α-mannosidase, and trypsin (API ZYM tests). Hippurate is hydrolyzed, and H_2S and oxidase are produced, but does not form indole. Tween 80 is degraded, but not adenine. L-Arabinose, fructose, glucose, *myo*-inositol, mannitol, raffinose, rhamnose (weakly), sucrose, and xylose are used as sole carbon sources, but not cellulose (all at 1%, w/v). Similarly, acetate, aconitate, benzoate (weakly), citrate, malate, and succinate are used as sole carbon sources, but not DL-tartrate (all at 0.2%, w/v).

Resistant (filter paper discs soak in µg/ml) to ampicillin (10), methicillin (5), norfloxacin (10), novobiocin (5), penicillin G (10 IU), and polymyxin B (300 IU), but sensitive to chloramphenicol (30), ciprofloxacin (5), imipenem (10), kanamycin sulfate (30), lincomycin hydrochloride (2), ofloxacin (10), oxytetracycline hydrochloride (30), rifampin (30), streptomycin sulfate (10), and vancomycin hydrochloride (30). Additional phenotypic properties are shown in Table 238.

Whole-organism hydrolysates are rich in arabinose and galactose. Muramic acid moieties are *N*-acetylated. Cellular fatty acid profiles contain major proportions of $C_{16:0}$ iso (41–42%), and smaller proportions of $C_{17:0}$ iso (8–10%), $C_{14:0}$ iso (7–9%), $C_{15:0}$ iso (8–9%), $C_{16:0}$ iso 2-OH (4–6%), $C_{15:0}$ (6%), $C_{17:0}$ (5–7%), and $C_{17:0}$ iso (8–10%). Mycolic acids are absent. The polar lipid pattern includes DPG, hydroxyphosphatidylethanolamine, PE, PG, and PS. The predominant menaquinone is MK-9(H_4) (86.9%); also contains minor amounts of MK-8(H_4) (4.5%), MK-9(H_6) (1.0–3.0%), and MK-10(H_4) (2.0%).

Source: isolated from the surface of rocks in a medieval alum slate mine.

DNA G+C content (mol%): not determined.

Type strain: HKI 0457, DSM 44993, JCM 14909, NRRL B-24474.

Sequence accession nos: DQ792500 (16S rRNA gene); EU822912 (*gyrB*).

Additional comments: grows on minimal medium supplemented with $CuSO_4$ (2 mM) and $NiCl_2$ (5 mM) (Carlsohn et al., 2007). The type strain DSM 44993 was mistakenly cited as DSM 44493 in the protologue of the valid publication.

32. **Amycolatopsis sacchari** Goodfellow, Kim, Minnikin, Whitehead, Zhou and Mattinson-Rose 2001, 191[VP]

sac′char.i. N.L. n. *Saccharum* generic name of sugar cane; N.L. gen. n. *sacchari* of sugar cane.

Forms branched substrate mycelium that fragments into rod-like elements when grown on modified Bennett's and Czapek Dox-yeast extract-casein (CYC) agars. The substrate mycelium carries moderate to abundant white aerial hyphae which differentiate into straight chains of spore-like structures on CYC agar. Diffusible pigments are not produced. Grows between 20 and 45°C, but not at 55°C, and between pH 5 and 8.

Positive for alkaline phosphatase, α-chymotrypsin, cystine arylamidase, esterase (C4), α-glucosidase, lysine (C14), naphthol-AS-BI-phosphohydrolase, and valine arylamidase, but negative for α- and β-galactosidases, α-mannosidase, and trypsin. Testosterone, and Tweens 20, 40, 60 and 80 are degraded. Adonitol, D- and L-arabinose, cellobiose, dextrin, *meso*-erythritol, fructose, D- and L-fucose, galactose, gentiobiose, glycerol, glycogen, lactose, maltose, mannitol, mannose, methyl α-D-glucoside, methyl β-D-glucoside, rhamnose, ribose, salicin, sorbose, sucrose, trehalose, xylitol, and xylose are used as sole carbon sources, but not amygdalin, arabitol, dulcitol, *myo*-inositol, melibiose, sorbitol, or tyrosine (all at 1%, w/v). Similarly, L-alanine, androsterone, arbutin, butan-1-ol, butyrate, ergosterol, ethanol, hippurate, propan-1-ol, propan-2-ol, propionate, propylase glycol, protocatechuic acid, pyruvate, quinic acid, L-serine, shikimic acid, succinate, testosterone, and tyrosine are used as sole carbon sources, but not acetamide, benzamide, catechol, L-citrulline, *p*-cresol, fumarate, *m*- or *p*- hydroxybenzoic acid, β-hydroxybutyric acid, 15-mandelic acid, squalene, syringealdehyde, tartrate, trimethylamine, or vanillin (at 0.1% w/v or v/v).

Grows in the presence of (μg/ml) bismuth citrate (10), crystal violet (1), phenol (100), phenyl ethanol (4000), potassium tellurite (10), sodium azide (10), sodium chloride (7%, w/v), teepol (100), tetrazolium (100), and thallous acetate (10), but sensitive to bismuth citrate (100), crystal violet (100), sodium chloride (10), tetrazolium (1000), and thallous acetate (100).

Resistant to (μg/ml) cephaloridine hydrochloride (250), chloramphenicol (50), demeclocycline hydrochloride(8), gentamicin sulfate (5), kanamycin sulfate (5), lincomycin hydrochloride (10), neomycin sulfate (10), nalidixic acid (30), oleandomycin phosphate (2), penicillin G (20), polymyxin B sulfate (50), rifampin (2), streptomycin sulfate (16), tobramycin sulfate (8), and vancomycin hydrochloride (0.25), but sensitive to kanamycin sulfate (50) and tetracycline hydrochloride (5). Additional phenotypic properties are shown in Table 238.

Whole-organism hydrolysates are rich in arabinose and galactose. The cellular fatty acid profile contains major proportions of $C_{17:0}$ anteiso (30–58%), and smaller proportions of $C_{16:0}$ (4–14%), $C_{17:0}$ (2–5%), $C_{17:1}$ (1–3%), $C_{17:0}$ anteiso (7–11%), and $C_{17:0}$ iso (7–11%). Mycolic acids are absent. The polar lipid pattern includes DPG, PE, PG, and PI. The predominant menaquinone is MK-9(H_4) (96%).

Source: isolated from sugar cane bagasse and from floor dust of a hemp factory.

DNA G+C content (mol%): not determined.

Type strain: K24, CIP 107029, DSM 44468, JCM 11272, KCTC 9863, NBRC 100339.

Sequence accession nos: AF223354 (16S rRNA gene); EU822913 (*gyrB*).

Additional comments: cleaves a broad range of 7-amino-4-methyl coumarin and 4-methylumbelliferone conjugated substrates (Goodfellow et al., 2001).

33. **Amycolatopsis sulphurea** Lechevalier, Prauser, Labeda and Ruan 1986, 35[VP] ("*Nocardia sulphurea*" Oliver and Sinclair 1964)

sul.phu′re.a. L. n. *sulphur* sulfur; L. masc. suff. *-eus* suffix used with various meanings; N.L. fem. adj. *sulphurea* of sulfur, referring to the yellow color of the substrate hyphae.

Forms a white to yellowish to olive substrate mycelium, which tends to break down into fragments. The aerial mycelium is light yellow. A dark brown diffusible pigment is produced on modified Bennett's agar. Grows between 10 to 37°C.

Does not degrade adenine. Acetate, citrate, lactate, malate, propionate, pyruvate, and succinate are used as sole carbon sources, but not benzoate, mucate, oxalate, or tartrate.

Resistant (μg/ml) to lysozyme, neomycin sulfate (4), tobramycin sulfate (8), novobiocin (10), polymyxin B (50), and vancomycin hydrochloride (0.25). Additional phenotypic properties are shown in Table 238.

Whole-organism hydrolysates are rich in arabinose and galactose. Mycolic acids are absent. The cellular fatty acid profile contains major proportions of $C_{16:0}$ iso (21.0%) and $C_{16:0}$ (17.0%), and smaller proportions (<10%) of $C_{14:0}$ iso (3–7%), $C_{15:0}$ iso (9–3%), $C_{15:0}$ anteiso 5.0%), $C_{15:0}$ (3–4%), *cis*9 $C_{16:0}$ (3–9%), $C_{17:0}$ iso (2.0%), $C_{17:0}$ anteiso (5–6%), $C_{17:1}$ (8–9%), $C_{17:0}$ iso (9.3%), and $C_{18:0}$ (3.2%). The polar lipid pattern includes PE and PME. The predominant menaquinones are MK-9(H_2,H_4).

Source: isolated from soil.

DNA G+C content (mol%): 67 (T_m).

Type strain: ATCC 27624, DSM 46092, NBRC 13270, IMET 7649, JCM 3142, VKM Ac-1244.

Sequence accession nos: AF051343 (16S rRNA gene); EU822914 (*gyrB*).

Additional comments: the name "*Nocardia sulphurea*" Oliver and Sinclair (1964) was described in a patent which means that the name has not been effectively published [Rule 25b (5) Bacteriological Code]. Consequently, this nomenclatural name cannot be revived (Rule 28a) and the citation of *Nocardia sulphurea* cannot refer to the original authors.

34. **Amycolatopsis taiwanensis** Tseng, Yang, Li and Jiang 2006, 1814[VP]

tai.wan.en′sis. N.L. fem. adj. *taiwanensis* of Taiwan, where the type strain was isolated.

The substrate mycelium is light yellow on Bennett's, peptone-yeast extract-iron, tyrosine and yeast extract-malt extract agars; yellowish-white on glucose-asparagine, inorganic salts-starch and oatmeal agars, and purple-yellow on tryptone-yeast extract agars. Short chains of oval spores are formed, albeit poorly, on glycerol-asparagine, inorganic

salts, starch, and tyrosine agars. Soluble pigments are not formed. Does not produce aerial hyphae. Grows at 20–40°C. Additional phenotypic properties are shown in Table 238.

Whole-organism hydrolysates are rich in arabinose and galactose. Muramic acid moieties are *N*-acetylated. The cellular fatty acid profile contains major proportions of $C_{16:0}$ iso (38.1%), and $C_{17:1}$ (25.4%), and smaller proportions (<10%) of $C_{17:0}$ anteiso (5.2%), $C_{16:0}$ (5.1%), and $C_{17:0}$ (4.0%). Mycolic acids are not present. The diagnostic polar lipid is PE. The predominant menaquinone is MK-9(H_4).

Source: isolated from soil collected from Yilan county, Taiwan.

DNA G+C content (mol%): 68.9 (HPLC).

Type strain: 0345M-7, BCRC 16802, JCM 14925, KCTC 19116, NBRC 102103.

Sequence accession nos: DQ160215 (16S rRNA gene); EU822915 (*gyrB*).

35. **Amycolatopsis thermoflava** Chun, Kim, Oh, Seong, Lee, Bae, Lee, Kang, Hah and Goodfellow 1999, 1372[VP]

ther.mo.fla′va. Gr. n. *thermê* heat; L. adj. *flavus* yellow; N.L. fem. adj. *thermoflava* thermophilic, yellow.

Forms an extensively branched substrate mycelium which fragments into squarish elements (0.6–0.7 × 6.5–14.0 µm). Aerial hyphae are sterile, sparse, and white; the substrate mycelium is brown and a brown diffusible pigment is produced on modified Bennett's agar. Grows at 28–55°C, but not at 10 or 60°C.

Resistant (µg/ml) to gentamicin sulfate (5), neomycin sulfate (8), novobiocin (10), rifampin (10), tobramycin sulfate (8), and vancomycin hydrochloride (0.25). Grows in the presence of lysozyme. Additional phenotypic properties are shown in Table 238.

Whole-organism hydrolysates are rich in arabinose and galactose. The cellular fatty acid profile contains major proportions of $C_{16:0}$ iso (29%), $C_{16:0}$ iso-α OH (16%), and $C_{17:0}$ anteiso (14%). Mycolic acids are not present. The predominant menaquinone is MK-9(H_4).

Source: isolated from a soil sample collected from Hainan Island, China.

DNA G+C content (mol%): 75.0 (T_m).

Type strain: N1165, CIP 106795, DSM 44574, NBRC 14333, JCM 10669, NRRL B-24140.

Sequence accession nos: AF052390 (16S rRNA gene); EU822916 (*gyrB*).

36. **Amycolatopsis tolypomycina** Wink, Kroppenstedt, Ganguli, Nadkarni, Schumann, Seibert and Stackebrandt 2003a, 935[VP] (Effective publication: Wink, Kroppenstedt, Ganguli, Nadkarni, Schumann, Seibert and Stackebrandt 2003b, 44.)

to.ly.po.my′ci.na. N.L. n. *tolypomycinum* tolypomycin (an antibiotic produced by the organism); L. fem. suff. *-ina* suffix used with the sense of belonging to; N.L. fem. adj. *tolypomycina* pertaining to tolypomycin.

The substrate mycelium is pure yellow on yeast extract-malt extract agar, melon yellow on oatmeal agar, yellow orange on inorganic salts-starch and tyrosine agars, and colorless on glycerol-asparagine agar; soluble pigments are not formed on any of these media. White, fragmenting aerial hyphae are produced on glycerol-asparagine, oatmeal, and tyrosine agars. Fragmentation of the substrate mycelium is seen on glycerol-asparagine, oatmeal, and tyrosine agars.

Positive for *N*-acetyl-β-glucosamidase, acid and alkaline phosphatases, cystine arylamidase, esterase (C8), α- and β-galactosidases, α-glucosidase, β-glucuronidase, leucine arylamidase, lipase (C14), α-mannosidase, trypsin, and valine arylamidase, but negative for esterase (C4), α-fucosidase, and naphthol-AS-BI-phosphohydrolase (API ZYM tests). Additional phenotypic properties are shown in Table 238.

Whole-organism hydrolysates are rich in arabinose and galactose. Cellular fatty acids contain major proportions of $C_{16:0}$ iso (14.5%), $C_{15:0}$ iso (10.3%), and $C_{17:0}$ anteiso (15.2%), and smaller proportions of $C_{14:0}$ (0.6%), $C_{15:0}$ (3.3%), $C_{16:0}$ (7.7%), $C_{17:0}$ (7.5%), $C_{15:1}$ (1.0%), $C_{16:1}$ (6.1%), $C_{17:1}$ (8.9%), $C_{18:1}$ *cis*9 (1.0%), $C_{14:0}$ iso (0.8%), $C_{15:0}$ anteiso (3.6%), $C_{16:1}$ (0.8%), $C_{17:0}$ iso (4.1%), $C_{15:1}$ anteiso (0.5%), $C_{16:0}$ 10-methyl iso (0.4%), $C_{16:0}$ 10-methyl (2.6%), $C_{17:0}$ 10-methyl (3.1%), 2-hydroxyl-$C_{15:0}$ anteiso (0.5%), 2-hydroxy-$C_{16:0}$ iso (3.1%), 2-hydroxyl-$C_{17:0}$ anteiso (0.5%), and 2-hydroxyl-$C_{17:0}$ anteiso (3.9%). Mycolic acids are absent. The polar lipid pattern includes DPG, HPE, PE, PG, and PI. The predominant menaquinone is MK-9(H_4).

Source: isolated from soil in Tokyo, Japan.

DNA G+C content (mol%): not determined.

Type strain: ATCC 21177, DSM 44544, NBRC 14664, NRRL B-24205.

Sequence accession nos: AJ293757 (16S rRNA gene); EU822917 (*gyrB*).

37. **Amycolatopsis tucumanensis** Albarracin, Alonso-Vega, Trujillo, Amoroso and Abate 2010, 400[VP]

tu.cu.ma.nen′sis. N.L. fem adj. *tucumanensis* of or pertaining to Tucumán, Argentina, the origin of the soil sample from which the type strain was isolated.

Forms an extensively branched honey-yellow substrate mycelium that fragments into squarish elements on glycerol-asparagine, inorganic salts-starch, peptone-yeast extract, tyrosine, and yeast extract-malt extract agars; diffusible pigments are not formed on these media. A white aerial mycelium is produced on glycerol-asparagine, tyrosine, and yeast extract-malt extract agars. Aerial hyphae differentiate into straight to flexuous chains of spore-like elements (0.3–0.8 × 1.5 µm). Grows between 15 and 55°C and from pH 5.0 to 10.

Produces β-galactosidase, *N*-acetyl-β-glucosamidase, and phosphatase. Raffinose is used as a sole source of carbon, but not cellobiose, rhamnose, or xylose.

Resistant to lysozyme (100 µg/ml) and high concentrations of copper (up to 3 mM). Additional phenotypic properties are shown in Table 238.

Whole-organism hydrolysates are rich in arabinose and galactose. The fatty acid profile contains major proportions of $C_{16:0}$ iso (23%), $C_{16:0}$ (12%), and $C_{17:0}$ anteiso (11%). Mycolic acids are absent. The polar lipid pattern includes DPG, PI, and HPE. The predominant menaquinone is MK-9(H_4); also contains minor amounts of MK-9(H_2), MK-9(H_6), and MK-10(H_2).

Source: isolated from a sediment sample polluted with copper in Tucumán, Argentina.

DNA G+C content (mol%): not determined.

Type strain: ABO, BCCM/LMG 24814, DSM 45259.
Sequence accession no. (16S rRNA gene): DQ886938.

38. **Amycolatopsis ultiminotia** Lee 2009, 1403[VP]

ul.ti.mi.no′ti.a. L. sup. adj. *ultimus* farthest, extreme; L. fem. adj. *notia* southern; N.L. fem. adj. *ultiminotia* farthest southern, implying that the type strain was isolated from the southernmost part of the Republic of Korea.

Forms a white aerial mycelium and a cream to yellow substrate mycelium which fragment into rod-shaped elements. Grows from 10–37°C, but not at 42°C, and from pH 5.1 and 12.1.

DNA and elastin are degraded, but not chitin or cellulose. Acid is produced from dextran and dulcitol, but not from D-arabinose, inulin, methyl α-D-mannoside, ribose, sorbose, or xylitol.

Whole-organism hydrolysates are rich in arabinose and galactose. The cellular fatty acid profile contains major proportions of $C_{17:0}$ (23.7%), $C_{15:0}$ (19.8%), $C_{15:0}$ iso (10.0%), and $C_{16:0}$ iso (13.2%), and smaller proportions of $C_{13:0}$ (0.8%), $C_{14:0}$ (0.8%), $C_{14:0}$ iso (7.9%), $C_{14.0}$ 2-OH (3.3%), $C_{15:1}$ (1.7%), $C_{15:0}$ anteiso (2.7%), $C_{15:0}$ iso 3-OH (2.1%), $C_{17:0}$ anteiso (2.0%), $C_{17:0}$ iso (0.7%), $C_{18:0}$ (4.1%), and $C_{18:1}$ 0.7%). Does not contain mycolic acids. The polar lipid pattern consists of PME and an unknown ninhydrin-positive phospholipid. The predominant menaquinone is MK-9(H_4).

Source: isolated from the rhizosphere of a cliff associated plant (*Peucedanmum japonicium* Thunb).
DNA G+C content (mol%): 67.5.
Type strain: DSM 45180, NRRL B-24662.
Sequence accession no. (16S rRNA gene): FM177516.

39. **Amycolatopsis vancoresmycina** Wink, Kroppenstedt, Ganguli, Nadkarni, Schumann, Seibert and Stackebrandt 2003a, 935[VP] (Effective publication: Wink, Kroppenstedt, Ganguli, Nadharni, Schumann, Seibert and Stackebrandt 2003b, 44.)

van.co.res.my′ci.na. N.L. n. *vancoresmycinum* an antibiotic, vancoresmycin, produced by the organism; L. fem. suff. *-ina* suffix used with the sense of belonging to: N.L. fem. adj. *vancoresmycina* pertaining to vancoresmycin.

Substrate mycelium is brown-beige on peptone-yeast extract-iron agar, saffron yellow on yeast extract-malt extract agar, and maize yellow on glycerol asparagine, inorganic salts-starch, oatmeal, and tyrosine agars; a brown soluble pigment is formed on yeast extract-malt extract agar. White fragmenting aerial hyphae are produced on inorganic salts-starch, oatmeal, and yeast extract-malt extract agars. Fragmentation of the substrate mycelium is seen on oatmeal and yeast extract-malt extract agars.

Positive for *N*-acetyl-β-glucosamidase, alkaline phosphatase, α-chymotrypsin, cystine arylamidase, esterase (C8), leucine arylamidase, lipase (C14), naphthol-AS-BI-phosphohydrolase, trypsin, and valine arylamidase, but negative for esterase (4), α-fucosidase, α- and β-galactosidases, α-glucosidase, β-glucuronidase, and α-mannosidase (API ZYM tests).

Whole-organism hydrolysates are rich in arabinose and galactose. The fatty acids profile contains major proportions of $C_{16:0}$ iso (41.9%) and smaller proportions (<10%) of $C_{15:0}$ (1.6%), $C_{16:0}$ (1.9%), $C_{17:0}$ (1.8%), $C_{15:1}$ (1.9%), $C_{14:0}$ iso (1.6%), $C_{15:0}$ iso (9.9%), $C_{16:1}$ iso (1.9%), $C_{17:0}$ iso (4.0%), $C_{15:0}$ anteiso (1.1%), $C_{17:0}$ anteiso (4.2%), *cis*9-$C_{16:1}$ (3.0%), $C_{17:1}$ *cis*9 (3.8%), $C_{16:0}$ 10-methyl (2.8%), $C_{16:1}$ 10-methyl (1.4%), $C_{17:0}$ 10-methyl iso (3.3%), $C_{17:0}$ 10-methyl (2.4%), 2-OH-$C_{15:0}$ iso (2.4%), and 2-hydroxy-$C_{16:0}$ iso (8.4%). Additional phenotypic properties are shown in Table 238.

Source: isolated from Indian soil.
DNA G+C content (mol%): not determined.
Type strain: ST 101170, DSM 44592, JCM 12675, NRRL B-24208.
Sequence accession nos: AJ508240 (16S rRNA gene); EU822918 (*gyrB*).

Species *incertae sedis*

1. "**Amycolatopsis lactamdurans**" Barreiro, Pisabarro and Martin 2000, 22

Forms a slight yellow substrate mycelium, a white aerial mycelium and a bright yellow substrate mycelium on modified Bennett's agar supplemented with mannitol and soybean flour. Grows from 25–37°C, but not at 10 or 45°C, and from pH 6.0–9.0.

Allantoin, esculin, and urea are hydrolyzed, but not arbutin. Does not produce H_2S or reduce nitrate. Elastin, hypoxanthine, L-tyrosine, Tween 40, uric acid, xanthine, and xylan are degraded, but not starch. Acid is produced from arabitol, cellobiose, galactose, glucose, glycerol, *myo*-inositol, mannitol, mannose, raffinose, salicin, sorbitol, trehalose, and xylose, but not from adonitol, cellobiose, dulcitol, *meso*-erythritol, glycogen, lactose, maltose, melezitose, melibiose, methyl α-D-glucoside, ribose, sucrose, turanose, or xylitol.

L-Arabinose, D-arabitol, cellobiose, dextrin, glycerol, *myo*-inositol, mannitol, sorbitol, trehalose, xylitol, and xylose are used as sole carbon sources, but not adonitol, *meso*-erythritol, glycogen, lactose, maltose, melezitose, melibiose, methyl α-D-glucoside, raffinose, ribose, or sucrose (all at 1%, w/v). Similarly, citrate, propionate, oxalate, and tartrate are used as sole carbon sources, but not benzoate, lactate, or mucate (all at 0.1%, w/v).

Resistant (μg/ml) to gentamicin sulfate (5), neomycin sulfate (8), streptomycin sulfate (16), novobiocin (10), polymixin B (50), and vancomycin hydrochloride (0.25), but sensitive to penicillin (20) and rifampin (10).

Source: soil.
DNA G+C content (mol%): not determined.
Type strain: ATCC 27382, NRRL 3802.
Sequence accession no. (16S rRNA gene): AJ243301.

Genus VI. **Crossiella** Labeda 2001, 1578^{VP}

DAVID P. LABEDA

Cros.si.el′la. N.L. fem. dim. n. *Crossiella* named for Thomas Cross, a microbiologist at the University of Bradford who made many contributions to actinomycete biology and systematics.

Aerobic. Gram-stain-positive, non-acid fast, nonmotile actinomycetes. Forms branched substrate mycelium (approx. 0.5 μm in diameter) and, on some media, aerial mycelia are produced. **Vegetative mycelium may fragment into rod-shaped elements and sclerotia-like pseudosporangia may be produced on the substrate mycelium. Swellings may be produced at or near the tip of aerial hyphae.** Mycolic acids are absent. Catalase-positive. **Contains *meso*-diaminopimelic acid as the diamino acid and acetylated peptidoglycan. The whole-cell sugar pattern consists of galactose, mannose, rhamnose, and ribose. The phospholipid pattern consists of phosphatidylethanolamine, phosphatidylmethylethanolamine, phosphatidylinositol, and phosphatidylinositol mannosides. The predominant menaquinone is MK-9(H_4)**. Has a fatty acid profile rich in branched-chain and saturated components. **Phylogenetically, the nearest neighbor is the genus *Kutzneria*.**

DNA G+C content (mol%): 71.4 (T_m).

Type species: **Crossiella cryophila** Labeda 2001, 1579^{VP}.

Further descriptive information

The type species of *Crossiella* was originally described by Takahashi et al. (1986) as *Nocardiopsis mutabilis* subsp. *cryophilis* for a novel soil isolate that produced the antibiotic dopsisamine. The authors observed that the morphological characteristics of the strain were somewhat different from those of *Nocardiopsis* but, based on their chemotaxonomic data, considered that the genus *Nocardiopsis* was the closest fit for their strain. The transfer of *Nocardiopsis mutabilis* and its subspecies to the genus *Saccharothrix* was proposed by two independent studies (Grund and Kroppenstedt, 1989; Labeda and Lechevalier, 1989) based on more detailed chemotaxonomic analyses. Evaluation of DNA relatedness of the type strain of *Nocardiopsis mutabilis* subsp. *cryophilis* with other species within the genus *Saccharothrix* (Labeda and Lechevalier, 1989) demonstrated that it was distinct and therefore constituted a novel species for which the name *Saccharothrix cryophila* was proposed. The DNA relatedness between this strain and strains of the other species was low (2–11%) and there was some question regarding its generic identity. Subsequent phylogenetic analysis of the type strains of many species of the genera within the suborder *Pseudonocardineae*, elevated in this volume to order *Pseudonocardiales*, based on 16S rRNA gene sequences (Labeda and Kroppenstedt, 2000) supported the existence of at least two families, *Actinosynnemataceae* and *Pseudonocardiaceae*, and also demonstrated that a number of *Saccharothrix* species were misclassified. *Saccharothrix cryophilus* is phylogenetically located outside of *Actinosynnemataceae sensu stricto*, far from *Saccharothrix* and closest to the genera *Kutzneria*, *Actinoalloteichus*, and *Streptoalloteichus* as seen in Figure 295. Consideration of the morphological, chemotaxonomic, and phenotypic properties of this strain resulted in the proposal for the creation of the new genus, *Crossiella*, for this species (Labeda, 2001).

Actinomycetes were reported as a significant emergent agent of placentitis and abortion in horses in Kentucky after first being observed in 1986 at the Livestock Disease Diagnostic Center at the University of Kentucky (Donahue and Williams, 2000; Giles et al., 1993; Hong et al., 1993). Nocardioform placentitis is the term used to describe this distinct type of placentitis in horses and the infection is diagnosed based on the location of the lesion on the chorionic surface of the placenta and the recovery of Gram-stain-positive branching micro-organisms upon culture. The actinomycete biomass may infiltrate up to 30% of the surface area of the placenta (light gray area in Figure 296), but does not invade fetal tissue and probably contributes to death or weakening of the unborn foal through competition for nutrients. Infections can result in spontaneous abortions, still birth, full-term deliveries of weak foals, or no apparent effect and healthy foals. The 16S rRNA gene sequences from strains isolated from placental tissues exhibit high similarity (98.1%) to that of the type strain of *Crossiella cryophila* and morphological and chemotaxonomic characteristics are also typical for the genus. Physiological characteristics for all strains are quite similar and 16S rRNA gene sequences are identical. A novel species, *Crossiella equi*, was therefore proposed for these equine isolates (Donahue et al., 2002). The manner in which *Crossiella equi* strains become introduced into the equine uterus and infect the placenta is still unknown, as are possible environmental reservoirs for this species. The incidence of *Crossiella equi* equine placentitis is quite variable and numbers of infected placentas observed are relatively low in most years.

Enrichment and isolation procedures

Crossiella grows well on typical actinomycete growth media such as yeast extract-malt extract agar (Shirling and Gottlieb, 1966) or NZamine medium [DSMZ medium 554 (DSMZ, 2001)]. Strains have been isolated from lesions on equine placentas on tryptic soy agar containing 5% blood, but other media without blood can be used. The type strain of *Crossiella cryophilus* was isolated from dilutions of a soil sample from Shosenkyo, Yamanashi Prefecture, Japan, but details of the isolation procedure are lacking and there have been no other reports of isolation of strains of *Crossiella* from environmental samples.

Maintenance procedures

Working cultures of *Crossiella* can be maintained as refrigerated (4°C) agar slants on an appropriate medium such as yeast extract-malt extract medium (Shirling and Gottlieb, 1966) or NZamine medium (DSMZ medium 554). The slants are generally incubated for 5–7 d at 28–30°C prior to refrigeration and then should be subcultured at monthly intervals. Longer term preservation of strains is best accomplished as frozen stocks in 20% aqueous glycerol at −80°C (mechanical freezer) to −172°C (liquid nitrogen vapor phase) or by using traditional lyophilization techniques.

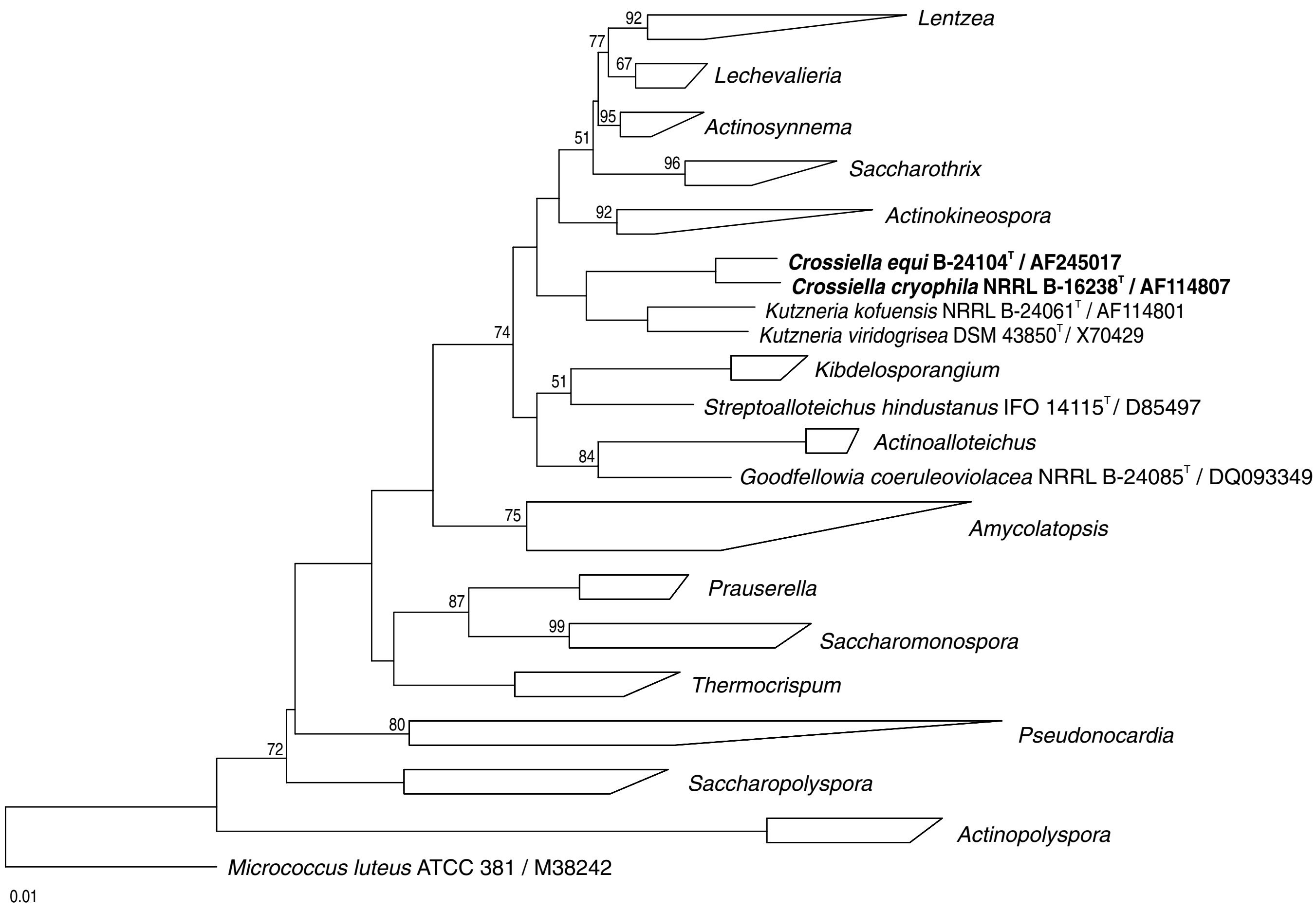

FIGURE 295. Phylogenetic tree for families of the order *Pseudonocardiales*, calculated from almost-complete 16S rRNA gene sequences using Kimura's evolutionary distance methods (Kimura, 1980) and the neighbor-joining method of Saitou and Nei (1987) illustrating the taxonomic position of *Crossiella cryophila* and *Crossiella equi* and the other taxa in the order. Bar = 0.01 nucleotide substitutions per site.

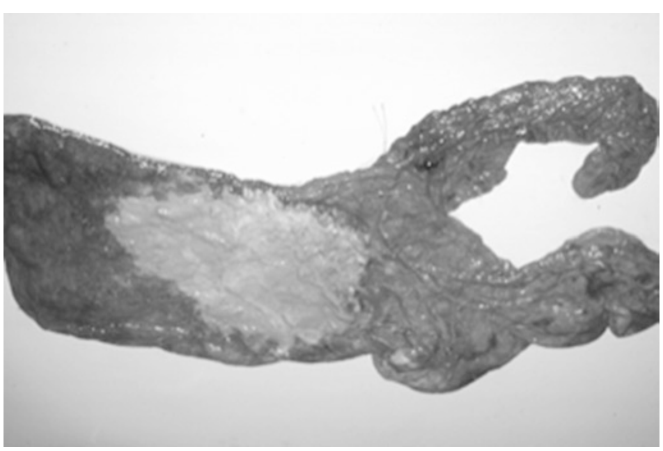

FIGURE 296. Photograph of an equine placenta exhibiting symptoms of *Crossiella equi* nocardioform placentitis. The light gray colored area is infiltrated with *Crossiella* mycelium. (Printed with permission of N.M. Williams, Livestock Disease Diagnostic Center, University of Kentucky, Lexington, KY, USA.)

Procedures for testing special characters

Strains are routinely cultivated on NZamine medium at 28°C. Morphological observations are made on the media of Shirling and Gottlieb (1966) and on NZamine medium.

Chemotaxonomic analyses of strains for polar lipids, menaquinones, and fatty acids are performed using methods previously described by Grund and Kroppenstedt (1989), Minnikin et al. (1984), and Sasser (1990).

Physiological tests, including production of acid from carbohydrates, utilization of organic acids, and hydrolysis and decomposition of adenine, casein, guanine, esculin, hypoxanthine, tyrosine, urea, and xanthine, are typically evaluated using the media of Gordon et al. (1974). Phosphatase activity is evaluated by using the method of Kurup and Schmitt (1973) substituting NZamine agar as the basal medium. Temperature range for growth is determined on slants of DSMZ medium 554 and salt tolerance is determined on slants of the same medium supplemented with 4% and 5% NaCl.

Differentiation of the genus *Crossiella* from other genera

The genus *Crossiella* is phylogenetically distinct from neighboring genera based on analyses of 16S rRNA gene sequences (Figure 295). Moreover, the micromorphology of *Crossiella* is distinct from that exhibited by neighboring taxa. *Crossiella* strains exhibit substrate mycelium fragmenting into rod-shaped elements, the presence of sclerotia or pseudosporangium-like bodies on the colony surface (Figure 297), and swellings near the tips of mycelium (Figure 297); the latter two properties have not been observed in members of the genus *Goodfellowiella*. Motile spores have not been observed and true sporangia such as those found in species of the genera *Kutzneria*, *Kibdelosporangium*, and *Streptoalloteichus* are not produced. Chains of spores typical of *Actinoalloteichus* species have not been observed in *Crossiella* species. The chemotaxonomic profile of *Crossiella* species is most similar to that of *Streptoalloteichus* (Table 239), but MK-10(H_4) menaquinones, while present in *Streptoalloteichus*, are not found in *Crossiella* strains.

Differentiation of the species of the genus *Crossiella*

The physiological characteristics of *Crossiella* species are summarized in Table 240. The physiological properties of six strains of *Crossiella equi* were evaluated for the original description of the species and there was some variation observed. Determination of acid production from the various sugars and utilization of organic acids in this species was complicated by the fact that all of the strains tested absorbed the pH indicator dye, bromothymol blue, into their substrate mycelium making it difficult to evaluate test results. The two species can be differentiated by the many physiological differences indicated in Table 240, but most obviously by the lack of growth of *Crossiella cryophila* at temperatures of 37°C or greater. The growth rate of *Crossiella equi* strains is also very much greater than that of *Crossiella cryophila* as well as most other actinomycetes. There are also differences between the fatty acid profiles of the species as can be seen in Table 241.

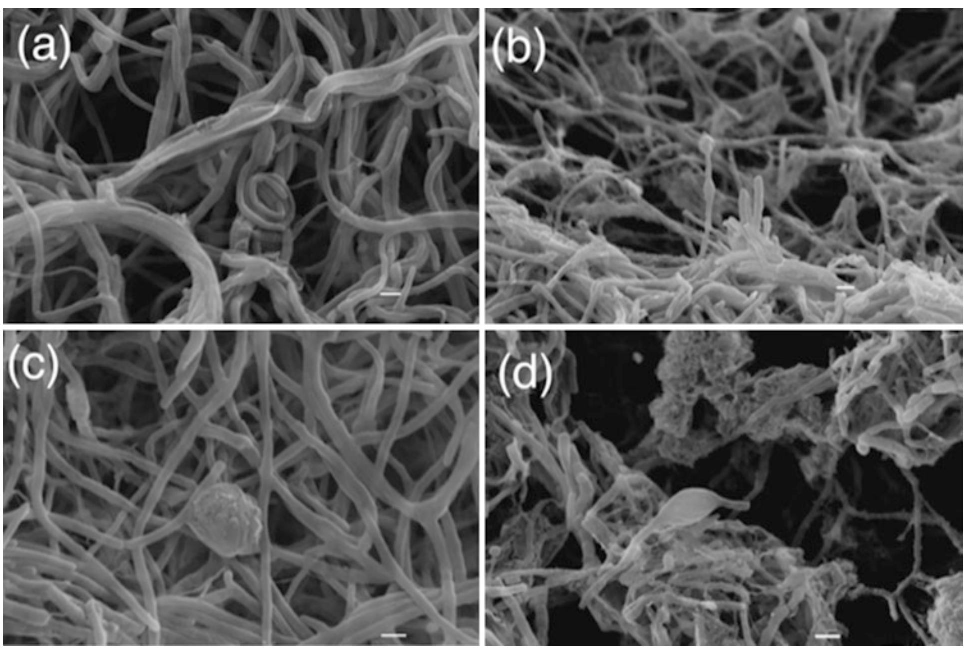

FIGURE 297. Comparison of the micromorphological properties of *Crossiella cryophilus* NRRL B-16238[T] (a, c) and *Crossiella equi* NRRL B-24104[T] (b, d). Note the pseudosporangia on the substrate mycelium in (a) and (b) and the swollen mycelial tips in (c) and (d). Bars = 1 µm.

TABLE 239. Comparison of chemotaxonomic profile of *Crossiella* with phylogenetically nearest genera

Character	*Crossiella*	*Actinoalloteichus*	*Goodfellowiella*	*Kibdelosporangium*	*Kutzneria*	*Streptoalloteichus*
Whole-cell sugar pattern[a]	Gal, Man, Rha, Rib	Glu, Gal, Man, Rib	Gal, Rib	Ara, Gal, Glu, Rha	Gal, Rha	Gal, Man, Rha, Rib
Phospholipids[b]	PE, DPG, PI, PIM, Methyl-PE	PIM, PI, PG, DPG, Methyl-PE	PE, DPG, OH-PE, Methyl-PE	PE, PME, PG, PI	PE, DPG, PI, PG, Methyl-PE	PE, DPG, PI, PIM, DPG, Methyl-PE
Predominant menaquinones	MK-9(H_4)	MK-9(H_4)	MK-9(H_4), MK-10(H_4)	MK-9(H_4)	MK-9(H_4)	MK-9(H_4), MK-10(H_4)

[a]Ara, Arabinose; Gal, galactose; Glu, glucose; Man, mannose; Rha, rhamnose; Rib, ribose.

[b]DPG, Diphosphatidylglycerol; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; PI, phosphatidylinositol; PIM, phosphatidylinositol mannosides.

TABLE 240. Physiological characteristics of species of the genus *Crossiella*[a]

Character	*Crossiella cryophila*	*Crossiella equi* (6 strains)
Decomposition of:		
Adenine	–	–
Allantoin	–	–
Casein	–	+
Esculin	+	+
Gelatin	+	+(4/6)
Hippurate	–	+ (5/6)
Hypoxanthine	–	– (4/6)
Starch	+	+
Tyrosine	+	+
Urea	+	–
Xanthine	–	–
Acid production from:		
Adonitol	–	–
Arabinose	–	+ (3/6)
Cellobiose	w	+
Dextrin	w	+
Dulcitol	–	–
Erythritol	–	–
Fructose	+	+
Galactose	+	+
Glucose	+	+
Glycerol	+	+
Inositol	+	+
Lactose	w	– (4/6)
Maltose	+	+
Mannose	+	+
Mannitol	–	– (4/6)
Melibiose	w	+
Methyl α-D-glucoside	–	–
Methyl β-D-xyloside	–	–
Raffinose	w	+
Rhamnose	–	+ (5/6)
Salicin	–	+ (5/6)
Sorbitol	–	– (5/6)
Sucrose	–	– (5/6)
Trehalose	+	+ (5/6)
Xylose	–	+ (5/6)
Utilization of:		
Acetate	+	w (5/6)
Benzoate	–	w (4/6)
Citrate	–	w (3/6)
Lactate	+	– (4/6)
Malate	+	–
Mucate	–	–
Oxalate	+	–
Propionate	+	w (3/6)
Succinate	+	w (4/6)
Tartrate	–	–
Production of:		
Nitrate reductase	+	+
Phosphatase	+	+
Growth in the presence of:		
4% NaCl	+	+
5% NaCl	–	+
Growth at:		
10°C	+	+
37°C	–	+
42°C	–	+
45°C	–	–

(continued)

[a]Symbol: w, weak positive.

TABLE 241. Fatty acid profiles of *Crossiella* species[a]

Fatty acid	*C. cryophila*	*C. equi* (6 strains)
$C_{14:0}$ iso	1.91	1.02
$C_{15:0}$ iso	44.28	57.51
$C_{15:0}$ anteiso	2.29	3.01
$C_{15:1}$ B	3.06	3.34
$C_{16:1}$ iso-H	3.16	1.98
$C_{16:0}$ iso	9.77	7.25
$C_{16:1}$ ω9*c*	3.11	0.80
$C_{16:0}$	1.76	0.66
$C_{16:0}$ iso 10-methyl	1.09	0.00
$C_{16:0}$ 9?-methyl	11.58	4.20
$C_{17:0}$ iso	11.36	4.54
$C_{17:0}$ anteiso	1.86	1.05
$C_{17:1}$ ω9*c*	1.86	0.82
$C_{17:0}$	1.21	0.50
$C_{17:0}$ iso 3-OH	0.00	4.68

[a]Fatty acids are listed as percentages of total fatty acids as determined by the Microbial Identification System software (MIDI Inc.) peak naming table.

List of species of the genus *Crossiella*

1. **Crossiella cryophila** (Labeda and Lechevalier 1989) Labeda 2001, 1579[VP] (*Saccharothrix cryophilus* Labeda and Lechevalier 1989, 420; "*Nocardiopsis mutabilis* subsp. *cryophilus*" Takahashi, Hotta, Saito, Morioka, Okami and Umezawa 1986, 179)

cry.o′phi.la. Gr. n. *kruos* icy cold, frost; N.L. adj. *philus* -*a* -*um* (from Gr. adj. *philos* -*ê* -*on*) friend, loving; N.L. fem. adj. *cryophila* cold loving, referring to the low permissive temperature range for growth.

Pale-yellow to light-brown substrate mycelium is produced on most media. White to yellowish-white aerial mycelium is formed, particularly on inorganic salts-starch agar or glycerol-asparagine agar. Soluble pigments are not produced. Physiological properties are shown in Table 240. Temperature range for growth is 10–33°C.

Source: isolated from soil.

DNA G+C content (mol%): 74.1 (T_m).

Type strain: NRRL B-16238, ATCC 51143, DSM 44230, IFO 14475, NBRC 14475, Okami TS-1980.

Sequence accession no. (16S rRNA gene): AF114806.

2. **Crossiella equi** Donahue, Williams, Sells and Labeda 2002, 2172[VP]

e′qui. L. gen. n. *equi* of the horse, referring to the source of isolation of this micro-organism, equine placentas.

Pale orange to light-brown substrate mycelium is formed on most media. Copious white aerial mycelium is produced on most media. Physiological properties are shown in Table 240. Temperature for growth is 10–42°C.

Source: isolated from equine placentas.

DNA G+C content (mol%): 74.1 (T_m).

Type strain: NRRL B-24104, CIP 107800, DSM 44580, LDDC 22291-98.

Sequence accession no. (16S rRNA gene): AF245017.

Genus VII. **Goodfellowiella** Labeda, Kroppenstedt, Euzéby and Tindall 2008, 1048[VP]

David P. Labeda

Good.fel.low.i.el′la. N.L. fem. dim. n. *Goodfellowiella* named for Michael Goodfellow, a microbiologist at the University of Newcastle-upon-Tyne, in recognition of his contributions to microbial systematics.

Aerobic. Gram-stain-positive, non-acid-fast, nonmotile actinomycetes. Branched substrate mycelium (approx. 0.5 µm in diameter) and, on some media, aerial mycelia are produced. **Ovoid conidia are produced by fragmentation of substrate mycelium.** Catalase-positive. **Contains *meso*-diaminopimelic acid as the diamino acid. The whole-cell sugar pattern consists of galactose and ribose. The phospholipid pattern consists of diphosphatidylglycerol, phosphatidylethanolamine, phosphatidylethanolamine with hydroxylated fatty acids, and traces of phosphatidylinositol and phosphatidylinositol mannosides. The predominant menaquinones are MK-9(H_4) and MK-10(H_4). Has a fatty acid profile rich in branched chain and saturated components including 10-methyl-branched heptadecanoic acid and anteiso-branched 2-hydroxy fatty acids.** Phylogenetically, its nearest neighbor is the genus *Actinoalloteichus*.

DNA G+C content (mol%): 68.2 (T_m).

Type species: **Goodfellowiella coeruleoviolacea** (Preobrazhenskaya and Terekhova 1987) Labeda, Kroppenstedt, Euzéby and Tindall 2008, 1048[VP].

Further descriptive information

The type species was originally described by Preobrazhenskaya et al. (1987, 1976) as *Actinomadura coeruleoviolacea* and was subsequently transferred by Kroppenstedt et al. (1990, 1991) to the genus *Saccharothrix* as *Saccharothrix coeruleoviolacea.* During a phylogenetic evaluation of species of the genus *Saccharothrix* with validly published names based on almost complete 16S rRNA gene sequences (Labeda and Kroppenstedt, 2000), it was noted that the type strain of *Saccharothrix coeruleoviolacea* was not related to other species of the genus and represented a new genus within the suborder *Pseudonocardineae*, elevated in this volume to order *Pseudonocardiales*. A polyphasic investigation of the characteristics of this strain demonstrated that it was also chemotaxonomically distinct from *Saccharothrix* and the other genera within the suborder and the new genus *Goodfellowia* was proposed by Labeda and Kroppenstedt (2006). It was later discovered that the genus name *Goodfellowia* had been used previously in as the name of an avian genus and thus *Goodfellowia* Labeda and Kroppenstedt 2006 was taxonomically illegitimate. A proposal was subsequently published to correct this situation by emending the genus name to *Goodfellowiella* (Preobrazhenskaya and Terekhova 1987) Labeda, Kroppenstedt, Euzéby and Tindall 2008.

Maintenance procedures

Working cultures of *Goodfellowiella* can be maintained as refrigerated (4°C) agar slants on appropriate media such as yeast extract-malt extract medium (Shirling and Gottlieb, 1966) or NZamine medium (DSMZ medium 554; DSMZ, 2001). The slants are generally incubated for 5–7 d at 28–30°C prior to refrigeration and then should be subcultured at monthly intervals. Longer term preservation of strains is best accomplished as frozen stocks in 20% (v/v) aqueous glycerol at −80°C (mechanical freezer) to −172°C (liquid nitrogen vapor phase) or by using traditional lyophilization techniques.

Procedures for testing special characters

Strains are routinely cultivated on NZamine medium at 28°C. Morphological observations are made on the media of Shirling and Gottlieb (1966) and NZamine medium.

Chemotaxonomic analyses of strains for fatty acids, menaquinones, and polar lipids are performed using methods described previously by Grund and Kroppenstedt (1989), Minnikin et al. (1984), Saddler et al. (1991), and Sasser (1990).

Physiological tests, including production of acid from carbohydrates, utilization of organic acids, and hydrolysis and decomposition of adenine, guanine, hypoxanthine, tyrosine, xanthine, casein, esculin, urea, and hippurate, are typically evaluated using the media of Gordon et al. (1974). Phosphatase activity is evaluated by using the method of Kurup and Schmitt (1973) substituting NZamine agar as the basal medium. Temperature range for growth is determined on slants of DSMZ medium 554 and salt tolerance is determined on slants of the same medium supplemented with 4% and 5% NaCl.

Differentiation of the genus *Goodfellowiella* from other genera

Goodfellowiella coeruleoviolacea is phylogenetically separate from the genus *Saccharothrix*, the genus in which this micro-organism was originally classified, and appears to be most closely related to the genus *Actinoalloteichus*, as can be seen in Figure 298. The chemotaxonomic properties of *Goodfellowiella* distinguish it from *Actinoalloteichus* and the other related taxa within the order *Pseudonocardiales*, as can be seen in Table 242. The whole-cell sugar pattern consisting of only galactose and ribose differs from those of other genera, as does the phospholipid pattern, i.e. a lack of phosphatidylinositol and the presence of both phosphatidyl-

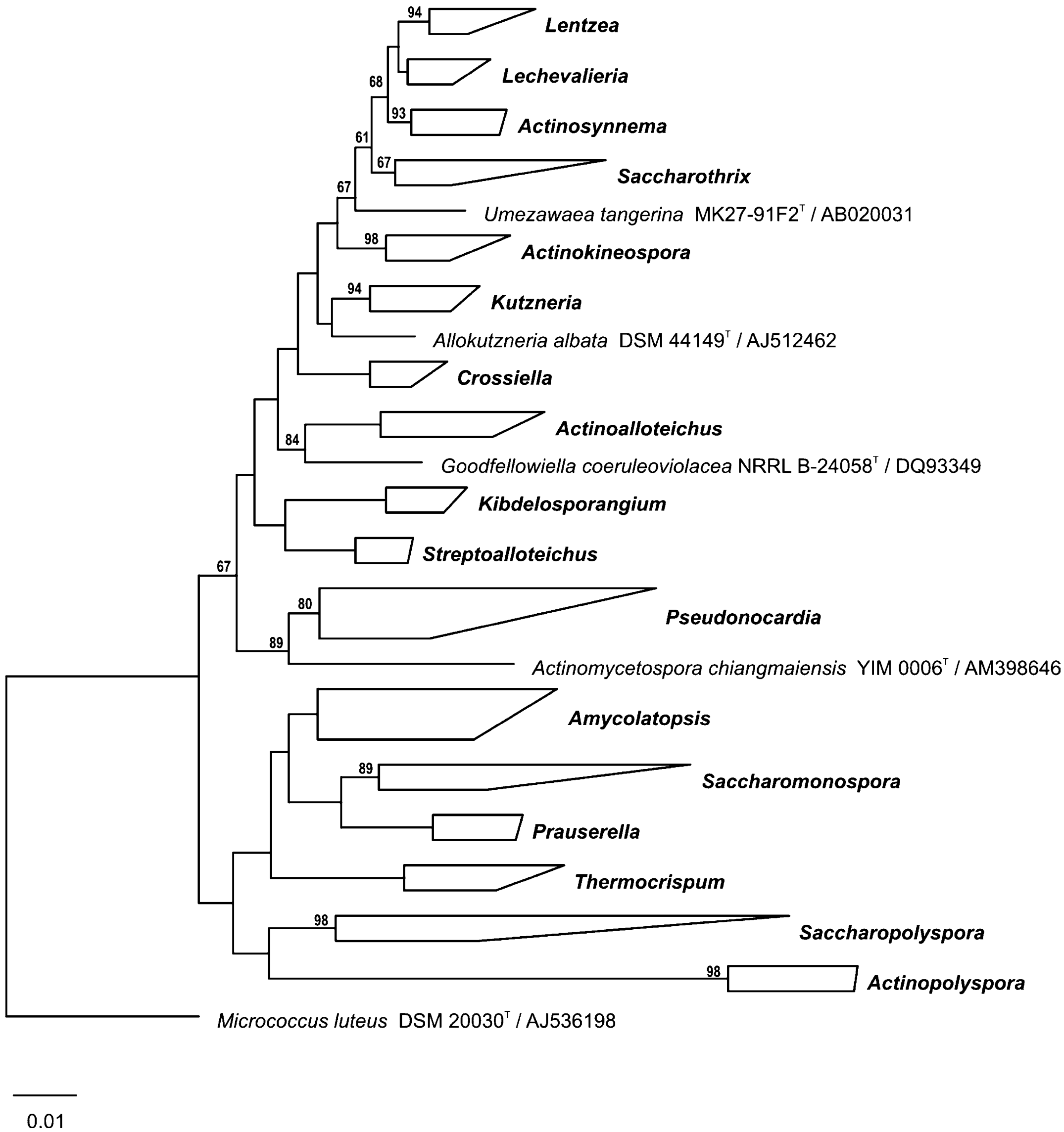

FIGURE 298. Phylogenetic tree for the genera of the order *Pseudonocardiales* calculated from almost-complete 16S rRNA gene sequences using the Kimura's evolutionary distance method (Kimura, 1980) and the neighbor-joining algorithm of Saitou and Nei (1987). Numbers at the nodes represent levels (%) of bootstrap support from 100 resampled datasets; values less than 60% are not shown. Bar = 0.01 nucleotide substitutions per site.

TABLE 242. Comparison of chemotaxonomic profile of *Goodfellowiella* with phylogenetically nearest genera and *Kibdelosporangium*

Character	*Goodfellowiella*	*Crossiella*	*Actinoalloteichus*	*Allokutzneria*	*Kibdelosporangium*	*Kutzneria*	*Streptoalloteichus*
Whole-cell sugar pattern[a]	Gal, Rib	Gal, Man, Rha, Rib	Glu, Gal, Man, Rib	Ara, Gal, Man	Ara, Gal, Glu, Rha	Gal, Rha	Gal, Man, Rha, Rib
Phospholipids[b]	PE, DPG, OH-PE, PME	PE, DPG, PI, PIM, PME	PIM, PI, PG, DPG, PME	PE, PME, OH-PE, PI, *lyso*-PME, DPG, PG, *lyso*-PE	PE, PME, PG, PI	PE, DPG, PI, PG, PME	PE, DPG, PI, PIM, DPG, PME
Predominant menaquinones	MK-9(H_4), MK-10(H_4)	MK-9(H_4)	MK-9(H_4)	MK-9(H_4)	MK-9(H_4)	MK-9(H_4)	MK-9(H_6), MK-10(H_6)
DNA G+C content (mol%)	69.2	71.4	72–72.5	71.6	66	70.3–70.7	nd

[a]Ara, Arabinose; Gal, galactose; Glu, glucose; Man, mannose; Rha, rhamnose; Rib, ribose.

[b]DPG, Diphosphatidylglycerol; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; PI, phosphatidylinositol; PIM, phosphatidylinositol mannosides; OH-PE, phosphatidylethanolamine with hydroxy fatty acids; *lyso*-PE, phosphatidylethanolamine where one fatty acid chain is missing from the glycerol backbone; *lyso*-PME, phosphatidylmethylethanolamine where one fatty acid chain is missing from the glycerol backbone; PME, phosphatidylmethylethanolamine.

TABLE 243. Fatty acid content of *Goodfellowiella coeruleoviolacea* and representative species of phylogenetically related genera

Fatty acid	*Goodfellowiella coeruleoviolacea* DSM 43935^T	*Actinoalloteichus cyanogriseus* DSM 43889^T	*Allokutzneria albata* DSM 44149^T	*Crossiella cryophila* DSM 44230^T	*Kibdelosporangium aridum* subsp. *aridum* DSM 43828^T	*Kutzneria viridogrisea* DSM 43850^T	*Streptoalloteichus hindustanus* DSM 44523^T
$C_{14:0}$ iso	–	5.0	6.4	0.7	5.2	–	–
$C_{14:0}$	–	–	–	–	1.8	–	–
$C_{15:0}$ iso	6.7	15.0	6.0	40.0	8.7	2.7	34.2
$C_{15:0}$ anteiso	5.0	10.0	0.5	2.2	12.7	–	10.7
$C_{15:1}$ (*cis*9)	–	–	3.2	3.8	–	–	–
$C_{15:0}$	6.2	trace	2.0	2.6	0.7	1.6	–
$C_{16:1}$ iso	4.5	8.0	10.5	3.2	6.0	0.6	–
$C_{16:0}$ iso	19.8	19.0	42.5	10.7	34.5	30.3	2.6
$C_{16:1}$ (*cis*9)	–	2.0	1.5	3.9	0.3	3.8	0.6
$C_{15:0}$ iso 2-OH	1.7	–	–	–	4.2	–	–
$C_{15:0}$ anteiso 2-OH	–	–	–	–	2.5	–	–
$C_{16:0}$	–	2.0	1.6	2.2	1.5	–	–
$C_{16:1}$ 10-methyl?	1.7	–	–	–	–	–	–
$C_{16:0}$ iso 10-methyl	–	–	–	0.9	–	–	–
$C_{16:0}$ 10-methyl	–	–	–	–	0.6	8.6	–
$C_{16:0}$ 2-OH	5.8	–	–	–	–	–	–
$C_{17:1}$ iso	–	–	–	–	–	–	14.5
$C_{17:1}$ anteiso	–	–	–	–	0.4	–	1.2
$C_{17:0}$ iso	1.7	3.0	2.3	10.3	1.0	6.2	10.6
$C_{17:0}$ anteiso	19.39	20.0	0.8	1.2	10.8	7.5	22.8
$C_{17:1}$ (*cis*9)	–	–	8.0	2.3	–	2.1	1.0
$C_{16:0}$ iso 2-OH	–	–	–	–	5.4	11.7	–
$C_{17:0}$	3.1	trace	3.8	1.8	0.4	4.3	–
$C_{17:0}$ 10-methyl	9.4	–	–	–	–	5.9	–
$C_{16:0}$ iso 3-OH	–	–	3.0	–	–	–	–
$C_{18:0}$ iso	–	–	1.0	–	0.3	0.9	–
$C_{18:1}$ (*cis*9)	–	–	1.0	–	–	0.8	–
$C_{17:0}$ anteiso 2-OH	6.5	–	–	–	–	4.5	–
$C_{17:0}$ iso 3-OH	–	–	3.8	–	0.9	–	–
$C_{17:0}$ anteiso 3-OH	–	–	–	4.1	–	–	0.3
$C_{17:0}$ 3-OH	–	–	0.7	–	2.1	–	–
$C_{18:0}$ 10-methyl	–	–	–	–	–	1.5	–
$C_{19:0}$ anteiso	–	–	–	–	–	–	0.5

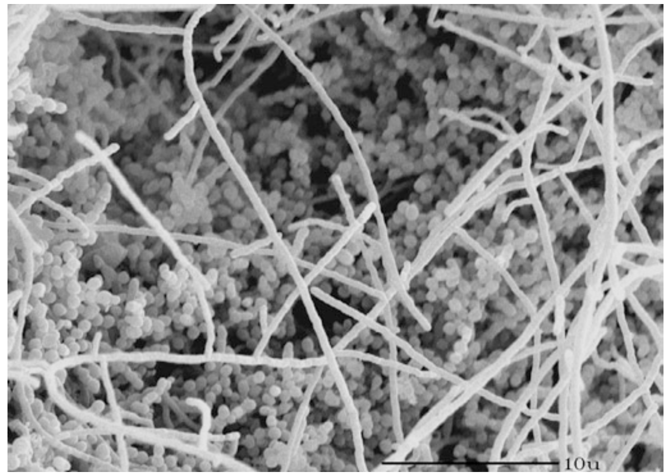

FIGURE 299. Scanning electron micrograph of 21-d growth of *Goodfellowiella coeruleoviolacea* NRRL B-24058^T on yeast extract-malt extract agar. Note that spores are produced by fragmentation of the vegetative mycelium. Bar = 10 μm.

monomethylethanolamine and phosphatidylethanolamine with 2-hydroxy fatty acids. Substantial quantities of menaquinone MK-10(H_4) are present and this is distinct from other taxa within the suborder. The fatty acid profile of *Goodfellowiella coeruleoviolacea* (Table 243) clearly distinguishes it from its nearest phylogenetic neighboring genera by the presence of significant quantities of $C_{17:0}$ 10-methyl fatty acid and various hydroxylated fatty acids. Scanning electron microscopic observations of colony growth on several different media have not revealed the presence of sporangia and the substrate mycelium appears to fragment into coccoidal rod elements (Figure 299). Spore chains typical of those observed in *Actinoalloteichus* species were not observed.

List of species of the genus *Goodfellowiella*

1. **Goodfellowiella coeruleoviolacea** (Preobrazhenskaya and Terekhova 1987) Labeda, Kroppenstedt, Euzeby and Tindall 2008, 1048^{VP} [*Actinomadura coeruleoviolacea* Preobrazhenskaya and Terekhova 1987, 179; *Saccharothrix coeruleoviolacea* (Preobrazhenskaya and Terekhova 1987) Kroppenstedt, Stackebrandt and Goodfellow 1990, 179; illegitimate synonym *Goodfellowia coeruleoviolacea* (Preobrazhenskaya and Terekhova 1987) Labeda and Kroppenstedt 2006, 1206]

co.e.ru.le.o.vi.o.la′ce.a. L. adj. *coeruleus* dark-colored, dark blue; L. adj. *violaceus* violet-colored, violet; N.L. fem. adj. *coeruleoviolacea* dark violet-colored.

Vegetative mycelium is pale yellow to dark brownish-yellow, depending on medium; white aerial hyphae are produced on most media becoming blue in color on several media including inorganic salts-starch (ISP4) agar and yeast extract-malt extract (ISP2) agar. Pale violet soluble pigment is produced on inorganic salts-starch agar and blue-green soluble pigment is produced on yeast extract-malt extract agar. Degrades or hydrolyzes casein, esculin, gelatin, hypoxanthine, starch, tyrosine, and urea. Does not degrade adenine, allantoin, or xanthine. Weakly reduces nitrates. Assimilates acetate, citrate, malate, oxalate, propionate, and succinate; does not assimilate benzoate, lactate, mucate, or tartrate. Acid is produced from adonitol, arabinose, cellobiose, dextrin, erythritol, fructose, galactose, glucose, glycerol, inositol, lactose, maltose, mannitol, mannose, melibiose, methyl α-D-glucoside, methyl β-xyloside, raffinose, rhamnose, salicin, sorbitol, sucrose, trehalose, and xylose; no acid is produced from dulcitol or melezitose. Grows weakly in the presence of 4% NaCl and not at all at higher salt concentrations.

Temperature range for growth is 15–45°C, with optimum growth around 30°C.

Source: isolated from soil.

DNA G+C content (mol%): 68.2 (T_m).

Type strain: NRRL B-24058, DSM 43935, INA 3564, JCM 9110, NBRC 14988, VKM Ac-1083.

Sequence accession no. (16S rRNA gene): DQ093349.

Genus VIII. **Kibdelosporangium** Shearer, Colman, Ferrin, Nisbet and Nash 1986a, 48[VP]

David P. Labeda

Kib.del.o.spo.ran′gi.um. Gr. adj. *kibdelos* false, ambiguous; Gr. n. *spora* seed, and in biology a spore; Gr. n. *angeion* a vessel; N.L. neut. n. *Kibdelosporangium* false or ambiguous sporangium.

Aerobic, catalase-positive, Gram-stain-positive, non-acid-fast, filamentous organisms that produce a substrate mycelium that penetrates the agar and forms a compact layer on top of the agar; aerial mycelium originates from the substrate mycelium. **Substrate mycelium may exhibit varying degrees of fragmentation and usually bears specialized structures that appear to be dichotomously branched, septate hyphae radiating from a common stalk. The aerial mycelia bear long chains of spores and sporangium-like structures. The sporangium-like structures are surrounded by well-defined walls, but do not contain spores and germinate directly, producing one or more germ tubes when placed on solid growth media. Motile elements or spores are not present. Contains *meso*-diaminopimelic acid as the diamino acid. Whole-cell sugar pattern consists of arabinose and D-galactose, with traces of madurose usually present**. The phospholipid pattern consists of diphosphatidylglycerol, phosphatidylethanolamine, phosphatidylinositol, phosphatidylinositol mannosides, and phosphatidylmethylethanolamine. **Mycolic acids are not present**.

DNA G+C content (mol%): 66 (T_m).

Type species: **Kibdelosporangium aridum** Shearer, Colman, Ferrin, Nisbet and Nash 1986a, 48[VP].

Further descriptive information

The genus *Kibdelosporangium* is placed phylogenetically within the family *Pseudonocardiaceae* in the order *Pseudonocardiales* (Figure 298) based on 16S rRNA gene sequences and shares similar chemotaxonomic characteristics with many of the genera within the family. The reproductive structures of members of the genus are quite distinct, however, and *Kibdelosporangium* colonies are frequently covered with straight to flexuous chains of rod-shaped spores (Figure 300) when viewed microscopically from above. Sporangium-like structures are observed beneath the spore chains, closer to the substrate mycelium (Figure 301). The sporangium-like structures and the spore chains can arise from the same aerial hyphae and may be borne apically on branched or unbranched hyphae, as well as terminally on short lateral hyphal branches.

The sporangium-like structures originate as small round swellings at the tips of the hyphae that continue to enlarge and, at maturity, are usually 9 to 35 µm in diameter (Figure 302). They are surrounded by a well-defined sporangial wall and contain septate, branched hyphae in an amorphous matrix (Figure 303). When placed on a suitable growth medium, the sporangium-like bodies germinate, usually within 24 to 48 h, and produce one or more germ tubes.

A third species, *Kibdelosporangium albatum*, was described by Tomita and others in 1993 because it appeared morphologically and chemotaxonomically most similar to the described members of the genus *Kibdelosporangium* (Tomita et al., 1993). This species has now been transferred by Labeda and Kroppenstedt (2008) to the new genus *Allokutzneria* as *Allokutzneria albata* on the basis of the phylogenetic position of this species, nearest to the genus *Kutzneria*, and its unique chemotaxonomic characteristics.

Enrichment and isolation procedures

Kibdelosporangium species can be isolated from soil by plating serial dilutions of the samples on starch-casein-nitrate agar (Küster and Williams, 1964) or arginine-glycerol-salt agar (El-Nakeeb and Lechevalier, 1963) media supplemented with

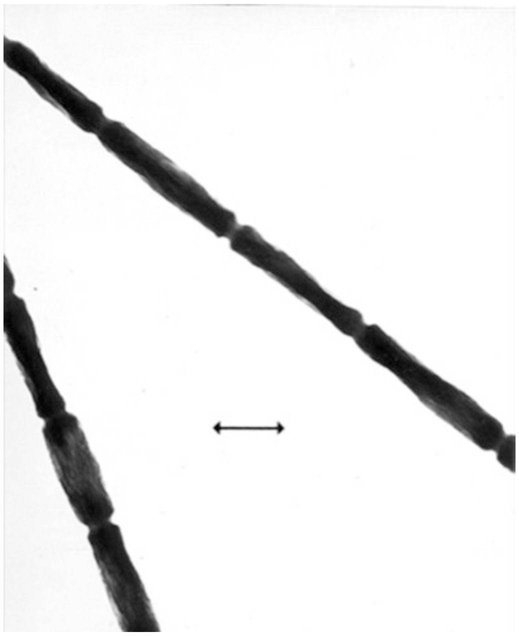

FIGURE 300. Transmission electron micrograph of *Kibdelosporangium aridum* spore chains (19-d-old culture on Bennett's agar). Bar = 1 µm. (Reproduced with permission from Shearer et al., 1986a. Int. J. Syst. Bacteriol. *36*: 47–54.)

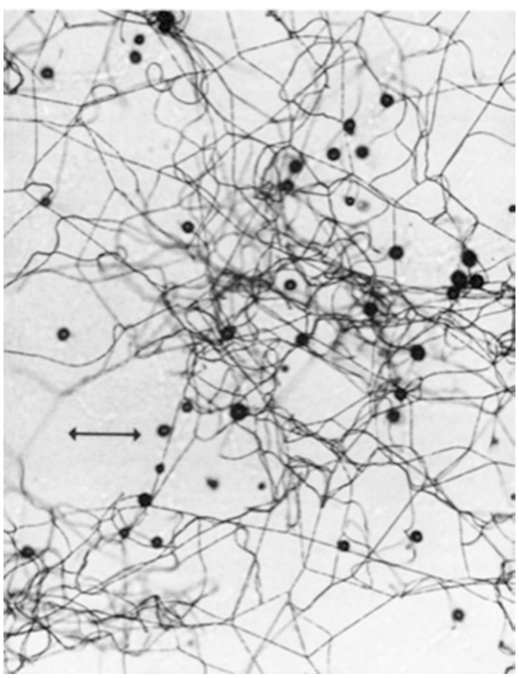

FIGURE 301. Micrograph of aerial mycelium with long, irregularly curved chains of spores and, nearer the agar surface, sporangium-like structures (*Kibdelosporangium aridum*; 26-d culture on water agar). Bar = 55 µm.

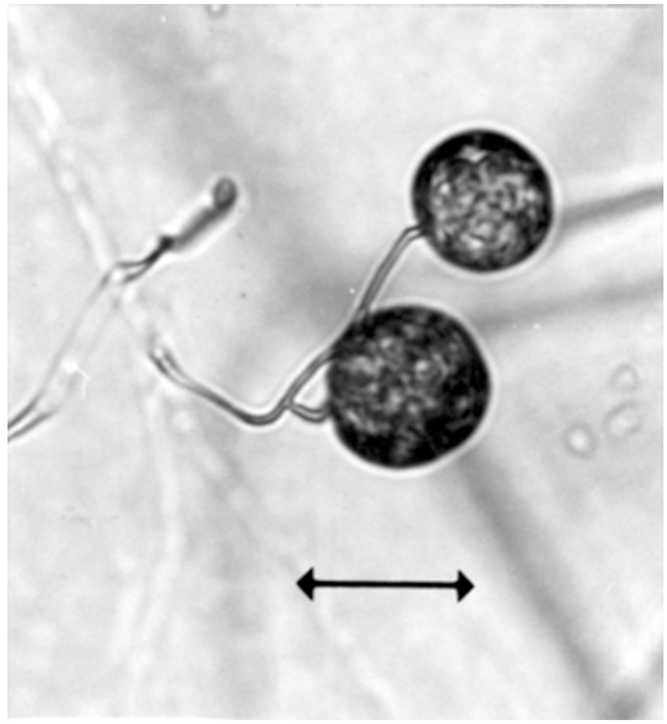

FIGURE 302. Micrograph of sporangium-like structures of *Kibdelosporangium aridum* (8-week-old culture on water agar). Bar = 14 µm. (Reproduced with permission from Shearer et al., 1986a. Int. J. Syst. Bacteriol. *36*: 47–54.)

FIGURE 303. Transmission electron micrograph of thin section of sporangium-like structure of *Kibdelosporangium aridum* (2.5-week-old culture on thin potato-carrot agar). Bar = 1.2 µm. (Reproduced with permission from Shearer et al., 1986a. Int. J. Syst. Bacteriol. *36*: 47–54.)

either vancomycin (1–25 μg/ml) or gentamicin (2.5–5.0 μg/ml) and antifungal antibiotics (e.g. cycloheximide, 25 μg/ml) (Shearer, 1987). Pre-treating air-dried soil samples by heating at 120°C for 60 min may also prove helpful by eliminating many competing soil micro-organisms. Plates are routinely incubated at 28°C for 4 weeks.

Kibdelosporangium colonies resemble those of many other actinomycetes and are therefore not easily identified on isolation plates. Observation of sporulating colonies on isolation plates microscopically (400×) is the best method to identify typical *Kibdelosporangium* colonies on these plates.

Maintenance procedures

Serial transfer of strains is not an acceptable method of maintenance for *Kibdelosporangium* species because they tend to lose the ability to produce sporangium-like structures after several transfers. Moderate to long-term preservation of strains is best accomplished as frozen stocks in 10% (v/v) aqueous glycerol at −80°C (mechanical freezer) to −172°C (liquid nitrogen vapor phase) or by using traditional lyophilization techniques.

Procedures for testing special characters

Strains are routinely cultivated on yeast extract-malt extract (ISP2) agar at 30°C. Morphological observations are made on the media of Shirling and Gottlieb (1966), thin potato-carrot agar (Higgins et al., 1967), soil extract agar (Shearer et al., 1983), or tap water agar.

Chemotaxonomic analysis of strains for fatty acids, menaquinones, and polar lipids are performed using the previously described methods of Grund and Kroppenstedt (1989), Minnikin et al. (1984), Sasser (1990), and Saddler et al. (1991).

Physiological tests, including production of acid from carbohydrates, utilization of organic acids, and hydrolysis and decomposition of adenine, casein, esculin, guanine, hippurate, hypoxanthine, tyrosine, xanthine, and urea, are typically evaluated using the media of Gordon et al. (1974). Phosphatase activity is determined by using the method of Kurup and Schmitt (1973) substituting NZamine agar as the basal medium. Temperature range for growth is determined on slants of DSMZ medium 554 (DSMZ, 2001) and salt tolerance is determined on slants of the same medium supplemented with 2 to 8% NaCl.

Differentiation of the genus *Kibdelosporangium* from other genera

Species of the genus *Kibdelosporangium* can be easily differentiated from the other sporangium- and pseudosporangium-forming genera within the order *Pseudonocardiales*, where they are firmly placed on the basis of 16S rRNA gene phylogeny (Figure 298), as well as on morphological and chemotaxonomic criteria. All *Kibdelosporangium* species produce sporangium-like bodies that do not appear to contain spores, similar to *Allokutzneria*, but are different from *Kutzneria* species whose sporangia do contain spores, see Table 244. Both *Kibdelosporangium* and *Allokutzneria* produce chains of spores on their aerial hyphae, but this is not observed in *Kutzneria* species. The chemotaxonomic profile for *Kibdelosporangium* species is also different from the other sporangiate or pseudosporangiate genera within the order, as can be seen in Table 244.

Differentiation of the species of the genus *Kibdelosporangium*

The physiological characteristics of *Kibdelosporangium* species and subspecies are summarized in Table 245 and can be used to differentiate between them. The strains are phylogenetically distinct based on 16S rRNA gene sequences (Figure 304) and gross colonial morphology provides some additional information for differentiation between the *Kibdelosporangium aridum* subspecies and *Kibdelosporangium philippinense*.

TABLE 244. Comparison of chemotaxonomic profiles of *Kibdelosporangium* with related genera classified in the order *Pseudonocardiales*

Character	*Kibdelosporangium*	*Allokutzneria*	*Kutzneria*	*Streptoalloteichus*	*Thermocrispum*
Sporangia/spores	Sporangia, 1–32 μm in diameter; spore chains formed on aerial hyphae	Sporangium-like bodies, 8–20 μm in diameter; spore chains on aerial hyphae	Sporangia, 10–40 μm in diameter; no spore chains.	Sporangium-like vessel (1 species), 1.5–4.5 × 2.7–7.0 μm containing 1–4 oval to rod-shaped spores; spore chains formed on aerial hyphae	Pseudosporangial fragment into rod-shaped spores
Motile spores	No	No	No	Variable (1 species)	No
Whole-cell sugar pattern[a]	Ara, Gal, Mad (v), Glu (v), Rha (v)	Ara, Gal, Man	Gal, Rha	Gal, Man, Rha (v)	Ara, Man, Glu, Gal (trace)
Phospholipids[b]	PE, PI, PME, PG, DPG, PIM	PE, PME, OH-PE, PI, *lyso*-PME, DPG, PG, *lyso*-PE	PE, DPG, PI, PG, PME	PE, DPG, PI, PIM, DPG, PME	PE, PI, OH-PE
Predominant menaquinones	MK-9(H_4), MK-9(H_6), MK-9(H_{10})	MK-9(H_4)	MK-9(H_4)	MK-9(H_6), MK-10(H_6)	MK-9(H_4)
DNA G+C content (mol%)	66	71.6	70.3–70.7	71.6	69.0–73.0

[a]Abbreviations: Ara, Arabinose; Gal, galactose; Glu, glucose; Mad, madurose; Man, mannose; Rha, rhamnose. v, Variable.

[b]Abbreviations: DPG, diphosphatidylglycerol; OH-PE, phosphatidylethanolamine with hydroxy fatty acids; *lyso*-PE, phosphatidylethanolamine where one fatty acid chain is missing from the glycerol backbone; *lyso*-PME, phosphatidylmethylethanolamine where one fatty acid chain is missing from the glycerol backbone; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; PI, phosphatidylinositol; PIM, phosphatidylinositol mannosides; PME, phosphatidylmethylethanolamine.

TABLE 245. Physiological characteristics of species of the genus *Kibdelosporangium*[a]

Character	*K. aridum* subsp. *aridum*	*K. aridum* subsp. *largum*	*K. philippinense*
Decomposition/hydrolysis of:			
Adenine	–	–	–
Allantoin	+	w	–
Casein	+	–	+
Cellulose	–	–	nd
Esculin	+	+	+
Gelatin	+	+	+
Hippurate	+	+	+
Hypoxanthine	+	+	+
Starch	–	–	–
Tyrosine	+	+	+
Urea	+	+	+
Xanthine	–	–	–
Acid from:			
Adonitol	–	nd	–
L-Arabinose	+	+	–
Cellobiose	+	+	+
Dextrin	+	+	–
Dulcitol	–	–	–
iso-Erythritol	–	–	–
D-Fructose	+	+	+
D-Galactose	+	+	+
Glucose	+	+	+
Glycerol	+	+	+
iso-Inositol	+	+	+
Inulin	–	–	–
Lactose	+	–	+
Maltose	+	+	+
D-Mannitol	+	+	+
D-Mannose	+	+	+
Melezitose	–	+	+
Melibiose	+	+	+
Methyl α-D-glucoside	+	+	+
Methyl β-D-xyloside	+	nd	nd
Raffinose	+	+	–
Rhamnose	+	+	+
D-Ribose	+	+	+
Salicin	v	v	–
D-Sorbitol	–	–	–
L-Sorbose	–	–	–
Sucrose	+	+	–
Trehalose	+	+	+
D-Xylose	+	+	+
Utilization of:			
Acetate	+	+	+
Benzoate	–	–	–
Citrate	+	+	+
Formate	+	+	+
Lactate	+	+	+
Malate	+	+	+
Oxalate	+	+	+
Propionate	+	+	+
Pyruvate	+	+	+
Succinate	+	+	+
Tartrate	–	–	–
Production of:			
Nitrate reductase	–	–	+
Phosphatase	+	+	+
Hydrogen sulfide	+	+	+
Melanin	+	+	+

(continued)

TABLE 245. (continued)

Character	*K. aridum* subsp. *aridum*	*K. aridum* subsp. *largum*	*K. philippinense*
Growth in:			
Lysozyme broth	–	–	–
2% NaCl	+	+	+
4% NaCl	+	v	–
5–7% NaCl	v	v	–
8% NaCl	–	–	–
Survival at 50°C/8 h	+	nd	nd
Growth at:			
10°C	v	–	–
15°C	+	+	–
42°C	+	+	–
45°C	tr	tr	–

[a]Symbols: w, weak positive; nd, not determined; v, variable; tr, trace amounts.

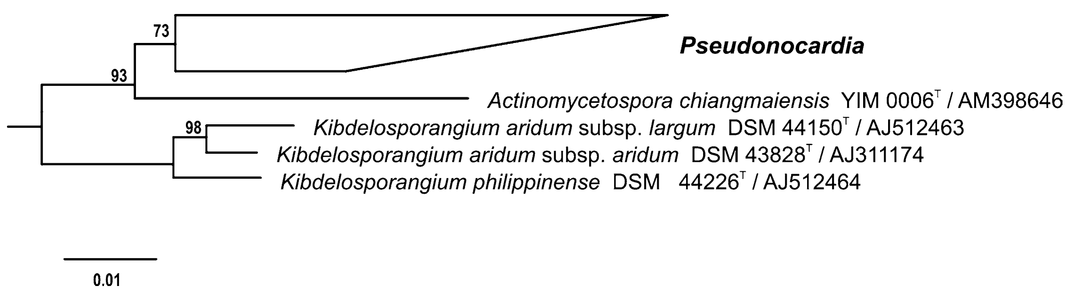

FIGURE 304. Phylogenetic tree for the genera *Kibdelosporangium*, *Actinomycetospora*, and *Pseudonocardia* calculated from almost-complete 16S rRNA gene sequences using the Kimura's evolutionary distance method (Kimura, 1980) and the neighbor-joining algorithm of Saitou and Nei (1987). Percentages at the nodes represent levels of bootstrap support (Felsenstein, 1993) from 100 resampled datasets; values less than 60% are not shown. Bar = 0.01 nucleotide substitutions per site.

List of species of the genus *Kibdelosporangium*

1. **Kibdelosporangium aridum** Shearer, Colman, Ferrin, Nisbet and Nash 1986a, 48[VP]

a′ri.dum. L. neut. adj. *aridum* dry, arid.

The substrate mycelium is off-white to grayish yellow-brown in color and is well developed with moderately branching, septate hyphae that are 0.4–1.0 µm in diameter. Fragmentation of substrate hyphae without hyphal displacement frequently occurs in plate cultures. Characteristic crystals are produced in the agar on many media. No pigments other than melanin or yellow-brown soluble pigments are produced. Produces the glycopeptides antibiotics aridicins A, B, and C (Shearer et al., 1985; Sitrin et al., 1985). Temperature for growth is 30°C.

Source: isolated from soil.

DNA G+C content (mol%): 66 (T_m).

Type strain: ATCC 39323, DSM 43828, JCM 7912, NBRC 14493, NRRL B-16436, SK&F AAD-216, VKM Ac-1316.

Sequence accession no. (16S rRNA gene): AJ311174.

Subsequently this species has been divided into subspecies.

1a. **Kibdelosporangium aridum subsp. aridum** Shearer, Colman, Ferrin, Nisbet and Nash 1986a, 48[VP]

The description is as for the species.

Type strain: ATCC 39323, DSM 43828, JCM 7912, NBRC 14493, NRRL B-16436, SK&F AAD-216, VKM Ac-1316.

Sequence accession no. (16S rRNA gene): AJ311174.

1b. **Kibdelosporangium aridum subsp. largum** Shearer, Giovenella, Grappel, Hedde, Mehta, Oh, Pan, Pitkin and Nisbet, 1988, 136[VP] (Effective publication: Shearer, Giovenella, Grappel, Hedde, Mehta, Oh, Pan, Pitkin and Nisbet 1986b, 1391.)

lar′gum. L. neut. adj. *largum* abundant, plentiful, numerous.

The substrate mycelium is off-white to yellow-brown in color and shows branching. The aerial mycelium is white to light gray in color, when produced. Pale yellow-brown to yellow-brown soluble pigments are produced on some media. Characteristic crystals are formed in the agar on some media. Temperature for growth is 30°C. Produces the glycopeptides antibiotics aridicins A, B, and C (Shearer et al., 1985; Sitrin et al., 1985) and the kibdelins A, B, C, and D (Folena-Wasserman et al., 1986; Shearer et al., 1986b).

Source: isolated from soil.

DNA G+C content (mol%): not determined.

Type strain: ATCC 39922, DSM 44150, JCM 9107, NBRC 15152, SK&F AAD-609.

Sequence accession no. (16S rRNA gene): AJ512463.

2. **Kibdelosporangium philippinense** Mertz and Yao 1988, 286[VP]

phil.ip.pi.nen′se. N.L. neut. adj. *philippinense* pertaining to the Philippines.

The branching substrate mycelium is generally pale yellow to orange-yellow in color. Aerial mycelium is white in color, when produced. Light brown to light reddish-brown soluble pigments are formed on some growth media. Production of crystals is not observed on any growth medium. Produces a ristocetin-like glycopeptide antibiotic. Temperature for growth is 30°C.

Source: isolated from soil.

DNA G+C content (mol%): not determined.

Type strain: A80407, ATCC 49844, DSM 44226, JCM 9918, NBRC 15154, NRRL 18198.

Sequence accession no. (16S rRNA gene): AJ512464.

Genus IX. **Kutzneria** Stackebrandt, Kroppenstedt, Jahnke, Kemmerling and Gürtler 1994, 267[VP]

David P. Labeda

Kutz.ne′ri.a. N.L. fem. n. *Kutzneria* named after Hans-Jürgen Kutzner, a German microbiologist.

Aerobic, Gram-stain-positive, mesophilic actinomycete which forms a stable, branched, cottony aerial mycelium. **Globose sporangia are large (diameter of 10–48 μm).** Sporangial walls are thick and strong. **Sporangiophores more than 50 μm long are formed by septation of coiled, unbranched hyphae within sporangiophores. Spores are spherical, rod-shaped, or ovoid and nonmotile.** Some species are thermotolerant. Chemo-organotrophic. **Cell walls contain *N*-acetylated muramic acid**, ***meso*-diaminopimelic acid, and generally galactose as the characteristic sugar.** Rhamnose may also be present as a whole-cell diagnostic sugar. **MK-9(II, III-H_4) are the major menaquinones. Major phospholipids include diphosphatidylglycerol, hydroxyphosphatidylethanolamine, phosphatidylethanolamine, and phosphatidylinositol. Major fatty acids are $C_{16:0}$ iso, $C_{16:0}$ iso 2-OH, $C_{16:0}$ 10-methyl, $C_{17:0}$ anteiso, and $C_{17:0}$ 2-OH anteiso; $C_{10:0}$, $C_{14:0}$ iso, and $C_{14:0}$ fatty acids are absent.** Natural habitat is soil.

DNA G+C content (mol%): 70.3–70.7 (T_m).

Type species: **Kutzneria viridogrisea** (Okuda, Furumai, Watanabe, Okugawa and Kimura 1966) Stackebrandt, Kroppenstedt, Jahnke, Kemmerling and Gürtler 1994, 268[VP].

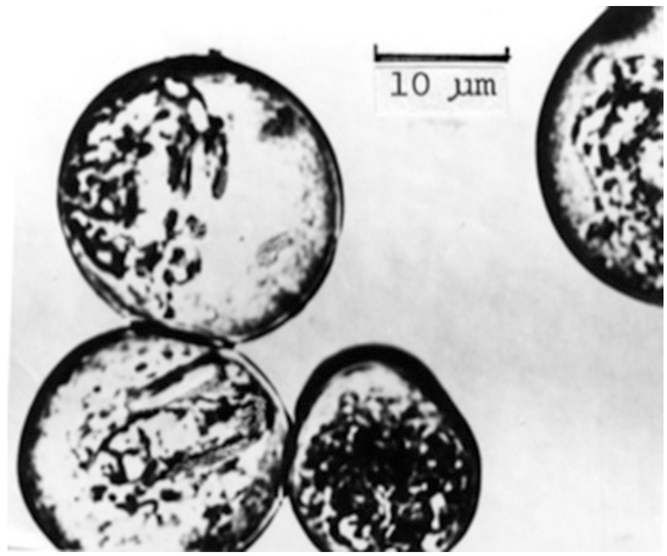

FIGURE 305. *Kutzneria kofuensis* sporangia. Note that sporangial walls are thick. Light micrograph stained with methylene blue. (Reproduced with permission from Nonomura and Ohara, 1969b. J. Ferment. Technol. *47*: 648.)

FIGURE 306. Light micrograph showing the long sporangiophores of *Kutzneria kofuensis*. (Reproduced with permission from Nonomura and Ohara, 1969b. J. Ferment. Technol. *47*: 648.)

Further descriptive information

All of the species currently classified within the genus *Kutzneria* were originally described as *Streptosporangium* species with validly published names based on their production of sporangia (Figure 305). The sporangia are produced on long sporophores (Figure 306) containing chains of nonmotile spores (Figure 307). Stackebrandt et al. (1994) proposed that these species be transferred to the new genus *Kutzneria* because phylogenetic analysis based on 16S rRNA genes (Kemmerling et al., 1993), as well as previous phylogenetic studies based on 5S rRNA genes (Kudo et al., 1993) and electrophoretic mobility of ribosomal protein AT-L30 (Ochi and Miyadoh, 1992), demonstrated that they were phylogenetically distinct from species of the genus *Streptosporangium*, as can be seen by their phylogenetic position within the suborder *Pseudonocardineae*, now the order *Pseudonocardiales*, shown in Figure 277 in the treatment of the family *Pseudonocardiaceae*. The chemotaxonomic characteristics of the species transferred to *Kutzneria* were also observed to be distinct from those of *Streptosporangium sensu stricto*, as can be seen in Table 246, particularly with regard to the lack of madurose in the whole-cell sugar pattern, the lack of ninhydrin-positive and sugar-positive phospholipids in their polar lipid patterns, and major differences in fatty acid and menaquinone profiles.

Enrichment and isolation procedures

Nonomura and Ohara (1969a) described a novel method for the isolation of *Microbispora* and *Streptosporangium* from soil

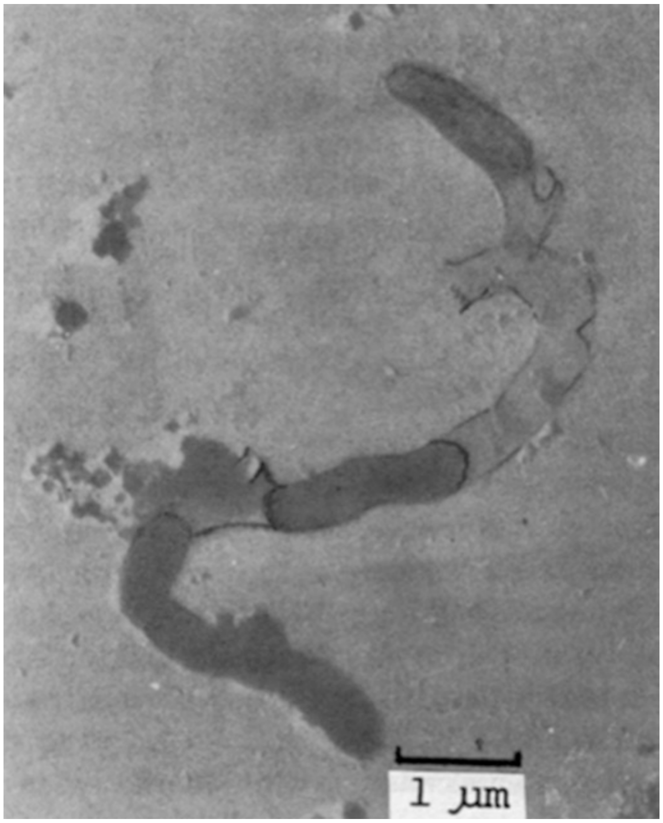

FIGURE 307. Electron micrograph showing *Kutzneria kofuensis* sheathed spores from within the sporangium. (Reproduced with permission from Nonomura and Ohara, 1969b. J. Ferment. Technol. *47*: 648.)

samples; the type strain of *Kutzneria kofuensis* was isolated using this procedure. Soil samples are pretreated by air drying, grinding in a mortar, and then heating at 120°C for 1 h. Aliquots of serial dilutions of the soil samples are spread onto the surface of arginine-vitamin (AV) agar typically containing antifungal antibiotics (e.g. cycloheximide and/or nystatin at 50 µg/ml) and plates are incubated for 40 d at 30°C. AV agar consists of (per l): L-arginine hydrochloride, 0.3 g; glucose, 1.0 g; glycerol, 1.0 g; K_2HPO_4, 0.3 g; $MgSO_4 \cdot 7H_2O$, 0.2 g; NaCl, 0.3 g; thiamine hydrochloride, 0.5 mg; riboflavin, 0.5 mg; niacin, 0.5 mg; pyridoxine hydrochloride, 0.5 mg; inositol, 0.5 mg; calcium pantothenate, 0.5 mg; *p*-aminobenzoic acid, 0.5 mg; biotin, 0.25 mg; $Fe_2(SO_4)_3$, 10 mg; $CuSO_4 \cdot 5H_2O$, 1 mg; $MnSO_4 \cdot H_2O$, 1 mg; $ZnSO_4 \cdot 7H_2O$, 1 mg; agar, 15 g. The pH should be adjusted to 6.4 prior to sterilization. The vitamins should be filter-sterilized and added to autoclaved and tempered medium prior to dispensing.

Maintenance procedures

Working cultures of *Kutzneria* can be maintained as refrigerated (4°C) agar slants on appropriate medium such as NZamine agar (DSMZ medium 554; DSMZ, 2001). The slants are generally incubated for 5–7 d at 28–30°C prior to refrigeration and then should be subcultured at monthly intervals. Longer term preservation of strains is best accomplished as frozen stocks in 20% (v/v) aqueous glycerol at −80°C (mechanical freezer) to −172°C (liquid nitrogen vapor phase) or by using traditional lyophilization techniques. Strains have also been successfully stored for shorter periods as quick-frozen stationary-phase broth cultures or mycelium suspensions in 20% aqueous glycerol at −20 to −72°C.

Procedures for testing special characters

Strains are routinely cultivated on NZamine agar at 28°C. Morphological observations are made on the media of Shirling and Gottlieb (1966) and NZamine agar.

Chemotaxonomic analysis of strains for fatty acids, menaquinones, and polar lipids are performed using methods previously described by Grund and Kroppenstedt (1989), Minnikin et al. (1984), Sasser (1990), and Saddler et al. (1991). Physiological tests, including production of acid from carbohydrates, utilization of organic acids, and hydrolysis and decomposition of adenine, casein, esculin, guanine, hippurate, hypoxanthine, tyrosine, xanthine, and urea, are typically evaluated using the media of Gordon et al. (1974). Phosphatase activity is determined by using the method of Kurup and Schmitt (1973), but substituting NZamine agar as the basal medium. Temperature range for growth is determined on slants of DSMZ medium 554 and salt tolerance is determined on slants of the same medium supplemented with 4% and 5% NaCl.

Differentiation of the genus *Kutzneria* from other genera

The genus *Kutzneria* is phylogenetically distinct from all of the other sporangium- or pseudosporangium-forming genera within the suborder *Pseudonocardineae*, now the order *Pseudonocardiales*, but is phylogenetically most closely related to the genus *Allokutzneria*, as can be seen in Figure 308. The morphology of the sporangia produced by *Kutzneria* species and their chemotaxonomic characteristics (Table 246) differentiate them from similar sporangium-producing genera. The chemotaxonomy of the genus *Kutzneria* is distinct from that of *Allokutzneria* in containing galactose and mannose as whole-cell sugars, i.e. arabinose, galactose, and mannose versus galactose and rhamnose that are found in *Allokutzneria*. The phospholipid profiles of these two genera are also quite different: *Allokutzneria* contains *lyso*-phosphatidylethanolamine, *lyso*-phosphatidylmethylethanolamine, and phosphatidylethanolamine containing hydroxylated fatty acids, whereas these are absent in *Kutzneria*. The sporangial morphology and chemotaxonomic profile, particularly in regard to the whole-cell sugar pattern and predominant menaquinones, between *Kutzneria* and *Kibdelosporangium*, are also quite different, as can be seen in Table 246.

Differentiation of the species of the genus *Kutzneria*

The morphological and physiological characteristics of *Kutzneria* species are summarized in Table 247 and can be used to differentiate between species, although a common set of physiological tests has not been used in the description of all species. The whole-cell sugar patterns of each species also appear to be distinct. The strains can also be distinguished based on their 16S rRNA gene sequences and phylogeny (Figure 308).

TABLE 246. Comparison of chemotaxonomic profile of *Kutzneria* with phylogenetically nearest genera and *Streptosporangium*

Character	*Kutzneria*	*Streptosporangium*	*Allokutzneria*	*Actinoalloteichus*	*Crossiella*	*Goodfellowiella*	*Kibdelosporangium*	*Saccharothrix*	*Streptoalloteichus*	*Umezawaea*
Production of sporangia and size	Diameter 11–50 μm, contain chains of sheathed spores	Diameter up to 10 μm, contains coiled chains of oval to rod-shaped spores	Diameter 8–20 μm, contains coiled hyphae, no spores	None	None	None	Diameter 9–35 μm, contains hyphae in an amorphous matrix	None	Pseudosporangia	None
Whole-cell sugar pattern[a]	Gal, Rha	Gal (v), Mad (v)	Ara, Gal, Man	Glu, Gal, Man, Rib	Gal, Man, Rha, Rib	Gal, Rib	Ara, Gal, Glu (v), Mad (v), Rha (v)	Gal, Rha, Man (tr)	Gal, Man, Rha, Rib	Gal, Man, Rib, Rha (tr)
Phospholipids[b]	PE, DPG, PI, PG, PME	PE, OH-PE, PI, DPG, GlN	PE, PME, OH-PE, PI, *lyso*-PME, DPG, PG, *lyso*-PE	PIM, PI, PG, DPG PME	PE, DPG, PI, PIM, PME	PE, DPG, OH-PE, PME	PE, PI, PME, PG, DPG, PIM	PE, OH-PE, DPG, PG, PI, PIM	PE, DPG, PI, PIM, DPG, PME	PE, PI, OH-PE, *lyso*-PE
Predominant menaquinones	MK-9(H_4)	MK-9(H_2), MK-9(II,VIII-H_4), and/or MK-9(H_6)	MK-9(H_4)	MK-9(H_4)	MK-9(H_4)	MK-9(H_4), MK-10(H_4)	MK-9(H_4), MK-9(H_6), MK-9(H_{10})	MK-9(H_4), MK-10(H_4)	MK-9(H_6), MK-10(H_6)	MK-9(H_4), MK-10(H_4)
DNA G+C content (mol%)	70.3–70.7	69–71	71.6	72–72.5	71.4	69.2	66	72.2–74.0	ndND	74.0

[a]Ara, Arabinose; Gal, galactose; Glu, glucose; Mad, madurose; Man, mannose; Rha, rhamnose; Rib, ribose. v, Variable; tr, trace.

[b]Abbreviations: DPG, diphosphatidylglycerol; GlN, *N*-acetylglucosamine-containing phospholipid; OH-PE, phosphatidylethanolamine with hydroxy fatty acids; *lyso*-PE, phosphatidylethanolamine where one fatty acid chain is missing from the glycerol backbone; *lyso*-PME, phosphatidylmethylethanolamine where one fatty acid chain is missing from the glycerol backbone; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; PI, phosphatidylinositol; PIM, phosphatidylinositol mannosides; PME, phosphatidylmethylethanolamine.

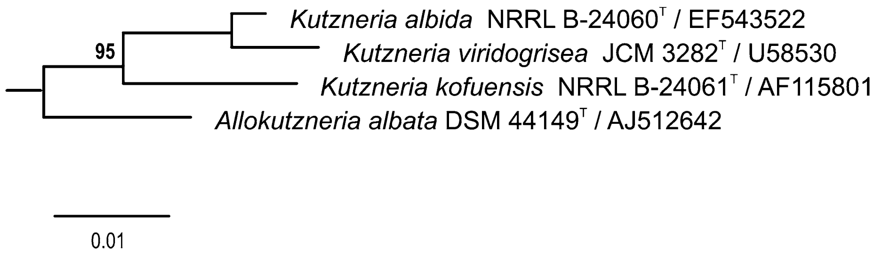

FIGURE 308. Phylogenetic tree for the genus *Kutzneria* and *Allokutzneria* calculated from almost-complete 16S rRNA gene sequences using the Kimura's evolutionary distance method (Kimura, 1980) and the neighbor-joining algorithm of Saitou and Nei (1987). Percentages at the nodes represent levels of bootstrap support (Felsenstein, 1989) from 100 resampled datasets; values less than 60% are not shown. Bar = 0.01 nucleotide substitutions per site.

TABLE 247. Morphological and physiological characteristics of *Kutzneria* species[a]

Characteristic	*K. viridogrisea*	*K. albida*	*K. kofuensis*
pH for growth	4–9	nd	nd
Sporangium:			
11–15 µm	–	(+)	+
16–20 µm	–	(+)	+
21–30 µm	+	+	–
31–50 µm	+	–	–
Sporangiospores:			
Short (10 µm)	–	–	+
Long (50 µm)	+	+	+
Spores:			
Spherical to ovoid	+	+	–
Rods	–	–	+
Color of spore mass	Greenish-gray	White	Greenish-gray
Color of substrate mycelium	Yellowish-brown to brown	Yellowish-brown to brown	Yellowish-brown to brown
Production of:			
Soluble pigments	–	–	–
Nitrate reductase	+	+	–
Hydrolysis of:			
Cellulose	–	–	nd
Gelatin	+	–	+
Milk (peptonization)	+	+	w
Starch	+	–	+
Growth as sole carbon source:			
Arabinose	w	+	+
Dextrin	nd	–	nd
Dulcitol	–	nd	nd
Fructose	+	+	nd
Galactose	+	nd	nd
Glucose	+	+	+
Glycerol	+	+	+
Inositol	w	+	+
Inulin	–	nd	nd
Lactose	w	+	nd
Maltose	+	nd	nd
Mannitol	+	+	nd
Mannose	+	+	nd
Raffinose	+	+	nd
Rhamnose	w	+	w
Salicin	–	–	nd
Sorbitol	+	nd	nd
Starch	+	nd	nd
Sucrose	+	+	nd
Xylose	w	+	nd

(continued)

TABLE 247. (continued)

Characteristic	*K. viridogrisea*	*K. albida*	*K. kofuensis*
Assimilation of:			
Malate	+	+	nd
Growth at:			
10°C	−	nd	nd
27°C	+	+	+
37°C	+	nd	+
42°C	nd	nd	+
50°C	v	nd	v

[a]Symbols: (+), sometimes observed; w, weak positive reaction; v, variable; nd, not determined.

List of species of the genus *Kutzneria*

1. **Kutzneria viridogrisea** (Okuda, Furumai, Watanabe, Okugawa and Kimura 1966) Stackebrandt, Kroppenstedt, Jahnke, Kemmerling and Gürtler 1994, 268[VP] (Basonym: *Streptosporangium viridogriseum* Okuda, Furumai, Watanabe, Okugawa and Kimura 1966, 126.)

vi.ri.do.gri′se.a. L. adj. *viridis* green; N.L. adj. *griseus* gray; N.L. fem. adj. *viridogrisea* greenish gray.

Substrate mycelium is yellowish-brown in color on most growth media. Cottony aerial mycelium is produced, initially white in color, then becoming greenish-gray to olive-gray, depending upon the growth medium. Soluble pigments are not produced on any medium. Temperature range for growth is 17–45°C, with an optimum at 37°C. Whole-cell sugars are galactose and rhamnose. Produces sporoviridin.

Source: isolated from soil.

DNA G+C content (mol%): 70.3 (HPLC).

Type strain: ATCC 25242, DSM 43850, JCM 3282, NBRC 15561, NRRL B-24059, VKM Ac-1297.

Sequence accession no. (16S rRNA gene): U58530.

2. **Kutzneria albida** (Furumai, Ogawa and Okuda 1968) Stackebrandt, Kroppenstedt, Jahnke, Kemmerling and Gürtler 1994, 268[VP] (Basonym: *Streptosporangium albidum* Furumai, Ogawa and Okuda 1968, 174)

al′bi.da. L. fem. adj. *albida* white.

Substrate mycelium is colorless to yellowish-brown on synthetic media with production of thin white aerial mycelia which become cottony or floccose and brownish-white. Sporangia (10–30 µm) can be easily observed microscopically. Substrate mycelium is pale yellow to reddish-brown on organic media. Soluble pigments are not produced on any medium. Temperature range for growth is 17°C to 45°C with an optimum at 37°C. Whole-cell sugar is rhamnose. Produces the antibiotic sporoviridin.

Source: isolated from soil.

DNA G+C content (mol%): 70.3 (HPLC).

Type strain: ATCC 25243, DSM 43870, JCM 3240, NBRC 13901, NRRL B-24060.

Sequence accession no. (16S rRNA gene): EF543522.

3. **Kutzneria kofuensis** (Nonomura and Ohara 1969b) Stackebrandt Kroppenstedt, Jahnke, Kemmerling and Gürtler 1994, 268[VP] (Basonym: *Streptosporangium viridogriseum* subsp. *kofuense* Nonomura and Ohara 1969b, 708)

ko.fu.en′sis. N.L. fem. adj. *kofuensis* belonging to Kofu, a district in Japan, where the organism was isolated.

Grows well on oatmeal-yeast extract-glucose agar and yeast-malt agar. Aerial mycelium is greenish-gray. Soluble pigments are not produced on any medium. Temperature range for growth is 17–45°C, with an optimum at 37°C. Whole-cell sugars are rhamnose and galactose. Produces chloramphenicol.

Source: isolated from soil.

DNA G+C content (mol%): 70.3 (HPLC).

Type strain: ATCC 27102, DSM 43851, JCM 3157, NBRC 13989, NRRL B-24061.

Sequence accession no. (16S rRNA gene): AF114801.

Genus X. **Lechevalieria** Labeda, Hatano, Kroppenstedt and Tamura 2001, 1049[VP]

David P. Labeda

Le.che.va.li.e′ri.a. N.L. fem. n. *Lechevalieria* named after the American microbiologists Hubert and Mary Lechevalier, who contributed substantially to the field of actinomycete biology during their careers at the Waksman Institute of Microbiology.

Branching vegetative mycelium (approx. 0.5 µm in diameter) is produced. **Very scant aerial mycelium is formed on some media.** Gram-stain-positive. Lysozyme resistant. Catalase-positive and aerobic. **The cell wall contains *meso*-diaminopimelic acid as the diamino acid. Whole-cell sugar pattern consists of galactose, mannose, and traces of rhamnose. Phospholipid pattern consists of significant quantities of phosphatidylethanolamine lacking hydroxylated fatty acids, as well as diphosphatidylglycerol, phosphatidylglycerol, and**

phosphatidylinositol. The major menaquinone is MK-9(H_4). Fatty acid profile consists of saturated and mono-unsaturated iso and anteiso fatty acids. **Phylogenetically represents a line of descent in the family *Pseudonocardiaceae*; adjacent to the genus *Saccharothrix* and close to the genera *Actinosynnema* and *Lentzea*. 16S rRNA gene sequence contains genus-specific diagnostic nucleotide signature pattern of TT (844–845) and GGT (1107–1109).**

DNA G+C content (mol%): 68–71.4 (T_m).

Type species: **Lechevalieria aerocolonigenes** (Shinobu and Kawato 1960) Labeda, Hatano, Kroppenstedt, and Tamura 2001, 1050[VP].

Further descriptive information

The genus *Lechevalieria* was described by Labeda et al. (2001) to contain strains formerly placed in the genus *Saccharothrix* that were found to be distinct by phylogenetic analysis of 16S rRNA gene sequences. Both of the constituent species, *Lechevalieria aerocolonigenes* and *Lechevalieria flava*, had been transferred previously into *Saccharothrix* from the genera *Streptomyces* and *Nocardiopsis*, respectively, although the latter taxon had been previously transferred first from the genus *Actinomadura* to the genus *Nocardiopsis*. Previously, the classification of these organisms had been based largely on morphology and then on chemotaxonomy. Subsequent phylogenetic analyses based on 16S rRNA gene sequences elucidated the significance of new chemotaxonomic markers which are essential for distinguishing *Lechevalieria* species from neighboring taxa.

Enrichment and isolation procedures

Lechevalieria strains, as well as those of the genera *Lentzea* and *Saccharothrix*, can be isolated from soil and plant residue samples by spreading soil dilutions onto the surface of routine media used for the general isolation of actinomycetes, such as 1.5% crude agar and 0.4% casein hydrolysate in tap water. Antibiotics can be used to more selectively isolate members of these genera as has been described by Shearer (1987). The recently described species *Lechevalieria fradiae* and *Lechevalieria xinjiangensis* were isolated from soil samples by serial plating on yeast extract-malt extract agar (Shirling and Gottlieb, 1966) and modified Bennett's agar (Jones, 1949), respectively.

Maintenance procedures

Working cultures of *Lechevalieria* can be maintained as refrigerated (4°C) agar slants on appropriate medium such as NZamine agar (DSMZ medium 554; DSMZ, 2001). The slants are generally incubated for 5 to 7 d at 28–30°C prior to refrigeration and then should be subcultured at monthly intervals. Longer term preservation of strains is best accomplished as frozen stocks in 20% (v/v) aqueous glycerol at −80°C (mechanical freezer) to −172°C (liquid nitrogen vapor phase) or by using traditional lyophilization techniques. Strains have also been successfully stored for shorter periods as quick-frozen stationary-phase broth cultures or mycelium suspensions in 20% aqueous glycerol at −20 to −72°C.

Procedures for testing special characters

Strains are routinely cultivated on NZamine agar (DSMZ medium 554) at 28°C. Morphological observations are made on the media of Shirling and Gottlieb (1966) and NZamine agar.

Chemotaxonomic analysis of strains for fatty acids, menaquinones, and polar lipids are performed using methods described previously by Grund and Kroppenstedt (1989), Minnikin et al. (1984), Sasser (1990), and Saddler et al. (1991).

Physiological tests, including production of acid from carbohydrates, utilization of organic acids, and hydrolysis and decomposition of adenine, casein, esculin, guanine, hippurate, hypoxanthine, tyrosine, xanthine, and urea, are typically evaluated using the media of Gordon et al. (1974). Phosphatase activity is determined by using the method of Kurup and Schmitt (1973) substituting NZamine agar as the basal medium. Temperature range for growth is determined on slants of DSMZ medium 554 and salt tolerance on slants of the same medium supplemented with 4% and 5% NaCl.

Differentiation of the genus *Lechevalieria* from other genera

The type strains of *Lechevalieria aerocolonigenes*, *Lechevalieria flava*, *Lechevalieria fradiae*, and *Lechevalieria xinjiangensis* form a monophyletic lineage distinct from the genus *Lentzea* (mean nucleotide similarity of 96.7%), and intermediary between *Lentzea* and the genus *Actinosynnema* (mean nucleotide similarity of 97.3%) as can be seen in Figure 290. The aligned sequences of the 16S rRNA genes for *Lechevalieria*, *Actinosynnema*, *Lentzea*, and *Saccharothrix* (Figure 309) illustrate that *Lechevalieria* strains can be clearly distinguished from the other genera on the basis of the diagnostic nucleotide signatures TT (844–845) and GGT (1107–1109). Note that the published 16S rRNA gene sequence for *Lechevalieria fradiae* contains the signature GGC (1107–1109) and this has not yet been confirmed to be accurate.

Species of the genus *Lechevalieria* can be distinguished from the genera *Actinosynnema* and *Saccharothrix* by the lack of hydroxy-substituted fatty acids in the phosphatidylethanolamine component of the diagnostic phospholipids (see Table 248), similar to species of the genus *Lentzea*. *Lechevalieria*, *Saccharothrix*, and *Umezawaea* strains tend to contain varying amounts of rhamnose in whole-cell hydrolysates, whereas *Lentzea* strains are observed to totally lack or contain only trace amounts of rhamnose and may also contain ribose. *Actinosynnema* species have only galactose and mannose as their diagnostic whole-cell sugar pattern.

Lechevalieria strains produce very sparse aerial mycelium on agar media, their substrate mycelium fragments into coccoid to coccoidal-rod-shaped elements, and they have not been observed to produce sporangia, coremia, or motile spores, which differentiates them from the genera *Actinokineospora* and *Actinosynnema*.

Differentiation of the species of the genus *Lechevalieria*

The physiological characteristics of *Lechevalieria* species are summarized in Table 249 and can be used to differentiate between species, although a common set of physiological tests has not been used in the description of all species. Gross colonial morphology provides some additional information for differentiation

	601	611	621	841
Lechevalieria aerocolonigenes NRRL B-3298^{T}	**AAACTTGGGG**	**CTTAACCCCG**	**AGCCTGCGGT**	**ACGTTCTCCG**
Lechevalieria flava NRRL B-16131^{T}	....	....	....	....
Lechevalieria fradiae CGMCC 4.3506^{T}	....	....	....	....**C**.....
Lechevalieria xinjiangensis CGMCC 4.3525^{T}	....	....	....	....
Lentzea albida IFO 16102^{T}	....	......**T**..**A**	...**T**......	...**CC**...**T**.
Lentzea albidocapillata DSM 44073^{T}	....	......**T**..**A**	...**T**......	...**CC**...**T**.
Lentzea californiensis NRRL B-16137^{T}	....	......**T**..**A**	...**T**...**C**..	...**CC**...**T**.
Lentzea flaviverrucosa AS4.0578^{T}	....	......**T**..**A**	...**T**......	...**CC**...**T**.
Lentzea kentuckyensis NRRL B-24416^{T}	....	......**T**..**A**	...**T**.......	...**CC**...**T**.
Lentzea violacea IMSNU 50388^{T}	....	......**T**..**A**	...**T**......	...**CC**...**T**.
Lentzea waywayandensis NRRL B-16159^{T}	....	......**T**..**A**	...**T**......	...**CC**...**T**.
Actinosynnema mirum DSM 43827^{T}	....	....	....	....**C**.....
Actinosynnema pretiosum subsp. *pretiosum* NRRL B-16060^{T}	....	....	...**T**......	....**C**.....
Saccharothrix australiensis NRRL 11239^{T}	......**CAC**.	......**GTG**.	....	....**C**.....
Saccharothrix algeriensis NRRL B-24137^{T}	......**CAC**.	......**GTG**.	....	....**C**.....
Saccharothrix coeruleofusca NRRL B-16115^{T}	......**CAC**.	......**GTG**.	....	....**C**.....
Saccharothrix espanaensis NRRL 15764^{T}	......**CAC**.	......**GTG**.	....	....**C**.....
Saccharothrix longispora NRRL B-16116^{T}	......**CAC**.	......**GTG**.	.......**A**..	....**C**.....
Saccharothrix mutabilis subsp. *capreolus* DSM 40225^{T}	......**CAC**.	...**N**..**GTG**.	....	....**C**.....
Saccharothrix mutabilis subsp. *mutabilis* DSM 43853^{T}	......**CAC**.	..**N**...**GTG**.	....	....**C**.....
Saccharothrix syringae NRRL B-16468^{T}	......**CAC**.	......**GTG**.	....	....**C**.....
Saccharothrix texasensis NRRL B-16134^{T}	......**CAC**.	......**GTG**.	.......**A**..	....**C**.....
Saccharothrix xinjinagensis AS.4.1731^{T}	......**CAC**.	......**GTG**.	....	....**C**.....
Umezawaea tangerinus MK27-91F2^{T}	....**C**.**ACA**.	......**TGT**.	**G**......**A**..	....

FIGURE 309. Nucleotide signatures in the 16S rRNA gene for the genera *Saccharothrix*, *Lechevalieria*, *Lentzea*, and *Umezawaea*.

TABLE 248. Chemotaxonomic characteristics of *Lechevalieria* and related genera[a]

Characteristic	*Lechevalieria*	*Actinosynnema*	*Lentzea*	*Saccharothrix*	*Umezawaea*
Whole-cell sugar pattern[b]	Gal, Man, Rha	Gal, Man	Gal, Man, Rib	Gal, Rha, Man (tr)	Gal, Rha
Phospholipids[c]	PE	PE, OH-PE, PI, PIM, DPG	PE, DPG, PG, PI	PE, OH-PE, PI, PIM, DPG, PG (v)	PE
Predominant menaquinone(s)	MK-9(H_4)	MK-9(H_4), MK-9(H_6)	MK-9(H_4)	MK-10(H_4), MK-9(H_4)	MK-9(H_4), MK-10(H_4) (tr)

[a]All genera have *meso*-diaminopimelic acid (A_2pm) as the cell-wall diamino acid, are of cell-wall chemotype III, and contain straight chain, mono-unsaturated, iso, and anteiso fatty acids. tr, Trace; v, variable.

[b]Gal, Galactose; Man, mannose; Rha, rhamnose; Rib, ribose.

[c]DPG, Diphosphatidylglycerol; OH-PE, phosphatidylethanolamine containing hydroxylated fatty acids; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; PI, phosphatidylinositol; PIM, phosphatidylinositolmannosides; PME, phosphatidylmethylethanolamine.

TABLE 249. Physiological characteristics of species of the genus *Lechevalieria*[a]

Characteristic	*L. aerocolonigenes*	*L. flava*	*L. fradiae*	*L. xinjiangensis*
Decomposition of:				
Adenine	–	–	–	nd
Allantoin	–	nd	nd	nd
Casein	+	–	+	+
Esculin	+	+	+	nd
Gelatin	+	nd	+	nd
Hippurate	–	nd	nd	nd
Hypoxanthine	+	+	–	–
Starch	+	nd	+	nd
Tyrosine	+	+	+	–
Urea	w	nd	+	nd
Xanthine	–	–	nd	nd
Acid from:				
Adonitol	–	–	–	nd
Arabinose	+	+	+	nd
Cellobiose	+	+	–	nd

(continued)

TABLE 249. (continued)

Characteristic	*L. aerocolonigenes*	*L. flava*	*L. fradiae*	*L. xinjiangensis*
Dextrin	+	+	–	nd
Dulcitol	–	nd	nd	nd
Erythritol	–	nd	+	nd
Fructose	+	+	–	nd
Galactose	+	nd	–	nd
Glucose	+	+	–	nd
Glycerol	+	nd	–	nd
Inositol	+	+	–	–
Lactose	+	+	–	–
Maltose	+	nd	+	nd
Mannose	+	nd	–	nd
Melibiose	+	+	–	nd
Methyl α-D-glucoside	w	nd	+	nd
Methyl β-D-xyloside	w	nd	nd	nd
Raffinose	+	+	+	nd
Rhamnose	+	+	–	nd
Salicin	+	nd	–	–
Sorbitol	–	–	–	nd
Sucrose	+	+	–	nd
Trehalose	+	nd	+	nd
Xylose	+	nd	+	nd
Utilization of:				
Acetate	+	–	+	+
Benzoate	–	–	–	nd
Citrate	+	+	–	+
Lactate	+	–	+	nd
Malate	+	nd	+	nd
Mucate	–	nd	nd	nd
Oxalate	+	nd	–	+
Propionate	+	nd	nd	+
Succinate	+	nd	+	nd
Tartrate	+	nd	–	nd
Production of:				
Nitrate reductase	+	+	+	+
Phosphatase	+	nd	nd	nd
Growth in the presence of:				
4% NaCl	+	+	+	+
5% NaCl	w	–	+	+
Growth at:				
10°C	+	+	–	+
37°C	+	+	+	+
42°C	w	+	+	+
45°C	–	–	+	+

[a]Symbols: w, weak positive; nd, not determined.

of the species based on the color of the substrate mycelium and production and color of soluble pigments, but some of the species appear quite similar when grown on agar media. The DNA relatedness between species, as summarized in Table 250, was determined during the characterization of the most recently described species (Wang et al., 2007; Zhang et al., 2007a) and provides evidence that these species are distinct.

TABLE 250. DNA relatedness (%) between *Lechevalieria* species[a]

	L. aerocolonigenes	*L. flava*	*L. fradiae*
L. flava	nd		
L. fradiae	45	37	
L. xinjiangensis	44	28	34

[a]Data are from Zhang et al. (2007a) and Wang et al. (2007).

List of species of the genus *Lechevalieria*

1. **Lechevalieria aerocolonigenes** (Shinobu and Kawato 1960) Labeda, Hatano, Kroppenstedt and Tamura 2001, 1050[VP] [*Saccharothrix aerocolonigenes* (Shinobu and Kawato 1960) Labeda 1986, 109; *Streptomyces aerocolonigenes* Shinobu and Kawato 1960, 215]

ae.ro.co.lo.ni′ge.nes. Gr. n. *aer* air; L. n. *colonia* a colony; N.L. suff. *-genes* (from Gr. v. *gennaô* to produce) producing; N.L. fem. adj. *aerocolonigenes* producing aerial colonies.

Branching substrate mycelium is produced (diameter approx. 0.5 µm); on some media extremely sparse aerial hyphae are formed. Some strains may appear to form clumps of interwoven hyphae or "colonies" in the aerial mycelium. Substrate and aerial mycelium fragment. Strains usually lose their capacity to form aerial mycelium during subcultivation.

Yellowish to brownish substrate mycelium is produced along with extremely sparse white aerial hyphae. Yellowish to brownish soluble pigment is produced on several media. Temperature for growth is 28°C.

Source: isolated from soil.

DNA G+C content (mol%): 70–71 (T_m).

Type strain: NRRL B-3298, ATCC 23870, CCRC 13661, DSM 40034, IFO 13195, JCM 4150.

Sequence accession no. (16S rRNA gene): AF114804.

2. **Lechevalieria flava** (Gauze, Maksimova, Ollkhovatova, Sveshnikova, Kochetkova and Ilchenko 1974) Labeda, Hatano, Kroppenstedt and Tamura 2001, 1050[VP] [*Saccharothrix flava* (Gauze, Maksimova, Ollkhovatova, Sveshnikova, Kochetkova and Ilchenko 1974) Grund and Kroppenstedt 1990a, 320 (Effective publication: Grund and Kroppenstedt 1989, 271.); *Nocardiopsis flava* (Gauze, Maksimova, Ollkhovatova, Sveshnikova, Kochetkova and Ilchenko 1974) Gauze and Sveshnikova 1985, 224; (Effective publication: Preobrazhenskaya, Sveshnikova and Gauze 1982, 111.); *Actinomadura flava* Gauze, Maksimova, Ollkhovatova, Sveshnikova, Kochetkova and Ilchenko 1974[AL]]

fla′va. L. fem adj. *flava* yellow (referring to the color of the substrate mycelium).

Substrate mycelium is usually yellowish in color and aerial mycelium, if present, is white. Special spore forming hyphae are absent. Long, straight aerial mycelium fragments completely into spores. Temperature for growth is 28°C.

Source: isolated from soil.

DNA G+C content (mol%): not determined.

Type strain: NRRL B-16131, ATCC 29533, CCRC 13328, DSM 43885, NBRC 14521, INA 2171, JCM 3296.

Sequence accession no. (16S rRNA gene): AF114808.

3. **Lechevalieria fradiae** Zhang, Xie, Liu and Goodfellow 2007a, 834[VP]

fra′di.ae. N.L. gen. n. *fradiae* of Fradia, a patronymic.

Substrate mycelium is yellow to orange in color. Aerial mycelium is not produced. Substrate mycelium fragments into coccoid to coccoidal–rod-shaped elements. No soluble pigments are produced. Temperature for growth is 28°C.

Source: isolated from soil.

DNA G+C content (mol%): 68.0 (T_m).

Type strain: Z6, CGMCC 4.3506, JCM 14205, NRRL B-24612.

Sequence accession no. (16S rRNA gene): AY114175.

4. **Lechevalieria xinjiangensis** Wang, Zhang, Tang, Mao, Wei, Huang, Liu, Shi and Goodfellow 2007, 2821[VP]

xin.ji.ang.en′sis. N.L. fem. adj. *xinjiangensis* referring to Xinjiang, north-western China, the source of the isolate.

Substrate mycelium is yellow to orange in color. Moderate amounts of white to yellow aerial mycelium produced on Bennett's or Gauze No. 1 agar, whereas brownish aerial mycelium is formed on other media. Substrate mycelium fragments into coccoid to coccoidal–rod-shaped elements. No soluble pigments are produced. Temperature for growth is 28°C.

Source: isolated from soil.

DNA G+C content (mol%): 68.6 (T_m).

Type strain: R24, CGMCC 4.3525, DSM 45081, NRRL B-24613.

Sequence accession no. (16S rRNA gene): DQ898283.

Genus XI. **Lentzea** Yassin, Rainey, Brzezinka, Jahnke, Weissbrodt, Budzikiewicz, Stackebrandt and Schaal 1995, 362[VP] emend. Labeda, Hatano, Kroppenstedt and Tamura 2001, 1049

David P. Labeda

Lent′ze.a. N.L. fem. n. *Lentzea* named after Friedrich A. Lentze, a German microbiologist who devoted a considerable part of his life to studying pathogenic actinomycetes.

Branched vegetative mycelia (diameter approx. 0.5 to 0.7 µm); aerial mycelium is produced and fragments into rod-shaped elements. Gram-stain-positive. Resistant to lysozyme. Catalase-positive. Aerobic. **The cell wall contains *meso*-diaminopimelic acid as the diamino acid. The characteristic whole-cell sugars are galactose, mannose, and ribose. The phospholipid pattern consists of significant amounts of phosphatidylethanolamine along with diphosphatidylglycerol, phophatidylglycerol, and phosphatidylinositol. The principal menaquinone is MK-9(H_4).** The fatty acid profile consists of straight-chain saturated, unsaturated, and branched-chain saturated fatty acids of the iso and anteiso types, in addition to tuberculostearic acid. **Phylogenetically, the genus *Lentzea* represents a line of descent adjacent to the genus *Actinosynnema* and close**

to the genera *Lechevalieria*, *Saccharothrix*, and *Umezawaea*. The 16S rRNA gene sequence contains genus-specific diagnostic nucleotide signature patterns of TCAA (617–620) and GCC (843–845).

DNA G+C content (mol%): 68.6–79.6 (HPLC, T_m)

Type species: **Lentzea albidocapillata** Yassin, Rainey, Brzezinka, Jahnke, Weissbrodt, Budzikiewicz, Stackebrandt and Schaal 1995, 362[VP].

Further descriptive information

The genus *Lentzea* was proposed by Yassin et al. (1995) for a single strain, which was isolated from a tissue sample taken from an abdominal mass of a patient with peritoneal carcinomatosis, on the basis of 16S rRNA gene phylogeny, and chemotaxonomic properties that distinguished it from the closely related genus *Saccharothrix*. Lee et al. (2000b) subsequently proposed, based on their chemotaxonomic and phylogenetic studies, that the genus *Lentzea* should be considered a later synonym of *Saccharothrix*. A subsequent study by Labeda et al. (2001), however, demonstrated that *Lentzea* was indeed a valid genus and could be differentiated from the genera *Saccharothrix* and *Lechevalieria* on the basis of phylogenetic position (see Figure 290 in the treatment of the genus *Lechevalieria*), diagnostic signature nucleotides TCAA (617–620) and GCC (843–845) in 16S rRNA gene sequences, and the presence of distinct chemotaxonomic characteristics. *Lentzea* species lack phosphatidylethanolamine containing 2-hydroxy-fatty acids in their polar lipid profiles, differentiating them from members of the genus *Saccharothrix*, and their whole-cell sugar pattern, consisting of galactose, mannose, and small quantities of ribose, differentiates them from both *Lechevalieria* and *Saccharothrix*.

Enrichment and isolation procedures

The first human clinical isolate of *Lentzea albidocapillata* was from a tissue sample; it was isolated from a streak culture on a Columbia blood agar plate. Subsequently, described species have generally been isolated from soil samples from China, Korea, and the United States, indicating that they most likely have a global distribution. Most recently, *Lentzea kentuckyensis* was isolated from an equine placenta, although this species is probably not pathogenic. Media typically used to isolate actinomycetes from environmental samples (i.e. soil and water samples), such as tap water agar or starch-casein agar (Küster and Williams, 1964) supplemented with antifungal antibiotics (cycloheximide, 50 µg/ml), can be used to obtain novel isolates of the genus *Lentzea*.

Maintenance procedures

Working cultures of *Lentzea* can be maintained as refrigerated (4°C) agar slants on an appropriate medium such as yeast extract-malt extract medium (Shirling and Gottlieb, 1966) or NZamine medium (DSMZ medium 554; DSMZ, 2001). The slants are generally incubated for 5 to 7 d at 28–30°C prior to refrigeration and then should be subcultured at monthly intervals. Longer term preservation of strains is best accomplished as frozen stocks in 20% (v/v) aqueous glycerol at −80°C (mechanical freezer) to −172°C (liquid nitrogen vapor phase) or by using traditional lyophilization techniques. Strains have also been successfully stored for shorter periods as quick-frozen stationary-phase broth cultures or mycelial suspensions in 20% aqueous glycerol at −20 to −72°C.

Procedures for testing special characters

Strains are routinely cultivated on NZamine medium (DSMZ medium 554) at 28°C. Morphological observations are made on the media of Shirling and Gottlieb (1966) and NZamine medium.

Chemotaxonomic analysis of strains for cell-wall diamino acid isomer and diagnostic whole-cell sugar content are determined by the methods of Staneck and Roberts (1974) and Saddler et al. (1991). Determination of cellular fatty acids, menaquinones, and polar lipids are performed using methods described previously by Grund and Kroppenstedt (1989), Minnikin et al. (1984), and Sasser (1990).

Physiological tests, including production of acid from carbohydrates, utilization of organic acids, and hydrolysis and decomposition of adenine, casein, esculin, guanine, hippurate, hypoxanthine, tyrosine, urea, and xanthine, are typically evaluated using the media of Gordon et al. (1974). Phosphatase activity is evaluated by using the method of Kurup and Schmitt (1973) substituting NZamine agar as the basal medium. The temperature range for growth is determined on slants of DSMZ medium 554 and salt tolerance is determined on slants of the same medium supplemented with 4% and 5% NaCl.

Differentiation of the genus *Lentzea* from other genera

Morphologically, *Lentzea* species are quite similar to species within the genera *Lechevalieria* and *Saccharothrix* producing aerial mycelium which may exhibit a "zig-zag" morphology (Figure 310a) and fragments into coccoidal-rod-shaped elements (Figure 310b). The chemotaxonomic characteristics of members of this genus (see Table 248 in the treatment of the genus *Lechevalieria*) include a polar lipid profile which lacks 2-hydroxy-fatty acid containing phosphatidylethanolamine and contains phosphatidylinositol and phosphatidylglycerol, differentiating them from *Saccharothrix* species. The sugars observed in their whole-cell sugar profiles include galactose, mannose, and small quantities of ribose, but no rhamnose, which differentiates them from both *Lechevalieria* and *Saccharothrix* species. The genus *Lentzea* can also be differentiated from the genera *Lechevalieria* and *Saccharothrix* phylogenetically and based on the genus-specific nucleotide signature patterns TCAA (617–620) and GCC (843–845) in their 16S rRNA gene sequence (see Figure 290 and Figure 309).

Differentiation of the species of the genus *Lentzea*

The physiological characteristics of *Lentzea* species are summarized in Table 251 and can be used to differentiate between species, although a common set of physiological tests has not been used in the description of all species. Gross colonial morphology provides some additional information of the species based on color of the substrate mycelium, production and color of aerial mycelium, and production and color of soluble pigments when grown on agar media. The distinct fatty acid profiles of each *Lentzea* species, as shown in Table 252, can also be used to differentiate between species.

TABLE 251. Physiological properties of *Lentzea* species[a]

Character	*L. albidocapillata*	*L. albida*	*L. californiensis*	*L. flaviverrucosa*	*L. kentuckyensis*	*L. violacea*	*L. waywayandensis*
Hydrolysis of:							
Adenine	−	−	−	−	−	−	−
Allantoin	nd	−	−	−	−	nd	−
Casein	+	+	+	−	+	+	+
Esculin	+	+	+	−	+	+	+
Gelatin	+	nd	nd	+	+	−	+
Hypoxanthine	+	+	+	+	+	+	+
Starch	+	+	+	+	+	+	+
Tyrosine	+	+	+	+	+	+	+
Urea	v	v	+	−	+	+	+
Xanthine	−	−	−	−	+	−	−
Production of:							
Nitrate reductase	−	−	+	+	−	−	+
Phosphatase	+	nd	nd	+	−	nd	+
Acid from:							
Adonitol	+	+	−	−	−	−	+
Arabinose	+	+	+	nd	+	+	+
Cellobiose	+	+	+	+	+	−	+
Dextrin	nd	+	+	+	nd	nd	+
Dulcitol	nd	−	−	nd	−	nd	−
Erythritol	nd	−	−	+	−	nd	+
Fructose	+	+	+	nd	+	+	+
Galactose	+	+	+	nd	+	+	+
Glucose	+	+	+	nd	+	+	+
Glycerol	nd	+	+	+	+	nd	+
Inositol	+	+	+	+	+	−	+
Lactose	+	w	+	−	+	+	+
Maltose	+	+	+	+	+	−	+
Mannitol	+	+	+	+	+	−	+
Mannose	nd	+	+	+	+	−	+
Melibiose	nd	+	+	+	+	−	+
Methyl β-xyloside	nd	−	−	nd	v	nd	−
Raffinose	v	−	+	+	+	+	+
Rhamnose	+	+	+	−	+	−	+
Salicin				+	+	−	+
Sucrose	+	+	+	+	+	−	+
Trehalose	+	+	+	−	+	−	+
Xylose	+	+	+	−	+	−	+
Assimilation of:							
Acetate	−	+	+	+	−	+	+
Benzoate	−	−	−	−	−	−	−
Citrate	−	+	+	−	v	−	+
Lactate	−	−	−	−	v	+	+
Malate	+	+	+	+	+	−	+
Mucate	nd	−	−	nd	−	nd	−
Oxalate	nd	−	−	−	+	+	+
Propionate	nd	+	+	+	−	+	+
Succinate	nd	+	+	−	+	+	+
Tartrate	nd	−	−	−	−	−	−
Growth in the presence of:							
4% (w/v) NaCl	+	+	+	−	+	+	+
5% (w/v) NaCl	nd	+	+	−	+	nd	+
Growth at:							
10°C	+	−	+	nd	+	+	+
37°C	+	+	+	+	+	+	w
42°C	−	+	−	+	−	−	−
45°C	−	+	−	−	−	−	−

[a]Symbols: nd, not determined; v, variable; w, weakly positive.

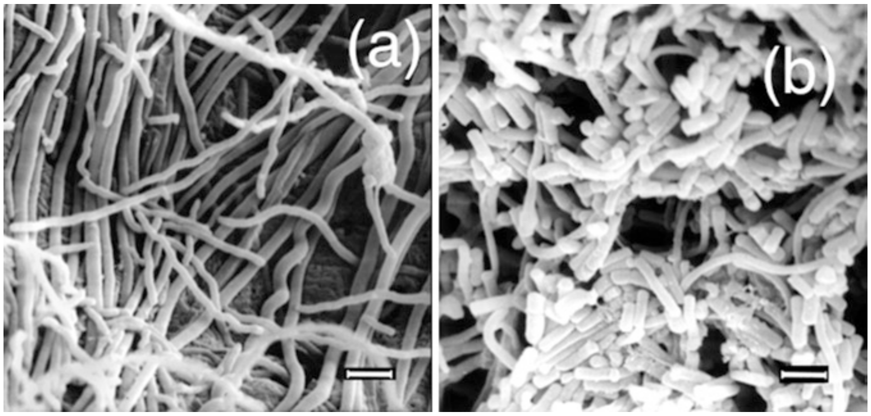

FIGURE 310. Scanning electron micrographs of *Lentzea albidocapillata* IMMIB D-958[T] grown for 14 d on yeast extract-malt extract agar illustrating "zig-zag" aerial mycelium (a) and coccoidal-rod-shaped fragmentation of the aerial mycelium (b). Bars = 2 μm. (Micrographs courtesy of A.F. Yassin, University of Bonn.)

TABLE 252. Fatty acid profiles of *Lentzea* species

Fatty acid (%)	*L. albidocapillata*	*L. albida*	*L. californiensis*	*L. flaviverrucosa*	*L. kentuckyensis*	*L. violacea*	*L. waywayandensis*
$C_{13:0}$ iso	–	0.46	–	–	0.19	–	–
$C_{13:0}$ anteiso	–	–	–	–	0.13	–	–
$C_{13:0}$	–	0.44	–	–	–	–	–
$C_{14:0}$ iso	8.00	3.28	10.28	5.87	8.88	8.90	12.70
$C_{14:1}$ *cis*9	–	–	–	–	–	0.21	–
$C_{14:0}$	0.56	1.91	1.85	1.55	0.52	1.27	–
$C_{15:1}$ iso	–	–	–	–	0.23	–	–
$C_{15:0}$ iso	3.58	14.45	9.90	5.63	10.07	6.15	6.55
$C_{15:0}$ anteiso	2.57	11.33	16.21	6.25	8.20	3.79	4.26
$C_{15:1}$ *cis*9	1.24	1.38	–	0.84	0.79	1.47	0.93
$C_{15:0}$	0.75	7.00	2.21	2.53	1.01	1.00	0.58
$C_{16:1}$ iso	9.27	0.62	–	1.85	3.83	4.05	10.07
$C_{16:0}$ iso	55.63	19.29	23.47	31.97	47.40	45.03	45.60
$C_{16:1}$ *cis*9	9.66	8.42	8.70	16.26	4.64	15.52	8.87
$C_{15:0}$ anteiso 2-OH	–	1.12	–	–	–	–	–
$C_{16:0}$	2.21	9.91	17.08	12.01	1.9	4.01	2.07
$C_{16:0}$ 10-methyl	3.04	–	4.14	5.48	–	1.40	4.10
$C_{17:1}$ iso	–	1.12	–	–	1.39	–	–
$C_{17:1}$ anteiso	–	–	–	–	0.68	–	–
$C_{17:0}$ iso	–	2.22	–	0.77	1.21	0.58	–
$C_{17:0}$ anteiso	0.97	5.64	4.01	2.32	4.61	1.18	1.42
$C_{17:1}$ *cis*9	1.57	4.14	–	2.09	1.95	1.87	1.48
$C_{17:1}$ *cis*11	–	–	–	–	1.03	2.49	–
$C_{16:0}$ iso 2-OH	0.95	0.63	–	1.08	–	–	1.35
$C_{17:0}$	–	4.75	2.16	1.62	0.65	0.39	–
$C_{17:0}$ 10-methyl	–	–	–	–	0.18	–	–
$C_{18:0}$ iso	–	–	–	–	0.34	–	–
$C_{18:1}$ *cis*9	–	0.75	–	1.03	0.17	0.69	–
$C_{18:0}$	–	1.05	–	0.84	–	–	–

List of species of the genus *Lentzea*

1. **Lentzea albidocapillata** Yassin, Rainey, Brzezinka, Jahnke, Weissbrodt, Budzikiewicz, Stackebrandt and Schaal 1995, 362[VP]

al.bi.do.ca.pil.la′ta. L. adj. *albidus* white; L. adj. *capillatus* hairy; N.L. fem. adj. *albidocapillata* white haired, referring to the abundant whitish aerial hyphae.

Substrate mycelium is yellow to yellowish-brown. Aerial mycelium is white to whitish-yellow. Soluble pigments are not produced. Temperature for growth is 30°C.

Source: isolated originally from the peritoneal cavity of a cancer patient.

DNA G+C content (mol%): 68.6 (HPLC).

Type strain: DSM 44073, AS 4.1519, CIP 107111, NBRC 15855, JCM 9732, NBRC 100372.

Sequence accession no. (16S rRNA gene): X84321.

Further comment: Lee et al. (2000b) suggested that *Lentzea albidocapillata* be transferred to the genus *Saccharothrix* Labeda et al. 1989, but phylogenetic and chemotaxonomic data do not support this proposal according to Labeda et al. (2001). *Lentzea* is considered to be the current correct name of a genus in which the type strain is *Lentzea albidocapillata*.

2. **Lentzea albida** Labeda, Hatano, Kroppenstedt and Tamura 2001, 1049[VP]

al.bi′da. L. fem. adj. *albida* whitish, referring to the color of the aerial mycelium.

Substrate mycelium is yellowish-orange in color on most media. Copious white aerial mycelium is produced. Soluble pigments are not produced. Temperature for growth is 28°C.

Source: isolated from soil.

DNA G+C content (mol%): not determined.

Type strain: NBRC 16102, AS 4.1727, DSM 44437, JCM 9734, KCTC 19911, NRRL B-24073.

Sequence accession no. (16S rRNA gene): AB006167.

3. **Lentzea californiensis** Labeda, Hatano, Kroppenstedt and Tamura 2001, 1049[VP]

cal.i.forn.i.en′sis. N.L. fem. adj. *californiensis* pertaining to California, referring to the source of this isolate, soil from California.

Substrate mycelium is yellow to orange-brown. White aerial mycelium is produced. An orange soluble pigment may be produced on Czapek's agar. Temperature for growth is 28°C.

Source: isolated from soil.

DNA G+C content (mol%): not determined.

Type strain: NRRL B-16137, DSM 43393, IMRU 550, JCM 11305, KCTC 19912.

Sequence accession no. (16S rRNA gene): AF174435.

4. **Lentzea flaviverrucosa** Xie, Wang, Huang, Wu, Ba and Liu 2002, 1818[VP]

fla.vi.ver.ru.co′sa. L. adj. *flavus* yellow; L. adj. *verrucosus* rough, rugged, verrucose; N.L. fem. adj. *flaviverrucosa* yellowish and verrucose, referring to the yellowish, verrucose colony morphology observed on the agar surface.

Substrate mycelium is pale yellow to yellowish-brown on most media. Sparse white to yellowish-white aerial mycelium is produced on some media (e.g. oatmeal agar). Neither melanin nor soluble pigments are produced. Temperature for growth is 28°C.

Source: isolated from soil.

DNA G+C content (mol%): 64.1 (T_m).

Type strain: AS 4.0578, CIP 107743, DSM 44664, JCM 11373, NBRC 100042.

Sequence accession no. (16S rRNA gene): AF183957.

5. **Lentzea kentuckyensis** Labeda, Donahue, Sells and Kroppenstedt 2007, 1782[VP]

ken.tuck.y.en′sis. N.L. fem. adj. *kentuckyensis* from Kentucky, named after the place of origin of the type strain, the state of Kentucky, USA.

Yellow to strong yellow substrate mycelium is produced on most media. Aerial mycelium ranging in color from white to yellowish-white is produced on most media. A faint brown soluble pigment is produced on some media. Temperature for growth is 28°C.

Source: isolated from an equine placenta.

DNA G+C content (mol%): not determined.

Type strain: NRRL B-24416, LDDC 2876-05, DSM 44909.

Sequence accession no. (16S rRNA gene): DQ291145.

6. **Lentzea violacea** (Lee, Kim, Roe, Kim, Kang and Hah 2000b) Labeda, Hatano, Kroppenstedt and Tamura 2001, 1049[VP] (*Saccharothrix violacea* Lee, Kim, Roe, Kim, Kang and Hah 2000b, 1320)

vi.o.la.ce′a. L. fem. adj. *violacea* violet-colored, violet.

Violet substrate mycelium and a white aerial mycelium produced on most media. Reddish-brown soluble pigment is produced. Melanin pigment is not produced. Temperature for growth is 28°C.

Source: isolated from soil.

DNA G+C content (mol%): 79.6 (HPLC).

Type strain: IMSNU 50388, DSM 44796, JCM 10975, KCTC 9948.

Sequence accession no. (16S rRNA gene): AJ242633.

7. **Lentzea waywayandensis** (Labeda and Lyons 1989) Labeda, Hatano, Kroppenstedt and Tamura 2001, 1049[VP] (*Saccharothrix waywayandensis* Labeda and Lyons 1989, 357)

way.way.an.den′sis. N.L. fem. adj. *waywayandensis* of or belonging to Lake Waywayanda, NJ, of the soil samples from which the organism was first isolated.

Substrate mycelium is pale yellow to dark yellow. Aerial mycelium is white when produced, particularly on inorganic salts-starch agar (ISP medium 4). Soluble pigments are not produced. Temperature for growth is 28°C.

Source: isolated from soil.

DNA G+C content (mol%): 71 (T_m).

Type strain: NRRL B-16159, AS 4.1646, ATCC 51594, DSM 44232, NBRC 14970, JCM 9114, NCIMB 13164, VKM Ac-1970.

Sequence accession no. (16S rRNA gene): AF114813.

Genus XII. **Prauserella** Kim and Goodfellow 1999, 510^{VP} emend. Li, Xu, Tang, Xu, Kroppenstedt, Stackebrandt and Jiang 2003c, 1547

Seung Bum Kim and Michael Goodfellow

Prau.se.rel′la. N.L. fem. dim. n. *Prauserella* named after Helmut Prauser, a German microbiologist who made many contributions to actinomycete systematics.

Aerobic, Gram-stain-positive, non-acid–alcohol-fast, nonmotile actinobacteria which form an extensively branched substrate mycelium (0.6–0.8 µm in diameter) that **fragments into irregular rod-shaped elements within 24–48 h on rich media**. Aerial hyphae, when formed, may differentiate into branched short or, at maturity, long chains which have a straight to flexuous arrangement. Spores are nonmotile. **Most strains grow optimally in the presence of 10% or between 10 and 15% NaCl at 28°C and pH 7.0.** Optimal growth occurs between pH 6.8 and 7.2. The temperature range for growth is 10–45°C, with optimal growth between 28 and 34°C. **Contains *meso*-diaminopimelic acid as the major diamino acid, an acetylated peptidoglycan, major amounts of arabinose and galactose, MK-9(H_4) as the predominant menaquinone, either phosphatidylcholine or phosphatidylethanolamine as the diagnostic phospholipid, and major amounts of branched chains and saturated fatty acids, but not mycolic acids.**

The genus *Prauserella*, as determined by 16S rRNA gene sequence analysis, is classified in the family *Pseudonocardiaceae.*

DNA G+C content (mol%): 65.8–70.1.

Type species: **Prauserella rugosa** (Lechevalier, Prauser, Labeda and Ruan 1986) Kim and Goodfellow 1999, 510^{VP}.

Further descriptive information

Phylogeny. The genus *Prauserella* forms a distinct branch within the evolutionary radiation occupied by the family *Pseudonocardiaceae* and is most closely related to the genus *Saccharomonospora* (Labeda et al., 2010a). The nine species assigned to the genus (Figure 311) share 16S rRNA gene sequence similarities which range from 95.8% between *Prauserella halophila* and *Prauserella muralis* to 100% between *Prauserella flava* and *Prauserella salsuginis.* The level of DNA–DNA relatedness between the latter two species is 56.9% (Li et al., 2009b).

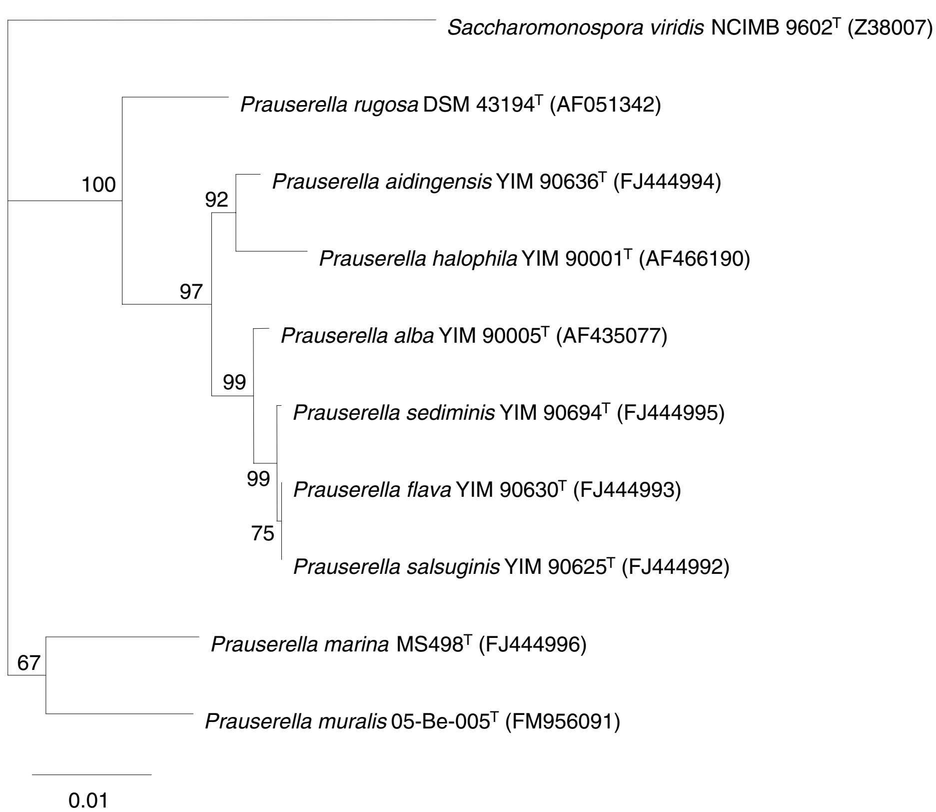

FIGURE 311. Neighbor-joining tree (Saitou and Nei, 1987) based on nearly complete 16S rRNA gene sequences showing the relationships between *Prauserella* species and representatives of some other genera classified in the family *Pseudonocardiaceae.* Numbers at the nodes indicate levels of bootstrap support (Felsenstein, 1985) based on analysis of 1000 datasets. Bar = 0.01 substitutions per nucleotide position.

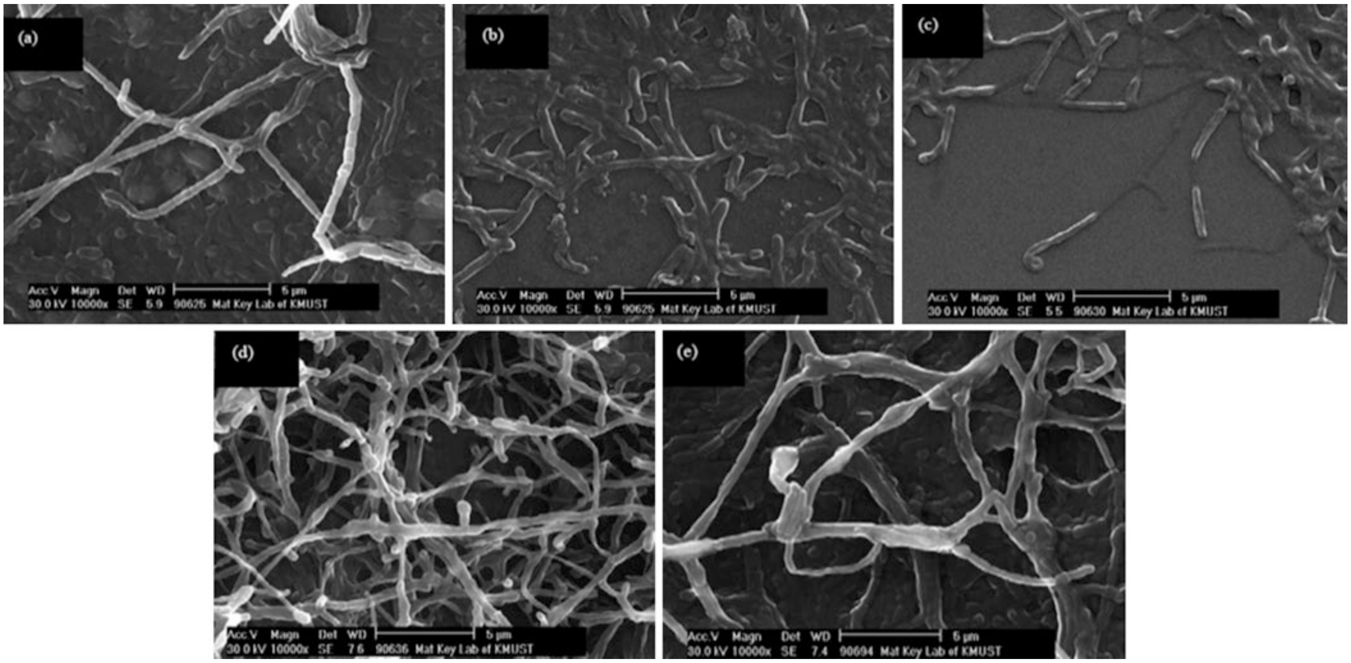

FIGURE 312. Scanning electron micrographs showing the morphology of *Prauserella aidingensis* YIM 90636^T, *Prauserella flava* YIM 90630^T, *Prauserella salsuginis* YIM 90625^T, and *Prauserella sediminis* YIM 90694^T grown on yeast extract-malt extract agar (ISP medium 2; Shirling and Gottlieb, 1966) at 28°C for 23 d (bars = 5 µm). (a) Spore chains of strain YIM 90625^T; (b) substrate mycelium of strain YIM 90625^T; (c) substrate mycelium of strain YIM 90630^T; (d) spore chains of strain YIM 90636^T; and (e) spore chains of strain YIM 90694^T. (Reproduced with permission from Li et al., 2009b. Int. J. Syst. Evol. Microbiol. *59*: 2928–2932.)

Cell morphology. *Prauserella* strains form a substrate mycelium which usually carries aerial hyphae, although *Prauserella flava* and *Prauserella rugosa* do not form an aerial mycelium (Lechevalier et al., 1986; Li et al., 2009b). Substrate mycelia fragment into rod-shaped elements and aerial hyphae may differentiate into branched short or, at maturity, long chains, which are straight to flexuous (Figure 312).

Chemotaxonomy. *Prauserella* strains contain *meso*-diaminopimelic acid as the diagnostic wall diamino acid and arabinose and galactose as major whole-cell sugars (Lechevalier et al., 1986; Li et al., 2009b; Mertz and Yao, 1993), i.e. they have wall chemotype IV *sensu* Lechevalier and Lechevalier (1970). Other major sugars may be detected in whole-cell hydrolysates, notably ribose (Table 253). In addition, all *Prauserella* strains have tetrahydrogenated menaquinones, MK-9(H_4), as the predominant isoprenologue, although *Prauserella rugosa* also contains a large amount of MK-9(H_2); small amounts of other menaquinones (<7%) may be detected (Lechevalier et al., 1986; Li et al., 2009b; Schäfer et al., 2010). *Prauserella rugosa* has *N*-acetylated muramic acid (Henssen et al., 1987).

Prauserellae can be assigned to two groups based on the discontinuous distribution of diagnostic polar lipids, although they all have patterns which include diphosphatidylglycerol, phosphatidylglycerol, phosphatidylinositol, phosphatidylethanolamine, and, in some instances, unknown phospholipids (Li et al., 2009b; Schäfer et al., 2010; Wang et al., 2010). *Prauserella alba*, *Prauserella halophila*, *Prauserella muralis*, and *Prauserella rugosa* contain phosphatidylethanolamine as the diagnostic polar lipid, whereas phosphatidylcholine is the diagnostic marker in *Prauserella aidingensis*, *Prauserella flava*, *Prauserella marina*, *Prauserella sediminis*, and *Prauserella salsuginis*; hence, these two groups have phospholipid patterns II and III, respectively (Lechevalier et al., 1977b, 1981). Hydroxyphosphatidylethanolamine and hydroxymethylphosphatidylethanolamine have been detected in *Prauserella rugosa* (Lechevalier et al., 1986; Yassin et al., 1993) and phosphatidylserine has been found in *Prauserella muralis* (Schäfer et al., 2010).

Prauserella strains contain complex mixtures of branched-chain and saturated fatty acids (Henssen et al., 1987; Li et al., 2003c; Mertz and Yao, 1993), but lack mycolic acids (Henssen et al., 1987; Lechevalier et al., 1986). The principal fatty acid, 14-methylpentadecanoic acid ($C_{16:0}$ iso), accounts for between 20 and 42% of total fatty acid composition; other major components (<10%) may include $C_{16:0}$, $C_{17:1}$ anteiso, and $C_{16:1}$ ω9*c* (Li et al., 2009b; Schäfer et al., 2010; Wang et al., 2010).

Nutritional and growth conditions. Prauserellae tend to grow well on standard media used to cultivate filamentous actinobacteria, such as Czapek's agar, glycerol-asparagine agar (ISP medium 5), inorganic salts-starch agar (ISP medium 4), nutrient agar, oatmeal agar (ISP medium 3) and yeast extract-malt extract agar (ISP medium 2) supplemented with 10% (w/v) NaCl and incubated at 28–37°C; the composition of the ISP media is given by Shirling and Gottlieb (1966). Most strains are either halophilic or halotolerant. However, *Prauserella marina* grows optimally on yeast extract-malt extract agar without NaCl at 28–37°C and pH 7.0 (Wang et al., 2010).

Metabolism and ecology. Little is known about the biological properties of *Prauserella* strains or of their distribution and activities in natural habitats. They are aerobic, have an oxida-

TABLE 253. Differential characteristics of *Prauserella* species[a,b]

Characteristic	*P. rugosa*	*P. aidingensis*	*P. alba*	*P. flava*	*P. halophila*	*P. marina*	*P. muralis*	*P. salsuginis*	*P. sediminis*
Aerial mycelium	–	+	+	–	+	+	+	+	+
Growth in NaCl:									
Range (%)	0–20	5–15	0–25	5–15	5–25	0–10	nd	5–15	5–20
Optimum (%)	5–10	8–10	10–15	8–10	10–15	0–5	nd	8–10	10
Degradation of:									
Gelatin	–	+	+	+	+	+	nd	+	+
Urea	+	–	–	–	+	–	nd	–	–
Carbon source utilization:									
L-Arabinose	+	–	+	–	–	+	+	+	–
Cellobiose	+	–	+	–	+	–	+	+	–
D-Fructose	+	+	+	+	+	–	+	+	–
D-Galactose	+	+	+	–	–	+	+	+	–
myo-Inositol	–	–	+	+	+	–	–	+	–
Lactose	+	+	nd	+	nd	–	nd	+	+
Maltose	+	–	+	–	–	+	+	–	–
D-Mannitol	+	–	+	–	+	+	+	+	–
D-Mannose	+	–	nd	–	nd	+	+	+	–
Raffinose	+	–	nd	–	nd	–	nd	–	–
L-Rhamnose	+	+	+	–	+	+	+	+	+
D-Ribose	+	+	+	+	+	+	+	+	–
Trehalose	+	+	–	–	+	+	+	+	–
D-Xylitol	+	–	+	–	+	–	nd	+	–
D-Xylose	+	+	+	+	+	+	+	+	–
Nitrogen source utilization:									
L-Arginine	–	–	+	–	+	+	nd	–	–
L-Glycine	+	–	+	–	+	nd	nd	–	–
L-Hydroxyproline	–	+	+	+	+	+	nd	+	+
L-Lysine	–	–	+	+	+	–	nd	+	+
L-Serine	–	–	+	–	+	+	nd	–	–
L-Threonine	–	+	+	+	+	+	nd	+	+
Cell-wall sugars[c]	nd	Ribose	Ribose	Ribose	Ribose	nd	Glucose	Ribose	Ribose
Phospholipids[d]	PE	PC	PE	PC	PC	PE, PC	PE, PS	PC	PC
DNA G+C content (mol%)	67.0–68.9	70.1	66.7	69.9	65.8	66.1	nd	69.1	69.1

[a]Symbols: +, positive; –, negative; nd, not determined.

[b]Data from Kim and Goodfellow (1999), Li et al. (2003c, 2009b), Schäfer et al. (2010), and Wang et al. (2010).

[c]In addition, all species contain arabinose and galactose.

[d]PC, Phosphatidylcholine; PE, phosphatidylethanolamine; PS, phosphatidylserine.

tive metabolism, grow on a broad range of sole carbon and sole nitrogen compounds, and tend to grow optimally in the presence of 8–15% (w/v) NaCl (Li et al., 2003c, 2009b; Schäfer et al., 2010; Wang et al., 2010). The type strain of *Prauserella rugosa* hydrolyzes arbutin, allantoin, and esculin, degrades several organic compounds, and is active against a broad range of 4-methylumbelliferone and 7-amino-4-methylcoumarin-conjugated fluorogenic compounds (De Boer et al., 1990); it also produces alkaline hydrolases when grown on *n*-dodecane (Smits et al., 1999). Most *Prauserella* strains have been isolated from saline habitats (Li et al., 2003c, 2009b; Wang et al., 2010), although *Prauserella muralis* was obtained from a wall colonized by fungi (Schäfer et al., 2010) and *Prauserella rugosa* was from the rumen of a cow (di Marco and Spalla, 1957).

Isolation procedures

Prauserella species have been isolated by plating serial dilutions of saline samples onto starch-casein agar supplemented with 20% (w/v) NaCl and incubating for about 4 weeks at 28°C (Li et al., 2003c) and by plating onto cellulose-casein multi-salt medium and incubating for 3 weeks at 37°C (Li et al., 2009b; Tang et al., 2008). *Prauserella marina* was obtained from a marine sediment sample following incubation at 22°C for 4 weeks on MOPS-proline agar (Wang et al., 2010) and *Prauserella muralis* was isolated by shaking a 1 g sample of plaster colonized by molds in 10 ml NaCl (0.9%, w/v) containing Tween 80 (0.01%, v/v) then plating aliquots of the suspension over starch-mineral agar plates (Gauze, 1985), which were then incubated for 2 weeks at 28°C (Schäfer et al., 2010).

Maintenance procedures

Working cultures of *Prauserella* can be maintained at 4°C on standard media such as modified Bennett's (Jones, 1949), organic medium M79 (Schäfer et al., 2010), and yeast extract-malt extract (Shirling and Gottlieb, 1966) agars supplemented with 10% (w/v) NaCl for halophilic and halotolerant strains. Moderate to long-term preservation can be achieved by storing mycelial spore suspensions in aqueous glycerol (10 or 20%, v/v) at −20 or −80°C (mechanical freezers), at −172°C (liquid nitrogen vapor phase), or by standard lyophilization techniques.

Procedures for testing special characters

Reliable and well established procedures are available for analysis of the isomers of diaminopimelic acid and whole-cell sugars (Hasegawa et al., 1983a; Staneck and Roberts, 1974), fatty acids (Sasser, 1990), and menaquinones and polar lipids (Minnikin et al., 1984). Physiological features, such as the production of acids from sugars, growth on sole carbon and nitrogen compounds, and degradation of complex organic substrates, can be determined using standard methods (De Boer et al., 1990; Shirling and Gottlieb, 1966; Williams et al., 1983).

Differentiation of the genus *Prauserella* from other genera

Prauserella strains can be distinguished from other actinobacteria classified in the family *Pseudonocardiaceae* by using a combination of chemotaxonomic and morphological features and by comparative 16S rRNA gene sequence analyses (Kim and Goodfellow, 1999; Labeda et al., 2010a), as shown in the treatment of the family *Pseudonocardiaceae* in this volume. The genus can be separated from other filamentous actinobacteria using genus-specific 16S rRNA gene restriction fragment patterns (Cook and Meyers, 2003).

Taxonomic comments

The monospecific genus *Prauserella*, with *Prauserella rugosa* as the type species, was proposed by Kim and Goodfellow (1999) for an organism which had been isolated from the rumen of a cow and designated "*Nocardia rugosa*" (di Marco and Spalla, 1957). The taxon was well described, although a type species was not designated until the species was transferred to the genus *Amycolatopsis* as *Amycolatopsis rugosa*, a move based on chemotaxonomic and morphological criteria (Lechevalier et al., 1986). This transfer proved to be a temporary one as the organism was shown to differ from *bona fide* members of the genus *Amycolatopsis* based on fatty acid (Henssen et al., 1987; Mertz and Yao, 1993), DNA–DNA relatedness (Labeda, 1995), and extensive phenotypic (De Boer et al., 1990) data. In addition, the organism did not give the characteristic amplification product with *Amycolatopsis* genus-specific primers (Tan et al., 2006b) and formed a distinct single-membered cluster in a numerical taxonomic study which included strains that were subsequently classified in the genus *Amycolatopsis* (Goodfellow, 1971). The genus *Prauserella* currently contains nine species, most of which were circumscribed in polyphasic studies which included DNA–DNA relatedness data (Li et al., 2003c, 2009b). The original description of the genus was emended by Li et al. (2003c).

Differentiation of the species of the genus *Prauserella*

Prauserella species can be distinguished using a combination of phenotypic properties (Table 253) and by DNA–DNA relatedness data (Li et al., 2003c, 2009b). Qualitative and quantitative differences have been found in the fatty acid profiles of some species (Li et al., 2009b; Schäfer et al., 2010; Wang et al., 2010).

List of species of the genus *Prauserella*

1. **Prauserella rugosa** (Lechevalier, Prauser, Labeda and Ruan 1986) Kim and Goodfellow 1999, 510[VP] (Basonym: *Amycolatopsis rugosa* Lechevalier, Prauser, Labeda and Ruan 1986, 35 *ex* di Marco and Spalla 1957; "*Nocardia rugosa*" di Marco and Spalla 1957, 28.)

ru.go′sa. L. fem. adj. *rugosa* wrinkled.

Forms an extensively branched substrate mycelium that fragments into irregular rods. Aerial hyphae are not formed. Cream colored, irregular, flat, and veined colonies (10 × 6 mm) are formed on Czapek Dox-yeast extract agar. Good growth occurs on Bennett's, modified Bennett's, and nutrient agars. Produces cream to yellowish glistening colonies which may be wrinkled or folded. Brownish soluble pigments are formed on some media. The temperature range for growth is 10–45°C, with an optimum at 34°C. Grows optimally between pH 6.8 and 7.2.

Allantoin and esculin are hydrolyzed. Produces phosphatase, but does not reduce nitrate or produce hydrogen sulfide. Acetate, benzoate, lactate, malate, propionate, pyruvate, and succinate are decarboxylated, but not citrate, mucate, oxalate, or tartrate. 2-Deoxythymidine-5-*para*-nitrophenyl (*p*NP) phosphate, *p*NP-α-D-glucopyranoside, and *p*NP-β-D-glucopyranoside are hydrolyzed, but not L-alanine-D-nitroanilide (*p*NA), *bis*-*p*NP phosphate, *o*NP-β-D-galactopyranoside, *p*NP-glucopyranoside, *p*NP-β-D-glucuronide, L-glutamate-γ-3-carbonyl-*p*NP, *p*NP-phenylphosphonate, *p*NP-phosphorylcholine, L-proline-*p*NA, or *p*NP-β-D-xylopyranoside.

Arbutin, casein, hypoxanthine, L-tyrosine, tributyrin, Tweens 20, 40, 60 and 80, and xanthine are degraded, but not adenine, elastin, starch, or testosterone.

Acid is formed from adonitol, L-arabinose, erythritol, fructose, galactose, glucose, glycerol, mannitol, mannose, rhamnose, salicin, trehalose, and xylose, but not from D-arabinose, cellobiose, dextrin, inositol, lactose, maltose, melibiose, methyl α-D-glucoside, raffinose, sorbitol, sucrose, or methyl β-D-xyloside.

Adonitol, amygdalin, arbutin, dextrin, erythritol, glycerol, glycogen, salicin, and sucrose are used as sole carbon sources for energy and growth, but not arabitol, dulcitol, D- or L-fucose, gentiobiose, glucose, sorbitol, or turanose (at 1%, w/v). L-Alanine, androsterone, cholesterol, ergosterol, L-glycine, L-proline, protocatechuic acid, shikimic acid, butyrate, propionate, pyruvate, succinate, and L-tyrosine are also used as sole carbon sources, but not α-D-alanine, L-arginine, L-asparagine, catechol, L-citrulline, creatine, L-cysteine, ferulic acid, gluconate, glucuronate, histamine, L-hydroxyproline, phthallic acid, progesterone, quinic acid, L-serine, fumarate, tartrate, syringealdehyde, L-threonine, or vanillin (all at 0.1%, w/v). Does not use L-histidine, L-ornithine, or L-phenylalanine as sole nitrogen sources.

Grows in the presence of (μg/ml) cephaloridine (2), demeclocycline (2), lincomycin (10), neomycin (3), oleandomycin (2), penicillin G (10), streptomycin (16), and vancomycin (0.25), but is sensitive to cephaloridine (25), chloramphenicol (25), gentamicin (0.5), kanamycin (5),

rifampin (2), tetracycline (5), and tobramycin (8). Similarly, growth occurs in the presence of (μg/ml) bismuth citrate (1), crystal violet (1), phenol (100), phenyl ethanol (0.1%, v/v), potassium tellurite (10), sodium azide (1), teepol (100), tetrazolium (100), thallous acetate (10), and sodium chloride (13%, w/v), but is sensitive to adenine (0.4%, w/v), crystal violet (10), phenyl ethanol (0.4%, v/v), potassium tellurite (100), sodium azide (100), and thallous acetate (100). Sensitive to lysozyme.

The type strain produces vitamin B_{12}.

Additional phenotypic properties are cited in the text and in Table 253.

Cellular fatty acids consist of major proportions of $C_{16:1}$ iso OH, $C_{16:0}$ iso, and $C_{17:1}$ ω6*c*; smaller proportions (<10%) of $C_{15:0}$ iso, $C_{15:1}$ ω6*c*, $C_{16:1}$ ω7*c* and/or $C_{15:0}$ iso 2-OH, $C_{15:0}$, $C_{16:0}$, $C_{16:0}$ 10-methyl, $C_{17:0}$ iso, $C_{17:1}$ ω8*c*, $C_{17:0}$, $C_{18:1}$ iso, and $C_{18:0}$ are found.

Source: isolated from the rumen of a cow.

DNA G+C content (mol%): 67.0–68.9 (T_m).

Type strain: ATCC 43014, CIP 106520, DSM 43194, NBRC 14506, IMRU 3760, JCM 9736, NCIMB 8926, NRRL B-2295, VKM Ac-1243.

Sequence accession no. (16S rRNA gene): AF051342.

2. **Prauserella aidingensis** Li, Tang, Chen, Wu, Zhi, Zhang and Li 2009b, 2926[VP]

ai.ding.en′sis. N.L. fem. adj. *aidingensis* of or belonging to Aiding Lake, where the type strain was isolated.

Forms a substrate mycelium that fragments and which carries a white aerial mycelium. Colonies are light gray-white on glycerol-asparagine agar (ISP medium 5), slightly gray-white on oatmeal agar (ISP medium 3), and brilliant yellow on potato and yeast extract-malt extract (ISP medium 2) agars. Grows at 15–45°C, pH 6.0–9.0, and 5–15% (w/v) NaCl on ISP 2 medium; optimal conditions are 28–37°C, pH 7, and 8–10% NaCl. Catalase-positive, but oxidase-negative. Coagulates milk, but is negative for H_2S production. L-Alanine, L-histidine, L-hydroxyproline, L-phenylalanine, L-proline, DL-tryptophan, L-tyrosine, L-xanthine, and L-valine are used as sole nitrogen sources, but not adenine or DL-methionine.

The DNA–DNA relatedness value between the type strain and that of *Prauserella sediminis* is 47.9%.

Additional phenotypic properties are cited in the text and in Table 253.

Cellular fatty acids contain major proportions of $C_{16:0}$ iso and $C_{16:1}$ ω9*c*; smaller proportions (<10%) of $C_{14:0}$, $C_{15:0}$, $C_{15:0}$ iso, $C_{15:1}$ B, $C_{16:0}$, $C_{16:0}$ iso 2-OH, $C_{16:1}$ iso H, $C_{17:0}$, $C_{17:0}$ iso 2-OH, $C_{17:0}$ anteiso, $C_{17:1}$ anteiso, and $C_{18:1}$ 2-OH, and traces of $C_{17:0}$ anteiso 2-OH are found.

Source: isolated from a salt lake.

DNA G+C content (mol%): 70.1 (HPLC).

Type strain: CCTCC AA 208053, DSM 45266, YIM 90636.

Sequence accession no. (16S rRNA gene): FJ444994.

3. **Prauserella alba** Li, Xu, Tang, Xu, Kroppenstedt, Stackebrandt and Jiang 2003c, 1548[VP]

al′ba. L. fem. adj. *alba* white, referring to the white aerial mycelium.

Forms a branched substrate mycelium which undergoes fragmentation. Substrate mycelia may be light orange-yellow (Czapek's and nutrient agars), gray-white (oatmeal agar; ISP medium 3), orange-yellow (yeast extract-malt extract agar; ISP medium 2), and ranges from yellow-white to light yellow on glucose-asparagine agar (ISP medium 5), inorganic salts-starch agar (ISP medium 4), and nutrient agar; all of these media were supplemented with 10% (w/v) NaCl. Diffusible pigments are not formed. White aerial hyphae differentiate into branched short or, at maturity, long, straight to flexuous spore chains on Czapek's agar. Optimal growth occurs on Czapek's agar supplemented with 10% (w/v) NaCl at 28°C and pH 7.0.

Does not hydrolyze esculin. *p*NP-α-D-Glucopyranoside is hydrolyzed, but not L-alanine-*p*NA, *bis*-*p*NP phosphate, 2-deoxythymidine-5-*p*NP phosphate, *o*NP-β-D-galactopyranoside, *p*NP-glucopyranoside, *p*NP-β-D-glucuronide, L-glutamate-γ-carbonyl-*p*NP, *p*NP-phenylphosphonate, *p*NP-phosphorylcholine, L-proline-*p*NA, or *p*NP-β-D-xylopyranoside. Utilizes acetate, *N*-acetyl-D-glucosamine, *cis*- and *trans*-aconitate, adipate, L-alanine, L-arabinose, citrate, L-histidine, 3-hydroxy-DL-butyrate, DL-lactate, L-leucine, L-malate, maltose, D-mannose, oxoglutarate, phenylacetate, L-phenylalanine, L-proline, pyruvate, L-serine, D-sorbitol, suberate, and trehalose, but not 4-aminobutyrate, L-aspartate, azelate, glutarate, itaconate, 4-hydroxybenzoate, or L-ornithine. DNA–DNA relatedness values between the type strain and those of other *Prauserella* species are as follows: *Prauserella aidingensis*, 41.4%; *Prauserella flava*, 43.6%; *Prauserella salsuginis*, 47.2%; and *Prauserella sediminis*, 51.6%.

Additional phenotypic features are cited in the text and in Table 253.

Cellular fatty acids contain major proportions of $C_{16:0}$ and $C_{16:0}$ 2-OH; smaller proportions (<10%) of $C_{14:0}$ iso, $C_{15:0}$ iso, $C_{15:1}$ B, $C_{16:0}$, $C_{16:1}$ *cis*9, $C_{16:1}$ iso H, $C_{17:0}$ iso, $C_{17:0}$ anteiso, $C_{17:0}$ anteiso 2-OH, and $C_{17:1}$ ω9*c*, and traces of $C_{15:0}$, $C_{17:0}$, and $C_{17:0}$ iso 2-OH are found.

Source: isolated from soil in hypersaline habitats.

DNA G+C content (mol%): 66.7 (T_m).

Type strain: CCTCC AA 001016, DSM 44590, YIM 90005.

Sequence accession no. (16S rRNA gene): AF435077.

Additional comments: in the original paper by Li et al. (2003c), strain CCTCC AA 001016 is wrongly cited as CCTCC AA001016.

4. **Prauserella flava** Li, Tang, Chen, Wu, Zhi, Zhang and Li 2009b, 2926[VP]

fla′va. L. fem. adj. *flava* yellow, referring to the color of the substrate mycelium.

Forms a substrate mycelium that undergoes fragmentation. Colonies are light yellow on glycerol-asparagine agar (ISP medium 5), brown on oatmeal agar (ISP medium 3), gray-yellow on potato agar, and brilliant yellow on yeast extract-malt extract agar (ISP medium 2). Does not form aerial hyphae on any of these media. Grows at 15–45°C, pH 6.0–9.0, and 5–15% (w/v) NaCl on ISP medium 2; optimal conditions are 28–37°C, pH 7, and 8–10% NaCl. Catalase-positive, but oxidase-negative. Coagulates milk, but is negative for H_2S production, nitrate reduction, and starch hydrolysis. L-Alanine, L-histidine, L-phenylalanine, L-proline, DL-tryptophan, L-tyrosine, xanthine, and L-valine are used as sole nitrogen sources, but not adenine, L-arginine, or DL-methionine.

DNA–DNA relatedness values of the type strain of *Prauserella flava* with those of *Prauserella aidingensis* and *Prauserella sediminis* are 55.3 and 40.9%, respectively.

Additional phenotypic properties are cited in the text and in Table 253.

Cellular fatty acids consist of major proportions of $C_{16:0}$ iso and $C_{17:1}$ anteiso; smaller proportions (<10%) of $C_{14:0}$, $C_{15:0}$, $C_{15:0}$ iso, $C_{15:1}$ B, $C_{16:0}$, $C_{16:0}$ iso 2-OH, $C_{16:1}$ ω9*c*, $C_{16:1}$ iso H, $C_{17:0}$, $C_{17:0}$ anteiso, and $C_{17:0}$ anteiso 2-OH, and traces of $C_{17:1}$ ω9*c* and $C_{18:1}$ 2-OH are found.

Source: isolated from a salt lake.

DNA G+C content (mol%): 69.9 (HPLC).

Type strain: CCTCC AA 208052, DSM 45265, YIM 90630.

Sequence accession no. (16S rRNA gene): FJ444993.

5. **Prauserella halophila** Li, Xu, Tang, Xu, Kroppenstedt, Stackebrandt and Jiang 2003c, 1548[VP]

ha.lo′phi.la. Gr. n. *hals halos* salt; N.L. adj. *philus -a -um* (from Gr. adj. *philos -ê -on*) friend, loving; N.L. fem. adj. *halophila* salt-loving, referring to the ability to grow at high NaCl concentrations.

Forms a branched substrate mycelium which undergoes fragmentation. Substrate mycelium is light orange-brown on Czapek's agar, light gray-white on arginine-glycerol agar (ISP medium 5), light yellow on inorganic salts-starch and yeast extract-malt extract agars (ISP media 4 and 2, respectively), deep gray-white on oatmeal agar (ISP medium 3), and deep yellow on potato agar [all media supplemented with 10% (w/v) NaCl]. Diffusible pigments are not formed. A white to yellow aerial mycelium is well developed on all of these media, apart from ISP medium 2. Aerial hyphae differentiate into branched short or, at maturity, long straight to flexuous spore chains. Optimal growth occurs on Czapek's agar supplemented with 10–15% (w/v) NaCl at 28°C and pH 7.0. Does not hydrolyze esculin, L-alanine-*p*NA, *bis*-*p*NP phosphate, 2-deoxythymidine-5-*p*NP phosphate, *o*NP-β-D-galactopyranoside, *p*NP-α-D-glucopyranoside, *p*NP-glucopyranoside, *p*NP-β-D-glucuronide, L-glutamate-γ-3-cabonyl-*p*NP, *p*NP-phenylphosphonate, *p*NP-phosphorylcholine, L-proline-*p*NP, or *p*NP-β-D-xylopyranoside. Acetate, adipate, L-alanine, azelate, fumarate, L-histidine, 3-hydroxy-DL-butyrate, L-malate, L-phenylalanine, propionate, L-proline, pyruvate, D-sorbitol, and suberate are used as sole carbon sources, but not *N*-acetyl-D-glucosamine, *cis*- or *trans*-aconitate, 4-aminobutyrate, L-arabinose, citrate, glutarate, 4-hydroxybenzoate, itaconate, DL-lactate, L-leucine, maltose, D-mannose, L-ornithine, oxoglutarate, L-phenylacetate, L-serine, or trehalose.

Additional phenotypic properties are cited in the text and in Table 253.

Cellular fatty acids contain major proportions of $C_{16:0}$ iso, $C_{17:1}$ ω6*c*, $C_{16:1}$ ω7*c* and/or $C_{15:0}$ iso 2-OH, and $C_{17:0}$ anteiso, and smaller proportions (<10%) of $C_{14:0}$ iso, $C_{15:0}$ iso, $C_{15:1}$ ω6*c*, $C_{16:1}$ iso H, $C_{16:0}$, $C_{17:0}$ 10-methyl, $C_{17:1}$ ω8*c*, $C_{17:0}$, and $C_{18:1}$ ω9*c*.

DNA–DNA relatedness values between the type strain and those of other *Prauserella* species are as follows: *Prauserella aidingensis*, 53.7%; *Prauserella alba*, 30.2%; *Prauserella flava*, 41.3%; *Prauserella salsuginis*, 20.8%; and *Prauserella sediminis*, 45.2%.

Source: isolated from soil in hypersaline habitats.

DNA G+C content (mol%): 65.8 (T_m).

Type strain: CCTCC AA 001015, DSM 44617, YIM 90001.

Sequence accession no. (16S rRNA gene): AF466190.

Additional comments: the etymology of the first compound of the specific epithet should be Gr. n. *halo halos* salt, not Gr. n. *halo* salt as cited by Li et al. (2003c). Similarly, the specific epithet *halophila* is a "N.L. fem. adj." not a "N.L. gen. adj." as cited by Li and his colleagues who also cited strain CCTCC AA 001015 as CCTCC AA001015.

6. **Prauserella marina** Wang, Li, Bian, Tang, Ren, Chen, Li and Zhang 2010, 988[VP]

ma.ri′na. L. fem. adj. *marina* of the sea, marine.

Forms a substrate mycelium which undergoes fragmentation. Substrate mycelium is light gray-white on glycerol-asparagine agar (ISP medium 5), moderate reddish-brown on yeast extract-malt extract agar (ISP medium 2), and pale pink on oatmeal (ISP medium 3) and potato agars. Optimal growth occurs on ISP 2 agar prepared without NaCl at 28–37°C and pH 7.0. Growth occurs at 15–45°C, pH 6.0–9.0 and 0–10% (w/v) NaCl. Catalase-positive, but oxidase-negative. Coagulates milk, but is negative for hydrogen sulfide production, nitrate reduction, and starch hydrolysis. L-Alanine, L-histidine, hypoxanthine, L-phenylalanine, L-proline, DL-tryptophan, L-tyrosine, L-valine, and xanthine are used as nitrogen sources, but not adenine or DL-methionine.

Additional phenotypic properties are cited in the text and in Table 253.

Cellular fatty acids contain major proportions of $C_{16:0}$ iso, $C_{16:0}$, and $C_{15:0}$ iso, and smaller proportions (<10%) of $C_{16:1}$ iso H and $C_{17:0}$.

Source: isolated from ocean sediment.

DNA G+C content (mol%): 66.1 (HPLC).

Type strain: CCTCC AA 208056, DSM 45268, MS498.

Sequence accession no. (16S rRNA gene): FJ444996.

7. **Prauserella muralis** Schäfer, Martin and Kämpfer 2010, 289[VP]

mu.ra′lis. L. fem. adj. *muralis* pertaining or belonging to walls.

Forms mycelial-like filaments (1.5 µm), and a white aerial mycelium which undergoes fragmentation. The substrate mycelium is gray to light orange on M79 agar. Grows well on nutrient and tryptone soy agars after 3 d at 25–30°C. Weakly oxidase-positive, but negative for esculin hydrolysis. *p*NP-α-D-Glucopyranoside and *p*NP-β-D-xylopyranoside are hydrolyzed, but not L-alanine-*p*NA, *bis*-*p*NP phosphate, 2-deoxythymidine-5-*p*NP phosphate, *o*NP-β-D-galactopyranoside, *p*NP-glucopyranoside, *p*NP-β-D-glucuronide, L-glutamate-γ-3-carbonyl-*p*NP, *p*NP-phenylphosphate, *p*NP-phosphorylcholine, or L-proline *p*NP.

Additional phenotypic properties are cited in the text or in Table 253.

Cellular fatty acids contain major proportions of $C_{16:0}$ iso; smaller proportions (<10%) of $C_{15:0}$ iso, $C_{15:1}$ ω6*c*, $C_{15:0}$, $C_{16:1}$ iso H, $C_{16:1}$ ω7*c* and/or $C_{15:0}$ iso 2-OH, $C_{16:0}$, $C_{16:0}$ 10-methyl, $C_{17:0}$ iso, $C_{17:0}$ anteiso, $C_{17:1}$ ω8*c*, $C_{17:1}$ ω6*c*, $C_{17:0}$, $C_{17:0}$ 10-methyl, and traces of $C_{14:0}$ iso and $C_{14:0}$ are found.

Source: isolated from the wall of a house colonized with molds.

DNA G+C content (mol%): not determined.

Type strain: 05-Be-005, CCM 7635, CCUG 57426, DSM 45305, NRRL B-24780.

Sequence accession no. (16S rRNA gene): FM956091.

8. **Prauserella salsuginis** Li, Tang, Chen, Wu, Zhi, Zhang and Li 2009b, 2926[VP]

sal.su′gi.nis. L. n. *salsugo -inis* brine, salt water, L. gen. n. *salsuginis* of salt water, from which the type strain was isolated.

Aerobic, Gram-stain-positive actinomycete which forms a substrate mycelium that undergoes fragmentation and produces a white aerial mycelium. Colonies are deep yellow on Czapek's agar, light gray-white on glycerol-asparagine agar (ISP medium 5), pale orange-yellow on nutrient and potato agars, and brilliant yellow on yeast extract-malt extract agar (ISP medium 2). Grows at 15–45°C, pH 6.0–9.0, and 5–15% (w/v) NaCl on ISP 2 medium; optimal conditions are 28–37°C, pH 7.0, and 8–10% NaCl.

Catalase-positive, but oxidase-negative. Coagulates milk, but is negative for melanin and H_2S production.

L-Alanine, L-histidine, L-phenylalanine, L-proline, DL-tryptophan, L-tyrosine, xanthine, and L-valine are used as sole nitrogen sources, but not adenine or DL-methionine.

DNA–DNA relatedness values between the type strain and those of other *Prauserella* species are: *Prauserella aidingensis*, 44.0%; *Prauserella flava*, 56.9%; and *Prauserella sediminis*, 60.3%.

Additional phenotypic properties are cited in the text and in Table 253.

Cellular fatty acids contain major proportions of $C_{16:0}$ iso; smaller proportions (<10%) of $C_{15:0}$, $C_{15:0}$ iso, $C_{15:1}$ B, $C_{16:0}$, $C_{16:0}$ iso 2-OH, $C_{16:1}$ ω9*c*, $C_{16:1}$ iso H, $C_{17:0}$ anteiso, $C_{17:0}$ anteiso 2-OH, $C_{17:1}$ anteiso, and $C_{18:1}$ 2-OH, and traces of $C_{17:1}$ ω9*c* are found.

Source: isolated from a salt lake.

DNA G+C content (mol%): 69.1 (HPLC).

Type strain: CCTCC AA 208051, DSM 45264, YIM 90625.

Sequence accession no. (16S rRNA gene): FJ444992.

9. **Prauserella sediminis** Li, Tang, Chen, Wu, Zhi, Zhang and Li 2009b, 2927[VP]

se.di′mi.nis. L. n. *sedimen -inis* sediment; L. gen. n. *sediminis* of sediment.

Forms a substrate mycelium that undergoes fragmentation and produces a white aerial mycelium on most media. Colonies are deep gray-white on glycerol-asparagine agar (ISP medium 5), pale yellow on oatmeal agar (ISP medium 3), gray-reddish orange on potato agar and orange-yellow on yeast extract-malt extract agar (ISP medium 2). Grows at 15–45°C, pH 6.0–9.0, and 5–20% (w/v) NaCl on ISP 2 medium; optimal conditions are 28–37°C, pH 7.0, and 10% NaCl.

Catalase-positive, but oxidase-negative. Coagulates milk, but is negative for H_2S production, nitrate reduction, and starch hydrolysis.

L-Alanine, L-histidine, L-phenylalanine, L-proline, DL-tryptophan, L-tyrosine, xanthine, and L-valine are used as sole nitrogen sources, but not adenine or DL-methionine.

Additional phenotypic properties are cited in the text and in Table 253.

Cellular fatty acids contain major proportions of $C_{16:0}$ iso; smaller proportions (<10%) of $C_{14:0}$ iso, $C_{15:0}$, $C_{15:0}$ iso, $C_{15:1}$ B, $C_{16:0}$, $C_{16:0}$ 2-OH, $C_{16:1}$ ω9*c*, $C_{16:1}$ iso H, $C_{17:0}$, $C_{17:0}$ iso 2-OH, $C_{17:0}$ anteiso, and $C_{17:1}$ anteiso, and traces of $C_{14:0}$, $C_{17:1}$ ω9*c*, and $C_{17:1}$ 2-OH are found.

Source: isolated from a salt lake.

DNA G+C content (mol%): 69.1 (HPLC).

Type strain: CCTCC AA 208054, DSM 45267, YIM 90694.

Sequence accession no. (16S rRNA gene): FJ444995.

Genus XIII. **Saccharomonospora** Nonomura and Ohara 1971, 899[AL]

Seung Bum Kim

Sac.cha.ro.mon.o.spo′ra. Gr. n. *sakchâr* sugar; Gr. adj. *monos* single, solitary; Gr. fem. n. *spora* seed; N.L. fem. n. *spora* a spore; N.L. fem. n. *Saccharomonospora* the sugar (-containing) single-spored (organism).

Gram-stain-positive, aerobic, and chemo-organotrophic. **Produces single or paired spores on aerial hyphae.** Spores may be formed on substrate mycelium. The aerial mycelium can be white, yellow-white, green, or light to dark blue; green pigmentation may also occur on the vegetative mycelium and diffuse into the surrounding medium. **Substrate mycelia are rarely fragmented**. Spores in pairs or short chains on vegetative or aerial hyphae are occasionally present. **The cell wall contains *meso*-diaminopimelic acid (*meso*-DAP), and the sugars arabinose and galactose (wall chemotype IV).** Mycolic acids are absent. Major amounts of iso- and anteiso- fatty acids are found; the main **menaquinone is MK-9(H_4)**. The diagnostic phospholipid is **phosphatidylethanolamine** (**phospholipid type II**), but some species may also contain glucosamine-containing phospholipids (type IV). **Mesophilic or thermophilic**; growth occurs between 24 and 60°C, and at neutral pH. **NaCl may be required for growth.** Isolated from soil, lake sediments, marsh soil, peat, manure, compost, and overheated fodder.

DNA G+C content (mol%): 68–74.

Type species: **Saccharomonospora viridis** (Schuurmans, Olson and San Clemente 1956) Nonomura and Ohara 1971, 899[AL] [*Thermoactinomyces viridis* Schuurmans, Olson and San Clemente 1956, 61; *Thermomonospora viridis* (Schuurmans, Olson and San Clemente 1956) Küster and Locci 1963].

Further descriptive information

Phylogeny. *Saccharomonospora* is phylogenetically related to the genera *Prauserella* and *Thermocrispum* (Kim and Goodfellow, 1999; Labeda and Kroppenstedt, 2006). In 16S rRNA gene sequence analyses, the species are divided into two main cluster groups, one including *Saccharomonospora azurea*, *Saccharomonospora cyanea*,

FIGURE 313. Aerial spores of *Saccharomonospora azurea* strain NA-128[T]. Magnification 4800×. (Reproduced with permission from Hu, 1997. Int. J. Syst. Bacteriol. *47*: 60–61.)

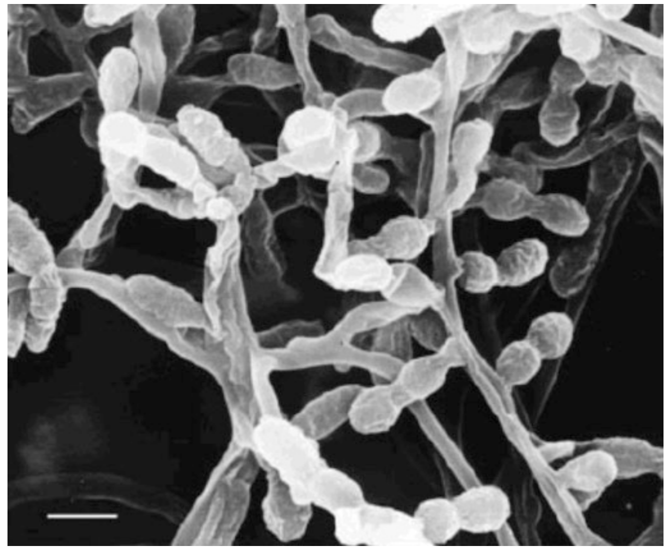

FIGURE 314. Aerial spore of *Saccharomonospora halophila* strain 8[T]. Bar = 1 µm. (Reproduced with permission from Al-Zarban et al., 2002. Int. J. Syst. Evol. Microbiol. *52*: 555–558.)

Saccharomonospora glauca, *Saccharomonospora viridis*, and *Saccharomonospora xinjiangensis*, and the other including *Saccharomonospora halophila*, *Saccharomonospora marina*, *Saccharomonospora paurometabolica*, and *Saccharomonospora saliphila*, respectively (Al-Zarban et al., 2002; Li et al., 2003b; Syed et al., 2008; Liu et al., 2010). The 16S rRNA gene sequence similarity among the type strains ranges from 95.2 to 98.5%. The levels of DNA–DNA relatedness between the type strain of *Saccharomonospora saliphila* and those of *Saccharomonospora azurea*, *Saccharomonospora halophila*, and *Saccharomonospora paurometabolica* were reported as 46.0, 41.0, and 42.5%, respectively (Syed et al., 2008). The value between *Saccharomonospora paurometabolica* and *Saccharomonospora halophila* was 53.8% (Li et al., 2003b).

Cellular morphology. The aerial and vegetative mycelia are well developed and irregularly branched. The substrate mycelium is, in most cases, non-fragmented. Aerial mycelium is generally abundant, but can be absent in some strains. The color of aerial mycelia is green to blue, except for *Saccharomonospora paurometabolica* and *Saccharomonospora xinjiangensis* which is white, orange-white, or yellow-white. Single spores are borne at the tip of aerial hyphae in most species (Figure 313), but spores in longitudinal pairs are also produced in *Saccharomonospora marina*, *Saccharomonospora saliphila* and *Saccharomonospora xinjiangensis* (Figure 314).The spores are ovoid, ellipsoidal, or round (0.7–1.1 × 1.0–1.8 µm); the surface of individual spores is smooth for *Saccharomonospora azurea*, *Saccharomonospora marina*, *Saccharomonospora paurometabolica*, *Saccharomonospora saliphila*, and *Saccharomonospora xinjiangensis*, and warty for the remaining four species. Wrinkled spores may be observed for *Saccharomonospora marina*, *Saccharomonospora paurometabolica* and *Saccharomonospora saliphila*. The spores of *Saccharomonospora viridis* are not viable at temperatures above 70°C.

Nutrition and growth conditions. Strains of *Saccharomonospora glauca*, *Saccharomonospora viridis*, and *Saccharomonospora xinjiangensis* are thermotolerant, preferring temperatures of 45–50°C, whereas the remaining species are mesophilic. *Saccharomonospora halophila* and *Saccharomonospora paurometabolica* require NaCl for growth, but *Saccharomonospora cyanea*, *Saccharomonospora glauca*, *Saccharomonospora viridis*, and *Saccharomonospora xinjiangensis* cannot grow in the presence of 5% (w/v) NaCl. *Saccharomonospora azurea* and *Saccharomonospora saliphila* do not require NaCl for growth, but the former can grow in the presence of up to 7%, and the latter in up to 20% (w/v) concentration.

Cell-wall composition. Phosphatidylethanolamine is the main phospholipid for all species and, in addition, hydroxyphosphatidylethanolamine, lysophosphatidylethanolamine, phosphatidylinositol, phosphatidylglycerol, and diphosphatidylglycerol are variably found (corresponding to the type II phospholipid pattern *sensu* Lechevalier et al., 1981). However, *Saccharomonospora xinjiangensis* contains phosphatidylcholine and glucosamine-containing phospholipid in addition to phosphatidylethanolamine, exhibiting a type IV phospholipid pattern. The fatty acid profile is highly variable among species, but is generally a mixture of branched and straight chain saturated or unsaturated fatty acids, with smaller amounts of hydroxylated fatty acids. The main menaquinone is MK-9(H_4), whereas MK-8(H_4), MK-9(H_2), and MK-7(H_4) are variably present as minor components.

Ecology. Saccharomonosporae occur in a wide range of natural or synthetic habitats including soil (Al-Zarban et al., 2002; Jin et al., 1998; Li et al., 2003b; Syed et al., 2008), compost (Amner et al., 1988; Dees and Ghiorse, 2001; Song et al., 2001; Steger et al., 2007), plant materials (Abdulla and El-Shatoury, 2007; Gangwar et al., 1989; Roussel et al., 2005; Unaogu et al., 1994; Liu et al., 2010), marine sediment (Maldonado et al., 2009), and marine sponge (Selvin et al., 2009). Compost is clearly a preferred habitat for thermophilic *Saccharomonospora* (Dees and Ghiorse, 2001; Khan et al., 1995; Song et al., 2001; Unaogu et al., 1994).

Other properties. *Saccharomonospora viridis* is strongly implicated as one of the causative agents of hypersensitivity

pneumonitis including farmer's lung disease (Greene et al., 1981; Harvey et al., 2001; Roberts et al., 1976; Treuhaft et al., 1980; Wenzel et al., 1974). Strains of *Saccharomonospora viridis*, *Saccharomonospora glauca*, "*Saccharomonospora caesia*", and "*Saccharomonospora internatus*" display antibiotic activities against Gram-stain-positive bacteria (Greiner-Mai et al., 1988). *Saccharomonospora viridis* is known to produce thermoviridin (Schuurmans et al., 1956). Many strains of *Saccharomonospora* produce degradative enzymes for natural or anthropogenic compounds, such as polyester, rice straw, mushroom compost, synthetic food waste compost, proteins, and starch (Abdulla and El-Shatoury, 2007; Collins et al., 1992; Dolashka et al., 1998; Song et al., 2001; Tseng et al., 2007).

Enrichment and isolation procedures

Mesophilic saccharomonosporae are present in soils, leaf litter, and manure; thermophilic strains can be found in compost, while marsh soils are a good source for halophilic strains. Conventional methods for the isolation of actinobacteria can be used for the isolation of saccharomonosporae, but improved recovery has been reported using selective isolation methods, such as dry heat treatment, addition of antibiotics to the isolation medium, use of sedimentation chamber with an Andersen air sampler, and a combination of the above methods (Amner et al., 1989; Andersen, 1958; Athalye et al., 1981; Kim et al., 1995; Nonomura and Ohara, 1971).

The isolation media include modified glycerol-asparagine agar [ISP medium 5 supplemented with 20% (w/v) NaCl] half-strength tryptone-soy agar, HV agar (Hayakawa, 1990), and R8 agar (Amner et al., 1989). For the prevention of fungal growth, antibiotics such as cycloheximide (50 µg/ml) may be added to the media.

Maintenance procedures

The following media can be used for the cultivation and maintenance of *Saccharmonospora* isolates: yeast extract-malt extract (ISP 2) agar, inorganic salts-starch (ISP 4) agar, Czapek–Dox yeast extract-Casamino acids (CYC) agar (Cross and Attwell, 1974), starch-nitrate agar with 10% (w/v) NaCl (Al-Zarban et al., 2002), and tryptic soy agar (TSA). *Saccharomonospora halophila* and *Saccharomonospora paurometabolica* require NaCl for growth at an optimal concentration of 10% (w/v). For long-term storage, spores and mycelial fragments are suspended in 10–20% (v/v) aqueous glycerol and stored at −20°C, or lyophilized.

Differentiation of the genus *Saccharomonospora* from other genera

Saccharomonospora can be distinguished from other members of the family *Pseudonocardiaceae* by the production of single spores on aerial hyphae. Fragmentation of the substrate mycelium is rarely observed, which is an important differential feature of the genus from closely related genera, including *Prauserella*, *Saccharopolyspora*, and *Thermoscrispum*. Sporangia-like structures have not been observed in *Saccharomonospora*, in contrast to *Crossiella*, *Kibdelosporangium*, *Kutzneria*, *Streptoalloteichus*, and *Thermocrispum*. The phospholipid pattern of *Saccharomonospora* is type II, with the exception of *Saccharomonospora xinjiangensis* (type IV), whereas that of *Saccharopolyspora* is type III.

Taxonomic comments

The genus *Saccharomonospora* currently contains nine species with validly published names (Figure 315). The type species *Saccharomonospora viridis* Nonomura and Ohara 1971 was first

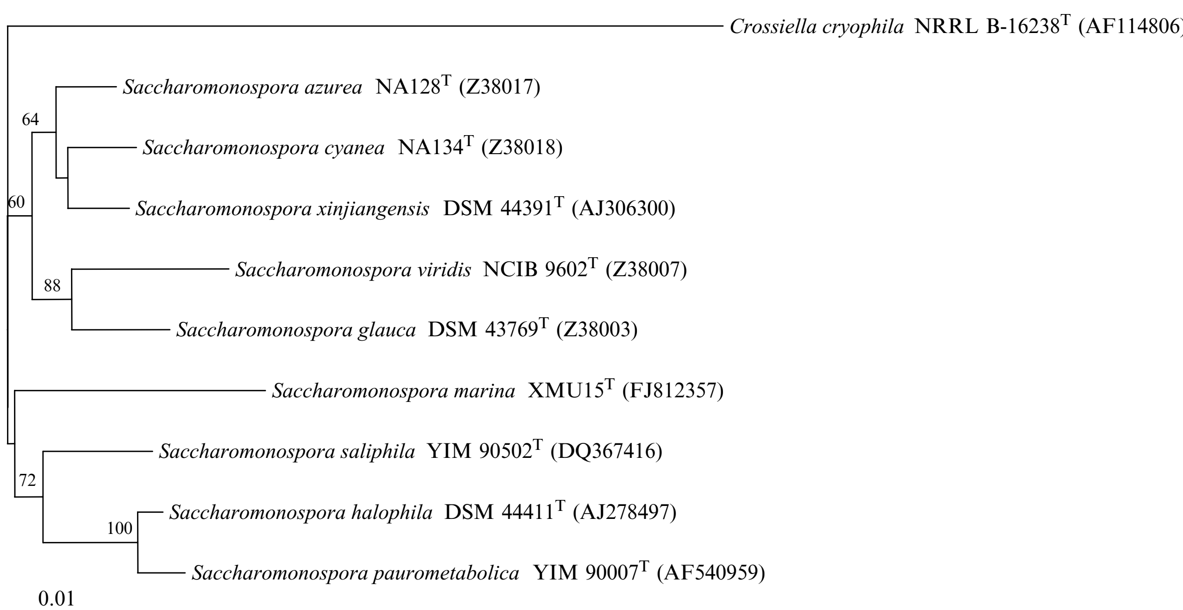

FIGURE 315. Phylogenetic tree of the genus *Saccharomonospora* based on 16S rRNA gene sequences. The Jukes–Cantor model was used in the estimation of evolutionary distances and neighbor-joining method was used for tree construction. Numbers at nodes indicate levels of bootstrap support (%). Bar = 0.01 substitutions per nucleotide position.

TABLE 254. Characteristics that differentiate the type strains of the genus *Saccharomonospora*[a]

Character	*S. viridis* NCIB 9602[T]	*S. azurea* NA-128[T]	*S. cyanea* NA-134[T]	*S. glauca* DSM 43769[T]	*S. halophila* DSM 44411[T]	*S. marina* KCTC 19701	*S. paurometabolica* YIM 90007[T]	*S. saliphila* YIM 90502[T]	*S. xinjiangensis* DSM 44391[T]
Aerial mycelium color	Green	Azure	Light to dark blue	Light blue to greenish	Light blue to greenish	White, gray to orange-white	White	Gray-red	Yellow-white
Spores in pairs	−	−	−	−	+	+	−	+	+
Spore ornamentation	Warty	Smooth	Warty	Warty	Warty	Smooth/ wrinkled	Smooth/ wrinkled	Smooth/ wrinkled	Smooth
Growth on sole carbon source (1%, w/v):									
L-Arabinose	−	−	nd	+	+	+	−	−	nd
Galactose	−	−	+	nd	+	+	−	+	nd
Glucose	Dbt	+	−	+	+	+	−	−	nd
Mannitol	Dbt	−	−	+	+	+	−	+	nd
Mannose	−	+	+	nd	+	+	nd	−	+
Melibiose	nd	+	−	nd	+	+	−	+	nd
Rhamnose	−	−	nd	−	+	+	−	+	+
Ribose	nd	+	+	nd	Dbt	−	−	nd	nd
Xylose	−	+	Dbt	+	−	+	−	+	+
Growth in NaCl (%, w/v):									
0	+	+	+	+	−	+	−	+	+
5	−	+	−	−	−	+	+	+	−
7	−	+	−	−	−	−	+	+	−
10	−	−	−	−	+	−	+	+	−
20	−	−	−	−	+	−	+	+	−
30	−	−	−	−	+	−	+	−	−
Growth temperature (°C[b])	35–50	24–40	24–40 (28–37)	37–60 (50)	(28–30)	(28–37)	(35–37)	(28–30)	45–50
Menaquinone	9(H_4)	nd	nd	9(H_4) (60%), 8(H_4) (20–30%)	9(H_4) (88%), 8(H_4) (12%)	9(H_4) (90%), 8(H_4) (10%)	9(H_4) (90%), 9(H_2) (10%)	9(H_4) (90%), 8(H_4) (10%)	9(H_2), 9(H_4), 7(H_4)
Phospholipid[c]	PI, PIM, DPG acyl-PG	nd	nd	PE-OH, lyso-PE	DPG, PI, PE-OH, lyso-PE	PI, PG, DPG	PI, PG, DPG, PE-OH	PI, PG, DPG	PC, GluNU
DNA G+C content (mol%)	69	nd	nd	nd	nd	68.1	71	71.8	nd

[a]+, Positive; −, negative; nd, not determined; Dbt, doubtful.

[b]Optimal temperatures are given in parentheses.

[c]PE-OH, hydroxyphosphatidylethanolamine; PI, phosphatidylinositol; PIM, phosphatidylinositol mannoside; PG, phosphatidylglycerol; DPG, diphosphatidylglycerol; PC, phosphatidylcholine; GluNU, glucosamine-containing phospholipid. All species contain phosphatidylethanolamine (PE).

proposed as "*Thermoactinomyces viridis*" by Schuurmans et al. (1956), and later reclassified as "*Thermomonospora viridis*" Küster and Locci 1963. *Saccharomonospora* was then proposed based on morphological and chemotaxonomic criteria (Nonomura and Ohara, 1971), which was further supported by numerical phenetic data (Goodfellow and Pirouz, 1982; McCarthy and Cross, 1984).

Phylogenetic studies on saccharomonosporae have been conducted using analysis of 16S rRNA gene sequences (Kim et al., 1995), internally transcribed spacer sequences of 16S–23S and 23S–5S rRNA genes (Yoon et al., 1997), and ribonuclease P (RNase P) RNA gene sequences (Cho et al., 1998). These data constantly suggested that "*Saccharomonospora caesia*" was a synonym of *Saccharomonospora azurea*. Additionally, DNA–DNA relatedness studies among the representative species of *Saccharomonospora* were also carried out (Yoon et al., 1999), confirming the former proposal. Ruan et al. (1994) used partial sequences of the 23S rRNA gene for the classification of *Saccharomonospora* and related taxa.

DNA–DNA hybridization data also confirmed that "*Saccharomonospora internatus*" (Greiner-Mai et al. 1987; Kurup 1981), formerly "*Micropolyspora internatus*" Agre et al. 1974, is a synonym of *Saccharomonospora viridis* Greiner-Mai et al. 1988, as the two taxa exhibited 90% DNA–DNA relatedness (Yoon et al., 1999).

A PCR-based method for the rapid detection of *Saccharomonospora* isolates has been developed using a set of genus-specific primers (Salazar et al., 2000). The sequence data indicate that these oligonucleotide primers are also applicable to the species that have been described subsequently, namely *Saccharomonospora halophila*, *Saccharomonospora paurometabolica*, and *Saccharomonospora xinjiangensis*. In another study, a rapid detection method based on fluorescence *in situ* hybridization (FISH) using 16S rRNA-targeted oligonucleotide probes was developed for the specific detection of *Saccharomonospora* spp. (Neef et al., 2003).

The primycin-producing actinobacterium, initially described as "*Thermomonospora galeriensis*" (Szabo et al., 1976), was reported to produce single spores and dark green aerial mycelium. This species contains a type IV cell wall and was considered to be a member of the genus *Saccharomonospora* by McCarthy and Cross (1984), but its correct taxonomic position needs to be clarified.

The strains known as "*Thermoactinomyces glaucus*" IFO 12530 (Henssen, 1957) and "*Thermoactinomyces monosporus*" IFO 14050 (Waksman and Cork, 1953) have been assigned to *Saccharomonospora glauca* based on chemotaxonomic and genotypic characterization (Yoon et al., 2000; Yoon and Park, 2000).

Further reading

Goodfellow, M. and T. Cross. 1984. Classification. *In* Goodfellow, Mordarski and Williams (Editors), The Biology of the Actinomycetes, Academic Press, London, pp. 7–164.

Differentiation of the species of the genus *Saccharomonospora*

The species of *Saccharomonospora* can be separated from one another by a combination of physiological and chemotaxonomic properties. *Saccharomonospora paurometabolica* produces white aerial mycelium, *Saccharomonospora xinjiangensis* yellow-white and *Saccharomonospora saliphila* gray-red, whereas most others produce blue to green aerial mycelia. The warty spore surface separates *Saccharomonospora cyanea*, *Saccharomonospora glauca*, *Saccharomonospora halophila*, and *Saccharomonospora viridis* from the remaining species. Unlike other species, *Saccharomonospora halophila* and *Saccharomonospora paurometabolica* can grow in the presence of up to 30% NaCl, but cannot grow without NaCl. Other differential properties are listed in Table 254.

List of species of the genus *Saccharomonospora*

1. **Saccharomonospora viridis** (Schuurmans, Olson and San Clemente 1956) Nonomura and Ohara 1971, 899[AL] [*Thermoactinomyces viridis* Schuurmans, Olson and San Clemente 1956, 61; *Thermomonospora viridis* (Schuurmans, Olson and San Clemente 1956) Küster and Locci 1963]. Subjective synonym: "*Saccharomonospora internatus*" (Agre et al. 1974) Greiner-Mai, Korn-Wendisch and Kutzner 1988.

   vir′i.dis. L. fem. adj. *viridis* green.

   Single spores are mainly produced on aerial mycelium, but short chains of spores may also be formed. The spores are heat-sensitive. Vegetative mycelia are branched and may produce spores. Leathery colonies are formed on agar media, with aerial hyphae covered by densely packed spores. Aerial mycelium is initially white, becoming gray-green to dark green. The aerial spore mass may be non-pigmented or lilac-colored. Vegetative mycelium may be green. Production of a green diffusible pigment is also observed. Amino acid and vitamin supplements (e.g. yeast extract) are required for good growth. Catalase, deaminase, and phosphatase are produced. Casein, gelatin, starch, xylan, and tyrosine are degraded, but not cellulose. A number of organic compounds can serve as sole sources of carbon, and the utilization of glycerol is characteristic. Optimal growth occurs at 35–50°C and pH 7.0–10. Growth occurs in the presence of up to 3% (w/v) NaCl, but not at concentrations of 5% or higher.

   Source: manure, compost, overheated fodder, soil, lake sediments, peat.

   DNA G+C content (mol%): 69–74 (T_m).

   Type strain: ATCC 15386, CCUG 5913, DSM 43017, JCM 3036, NBRC 12207, NCIB 9602, NRRL B-3044, VKM Ac-681.

   Sequence accession no. (16S rRNA gene): Z38007.

2. **Saccharomonospora azurea** Hu 1987, 61[VP]

   Subjective synonym: "*Saccharomonospora caesia*" (Kalakoutskii 1964) Greiner-Mai, Kroppenstedt, Korn-Wendisch and Kutzner 1987.

   a.zu.re′a. N.L. fem. adj. *azurea* azure, referring to the color of the aerial mycelium.

Substrate mycelium is non-fragmenting. Sporangia are not produced. Single spores are borne mainly on the aerial mycelium. Spores are oval or round, 0.8 to 1.0 µm. Sporophores are very short or sessile. Spore surface is smooth. The color of the aerial mycelium is azure on oatmeal and Czapek–Dox sucrose agars. No distinct soluble pigment is formed. D-Fructose, D-glucose, glycerol, lactose, maltose, mannose, melibiose, raffinose, L-rhamnose, ribose, sucrose, trehalose, and D-xylose are utilized as sole carbon sources, but not L-arabinose, galactose, inositol, or D-mannitol. Growth occurs between 24 and 40°C.

Source: soil.

DNA G+C content (mol%): not determined.

Type strain: NA-128, ATCC 43670, DSM 44631, JCM 7551, NBRC 14651, SIIA 86128.

Sequence accession no. (16S rRNA gene): Z38017.

3. **Saccharomonospora cyanea** Hu, Cheng and Wei 1988, 445[VP]

cy.a′ne.a. L. fem. adj. *cyanea* dark blue, referring to the color of aerial mycelium.

Substrate mycelium is non-fragmenting. No sporangium is observed. Single spores are borne mainly on aerial mycelium. Spores are oval to ellipsoidal (0.8–1.0 ×1.0–1.8 µm). Sporophores are very short or sessile. The color of the aerial mycelium is light to dark blue on various agar media. No distinct soluble pigment is formed. D-Fructose, glycerol, lactose, maltose, mannose, raffinose, L-rhamnose, ribose, sucrose, trehalose, and D-xylose are utilized as sole carbon sources, but not L-arabinose, D-glucose, *i*-inositol, D-mannitol, or melibiose. The temperature range for growth is between 24 and 40°C; optimal growth occurs between 28 and 37°C.

Source: soil.

DNA G+C content (mol%): not determined.

Type strain: NA-134, ATCC 43724, DSM 44106, JCM 7552, NBRC 14841, SIIA 86134.

Sequence accession no. (16S rRNA gene): Z38018.

4. **Saccharomonospora glauca** Greiner-Mai, Korn-Wendisch and Kutzner 1988, 403[VP]

glau′ca. L. fem. adj. *glauca* bluish, greenish, grayish blue, referring to the color of the aerial mycelium.

Branching, non-fragmenting aerial and substrate mycelia are formed. Single spores are produced tightly packed on the aerial hyphae. Spores are smooth or slightly roughened, round to oval, and 0.8 to 1.0 µm in diameter. Colonies produce light green to bluish green (turquoise) aerial mycelium, dark green substrate mycelium, and soluble pigment on glycerol-cornsteep (GC) and glycerol-yeast extract-malt extract (GYM) agars. The temperature range for growth is 37–60°C and the optimum temperature is 50°C. Strains are sensitive to lysozyme (200 U/ml) and tolerant to 7% (w/v) NaCl. No melanin is produced. Tyrosine, starch, triglycerides, blood cells (hemolysis), casein, collagen, and esculin are degraded. Arabinose, dextrin, D-glucose, and mannitol are used as sole carbon sources. Antibiotic activity is displayed against Gram-stain-positive bacteria.

All strains of *Saccharomonospora glauca* are sensitive to phage ϕ771 and, in addition, several strains are sensitive to phage ϕL1g. All strains are resistant to the Tm_1 family of phages, which is specific for *Saccharomonospora viridis*. All strains show identical total protein and DNA restriction patterns; esterase pattern III (four main bands) is found. The R_f value of malate dehydrogenase is 0.50.

Source: moldy hay, soil, compost, and manure.

DNA G+C content (mol%): not determined.

Type strain: K62, DSM 43769, JCM 7444, NBRC 14831.

Sequence accession no. (16S rRNA gene): Z38003.

5. **Saccharomonospora halophila** Al-Zarban, Al-Musallam, Abbas, Stackebrandt and Kroppenstedt 2002, 557[VP]

ha.lo.phi′la. L. n. *hals, halos* salt; N.L. adj. *philus -a -um* (from Gr. adj. *philos -ê -on*) friend, loving; N.L. fem. adj. *halophila* salt-loving, referring to the ability to grow at high NaCl concentration.

Light blue aerial mycelium is produced. Specific endo- or exo-pigments are not observed. Optimal growth is obtained on starch-nitrate agar supplemented with 10% (w/v) NaCl at 28°C. Grows in the presence of 10–30% NaCl. Feathers can be utilized as sole C and N source in the presence of 10% (w/v) NaCl. L-Arabinose, D-galactose, D-glucose, mannitol, mannose, melibiose, and L-rhamnose are utilized as sole carbon sources, but not D-xylose. The utilization of D-ribose is doubtful. Major cellular fatty acids are $C_{16:0}$ iso (22.5%), $C_{16:0}$ (15.8%), and $C_{16:1}$ (14.1%).

Source: salt marsh soil.

DNA G+C content (mol%): not determined.

Type strain: strain 8, DSM 44411, JCM 11761, NRRL B-24125.

Sequence accession no. (16S rRNA gene): AJ278497.

6. **Saccharomonospora marina** Liu, Li, Zheng, Huang and Li 2010, 1856[VP]

ma.ri′na. L. fem. adj. *marina* of the sea.

Nonmotile smooth or wrinkled spores are produced on the branched aerial mycelium singly, in pairs and occasionally in short chains. Optimal growth occurs at 28–37°C and at pH 7.0 on ISP medium 2. Growth occurs in the presence of up to 5% NaCl (w/v), with an optimum concentration of 0–3% (w/v). D-Arabinose, cellobiose, D-galactose, D-glucose, *myo*-inositol, maltose, D-mannitol, D-mannose, melibiose, L-rhamnose, sucrose and D-xylose are utilized as carbon sources, but not lactose, sorbitol, L-sorbose, raffinose and ribose. L-Alanine, L-arginine, L-cystine, L-glutamate, glycine, L-leucine, L-lysine, L-methionine, L-phenylalanine, L-proline, L-serine, L-threonine, L-tyrosine and L-valine are used as nitrogen sources, but not DL-asparagine, L-histidine, L-hydroxyproline and DL-tryptophan. Gelatin liquefaction, milk coagulation and nitrate reduction are positive, but cellulose and starch hydrolysis, hydrogen sulfide and melanin production are negative. Catalase, urease and oxidase activities are negative. Resistant to ampicillin (10 mg), carbenicillin (10 mg), cefuroxime (30 mg), ceftriaxone (30 mg), cephalothin (V) (30 mg), cephalothin (VI) (30 mg), chloramphenicol (30 mg), furoxone (30 mg), penicillin (10 mg), piperacillin (10 mg) and oxacillin (1 mg), but not to cephalothin (IV) (300 mg) and fortum (30 mg).

Major cellular fatty acids are $C_{16:0}$ iso (26.3%), $C_{17:1}$ ω6*c* (16.8%), $C_{15:0}$ (15.2%), $C_{16:0}$ (8.9%), $C_{17:1}$ ω8*c* (7.7%), and $C_{16:1}$ iso H (6.0%).

Habitat: ocean sediment.

DNA G+C content (mol%): 68.1 (HPLC).

Type strain: XMU15, KCTC 19701, CCTCC AA 209048.

Sequence accession no. (16S rRNA gene): FJ812357.

7. **Saccharomonospora paurometabolica** Li, Tang, Stackebrandt, Kroppenstedt, Schumann, Xu and Jiang 2003b, 1593[VP]

pau.ro.me.ta.bo′li.ca. Gr. adj. *pauros* little; Gr. adj. *metabolikos* changeable; N.L. fem. adj. *paurometabolica* little changeable, referring to the poor utilization of carbon sources.

Aerial mycelium is well developed on yeast extract-malt extract agar, glycerol-asparagine agar, nutrient agar, and Czapek's agar; moderate on oatmeal agar and poor on inorganic salts-starch agar and potato agar. White aerial mycelium is produced on all media except on nutrient agar where it is green-yellow. Sporulation is good on ISP 2, ISP 5, nutrient agar, and Czapek's agar, and moderate on ISP 3 agar, but poor on ISP 4 agar. Substrate mycelium is well developed on most media tested. The color of the substrate mycelium is deep orange-yellow (ISP 2), light yellow-brown (nutrient agar), light yellow-orange (potato agar), or white (ISP 4, ISP 5, and Czapek's agar). Nonmotile, single spores with smooth or wrinkled surface are borne on either the aerial or substrate mycelium. Optimum growth temperature is 35–37°C. Optimum NaCl concentration for growth is 10% (w/v). Nitrate is reduced. Milk peptonization and coagulation, gelatin liquefaction, growth in cellulose, H_2S and melanin production, starch hydrolysis, and urease production are not observed. Major cellular fatty acids are $C_{18:1}$ (44.3%), $C_{16:0}$ (20.7%), and $C_{16:0}$ iso (11.2%).

Source: saline soil.

DNA G+C content (mol%): 71 (T_m).

Type strain: BCRC (formerly CCRC) 16315, CCTCC AA 001018, DSM 44619, JCM 13241, YIM 90007.

Sequence accession no. (16S rRNA gene): AF540959.

8. **Saccharomonospora saliphila** Syed, Tang, Cai, Zhi, Agasar, Lee, Kim, Jiang, Xu and Li 2008, 572[VP]

sa.li′phi.la. L. n. *sal*, *salis* salt; N.L. adj. *philus -a -um* (from Gr. adj. *philos -ê -on*) friend, loving; N.L. fem. adj. *saliphila* salt-loving.

Aerial mycelium is well developed on yeast extract-malt extract agar (ISP 2), inorganic salts-starch agar (ISP 4), glycerol-asparagine agar (ISP 5), potato agar, and Czapek's agar; no growth is observed on oatmeal agar (ISP 3) or nutrient agar. Grayish to reddish-gray aerial mycelium is produced on the above media. Good sporulation is observed on ISP 2, ISP 4, ISP 5, potato agar, and Czapek's agar. Substrate mycelium is well developed on most media tested. The color of substrate mycelium is grayish red on Czapek's agar, dark red on ISP 2 and ISP 5, and blackish red on ISP 4 and potato agar. Nonmotile, single or pairs of spores with smooth or wrinkled surfaces are borne on aerial mycelium. Optimum growth temperature is 28°C; grows well at temperatures up to 40°C. Optimum growth is observed in 10% (w/v) NaCl, although it is not essential for growth. H_2S is produced. Milk peptonization and coagulation, gelatin liquefaction, growth in cellulose, melanin production, starch hydrolysis, and urease production are not observed. Cellobiose, fructose, D-galactose, D-glucose, maltose, raffinose, sorbitol, sucrose, and D-xylose are utilized as sole carbon sources, but not L-arabinose, *myo*-inositol, lactose, mannitol, rhamnose, trehalose, or xylitol. Major cellular fatty acids are $C_{16:0}$ iso (49.2%), $C_{17:1}$ ω6*c* (9.1%), $C_{15:0}$ (5.4%), and $C_{16:1}$ iso OH (5.0%).

Source: muddy soil.

DNA G+C content (mol%): 71.8 (T_m).

Type strain: DSM 45087, KCTC 19234, YIM 90502.

Sequence accession no. (16S rRNA gene): DQ367416.

9. **Saccharomonospora xinjiangensis** Jin, Xu, Mao, Hseu and Jiang 1998, 1097[VP]

xin.ji.ang.en′sis. N.L. fem. adj. *xinjiangensis* pertaining to Xinjiang, a province of north-west China.

Yellow-white aerial mycelium is formed on ISP 2, ISP 3, and nutrient agar (light green-gray on Czapek's agar), and the vegetative mycelium is light yellowish. The sporulation of both aerial and vegetative mycelia is good on most media tested. Spores are borne in longitudinal pairs on vegetative hyphae, and in longitudinal pairs (or in a few cases singly) on aerial hyphae. Light yellow-brown diffusible pigment is produced on potato extract-glucose agar, but melanin pigment is not produced on tyrosine agar. Adonitol, cellobiose, fructose, inositol, inulin, mannitol, raffinose, rhamnose, and xylose are utilized, but no acid is produced from these carbon sources. Alanine, histidine, and proline are utilized. Starch, cellulose, and lecithin are degraded. Hydrogen sulfide is produced. Autolysis of aerial hyphae is observed on yeast extract-malt extract agar and nutrient agar. Growth occurs between 45 and 50°C. Unlike other species of the genus, the phospholipid pattern is type IV, containing phosphatidylcholine and glucosamine-containing phospholipids in addition to phosphatidylethanolamine.

Source: soil.

DNA G+C content (mol%): not determined.

Type strain: XJ-54, CCTCC AA 97021, DSM 44391, JCM 11270.

Sequence accession no. (16S rRNA gene): AJ306300.

Genus XIV. **Saccharopolyspora** Lacey and Goodfellow 1975, 76[AL] emend. Korn-Wendisch, Kempf, Grund, Kroppenstedt and Kutzner 1989, 438

Seung Bum Kim and Michael Goodfellow

Sac.cha.ro.po.ly.spo′ra. N.L. n. *Saccharum* generic name of sugar cane; Gr. adj. *polus* many; Gr. n. *spora* a seed, and in biology a spore; N.L. fem. n. *Saccharopolyspora* the many spored (organism) from sugar cane.

Aerobic, Gram-stain-positive, non-acid-fast, nonmotile, catalase-positive actinobacteria which form an extensively branched substrate mycelium **that typically fragments into coccoid and/or rod-shaped elements**. In some species, the substrate hyphae remain intact or are partially transformed into chains of spores. **Aerial hyphae, when present, generally differentiate into bead-like chains of spores contained within a smooth sheath.** Spores are borne in straight, flexuous, hooked, looped, or spiral chains.

Spore surfaces can be hairy, smooth, spiny, rough, or warty. Substrate mycelia may be buff, brownish red, orange, or yellow and aerial mycelia are white to gray or pinkish white. Diverse compounds are used as sole carbon sources for energy and growth. **Whole-organism hydrolysates contain *meso*-diaminopimelic acid, arabinose, and galactose. Muramic acid moieties are *N*-acetylated. Cells contain tetrahydrogenated menaquinones with nine isoprene units as the predominant menaquinone, phosphatidylcholine, phosphatidylethanolamine, and phosphatidylmethylethanolamine as major polar lipids, and fatty acid profiles rich in iso- and anteiso-branched chain components, but lack mycolic acids.** The phylogenetic position of *Saccharopolyspora*, as determined by 16S rRNA gene sequencing, is in the family *Pseudonocardiaceae*.

DNA G+C content (mol%): 66–77.

Type species: **Saccharopolyspora hirsuta** Lacey and Goodfellow 1975, 78[AL].

Further descriptive information

Phylogeny. The genus *Saccharopolyspora* forms a distinct line of descent in the 16S rRNA *Pseudonocadiaceae* tree (Labeda et al., 2010a; Lu et al., 2001). The relationships between *Saccharopolyspora* species with validly published names are shown in Figure 316. The two most closely related taxa, *Saccharopolyspora hirsuta* subsp. *kobensis* and *Saccharopolyspora jiangxiensis*, share a 16S rRNA gene sequence similarity of 99% and the two most distantly related species, *Saccharopolyspora erythraea* and *Saccharopolyspora thermoflava*, share a similarity of 92%.

Cell morphology. Most saccharopolysporae, like the well-studied type strains of *Saccharopolyspora hirsuta* and *Saccharopolyspora rectivirgula* (formerly *Faenia rectivirgula*), form an extensively branched substrate mycelium, carrying aerial hyphae (Lacey, 1989a, 1989c; Locci, 1976). In *Saccharopolyspora hirsuta*, some substrate hyphae, like those of *Nocardia* strains, fragment into chains of cells in angular opposition (Figure 317), whereas others remain stable (Figure 318). Fragmented hyphae are most abundant in older parts of cultures, although they usually occur together with sterile hyphae. Substrate hyphae remain intact in some species such as *Saccharopolyspora halophila* (Tang et al., 2009a) and *Saccharopolyspora tripterygii* (Li et al., 2009a). In *Saccharopolyspora rectivirgula*, branching of the substrate hyphae is almost at right angles with chains of spores, mostly on short unbranched lateral and terminal sporophores (Figure 319). The developmental micromorphology of the organism has been studied by Locci (1976).

Aerial hyphae which differentiate into chains of spores are typical of *Saccharopolyspora* strains, as illustrated in Figure 320 and Figure 321. The spores of some species, such as *Saccharopolyspora hirsuta*, *Saccharopolyspora hordei*, and *Saccharopolyspor aspinosa*, are separated by lengths of apparently empty hyphae giving a characteristic bead-like appearance (Goodfellow et al., 1989b; Lacey and Goodfellow, 1975; Mertz and Yao, 1990). The spore chains of *Saccharopolyspora hirsuta* may be straight but are usually in loops or spirals (Korn-Wendisch et al., 1989; Lacey and Goodfellow, 1975). In contrast, straight to flexuous chains of 6–10 spores are seen in *Saccharopolyspora jiangxiensis*

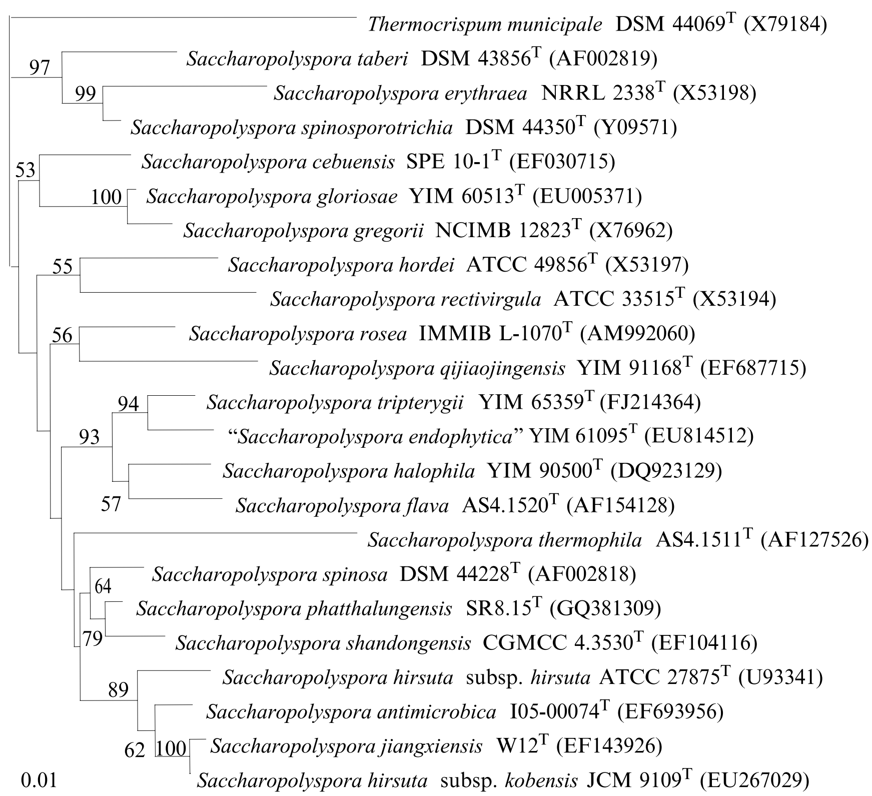

FIGURE 316. Neighbor-joining tree (Saitou and Nei, 1987) based on nearly complete 16S rRNA gene sequences showing relationships between *Saccharopolyspora* species. Numbers at the nodes indicate levels of bootstrap support (Felsenstein, 1985) based on an analysis of 1000 resampled datasets. Only values over 50% are given. Bar = 0.01 substitutions per nucleotide position.

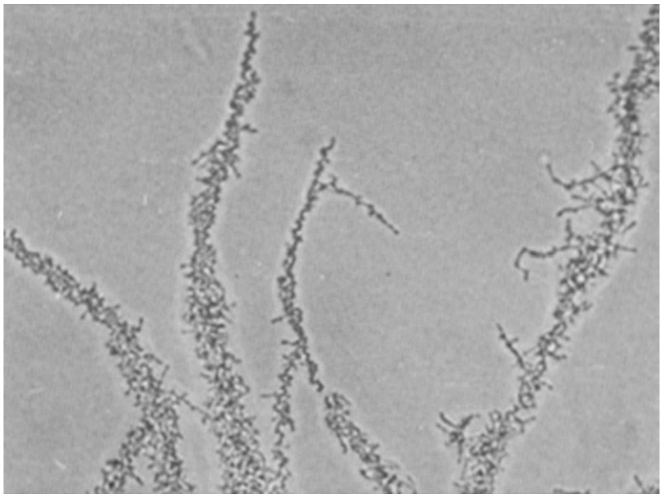

FIGURE 317. Fragmentation of substrate mycelium of *Saccharopolyspora hirsuta*. Glycerol-asparagine agar, incubation 40°C (500×). (Reproduced with permission from Lacey and Goodfellow, 1975. J. Gen. Microbiol. *88*: 75–85.)

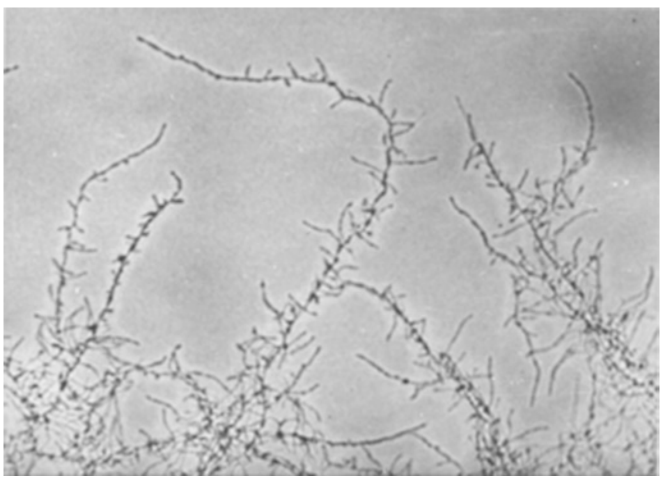

FIGURE 318. Morphology of substrate mycelium of *Saccharopolyspora hirsuta*. Glycerol-asparagine agar, incubation 40°C (550×). (Reproduced with permission from Lacey and Goodfellow, 1975. J. Gen. Microbiol. *88*: 75–85.)

(Zhang et al., 2009), hooked or flexuous chains of 4–6 spores in *Saccharopolyspora thermoflava* (Lu et al., 2001), spiral chains in *Saccharopolyspora spinosporotrichia* (Zhou et al., 1998), and short, straight chains in *Saccharopolyspora rectivirgula* (Figure 322).

Most *Saccharopolyspora* species have spores with smooth surfaces (Table 255). However, ornamented spores are not uncommon, as exemplified by the presence of hairy spores in *Saccharopolyspora hirsuta* (Lacey and Goodfellow, 1975), spiny spores in *Saccharopolyspora shandongensis* (Zhang et al., 2008b), warty spores in *Saccharopolyspora spinosporotrichia* (Zhou et al., 1998), and rough spores in *Saccharopolyspora rectivirgula*, as shown in Figure 322. Spores may be spherical as in *Saccharopolyspora spinosporotrichia* (Zhou et al., 1998), round to oval as in *Saccharopolyspora hirsuta* (Lacey and Goodfellow, 1975), or vesicular as shown by *Saccharopolyspora thermophila* (Lu et al., 2001). The spores of some species are covered by a sheath, as in *Saccharopolyspora gregorii*, *Saccharopolyspora hirsuta*, *Saccharopolyspora hordei*, and *Saccharopolyspora spinosa* (Goodfellow et al., 1989b; Lacey and Goodfellow, 1975; Mertz and Yao, 1990).

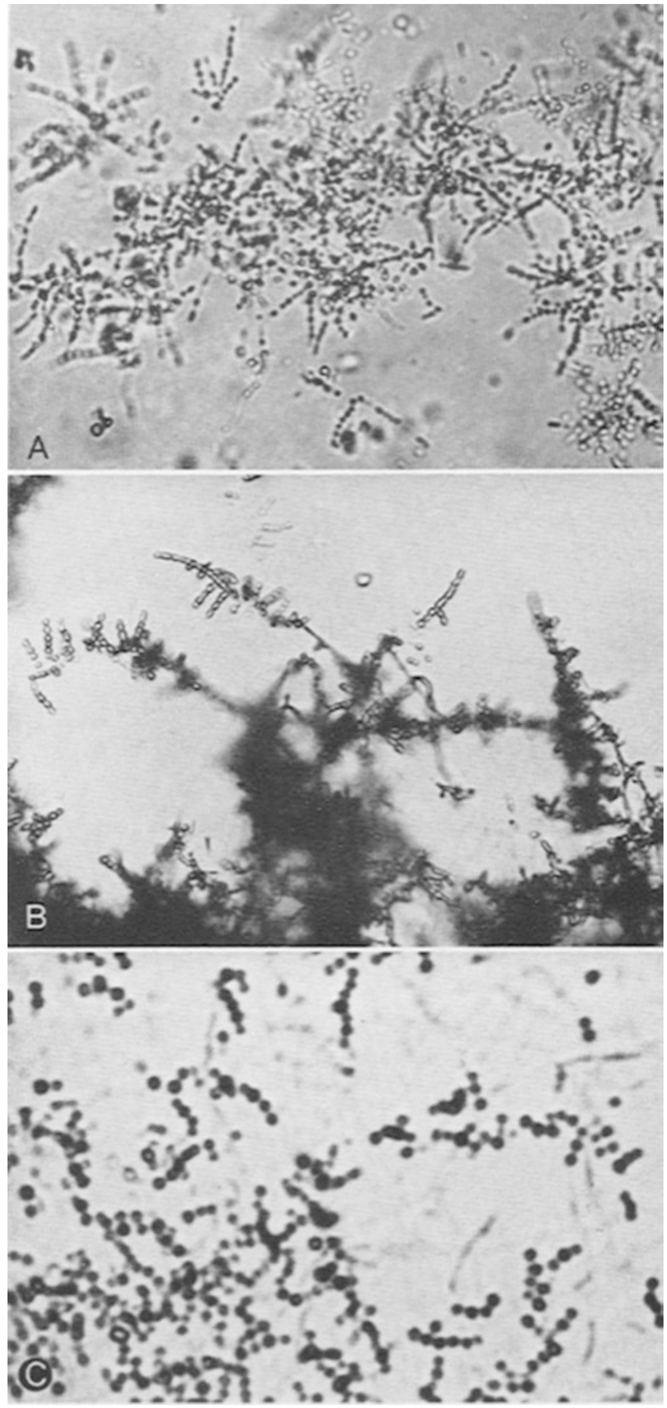

FIGURE 319. Morphology of substrate mycelium of *Saccharopolyspora rectivirgula*. (A) Appearance near growing margin (×650). (B) Typical right angle branching (650×). (C) Spore chains in older part of colony (1300×). Half-strength nutrient agar, 55°C.

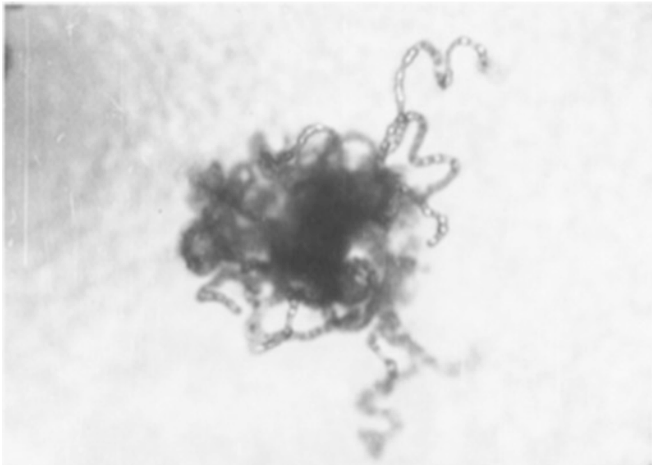

FIGURE 320. Spore chains on aerial mycelium of *Saccharopolyspora hirsuta* showing tufted appearance and typical curved chains. Half-strength nutrient agar, incubation 40°C (800×). (Reproduced with permission from Lacey and Goodfellow, 1975. J. Gen. Microbiol. *88*: 75–85.)

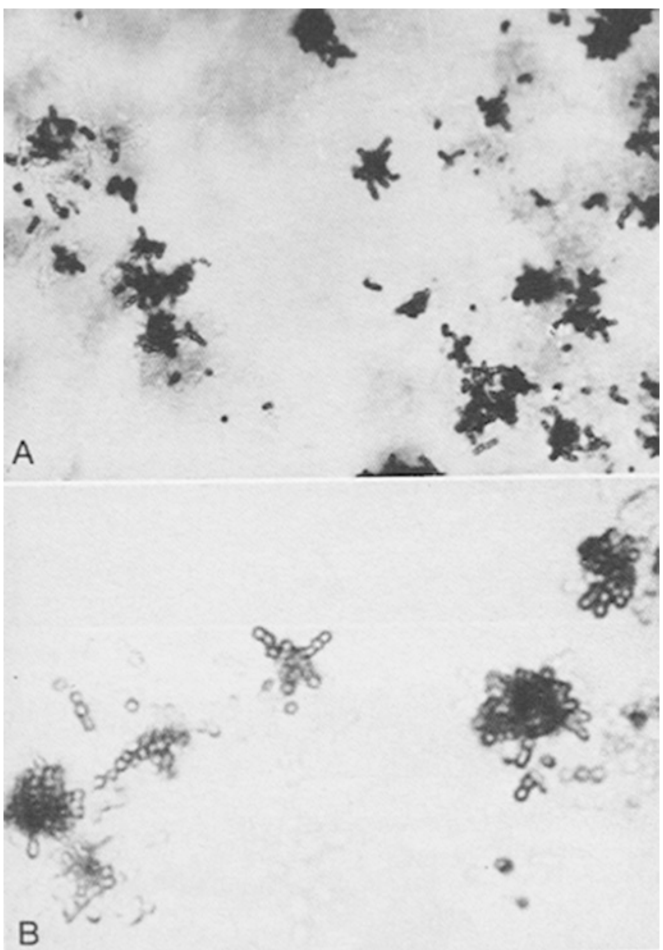

FIGURE 321. Aerial mycelium of *Saccharopolyspora rectivirgula* showing (A) sparse, tufted appearance (×300) and (B) formation of spore chains (780×). Half-strength nutrient agar, 55°C.

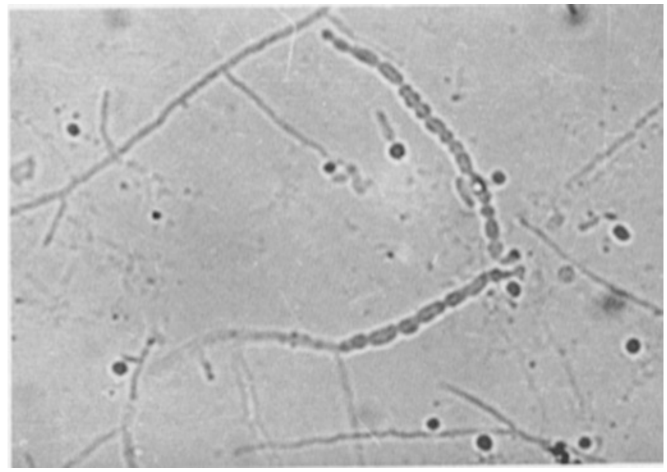

FIGURE 322. Scanning electron micrographs of (A) sporulating hyphae (3000×) and (B) spores (13,000×) of *Saccharopolyspora rectivirgula.*

Fine structure. In general, studies on *Saccharopolyspora hirsuta* and *Saccharopolyspora rectivirgula* show that their cell-wall structure resembles that of other actinobacteria (Lacey, 1989c; Lacey and Goodfellow, 1975). In thin section, *Saccharopolyspora hirsuta* hyphae (Figure 323) are bound by a wall 22–30 nm thick. Within this, a typical unit membrane encloses granular cytoplasm with axial diffuse nuclear material. Electron-transparent vacuoles, up to 0.3 µm in diameter and resembling lipid accumulations in other filamentous actinobacteria which undergo fragmentation (Williams et al., 1976), are sometimes abundant. Also occasionally present are electron-dense granules, up to 0.1 µm in diameter, resembling metachromatic or polyphosphate granules. Septation occurs by double ingrowth of the wall leading to fragmentation (type II; Williams et al., 1973). This may be associated with lamellar mesosomes up to 0.25 µm in diameter.

The sheath surrounding the spores of *Saccharopolyspora hirsuta* is 18–36 nm thick. It carries tufts of structureless hairs, triangular and 0.2–0.3 µm across at the base, which extend into apical filaments about 20 nm in diameter. The hairs are long, straight, or curved, and brittle (Figure 324). The morphology of the hairs is best seen on lengths of empty sheath (Figure 325) or by scanning electron microscopy (Figure 326). Spore walls are thickened uniformly to 50–60 nm, but their internal structure resembles that of hyphae, though they contain few vacuoles (Figure 327).

Two types of hyphae have been distinguished in thin sections of *Saccharopolyspora rectivirgula*, one having walls 19–25 nm thick and the other with walls 11–15 nm thick (Dorokhova et al., 1970). The cytoplasm in the thicker-walled cells is uniformly fine grained with a large nuclear zone extending the full-length of the cell. In thinner walled cells, the cytoplasm is less compact and homogeneous and the nuclear zone appears as small areas of low density. Mesosomes are less developed than in the thicker wall cells. Hyphae tend to autolyze during prolonged incubation at 55°C or at room temperature.

The spore chains of *Saccharopolyspora rectivirgula* are surrounded by a multilayered sheath, although this is less evident on spore chains formed on the substrate mycelium than on those produced on the aerial mycelium (Dorokhova et al., 1969; Williams et al., 1976). The spores are covered by a wall

TABLE 255. Differential properties of the type strains of species belonging to the genus *Saccharopolyspora*[a,b]

Property	1. *S. hirsuta*	2. *S. antimicrobia*	3. *S. cebuensis*	4. "*S. endophytica*"	5. *S. erythraea*	6. *S. flava*	7. *S. gloriosae*	8. *S. gregorii*	9. *S. halophila*	10. *S. hordei*	11. *S. jiangxiensis*	12. *S. phatthalungensis*	13. *S. qijiaojingensis*	14. *S. rectivirgula*	15. *S. rosea*	16. *S. shandongensis*	17. *S. spinosa*	18. *S. spinosporotrichia*	19. *S. taberi*	20. *S. thermo phila*	21. *S. tripterygii*
Spore chains	Straight to loose spirals	Straight	Straight	Straight to loose spirals	Open spirals	Straight	Hooks/curved	Hooks/flexuous	Straight	Hooks/spirals	Straight to flexuous	Hooks/open loops	Straight	Straight	Straight	Spiral	Hooks/open loops	Spiral	−	Hooked/flexuous	Straight
Spore surfaces	Hairy	Rough	Smooth	Smooth	Spiny	Smooth	Smooth	Smooth	Smooth	Smooth	Smooth or irregularly rough	Spiny	Smooth	Smooth or irregularly rough	Smooth	Spiny	Spiny	Warty	−	Smooth	Smooth
Degradation of:																					
Adenine	+	+	−	+	+	+	+	−	−	+	+	−	−	−	+	+	−	−	+	+	nd
Casein	+	+	−	−	−	−	−	+	+	+	−	+	−	−	+	+	−	+	+	−	nd
Chitin	−	−	−	+	+	−	−	−	−	+	−	−	−	−	nd	−	−	−	+	−	nd
Esculin	+	+	+	−	+	+	+	+	+	+	+	−	−	+	+	+	+	+	+	+	+
Elastin	+	−	nd	nd	+	−	nd	+	nd	+	+		nd	−	−	+	−	+	+	−	nd
Hypoxanthine	+	+	−	+	+	+	+	+	+	−	−	+	+	+	+	+	+	nd	+	+	nd
Starch	+	+	+	+	+	+	+	+	−	+	+	−	−	+	nd	+	−	+	+	+	−
Tyrosine	+	+	+	+	+	−	+	+	+	+	+	+	−	+	+	+	+	−	+	+	nd
Urea	+	+	nd	−	nd	+	nd	−	+	−	+	+	−	+	+	+	+	+			+
Xanthine	+	−	nd	+	+	+	+	+	nd	+	+	−	+	+	−	+	−	−	+	−	nd
Nitrate reduction	−	+	−	+	+	+	−	−	+	−	+	−	−	+	−	+	+	−	+	−	−
NaCl tolerance (w/v)	<7	≤7	2.5–12.5	≤15	<5	7	≤11	13	3–20	<13	<11	<7	6–22	<10	nd	<7	<11	2–3	7	7	≤12
Temperature range (°C)	25–50	20–45	15–37	20–45	20–42	28–37	10–32	10–35	10–45	20–60	15–45	18–42	20–40	37–63	22–42	15–38	15–37	28–37	20–45	45–55	10–37
Growth on carbon sources:																					
L-Arabinose	−	+	+	+	+	−	+	+	+	+	+	−[c]	−	−	+	+	+	−	−	−	+
D-Galactose	+	+	+	+	+	+	−	+	+	+	+	+[c]	+	+	+	+	−	+	+	+	+
D-Lactose	+	+	+	+	−	+	−	−	+	+	+	nd[c]	+	+	−	−	−	−	+	+	+
Maltose	+	+	+	+	+	+	+	+	+	+	+	−[c]	+	+	+	+	−	+	+	+	+
Mannitol	+	+	−	+	+	+	+	+	+	+	+	+[c]	+	+	+	+	+	+	+	+	+
Raffinose	+	+	+	+	+	+	−	+	+	+	+	−[c]	nd	+	−	+	−	+	+	+	+
L-Rhamnose	+	+	+	+	+	+	+	+	+	+	+	−[c]	+	+	−	+	−	+	+	+	−
Sucrose	+	+	+	+	+	+	−	+	+	+	+	−[c]	−	+	+	+	−	+	+	+	−
D-Xylose	+	+	+	+	+	+	+	+	+	+	+	+[c]	−	+	+	+	−	+	+	−	+

[a]Data for type strains. All strains were positive for the utilization of fructose, glucose, and mannose as the sole carbon sources for energy and growth.

[b]+, Positive; −, negative; nd, not determined.

[c]Determined by acid production from substrate.

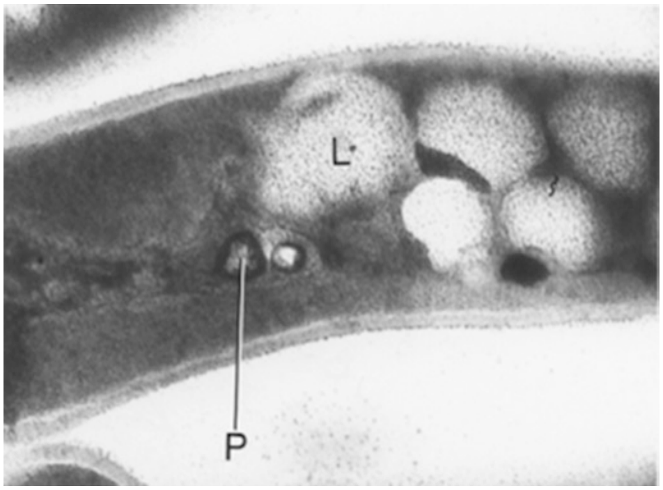

FIGURE 323. Longitudinal section of hyphae of *Saccharopolyspora hirsuta* showing possible lipid accumulation (L) and polyphosphate granules (P) (70,000×). (Reproduced with permission from Lacey and Goodfellow, 1975. J. Gen. Microbiol. *88*: 75–85.)

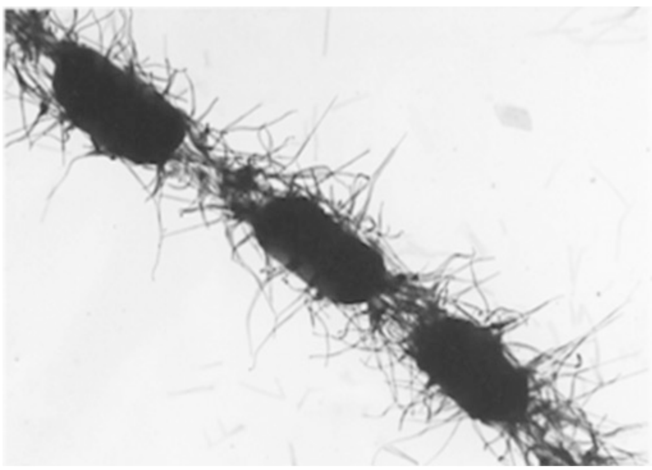

FIGURE 324. Electron micrograph of spore chain of *Saccharopolyspora hirsuta* (18,400×). (Reproduced with permission from Lacey and Goodfellow, 1975. J. Gen. Microbiol. *88*: 75–85.)

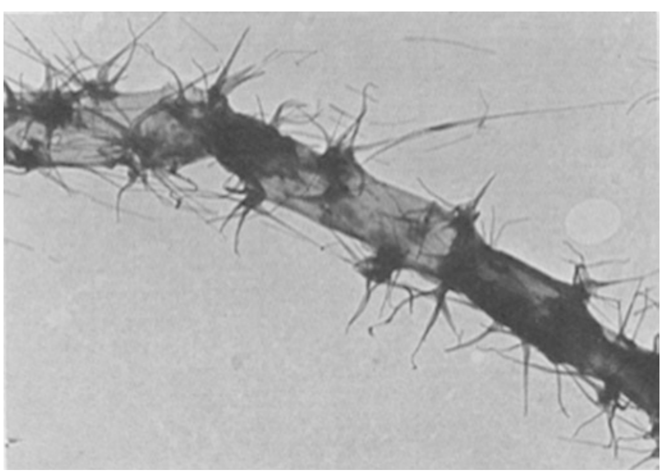

FIGURE 325. Electron micrograph of spore sheath of *Saccharopolyspora hirsuta* showing tufted production of hairs (17,600×). (Reproduced with permission from Lacey and Goodfellow, 1975. J. Gen. Microbiol. *88*: 75–85.)

FIGURE 326. Scanning electron micrograph of spores of *Saccharopolyspora hirsuta* (15,000×). (Reproduced with permission from Lacey and Goodfellow, 1975. J. Gen. Microbiol. *88*: 75–85.)

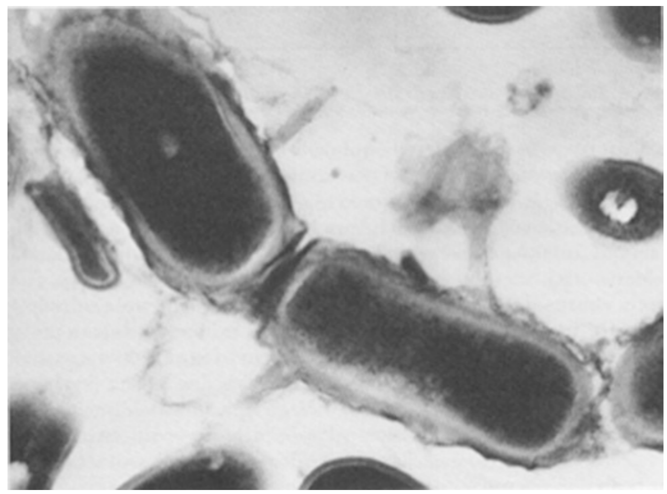

FIGURE 327. Longitudinal section of mature spore chain of *Saccharopolyspora hirsuta* showing sheath and hair bases (34,000×). (Reproduced with permission from Lacey and Goodfellow, 1975. J. Gen. Microbiol. *88*: 75–85.)

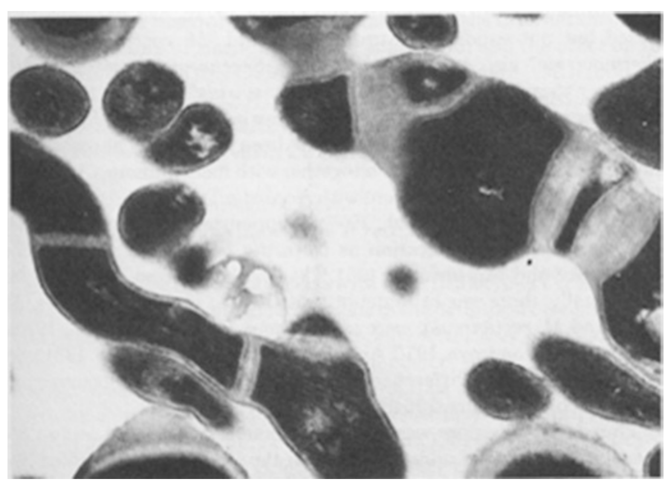

FIGURE 328. Sections of mycelium of *Saccharopolyspora rectivirgula* showing double septa in normal hyphae and irregular thickened septa in enlarged hyphae or aberrant spore chains (25,000×).

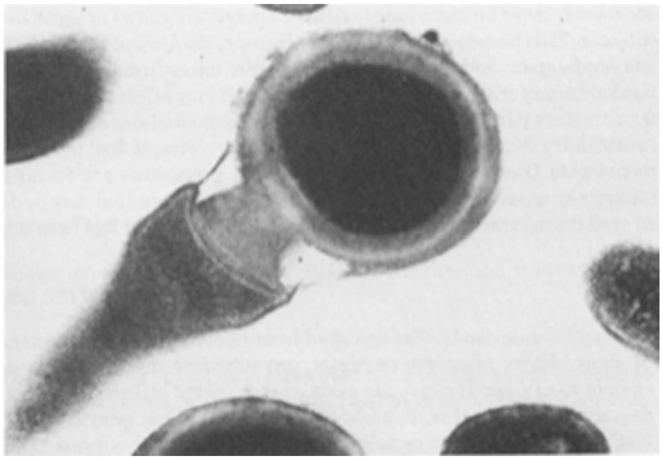

FIGURE 329. Longitudinal section through a developing spore of *Saccharopolyspora hirsuta* (40,000×).

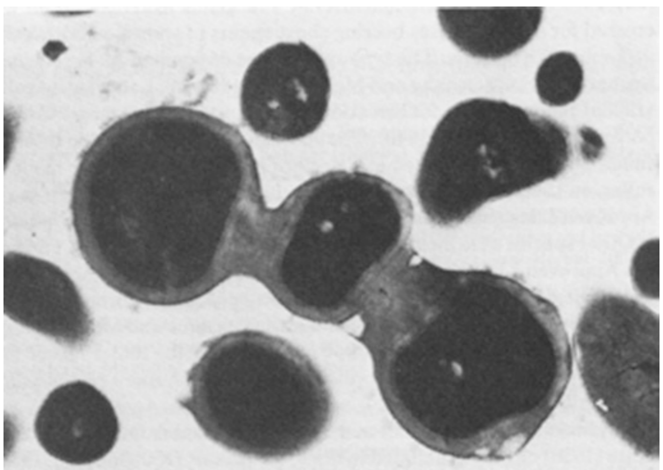

FIGURE 330. Longitudinal section through a developing spore of *Saccharopolyspora rectivirgula* showing interspore pads (25,000×).

70–100 nm thick in which two layers may be distinguished (Figure 328 and Figure 329), differing in thickness and electron density. Additional thickening of the cross-walls usually occurs giving characteristic interspore pads (Dorokhova et al., 1969) (Figure 330). These may sometimes be seen by light microscopy of stained preparations as conspicuous non-staining areas (Cross et al., 1968), but they may break down as the spore matures (Dorokhova et al., 1969). Plasmodesmata have been observed within the interspore pads. The protoplast is separated from the wall by a membrane and contains small, dark, densely packed ribosomes. Mesosomes are well developed and often adjoin the nuclear material. Although the spores are characteristically round or oval, spores of irregular shape are often seen in sections.

Colony morphology. Saccharopolysporae grow well on most standard media used to cultivate filamentous actinomycetes, such as Czapek's, glucose-asparagine (ISP medium 5), inorganic salts-starch (ISP medium 4), Sauton's, and V-8 vegetable juice agars. Colonies vary in size, in the extent of aerial hyphae production, and in aerial and substrate mycelia pigments. Those of *Saccharopolyspora hirsuta*, for instance, are thin,

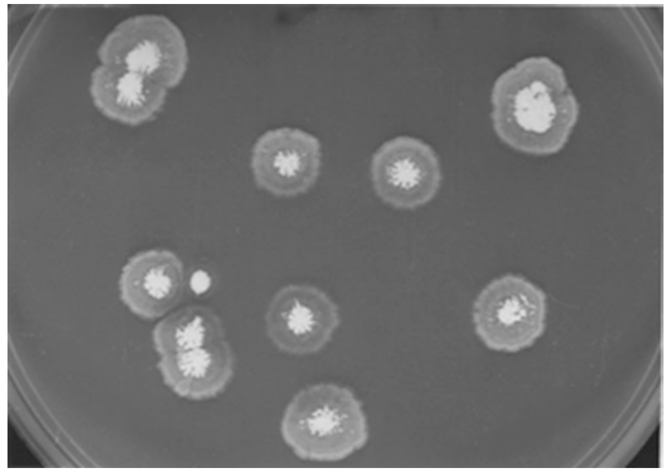

FIGURE 331. Colonies of *Saccharopolyspora hirsuta*. Half-strength nutrient agar, incubation 37°C (×1). (Reproduced with permission from Lacey and Goodfellow, 1975. J. Gen. Microbiol. *88*: 75–85.)

round or convex, wrinkled, and grow to about 1 cm in diameter in 7 d at 40°C, with a central area of white aerial mycelium on an almost colorless substrate mycelium (Lacey, 1975), as illustrated in Figure 331. In contrast, colonies of *Saccharopolyspora rectivirgula* grow to 5 mm in diameter in 7 d at 40–50°C; the substrate mycelium may be colorless, brown-yellow, or orange-yellow and the aerial mycelium may be white to light pink, though it is often sparse or absent (Korn-Wendisch et al., 1989; Lacey, 1989c). The aerial mycelium of *Saccharopolyspora gregorii* is also at best sparse (Goodfellow et al., 1989b), whereas macroscopically visible aerial hyphae have not been observed in *Saccharopolyspora taberi* (Labeda, 1987). *Saccharopolyspora flava* only produces aerial hyphae after prolonged incubation on oatmeal agar (Lu et al., 2001). Additional details on other species can be found in the species descriptions.

Chemotaxonomy. *Saccharopolyspora* species have a wall peptidoglycan which is characterized by the presence of *meso*-diaminopimelic acid, arabinose, and galactose (wall chemotype IV *sensu* Lechevalier and Lechevalier, 1970), an A1γ peptidoglycan (Schleifer and Kandler, 1972), and *N*-acetylated muramic acid (Duangmal et al., 2010). They have fatty acid profiles rich in iso- and anteiso-branched components in which $C_{15:0}$ iso, $C_{16:0}$ iso, and $C_{17:0}$ anteiso tend to predominate (Embley et al., 1987; Goodfellow et al., 1989b; Tang et al., 2009a, 2009b) and lack mycolic acids (Mertz and Yao, 1990; Minnikin et al., 1975; Pimentel-Elardo et al., 2008; Zhou et al., 1998), but typically contain major amounts of phosphatidylcholine, phosphatidylethanolamine, and phosphatidylmethylethanolamine (Embley et al., 1988b; Korn-Wendisch et al., 1989; Pimentel-Elardo et al., 2008) with a variable distribution of diphosphatidylglycerol, phosphatidylglycerol, phosphatidylinositol, and phosphatidylinositol mannosides (Li et al., 2009a; Yassin, 2009; Yuan et al., 2008) and, hence, have polar lipid pattern type III after Lechevalier et al. (1981, 1977b). A major glycolipid has been detected in *Saccharopolyspora erythraea*, *Saccharopolyspora hirsuta*, and *Saccharopolyspora rectivirgula* (Gamian et al., 1996). The predominant menaquinones are tetrahydrogenated with nine isoprene units [MK-9(H_4)] (Embley et al., 1988b; Korn-Wendisch et al., 1989; Tang et al., 2009a, 2009b), though smaller amounts

of MK-9(H_2, H_6, H_8) may be present (Alderson et al., 1985; Collins et al., 1977; Lu et al., 2001; Qin et al., 2008a). The type strain of *Saccharopolyspora thermophila* contains MK-9(H_6, H_8) as the predominant isoprenologs (Lu et al., 2001), though this apparently anomalous result may reflect the time when the culture was sampled as there is evidence that the menaquinone composition of *Amycolatopsis* and *Streptomyces* strains can be age-dependent (Saddler et al., 1986; Yassin et al., 1991). There is preliminary evidence that the esterase patterns of *Saccharopolyspora* strains may be species-specific (Korn-Wendisch et al., 1989).

Nutrition and growth conditions. In general, saccharopolysporae do not have any specific growth requirements and can grow on a diverse range of compounds as sole sources of carbon and nitrogen (Lacey and Goodfellow, 1975; Li et al., 2009a; Zhang et al., 2009). They grow well in media supplemented with salt, notably *Saccharopolyspora halophila* and *Saccharopolyspora qijiaojingensis*, which grow in the presence of 20% (w/v) NaCl (Tang et al., 2009a, 2009b). The type strain of *Saccharopolyspora cebuensis* has a strict requirement for salt which suggests that it is an obligate marine actinomycete (Pimentel-Elardo et al., 2008). Saccharopolysporae show a range of temperature requirements; some, such as *Saccharopolyspora hirsuta*, *Saccharopolyspora hordei*, *Saccharopolyspora rectivirgula*, and *Saccharopolyspora thermophila*, are moderate thermophiles (Goodfellow et al., 1989b; Lacey and Goodfellow, 1975; Lu et al., 2001). Most strains grow within the pH range 5.0–9.0 (Qin et al., 2008a; Tang et al., 2009b; Zhang et al., 2009).

Metabolism. Saccharopolysporae are aerobic, catalase-positive, chemo-organotrophic actinomycetes which have an oxidative metabolism. They degrade a broad range of organic substrates and use diverse compounds as sole carbon sources (Goodfellow et al., 1989b; Lacey and Goodfellow, 1975; Yuan et al., 2008). Isolates from fodder and other plant material typically degrade hypoxanthine, starch, Tweens, tyrosine, and xanthine, but not xylan (Goodfellow et al., 1989b; Lacey, 1989c).

Members of the genus are best known as a source of secondary metabolites, notably *Saccharopolyspora erythraea*, which is used for the industrial scale production of the clinically important macrolide antibiotic, erythromycin A, and *Saccharopolyspora spinosa*, the source of spinosyns A and D, glycosylated polyketide-derived macrolides which are commercially marketed as the insecticide spinosad (Hong et al., 2006, 2008; Huang et al., 2009). However, despite the commercial importance of these organisms, relatively little is known about their metabolic properties, apart from studies on erythromycin (Chen et al., 2008; Katz and Donadio, 1995; Reeves et al., 2006, 2007; Staunton and Weissman, 2001; Weissman et al., 2004) and spinosyn (Gaisser et al., 2001; Hong et al., 2008; Huang et al., 2009; Kim et al., 2007; Kirst et al., 1991, 1992; Waldron et al., 2001) biosynthesis and on host–vector systems developed for *Saccharopolyspora erythraea* (Gaisser et al., 2000). "*Saccharopolyspora pogona*" (Lewer et al., 2002) synthesizes pogonins (butylene-spinosyns), pericidal macrolides which are similar to the spinosyns produced by *Saccharopolyspora spinosa* (Hahn et al., 2006). Another taxon that does not have a validly published name, "*Saccharopolyspora aurantica*", forms a complex of pesticidal compounds designated CL307-24 (Etienne et al., 1993).

The erythromycins are broad-spectrum antibiotics active against Gram-stain-positive bacteria (Labeda, 1987; Staunton and Weissman, 2001). Erythromycin A, the most widely used and clinically effective member of the family, contains a characteristic 14-membered macrolide to which are attached two unusual deoxysugars, desosamine and mycarose (Chen et al., 2008). The entire gene cluster governing the biosynthesis of erythromycin A has been cloned and each open reading frame has been characterized by targeted gene inactivation. Erythromycins B and C, which are biologically less active and cause greater side effects, are intermediates in erythromycin A biosynthesis.

Spinosyns are unique macrolides with a tetracyclic core, which consists of a 12-membered macrocyclic lactone fused to a 5,6,5-*cis*-anti-*trans* tricyclic ring system, to which the deoxysugars forosamine and tri-*O*-methylated rhamnose are attached (Huang et al., 2009). The biosynthetic pathway of spinosyns have been determined, notably in studies based on precursor-labeling, identification of intermediate metabolites using blocked mutants, and by *in vitro* analysis of enzymes involved in spinosyn biosynthesis (Kirst et al., 1993). To date, more than 25 spinosyns have been isolated and identified from *Saccharopolyspora spinosa* (Crouse et al., 2001). The most abundant spinosyns from fermentation broths of this organism are spinosyn A (approx. 85% of spinosad) and spinosyn D (approx. 15% of spinosad). Spinosad kills susceptible insects by causing rapid excitation of the insect nervous system, probably through the interaction and binding of the δ-aminobutyric acid and nicotinacetylcholine receptor sites (Millar and Denholm, 2007).

Bioactive compounds are also produced by other *Saccharopolyspora* species. *Saccharopolyspora hirsuta* synthesizes a macrolide, nargenicin A (Ikeda et al., 1985), an aminoglycoside complex, apramycin and derivatives (Kamiya et al., 1983; O'Connor et al., 1976), and a cyclic polyketide, nodusmicin (Whaley et al., 1980). *Saccharopolyspora hirsuta* subsp. *kobensis* produces sporaricin and related aminoglycoside antibiotics (Deushi et al., 1979; Umezawa et al., 1987) and *Saccharopolyspora* sp. strain AC 3440 produces 4-deamino-4-hydroxyapromycin (Awata et al., 1983). *Saccharopolyspora erythraea* is a rich source of bioactive compounds other than erythromycin, including erythronolide B (Martin and Rosenbrook, 1967), an *N*-acetylmuramidase (Morita et al., 1978), a trypsin-like protease (Yoshida et al., 1971), and a rennin-like enzyme (Sternberg, 1976).

The genome of *Saccharopolyspora erythraea* NRRL 2338^T has been sequenced (Oliynyk et al., 2007), a development which is promoting interest in the metabolism and biotechnological exploitation of this and related actinobacteria (Katz and Khosla, 2007; Peano et al., 2007). The genome of this organism shows considerable divergence from those of streptomycetes in gene organization and function thereby confirming previous taxonomic insights (Labeda, 1987). Oliynyk and his colleagues reported that the *Saccharopolyspora erythraea* genome contained at least 25 gene clusters for the production of known and predicted secondary metabolites, at least 72 genes predicted to confer resistance to a range of common antibiotic classes and many sets of duplicated genes to support the saprophytic way of life of the organism. The latter included genes involved in defense and stress responses, in ensuring or preserving correct protein folding, and those encoding a wide range of degradative enzymes, including seven chitinases and multiple glucanases and proteinases. The genome sequence of *Saccharopolyspora erythraea* sets the stage for understanding the biology of this organism both in nature and in the fermentation process and for explaining

the higher levels of erythromycin production in industrially significant strains (Katz and Khosla, 2007).

Genetics. Most studies have been directed towards mapping and cloning of the erythromycin biosynthesis and resistance genes of *Saccharopolyspora erythraea* (Baltz et al., 1986; Bibb et al., 1986; Stanzak et al., 1986; Tuan et al., 1986; Vanden Boom, 2000; Weber et al., 1985) and on the cloning and analysis of the *Saccharopolyspora spinosa* spinosad biosynthetic gene cluster (Hong et al., 2008; Matsushima and Baltz, 1994; Matsushima et al., 1994; Waldron et al., 2001). The erythromycin genes are located on the chromosome, together with resistance and regulatory elements (Oliynyk et al., 2007; Stanzak et al., 1986; Weber et al., 1985). Most of the *Saccharopolyspora spinosa* genes involved in spinosyn biosynthesis are present in a 74 kb cluster with characterization studies suggesting that spinosyns are synthesized by mechanisms similar to those used to assemble complex macrolides from primary metabolic precursors as in other actinobacteria (Waldron et al., 2001). These workers noted that several unusual genes in the spinosyn gene cluster might encode enzymes which generate the tetracyclic polyketide aglycone nucleus, the most striking structural feature of spinosyns.

Integrated and conjugative elements (AICEs) have been detected in *Saccharopolyspora erythraea* (Brown et al., 1988, Brown et al., 1994; te Poele et al., 2008). te Poele and her colleagues found two novel putative AICEs in the *Saccharopolyspora erythraea* genome, one of which (PSE102) encoded a putative aminoglycoside phosphotransferase which may confer antibiotic resistance to the host. They also found that the AICEs of *Saccharopolyspora erythraea*, *Amycolatopsis mediterranei*, and *Amycolatopsis methanolica* have a highly conserved structural organization which consists of four functional modules (conjugative transfer, excision/integration, regulation, and replication). Identification and characterization of mobile genetic elements is important for the manipulation of commercially significant *Saccharopolyspora* strains.

The genes responsible for the biosynthesis of erythromycin were cloned into but not well expressed in "*Streptomyces lividans*" (Stanzak et al., 1986; Thompson et al., 1982), a problem reflecting the phylogenetic gulf between *Saccharopolyspora* and *Streptomyces* strains (Embley et al., 1988a) and, thereby, emphasizing the need to develop cloning systems based on indigenous *Saccharopolyspora* vectors (Gayer-Herkert et al., 1989; Katz et al., 1988). There is evidence that *Streptomyces* phages are unstable in *Saccharopolyspora* strains (Gayer-Herkert et al., 1989; Yamamoto et al., 1986). Plasmids have been detected in the type strain of *Saccharopolyspora erythraea* (Brown et al., 1986; Chiang et al., 1985) and methods for the transformation of *Saccharopolyspora erythraea* (Yamamoto et al., 1986) and *Saccharopolyspora rectivirgula* (Gayer-Herkert et al., 1989) have been described.

The *Saccharopolyspora spinosa* spinosad biosynthetic gene cluster has been cloned, analysed, and shown to contain five large open reading frames which encode a multifunctional type 1 polyketide synthase (Waldron et al., 2001). The enzymes involved in D-forosamine [(4-dimethylamino)-2,3,4,6-tetradeoxy-β-D-*threo*-hexopyranose] biosynthesis in the spinosyn pathway have been cloned and expressed heterologically and the corresponding proteins have been purified and their activities examined *in vitro,* developments which have elucidated the mechanisms for the synthesis of this highly deoxygenated sugar (Hong et al., 2008). Indeed, these studies have provided the basis for future work on the biosynthesis of 2,3,6-trideoxy- and 2,3,4,6-tetradeoxyhexoses present in many bioactive natural products.

The sequenced chromosome of the type strain of *Saccharopolyspora erythraea* (8.2 kb) is circular like those of *Amycolatopsis mediterranei*, *Corynebacterium diphtheriae*, and *Mycobacterium tuberculosis*, but unlike the linear chromosomes of "*Streptomyces coelicolor*" A(3)2 and *Streptomyces avermitilis* MA-4680 (Oliynyk et al., 2007; Peano et al., 2007; Zhao et al., 2010). Approximately, half of the chromosome, which contains more than 7200 genes, consists of a core region which includes most of the genes required for primary metabolism, cell division, information transfer, and sporulation (Katz and Khosla, 2007). Erythromycin biosynthesis is encoded in the core region, which suggests that this natural product has been integral to the evolutionary success of this organism. The remaining half of the chromosome contains most of the remaining gene clusters involved in secondary metabolism, including genes for the biosynthesis of unknown polyketides, ribosomal peptides, and terpenoids.

It is clear that *Saccharopolyspora erythraea* has a remarkable capacity for the production of secondary metabolites, a trait which seems likely to confer substantial advantages to the organism in the soil milieu. Indeed, it has been suggested that the ability to deploy a differential chemical arsenal may be a general evolutionary strategy used by actinobacteria which grow as filamentous mycelia in highly competitive environments (Challis and Hopwood, 2003; Jenke-Kodama et al., 2006). A detailed understanding of the transcriptional organization of the *Saccharopolyspora erythraea* chromosome has been attained by using GeneChip DNA microarrays derived from the complete gene sequence of this organism (Peano et al., 2007). The use of the *Saccharopolyspora erythraea* DNA microarray improved the specificity and sensitivity of gene expression analysis, allowing a global and, at the same time, detailed understanding of how *Saccharopolyspora erythraea* genes are modulated. The results confirmed that the erythromycin gene cluster is upregulated during the initial growth phase of the organism, and identified six additional clusters – for non-ribosomal peptides and terpenes – that are regulated in later growth phases.

Bacteriophages. Until recently, considerable interest was shown in *Saccharopolyspora* phages both as cloning vectors (Katz et al., 1988; Schneider and Kutzner, 1989) and taxonomic markers (Korn-Wendisch et al., 1989; Labeda, 1987; Prauser and Momirova, 1970). Most attention was focused on phages isolated from *Saccharopolyspora erythraea* and *Saccharopolyspora rectivirgula* (Donadio et al., 1986; Grund and Hutchinson, 1987; Katz et al., 1988; Kempf et al., 1987; Schneider et al., 1987) using methods similar to those developed for *Streptomyces* species (Hopwood et al., 1985; Lanning and Williams, 1982), albeit with minor modifications and the use of appropriate baiting and indicator strains (Greiner-Mai et al., 1987; Grund and Hutchinson, 1987; Kurup and Heinzen, 1978).

Some phages isolated from *Saccharopolyspora erythraea* were found to infect and multiply in *Saccharopolyspora rectivirgula* and *vice versa* (Korn-Wendisch et al., 1989; Smorawinska et al., 1988). Some *Saccharopolyspora erythraea* phages have been shown to infect *Saccharopolyspora hirsuta* and *Saccharopolyspora taberi* (Grund and Hutchinson, 1987; Korn-Wendisch et al., 1989). Phage life cycle and plaque morphology are also host-dependent, whereas phages lysogenic for one host may lyse another (Brzezinski et al., 1986; Kempf et al., 1987; Schneider and Kutzner, 1989).

Saccharopolyspora phages have a tail of variable length attached to an icosahedron capsid which encloses a double-stranded genome with *cos* termini present in some strains (Grund and Hutchinson, 1987; Katz et al., 1988; Kurup and Heinzen, 1978; Schneider et al., 1987; Schneider and Kutzner, 1989). The central regions of representative phages from *Saccharopolyspora erythraea* (Brzezinski et al., 1986) and *Saccharopolyspora rectivirgula* (Schneider et al., 1987) are variable due to the deletion or insertion of large gene fragments (Brzezinski et al., 1986; Schneider et al., 1987; Schneider and Kutzner, 1989; Smorawinska et al., 1988). Hybridization studies (Schneider and Kutzner, 1989) have shown that the same or related elements occur in the genomes of *Saccharopolyspora rectivirgula* phages representing different compatability groups and in a phage isolated from *Saccharopolyspora erythraea* (Brzezinski et al., 1986).

Antibiotic sensitivity patterns. In general, saccharopolysporae are resistant to a broad range of antibiotics though studies have been limited to species such as *Saccharopolyspora antimicrobica*, *Saccharopolyspora gregorii*, *Saccharopolyspora hirsuta*, *Saccharopolyspora hordei*, and *Saccharopolyspora spinosa*. The antibiotic sensitivity profiles of these and other *Saccharopolyspora* species are given in the species descriptions.

Pathogenicity. Saccharopolysporae are not known to cause infections though *Saccharopolyspora rectivirgula* is the chief agent of the extrinsic allergic alveolitis condition known as farmer's lung (Campbell, 1932; Pepys et al., 1963). This disease, which occurs widely in China, Europe, Japan, and the USA, is caused by the inhalation of large numbers of spores released when moldy substrates are disturbed in the presence of sensitized individuals (Lacey, 1981). In western Scotland and the Orkneys, it has been reported to affect up to 8.6% of farm workers (Grant et al., 1972), whereas in the US, 8.4% of Wisconsin dairy farmers had precipitins to actinobacteria, nearly 90% of them to *Saccharopolyspora rectivirgula* (Roberts et al., 1976). Farmer's lung is normally a chronic rather than an acute disease, although fatalities have been recorded (Lacey, 1988). *Saccharopolyspora hordei* spores are also found in high numbers in grain stores and may be involved as an agent of extrinsic allergic alveolitis (Lacey and Crook, 1988). Respiratory diseases resembling farmer's lung have been reported in cattle and horses exposed to moldy hay (Lacey, 1988; Pirie et al., 1971) and an acute outbreak resulting in several fatalities has been reported in Canadian cattle (Wilkie, 1978).

The criteria used for the diagnosis of extrinsic allergic alveolitis, including farmer's lung, have been considered by Lacey (1988). Antigens from *Saccharopolyspora rectivirgula* have been purified, which can be used for the detection of circulating antibodies in the sera of patients (Brummund et al., 1988; Mäntyjärvi and Kurup, 1988). An enzyme-linked immunosorbent assay has also been developed for this purpose (Ramasamy et al., 1987).

Antigenicity. The antigenicity of *Saccharopolyspora rectivirgula* has received a lot of attention since the organism, first known as *Thermopolyspora polyspora*, then as *Micropolyspora faeni* and *Faenia rectivirgula*, was implicated in farmer's lung (Pepys et al., 1963). Initially, three precipitin arcs were recognized in gel diffusion and immunoelectrophoresis tests using extracts of *Saccharopolyspora rectivirgula* and sera from farmer's lung patients. Using gel filtration, adsorption on DEAE columns and elution with crossed immunoelectrophoresis and immunodiffusion, the precipitins have been resolved into up to 75 individual antigenic components, as outlined by Lacey (1989a).

Ecology. Members of the genus *Saccharopolyspora* have been isolated from a broad range of habitats, notably plant material which has been stored and allowed to decay (Lacey, 1971, 1974, 1978). *Saccharopolyspora rectivirgula* is common in spontaneously heating vegetable material. It was first isolated from moldy hay which had been baled containing more than 35% water content and heated to 50–65°C (Gregory et al., 1963). Subsequently, it has been found in cereal grain, cotton bales, mushroom compost, straw, sugar corn bagasse, and the air over pastures (Lacey, 1978). Heating of the substrate is initiated by plant cells and mesophilic bacteria and fungi. The organism begins to grow at 30 to 35°C, but larger numbers are found in hays that heat to around 60°C after baling at about 39% water content. The change in pH from 5.5–6.0 to 7.0–8.0 caused by fungal proteolysis probably favors actinobacterial colonization though *Saccharopolyspora rectivirgula* can sometimes grow in hay without pretreatment (Gregory et al., 1963). It can survive in moist grains stored anaerobically in silos and its spores can withstand up to 20 min at 70°C.

Other species isolated from plant material include *Saccharopolyspora gregorii*, *Saccharopolyspora hirsuta*, and *Saccharopolyspora hordei*. *Saccharopolyspora hirsuta* is known mostly from moldy sugar cane bagasse that has heated spontaneously during storage. It was found in 12% of samples originating in India, Jamaica, Puerto Rico, and Trinidad (Lacey, 1974). Strains of *Saccharopolyspora hordei* are common in stored barley and hay and are found less frequently in sugar cane bagasse. This organism, together with *Saccharopolyspora rectivirgula*, was present in 15% of freshly harvested grain samples, but not in grass used to seal moist barley silos (Lacey, 1971). However, after storage they occurred in 33% of grass and straw samples and in 57% and 32% of grain samples in two seasons. *Saccharopolyspora rectivirgula* has been isolated from a range of vegetable material collected from different sites in the Nigerian states of Anambra and Enugu (Unaogu et al., 1994). *Saccharopolyspora gregorii* has been infrequently isolated from barley, stored hay, and straw (Goodfellow et al., 1989b). Saccharopolysporae may also be present on lichens (Gonzalez et al., 2005).

Developments in the systematics of *Saccharopolyspora* provide good grounds for the recognition of novel species isolated from diverse man-made and natural habitats. Novel species from terrestrial habitats include *Saccharopolyspora flava* and *Saccharopolyspora thermophila* from garden soil (Lu et al., 2001), *Saccharopolyspora phatthalungensis* from the rhizosphere of *Hevea brasiliensis* (Duangmal et al., 2010), *Saccharopolyspora shandongensis* from wheat field soil (Zhang et al., 2008b), *Saccharopolyspora tripterygii* from a surface-sterilized stem sample from *Trypterygium hypoglaucum* (Li et al., 2009a), and "*Saccharopolyspora endophytica*" from a root of *Mytenus austroyunnanensis* (Qin et al., 2008a). In addition, *Saccharopolyspora halophila* and *Saccharopolyspora qijiaojingensis* were isolated from saline salt lakes (Tang et al., 2009a, 2009b), *Saccharopolyspora cebuensis* was from a sponge (Pimentel-Elardo et al., 2008), *Saccharopolyspora rosea* was from a patient with bronchial carcinoma (Yassin, 2009), and *Saccharopolyspora spinosa* was from soil collected from a sugar mill rum still (Mertz and Yao, 1990).

Isolation procedures

The isolation of *Saccharopolyspora* from airborne dust, grain, fodder, and other vegetable material is best achieved by using

an Andersen sampler (Andersen, 1958) to examine spore clouds generated using a wind tunnel or sedimentation chamber (Gregory and Lacey, 1963; Lacey and Dutkiewicz, 1976a, 1976b). Sedimentation of spore clouds should be allowed to proceed for 10–30 min in order to allow larger particles to settle as the lighter actinomycete spores remain airborne. The Andersen sampler should be loaded with appropriate isolation media supplemented with cycloheximide (50 μg/ml) to inhibit the growth of fungi (Cross et al., 1968).

Colonies of *Saccharopolyspora hirsuta* were first isolated from airborne sugar cane bagasse dust (Lacey, 1974) using a wind tunnel and an Andersen sampler (Lacey and Goodfellow, 1975). The medium, half-strength nutrient agar supplemented with cycloheximide, was incubated at 40°C following inoculation. The resultant colonies were colorless to pale brown, thin, and raised or convex and carried small tufts of white aerial mycelia in the centers. The same procedure was used to isolate *Saccharopolyspora gregorii* and *Saccharopolyspora hordei* from cereal grain and hay (Goodfellow et al., 1989b; Lacey, 1971). Isolation of *Saccharopolyspora gregorii* is enhanced at 30°C.

Dilution and direct plating procedures have been used to isolate *Saccharopolyspora rectivirgula*, but enumeration in hay and other vegetable matter is best achieved by using an Andersen sampler to impact airborne spores onto appropriate media. A range of media can be used, including half-strength nutrient agar (Lacey, 1971, 1974), half-strength tryptone soy agar supplemented with casein hydrolysates (Lacey, 1989a), R8 agar (Amner et al., 1989), and starch-casein-arginine agar (Iwasaki et al., 1979). Hippurate has been used as a selective carbon source for the isolation of *Saccharopolyspora rectivirgula* (Mattinson-Rose, 1986). This investigator used the membrane-filter method of Hirsch and Christensen (1983) to isolate *Saccharopolyspora hirsuta* from dilutions of substrate suspensions; only mycelial actinomycetes can grow through the pores in membrane filters and thereby reach and grow on the surface of isolation media.

Small numbers of taxonomically diverse saccharopolysporae have been isolated from aquatic and terrestrial habitats by plating suspensions of substrates onto non-selective media, as exemplified by the isolation of *Saccharopolyspora antimicrobica* on yeast extract-malt extract agar (Yuan et al., 2008) and *Saccharopolyspora flava* on oatmeal agar (Lu et al., 2001). Additional strains have been isolated using more selective, but nevertheless empirical procedures, as illustrated by the isolation of *Saccharopolyspora halophila* on cellulose-casein multi-salts agar (Tang et al., 2009a) and *Saccharopolyspora phatthalungensis* on starch-casein agar supplemented with ketokamazole and nalidixic acid (Duangmal et al., 2010). In general, inoculated plates were incubated at 28°C for between 1 and 4 weeks.

Maintenance procedures

Working cultures of *Saccharopolyspora* strains can be maintained using refrigerated (4°C) agar slopes on media such as glucose-yeast extract agar (Gordon and Mihm, 1962), V8 vegetable juice agar (Corbaz et al., 1963), and yeast extract-malt extract agar (ISP medium 2; Shirling and Gottlieb, 1966). Similarly, halophilic strains can be maintained on inorganic salts-starch agar (ISP medium 4; Shirling and Gottlieb, 1966). Longer term preservation of strains is best achieved as frozen suspensions of mycelia and spores at −80°C (mechanical freezer) to −172°C (liquid nitrogen vapor phase) or by using standard lyophilization techniques.

Differentiation of the genus *Saccharopolyspora* from other genera

Saccharopolyspora strains can be distinguished from members of other genera classified in the family *Pseudonocardiaceae* by using a combination of chemical and morphological markers and by comparative 16S rRNA gene sequence studies (see the family *Pseudonocardiaceae*, above). It can be separated from other genera in the family on the basis of PAGE and sequence analysis of ribosomal AT-L30 proteins (Ochi, 1995; Ochi and Yoshida, 1991) and by the use of a genus-specific primer (Morón et al., 1999).

Taxonomic comments

The genus *Saccharopolyspora* was proposed by Lacey and Goodfellow (1975) to encompass actinobacteria from sugar cane bagasse that produced aerial mycelia with bead-like chains of spores enclosed in a characteristic hairy sheath. Subsequently, Labeda (1987) transferred the type species of *Streptomyces erythraeus* to the genus *Saccharopolyspora* as *Saccharopolyspora erythraea* because its cell walls contained *meso*-diaminopimelic acid, arabinose, and galactose. He also proposed that "*Nocardia taberi*" be reclassified as *Saccharopolyspora hirsuta* subsp. *taberi*, an organism later given species status as *Saccharopolyspora taberi* (Korn-Wendisch et al., 1989). Another taxon, "*Saccharopolyspora hirsuta* subsp. *kobensis*" (Iwasaki et al., 1979) was formally recognized by these investigators. Two other taxa, "*Saccharopolyspora aurantiaca*" and "*Saccharopolyspora hirsuta* subsp. *kunmingensis*" were described for strains isolated from soil taken from a rice field (Jiang and Xu, 1986). Three other species, *Saccharopolyspora gregorii*, *Saccharopolyspora hordei*, and *Saccharopolyspora spinosa*, were early additions to the genus (Goodfellow et al., 1989b; Mertz and Yao, 1990).

There have been numerous twists and turns in the classification and nomenclature of *Saccharopolyspora rectivirgula*, an organism designated *Faenia rectivirgula* in the last edition of *Bergey's Manual of Systematic Bacteriology* (Lacey, 1989a). The name *Faenia rectivirgula* was proposed by Kurup and Agre (1983) for the thermophilic actinobacterium *Micropolyspora faeni* (Cross et al., 1968) which had been previously named "*Thermopolyspora polyspora*" (Corbaz et al., 1963) and "*Thermopolyspora rectivirgula*" (Krasil'nikov and Agre, 1964). The genus name *Micropolyspora* lost its standing in nomenclature when its type strain, *Micropolyspora brevicatena*, was reclassified as *Nocardia brevicatena* (Goodfellow and Pirouz, 1982). The subsequent reclassification of *Faenia rectivirgula* as *Saccharopolyspora rectivirgula* (Korn-Wendisch et al., 1989) was supported by a wealth of taxonomic information, including data derived from chemotaxonomic (Collins et al., 1977; Embley et al., 1988b; Kroppenstedt, 1985; Mordarskaia et al., 1973), phage host range (Schneider and Kutzner, 1989; Smorawinska et al., 1988), and 16S rRNA gene sequencing (Bowen et al., 1989; Embley et al., 1988a, 1988c) studies. The name of the mesophilic species "*Faenia rectivirgula*", proposed by Okazaki et al. (1987), has not been validly published.

The genus *Saccharopolyspora* currently contains 20 species with validly published names, most of which have been delineated using a broad range of genotypic and phenotypic features, in some instances including DNA–DNA relatedness data

(Li et al., 2009a; Qin et al., 2008a; Yuan et al., 2008; Zhang et al., 2008b, 2009). However, despite the recent rise in the number of *Saccharopolyspora* species, mainly based on the description of single isolates, there is evidence that the genus is underspeciated (Goodfellow et al., 1989b).

Differentiation of the species of the genus *Saccharopolyspora*

Saccharopolyspora species can be distinguished by using a range of phenotypic markers (Table 255), though results need to be interpreted with care as a common set of tests are not yet available for the delineation of all species and because many of the latter are based on the description of single isolates. In practice, closely related *Saccharopolyspora* species are delineated using a combination of 16S rRNA gene sequence, DNA–DNA relatedness, and phenotypic data (Duangmal et al., 2010; Qin et al., 2008a). Some *Saccharopolyspora* species have been distinguished by differences in quantitative fatty acid composition (Embley et al., 1988b; Mertz and Yao, 1990) and on the basis of esterase patterns, plasmid profiles, and phage host range studies (Korn-Wendisch et al., 1989; Schneider and Kutzner, 1989).

List of species of the genus *Saccharopolyspora*

1. **Saccharopolyspora hirsuta** Lacey and Goodfellow 1975, 78[VP]

hir.su′ta. L. fem. adj. *hirsuta* hairy, rough, shaggy, bristly, referring to the hairy spore chains produced by the organism.

Forms branched, substrate hyphae (0.4–0.6 μm diameter) which tend to fragment into rod-shaped elements towards the edges and in older parts of colonies. Aerial hyphae (0.5–0.7 μm diameter) differentiate into bead-like spores which tend to be separated by short lengths of apparently "empty" hyphae. Looped or loosely spiral chains of spores are produced, though long straight chains of spores are sometimes formed between tufts of aerial hyphae. Spores are round to oval (0.7–1.3 × 0.5–0.7 μm) and are covered by a sheath bearing tufts of long straight or curved hairs. Thin, raised or convex, and usually slightly wrinkled colonies are formed in 7 d at 40°C. Sparse white aerial hyphae are produced in tufts at the center of colonies. The substrate mycelium is colorless to cartridge buff and usually gelatinous or mucoid. Good growth occurs on V-8 vegetable juice and yeast extract-malt extract (ISP medium 2) agars; a yellow diffusible pigment is formed on the latter. Grows on agar media between 25 and 50°C, with an optimal temperature of about 37 to 40°C; does not grow at 10°C. Aerial hyphae are only formed at the optimal temperature.

Degrades adenine, but not xylan. Adonitol, cellobiose, erythritol, glycerol, inositol, lactose, methyl α-D-glucoside, methyl β-D-glucoside, sorbitol, and trehalose are used as sole carbon sources, but not arabinose, melezitose, or salicin (all at 1%, w/v). Similarly, acetate, benzoate, butyrate, citrate, fumarate, H-malate, succinate, and sebacate acid are used as sole carbon sources, but not adipate or tartrate (all at 0.1%, w/v).

Resistant (filter paper discs soaked in μg/ml antibiotics) to erythromycin (50), gentamicin (100), kanamycin (10), streptomycin (100), neomycin (50), tobramycin (100), and rifampin (50), but susceptible to dapsone (500) and septrin (500). Sensitive to lysozyme. Additional phenotypic properties are shown in Table 255.

Whole-organism hydrolysates contain arabinose and galactose as characteristic sugars and *meso*-diaminopimelic acid as the principal diamino acid. Cellular fatty acids contain major proportions of iso- and anteiso-branched chain components. The predominant menaquinone is MK-9(H_4).

Source: isolated from moldy sugarcane bagasse spontaneously heated during storage.

DNA G+C content (mol%): not determined.

Type strain: ATCC 27875, CBS 420.74, DSM 43463, IFO (now NBRC) 13919, JCM 3170, NCIB 11079, NRRL B-5792.

Sequence accession no. (16S rRNA gene): U93341.

Taxonomic comments: subsequently, this species has been divided into subspecies.

1a. **Saccharopolyspora hirsuta subsp. hirsuta** Lacey and Goodfellow 1975, 78[VP]

Description is as for the species.

Additional comments: this subspecies was automatically created by the valid publication of *Saccharopolyspora hirsuta* subsp. *taberi* Labeda (1987) (Howey et al., 1990), a taxon subsequently raised to species status (Korn-Wendisch et al., 1989).

1b. **Saccharopolyspora hirsuta subsp. kobensis** (*ex* Iwasaki, Itoh and Mori 1979) Lacey 1989b, 496[VP] (Effective publication: Lacey 1989c, 2385.) (*Saccharopolyspora hirsuta* subsp. *kobensis* Iwasaki, Itoh and Mori 1979, 185)

ko.ben′sis. N.L. fem. adj. *kobensis* belonging to Kobe, a city in Japan where the organism was isolated.

Differs from *Saccharopolyspora hirsuta* subsp. *hirsuta* in having a yellow to pink substrate mycelium and a yellow to red soluble pigment, in reducing nitrate to nitrite, and by using inositol, rhamnose, sorbitol, and xylose. Source of the antibiotic sporacin.

Source: isolated from soil.

DNA G+C content (mol%): not determined.

Type strain: ATCC 20501, FERM-P 3912, NBRC 15151, JCM 9109, KC 6606.

Sequence accession no. (16S rRNA gene): DQ381814.

Additional comments: a revised name (Lacey, 1989b), cited in Validation List 31.

2. **Saccharopolyspora antimicrobica** Yuan, Zhang, Guan, Wei, Li, Yu, Li and Zhang 2008, 1184[VP]

an.ti.mi.cro′bi.ca. Gr. prep. *anti* against; N.L. n. *microbium* microbe; L. adj. suff. *-cus -a -um* suffix used with various meanings; N.L. fem. adj. *antimicrobica* antimicrobial.

Forms extensively branched, white, buff to pink substrate mycelia that later fragment into rod-shaped elements. Aerial hyphae differentiate into long straight chains of rough-surfaced spores. Grows well on glycerol-asparagine (ISP medium 5), inorganic salts-starch (ISP medium 4), oatmeal (ISP

medium 3), and yeast extract-malt extract (ISP medium 2) agars. White aerial hyphae are produced on ISP 2 agar. Diffusible pigments, buff, pink to brown, are produced on some agar media. Grows in the presence of 0–7% (w/v) NaCl. Temperature and pH ranges for growth are 20–45°C and pH 6.0–8.5, respectively. Optimal temperature and pH for growth are 28–37°C and pH 7.0–7.5, respectively.

Gelatin and xylan are degraded, but not cellulose, chitin, or elastin. Milk is coagulated and peptonized, but does not produce H_2S. Positive for acid phosphatase, alkaline phosphatase, β-galactosidase, α-glucosaccharase, β-glucosaccharase, and *N*-acetylglucosaminidase. Adonitol, cellobiose, dutcitol, inositol, melezitose, melibiose, D-ribose, salicin, sorbitol, turanose, acetate, citrate, malonate, phenylalanine, glyconate, glucosamine, and tartrate are used as sole carbon sources, but not erythritol, methyl α-D-glucoside, or trehalose.

Resistant (μg/ml) to amikacin (30), ampicillin (10), aztreonam (30), cefotaxime (30), ceftazidime (30), chloromycetin (30), erythromycin (15), furadantin (300), gentamicin (10), oxacillin (1), penicillin G (10 U), streptomycin (10), and tobramycin (10). Inhibited by lysozyme. Shows antimicrobial activity against *Escherichia coli* and *Staphylococcus aureus*. Additional phenotypic features are shown in Table 255.

Whole-organism hydrolysates contain arabinose and galactose as characteristic sugars and *meso*-diaminopimelic acid as the principal diamino acid. Cellular fatty acids contain major proportions of $C_{15:0}$ iso, $C_{16:0}$ iso, $C_{17:0}$ iso, and $C_{17:0}$ anteiso. The predominant menaquinone is MK-9(H_4) and the major phospholipids are diphosphatidylglycerol, phosphatidylcholine, phosphatidylglycerol, and phosphatidylinositol.

Source: isolated from soil.

DNA G+C content (mol%): 69.3 (T_m).

Type strain: I05-00074, CCM 7463, DSM 45119, KCTC 19303.

Sequence accession no. (16S rRNA gene): EF693956.

3. **Saccharopolyspora cebuensis** Pimentel-Elardo, Tiro, Grozdanov and Hentschel 2008, 631[VP]

ce.bu.en′sis. N.L. fem. adj. *cebuensis* pertaining to the province of Cebu in the Philippines where the type strain was collected.

Forms extensively branched white substrate hyphae which fragment into rod-shaped elements. Aerial hyphae bear short chains of round to oval spores with smooth surfaces. Brown diffusible pigment is observed. Grows at 15–37°C on yeast extract-malt extract (ISP medium 2) or M1 agars which contain 2.5–12.5% (w/v) NaCl or 25–100% artificial seawater. Does not grow anaerobically.

Positive for acid phosphatase, alkaline phosphatase, esterase (C4), esterase lipase (C8), α-glucosidase, leucine arylamidase, lipase (C14), α-mannosidase, naphthol-A-BI-phosphohydratase, *N*-acetyl-β-glucosaminidase, and valine arylamidase (API ZYM tests). Catalase-positive and oxidase-negative.

Amygdalin, D-arabinose, arabitol, cellobiose, dulcitol, erythritol, esculin, fucose, gentiobiose, glycerol, glycogen, inulin, *N*-acetylglucosamine, potassium gluconate, ribose, starch, and trehalose are used as sole carbon sources. Additional phenotypic features are shown in Table 255.

Whole-organism hydrolysates contain arabinose and galactose as characteristic sugars and *meso*-diaminopimelic acid as the principal diamino acid. Cellular fatty acids contain major proportions of iso-, anteiso-, and 10-methyl-branched fatty acids. The predominant menaquinone is MK-9(H_4) and the major polar lipids are diphosphatidylglycerol, phosphatidylcholine, phosphatidylethanolamine, phosphatidylglycerol, phosphatidylinositol, and phosphatidylmethylethanolamine.

Source: isolated from a marine sponge (*Haliclona* sp.).

DNA G+C content (mol%): 72.6 (HPLC).

Type strain: DSM 45019, CIP 109355, SPE 10-1.

Sequence accession no. (16S rRNA gene): EF030715.

4. **Saccharopolyspora erythraea** (Waksman 1923) Labeda 1987, 21[VP] [*Actinomyces erythreus* Waksman 1923, 370; *Streptomyces erythreus* (Waksman 1923) Waksman and Henrici 1948, 938]

e.ry.thra′e.a. L. fem. adj. *erythraea* reddish, referring to colony color.

Forms an extensively branched substrate mycelium. Spore chains are usually short so that imperfect spirals and short to flexuous chains are common. Spore surfaces are spiny. Substrate mycelium ranges from pale orangish yellow to reddish brown, depending on the medium. Pale pink to moderately brownish gray aerial hyphae are formed on many media, including glycerol-asparagine agar (ISP medium 5). White aerial mycelia are seen. A faint yellow to pinkish orangish brown soluble pigment is produced on most media. Melanin pigments are not produced. Grows between 20 and 42°C.

Degrades gelatin, but does not produce phosphatase or hydrolyze allantoin. Acid is produced from adonitol, arabinose, cellobiose, dextrin, erythritol, fructose, galactose, glucose, glycerol, inositol, maltose, mannitol, mannose, melezitose, melibiose, raffinose, rhamnose, salicin, sucrose, and xylose, but not from dutcitol, lactose, methyl α-D-glucoside, methyl β-D-xyloside, or sorbitol. Acetate, citrate, lactate, malate, propionate, pyruvate, and succinate are used as sole carbon sources, but not benzoate, mucate, oxalate, or tartrate. Many strains produce erythromycins A or B. Additional phenotypic properties are shown in Table 255.

Whole-organism hydrolysates contain arabinose and galactose as characteristic sugars and *meso*-diaminopimelic acid as the predominant principal diamino acid. The predominant menaquinone is MK-9(H_4).

Source: isolated from soil.

DNA G+C content (mol%): 76.9 (T_m).

Type strain: ATCC 11635, DSM 40517, ISP 5517, NRRL 2338.

Sequence accession no. (16S rRNA gene): X53198.

Additional comments: Saccharopolyspora erythraea (Waksman 1923) Labeda 1987 comb. nov. was initially described as *Saccharopolyspora erythraea* sp. nov. However, since *Saccharopolyspora erythraea* Labeda 1987 contains the type strain of *Streptomyces erythraeus* (Waksman 1923) Waksman and Henrici 1948 (Approved Lists, Skerman et al., 1980), the taxon has to be considered as a new combination, *Saccharopolyspora erythraea* (Waksman 1923) Labeda 1987.

5. **Saccharopolyspora flava** Lu, Liu, Wang, Zhang, Qi and Goodfellow 2001, 322[VP]

fla′va. L. fem. adj. *flava* yellow, referring to the color of the substrate mycelium.

Forms an extensively branched, yellow substrate mycelium which fragments into rod-shaped elements after 3–4 d at 28°C. Aerial hyphae are produced after prolonged cultivation on oatmeal agar. The aerial mycelium carries abundant straight chains of 3–5 smooth surfaced spores (0.4–0.5 × 0.6 µm). The organism grows well on glucose-asparagine, modified Sauton's, and yeast extract-malt extract (ISP medium 2) agars. Does not produce diffusible pigments. Grows between 28 and 37°C. Weak growth occurs in the presence of 7% (w/v) NaCl.

Cellulose and xylan are degraded, but not guanine. Utilizes adonitol, cellobiose, erythritol, glycerol, inositol, methyl α-D-glucoside, salicin, sorbitol, acetate, adipate, benzoate, butyrate, citrate, fumarate, H-malate, propionate, pyruvate, and succinate as sole carbon sources, but not melezitose, sebacic acid, or tartrate.

Sensitive (discs soaked in 100 µg/ml antibiotic) to gentamicin, streptomycin, and rifampin. Sensitive to lysozyme. Additional phenotypic features are shown in Table 255.

Whole-organism hydrolysates contain arabinose and galactose as characteristic sugars and *meso*-diaminopimelic acid as the principal diamino acid. The predominant menaquinone is MK-9(H_4) and the polar lipid pattern includes phosphatidylcholine.

Source: isolated from garden soil.

DNA G+C content (mol%): 67 (T_m).

Type strain: 07, AS4.1520, DSM 44771, NBRC 16345, JCM 10665.

Sequence accession no. (16S rRNA gene): AF154128.

6. **Saccharopolyspora gloriosae** Qin, Chen, Klenk, Kim, Xu and Li 2010, 1150[VP]

glo.ri.o′sa.e. N.L. fem. gen. n. *gloriosae* of the plant genus *Gloriosa*, referring to the isolation of the type strain from a stem of *Gloriosa superba*.

Forms an extensively branched substrate mycelium. Curved and hooked chains of smooth-surfaced spores are formed on the aerial mycelium. Grows well on glycerol-asparagine (ISP medium 5), oatmeal (ISP medium 3), and yeast extract-malt extract (ISP medium 2) agars and moderately well on Czapek's and inorganic salts-starch (ISP medium 4) agars. Grows between 10 and 32°C (optimally at 28°C), pH 6.0 and 8.0, and in the presence of 0–11% NaCl (optimally with 0–5% salt).

Oxidase-negative. Adonitol, D-arabinose, arabitol, cellobiose, erythritol, esculin, fructose, gentiobiose, glycerol, glycogen, inositol, mannose, melezitose, starch, and trehalose are used as sole carbon sources. L-Alanine, D-arginine, L-asparagine, L-leucine, L-histidine, L-ornithine, L-proline, L-serine, and L-threonine are used as sole nitrogen sources. Additional phenotypic features are shown in Table 255.

Whole-organism hydrolysates contain arabinose and galactose as characteristic sugars and *meso*-diaminopimelic acid as the principal diamino acid. Cellular fatty acids contain major proportions of $C_{16:0}$ iso, $C_{17:0}$ anteiso, and $C_{17:1}$ *cis*9. The predominant menaquinone is MK-9(H_4) and the major polar lipids are diphosphatidylglycerol, phosphatidylcholine, phosphatidylethanolamine, phosphatidylglycerol, phosphatidylinositol, phosphatidylinositol mannosides, and several unknown phospholipids.

Source: isolated from a surface-sterilized stem of *Gloriosa superba* L.

DNA G+C content (mol%): 71.6 (HPLC).

Type strain: YIM 60513, KCTC 19243, CCTCC AA 207006.

Sequence accession no. (16S rRNA gene): EU005371.

7. **Saccharopolyspora gregorii** Goodfellow, Lacey, Athalye, Embley and Bowen 1989a, 496[VP] (Effective publication: Goodfellow, Lacey, Athalye, Embley and Bowen 1989b, 2137.)

gre.gor′i.i. N.L. gen. masc. n. *gregorii* of Gregory, named after P.H. Gregory, a British mycologist and aerobiologist.

Forms an extensively branched substrate mycelium, which fragments into coccoid elements. Sporulation or fragmentation of the aerial mycelium may sometimes be seen, forming hooks or flexuous chains of spores with smooth surfaces. Transmission electron micrographs show mainly vegetative rods and cocci, but occasionally spores with a smooth sheath are seen. Colonies are pale yellowish to buff with sparse white aerial mycelium at growth temperatures of about 20°C. Temperature range for growth is 10–35°C.

Degrades arbutin, gelatin, and Tweens 20 and 80. Adonitol, glycerol, D- and L-alanine, L-proline, propionate, and pyruvate are used as sole carbon sources, but not glycogen, trehalose, starch, or L-serine.

Resistant (µg/ml) to cephaloridine (2), lincomycin (16), oleandomycin (2), and benzylpenicillin (10), but sensitive to demecylocyline (2), gentamicin (4), neomycin (3), rifampin (2), streptomycin (4), tobramycin (8), and vancomycin (2). Grows in the presence of (%, w/v) bismuth citrate (0.001), crystal violet (0.0001), phenol (0.01), phenyl ethanol (0.1%, v/v), potassium tellurite (0.001), sodium azide (0.001), sodium chloride (13), tetrazolium (0.01), teepol (0.01), and thallous acetate (0.0001), but is sensitive to bismuth citrate (0.01), phenyl ethanol (0.4%, v/v), potassium tellurite (0.01), and thallous acetate (0.01). Additional phenotypic features are shown in Table 255.

Whole-organism hydrolysates contain arabinose and galactose as characteristic sugars and *meso*-diaminopimelic acid as the principal diamino acid. Cellular fatty acids contain major proportions of $C_{16:0}$ iso, $C_{17:0}$ anteiso, and $C_{17:0}$ iso. The predominant menaquinone is MK-9(H_4) and the major polar lipids are diphosphatidylglycerol, phosphatidylcholine, phosphatidylglycerol, phosphatidylinositol, and uncharacterized phospho- and glycolipids.

Source: isolated from grass, hay, straw, barley grain, and soil.

DNA G+C content (mol%): 74 (T_m).

Type strain: A85, ATCC 51265, DSM 44324, NBRC 15045, NCIMB 12823, JCM 9687, NRRL B-16506.

Sequence accession no. (16S rRNA gene): X76962.

8. **Saccharopolyspora halophila** Tang, Wang, Cai, Zhi, Lou, Xu, Jiang and Li 2009a, 557[VP]

ha.lo′phi.la. Gr. n. *hals halos* salt; N.L. adj. *philus -a -um* (from Gr. adj. *philos -ê -on*) friend, loving; N.L. fem. adj. *halophila* salt-loving, referring to the ability to grow at high NaCl concentrations.

Moderately halophilic. Forms a well-developed substrate mycelium that shows no evidence of fragmentation. Aerial hyphae differentiate into long straight chains of oval or spherical spores (0.6–0.7 × 0.6–1.1 µm) which have smooth

surfaces. The aerial mycelium is white-yellow in color and the substrate mycelium is yellow to moderate orange-yellow. Does not produce diffusible pigments. Grows well on Czapek's, glycerol-asparagine (ISP medium 5), inorganic salts-starch (ISP medium 4), potato, and yeast extract-malt extract (ISP medium 2) agars. Moderate growth occurs on nutrient and oatmeal (ISP medium 3) agars. Temperature, pH, and NaCl tolerance ranges are 10–45°C, pH 6–8.5, and 3–20% (w/v), respectively. Good growth occurs at 28–37°C, pH 7–8, and with 10–15% (w/v) NaCl.

Does not degrade cellulose. Liquefies gelatin. Milk peptonization and coagulation are positive. Does not produce H_2S. Acid is produced on L-arabinose, glucose, *myo*-inositol, rhamnose, and xylose. Cellobiose, *myo*-inositol, xylitol, and acetate are used as sole carbon sources, but not sorbitol or trehalose. Alanine, asparagine, arginine, cystine, glycine, histidine, homocysteine, hypoxanthine, lysine, proline, threonine, tyrosine, valine, and urea are utilized as sole nitrogen sources, but not adenine, hydroxyproline, or glutamate. Additional phenotypic features are shown in Table 255.

Whole-organism hydrolysates contain arabinose and galactose as characteristic sugars and *meso*-diaminopimelic acid as the principal diamino acid. Cellular fatty acids contain major proportions of $C_{15:0}$ iso, $C_{16:0}$ iso, and $C_{17:0}$ anteiso. The predominant menaquinone is MK-9(H_4) and the major polar lipids are diphosphatidylglycerol and phosphatidylcholine; large amounts of phosphatidylinositol are also present.

Source: isolated from a saline lake.

DNA G+C content (mol%): 66.3 (HPLC).

Type strain: DSM 45007, KCTC 19162, YIM 90500.

Sequence accession no. (16S rRNA gene): DQ923129.

9. **Saccharopolyspora hordei** Goodfellow, Lacey, Athalye, Embley and Bowen 1989a, 496[VP] (Effective publication: Goodfellow, Lacey, Athalye, Embley and Bowen 1989b, 2137.)

hor′de.i. L. n. *hordeum* barley, and also the generic name of barley (*Hordeum*); L. gen. n. *hordei* from barley, referring to the isolation source of the strains.

Forms an extensively branched substrate mycelium which sometimes fragments into coccoid elements. Aerial mycelium is either not produced or is sparse and is white with short flexuous, hooked, or spiral spore chains often with a beaded appearance. Transmission electron micrographs show that the spores are covered by a smooth sheath with adjacent spores often separated by a short length of empty hypha. Colonies are colorless to light buff, conical, rounded, or wrinkled. Grows at 20–60°C.

Resistant (μg/ml) to lincomycin (16), neomycin (3), oleandomycin (16), benzylpenicillin (10), and tobramycin (4), but sensitive to cephaloridine (10), demeclocycline (2), gentamicin (16), rifampin (2), streptomycin (4), and vancomycin (2). Arbutin, DNA, guanine, RNA, testosterone, and Tweens 20 and 80 are degraded. Does not hydrolyze allantoin. Cellobiose, glycogen, glycerol, and starch are used as sole carbon sources. Grows well in the presence of (%, w/v) bismuth citrate (0.0001), crystal violet (0.0001), phenol (0.1), phenyl ethanol (0.2%, v/v), potassium tellurite (0.001), sodium azide (0.001), sodium chloride (10), teepol (0.01), tetrazolium (0.01), and thallous acetate (0.0001), but is sensitive to bismuth citrate (0.01), phenyl ethanol (0.4%, v/v), potassium tellurite (0.01), sodium azide (0.02), and thallous acetate (0.01). Additional phenotypic features are shown in Table 255.

Whole-organism hydrolysates contain arabinose and galactose as characteristic sugars and *meso*-diaminopimelic acid as the principal diamino acid. Cellular fatty acids contain major proportions of $C_{16:0}$ iso, $C_{17:0}$ anteiso, and $C_{17:0}$ iso. The predominant menaquinone is MK-9(H_4) and the major polar lipids are diphosphatidylglycerol, phosphatidylcholine, phosphatidylglycerol, phosphatidylinositol, and uncharacterized phospho- and glycolipids.

Source: isolated from barley and oat grains, grass hay, straw, and sugar cane bagasse.

DNA G+C content (mol%): 72 (T_m).

Type strain: A54, ATCC 49856, DSM 44065, NBRC 15046, JCM 8090, NCIMB 12824, NRRL B-16507.

Sequence accession no. (16S rRNA gene): X53197.

10. **Saccharopolyspora jiangxiensis** Zhang, Wu and Liu 2009, 1078[VP]

ji.ang.xi.en′sis. N.L. fem. adj. *jiangxiensis* pertaining to Jiangxi Province, China, the source of the grass-field soil from which the organism was isolated.

Produces an extensively branched (0.4–0.7 μm diameter), colorless to buff substrate mycelium which fragments *in situ* into coccoid- and rod-shaped elements after 3–4 d at 28°C. White to buff aerial hyphae (0.6–1.0 μm in diameter) are produced upon prolonged cultivation on GYM agar. The aerial mycelium carries long straight to flexuous chains of 6–10 spores (spore size 0.8–1.1 × 1.0–1.3 μm). The spore surface is smooth or irregularly rough. Colony elevation is convex to irregular and colony margins are filamentous. Does not form diffusible pigments. Grows well on GYM, inorganic salts-starch (ISP medium 4), oatmeal (ISP medium 3), and modified Sauton's agars, and at 15–45°C and pH 5.5–9.5. Weak growth occurs in the presence of NaCl at 11%, but not at 12% (w/v).

Does not degrade cellulose, guanine, or xylan. Catalase- and arbutin-positive. Acid is formed from adonitol, D-arabinose, dextrin, D-fructose, D-galactose, D-glucose, glycerol, inulin, maltose, D-mannitol, D-mannose, D-ribose, sucrose, and trehalose, but not from L-arabinose, cellobiose, ethanol, *meso*-erythritol, glycogen, *myo*-inositol, D-lactose, melezitose, melibiose, methyl α-D-glucoside, raffinose, α-L-rhamnose, D-salicin, D-sorbitol, or D-xylose. Adonitol, D-arabinose, *meso*-erythritol, methyl α-D-glucoside, *myo*-inositol, melezitose, sorbitol, trehalose, acetate, benzoate, citrate, fumarate, malate, succinate, and tartrate are used as sole carbon sources, but not salicin, malonate, or oxalate.

Resistant (μg per disc) to amoxycillin plus clavulanic acid (10), ampicillin (10), aztreonam (30), clindamycin (2), erythromycin (15), kanamycin (30), penicillin G (10 U), and tobramycin (10), but susceptible to amikacin (30), cefotaxime (30), chloramphenicol (30), ciprofloxacin (5), gentamicin (10), mezlocillin (75), ofloxacin (5), rifampin (5), streptomycin (10), and tetracycline (30). Grows in the presence of lysozyme (0.005%, w/v). Additional phenotypic properties are shown in Table 255.

Whole-organism hydrolysates contain arabinose and galactose as characteristic sugars and *meso*-diaminopimelic

acid as the major diamino acid. Cellular fatty acids contain major proportions of $C_{15:0}$ iso, $C_{16:0}$ iso, $C_{17:0}$ iso, and $C_{17:0}$ anteiso. The predominant menaquinone is MK-9(H_4) and the major polar lipids are phosphatidylcholine, phosphatidylethanolamine, and phosphatidylglycerol.

DNA relatedness values between the type strain and corresponding strains of closely related species are as follows: *Saccharopolyspora antimicrobica*, 56.3%; *Saccharopolyspora hirsuta*, 47.8%; *Saccharopolyspora shangdongensis*, 25.5%; and *Saccharopolyspora spinosa*, 21.7%.

Source: isolated from a soil sample from a grass field.

DNA G+C content (mol%): 70.3 (T_m).

Type strain: CGMCC 4.3529, JCM 14613, W12.

Sequence accession no. (16S rRNA gene): EF143926.

11. **Saccharopolyspora phatthalungensis** Duangmal, Mingma, Thamchaipenet, Matsumoto and Takahashi 2010, 1907[VP]

phat.tha.lun.gen′sis. N.L. fem. adj. *phatthalungensis* referring to Phatthalung Province, Thailand, the source of the rhizospheric soil from which the organism was isolated.

Forms an extensively branched substrate mycelium which is yellowish to yellowish brown on glycerol-asparagine (ISP medium 5), glucose-yeast extract, inorganic salts-starch (ISP medium 4), peptone-yeast extract iron (ISP medium 5), tyrosine (ISP medium 7), tryptone-yeast extract (ISP medium 1), and yeast extract-malt extract (ISP medium 2) agars. A white aerial mycelium is produced on these media and a brownish-black soluble pigment is produced on ISP medium 2. Spores with spiny surfaces are borne in hooks/open loops. Grows between 18 and 42°C (optimally at 28–34°C) and between pH 5.0 and 9.0. Grows well on ISP medium 2 supplemented with 1–5% (w/v) NaCl, moderately well with 6% (w/v) NaCl, and poorly in the presence of 7% (w/v).

Gelatin is degraded, but not arbutin, cellulose, or guanine. Catalase-positive, but does not hydrolyze allantoin. H_2S is produced. Acid is produced from adonitol, fructose, galactose, glucose, glycerol, *myo*-inositol, mannitol, mannose, ribose, trehalose, and xylose (weakly), but not from arabinose, cellobiose, fucose, β-lactose, maltose, melibiose, rhamnose, raffinose, sorbitol, sorbose, sucrose, or xylitol. Positive for alkaline phosphatase and leucine aminopeptidase, but negative for acid phosphatase, α-chymotrypsin, cystine aminopeptidase, esterase (C4), α-fucosidase, α-galactosidase, β-galactosidase, α-glucosidase, β-glucosidase, β-glucuronidase, lipase (C8), lipase (C14), α-mannosidase, *N*-acetyl-β-glucosaminidase, trypsin phosphoamidase, and valine aminopeptidase (API ZYM tests). Additional phenotypic properties are shown in Table 255.

Whole-organism hydrolysates contain arabinose and galactose as characteristic sugars and *meso*-diaminopimelic acid as the principal diamino acid. The glycan moiety of the peptidoglycan is acetylated. Cellular fatty acids contain major proportions of $C_{16:0}$ and 10-methyl $C_{17:0}$. The predominant menaquinone is MK-9(H_4) and the major polar lipids are phosphatidylcholine, phosphatidylglycerol, and phosphatidylinositol.

Source: isolated from rhizospheric soil.

DNA G+C content (mol%): 70.3 (HPLC).

Type strain: BCC 35844, NRRL B-24798, SR8.15, TISTR 1921.

Sequence accession no. (16S rRNA gene): GQ381309.

12. **Saccharopolyspora qijiaojingensis** Tang, Wang, Wu, Cao, Lou, Xu, Jiang and Li 2009b, 2168[VP]

qi.ji.a.o.jing.en′sis. N.L. fem. adj. *qijiaojingensis* pertaining to Qijiaojing Lake, Xinjiang Province, north-west China, where the sample from which the type strain was isolated was collected.

Moderately halophilic. Forms a well-developed substrate mycelium that fragments into rod-shaped elements. Bead-like straight chains of smooth surfaced spores (0.6–0.7 × 0.7–1.1 µm in size) are formed on the aerial mycelium. Temperature, pH, and NaCl ranges for growth are 20–40°C, pH 5.0–8.0, and 6–22% (w/v) NaCl; optimal growth occurs at 28°C, pH 7.0, and 10–15% (w/v) NaCl.

Gelatin and Tween 20 are degraded, but not cellulose or Tween 80. Does not peptonize or coagulate milk or produce H_2S. Trehalose is used as a sole carbon source, but not cellobiose, glycerol, glycogen, or starch. DL-Alanine, L-hydroxyproline, hypoxanthine, L-histidine, L-asparagine, and xanthine are utilized as nitrogen sources, but not L-lysine, L-serine, adenine, L-tyrosine, or L-phenylalanine.

Resistant (µg per disc) to gentamicin (10), streptomycin (10), sulfamethoxazole/trimethoprin (23.75/1.25), and tobramycin, but sensitive to amoxycillin (10), ampicillin (10), chloroamphenicol (30), ciprofloxacin (5), erythromycin (15), rifampin (5), tetracycline (30), and vancomycin (30). Additional phenotypic properties are shown in Table 255.

Whole-organism hydrolysates contain arabinose and galactose as characteristic sugars and *meso*-diaminopimelic acid as the principal diamino acid. Cellular fatty acids contain major proportions of $C_{15:0}$ iso, $C_{16:0}$ iso, and $C_{17:0}$ iso. The predominant menaquinone is MK-9(H_4) and the major polar lipids are diphosphatidylglycerol, phosphatidylcholine, phosphatidylethanolamine, phosphatidylinositol, phosphatidylinositol mannosides, and an unknown phospholipid.

Source: isolated from a salt lake.

DNA G+C content (mol%): 70.1 (HPLC).

Type strain: DSM 45088, KCTC 19235, YIM 91168.

Sequence accession no. (16S rRNA gene): EF687715.

13. **Saccharopolyspora rectivirgula** (Krasil'nikov and Agre 1964) Korn-Wendisch, Kempf, Grund, Kroppenstedt and Kutzner 1989, 439[VP] [*Faenia rectivirgula* (Cross, Maciver and Lacey 1968) Kurup and Agre 1983, 664; *Micropolyspora faeni* (Corbaz, Gregory and Lacey 1963) Cross, Maciver and Lacey 1968, 354; Basonym: *Micropolyspora rectivirgula* (Krasil'nikov and Agre 1964) Prauser and Momirova 1970, 220; *Thermopolyspora polyspora* Corbaz, Gregory and Lacey 1963, 450; *Thermopolyspora polyspora* Henssen 1957, 396; *Thermopolyspora rectivirgula* Krasil'nikov and Agre 1964, 106]

rec.ti.vir′gu.la. L. adj. *rectus* straight; L. n. *virgula* twig; N.L. n. *rectivirgula* straight twig.

Forms chains of spores on both substrate and aerial hyphae. The spores are borne on short, unbranched, lateral, or terminal sporophores. Spore formation is basipetal and spore surfaces are rough. Substrate hyphae are branched, septate, 0.5–0.8 µm in diameter, and yellow to orange on GYM and TS agars. Similarly, a white to pink aerial mycelium is formed on these media: aerial hyphae are especially well developed in the presence of 5% (w/v) NaCl. Grows

at 37–63°C (optimally at 50°C) and in the presence of 10% (w/v) NaCl.

Degrades gelatin, but not xylan. Allantoin and uric acid are hydrolyzed, but hemolysis and egg yolk reactions are negative. Sensitive to lysozyme. Fructose and inositol are used as sole carbon sources. Lysed by the five lytic *Saccharopolyspora rectivirgula* phages (e.g. P113); nonlysogenic strains are lysed by all temperate *Saccharopolyspora rectivirgula* phages (e.g. ϕFR114 and ϕFR-C) and three *Saccharopolyspora erythraea* phages (e.g. P517). Additional phenotypic properties are shown in Table 255.

Whole-organism hydrolysates contain arabinose and galactose as characteristic sugars and *meso*-diaminopimelic acid as the principal diamino acid. The predominant menaquinone is MK-9(H_4) and the major polar lipids are phosphatidylcholine, phosphatidylethanolamine, and phosphatidylmethylethanolamine.

Causative agent of farmer's lung disease.

Source: isolated from moldy hay, soil, compost, and manure.

DNA G+C content (mol%): 70.4 (HPLC).

Type strain: ATCC 33515, BKM A-810, DSM 43747, NBRC 12464, INMI 683, JCM 3057, NRRL B-16280, VKM Ac-810.

Sequence accession no. (16S rRNA gene): X53194.

14. **Saccharopolyspora rosea** Yassin 2009, 1151[VP]

ro.se′a. L. fem. adj. *rosea* rose-colored, pink, referring to the color of the diffusible pigment produced by the organism.

Forms extensively branched, brownish-yellow substrate hyphae (0.4–0.5 µm in diameter) which fragment into rod-shaped elements. Yellowish to white aerial hyphae (0.5–0.7 µm in diameter) differentiate into long straight chains of smooth-surfaced spores. Pink colored diffusible pigments are produced on oatmeal (ISP medium 3), inorganic salts-starch (ISP medium 4), and yeast extract-malt extract (ISP medium 2) agars, but does not form melanin pigments on peptone-yeast extract iron (ISP medium 6) or tyrosine (ISP medium 7) agars. Grows at 22–42°C and pH 6.0–9.0.

Gelatin is degraded, but not guanine, keratin, or testosterone. Catalase-positive and oxidase-negative. Acetate, adipate, adonitol, isoamyl alcohol, 2,3-butanediol, citrate, *meso*-erythritol, D-gluconate, *myo*-inositol, L-lactate, sorbitol, and trehalose are used as sole carbon sources, but not cellobiose, *m*-hydroxybenzoate, *p*-hydroxybenzoate, lactose, melezitose, or 1,2-propanediol. Acetamide, L-alanine, arginine, gelatin, ornithine, L-proline, and L-serine are used as simultaneous sources of carbon and nitrogen. Additional phenotypic properties are shown in Table 255.

Whole-organism hydrolysates contain arabinose and galactose as characteristic sugars and *meso*-diaminopimelic acid as the principal diamino acid. Cellular fatty acids contain major proportions of $C_{16:0}$ iso, $C_{17:0}$ iso, and $C_{17:0}$ anteiso. The predominant menaquinone is MK-9(H_4) and the major polar lipids are diphosphatidylglycerol, phosphatidylcholine, phosphatidylglycerol, and phosphatidylinositol.

Source: isolated from a bronchial lavage of patient with bronchial carcinoma.

DNA G+C content (mol%): unknown.

Type strain: DSM 45226, CCUG 56401, IMMIB L-1070.

Sequence accession no. (16S rRNA gene): AM992060.

15. **Saccharopolyspora shandongensis** Zhang, Wu, Zhang, Liu and Song 2008b, 1096[VP]

shan.dong.en′sis. N.L. fem. adj. *shandongensis* referring to Shandong Province, China, the source of the wheat-field soil from which the type strain was isolated.

Forms an extensively branched substrate mycelium (0.5–0.8 µm in diameter) which fragments *in situ* into coccoid- and rod-shaped elements after 3–4 d at 28°C. White aerial hyphae (0.7–1.0 µm in diameter) are produced upon prolonged cultivation on glucose-yeast extract-malt agar. The aerial mycelium carries abundant spiral chains of ten or more spiny-surfaced spores (0.8–1.0 × 1.0–1.2 µm) in a spiral arrangement. Grows well on oatmeal (ISP medium 3) and Sauton's agars. A brown diffusible pigment is formed on standard media. Grows at 15–38°C and pH 5.5–9.5. Weak growth occurs in the presence of 7% (w/v) NaCl, but no growth occurs in the presence of 8% (w/v) NaCl.

Does not degrade cellulose, guanine, or xylan. Catalase- and arbutin-positive. Acid is formed from adonitol, D-arabinose, cellobiose, dextrin, D-fructose, D-glucose, glycerol, maltose, D-mannitol, D-mannose, melibiose, D-ribose, and trehalose, but not from L-arabinose, ethanol, *meso*-erythritol, D-galactose, glycogen, *myo*-inositol, inulin, D-lactose, melezitose, methyl α-D-glucoside, raffinose, α-L-rhamnose, D-salicin, D-sorbitol, sucrose, or D-xylose. Adonitol, D-arabinose, cellobiose, *meso*-erythritol, D-fructose, D-glucose, glycerol, D-mannitol, D-mannose, methyl α-D-glucoside, melibiose, D-sorbitol, trehalose, acetate, benzoate, citrate, succinate, and tartrate are used as sole carbon sources, but not *myo*-inositol, melezitose, D-salicin, fumarate, malate, malonate, or oxalate.

Resistant (µg per filter paper disc) to aztreonam (30), clindamycin (2), and penicillin G (10 IU), but susceptible to amikacin (30), amoxycillin plus clavulanic acid (10), ampicillin (10), cefotaxime (30), chloramphenicol (30), ciprofloxacin (5), erythromycin (15), gentamicin (10), kanamycin (30), mezlocillin (75), ofloxacin (5), rifampin (5), streptomycin (10), tetracycline (30), and tobramycin (10). Grows in the presence of lysozyme (0.0005%, w/v). Additional phenotypic properties are shown in Table 255.

Whole-organism hydrolysates contain arabinose and galactose as characteristic sugars and *meso*-diaminopimelic acid as the major diamino acid. Cellular fatty acids contain major proportions of $C_{16:0}$ iso, $C_{17:0}$ anteiso, $C_{18:0}$ iso, $C_{17:1}$ ω8*c*, and $C_{17:0}$ iso. The predominant menaquinone is MK-9(H_4) and the diagnostic polar lipid is phosphatidylcholine.

The DNA–DNA relatedness value between the type strain and the corresponding strain of *Saccharopolyspora spinosa*, a closely related species, is 32.8%.

Source: isolated from wheat-field soil.

DNA G+C content (mol%): 70.1 (T_m).

Type strain: 88, CGMCC 4.3530, JCM 14614.

Sequence accession no. (16S rRNA gene): EF104116.

16. **Saccharopolyspora spinosa** Mertz and Yao 1990, 38[VP]

spi.no′sa. L. fem. adj. *spinosa* spiny, referring to the spiny spore sheath surface.

Forms an extensive substrate mycelium which fragments in liquid media. Well-formed aerial hyphae segment into long chains of spores arranged in hooks and open loops. Short and incomplete spiral spore chains are also seen. The

spores (1.1 × 1.5 µm) have spiny surfaces. Aerial hyphae have a distinctive bead-like appearance with many empty spaces in the spore chains. The aerial mycelium is yellowish pink and the vegetative mycelium is yellow to yellowish brown. A soluble brown pigment is produced on some media. The temperature range for growth is 15 to 37°C. Does not survive a temperature of 50°C for 8 h. Growth occurs in the presence of 11% NaCl.

Allantoin, malate, and testosterone are degraded, but not guanine. Catalase- and phosphatase-positive. Acid is produced from adonitol, D-arabinose, *meso*-erythritol, D-fructose, D-glucose, glycerol, D-mannitol, D-mannose, D-ribose, and trehalose, but not from L-arabinose, cellobiose, cellulose, dextrin, dulcitol, ethanol, D-galactose, glycogen, inositol, inulin, lactose, maltose, melezitose, melibiose, methyl α-D-glucoside, raffinose, L-rhamnose, salicin, D-sorbitol, L-sorbose, sucrose, xylitol, or D-xylose. Acetate, butyrate, citrate, formate, lactate, malate, propionate, pyruvate, and succinate are used as sole carbon sources, but not benzoate, mucate, oxalate, or tartrate.

Resistant to (µg per disc) cephalothin (20), kasugamycin (500), nalidixic acid (30), novobiocin (20), oligomycin (100), oxytetracycline (10), penicillin (10 U), polymixin B (300 U), rifampin (5), and trimethoprim (30), but sensitive to bacitracin (10 U), chloromycetin (30), erythromycin (15), gramicidin S (10), gentamicin (10), kanamycin (10), lincomycin (2), mandelamine (3), mikamycin A and B (10), neomycin (30), oleandomycin (15), spiramycin (10), streptomycin (10), tetracycline (30), thiostreptin (10), tobramycin (10), timicamycin (5), valinomycin (10), and vancomycin (30). Additional phenotypic properties are shown in Table 255.

Whole-organism hydrolysates contain arabinose and galactose as characteristic sugars and *meso*-diaminopimelic acid as the principal diamino acid. The predominant menaquinone is MK-9(H_4) and the major polar lipids are cardiolipin and phosphatidylcholine.

Source: isolated from soil collected from a sugar mill rum still.

DNA G+C content (mol%): not known.

Type strain: A83543.1, ATCC 49460, DSM 44228, JCM 9375, NBRC 15153, NRRL 18395.

Sequence accession no. (16S rRNA gene): AF002818.

17. **Saccharopolyspora spinosporotrichia** Zhou, Liu, Qian, Kim and Goodfellow 1998, 56[VP]

spi.no.spo.ro.tri′chi.a. L. adj. *spinosus* thorny; N.L. n. *spora* a spore; N.L. n. *trichia* trichite; N.L. n. *spinosporotrichia* spores bearing needle-like spines.

Forms an extensively branched, reddish-brown substrate mycelium that fragments into rod-shaped elements. An abundant white to gray aerial mycelium bears long spiral chains of spherical spores with warty surfaces. Grows well on Bennett's, Czapek's, Gause's synthetic, inorganic salts-starch (ISP medium 4), oatmeal (ISP medium 3), and sucrose-yeast extract agars. A brown diffusible pigment is formed on most standard media. Grows between 25 and 37°C and in the presence of 2–3% (w/v) sodium chloride.

Glycerol, *myo*-inositol, melibiose, ribose, and sorbitol are used as sole carbon sources, but not sorbose. Additional phenotypic properties are shown in Table 255.

Whole-organism hydrolysates contain arabinose and galactose as characteristic sugars and *meso*-diaminopimelic acid as the predominant diamino acid. The predominant menaquinone is MK-9(H_4); the polar lipid profile includes major amounts of phosphatidylcholine.

Source: isolated from soil.

DNA G+C content (mol%): 70.4 (T_m).

Type strain: AS4.198, DSM 44350, NBRC 16190, JCM 10303.

Sequence accession no. (16S rRNA gene): Y09571.

18. **Saccharopolyspora taberi** (Labeda 1987) Korn-Wendisch, Kempf, Grund, Kroppenstedt and Kutzner 1989, 439[VP] (Basonym: *Saccharopolyspora hirsuta* subsp. *taberi* Labeda 1987, 21; *Saccharopolyspora hirsuta* subsp. *kobensis* Iwasaki, Itoh and Mori 1979, 185.)

ta′ber.i. N.L. gen. masc. n. *taberi* of Taber, named after Willard A. Taber, the American microbiologist who first isolated the organism.

Forms a substrate mycelium that ranges from yellow to orange and from reddish to brownish red on glucose-yeast extract and tryptic soy agars. Neither aerial hyphae nor spores have been detected. Grows from 20 to 45°C, optimally at 37°C. Grows poorly in the presence of 7% NaCl.

Degrades gelatin, but not DNA. Allantoin, arbutin, and uric acid are hydrolyzed. Negative for hemolysis and for the egg yolk reaction. Inositol is used as a sole carbon source.

The dark red metabolite texazone [2-(*N*-methylamino)-3H-phenoxazin-3-one-8-carboxylic acid] is produced. Lysed by three *Saccharopolyspora erythraea* phages (e.g. P517) and four temperate *Saccharopolyspora rectivirgula* phages (e.g. ϕFR-C). Additional phenotypic properties are shown in Table 255.

Whole-organism hydrolysates contain arabinose and galactose as characteristic sugars and *meso*-diaminopimelic acid as the principal diamino acid. Cellular fatty acids contain major proportions of $C_{16:0}$ iso, $C_{17:0}$ iso, $C_{15:0}$ iso, and $C_{17:0}$ anteiso. The predominant menaquinone is MK-9(H_4) and the major polar lipids are diphosphatidylglycerol, phosphatidylcholine, phosphatidylethanolamine, phosphatidylglycerol, phosphatidylmethylethanolamine, and hydroxyphosphatidylethanolamine.

Source: isolated from soil.

DNA G+C content (mol%): 70.8 (HPLC).

Type strain: ATCC 49842, DSM 43856, NBRC 15061, JCM 9383, LL-WRAT-210, NRRL B-16173.

Sequence accession no. (16S rRNA gene): AF002819.

19. **Saccharopolyspora thermophila** Lu, Liu, Wang, Zhang, Qi and Goodfellow 2001, 322[VP]

ther.mo′phi.la. Gr. n. *thermê* heat; N.L. adj. *philus -a -um* (from Gr. adj. *philos -ê -on*) friend, loving; N.L. fem. adj. *thermophila* heat-loving.

Forms an extensively branched colorless to buff substrate mycelium that fragments into rod-shaped elements after 4–5 d at 45°C and an abundant aerial mycelium which carries long hooked to flexuous chains of 4–6 smooth-surfaced, vesicular spores (0.7–1.1 × 0.85–1.5 µm). Grows well on oatmeal, modified Sauton's, and yeast extract-malt extract (ISP medium 2) agars. Does not produce diffusible pigments. The temperature growth range is 45–55°C. Growth occurs in the presence of 7% (w/v) NaCl.

Degrades guanine and xylan, but not cellulose. Adonitol, cellobiose, erythritol, glycerol, inositol, melezitose, methyl α-D-glucoside, salicin, sorbitol, trehalose, acetate, adipate, benzoate, butyrate, citrate, fumarate, H-malate, propionate, pyruvate, sebacic acid, and succinate are used as sole carbon sources, but not tartrate.

Inhibited by lysozyme, gentamicin, streptomycin, and rifampin. Additional phenotypic properties are shown in Table 255.

Whole-organism hydrolysates contain arabinose and galactose as characteristic sugars and *meso*-diaminopimelic acid as the principal diamino acid. The predominant menaquinones are MK-9(H_6) and MK-9(H_8); the polar lipid pattern contains phosphatidylcholine.

Source: isolated from grassland soil.

DNA G+C content (mol%): 73.1 (T_m).

Type strain: 216, AS4.1511, DSM 44575, NBRC 16346, JCM 10664.

Sequence accession no. (16S rRNA gene): AF127526.

20. **Saccharopolyspora tripterygii** Li, Zhao, Qin, Huang, Zhu, Xu and Li 2009a, 3042[VP]

trip.te.ry′gi.i. N.L. n. *Tripterygium* a botanical genus name; N.L. gen. n. *tripterygii* of *Tripterygium*, the plant genus from which this species was isolated.

Forms an extensively branched substrate mycelium and an aerial mycelium which carries long spore chains. Spores are elliptical or short rods (0.5–0.8 × 1.0–1.5 µm) with smooth surfaces. Grows well on glycerol-asparagine (ISP medium 5), inorganic salts-starch (ISP medium 4), potato, and yeast extract-malt extract (ISP medium 2) agars, and moderately well on Czapek's, nutrient, and oatmeal (ISP medium 3) agars. Soluble pigments are not produced. The substrate mycelium is orange-yellow and the aerial mycelium is white on most of the media cited above. Grows at 10–37°C and pH 7.0–8.0. The NaCl tolerance range is up to 12% (w/v).

Degrades Tweens 20 and 40, but not cellulose or gelatin. Catalase-positive, but does not coagulate or peptonize milk or produce H_2S. Acid is produced from amygdalin and esculin, but not from cellobiose, dulcitol, inositol, mannose, oxalate, or sorbitol. D-Arabinose, ribose, acetate, and citrate are used as sole carbon sources. Adenine, L-arginine, L-hydroxyproline, hypoxanthine, L-lysine, L-phenylalanine, L-serine, L-tyrosine, and L-valine are used as sole nitrogen sources, but not L-alanine, glycine, or xanthine. Additional phenotypic properties are shown in Table 255.

Whole-organism hydrolysates contain arabinose and galactose as characteristic sugars and *meso*-diaminopimelic acid as the principal acid. Cellular fatty acids contain major proportions (>10%) of $C_{15:0}$ iso, $C_{17:0}$ anteiso, $C_{18:0}$ iso, and $C_{17:0}$ iso. The predominant menaquinone is MK-9(H_4) and the major polar lipids are phosphatidylcholine, phosphatidylethanolamine, phosphatidylglycerol, phosphatidylinositol, phosphatidylinositol mannosides, phosphatidylmethylethanolamine, and one unknown phospholipid.

DNA–DNA relatedness values (± SD) between the type strain and corresponding strains of closely related species are as follows: "*Saccharopolyspora endophytica*", 57.5 ± 4.5%; *Saccharopolyspora flava*, 44.9 ± 3.5%; and *Saccharopolyspora spinosa*, 48.5 ± 3.0%.

Source: isolated from a surface-sterilized stem sample of *Tripterygium hypoglaucum*.

DNA G+C content (mol%): 70.5 (HPLC).

Type strain: CCTCC AA 208062, DSM 45269, YIM 65359.

Sequence accession no. (16S rRNA gene): FJ214364.

Species *incertae sedis*

1. **"Saccharopolyspora endophytica"** Qin, Li, Zhao, Chen, Xu and Li 2008a, 354

en.do.phy′ti.ca. Gr. adj. *endo* inside; Gr. *phyton* plant; L. fem. suff. *-ica*, adjectival suffix used with the sense of belonging to; N.L. fem. adj. *endophytica* within plant, pertaining to the original isolation from plant tissues.

Forms an extensively branched substrate mycelium. Long, smooth-surfaced spores are borne in straight chains or loose spirals on the aerial mycelium. Grows well on Czapek's, glycerol-asparagine (ISP medium 5), potato, and yeast extract-malt extract (ISP medium 2) agars. White aerial hyphae are formed on most of these media and pink diffusible pigments are formed on some agar media. NaCl tolerance range is 0–15% (w/v). Temperature and pH ranges for growth are 20–45°C and pH 5.0–9.0, respectively. Optimal temperature and pH for growth are 37°C and pH 7.0–7.5, respectively.

Cellulose, chitin, hypoxanthine, starch, tyrosine, Tweens 20 and 80, and xanthine are degraded, but not casein. Milk is not coagulated or peptonized. Does not hydrolyze esculin or urea, or produce H_2S. Cellobiose, dextrin, erythritol, inositol, malate, ribose, sorbitol, starch, trehalose, and xylitol are used as sole carbon sources, but not dulcitol, glycine, oxalate, propionate, sorbose, or succinate. Additional phenotypic features are shown in Table 255.

Whole-organism hydrolysates contain arabinose and galactose as characteristic sugars and *meso*-diaminopimelic acid as the principal diamino acid. Cellular fatty acids contain major proportions of $C_{15:0}$ iso, $C_{16:0}$ iso, $C_{17:0}$ iso, and $C_{17:0}$ anteiso. The predominant menaquinone is MK-9(H_4) and the major polar lipds are diphphatidylglycerol, phosphatidylcholine, phosphatidylethanolamine, phosphatidylglycerol, phosphatidylinositol, and phosphatidylinositol mannosides.

The DNA–DNA relatedness value between the type strain and the corresponding strain of *Saccharopolyspora flava*, a closely related species, is 31%.

Source: isolated from surface-sterilized root of *Maytenus austroyunnanensis*.

DNA G+C content (mol%): 66.2 (HPLC).

Type strain: YIM 61095, KCTC 19397, CCTCC AA 208003.

Sequence accession no. (16S rRNA gene): EU814512.

Genus XV. **Saccharothrix** Labeda, Testa, Lechevalier and Lechevalier 1984, 429[VP] emend. Labeda and Lechevalier 1989, 422

David P. Labeda

Sac.cha.ro′thrix. Gr. n. *sakchâr* sugar; Gr. fem. n. *thrix* hair; N.L. fem. n. *Saccharothrix* sugar-containing hair.

Branched vegetative mycelia (approximately 0.5–0.7 μm diameter); aerial mycelium is produced on some growth media. Both the vegetative and aerial hyphae fragment into coccoid to coccoid-rod, nonmotile elements. A "zig-zag" morphology of the aerial hyphae is typically observed during sporulation of most species. Gram-stain-positive. Resistant to lysozyme. Catalase-positive and aerobic. **Cell wall contains *meso*-diaminopimelic acid. Characteristic whole-cell sugars consist of galactose, rhamnose, and a trace of mannose. The phospholipid pattern contains significant amounts of phosphatidylethanolamine and phosphatidylethanolamine containing 2-hydroxy fatty acids, as well as diphosphatidylglycerol, phosphatidylglycerol, phosphatidylinositol, and phosphatidylinositol mannosides. The principal menaquinones are MK-9(H_4) and MK-10(H_4).** The fatty acid profile consists predominantly of iso- and anteiso-pentadecanoic, hexadecanoic, and heptadecanoic saturated fatty acids along with smaller quantities of unsaturated, straight-chain pentadecanoic, hexadecanoic, and heptadecanoic fatty acids. Phylogenetically, the genus *Saccharothrix* represents a line of descent closest to the genera *Lentzea* and *Lechevalieria*. **The 16S rRNA gene sequence contains the genus-specific diagnostic nucleotide signature pattern of CAC (607–609) and GTG (617–619).**

DNA G+C content (mol%): 67–76 (T_m).

Type species: **Saccharothrix australiensis** Labeda, Testa, Lechevalier and Lechevalier 1984, 430[VP].

TABLE 256. Secondary metabolites produced by the type strains of *Saccharothrix* species

Species	Compound or activity
Saccharothrix algeriensis	Dithiolopyrrolone antibiotics
Saccharothrix australiensis	Antibiotic LL-BM-782-β
Saccharothrix espanaensis	Antibiotic LL-C19004-α
Saccharothrix mutabilis subsp. *capreolus*	Antibiotic capreomycin
Saccharothrix mutabilis subsp. *mutabilis*	Antibiotic polynitoxin
Saccharothrix syringae	Antibiotic nocamycin
Saccharothrix xinjiangensis	Degrades pyrene

Further descriptive information

After the initial description of the genus *Saccharothrix* by Labeda et al. (1984), numerous species were transferred to this genus from other taxa, such as *Actinomadura* and *Nocardiopsis*, based on chemotaxonomic and morphological criteria. The number of species in this genus with validly published names has decreased in recent years as a result of taxonomic reclassifications based on molecular phylogenetic studies and reassessment of chemotaxonomic data. In this regard, *Crossiella cryophila*, *Goodfellowiella coeruleoviolacea*, *Lechevalieria aerocolonigenes*, *Lechevalieria flava*, *Lentzea waywayandensis*, and *Umezawaea tangerina* were all valid species within the genus *Saccharothrix* but were subsequently reclassified (Labeda, 2001; Labeda et al., 2001; Labeda and Kroppenstedt, 2006, 2007) as phylogenetic relationships based on analyses of the 16S rRNA gene were understood. In all of these taxa, a careful re-examination of the chemotaxonomic profiles found differences from that of *Saccharothrix sensu stricto* which were supportive of description as members of new or other existing genera.

Members of the genus *Saccharothrix* have been of great interest as sources of novel secondary metabolites and many of the species were isolated and described as a function of natural products discovery programs in the pharmaceutical industry. The antibiotics produced and the biotechnological activities shown by the type strains of several species of *Saccharothrix* can be seen in Table 256.

Enrichment and isolation procedures

Strains of *Lechevalieria*, *Lentzea*, and *Saccharothrix* have been isolated from soil samples by spread-plating serial soil dilutions onto routine selective media (such as 1.5% crude agar and 0.4% casein hydrolysate in tap water) used for the general isolation of actinomycetes. Shearer (1987) reported the use of typical actinomycete isolation media, such as Gauze mineral medium no. 1 (Gauze et al., 1957) or starch-casein agar (Küster and Williams, 1964), supplemented with 5–10 μg/ml penicillin G and 15 μg/ml nalidixic acid antibiotics to selectively isolate *Saccharothrix* strains. *Saccharothrix algeriensis* was isolated from a Saharan soil sample by plating soil dilutions on the humic acid-vitamin medium of Hayakawa and Nonomura (1987) supplemented with streptomycin sulfate (10 μg/ml) and cycloheximide (50 μg/ml) (Zitouni et al., 2004). More recently, the pyrene-degrading species *Saccharothrix xinjiangensis* was isolated from filtered water samples (0.22 μm pore size) from Tianchi Lake by plating serial dilutions onto agar plates with anthracene, benzene, phenathrene, or pyrene as the sole carbon source (Hu et al., 2004).

Maintenance procedures

Working cultures of *Saccharothrix* can be maintained as refrigerated (4°C) agar slants on an appropriate medium such as NZamine medium (DSMZ medium 554; DSMZ, 2001). The slants are generally incubated for 5–7 d at 28–30°C prior to refrigeration and then should be subcultured at monthly intervals. Longer term preservation of strains is best accomplished as frozen stocks in 20% (v/v) aqueous glycerol at −80°C (mechanical freezer) to −172°C (liquid nitrogen vapor phase) or by using traditional lyophilization techniques.

Procedures for testing special characters

Strains are routinely cultivated on NZamine medium (DSMZ medium 554) at 28°C. Morphological observations are made on the media of Shirling and Gottlieb (1966) and NZamine medium.

Chemotaxonomic analyses of strains for fatty acids, polar lipids, and menaquinones are performed using methods previously described by Grund and Kroppenstedt (1989), Minnikin et al. (1984), Saddler et al. (1991), and Sasser (1990).

Physiological tests, including production of acid from carbohydrates, utilization of organic acids, and hydrolysis and decomposition of adenine, casein, esculin, guanine, hippurate, hypoxanthine, tyrosine, xanthine, and urea, are typically determined using the media of Gordon et al. (1974). Phosphatase activity is evaluated by using the method of Kurup and Schmitt

(1973) substituting NZamine agar as the basal medium. Temperature range for growth is determined on slants of NZamine medium and salt tolerance is determined on slants of the same medium supplemented with 4% and 5% NaCl.

Differentiation of the genus *Saccharothrix* from other genera

The type strains of the species of the genus *Saccharothrix* form a monophyletic lineage that is distinct from the genera *Actinosynnema*, *Lechevalieria*, and *Lentzea* and closest to, but distinct from, the genus *Umezawaea* (see Figure 290). The aligned sequences of the 16S rRNA genes for *Saccharothrix*, *Actinosynnema*, *Lechevalieria*, *Lentzea*, and *Umezawaea* (see Figure 309 in the treatment of the genus *Lechevalieria*) illustrate that *Saccharothrix* strains can easily be distinguished from those of other genera on the basis of the diagnostic nucleotide signatures CAC (607–609) and GTG (617–619). Salazar et al. (2002) also reported a set of specific PCR primers for the genus *Saccharothrix*, Stx2 (5′-AAGGCCCTTCGGGGTACACGAG-3′) and Stx1 (5′-TCGACCGCAGGCTCCACG-3′), that with a PCR annealing temperature of 66°C permitted the rapid detection of all species except *Saccharothrix texasensis*.

The species of the genus *Saccharothrix* can be distinguished chemotaxonomically from those of the genera *Lechevalieria*, *Lentzea*, and *Umezawaea* by the presence of phosphatidylethanolamine containing 2-hydroxy fatty acids in their phospholipid profiles (see Table 257), similar to the genus *Actinosynnema* from which they can be easily distinguished. *Saccharothrix* species can be differentiated from other phylogenetically related genera by the presence of galactose, rhamnose, and a trace of mannose as their whole-cell sugar pattern and the presence of both MK-9(H_4) and MK-10(H_4) as their predominant menaquinones (see Table 257).

Saccharothrix strains produce aerial mycelium on some growth media and both the substrate and aerial hyphae fragment into coccoid to coccoidal-rod-shaped elements. Sporangia, coremia, or motile spores are not produced, which differentiates *Saccharothrix* strains from members of the genera *Actinosynnema* and *Actinokineospora*.

Differentiation of the species of the genus *Saccharothrix*

The physiological characteristics of *Saccharothrix* species are summarized in Table 258 and can be used to differentiate between species, although a common set of physiological tests has not been used in the description of all species. Gross colonial morphology provides some additional information on the species based on the color of the substrate mycelium, production and color of aerial mycelium, and production and color of soluble pigments when grown on agar media.

TABLE 257. Chemotaxonomic characteristics of *Saccharothrix* and related genera[a]

Character	*Saccharothrix*	*Actinosynnema*	*Lechevalieria*	*Lentzea*	*Umezawaea*
Whole-cell sugar pattern[b]	Gal, Rha, Man (trace)	Gal, Man	Gal, Man, Rha	Gal, Man, Rib	Gal, Rha
Phospholipids[c]	PE, OH-PE, PI, PIM, DPG, PG (v)	PE, OH-PE, PI, PIM, DPG	PE	PE, DPG, PG, PI	PE
Predominant menaquinones	MK-9(H_4), MK-10(H_4)	MK-9(H_4), MK-9(H_6)	MK-9(H_4)	MK-9(H_4)	MK-9(H_4), trace MK-10(H_4)

[a]All genera have *meso*-diaminopimelic acid (A_2pm) as the cell-wall diamino acid, are of cell-wall chemotype III, and contain straight-chain, mono-unsaturated, iso, and anteiso fatty acids.

[b]Gal, Galactose; Man, mannose; Rib, ribose; Rha, rhamnose.

[c]DPG, Diphosphatidylglycerol; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; PI, phosphatidylinositol; PIM, phosphatidylinositol mannosides; OH-PE, phosphatidylethanolamine containing hydroxylated fatty acids. v, Variable.

TABLE 258. Physiological properties of *Saccharothrix* species[a]

Character	*S. australiensis*	*S. algeriensis*	*S. coeruleofusca*	*S. espanaensis*	*S. longispora*	*S. mutabilis* subsp. *capreolus*	*S. mutabilis* subsp. *mutabilis*	*S. syringae*	*S. texasensis*	*S. xinjiangensis*
Hydrolysis of:										
Adenine	–	–	–	–	+	–	–	–	–	+
Casein	+	+	+	+	+	+	+	+	+	nd
Esculin	+	+	+	+	+	+	+	+	+	nd
Gelatin	+	+	+	+	+	+	+	+	+	nd
Hippurate	–	nd	–	+	–	+	+	+	+	nd
Hypoxanthine	–	–	–	+	–	+	+	–	–	nd

(continued)

TABLE 258. (continued)

Character	*S. australiensis*	*S. algeriensis*	*S. coeruleofusca*	*S. espanaensis*	*S. longispora*	*S. mutabilis* subsp. *capreolus*	*S. mutabilis* subsp. *mutabilis*	*S. syringae*	*S. texasensis*	*S. xinjiangensis*
Starch	–	–	+	–	+	+	+	+	+	–
Tyrosine	+	+	–	–	+	+	+	+	+	–
Urea	–	nd	–	–	+	–	–	–	–	nd
Xanthine	–	–	–	–	–	–	–	–	–	nd
Production of:										
Soluble pigments	+	+	–	–	–	–	–	+	–	+
Nitrate reductase	+	+	–	w	+	–	+	–	–	–
Phosphatase	–	nd	nd	+	nd	nd	+	nd	+	nd
Acid from:										
Adonitol	–	–	–	–	–	–	–	–	–	nd
Arabinose	–	–	+	–	+	+	+	+	+	+
Cellobiose	+	–	+	+	+	+	+	+	+	nd
Dextrin	+	–	+	–	+	+	+	+	+	nd
Dulcitol	–	–	nd	–	nd	nd	–	nd	–	nd
Erythritol	+	–	nd	–	nd	nd	–	nd	–	nd
Fructose	+	+	+	+	+	+	+	+	v	nd
Galactose	+	+	nd	nd	nd	nd	+	nd	+	nd
Glucose	+	+	+	+	+	+	+	+	+	nd
Glycerol	+	+	nd	+	+	nd	+	nd	+	nd
Inositol	–	–	–	–	–	+	+	–	+	nd
Lactose	–	–	+	–	+	–	+	+	+	+
Maltose	+	+	nd	v	nd	nd	nd	nd	nd	nd
Mannitol	nd	–	nd	–	nd	nd	nd	nd	+	nd
Mannose	+	–	nd	+	nd	nd	+	nd	+	nd
Melibiose	–	–	–	–	–	+	+	+	+	+
Methyl α-D-glucoside	–	–	+	–	–	–	+	–	+	+
Methyl β-xyloside	–	nd	nd	–	nd	nd	nd	nd	v	nd
Raffinose	–	–	+	–	–	–	+	+	–	+
Rhamnose	–	–	+	–	+	–	–	+	+	+
Salicin	–	nd	nd	–	nd	nd	+	nd	+	nd
Sorbitol	+	–	+	–	–	–	–	–	–	–
Sucrose	–	–	+	+	+	–	+	+	+	+
Trehalose	+	nd	nd	+	nd	nd	+	nd	+	nd
Xylose	–	–	+	v	+	+	+	+	+	+
Assimilation of:										
Acetate	+	+	nd	+	nd	nd	+	nd	+	nd
Benzoate	–	–	nd	–	–	nd	–	nd	–	–
Citrate	–	+	–	v	+	–	+	–	–	–
Lactate	v	nd	–	+	+	–	+	+	+	–
Malate	+	nd	–	+	+	+	+	+	+	nd
Mucate	–	nd	nd	–	nd	nd	–	–	–	nd
Oxalate	–	–	nd	–	nd	nd	–	nd	–	nd
Propionate	+	–	nd	v	nd	nd	+	nd	v	nd
Succinate	+	+	nd	+	nd	nd	+	nd	+	nd
Tartrate	–	–	nd	–	nd	nd	–	nd	–	–
Growth in the presence of:										
4% NaCl	+	+	+	+	+	+	–	+	–	–
5% NaCl	–	–	+	–	+	+	–	+	–	–
Growth at:										
10°C	+	+	+	+	+	+	+	+	+	+
37°C	+	+	+	+	+	+	+	+	+	+
45°C	+	+	+	–	–	+	+	+	–	+
50°C	nd	nd	nd	nd	nd	nd	nd	nd	–	+

[a]Symbols: nd, not determined; v, variable positive reaction; w, weak reaction.

List of species of the genus *Saccharothrix*

1. **Saccharothrix australiensis** Labeda, Testa, Lechevalier and Lechevalier 1984, 430[VP]

aus.tra.li.en′sis. N.L. fem. adj. *australiensis* of or belonging to Australia, referring to the location of the soil sample from which the organism was first isolated.

Substrate mycelium is brownish to grayish-yellow in color. Sparse to abundant white to yellowish-gray aerial mycelium produced on many media. Brownish soluble pigments are formed on several media. Temperature for growth is 28°C.

Source: isolated from soil.

DNA G+C content (mol%): 76 (T_m).

Type strain: NRRL 11239, ATCC 31497, DSM 43800, IAM 14291, NBRC 14444, IMSNU 21246, JCM 3370, KCTC 9193, KCTC 9388, NCIMB 13188, VKM Ac-894, LL-BM782Ce82.

Sequence accession nos (16S rRNA gene): X53193.1, AF114803.

2. **Saccharothrix algeriensis** Zitouni, Lamari, Boudjella, Badji, Sabaou, Gaouar, Matthieu, Lebrihi and Labeda 2004, 1380[VP]

al.ge.ri.en′sis. N.L. fem. adj. *algeriensis* of Algeria, where the type strain originated.

Substrate mycelium is vivid yellow, orange-yellow, or yellowish-brown. Copious yellow-orange aerial mycelium is produced. A bright yellow soluble pigment is produced on some media. Temperature for growth is 28°C.

Source: isolated from soil.

DNA G+C content (mol%): not determined.

Type strain: DSM 44581, JCM 13242, NRRL B-24137, SA 233.

Sequence accession no. (16S rRNA gene): AY054972.

3. **Saccharothrix coeruleofusca** (Preobrazhenskaya and Sveshnikova 1974) Grund and Kroppenstedt 1990a, 320[VP] (Effective publication: Grund and Kroppenstedt 1989, 270.) [*Actinomadura coeruleofusca* Preobrazhenskaya and Sveshnikova 1974, 864; *Nocardiopsis coeruleofusca* (Preobrazhenskaya and Sveshnikova 1974) Preobrazhenskaya and Sveshnikova 1985, 224]

co.e.ru.le.o.fus′ca. L. adj. *coeruleus* dark-colored, dark blue; L. adj. *fuscus* dark, swarthy, dusky, tawny; N.L. fem. adj. *coeruleofusca* blue-brown (referring to the color of aerial and substrate mycelium).

Substrate mycelium is yellowish in color. Only sparse aerial mycelia, if any, are produced on complex growth media. On glycerol-nitrate or starch-nitrate media, a blue to dark blue aerial mycelium is produced. Temperature for growth is 28°C.

Source: isolated from soil.

DNA G+C content (mol%): 67.0 (T_m) (Poschner et al., 1985).

Type strain: ATCC 35108, BCRC 13313, DSM 43679, IFO 14520, IMET 9602, IMSNU 21357, INA 1335, JCM 3313, KCTC 9389, NRRL B-16115, VKM Ac-855.

Sequence accession no. (16S rRNA gene): AF114805.

4. **Saccharothrix espanaensis** Labeda and Lechevalier 1989, 422[VP]

es.pa.na.en′sis. N.L. fem. adj. *espanaensis* of or belonging to Spain, referring to the source of the soil sample, Puerto Llano (Spain) from which the micro-organism was first isolated.

Substrate mycelium is grayish-yellow to yellowish-brown. Sparse white aerial mycelium is produced on some media, particularly glycerol-asparagine agar and inorganic salts-starch agar (ISP 3) and a yellow soluble pigment is also produced on these media. A brown to reddish-brown soluble pigment is formed on rich complex media. Temperature for growth is 28°C.

Source: isolated from soil.

DNA G+C content (mol%): 72.2 (T_m).

Type strain: ATCC 51144, CGMCC 4.1714, DSM 44229, IFO 15066, IMSNU 21342, JCM 9112, KCTC 9392, NBRC 15066, NRRL 15764, VKM Ac-1969.

Sequence accession no. (16S rRNA gene): AF114807.

5. **Saccharothrix longispora** (Preobrazhenskaya and Sveshnikova 1974) Grund and Kroppenstedt 1990a, 320[VP] (Effective publication: Grund and Kroppenstedt 1989, 272.) [*Actinomadura longispora* Preobrazhenskaya and Sveshnikova 1974, 866; *Nocardiopsis longispora* (Preobrazhenskaya and Sveshnikova 1974) Preobrazhenskaya and Sveshnikova 1985, 224]

lon.gi.spo′ra. L. adj. longus long; Gr. n. spora a seed and in biology a spore; N.L. n. *longispora* (nominative in apposition) the long spore, referring to the oblong shape of the spores.

Substrate mycelium has a yellow or red color and aerial mycelium is normally absent. On glycerol-nitrate agar a blue aerial mycelium is formed. Temperature for growth is 28°C.

Source: isolated from soil.

DNA G+C content (mol%): 68.0 (T_m) (Poschner et al., 1985).

Type strain: INA 10222, ATCC 35109, BCRC 13395, DSM 43749, HUT-6594, NBRC 14522, IMET 9603, IMSNU 21359, JCM 3314, KCTC 9394, NRRL B-16116, VKM Ac-907.

Sequence accession no. (16S rRNA gene): AF114809.

6. **Saccharothrix mutabilis** (Shearer, Colman and Nash 1983) Labeda and Lechevalier 1989, 422[VP] (*Nocardiopsis mutabilis* Shearer, Colman and Nash 1983, 374)

mu.ta′bi.lis. L. fem. adj. *mutabilis* changeable, variable, inconstant, referring to the variety of colony morphologies observed, particularly on rich organic media.

Substrate mycelium is yellow to yellowish-brown. White aerial mycelium is produced on most media. Upon exposure to light, the aerial mycelium may turn orange-yellow. Light yellow to yellowish-brown soluble pigments are produced on some media. Temperature for growth is 28°C.

Source: isolated from soil.

DNA G+C content (mol%): 73.1 (T_m).

Type strain: ATCC 31520, BCRC 12528, DSM 43853, IFM 240, NBRC 14310, IMSNU 21336, JCM 3380, KCTC 9397, NRRL B-16077, SKF AAA-025, VKM Ac-2023.

Sequence accession no. (16S rRNA gene): X76966.

This species has subsequently been divided into subspecies as follows.

6a. **Saccharothrix mutabilis subsp. mutabilis** (Shearer, Colman and Nash 1983) Labeda and Lechevalier 1989, 422[VP] (*Nocardiopsis mutabilis* Shearer, Colman and Nash 1983, 374)

Characteristics as above for *Saccharothrix mutabilis*.

6b. **Saccharothrix mutabilis subsp. capreolus** (*ex* Stark, Higgens, Wulfe, Hoehn and McGuire 1963) Grund and Kroppenstedt 1990a, 320[VP] (Effective publication: Grund and Kroppenstedt 1989, 273.) (*Streptomyces capreolus* Stark, Higgens, Wulfe, Hoehn and McGuire 1963, 596)

ca.pre′o.lus. L. masc. n. *capreolus* roebuck or chamois, two-pronged like the chamois, bifurcate.

Substrate mycelium is yellowish to brownish in color. Sparse, white aerial mycelium is produced on some media. Temperature for growth is 28°C.

Source: isolated from soil.

DNA G+C content (mol%): not determined.

Type strain: ATCC 23892, BCRC 13692, CBS 678.68, DSM 40225, NBRC 12847, IMSNU 21351, ISP 5225, JCM 4248, JCM 4630, KCTC 9395, NCIMB 9611, NCIMB 9801, NRRL 2773, RIA 1167, VKM Ac-1848.

Sequence accession no. (16S rRNA gene): X76865.

7. **Saccharothrix syringae** (Gauze and Sveshnikova 1985) Grund and Kroppenstedt 1990a, 320[VP] (Effective publication: Grund and Kroppenstedt 1989, 273.) (*Nocardiopsis syringae* Gauze, Sveshnikova, Ukholina, Komarova and Bazhanov 1977, 483)

sy.rin′ga.e. N.L. fem. n. *Syringa* generic name of lilac; N.L. fem. gen. n. *syringae* of the lilac (referring to the color of aerial mycelium).

Substrate mycelium is yellowish to brownish in color. Aerial mycelium is sparse and white in color on most media. The aerial mycelium is reported (Gauze et al., 1977) to be pale to dark lilac in color on Gauze no. 1 mineral agar (Gauze et al., 1957) or glycerol-nitrate agar (Lindenbein, 1952), hence the species name. Temperature for growth is 28°C.

Source: isolated from soil.

DNA G+C content (mol%): not determined.

Type strain: INA 2240, AS 4.1716, ATCC 51364, DSM 43886, NBRC 14523, IMET 9675, JCM 6844, KCTC 9398, NRRL B-16468, VKM Ac-1858.

Sequence accession no. (16S rRNA gene): AF114812.

8. **Saccharothrix texasensis** Labeda and Lyons 1989, 357[VP]

tex.as.en′sis. N.L. fem. adj. *texasensis* of or belonging to the state of Texas, referring to the source of the soil samples from which the species was first isolated.

Substrate mycelium is dark yellow to brownish-yellow. Sparse, white aerial mycelium is produced on some media. A brown to reddish-brown soluble pigment is formed on some media. Temperature for growth is 28°C.

Source: isolated from soil.

DNA G+C content (mol%): 74 (T_m).

Type strain: NRRL B-16134, AS 4.1713, ATCC 51593, DSM 44231, NBRC 14971, IMSNU 21343, JCM 9113, KCTC 9399, NCIMB 13186, VKM Ac-1968.

Sequence accession no. (16S rRNA gene): AF114814.

9. **Saccharothrix xinjiangensis** Hu, Zhou, Zhou, Liu and Liu 2004, 2094[VP]

xin.ji.ang.en′sis. N.L. fem. adj. *xinjiangensis* pertaining to Xinjiang, where the type strain was isolated.

The substrate mycelium is pinkish-buff in color on yeast extract-malt extract agar and pale orange to pale brown on other media. The aerial mycelium is grayish-white in color. Pale brown soluble pigments are produced on some media. Temperature for growth is 28°C.

Source: isolated from lake water.

DNA G+C content (mol%): 70.4 (T_m).

Type strain: AS 4.1731, DSM 44896, JCM 12329, NRRL B-24321, PYX-6.

Genus XVI. **Streptoalloteichus** Tomita, Nakakita, Hoshino, Numata and Kawaguchi 1987, 211[VP] emend. Tamura, Ishida, Otoguro, Hatano and Suzuki 2008b, 689

Seung Bum Kim and Tomohiko Tamura

Strep.to.al.lo.tei′chus. Gr. adj. *streptos* bent; Gr. adj. *allos* different; Gr. n. *teichos* wall; N.L. masc. n. *Streptoalloteichus* intended to mean streptomycete with different wall.

Gram-stain-positive, non-acid-fast, aerobic actinomycetes that show well-branched and non-fragmenting substrate hyphae. **Aerial mycelium bears chains of five to 50 spores** (0.5–1.2 µm wide) at the tip of well-branched hyphae. **Sporangia-like structures are formed**. The predominant menaquinones are **MK-9(H_6) and MK-10(H_6)**. The cell-wall peptidoglycan contains a major amount of ***meso*-diaminopimelic acid**. The predominant cellular fatty acids are **$C_{15:0}$ iso and $C_{17:0}$ anteiso**. Whole-cell hydrolysates contain **D-galactose and D-mannose**, whereas L-rhamnose and D-glucose are variably present. **Phosphatidylethanolamine** is present as the diagnostic phospholipid (phospholipid pattern type PII). Habitat: soil.

Type species: **Streptoalloteichus hindustanus** Tomita, Nakakita, Hoshino, Numata and Kawaguchi 1987, 211[VP].

Further descriptive information

The genus *Streptoalloteichus* currently belongs to the family *Pseudonocardiaceae* (Stackebrandt et al., 1997) and contains two species, *Streptoalloteichus hindustanus* and *Streptoalloteichus tenebrarius* (Table 259). However, the taxonomic affiliation of the genus at the family level looks rather unclear. *Streptoalloteichus*, together with *Crossiella* and *Kutzneria*, have been consistently placed outside both the *Pseudonocardiaceae* and *Actinosynnemataceae* clades, still residing within the former suborder *Pseudonocardineae* (Labeda, 2001; Labeda and Kroppenstedt, 2006), elevated in this volume to order *Pseudonocardiales*. Further phylogenetic studies on these genera and related taxa might be necessary to clarify their taxonomic positions within the order.

TABLE 259. Differential characteristics that distinguish the two species of the genus *Streptoalloteichus*[a]

Characteristic	*S. hindustanus* C677-91^T	*S. tenebrarius* NBRC 16177^T
Morphology:		
Sporangia-like vessels	+	–
Motile spores	+	–
Cell chemistry:		
Whole-cell sugars[b]	Gal, Man, Rha	Gal, Man, Glc
Menaquinones (MK-)	9(H_6), 10(H_6)	10(H_6), 10(H_4), 9(H_6), 9(H_4)
DNA G+C content (mol%)	nd	71.6
Cultural characteristics on inorganic salts-starch agar (ISP 4):		
Vegetative mycelium	Thin, colorless to grayish yellow	Grayish pink
Aerial mycelium	Pale pinkish yellow	Light grayish-yellow with white areas
Diffusible pigment	None	Grayish pink
Cultural characteristics on glycerol-asparagine agar (ISP 5):		
Growth	Restricted	Moderate
Vegetative mycelium	Thin, colorless to grayish yellow	Pale yellow
Aerial mycelium	Patches, white, turning yellowish gray later	Pale yellow with white areas
Cultural characteristics on Bennett's agar:		
Vegetative mycelium	Pale olivaceous yellow to light brown	Pale yellow
Aerial mycelium	Velvety, light yellowish beige	White
Utilization of:		
Maltose	–	+
L-Arabinose	–	+
D-Xylose	–	+
Salicin	–	+
myo-Inositol	–	+
Lactose	Weak	–
Antibiotic production	Tallysomycins A, B, and C; nebramycin factors II, IV′, and V′	Nebramycin factors I to XIII
Tolerance to 7% (w/v) NaCl[c]	–	+
Light sensitivity for formation of aerial mycelium	–	+

[a]Modified from Table 1 of Tamura et al. (2008b); nd, not determined.

[b]Gal, galactose; Man, mannose; Rha, rhamnose; Glc, glucose.

[c]Both grow at 5%, neither grows at 10%.

Streptoalloteichus hindustanus and *Streptoalloteichus tenebrarius* share 99.5% 16S rRNA gene sequence similarity, but only 24.3–37.9% DNA–DNA relatedness (Tamura et al., 2008b). The two species are clearly distinguishable by their morphological properties: *Streptoalloteichus hindustanus* produces oval or spherical sporangia-like vessels on the vegetative hyphae (Figure 332) and motile spores with a single polar flagellum. Sporangia-like structures can also be found, albeit with lower frequency, in *Streptoalloteichus tenebrarius* (Figure 333). However, the spores of *Streptoalloteichus tenebrarius* are nonmotile.

Purified cell wall preparations contain *meso*-diaminopimelic acid, but not the L-isomer or the hydroxy analogs. Alanine and glutamic acid are found in *Streptoalloteichus tenebrarius*. Whole-cell hydrolysates contain galactose and mannose; in addition, rhamnose and glucose are present in *Streptoalloteichus hindustanus* and *Streptoalloteichus tenebrarius*, respectively. The diagnostic phospholipid is phosphatidylethanolamine (phospholipid type PII). The predominant fatty acids of *Streptoalloteichus* are $C_{15:0}$ iso and $C_{17:0}$ anteiso.

Both species of *Streptoalloteichus* are known to produce nebramycins and *Streptoalloteichus hindustanus* is also known to produce tallysomycins.

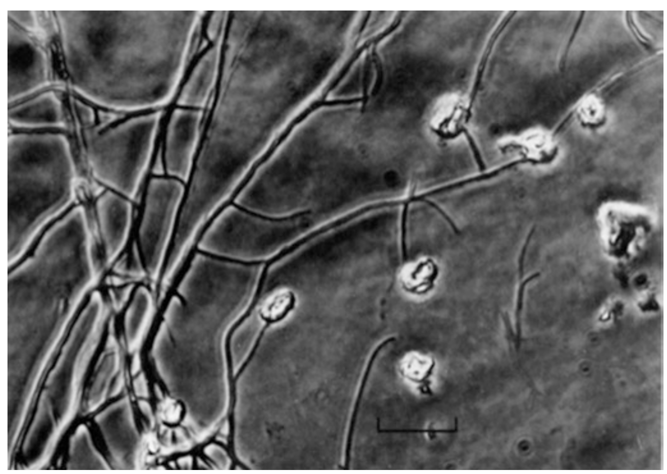

FIGURE 332. Sporangia-like vessels enveloping a single spore or a row of two to four spores of *Streptoalloteichus hindustanus*, which are formed on the sporangiophore of the substrate mycelium. Bar = 10 µm.

Enrichment and isolation procedures

Streptoalloteichus hindustanus was isolated from dry soil samples collected in Gujarat or adjacent states where the natural vegetation is dry tropical forest and scrub using nutrient agar supplemented with butirosin (50 µg/ml). Yeast extract-malt extract agar (ISP 2) or Bennett's agar, supplemented with kanamycin or gentamicin (10 µg/ml) and nystatin (100 µg/ml), can also be used. The agar plates are incubated for 1–2 weeks at 43°C. Colonies of *Streptoalloteichus hindustanus*

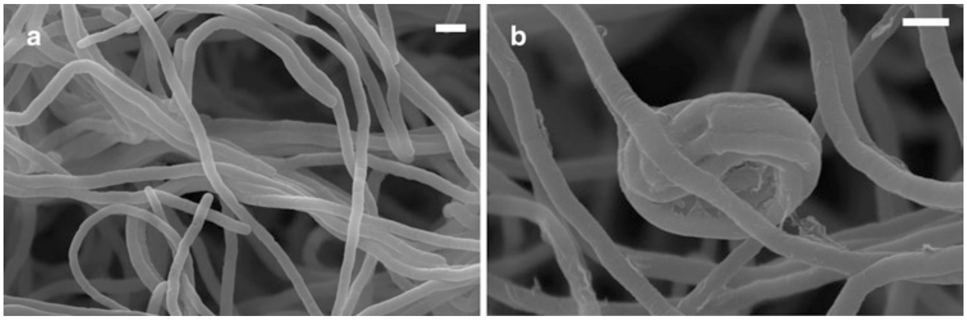

FIGURE 333. Scanning electron micrographic images of *Streptoalloteichus tenebrarius* showing the formation of arthrospore chains (a) and sporangia-like structure (b). Bars = 1 µm.

are distinguishable from those of most species of the genus *Streptomyces* by pale pinkish-yellow aerial mycelium, and the lack of a distinct reverse-side color.

Streptoalloteichus tenebrarius can be isolated from soil and grows on various media such as ISP 2, 4, and 5, and Bennett's agar medium.

Maintenance procedures

Streptoalloteichus strains can be maintained on media that is used universally for actinobacteria. The mixture of spores and mycelial fragments can be stored in 20% (v/v) glycerol solution at temperatures below −20°C or as lyophilized powder. Alternatively, the mixture is suspended in sterilized skim milk (10%) and stored at −80°C in screw-capped tubes. Lyophilization of the spore suspension is carried out by standard procedures for actinobacteria and the lyophilized culture is preserved under a vacuum of between 0.05 and 0.005 mmHg.

Procedures for testing special characters

Sporangia formation may be induced by growing the strains at 28°C for 3–4 weeks on ISP 2 or ISP 5 agar. Microscopic observation of sporangia and other structures in the vegetative mycelium can be achieved by preparing the cultures using the coverslip technique (Kawato and Shinobu, 1959).

Alternatively, ISP 5 broth or soil extract broth (Tomita, 1989) may be used. Media in metal-capped tubes (18×180 mm in size; 3 ml medium per tube) are autoclaved, inoculated heavily with a spore suspension, and incubated as slants for 4–6 weeks at 28°C. The sporangia can be observed at hyphal tips of mycelial masses.

Differentiation from closely related taxa

Streptoalloteichus is phylogenetically mostly related to members of the genera *Goodfellowiella*, *Crossiella*, and *Kibdelosporangium*, with 16S rRNA gene sequence similarities between strains of 95 to 96% (Figure 334). However, *Streptoalloteichus* can be differentiated from the other three genera based on chemotaxonomic properties. The major isoprenoid quinones of *Streptoalloteichus* are MK-9(H_6) and MK-10(H_6), whereas those of *Goodfellowiella* are MK-9(H_4) and MK-10(H_4), and that of *Crossiella* and *Kibdelosporangium* is MK-9(H_4). Mannose is one of the diagnostic sugars for *Streptoalloteichus*, but not for *Goodfellowiella* or *Kibdelosporangium*.

Streptoalloteichus can be distinguished from other genera of the family *Pseudonocardiaceae* by a combination of morphological and chemotaxonomic properties, such as spore chains, sporangia-like structures, phospholipid type, menaquinone profiles, and diagnostic sugars. In particular, the menaquinone profiles, consisting of MK-9(H_6) and MK-10(H_6), distinguish the genus from the rest of the family *Pseudonocardiaceae*. The presence of galactose and mannose in the cell wall also separates the genus from the genera *Actinoalloteichus*, *Actinopolyspora*, *Amycolatopsis*, *Goodfellowiella*, *Kibdelosporangium*, *Pseudonocardia*, *Prauserella*, *Saccharomonospora*, and *Saccharopolyspora*. The phospholipid pattern of *Streptoalloteichus* is type II *sensu* Lechevalier et al. (1977a), which is different from those of *Actinopolyspora* (type III), *Saccharopolyspora* (type III), and *Thermobispora* (type IV).

Formation of sporangia-like structures separates the genus from *Actinoalloteichus*, *Actinopolyspora*, *Goodfellowiella*, *Prauserella*, *Saccharopolyspora*, and *Thermobispora*. Mycelial fragmentation is not observed in *Streptoalloteichus*, which is different from *Actinoalloteichus*, *Amycolatopsis*, *Crossiella*, *Goodfellowiella*, *Kibdelosporangium*, *Prauserella*, *Saccharopolyspora*, and *Thermoscrispum*.

Streptoalloteichus can also be differentiated from related genera using 16S rRNA gene restriction fragment length polymorphism (RFLP) patterns, as suggested by Cook and Meyers (2003). All actinobacterial taxa can be divided into three distinct groups based on the RFLP patterns of 16S rRNA gene with *Sau*3A1. *Streptoalloteichus* belongs to Group 3; additional RFLP patterns with restriction enzymes *Asn*1 and *Sph*1 separate the genus from other genera of Group 3.

Taxonomic comments

The genus *Streptoalloteichus* with *Streptoalloteichus hindustanus* as the type and only species was formally published by Tomita et al. (1987), although the genus was originally proposed in 1978 (Tomita et al., 1978). *Streptoalloteichus tenebrarius* was originally described as "*Streptomyces tenebrarius*" (Higgins and Kastner, 1967). *Streptoalloteichus hindustanus* and *Streptoalloteichus tenebrarius* share a number of properties in common, e.g. cell-wall type, production of short spore chain clusters, smooth-surfaced spores, sclerotium formation, and pale pinkish-yellow aerial mycelium. On the other hand, the two species can be differentiated from each other based on various phenotypic characters. For example, the sporangia-like vessels enveloping motile spores are rarely observed in *Streptoalloteichus tenebrarius* and light sensitivity in the formation of aerial mycelium is only observed in *Streptoalloteichus tenebrarius*. Growth at 45°C has been reported for *Streptoalloteichus hindustanus*, but not for *Streptoalloteichus tenebrarius*. Similarly, *Streptoalloteichus tenebrarius* can grow in the presence of 7% NaCl, but *Streptoalloteichus hindustanus* cannot. The latter produces tallysomycins A, B, and C, and nebramycin factors II, IV′, and V′, whereas *Streptoalloteichus tenebrarius* produces nebramycin factors I to XIII.

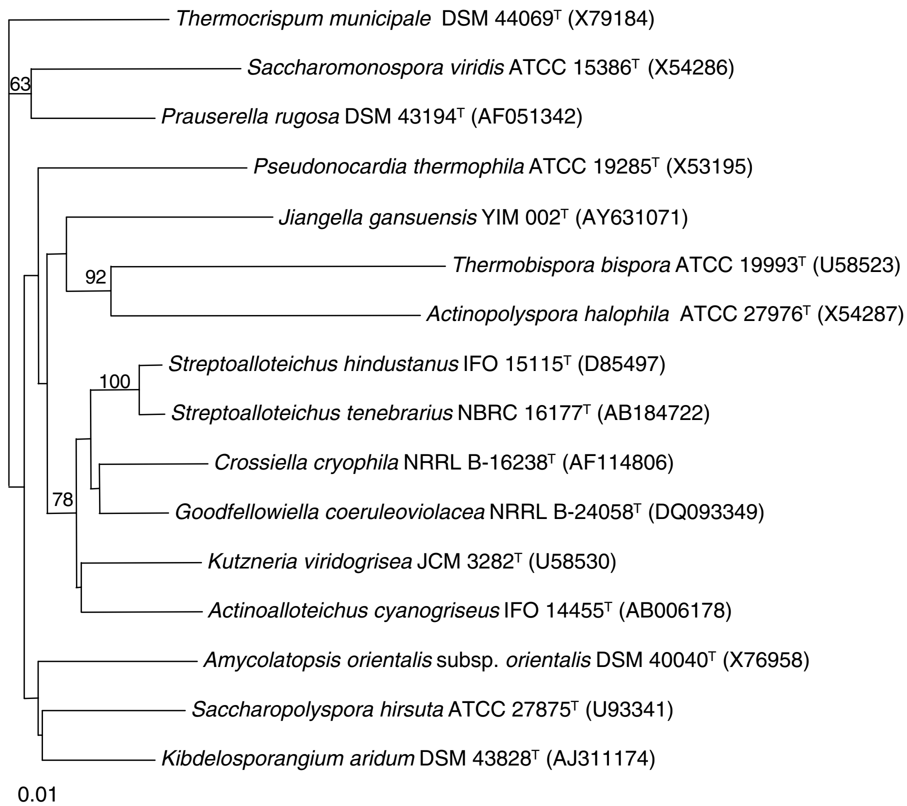

FIGURE 334. Phylogenetic tree of the genus *Streptoalloteichus* and related taxa based on 16S rRNA gene sequences. The Jukes–Cantor model was used in the estimation of evolutionary distances and the neighbor-joining method was used for tree construction. Numbers at nodes indicate levels of bootstrap support (%). Bar = 0.01 substitutions per nucleotide position.

Miscellaneous comments

Streptoalloteichus hindustanus has the ability to bear both non-motile conidiospores and motile sporangiospores. This morphology may be derived from adaptation to the original environments of this micro-organism, which have dry and rainy seasons in a year. *Streptoalloteichus hindustanus* was reported to produce tallysomycins A, B, and C, antitumor compounds, and BMY-28190, an antiviral agent (Ohkuma et al., 1988). *Streptoalloteichus* sp. 1454-19 was reported to produce siderophores IC202A, B, and C (Iijima et al., 1999a, 1999b). The bleomycin resistance (*ble*) gene from *Streptoalloteichus hindustanus* has been used as a dominant selection marker to construct transformation systems in animal, plant, fungal, and bacterial cells (Drocourt et al., 1990).

List of species of the genus *Streptoalloteichus*

1. **Streptoalloteichus hindustanus** Tomita, Nakakita, Hoshino, Numata and Kawaguchi 1987, 211[VP]

hin.du.stan′us. N.L. masc. adj. *hindustanus* of Hindustan, northwest region of India.

The aerial mycelium is abundant on most culture media. After sporulation, the aerial mycelium is pale in color and yellow, pink, or gray depending on the medium. The vegetative mycelium is colorless to light yellowish brown. Both substrate and aerial mycelia are well developed and branched, averaging 0.5 µm in diameter. Chains of spores are formed only at the tip of the aerial mycelium. These may be either long (10–50 spores in a chain) or short, hooked, branching spore chains. The individual arthrospores in the long chains are oval to cylindrical (0.5–2.0 µm in diameter) and the conidiospores in the short chains are barrel shaped. The spore surface is smooth in both cases. Spores are motile with a single polar flagellum. The spore chain cluster consists of curved or L-shaped conidiophore chains with many branches and often develops into a thick mass. The substrate mycelium does not fragment. The substrate mycelium penetrating the agar is thin, especially in chemically defined media; it is covered with a thick mass of white aerial mycelium that turns to pale yellow after sporulation. Single, oval, or spherical sporangia-like vessels enveloping one spore or a single row of two to four spores are randomly formed among the vegetative hyphae. Sporangia are 1.5–4.5 × 2.7–7.0 µm in size and sporangiospores are 0.9–1.5 × 1.2–4.0 µm. Globose dense bodies consisting of coalesced vegetative mycelium and sclerotia may be formed on the aerial mycelium. The temperature range for growth is 20–54°C; no growth is observed at 56°C. The optimal temperature for growth is 45°C. Additional cultural and physiological characteristics are shown in Table 260.

TABLE 260. Cultural characteristics of *Streptoalloteichus hindustanus*

Medium	Aerial mycelium	Vegetative mycelium	Diffusible pigments
Yeast extract-malt extract (ISP 2)	Thick pale yellowish pink	Light yellowish brown	None
Inorganic salts-starch (ISP 4)	Pale pinkish yellow	Thin, colorless to grayish yellow	None
Glycerol-asparagine (ISP 5)	Patches, white, turning yellowish gray later	Thin, colorless to grayish yellow	None
Peptone-yeast extract-iron (ISP 6)	Scant, white	Moderate brown	Light yellowish brown

Gelatin, casein, and starch are hydrolyzed; skim milk is coagulated and slightly peptonized. Melanoid pigments are not formed on ISP 1, 6, or 7. Catalase-positive, but tyrosinase-negative. Nitrate reduction occurs. Abundant growth is observed at 30–50°C, but no growth is seen at 56°C. Growth occurs with up to 5% (w/v) NaCl, but not with 7% (w/v). Utilizes D-fructose, D-galactose, D-glucose, and D-mannose as sole carbon sources, but not L-arabinose, inositol, D-mannitol, D-raffinose, L-rhamnose, D-sorbitol, or D-xylose. Weak growth occurs on lactose and sucrose. Resistant to ampicillin, cephalothin, erythromycin, gentamicin, kanamycin, and tetracycline at 100 µg/ml, and to novobiocin at 25 µg/ml. Less resistant to chloramphenicol and rifampin. Major menaquinones are MK-9(H_6) and MK-10(H_6).

Source: soil.

DNA G+C content (mol%): Not known.

Type strain: ATCC 31217, IFO 15115.

Sequence accession no. (16S rRNA gene): D85497.

2. **Streptoalloteichus tenebrarius** (*ex* Higgins and Kastner 1967) Tamura, Ishida, Otoguro, Hatano and Suzuki 2008b, 689[VP] (Subjective synonym: "*Streptomyces tenebrarius*" Higgins and Kastner 1967.)

te.ne.bra′ri.us. L. masc. adj. *tenebrarius* of or belonging to darkness, reflecting the sensitivity of the culture to light.

Straight spore chains are formed on short sporophores on the substrate mycelium. Spores have a smooth surface and are short, nonmotile rods (0.6–0.9 × 1.0–1.5 µm). Sporangia-like structures, though not common, can be formed. No soluble pigment is produced on ISP 7 agar medium.

Starch is hydrolyzed weakly and gelatin is hydrolyzed. Calcium malate is not decomposed and milk is not coagulated or peptonized. Optimum temperature for growth is 20–30°C; grows at 37°C, but not at 45°C. Grows in the presence of up to 8% (w/v) NaCl, but not in 10% (w/v) NaCl.

N-Acetyl-D-galactosamine, *N*-acetyl-D-glucosamine, arabonic acid, *p*-arbutin, D-fructose, gluconate, D-glucosamine, D-glucosaminic acid, D-glucose, glycogen, maltose, D-mannose, D-ribose, L-arabinose, D-xylose, sucrose, raffinose, salicin, starch, trehalose, adonitol, glycerol, *myo*-inositol, acetate, butyrate, caprate, isobutyrate, propionate, *cis*-aconitate, azelate, citrate, fumarate, DL-3-hydroxybutyrate, D-lactate, DL-lactate, DL-malate, L-malate, oxaloacetate, 2-oxoglutarate, pyruvate, acetamidocaprate, acetyl L-glutamine, acetyl glycine, D-alanine, β-alanine, L-arginine, L-asparagine, L-aspartate, casein, L-citrulline, L-glutamate, L-glutamine, L-histidine, L-isoleucine, L-leucine, L-ornithine, L-proline, L-serine, L-tryptophan, L-valine, and ethanolamine are utilized as sole carbon sources (as sodium salts where applicable). Major menaquinones are MK-10(H_6), MK-10(H_4), MK-9(H_6), and MK-9(H_4).

Source: soil.

DNA G+C content (mol%): 71.6 (HPLC)

Type strain: NBRC 16177, ATCC 17920, DSM 40477, JCM 4838, NRRL B-12390, ISP 5477.

Sequence accession no. (16S rRNA gene): AB184722.

Genus XVII. **Thermocrispum** Korn-Wendisch, Rainey, Kroppenstedt, Kempf, Majazza, Kutzner and Stackebrandt 1995, 73[VP]

Seung Bum Kim

Ther.mo.cris′pum. Gr. adj. *thermos* warm, hot; L. neut. adj. *crispum* tightly curled; N.L. neut. n. *Thermocrispum* a heat-loving, tightly curled organism.

Thermophilic, Gram-stain-positive, aerobic, catalase-positive, and non-acid-fast. Produces filamentous, branched hyphae. The **aerial mycelium is white**, and the **vegetative mycelium is yellow to light brown**. Soluble pigments are not produced. **Aerial hyphae are straight to flexuous and often aggregate into clusters (pseudosporangia) that fragment into rod-like structures**. Good growth occurs on Czapek Dox-yeast extract-Casamino acids (CYC) agar, glucose-yeast extract-malt extract (GYM) agar, Hickey–Tresner agar, oatmeal agar, potato-carrot agar, peptone-maize extract (PM) agar, R2A agar, and R8 agar, as well as on tryptic soy agar (TSA). **The temperature range for growth is 20–62.5°C**; the optimum range is 45–55°C. Growth occurs between pH 6.0 and 11.0, and also in the presence of NaCl (5%, w/v), novobiocin (25 µg/ml), and crystal violet (0.2 µg/ml). Nitrate is reduced to nitrite under aerobic conditions. The **cell wall contains *meso*-diaminopimelic acid and the whole-cell sugar pattern is type C**; **major amounts of arabinose, mannose, and glucose are present**, but only traces of galactose are detected. **Phospholipid pattern is type PII**, containing phosphatidylethanolamine as the diagnostic phospholipid. Mycolic acids are not present. The **predominant menaquinone is MK-9(H_4)**. Resistant to a set of phages that infect the genera *Amycolatopsis*, *Pseudonocardia*, *Saccharomonospora*, *Saccharopolyspora*, and *Saccharothrix*. Sensitive to genus-specific phages isolated from different habitats.

DNA G+C content (mol%): 69–73 (HPLC).

Type species: **Thermocrispum municipale** Korn-Wendisch, Rainey, Kroppenstedt, Kempf, Majazza, Kutzner and Stackebrandt 1995, 73[VP].

Further descriptive information

The genus *Thermocrispum* belongs to the family *Pseudonocardiaceae* and currently contains two species, *Thermocrispum agreste* and *Thermocrispum municipale* (Figure 335). The 16S rRNA gene sequence similarity between the type strains of the two species was reported as 98.2% (Korn-Wendisch et al., 1995), but BLAST (http://blast.ncbi.nlm.nih.gov/blast.cgi) results indicate a lower similarity value (96.0%).

Good growth of all *Thermocrispum* strains can be observed on the media mentioned in the genus description. Macroscopically, the isolates are similar to members of the genus *Saccharopolyspora*. All strains produce abundant white aerial mycelium and yellow to light brown substrate mycelium on CYC agar (Cross and Attwell, 1974) and potato-carrot agar (Cross et al., 1963), whereas sparse to moderate aerial mycelium is formed on TSA, GYM agar (ISP medium 4), oatmeal agar (ISP medium 3), PM agar (Agre, 1964), and R2A agar (Reasoner and Geldreich, 1985). Growth is poor on glycerol-arginine agar (El-Nakeeb and Lechevalier, 1963). The surfaces of colonies are covered with straight to flexuous aerial hyphae and both substrate and aerial mycelia often form aggregates (Figure 336). The aerial mycelium fragments into rod-like structures (Figure 337). Pseudosporangia similar to the structures of *Kibdelosporangium* can be observed in all strains. However, these structures are not surrounded by a well-defined wall and contain septate hyphae which fragment into rod-like structures (Figure 338).

Thermocrispum strains can degrade or hydrolyze casein, tyrosine, gelatin, DNA, esculin, Tween 80, and tributyrin, but not adenine, allantoin, cellulose, chitin, poly-β-hydroxybutyric acid, hypoxanthine, starch, urea, xanthine, or xylan. Pectin is weakly degraded and arbutin is hydrolyzed slowly. Melanoid pigments are not produced. Cellobiose, dextrin, galactose, glucose, inositol, mannose, sucrose, trehalose, sodium malonate, and sodium pyruvate are utilized as sole carbon sources, but adonitol, inulin, lactose, melibiose, raffinose, rhamnose, xylitol, xylose, and sodium propionate are not. Mannitol, melezitose, and sorbitol are utilized poorly.

All strains grow well at temperatures ranging from 28 to 60°C and between pH 6.0 and 11.0. Growth is weak at 20°C and inhibited at 65°C. All strains are resistant to novobiocin and crystal violet, and also to 5% (w/v) NaCl when the basal medium is GYM agar or TSA. All strains are susceptible to lysozyme degradation. *Thermocrispum* strains do not exhibit antibiotic activity against *Escherichia coli*, *Corynebacterium glutamicum*, *Bacillus subtilis*, or *Staphylococcus aureus*. The type strain of *Thermocrispum agreste* is slightly active against *Rhodococcus rhodochrous* and *Micrococcus luteus*, and also inhibits *Azotobacter chroococcum*, *Candida albicans*,

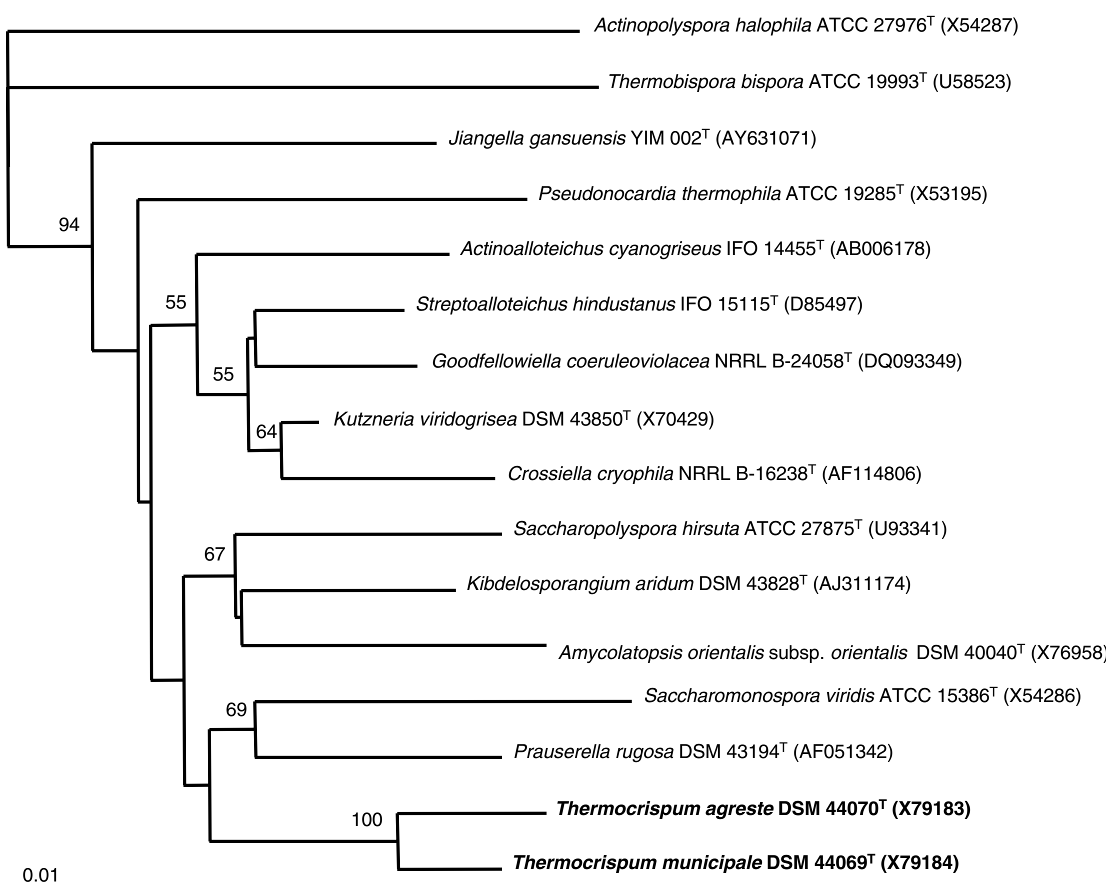

FIGURE 335. Phylogenetic tree of the genus *Thermocrispum* and related taxa based on 16S rRNA gene sequences. The Jukes–Cantor model was used in the estimation of evolutionary distances and the neighbor-joining method was used for tree construction. Numbers at nodes indicate levels of bootstrap support (%). Bar = 0.01 substitutions per nucleotide position.

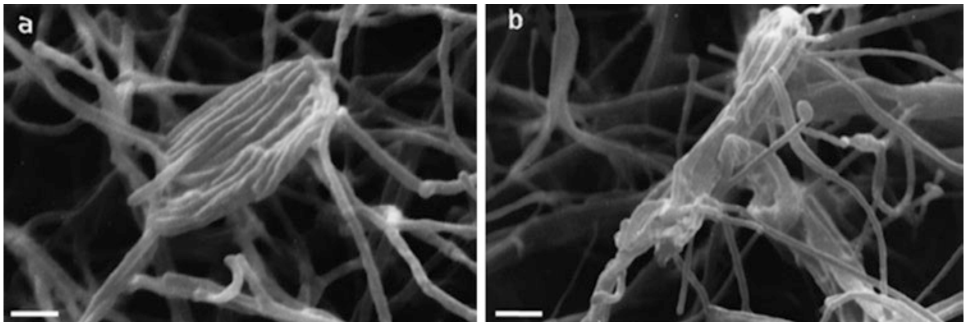

FIGURE 336. Scanning electron micrographic images of aerial mycelia of *Thermocrispum municipale* MKD35^T (a; bar = 1 μm) and *Thermocrispum agreste* CHB77^T (b; bar = 2 μm). (Reproduced with permission from Korn-Wendisch et al., 1995. Int. J. Syst. Bacteriol. *45*: 67–77.)

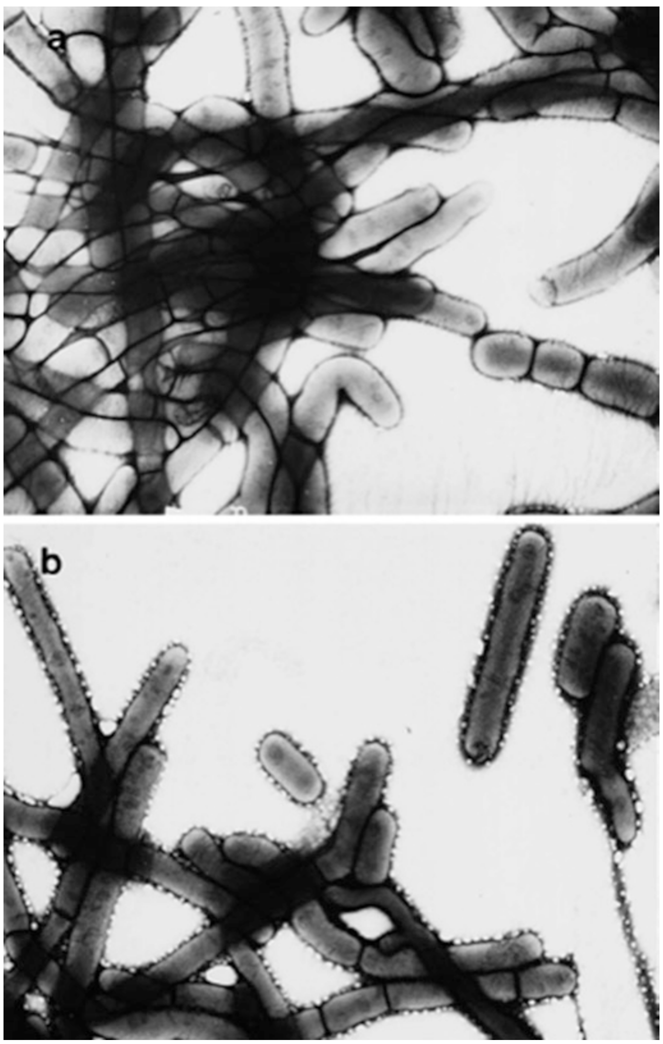

FIGURE 337. Transmission electron micrographic images of aerial mycelia of *Thermocrispum municipale* MKD35^T (a) and *Thermocrispum agreste* CHB77^T (b). Magnification 25,000×. (Reproduced with permission from Korn-Wendisch et al., 1995. Int. J. Syst. Bacteriol. *45*: 67–77.)

Saccharomyces cerevisiae, and *Geotrichum candidum*. A plasmid of about 35 kb was detected in *Thermocrispum municipale* MKD19.

All strains have similar phospholipid patterns and contain phosphatidylethanolamine, phosphatidylinositol, hydroxy-phosphatidylethanolamine, and an unknown ninhydrin-positive lipid (phospholipid type PII). This phospholipid pattern is also found in the genera *Saccharothrix* and *Kutzneria* and in some other genera of the family *Pseudonocardiaceae*, including the genera *Amycolatopsis*, *Kibdelosporangium*, and *Saccharomonospora*. MK-9(H_4) is the predominant menaquinone (95–100%) in all strains of *Thermocrispum* studied. The DNA G+C contents of *Thermocrispum* strains range from 69 to 70 mol%, except for *Thermocrispum municipale* MKD19 (73 mol%). All strains exhibit qualitatively similar fatty acid profiles consisting mainly of 14-methylpentadecanoic acid ($C_{16:0}$ iso) and 2-hydroxy fatty acids (fatty acid type 3f); iso-, anteiso-, and 10-methyl branched fatty acids with 17 carbons are found in smaller amounts.

On the basis of the typing results using actinophages, the two species of *Thermocrispum* can be separated from each other. *Thermocrispum agreste* CHB77^T can be lyzed by its corresponding phage, f77, but is sensitive to only two of the five phages isolated for *Thermocrispum municipale* strains MKD8, MKD10, MKD19, MKD35^T, and MKD38, even if high phage titers are used. In contrast, only four of the nine *Thermocrispum municipale* strains can be lyzed by a high titer of f77. The strains of *Thermocrispum municipale* form three subgroups; group 1, strains MKD8, TMK2, and TMD78, can be lyzed by all five phages isolated for strains MKD8, MKD10, MKD19, MKD35^T, and MKD38; group 2, strains TMS14 and MKD38, are not lyzed by f10 even if high phage titers are used, and strain MKD35^T can be lyzed by this phage only if a high phage titer is used; group 3, strains MKD10, MKD19, and MKD57, are sensitive to phages f35 and f38 only if high phage titers are used.

The band patterns of esterases were shown to differentiate *Thermocrispum agreste* from strains of *Thermocrispum municipale* (Korn-Wendisch et al., 1995).

Enrichment and isolation procedures

Strains of *Thermocrispum municipale* can be isolated from municipal waste compost, and air of compost plants and refuse incineration plants. *Thermocrispum agreste* can be isolated from mushroom compost. Isolation methods include the dilution plate technique for isolation from various environments and use of an Andersen sampler for collecting isolates from aerosols at waste-composting plants. Since the organisms are thermophilic, high temperatures such as 50°C are used for isolation. *Thermocrispum* strains have been found to grow on the following six media combinations: R8 agar and TSA without antibiotics, TSA supplemented with rifampin (10 mg/ml), TSA supplemented with erythromycin and oleandomycin (100 mg/ml each), TSA supplemented with novobiocin (25 mg/ml), and PM agar supplemented with

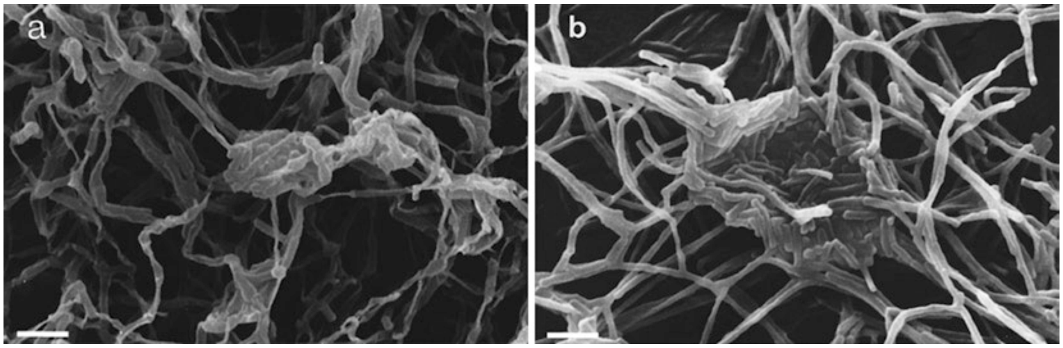

FIGURE 338. Scanning electron micrographic images of *Thermocrispum municipale* MKD35^T showing pseudosporangia (a) and a pseudosporangium containing septate hyphae which fragmented into rod-like structures (b). Bars = 1 μm. (Reproduced with permission from Korn-Wendisch et al., 1995. Int. J. Syst. Bacteriol. *45*: 67–77.)

novobiocin (25 mg/ml). To inhibit fungal growth, cycloheximide and nystatin (50 mg/ml each) are added to the media.

Maintenance procedures

Strains of *Thermocrispum* can be maintained on CYC agar, GYM agar, Hickey–Tresner agar (Hickey and Tresner, 1952), oatmeal agar, potato-carrot agar, PM agar, R2A agar, and R8 agar (Amner et al., 1989), and TSA. The optimum temperature for growth is between 45 and 55°C at neutral pH. For long-term preservation, storage of mycelial or spore suspensions in 20% (v/v) glycerol at −20°C, or lyophilization is recommended.

Differentiation of the genus *Thermocrispum* from closely related taxa

Thermocrispum can be differentiated from related genera of the family *Pseudonocardiaceae* based on chemotaxonomic profiles (Korn-Wendisch et al., 1995). The cell-wall chemotype of *Thermocrispum* (type III) is different from that of *Actinopolyspora*, *Amycolatopsis*, *Kibdelosporangium*, *Pseudonocardia*, *Saccharomonospora*, and *Saccharopolyspora* (type IV). The phospholipid pattern of *Thermocrispum* (PII) is different from that of *Actinopolyspora*, *Pseudonocardia*, and *Saccharopolyspora*. MK-8(H_4), found among *Pseudonocardia* and *Saccharomonospora*, is not present in *Thermocrispum*.

From the phylogenetic analysis, *Amycolatopsis*, *Kibdelosporangium*, *Prauserella*, *Saccharomonospora*, and *Saccharopolyspora* are shown as the neighboring genera (Figure 335), but the levels of 16S rRNA gene sequence similarity between the species of *Thermocrispum* and the type species of these taxa are below 94.4% based on BLAST results (http://blast.ncbi.nlm.nih.gov/blast.cgi). *Thermocrispum* can also be differentiated from related genera using 16S rRNA gene restriction fragment patterns, as suggested by Cook and Meyers (2003).

Taxonomic comments

A recent report suggested that *Sciscionella marina* is loosely associated with *Thermocrispum municipale* sharing 93% sequence similarity (Tian et al., 2009); however, BLAST results indicate that the similarity level should be lower (91.9%). Since the initial description of the genus in 1995, no additional species have been described. The differences in phage sensitivity and fatty acid profiles among the strains of *Thermocrispum municipale* imply possible heterogeneity within the species (Korn-Wendisch et al., 1995). However, the 16S rRNA gene sequences of only two strains, including the type strain, are publicly available to date, and thus the phylogenetic relationship among the isolates is not clear. Further work on the isolates may be able to clarify the taxonomic positions of the *Thermocrispum municipale* strains.

TABLE 261. Differential characteristics of the two species of the genus *Thermocrispum*[a]

Properties	*T. municipale*	*T. agreste*
Growth at:		
20°C	w	tr
28°C	+++	++
62.5°C	(+)	++
65°C	−	−
Growth in the presence of NaCl at:		
7%	+++	++
10%	++	−
13%	−	−
Resistance to 25 μg/ml kanamycin	(+)	+
Degradation of:		
Elastin	−	+
Guanine	(+)	−
Utilization of:		
Fructose	+	tr
Maltose	+	(+)
Melezitose	v	tr
Mannitol	(+)	tr
Sorbitol (=glucitol)	v	tr
Salicin	(+)	+
Sodium acetate	+	tr
Sodium citrate	v	−
Hemolysis	−	+
Egg yolk reaction	−	+

[a]Data taken from Korn-Wendisch et al. (1995). Symbols: +++, good growth with abundant aerial mycelium; ++, good growth with moderate aerial mycelium; +, positive growth or reaction; (+), poor growth or moderate reaction; −, negative; w, weak; v, variable; tr, traces of growth.

Differentiation of the species of the genus *Thermocrispum*

Thermocrispum species can be distinguished from one another by using a combination of nutritional and physiological characteristics (Table 261).

List of species of the genus *Thermocrispum*

1. **Thermocrispum municipale** Korn-Wendisch, Rainey, Kroppenstedt, Kempf, Majazza, Kutzner and Stackebrandt 1995, 73[VP]

mu.ni.ci.pa′le. L. neut. adj. *municipale* municipal, referring to the environment from which strains were isolated.

White aerial mycelium with long, straight, or flexuous chains of spores are formed. Aerial mycelia fragment into rod-like structures and both aerial and substrate mycelia often form aggregates. Sporangia-like structures are formed. Good growth occurs between 28 and 60°C, but weak growth is observed at 20 and 62.5°C. No growth occurs at 65°C. Good growth is also observed in the presence of up to 10% (w/v) NaCl, but not at 13%. Moderately resistant to kanamycin (25 μg/ml). Casein, DNA, esculin, gelatin, tributyrin, Tween 80, and tyrosine are degraded or hydrolyzed. Guanine is moderately degraded, pectin is weakly degraded, and arbutin is slowly hydrolyzed. In contrast, adenine, allantoin, cellulose, chitin, elastin, hypoxanthine, poly-β-hydroxybutyric acid, starch, urea, xanthine, and xylan are not degraded or hydrolyzed. Melanin is not produced. Dextrin, fructose, glucose, galactose, inositol, maltose, mannose, cellobiose, sucrose, trehalose, sodium acetate, sodium malonate, and sodium pyruvate are utilized as sole carbon sources, and mannitol and salicin are utilized weakly. In contrast, adonitol, inulin, lactose, melibiose, oxalate, raffinose, rhamnose, xylitol, xylose, and sodium propionate are not utilized as sole carbon sources. Melezitose, sorbitol, and sodium citrate may or may not be utilized as sole carbon sources. Hemolysis and egg yolk reaction are negative. Nitrate is reduced to form nitrite under aerobic conditions. Resistant to novobiocin and crystal violet.

Source: municipal waste compost, air of compost plants, and air of a refuse incineration plant.

DNA G+C content (mol%): 69–73 (HPLC).

Type strain: MKD35, DSM 44069.

Sequence accession no. (16S rRNA gene): X79184.

2. **Thermocrispum agreste** Korn-Wendisch, Rainey, Kroppenstedt, Kempf, Majazza, Kutzner and Stackebrandt 1995, 73[VP]

a.gre′ste. L. neut. adj. *agreste* rural, referring to the origin of the compost from which the organism was isolated.

White aerial mycelium with long, straight, or flexuous chains of spores are formed. Aerial mycelia fragment into rod-like structures and both aerial and substrate mycelia often form aggregates. Sporangia-like structures are formed. Good growth occurs between 28 and 62.5°C; traces of growth are observed at 20°C, but not at 65°C. Good growth is also observed in the presence of up to 7% (w/v) NaCl, but not at 10%. Resistant to kanamycin (25 μg/ml). Casein, DNA, elastin, esculin, gelatin, tributyrin, Tween 80, and tyrosine are degraded or hydrolyzed. In contrast, adenine, allantoin, cellulose, chitin, guanine, hypoxanthine, poly-β-hydroxybutyric acid, starch, urea, xanthine, and xylan are not degraded or hydrolyzed. Melanin is not produced. Cellobiose, dextrin, glucose, galactose, inositol, mannose, salicin, sucrose, trehalose, sodium malonate, and sodium pyruvate are utilized as sole carbon sources, and maltose is utilized weakly. In contrast, adonitol, inulin, lactose, melibiose, oxalate, raffinose, rhamnose, xylitol, xylose, sodium citrate, and sodium propionate are not utilized as sole carbon sources. Only traces of growth occur in the presence of fructose, melezitose, mannitol, sorbitol, and sodium acetate as sole carbon sources. Hemolytic activity and egg yolk reactions are positive. Nitrate is reduced to form nitrite under aerobic conditions. Resistant to novobiocin and crystal violet. Inhibits growth of *Azotobacter chroococcum*, *Candida albicans*, *Saccharomyces cerevisiae*, and *Geotrichum candidum*, whereas slightly antagonistic against the Gram-stain-positive bacteria *Rhodococcus rhodochrous*, and *Micrococcus luteus*. No antagonistic activity is observed against *Escherichia coli*, *Corynebacterium glutamicum*, *Bacillus subtilis*, or *Staphylococcus aureus*.

Source: mushroom compost.

DNA G+C content (mol%): unknown.

Type strain: CHB77, DSM 44070.

Sequence accession no. (16S rRNA gene): X79183.

Genus XVIII. **Umezawaea** Labeda and Kroppenstedt 2007, 2761[VP]

David P. Labeda

Um.e.za.wa′e.a. N.L. fem. n. *Umezawaea* named for Hamao Umezawa (1914–1986), of the Institute of Microbial Chemistry, Tokyo, in recognition of his leadership and contributions to the study of the biology and natural products of actinomycetes.

Aerobic. Gram-stain-positive, non-acidfast, nonmotile actinomycetes. Branched substrate mycelium (approx. 0.3–0.5 μm in diameter) is produced; aerial mycelia are formed on some media. **Ovoid or cylindrical conidia (0.3–0.5 × 1.1–1.9 μm) are produced by fragmentation of substrate mycelium. Pseudosporangia are produced on some media. Contains *meso*-diaminopimelic acid as the diamino acid. The muramic acid in the cell-wall peptidoglycan is acetylated. The whole-cell sugar pattern consists of galactose, mannose, and ribose, with a trace of rhamnose. The phospholipid pattern consists predominantly of phosphatidylethanolamine, phosphatidylinositol, phosphatidylethanolamine containing hydoxylated fatty acids, and *lyso*-phosphatidylethanolamine. The predominant menaquinone is MK-9(H_4), with a trace of MK-10(H_4).** Mycolic acids are absent. Has a fatty acid profile consisting predominantly of $C_{16:0}$ iso fatty acids, with $C_{14:0}$ iso, $C_{15:0}$ iso, $C_{16:0}$, $C_{16:1}$, $C_{17:1}$, and $C_{16:1}$ iso as minor components. Phylogenetically, nearest neighbor is the genus *Saccharothrix*.

DNA G+C content (mol%): 74 (HPLC).

Type species: **Umezawaea tangerina** (Kinoshita, Igarashi, Ikeno, Hori and Hamada 2000) Labeda and Kroppenstedt 2007, 2761[VP].

Further descriptive information

Umezawaea tangerina was originally described as a species of the genus *Saccharothrix* (Kinoshita et al., 1999), although the production of pseudosporangia on the aerial mycelium, a feature not detected in other species in this genus, was noted. Phylogenetic analysis of species of the genus *Saccharothrix* and related genera revealed that *Saccharothrix tangerinus* consistently appeared distant from *Saccharothrix sensu stricto* (see Figure 290). A subsequent re-evaluation of the chemotaxonomic characteristics of this strain determined that it was different from the other taxa in the family *Pseudonocardiaceae*, particularly in regard to the presence of significant quantities of *lyso*-phosphatidylethanolamine in the phospholipid profile (Table 262). The nucleotide signatures in the 16S rRNA gene of *Umezawaea tangerina* are also different from those of *Saccharothrix* and related taxa (see Figure 309).

Enrichment and isolation procedures

Strains of *Lechevalieria*, *Lentzea*, and *Saccharothrix* have been isolated from soil samples by spread-plating serial soil dilutions onto routine selective media (such as 1.5% crude agar and 0.4% casein hydrolysate in tap water) used for the general isolation of actinomycetes. Shearer (1987) reported the use of typical actinomycete isolation media, such as Gauze mineral medium no. 1 (Gauze et al., 1957) or starch-casein agar (Küster and Williams, 1964), supplemented with 5–10 µg/ml penicillin G and 15 µg/ml nalidixic acid antibiotics to selectively isolate *Saccharothrix* strains that are phylogenetically close to *Umezawaea*.

Maintenance procedures

Working cultures of *Umezawaea* can be maintained as refrigerated (4°C) agar slants on an appropriate medium such as yeast extract-malt extract medium (Shirling and Gottlieb, 1966) or NZamine medium (DSMZ medium 554; DSMZ, 2001). The slants are generally incubated for 5–7 d at 28–30°C prior to refrigeration and then should be subcultured at monthly intervals. Longer term preservation of strains is best accomplished as frozen stocks in 20% aqueous glycerol at −80°C (mechanical freezer) to −172°C (liquid nitrogen vapor phase) or by using traditional lyophilization techniques.

Procedures for testing special characters

Strains are routinely cultivated on NZamine medium (DSMZ medium 554) at 28°C. Morphological observations are made on the media of Shirling and Gottlieb (1966) and NZamine medium.

Chemotaxonomic analyses of strains for whole-cell sugars, polar lipids, menaquinones, and fatty acids are performed using methods described previously by Saddler et al. (1991), Grund and Kroppenstedt (1990b), Minnikin et al. (1984), and Sasser (1990).

Physiological tests, including production of acid from carbohydrates, utilization of organic acids, and hydrolysis and decomposition of adenine, casein, esculin, hippurate, guanine, hypoxanthine, tyrosine, urea, and xanthine, are typically evaluated using the media of Gordon et al. (1974). Phosphatase activity is evaluated by using the method of Kurup and Schmitt (1973) substituting NZamine agar as the basal medium. Temperature range for growth is determined on slants of DSMZ medium 554 and salt tolerance on slants of the same medium supplemented with 4% and 5% NaCl.

Differentiation of the genus *Umezawaea* from other genera

Umezawaea strains can easily be differentiated from morphologically similar members of the suborder *Pseudonocardineae* by the presence of *lyso*-phosphatidylethanolamine as a predominant component in the polar lipid profiles in addition to phosphatidylethanolamine containing 2-hydroxy fatty acids (Table 262). The nucleotide signatures in the 16S rRNA gene sequences can be used to differentiate *Umezawaea* strains from *Saccharothrix* and other closely related genera in the family *Pseudonocardiaceae* (see Figure 309).

List of species of the genus *Umezawaea*

1. **Umezawaea tangerina** (Kinoshita, Igarashi, Ikeno, Hori and Hamada 2000) Labeda and Kroppenstedt 2007, 2761[VP] (*Saccharothrix tangerinus* Kinoshita, Igarashi, Ikeno, Hori and Hamada 2000, 949; effective publication: Kinoshita, Igarashi, Ikeno, Hori and Hamada 1999, 27.)

   tan.ger.i′na. N.L. fem. adj. *tangerina* tangerine-colored, referring to the color of the vegetative growth.

   The pale yellow, pale yellow-orange, or pale yellowish-brown substrate mycelium fragments into coccoidal-rod elements. White to brownish-white aerial mycelia are produced on some media and fragment into coccoid to coccoidal-rod elements. Pseudosporangia are sometimes produced on the aerial mycelia. Soluble pigments are faint brown or not produced. Melanin pigments are not produced. L-Arabinose, D-xylose, D-glucose, D-fructose, and inositol are utilized as sole carbon sources. Casein, hypoxanthine, starch, and tyrosine are hydrolyzed; adenine and urea are not hydrolyzed. Acid is produced from L-arabinose, cellobiose, dextrin, D-glucose, glycerol, and inositol, but not from adonitol or *meso*-erythritol. Citrate and oxalate are assimilated, but not DL-tartrate. Nitrate is not reduced. Phosphatase is produced. Grows in the presence of 4% (w/v) NaCl. Temperature range for growth is 20–30°C. Produces the antibiotic formamicin. Grows at 28°C.

   Source: isolated from soil.

   DNA G+C content (mol%): 74 (HPLC).

   Type strain: MK27-91F2, DSM 44720, FERM P-16053, JCM 10302, NBRC 16184, NRRL B-24463.

   Sequence accession no. (16S rRNA gene): AB020031.

TABLE 262. Chemotaxonomic characteristics of *Umezawaea* compared to other similar members of the family *Pseudonocardiaceae*[a]

Characteristic[b]	*Umezawaea*	*Actinokineospora*	*Actinosynnema*	*Crossiella*	*Goodfellowiella*	*Lechevalieria*	*Lentzea*	*Saccharothrix*
Whole-cell sugar pattern[c]	Gal, Man, Rib, Rha (tr)	Gal, Man, Rha	Gal, Man	Gal, Rha, Rib	Gal, Rib	Gal, Man, Rha	Gal, Man, Rib	Gal, Rha, Man (tr)
Phospholipids[d]	PE, PI, OH-PE, *lyso*-PE, DPG, DPG, PIM	PE	PE, OH-PE, PI, PIM, DPG	PE, DPG, PI, PIM, PME	PE, OH-PE, DPG	PE	PE, DPG, PG, PI	PE, OH-PE, PI, PIM, DPG, PG (v)
Predominant menaquinones	MK-9(H_4), (tr), MK-10(H_4)	MK-9(H_4)	MK-9(H_4), MK-9(H_6)	MK-9(H_4)	MK-10(H_4), MK-9(H_4)	MK-9(H_4)	MK-9(H_4)	MK-9(H_4), MK-10(H_4)

[a]tr, Trace; v, variable.

[b]All genera have *meso*-diaminopimelic acid as the cell-wall diamino acid and are of cell-wall chemotype III.

[c]Gal, Galactose; Man, mannose; Rha, rhamnose; Rib, ribose.

[d]DPG, diphosphatidylglycerol; OH-PE, phosphatidylethanolamine with hydroxy fatty acids; *lyso*-PE, phosphatidylethanolamine where one fatty acid chain is missing from the glycerol backbone; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; PI, phosphatidylinositol; PIM, phosphatidylinositol mannosides; PME, phosphatidylmethylethanolamine.

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Order XIV. **Streptomycetales** ord. nov.

PETER KÄMPFER

Strep.to.my.cet.al′es. N.L. masc. n. *Streptomyces* type genus of the order; suff. *-ales* ending to denote an order; N.L. fem. pl. n. *Streptomycetales* the *Streptomyces* order.

The order was created by elevation of suborder *Streptomycineae* Rainey et al. 1997.

The description is that of the type family *Streptomycetaceae*.

Type genus: **Streptomyces** Waksman and Henrici 1943, 339^AL^.

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Family I. **Streptomycetaceae** Waksman and Henrici 1943, 339^AL^ emend. Rainey, Ward-Rainey and Stackebrandt 1997, 486 emend. Kim, Lonsdale, Seong and Goodfellow 2003b, 113 emend. Zhi, Li and Stackebrandt 2009, 600

PETER KÄMPFER

Strep.to.my.ce.ta.ce′a.e. N.L. masc. n. *Streptomyces* type genus of the family; L. suff. *-aceae* ending to denote a family; N.L. fem. pl. n. *Streptomycetaceae* the *Streptomyces* family.

Aerobic, Gram-stain-positive, non-acid–alcohol-fast actinomycetes that form an extensively branched substrate mycelium which rarely fragments. At maturity, the **aerial mycelium forms chains of three to many spores**. Members of a few species bear short chains of spores on the substrate mycelium. The organisms produce a **wide range of pigments**, which are responsible for the colors of the substrate and aerial mycelia. The organisms grow within different pH ranges and are chemo-organotrophic with an oxidative type of metabolism. **Walls of cells from the substrate mycelium contain either LL- or *meso*-diaminopimelic acid (A_2pm) as the predominant diamino acid; aerial or submerged spores contain LL-A_2pm (peptidoglycan type A3γ)**. Whole-organism **sugar profiles may contain major amounts of either galactose or galactose and rhamnose. Lipid profiles typically contain: hexa- and octahydrogenated menaquinones with nine isoprene units as the predominant isoprenologs; diphosphatidylglycerol, phosphatidylethanolamine, phosphatidylinositol, and phosphatidylinositol mannosides as major polar lipids; and complex mixtures of saturated, and iso- and anteiso-fatty acids. Mycolic acids are not present**. The pattern of 16S rRNA gene sequence signatures consists of nucleotides at positions: 127:234 (G–C), 449 (A), 672:734 (C–G), 950:1231 (U–G), 952:1229 (U–A), 955:1225 (C–G), 965 (C), 986:1219 (A–U), and 1362 (C). The family *Streptomycetaceae* belongs to the order *Streptomycetales*.

DNA G+C content (mol%): generally 66–74.

Type genus: **Streptomyces** Waksman and Henrici 1943, 339^AL^.

Taxonomic comments

The family *Streptomycetaceae* was established in 1943 by Waksman and Henrici to accommodate actinomycetes with branched slender mycelia, rarely or not septate, spores on aerial hyphae, and not fragmenting into oidia (Waksman and Henrici, 1943). At that time, the description was mainly based on morphology. In the 8th edition of *Bergey's Manual of Determinative Bacteriology*, Pridham and Tresner (1974b) listed *Streptomyces*, *Streptoverticillium*, *Sporichthya*, and *Microellobosporia* as members of the family. Over the years, additional genera, like *Actinopycnidium*, *Actinosporangium*, *Chainia*, *Elytrosporangium*, *Kitasatoa*, and *Microellobosporia*, have been distinguished from *Streptomyces* by morphological criteria, but they have many phenotypic and genotypic characters in common with *Streptomyces* and have therefore been proposed as synonyms of this genus (Goodfellow et al., 1986a, 1986b, 1986c, 1986d, 1986e). The genus *Sporichthya* is now classified in the family *Sporichthyaceae* of the order *Frankiales* (Stackebrandt et al., 1997).

The genus *Streptoverticillium* was found to be distinguishable from *Streptomyces* by its verticillate sporophores, but also shared many characteristics with streptomycetes; numerical phenetic

(Kämpfer et al., 1991; Williams et al., 1983a) and rRNA–DNA similarities (Gladek et al., 1985; Witt and Stackebrandt, 1990) supported the proposal that the genus was a synonym of *Streptomyces* (Witt and Stackebrandt, 1990). Wellington et al. (1992) proposed the unification of *Kitasatospora* with *Streptomyces* on the basis of chemotaxonomic, biochemical, and 16S rRNA gene sequence similarities. However, Zhang et al. (1997) revived the genus mainly on the basis of the ratio of *meso*-A_2pm to LL-A_2pm in whole-cell hydrolysates. The *meso*-A_2pm content is 49–89% in *Kitasatospora* strains and 1–16% in *Streptomyces* strains. Furthermore, galactose is present in the whole-cell hydrolysates of *Kitasatospora* strains, but not in whole-cell hydrolysates of *Streptomyces* strains.

Kim et al. (2003b) proposed an additional member of the family, the genus *Streptacidiphilus*, which contained acidophilic actinomycetes isolated from acidic soils and litter. These actinomycetes grow over a pH range of 3.5–6.5, with optimum growth at pH 4.5–5.5.

The genera *Streptomyces*, *Kitasatospora*, and *Streptacidiphilus* are very difficult to differentiate on the basis of phenotypic features (including the few distinguishing chemotaxonomic markers given above). The few characteristic differentiating features are shown in Table 263. Although 16S rRNA gene sequence analyses have provided a framework for prokaryotic classification, the current classification based on this molecule has not clarified the taxonomic problems within the family *Streptomycetaceae* (Kämpfer, 2006). *Kitasatospora* and *Streptacidiphilus* form stable, separate sub-branches on the basis of phylogenetic analyses within the family *Streptomycetaceae*, but they are grouped within the large *Streptomyces* tree (Figure 339) and 16S rRNA gene sequence similarities are equally high to many *Streptomyces* species/groups and *Streptacidiphilus* species. On the basis of DNA–DNA microarray hybridizations, Hsiao and Kirby (2008) compared the genome content of *Streptomyces avermitilis*, "*Streptomyces cattleya*", "*Streptomyces maritimus*", and *Kitasatospora* (*Streptomyces*) *aureofaciens* with that of *Streptomyces coelicolor* A3(2) and found a very high agreement with the genome sequence data for *Streptomyces* and *Kitasatospora*.

For all these reasons, it is questionable whether a separate generic status for *Kitasatospora* and *Streptacidiphilus* is justified. In this edition of *Bergey's Manual*, these genera are cited as genera *incertae sedis*.

TABLE 263. Chemotaxonomic, morphological and physiological characteristics of *Kitasatospora*, *Streptacidiphilus*, and *Streptomyces* strains (according to Kim et al., 2003b)[a]

	Streptomyces	*Kitasatospora*	*Streptacidiphilus*
Long chains of spores formed on aerial hyphae	+	+	+
Optimal pH range	6.5–8.0[b]	nd	4.5–5.5
pH range for growth	5.0–11.5	5.5–9.0	3.5–6.0
A_2pm isomer(s) in whole-organism hydrolysates	LL-A_2pm	LL- and *meso*-A_2pm[c]	LL-A_2pm
Diagnostic sugars in whole-organism hydrolysates	None	Galactose[d]	Galactose, rhamnose
Predominant phospholipids[e]	DPG, PE, PI, PIMs	DPG, PE, PI, PIMs	DPG, PE, PI, PIMs
Major menaquiones[f]	MK-9(H_6,H_8)	MK-9(H_6,H_8)	MK-9(H_6,H_8)
Fatty acid pattern[g]	2c	2c	2c
DNA G+C content (mol%)	66–73	70–74	70–72

[a]Data taken from this and previous studies (Antony-Babu and Goodfellow, 2008; Lonsdale, 1985; Nakagaito et al., 1992a, 1992b; Ōmura et al., 1989; Shirling and Gottlieb, 1977; Williams et al., 1989).

[b]Alkaliphilic strains, which grow between pH 5.0 and 11.0, have an optimum at pH 9–9.5 (Mikami et al., 1982; Antony-Babu and Goodfellow, 2008).

[c]Aerial and submerged spores contain LL-A_2pm and vegetative mycelia *meso*-A_2pm.

[d]Rhamnose was detected in whole-organism hydrolysates of *Kitasatospora mediocidica* (Labeda, 1988).

[e]DPG, Diphosphatidylglycerol; PE, phosphatidylethanolamine; PI, phosphatidylinositol; PIMs, phosphatidylinositol mannosides.

[f]MK-9(H_6,H_8), hexa-and octa-hydrogenated menaquinones with nine isoprene units.

[g]Fatty acid group *sensu* Kroppenstedt (1985).

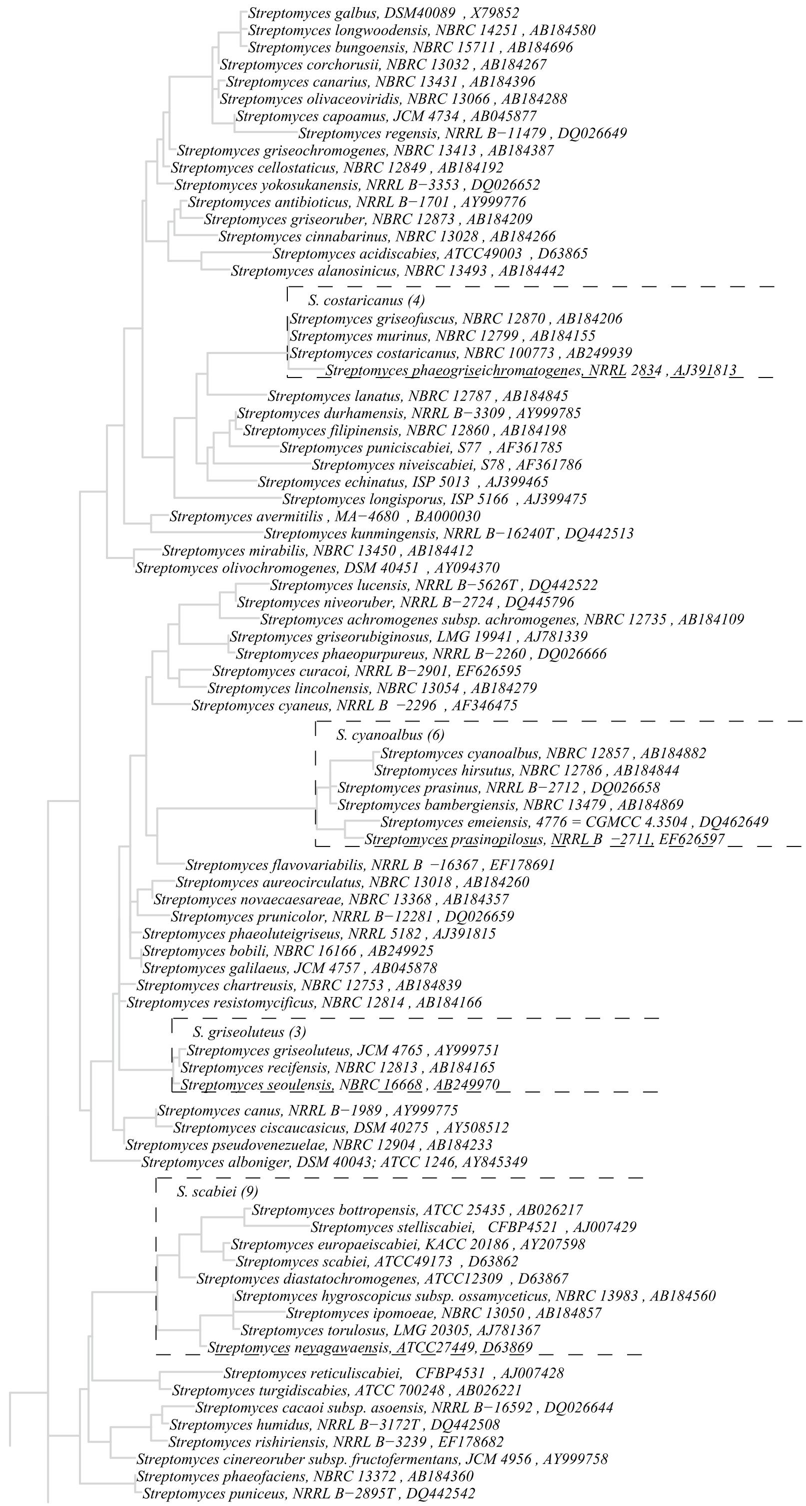

FIGURE 339. Phylogenetic analysis based on 16S rRNA gene sequences available from the EMBL database (accession nos are given in parentheses). The phylogenetic tree was constructed using the ARB software package (version December 2007; Ludwig et al., 2004) and the corresponding SILVA SSURef 95 database (version July 2008; Pruesse et al., 2007). Tree building was performed with all *Streptomycetaceae* sequences available in the SILVA database using the maximum-likelihood method with fastDNAml (Olsen et al., 1994) and an *Actionbacteria* conservatory filter. All strains in the figure are type strains.

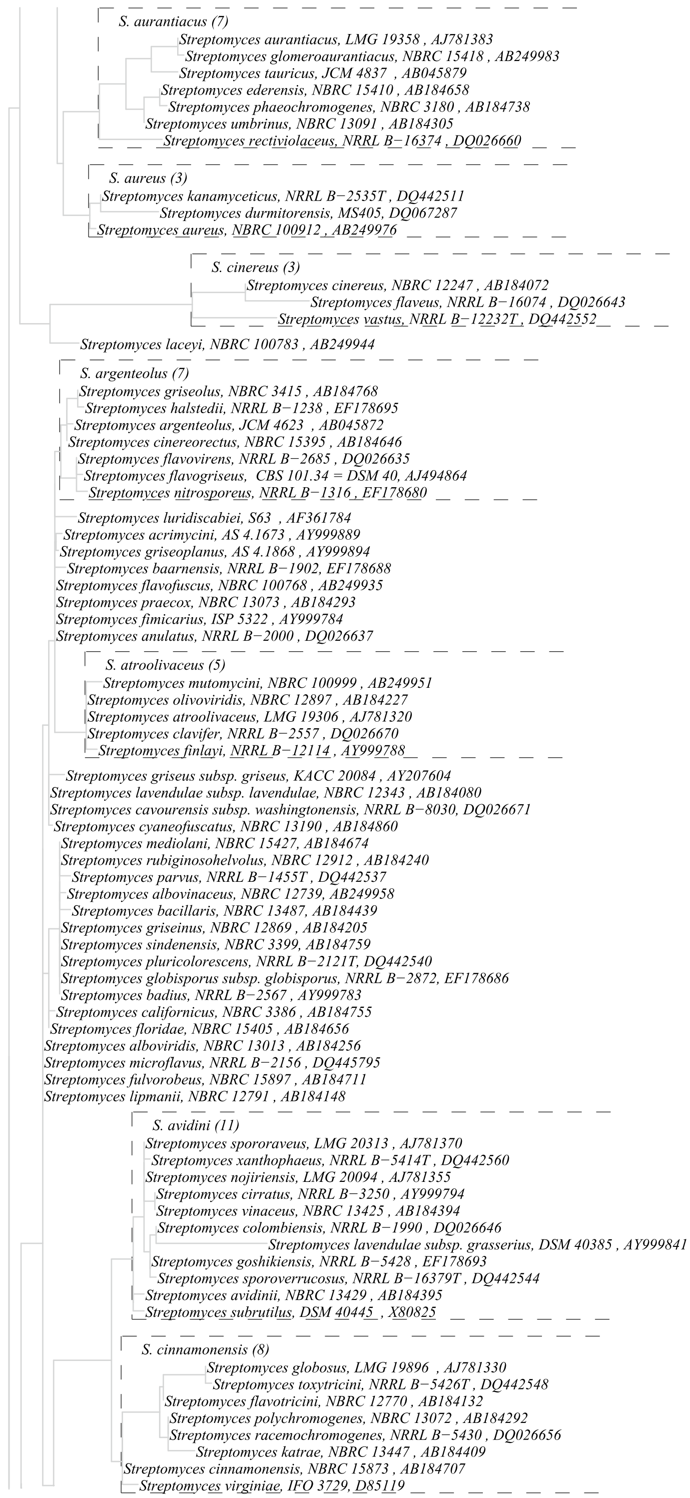

FIGURE 339. (continued)

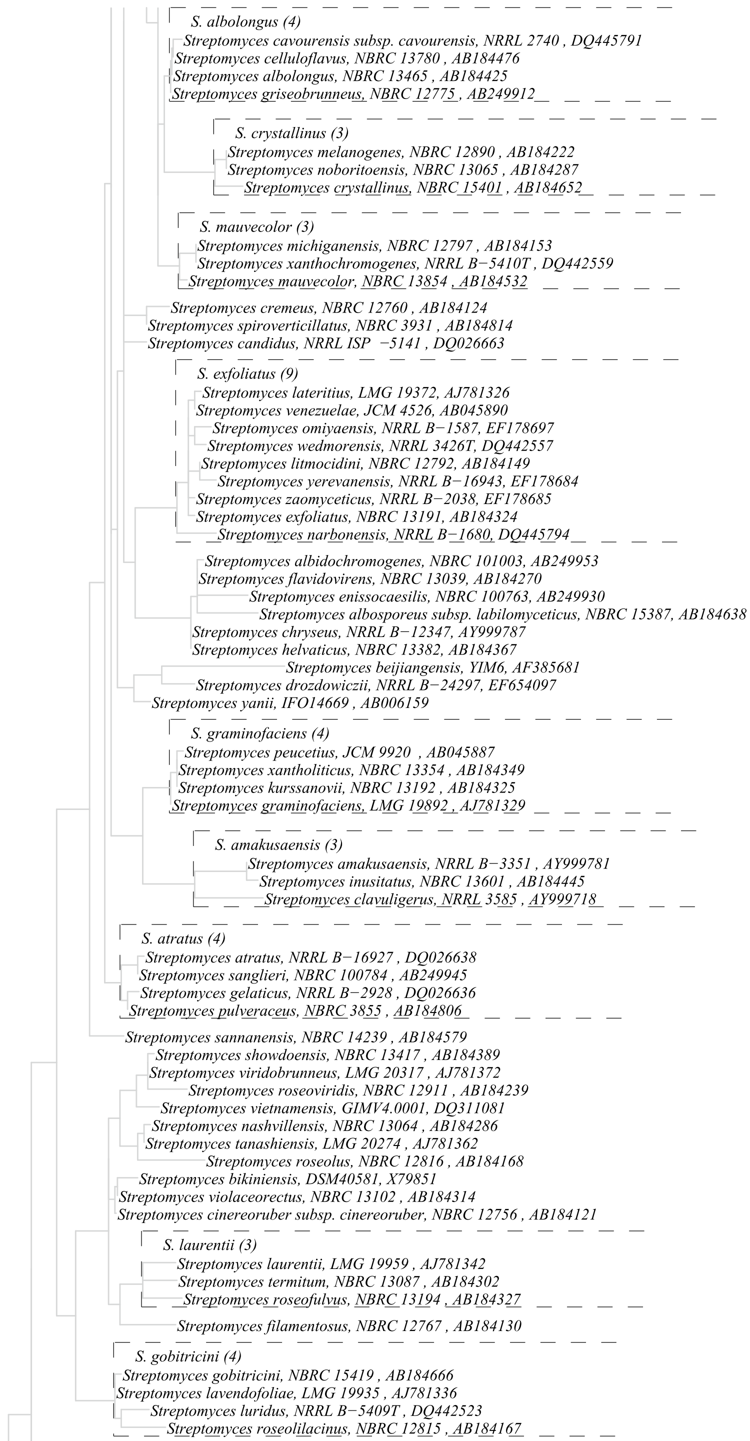

FIGURE 339. (continued)

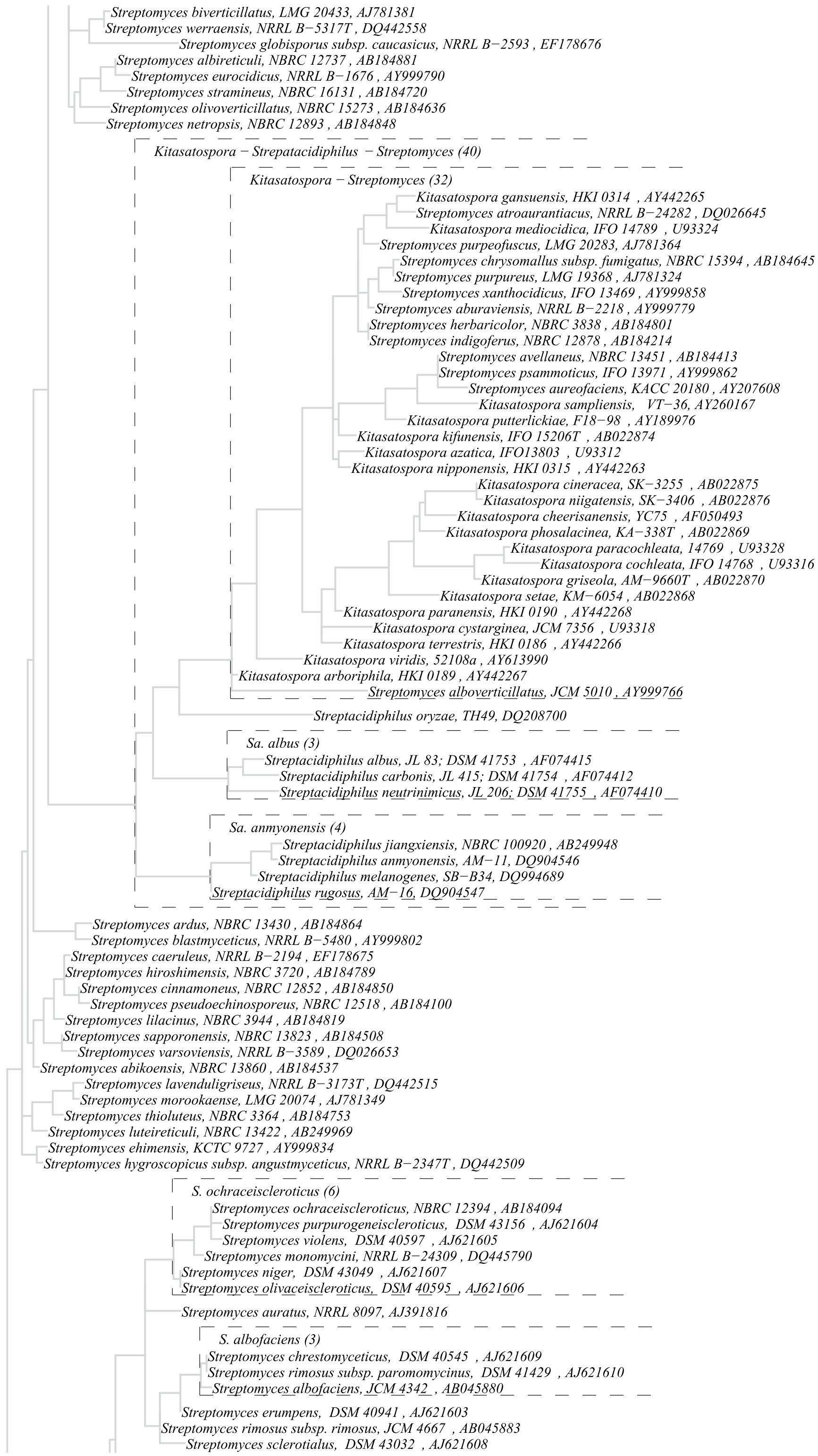

FIGURE 339. (continued)

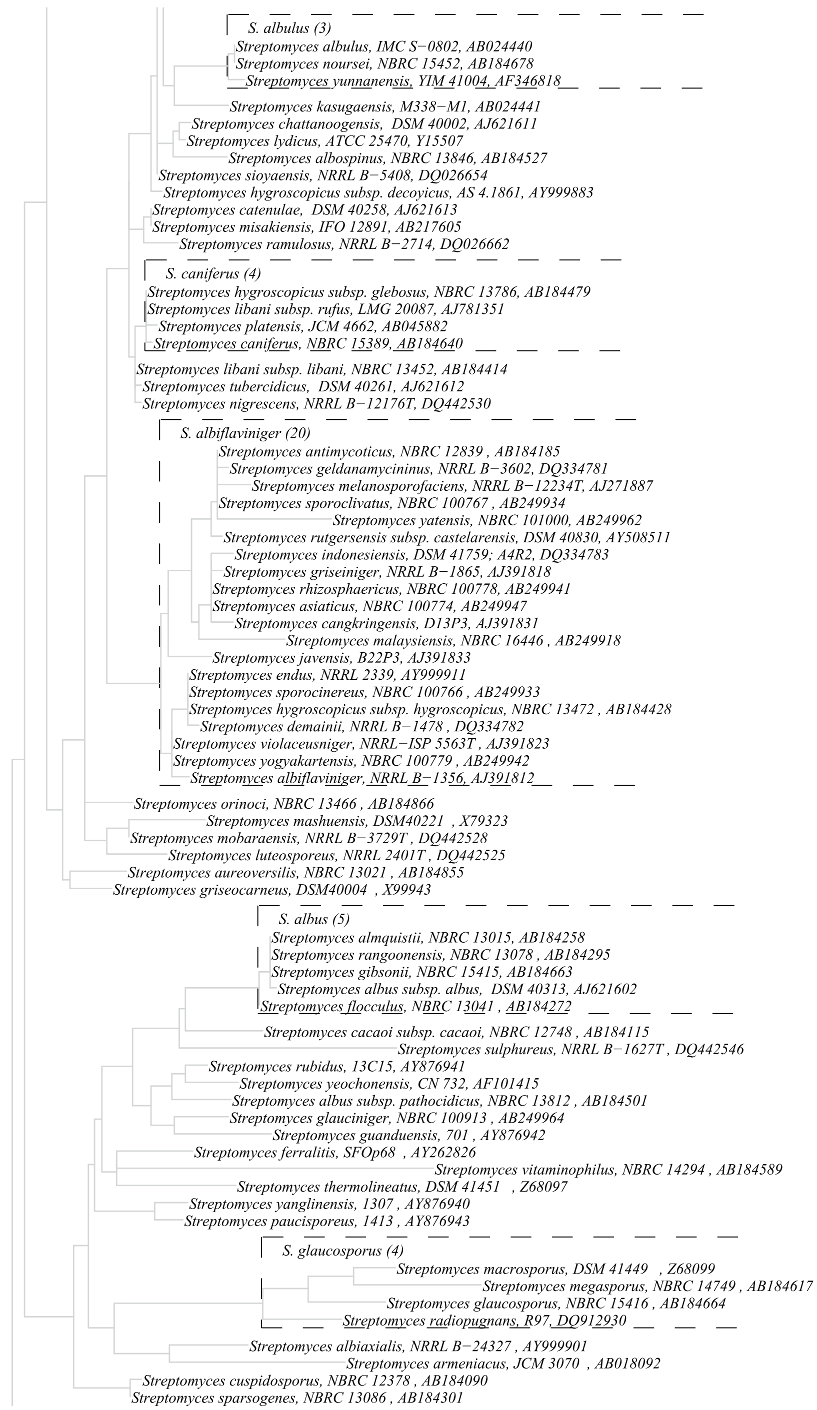

FIGURE 339. (continued)

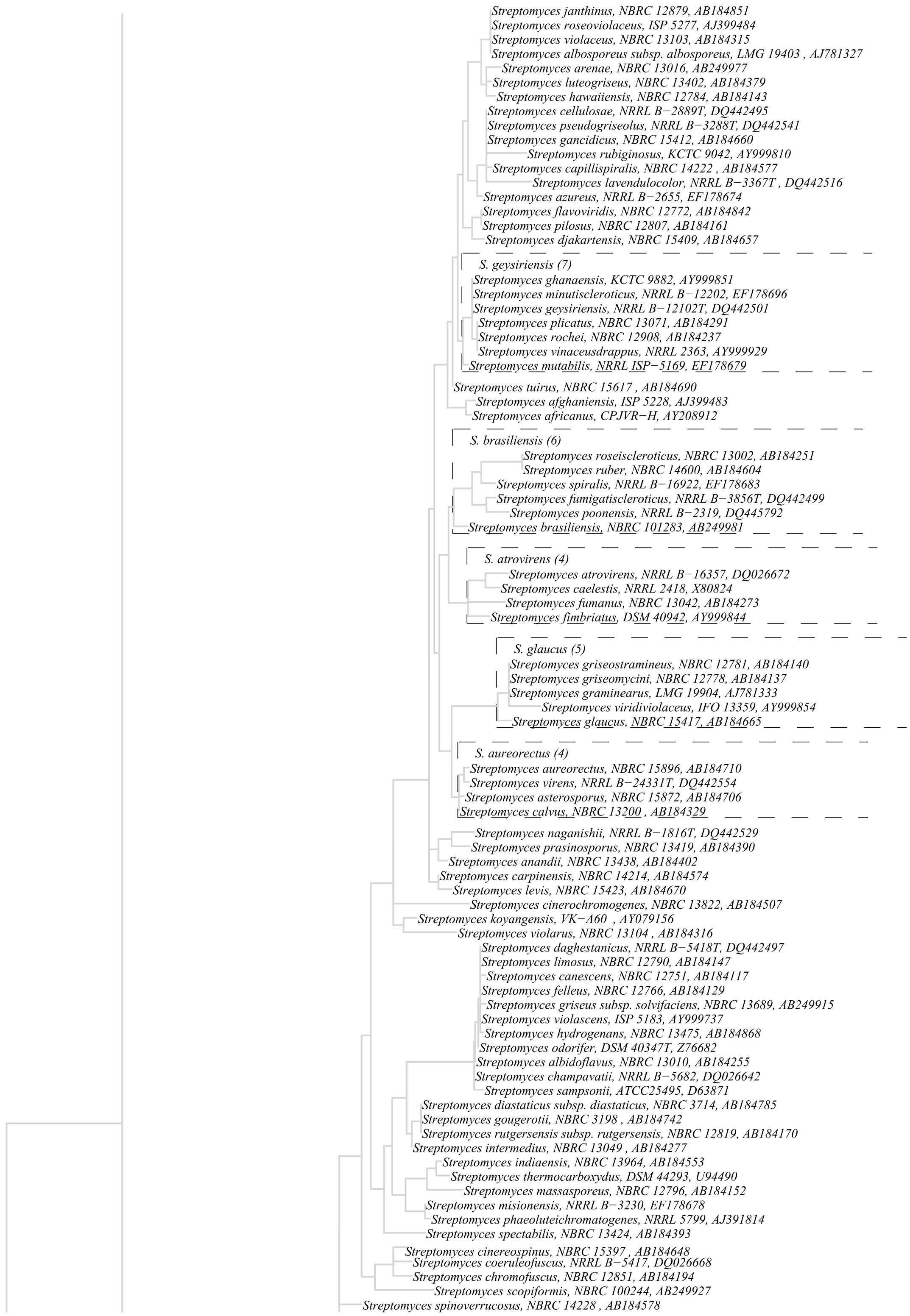

FIGURE 339. (continued)

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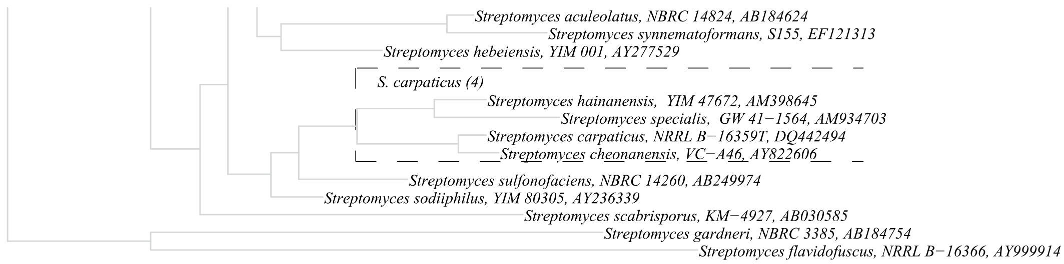

FIGURE 339. (continued)

Genus I. **Streptomyces** Waksman and Henrici 1943, 339^AL emend. Witt and Stackebrandt 1990, 370 emend. Wellington, Stackebrandt, Sanders, Wolstrup and Jorgensen 1992, 159

Peter Kämpfer

Strep.to.my′ces. Gr. adj. *streptos* pliant, bent; Gr. masc. n. *mukês* fungus; N.L. masc. n. *Streptomyces* pliant or bent fungus.

Aerobic, Gram-stain-positive, non-acid-fast bacteria that form extensively branched substrate and aerial mycelia. Chemo-organotrophic, having an oxidative type of metabolism. The vegetative hyphae (0.5–2.0 µm in diameter) rarely fragment. **The aerial mycelium forms chains of three to many spores at maturity**. Some species show short chains of spores on the substrate mycelium and others form sclerotia, pycnidial-, sporangia-, and synnemata-like structures. The spores are nonmotile. **Colonies are discrete and lichenoid, leathery, or butyrous.** Often, colonies initially show a smooth surface, but later develop a weft of aerial mycelium that may appear floccose, granular, powdery, or velvety. **Can produce a wide variety of pigments responsible for the color of the vegetative and aerial mycelia. Colored diffusible pigments may also be formed.** Many strains are able to produce one or more antibiotic substances.

Catalase-positive. Generally reduce nitrates to nitrites and degrade polymeric substrates such as casein, gelatin, hypoxanthine, and starch, in addition to adenine and L-tyrosine. **Most species use a wide range of organic compounds as sole sources of carbon for energy and growth.** The temperature optimum for most species is in the range 25–35°C; some species, however, can grow at temperatures within the psychrophilic and thermophilic range; optimum pH range for growth is 6.5–8.0. The cell-wall peptidoglycan contains major amounts of **LL-A_2pm**. In some cases, low amounts of *meso*-A_2pm can be detected. **They lack mycolic acids, contain major amounts of saturated, iso- and anteiso-fatty acids, and typically possess either hexa- or octahydrogenated menaquinones with nine isoprene units as the predominant isoprenolog, although menaquinones with eight and ten isoprene units are also found. A complex polar lipid pattern is observed, containing typically diphosphatidylglycerol, phosphatidylethanolamine, phosphatidylinositol, and phosphatidylinositol mannosides. Widely distributed and abundant in soil, including composts.** A few species are pathogenic for animals and man, whereas some are phytopathogens.

DNA G+C content (mol%): 66–78 (T_m).

Type species: **Streptomyces albus** (Rossi Doria 1891) Waksman and Henrici 1943, 339^AL.

Further descriptive information

Morphology, fine structure, and life cycle. Early investigations of streptomycetes were dominated by a strong emphasis on morphology. The complex life cycle of streptomycetes (see below) offers three features for detailed microscopic characterization: (a) vegetative (substrate) mycelium (on solid and in liquid media); (b) aerial mycelium bearing chains of arthrospores (sometimes called "sporophores"); and (c) the arthrospores themselves (Kutzner, 1981). It is the last two categories that have provided most diagnostic information for taxonomists.

Early reports indicated that streptomycetes formed spore chains on the vegetative mycelium in both solid and liquid culture (e.g. Carvajal, 1947; Glauert and Hopwood, 1960; Tresner et al., 1967). However, it was never clarified whether these structures were analogous to the arthrospores formed on the aerial mycelium rather than deformations of the hyphae produced in staling cultures.

The fine structure and development of the aerial arthrospores have been studied extensively (Locci and Sharples, 1984). They are formed by septation and disarticulation of pre-existing hyphal elements within a thin fibrous sheath. The spore wall is formed, at least in part, from wall layers of the parent hypha; this is termed *holothallic development* (Locci and Sharples, 1984), and was found to be typical for many other sporo actinomycetes (Williams et al., 1973). The configuration of the spore chains (or sporophores) has played a very important role in species descriptions for many years. Often, the chains are long and contain more than 50 arthrospores. It is known that a number

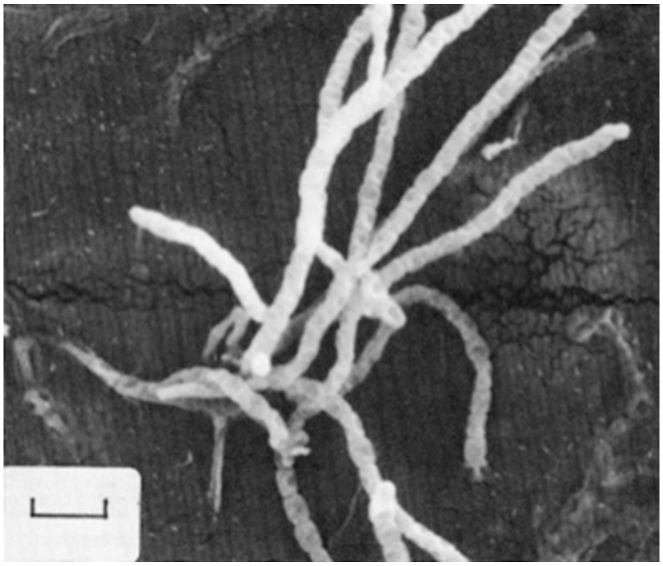

FIGURE 340. Straight to flexuous (*Rectiflexibles*) spore chains of *Streptomyces griseus*. Bar, 2 µm.

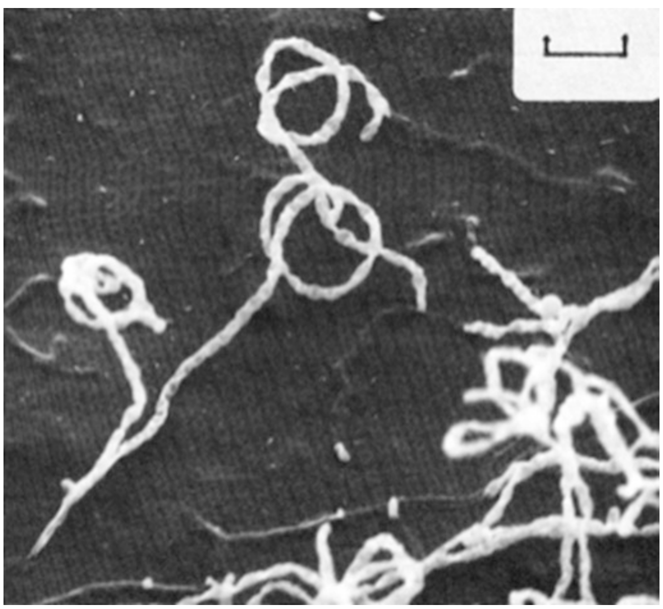

FIGURE 341. Looped (*Retinaculiaperti*) spore chains of *Streptomyces vinaceus*. Scanning electron microscopy. Bar, 5 µm.

of different genes are involved in spore formation (Chater, 1979) and that different cultivation conditions can have an influence on spore formation. The range of spore chain morphologies is extensive and some workers have recognized many categories, for example, Ettlinger et al. (1958a) grouped strains into 15 morphological types. A simpler and practical scheme was proposed by Pridham et al. (1958) and adopted for the International *Streptomyces* Project (ISP; Shirling and Gottlieb, 1966). Three categories recognized were: (a) straight to flexuous (*Rectiflexibiles*) (Figure 340); (b) hooks, loops, or spirals with one to two turns (*Retinaculiaperti*) (Figure 341); and (c) spirals (*Spirales*) (Figure 342).

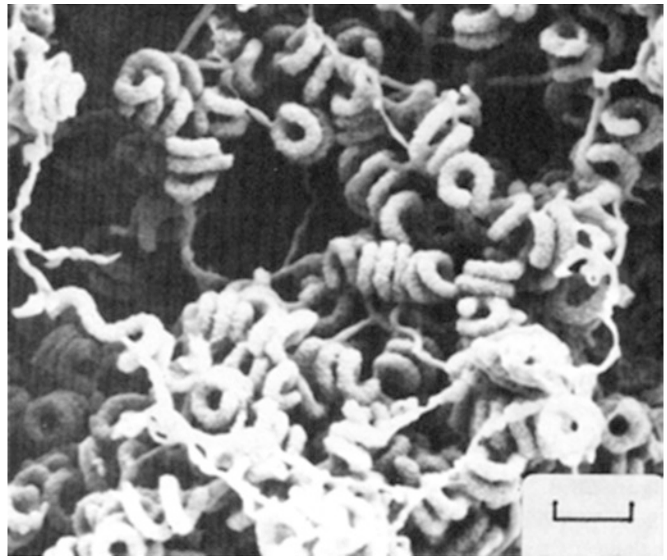

FIGURE 342. Spiral (*Spirales*) spore chains of *Streptomyces hygroscopicus*. Scanning electron microscopy. Bar, 5, µm.

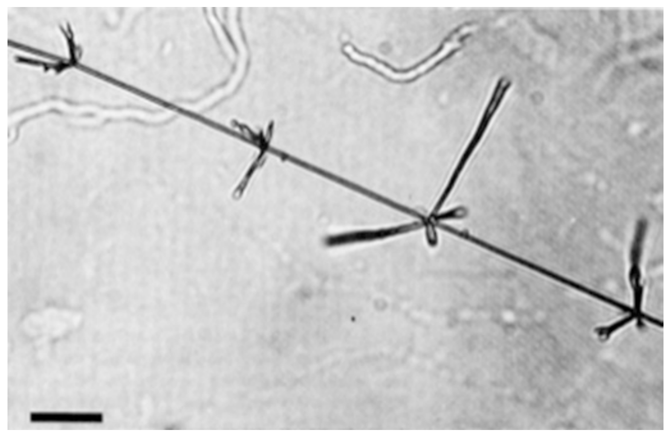

FIGURE 343. Aerial filament and initial verticils of *Streptoverticillium cinnamineum*. Light microscopy. Bar, 20 µm.

It should be noted, however, that even this simple system can pose problems, because it is not unusual that more than one category is seen in the same culture, and the distinction between *Retinaculiaperti* and *Spirales* is not always clear (Shirling and Gottlieb, 1977; Williams and Wellington, 1980). Aerial filaments can also differentiate into verticils (Figure 343, Figure 344, Figure 345, Figure 346, Figure 347, Figure 348, and Figure 349) as for most of species formerly grouped into the genus *Streptoverticillium* (Locci and Schofield, 1989).

Spore surface ornamentation has also been adopted as a taxonomic character. The ornaments, which are in fact borne on the spore sheath, can be grouped into the categories smooth, spiny, hairy, and warty. A further type, rugose, was proposed by Dietz and Mathews (1971) (Figure 350, Figure 351, Figure 352, Figure 353, and Figure 354). Spore surface ornamentation is a stable character, but the differences between smooth, warty, and rugose types can be difficult to observe. However, these problems can be resolved by using scanning electron microscopy.

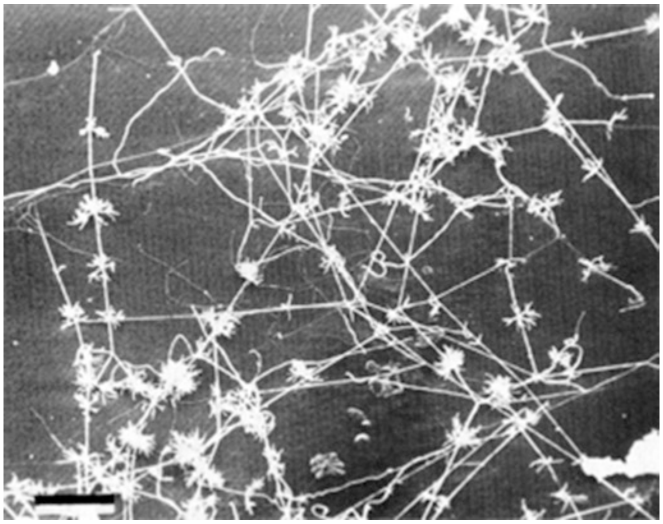

FIGURE 344. Aerial mycelium of *Streptoverticillium baldaccii*. Scanning electron microscopy. Bar, 50 μm.

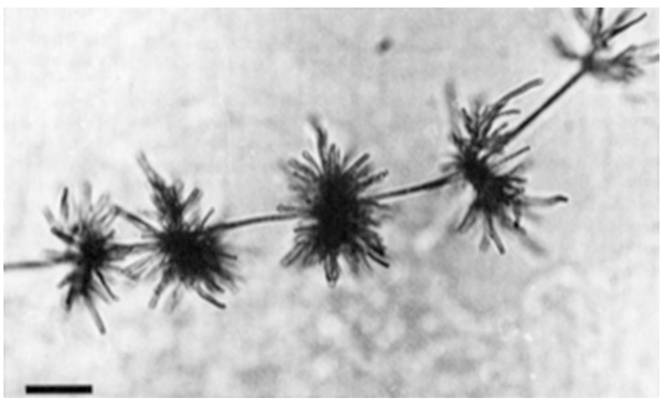

FIGURE 345. Sporulated aerial filament of *Streptoverticillium roseoverticillatum* subsp. *albosporum*. Light microscopy. Bar, 20 μm.

Normally, streptomycetes grow by tip extension as long, branching vegetative hyphae, which rarely have septae. Often, the compartments within the substrate hyphae contain numerous copies of the chromosomal DNA (Schrempf, 2006). The formation of aerial hyphae starts in response to nutrient depletion. The aerial structures can contain several different surface layers. The hydrophobic rodlet layer, which is one of them, comprises the proteins RdlA and RdlB. The corresponding genes have been studied in *Streptomyces coelicolor* M145 and "*Streptomyces lividans*" TK23. They are expressed within growing aerial hyphae, but not within spores (Claessen et al., 2002).

Comparisons of *Streptomyces coelicolor* A3(2) and *Streptomyces griseus* have shown that these strains share several orthologous genes that are important for the development of the aerial hyphae (Chater and Horinouchi, 2003). Some of these genes, e.g. *ramC* and *ramR*, are necessary for the production of the aerial hyphae, but are not essential for vegetative growth. The production of RamC requires additional developmental regulatory genes (*bldD*, *crpA*, and *ramR*, but not *bldN* and *bldM*; O'Connor et al., 2002). The expression of different *whi* (white) genes is important for induction of curling of the aerial hyphae, their septation, and finally spore formation. In studies on *Streptomyces coelicolor* A3(2), it has been shown that some early regulatory *whi* genes (*A*, *B*, *G*, *H*, *I*, and *J*) are required for septation

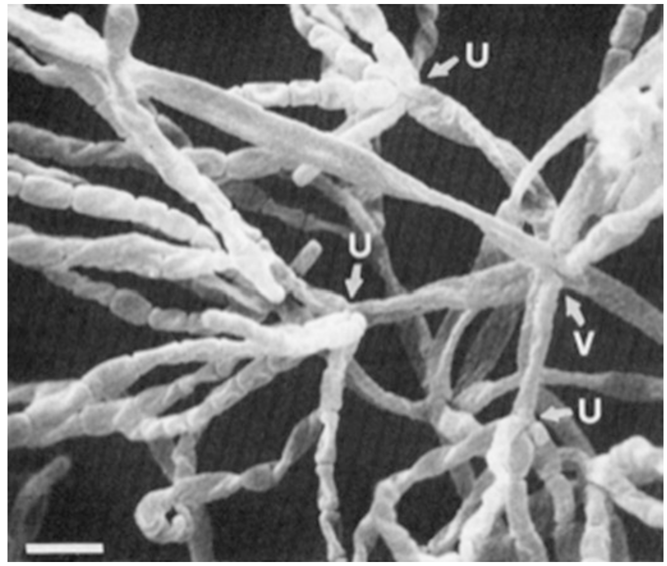

FIGURE 346. Verticil (V) and umbels (U) of spore chains of *Streptoverticillium kentuckense*. Scanning electron microscopy. Bar, 2 μm. (Reproduced with permission from Locci and Petrolini Baldan, 1971. Rivista di Patolgia Vegetale *7 (Suppl.)*: 3–19.)

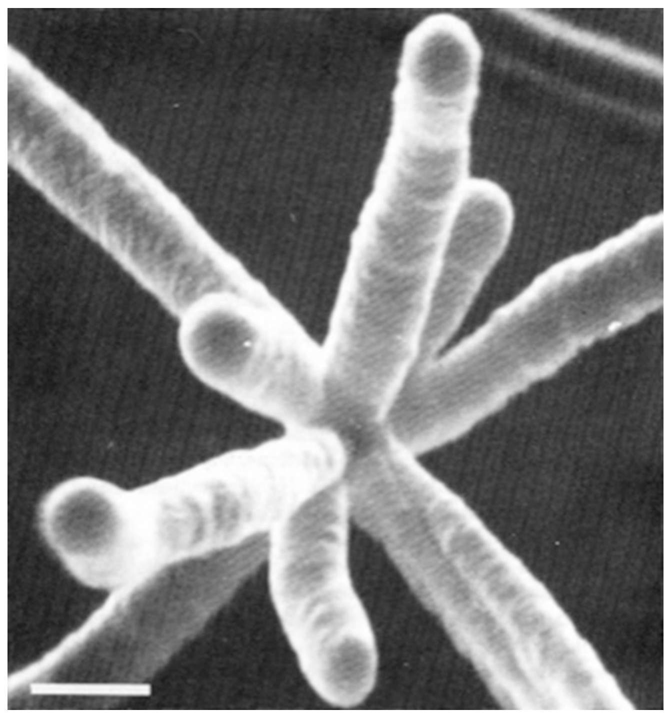

FIGURE 347. Verticil formation in *Streptoverticillium cinnamoneum*. Scanning electron microscopy. Bar, 1 μm. (Reproduced with permission from Locci and Petrolini Baldan, 1971. Rivista di Patolgia Vegetale *7 (Suppl.)*: 3–19.)

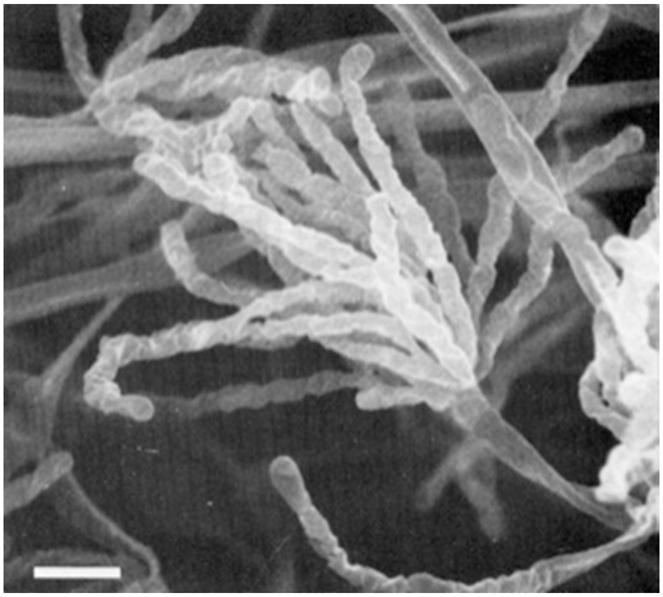

FIGURE 348. Mature umbel of spore chains of *Streptoverticillium baldaccii*. Scanning electron microscopy, Bar, 2 µm.

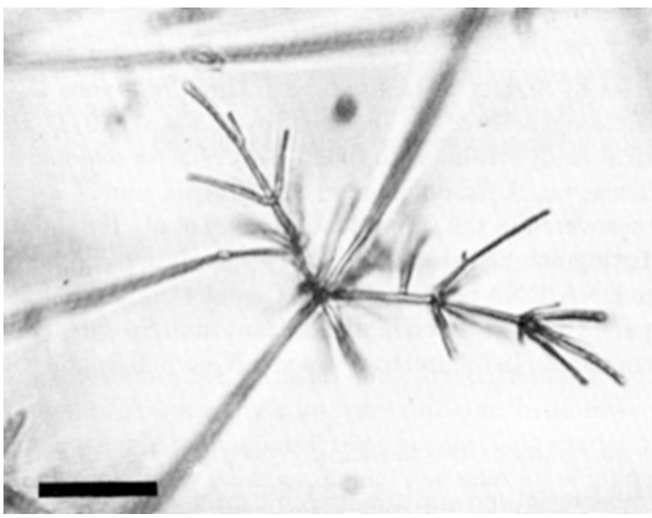

FIGURE 349. Biverticillate sporophore structure of *Streptoverticillium ehimense*. Light microscopy. Bar, 20 µm.

during sporulation. Furthermore, the *whiA* gene plays a major role in the extension process of the development of aerial hyphae towards septation (Ainsa et al., 2000).

Studies of the full genome of *Streptomyces coelicolor* A3(2) have shown that this strain encodes about 60 sigma (σ) factors. A subfamily of nine of these σ factors is involved in the late sporulation process and σ^H is involved in the development of aerial hyphae (in addition to responses to various stresses), whereas spore maturation is governed by σ^F (Schrempf, 2006). A recently published comparative genomic analysis (Chater and Chandra, 2006) gives a more detailed insight into this complex process. The partitioning of chromosomes is observed within the aerial hyphae; a comparatively synchronous septation leads

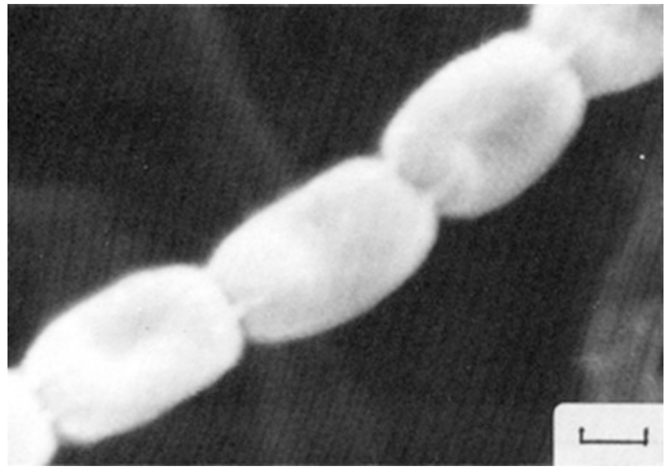

FIGURE 350. Smooth spores of *Streptomyces niveus*. Scanning electron microscopy. Bar, 0.25 µm.

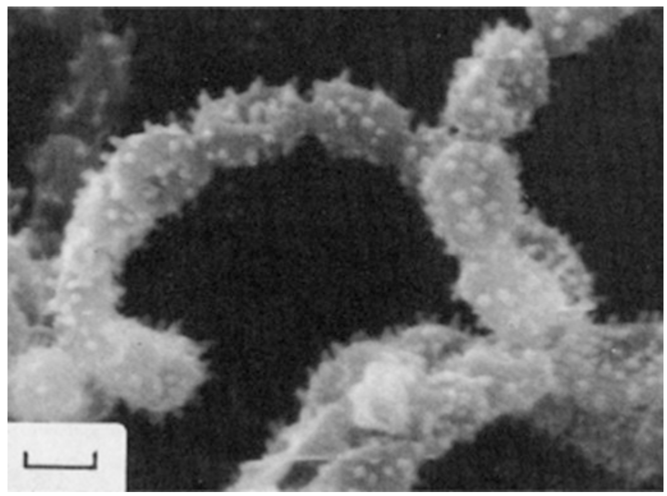

FIGURE 351. Spiny spores of *Streptomyces viridochromogenes*. Scanning electron microscopy. Bar, 0.5 µm.

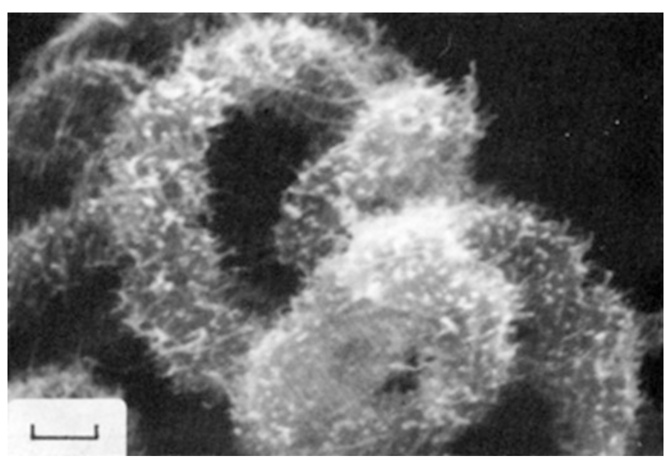

FIGURE 352. Hairy spores of *Streptomyces glaucescens*. Scanning electron microscopy. Bar, 0.5 µm.

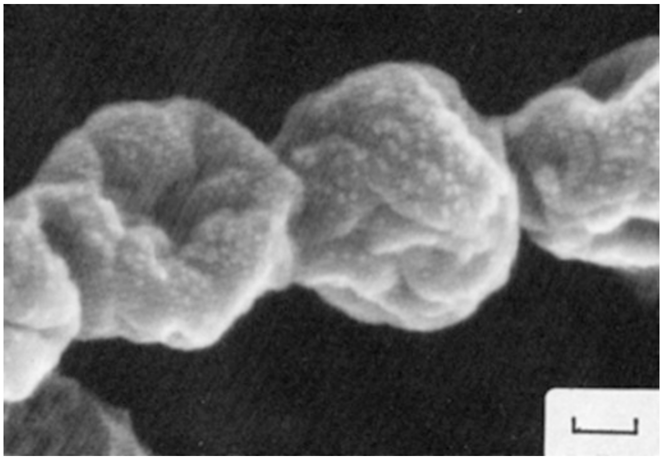

FIGURE 353. Warty spores of "*Streptomyces pulcher*". Scanning electron microscopy. Bar, 0.25 μm.

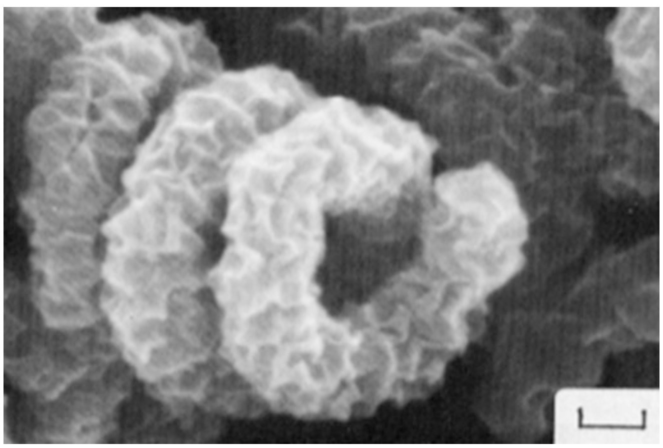

FIGURE 354. Rugose spores of *Streptomyces hygroscopicus*. Scanning electron microscopy. Bar, 0.5 μm.

to the compartment formation, which finally results in pore formation after maturation. Spores contain single chromosomes.

Cell division in *Streptomyces* is also a complex process. Normally, this is initiated by polymerization of the FtsZ protein on the inner surface of the cytoplasma membrane to form the Z-ring structure at the future division site (Lutkenhaus, 1997; Margolin, 2003). Studies on the transcription of genes in *Streptomyces griseus* suggest that *ftsZ* is expressed during both vegetative growth and sporulation (Dharmatilake and Kendrick, 1994). For *Streptomyces coelicolor* A3(2), it has been shown that FtsZ is required for septation within the vegetative substrate mycelium as well as for the synchronous formation of septae within the developing aerial hyphae prior to detectable partitioning of nucleoids (Grantcharova et al., 2003). The cycle of differentiation is usually observed on solid media, although for some strains [i.e. *Streptomyces griseus* (McCue et al., 1996); *Streptomyces coelicolor* A3(2) (Van Keulen et al., 2003)], sporulation in liquid culture is also reported. At the air interface of standing liquid cultures, gas vesicles may be present within the hyphae. For *Streptomyces coelicolor* A2(3), a gene cluster was reported that encoded proteins resembling gas vesicles of cyanobacteria and their homologs within halophilic archaea (Schrempf, 2006). For several *Streptomyces* strains, a transient slow down during growth in liquid culture is reported before entering the stationary phase. In this transition phase, an increase in ppGpp (guanosine 3′,5′-bispyrophosphate) and a decrease in GTP, as well as the activation of genes required for secondary metabolism, is observed. The synthesis of two ribosomal proteins is drastically reduced when the culture approaches the stationary phase (Blanco et al., 1994).

Cell-envelope composition.

Peptidoglycan. The ultrastructure and chemical composition of the cell walls of streptomycetes are typical for Gram-stain-positive bacteria (Schleifer and Kandler, 1972). Under the electron microscope, they appear as homogeneous electron-dense layers of about 16–35 nm. The cell walls are composed of peptidoglycan strands, which are multilayered. The heteropolymer peptidoglycan consists of heteropolysaccharide chains (the so-called "sugar backbone"), which are connected by peptide cross-links. The sugar backbone is composed of alternating β-1,4-linked units of the sugar derivatives *N*-acetylglucosamine and *N*-acetylmuramic acid. An oligopeptide of alternating D- and L-amino acids substitute the carboxyl group of the muramic acid (Schleifer and Kandler, 1972). In *Streptomyces*, the substitution is a tetrapeptide L-Ala–D-Glu–LL-A_2pm–D-Ala, which is cross-linked by a pentaglycine bridge extending from the C-terminal D-alanine of the peptide unit to the amino group located on the D carbon of LL-A_2pm. The resulting macromolecular structure forms the cell envelope. This LL-A_2pm–Gly_5 is also called the A3γ peptidoglycan type (Schleifer and Kandler, 1972) and is diagnostic for streptomycetes as well as some other combined-wall chemotype I actinomycetes (Lechevalier and Lechevalier, 1970a, 1970b, 1970c).

Lechevalier and coworkers used specific amino acids in purified cell walls to group aerobic actinomycetes into four so-called "wall chemotypes". Cell walls with *meso*-A_2pm and LL-A_2pm were the first to be detected. Cell-wall composition can vary with the developmental stage of streptomycetes. Takahashi et al. (1984) reported that submerged mycelium of strains having a cell wall with *meso*-A_2pm and LL-A_2pm consists of LL-A_2pm and glycine (wall chemotype I), whereas in the spores only *meso*-A_2pm could be detected (wall chemotype III according to Lechevalier and Lechevalier, 1970a, 1970b, 1970c). The quantitative distribution of cell-wall amino acids and cell-wall sugars differed in the cell-wall composition of aerial, substrate, and submerged mycelia of 11 streptomycetes. *N*-Acetylmuramic acid is found in the glycolyl type in the cell walls of *Streptomyces*, as in all other actinomycetes (Uchida and Aida, 1977).

Muramic acid phosphate residues are essential as attachment points to teichoic acids; the latter are polymeric substances containing repeating phosphodiester groups. They consist of polyols (i.e. the sugar alcohols glycerol and ribitol) or *N*-acetylamino sugars or both and are valuable for the identification of Gram-stain-positive bacteria. The structure of teichoic acids does not differ between streptomycetes and other Gram-stain-positive bacteria. The polymers consist either of ribitol phosphate or glycerol phosphate. Significant for the teichoic acids of actinomycetes is the absence of ester-bound D-alanine; instead, ester-linked acetic acid and sometimes succinic acid residues are present (Naumova et al., 1980).

The synthesis of either ribitol phosphate (e.g. *Streptomyces streptomycinii* and *Streptomyces violaceus*) or glycerol phosphate polymers (e.g. *Streptomyces antibioticus, Streptomyces levoris, Streptomyces rimosus*, and *Streptomyces thermovulgaris*) has been reported in streptomycetes (Naumova et al., 1980). In ribitol teichoic acids, positions 1 and 5 of ribitol are connected to the phosphates, but in glycerol teichoic acids, position 1 is commonly connected to 3, and, in other types, links to 2 (as in *Streptomyces antibioticus*) are uncommon. Polyol phosphates can be substituted with various combinations of sugars or amino sugars or both. The sugars or amino sugars are linked to glycerol or ribitol via glycosidic bonds. The role of teichoic acid in the taxonomy of *Streptomyces* is not clear as only a few strains have been analyzed in detail (Naumova et al., 1980).

Cell-wall polysaccharides. Cell-wall polysaccharides seem to be of no diagnostic value (Lechevalier et al., 1971) for strains that have LL-A_2pm in their cell wall. Occasionally, diagnostic sugars found in actinomycetes (e.g. arabinose, galactose, and xylose) have been reported in streptomycetes. The presence of diagnostic sugars in streptomycetes was extensively sought by Kroppenstedt (1977) who analyzed hundreds of strains. Glucose, mannose, and ribose are usually found in small amounts.

Phospho- and glycolipids. The lipids of streptomycetes consist mainly of diphosphatidylglycerol, phosphatidylethanolamine, phosphatidylinositol, and phosphatidylinositol mannosides. A summary of the lipid composition of actinomycetes can be found in Lechevalier et al. (1977). Glycolipids cannot be used for the identification of streptomycetes because they do not occur consistently and culture conditions largely determine their qualitative and quantitative lipid composition. Glycolipid content increases significantly under phosphate-limiting conditions.

Polar lipids have a significant taxonomic value in actinomycetes, as demonstrated by Lechevalier et al. (1977). The phospholipids of 97 actinomycete strains, representing 20 genera, were analyzed and assigned to five phospholipid types by Lechevalier et al. (1977). The phospholipid groups are characterized by the absence or presence of certain nitrogenous phospholipids. Members of the family *Streptomycetaceae* have phospholipid type II. The marker lipids of this type are phosphatidylethanolamine, methyl-phosphatidylethanolamine, hydroxy-phosphatidylethanolamine, and lyso-phosphatidylethanolamine, although differentiation can be made using additional lipids (e.g. phosphomonoester and hydroxy-phosphatidylethanolamine) and the presence or absence of phophatidylglycerol and phosphatidylinositol.

Menaquinones. Streptomycetes contain only menaquinones (Collins and Jones, 1981). The synthesized quinones have a partly saturated isoprenoid side chain at position 3 of the naphthoquinone ring. In this, streptomycetes resemble the majority of actinomycetes. Menaquinone composition has a great taxonomic value for the differentiation of actinomycetes. The following three variations are useful for classification and identification: 1) the different numbers of isoprene units; 2) the different degree of hydrogenation; 3) and the position of hydrogenated isoprene units (Figure 355, Table 264). The

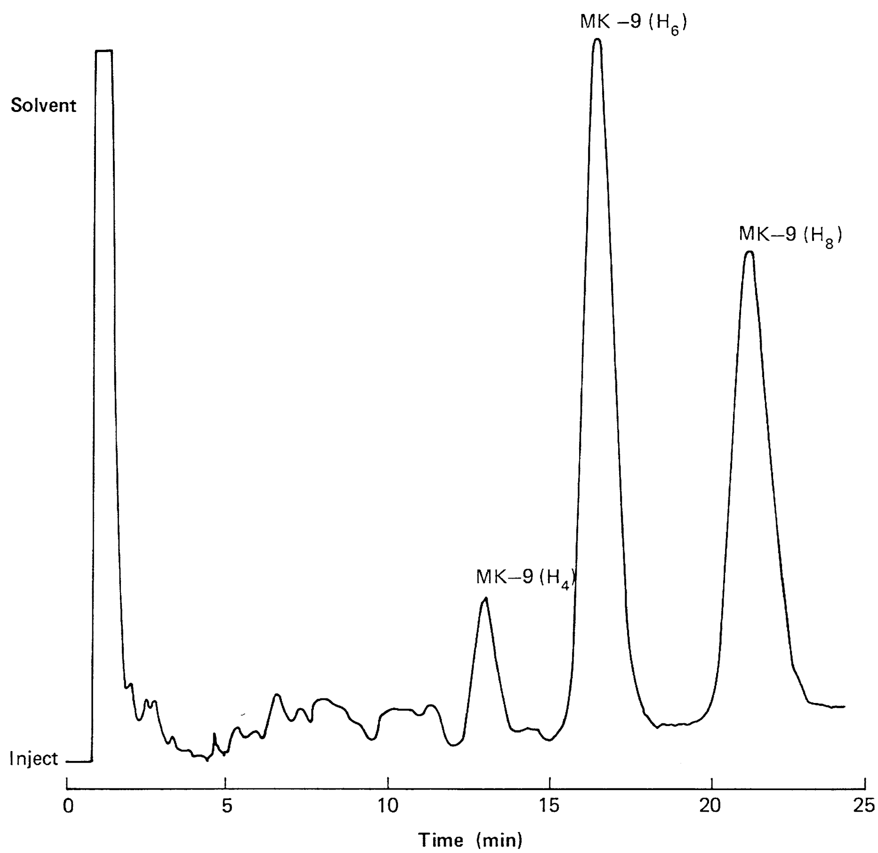

FIGURE 355. High-performance liquid chromatogram of menaquinones from *Streptomyces cyaneus* NCIB 9616.

TABLE 264. Key biochemical markers of genera assigned to the family *Streptomycetaceae* and to other families, which include strains producing an aerial mycelium (modified from Korn-Wendisch and Kutzner, 1992)[a]

Family and genus	A_2pm[b]	Glycine in IPB[b]	Peptidoglycan type[b]	Sugar type[b]	Phospholipid type[c]	Mycolic acids[b]	Fatty acid pattern[d]	Menaquinones[e]	DNA G+C content (mol%)
Streptomycetaceae:									
Streptomyces	LL	+	A3γ	–	PII	–	2c	$9(H_6)/(H_8)$	69–78
Kitasatospora	LL/*meso*	+	A3γ	C/E	PII	–	2c	$9(H_6)/(H_8)$	66–73
Streptacidiphilus	LL	+	A3γ	E	PII	–	2c	$9(H_6)/(H_8)$	70–72
Pseudonocardiaceae:									
Amycolatopsis	*meso*	–	A1γ	A	PII	–	3f	$9(H_4)(H_2)$	66–69
Kibdelosporangium[f]	*meso*	–	A1γ	A	PII	–	3f	nd	66
Pseudonocardia	*meso*	–	A1γ	A	PIII	–	2f	$8(H_4)$	79
Saccharopolyspora	*meso*	–	A1γ	A	PIII	–	2c/3e	$9(H_4)/10(H_4)/9(H_2)$	70–72
Saccharomonospora	*meso*	–	A1γ	A	PII	–	2a	$9(H_4)/8(H_4)$	69–74
Actinopolyspora	*meso*	–	A1γ	A	PIII	–	2c	$9(H_6)/9(H_4)$	64
Genera belonging to different families of the *Actinobacteria*[j]:									
Sporichthya	LL	+	A3γ	–	nd	–	3a	$9(H_6)/9(H_8)$	nd
Kineosporia[g]	LL/*meso*	(+)	nd	C	PIII	–	1	$9(H_4)$	69
Nocardioides	LL	+	A3γ	–	PI	–	3c	$8(H_4)$	66–73
Actinomadura[h]	*meso*	–	A1γ	B	PI	–	3a	$9(H_6)/(H_4)/(H_8)$	66–72
Microtetraspora[h]	*meso*	–	A1γ	B	PIV	–	3c	$9(H_4)/(H_2)/(H_0)$	66–69
Glycomyces	*meso*	+	nd	D	PI	–	2c	$9(H_4)/10(H_4)$	71–73
Saccharothrix	*meso*	–	nd	C/E	PII	–	3f	$9(H_4)/10(H_4)$	70–76
Nocardia[i]	*meso*	–	A1γ	A	PII	+	1b	cyclo $8(H_4)/9(H_2)$	64–72
Nocardiopsis	*meso*	–	nd	C	PIII	–	3d	$10(H_2)/(H_4)/(H_6)$	64–69
Streptoalloteichus	*meso*	–	nd	C	PII	–	nd	$9(H_6)/10(H_6)$	nd

[a]Abbreviations: IPB; interpeptide bridge. Symbols of sugar type: A, arabinose and galactose; B, madurose; C, no diagnostic sugars; D, arabinose and xylose; E, rhamnose and galactose; –, not applicable for LL-A_2pm. Phospholipid type: PI, phosphatidylglycerol (variable); PII, only phosphatidylethanolamine, PIII, phosphatidylcholine (with phosphatidylethanolamine, phosphatidylmethylethanolamine, and phosphatidylglycerol variable, no phospholipids containing glucosamine); PIV, phospholipids containing glucosamine (with phosphatidylethanolamine and phosphatidylmethylethanolamine variable). Menaquinones: number indicates number of isoprene units, H_x indicates presence of x hydrogenated menaquinones.

[b]Data from Goodfellow (1989).

[c]Data from Lechevalier et al. (1981, 1977).

[d]Data from Kroppenstedt(1985).

[e]Data from Kroppenstedt (1987) and R. Kroppenstedt (personal communication).

[f]Data from Bowen et al. (1989).

[g]Data from Itoh et al. (1989). The genus *Kineosporia* does not produce aerial mycelium.

[h]Data from R. Kroppenstedt (personal communication).

[i]Data from menaquinones from Howarth et al. (1986).

[j]For details see Stackebrandt and Schumann (2006).

menaquinones of streptomycetes have a highly hydrogenated isoprenoid chain and three to four (rarely five) saturated isoprene units. The actinomycetes, which belong to this type, can be differentiated by a different degree of saturation.

Fatty acids. *Streptomyces* species synthesize terminally branched fatty acids. 2-Methylbutyrate as a starting compound results in anteiso-branched fatty acids with an odd number of carbon atoms. In contrast, isovalerate and isobutyrate as starting compounds lead to the formation of iso-branched fatty acids with even and odd numbers of carbon atoms, respectively. For this reason, iso- and anteiso-branched fatty acids appear in pairs with odd numbers of carbon atoms only (Figure 356).

Colonial characteristics. Streptomycetes have many differential colonial features, such as pigmentation of spores, substrate mycelium, and diffusible exopigments, together with the morphology of colonies and the texture of the aerial mycelium. The production of different pigments has been widely used in classification and identification, but it is important to mention that colony morphology is too variable for use as a taxonomic character.

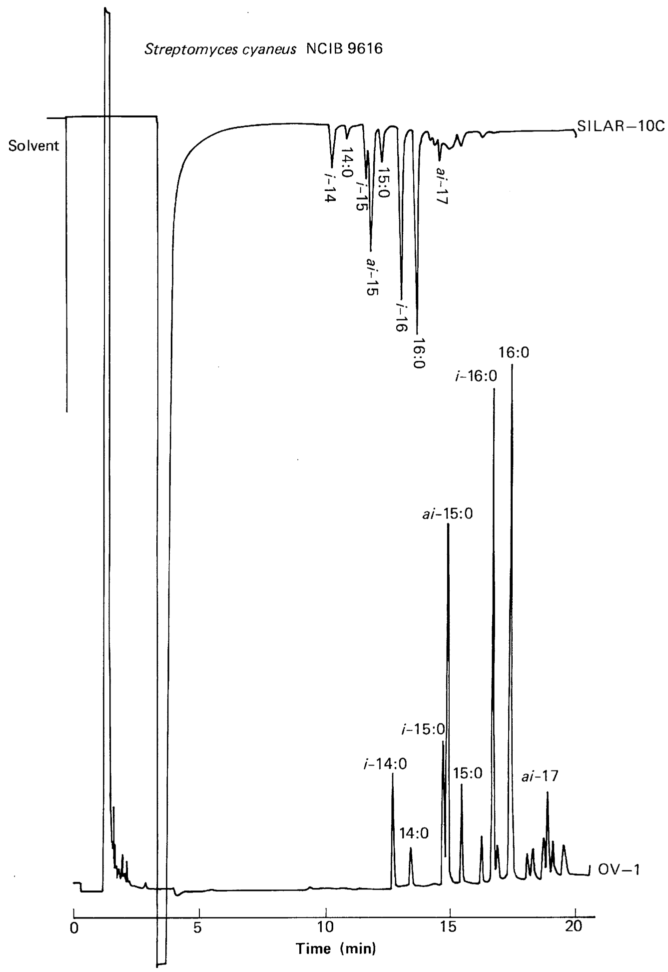

FIGURE 356. Gas chromatographic analysis of the fatty acid methyl esters of *Strepotomyces cyaneus* NCIB 9616.

One feature, widely used in streptomycete taxonomy, is spore mass color. Strains showing different spore colors have been assigned to "sections", "series", and "species-groups" (Burkholder et al., 1954; Ettlinger et al., 1958a; Flaig and Kutzner, 1954, 1960b; Gauze et al., 1957; Hesseltine et al., 1954; Krasil'nikov, 1960; Pridham et al., 1958). In the 8th edition of *Bergey's Manual* (Pridham and Tresner, 1974a), *Streptomyces* species were assigned to seven color series: Blue, Gray, Green, Red, Violet, White, and Yellow. In a later survey, the series were extended to accommodate additional colors (Kutzner, 1981). The color of the spore mass is still useful, but its determination may be difficult, because the color can be influenced by factors such as the medium, growth regime, and age of the culture. Sometimes, the color cannot clearly be attributed to any established category.

The color of the substrate mycelium and the soluble pigment are of high value when they are striking, e.g. blue, dark green, red, and violet. The color of the substrate mycelium has been used in a preliminary approach to group streptomycetes (Baldacci, 1958; Baldacci et al., 1954; Krasil'nikov et al., 1961), but again, the expression of the various pigments is often influenced by the medium composition, temperature, pH, and age of culture (Kutzner, 1981). Also, diffusible pigments and their pH sensitivity have been used as taxonomic characters (Jensen, 1930; Shirling and Gottlieb, 1970; Waksman and Curtis, 1916), but it may be that chemically different pigments exhibit the same color (Krasil'nikov, 1970a; Kutzner, 1981). Some *Streptomyces* strains produce anthracyclinglycoside, diazaindophenol, naphthoquinone, phenoxazinone, and prodigiosin pigments (Kutzner, 1981).

Despite the problems, determinations of aerial spore mass color, substrate mycelium color, and diffusible pigments were used for the descriptions of *Streptomyces* species in the ISP (Shirling and Gottlieb, 1966). It was important that the color determinations were based on standardized media and methods, but there was some disagreement on aerial spore color determination using the Tresner and Backus (1963) color chart. The diagnostic value of spore color was limited because over half of the test strains were placed in the Gray series. Good agreement was found for the determinations of pH sensitivity of the diffusible pigments, but not in the interpretation of substrate mycelium color (Shirling and Gottlieb, 1970).

Genetics and genomics. The genetics of streptomycetes is a rapidly developing topic and a wealth of information has been published within the last few years (for reviews and more information see, for example: Chen et al., 2002; Donadio et al., 2002; Paradkar et al., 2003; Schrempf, 2006; Ventura et al., 2007; Hopwood, 2003, 2007; Hsiao and Kirby, 2008; Dyson, 2010; and references therein). It is, of course, far beyond the scope of this short chapter to give a comprehensive summary of all the recent findings in this exciting area of research; however, despite this, it is not clear at present how this information can be used for taxonomic purposes.

The literature on *Streptomyces* genomes is extensive and information on the complex structure of genomes is increasing, mainly because streptomycetes are very abundant and important as soil inhabitants, where they are regarded as major agents in the cycling of organic carbon compounds. They are also able to produce many and diverse hydrolytic exoenzymes, like chitinases and cellulases. Summaries on their genomics and genetics are available (Paradkar et al., 2003; Schrempf, 2006; Ventura et al., 2007) and, hence, this paragraph is intended to give only a very short summary of existing knowledge on *Streptomyces* genetics.

In particular, one strain, *Streptomyces coelicolor* A3(2), is of major importance as it represents a major model organism for studying developmental complexity. In addition, streptomycetes are the most important natural source of antibiotic compounds and other bioactive metabolites. The whole genome sequences of strains of *Streptomyces* species have been studied and are published or available online: *Streptomyces coelicolor* A3(2) (representing the model streptomycete), *Streptomyces ambofaciens* strains ATCC 15154, DSM 40697, ETH 9247, and ETH 11317 (*Streptomyces ambofaciens* is known for its remarkable genetic instability), *Streptomyces avermitilis* MA-4680^T (the producer of avermectin), *Streptomyces griseus* subsp. *griseus* NBRC 13350 (producer of bioactive secondary metabolites), and *Streptomyces scabiei* 87.22 (causing potato scab).

All *Streptomyces* strains studied so far contain a large genome, which can be circular or linear. The linearity cannot be simply deduced from the extensive genetic linkage mapping of *Streptomyces coelicolor* and is not regarded to be linked with mycelial growth.

The *Streptomyces coelicolor* A3(2) genome was considered to be circular, but on the basis of studies on cosmid libraries in combination with comparisons of physical maps from the wild-type and mutant strains of *Streptomyces coelicolor* A3(2) and "*Streptomyces lividans*", it is quite likely that the chromosome occurs in circular and linear forms (Lin et al., 1993; Redenbach et al., 1996). Pulse-field gel electrophoresis (PFGE) studies have revealed the presence of a linear chromosome in other streptomycetes, including *Streptomyces ambofaciens* (Leblond et al., 1996), *Streptomyces antibioticus*, *Streptomyces moderatus*, *Streptomyces lipmanii*, *Streptomyces parvulus*, *Streptomyces rochei* (Lin et al., 1993), *Streptomyces griseus* (Lezhava et al., 1995), and *Streptomyces hygroscopicus* (Pang et al., 2002a, 2002b). A linear arrangement of the chromosome has also been shown for a *Streptoverticillium* sp. (Redenbach et al., 1998).

The *Streptomyces coelicolor* A3(2) chromosome contains about 8667 Mbp, which corresponds to 7825 genes. Twenty gene clusters encode known or predicted secondary metabolites (Bentley et al., 2002). It is noteworthy that the *Streptomyces coelicolor* chromosome was shown to carry more genes (i.e. 7825) than the eukaryote *Saccharomyces cerevisiae* (containing 6203 genes). The *Streptomyces avermitilis* MA-4680^T genome was shown to comprise about 9025 Mbp (mean G+C content 70.7 mol%), which corresponds to 7574 potential open reading frames, 35% of which constitute 721 paralogous families (Ōmura et al., 2001; Ventura et al., 2007). Thirty gene clusters encode secondary metabolites. It was found that one region of 6500 Mbp has been highly conserved with respect to gene order in the *Streptomyces avermitilis* MA-4680^T and *Streptomyces coelicolor* A3(2) genomes and, hence, may contain essential genes. The terminal regions are not conserved and contain "nonessential genes" (Ikeda et al., 2003). It is interesting to note that an ancient synteny (conservation of gene order) has been revealed between the central core of the *Streptomyces coelicolor* A3(2) chromosome and the whole chromosomes of *Corynebacterium diphtheriae* and *Mycobacterium tuberculosis* (Bentley et al., 2002).

Whole-genome synteny plots have shown a high conservation of the overall position and orientation of common genes in the chromosomes of *Streptomyces coelicolor* A3(2) and *Streptomyces avermitilis* MA-4680^T (Ventura et al., 2007). Ikeda et al. (2003) showed that about two-thirds (5283) of the genes of these strains represent conserved orthologs (estimated by reciprocal BLAST analysis). Similarly, 4837 genes were found to be orthologous between these two strains (Ventura et al., 2007). When *Streptomyces scabiei* ATCC 49173^T is added to this comparison, the number of genes conserved among the three strains drops to 4190 and, as pointed out by Ventura et al. (2007), a four-way analysis, including the hitherto unpublished sequence of *Streptomyces venezuelae* ATCC 10595, reduces the number to 3566. It can be expected that the number will fall further as more *Streptomyces* genomes become available. Ventura et al. (2007) pointed out that only about 17% of the 3566 genes common to the four *Streptomyces* genomes are present in *Escherichia coli* K-12 and *Bacillus subtilis* 168.

Hsiao and Kirby (2008) used DNA–DNA microarray hybridization to compare the genome content of *Streptomyces avermitilis* ATCC 31267^T, "*Streptomyces cattleya*" ATCC 35852, "*Streptomyces maritimus*" Yang-Ming and *Kitasatospora* (*Streptomyces*) *aureofaciens* ATCC 10762^T with that of *Streptomyces coelicolor* A3(2). About 93% agreement with the genome sequence data available for *Streptomyces avermitilis* ATCC 31267^T was shown and a number of trends in the genome structure for *Streptomyces* and closely related *Kitasatospora* species could be detected. The core central region was well conserved and a low degree of gene conservation in the terminal regions of the linear chromosome was observed across all four strains. Between these regions, two areas of intermediate gene conservation were detected by microarray analysis though some conserved genes were also identified within the terminal regions.

The replication process of the *Streptomyces* chromosome has been summarized by Schrempf (2006) and Ventura et al. (2007). The replication of linear *Streptomyces* chromosomes and plasmids is initiated from a fairly centrally located replication origin rich in DnaA box sequences and proceeds bidirectionally towards the telomeres. The chromosomal replication origin (*oriC*) region was found to be highly conserved in *Streptomyces coelicolor* A3(2) (Calcutt and Schmidt, 1992), "*Streptomyces lividans*" 66 (Zakrzewska-Czerwinska and Schrempf, 1992), "*Streptomyces lividus*" TK21, *Streptomyces antibioticus* ETH 7451,

and *Streptomyces chrysomallus* ATCC 11523^T (Jakimowicz et al., 1998). Interestingly, it was found that contrary to the high overall G+C content (69–73 mol%) of *Streptomyces* DNA, the region of the origin (*oriC*) is rich in A+T (64 mol%). The chromosomal ends of some *Streptomyces* species have been shown to contain terminal inverted repeats (TIRs), which are covalently bound to proteins, most likely at their 5′ ends. TIR lengths among available sequenced *Streptomyces* chromosomes vary considerably: 174 bp for *Streptomyces avermitilis*; 18,488 bp for *Streptomyces scabiei*; 21,653 bp for *Streptomyces coelicolor* M145; and approximately 198 kb for *Streptomyces ambofaciens* (Ventura et al., 2007).

The telomeres are replicated by a special mechanism which is initiated by priming from a terminal protein covalently bound to the 5′ ends. Approximately 250–320 nucleotides at these ends are characteristic and possess a complex secondary structure (Ventura et al., 2007).

The origin of the linearity of the *Streptomyces* chromosome is thought to have occurred by single-crossover recombination between an initially circular chromosome and a linear plasmid. Several examples of exchange of ends between chromosomes and linear plasmids, resulting in hybrid molecules with different right and left ends, have been reported.

The genomes of streptomycetes contain several examples of apparent redundancy of metabolic genes, which is thought to be due to the complex morphological and physiological differentiation of streptomycetes. The metabolic genes comprise genes that encode functions involved in carbon storage transactions, genes specific for different hyphal cell types, genes comprising enzymes of the pentose phosphate pathway, and multiple *fabH*-like genes, which are important for the first step in fatty acid biosynthesis (some of these are linked with secondary metabolism gene sets) (Ventura et al., 2007). Interestingly, some genes are not present in streptomycetes. Two of the three subunits of exonuclease V (the *recB* and *recC* genes), for instance, are not found in streptomycetes, though they are present in other actinobacteria such as mycobacteria. The XerCD pathway, which is responsible for the resolution of circular chromosomes after replication, is absent from streptomycetes, a result in line with the linearity of *Streptomyces* chromosomes. The conserved *ftsA* gene, which is widely distributed in the domain *Bacteria* and involved in the complex cell division process, is generally absent from actinobacteria. In addition, the *minC* and *minE* genes, which are involved in the choice of division site in many unicellular bacteria are not found in *Streptomyces* strains (Ventura et al., 2007).

Streptomyces colonies often show a high spontaneous variability in antibiotic biosynthesis, pigmentation, and sporulation. The various antibiotic resistances, A-factor formation, and synthesis of tyrosinase or arginosuccinate are encoded by unstable genes. It is possible to stimulate this genetic instability by mutagens, such as ethidium bromide, mitomycin, ultraviolet light, and by gyrase- (topoisomerase II) inhibiting antibiotics. Often, these variations can be attributed to large chromosomal deletions, preferentially occurring at the telomeric and subtelomeric regions, and including up to 2 Mbp of DNA (Chen, 1995; Hütter and Eckhardt, 1988; Leblond and Decaris, 1994; Schrempf et al., 1989). More details about these processes are given by Schrempf (2006). The variability of the chromosomal DNA is further increased by its interaction with linear and circular plasmids, phages, transposons, and insertion elements. Streptomycetes inhabit quickly changing environments and, hence, the high plasticity of the genome is likely an effective prerequisite for quick adaptation.

Streptomyces extrachromosomal elements. Many plasmids have been described (for a review, see Kieser et al., 2000). Linear plasmids, often very large, are widespread and diverse among streptomycetes, but circular plasmids are also found. The various types of circular plasmids differ in size. Schrempf et al. (1989) isolated the low-copy number circular plasmid SCP2 from a streptomycete and this is used as a cloning vector. The plasmid SCP2* (a variant of SCP2; Hopwood et al., 1985) has been sequenced and encodes 34 proteins, most of them of unknown function. The replication region was shown to contain the *repI* and the *repH* genes, both encoding small proteins. The *traA* gene, essential for DNA transfer and pock-formation, was identified in addition to 10 additional genes, which are thought to be involved in conjugation and DNA spreading. Plasmid pIJ101 (about 8.8 kb) is a high-copy-number natural conjugative *Streptomyces* plasmid. On this plasmid, regions for its replication, stability, transfer and distribution have been identified. Efficient conjugation among streptomycetes necessitates the plasmid-encoded *tra* gene and the *cis*-acting locus of transfer (Ducote et al., 2000). A further natural plasmid, pSG5 (initially found in *Streptomyces ghanaensis* DSM 2932), of about 12.3 kb, is naturally temperature-sensitive and cells may contain approximately 50 copies per chromosome. Additional multicopy plasmids pSN22 (Kataoka et al., 1991), pJV1 (Bailey et al., 1986), and pSMA2 (Pernodet et al., 1984) replicate via a rolling circle mechanism (Hagege et al., 1993; Servín-González, 1993).

Linear plasmids are also common in streptomycetes; however, knowledge of their encoding functions is still restricted to few functions. These include antibiotic production (Gravius et al., 1994; Kinashi et al., 1991) and mercury resistance (Ravel et al., 1998). Some of the linear plasmids are efficiently transferred during conjugation. The replication mechanism is best understood for pSLA2, initiated bidirectionally near the center and proceeding towards its telomeric ends, generating 3′ leading-strand overhangs. It should be mentioned that several other members of the order *Actinomycetales* harbor genes on linear plasmids, among them those required for isopropylbenzene and trichlorethylene catabolism (*Rhodococcus erythropolis*; Kebeler et al., 1996), biphenyl degradation (*Rhodococcus erythropolis* and *Rhodococcus globerulus*; Kosono et al., 1997), hydrogen autotrophy (*Rhodococcus opacus*, formerly "*Nocardia opaca*"; Kalkus et al., 1993), and fasciation in plants (*Rhodococcus fascians*; Crespi et al., 1992). Linear plasmids have also been discovered for mycobacteria (*Mycobacterium avium*, *Mycobacterium branderi*, *Mycobacterium celaturum*, and *Mycobacterium xenopi*). Their termini are similar to those of linear plasmids from *Streptomyces* and *Rhodococcus* species (Picardeau and Vincent, 1998); it is an open question whether circular and linear plasmids are exchanged during conjugation amongst actinomycetes. Relatively little is known about *Streptomyces* transposons; a summary of this topic is available (Schrempf, 2006). Phages with broad or narrow host ranges can be obtained from soil and several of them have been used for classifying strains (for review, see Kutzner, 1981). However, in current taxonomic studies, phage host range studies are not carried out.

DNA regions in mycelial actinobacterial genomes acquired by HGT. Despite the gross synteny between the central regions

of *Streptomyces* genomes, there are hundreds of insertion-deletion (indel) differences between *Streptomyces coelicolor* A3(2) and *Streptomyces avermitilis* MA-4680^T, most of them involving one or a few genes. This often makes it difficult to recognize synteny at the level of small groups of genes. Streptomycetes also have numerous larger islands of species-specific DNA (Ventura et al., 2007). Prior to the publication of other *Streptomyces* genome sequences, 14 islands of likely laterally acquired DNA were found by Bentley et al. (2002) in the *Streptomyces coelicolor* A3(2) genome on the basis of gene content, atypical G+C content, and location next to a tRNA determinant. Around 50% of these islands were shared with *Streptomyces ambofaciens*. From this, it is clear that in pairwise synteny plots the genes in the "subtelomeric arms" of *Streptomyces* chromosomes are much less conserved between species than those in the central regions or "cores" and that the cores contain most of the genes conserved with other actinobacteria (Bentley et al., 2002; Choulet et al., 2006; Ikeda et al., 2003).

Production of extracellular enzymes. Streptomycetes are widely distributed in soil and play an important role in the recycling of organic matter. It is not surprising, therefore, that *Streptomyces* genomes encode high numbers of predicted secreted proteins; the approximately 800 proteins in *Streptomyces coelicolor* A3(2) have been found to comprise 147 hydrolases, of which seven are cellulases and five are chitinases (Bentley et al., 2002; Ventura et al., 2007). Almost all *Streptomyces* strains studied so far can use chitin not only as a carbon, but also as a nitrogen source (Blaak and Schrempf, 1995) and, often, several chitinases are produced (Miyashita et al., 1991; Robbins et al., 1988). For more details, see Schrempf (2006). Amylases and their inhibitors are also common in streptomycetes. An α-amylase gene (*aml*) of *Streptomyces limosus* ATCC 19778^T has been cloned by Virolle and Bibb (1988). It can be deduced from sequence information that the *Streptomyces coelicolor* A3(2) genome has many genes which code for glucosyltransferases. In addition, xylanases and their genes have been identified from several streptomycetes, i.e. "*Streptomyces lividans*" 10-164 (Pagé et al., 1996), *Streptomyces halstedii* JM8 (Ruiz-Arribas et al., 1998), and the thermophilic *Streptomyces thermoviolaceus* OPC-520 (Tsujibo et al., 1997). Laccases, including those produced by *Streptomyces cyaneus* CECT 3335, can be efficiently applied for biobleaching of kraft pulps (Arias et al., 2003).

Extracellular proteases are widely distributed among streptomycetes and several corresponding genes have been characterized (Kim and Lee, 1995). Streptomycetes also contain many genes for protease inhibitors (Taguchi et al., 1996), including leupeptin and subtilisin (Hiraga et al., 2000). In addition, keratinases are frequently found (for review, see Kutzner, 1981). A few extracellular lipases and their genes have been studied from different *Streptomyces* strains (Servín-González et al., 1997; Sommer et al., 1997), among them lipolytic enzymes expressed by *Streptomyces rimosus* R6-554W (Vujaklija et al., 2002).

Jendrossek et al. (1997) found out that *Streptomyces* strains are the predominant community members of latex rubber-degrading actinomycetes. Some streptomycetes can produce enzymes involved in the modification of pharmacologically relevant compounds and xenobiotics (Peczynska-Czoch and Mordarski, 1988).

Primary metabolism. In contrast to secondary metabolism, relatively few studies have been published on the primary metabolism of streptomycetes. Some genes, among them those encoding key enzymes, like fructose-1,6-bisphosphate aldolase and glucose-6-phosphate dehydrogenases, have been identified. Butler et al. (2002) identified two *zwf* genes determining isozymes of glucose-6-phosphate dehydrogenases [the first enzyme in the oxidative pentose phosphate pathway (PPP)] and one gene (*devB*) encoding 6-phosphogluconolactonase in "*Streptomyces lividans*" 66. The PPP and the tricarboxylic acid cycle relative to glucose uptake have been studied in *Streptomyces noursei* ATCC 11455^T (Jonsbu et al., 2001).

Similar to enteric bacteria, glutamine synthetase I (GSI) in *Streptomyces coelicolor* A3(2) is post-translationally controlled by adenylyltransferase (Hesketh et al., 2002a). A novel class of glutamate dehydrogenases (GDHs) has been detected in *Streptomyces clavuligerus* NRRL 3585^T (Minambres et al., 2000). In addition, *Streptomyces coelicolor* A3(2) can utilize fatty acids (C4 to C18) as sole carbon sources (Banchio and Gramajo, 1997) and the glyoxylate cycle also seems to be present, at least in *Streptomyces clavuligerus* NRRL 3585^T (Soh et al., 2001). Malonate is a well-known competitive inhibitor of succinate dehydrogenase. Kim and Goodfellow (2002) used the genes *matB* and *matC* in generating strain variants of *Streptomyces* used for the production of antibiotics.

The pathways and genes required for the biosynthesis of primary compounds, including their regulation pattern (Rodríguez-García et al., 1997), should be studied in more detail, in order to improve knowledge on metabolic fluxes (Obanye et al., 1996). This will certainly improve the biotechnological production of pharmacologically active compounds derived from primary metabolites. Detailed analyses of proteins involved in primary and secondary metabolism are available (Hesketh et al., 2002b; Huang et al., 2001). Proteomic and metabolomic data are currently being studied (e.g. Novotna et al., 2003).

Secondary metabolism. Streptomycetes have been the most important source of antibiotics since the discovery of actinomycin D, streptothricin, and streptomycin in the 1940s by Waksman and coworkers (for a review, see Hopwood, 2007). Streptomycetes synthesize a large variety of chemically different compounds, many of them acting as antibiotics, cytostatics, fungicides, or as modulators of immune responses (see e.g. Horinouchi, 2002; Bérdy, 2005; Challis and Hopwood, 2003; Van Wezel and Vijgenboom, 2004; and Hopwood, (2007) for more detailed information). Consequently, the study of *Streptomyces* genomes has been of great interest and has led to the discovery of 23 gene sets that code for these compounds in the *Streptomyces coelicolor* A3(2) chromosome and 30 in that of *Streptomyces avermitilis* MA-4680^T (Ventura et al., 2007). It is interesting that many of these gene sets are present in one genome, but not in others, and that the same position in different chromosomes can be occupied by different secondary metabolism clusters. For example, the *pks1* cluster of *Streptomyces avermitilis* MA-4680^T is replaced in *Streptomyces ambofaciens* ATCC 23877^T by a different secondary metabolism cluster of 28 genes and in *Streptomyces coelicolor* A3(2) by a 31-gene insertion (Choulet et al., 2006).

The subtelomeric chromosome arms often contain gene clusters for secondary metabolism, especially those that are

species-specific. The more abundant genes for secondary metabolism, such as those for the production of pentalenolactone, different siderophores, and the odor compound geosmin, typically fall in syntenous locations within the central core region (Bentley et al., 2002; Ikeda et al., 2003). It is known that certain linear plasmids may also carry such clusters, thereby explaining the impact of lateral gene transfer between chromosomes present in different streptomycetes (Ventura et al., 2007).

The genes encoding different pharmacologically active substances of importance are located within DNA stretches of 20 kb to more than 100 kb. Successful cloning has been achieved by complementing mutants, by screening total genomic DNA or gene libraries with homologous or heterologous gene probes generated by cloning, or with the help of PCR, as well as by transposon mutagenesis (Schrempf, 2006). It is interesting that the genes for the biosynthesis of antibiotics are frequently located near one or more genes mediating resistance to the corresponding antibiotic. The following listing has been adapted from Schrempf (2006). The gene-cluster for the synthesis of the polyketide actinorhodin was achieved by complementation of mutants (Malpartida and Hopwood, 1984). Other gene clusters for polyketides were cloned using a gene-probe for the predicted key step for polyketide synthesis. These polyketides include: daunorubicin (Stutzman-Engwall and Hutchinson, 1989), frenolicin (Bibb et al., 1994), granaticin (Sherman et al., 1989), griseusin B (Yu et al., 1994), jadomycin B (Han et al., 1994), mithramycin (Lombo et al., 1996), tetracyclines (Binnie et al., 1989), tetracenomycin C (Motamedi and Hutchinson, 1987), tetrangomycin (Hong et al., 1997), and urdamycin A (Decker and Haag, 1995).

Genes for several clusters of macrolides have been identified, including the genes for carbomycin (Epp et al., 1987), tylosin (Fishman et al., 1987), oleandomycin (Swan et al., 1994), and rapamycin (Schwecke et al., 1995). Genes for peptide antibiotics [such as actinomycin (Hsieh and Jones, 1995; Stindl and Keller, 1994) and biolaphos (Murakami et al., 1986)], and cyclopentenoid antibiotics (such as methylenomycin; Chater and Bruton, 1985) have also been found. Additionally, genes have been detected for the synthesis of nikkomycin (a nucleoside-peptide; Bormann et al., 1996), nosiheptide (a thiopeptide; Dosch et al., 1988), undecylprodigiosin (a pyrrole; Feitelson and Hopwood, 1983; Malpartida et al., 1990), ansamycins [such as rubradirin (Sohng et al., 1997) and rifamycin (August et al., 1998)], aminoglycosides [such as puromycin (Lacalle et al., 1992) and streptomycin (Distler et al., 1987; Ohnuki et al., 1985)], carbapenems (Nakata et al., 1989), cephamycin (Aharonowitz et al., 1992; Paradkar et al., 1996), and cyclopilins (Pahl et al., 1997). Again, it is far beyond the scope of this chapter to give a comprehensive overview on the secondary metabolism of streptomycetes, which are covered in text books and/or reviews (e.g. Bérdy, 2005; Challis and Hopwood, 2003; Van Wezel and Vijgenboom, 2004; Hopwood, 2007; Dyson, 2010).

Ecology. Streptomycetes can be isolated in high numbers from soil, which is their primary natural habitat. As mentioned above, most streptomycetes can degrade complex and recalcitrant plant and animal materials, often polymeric residues including polysaccharides (e.g. cellulose, chitin, pectin, and starch), proteins (e.g. elastin and keratin), aromatic compounds, and lignocellulose. The biodegradative activities of actinomycetes have been the subject of several reviews (Crawford, 1988; Lechevalier, 1988; Peczynska-Czoch and Mordarski, 1988). Streptomycetes are able to degrade lignin, which occurs in nature together with cellulose and xylan (hemicellulose) in a lignocellulose complex. Experiments with ^{14}C-labeled lignin showed that streptomycetes (Antai and Crawford, 1981; Crawford, 1978), as well as other genera of actinomycetes, are involved in lignin decomposition (McCarthy et al., 1984, 1986; McCarthy and Broda, 1984), although fungi play a more important role in this process (Crawford, 1981; Janshekar and Fiechter, 1983; Kirk and Farrell, 1987). Ligniolytic streptomycetes can degrade the cellulose of the lignocellulose complex. For more details, see Ramachandra et al. (1988), Wang et al. (1990), Crawford et al. (1993), Chamberlain and Crawford (2000), Kormanec et al. (2001), Gottschalk et al. (2003), and Kaneko et al. (2003).

In addition, mesophilic and thermophilic streptomycetes have been reported to contain multicomponent cellulases, which consist of several endoglucanases and exoglucanases (Crawford and McCoy, 1972; Enger and Sleeper, 1965; Harchand and Singh, 1997; MacKenzie et al., 1984; Marri et al., 1997; Ulrich and Wirth, 1999; Wirth and Ulrich, 2002). Xylanases, which are involved in the decomposition of the lignocellulose complex, seem to be widespread among thermophilic actinomycetes, although they have also been found in mesophilic streptomycetes (Deobald and Crawford, 1987; Godden et al., 1989; Kluepfel and Ishaque, 1982; Kluepfel et al., 1986; McCarthy et al., 1985; Morosoli et al., 1999; Schäfer et al., 1996). Other polymeric compounds found in streptomycetes include pectinolytic complexes (Sato and Kaji, 1975, 1977, 1980a, 1980b) and chitinolytic complexes, which consist of chitinase and chitobiase, and have generally been isolated in full from *Streptomyces griseus* (Berger and Reynolds, 1958), *Streptomyces antibioticus* (Jeuniaux, 1966), and other streptomycetes (Beyer and Diekmann, 1985). More details are given by Schrempf (2006).

Starch, which is the primary material for the textile, paper, and food industries, can be degraded by a wide variety of fungi and bacteria. The enzymes involved are amylases, some of which have been found in several streptomycetes (Fairbairn et al., 1986; McKillop et al., 1986; Mordarski et al., 1970; Suganuma et al., 1980).

Next to degrading polymeric compounds, streptomycetes have the ability to degrade other organic materials, e.g. cotton and plant fibers (Khan et al., 1978; Lacey and Lacey, 1987), wool (Noval and Nickerson, 1959), hydrocarbons in jet fuel and emulsions (Genner and Hill, 1981), rubber (Cundell and Mulcock, 1975; Hutchinson et al., 1975), and plastics (Pommer and Lorenz, 1986). Lacey (1988) and Behal (2000) give a detailed review about the biodegradation of natural and synthetic substances. More details are given by Schrempf (2006).

In soil, streptomycetes can show pronounced mycelial growth. In this habitat, they are adapted to various, often quickly changing physical conditions (e.g. shifts in aeration, drought, frost, hydrostatic pressure and anaerobic conditions, moisture tension, and pH) by the formation of spores, which are semidormant stages in the life cycle and can survive in soil for long periods (Ensign, 1978; Mayfield et al., 1972). Viable cultures of cells have been reported by Morita (1985) from 70-year-old soil samples. Streptomycetes are almost always present as inactive spores in soil. One disadvantage of persisting as a spore is

the very low germination efficiency, which may be caused by competition with other micro-organisms. Spores which pre-germinate can grow for a short time and then resporulate (Lloyd, 1969). Several factors may be responsible for the germination of spores. Next to special signaling factors, the presence of exogenous nutrients, water, and Ca^{2+} seems to be necessary (Ensign, 1978). In addition to germination, nutrients influence the extent of hyphal growth and the time of differentiation into aerial hyphae. Fodders and other organic material, freshwater, and marine habitats, as well as potable water systems, can come into contact with soil (Korn-Wendisch and Kutzner, 1992), e.g. through human and other activities. Natural substrates (e.g. grain, hay, fodder, and wood) and synthetic products (e.g. cotton textiles, fabric, paper, rubber, plastics, and plasticizers), which can be found in or transported to soil, can be degraded with the help of mesophilic and especially thermophilic streptomycetes. The contamination of creeks and rivers with soil streptomycetes is due to drainage, e.g. after heavy rainfalls. Streptomycetes find their way into the sediments of the lakes, rivers, and, after transport to the sea, into marine sediments. It may be possible that drinking water supplies become contaminated with streptomycetes. This is a problem because the compounds produced by some streptomycetes are odorous and lead to the spoilage of the water.

Streptomycetes as plant pathogens. Some of the many saprophytic *Streptomyces* species are plant pathogens which may cause economically important diseases, including potato scab. *Streptomyces scabiei* can still be regarded as the dominant pathogenic species worldwide, but is only one of many streptomycetes which cause very similar disease symptoms on plants. In addition to *Streptomyces scabiei* (Lambert and Loria, 1989b), *Streptomyces acidiscabies* (Lambert and Loria, 1989a), *Streptomyces turgidiscabies* (Miyajima et al., 1998), *Streptomyces europaeiscabiei*, *Streptomyces stelliscabiei* (Bouchek-Mechiche et al., 2000), *Streptomyces luridiscabiei*, *Streptomyces puniciscabiei*, and *Streptomyces niveiscabiei* (Park et al., 2003) have been shown to be plant pathogens that cause either common scab or netted scab, mostly in potatoes.

Streptomyces scabiei (previously know as "*Streptomyces scabies*"), which is the most important and oldest characterized potato scab pathogen, has been isolated from beets, carrot, peanut, and radish, among other crops (Loria et al., 2006). Strains of *Streptomyces scabiei* are phenotypically similar to *Streptomyces bottropensis*, *Streptomyces diastatochromogenes*, and *Streptomyces neyagawaensis*, a result confirmed by 16S rRNA gene sequence analyses. *Streptomyces acidiscabies* has been isolated from low pH soils in the north-eastern United States, amongst other locations. *Streptomyces turgidiscabies* has been isolated from cases of potato scab in Finland, but also from Japan and Korea (Loria et al., 2006). *Streptomyces europaeiscabiei*, the most closely related species to *Streptomyces scabiei* has been isolated from various locations in Europe. Three species, *Streptomyces luridiscabiei*, *Streptomyces niveiscabiei*, and *Streptomyces puniciscabiei*, are the causal agents of potato scab in Korea.

16S rRNA gene sequence analyses and DNA–DNA hybridization studies show that the documented pathogenic strains fall outside the described species listed above (Loria et al., 2006). This can be attributed to the polyphyletic nature of scab-causing species and the existence of a transmissible pathogenicity island, which seems to confer the pathogenic phenotype on some otherwise non-pathogenic species, as reviewed by Loria et al. (2006). The mechanisms used by plant-pathogenic species to manipulate their hosts have been studied in detail and summarized by Loria et al. (2008, 2006). The nitrated dipeptide phytotoxin, thaxtomin, plays an important role in inhibiting cellulose biosynthesis in expanding plant tissues, stimulating Ca^{2+} spiking, and causing cell death. In addition, a secreted necrogenic protein, Nec1, contributes to virulence on diverse plant species. A detailed genetic analysis revealed that the thaxtomin biosynthetic genes and *nec1* lie on a large mobilizable plasmid PAI, along with other putative virulence genes, including a cytokinin biosynthetic pathway and a saponinase homolog. The PAI is mobilized during conjugation and site-specifically inserts itself into the linear chromosome of recipient species, thereby accounting for the emergence of new pathogens in agricultural systems.

Streptomycetes as human pathogens. So far, only very few streptomycetes have been isolated from human pathological material. They include organisms that cause actinomycetoma, which is a localized chronic, destructive, and progressive infection of skin, subcutaneous tissue, and eventually bone (Develoux et al., 1999; McNeil and Brown, 1994). In certain tropical and subtropical regions, this disease is endemic and has a devastating effect on patients, as it frequently leads to deformities, disabilities, and eventually amputation of the affected organs. Although some of the main causal agents belong to other genera and species, i.e. *Actinomadura madurae*, *Actinomadura pelletieri*, *Nocardia brasiliensis*, *Nocardia otitidiscaviarum*, and *Nocardia transvalensis*, *Streptomyces somaliensis* is often implicated, notably in parts of the Sudan (Trujillo and Goodfellow, 2003). However, the recognition of a second species, *Streptomyces sudanensis*, by Quintana et al. (2008) suggests that some strains identified as *Streptomyces somaliensis* (Fahal, 2006; Gumaa, 1994; Gumaa and Mahgoub, 1975; Taha, 1983) may have been misclassified. Indeed, there is evidence that streptomycetes associated with cases of actinomycetoma in the Sudan (Fahal, 2004, 2006; Fahal and Hassan, 1992; Mahgoub, 1985) may be underspeciated (Quintana et al., 2008; Trujillo and Goodfellow, 2003).

Soil as a habitat. Abiotic and biotic factors, especially vegetation, content and kind of organic matter, soil type, season and climate, temperature, circulation of water and air, and pH influence the character and community composition of any habitat, including the microbial community. Streptomycetes are common in soils and have been the subject of several reviews: Goodfellow and Simpson (1987), Korn-Wendisch and Kutzner (1992), Lechevalier (1988), Williams (1982), Williams et al. (1982a). The most extensive studies have been carried out by Stan Williams and his colleagues (Flowers and Williams, 1977a, 1977b; Khan and Williams, 1975; Mayfield et al., 1972; Ruddick and Williams, 1972; Watson and Williams, 1974; Williams et al., 1971; Williams and Mayfield, 1971; Williams and Robinson, 1981). The typical life cycle of streptomycetes in soil, including its genetic control, can be found in Kieser et al. (2000). In most soils, streptomycetes constitute about 1–20% of the total viable count, that is 10^4–10^7 colony-forming units (c.f.u.) per g soil (Korn-Wendisch and Kutzner, 1992), and in some soils form the dominant population. Further details in the numbers and distribution of streptomycetes in soil can be found elsewhere (Flaig and Kutzner, 1960a; Küster, 1976; Misiek, 1955; Szabó and Marton, 1964).

The detection and localization of different *Streptomyces* species in their natural habitat are based mainly on cultivation-dependent techniques. Intrageneric classification of the genus *Streptomyces* is difficult and, hence, ecophysiological studies are often difficult to compare. Williams et al. (1969) assigned soil streptomycetes to color-groups, based on diffusible pigment colors formed on oatmeal agar, and on their capacity to produce melanin pigments on peptone-yeast extract-iron agar. This classification into color-groups was then used as a tool by other researchers to study the diversity of streptomycetes in natural habitats (e.g. Goodfellow and Haynes, 1984; Atalan et al., (2000) Sembiring et al., 2000). However, this color-grouping is a subjective method and comparison of data between different studies is difficult. Recently, a computer-assisted numerical analysis was carried out with 321 alkaliphilic streptomycetes that were assigned to color-groups (Antony-Babu et al., 2010). The authors argue that, with this method, distances between individual colors could be calculated more objectively and that the data can be compared with computer-assisted numerically defined color-groups in future investigations on streptomycete taxonomy in natural habitats.

Williams et al. (1972) have shown that streptomycetes resist desiccation because of their ability to form arthrospores. In addition, the water tension they need for growth can be much lower than for other bacteria, but on the other hand, they may be very sensitive to water-logged conditions.

Most attention has been focused on neutrophilic streptomycetes, which are common in neutral to alkaline soils (e.g. Flaig and Kutzner, 1960a), although acidotolerant and acidophilic streptomycetes are abundant in acidic soils and can be isolated using starch-casein agar adjusted to pH 5.0 supplemented with anti-fungal agents (Hagerdorn, 1976; Khan and Williams, 1975). Acidophilic streptomycetes produce specific and stable amylases (Williams and Flowers, 1978; Williams and Robinson, 1981). In contrast, alkalitolerant and alkaliphilic streptomycetes are common in alkaline soils (Antony-Babu and Goodfellow, 2008; Mikami et al., 1982, 1985; Taber, 1959, 1960).

Streptomycetes, as well as other soil bacteria, have been isolated from the intestinal tract of earthworms (Brüsewitz, 1959; Parle, 1963a, 1963b), the gut of arthropods (Bignell, 1984; Bignell et al., 1980, 1981; Szabó et al., 1967), and pellets produced by millipedes and woodlice (Márialigeti et al., 1984). Streptomycetes are also found in the rhizosphere (Goodfellow and Williams, 1983; Sembiring et al., 2000) where they may have an important role.

It has been suggested ever since the discovery of antibiotics from streptomycetes that antibiotic-producing organisms have a competitive advantage over nonproducing organisms. However, there is no clear evidence for the *in situ* production of antibiotics in soil (Williams, 1982). Antibiotics are difficult to detect in soil as they are found in low concentrations and may be unstable (Brian, 1957; Williams, 1982). In addition, they may be adsorbed onto soil colloids (Williams, 1982) and may also be produced at certain stages of the growth cycle (Williams, 1982; Williams and Khan, 1974).

However, antibiotic production in soil was reported by Rothrock and Gottlieb (1984) who supplemented sterilized soil with nutrients prior to adding a potent producer. In the control of fungal root pathogens, streptomycetes seem to play an important role (Goodfellow et al., 2007; Rothrock and Gottlieb, 1981; Sing and Mehrotra, 1980; Williams, 1978, 1982). It has also been observed that many streptomycetes are often successful in competition with other rhizosphere bacteria such as pseudomonads and bacilli, especially in relatively dry soils.

Thermophilic streptomycetes. The genus *Streptomyces* contains mainly mesophilic species, though some streptomycetes are thermotolerant (growing up to 45°C) and a few are thermophilic. So far, all described thermophilic streptomycetes grow at temperatures between 28–55°C and several grow at even higher temperatures. Kim et al. (1999) studied the taxonomy of thermophilic streptomycetes in detail. Additional thermophilic species (*Streptomyces thermocoprophilus* and *Streptomyces thermospinisporus*) were described by Kim et al. (2000) and Kim and Goodfellow (2002). The life cycle of thermophilic streptomycetes includes active growth at sites of high temperatures, e.g. compost, manure, and self-heating hay or grain. When the vegetative phase ends, the formation of large numbers of spores begins. The spores are returned with the compost or manure to the fields and pastures and can colonize plant material and hay directly or via soil dust (Korn-Wendisch and Kutzner, 1992). Therefore, the genus *Streptomyces* accounts for the majority of actinomycetes isolated from bioaerosols in the surroundings of composting facilities (P. Kämpfer and others, unpublished observation). Thermophilic actinomycetes are widespread and can be isolated from various sources like soils (Craveri and Pagani, 1962; Tendler and Burkholder, 1961), pig feces (Ohta and Ikeda, 1978), sewage-sludge compost (Millner, 1982), and freshwater habitats (Cross, 1981a, 1981b).

Freshwater environments, water supplies, and marine environments. Actinomycetes can easily be isolated from fresh water and especially from sediments of rivers and lakes. However, it is assumed that most of these organisms do not live naturally at these sites and are therefore inactive (Cross, 1981a, 1981b). Instead, they are wash-in forms ("aliens") from surrounding terrestrial environments. In particular, rivers carry vast amounts of various actinomycetes, including streptomycetes. Nevertheless, actinomycetes can survive as dormant spores in aquatic habitats for a long time (Al-Diwany and Cross, 1978). Burman (1973) found 59–200 streptomycetes and 10–20 micromonosporae per ml river water sampled from the River Thames, UK. The streptomycetes grew on decaying vegetation on riverbanks and mud flats at low water or on floating mats of decaying algae or other vegetation. They produce odorous substances which are washed into the water when river levels increase giving rise to "earthy tastes" in drinking water. Geosmin and methyl-isoborneol are the two most frequently detected odorous compounds (Gerber, 1979a, 1979b). Wood et al. (1983) noted that preventing the contamination of potable water with these compounds and, thus, the earthy tastes in reservoirs and water supply systems, depends on locating the production sites and determining the patterns of distribution of these compounds (Lechevalier et al., 1980; Silvey and Roach, 1975). Burman (1973) found that the number of streptomycetes in drinking water was reduced by filtration processes. He also detected a new, aquatic strain of *Streptomyces* in the distribution system (for details, see Burman, 1973).

The occurrence of streptomycetes in marine habitats, including sediments, has been considered by several workers (Cross, 1981b; Goodfellow and Haynes, 1984; Okazaki and Okami,

1976; Weyland, 1981a, 1981b; Weyland and Helmke, 1988). Streptomycetes have been found in the littoral and inshore zone and in deep-sea sediments. Although streptomycetes can be isolated from both localities, they are not necessarily part of the autochthonous microflora, but are probably derived from terrestrial habitats. Streptomycetes isolated from sediments (Roach and Silvey, 1959) and from decaying seaweed (Siebert and Schwartz, 1956) in littoral zones were able to grow on polymeric substances, such as agar and chitin (Humm and Shepard, 1946), alginate and laminarin (Chesters et al., 1956), and cellulose (Chandramohan et al., 1972), which are substances characteristic of these habitats.

In sediments, the ratio of different actinomycete taxa is dependent on the depth and the location of the sampling sites (Weyland, 1981b; Weyland and Helmke, 1988). In the open sea, only low numbers of actinomycetes are generally detected (viable counts about 100 c.f.u. per ml of wet sediment). It is assumed that the distribution of streptomycetes is correlated with barotolerance (Helmke, 1981), halotolerance, and psychrophilism (Weyland, 1981a) (horizontal as well as vertical) of streptomycetes, micromonosporae, and rhodococci. On the other hand, Goodfellow and Haynes (1984) did not find any correlation between salinity, pH, or depth and the number of actinomycetes recovered from marine sediments. These workers studied 732 isolates; 250 belonged to *Streptomyces*, 250 to *Micromonospora*, 140 to *Rhodococcus*, and 92 were assigned to the genus *Thermoactinomyces*. One of the streptomycetes was subsequently identified using a computer-assisted approach (Williams et al., 1983b) and about half of them were assigned to a cluster equated with *Streptomyces albidoflavus* (Williams et al., 1983a).

Streptomycetes are mainly found in sediments of shallow seas (70–520 m deep) with 300–1270 colonies per cm^3, whereas *Micromonospora* are dominant in samples 700–1600 m deep (Okami and Okazaki, 1978). However, these authors did not detect actinomycetes from depths of 2800 and 5000 m in the Pacific Ocean. On the other hand, Pathom-aree et al. (2006) isolated actinomycetes, including streptomycetes, from the Mariana Trench in the Pacific at a depth of 10,898 m. Marine streptomycetes showed a higher salt tolerance than their terrestrial counterparts, though salt tolerance among streptomycetes is widespread (Tresner et al., 1968). A few of the isolated marine streptomycetes were found to be obligate halophiles (Okazaki and Okami, 1976).

Many antibiotic-producing streptomycetes have been isolated from marine habitats (Goodfellow and Fiedler, 2010; Hotta et al., 1980; Okami and Okazaki, 1972; Okami et al., 1976) including seaweed (Nissen, 1963). Recently, Goodfellow and Fiedler (2010) provided a review on a bioprospecting strategy based upon the premise that new secondary metabolites can be found by screening relatively small numbers of dereplicated, novel actinomycetes isolated from marine sediments.

Enrichment, isolation, and cultivation

Korn-Wendisch and Kutzner (1992) summarized extensively the procedures used to isolate streptomycetes. These procedures are briefly described here. Further information about isolation for special purposes, growth, and preservation of streptomycetes can be found in the excellent textbook *Practical Streptomyces Genetics* (Kieser et al., 2000).

Generally, isolation procedures for micro-organisms are dependent on the nature of the micro-organism, as well as the number of individuals relative to the number of other microbes within the habitat (Stolp and Starr, 1981). Direct plating of a serial dilution on a nutrient agar medium can readily lead to a pure culture, if the chosen organism is best adapted to the selected isolation conditions. However, this procedure does not work well for isolation of streptomycetes. Instead enrichment cultures or selective media and/or specific isolation conditions are usually used.

Members of the family *Streptomycetaceae* can be isolated using the following selective criteria (Korn-Wendisch and Kutzner, 1992; Williams et al., 1984a; Williams and Wellington, 1982a, 1982b) (1) choice of the material containing the selected micro-organisms; (2) pretreatment of the sample, and in some cases, enrichment of the chosen microbial groups; (3) use of selective media or selective incubation conditions or both; and (4) colony selection on the basis of colony morphology.

Streptomycetes occur in and can be isolated from a wide variety of habitats. In most cases, the organisms are extracted from soil or another environmental sample, followed by dilution of cells (cell aggregates) to allow cultivation on solid media.

Isolation and enrichment from soil. Vegetative mycelia and spore chains are often closely associated with soil mineral and organic particles. For isolation, vigorous shaking of the sample with the diluent is often needed to suspend the spores or mycelial fragments. It can be helpful to use glass beads and agitate the sample on a shaker. Additional methods are described in the literature, including the use of mechanical devices such as the Ultrasonics sonicator-disrupter, Ultra-Turrax homogenizer, Turmix blender, Waring blender, or a mortar and pestle. However, the efficiency of these pretreatments has not been compared in detail. Other procedures described include the use of chemical disruption methods to separate mycelia from spores. Herron and Wellington (1990) gently shook soil samples with an ion-exchange resin Chelex-100 (Bio-Rad) followed by differential centrifugation and filtration. For increasing the yield and diversity of actinobacteria from natural habitats, the dispersion and differential centrifugation (DDC) technique can be used, which is a multistage procedure that combines several physicochemical treatments (Goodfellow and Fiedler, 2010). The DDC technique was introduced by Hopkins et al. (1991).

Subsequent treatment of samples (i.e. preparing dilutions and plating) differs little from usual bacteriological practice. Before dilutions are made, coarse particles of the soil suspensions should be allowed to settle. Another possibility is the use of the soil particles for the incubation of "soil plates" (Warcup, 1950); this method is also used to isolate fungi. For streptomycetes, the addition of lime to soil can be a helpful enrichment factor [see chapter 2 of Kieser et al. (2000) and references therein]. Surface-inoculation of isolation plates may be carried out with a sterile glass rod (or Drigalski spatula).

To avoid the spread of motile bacteria via water films, plates can be dried at 45°C before incubation (Vickers and Williams, 1987). Another highly recommended procedure is to mix the soil suspension with the molten agar (Korn-Wendisch and Kutzner, 1992). A 100-fold increase in streptomycete colonies on isolation plates can be achieved by the addition of $CaCO_3$ to air-dried soil samples (10:1, w/w) and subsequent incubation at

26°C for 7–9 d in a water-saturated atmosphere (El-Nakeeb and Lechevalier, 1963; Tsao et al., 1960).

Jensen (1930) enriched streptomycetes by amending soil with keratin; it is also known that adding chitin to soil enhances the growth of streptomycetes (Williams and Mayfield, 1971). In addition, enrichment of acidophilic and neutrophilic streptomycetes in acidic soil and litter can be achieved using fungal chitin (Williams and Robinson, 1981). Chitin in the form of insect wings has also been used as an isolation strategy (Jagnow, 1957; Okafor, 1966; Veldkamp, 1955). Other selective isolation methods studied by Porter and Wilhelm (1961) include the use of various other organic materials, such as salmon viscera meal, peanut meal, cottonseed meal, and dried blood flour (15 mg/g of soil), as described by Porter and Wilhelm (1961). Additionally, the authors recorded an increase in the number of streptomycetes (up to 1000-fold) when the enrichment cultures were incubated under moist conditions.

Arginine glycerol agar is frequently used for the selective isolation of streptomycetes (El-Nakeeb and Lechevalier, 1963), as are: HV agar (Hayakawa and Nonomura, 1987a, 1987b), colloidal chitin agar (Hsu and Lockwood, 1975), and reduced arginine starch salts agar.

To reduce or inhibit other microbes, several biological, chemical, and physical methods have been studied (see reviews by: Goodfellow and Williams, 1986; Goodfellow and Fiedler, 2010). The centrifugation of soil suspensions for 20 min at 1600 × *g* separates streptomycetes spores (in the supernatant) from other bacteria and fungal spores (in the sediment) as applied by Nüesch (1965), though this method has not been very successful. El-Nakeeb and Lechevalier (1963) used a similar approach, but obtained a significantly smaller number of streptomycete colonies as compared with the control. A simple sedimentation method was described by Voelskow (1988/89), who suspended 1 g soil in 15 ml salt solution and mixed the preparation by vigorously shaking followed by ultrasonic vibrations. Samples were taken from different levels of this solution after 1, 2, and 4 h of sedimentation, further diluted, and plated onto agar surfaces.

Arthrospores have a relatively high resistance to low moisture tension; hence, initial drying and heating procedures can be applied to environmental samples to reduce the number of unwanted bacteria. A relative increase in streptomycete concentrations can be obtained by drying samples, or by prolonged storage at ambient temperatures for mesophiles and at 50–60°C for thermophiles. A significant reduction in the vegetative bacterial proportion without affecting the colony counts of streptomycetes can be maintained by heat treatment of soil (40–50°C, 2–16 h), as reported by Williams et al. (1972).

Membrane filtration has been used for the enrichment of streptomycetes from water (Burman et al., 1969), and from seawater and mud (Okami and Okazaki, 1972) samples. This method was used as a first step in the isolation of streptomycetes from soil. Trolldenier (1966) filtered 1 ml of a series of 10-fold dilutions through membranes (0.3 µm pore size) prior to placing the latter upside down on a suitable agar medium supplemented with 10% compost soil. Streptomycetes were able to grow through the pores and developed colonies between the agar surface and the membrane filter, whereas other bacteria and fungi were unable to grow through the pores. The application of this procedure led to a three to fivefold increase in the number of streptomycete colonies compared with poured plates without soil in the medium.

The use of cellulose ester membrane filters (pore size 0.01–3.0 µm) was introduced by Hirsch and Christensen (1983). The membrane filters were placed onto nutrient agar containing anti-fungal antibiotics (cycloheximide and candicidin) and samples of soil, water, and vegetable material were used to inoculate the plates. The hyphae of actinomycetes were able to penetrate the pores in the membrane filters and grow on the underlying agar medium after 4 d, whereas the growth of other bacteria was restricted to the surface of the filters. Afterwards, the membrane filters were removed and the plates were reincubated to allow further development of actinomycete colonies. Filters (0.22–0.45 µm) can also be used for the exclusive recovery of actinomycetes, as described by Polsinelli and Mazza (1984) and Hanka et al. (1985).

Several authors have added chemicals to environmental samples to improve isolation efficiency. One recommended method to eliminate bacteria and fungi involved phenol treatment of a dense soil suspension (1.4% for 10 min) though El-Nakeeb and Lechevalier (1963) obtained less favorable results with this method. Burman et al. (1969) found that streptomycetes and other actinomycetes were slightly more resistant to ammonia, chloramine, and sodium hypochlorite than other bacteria. Hence, they used these agents for the treatment of water samples.

Isolation of airborne spores. *Streptomyces* spores from self-heating material such as hay or compost can be agitated in a wind tunnel (Lacey and Dutkiewicz, 1976b) or sedimentation chamber (see below; Lacey and Dutkiewicz, 1976a), prior to using an Andersen sampler to inoculate plates with the resultant aerosol (Goodfellow and Williams, 1986). This method is widely employed for the isolation of thermophilic actinomycetes, but can also be used for the isolation of mesophilic streptomycetes from soil. Other devices such as filtration samplers (e.g. Sartorius MD 8) can be used for the sampling of airborne streptomycetes.

Use of selective media. For the isolation of desired micro-organisms, selective media are widely used. By varying a number of factors, the media are favorable for some microbes but not for others. Factors that can be varied are as follows: (1) the nutrient composition and concentration of the isolation medium can be adjusted to the preferred micro-organisms, i.e. choice of carbon and nitrogen sources preferred by the organisms; (2) chemical substances can be added to the medium to inhibit selectively the accompanying flora of the natural habitat or to stimulate the desired organisms; (3) adequate pH values for acidophilic, neutrophilic, and alkaliphilic organisms can be chosen; (4) various temperatures can be used, depending on the temperature optima of the organisms, e.g. thermophiles or psychrophiles.

For the isolation of streptomycetes, many different media have been empirically formulated. In Table 265, selected carbon and nitrogen compounds are listed that are especially suitable for the isolation of these organisms. Table 266 and Table 267 list the most frequently used media with their formulae. On the other hand, streptomycetes can also be grown on very poor media such as water agar.

Different carbon and nitrogen sources for enrichment. It was recognized early on that streptomycetes can degrade chitin (Jagnow, 1957; Veldkamp, 1955). On this basis, Lingappa and Lockwood (1962) described a chitin medium for selective

TABLE 265. Nutrients and selective agents recommended for isolation of streptomycetes from soil (according to Korn-Wendisch and Kutzner, 1992)

	Selective agents in the medium		
Preferred C and N source	Antibiotic	Others	Reference
Starch, KNO_3			Flaig and Kutzner (1960a)
Starch, casein, KNO_3			Küster and Williams (1964a)
Chitin			Lingappa and Lockwood (1962)
Glycerol, arginine			El-Nakeeb and Lechevalier (1963)
Glycerol, casein, KNO_3			Küster and Williams (1964a)
Raffinose, histidine			Vickers et al. (1984)
Starch, casein, KNO_3	Rifampin		Vickers et al. (1984)
Starch, casein, KNO_3	Cycloheximide, nystatin, penicillin, polymyxin		Williams and Davies (1965)
Glycerol, arginine	Cycloheximide, pimaricin, nystatin		Porter et al. (1960)
Glucose, asparagine	Cycloheximide		Corke and Chase (1956)
Asparagine		Propionate	Crook et al. (1950)
Starch, casein, KNO_3	Cycloheximide	Rose Bengal	Ottow (1972)

TABLE 266. Some media recommended for the selective isolation of streptomycetes[a]

	1	2	3	4	5
Ingredients (g/l)	Starch-casein-KNO_3 agar	Glycerol-arginine agar	*Actinomyces* isolation agar	Chitin agar	Raffinose-histidine agar
Chitin (colloidal)	–	–	–	4.0	–
Starch	10.0[b]	–	–	–	–
Glycerol	–	12.5	5.0[c]	–	–
Raffinose	–	–	–	–	10.0
Sodium propionate	–	–	4.0	–	–
KNO_3	2.0	–	–	–	–
Casein	0.3	–	–	–	–
Sodium caseinate	–	–	2.0	–	–
Asparagine	–	–	0.1	–	–
Arginine	–	1.0	–	–	–
Histidine	–	–	–	–	1.0
NaCl	2.0	1.0	–	–	–
KH_2PO_4	–	–	–	0.3	–
K_2HPO_4	2.0	1.0	0.5	0.7	1.0
$MgSO_4 \cdot 7H_2O$	0.05	0.5	0.1	0.5	0.5
$CaCO_3$	0.02	–	–	–	–
$Fe_2(SO_4)_3 \cdot 6H_2O$	–	0.01	–	–	–
$FeSO_4 \cdot 7H_2O$	0.01	–	0.001	0.01	0.01
$CuSO_4 \cdot 5H_2O$	–	0.001	–	–	–
$ZnSO_4 \cdot 7H_2O$	–	0.001	–	0.001	–
$MnSO_4 \cdot H_2O$	–	0.001	–	–	–
$MnCl_2 \cdot 4H_2O$	–	–	–	0.001	–
Agar[d]	18.0	15.0	15.0	20.0	12.0
pH				Adjusted to 7.0–7.5 or lower or higher depending on the flora to be isolated.	

[a]References for media: 1, according to Küster and Williams (1964a); 2, El-Nakeeb and Lechevalier (1963); 3, Difco; 4, Hsu and Lockwood (1975); 5, Vickers et al. (1984).

[b]Alternatively, glycerol at 10 g/l can be used.

[c]Not contained in the dehydrated medium; added at the time of preparation.

[d]The different amounts of the agar are due to the varying quality used by the individual authors.

TABLE 267. Composition of some media suitable for the cultivation of streptomycetes (according to Korn-Wendisch and Kutzner, 1992)[a]

Medium	Ingredients	Comments
1. Glucose-yeast extract-malt extract (GYM) agar	Glucose, 4.0 g	Addition of $CaCO_3$, 2.0 g/liter, is advantageous for streptomycetes. Adjust medium to pH 7.2
	Yeast extract, 4.0 g	
	Malt extract, 10.0 g	
	Agar, 12.0 g	
	Distilled water, 1 l	
2. Oatmeal agar	Oatmeal, 20.0 g	Cook 20.0 g oatmeal in 1 liter distilled water for 20 min. Filter through cheesecloth. Add distilled water to restore volume of filtrate to 1 liter, then add trace salts solution and agar. Adjust to pH 7.2.
	Agar, 12.0 g	
	Trace salts solution (see no. 5), 1.0 ml	
	Distilled water, 1 l	
3. Inorganic salts-starch agar	Starch (soluble), 10.0 g	Make a paste of the starch with a small amount of cold distilled water and bring to a volume of 1 liter; then add the other ingredients. The pH should be between 7.0 and 7.4. Do not adjust it if it is within this range.
	$(NH_4)_2SO_4$, 2.0 g	
	K_2HPO_4 (anhydrous basis), 1.0 g	
	$MgSO_4 \cdot 7H_2O$, 1.0 g	
	NaCl, 1.0 g	
	$CaCO_3$, 2.0 g	
	Trace salts solution (see no. 5), 1.0 ml	
	Agar, 12.0 g	
	Distilled water, 1 l	
4. Glycerol-asparagine agar	Glycerol, 10.0 g	The pH should be about 7.0–7.4. Do not adjust if it is within this range
	L-Asparagine (anhydrous basis), 1.0 g	
	K_2HPO_4, 1.0 g	
	Trace salts solution (see no. 5), 1.0 ml	
	Agar, 12.0 g	
	Distilled water, 1 l	
5. Trace salts solution	$FeSO_4 \cdot 7H_2O$, 0.1 g	
	$MnCl_2 \cdot 4H_2O$, 0.1 g	
	$ZnSO_4 \cdot 7H_2O$, 0.1 g	
	Distilled water, 100.0 ml	
6. Trace elements solution SPV-4	$CaCl_2 \cdot 2H_2O$, 4.0 g	SPV-4 is used as an alternative to (5). 5 ml of this stock solution is added to 1 liter of medium
	Fe (III) citrate, 1.0 g	
	$MnSO_4$, 0.2 g	
	$ZnCl_2$, 0.1 g	
	$CuSO_4 \cdot 5H_2O$, 0.04 g	
	$CoCl_2$, 0.022 g	
	$Na_2MoO_4 \cdot 2H_2O$, 0.025 g	
	$Na_2B_4O_7 \cdot 10H_2O$, 0.1 g	
	Distilled water, 1 l	

[a]Recipes 1–5 are from Shirling and Gottlieb (1966) and recipe 6 is from Voelskow (1988/89).

isolation. However, this chitin medium was only a little better than water agar, which was recognized by the authors and later also by El-Nakeeb and Lechevalier (1963). A useful medium for the isolation of actinomycetes (*Streptomyces*, *Nocardia*, and *Micromonospora*) from water samples was developed by Hsu and Lockwood (1975), who added mineral salts to the chitin medium (Table 266). Unfortunately, it had little effect when isolating actinomycetes from soil. Note that chitinolytic activity is not a genus-specific feature for *Streptomyces*. Only 25% of over 300 strains were strongly chitinolytic (Williams et al., 1983a). Consequently, this widely used medium selects chitinolytic streptomycete strains which may not be the most abundant in soil. Starch is a suitable selective carbon source for streptomycetes, as it is degraded by the vast majority of streptomycetes. The combination of starch with nitrate is used by many streptomycetes in contrast to other bacteria (Flaig and Kutzner, 1960a). Küster and Williams (1964a, 1964b), who improved this medium, stated: "The three best media, allowing good development of streptomycetes while suppressing bacterial growth, were those containing starch or glycerol as the carbon source with casein, arginine, or nitrate as the nitrogen source." Streptomycete isolation is also favored by the combination of

glycerol and arginine (Benedict et al., 1955). Further studies by El-Nakeeb and Lechevalier (1963) revealed that this medium (Table 266 and Table 267) was superior to nine other media, resulting in higher numbers and proportions of streptomycete colonies.

Other compounds that have been used successfully for the selective isolation of streptomycetes are, e.g. cholesterol (Brown and Peterson, 1966), elemental sulfur (Wieringa, 1966), pectin (Wieringa, 1955), poly-β-hydroxybutyrate (Delafield et al., 1965), rubber (Nette et al., 1959), and natural and artificial humic acids (Hayakawa and Nonomura, 1987a, 1987b). Most of these compounds strongly select organisms which produce visible zones of clearing or other changes in the medium.

Compounds with anti-fungal activity (antibiotics) are generally used to supplement isolation media to suppress fungal growth (Table 265), notably, cycloheximide (actidione, 50–100 μg/ml), as described by Williams and Davies (1965). Additionally, these authors found that pimaricin and nystatin (each at 10–50 μg/ml) were even more effective.

Compounds with anti-bacterial activity need to be used with care as some actinomycetes may also be sensitive to them. Polymyxin (5 μg/ml) and penicillin (1 μg/ml), for instance, suppress the growth of bacteria, but also inhibit some streptomycetes (Williams and Davies, 1965). Actinobacterial genera differ significantly in their sensitivity to anti-bacterial compounds, notably streptomycetes (Preobrazhenskaya et al., 1978). Thus, it may be more helpful to use anti-bacterial compounds for the isolation of other actinobacterial genera (Cross, 1982).

However, the selective isolation of certain species or groups of *Streptomyces* can be facilitated by supplementary media with antibiotics, as exemplified by the use of starch-casein agar containing rifampin (50 μg/ml) for the selective isolation of members of the *Streptomyces diastaticus* cluster by Williams et al. (1983a) and Vickers et al. (1984). A similar effect was described by Wellington et al. (1987), who used several media containing different C and N sources, as well as media supplemented with inhibitors.

Streptoverticil-producing *Streptomyces* species were isolated by Hanka et al. (1985) using a selective isolation medium supplemented with cycloheximide and nystatin (each at 50 μg/ml), to control fungal growth, and oxytetracycline (25 μg/ml) to suppress that of other actinomycete genera, including *Streptomyces* groups. By adding lysozyme (1000 μg/ml), Hanka and Schaadt (1988) enhanced the selectivity of this medium. The selectivity of this medium was enhanced by the addition of sodium propionate to suppress the growth of fungi (Crook et al., 1950; Table 265). Starch-casein-nitrate agar (Ottow, 1972) containing Rose Bengal (35 mg/l) suppresses the growth of most bacteria and inhibits spreading of fungi across isolation plates.

pH of isolation media and incubation temperatures. Most streptomycetes grow optimally at neutral pH values, i.e. they are neutrophilic organisms. Consequently, most isolation media have pH values of 7.0–7.5. In contrast, acidophilic streptomycetes grow best on media with a pH of 4.5 (Khan and Williams, 1975) and alkaliphilic strains show optimum growth on media with pH values of 10–11 (Mikami et al., 1982). Most streptomycetes isolated from soils are mesophilic; hence, isolation plates are usually incubated at 22–37°C (mostly at 28°C). In contrast, psychrophilic strains (e.g. from marine environments) grow best at 15–20°C, while thermotolerant and thermophilic strains grow well at higher temperatures (40, 45, 50, or 55°C). Thermophilic actinomycetes often form colonies within 2–5 d of incubation, whereas their mesophilic counterparts tend to produce visible colonies within 7–14 d. Marine and other psychrophilic actinomycetes may need several weeks (up to 10) for visible colonies to appear on isolation media.

In most cases, colonies of *Streptomyces* can be readily recognized by their macroscopic and microscopic appearance. *Streptomyces* can usually be purified by transferring colonies from isolation plates into nonselective medium. Williams and Wellington (1982b) stated that purification is "undoubtedly the most time-consuming and often the most frustrating stage of the isolation procedure". Acidiphilic streptomycetes can be readily isolated on acidified starch-casein agar supplemented with cycloheximide and nystatin (Kim et al., 2003b).

Isolation of antibiotic-producing actinomycetes. Antibiotic-producing streptomycetes are isolated following the same procedures as given above.

The activity of streptomycetes is normally tested after the isolation of pure cultures, though procedures are available to detect them on isolation plates. Antibiotic exhibiting strains can, for instance, be detected on initial dilution plates by flooding or spraying them with appropriate indicator organisms then incubating plates until zones of inhibition are detected (Lindner and Wallhausser, 1955; Wilde, 1964). Alternatively, antibiotic activity of the colonies against selected sensitive organisms can be examined by using a simple replication procedure (Lechevalier and Corke, 1953). Further information about selective techniques that can be used for the isolation and screening of antibiotic producing actinomycetes can be found in Nolan and Cross (1988). Protocols for the selective isolation of streptomycetes for the generation of spore suspensions and for more sophisticated experimental procedures are described by Kieser et al. (2000).

Isolation of thermophilic streptomycetes. As stated earlier, most actinomycetes, including thermophilic streptomycetes, originate from samples taken from high temperature environments (e.g. compost materials, manure heaps, and fodders). Consequently, high temperatures (45–60°C) should be used for the selective isolation of such organisms (Festenstein et al., 1965).

It is also important to incubate thermophilic streptomycetes in a humid atmosphere (Greiner-Mai et al., 1987) by incubating plates in large jars with water at the bottom; another effective method is to seal Petri dishes with masking tape.

Interestingly, the media recommended for the isolation of thermophilic actinomycetes, including streptomycetes, contain higher nutrient concentrations than those used for mesophilic strains. Sometimes, anti-fungal and anti-bacterial agents are added as supplements to such media (Goodfellow et al., 1987b; Lacey and Dutkiewicz, 1976b). Special procedures recommended for the isolation of thermophilic actinomycetes are available (Cross, 1968; Fergus, 1964; Gregory and Lacey, 1963; B. Kim et al., 2000; D. Kim et al., 1996; S.B. Kim et al., 1998; Uridil and Tetrault, 1959).

Isolation from aquatic habitats. The media listed in Table 266 can be used to isolate streptomycetes from water. Hsu and Lockwood (1975) found that chitin-agar was more effective than egg albumin, glycerol-arginine, starch-casein, and *Actinomyces* isolation agars for the incubation of actinomycetes from aquatic habitats (see also Table 266).

After dilution, water samples can be streaked directly onto solid medium. When low numbers of actinomycete samples are expected, they can be concentrated by membrane filtration [for details, see Burman et al. (1969)].

The selective isolation of streptomycetes from marine habitats can be enhanced when media are prepared using seawater or an equivalent. Media containing 25 or 75% seawater (Weyland, 1981a, 1981b), artificial seawater (Goodfellow and Haynes, 1984), and deionized water supplemented with 3.0% NaCl (Okami and Okazaki, 1978) have all proved to be effective. See Weyland (1981b) and Goodfellow and Haynes (1984) for further details.

Isolation from diseased plants. For the isolation of streptomycetes from diseased plant tissues, e.g. scabby potatoes or beet surface layers, three general steps have been recommended (see also Korn-Wendisch and Kutzner, 1992): sterilization of the surfaces of tubers, beets, or roots; maceration of plant tissues; and use of appropriate isolation media. Several authors have described detailed methods for the isolation of *Streptomyces scabiei* from potatoes (Adams and Lapwood, 1978; Archuleta and Easton, 1981; KenKnight and Munzie, 1939; Menzies and Dade, 1959; Taylor, 1936).

Nutritional requirements and media for sporulation. The vast majority of streptomycetes are nonfastidious organisms with a chemo-organotrophic metabolism. The nutritional requirements of streptomycetes are appropriate to an organic carbon source, such as starch, glucose, glycerol, and lactate, and usually met by the provision of a suitable inorganic nitrogen source, like ammonium or nitrate (Kutzner, 1981). However, different isolates can vary considerably in their carbon and nitrogen source utilization patterns, which are often used as taxonomic characters (e.g. Shirling and Gottlieb, 1966; Pridham and Tresner, 1974a; Williams et al., 1983a; Kämpfer et al., 1991). Widely used carbon sources include cellobiose, glucose, glycerol, D-mannose, and trehalose; useful nitrogen sources are ammonium, L-arginine, L-asparagine, and nitrate. Relatively few strains use organic acids, inulin, L-methionine, nitrite, or xylitol, but most can degrade casein, esculin, gelatin, and hypoxanthine. Streptomycetes grow well on many different media, but spore production is usually most prolific on those with a high carbon:nitrogen ratio (Kutzner, 1981). Streptomycetes generally require a good supply of free water for growth, but are unable to develop at high osmotic or matric potentials. Survival of streptomycetes in dry conditions is aided by the high resistance of arthrospores, in contrast to vegetative mycelia, to desiccation (Williams et al., 1972).

The need to add specific trace elements to culture media has not been studied in detail. Spicher (1955) described the positive effect of trace elements in soil on the growth of streptomycetes. However, many of the early media used (even the "synthetic" media) were not supplemented with trace elements. Indeed, recipes of many authors (Table 266 and Table 267) contained only a selected number of metal ions. A rather complete mixture (SPV-4; Table 267) has been found to be optimal for the growth of actinomycetes and other bacteria (Voelskow, 1988/89).

"Synthetic media" can be used for the cultivation of streptomycetes, though the need for specific nutritional requirements with respect to vitamins and organic growth factors has not been addressed. Growth rates and biomass production can be enhanced by using complex organic substrates (e.g. malt extract, oatmeal, or yeast extract). A combination of a complex organic carbon source with a single amino acid as nitrogen source (e.g. glutamic acid, arginine, or asparagine) is also suitable.

"General media" have been proposed for the growth of streptomycetes as they allow the completion of the *Streptomyces* life cycle, i.e. germination of spores, growth of substrate and aerial mycelium, and visible formation of spores (visible, because of the typical color of the spores). Some of these media were used in the International *Streptomyces* Project (ISP), among them glucose-yeast extract-malt extract, oatmeal, inorganic salts-starch, and glycerol-asparagine agars (Shirling and Gottlieb, 1966). Innumerable general media have been recommended for the growth of streptomycetes, four of which are of considerable practical value (Table 267). For additional media formulations, see Waksman (1961) and Williams and Cross (1971). $CaCO_3$ is added to some media, as Ca^{2+} promotes growth and neutralizes acids produced by many streptomycetes; such media also allow good sporulation. Cultures should be checked microscopically to detect the extent of sporulation as macroscopically heavy aerial mycelia may contain very few spores while aerial mycelia which are hardly detectable by the naked eye may be a good source of spores.

A list of specialized media especially for genetic studies on streptomycetes is given by Kieser et al. (2000).

Media containing soil, clay, minerals, and calcium humate. Soil promotes growth, sporulation, and pigmentation of actinomycetes/streptomycetes (Trolldenier, 1966) and is therefore often added to isolation media to increase the number of colonies. The addition of montmorillonite or calcium humate to liquid media stimulates the growth and metabolic activity of some actinomycetes (Martin et al., 1976). A similar effect has been observed for clay in dialysis tubes after a short lag period, an observation which may be explained by the adsorption of one or more inhibitory substances produced during growth. Adsorbing materials have a positive effect on the genetic stability of other bacteria and on fungi (Martin et al., 1976).

Temperature, pH, and oxygen. The environmental requirements and tolerances of streptomycetes have been described in detail by Kutzner (1981). Most streptomycetes grow at temperatures between 10 and 37°C and, hence, are regarded as mesophiles. Nevertheless, several species can grow at temperatures above 37°C, although most of them are thermotolerant rather than thermophilic in their responses. A variety of type strains studied by Williams et al. (1983a) grew at 10, 37, and 45°C, although a few grew slowly at 4°C (1983a). However, in many instances, the optimal temperature for rapid growth or maximal yield may not be the best choice for studying the production of secondary metabolites (e.g. antibiotics and pigments). This means that culture conditions are influenced by the aims of the study.

Most *Streptomyces* strains behave as neutrophiles in culture, growing between pH 5.0 and 9.0, with an optimum close to pH 7.0. Only a few of the type strains studied by Williams et al. (1983a) were able to grow at pH 4.3, though large populations of acidophilic and acidoduric strains have been reported from acidic soils (Hagedorn, 1976; Khan and Williams, 1975; Williams et al., 1971). Acidophilic streptomycetes grow from about

pH 3.5 to 6.5, and optimally between pH 4.5–5.5. However, a wide spectrum of pH requirements exists among streptomycetes from acidic environments (Flowers and Williams, 1977b). Acidophilic strains are able to produce diastases (Williams and Flowers, 1978) and chitinases which have optima pH below that of corresponding enzymes from neutrophilic counterparts.

Populations of alkaliphilic streptomycetes with optimal growth at pH 9.0–9.5 have been isolated from soils in Japan by Mikami et al. (1982), who found that six of the type strains tested were able to grow at pH 11.5.

In addition, large populations of alkaliphilic streptomycetes have been isolated from a beach and dune sand system at Ross Links in Northumberland, UK (Antony-Babu and Goodfellow, 2008). Streptomycetes are generally regarded as obligate aerobes with a limited capacity for microaerophilic growth in culture (Kutzner, 1981) and dissimilatory reduction of nitrate is common. Whether streptomycetes grow aerobically or microaerophilically depends on the nutritional status of the medium.

In a nutrient medium, streptomycetes grow aerobically at the surface of the semisolid agar column, but in poor media or in a medium with a non-utilizable carbon source, they grow microaerophilically in semisolid agar. In stationary liquid culture, streptomycetes grow as pellicles at the surface, while the medium itself remains completely clear.

Cultivation and preparation of inoculum. Solid media in dishes or slants should be used for the growth of streptomycetes for subcultivation and maintenance as well as for most diagnostic tests. Many strains produce aerial mycelia and spores on solid media when the entire surface is covered by confluent growth. In contrast to most molds, *Streptomyces* colonies spread over a limited distance and, hence, a point inoculation will not usually lead result in confluent growth. However, if streaked onto a plate, some strains need empty spaces between the streaks (cross-hatch inoculation) to sporulate. Dry conditions are generally more suitable for sporulation. A horizontal incubation of slants for the first 2 d allows liquid to soak into the surface of the agar (Hopwood et al., 1985). A suspension of inoculum in liquid should be used as starting material for sporulation (Kieser et al., 2000).

For the propagation of cultures, single colonies should be selected and streaked onto fresh media. Successive rounds of mass culture should be avoided, especially in genetic studies, because this technique reduces the accumulation of revertants or the gradual loss of selected plasmids or both (Kieser et al., 2000). When streptomycetes are cultivated on solid media, morphological heterogeneity is often observed. More details can be found in Kieser et al. (2000).

Precultivation of grown colonies in liquid media is required to obtain a homogeneous suspension for some diagnostic tests (Kämpfer et al., 1991). Streptomycetes should be cultivated in liquid medium without agitation. This precultivation is necessary for certain physiological studies (e.g. degradation tests), for the provision of cell material for biochemical analysis, and for the production of secondary metabolites (e.g. antibiotics) or enzymes. For many detailed studies, e.g. for preparation of protoplasts for fusion, transformation or transfection, liquid cultures should also be started from an inoculum of spores.

The multicellular lifestyle of streptomycetes causes some problems in the study of metabolic properties because not all cells of the initial suspension are in the same physiological condition. In general, streptomycetes grow by mycelial elongation and branching. However, physiological homogeneity cannot be sustained when central parts of the colony become nutrient limited. Therefore, spore germlings are used in physiological studies, although large numbers of spores are needed. To avoid this problem, liquid cultures can be supplemented with, for example, dispersants, like agar, carboxymethylcellulose, Junlon, polyethylene glycol, starch, and sucrose. A summary of the advantages and disadvantages of these methods can be found in chapter 2 of Kieser et al. (2000). Cultures need to be shaken during incubation due to the highly aerobic nature of streptomycetes. Recommended procedures include Erlenmeyer flasks with the use of indentations or stainless steel springs, but tubes in a slanted position on a shaker or roller also allow an excellent supply of oxygen for small quantities of broth, 3–5 ml being enough for some physiological tests. However, it should be noted that some secondary metabolites (e.g. antibiotics and pigments, which are produced on solid media) may not be synthesized under these conditions.

Two media recommended by Korn-Wendisch and Kutzner (1992) have been widely used for submerged cultivation of streptomycetes (g/l): 1) GPYB broth (glucose, 10.0; peptone from casein, 5.0; yeast extract, 5.0; beef extract, 5.0; $CaCl_2 \cdot 2H_2O$, 0.74; pH 7.2); and 2) soybean meal-mannitol nutrient medium (soybean meal, 20.0; mannitol, 20.0; pH 7.2). Arthrospores and vegetative mycelium can be used as inoculation material for subculturing streptomycetes; vegetative mycelium occasionally includes "submerged spores" (Wilkin and Rhodes, 1955). Similar procedures are recommended by Kieser et al. (2000).

Spore suspensions stored at 4°C can be used for several weeks. A few glass beads should be added to the screw-cap tubes, as spores tend to settle and clump. Glass beads help to resuspend spores before use. The preparation of mycelia for detailed DNA or RNA studies is described in chapters 8 and 9 of Kieser et al. (2000).

Maintenance procedures

Several different procedures have been employed (Kirsop and Snell, 1984) for the short- and long-term preservation of microorganisms. Korn-Wendisch and Kutzner (1992) described three short-term preservation methods: (1) agar slope cultures may be stored at 4°C for few months; (2) spore suspensions can be mixed with soft water agar and kept at 4°C (Kutzner, 1972); and (3) glycerol can be added to spore suspensions (final concentration, 10%, v/v) which are then stored at −20°C (Wellington and Williams, 1978). After thawing, these cultures can serve as inocula for most diagnostic tests, except carbon utilization (Williams et al., 1983a).

For long-term preservation, Kieser et al. (2000) recommended the preparation of spore suspensions in 20% glycerol which can be frozen and maintained at −20°C. In another method, strains are grown in complex media like trypticase soy broth (TSB) agar, 20% glycerol plus 10% lactose are added, and the samples are stored in the vapor phase of liquid nitrogen. A third method uses drying on unglazed porcelain beads (Lange and Boyd, 1968), followed by soil culture (Pridham et al., 1973), and lyophilization (Hopwood and Ferguson, 1969).

Spore suspensions or homogenized mycelia mixed with glycerol to give a final concentration of 25% can be kept at −25°C

for longer term preservation (Wellington and Williams, 1978). Alternatively, spores and mycelia suspended in 10% skim milk can be lyophilized. Liquid nitrogen cryopreservation is a very simple, reliable, and time-saving method. In this method, living cells are stored in small polyvinyl chloride (PVC) tubes ("straws") at −196°C; this procedure has been tested for various actinomycetes. First, strains are harvested from well-sporulated cultures grown on suitable agar media in Petri dishes. A 2 × 25 mm piece of sterile PVC tubing is pressed into the mycelial mat and agar, and carefully raised to excise the agar plug. This procedure is repeated until the tube is filled with agar. The tube is then placed in a sterile cryovial (the screw cap marked with the strain accession number); up to 13 tubes can be placed in a 1.8 ml vial. Two vials prepared for each strain are then fixed to a metal clamp for freezing in the gas phase of a liquid nitrogen container. After 10–15 min, when the temperature falls below −130°C, the clamp can be immersed in the liquid phase at −196°C. A container with a capacity of 250 l will hold at least 8000 vials or 4000 strains. For viability testing, one tube is removed from the vial within the nitrogen gas atmosphere of the container and placed directly and thawed on a suitable agar medium. The mycelium will be visible after a few days of incubation. Plugs may be pushed out of the tubes with a sterile needle when strains do not produce abundant mycelium.

Differentiation of the genus *Streptomyces* from other genera

It is standard practice to assign unknown actinomycetes to genera based on 16S rRNA gene sequence analyses. However, it can be difficult to distinguish between species using this approach, especially in the case of streptomycetes (e.g. Stackebrandt et al., 1991a, 1991b, 1992); Kumar and Goodfellow, 2008).

Members of the genus *Streptomyces* can often be distinguished from other filamentous actinomycetes on the basis of colony morphology (Table 268 and Table 269), in particular by aerial spore mass substrate mycelium and soluble pigment colors. Traditional methods highly recommended for this purpose are described by Korn-Wendisch and Kutzner (1992). Antony-Babu et al. (2010) used a computer-assisted numerical analysis based on the color-grouping procedure of Williams et al. (1969) to group 321 alkaliphilic streptomycetes grown on oatmeal agar (ISP 3) and peptone-yeast extract-iron agar (ISP 6). With this method, large numbers of streptomycetes can be assigned without using polyphasic taxonomic approaches.

In addition, streptomycetes can often be distinguished from other filamentous actinomycetes on the basis of morphological properties, notably aerial mycelium, arthrospores, and vegetative mycelium (Figure 357, Figure 358, and Figure 359). Details on the procedure used to detect such properties can be found in Korn-Wendisch and Kutzner (1992) and chapter 3 of Kieser et al. (2000).

Members of the genus *Streptomyces* can also be distinguished from related taxa using chemotaxonomic procedures (Lechevalier and Lechevalier, 1970b). Streptomycetes typically contain LL-A_2pm in cell wall or whole-cell hydrolysates (Lechevalier and Lechevalier, 1970b, 1970c), they lack mycolic acids, and produce major amounts of iso- and anteiso-methyl branched fatty acids (Kroppenstedt, 1985) (Table 263). Major menaquinones are hexa- and octa-hydrogenated menaquinones with nine isoprene units (Kim et al., 2003b). An important chemotaxonomic character for the differentiation of *Kitasatospora* from *Streptomyces*

TABLE 268. Spore colors for the grouping of streptomycetes and representatives of each color group (according to Korn-Wendisch and Kutzner, 1992)

Color of aerial mycelium	Representative species (DSM no.)[a]
Yellow-gray: "griseus"	*S. griseus* (40236); *S. coelicolor* (40233)
Pink/light violet	*S. fradiae* (40063); *S. toxytricini* (40178)
Gray-pink/lavender: "cinnamomeus"	*S. lavendulae* (40069); *flavotricini* (40152)
Brown (plus gray or red)	*S. eurythermus* (40014); *S. fragilis* (40044)
Blue: "azureus"	*S. viridochromogenes* (40110); *S. cyaneus* (40108)
Blue-green: "glaucus"	*S. glaucescens* (40155)
Green: "prasinus"	*S. prasinus* (40099); *S. hirsutus* (40095)
Gray: "cinereus"	*S. violaceoruber* (40049); *S. echinatus* (40013)
White: "niveus"	*S. albus* (40313); *S. longisporus* (40166)
Not definable: white plus various plus various light-colored shades	*S. alboniger* (40043); *S. rimosus* (40260)

[a]DSM no. 40XXX, ISP no. 5XXX; e.g. 40236, ISP 5236.

TABLE 269. Colors of substrate mycelium and soluble pigment occurring in streptomycetes (according to Korn-Wendisch and Kutzner, 1992)

Color	Representative species (DSM no.)[a]
Orange to dark red (mainly endopigment)	*S. aurantiacus* (40412); *S. griseoruber* (40275) *S. longispororuber* (40599); *S. spectabilis* (40512)
Red to blue/violet (mainly endopigment)	*S. californicus* (40058); *S. cinereoruber* (40012) *S. violaceus* (40082); *S. purpurascens* (40310)
Red-violet to blue (endo- and/or exopigment)	*S. coelicolor* (40233); *S. cyaneus* (40108) *S. violaceoruber* (40049); *S. lateritius* (40163)
Yellow-orange/greenish-yellow (endo- and exopigment)	*S. atroolivaceus* (40137); *S. canarius* (40528) *S. galbus* (40089); *S. tendae* (40101)
Green to gray-olive (endo- and exopigment)	*S. flavoviridis* (40210); *S. olivoviridis* (40211) *S. viridochromogenes* (40110); *S. nigrifaciens* (40071)
Green (endopigment)	"*S. malachiticus*" (40167); "*S. malachitorectus*" (40333)
Red-brown to dark-brown (endo- and exopigment)	*S. badius* (40139); *S. eurythermus* (40014) *S. phaeochromogenes* (40073); *S. ramulosus* (40100)
Gray-brown to black (mainly endopigment)	*S. alboniger* (40043); *S. hygroscopicus* (40578) *S. purpeofuscus* (40283); *S. mirabilis* (40553)

[a]DSM no. 40XXX = ISP no. 5 XXX.

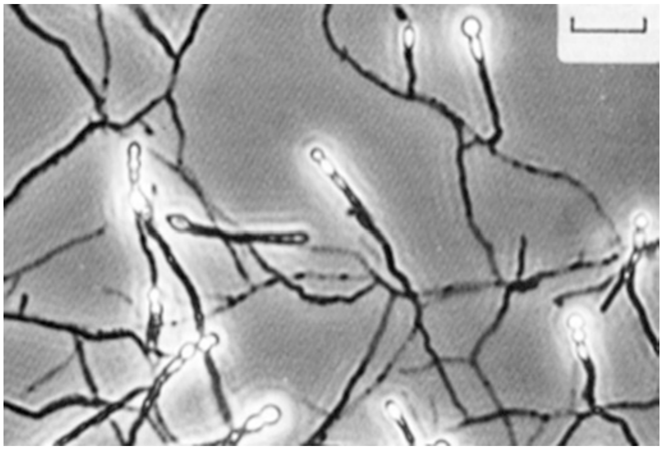

FIGURE 357. Spore chains *Streptomyces* (*Microellobosporia*) species on aerial mycelium. Light microscopy. Bar, 5 μm. (Courtesy of T. Cross, University of Bradford, Bradford, U.K.)

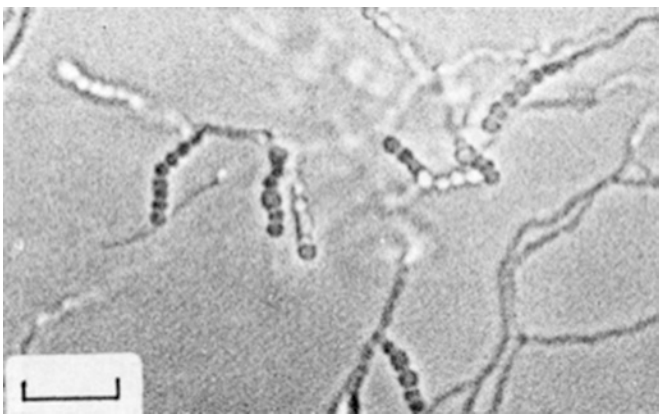

FIGURE 358. Spore chains of *Streptomyces carpinensis* (*Elytrosporangium carpinese*) on substrate mycelium. Light microscopy. Bar, 5 μm. (Courtesy of T. Cross, University of Bradford, Bradford, U.K.)

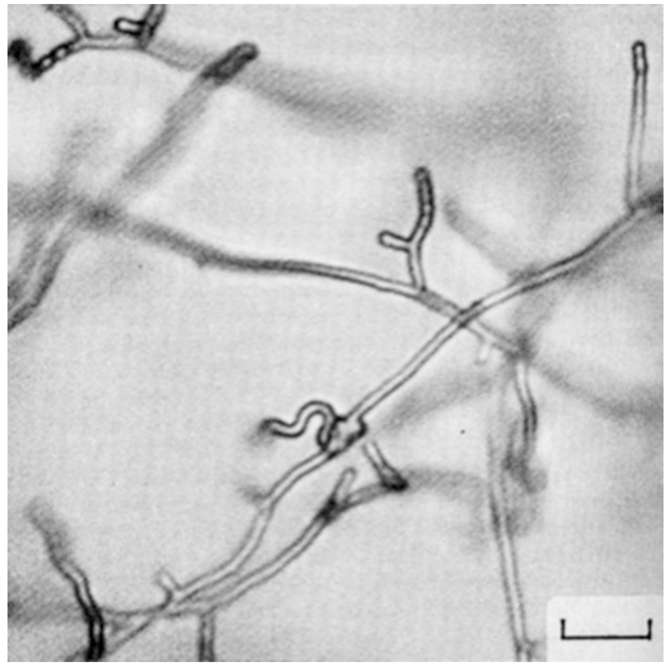

FIGURE 359. Spore chains of *Streptomyces carpinensis* (*Elytrosporangium carpinense*) on aerial mycelium. Light microscopy. Bar, 5 μm. (Courtesy of T. Cross, University of Bradford, Bradford, U.K.)

is the presence of *meso*-A_2pm in whole-cell hydrolysates (Table 263). In *Kitasatospora* strains, the *meso*-A_2pm content is 49–89%, whereas in *Streptomyces* strains it is 1–16% (Zhang et al., 1997). The predominant diamino acid of strains belonging to the genus *Streptacidiphilus* is (like *Streptomyces*) LL-A_2pm (Kim et al., 2003b). Further useful characters for species identification are shown in Table 274.

Taxonomic comments

Phenotypic methods comprise all procedures that are not directed towards analyses of DNA or RNA, and include chemotaxonomic techniques. Most early studies on streptomycetes (between 1916 and 1943) were carried out by soil microbiologists who were mainly interested in ecological questions; hence, only a few species were described at that time. Descriptions were mainly based on morphological criteria, pigmentation, and ecological requirements (Jensen, 1930; Waksman, 1919; Waksman and Curtis, 1916). However, the discovery of actinomycin from *Streptomyces antibioticus* (Waksman and Woodruff, 1940) promoted widespread interest in streptomycetes as a source of novel bioactive compounds. The focus on screening streptomycetes for novel bioactive compounds led to a widespread tendency for streptomycetes to be assigned to novel species on the basis of their ability to produce new natural products. This tendency led to an explosion of species descriptions and resulted in an overclassification of the genus with over 3000 species being recognized (Trejo, 1970).

The International *Streptomyces* Project (ISP) introduced 1964 standard criteria for determining species (Shirling and Gottlieb, 1968a, 1968b, 1969, 1972). These descriptions were based mainly on morphology (i.e. spore chain arrangement, spore surface ornamentation, color of spores, substrate mycelium, soluble pigments, and production of melanin pigments), and a few physiological properties, which were mainly restricted to utilization tests of different carbon sources. As a result, more than 450 *Streptomyces* species were redescribed and their type strains were deposited in internationally recognized culture collections. However, these studies did not directly lead to the generation of schemes for the identification of *Streptomyces* species.

Numerical taxonomic methods were developed in the 1960s for both the classification and identification of bacteria, including streptomycetes. Silvestri et al. (1962) carried out the first numerical taxonomic studies on streptomycetes and found considerable diversity within the genus, as well as groups which corresponded to initial morphological descriptions. However, these studies had little impact on *Streptomyces* systematics and did not

TABLE 270. Numerical classifications of streptomycetes (modified according to Korn-Wendisch and Kutzner, 1992)

Number of strains	No. characters (features)	Nature of material	No. clusters	No. unclustered strains	Reference
159	105	"Species"	24	16	Silvestri et al. (1962)
18	46	Isolates	5		Williams et al. (1969)
448	31	ISP "species"	14/21	168/37	Kurylowicz et al. (1967)[a]
618	24	ISP "species"	15	218	Gyllenberg (1976)
111	185	*Streptomyces* with verticals and pseudoverticils, formerly *Streptoverticillium*	24		Locci et al. (1981)
475	139	394 ISP species plus others	73	28	Williams et al. (1983a)
821	329	394 ISP species plus others	15 (major), 34 (minor)	40	Kämpfer et al. (1991)

[a]14 and 168 were obtained by the Wroclaw dendrite method. 21 and 37 were obtained by the centrifugal correlation method.

result in any nomenclatural changes, despite the development of other small databases for the identification of streptomycetes (Kurylowicz et al., 1975; Gyllenberg, 1976; Table 270).

A large-scale numerical taxonomic study was undertaken by Williams et al. (1983a) who analyzed 475 strains (including 394 *Streptomyces* type cultures from the ISP) for 139 unit characters using the simple matching (S_{SM}) and Jaccard coefficients (Sj), and the mean linkage algorithm UPGMA (unweighted pair group method with arithmetic means). The 394 type strains were assigned to 19 major (6–71 strains), 40 minor (2–5 strains), and 18 single clusters recovered at the 77±5% S_{SM} level. The minor and single-membered clusters were equated with species and the major clusters with species-groups.

The largest species group, *Streptomyces albidoflavus* (cluster 1), contained 71 strains, including 44 type strains, 15 representatives of species with names that were not validly published, and 12 unnamed strains. This taxon was subsequently subdivided into three subclusters: cluster 1a, *Streptomyces albidoflavus* subsp. *albidoflavus* (20 strains); cluster 1b, *Streptomyces albidoflavus* subsp. *anulatus* (38 strains); and cluster 1c, *Streptomyces albidoflavus* subsp. *halstedii* (13 strains; Williams et al., 1989). Cluster 1 included strains which showed considerable phenotypic diversity, though almost all of the strains formed yellow gray colonies, smooth spores in straight chains, did not produce melanin pigments, and were resistant to several antibiotics, including cephaloridine, lincomycin, and penicillin. Nearly 40% of the strains produced compounds with anti-fungal activity, 32% of the compounds were active against Gram-stain-positive micro-organisms, and 10% inhibited Gram-stain-negative micro-organisms (Williams et al., 1983b). This example highlights the extensive diversity found within a single cluster and exemplifies problems associated with streptomycete systematics (Anderson and Wellington, 2001).

The comprehensive survey of Williams et al. (1983a) led to a reduction in the number of described *Streptomyces* species (Williams et al., 1989), although the problem of overspeciation remained. Additionally, isolates from natural habitats did not match the reference strains used to construct identification schemes (Goodfellow and Dickenson, 1985). The problem was addressed by the generation of probability matrices for the identification of streptomycetes (Langham et al., 1989; Williams et al., 1983b), but these developments were not widely adopted by the scientific community.

The chapter on *Streptomyces* in the 1989 edition of *Bergey's Manual of Systematic Bacteriology* (Williams et al., 1989) was based on the numerical taxonomy studies of Williams and his colleagues. This chapter included descriptions of 142 *Streptomyces* species, in contrast to the 463 species described in the 1974 edition of *Bergey's Manual of Determinative Bacteriology* (Pridham and Tresner, 1974a).

Kämpfer et al. (1991) carried out an extensive numerical taxonomic analysis of the genus *Streptomyces* and, where possible, included more than one strain of each species. These workers examined 821 strains for 329 physiological properties and compared their data with those of Williams et al. (1983a). They also examined their strains for genetic and chemotaxonomic properties and compared them with the numerical data. They recognized many of the clusters defined by Williams et al. (1983a); for example, the *Streptomyces albidoflavus*, *Streptomyces anulatus*, *Streptomyces griseus*, and *Streptomyces halstedii* groups were recovered in both studies; 28 of the *Streptomyces griseus* strains were assigned to cluster 1. Interestingly, groups often contained strains which shared the same specific epithet indicating that some previous classifications were reliable. However, exceptions were also observed, for example, *Streptomyces hygroscopicus* strains were recovered in cluster 1 and in several other clusters and subclusters.

Data generated in this study were used to construct a probability matrix for the identification of streptomycetes (Kämpfer and Kroppenstedt, 1991), but this identification scheme was not widely used by other research groups.

Chemotaxonomic and molecular methods were used in parallel with the application of numerical taxonomic procedures for the classification of streptomycetes. The additional phenotypic methods used to study streptomycetes included cell-wall analysis (Lechevalier and Lechevalier, 1970a, 1970b, 1970c), fatty acid profiling (Hofheinz and Grisebach, 1965; Kroppenstedt, 1992; Lechevalier, 1977; Saddler et al., 1986, 1987), rapid biochemical tests based on the use of 4-methyl-umbelliferone-linked substrates (Goodfellow et al., 1987b), serological assays (Ridell et al., 1986), phage typing (Korn-Wendisch and Schneider, 1992; Wellington and Williams, 1981b), Curie-point pyrolysis MS of whole cells (Ferguson et al., 1997; Sanglier et al., 1992), whole organism protein profiling (Goodfellow and O'Donnell, 1993; Lanoot et al., 2002; Manchester et al.,

1990), and comparison of ribosomal protein patterns (Ochi, 1989, 1992, 1995).

Fatty acids. Hofheinz and Grisebach (1965) examined selected *Saccharopolyspora erythraeus* (formerly "*Streptomyces erythraeus*") and *Streptomyces halstedii* strains to clarify the biosynthetic pathway of branched-chain fatty acid synthesis. They found that *Streptomyces* strains synthesized terminally branched fatty acids; the starting compound, 2-methylbutyrate, led to the synthesis of anteiso-branched fatty acids with an odd number of carbon atoms. In contrast, isovalerate and isobutyrate were the starting compounds that lead to the formation of iso-branched fatty acids with even and odd numbers of carbon atoms, respectively. Consequently, iso- and anteiso-branched fatty acids appear in pairs with odd numbers of carbon atoms.

Hofheinz and Grisebach also identified individual fatty acids by first separating them as their methyl esters by GC on different stationary phases, then analyzing the results by comparing the equivalent chain-lengths of unknown fatty acids with those of standard mixtures. Preparative GC and physical methods, such as MS and NMR spectrometry, confirmed these results. Iso- and anteiso-branched fatty acids with chain lengths of 15 and 17 carbon atoms were detected in both *Saccharopolyspora erythraeus* and *Streptomyces halstedii*. In addition, high amounts of 14-methylpentadecanoic acid ($C_{16:0}$ iso) were found, whereas minor amounts of unbranched fatty acids, tuberculostearic acid and their homologs were detected in the *Saccharopolyspora erythraea* strains, but not in *Streptomyces halstedii*.

A limited number of streptomycetes synthesize small amounts of hydroxy fatty acids in the presence of optimal amounts of oxygen. These fatty acids are easily destroyed in a non-deactivated injection port of capillary GC systems. Hence, they are not always detected. However, some streptomycetes produce hydroxy fatty acids which are highly diagnostic when strains are grown under reproducible culture conditions. Hydroxy fatty acids have been detected in all strains of *Streptomyces coelicolor* (30), in 20 of 27 *Streptomyces hygroscopicus* strains, *Streptomyces rimosus* (14), and *Streptomyces violaceusniger* (18), but not in *Streptomyces albus* (33), *Streptomyces fradiae* (25), *Streptomyces glaucescens* (8), *Streptomyces griseus* (22), *Streptomyces lavendulae* (18), *Streptomyces violaceoruber* (16), or *Streptomyces viridochromogenes* (25; Kroppenstedt, 1992; R.M. Kroppenstedt, unpublished observations).

Standardized growth and cultivation conditions are prerequisites for generating fatty acid patterns for classification below the genus level (Saddler et al., 1986). These workers examined the fatty acid profiles of *Streptomyces cyaneus* strains and associated soil isolates, which produced a blue aerial spore mass; 13 of 19 blue-spored strains belonged to the *Streptomyces cyaneus* cluster (Hütter, 1962; Korn et al., 1978; Pridham and Tresner, 1974a). Saddler et al. (1987) showed that 8 of 10 blue-spored isolates were grouped together based on fatty acid data, whereas 17 of 34 *Streptomyces cyaneus* strains were assigned to a separate cluster. Saddler and his colleagues concluded that conventional features, like spore chain morphology, color, and ornamentation of spores, were not reliable for the classification of streptomycetes, but would be helpful for presumptive identification, a point also made by Williams et al. (1983a). However, Saddler and his colleagues demonstrated that the *Streptomyces cyaneus* taxon as defined by Williams et al. (1983a) was heterogeneous.

In general, fatty acid patterns cannot be used to delimit *Streptomyces* species (Phillips, 1992; R.M. Kroppenstedt, unpublished observation), but are still useful for the rapid characterization (independent of the taxonomic status) of large numbers of wild-type streptomycetes isolated from the environment when used under standardized conditions (Saddler et al., 1987). Identification and quantification can be done by using the automated commercially available MIDI system consisting of a Hewlett Packard model 5890 capillary GC and a computer with specific software (Microbial ID, Inc., Newark, DE). The MIDI system automatically identifies fatty acids using fatty acid standard mixtures for comparison. However, comparisons of different analytical methods show that numerical methods cannot be used for classification, because different methods give different groupings. Additionally, the resolution is not good; hence, too many strains are grouped together into some clusters (Kämpfer et al., 1991; Williams et al., 1983a).

Curie-point pyrolysis MS. Another method that has been applied to the classification and identification of actinomycetes is pyrolysis MS (PyMS; Sanglier et al., 1992; Ferguson et al., 1997). Whole cells exposed to high temperatures are degraded in a nonoxidative environment leading to the generation of pyrolysate, which can be analyzed by MS. This method needs to be rigorously standardized and results in the production of a fingerprint for each organism.

Sanglier et al. (1992) applied this method to strains belonging to the *Streptomyces albidoflavus* species-group defined by Williams et al. (1983a) and recovered *Streptomyces albidoflavus* and *Streptomyces anulatus* strains in distinct groups. The six *Streptomyces halstedii* strains (the third subgroup) were assigned to three groups. *Streptomyces albidoflavus* and *Streptomyces anulatus* strains were also recovered in different groups by Kämpfer et al. (1991). They also found that *Streptomyces anulatus* ISP 5361^T, the strain used to name the *Streptomyces anulatus* cluster, formed a single-membered cluster (Kämpfer et al., 1991).

Serology. Serological methods have rarely been used in *Streptomyces* systematics. Ridell et al. (1986) used antisera against the mycelia of streptomycetes, streptoverticillia, and *Nocardiopsis* species to confirm the high similarity between *Streptomyces lavendulae* and streptoverticillia (Kämpfer et al., 1991; Witt and Stackebrandt, 1990). The antisera of Kirby and Rybick (1986) were shown to be genus-specific and to a certain degree also group-specific when tested against *Streptomyces griseus* (*Streptomyces anulatus*, cluster 1B of Williams et al., 1983a) and "*Streptomyces cattleya*" (cluster 47 of Williams et al., 1983a). Wipat et al. (1994) generated a monoclonal antibody to "*Streptomyces lividans*" 1326 which was specific for "*Streptomyces lividans*" strain 1326 and for strains assigned to cluster 21 by Williams et al. (1983a).

Phage typing. Phage typing can be used for host identification at the genus and species levels (Korn et al., 1978; Kutzner, 1961a, 1961b; Wellington and Williams, 1981b; Welsch et al., 1957). Many actinophages, mainly virulent, have been used for phage typing. Two different groups of streptomycete phages exist, namely polyvalent phages (e.g. φC31; Chater et al., 1986) and species-specific phages (Anderson and Wellington, 2001; Table 271). Actinophages are specific at the genus level (e.g. Wellington and Williams, 1981b; Korn-Wendisch, 1982;

TABLE 271. Species-specific actinophages of the genus *Streptomyces* (modified according to Anderson and Wellington, 2001)

Phage	Host	Host species group	Host cluster no.[a]	Cluster no.[b]	Reference
S3	*S. albus* DSM 40313[T]	*S. albus*	16	32	Korn-Wendisch and Schneider (1992)
SAt1	*S. azureus* ATCC 14921[T]	*S. cyaneus*	18	9	Ogata et al. (1985)
100	"*S. caesius*" ATCC 19828	*S. griseoruber*	21	6	Wellington and Williams (1981b)
98	*S. coelicolor* Müller ATCC 23899[T]	*S. albidoflavus*	1A	1-1	Wellington and Williams (1981b)
14, 24, 233	*S. coelicolor* Müller ATCC 23899[T]	*S. albidoflavus*	1A	1-1	Korn-Wendisch and Schneider (1992)
90	*S. griseinus* ATCC 23915[T]	*S. albidoflavus*	1B	1-3	Wellington and Williams (1981b)
89, DP 9	*S. griseus* ATCC 23345[T]	*S. albidoflavus*	1B	1-3	Wellington and Williams (1981b)
41	*S. matensis* ATCC 23935[T]	*S. rochei*	12	6	Wellington and Williams (1981b)
33	"*S. scabies*" ATCC 23962	*S. atroolivaceus*	3	1-3	Wellington and Williams (1981b)
SV1, SV2	*S. venezuelae* ATCC 10712[T]	*S. violaceus*	6	2	Stuttard (1982)
4, 5a, 5b, 49	*S. violaceoruber* DSM 40049[T]	*S. violaceoruber*	SMC	69	Korn-Wendisch and Schneider (1992)

[a]Clusters according to Williams et al. (1983a, 1983b); SMC, Single-member cluster (Williams et al., 1989).

[b]Clusters according to Kämpfer et al. (1991).

Prauser, 1984). Actinophage host range studies helped to justify the transfer of the genera *Actinopycnidium*, *Actinosporangium*, *Chainia*, *Elytrosporangium*, *Microellobosporia*, *Kitasatoa*, and *Streptoverticillium* to the genus *Streptomyces* (Goodfellow et al., 1986a, 1986c, 1986d, 1986e; Witt and Stackebrandt, 1990). Similar studies supported the transfer of *Actinoplanes armeniacus* to the genus *Streptomyces* (Kroppenstedt et al., 1981; Wellington and Williams, 1981a) and "*Streptomyces erythraeus*" to the genus *Saccharopolyspora* (Labeda, 1987). Phage typing has been shown to be less useful for species or group identification of *Streptomyces*, though there are a few exceptions (Table 271).

Phages are widely used in industrial microbiology (Carvajal, 1953; Ogata, 1980) and in genetic studies (see Chater, 1986), as exemplified in chapter 12 of Kieser et al. (2000). The temperate phage, φC31, is one of the best-investigated temperate actinophages and has a broad host range within the genus *Streptomyces* (Lomovskaya et al., 1980). This phage has been employed for many purposes (e.g. transfection, transduction, detection of transposon-like elements of host DNA, and cloning); details can be found in chapter 12 of Kieser et al. (2000).

Protein profiling. PAGE is used to analyze total protein extracts resulting in more or less complex banding patterns. These patterns have been used to clarify relationships between the species and subspecies levels of various bacterial genera. One-dimensional (1-D) and two-dimensional (2-D) protein electrophoresis can be used to determine protein patterns. Manchester et al. (1990) used one-dimensional protein electrophoresis to examine 37 *Streptomyces* strains, including five streptoverticillia, and found some taxonomic correlations between the resultant profiles and groups based on phenotypes (Kämpfer et al., 1991; Williams et al., 1983a) and DNA hybridization data (Table 272). However, only a few of these correlations were confirmed by Lanoot et al. (2002).

PAGE and DNA–DNA hybridizations were used to elucidate the taxonomy of *Streptomyces* isolates that caused common potato scab (Paradis et al., 1994). The isolates were the subject of an SDS-PAGE analysis and found to belong to two groups with a correlation coefficient of 0.75. The same groups were recovered in the DNA–DNA relatedness study, though not in the corresponding fatty acid analysis. This lack of correlation can be attributed to the influence of growth conditions on fatty acid profiles (Saddler et al., 1986, 1987). The protein profiling did not allow pathogenic and nonpathogenic strains to be differentiated. Lanoot et al. (2002) used SDS-PAGE of whole-cell proteins in an examination of 93 *Streptomyces* reference strains. Subsequent computer-assisted numerical analysis revealed 24 clusters, which included strains with very similar protein profiles. Several type strains were assigned to five clusters, which had visually identical patterns. DNA–DNA hybridizations of these type strains revealed similarities higher than 70%. On the basis of these results, *Streptomyces albosporeus* subsp. *albosporeus* LMG 19403[T] was considered to be a subjective synonym of *Streptomyces aurantiacus* LMG 19358[T], *Streptomyces aminophilus* LMG 19319[T] was a subjective synonym of *Streptomyces cacaoi* subsp. *cacaoi* LMG 19320[T], *Streptomyces niveus* LMG 19395[T] and *Streptomyces spheroides* LMG 19392[T] were subjective synonyms of *Streptomyces caeruleus* LMG 19399[T], and *Streptomyces violatus* LMG 19397[T] was a subjective synonym of *Streptomyces violaceus* LMG 19360[T] (Table 272).

Two-dimensional PAGE of the total cellular proteins gives greater resolution than one-dimensional studies. Very complex patterns can be obtained with 2-D PAGE, though this method seems to be too sensitive to differentiate between proteins with high rates of evolution (Hori and Osawa, 1987). Two-dimensional PAGE studies designed to distinguish between ribosomal proteins of streptomycetes were first described by Mikulik et al. (1982) and later by Ochi (1989). Subsequently, AT-L30 proteins were found to give genus-specific profiles (Ochi, 1992), while analyses of the N-terminal sequences of the ribosomal AT-L30 protein allowed streptomycete strains to be assigned to different taxonomic groups (Ochi, 1995); these groups were assigned to phylogenetic groupings which suggested that the genus *Streptomyces* was well described. However, no correlation was found between Ochi's groupings and numerical phenetic groups (Kämpfer et al., 1991; Williams et al., 1983a). Details of these groupings are in Table 272.

Multilocus enzyme electrophoresis (MLEE) results in more specific patterns than protein profiling. MLEE depends on the relative mobilities of cellular enzymes in a gel matrix. In a small study of 24 *Streptomyces* strains (Oh et al., 1996), both inter- and intraspecific characterization of the organisms was achieved, if the appropriate enzymes were used.

The isolation and sequencing of specific proteins led to more detailed taxonomic studies of some *Streptomyces*. The *Streptomyces* subtilisin inhibitor protein (SSI), for example, was used by Taguchi et al. (1996) to determine the taxonomic status of "*Streptomyces lividans*" 66, *Streptomyces coelicolor* Müller ISP 5233^{T}, and *Streptomyces coelicolor* A3(2); this protein plays unidentified role(s) in physiological or morphological regulation. Ribosomal sequence comparisons were supported by the alignments of the SSI indicating that *Streptomyces coelicolor* A3(2) is more closely related to "*Streptomyces lividans*" 66 [cluster 21 of Williams et al. (1983a)] than to the type strain, *Streptomyces coelicolor* Müller ISP 5233^{T} (cluster 1).

Genotypic methods. Genotypic methods encompass all procedures that are directed towards DNA and RNA molecules (Schleifer and Stackebrandt, 1983; Vandamme et al., 1996). The analysis of bacterial genomes by molecular methods has provided a new basis for studying bacterial taxonomy. In some cases, phylogenetic relationships of prokaryotes could be studied at the genus, species, and even subspecies level, leading to a wide use of these methods in modern taxonomic studies. Vandamme et al. (1996) described the general taxonomic values of different molecular techniques, while Anderson and Wellington (2001) considered their use in streptomycete systematics. In the following, the usefulness of these methods in the delineation of species within the genus *Streptomyces* is discussed briefly.

By comparative analysis of sequences of homologous and genetically stable semantides, it was shown that several classification systems based on morphology and physiology did not reflect the natural relationships among actinomycetes and related organisms (Stackebrandt and Schumann, 2006). In this respect, sequence analysis of the rRNA gene has revolutionized our insight into phylogenetic lineages of major taxonomic groups. Nevertheless, 16S rRNA gene sequencing does not always have the resolving power to delimit species. Additionally, while new strains should not be assigned to existing species solely on the basis of this molecule, rRNA gene sequence comparisons do play an important role in the taxonomy of *Streptomyces* and in studying horizontal gene transfer within the genus (Huddleston et al., 1997).

Three regions within 16S rRNA genes have been found to show enough variability to be useful as genus-specific (*a* and *b* regions) and species-specific (*c* regions) probes [see Stackebrandt et al. (1991a, 1991b, 1992) and Anderson and Wellington (2001)]. Next to 16S rRNA genes, 23S rRNA and 5S rRNA genes (Mehling et al., 1995), 16S–23S rRNA internally transcribed spacer (ITS) sequences (Song et al., 2003), and ribosomal protein sequences have been used to investigate relationships between *Streptomyces* (Liao and Dennis, 1994; Ochi, 1995). The ITS sequences of the six rDNA operons from two *Streptomyces ambofaciens* strains were analyzed by Wenner et al. (2002), who confirmed that a high degree of ITS variability was a common characteristic amongst *Streptomyces* species. They also showed that recombination frequently occurs between rDNA loci leading to the exchange of nucleotide blocks. Given such intraspecific variation and intragenomic heterogeneity rRNA gene sequences cannot solely be used for taxonomic studies.

DNA hybridization. The DNA–DNA hybridization values (%) and the decrease in thermal stability of hybrids are currently used as the "gold standards" for species delineation in bacteriology (Wayne et al., 1987), despite the recommendations of the Ad hoc committee for the re-evaluation of the species definition in light of the application of other methods (Stackebrandt et al., 2002), DNA–DNA similarity and changes in melting temperature (ΔT_m; Wayne et al., 1987) still remain the acknowledged standards for the definition of species.

DNA–DNA hybridizations of total chromosomal DNA have been widely used in the classification of *Streptomyces* species. In an initial study, strains of the *Streptomyces albidoflavus* cluster 1, defined by Williams et al. (1983a) using numerical phenetic methods, were the subject of DNA–DNA hybridization studies, performed by reassociation of labeled DNA on nitrocellulose filters (Mordarski et al., 1986). Good congruence was found between the results of the two approaches and the homogeneity of the *Streptomyces albidoflavus* subcluster *albidoflavus* was confirmed. However, two further subclusters obtained by DNA–DNA hybridization were not congruent with the *Streptomyces anulatus* or *Streptomyces halstedii* subclusters of Williams et al. (1983a), though some correlation was found with the groupings of Kämpfer et al. (1991). DNA–DNA pairing data supported the assignment of *Streptoverticillium* strains to the genus *Streptomyces* (reassociation of labeled DNA on filters; Witt and Stackebrandt, 1990), a result that was confirmed in the numerical phenetic study of Kämpfer et al. (1991).

The most extensive DNA–DNA hybridization (thermal renaturation method) studies on strains assigned to some of the major phenetic groups of Williams et al. (1983a) were carried out by Labeda and his colleagues (Labeda, 1993, 1996, 1998; Labeda and Lyons, 1991a, 1991b). They found little correlation between the DNA–DNA pairing and numerical phenetic data with respect to the *Streptomyces cyaneus* (Labeda and Lyons, 1991a), *Streptomyces violaceusniger* (Labeda and Lyons, 1991b), *Streptomyces lavendulae* (Labeda, 1993), the verticil-forming streptomycetes (formerly *Streptoverticillium* species; Labeda, 1996; Hatano et al., 2003), and *Streptomyces fulvissimus* and *Streptomyces griseoviridis* phenotypic clusters (Labeda, 1998), though some correlation was found with phenotypic groups circumscribed by Kämpfer et al. (1991) (Table 272). The fact that certain regions within the *Streptomyces* chromosome show considerable genetic instability supports the continued use of DNA–DNA pairing (Redenbach et al., 1993). However, DNA–DNA hybridization data can be influenced by the presence of large plasmids in *Streptomyces* strains. The general properties of *Streptomyces* plasmids and their use for gene cloning are considered in chapter 11 of Kieser et al. (2000).

TABLE 272. List of *Streptomyces* species (including *Kitasatospora* and *Streptacidiphilus* species) arranged according to the grouping given in Figure 339

Species names and groups[1]	No. in lists of species[2]	Type strain	Accession no.	Wil 83a[3]	Wil 89[4]	Käm 91[5]	Hat 03[6]	Lan 02[7]	Ful 95[8]	Och 95[9]	Kat 97[10]	Lab[11]	Sch[12]	Lan 04[13]	Lan 02[14]	Lan 04[15]	Lan 04[16]	Lan 04[17]	Lan 04[18]	Lan 04[19]	Lan 04[20]
Most closely related to group S. costaricanus et rel.:																					
S. galbus	195	DSM 40089, ATCC 23910, LMG 19879, ISP 5089	X79852	A 15	I 08	006 1-10							Sch00	Lan2-00	BENP	+		+			cl22
S. longwoodensis	295	DSM 41677, LMG 20096, NBRC 14251	AB184580										Sch00	Lan2-00	BENP	+		+			cl22
S. bungoensis	78	DSM 41781, LMG 20439, NBRC 15711	AB184696											Lan2-00	BENP	+		+			cl22
S. corchorusii	133	DSM 40340, ATCC 25444, LMG 20488, ISP 5340, NBRC 13032	AB184267	A 20	I 13	009 1-19							Sch04	Lan2-26	BENP	+	(a)	+			cl52
S. canarius	84	DSM 40528, ATCC 27423, LMG 20443, ISP 5528, NBRC 13431	AB184396	A 20	I 13	009 1-19							Sch00	Lan2-00	BENP	+		+			cl22
S. olivaceoviridis	351	DSM 40334, ATCC 23630, LMG 19324, ISP 5334, NBRC 13066	AB184288	A 20	I 13	009 1-19		La-21		OC-III			Sch04	Lan2-00	BENP +	+		+		+	cl53
S. capoamus	91	DSM 40494, ATCC 19006, LMG 20447, ISP 5494, JCM 4734	AB045877	C 45	II 13	1-7 1-15							Sch00		BENP	+		+		+	+
S. regensis	405	DSM 40551, ATCC 27461, LMG 20300, ISP 5551, NRRL B-11479	DQ026649	A 20	I 13	009 1-19							Sch00	Lan2-00	BENP	+		+			cl52
S. griseochromogenes	222	DSM 40499, ATCC 14511, LMG 19891, ISP 5499, NBRC 13413	AB184387	A 18	I 11	1-5 011						L2	Sch00	Lan2-00	BENP	+		+		+	cl22
S. cellostaticus	97	DSM 40189, ATCC 23894, LMG 20452, ISP 5189, NBRC 12849	AB184192	A 06	I 05	007 003							Sch15	Lan2-00	BENP	+		+			cl55
S. yokosukanensis	531	DSM 40224, ATCC 25520, LMG 21040, ISP 5224, NRRL B-3353	DQ026652	A 30	II 06	009 1-19							Sch00	Lan2-00	BENP	+		+			cl22
S. antibioticus	35	DSM 40234, ATCC 8663, LMG 20412, ISP 5234, NRRL B-1701	AY999776	A 31	I 21	1-7 1-15				OC-IV			Sch00	Lan2-00	BENP	+		+			cl13
S. griseoruber	232	DSM 40281, ATCC 23919, LMG 19325, ISP 5281, NBRC 12873	AB184209	A 21	I 14	018 023		La-00		OC-I			Sch00	Lan2-00	BENP +	+		+	cl15	+	+
S. cinnabarinus	115	DSM 40467, ATCC 23617, LMG 20467, ISP 5467, NBRC 13028	AB184266	A 18	I 11	009 1-19						L2	Sch00	Lan2-00	BENP	+		+		+	+
S. acidiscabies	5	DSM 41668, ATCC 49003, LMG 19856	D63865										Sch00	Lan2-00	BENP	+		+		+	+

(continued)

"Footnotes are given on pp. 1558-1559."

TABLE 272. (continued)

								Morphological characters[24]				Physiological tests[25]										
Guo 08[21] 16S rRNA	Guo 08[21] *atpD*	Guo 08[21] *gyrB*	Guo 08[21] *recA*	Guo 08[21] *rpoB*	Guo 08[21] *trpB*	Guo 08[21] concat.	Kim 04[23] *rpoB*	I	II	III	IV	I	II	III	IV	V	VI	VII	VIII	IX	X	XI
								Gy	S	C+	SM	+	+	+	−	+	n	−	+	+	n	−
								Gy	S	C−	SM	+	+	+	−	+	+	±	+	+	+	−
								Gy	S	C+	SPY	+	+	+	−	+	+	n	+	−	−	n
							+	Gy	S	C−	SM	+	+	+	+	+	n	+	+	+	n	+
								Y	S	C−	SM	+	+	+	+	+	+	+	+	+	−	+
							+	Gy	S	C−	SM	+	+	+	+	+	n	+	+	+	n	+
								R	RF	C+	SM	+	+	+	−	+	+	n	+	+	+	+
								Gy	S	C+	n	+	+	+	−	+	−	n	+	+	n	+
							+	Gy	S	C+	SPY	+	+	+	−	+	+	+	+	+	−	+
								Gy/R	S	C+	SPY	+	+	+	+	+	n	+	+	+	n	+
								R	S	C+	SPY	+	+	+	+	+	+	+	+	+	+	+
							Group A18	Gy	RF	C+	SM	+	+	+	+	+	+	−	+	+	−	−
								Gy	S	C+	SM	+	+	+	+	+	+	−	−	+	+	−
								R	RF	C+	SM	+	+	+	+	+	n	+	+	+	n	+
								R	RF	C−	SM	+	+	+	+	+	n	−	+	n	n	+

(continued)

TABLE 272. List of *Streptomyces* species continued

Species names and groups[1]	No. in lists of species[2]	Type strain	Accession no.	Wil 83a[3]	Wil 89[4]	Käm 91[5]	Hat 03[6]	Lan 02[7]	Ful 95[8]	Och 95[9]	Kat 97[10]	Lab[11]	Sch[12]	Lan 04[13]	Lan 02[14]	Lan 04[15]	Lan 04[16]	Lan 04[17]	Lan 04[18]	Lan 04[19]	Lan 04[20]
S. alanosinicus	10	DSM 40606, ATCC 15710, LMG 20391, ISP 5606, NBRC 13493	AB184442		IV 01 (gray series)	009 1-19							Sch34	Lan2-00	BENP	+		+			cl12
Group S. costaricanus et rel.:																					
S. griseofuscus	224	DSM 40191, ATCC 23916, LMG 19885, ISP 5191, NBRC 12870	AB184206	A 12	I 07	1-6 1-16			FU-6		KA-G		Sch00	Lan2-00	BENP	+		+		+	cl06
S. murinus	327	DSM 40091, ATCC 19788, LMG 10475, ISP 5091, NBRC 12799	AB184155	A 17	I 10	1-6 1-16						L3	Sch00	Lan2-00	BENP	+		+		+	cl59
S. costaricanus	134	DSM 41827, ATCC 55274, NBRC 100773	AB249939																		
S. phaeogriseichromatogenes	369	DSM 40710, NRRL 2834	AJ391813																		
Most closely related to group S. costaricanus et rel.:																					
S. lanatus	276	DSM 40090, ATCC 19775, LMG 19380, ISP 5090, NBRC 12787	AB184845	A 18	I 11	016 1-19		La-21				L2	Sch00	Lan2-00	BENP +	+		+	cl09		cl22
S. durhamensis	149	DSM 40539, ATCC 23194, LMG 20501, ISP 5539, NRRL B-3309	AY999785	A 30	II 06	009 1-19							Sch00	Lan2-00	BENP	+		+			cl22
S. filipinensis	169	DSM 40112, ATCC 23905, LMG 19333, ISP 5112, NBRC 12860	AB184198	A 30	II 06	009 1-19		La-10		OC-III			Sch00	Lan2-00	BENP +	+		+	cl09	+	cl23
S. puniciscabiei	392	KACC 20253, LMG 21391, S77	AF361785																	+	
S. niveiscabiei	339	LMG 21392, S78	AF361786																	+	
S. echinatus	151	DSM 40013, ATCC 19748, LMG 5972, ISP 5013	AJ399465	A 18	I 11	1-6 1-10			FU-1			L2	Sch00	Lan2-00	BENP	+		+			+
S. longisporus	294	DSM 40166, ATCC 23931, LMG 20053, ISP 5166	AJ399475	A 18	I 11	009 1-19						L2	Sch00	Lan2-00	BENP	+		+		+	cl22
S. avermitilis	60	ATCC 31267, MA-4680	BA000030																		
S. kunmingensis	271	DSM 41681, LMG 20521, NRRL B-16240	DQ442513										Sch00	Lan2-00	BENP	+		+			cl22
S. mirabilis	321	DSM 40553, ATCC 27447, LMG 20076, ISP 5553, NBRC 13450	AB184412	A 19	I 12	1-7 1-19							Sch00	Lan2-00	BENP	+		+		+	cl20
S. olivochromogenes	353	DSM 40451, ATCC 3336, LMG 20071, ISP 5451	AY094370	A 19	I 12	009 1-19							Sch00	Lan2-00	BENP	+		+			cl20
Most closely related to group S. cyanoalbus et rel.:																					
S. lucensis	296	DSM 40317, ATCC 17804, LMG 20065, ISP 5317, NRRL B-5626	DQ442522	A 31	I 21	1-5 1-16							Sch00	Lan2-00	BENP	+		+		+	+

(continued)

TABLE 272. (continued)

								Morphological characters[24]				Physiological tests[25]										
Guo 08[21] 16S rRNA	Guo 08[21] *atpD*	Guo 08[21] *gyrB*	Guo 08[21] *recA*	Guo 08[21] *rpoB*	Guo 08[21] *trpB*	Guo 08[21] concat.	Kim 04[23] *rpoB*	I	II	III	IV	I	II	III	IV	V	VI	VII	VIII	IX	X	XI
								Gy	S	C+	SPY	+	+	+	−	+	+	+	+	+	+	n
								Gy	S	C−	SM	+	+	+	−	+	n	−	+	−	n	−
								Gy	S	C−	SM	+	+	−	−	+	n	−	+	n	n	−
								Gy	S	C−	SM	+	+	−	−	+	+	−	n	n	+	−
								B	S	C+	SPY	+	+	+	+	+	n	+	+	+	n	+
								Gy	S	C+	SPY	+	+	+	−	+	+	+	+	+	−	n
								Gy	S	C+	SPY	+	+	+	−	+	+	+	+	+	−	+
									RF	C+/C−	SPY	+	+	+	+	+	n	+	+	+		+
								W/Gy	RF	C−	SM	+	+	+	+	+	n	+	+	+	n	+
								Gy	S	C+	SPY	+	+	+	+	+	+	+	+	+	−	−
								W	S	C+	SPY	+	+	+	+	+	n	+	+	+	n	+
								GY	S	C+	SM	+	+	+	+	+	n	n	n	+	n	−
								W	S	C−	n	n	+	+	+	n	n	+	+	−	n	−
								Gy	S	C+	SM	+	+	+	+	n	n	n	n	n	n	n
							+	Gy	S	C+	SM	+	+	+	−	+	+	+	+	+	+	n
								Gy	S	C+	SPY	+	+	+	−	+	n	−	+	−	n	+

(continued)

TABLE 272. List of *Streptomyces* species continued

Species names and groups[1]	No. in lists of species[2]	Type strain	Accession no.	Wil 83a[3]	Wil 89[4]	Käm 91[5]	Hat 03[6]	Lan 02[7]	Ful 95[8]	Och 95[9]	Kat 97[10]	Lab[11]	Sch[12]	Lan 04[13]	Lan 02[14]	Lan 04[15]	Lan 04[16]	Lan 04[17]	Lan 04[18]	Lan 04[19]	Lan 04[20]
S. niveoruber	340	DSM 40638, ATCC 14971, LMG 19379, NRRL B-2724	DQ445796		IV 08 (red series)	013 1-19		La-01					Sch00	Lan2-00	BENP +	+		+	cl04		+
S. achromogenes subsp. *achromogenes*	4a	DSM 40028, ATCC 12767, LMG 20387, ISP 5028, NBRC 12735	AB184109	A 19	I 12	1-5 009			FU-1		KA-B	L2	Sch00	Lan2-00	BENP	+		+		+	cl22
S. griseorubiginosus	233	DSM 40469, ATCC 23627, LMG 19941, ISP 5469	AJ781339	A 18	I 11	009 1-19						L2	Sch23	Lan2-00	BENP	+		+		+	cl19
S. phaeopurpureus	372	DSM 40125, ATCC 23946, LMG 20051, ISP 5125, NRRL B-2260	DQ026666	A 09	II 02	009 1-19							Sch23	Lan2-02	BENP	+	(b)	+			cl51
S. curacoi	137	DSM 40107, ATCC 13385, LMG 20491, ISP 5107, NRRL B-2901	EF626595	A 18	I 11	009 0-19						L2	Sch00	Lan2-00	BENP	+		+		+	cl55
S. lincolnensis	288	DSM 40355, ATCC 25466, LMG 20068, ISP 5355, NBRC 13054	AB184279	A 19	I 12	009 1-19							Sch00	Lan2-00	BENP	+		+		+	cl13
S. cyaneus	140	DSM 40108, ATCC 14923, LMG 20494, ISP 5108, NRRL B-2296	AF346475	A 18		009 1-19						L2	Sch00	Lan2-00	BENP	+	(g)	+		+	+
Group S. cyanoalbus et rel.:																					
S. cyanoalbus	141	DSM 40198, ATCC 15859, LMG 19343, ISP 5198, NBRC 12857	AB184882	A 37	I 17	007 003		La-17				Sch15	Lan2-00	MG-016	BEN-BOX +			+	cl14	+	+
S. hirsutus	249	DSM 40095, LMG 19927, ISP 5095, NBRC 12786	AB184844										Sch00	Lan2-00	BENP	+		+		+	cl49
S. prasinus	384	DSM 40099, ATCC 19800, LMG 20259, ISP 5099, NRRL B-2712	DQ026658	A 37	I 17	007 003							Sch00		BENP	+		+	cl14	+	cl49
S. bambergiensis	67	DSM 40590, ATCC 13879, LMG 19299, ISP 5590, NBRC 13479	AB184869	A Sm	III 10	075 1-25		La-20		OC-non			Sch00		BENP +	+		+	cl14		+
S. emeiensis	155	DSM 41884, CGMCC 4.3504	DQ462649																		
S. prasinopilosus	382	DSM 40098, ATCC 19799, LMG 19345, ISP 5098, NRRL B-2711	EF626597	A 37	I 17	007 003		La-20					Sch00	Lan2-00	BENP +	+		+	cl14	+	cl49
Most closely related to group S. cyanoalbus et rel.:																					
S. flavovariabilis	183	DSM 41479, LMG 19905, NRRL B-16367	EF178691										Sch00	Lan2-00	BENP	+		+		+	+
S. aureocirculatus	52	DSM 40386, ATCC 19823, LMG 21794, ISP 5386, NBRC 13018	AB184260	A 03	II 20	033 1-33							Sch00	Lan2-00	BENP	+		+		+	cl22

(continued)

TABLE 272. (continued)

								Morphological characters[24]				Physiological tests[25]										
Guo 08[21] 16S rRNA	Guo 08[21] *atpD*	Guo 08[21] *gyrB*	Guo 08[21] *recA*	Guo 08[21] *rpoB*	Guo 08[21] *trpB*	Guo 08[21] concat.	Kim 04[23] *rpoB*	I	II	III	IV	I	II	III	IV	V	VI	VII	VIII	IX	X	XI
								R	S	C–	SM	+	+	+	+	n	n	n	n	n	n	n
							+	Gy	RF	C+	SM	+	+	+	+	+	+	–	+	+	+	–
								Gy	RF	C+	SM	+	+	+	+	+	n	+	+	+	n	+
								Gy	RF	C+	SM	+	+	+	+	+	+	+	+	+	–	n
								B	S	C+	SPY	+	+	+	+	+	+	+	+	+	+	+
							+	R	RF	C+	SM	+	+	+	+	+	+	+	+	+	+	+
+	+	+	+	+	+	+	+	B	S	C+	SPY	+	+	+	+	n	n	n	n	n	n	n
								Gy	S	C–	H	+	+	+	+	+	+	+	+	–	–	+
								G	S	C–	SPY	+	+	+	+	+	n	+	+	+	n	+
								G	S	C–	SPY	+	+	+	+	+	+	–	+	+	–	+
							+	G	S	C–	H	n	n	n	n	n	n	n	n	n	n	n
								Gy	RF	C–	SPY	+	+	+	+	+	+	+	n	+	n	+
								G	S	C–	H	+	+	+	+	+	+	–	+	+	–	n
								R	S	C+	SPY	+	+	+	+	+	+	+	+	n	n	+
								W	RF	C–	SM	+	n	–	–	+	+	–	+	+	n	–

(continued)

TABLE 272. List of *Streptomyces* species continued

Species names and groups[1]	No. in lists of species[2]	Type strain	Accession no.	Wil 83a[3]	Wil 89[4]	Käm 91[5]	Hat 03[6]	Lan 02[7]	Ful 95[8]	Och 95[9]	Kat 97[10]	Lab[11]	Sch[12]	Lan 04[13]	Lan 02[14]	Lan 04[15]	Lan 04[16]	Lan 04[17]	Lan 04[18]	Lan 04[19]	Lan 04[20]
S. novaecaesareae	347	DSM 40358, ATCC 27452, LMG 20069, ISP 5358, NBRC 13368	AB184357	J Sm	III 25	004 006				OC-IV			Sch00	Lan2-00	BENP	+		+		+	cl22
S. prunicolor	385	DSM 40335, ATCC 25487, LMG 19311, ISP 5335, NRRL B-12281	DQ026659	A 11	III 01	1-1 1-1		La-00		OC-II			Sch09		BENP +	+		+		+	+
S. phaeoluteigriseus	371	DSM 41896, NRRL 5182, NRRL ISP-5182	AJ391815																	+	
S. bobili	75	DSM 40056, ATCC 3310, LMG 20436, ISP 5056, NBRC 16166	AB249925		IV 02 (white series)	1-7 1-15							Sch37	Lan2-00	BENP	+		+		+	cl22
S. galilaeus	196	DSM 40481, ATCC 14969, LMG 21790, ISP 5481, JCM 4757	AB045878	A 19	I 12	1-7 1-15							Sch37	Lan2-00	BENP	+		+		+	cl22
Most closely related to groups S. cyanoalbus et rel.: and S. griseoluteus et rel.:																					
S. chartreusis	102	DSM 40085, ATCC 14922, LMG 20455, ISP 5085, NBRC 12753	AB184839	A 18	I 11	009 1-19						L2	Sch30	Lan2-00	BENP	+		+		+	+
S. resistomycificus	406	DSM 40133, ATCC 19804, ISP 5133, NBRC 12814	AB184166	A 18	I 11	009 1-19			FU-12a			L2	Sch00	Lan2-00	BENP	+		+		+	+
Most closely related to group S. griseoluteus et rel.:																					
S. griseoluteus	228	DSM 40392, ATCC 12768, LMG 19356, ISP 5392, JCM 4765	AY999751	C 43	II 11	1-5 1-16		La-24		OC-III			Sch00		BENP +	+		+		+	cl41
S. recifensis	402	DSM 40115, ATCC 19803, LMG 20261, ISP 5115, NBRC 12813	AB184165	A 23	I 20	1-5 059							Sch00		BENP	+		+		+	cl41
S. seoulensis	437	NBRC 16255, NBRC 16668, JCM 10116	AB249970																		
Most closely related to groups S. cyanoalbus et rel. and S. griseoluteus et rel.:																					
S. canus	89	DSM 40017, ATCC 12237, LMG 19329, ISP 5017, NRRL B-1989	AY999775	A 25	III 02	009 1-19		La-21		OC-IV			Sch00	Lan2-00	BENP +	+		+	cl09	+	+
S. ciscaucasicus	119	DSM 40275, LMG 20474, ISP 5275	AY508512										Sch00	Lan2-05	BENP	+		+		+	cl19
S. pseudovenezuelae	389	NBRC 12904	AB184233																		
S. alboniger	21	DSM 40043, ATCC 12461, LMG 20397, ISP 5043	AY845349	A 1B	I 02	1-6 1-31							Sch00	Lan2-00	BENP	+		+		+	+
Most closely related to group S. scabiei et rel.:																					
S. bottropensis	76	DSM 40262, ATCC 25435, LMG 20437, ISP 5262	AB026217	A 19	I 12	009 1-19							Sch00	Lan2-00	BENP	+		+		+	+

(continued)

TABLE 272. (continued)

Guo 08[21] 16S rRNA	Guo 08[21] *atpD*	Guo 08[21] *gyrB*	Guo 08[21] *recA*	Guo 08[21] *rpoB*	Guo 08[21] *trpB*	Guo 08[21] concat.	Kim 04[23] *rpoB*	Morphological characters[24] I	II	III	IV	Physiological tests[25] I	II	III	IV	V	VI	VII	VIII	IX	X	XI
								n	n	C–	n	+	+	+	+	+	n	+	+	+	n	+
								R	RF	C–	SM	+	+	+	+	+	n	+	+	+	n	n
+	+	+	+	+	+	+		W	S	C+	SM	+	+	+	+	+	+	+	–	+	–	+
							Asn (AAC)[442]	Gy	S	C+	SM	+	+	+	+	n	n	n	n	n	n	n
								B	S	C+	SPY	+	+	+	+	+	+	+	+	+	+	+
								Gy	S	C+	SM	+	+	+	+	+	n	+	+	+	n	+
								Gy	RF	C+	SM	+	+	+	–	+	n	–	+	–	n	–
								Gy	S	C–	SM	+	+	+	–	+	+	+	+	–	+	+
								Gy	RF	C–	SM	+		–		+		+	+	–	+	+
								Gy	S	C–	SPY	+	+	+	+	+	+	±	+	+	n	+
								Gy	S	C–	SPY	+	+	+	+	+	n	+	n	n	n	n
							+	W	RF	C–	SM	+	+	+	–	+	+	–	+	+	+	–
								Gy	S	C+	SM	+	+	+	+	+	n	+	+	+	n	+

(continued)

TABLE 272. List of *Streptomyces* species continued

Species names and groups[1]	No. in lists of species[2]	Type strain	Accession no.	Wil 83a[3]	Wil 89[4]	Käm 91[5]	Hat 03[6]	Lan 02[7]	Ful 95[8]	Och 95[9]	Kat 97[10]	Lab[11]	Sch[12]	Lan 04[13]	Lan 02[14]	Lan 04[15]	Lan 04[16]	Lan 04[17]	Lan 04[18]	Lan 04[19]	Lan 04[20]
S. stelliscabiei	459	DSM 41803, NCPPB 4040, CFBP 4521	AJ007429																		
S. europaeiscabiei	162	DSM 41802, KACC 20186	AY207598																	+	
S. scabiei	433	DSM 41658, ATCC 49173, LMG 20323	D63862										Sch00	Lan2-00	BENP	+		+			cl25
S. diastatochromogenes	145	DSM 40449, ATCC 12309, LMG 20498, ISP 5449	D63867	A 19	I 12	009 1-19							Sch00	Lan2-00	BENP	+		+			cl25
S. hygroscopicus subsp. *ossamyceticus*	253e	DSM 40824, ATCC 15420, LMG 19951, NBRC 13983	AB184560		I 16	009 1-19							Sch20	Lan2-00	BENP	+		+			cl26
S. ipomoeae	260	DSM 40383, ATCC 25462, LMG 20520, ISP 5383, NBRC 13050	AB184857		IV 02 (blue series)	077 074						L2	Sch00	Lan2-00	BENP	+		+		+	cl26
S. torulosus	483	DSM 40894, NRRL B-3889, LMG 20305	AJ781367		IV 31 (gray series)	009 1-19							Sch00	Lan2-00	BENP	+		+		+	cl26
S. neyagawaensis	334	DSM 40588, ATCC 27449, LMG 20080, ISP 5588	D63869	A 18	I 11	009 1-19			FU-24			L2	Sch00	Lan2-00	BENP	+		+		+	cl26
Most closely related to group S. scabiei et rel.:																					
S. reticuliscabiei	407	DSM 41804, CIP 107061, CFBP 4531	AJ007428																		
S. turgidiscabies	488	ATCC 702348, ATCC 700248	AB026221																	+	
S. cacaoi subsp. *asoensis*	79b	DSM 41440, ATCC 19093, LMG 20440, NRRL B-16592	DQ026644										Sch00	Lan2-00	BENP	+		+		+	+
S. humidus	250	DSM 40263, ATCC 12760, LMG 19936, ISP 5263, NRRL B-3172	DQ442508	A 19	I 12	009 1-19							Sch00	Lan2-00	BENP	+		+			cl22
S. rishiriensis	410	DSM 40489, ATCC 14812, LMG 20297, ISP 5489, NRRL B-3239	EF178682	A 19	I 12	1-7 1-15			FU-12a				Sch00	Lan2-00	BENP	+		+			+
S. cinereoruber subsp. *fructofermentans*	111b	DSM 40692, NRRL 2588, LMG 20463, JCM 4956	AY999758		I 04	006 1-18							Sch00	Lan2-00	BENP	+		+		+	cl20
S. phaeofaciens	368	DSM 40367, LMG 20070, ISP 5367, NBRC 13372	AB184360										Sch00	Lan2-00	BENP	+		+		+	+
S. puniceus	391	DSM 40083, ATCC 19801, LMG 20258, ISP 5083, NRRL B-2895	DQ442542	A 09	II 02	005 029							Sch00	Lan2-00	BENP	+		+		+	cl22
Group S. aurantiacus et rel.:																					
S. aurantiacus	49	DSM 40412, ATCC 19822, LMG 19358, ISP 5412	AJ781383	C 45	II 13	012 019		La-01		OC-non			Sch00	Lan2-00	BENP cl01	+	(c)	+	cl05	+	cl24
S. glomeroaurantiacus	211	DSM 41782, NBRC 15418	AB249983																		

(continued)

TABLE 272. (continued)

Guo 08[21] 16S rRNA	Guo 08[21] *atpD*	Guo 08[21] *gyrB*	Guo 08[21] *recA*	Guo 08[21] *rpoB*	Guo 08[21] *trpB*	Guo 08[21] concat.	Kim 04[23] *rpoB*	Morphological characters[24] I	II	III	IV	Physiological tests[25] I	II	III	IV	V	VI	VII	VIII	IX	X	XI
								Gy	S	C+		+	+	+	+	+	n	+	+	+	n	+
								Gy	S	C+	n	+	+	+	+	+	n	+	+	+	n	+
								Gy	RF	C+/C–	SM	+	+	+	+	+	n	–	+	–	n	–
								Gy	S/RA	C+	SM	+	+	+	+	+	n	+	+	+	n	+
								Gy	S	C+	SM	+	+	+	+	+	+	+	+	+	–	+
								B	S	C–	SPY	+	+	+	+	+	n	+	+	+	n	+
								Gy	S	C+	WTY	+	+	+	+	+	+	+	+	+	–	n
								Gy	S	C+	SM	+	+	+	–	+	+	+	+	+	+	+
								Gy	RF	C–		+	+		+	+		+	+	+		+
								Gy	RF	C–	SM	+	+	+	+	+	n	+	+	+	n	+
								Gy	RF	C+	SM	+	+	+	n	+	+	+	n	+	n	+
								Gy	S	C–	SM	+	+	+	+	+	+	–	+	+	+	–
								Gy	S	C+	SM	+	+	+	+	+	+	+	–	+	+	+
							+	Gy	RF	C+	SM	+	+	+	+	+	+	–	–	–	+	n
								Gy	S	C+	SM	+	+	+	+	n	n	n	n	n	n	n
								Y	RF	C–	SM	+	+	+	–	+	+	–	+	–	+	±
								R	S	C–	SM	+	±	+	+	+	+	±	+	+	n	±
								R	S	C–	SM	+	+	–	–	+	+	–	+	+	–	–

(continued)

TABLE 272. List of *Streptomyces* species continued

Species names and groups[1]	No. in lists of species[2]	Type strain	Accession no.	Wil 83a[3]	Wil 89[4]	Käm 91[5]	Hat 03[6]	Lan 02[7]	Ful 95[8]	Och 95[9]	Kat 97[10]	Lab[11]	Sch[12]	Lan 04[13]	Lan 02[14]	Lan 04[15]	Lan 04[16]	Lan 04[17]	Lan 04[18]	Lan 04[19]	Lan 04[20]
S. tauricus	467	DSM 40560, ATCC 27470, LMG 20301, ISP 5560, JCM 4837	AB045879	A 19		012 019							Sch00	Lan2-00	BENP	+		+		+	cl24
S. ederensis	153	DSM 40741, ATCC 15304, LMG 20504, NBRC 15410	AB184658		IV 14 (gray series)	013 1-19							Sch00	Lan2-00	BENP	+		+			cl19
S. phaeochromogenes	367	DSM 40073, ATCC 3338, LMG 19348, ISP 5073, NBRC 3180	AB184738	A 40	I 18	009 0-19		La-01		OC-II			Sch00	Lan2-00	BENP +	+		+	cl06		-
S. umbrinus	489	DSM 40278, ATCC 19929, LMG 20280, ISP 5278, NBRC 13091	AB184305	A 05	I 04	1-6 1-16							Sch00	Lan2-15	BENP	+		+			cl54
S. rectiviolaceus	404	DSM 41459, LMG 20310, NRRL B-16374	DQ026660										Sch00	Lan2-00	BENP	+		+		+	-
Group S. aureus et rel.:																					
S. kanamyceticus	264	DSM 40500, LMG 19351, ISP 5500, NRRL B-2535	DQ442511					La-11					Sch00	Lan2-00	BENP +	+		+	cl02		cl23
S. durmitorensis	150	DSM 41863, MS405	DQ067287																		
S. aureus	57	DSM 41785, NCIMB 13927, NBRC 100912	AB249976																		
Group S. cinereus et rel.:																					
S. cinereus	113	DSM 43033, LMG 21310, NBRC 12247	AB184072										Sch00	Lan2-00	BENP	+		+		+	+
S. flaveus	174	DSM 43153, ATCC 15332, LMG 19323, NRRL B-16074	DQ026643					La-21							BENP +	+		+			-
S. vastus	493	DSM 40309, LMG 21043, NRRL B-12232	DQ442552										Sch21	Lan2-00	BENP	+		+		+	+
Most closely related to group S. cinereus et rel.:																					
S. laceyi	274	DSM 41788, NBRC 100783	AB249944																	+	
Group S. argenteolus et rel.:																					
S. griseolus	227	DSM 40067, ATCC 3325, LMG 19878, ISP 5067, NBRC 3415	AB184768	A 1C	I 03	1-2 015			FU-24		KA-B		Sch06		BENP	+		+		+	cl53
S. halstedii	242	DSM 40068, ATCC 10897, ISP 5068, NRRL B-1238	EF178695	A 1C	I 03	1-2 015			FU-24	OC-I	KA-B		Sch06							+	
S. argenteolus	41	DSM 40226, ATCC 11009, LMG 5967, ISP 5226, JCM 4623	AB045872	A 15	I 08	1-5 011					KA-B		Sch00	Lan2-00	BENP	+		+		+	cl23
S. cinereorectus	110	DSM 41469, LMG 20461, NBRC 15395	AB184646										Sch00	Lan2-30	BENP	+	(b)	+		+	cl28
S. flavovirens	184	DSM 40062, ATCC 3320, LMG 20516, ISP 5062, NRRL B-2685	DQ026635	A 1C		1-2 015							Sch05	Lan2-09	BENP	+	(a)	+		+	cl53

(continued)

TABLE 272. (continued)

								Morphological characters[24]				Physiological tests[25]										
Guo 08[21] 16S rRNA	Guo 08[21] *atpD*	Guo 08[21] *gyrB*	Guo 08[21] *recA*	Guo 08[21] *rpoB*	Guo 08[21] *trpB*	Guo 08[21] concat.	Kim 04[23] *rpoB*	I	II	III	IV	I	II	III	IV	V	VI	VII	VIII	IX	X	XI
								R	S	C–	SM	n	+	+	+	+	n	+	–	n	n	n
								Gy	RF	C+	SM	n	n	n	n	n	n	n	n	n	n	n
							+	R	RF	C+	SM	+	+	+	+	+	+	+	+	+	+	n
								R	RF	C+	SM	+	+	+	+	+	+	+	+	+	–	n
								V	RF	C–	SM	+	+	+	+	+	+	+	+	+	n	+
+	+	+	+	+	+	+		Y	RF	C–	SM	+	+	+	–	+	+	+	+	–	+	n
								Y	RF	C–	SM	+	+	–	–	+	+	+	+	–	n	+
+	+	+	+	+	+	+		Gy	S	C+	SM	n	n	n	n	n	n	n	+	+	n	n
								W	RF	C–	SM	+	+	+	+	+	+	+	+	+	+	+
								Gy	RF	C–	SM	+	+	+	+	+	+	+	+	+	+	+
								Gy	S	C–	SM	+	+	+	+	+	+	+	+	+	+	+
+	+	+	+	+	+	+		Gy/Y/R	S		SM								+	–		
+	+	+	+	+	+	+	+	Gy	RF	C–	SM	+	+	+	–	+	+	+	–	–	–	n
								Gy	RF	C–	SM	+	+	+	–	+	n	–	–	–	n	–
+	+	+	+	+	+	+	+	Gy	S	C–	SM	+	+	+	+	+	+	–	+	–	+	–
								Gy	RF	C–	SM	+	–	n	–	+	n	+	+	–	n	n
								Gy	RF	C–	SM	+	+	+	+	+	+	+	+	–	–	–

(continued)

TABLE 272. List of *Streptomyces* species continued

Species names and groups[1]	No. in lists of species[2]	Type strain	Accession no.	Wil 83a[3]	Wil 89[4]	Käm 91[5]	Hat 03[6]	Lan 02[7]	Ful 95[8]	Och 95[9]	Kat 97[10]	Lab[11]	Sch[12]	Lan 04[13]	Lan 02[14]	Lan 04[15]	Lan 04[16]	Lan 04[17]	Lan 04[18]	Lan 04[19]	Lan 04[20]
S. flavogriseus	180	DSM 40323, ATCC 25452, LMG 19887, ISP 5323, CBS 101.34	AJ494864	A 1C	I 03	1-2 015			FU-19b		KA-B		Sch00/0	Lan2-09	BENP	+		+			cl53
S. nitrosporeus	338	DSM 40023, LMG 20044, ISP 5023, NRRL B-1316	EF178680										Sch26	Lan2-00	BENP	+		+		+	+
Most closely related to groups S. argenteolus et rel.: and S. atroolivaceus et rel.:																					
S. luridiscabiei	297	KACC 20252, LMG 21390, S63	AF361784																	+	
S. acrimycini	6	DSM 40135, ATCC 19885, LMG 21798, ISP 5135, AS 4.1673	AY999889		IV 04 (green series)	1-3 010							Sch00	Lan2-00	BENP	+		+			cl05
S. griseoplanus	230	DSM 40009, ATCC 19766, LMG 19923, ISP 5009, AS 4.1868	AY999894	A 29	I 15	078 060							Sch00	Lan2-00	BENP	+		+		+	cl28
S. baarnensis	63	DSM 40232, ATCC 23885, LMG 20431, ISP 5232, NRRL B-1902	EF178688	A 1B	I 02	006 1-2					KA-B		Sch01		BENP	+		+		+	cl23
S. flavofuscus	179	DSM 41426, ATCC 19908, LMG 19900, NBRC 100768	AB249935										Sch00	Lan2-19	BENP	+		+		+	cl23
S. praecox	381	DSM 40393, ATCC 3374, LMG 20290, ISP 5393, NBRC 13073	AB184293		IV 08 (yellow series)	1-3 1-2							Sch01		BENP	+		+		+	cl23
S. fimicarius	171	DSM 40322, ATCC 25449, LMG 21044, ISP 5322	AY999784	A 1B	I 02	1-3 1-2			FU-9				Sch01		BENP	+		+		+	cl23
S. anulatus	37	DSM 40361, ATCC 27416, LMG 19301, ISP 5361, NRRL B-2000	DQ026637	A 1B	I 02	047 1-35		La-22		OC-I	KA-B		Sch01	Lan2-18	BENP +	+	(a) (e)	+	cl02	+	cl23
Group S. atroolivaceus et rel.:																					
S. mutomycini	329	DSM 41691, LMG 20098, NBRC 100999	AB249951										Sch00	Lan2-00	BENP	+		+		+	cl23
S. olivoviridis	357	DSM 40211, ATCC 15882, LMG 20057, ISP 5211, NBRC 12897	AB184227	A 03	II 20	1-3 010							Sch16		BENP	+		+		+	cl23
S. atroolivaceus	47	DSM 40137, ATCC 19725, LMG 19306, ISP 5137	AJ781320	A 03	II 20	006 0-10		La-23		OC-I			Sch16		BENP +	+		+	cl02		cl23
S. clavifer	121	DSM 40843, LMG 20476, NRRL B-2557	DQ026670										Sch00	Lan2-05	BENP	+		+			cl19
S. finlayi	172	DSM 40218, ATCC 23340, LMG 19373, ISP 5218, NRRL B-12114	AY999788	I Sm	III 24	22-4 043		La-00		OC-I			Sch00	Lan2-00	BENP +	+		+	cl02	+	cl23

(continued)

TABLE 272. (continued)

								Morphological characters[24]				Physiological tests[25]										
Guo 08[21] 16S rRNA	Guo 08[21] *atpD*	Guo 08[21] *gyrB*	Guo 08[21] *recA*	Guo 08[21] *rpoB*	Guo 08[21] *trpB*	Guo 08[21] concat.	Kim 04[23] *rpoB*	I	II	III	IV	I	II	III	IV	V	VI	VII	VIII	IX	X	XI
+	+	+	+	+	+	+		Gy	RF	C–	SM	+	+	+	+	+	n	–	+	–	n	–
								Gy	RF	C–	SM	+	+	+	+	–	+	–	–	–	–	–
+	+	+	+	+	+	+		Y/W	RF	C+	SM		+	+	+	+		+	+	+		+
Group I	Yes[22]	Yes	Yes	Yes	Yes	Yes		G	S	C–	H	+	+	–	+	+	n	–	+	+	n	–
Group I	No[22]	No	No	No	No	No		Gy	S	C–	WTY	+	+	+	–	+	+	+	–	–	–	–
								W	RF	C–	SM	+	+	+	+	+	+	–	+	+	n	n
								Y	RF	C–	SM											
Group I	No	No	No	No	No	No		Y	RF	C–	SM	+	+	+	+	+	+	+	+	–	+	n
Group I	Yes	Yes	Yes	Yes	Yes	Yes		Y	RF	C–	SM	+	+	+	+	+	+	–	+	–	–	n
Group I	No	No	No	No	No	No		Y	RF	C–	SM	+	+	+	+	+	n	–	+	–	n	–
+	+	+	+	+	+	+		Gy	S	C–	SPY	+	+	+		+			+			
								Gy	S	C–	SPY	+	+	+	+	+	+	–	+	–	–	–
+[2]	+	+	+	+	+	+		Gy	S	C–	WTY	+	+	+	+	+	n	n	n	n	n	n
								W	RF	C–	SM	+	+	–	+	+	+	–	+	–	–	n
+	+	+	+	+	+	+		Gy	S	C–	H	+	+	+	+	–	n	–	–	–	n	±

(continued)

TABLE 272. List of *Streptomyces* species continued

Species names and groups[1]	No. in lists of species[2]	Type strain	Accession no.	Wil 83a[3]	Wil 89[4]	Käm 91[5]	Hat 03[6]	Lan 02[7]	Ful 95[8]	Och 95[9]	Kat 97[10]	Lab[11]	Sch[12]	Lan 04[13]	Lan 02[14]	Lan 04[15]	Lan 04[16]	Lan 04[17]	Lan 04[18]	Lan 04[19]	Lan 04[20]
Most closely related to groups S. argenteolus et rel. and S. atroolivaceus et rel.:																					
S. griseus subsp. *griseus*	238a	DSM 40236, ATCC 23345, LMG 19302, ISP 5236, KACC 20084	AY207604	A 1B	I 02	1-3 1-2		La-22	FU-19		KA-B		Sch00	Lan2-00	BENP +	+		+	cl02	+	cl23
S. lavendulae subsp. *lavendulae*	280a	DSM 40069, ATCC 8664, LMG 19925, ISP 5069, NBRC 12343	AB184080	F 61	I 22	22-3 042			FU-12b	OC-I		L3/L	Sch00	Lan2-00	BENP	+	(e) (l)	+			-
S. cavourensis subsp. *washingtonensis*	96b	DSM 41423, LMG 20451, NRRL B-8030	DQ026671										Sch00	Lan2-00	BENP	+		+			cl23
S. cyaneofuscatus	139	DSM 40148, ATCC 23619, LMG 20493, ISP 5148, NBRC 13190	AB184860	A 1B	I 02	1-3 1-2							Sch00	Lan2-00	BENP	+		+		+	cl23
Not closely related to one of the groups:																					
S. mediolani	313	DSM 41058, DSM 41647, LMG 20093, NBRC 15427	AB184674										Sch20	Lan2-16	BENP	+		+			cl23
S. rubiginosohelvolus	424	DSM 40176, ATCC 19926, LMG 20267, ISP 5176, NBRC 12912	AB184240		IV 12 (red series)	006 1-2							Sch03		BENP	+		+			cl23
S. parvus	364	DSM 40348, ATCC 12433, LMG 20524, ISP 5348, NRRL B-1455	DQ442537	A 1B	I 02	1-3 1-2			FU-6		KA-B		Sch00	Lan2-00	BENP	+		+	cl02		cl23
S. albovinaceus	25	DSM 40136, ATCC 15823, LMG 20402, ISP 5136, NBRC 12739	AB249958	A 1B	I 02	1-3 008					KA-B		Sch03	Lan2-16	BENP	+		+		+	cl23
S. bacillaris	64	DSM 40598, ATCC 15855, LMG 8585, ISP 5598, NBRC 13487	AB184439	A 1B	I 02	1-3 1-2					KA-B		Sch00	Lan2-00	BENP	+		+		+	cl50
S. griseinus	218	DSM 40047, ATCC 23915, LMG 19875, ISP 5047, NBRC 12869	AB184205	A 1B	I 02	1-3 1-2			FU-6		KA-B		Sch03	Lan2-00	BENP	+		+			-
S. sindenensis	441	DSM 40255, ATCC 23963, LMG 21041, ISP 5255, NBRC 3399	AB184759	A 1B		1-3 1-2					KA-B		Sch00		BENP	+		+		+	cl23
S. pluricolorescens	378	DSM 40019, ATCC 19798, LMG 8576, ISP 5019, NRRL B-2121	DQ442540	A 1B	I 02	1-3 1-2					KA-B		Sch03		BENP	+		+		+	-
S. globisporus subsp. *globisporus*	208a	DSM 40199, ATCC 15864, LMG 8578, ISP 5199, NRRL B-2872	EF178686	A 1B	I 02	1-3 1-2					KA-B		Sch20	Lan2-00	BENP	+		+			cl23
S. badius	65	DSM 40139, ATCC 19888, LMG 19353, ISP 5139, NRRL B-2567	AY999783	C Sm	III 15	1-1 1-1		La-00		OC-I			Sch00		BENP +	+		+	cl02	+	cl23

(continued)

TABLE 272. (continued)

								Morphological characters[24]				Physiological tests[25]										
Guo 08[21] 16S rRNA	Guo 08[21] *atpD*	Guo 08[21] *gyrB*	Guo 08[21] *recA*	Guo 08[21] *rpoB*	Guo 08[21] *trpB*	Guo 08[21] concat.	Kim 04[23] *rpoB*	I	II	III	IV	I	II	III	IV	V	VI	VII	VIII	IX	X	XI
Group IV	Yes	Yes	Yes	Yes	No	Yes	Group A1B	Y	RF	C–	SM	+	+	–	–	+	+	–	+	–	+	–
								R	S	C+	SM	+	–	–	–	–	+	+	–	–	+	–
								Y	RF	C+	SM	+	+	+	–	+	n	–	+	–	n	–
+	+	+	+	+	+	+		Y	RF	C+	SM	+	+	–	+	+	+	–	+	–	+	+
Group II	Yes	Yes	Yes	Yes	Yes	Yes		Y	RF													
								R	RF	C–	SM	+	+	+	+	+	n	–	+	–	n	–
								Y	RF	C–	SM	+	+	+	+	+	+	–	+	–	–	n
Group II	Yes	Yes	Yes	Yes	Yes	Yes	+	W	RF	C–	SM	+	+	+	+	+	+	–	+	–	+	–
								Y	RF	C+	SM	+	+	–	–	+	+	–	+	+	+	n
Group II	Yes	Yes	Yes	Yes	Yes	Yes		Y	RF	C–	SM	+	+	+	+	+	+	–	+	–	–	–
Group II	No	No	Yes	Yes	Yes	No		Y	RF	C–	SM	+	+	+	–	+	+	–	+	–	+	n
								Y	RF	C–	SM	+	+	–	+	+	n	–	+	–	n	–
							Group A1B	Y	RF	C–	SM	+	+	+	+	+	n	–	+	–	n	–
Group II	No	No	Yes	Yes	Yes	No		Y	RF	C–	SM	+	+	+	–	+	n	–	+	–	n	–

(continued)

TABLE 272. List of *Streptomyces* species continued

Species names and groups[1]	No. in lists of species[2]	Type strain	Accession no.	Wil 83a[3]	Wil 89[4]	Käm 91[5]	Hat 03[6]	Lan 02[7]	Ful 95[8]	Och 95[9]	Kat 97[10]	Lab[11]	Sch[12]	Lan 04[13]	Lan 02[14]	Lan 04[15]	Lan 04[16]	Lan 04[17]	Lan 04[18]	Lan 04[19]	Lan 04[20]
S. californicus	82	DSM 40058, ATCC 3312/ ATCC 19734, LMG 19309, ISP 5058, NBRC 3386	AB184755	A 09	II 02	1-3 030		La-22	FU-6	OC-I			Sch32	Lan2-00	BENP +	+		+	cl02		cl23
S. floridae	187	DSM 40938, NCIB 9345, LMG 19899, NBRC 15405	AB184656		IV 04 (yellow series)	1-3 1-2							Sch32	Lan2-00	BENP	+		+		+	cl23
S. alboviridis	26	DSM 40326, ATCC 25425, LMG 20403, ISP 5326, NBRC 13013	AB184256	A 1B	I 02	1-3 1-2					KA-B		Sch02	Lan2-00	BENP	+		+		+	cl23
S. microflavus	319	DSM 40331, ATCC 13231, LMG 19327, ISP 5331, NRRL B-2156	DQ445795	A 23	I 20	1-3 1-2		La-22		OC-I			Sch02	Lan2-12	BENP +	+	(a)	+	cl02	+	cl23
S. fulvorobeus	192	DSM 41455, LMG 19901, NBRC 15897	AB184711										Sch00	Lan2-00	BENP	+		+		+	cl23
S. lipmanii	289	DSM 40070, ATCC 3331, LMG 20047, ISP 5070, NBRC 12791	AB184148	A 1B	I 02	1-3 1-2			FU-9		KA-B		Sch02	Lan2-12	BENP	+	(a)	+			cl23
Group S. avidini et rel.:																					
S. spororaveus	457	DSM 41462, LMG 20313	AJ781370										Sch00	Lan2-00	BENP	+		+		+	cl22
S. xanthophaeus	524	DSM 40134, ATCC 19819, LMG 21039, ISP 5134, NRRL B-5414	DQ442560	F 61	I 22	084 067						L5	Sch00	Lan2-00	BENP	+		+		+	cl22
S. nojiriensis	345	DSM 41655, LMG 20094	AJ781355										Sch00	Lan2-00	BENP	+		+		+	cl22
S. cirratus	118	DSM 40479, ATCC 14699, LMG 20473, ISP 5479, NRRL B-3250	AY999794	F 62	II 14	22-3 042							Sch00	Lan2-00	BENP	+		+			cl22
S. vinaceus	496	DSM 40515, ATCC 27476, LMG 20533, ISP 5515, NBRC 13425	AB184394	A 06	I 05	22-3 042					KA-A		Sch00	Lan2-23	BENP	+	(b)	+			cl08
S. colombiensis	132	DSM 40558, ATCC 27425, LMG 20487, ISP 5558, NRRL B-1990	DQ026646	F 61	I 22	22-3 042			FU-12b			L5	Sch00	Lan2-13	BENP	+	(b) (l)	+		+	-
S. lavendulae subsp. *grasserius*	280b	DSM 40385, LMG 19938	AY999841											Lan2-00	BENP	+		+			-
S. goshikiensis	213	DSM 40190, ATCC 23914, LMG 19884, ISP 5190, NRRL B-5428	EF178693	F 61	I 22	22-3 042						L3/L	Sch00		BENP	+		+			cl22
S. sporoverrucosus	458	DSM 41463, LMG 20314, NRRL B-16379	DQ442544										Sch00		BENP	+		+		+	cl22
S. avidinii	61	DSM 40526, ATCC 27419, LMG 20428, ISP 5526, NBRC 13429	AB184395	F 56		023 004							Sch00	Lan2-00	BENP	+		+			cl22
S. subrutilus	461	DSM 40445, ATCC 27467, LMG 20294, ISP 5445	X80825	F 61		22-3 042						L5	Sch00	Lan2-00	BENP	+		+			cl22

(continued)

TABLE 272. (continued)

								Morphological characters[24]				Physiological tests[25]										
Guo 08[21] 16S rRNA	Guo 08[21] *atpD*	Guo 08[21] *gyrB*	Guo 08[21] *recA*	Guo 08[21] *rpoB*	Guo 08[21] *trpB*	Guo 08[21] concat.	Kim 04[23] *rpoB*	I	II	III	IV	I	II	III	IV	V	VI	VII	VIII	IX	X	XI
+	+	+	+	+	+	+		Y	RF	C–	SM	+	+	–	–	+	+	–	+	–	–	–
+	+	+	+	+	+	+		Y	RF	C–	SM	+	+	–	–	+	+	–	+	–	–	n
Group III	Yes	Yes	Yes	Yes	Yes	Yes	Group A1B	Y	RF	C–	SM	+	+	–	+	+	n	–	+	–	n	–
Group III	Yes	Yes	Yes	Yes	Yes	Yes		Y	RF	C–	SM	+	+	–	+	+	+	–	+	–	+	–
Group III	No	No	No	No	No	No		R–Y	S	C–	SM	+	–	+	–	n	n	n	–	–	n	n
								Y	RF	C–	SM	+	+	–	+	+	+	+	+	–	+	±
+	+	+	+	+	+	+		Gy	S/RA	C+	WTY/ SM	+	–	–	–	–	–	–	n	n	n	n
							+	R	RF	C+	SM	+	–	–	–	–	n	–	–	–	n	–
+	+	+	+	+	+	+		Gy	S	C+	SM	+	–	–	–	–	–	–	–	–	+	–
+	+	+	+	+	+	+	Group F	Gy	S	C+	SM	+	+	+	–	+	+	–	–	–	–	+
								R	S	C+	SM	+	–	–	–	n	n	n	n	n	n	n
								R	S	C+	SM	+	–	–	–	n	n	n	n	n	n	n
								R	S	C+	SM	+	–	–	–	–	+	–	–	–	+	–
								R	S	C+	SM	+	–	–	–	+	n	–	–	–	n	–
								Gy/Y	S	C+	WTY											
							Asn(AAC)[442]	Gy	S	C+		n	–	–	n	n	n	–	n	n	n	n
+	+	+	+	+	+	+		R	RF	C+	SM	+	–	–	–	+	+	–	–	–	–	+

(continued)

TABLE 272. List of *Streptomyces* species continued

Species names and groups[1]	No. in lists of species[2]	Type strain	Accession no.	Wil 83a[3]	Wil 89[4]	Käm 91[5]	Hat 03[6]	Lan 02[7]	Ful 95[8]	Och 95[9]	Kat 97[10]	Lab[11]	Sch[12]	Lan 04[13]	Lan 02[14]	Lan 04[15]	Lan 04[16]	Lan 04[17]	Lan 04[18]	Lan 04[19]	Lan 04[20]
Group S. cinnamonensis et rel.:																					
S. globosus	209	DSM 40815, ATCC 14979, LMG 19896	AJ781330		IV 19 (gray series)	22-3 042							Sch00	Lan2-00	BENP	+		+		+	cl31
S. toxytricini	484	DSM 40178, ATCC 19813, LMG 20269, ISP 5178, NRRL B-5426	DQ442548	F 61		22-3 042						L3/L	Sch00	Lan2-00	BENP	+		+		+	cl31
S. flavotricini	182	DSM 40152, ATCC 23621, LMG 19880, ISP 5152, NBRC 12770	AB184132	F 61	I 22	22-3 042			FU-1			L3/L	Sch00	Lan2-00	BENP	+		+			cl22
S. polychromogenes	379	DSM 40316, ATCC 12595, LMG 20287, ISP 5316, NBRC 13072	AB184292	F 61	I 22	22-3 042						L3/L	Sch00	Lan2-00	BENP	+		+		+	cl22
S. racemochromogenes	397	DSM 40194, ATCC 23954, LMG 20273, ISP 5194, NRRL B-5430	DQ026656	F 61	I 22	22-3 042						L5	Sch00	Lan2-00	BENP	+		+			cl22
S. katrae	267	DSM 40550, ATCC 27440, LMG 19945, ISP 5550, NBRC 13447	AB184409	F 61	I 22	22-3 042						L5	Sch26	Lan2-00	BENP	+		+		+	cl22
S. cinnamonensis	116	DSM 40803, ATCC 12308, LMG 20468, NBRC 15873	AB184707		IV 02 (red series)	22-3 042							Sch00	Lan2-00	BENP	+		+			cl22
S. virginiae	510	DSM 40094, ATCC 19817, LMG 20534, ISP 5094, IFO 3729	D85119	F 61	I 22	22-3 042			FU-12b			L3/L	Sch00	Lan2-00	BENP	+		+			cl22
Group S. albolongus et rel.:																					
S. cavourensis subsp. *cavourensis*	96a	DSM 40300, ATCC 14889, LMG 20450, ISP 5300, NRRL 2740	DQ445791	A 1B	I 02	1-3 1-2			FU-6		KA-A		Sch00	Lan2-00	BENP	+		+		+	cl22
S. celluloflavus	98	DSM 40839, ATCC 29806, LMG 21796, NBRC 13780	AB184476		IV 01 (yellow series)	020 032							Sch00	Lan2-00	BENP	+		+		+	cl42
S. albolongus	20	DSM 40570, ATCC 27414, LMG 20396, ISP 5570, NBRC 13465	AB184425	F 63	II 15	22-4 043						L4	Sch00	Lan2-00	BENP	+		+			+
S. griseobrunneus	220	DSM 40066, ATCC 19762, LMG 19877, ISP 5066, NBRC 12775	AB249912	A 1B	I 02	1-3 1-2			FU-6		KA-A		Sch00	Lan2-00	BENP	+		+			cl50
Group S. crystallinus et rel.:																					
S. melanogenes	315	DSM 40192, ATCC 23937, LMG 20056, ISP 5192, NBRC 12890	AB184222	A 33	II 07	009 1-09							Sch00	Lan2-00	BENP	+		+		+	cl13
S. noboritoensis	342	DSM 40223, ATCC 25477, LMG 19337, ISP 5223, NBRC 13065	AB184287	A 33	II 07	009 1-09		La-19		OC-I			Sch00	Lan2-00	BENP +	+		+	cl06		cl34

(continued)

TABLE 272. (continued)

Guo 08[21] 16S rRNA	Guo 08[21] *atpD*	Guo 08[21] *gyrB*	Guo 08[21] *recA*	Guo 08[21] *rpoB*	Guo 08[21] *trpB*	Guo 08[21] concat.	Kim 04[23] *rpoB*	Morphological characters[24] I	II	III	IV	Physiological tests[25] I	II	III	IV	V	VI	VII	VIII	IX	X	XI
								Gy	RF	C+	SM	+	+	+	−	n	n	n	n	n	n	n
								R	S	C+	SM	+	−	−	−	±	n	−	−	−	n	−
								R	RF	C+	SM	+	−	−	−	±	n	−	−	−	n	−
								B	RF	C+	SM	+	+	+	−	+	+	−	−	−	+	n
								R	S	C+	SM	+	−	+	−	−	n	−	−	−	n	+
								R	S	C+	SM	+	−	−	−	+	+	+	−	−	−	n
								R	S	C+	SM	+	−	−	−	+	n	−	−	−	+	n
+	+	+	+	+	+	+	Group F	R	S	C+	SM	+	−	−	−	+	n	−	−	+	+	n
								Y/R	RF	C+	SM	+	+	−	−	+	n	−	+	−	n	n
								Y	RF	C−	SM	+	−	−	−	n	n	n	n	n	n	n
							+	W	RF	C−	SM	+	+	+	−	+	+	−	+	+	+	−
+	+	+	+	+	+	+		Y	RF	C+	SM	+	+	−	−	+	+	+	+	−	+	+
								R	RF	C+	SM	+	+	+	−	+	n	+	+	+	n	±
								Gy	RF	C+	SM	+	+	+	−	+	n	+	+	+	+	±

(continued)

TABLE 272. List of *Streptomyces* species continued

Species names and groups[1]	No. in lists of species[2]	Type strain	Accession no.	Wil 83a[3]	Wil 89[4]	Käm 91[5]	Hat 03[6]	Lan 02[7]	Ful 95[8]	Och 95[9]	Kat 97[10]	Lab[11]	Sch[12]	Lan 04[13]	Lan 02[14]	Lan 04[15]	Lan 04[16]	Lan 04[17]	Lan 04[18]	Lan 04[19]	Lan 04[20]
S. crystallinus	136	DSM 40945, LMG 20490, NBRC 15401	AB184652		IV 03 (red series)	009 1-09							Sch00	Lan2-00	BENP	+		+		+	cl34
Group S. mauvecolor et rel.:																					
S. michiganensis	318	DSM 40015, ATCC 14970, LMG 20042, ISP 5015, NBRC 12797	AB184153	A 06	I 05	005 029							Sch00	Lan2-00	BENP	+		+			cl14
S. xanthochromogenes	521	DSM 40111, ATCC 19818, LMG 19366, ISP 5111, NRRL B-5410	DQ442559	F 63	II 15	005 029		La-23		OC-I			Sch00	Lan2-00	BENP +	+		+	cl02	+	+
S. mauvecolor	312	DSM 41702, LMG 20100, NBRC 13854	AB184532										Sch00	Lan2-00	BENP	+		+		+	cl34
Not closely related to one of the groups:																					
S. cremeus	135	DSM 40147, ATCC 19897, LMG 20489, ISP 5147, NBRC 12760	AB184124	A 1B	I 02	002 1-7			FU-21				Sch00	Lan2-00	BENP	+		+			cl22
S. spiroverticillatus	453	DSM 40036, ATCC 19811, LMG 20254, ISP 5036, NBRC 3931	AB184814	A 06	I 05	002 1-7					KA- A		Sch00	Lan2-00	BENP	+		+		+	cl22
S. candidus	85	DSM 40141, ATCC 19891, ISP 5141	DQ026663	A 03		002 1-7							Sch00							+	
Group S. exfoliatus et rel.:																					
S. lateritius	277	DSM 40163, ATCC 19913, LMG 19372, ISP 5163	AJ781326	H Sm	III 23	22-3 1-08		La-00		OC-II			Sch00	Lan2-00	BENP +	+		+	cl07	+	cl22
S. venezuelae	494	DSM 40230, ATCC 10712, LMG 19308, ISP 5230, JCM 4526	AB045890	A 06	I 05	002 1-7		La-00			KA- C		Sch00	Lan2-00	BENP +	+		+	cl07		cl22
S. omiyaensis	358	DSM 40552, ATCC 27454, LMG 20075, ISP 5552, NRRL B-1587	EF178697	A 05	I 04	002 1-7					KA- C		Sch00	Lan2-00	BENP	+		+		+	cl23
S. wedmorensis	518	DSM 41676, ATCC 21239, LMG 21050, NRRL 3426	DQ442557										Sch00	Lan2-00	BENP	+		+			cl23
S. litmocidini	290	DSM 40164, ATCC 19914, LMG 20052, ISP 5164, NBRC 12792	AB184149	A 05	I 04	002 1-7					KA- C		Sch00		BENP	+		+			cl22
S. yerevanensis	529	DSM 43167, LMG 21053, NRRL B-16943	EF178684		III 18	080 066				OC-1					BENP	+		+			+
S. zaomyceticus	533	DSM 40196, ATCC 27482, LMG 19853, ISP 5196, NRRL B-2038	EF178685	A 05	I 04	002 1-7					KA- C		Sch00	Lan2-00	BENP	+		+			cl23
S. exfoliatus	164	DSM 40060, ATCC 12627, LMG 19307, ISP 5060, NBRC 13191	AB184324	A 05	I 04	002 1-7		La-00		OC-II	KA- C		Sch00	Lan2-00	BENP +	+		+	cl01	+	cl23

(continued)

TABLE 272. (continued)

Guo 08[21] 16S rRNA	Guo 08[21] *atpD*	Guo 08[21] *gyrB*	Guo 08[21] *recA*	Guo 08[21] *rpoB*	Guo 08[21] *trpB*	Guo 08[21] concat.	Kim 04[23] *rpoB*	Morphological characters[24] I	II	III	IV	Physiological tests[25] I	II	III	IV	V	VI	VII	VIII	IX	X	XI
								R	RF	C+	SM	+	n	n	n	n	n	n	n	n	n	–
								Y	RF	C+	SM	+	+	–	–	+	n	±	+	+	n	–
								Y	RF	C+	SM	+	+	±	±	+	n	±	+	±	n	±
+	+	+	+	+	+	+		Vi	S	C+	SPY	+	–	+	–	–	+	+	–	–	+	–
+	+	+	+	+	+	+		R	S	C–	SM	+	+	+	–	+	+	–	–	–	–	–
+	+	+	+	+	+	+		W	S	C–	SM	+	+	+	–	+	n	–	–	–	n	n
								W	RF	C–	SM	+	+	+	+	n	n	–	+	–	n	–
								R	S	C+	WTY	+	+	+	+	+	n	–	–	±	n	–
+	+	+	+	+	+	+		R	RF	C+	SM	+	–	–	–	–	n	–	–	–	n	–
								Gy	RF	C–	SM	+	+	–	+	–	+	–	–	–	–	n
								Gy	RF	C–	SM	+	+	+	+	+			+			
								Gy	RF	C+	SM	+	±	+	–	±	n	–	–	–	n	–
								Gy		C–	SM	+	+	+	+	+	–	+	+	+	+	+
								Gy	RF	C+	SM	+	+	+	–	–	+	–	–	–	+	+
+	+	+	+	+	+	+		R	RF	C–	SM	+	+	+	+	+	+	+	–	–	+	+

(continued)

TABLE 272. List of *Streptomyces* species continued

Species names and groups[1]	No. in lists of species[2]	Type strain	Accession no.	Wil 83a[3]	Wil 89[4]	Käm 91[5]	Hat 03[6]	Lan 02[7]	Ful 95[8]	Och 95[9]	Kat 97[10]	Lab[11]	Sch[12]	Lan 04[13]	Lan 02[14]	Lan 04[15]	Lan 04[16]	Lan 04[17]	Lan 04[18]	Lan 04[19]	Lan 04[20]
S. narbonensis	331	DSM 40016, ATCC 19790, LMG 20043, ISP 5016, NRRL B-1680	DQ445794	A 04	I 04	002 1-7					KA-C		Sch00	Lan2-00	BENP	+		+			cl44
Most closely related to group S. exfoliatus et rel.:																					
S. albidochromogenes	13	DSM 41800, NBRC 101003	AB249953																		
S. flavidovirens	176	DSM 40150, ATCC 19900, LMG 19387, ISP 5150, NBRC 13039	AB184270		IV 03 (yellow series)	026 033		La-22					Sch00	Lan2-00	BENP +	+		+		+	+
S. enissocaesilis	157	DSM 41454, LMG 20506, NBRC 100763	AB249930										Sch00	Lan2-06	BENP	+		+		+	cl58
S. albosporeus subsp. *labilomyceticus*	23b	DSM 41672, LMG 20400, NBRC 15387	AB184638										Sch00	Lan2-00	BENP	+		+		+	cl23
S. chryseus	108	DSM 40420, ATCC 19829, LMG 20458, ISP 5420, NRRL B-12347	AY999787	A 17	I 10	22-3 1-08						L3	Sch28	Lan2-00	BENP	+		+			cl23
S. helvaticus	246	DSM 40431, ATCC 19841, LMG 19940, ISP 5431, NBRC 13382	AB184367	F 62	II 14	22-3 043							Sch28	Lan2-00	BENP	+		+		+	cl23
Not closely related to one of the groups:																					
S. beijiangensis	69	DSM 41794, NBRC 100044, YIM6	AF385681																	+	
S. drozdowiczii	148	NRRL B-24297	EF654097																	+	
S. yanii	526	AS 4.1146, JCM 3331, IFO 14669	AB006159																		
Group S. graminofaciens et rel.:																					
S. peucetius	366	DSM 40754, NCIB 10972, LMG 20084, JCM 9920	AB045887		IV 09 (red series)	035 1-33							Sch00	Lan2-00	BENP	+		+			cl21
S. xantholiticus	523	DSM 40244, ATCC 27481, LMG 19402, ISP 5244, NBRC 13354	AB184349	C 24	II 05	062 024		La-21					Sch00	Lan2-00	BENP +	+		+	cl04	+	cl21
S. kurssanovii	272	DSM 40162, ATCC 15824, LMG 19933, ISP 5162, NBRC 13192	AB184325	F 60	IV 20 (gray series)	025 1-15							Sch00	Lan2-00	BENP	+		+		+	cl21
S. graminofaciens	216	DSM 40559, ATCC 12705, LMG 19892, ISP 5559	AJ781329	A 26	III 03	004 1-23				OC-I			Sch00	Lan2-00	BENP	+		+		+	+
Group S. amakusaensis et rel.:																					
S. amakusaensis	30	DSM 40219, ATCC 23876, LMG 19350, ISP 5219, NRRL B-3351	AY999781	B Sm	III 12	079 063		La-00		OC-I		L2	Sch00	Lan2-00	BENP +	+		+	cl14	+	+
S. inusitatus	259	DSM 41441, LMG 19955, NBRC 13601	AB184445										Sch00	Lan2-00	BENP	+		+		+	cl13

(continued)

TABLE 272. (continued)

Guo 08[21] 16S rRNA	Guo 08[21] *atpD*	Guo 08[21] *gyrB*	Guo 08[21] *recA*	Guo 08[21] *rpoB*	Guo 08[21] *trpB*	Guo 08[21] concat.	Kim 04[23] *rpoB*	Morphological characters[24] I	II	III	IV	Physiological tests[25] I	II	III	IV	V	VI	VII	VIII	IX	X	XI
								Gy	RF	C+	SM	+	+	+	+	+	n	+	−	−	n	+
								W	S	C+	SM	+	+	+	−	+	n	−	+	−	n	n
								Y/W	RF/ RA/S	C+	SM	+	+	+	+/−	+/−	n	−	+/−	+/−	n	+/−
								n	S	C−	SM	+	+	+	−	n	n	−	+	n	n	n
								W	RF	C−	SM	+	+	−	−	−	+	+	n	−	+	+
								Y	S	C−	SM	+	n	+	−	n	+	−	−	−	n	−
								Y	S	C−	SM	+	n	+	−	n	+	−	−	−	n	−
								n	RF–RA	C−	n	+	+	−	−	−	+	n	−	n	n	−
+	+	+	+	+	+	+		Gy Gy	S RF	C+ C−	SM	+	+	n	n	+	n	+	+	n	n	n
+	+	+	+	+	+	+		R	S	C−	SM	+	+	−	−	+	n	+	+	n	n	+
								W	S	C−	SM	+	−	−	−	n	+	−	n	n	n	−
								Gy	S	C+	SM	+	+	+	−	+	+	+	−	−	−	+
+	+	+	+	+	+	+		Gy	S	C−	WTY	+	+	+	+	n	n	n	n	n	n	n
								B	S	C+	SM	+	−	±	−	−	−	−	−	−	−	±
								B/Gy	S	C−	SM	+					+					

(continued)

TABLE 272. List of *Streptomyces* species continued

Species names and groups[1]	No. in lists of species[2]	Type strain	Accession no.	Wil 83a[3]	Wil 89[4]	Käm 91[5]	Hat 03[6]	Lan 02[7]	Ful 95[8]	Och 95[9]	Kat 97[10]	Lab[11]	Sch[12]	Lan 04[13]	Lan 02[14]	Lan 04[15]	Lan 04[16]	Lan 04[17]	Lan 04[18]	Lan 04[19]	Lan 04[20]
S. clavuligerus	122	DSM 40751, ATCC 27064, LMG 20477, DSM 738, NRRL 3585	AY999718		IV 10 (gray series)	22-5 036							Sch00	Lan2-00	BENP	+		+		+	+
Group S. atratus et rel.:																					
S. atratus	45	DSM 41673, LMG 20420, NRRL B-16927	DQ026638										Sch00	Lan2-00	BENP	+		+		+	cl23
S. sanglieri	430	DSM 41791, NBRC 100784	AB249945																	+	
S. gelaticus	199	DSM 40065, ATCC 3323, LMG 19376, ISP 5065, NRRL B-2928	DQ026636	A Sm	III 11	003 1-3		La-00					Sch00	Lan2-00	BENP +	+		+		+	+
S. pulveraceus	390	DSM 41657, LMG 20322, NBRC 3855	AB184806										Sch00	Lan2-00	BENP	+		+			cl23
Not closely related to one of the groups:																					
S. sannanensis	431	DSM 41705, LMG 20329, NBRC 14239	AB184579										Sch07	Lan2-04	BENP	+		+			cl22
Most closely related to group S. laurentii et rel.:																					
S. showdoensis	440	DSM 40504, ATCC 15105, LMG 20298, ISP 5504, NBRC 13417	AB184389	A 06	I 05	22-2 037							Sch00	Lan2-00	BENP	+		+		+	cl23
S. viridobrunneus	513	DSM 41466, LMG 20317	AJ781372										Sch00	Lan2-00	BENP	+		+		+	cl22
S. roseoviridis	421	DSM 40175, ATCC 23959, LMG 20266, ISP 5175, NBRC 12911	AB184239	A 05	I 04	22-2 037							Sch00	Lan2-00	BENP	+		+		+	cl44
S. vietnamensis	495	CCTCC M 205143, JCM 21785, GIMV4.0001	DQ311081																		
S. nashvillensis	332	DSM 40314, ATCC 25476, LMG 20064, ISP 5314, NBRC 13064	AB184286	A 05	I 04	002 1-7							Sch00	Lan2-00	BENP	+		+		+	cl23
S. tanashiensis	466	DSM 40195, ATCC 23967, LMG 20274, ISP 5195	AJ781362		IV 30 (gray series)	002 1-7							Sch00	Lan2-00	BENP	+		+			cl22
S. roseolus	417	DSM 40174, ATCC 23210, LMG 20265, ISP 5174, NBRC 12816	AB184168	A 05	I 04	002 1-7							Sch00	Lan2-00	BENP	+		+		+	cl22
S. bikiniensis	71	DSM 40581, ATCC 11062, LMG 19367, ISP 5581	X79851	F 64	III 21	22-4 1-07		La-00		OC-II			Sch00	Lan2-00	BENP +	+		+	cl01		cl23
S. violaceorectus	500	DSM 40279, ATCC 25514, LMG 20281, ISP 5279, NBRC 13102	AB184314	A 05	I 04	002 1-7							Sch00		BENP	+		+		+	cl23
S. cinereoruber subsp. *cinereoruber*	111a	DSM 40012, ATCC 19740, LMG 20462, ISP 5012, NBRC 12756	AB184121	A 05	I 04	002 038			FU-6				Sch00		BENP	+		+		+	cl23

(continued)

TABLE 272. (continued)

Guo 08[21] 16S rRNA	Guo 08[21] *atpD*	Guo 08[21] *gyrB*	Guo 08[21] *recA*	Guo 08[21] *rpoB*	Guo 08[21] *trpB*	Guo 08[21] concat.	Kim 04[23] *rpoB*	Morphological characters[24] I	II	III	IV	Physiological tests[25] I	II	III	IV	V	VI	VII	VIII	IX	X	XI
								Gy	RF		SM											
								Gy	S	C–	SM	+	+	–	+	+	+	+	–	–	+	n
								Gy	S	C+	SM	n	+	+	+	+	+	+	n	n	n	+
								Gy	RF	C–	SM	+	+	–	+	–	+	+	–	–	+	+
+	+	+	+	+	+	+		Gy	S	C+	SM	n	+	–	+	+	+	+	–	–	+	–
								Gy	S	C–	SM	±	±	–	–	–	–	–	–	–	–	–
								Gy	RF	C+	SM	+	+	±	–	+	+	–	–	–	+	±
								Gy	RF	C+	SM	+		–	–			+	–	–		
								R	RF	C+	SM	+	+	+	–	–	+	–	–	–	–	n
								W	RF	C+	n	+	+	+	+	+	+	n	n	n	n	+
								Gy	RF	C+	SM	+	+	+	–	–	+	–	–	–	+	±
+	+	+	+	+	+	+		Gy	RF	C+	SM	+	+	+	–	–	+	–	–	–	+	–
								R	RF	C–	SM	+	+	+	+	±	n	–	–	–	n	–
							+	Gy	RF	C+	SM	+	+	–	–	–	+	–	–	–	±	±
								Gy	RF	C+	SM	+	+	+	–	+	n	–	–	–	n	+
								Gy	RF	C+	SM	+	+	+	–	–	+	–	–	–	+	–

(continued)

TABLE 272. List of *Streptomyces* species continued

Species names and groups[1]	No. in lists of species[2]	Type strain	Accession no.	Wil 83a[3]	Wil 89[4]	Käm 91[5]	Hat 03[6]	Lan 02[7]	Ful 95[8]	Och 95[9]	Kat 97[10]	Lab[11]	Sch[12]	Lan 04[13]	Lan 02[14]	Lan 04[15]	Lan 04[16]	Lan 04[17]	Lan 04[18]	Lan 04[19]	Lan 04[20]
Group S. laurentii et rel.:																					
S. laurentii	278	DSM 41684, LMG 19959	AJ781342										Sch00	Lan2-00	BENP	+		+		+	cl22
S. termitum	469	DSM 40329, ATCC 25499, LMG 20289, ISP 5329, NBRC 13087	AB184302	A 05	I 04	22-2 037							Sch00	Lan2-00	BENP	+		+			cl22
S. roseofulvus	415	DSM 40172, ATCC 19921, LMG 20263, ISP 5172, NBRC 13194	AB184327	A 14	II 04	002 1-7							Sch00	Lan2-00	BENP	+		+			cl22
Most closely related to group S. laurentii et rel.:																					
S. filamentosus	168	DSM 40022, ATCC 19753, LMG 20512, ISP 5022, NBRC 12767	AB184130	A 05	I 04	002 1-7							Sch00	Lan2-24	BENP	+	(b)	+		+	cl23
Group S. gobitricini et rel.:																					
S. gobitricini	212	DSM 41701, LMG 19910, NBRC 15419	AB184666										Sch00	Lan2-00	BENP	+		+			cl14
S. lavendofoliae	279	DSM 40217, ATCC 15872, LMG 19935, ISP 5217	AJ781336		IV 07 (red series)	22-3 1-08							Sch00	Lan2-00	BENP	+		+		+	cl14
S. luridus	298	DSM 40081, ATCC 19782, LMG 19365, ISP 5081, NRRL B-5409	DQ442523	F 62	II 14	22-3 1-08		La-17		OC-II			Sch00	Lan2-00	BENP +	+		+			-
S. roseolilacinus	416	DSM 40173, ATCC 19922, LMG 20264, ISP 5173, NBRC 12815	AB184167	G 68	II 18	22-5 039							Sch00	Lan2-00	BENP	+		+			cl12
Not closely related to one of the groups:																					
S. biverticillatus	72	DSM 40272, ATCC 23615, LMG 20433, ISP 5272	AJ781381		Sv. 01	22-1 040	Ha7					L4		Lan2-00	BENP	+	(j)	+		+	cl13
S. werraensis	519	DSM 40486, ATCC 14424, LMG 21047, ISP 5486, NRRL B-5317	DQ442558	A 12	I 07	006 1-18					KA-G		Sch00	Lan2-00	BENP	+		+		+	cl04
S. globisporus subsp. *caucasicus*	208b	DSM 40814, ATCC 19907, LMG 19895, NRRL B-2593	EF178676		I 02	1-1 1-1							Sch10		BENP	+		+		+	cl08
S. albireticuli	16	DSM 40051, ATCC 19721, LMG 20393, ISP 5051, NBRC 12737	AB184881	F SM	Sv. 11	076 069	Ha5						Sch00	Lan2-00	BENP	+	(j)	+			cl13
S. eurocidicus	161	DSM 40604, ATCC 27428, LMG 20509, ISP 5604, NRRL B-1676	AY999790	F 56	Sv. 02	22-1 040	Ha5					L4	Sch00	Lan2-00	BENP	+	(j)	+			cl13
S. stramineus	460	DSM 41783, NBRC 16131	AB184720				Ha16														

(continued)

TABLE 272. (continued)

								Morphological characters[24]				Physiological tests[25]										
Guo 08[21] 16S rRNA	Guo 08[21] *atpD*	Guo 08[21] *gyrB*	Guo 08[21] *recA*	Guo 08[21] *rpoB*	Guo 08[21] *trpB*	Guo 08[21] concat.	Kim 04[23] *rpoB*	I	II	III	IV	I	II	III	IV	V	VI	VII	VIII	IX	X	XI
									RF	C–	SM	+	+	–	–	–	+	–	–	–	n	+
								R	RF	C–	SM	+	+	–	n	–	n	–	–	–	n	n
								R	RF	C–	SM	+	+	+	+	+	+	+	–	–	+	+
								R	RF	C–	SM	+	+	+	–	–	+	–	–	–	–	+
								R	RF	C+	SM	+	+	+	+	+	+	–	–	+	–	n
								R	S	C+	SM	+	+	+	–	–	+	–	–	+	–	–
								R	S	C+	SM	+	+	+	–	±	n	–	–	+	n	–
								R	S	C–	SM	+	–	+	–	±	n	–	–	–	n	–
								Bi	VE	C+	SM	+	±	–	±	±	n	±	–	±	n	±
								Gy	S	C–	n	n	n	n	n	n	n	n	n	n	n	n
								Y	RF	C–	SM	+	+	+	–	n	n	n	n	n	n	n
							Group F	Ar	VE	C+	SM	+	±	±	±	±	n	±	±	+	n	+
								Ar	VE	C+	SM	+	–	–	–	±	n	–	–	±	n	+
								Y	VER	C+	SM	+	–	n	–	+	–	–	+	+	–	n

(continued)

TABLE 272. List of *Streptomyces* species continued

Species names and groups[1]	No. in lists of species[2]	Type strain	Accession no.	Wil 83a[3]	Wil 89[4]	Käm 91[5]	Hat 03[6]	Lan 02[7]	Ful 95[8]	Och 95[9]	Kat 97[10]	Lab[11]	Sch[12]	Lan 04[13]	Lan 02[14]	Lan 04[15]	Lan 04[16]	Lan 04[17]	Lan 04[18]	Lan 04[19]	Lan 04[20]
S. olivoverticillatus	356	DSM 40250, NRRL B-1994, LMG 20058, NBRC 15273	AB184636				Ha18					L4	Sch00	Lan2-00	BENP	+	(j)	+		+	cl13
S. netropsis	333	DSM 40259, ATCC 23940, LMG 5979, ISP 5259, NBRC 12893	AB184848	F 56	Sv. 01	22-1 040	Ha14		FU-21			L4	Sch00	Lan2-00	BENP	+	(j)	+		+	cl13
Group Kitasatospora–Streptacidiphilus–Streptomyces																					
Subgroup Kitasatospora–Streptomyces:																					
K. gansuensis	8*	DSM 44786, NBRC 101835, HKI 0314	AY442265																		
S. atroaurantiacus	46	DSM 41649, LMG 20421, NRRL B-24282	DQ026645										Sch00	Lan2-00	BENP	+		+		+	cl30
K. mediocidica	11*	DSM 43929, IFO 14789, IFO 14789	U93324																		
S. purpeofuscus	393	DSM 40283, ATCC 23952, LMG 20283, ISP 5283	AJ781364		IV 26 (gray series)	22-3 043							Sch00	Lan2-00	BENP	+		+			cl30
S. chrysomallus subsp. *fumigatus*	109b	DSM 41424, LMG 21793, NBRC 15394	AB184645										Sch00	Lan2-00	BENP	+		+		+	+
S. purpureus	395	DSM 43362, LMG 19368	AJ781324		I 23	22-3 1-05		La-18		OC-I			Sch00	Lan2-00						+	
S. xanthocidicus	522	DSM 40575, ATCC 27480, LMG 19370, ISP 5575, IFO 13469	AY999858	F 66	II 16	22-4 043		La-18					Sch00	Lan2-00	BENP +	+		+	cl03	+	cl29
S. aburaviensis	3	DSM 40033, ATCC 23869, LMG 19305, ISP 5033, NRRL B-2218	AY999779	A 02	II 01	22-3 043		La-00		OC-I			Sch00	Lan2-00	BENP +	+		+	cl03	+	+
S. herbaricolor	247	DSM 40123, ATCC 23922, LMG 19929, ISP 5123, NBRC 3838	AB184801	A 02	II 01	22-4 043							Sch00	Lan2-00	BENP	+		+		+	cl30
S. indigoferus	256	DSM 40124, LMG 19930, ISP 5124, NBRC 12878	AB184214										Sch00	Lan2-00	BENP	+		+			cl30
S. avellaneus	58	DSM 40554, ATCC 23730, LMG 20427, ISP 5554, NBRC 13451	AB184413		II 17	002 1-7							Sch00	Lan2-28	BENP	+		+			cl29
S. psammoticus	386	DSM 40341, ATCC 25488, LMG 20525, ISP 5341, IFO 13971	AY999862	F 67	II 17	011 1-21				OC-I			Sch09	Lan2-28	BENP	+		+		+	cl29
S. aureofaciens	53	DSM 40127, ATCC 10762, LMG 5968, ISP 5127, KACC 20180	AY207608	A 14	II 04	22-4 043				OC-I			Sch00	Lan2-28	BENP	+		+		+	cl29
K. sampliensis	18*	DSM 44898, NBRC 102069, VT-36	AY260167																		
K. putterlickiae	17*	DSM 44665, NBRC 100917, F18-98	AY189976																	+	

(continued)

TABLE 272. (continued)

								Morphological characters[24]				Physiological tests[25]										
Guo 08[21] 16S rRNA	Guo 08[21] *atpD*	Guo 08[21] *gyrB*	Guo 08[21] *recA*	Guo 08[21] *rpoB*	Guo 08[21] *trpB*	Guo 08[21] concat.	Kim 04[23] *rpoB*	I	II	III	IV	I	II	III	IV	V	VI	VII	VIII	IX	X	XI
								Ar	VE	C+	SM	+	−	±	−	−	n	±	−	±	n	+
							Group F	Ke	VE	C−	SM	+	±	−	±	−	n	−	−	+	n	−
								W	RF	C+	SM	+	+	+	−	+	n	−	−	−	n	+
								Gy	RF	C+	SM	+	+	+	−	−	+	−	−	−	−	n
								Gy	RF	C−	SM	+	+	+	−	−	+	−	−	−	−	n
							+	Gy/R	RF	C+	SM	+	−	+	−	+	n	−	−	+	+	−
								Gy	RF	C−	SM	+	+	+	−	+	+	−	−	−	−	+
								Gy	RF	C−	SM	+	±	−	−	±	−	−	−	−	−	−
								Gy	RF	C+	SM	+	+	+	−	+	+	+	−	−	−	+
								Gy	RF	C+	SM	+	+	+	−	−	+	−	−	−	−	n
								Gy	S	C−	SM	+	±	−	−	+	n	−	n	−	n	+
								Gy	S	C−	SM	+	−	−	−	+	n	−	−	−	−	+
								Gy	S	C−	SM	+	±	+	−	+	+	−	−	−	−	+

(continued)

TABLE 272. List of *Streptomyces* species continued

Species names and groups[1]	No. in lists of species[2]	Type strain	Accession no.	Wil 83a[3]	Wil 89[4]	Käm 91[5]	Hat 03[6]	Lan 02[7]	Ful 95[8]	Och 95[9]	Kat 97[10]	Lab[11]	Sch[12]	Lan 04[13]	Lan 02[14]	Lan 04[15]	Lan 04[16]	Lan 04[17]	Lan 04[18]	Lan 04[19]	Lan 04[20]
K. kifunensis	10*	DSM 41654, IFO 15206	AB022874																		
K. azatica	3*	DSM 41650, LMG 20429, IFO 13803	U93312											Lan2-00	BENP	+		+		+	cl30
K. nipponensis	13*	DSM 44787, NBRC 101836, HKI 0315	AY442263																		
K. cineracea	5*	NRRL B-24134, SK-3255	AB022875																		
K. niigatensis	12*	IFO 16453, SK-3406	AB022876																		
K. cheerisanensis	4*	KCTC 2395, YC75	AF050493																		
K. phosalacinea	16*	DSM 43860, NRRL B-16230, LMG 20102, KA-338	AB022869											Lan2-00	BENP	+		+			cl27
K. paracochleata	14*	DSM 41656, NBRC 14769	U93328												BENP	+		+			cl28
K. cochleata	6*	DSM 41652, NBRC 14768	U93316											Lan2-30	BENP	+	(b)	+		+	cl28
K. griseola	9*	DSM 43859, NRRL B-16229, AM-9660	AB022870																		
K. setae	1*	DSM 43861, NBRC 14216, LMG 20529, KM-6054	AB022868											Lan2-00	BENP	+		+			cl27
K. paranensis	15*	DSM 44788, NBRC 101837, HKI 0190	AY442268																		
K. cystarginea	7*	DSM 41680, IFO 14836, JCM 7356	U93318																		
K. terrestris	19*	DSM 44789, NBRC 101838, HKI 0186	AY442266																		
K. viridis	20*	DSM 44826, 52108a	AY613990																		
K. arboriphila	2*	DSM 44785, NBRC 101834, HKI 0189	AY442267																		
S. alboverticillatus	24	DSM 41678, DSM 41500, LMG 20401, JCM 5010	AY999766				Ha6						Sch00	Lan2-00	BENP	+	(j)	+		+	cl17
Group Kitasatospora–Streptacidiphilus–Streptomyces:																					
Streptacidiphilus oryzae	7†	CGMCC 4.2012, JCM 13271, TH49	DQ208700																		
Subgroup Streptacidiphilus albus et rel.:																					
Streptacidiphilus albus	1†	DSM 41753, JL 83	AF074415																	+	
Streptacidiphilus carbonis	3†	DSM 41754, JL 415	AF074412																		
Streptacidiphilus neutrinimicus	6†	DSM 41755, NBRC 100921, JL 206	AF074410																		
Subgroup Streptacidiphilus anmyonensis et rel.:																					
Streptacidiphilus jiangxiensis	4†	NBRC 100920, JCM 12277	AB249948																		
Streptacidiphilus anmyonensis	2†	NBRC 103185, AM-11	DQ904546																		
Streptacidiphilus melanogenes	5†	NBRC 103184, SB-B34	DQ994689																		
Streptacidiphilus rugosus	8†	NBRC 103186, AM-16	DQ904547																		

(continued)

TABLE 272. (continued)

Guo 08[21] 16S rRNA	Guo 08[21] *atpD*	Guo 08[21] *gyrB*	Guo 08[21] *recA*	Guo 08[21] *rpoB*	Guo 08[21] *trpB*	Guo 08[21] concat.	Kim 04[23] *rpoB*	Morphological characters[24] I	II	III	IV	Physiological tests[25] I	II	III	IV	V	VI	VII	VIII	IX	X	XI
							Kitasatospora															
							Kitasatospora															
							Kitasatospora															
								Gy	S	C+	SM	+	−	+	−	−	n	n	n	−	n	−
							Kitasatospora															
							Kitasatospora															
								W		C−								−	−			

(continued)

TABLE 272. List of *Streptomyces* species continued

Species names and groups[1]	No. in lists of species[2]	Type strain	Accession no.	Wil 83a[3]	Wil 89[4]	Käm 91[5]	Hat 03[6]	Lan 02[7]	Ful 95[8]	Och 95[9]	Kat 97[10]	Lab[11]	Sch[12]	Lan 04[13]	Lan 02[14]	Lan 04[15]	Lan 04[16]	Lan 04[17]	Lan 04[18]	Lan 04[19]	Lan 04[20]
Not closely related to one of the groups:																					
S. ardus	39	DSM 40527, ATCC 27417, LMG 20415, ISP 5527, NBRC 13430	AB184864		Sv. 03	22-1 040	Ha2					L4	Sch00	Lan2-00	BENP	+	(j)	+			cl17
S. blastmyceticus	73	DSM 40029, ATCC 19731, LMG 20434, ISP 5029, NRRL B-5480	AY999802	F 58	Sv. 02	22-1 040	Ha3					L4	Sch00	Lan2-00	BENP	+	(j)	+		+	cl17
S. caeruleus	81	DSM 40103, ATCC 27421, LMG 19399, ISP 5103, NRRL B-2194	EF178675		IV 07 (gray series)	058 050		La-19					Sch00	Lan2-14	BENP cl19	+	(c)	+	cl09	+	cl47
S. hiroshimensis	248	DSM 40037, ATCC 19772, LMG 19924, ISP 5037, NBRC 3720	AB184789	F 57	Sv. 01	22-1 040	Ha7		FU-NC			L4	Sch00	Lan2-00	BENP	+	(j)	+			cl12
S. cinnamoneus	117	DSM 40005, ATCC 11874, LMG 8602, ISP 5005, NBRC 12852	AB184850	F 55	Sv. 02	22-1 040	Ha4					L4	Sch00		BENP	+	(j)	+			cl17
S. pseudoechinosporeus	387	DSM 43035, LMG 21052, NBRC 12518	AB184100										Sch00	Lan2-00	BENP	+		+		+	+
S. lilacinus	286	DSM 40254, ATCC 23930, LMG 20059, ISP 5254, NBRC 3944	AB184819		Sv. 16	22-1 040	Ha8						Sch00	Lan2-00	BENP	+	(j)	+		+	cl12
S. sapporonensis	432	DSM 41675, LMG 20324, NBRC 13823	AB184508				Ha4						Sch17	Lan2-00	BENP	+	(j)	+		+	cl17
S. varsoviensis	492	DSM 40346, ATCC 25505, LMG 20083, ISP 5346, NRRL B-3589	DQ026653	C 46	III 13	037 028		La-12		OC-II			Sch00	Lan2-00	BENP cl12	+	(c)	+		+	cl13
S. abikoensis	2	DSM 40831, NRRL B-2113, LMG 20386, NBRC 13860	AB184537				Ha1					L4	Sch00	Lan2-00	BENP	+	(j)	+		+	cl12
S. lavenduligriseus	281	DSM 40487, ATCC 13306, LMG 19943, ISP 5487, NRRL B-3173	DQ442515	A 34	Sv. 02	1-5 009						L4	Sch00	Lan2-00	BENP	+		+		+	cl59
S. morookaense	345	DSM 40503, ATCC 19166, LMG 20074, ISP 5503	AJ781349	F 59	Sv. 08	22-1 040	Ha13						Sch00	Lan2-00	BENP	+	(j)	+			+
S. thioluteus	482	DSM 40027, ATCC 12310, LMG 21037, ISP 5027, NBRC 3364	AB184753	F Sm	Sv. 21	22-1 040	Ha17						Sch24	Lan2-00	BENP	+	(j)	+		+	cl12
S. luteireticuli	300	DSM 40509, ATCC 27446, ISP 5509, NBRC 13422	AB249969		Sv.	1-8 1-17	Ha9					L4									
S. ehimensis	154	DSM 40253, ATCC 23903, LMG 20505, ISP 5253, KCTC 9727	AY999834		Sv. 09	22-1 040	Hal						Sch00	Lan2-00	BENP	+	(j)	+		+	cl12
S. hygroscopicus subsp. *angustmyceticus*	253b	DSM 41683, LMG 19958, NRRL B-2347	DQ442509										Sch00	Lan2-00	BENP	+		+			cl23

(continued)

TABLE 272. (continued)

								Morphological characters[24]				Physiological tests[25]										
Guo 08[21] 16S rRNA	Guo 08[21] *atpD*	Guo 08[21] *gyrB*	Guo 08[21] *recA*	Guo 08[21] *rpoB*	Guo 08[21] *trpB*	Guo 08[21] concat.	Kim 04[23] *rpoB*	I	II	III	IV	I	II	III	IV	V	VI	VII	VIII	IX	X	XI
								Ar	VE	C+	SM	+	–	–	–	+	n	±	–	+	n	+
								Mo	VE	C+	SM	+	–	–	±	–	n	±	–	±	n	+
								Gy	RF	C–	SM	+	–	–	–	n	n	n	n	n	n	n
							Group F	Hi	VE	C+	SM	+	–	–	–	–	n	–	–	+	n	±
								Ci	VE	C–	SM	+	±	–	±	±	n	±	±	+	n	+
								W/Gy		C+	SM	+	+	+	+	+	+	+	+			+
							Group F	Li	VE	C+	SM	+	–	–	–	–	n	–	–	±	n	±
								R		C–								–	–			
								W	S	C–	SM	+	–	–	–	+	+	–	+	+	+	–
							Group F	Ar	VE	C+	SM	–	–	–	–	–	n	–	–	–	n	–
								Mo	VE	C–	SM	+	±	±	±	+	n	+	±	+	n	+
								Y	VE	C–	SM	+	–	–	?	+	n	+	+	+	n	–
								Th	VE	C–	SM	+	–	–	–	–	n	–	–	±	n	±
								Y/Gy	VE	C+	SM	+	?	?	?	?	n	?	?	+	n	?
							Group F	Ke	VE	C+	SM	+	±	–	±	±	n	–	±	±	n	±
								Gy	S	C–	SM	+	–	–	–	n	±	±	+	–	–	+

(continued)

TABLE 272. List of *Streptomyces* species continued

Species names and groups[1]	No. in lists of species[2]	Type strain	Accession no.	Wil 83a[3]	Wil 89[4]	Käm 91[5]	Hat 03[6]	Lan 02[7]	Ful 95[8]	Och 95[9]	Kat 97[10]	Lab[11]	Sch[12]	Lan 04[13]	Lan 02[14]	Lan 04[15]	Lan 04[16]	Lan 04[17]	Lan 04[18]	Lan 04[19]	Lan 04[20]
Group S. ochraceiscleroticus et rel.:																					
S. ochraceiscleroticus	348	DSM 40594/ DSM 43155, ATCC 15814, LMG 19349, NBRC 12394	AB184094		III 08	069 1-26				OC-non			Sch00	Lan2-00	BENP +	+		+	cl08	+	+
S. purpurogeneiscleroticus	396	DSM 40271, DSM 43156, LMG 20331	AJ621604	A 40		069 1-26							Sch00	Lan2-00	BENP	+		+		+	+
S. violens	508	DSM 40597, ATCC 15898, LMG 20303, ISP 5597	AJ621605	A 40	I 18	069 1-26							Sch00	Lan2-00	BENP	+		+		+	+
S. monomycini	325	DSM 41801, NRRL B-24309	DQ445790																		
S. niger	335	DSM 40302, DSM 43049, LMG 20101	AJ621607	A 40	I 18	069 1-26							Sch00	Lan2-00	BENP	+		+		+	+
S. olivaceiscleroticus	350	DSM 40595, ATCC 15722, LMG 20081, ISP 5595	AJ621606		IV 24 (gray series)	069 1-26							Sch00	Lan2-00	BENP	+		+			+
Most closely related to groups S. ochraceiscleroticus et rel. and S. albofaciens et rel.:																					
S. auratus	51	DSM 41897, NRRL 8097	AJ391816																		
Group S. albofaciens et rel.:																					
S. chrestomyceticus	106	DSM 40545, ATCC 14947, LMG 20457, ISP 5545	AJ621609	B 42	I 19	035 1-33							Sch00	Lan2-00	BENP	+		+		+	cl23
S. rimosus subsp. *paromomycinus*	409b	DSM 41429, LMG 20308	AJ621610										Sch00	Lan2-00	BENP	+		+		+	cl23
S. albofaciens	17	DSM 40268, ATCC 25184, LMG 20394, ISP 5268, JCM 4342	AB045880	B 42	I 19	035 1-33							Sch00	Lan2-00	BENP	+		+		+	cl10
Most closely related to groups S. ochraceiscleroticus et rel. and S. albofaciens et rel.:																					
S. erumpens	158	DSM 40941, ATCC 23266, LMG 20507	AJ621603		IV 15 (gray series)	035 1-33							Sch21	Lan2-00	BENP	+		+		+	cl23
S. rimosus subsp. *rimosus*	409a	DSM 40260, ATCC 10970, LMG 19352, ISP 5260, JCM 4667	AB045883	B 42	I 19	035 1-33		La-09		OC-non			Sch00	Lan2-00	BENP +	+		+	cl13	+	cl10
S. sclerotialus	435	DSM 40269, DSM 43032, LMG 20528	AJ621608		I 18	069 1-26							Sch00	Lan2-00	BENP	+		+			cl02
Group S. albulus et rel.:																					
S. albulus	27	DSM 40492, ATCC 12757, LMG 20404, ISP 5492, IMC S-0802	AB024440	A 29	I 15	025 109							Sch00	Lan2-00	BENP	+		+		+	+
S. noursei	346	DSM 40635, ATCC 11455, LMG 5982, NBRC 15452	AB184678		IV 23 (gray series)	025 1-09								Lan2-00	BENP	+		+		+	+
S. yunnanensis	532	DSM 41793, CGMCC 4.1004, JCM 12115, YIM 41004	AF346818																	+	

(continued)

TABLE 272. (continued)

								Morphological characters[24]				Physiological tests[25]										
Guo 08[21] 16S rRNA	Guo 08[21] *atpD*	Guo 08[21] *gyrB*	Guo 08[21] *recA*	Guo 08[21] *rpoB*	Guo 08[21] *trpB*	Guo 08[21] concat.	Kim 04[23] *rpoB*	I	II	III	IV	I	II	III	IV	V	VI	VII	VIII	IX	X	XI
								W	S	C–	SM	+	+	+	+	+	+	+	+	+	+	n
								Gy	S	C–	SM	+	+	+	+	+	+	+	+	+	+	n
								n	n	C–	n	+	+	+	+	+	n	+	+	+	n	+
								W/Gy	S	C–	SM	+	+	–	–	+	n	–	+	–	n	n
								W/Gy	S	C–	SM	+	+	+	+	+	+	+	+	+	+	+
								Gy	S	C–	SM	+	+	+	+	n	n	n	n	n	n	n
								Gy	S	C+	SM	+	n	n	+	+	+	+	+	n	+	+
								W	S	C–	SM	+	–	–	–	+	+	–	+	–	–	n
								W	S	C–	SM	+	–	–	–	+	+	+	+	+	–	–
							+	W	S	C–	SM	+	±	+	–	+	n	+	+	+	n	±
Group IV	Yes	Yes	Yes	Yes	Yes	Yes		Gy	S	C–	SM	+	–	+	–	+	+	+	+	+	–	–
								W	S	C–	SM	+	–	+	–	+	+	+	+	+	–	n
								W	S	C–	SM	+	+	+	+	+	n	+	+	+	n	+
							+	Gy	S	C–	SPY	+	–	–	–	+	+	–	+	+	+	n
								Gy	S	C–	SPY	+	–	–	–	+	+	–	+	+	–	+
									S	C–	RU	+	–	+	+	+	n	+	+	+	n	–

(continued)

TABLE 272. List of *Streptomyces* species continued

Species names and groups[1]	No. in lists of species[2]	Type strain	Accession no.	Wil 83a[3]	Wil 89[4]	Käm 91[5]	Hat 03[6]	Lan 02[7]	Ful 95[8]	Och 95[9]	Kat 97[10]	Lab[11]	Sch[12]	Lan 04[13]	Lan 02[14]	Lan 04[15]	Lan 04[16]	Lan 04[17]	Lan 04[18]	Lan 04[19]	Lan 04[20]
Most closely related to groups S. ochraceiscleroticucs et rel., S. albofaciens et rel. and S. albulus et rel.:																					
S. kasugaensis	266	DSM 40819, LMG 19949, ISP 5819, M338-M1	AB024441										Sch00	Lan2-00	BENP	+		+		+	cl42
S. chattanoogensis	103	DSM 40002, ATCC 19739, LMG 19339, ISP 5002	AJ621611					La-00		OC-non			Sch00	Lan2-00	BENP +	+		+	cl12	+	cl23
S. lydicus	304	DSM 40461, ATCC 25470, LMG 19331, ISP 5461	Y15507	A 29	I 15	025 005		La-09	FU-21	OC-non			Sch00	Lan2-00	BENP +	+		+	cl12	+	cl23
S. albospinus	22	DSM 41674, LMG 20398, NBRC 13846	AB184527										Sch00	Lan2-00	BENP	+		+			+
S. sioyaensis	442	DSM 40032, ATCC 13989, LMG 20531, ISP 5032, NRRL B-5408	DQ026654	A 29	I 15	025 005							Sch00	Lan2-06	BENP	+		+		+	cl58
S. hygroscopicus subsp. *decoyicus*	253c	DSM 41427, LMG 19954, AS 4.1861	AY999883										Sch00	Lan2-00	BENP	+		+		+	cl23
Most closely related to groups S. ochraceiscleroticucs et rel., S. albofaciens et rel., S. albulus et rel. and S. caniferus et rel.:																					
S. catenulae	94	DSM 40258, ATCC 12476, LMG 20449, ISP 5258	AJ621613	C 43	II 11	035 041							Sch00	Lan2-00	BENP	+		+		+	cl23
S. misakiensis	322	DSM 40222, ATCC 23938, LMG 19369, ISP 5222, IFO 12891	AB217605	F 66	II 16	22-4 043		La-18		OC-non			Sch00	Lan2-00	BENP +	+		+		+	cl29
S. ramulosus	400	DSM 40100, ATCC 19802, LMG 19354, ISP 5100, NRRL B-2714	DQ026662	C Sm	III 16	035 041		La-00		OC-non			Sch00	Lan2-00	BENP +	+		+	cl12	+	cl23
Group S. caniferus et rel.:																					
S. hygroscopicus subsp. *glebosus*	253d	DSM 40823, LMG 19950, NBRC 13786	AB184479										Sch22	Lan2-00	BENP	+		+			cl23
S. libani subsp. *rufus*	284b	DSM 41230, LMG 20087	AJ781351										Sch22	Lan2-00	BENP	+		+		+	cl23
S. platensis	376	DSM 40041, ATCC 13865, LMG 20046, ISP 5041, JCM 4662	AB045882	A 29	I 15	025 005			FU-21				Sch22	Lan2-00	BENP	+		+		+	cl23
S. caniferus	88	DSM 41453, LMG 20446, NBRC 15389	AB184640										Sch00	Lan2-00	BENP	+		+			cl23
Most closely related to group S. caniferus et rel.:																					
S. libani subsp. *libani*	284a	DSM 40555, ATCC 23732, LMG 20077, ISP 5555, NBRC 13452	AB184414	A 29	I 15	025 005							Sch00		BENP	+		+			cl23

(continued)

TABLE 272. (continued)

Guo 08[21] 16S rRNA	Guo 08[21] *atpD*	Guo 08[21] *gyrB*	Guo 08[21] *recA*	Guo 08[21] *rpoB*	Guo 08[21] *trpB*	Guo 08[21] concat.	Kim 04[23] *rpoB*	Morphological characters[24] I	II	III	IV	Physiological tests[25] I	II	III	IV	V	VI	VII	VIII	IX	X	XI
								Gy	S	C–	SM	+	–	–	–	+		+	–	+		–
							+	Gy	S	C–	SPY	+	–	–	–	+	n	+	+	+	n	+
								Gy	S	C–	SM	+	+	+	–	+	+	+	+	–	n	+
								Gy	S	C–	SPY	+	±	–	–	+	+	+	+	+	+	–
							+	Gy	S	C–	SM	+	+	–	–	+	+	+	+	+	–	+
								Gy	S	C–	SM	+	+	–	–	+	+	–	+	+	–	n
							+	Gy	RF	C–	SM	+	–	–	–	+	+	–	+	–	–	n
								Gy	RF	C–	SM	+	–	–	–	+	n	+	+	+	n	+
								Gy	RF	C–	SM	+	–	–	–	±	+	+	+	–	–	–
								Gy	S	C–	SM	+	+	–	–	+	+	+	+	+	–	+
								Gy	S	C–	SM	+	+	+	–	+	n	+	n	+	n	+
								Gy	S	C–	SM	+	–	–	–	+	n	+	+	+	n	n
								Gy	S	C–	SM	+	–	–	+	–	–	–	+	+	n	n
							+	Gy	S	C–	SM	+	+	–	–	+	n	+	n	+	n	+
							+	Gy	S	C–	SM	+	–	–	–	+	n	+	+	+	n	+

(continued)

TABLE 272. List of *Streptomyces* species continued

Species names and groups[1]	No. in lists of species[2]	Type strain	Accession no.	Wil 83a[3]	Wil 89[4]	Käm 91[5]	Hat 03[6]	Lan 02[7]	Ful 95[8]	Och 95[9]	Kat 97[10]	Lab[11]	Sch[12]	Lan 04[13]	Lan 02[14]	Lan 04[15]	Lan 04[16]	Lan 04[17]	Lan 04[18]	Lan 04[19]	Lan 04[20]
S. tubercidicus	486	DSM 40261, ATCC 25502, LMG 19361, ISP 5261	AJ621612	C 47	III 14	025 005		La-02		OC-non			Sch00	Lan2-00	BENP +	+		+	cl12		cl23
S. nigrescens	336	DSM 40276, ATCC 23941, LMG 19332, ISP 5276, NRRL B-12176	DQ442530	A 29	I 15	025 005		La-02					Sch00		BENP +	+		+	cl12	+	cl23
Group S. albiflaviniger et rel.:																					
S. antimycoticus	36	DSM 40284, ATCC 23880, LMG 20413, ISP 5284, NBRC 12839	AB184185		IV 05 (gray series)	051 018							Sch27		BENP	+		+		+	cl16
S. geldanamycininus	200	DSM 41894, NRRL 3602, NRRL B-3602	DQ334781																	+	
S. melanosporofaciens	316	DSM 40318, ATCC 25473, LMG 20066, ISP 5318, NRRL B-12234	AJ271887	A 32	I 16	051 018						L1	Sch00	Lan2-00	BENP	+		+		+	cl16
S. sporoclivatus	456	DSM 41461, LMG 20312, NBRC 100767	AB249934										Sch27		BENP	+		+		+	cl16
S. yatensis	527	DSM 41771, NBRC 101000	AB249962																	+	
S. rutgersensis subsp. *castelarensis*	427b	DSM 40830, ATCC 15191, LMG 20304	AY508511		I 01	055 018							Sch00	Lan2-00	BENP	+		+			cl16
S. indonesiensis	257	DSM 41759, A4R2	DQ334783																	+	
S. griseiniger	217	DSM 41895, NRRL B-1865	AJ391818																	+	
S. rhizosphaericus	408	DSM 41760, NBRC 100778	AB249941																		
S. asiaticus	43	DSM 41761, NBRC 100774	AB249947																		
S. cangkringensis	87	DSM 41769, D13P3	AJ391831																	+	
S. malaysiensis	308	DSM 41697, LMG 20099, NBRC 16446	AB249918										Sch00	Lan2-00	BENP	+		+			l15
S. javensis	262	DSM 41764, B22P3	AJ391833																	+	
S. endus	156	DSM 40187, NRRL 2339, LMG 19393	AY999911					La-08				L1	Sch36	Lan2-29	BENP cl08	+	(f)	+	cl08	+	cl16
S. sporocinereus	455	DSM 41460, LMG 20311, NBRC 100766	AB249933										Sch36	Lan2-00	BENP	+		+			cl16
S. hygroscopicus subsp. *hygroscopicus*	253a	DSM 40578, ATCC 27438, LMG 19335, ISP 5578, NBRC 13472	AB184428	A 32	I 16	085 012		La-08	FU-6			L1	Sch00	Lan2-29	BENP cl08	+	(f)	+	cl08		cl16
S. demainii	143	DSM 41600, NRRL B-1478	DQ334782																		
S. violaceusniger	504	DSM 40563, ATCC 27477, LMG 19336, ISP 5563	AJ391823	A 32	I 16	051 018		La-07		OC-I		L1	Sch00	Lan2-00	BENP +	+	(f)	+	cl09		cl15
S. yogyakartensis	530	DSM 41766, NBRC 100779	AB249942																	+	
S. albiflaviniger	15	DSM 41598, NRRL B-1356	AJ391812																		

(continued)

TABLE 272. (continued)

								Morphological characters[24]				Physiological tests[25]										
Guo 08[21] 16S rRNA	Guo 08[21] *atpD*	Guo 08[21] *gyrB*	Guo 08[21] *recA*	Guo 08[21] *rpoB*	Guo 08[21] *trpB*	Guo 08[21] concat.	Kim 04[23] *rpoB*	I	II	III	IV	I	II	III	IV	V	VI	VII	VIII	IX	X	XI
								Gy	S	C–	SM	+	+	−	−	+	n	+	+	+	n	+
								Gy	S	C–	SM	+	+	+	+	+	n	−	+	+	+	+
								Gy	S	C–	RU	n	n	+	n	−	n	n	n	n	−	n
								Gy	S	C–	SM	+	+	+	+	+	n	+	+	+	n	−
								Gy	S	C–	WTY	+	+	−	−	+	+	+	+	n	n	n
								Gy	S	C–	RU	+	+	+	+	+	+	+	+	+	+	+
								Gy	S	C–	SM	+	+	+	+	+	+	+	+	−	+	−
								Gy	S	C+	RU	n	n	n	n	n	n	n	n	n	n	+
								Gy	S	C–	RU	n	n	−	n	+	n	n	n	n	−	n
								Gy	S	C–	RU											+
								Gy	S	C–	RU	n	n	n	n	n	n	n	n	n	n	+
								Gy	S	C–	RU	n	n	n	n	n	n	n	n	n	n	+
								W/Gy	S	C+	RU	+	+	+	+	+	+	+	+	+	n	−
								Gy	S	C–	RU											+
								Gy	S	C–	SM	+	+	+	+	+	+	−	+	−	+	−
								Gy	S	C–	WTY	+	n	n	n	−	+	n	n	n	n	n
								Gy	S	C–	SM	+	+	+	+	+	n	−	n	−	+	n
								Gy–Y	S	C–	RU	n	n	+	n	−	n	n	n	n	−	n
								Gy	S	C–	SM	+	+	+	+	+	n	+	+	+	+	n
								Gy	S	C–	RU	n	n	n	n	n	n	n	n	n	n	+
								W	S	C–	RU	n	n	+	n	+	n	n	n	n	−	n

(continued)

TABLE 272. List of *Streptomyces* species continued

Species names and groups[1]	No. in lists of species[2]	Type strain	Accession no.	Wil 83a[3]	Wil 89[4]	Käm 91[5]	Hat 03[6]	Lan 02[7]	Ful 95[8]	Och 95[9]	Kat 97[10]	Lab[11]	Sch[12]	Lan 04[13]	Lan 02[14]	Lan 04[15]	Lan 04[16]	Lan 04[17]	Lan 04[18]	Lan 04[19]	Lan 04[20]
Most closely related to groups S. ochraceiscleroticus et rel., S. albofaciens et rel., S. albulus et rel., S. caniferus et rel. and S. albiflaviniger et rel.:																					
S. orinoci	359	DSM 40571, ATCC 23202, LMG 20079, ISP 5571, NBRC 13466	AB184866	F 58	Sv. 17	22-1 040	Ha15						Sch00	Lan2-00	BENP	+	(j)	+		+	cl15
S. mashuensis	309	DSM 40221, ATCC 23934, LMG 8603, ISP 5221	X79323	F 55	Sv. 03	22-1 040	Ha11					L4	Sch31		BENP	+	(j)	+			cl11
S. mobaraensis	324	DSM 40847, ATCC 29032, LMG 20086, NRRL B-3729	DQ442528		Sv. 07	22-1 040	Ha12		FU-12b			L4	Sch00		BENP	+	(j)	+			cl56
S. luteosporeus	302	DSM 40833, LMG 20085, NRRL 2401	DQ442525				Ha10						Sch00	Lan2-00	BENP	+	(j)	+			+
S. aureoversilis	55	DSM 40387, ATCC 15853, LMG 20425, ISP 5387, NBRC 13021	AB184855		Sv. 05	22-1 040	Ha7					L4	Sch00	Lan2-00	BENP	+	(j)	+		+	cl48
S. griseocarneus	221	DSM 40004, ATCC 12628, LMG 5973, ISP 5004	X99943	F 55	Sv. 03	22-1 040	Ha6	La-00	FU-12b			L4	Sch00	Lan2-00	BENP +	+	(j) €	+		+	cl17
Group S. albus et rel.:																					
S. almquistii	28	DSM 40447, ATCC 618, LMG 21307, ISP 5447, NBRC 13015	AB184258	A 16	I 09	030 1-34							Sch24	Lan2-20	BENP	+		+		+	cl18
S. rangoonensis	401	DSM 40452, ATCC 6860, LMG 20295, ISP 5452, NBRC 13078	AB184295		IV 07 (white series)	030 1-34							Sch24	Lan2-20	BENP	+		+		+	cl18
S. gibsonii	203	DSM 43284, ATCC 6852, LMG 19912, NBRC 15415	AB184663		IV 05 (white series)	030 1-34							Sch24	Lan2-20	BENP	+		+		+	cl18
S. albus subsp. *albus*	1a	DSM 40313, ATCC 3004, ISP 5313	AJ621602	A 16	I 09	032 027			FU-6	OC-non			Sch24	Lan2-20						+	
S. flocculus	186	DSM 40327, ATCC 25453, LMG 19889, ISP 5327, NBRC 13041	AB184272	A 16	I 09	030 1-34							Sch24	Lan2-00	BENP	+		+			cl18
Most closely related to group S. albus et rel.:																					
S. cacaoi subsp. *cacaoi*	79a	DSM 40057, ATCC 3082, LMG 19320, ISP 5057, NBRC 12748	AB184115	A 16	I 09	031 1-34		La-05					Sch00	Lan2-17	BENP cl05	+	(c)	+	cl08	+	cl36
S. sulphureus	463	DSM 40104, ATCC 27468, LMG 19355, ISP 5104, NRRL B-1627	DQ442546	C Sm	III 17	068 002		La-00		OC-non			Sch00		BENP +	+		+		+	-
S. rubidus	423	CGMCC 4.2026, 13C15	AY876941																		

(continued)

TABLE 272. (continued)

								Morphological characters[24]				Physiological tests[25]										
Guo 08[21] 16S rRNA	Guo 08[21] *atpD*	Guo 08[21] *gyrB*	Guo 08[21] *recA*	Guo 08[21] *rpoB*	Guo 08[21] *trpB*	Guo 08[21] concat.	Kim 04[23] *rpoB*	I	II	III	IV	I	II	III	IV	V	VI	VII	VIII	IX	X	XI
								Ar	VE	C–	SM	+	–	–	–	±	n	±	–	–	n	±
								Ar	VE	C+	SM	+	–	–	–	+	n	±	–	+	n	+
								Mo	VE	C–	SM	+	±	±	–	+	n	–	±	±	n	+
								W	S	C–	SM	+	+	–	–	±	+	–	+	–	+	n
								Bi	VE	C+	SM	+	–	–	±	–	n	±	–	+	n	+
								Gr	VE	C+	SM	+	–	–	–	±	n	–	–	+	n	±
							Group A16	W	S	C–	SM	+	+	–	–	+	n	–	+	–	+	–
								W	S	C–	SM	+	+	±	–	+	n	–	+	–	n	–
								W	S	C–	SM	+	+	+	–	–	n	–	+	–	+	n
							Group A16	W	S	C–	SM	+	+	–	–	±	+	–	+	–	+	n
								W	S	C–	SM	+	+	+	–	+	+	+	+	+	+	n
								W	S	C–	SM	+	+	+	–	+	n	±	+	–	n	±
								Y	RF	C–	SM	+	+	+	–	+	n	+	n	–	+	n
									RF	C–	SM	+					+		+		+	

(continued)

TABLE 272. List of *Streptomyces* species continued

Species names and groups[1]	No. in lists of species[2]	Type strain	Accession no.	Wil 83a[3]	Wil 89[4]	Käm 91[5]	Hat 03[6]	Lan 02[7]	Ful 95[8]	Och 95[9]	Kat 97[10]	Lab[11]	Sch[12]	Lan 04[13]	Lan 02[14]	Lan 04[15]	Lan 04[16]	Lan 04[17]	Lan 04[18]	Lan 04[19]	Lan 04[20]
S. yeochonensis	528	NBRC 100782, JCM 12366, CN 732	AF101415																		
S. albus subsp. *pathocidicus*	1b	DSM 40799, LMG 20406, NBRC 13812	AB184501										Sch00	Lan2-00	BENP	+		+			cl07
S. glauciniger	205	LMG 22082, NBRC 100913	AB249964																		
S. guanduensis	239	CGMCC 4.2022, 701	AY876942																		
Most closely related to groups S. albus et rel. and S. glaucosporus et rel.:																					
S. ferralitis	166	DSM 41836, SFOp68	AY262826																	+	
S. vitaminophilus	517	DSM 41686, LMG 21051, NBRC 14294	AB184589										Sch00	Lan2-00	BENP	+		+		+	+
S. thermolineatus	477	DSM 41451, LMG 20309	Z68097										Sch00	Lan2-00	BENP	+		+		+	+
S. yanglinensis	525	CGMCC 4.2023, JCM 13275, 1307	AY876940																		
S. paucisporeus	365	CGMCC 4.2025, 1413	AY876943																		
Group S. glaucosporus et rel.:																					
S. macrosporus	305	DSM 41449	Z68099										Sch00	Lan2-00						+	
S. megasporus	314	DSM 41476, LMG 20092, NBRC 14749	AB184617										Sch00	Lan2-00	BENP	+		+			-
S. glaucosporus	206	DSM 41689, LMG 19907, NBRC 15416	AB184664										Sch00	Lan2-00	BENP	+		+			cl44
S. radiopugnans	398	DSM 41901, CGMCC 4.3519, R97	DQ912930																		
Most closely related to group S. glaucosporus et rel.:																					
S. albiaxialis	12	DSM 41799, NBRC 101002, NRRL B-24327	AY999901																	+	
S. armeniacus	42	DSM 43125, LMG 20418, JCM 3070	AB018092										Sch00	Lan2-00	BENP	+		+			+
Most closely related to groups S. albus et rel. and S. glaucosporus et rel.:																					
S. cuspidosporus	138	DSM 41425, LMG 20492, NBRC 12378	AB184090		IV 11 (gray series)	22-4 1-06							Sch08	Lan2-00	BENP	+		+		+	cl56
S. sparsogenes	445	DSM 40356, ATCC 25498, LMG 19378, ISP 5356, NBRC 13086	AB184301	A 32	I 16	010 1-19		La-07				L1	Sch00		BENP +	+		+			-
Most closely related to group S. geysiriensis et rel.:																					
S. janthinus	261	DSM 40206, ATCC 15870, LMG 8591, ISP 5206, NBRC 12879	AB184851	A 18	I 11	009 1-19						L2	Sch00		BENP	+		+			cl01
S. roseoviolaceus	420	DSM 40277, ATCC 25493, LMG 8594, ISP 5277	AJ399484	A 18	I 11	009 1-19			FU-1			L2	Sch00		BENP	+		+			cl01

(continued)

TABLE 272. (continued)

Guo 08[21] 16S rRNA	Guo 08[21] *atpD*	Guo 08[21] *gyrB*	Guo 08[21] *recA*	Guo 08[21] *rpoB*	Guo 08[21] *trpB*	Guo 08[21] concat.	Kim 04[23] *rpoB*	Morphological characters[24] I	II	III	IV	Physiological tests[25] I	II	III	IV	V	VI	VII	VIII	IX	X	XI
								Gy	RF	n	SM	n	n	n		n	n	n	n	n	+	n
								W	S	C–	SM	+	+	+	+	–	+	–	–	+	–	n
								Gy	S	C–	SM	+	+	+	+	+	+	+	+	+	n	+
								Gy/W	RF	C–	SM	+					+		+		+	
								W	S	C–	SM	+	n	–	n	n	+	n	+	n	n	n
										C–	SM	+	+	–	+	–	n	–	–	–	n	–
								G	RF	C–	SM	n	n	n	n	n	n	n	+	n	n	n
								W/Gy	RF	C–	SM	+	n	n	n	n	n	n	+	n	+	n
								W/Gy	RF	C+/C–	SM	+					+		+			
								Gy	S	C–	SPY	+	+	+	+	+	n	–	+	+	n	–
								G	S	C–	SPY/ WTY	+	+	+	+							
								G	S	C–	WTY	+	+	n	–	n	n	n	n	–	n	n
								W	S	C–	WTY	n	+	n	+	n	n	n	+	–	n	+
								W	S	C–	SM	+	+	+	+	n	n	+	–	–	n	+
								W	S	C–	n	+	+	+	+	+	+	+	–	n	+	+
								Gy	S	C–	SPY	+	+	+	+	+	+	+	+	+	+	+
								Gy	S	C–	SPY	+	+	+	+	+	±	+	+	±	–	+
								R	S	C+	SPY	+	+	+	+	+	+	+	+	+	+	+
								R	S	C+	SPY	+	+	+	+	+	n	+	+	+	n	+

(continued)

TABLE 272. List of *Streptomyces* species continued

Species names and groups[1]	No. in lists of species[2]	Type strain	Accession no.	Wil 83a[3]	Wil 89[4]	Käm 91[5]	Hat 03[6]	Lan 02[7]	Ful 95[8]	Och 95[9]	Kat 97[10]	Lab[11]	Sch[12]	Lan 04[13]	Lan 02[14]	Lan 04[15]	Lan 04[16]	Lan 04[17]	Lan 04[18]	Lan 04[19]	Lan 04[20]
S. violaceus	503	DSM 40082, ATCC 15888, LMG 20257, ISP 5082, NBRC 13103	AB184315	A 06	I 05	009 0-19				OC-III			Sch00	Lan2-00	BENP	+		+		+	cl01
S. albosporeus subsp. *albosporeus*	23a	DSM 40795, ATCC 15394, LMG 19403	AJ781327		IV 01 (red series)	063 049		La-01					Sch00	Lan2-00	BENP cl01	+	(c)	+	cl05	+	cl24
S. arenae	40	DSM 40293, ATCC 25428, LMG 20416, ISP 5293, NBRC 13016	AB249977	A 18	I 11	009 1-19						L2	Sch00	Lan2-00	BENP	+		+		+	cl01
S. luteogriseus	301	DSM 40483, ATCC 15072, LMG 20073, ISP 5483, NBRC 13402	AB184379	A 18	I 11	009 1-19						L2	Sch25	Lan2-00	BENP	+		+		+	cl01
S. hawaiiensis	243	DSM 40042, ATCC 12236, LMG 5975, ISP 5042, NBRC 12784	AB184143	A 18	I 11	009 1-19						L2	Sch34	Lan2-00	BENP	+		+			cl01
S. cellulosae	100	DSM 40362, ATCC 25439, LMG 19315, ISP 5362, NRRL B-2889	DQ442495	A 13	II 03	006 1-18		La-15		OC-non			Sch00	Lan2-00	BENP +	+		+	cl17		cl04
S. pseudogriseolus	388	DSM 40026, ATCC 12770, ISP 5026, NRRL B-3288	DQ442541	A 12	I 07	006 1-18					KA-G		Sch13	Lan2-00						+	
S. gancidicus	197	DSM 40935, NRRL B-1872, LMG 19898, NBRC 15412	AB184660		IV 17 (gray series)	006 1-18							Sch13	Lan2-00	BENP	+		+		+	cl04
S. rubiginosus	425	DSM 40177, ATCC 19927, LMG 20268, ISP 5177, KCTC 9042	AY999810	A 12	I 07	006 1-18							Sch13	Lan2-00	BENP	+		+		+	cl04
S. capillispiralis	90	DSM 41695, LMG 19909, NBRC 14222	AB184577										Sch00	Lan2-00	BENP	+		+		+	+
S. lavendulocolor	282	DSM 40216, ATCC 15871, LMG 19934, ISP 5216, NRRL B-3367	DQ442516	F 61	I 22	22-3 1-08						L5	Sch00	Lan2-00	BENP	+		+		+	cl12
S. azureus	62	DSM 40106, ATCC 14921, LMG 20430, ISP 5106, NRRL B-2655	EF178674	A 18	I 11	009 1-19			FU-1			L2	Sch00	Lan2-00	BENP	+		+		+	cl01
S. flavoviridis	185	DSM 40153, ATCC 19903, LMG 19881, ISP 5153, NBRC 12772	AB184842	A 28		006 1-10							Sch00		BENP	+		+		+	cl35
S. pilosus	375	DSM 40097, ATCC 19797, LMG 20049, ISP 5097, NBRC 12807	AB184161	A 37	I 17	006 1-10							Sch00		BENP	+		+		+	cl35
S. djakartensis	147	DSM 40743, ATCC 13441, LMG 21795, NBRC 15409	AB184657		IV 12 (gray series)	035 1-33							Sch00	Lan2-00	BENP	+		+		+	cl04
Group S. geysiriensis et rel.:																					
S. ghanaensis	202	DSM 40746, ATCC 14672, LMG 19894, KCTC 9882	AY999851		IV 05 (green series)	1-7 1-21							Sch00		BENP	+		+		+	+

(continued)

TABLE 272. (continued)

								Morphological characters[24]				Physiological tests[25]										
Guo 08[21] 16S rRNA	Guo 08[21] *atpD*	Guo 08[21] *gyrB*	Guo 08[21] *recA*	Guo 08[21] *rpoB*	Guo 08[21] *trpB*	Guo 08[21] concat.	Kim 04[23] *rpoB*	I	II	III	IV	I	II	III	IV	V	VI	VII	VIII	IX	X	XI
								R	S	C+	SY	+	+	+	+	+	+	+	n	+	n	+
								R	S	C–	SM	+	+	+	+	+	+	+	+	+	–	n
								Gy	S	C+	SPY	+	+	+	+	+	+	+	+	+	n	+
								Gy	S	C+	SM	+	+	+	+	+	+	+	+	+	+	+
								W	S/RA	C+	SPY	+	?	+	+	+	n	+	+	+	n	+
								Y	RF	C–	SM	+	+	+	+	+	n	–	+	+	n	n
								Gy	S	C–	SPY	+	+	+	+	+	+	–	+	+	+	n
								Gy	S	C–	SPY	+	+	+	+	+	+	–	+	+	–	–
								Gy	S	C–	SPY	+	+	±	+	+	n	–	+	+	n	+
								Gy	S	C–	H	n	n	–	n	n	n	–	n	n	–	–
								R	S	C+	SM	+	+	+	–	–	+	–	–	+	–	–
								B	S	C+	WTY	+	+	+	+	+	+	+	+	+	n	+
								Gy/G	S	C+	H	+	+	+	+	+	n	–	+	+	n	–
								Gy	S	C+	H	+	+	+	+	+	n	–	+	+	n	–
								Gy	S	C+	n	n	n	n	n	n	n	n	n	n	n	n
								G	S	C–	SPY	n	n	n	n	n	n	n	n	n	n	n

(continued)

TABLE 272. List of *Streptomyces* species continued

Species names and groups[1]	No. in lists of species[2]	Type strain	Accession no.	Wil 83a[3]	Wil 89[4]	Käm 91[5]	Hat 03[6]	Lan 02[7]	Ful 95[8]	Och 95[9]	Kat 97[10]	Lab[11]	Sch[12]	Lan 04[13]	Lan 02[14]	Lan 04[15]	Lan 04[16]	Lan 04[17]	Lan 04[18]	Lan 04[19]	Lan 04[20]
S. minutiscleroticus	320	DSM 40301, ATCC 17757, LMG 20062, ISP 5301, NRRL B-12202	EF178696	A 15	I 08	006 1-18					KA-G		Sch00	Lan2-03	BENP	+	(a)	+			cl46
S. geysiriensis	201	DSM 40742, ATCC 15303, LMG 19893, NRRL B-12102	DQ442501		IV 18 (gray series)	006 1-18							Sch14	Lan2-00	BENP	+		+			cl39
S. plicatus	377	DSM 40319, ATCC 25483, LMG 20288, ISP 5319, NBRC 13071	AB184291	A 12	I 07	006 1-18					A-E		Sch14	Lan2-00	BENP	+		+		+	cl39
S. rochei	411	DSM 40231, ATCC 10739, LMG 19313, ISP 5231, NBRC 12908	AB184237	A 12	I 07	006 1-18		La.13		OC-III	A-E		Sch00	Lan2-00	BENP +	+		+	cl17	+	cl39
S. vinaceusdrappus	497	DSM 40470, ATCC 25511, LMG 20296, ISP 5470, NRRL 2363	AY999929	A 12	I 07	006 1-18					A-E		Sch14	Lan2-00	BENP	+		+			cl39
S. mutabilis	328	DSM 40169, ATCC 19919, LMG 20054, ISP 5169	EF178679	A 12	I 07	006 1-18					A-E		Sch00	Lan2-00	BENP	+		+		+	+
Most closely related to group S. geysiriensis et rel.:																					
S. tuirus	487	DSM 40505, LMG 20299, NBRC 15617	AB184690	A 21	I 14	006 1-18							Sch00	Lan2-00	BENP	+		+			cl01
S. afghaniensis	8	DSM 40228, ATCC 23871, LMG 20390, ISP 5228	AJ399483	A 18	I 11	009 1-19			FU-1			L2	Sch00	Lan2-00	BENP	+		+		+	cl01
S. africanus	9	DSM 41829, NBRC 101005, CPJVR-H	AY208912																	+	
Group S. brasiliensis et rel.:																					
S. roseiscleroticus	412	DSM 40303, ATCC 17755, LMG 20284, ISP 5303, NBRC 13002	AB184251		II 19	049 022							Sch29	Lan2-01	BENP	+		+			cl38
S. ruber	422	DSM 40304, LMG 20285, NBRC 14600	AB184604		IV 11 (red series)	049 022							Sch29	Lan2-01	BENP	+		+			cl38
S. spiralis	452	DSM 43836, LMG 20332, NRRL B-16922	EF178683										Sch00	Lan2-00	BENP	+		+		+	+
S. fumigatiscleroticus	194	DSM 43154, LMG 19911, NRRL B-3856	DQ442499										Sch00	Lan2-00	BENP	+		+		+	+
S. poonensis	380	DSM 40596, ATCC 15723, LMG 19326, ISP 5596, NRRL B-2319	DQ445792	A 22	II 19	071 1-19		La-04		OC-III			Sch00	Lan2-00	BENP +	+		+	cl14	+	+
S. brasiliensis	70	DSM 43159, ATCC 23727, LMG 20438, NBRC 101283	AB249981										Sch38	Lan2-00	BENP	+		+		+	+
Group S. atrovirens et rel.:																					
S. atrovirens	48	DSM 41467, LMG 20422, NRRL B-16357	DQ026672										Sch00	Lan2-00	BENP	+		+		+	+

(continued)

TABLE 272. (continued)

Guo 08[21] 16S rRNA	Guo 08[21] *atpD*	Guo 08[21] *gyrB*	Guo 08[21] *recA*	Guo 08[21] *rpoB*	Guo 08[21] *trpB*	Guo 08[21] concat.	Kim 04[23] *rpoB*	Morphological characters[24] I	II	III	IV	Physiological tests[25] I	II	III	IV	V	VI	VII	VIII	IX	X	XI
								Gy	S	C–	n	+	n	+	+	+	+	–	+	n	+	–
								Gy	S	C–	H	n	n	n	n	n	n	n	n	n	n	n
								Gy	S	C–	SM	+	+	+	+	+	+	–	+	+	n	–
								Gy	S	C–	SM	+	+	+	+	+	+	–	+	+	+	–
								R	S	C–	SM	+	+	+	+	+	n	+	+	+	+	n
								Gy	S	C–	SM	+	+	+	+	+	+	–	+	+	–	±
								R	S	C+	SM	+	+	+	+	+	+	+	+	+	–	+
								Gy	S	C+	SPY	+	+	+	+	+	+	+	±	±	n	+
								B	S	C–	SPY	n	n	+	+	+	+	+	+	+	+	–
								R	S	C–	SM	+	+	+	+	+	+	–	+	–	–	n
								W/R	S	C–	SM	+	+	+	+	+		–	+			
								Y–Gy	S	C–	SM	+	–	+	+	+	+	+	+	+	+	+
									S	C–		+	+	+	–	n	–	n	+	–	–	?
								Gy	S	C–	SM	+	+	+	+	+	+	+	+	+	+	n
								Gy	S	C–	SM	+	n	+	+	+	+	+	+	+	+	+
								Gy	S	C–	H	n	+	–	+	+	n	+	+	+	n	n

(continued)

TABLE 272. List of *Streptomyces* species continued

Species names and groups[1]	No. in lists of species[2]	Type strain	Accession no.	Wil 83a[3]	Wil 89[4]	Käm 91[5]	Hat 03[6]	Lan 02[7]	Ful 95[8]	Och 95[9]	Kat 97[10]	Lab[11]	Sch[12]	Lan 04[13]	Lan 02[14]	Lan 04[15]	Lan 04[16]	Lan 04[17]	Lan 04[18]	Lan 04[19]	Lan 04[20]
S. caelestis	80	DSM 40084, ATCC 15084, LMG 5970, ISP 5084, NRRL 2418	X80824	A 18	I 11	009 1-19						L2	Sch00	Lan2-00	BENP	+		+		+	+
S. fumanus	193	DSM 40154, ATCC 19904, LMG 19882, ISP 5154, NBRC 13042	AB184273	A 18	I 11	1-7 1-19							Sch00	Lan2-00	BENP	+		+			cl12
S. fimbriatus	170	DSM 40942, ATCC 15051, LMG 20513	AY999844		IV 16 (gray series)	006 1-18							Sch00	Lan2-00	BENP	+		+		+	+
Group S. glaucus et rel.:																					
S. griseostramineus	235	DSM 40161, ATCC 23628, LMG 19932, ISP 5161, NBRC 12781	AB184140	F 60	IV 06 (green series)	006 1-10							Sch00	Lan2-10	BENP	+		+		+	cl04
S. griseomycini	229	DSM 40159, ATCC 23625, LMG 19883, ISP 5159, NBRC 12778	AB184137	A 12	I 07	006 1-10							Sch00	Lan2-10	BENP	+		+		+	cl04
S. graminearus	215	DSM 41747, LMG 19904	AJ781333										Sch00	Lan2-10	BENP	+		+		+	cl04
S. viridiviolaceus	512	DSM 40280, ATCC 27478, LMG 20282, ISP 5280, IFO 13359	AY999854		IV 35 (gray series)	006 1-18							Sch00	Lan2-00	BENP +	+		+			-
S. glaucus	207	DSM 41456, LMG 19902, NBRC 15417	AB184665										Sch00	Lan2-00	BENP	+		+		+	+
Group S. aureorectus et rel.:																					
S. aureorectus	54	DSM 41692, LMG 19908, NBRC 15896	AB184710										Sch19	Lan2-08	BENP	+		+			cl40
S. virens	509	DSM 41465, LMG 20316, NRRL B-24331	DQ442554										Sch00	Lan2-08	BENP	+		+			cl40
S. asterosporus	44	DSM 41452, LMG 20419, NBRC 15872	AB184706										Sch00	Lan2-08	BENP	+		+			cl40
S. calvus	83	DSM 40010, ATCC 13382, LMG 20442, ISP 5010, NBRC 13200	AB184329	A 12	I 07	006 1-18							Sch19	Lan2-08	BENP	+		+		+	cl40
Most closely related to groups S. geysiriensis et rel., S. brasiliensis et rel., S. atrovirens et rel., S. glaucus et rel. and S. aureorectus et rel.:																					
S. naganishii	330	DSM 40282, ATCC 23939, LMG 21042, ISP 5282, NRRL B-1816	DQ442529	A 31	I 21	1-6 1-15							Sch00	Lan2-00	BENP	+		+		+	+
S. prasinosporus	383	DSM 40506, ATCC 17918, LMG 19346, ISP 5506, NBRC 13419	AB184390	A 38	III 07	22-2 1-15		L.10		OC-III			Sch25	Lan2-00	BENP +	+		+	cl10	+	cl54
S. anandii	33	DSM 40535, ATCC 19388, LMG 8600, ISP 5535, NBRC 13438	AB184402	B 42	I 19	021 1-05							Sch00	Lan2-00	BENP	+		+		+	cl08

(continued)

TABLE 272. (continued)

								Morphological characters[24]				Physiological tests[25]										
Guo 08[21] 16S rRNA	Guo 08[21] *atpD*	Guo 08[21] *gyrB*	Guo 08[21] *recA*	Guo 08[21] *rpoB*	Guo 08[21] *trpB*	Guo 08[21] concat.	Kim 04[23] *rpoB*	I	II	III	IV	I	II	III	IV	V	VI	VII	VIII	IX	X	XI
								B	S	C+	SM	+	+	+	+	+	+	+	−	+	−	+
								R	S	C–	SM	+	+	+	+	+	n	+	+	−	n	−
								Gy	S	C+	SPY	+	+	+	+	+	+	+	+	+	−	n
								G	S	C+	H	+	+	+	+	+	n	n	+	+	n	−
								G	S/RA	C+	H/SM	+	+	+	+	+	n	−	+	+	n	−
								Gy	S	C–	SM	+		+	+	+		+	+	+		
								Gy	S	C–	SPY	n	n	n	n	n	n	n	n	n	n	n
								B/G	S	C–	H	+	+	+	+	n	+	n	+	+	n	n
								W	RF	C–	SM	n	+	n	n	n	+	+	n	n	n	n
								Gy	S	C–	SPY/ WTY	n	−	+	−	+	n	−	+	n	n	n
								Gy	S	C–	SPY	+	−	+	+	+	+	−	+	n	n	n
								Gy	S	C–	H	+	+	+	+	+	+	+	+	+	+	+
								Gy	S	C+	SM	+	+	+	+	+	+	+	+	+	+	−
								G	S	C+	H	+	+	+	+	+	n	n	+	+	+	−
								Gy	S	C+	SM	+	+	+	−	+	+	+	+	+	−	n

(continued)

TABLE 272. List of *Streptomyces* species continued

Species names and groups[1]	No. in lists of species[2]	Type strain	Accession no.	Wil 83a[3]	Wil 89[4]	Käm 91[5]	Hat 03[6]	Lan 02[7]	Ful 95[8]	Och 95[9]	Kat 97[10]	Lab[11]	Sch[12]	Lan 04[13]	Lan 02[14]	Lan 04[15]	Lan 04[16]	Lan 04[17]	Lan 04[18]	Lan 04[19]	Lan 04[20]
S. carpinensis	93	DSM 43835, LMG 19913, NBRC 14214	AB184574										Sch00	Lan2-00	BENP	+		+		+	+
S. levis	283	DSM 41458, LMG 20090, NBRC 15423	AB184670										Sch00	Lan2-00	BENP	+		+			cl01
S. cinerochromogenes	114	DSM 41651, LMG 20466, NBRC 13822	AB184507										Sch00	Lan2-00	BENP	+		+		+	cl57
S. koyangensis	270	NBRC 100598, VK-A60	AY079156																		
S. violarus	505	DSM 40205, ATCC 15891, LMG 20275, ISP 5205, NBRC 13104	AB184316	A 18	I 11	009 1-19						L2	Sch00		BENP	+		+			cl01
Not closely related to one of the groups:																					
S. daghestanicus	142	DSM 40149, ATCC 23620, LMG 20496, ISP 5149, NRRL B-5418	DQ442497	A 17		006 010						L3	Sch40	Lan2-11	BENP	+		+			cl37
S. limosus	287	DSM 40131, ATCC 19778, LMG 8570, ISP 5131, NBRC 12790	AB184147	A 1A	I 01	1-1 1-1			FU-1		KA-D		Sch00	Lan2-22	BENP	+	(k)	+		+	cl08
S. canescens	86	DSM 40001, ATCC 19736, LMG 20445, ISP 5001, NBRC 12751	AB184117	A 1A	I 01	1-1 1-1					KA-D		Sch10	Lan2-22	BENP	+	(k)	+			cl08
S. felleus	165	DSM 40130, ATCC 19752, LMG 20511, ISP 5130, NBRC 12766	AB184129	A 1A	I 01	1-1 1-1					KA-D		Sch00	Lan2-22	BENP	+	(k)	+			cl08
S. griseus subsp. *solvifaciens*	238d	DSM 40933, NRRL B-1561, LMG 19952, NBRC 13689	AB249915		I 02	1-1 1-1							Sch10	Lan2-00	BENP	+		+			cl08
S. violascens	506	DSM 40183, ATCC 23968, LMG 20272, ISP 5183	AY999737	A 06	I 05	002 1-7							Sch00	Lan2-00	BENP	+		+		+	cl23
S. hydrogenans	252	DSM 40586, ATCC 19631, LMG 19948, ISP 5586, NBRC 13475	AB184868	A 05	I 04	002 1-7							Sch00	Lan2-00	BENP	+		+		+	-
S. odorifer	349	DSM 40347, ATCC 6246, LMG 8572, ISP 5347	Z76682	A 1A	I 01	1-1 1-1					KA-D		Sch00		BENP	+	(k)	+			cl08
S. albidoflavus	14	DSM 40455, ATCC 25422, LMG 19300, ISP 5455, NBRC 13010	AB184255	A 1A	I 01	1-1 1-1		La-00	FU-1	OC-non	KA-D		Sch10		BENP +	+	(e) (k)	+	cl06		cl08
S. champavatii	101	DSM 40841, NRRL B-5682, LMG 20454	DQ026642		IV 02 (yellow series	)1-1 1-1							Sch00		BENP	+		+			cl08
S. sampsonii	429	DSM 40394, ATCC 25495, LMG 8574, ISP 5394	D63871	A 1A	I 01	1-1 1-1					KA-D		Sch00	Lan2-22	BENP	+	(k)	+		+	cl08
S. diastaticus subsp. *diastaticus*	144a	DSM 40496, ATCC 3315, LMG 19322, ISP 5496, NBRC 3714	AB184785	A 19	I 12	1-1 1-1		La-00	FU-1	OC-non			Sch00		BENP +	+		+		+	cl09

(continued)

TABLE 272. (continued)

Guo 08[21] 16S rRNA	Guo 08[21] *atpD*	Guo 08[21] *gyrB*	Guo 08[21] *recA*	Guo 08[21] *rpoB*	Guo 08[21] *trpB*	Guo 08[21] concat.	Kim 04[23] *rpoB*	Morphological characters[24] I	II	III	IV	Physiological tests[25] I	II	III	IV	V	VI	VII	VIII	IX	X	XI
								Gy	S	C–	SM	+	–	n	+	+	+	+	+	–	+	–
								V	S	C–	SM	+	+	+		+		+	+			
								Gy	S	C+	SM	n	–	+	+	n	n	–	–	–	+	+
								W/Gy	RF	C–	SM		+	+	–	+		–	+	–		–
								R	S	C+	SPY	+	n	+	+	+	+	+	n	+	n	+
								R	S	C–	SM	+	+	+	+	+	n	–	+	–	n	–
							+	Y	RF	C–	SM	+	+	+	–	+	n	–	+	–	n	–
								Y	RF	C–	SM	+	–	+	–	+	n	–	–	–	–	–
							Asn(AAC)[442]	Y	RF	C–	SM	+	+	+	+	n	n	–	+	–	+	n
+	+	+	+	+	+	+		Y	RF	C–	SM	+	+	+	–	+	n	–	+	–	+	n
							+	V	S	C+	SPY	+	+	+	–	+	n	+	–	±	n	±
								W/Y/Gy	RF	C–	SM	+	+	+	+	–	n	–	–	–	n	–
								Y	RF	C–	SM	+	+	+	–	+	n	–	+	+	+	n
								Y	RF	C–	SM	+	+	+	–	+	n	–	+	–	n	–
								Y	RF	C–	SM	+	+	+	–	+	+	–	+	n	n	–
								Y	RF	C–	SM	+	+	+	–	+	n	–	+	–	n	–
								Gy/Y	RF	C–	SM	+	+	+	–	+	n	–	+	–	n	+

(continued)

TABLE 272. List of *Streptomyces* species continued

Species names and groups[1]	No. in lists of species[2]	Type strain	Accession no.	Wil 83a[3]	Wil 89[4]	Käm 91[5]	Hat 03[6]	Lan 02[7]	Ful 95[8]	Och 95[9]	Kat 97[10]	Lab[11]	Sch[12]	Lan 04[13]	Lan 02[14]	Lan 04[15]	Lan 04[16]	Lan 04[17]	Lan 04[18]	Lan 04[19]	Lan 04[20]
S. gougerotii	214	DSM 40324, ATCC 10975, LMG 19888, ISP 5324, NBRC 3198	AB184742	A 1A	I 01	1-1 1-1					KA-D		Sch00		BENP	+	(k)	+		+	cl09
S. rutgersensis subsp. *rutgersensis*	427a	DSM 40077, ATCC 3350, LMG 8568, ISP 5077, NBRC 12819	AB184170	A 1A	I 01	1-1 1-1					KA-D		Sch00		BENP	+	(k)	+		+	cl09
S. intermedius	258	DSM 40372, ATCC 3329, LMG 19304, ISP 5372, NBRC 13049	AB184277	A 1A	I 01	1-1 1-1		La-03			KA-D		Sch10	Lan2-00	BENP +	+		+	cl14		cl08
S. indiaensis	255	DSM 43803, LMG 19961, NBRC 13964	AB184553										Sch00	Lan2-00	BENP	+		+		+	cl04
S. thermocarboxydus	473	DSM 44293	U94490										Sch00							+	
S. massasporeus	310	DSM 40035, ATCC 19785, LMG 19362, ISP 5035, NBRC 12796	AB184152	D SM	III 19	015 1-19		La-12		OC-III			Sch00	Lan2-00	BENP +	+		+	cl17	+	cl01
S. misionensis	323	DSM 40306, ATCC 14991, LMG 20063, ISP 5306, NRRL B-3230	EF178678	A 31	I 21	1-6 1-16							Sch00	Lan2-15	BENP	+		+		+	cl54
S. phaeoluteichromatogenes	370	NRRL B-5799	AJ391814																		
S. spectabilis	447	DSM 40512, NRRL 2494, LMG 5986, ISP 5512, NBRC 13424	AB184393									L3	Sch00	Lan2-00	BENP	+		+		+	+
S. cinereospinus	112	DSM 41470, LMG 20464, NBRC 15397	AB184648										Sch00	Lan2-00	BENP	+		+		+	cl22
S. coeruleofuscus	127	DSM 40144, ATCC 23618, LMG 20482, ISP 5144, NRRL B-5417	DQ026668	A 18	I 11	009 1-19						L2	Sch00	Lan2-00	BENP	+		+			cl01
S. chromofuscus	107	DSM 40273, ATCC 23896, LMG 19317, ISP 5273, NBRC 12851	AB184194	A 15	I 08	006 1-18		La-06		OC-III			Sch00	Lan2-00	BENP +	+		+	cl10	+	cl09
S. scopiformis	436	DSM 41825, LMG 20251, NBRC 100244	AB249927																	+	
S. spinoverrucosus	451	DSM 41648, LMG 20321, NBRC 14228	AB184578										Sch00	Lan2-00	BENP	+		+		+	+
Most closely related to group S. mexicanus et rel.:																					
S. thermospinosisporus	479	DSM 41779, NBRC 100043, JCM 11756, AT10	AF333113																		
S. thermodiastaticus	475	DSM 40573, ATCC 27472, LMG 20302, ISP 5573, JCM 4840	AB018095	A 1C	I 03	006 1-18							Sch00	Lan2-00	BENP	+		+		+	cl04
S. thermocarboxydovorans	472	DSM 44296, LMG 19860	U94489										Sch00		BENP	+		+			cl04
S. thermoviolaceus subsp. *apingens*	480b	DSM 41392, LMG 20307	Z68095										Sch00		BENP	+		+		+	cl03
S. thermoviolaceus subsp. *thermoviolaceus*	480a	DSM 40443, ATCC 19283, LMG 19359, ISP 5443	Z68096	C 45	II 13	004 006		La-13					Sch00		BENP +	+		+	cl11	+	cl03

(continued)

TABLE 272. (continued)

								Morphological characters[24]				Physiological tests[25]										
Guo 08[21] 16S rRNA	Guo 08[21] *atpD*	Guo 08[21] *gyrB*	Guo 08[21] *recA*	Guo 08[21] *rpoB*	Guo 08[21] *trpB*	Guo 08[21] concat.	Kim 04[23] *rpoB*	I	II	III	IV	I	II	III	IV	V	VI	VII	VIII	IX	X	XI
								Y	RF	C–	SM	+	–	+	–	n	n	n	n	n	n	n
								Y	RF	C–	SM	+	+	+	–	+	+	+	+	–	+	–
								Y	RF	C–	SM	+	+	+	–	+	+	+	+	–	+	+
								Gy	S	C+	SM	+	+	+	–	+	n	–	+	+	–	+
								Gy	RA	C–	WTY	+	–	n	n	+	n	–	+	+	n	–
								Gy	S	C+	SM	+	+	+	+	+	+	+	+	+	+	+
								Gy	S	C–	SM	+	+	+	+	+	+	+	+	+	–	–
							Asn (AAC)[442]	R	RF	C+	SM	+	+	–	–	+	+	+	+	+	–	–
								Gy	S	C–	SPY	+	–	–	+	+	n	+	n	–	n	n
								B	S	C+	SPY	+	+	+	+	+	n	+	+	+	n	+
								Gy	S	C+	SPY	+	+	+	+	+	n	+	+	+	n	–
								Gy	RF		SPY	+	+	+	+	+	+	–	–	+	n	+
								G	S	C+	SPY/ WTY	+	+	+	+	+	n	+	+	+	n	+
								Gy	RF	C–	SPY							+	+	+		+
								Gy	S	C–	WTY/ SPY	+	+	+	+	+	n	+	+	+	n	–
								Gy	RF	C–	SM	n	n	n	n	+	n	n	n	n	n	n
								Gy	S	??	WTY	n	n	n	n	n	n	n	n	n	n	n
								Gy	S	C+	SM	n	n	n	n	n	n	n	n	n	n	n

(continued)

TABLE 272. List of *Streptomyces* species continued

Species names and groups[1]	No. in lists of species[2]	Type strain	Accession no.	Wil 83a[3]	Wil 89[4]	Käm 91[5]	Hat 03[6]	Lan 02[7]	Ful 95[8]	Och 95[9]	Kat 97[10]	Lab[11]	Sch[12]	Lan 04[13]	Lan 02[14]	Lan 04[15]	Lan 04[16]	Lan 04[17]	Lan 04[18]	Lan 04[19]	Lan 04[20]
S. nodosus	343	DSM 40109, ATCC 14899, LMG 19430, ISP 5109	AF114033	A 35	II 08	006 1-11							Sch00	Lan2-00	BENP +	+		+	cl11	+	cl04
S. viridosporus	516	DSM 40243, ATCC 27479, LMG 20278, ISP 5243, NRRL 2414	DQ442556	A 15	I 08	006 1-18							Sch00		BENP	+		+			+
Group S. mexicanus et rel.:																					
S. thermogriseus	476	DSM 41756, LMG 20532, NBRC 100772	AB249980										Sch00	Lan2-07	BENP	+		+			cl32
S. thermovulgaris	481	DSM 40444, ATCC 19284, LMG 19342, ISP 5444	Z68094	A 36	II 09	021 002		La-00		OC-non			Sch00	Lan2-07	BENP +	+		+	cl10	+	cl32
S. thermoalcalitolerans	470	DSM 41741, LMG 19858, NBRC 16322	AB249909										Sch00	Lan2-00	BENP	+		+			+
S. mexicanus	317	DSM 41796, NBRC 100915	AB249966																		
S. thermocoprophilus	474	DSM 41700, LMG 19857, B19	AJ007402										Sch00	Lan2-00	BENP	+		+		+	+
Most closely related to group S. mexicanus et rel.:																					
S. bangladeshensis	68	NRRL B-24326, LMG 22738, AAB-4	AY750056																		
S. rameus	399	DSM 41685, LMG 20326, KCTC 9767	AY999821										Sch33		BENP	+		+		+	cl02
S. griseosporeus	234	DSM 40562, ATCC 27435, LMG 19947, ISP 5562, NBRC 13458	AB184419	A 23	I 20	1-7 1-19							Sch00	Lan2-00	BENP	+		+		+	+
S. achromogenes subsp. *rubradiris*	4b	DSM 40789, NRRL 3061, LMG 20388, KCTC 9742	AY999846		I 12	028 009							Sch00	Lan2-00	BENP	+		+		+	+
S. glomeratus	210	DSM 41457, LMG 19903, NBRC 15898	AB249917											Lan2-00	BENP	+		+		+	cl09
S. eurythermus	163	DSM 40014, ATCC 14975, LMG 20510, ISP 5014	D63870	A 23	I 20	1-5 009							Sch00	Lan2-00	BENP	+		+			cl08
S. nogalater	344	DSM 40546, ATCC 27451, LMG 19338, ISP 5546, JCM 4799	AB045886	A 34	III 06	1-5 009		La-14		OC-III			Sch00	Lan2-00	BENP +	+		+	cl14	+	cl04
S. fragilis	190	DSM 40044, ATCC 23908, LMG 19874, ISP 5044, NRRL 2424	AY999917	G SM	III 22	078 058				OC-III			Sch38	Lan2-00	BENP	+		+		+	+
Group S. erythrogriseus et rel.:																					
S. erythrogriseus	160	DSM 40116, ATCC 27427, LMG 19406, ISP 5116	AJ781328		IV 04 (red series)	074 1-27		La-15					Sch35	Lan2-25	BENP +	+		+	cl17	+	cl01
S. labedae	273	DSM 41446, LMG 19956, NBRC 15864	AB184704										Sch35	Lan2-25	BENP	+		+		+	cl01

(continued)

TABLE 272. (continued)

								Morphological characters[24]				Physiological tests[25]										
Guo 08[21] 16S rRNA	Guo 08[21] *atpD*	Guo 08[21] *gyrB*	Guo 08[21] *recA*	Guo 08[21] *rpoB*	Guo 08[21] *trpB*	Guo 08[21] concat.	Kim 04[23] *rpoB*	I	II	III	IV	I	II	III	IV	V	VI	VII	VIII	IX	X	XI
							+	Gy	S	C–	SM	+	+	–	+	+	n	–	+	+	n	–
								G	S	C–	SPY	+	+	+	+	+	+	–	+	+	–	±
										C–	SM	±	±		+	±		±	+	–		
								Gy	S	C–	SM	+	+	+	+	+	n	+	+	+	n	+
								Gy	n	C–	WTY	+	+	+	+	+	+	–	+	+	n	+
								Gy	RF	C–	SM	+	+	+	–	+	+	+	+	–	n	–
								Gy	RF	C+	SM	+	+	+	n	+	n	–	+	n	n	–
								Y–G	RF	C+	SM	+	–	+	+	+	n	+	+	+	+	n
							+	Gy	S	C+	SM	n	+	+	–	+	+	+	+	–	+	+
								Gy	S	C+	SM	+	+	+	+	+	+	+	+	+	+	+
								Gy	S	C+	SM	+	+	+	+	+	+	+	+	±	±	+
								Gy	S	C+	SM		+	+	+	+		+	+			
								Gy	S	C+	SM	+	+	+	–	+	+	+	+	–	–	+
								Gy	S	C–	SM	+	+	+	+	+	+	+	+	+	–	–
								R	S	C–	SM	+	+	+	–	–	+	–	–	–	–	±
								Gy/ R/W	S	C–	SPY/SM	+	+	+	+	+	n	–	+	+	n	–
								Gy	S	C–	SPY	+	+	–	+	+	+	–	+	+	–	–

(continued)

TABLE 272. List of *Streptomyces* species continued

Species names and groups[1]	No. in lists of species[2]	Type strain	Accession no.	Wil 83a[3]	Wil 89[4]	Käm 91[5]	Hat 03[6]	Lan 02[7]	Ful 95[8]	Och 95[9]	Kat 97[10]	Lab[11]	Sch[12]	Lan 04[13]	Lan 02[14]	Lan 04[15]	Lan 04[16]	Lan 04[17]	Lan 04[18]	Lan 04[19]	Lan 04[20]
S. griseoincarnatus	225	DSM 40274, ATCC 23623, LMG 19316, ISP 5274	AJ781321	A 13	II 03	006 1-18		La-15					Sch35	Lan2-25	BENP +	+		+	cl17	+	cl01
S. variabilis	490	DSM 40179, ATCC 19930, LMG 20270, ISP 5179, NRRL B-3984	DQ442551	A 12	I 07	006 1-18					KA-F		Sch35	Lan2-25	BENP	+		+			cl01
Most closely related to group S. erythrogriseus et rel.:																					
S. althioticus	29	DSM 40092, ATCC 19724, LMG 20408, ISP 5092, KCTC 9752	AY999808	A 12	I 07	006 1-18							Sch00	Lan2-00	BENP	+		+			+
S. matensis	311	DSM 40188, ATCC 23935, LMG 20055, ISP 5188, NBRC 12889	AB184221	A 12	I 07	006 1-18			FU-1				Sch00	Lan2-00	BENP	+		+		+	cl01
S. griseorubens	231	DSM 40160, ATCC 19909, LMG 19931, ISP 5160, NBRC 12780	AB184139	A 12	I 07	006 1-18					KA-F		Sch30	Lan2-00	BENP	+		+			cl01
S. viridochromogenes	514	DSM 40110, ATCC 14920, LMG 20260, ISP 5110, NRRL B-1511	DQ442555	A 27	III 04	009 1-19				OC-III		L2	Sch00	Lan2-00	BENP	+		+		+	cl54
S. iakyrus	254	DSM 40482, ATCC 15375, LMG 19942, ISP 5482, NBRC 13401	AB184877	A 18	I 11	009 1-19						L2	Sch00	Lan2-00	BENP	+		+		+	cl01
S. violaceochromogenes	498	DSM 40181, LMG 20271, IFO 13100	AY999867												BENP	+		+		+	cl01
S. collinus	131	DSM 40129, ATCC 19743, LMG 20486, ISP 5129, NBRC 12759	AB184123	A 18	I 11	009 1-19			FU-1			L2	Sch39	Lan2-00	BENP	+		+		+	+
S. malachitofuscus	306	DSM 40332, ATCC 25471, LMG 20067, ISP 5332, NBRC 13059	AB184282			006 1-18							Sch00	Lan2-00	BENP +	+		+		+	cl04
S. paradoxus	361	DSM 43350, LMG 20523, NBRC 14887	AB184628										Sch00	Lan2-00	BENP	+		+		+	cl01
S. griseoflavus	223	DSM 40456, ATCC 25456, LMG 19344, ISP 5456	AJ781322	A 37	I 17	006 1-18		La-04		OC-non			Sch00	Lan2-00	BENP +	+		+			cl01
S. flaveolus	173	DSM 40061, ATCC 3319, LMG 19328, ISP 5061, NBRC 3408	AB184764	A 24	II 05	1-6 1-13		La-12		OC-III			Sch00	Lan2-00	BENP +	+		+	cl17	+	cl59
S. glaucescens	204	DSM 40155, ATCC 23622, LMG 19330, ISP 5155, NBRC 12774	AB184843	A 28	III 05	006 1-10		La-16		OC-III		L2	Sch00	Lan2-00	BENP +	+		+		+	cl57
S. pharetrae	374	DSM 41856, NRRL B-24333, CZA14	AY699792																		
S. malachitospinus	307	IFO 101004, NBRC 101004	AB249954																		

(continued)

TABLE 272. (continued)

								Morphological characters[24]				Physiological tests[25]										
Guo 08[21] 16S rRNA	Guo 08[21] *atpD*	Guo 08[21] *gyrB*	Guo 08[21] *recA*	Guo 08[21] *rpoB*	Guo 08[21] *trpB*	Guo 08[21] concat.	Kim 04[23] *rpoB*	I	II	III	IV	I	II	III	IV	V	VI	VII	VIII	IX	X	XI
								Gy	S	C–	SPY	+	+	+	+	+	n	–	+	±	n	+
								Gy/R	S/RA	C–	SPY	+	+	+	+	+	n	–	+	+	n	–
								Gy	S	C–	SPY	+	+	+	+	+	+	–	+	+	n	±
								Gy	S	C–	SPY	+	+	+	+	+	+	–	+	+	n	–
								Gy	S	C–	SPY	+	+	±	+	+	n	–	+	±	n	–
								B	S	C+	SPY	+	+	+	+	+	+	+	+	+	+	+
								Gy	S	C+	SPY	+	+	+	+	+	+	+	+	+	+	n
								Gy	S	C+	SM	+	+	+	+	+	n	+	+	+	n	+
							Group A18	Gy	S	C+	SM	+	+	+	+	+	n	+	+	+	n	+
								Gy	S	C+	SPY	+	+	+	+	+	n	–	+	+	n	+
								Gy	RA	C+	SM	+	+	+	+	+	+	+	+	+	n	+
								Gy	S	C–	SPY	+	+	+	+	+	n	–	+	+	n	–
								Gy	S	C–	H	+	+	+	+	+	+	+	+	+	+	+
								B/G	S	C+	H	+	+	+	+	+	n	–	+	+	n	–
								Gy		C+	H			+	+				+			
								Gy	S	C–	SPY	+		+			+					

(continued)

TABLE 272. List of *Streptomyces* species continued

Species names and groups[1]	No. in lists of species[2]	Type strain	Accession no.	Wil 83a[3]	Wil 89[4]	Käm 91[5]	Hat 03[6]	Lan 02[7]	Ful 95[8]	Och 95[9]	Kat 97[10]	Lab[11]	Sch[12]	Lan 04[13]	Lan 02[14]	Lan 04[15]	Lan 04[16]	Lan 04[17]	Lan 04[18]	Lan 04[19]	Lan 04[20]
S. parvulus	363	DSM 40048, ATCC 12434, ISP 5048, NBRC 13193	AB184326	A 12		006 1-18		La-24					Sch00	Lan2-00	BENP +	+		+	cl17		cl06
S. tendae	468	DSM 40101, ATCC 19812, LMG 19314, ISP 5101	D63873	A 12	I 07	006 1-18		La-14			KA-E		Sch00	Lan2-00	BENP +	+		+	+	+	cl06
S. violaceorubidus	502	DSM 41478, LMG 20319	AJ781374										Sch00	Lan2-00	BENP	+		+			cl08
S. albaduncus	11	DSM 40478, ATCC 14698, LMG 20392, ISP 5478, JCM 4715	AY999757		IV 02 (gray series)	006 1-10							Sch00	Lan2-00	BENP	+		+		+	cl05
S. griseoloalbus	226	DSM 40468, ATCC 23624, LMG 21308, ISP 5468, NBRC 13046	AB184275		IV 05 (yellow series	)017 007							Sch00	Lan2-00	BENP	+		+		+	cl22
S. heliomycini	245	DSM 41690, IFO 15899, LMG 19960, NBRC 15899	AB184712										Sch00	Lan2-00	BENP	+		+			cl05
S. ambofaciens	31	DSM 40053, ATCC 23877, LMG 20409, ISP 5053	M27245	A 23	I 20	006 1-18			FU-6				Sch00	Lan2-00	BENP	+		+		+	+
Most closely related to group S. coelescens et rel.:																					
S. rubrogriseus	426	DSM 41477, LMG 20318, NBRC 15455	AB184681										Sch00	Lan2-00	BENP	+		+			cl22
S. tricolor	485	DSM 41704, LMG 20328, NBRC 15461	AB184687										Sch33	Lan2-21	BENP	+	(b)	+			cl02
S. lienomycini	285	DSM 41475, LMG 20091	AJ781353										Sch00	Lan2-00	BENP	+		+		+	cl22
S. anthocyanicus	34	DSM 41422, LMG 20411, NBRC 14892	AB184631		IV 03 (gray series)	013 1-19							Sch00	Lan2-04	BENP	+		+		+	cl22
S. olivaceus	352	DSM 40072, ATCC 3335, LMG 19394, ISP 5072, NBRC 3200	AB184743	A 1C	I 03	042 014		La-23	FU-1				Sch00	Lan2-00	BENP +	+		+	cl16	+	cl13
S. pactum	360	DSM 40530, ATCC 27456, LMG 19357, ISP 5530, NBRC 13433	AB184398	C 44	II 12	22-4 035		La-11		OC-II			Sch00	Lan2-00	BENP +	+		+			+
Group S. coelescens et rel.:																					
S. coelescens	123	DSM 40421, ATCC 19830, LMG 20479, ISP 5421, ICSSB 1021	AF503496	A 21	I 14	006 1-18							Sch07		BENP	+	(k)	+		+	cl22
S. humiferus	251	DSM 43030, LMG 20519	AF503491										Sch08		BENP	+	(k)	+			cl22
S. violaceolatus	499	DSM 40438, ATCC 19847, LMG 20293, ISP 5438, ICSSB 1022	AF503497	A 21	I 14	006 1-18							Sch08		BENP	+	(k)	+		+	cl22
S. violaceoruber	501	DSM 40049, ATCC 14980, LMG 20256, ISP 5049, ICSSB 1016	AF503492		IV 34 (gray series)	069 1-26							Sch07		BENP	+	(d) (k)	+		+	cl22

(continued)

TABLE 272. (continued)

								Morphological characters[24]				Physiological tests[25]										
Guo 08[21] 16S rRNA	Guo 08[21] *atpD*	Guo 08[21] *gyrB*	Guo 08[21] *recA*	Guo 08[21] *rpoB*	Guo 08[21] *trpB*	Guo 08[21] concat.	Kim 04[23] *rpoB*	I	II	III	IV	I	II	III	IV	V	VI	VII	VIII	IX	X	XI
								Gy	S	C–	SM	+	+	+	+	+	n	–	+	+	+	+
								Gy	S	C–	SM	+	+	+	+	+	n	–	+	+	n	+
								Gy/W	S	C–	SM	+	+	+	+	+		+	+	+		
								Gy	S	C–	SPY	+	+	+	+	+	+	+	+	+	+	±
								Y	RF	C–	SM	+	+	+	+	+	n	n	+	+	n	+
								Gy	S	C–	WTY/ SPY/H	+	+		+	+			+	+		
								Gy	S	C–	SM	+	+	+	+	+	n	–	+	+	n	+
								Gy/R	S	C–	SM											
								Gy	S	C–	SM	n	n	n	n	n	n	n	n	n	n	n
								Gy	S	C+	SM		+	+	+	+		+	+	+		
								Gy	S	C–	SM	+	n	+	+	+	+	–	–	+	n	–
								Gy	S	C–	SM	+	+	+	+	+	+	+	+	+	–	–
								Gy	S	C–	H	+	–	–	–	–	+	–	–	–	–	–
								Gy	S	C–	SM	+	n	+	n	n	n	n	–	n	n	n
								Gy	S	C–	SM	+	+	+	+	+	+	+	+	+	n	–
								Gy	S	C–	SM	+	+	+	+	+	n	+	+	+	n	+
								Gy	S	C–	SM	+	+	+	+	+	+	–	+	+	+	–

(continued)

TABLE 272. List of *Streptomyces* species continued

Species names and groups[1]	No. in lists of species[2]	Type strain	Accession no.	Wil 83a[3]	Wil 89[4]	Käm 91[5]	Hat 03[6]	Lan 02[7]	Ful 95[8]	Och 95[9]	Kat 97[10]	Lab[11]	Sch[12]	Lan 04[13]	Lan 02[14]	Lan 04[15]	Lan 04[16]	Lan 04[17]	Lan 04[18]	Lan 04[19]	Lan 04[20]
Most closely related to group S. coelescens et rel.:																					
S. coelicoflavus	124	DSM 41471, LMG 20480, NBRC 15399	AB184650										Sch00	Lan2-00	BENP	+		+			cl23
S. diastaticus subsp. *ardesiacus*	144b	DSM 40934, IFO 15402, LMG 20497, NRRL B-1773	DQ026631												BENP	+		+		+	+
Most closely related to group S. coeruleorubidus et rel.:																					
S. lomondensis	291	DSM 41428, LMG 20088, NBRC 15426	AB184673		IV 03 (blue series)	009 1-19							Sch00	Lan2-00	BENP	+		+			cl04
S. lusitanus	299	DSM 40568, ATCC 15842, LMG 20078, ISP 5568, NBRC 13464	AB184424	C 44	II 12	006 1-18							Sch00	Lan2-00	BENP	+		+		+	-
S. purpurascens	394	DSM 40310, ATCC 25489, LMG 20526, ISP 5310	AB045888	A 18	I 11	009 1-19						L2	Sch00	Lan2-00	BENP	+	(g)	+		+	cl01
S. bellus	70	DSM 40185, ATCC 14925, LMG 19401, ISP 5185, NBRC 12844	AB184849	A 18	I 11	061 1-22		La1-02				L2	Sch00		BENP +	+		+	cl17	+	cl01
S. coerulescens	130	DSM 40146, ATCC 19896, LMG 8590, ISP 5146	AY999720	A 18	I 11	009 1-19						L2	Sch00	Lan2-00	BENP	+		+		+	cl01
S. speibonae	448	DSM 41797, ATCC BAA-411, PK-Blue	AF452714																	+	
S. longispororuber	293	DSM 40599, ATCC 27443, LMG 20082, ISP 5599, NBRC 13488	AB184440	A 10	I 06	033 1-33					KA-F		Sch00	Lan2-00	BENP	+		+			cl01
Group S. coeruleorubidus et rel.:																					
S. albogriseolus	19	DSM 40003, ATCC 23875, LMG 20395, ISP 5003, NRRL B-1305	AJ494865	A 12	I 07	006 1-18					KA-F		Sch00	Lan2-00	BENP	+		+		+	+
S. viridodiastaticus	515	DSM 40249, ATCC 25518, LMG 20279, ISP 5249, NBRC 13106	AY999852		IV 36 (gray series)	006 1-18							Sch00	Lan2-00	BENP	+		+			+
S. coeruleorubidus	129	DSM 40145, ATCC 13740, LMG 20484, ISP 5145	AY999719	A 18	I 11	009 1-19						L2	Sch00		BENP	+	(g)	+			cl01
Group S. aurantiogriseus et rel.:																					
S. coelicolor	125	DSM 40233, ATCC 23899, LMG 8571, ISP 5233, NRRL B-2812	DQ442496	A 1A	I 01	1-1 1-1			FU-1		KA-D		Sch09	Lan2-00	BENP	+	(d) (k)	+		+	cl08
S. griseoviridis	237	DSM 40229, ATCC 23920, LMG 19321, ISP 5229, KCTC 9780	AY999807	A 17	I 10	006 010		La-06		OC-III		L3	Sch40	Lan2-11	BENP +	+	(h)	+		+	cl37

(continued)

TABLE 272. (continued)

Guo 08[21] 16S rRNA	Guo 08[21] *atpD*	Guo 08[21] *gyrB*	Guo 08[21] *recA*	Guo 08[21] *rpoB*	Guo 08[21] *trpB*	Guo 08[21] concat.	Kim 04[23] *rpoB*	Morphological characters[24] I	II	III	IV	Physiological tests[25] I	II	III	IV	V	VI	VII	VIII	IX	X	XI
								n	S	C–	SM	+	+	+	+	+	n	n	+	+	n	n
								Gy	S	C–	SM	+	+	+	+	n	n	n	n	n	n	n
								R/B	RF/S	C+	WTY/ SPY	+	+	+	+	+	+	+	+	+	n	+
								Gy	S	C–	SM	+	–	±	–	+	n	–	–	±	–	+
								R	S	C+	SPY	+	+	+	+	+	n	+	+	+	+	+
								B	S	C+	SPY	+	+	+	+	+	n	+	+	+	n	+
								B	S	C+	SPY	+	+	+	+	+	n	+	+	+	n	+
								Gy	S	C+	H		+	+	+	+	+	–	+	+	–	+
								W	S	C+	SM	n	n	n	n	n	n	n	n	n	n	n
							Asn (AAC)^{442}	Gy	S	C–	SM	+	+	+	+	+	+	+	+	+	+	+
								Gy	S	C–	SPY	+	+	+	+	+	n	n	+	+	n	n
							+	B	S	C+	SPY	+	+	+	+	+	+	+	+	+	+	+
								Y	RF	C–	SM	+	+	+	–	+	n	–	+	–	n	n
							+	R	S	C–	SM	+	+	+	+	+	+	–	+	–	n	–

(continued)

TABLE 272. List of *Streptomyces* species continued

Species names and groups[1]	No. in lists of species[2]	Type strain	Accession no.	Wil 83a[3]	Wil 89[4]	Käm 91[5]	Hat 03[6]	Lan 02[7]	Ful 95[8]	Och 95[9]	Kat 97[10]	Lab[11]	Sch[12]	Lan 04[13]	Lan 02[14]	Lan 04[15]	Lan 04[16]	Lan 04[17]	Lan 04[18]	Lan 04[19]	Lan 04[20]
S. aurantiogriseus	50	DSM 40138, ATCC 23883, LMG 19298, NRRL-ISP 5138, NRRL B-5416	AY999793																		
Most closely related to group S. aurantiogriseus et rel.:																					
S. griseoaurantiacus	219	DSM 40430, ATCC 19840, LMG 21045, ISP 5430, NBRC 15440	AB184676	A 12	I 07	1-7 1-15							Sch00	Lan2-00	BENP	+		+		+	+
S. jietaisiensis	263	AS 4.1859, JCM 12279, FXJ46	AY314783																		
Group S. coeruleoprunus et rel.:																					
S. coeruleoprunus	128	DSM 41472, LMG 20483, NBRC 15400	AB184651										Sch00	Lan2-00	BENP	+		+		+	cl33
S. somaliensis	444	DSM 40738	AJ007403										Sch00	Lan2-00						+	
S. fradiae	189	DSM 40063, ATCC 10745, LMG 19371, ISP 5063, NRRL B-1195	DQ026630	G 68	II 18	22-5 039		La-00		OC-I			Sch12	Lan2-27	BENP +	+	(b)	+	cl09	+	cl45
Most closely related to group S. coeruleoprunus et rel.:																					
S. bluensis	74	DSM 40564, ATCC 27420, LMG 5969, ISP 5564	X79324	A 39	II 10	052 017						L2	Sch00	Lan2-00	BENP	+		+		+	cl07
Not closely related to one of the groups:																					
S. variegatus	491	DSM 41464, LMG 20315	AJ781371										Sch00	Lan2-00	BENP	+		+		+	+
S. fulvissimus	191	DSM 40593, ATCC 27431, LMG 19310, ISP 5593, NBRC 13482	AB184434	A 10		034 1-33		La-00		OC-IV		L3	Sch00	Lan2-00	BENP +	+	(h)	+	cl14		cl43
S. aureoverticillatus	56	DSM 40080, ATCC 15854, LMG 20426, ISP 5080, NRRL B-3326	AY999774	A 10	I 06	033 1-33						L3	Sch00	Lan2-00	BENP	+		+		+	+
S. flavofungini	178	DSM 40366, ATCC 27430, LMG 21799, ISP 5366, NBRC 13371	AB184359	B 42		033 1-33							Sch00	Lan2-00	BENP	+		+			+
S. alboflavus	18	DSM 40045, ATCC 12626, LMG 21038, ISP 5045, NRRL B-2373	EF178699	E 54	III 20	033 1-33		La-00		OC-IV			Sch00	Lan2-00	BENP +	+		+			cl43
S. aculeolatus	7	DSM 41644, LMG 19906, NBRC 14824	AB184624										Sch00	Lan2-00	BENP	+		+			+
S. synnematoformans	464	DSM 41902, CGMCC 4.2055, S155	EF121313																		
S. hebeiensis	244	DSM 41837, CCTCC AA-203005, YIM 001	AY277529																	+	

(continued)

TABLE 272. (continued)

								Morphological characters[24]				Physiological tests[25]										
Guo 08[21] 16S rRNA	Guo 08[21] *atpD*	Guo 08[21] *gyrB*	Guo 08[21] *recA*	Guo 08[21] *rpoB*	Guo 08[21] *trpB*	Guo 08[21] concat.	Kim 04[23] *rpoB*	I	II	III	IV	I	II	III	IV	V	VI	VII	VIII	IX	X	XI
								Gy	S	C+	SM	+	+	+	+	+	n	+	+	+	n	+
								Gy	S	C–	WTY	+	+	+	+	n	n	n	n	n	n	n
									RF	C–	SM	+	+				+		+		+	
								B	RF	C–	SM	+	+	+	+	+	–	–	n	+	n	n
							+	Y	RF	C–	SM	+	+	+	–	+	n	–	+	–	n	+
							+	R	S	C–	SM	+	+	+	–	–	+	+	–	–	–	–
								B	S	C–	SPY	+	+	+	+	+	+	+	+	+	+	+
								W/R	S	C–	SM		+	+	–	+		–				
								Y	RF	C+	SM	+	+	+	–	+	n	–	+	+	+	n
								R	S	C–	SM	+	+	+	–	+	+	+	+	+	+	n
								W/Y	RF	C–	SM	+	+	+	?	+	n	+	+	+	n	–
							+	Y	RF	C+	SM	+	+	+	–	+	+	+	+	+	–	+
								W,Y,R	S	n	WTY–SPY	+	+	+	+	+	n	+	+	–	n	–
								Gy–R	RF	C+	SM	+	n	n	n	+	+	n	n	n	–	–
									RF	C+	WTY		+				+					

(continued)

TABLE 272. List of *Streptomyces* species continued

Species names and groups[1]	No. in lists of species[2]	Type strain	Accession no.	Wil 83a[3]	Wil 89[4]	Käm 91[5]	Hat 03[6]	Lan 02[7]	Ful 95[8]	Och 95[9]	Kat 97[10]	Lab[11]	Sch[12]	Lan 04[13]	Lan 02[14]	Lan 04[15]	Lan 04[16]	Lan 04[17]	Lan 04[18]	Lan 04[19]	Lan 04[20]
Group S. carpaticus et rel.:																					
S. hainanensis	240	DSM 41900, CCTCC AA-205017, YIM 47672	AM398645																		
S. specialis	446	DSM 41924, CCM 7499, GW 41-1564	AM934703																		
S. carpaticus	92	DSM 41468, ATCC 43678, LMG 20448, NRRL B-16359	DQ442494										Sch00	Lan2-00	BENP	+		+			+
S. cheonanensis	104	NBRC 100940, VC-A46	AY822606																		
Most closely related to group S. carpaticus et rel.:																					
S. sulfonofaciens	462	DSM 41679, ATCC 31892, LMG 20325, NBRC 14260	AB249974										Sch00	Lan2-00	BENP	+		+		+	+
S. sodiiphilus	443	CCTCC AA-203015, JCM 13581, YIM 80305	AY236339																		
Not closely related to one of the groups:																					
S. scabrisporus	434	NBRC 100760, KM-4927	AB030585																		
S. gardneri	198	DSM 40064, ATCC 9604, LMG 19876, ISP 5064, NBRC 3385	AB184754	A 04		002 1-07			FU-23		KA-C		Sch00	Lan2-00	BENP	+		+		+	cl44
S. flavidofuscus	175	DSM 41473, ATCC 43683, NRRL B-16366	AY999914										Sch00	Lan2-00	BENP	+		+		+	-
Regarded as later heterotypic synonym of Streptomyces abikoensis (for references, see list of type strains):																					
S. luteoverticillatus	303	DSM 40038, ATCC 23933, LMG 20045, ISP 5038	AB184803	F 55	Sv. 03	22-1 040	Ha1		FU-12b				Sch00	Lan2-00	BENP	+	(j)	+		+	cl12
S. olivoreticuli subsp. *olivoreticuli*	355	DSM 40105, LMG 20050, ISP 5105	AB184853				Ha1						Sch00	Lan2-00	BENP	+	(j)	+		+	cl12
S. parvisporogenes	362	DSM 40473, ATCC 12568, LMG 20072, ISP 5473	AB249913		Sv. 02	22-1 040	Ha1						Sch00	Lan2-00	BENP	+	(j)	+			cl12
Regarded as later heterotypic synonym of Streptomyces anulatus (for references, see list of type strains):																					
S. chrysomallus subsp. *chrysomallus*	109a	DSM 40128, ATCC 11523, LMG 20459, ISP 5128	AB184644	A 1B		1-3 1-2			FU-22		KA-B		Sch01	Lan2-18	BENP	+	(a)	+			cl23
S. citreofluorescens	120	DSM 40265, ATCC 15858, LMG 20475, ISP 5265	AB184195	A 1B	I 02	1-3 1-2			FU-19b		KA-B		Sch01	Lan2-18	BENP	+	(a)	+		+	cl23
S. fluorescens	188	DSM 40203, ATCC 15860, LMG 8579, ISP 5203	AB184199	A 1B	I 02	1-3 1-2					KA-B		Sch01	Lab2-18	BENP	+	(a)	+		+	cl23

(continued)

TABLE 272. (continued)

								Morphological characters[24]				Physiological tests[25]										
Guo 08[21] 16S rRNA	Guo 08[21] *atpD*	Guo 08[21] *gyrB*	Guo 08[21] *recA*	Guo 08[21] *rpoB*	Guo 08[21] *trpB*	Guo 08[21] concat.	Kim 04[23] *rpoB*	I	II	III	IV	I	II	III	IV	V	VI	VII	VIII	IX	X	XI
								W	S	C+	SM	+	–	–	–	–	–	–	–	–	–	–
								W	S	C+	n	+	–	n	–	–	–	n	n	+	–	+
								Gy	S	C–	SM	+	+	+	+	+	n	+	+	+	n	n
								Gy	RF	C+	SM	n	+	+	+	+	n	+	+	+	–	+
								R/V	RF		SM	+	+	+	+	+	n	–	+	–	n	+
									RF	C–		–	–	–	+	–	–	–	–	–	n	–
								Gy	S	C–	RU	+	+	–	+	+	n	–	–	+	–	–
								Gy	RF	C+	SM	+	+	+	+	+	+	+	–	–	+	n
+	+	+	+	+	+	+		Y	S	C+	SM	+	n	+	+	+	n	+	+	+	n	n
								Lu	VE	C+	SM	+	–	–	–	+	n	±	+	–	n	+
								Lu	VE	C+	SM	+	–	–	–	–	n	±	–	±	n	+
								Ar	VE	C+	SM	+	–	–	–	±	n	–	–	±	n	±
								Y	RF	C–	SM	+	+	+	+	+	+	–	+	–	+	–
								Y	RF	C–	SM	+	+	+	+	+	+	–	+	–	–	–
								Y	RF	C–	SM	+	+	+	–	+	+	–	+	–	+	–

(continued)

TABLE 272. List of *Streptomyces* species continued

Species names and groups[1]	No. in lists of species[2]	Type strain	Accession no.	Wil 83a[3]	Wil 89[4]	Käm 91[5]	Hat 03[6]	Lan 02[7]	Ful 95[8]	Och 95[9]	Kat 97[10]	Lab[11]	Sch[12]	Lan 04[13]	Lan 02[14]	Lan 04[15]	Lan 04[16]	Lan 04[17]	Lan 04[18]	Lan 04[19]	Lan 04[20]
Regarded as later heterotypic synonym of Streptomyces avermitilis (for references, see list of type strains):																					
S. avermectinius	59																				
Regarded as later heterotypic synonym of Streptomyces cacaoi (for references, see list of type strains):																					
S. aminophilus	32	DSM 40186, ATCC 14961, LMG 19319, ISP 5186	AB184183	A 16	I 09	031 1-34		La-05					Sch00	Lan2-17	BENP cl05	+	(c)	+	cl08	+	cl36
Regarded as later heterotypic synonym of Streptomyces caeruleus (for references, see list of type strains):																					
S. niveus	341	DSM 40088, ATCC 19793, LMG 19395, ISP 5088	AB184160	A 1B	I 02	043 013		La-19					Sch11	Lan2-14	BENP cl19	+	(c)	+	cl09	+	cl47
S. spheroides	450	DSM 40292, ATCC 23965, LMG 19392, ISP 5292	EF178698	A 1B	I 02	040 048		La-19					Sch11	Lan2-00	BENP cl19	+	(c)	+	cl09		cl47
Regarded as later heterotypic synonym of Streptomyces cinnamoneus (for references, see list of type strains):																					
S. griseoverticillatus	236	DSM 40507, ATCC 27436, LMG 19944, ISP 5507	AB184862	F 58		22-1 040	Ha4						Sch17	Lan2-00	BENP	+	(j)	+		+	cl17
S. hachijoensis	240	DSM 40114, ATCC 19769, LMG 19928, ISP 5114	AB184141	F 55	Sv. 04	22-1 040	Ha4		FU-NC			L4	Sch00	Lan2-00	BENP	+	(j)	+		+	cl13
Regarded as later heterotypic synonym of Streptomyces chibaensis (for references, see list of type strains):																					
S. chibaensis	105	DSM 40220, ATCC 23895, LMG 20456, ISP 5220	AB184193	A 24	II 05	009 1-19							Sch04	Lan2-26	BENP	+	(a)	+		+	cl52
Regarded as later heterotypic synonym of Streptomyces filamentosus (for references, see list of type strains):																					
S. roseosporus	418	DSM 40122, ATCC 23958, LMG 20262, ISP 5122	AB184238	A 05	I 04	002 1-7							Sch00	Lan2-24	BENP	+	(b)	+			cl23
Regarded as later heterotypic synonym of Streptomyces flavofuscus (for references, see list of type strains):																					
S. globisporus subsp. *flavofuscus*	208c	ATCC 19908	DQ026648																	+	

(continued)

TABLE 272. (continued)

								Morphological characters[24]				Physiological tests[25]										
Guo 08[21] 16S rRNA	Guo 08[21] *atpD*	Guo 08[21] *gyrB*	Guo 08[21] *recA*	Guo 08[21] *rpoB*	Guo 08[21] *trpB*	Guo 08[21] concat.	Kim 04[23] *rpoB*	I	II	III	IV	I	II	III	IV	V	VI	VII	VIII	IX	X	XI
								W	S	C–	SM	+	+	+	−	+	+	−	+	−	+	n
								Y	S	C–	SM	+	+	+	+	+	+	−	+	−	−	n
								Y	S	C–	SM	+	+	−	+	+	n	−	+	−	n	n
								Ke	VE	C–	SM	+	−	−	−	−	n	±	−	+	n	+
							Group F	Ci	VE	C–	SM	+	±	−	±	±	n	±	±	+	n	±
								Gy	S	C–	SM	+	+	+	+	+	+	+	+	+	−	+
								R	RF	C–	SM	+	+	+	+	−	n	−	−	−	+	−
								Y	RF	C–	SM	+	+	+	+	n	n	n	n	n	n	n

(continued)

TABLE 272. List of *Streptomyces* species continued

Species names and groups[1]	No. in lists of species[2]	Type strain	Accession no.	Wil 83a[3]	Wil 89[4]	Käm 91[5]	Hat 03[6]	Lan 02[7]	Ful 95[8]	Och 95[9]	Kat 97[10]	Lab[11]	Sch[12]	Lan 04[13]	Lan 02[14]	Lan 04[15]	Lan 04[16]	Lan 04[17]	Lan 04[18]	Lan 04[19]	Lan 04[20]
Regarded as later heterotypic synonym of Streptomyces flavovirens (for references, see list of type strains):																					
S. nigrifaciens	337	DSM 40071, ATCC 19791, LMG 20048, ISP 5071	AB184158	A 1C	I 03	1-2 015					KA-B		Sch05	Lan2-09	BENP	+	(a)	+		+	cl53
Regarded as later heterotypic synonym of Streptomyces fradiae (for references, see list of type strains):																					
S. roseoflavus	414	DSM 40536, ATCC 13167, LMG 20535, ISP 5536			IV 10 (red series)	22-5 105							Sch12	Lan2-27	BENP	+	(b)	+			cl45
Regarded as later heterotypic synonym of Streptomyces griseocarneus (for references, see list of type strains):																					
S. septatus	438	DSM 40577, ATCC 27464, LMG 8604, ISP 5577	AB184883	F 55	Sv. 02	22-1 040	Ha6						Sch00	Lan2-00	BENP	+	(j)	+			+
Regarded as later heterotypic synonym of Streptomyces griseus (for references, see list of type strains):																					
S. setonii	439	DSM 40395, ATCC 25497, LMG 20291, ISP 5395	D63872	A 1B	I 02	1-3 1-2					KA-B		Sch00	Lan2-19	BENP	+		+		+	cl23
Regarded as later heterotypic synonym of Streptomyces hiroshimensis (for references, see list of type strains):																					
S. rectiverticillatus	403	DSM 40436, ATCC 19845, LMG 20292, ISP 5436	AB184296	F 57	Sv. 18	22-1 040	Ha7						Sch00	Lan2-00	BENP	+	(j)	+		+	cl48
S. roseoverticillatus	419	DSM 40039, ATCC 19807, LMG 20255, ISP 5039	AB184169		Sv. 01	22-1 040	Ha7					L4	Sch00	Lan2-00	BENP	+	(j)	+			cl13
S. salmonis	428	DSM 40895, NRRL B-1472, LMG 20306	X53169		Sv. 05	22-1 040	Ha7					L4	Sch00	Lan2-00	BENP	+	(j)	+		+	cl13
S. spitsbergensis	454	ATCC 51269, JCM 8881	AB184700				Ha7													+	
S. fervens	167	DSM 40086, ATCC 27429, ISP 5086	AB184871		Sv. 01	22-1 040						L4	Sch00								
Regarded as later heterotypic synonym of Streptomyces lilacinus (for references, see list of type strains):																					
S. kashmirensis	265	DSM 40336, LMG 19937, ISP 5336	AB184546				Ha8						Sch00	Lan2-00	BENP	+	(j)	+			cl12

(continued)

TABLE 272. (continued)

								Morphological characters[24]				Physiological tests[25]										
Guo 08[21] 16S rRNA	Guo 08[21] *atpD*	Guo 08[21] *gyrB*	Guo 08[21] *recA*	Guo 08[21] *rpoB*	Guo 08[21] *trpB*	Guo 08[21] concat.	Kim 04[23] *rpoB*	I	II	III	IV	I	II	III	IV	V	VI	VII	VIII	IX	X	XI
								Gy	RF	C–	SM	+	+	+	+	+	+	+	+	+	–	n
								R	S	C–	SM	+	+	+	–	–	–	–	–	–	n	–
								Y/R	VE	C+	SM	+	–	–	–	?	n	–	–	+	n	–
Group I	No	No	No	No	No	No	+	Y	RF	C–	SM	+	+	+	+	+	n	–	+	–	n	–
								Ke	VE	C+	SM	+	±	–	±	+	n	±	+	+	n	±
								Bi	VE	C+	SM	+	–	–	–	–	n	–	–	–	n	+
								Sa	VE	C+	SM	+	–	–	–	–	n	–	–	±	n	±
								R/V	RF		SM											
								Ba	VE	C+	SM	+	±	–	±	±	n	±	±	±	n	+
								Li	VE	C+	SM	–	–	–	–	–	n	–	–	–	n	–

(continued)

TABLE 272. List of *Streptomyces* species continued

Species names and groups[1]	No. in lists of species[2]	Type strain	Accession no.	Wil 83a[3]	Wil 89[4]	Käm 91[5]	Hat 03[6]	Lan 02[7]	Ful 95[8]	Och 95[9]	Kat 97[10]	Lab[11]	Sch[12]	Lan 04[13]	Lan 02[14]	Lan 04[15]	Lan 04[16]	Lan 04[17]	Lan 04[18]	Lan 04[19]	Lan 04[20]
Regarded as later heterotypic synonym of Streptomyces mashuensis (for references, see list of type strains):																					
S. kishiwadensis	269	DSM 40397, ATCC 25464, LMG 19939, ISP 5397	AB184858		Sv. 15	22-1 040	Hal1						Sch31		BENP	+	(j)	+		+	cl11
Regarded as later heterotypic synonym of Streptomyces microflavus (for references, see list of type strains):																					
S. griseus subsp. *alpha*	238b	DSM 40937, NRRL B-2249, LMG 19953	AB184668		I 02	1-3 1-2							Sch02	Lan2-12	BENP	+	(a)	+			cl23
S. griseus subsp. *cretosus*	238c	DSM 40561, ISP 5561	AB184418										Sch02	Lan2-12						+	
S. willmorei	520	DSM 40459, ATCC 6867, LMG 21046, ISP 5459	AB184374	A 1B	I 02	1-3 1-2					KA-B		Sch02	Lan2-12	BENP	+	(a)	+		+	cl23
Regarded as later heterotypic synonym of Streptomyces minutiscleroticus (for references, see list of type strains):																					
S. flaviscleroticus	177	DSM 40270, ATCC 19347, LMG 19886, ISP 5270	AB184634		I 08	017 007					KA-G		Sch00	Lan2-03	BENP	+	(a)	+		+	cl46
Regarded as later heterotypic synonym of Streptomyces mobaraensis (for references, see list of type strains):																					
S. ladakanum	275	DSM 40587, NRRL 3191	AB184430				Ha12					L4									
Regarded as later heterotypic synonym of Streptomyces netropsis (for references, see list of type strains):																					
S. distallicus	146	DSM 40846, NCIB 8936, LMG 20499	AB184703		Sv. 01	22-1 040	Hal4					L4		Lan2-13	BENP	+	(b) (j)	+		+	cl13
S. flavopersicus	181	DSM 40093, ATCC 19756, ISP 5093	AB249911	F 56		22-1 040	Ha14					L4	Sch00							+	
S. kentuckensis	268	DSM 40052, ATCC 12691, ISP 5052	AB184215	F SM	Sv. 11	22-1 040	Hal4					L4	Sch00							+	
S. syringium	465	DSM 41480, LMG 20320	AJ781375				Ha14						Sch00	Lan2-00	BENP	+	(j)	+		+	cl13
Regarded as later heterotypic synonym of Streptomyces phaeopurpureus (for references, see list of type strains):																					
S. phaeoviridis	373	DSM 40285, ATCC 23947, LMG 20061, ISP 5285	AB184230	A 19	I 12	009 1-19							Sch23	Lan2-02	BENP	+	(b)	+		+	cl51

(continued)

TABLE 272. (continued)

Guo 08[21] 16S rRNA	Guo 08[21] *atpD*	Guo 08[21] *gyrB*	Guo 08[21] *recA*	Guo 08[21] *rpoB*	Guo 08[21] *trpB*	Guo 08[21] concat.	Kim 04[23] *rpoB*	Morphological characters[24] I	II	III	IV	Physiological tests[25] I	II	III	IV	V	VI	VII	VIII	IX	X	XI
								Ar	VE	C+	SM	+	−	±	−	+	n	±	−	+	n	+
Group III	Yes	Yes	Yes	Yes	Yes	Yes		Y	RF	C−	SM	+	+	−	+	n	n	n	n	n	n	n
Group III	Yes	Yes	Yes	Yes	Yes	Yes		Y	RF	C−	SM	+	+	−	+	+	n	−	n	−	+	n
								Y	RF	C−	SM	+	+	−	+	n	n	n	n	n	n	n
								n	n	C−/C+	n	+	+	+	+	+	n	−	+	−	n	?
								W/Y	VE	C−	SM	+	−	−	−	+	n	−	−	−	n	−
								Ke	VE	C+	SM	+	±	−	±	±	n	±	−	+	n	±
								Ke	VE	C+	SM	+	−	−	−	±	n	−	−	+	n	±
								Ke	VE	C−	SM	+	±	−	−	±	n	±	±	+	n	+
								R	S	C+	SM	+	−	−	−	+	−	−	−	−	n	−
								R	S	C−	SM	+	+	+	+	+	n	+	+	n	n	+

(continued)

TABLE 272. List of *Streptomyces* species continued

Species names and groups[1]	No. in lists of species[2]	Type strain	Accession no.	Wil 83a[3]	Wil 89[4]	Käm 91[5]	Hat 03[6]	Lan 02[7]	Ful 95[8]	Och 95[9]	Kat 97[10]	Lab[11]	Sch[12]	Lan 04[13]	Lan 02[14]	Lan 04[15]	Lan 04[16]	Lan 04[17]	Lan 04[18]	Lan 04[19]	Lan 04[20]
Regarded as later heterotypic synonym of Streptomyces thermovulgaris (for references, see list of type strains):																					
S. thermonitrificans	478	DSM 40579, ATCC 23385, ISP 5579	Z68098	A 36	II 09	021 002														+	
Regarded as later heterotypic synonym of Streptomyces tricolor (for references, see list of type strains):																					
S. roseodiastaticus	413	DSM 41703, LMG 20327	AB184683										Sch33	Lan2-21	BENP	+	(b)	+			cl02
Regarded as later heterotypic synonym of Streptomyces olioverticillatus (for references, see list of type strains):																					
S. viridiflavus	511	LMG 20277	AB184702												BENP	+	(j)	+			cl13
Regarded as later heterotypic synonym of Streptomyces violaceus (for references, see list of type strains):																					
S. violatus	507	DSM 40209, ATCC 15892, LMG 19397, ISP 5209	AJ399480	A 18	I 11	050 019		La-12					Sch00		BENP cl12	+	(c)	+	cl14	+	cl01
No detailed sequence information available:																					
S. caviscabies	95	DSM 41811, ATCC 51928	AF112160																		
S. coeruleoflavus (no sequence available)	126																				
S. arabicus	38	DSM 40252, ATCC 23881, LMG 20414, ISP 5252	D44271	A 12	I 07	006 1-18							Sch00	Lan2-23	BENP	+	(b)	+		+	cl08
S. baldaccii	66	DSM 40845, ATCC 23654	X53164		Sv. 01	22-1 040	Ha7		FU-12b			L4								+	
S. cellulolyticus	99																			+	
S. echinoruber	152	DSM 41696, IFO 14238											Sch00							+	
S. erythraeus	159	DSM 40517, LMG 20508												Lan2-00	BENP	+		+		+	+
S. longisporoflavus	292	DSM 40165, ATCC 19915, LMG 19347, ISP 5165	AB184220	A 39	II 10	005 010		La-00		OC-non			Sch00	Lan2-00	BENP +	+		+	cl06	+	cl19
S. olivomycini	354																				
S. speleomycini	449																				
S. thermoautotrophicus	471	DSM 41605																		+	
Not in tree:																					
S. aldersoniae		DSM 41909, NRRL 18513	EU170123																		
S. alni		D65, CGMCC 4.3510, NRRL B-24611	DQ460470																		
S. angustmycinicus		DSM 41683, NRRL B-2347	EU170119																		
S. ascomycinicus		DSM 40822, NBRC 13981	EU170121																		

(continued)

TABLE 272. (continued)

Guo 08[21] 16S rRNA	Guo 08[21] *atpD*	Guo 08[21] *gyrB*	Guo 08[21] *recA*	Guo 08[21] *rpoB*	Guo 08[21] *trpB*	Guo 08[21] concat.	Kim 04[23] *rpoB*	Morphological characters[24] I	II	III	IV	Physiological tests[25] I	II	III	IV	V	VI	VII	VIII	IX	X	XI
								Gy	RF	C+	SM	+	−	−	−	−	+	−	+	+	−	−
								Gy	S	C−	SM	+	+	+	+	n	n	n	n	n	n	n
								W		C−								−	−			
							Group A18	R	S	C+	SPY	+	+	+	+	+	+	+	n	+	n	+
Group I	Yes	Yes	Yes	Yes	Yes	Yes		W	RF	C−	SM	n	n	n	n	n	n	+	n	n	n	n
								B	S	C−	SPY	n	+	+	+	+	n	+	n	+	n	n
								Gy	S	C−	SPY	+	+	+	+	+	n	−	+	+	n	n
								Ba	VE	C+	SM	+	−	−	−	−	n	±	±	±	n	+
								W Gy	RF S	C−	WTY SPY	+	+	+	−	+	n	n	n	+	n	+
								R	S	C−	SPY	+	+	+	+	+	+	+	+	+	+	n
								Y	S	C−	SM	+	+	+	+	+	+	−	+	−	+	−
									RF	C−	SM		+	+	+	+		+	+			
								Y/Gy Gy	RF	C−	SM		+	+	+	+	+			+		
								Gy/W	L	C−	SM	n	+	+	−	+	+	+	n	n	+	n
								W/Gy	RF	C−	SM	n	+	+	+	(+)	n	+	n	−	+	+
								Gy/W	S	C−	SM	n	−	−	−	+	+	+	n	n	−	n
								Gy/W	L	C−	SPY	n	+	+	+	+	+	+	n	n	+	n

(continued)

TABLE 272. List of *Streptomyces* species continued

Species names and groups[1]	No. in lists of species[2]	Type strain	Accession no.	Wil 83a[3]	Wil 89[4]	Käm 91[5]	Hat 03[6]	Lan 02[7]	Ful 95[8]	Och 95[9]	Kat 97[10]	Lab[11]	Sch[12]	Lan 04[13]	Lan 02[14]	Lan 04[15]	Lan 04[16]	Lan 04[17]	Lan 04[18]	Lan 04[19]	Lan 04[20]
S. atriruber		DSM 41860, LDDC6330-99, NRRL B-24165	EU812169																		
S. avicenniae		DSM 41943, MCCC 1A01535, CGMCC 4.5510	EU399234																		
S. axinellae		DSM 41948, Pol001, CIP 109838	EU683612																		
S. baliensis		ID03-0915, BTCC B-608, NBRC 104276	AB441718																		
S. castelarensis		DSM 40830, ATCC 15191	EF408732																		
S. deccanensis		DAS-139, KCTC 19241, CCTCC AA-207004	EF219459																		
S. decoyininicus		DSM 41427, NRRL 2666	EU170127																		
S. gulbargensis		DAS 131, KCTC 19179, CCTCC AA-206001	DQ317411																		
S. haliclonae		DSM 41970, Sp080513SC-31, NBRC 105049	AB473556																		
S. himastatinicus		DSM 41914, ATCC 53653	EF408736																		
S. hypolithicus		DSM 41950, HSM#10, NRRL B-24669	EU196762																		
S. iranensis		DSM 41954, HM 35, CCUG 57623	FJ472862																		
S. lunalinharesii		DSM 41876, ATCC BAA-1231, RCQ1071, CIP 108852	DQ094838																		
S. marinus		DSM 41968, Sp080513GE-26, NBRC 105047	AB473554																		
S. marokkonensis		DSM 41918, LMG 23016, R-22003, Ap1	AJ965470																		
S. mayteni		YIM 60475, KCTC 19383, CCTCC AA-207005	EU200683																		
S. milbemycinicus		DSM 41911, NRRL 5739	EU170126																		
S. modarskii		DSM 40771, NRRL B-1346	EF408735																		
S. nanshensis		SCSIO 01066, KCTC 19400, CCTCC AA-208005	EU589334																		
S. osmaniensis		OU-63, PCM 2690, CCTCC AA-209025	FJ613126																		
S. plumbiresistens		CCNWHX 13-160, ACCC 41207, HAMBI 2991	EU526954																		
S. polyantibioticus		DSM 44925, SPR, NRRL B-24448	DQ141528																		
S. rapamycinicus		ATCC 29253, NRRL 5491	EF408733																		
S. ruanii		DSM 40276, ISP 5276	EF408737																		
S. sedi		DSM 41942, YIM 65188, CCTCC AA-208020	EU925562																		

(continued)

TABLE 272. (continued)

								Morphological characters[24]				Physiological tests[25]										
Guo 08[21] 16S rRNA	Guo 08[21] *atpD*	Guo 08[21] *gyrB*	Guo 08[21] *recA*	Guo 08[21] *rpoB*	Guo 08[21] *trpB*	Guo 08[21] concat.	Kim 04[23] *rpoB*	I	II	III	IV	I	II	III	IV	V	VI	VII	VIII	IX	X	XI
								Gy	RF	C–	SM	+	+	+	+	+	+	+	+	+	+	±
									S		SM	n	+	+	+	n	+	+	+	–	n	+
									S		SM	+	+	–	+	+	+	–	+	n	–	–
									RF	C+	WTY/ SM	+	+	+	+	+	+	+	+	n	n	+
								Gy/Bl	S	C–	RU	+	+	n	+	+	+	+	+	n	n	–
								W	SC	C+	H	+	+	+	+	+	n	+	+	n	n	+
								Gy	S	C–	SM	n	+	–	–	+	+	–	n	n	–	n
									SC	C+	SM	+	+	+	+	n	+	+	+	n	n	+
								W	S	C–	SM	+	–	–	–	+	n	+	+	n	n	+
								Gy/Bl	S	C–	RU	+	+	n	+	+	+	+	+	n	n	+
									SC/RF	C–	SM	+	–	–	n	–	+	–	–	n	±	–
								Y–Gy/ Gy	S		RU	n	+	+	+	n	+	+	+	+	n	+
								Gy	S		SPY	n	–	+	+	n	n	–	n	n	n	+
									S	C–	SM	+	+	+	–	+	n	–	+	n	n	+
								Gy	S	C–	SM	+	+	±	+	+	+	–	+	±	+	+
									S/L		SM	–	–	–	–	+	+	–	n	–	n	+
								Gy	S/L	C–	WTY	n	+	+	+	–	–	–	n	n	–	n
								Gy/Bl	S	C–	RU	+	+	n	+	+	+	+	n	n	n	+
									S	C+	SM	+	+	n	+	+	+	+	+	+	n	+
								Gy–B	S	C+	SPY	n	+	+	+	+	+	+	+	n	n	+
								G–W	RF	C–	SM	+	+	+	+	+	+	+	n	n	n	+
									RF	C+	SM	+	+	–	–	+	+	+	–	n	+	–
								Gy/Bl	S	C–	RU	+	+	–	+	+	n	+	+	n	n	+
								Gy/Bl	S	C–	RU	n	+	–	+	+	+	n	n	n	n	+
									S		SM	n	–	n	–	+	–	–	–	–	n	n

(continued)

TABLE 272. List of *Streptomyces* species continued

Species names and groups[1]	No. in lists of species[2]	Type strain	Accession no.	Wil 83a[3]	Wil 89[4]	Käm 91[5]	Hat 03[6]	Lan 02[7]	Ful 95[8]	Och 95[9]	Kat 97[10]	Lab[11]	Sch[12]	Lan 04[13]	Lan 02[14]	Lan 04[15]	Lan 04[16]	Lan 04[17]	Lan 04[18]	Lan 04[19]	Lan 04[20]
S. silaceus		DSM 41861, LDDC 6638-99, NRRL B-24166	EU812170																		
S. tateyamensis		DSM 41969, Sp080513SC-30, NBRC 105048	AB473555																		
S. thinghirensis		DSM 41919, S10, CCMM B35	FM202482																		
S. tritolerans		DAS 165	DQ345779																		
S. wellingtoniae		DSM 40632, NRRL B-1503	EU170124																		
S. xiamenensis		DSM 41903, MCCC 1A01550, CGMCC 4.3534	EF012099																		
S. xinghaiensis		S187, KCTC 19546, CCTCC AA 208049, NRRL B-24674	EF577247																		
K. kazusensis		SK 60, KCTC 19565, JCM 14560	AB278569																		
K. saccharophila		SK 15, KCTC 19566, JCM 14559	AB278568																		

1. Species are grouped according to the maximum-likelihood tree in Figure 339.

2. Without symbols: list of type strains of *Streptomyces*; † list of type strains of *Streptacidiphilus*; * list of type strains of *Kitasatospora*.

3. Groups as described by Williams et al. (1983a), grouping on the basis of numerical identification.

4. Groups as described by Williams et al. (1989), grouping mainly on the basis of numerical identification according to Williams et al. (1983a).

5. Groups as described by Kämpfer et al. (1991), grouping on the basis of numerical identification.

6. Groups as described by Hatano et al. (2003), grouping on the basis of phenotypes, DNA–DNA hybridization and sequences of *gyrB*.

7. Groups as described by Lanoot et al. (2002), grouping on the basis of protein profiles.

8. Groups as described by Fulton et al. (1995), grouping on the basis of fingerprints of the rRNA operon.

9. Groups as described by Ochi (1995), grouping on the basis of the ribosomal AT-L30 protein.

10. Groups as described by Kataoka et al. (1997), grouping on the basis of partial 16S rRNA gene sequences containing a variable α region.

11. Groups as described by: Labeda and Lyons (1991b), L1; Labeda and Lyons (1991a), L2; Labeda (1998), L3; Labeda (1996), L4; Labeda (1993), L5 (grouping on the basis of DNA relatedness).

12. Groups as described by P. Schumann (unpublished), grouping on the basis of ribotyping.

13. Groups as described by Lanoot et al. (2004), grouping on the basis of Box-PCR.

14. Groups as described by Lanoot et al. (2002), grouping on the basis of protein profiles.

15. Groups as described by Lanoot (2004), grouping on the basis of DNA–DNA hybridization.

16. Groups as described by Lanoot (2004), grouping on the basis of DNA–DNA hybridization.

17. Groups as described by Lanoot (2004), grouping on the basis of ARDRA.

18. Groups as described by Lanoot (2004), grouping on the basis of ARDRA.

19. Groups as described by Lanoot (2004), grouping on the basis of analysis of the ITS region.

20. Groups as described by Lanoot (2004), grouping on the basis of 16S rRNA-ITS RFLP.

21. Groups as described by Guo et al. (2008), grouping on the basis of multilocus phylogeny calculated with the sequences of five housekeeping genes (*atpD, gyrB, recA, rpoB, trpB*) and the 16S rRNA gene.

22. +, Strains that were used in the study of Guo et al. (2008) but did not belong to one of the four detected groups; yes, strains that show the same grouping (I–IV) as in the 16S rRNA gene sequence tree; no, strains do not show the same grouping (I–IV) as in the 16S rRNA gene sequence tree.

23. Groups as described by Kim et al. (2004), grouping on the basis of *rpoB* gene sequences; +, strains that were used in this study; Group A16, group A18, group A1B, group F, *Kitasatospora* and Asn(AAC), names of species groups based on the *rpoB* gene according to Kim et al. (2004).

24. Morphological characters of species described before 1974 according to Pridham and Tresner (1974a, 1974b) and Baldacci and Locci (1974). n, Not determined.

I. Spore color en masse indicated as W (White), Gy (Gray), Y (Yellow), R (Red), B (Blue), G (Green), V (Violet), Bl (Black), Ba (substrate mycelium pink-red to orange-red, aerial mycelium pink, gray-pink, and violet-pink), Bi (substrate mycelium colorless, reddish and orange, yellow to brick red, aerial mycelium pinkish white), Hi (substrate mycelium brick red, aerial mycelium beige to pink-beige), Sa (substrate mycelium brick-red to orange, aerial mycelium white with pink and yellow shades), Lu (substrate mycelium yellow, yellowish to brown, aerial mycelium light yellow, yellowish to beige), Gr (substrate mycelium brownish yellow, aerial mycelium pinkish beige with lilac shades), Ci (substrate mycelium yellow to greenish, yellow and brown-yellow, aerial mycelium pinkish with beige and lilac shades), Ar (substrate mycelium light yellow to yellowish to pinkish yellow, aerial mycelium basically white with yellow, pink and gray), Ke (substrate mycelium yellow, yellowish to hazel-nut yellow, aerial mycelium beige

TABLE 272. (continued)

								Morphological characters[24]				Physiological tests[25]										
Guo 08[21] 16S rRNA	Guo 08[21] *atpD*	Guo 08[21] *gyrB*	Guo 08[21] *recA*	Guo 08[21] *rpoB*	Guo 08[21] *trpB*	Guo 08[21] concat.	Kim 04[23] *rpoB*	I	II	III	IV	I	II	III	IV	V	VI	VII	VIII	IX	X	XI
								W/Y–W	RF	C–	SM	+	+	−	−	+	+	+	+	+	+	−
								Gy	S	C–	SM	+	−	−	−	−	n	−	+	n	n	+
									S	C–	SM	+	−	n	+	+	+	−	+	n	n	±
								W/Gy	SC/RF	C+	SM	+	+	+	+	+	+	+	+	−	n	+
								R–Gy	S/L	C–	SM	n	+	+	+	+	+	+	n	n	−	n
									SC/RF	C–	SM	n	−	+	+	n	−	−	+	n	n	+
									SC/RF		SM	+	n	−	+	+	+	−	+	n	n	+
								W		C–	SM	+	+	+	−	−	+	−	−	−	n	−
								Gy	SC/RF	C–	SM	+	+	+	+	+	+	+	−	−	n	+

with shades toward yellow, pink and cinnamon), Mo (substrate mycelium yellow to greenish yellow, aerial mycelium grayish green), Li (substrate mycelium brown, aerial mycelium pinkish white), Th (substrate mycelium brown-yellow to greenish, aerial mycelium light yellow).

II. Spore chain morphology indicated as RF (Rectus Flexibilis), S (Spira), VE (Verticil), RA (Retinaculum-Apertum), SC (straight chains), L (loop).

III. C+, Melanoid pigments produced; C–, pigments not produced.

IV. Spore wall ornamentation indicated as SM (smooth), SPY (spiny), H (hairy), WTY (warty), RU (rugose).

25. Physiological tests of species described before 1974 according to Pridham and Tresner (1974a, 1974b) and Baldacci and Locci (1974). +, Positive for utilization of carbon compounds; –, negative for utilization of carbon compounds; n, not determined.

I. D-Glucose

II. D-Xylose

III. L-Arabinose

IV. L-Rhamnose

V. D-Fructose

VI. D-Galactose

VII. Raffinose

VIII. D-Mannitol

IX. i-Inositol

X. Salicin

XI. Sucrose

Fingerprinting techniques.

Randomly amplified polymorphic DNA (RAPD) PCR. In RAPD-PCR, single primers with arbitrary nucleotide sequences are used to amplify DNA at low annealing temperatures in order to detect polymorphisms. The method is used as a rapid screening method to detect similarities among streptomycetes. For meaningful results, a stringent standardization of reaction parameters is required; the latter include primer sequence, annealing temperature, buffer components, and concentration and quality of template DNA. RAPD-PCR results in a characteristic fingerprint of PCR products. The standardized procedure allows the detection of chromosomal differences between individual isolates without having any prior knowledge of chromosomal sequences.

Mehling et al. (1995) used this technique in a study of actinomycete species, but did not detect any characteristic banding patterns for closely related species unless they used a highly specific actinomycete primer. Even so, the resulting fingerprints contained only four bands. Similar results were reported by Huddleston et al. (1995), who used the method to try and determine interspecific relationships among members of the *Streptomyces albidoflavus* cluster of Williams et al. (1983a). Anzai et al. (1994) investigated 11 primers with various fragment patterns from zero to 20 and found that variations in fingerprint patterns can be obtained by substitution of a single base on the arbitrary primer; the most significant differences were observed when the sequence at the 3′ end was altered. Anzai and colleagues used an optimized procedure to study the relationship of *Streptomyces virginiae* and *Streptomyces lavendulae* strains; members of these taxa were assigned to the same numerically defined clusters by Williams et al. (1983a) and Kämpfer et al. (1991). Good correlation was found between the RAPD-PCR data and corresponding DNA–DNA hybridization, low-frequency restriction fragment analysis, and cultural and physiological tests, though the interspecific relationship of *Streptomyces lavendulae* and *Streptomyces virginiae* strains was not clarified.

Restriction digests of total chromosomal DNA. Low-frequency restriction fragment analysis is based on the digestion of the entire bacterial chromosome with restriction endonucleases that cut infrequently. Rare AT cutters are used for streptomycetes given their high DNA G+C content. PFGE is used to separate the resultant fragments. In the first study, Beyazova and Lechevalier (1993) examined 59 strains from eight species-groups and found that the method was useful as related strains clustered together. However, some discrepancies were found, for example, for strains grouped into the *Streptomyces cyaneus* cluster of Williams et al. (1983a). Like RAPD-PCR, the method seems to be useful for the detection of very closely related strains, but cannot be used to resolve interspecific relationships. A misinterpretation of banding patterns can result from large chromosomal amplifications or deletions (Rauland et al., 1995).

Nucleic acid sequence comparisons of 16S rRNA and other genes. In an early review of the application of 16S rRNA gene sequence analysis to the classification of streptomycetes, Stackebrandt et al. (1992) highlighted the importance of the region selected for comparison. They found that relationships between strains were influenced by which variable region (*a*, *b*, or *c*) was studied. Kataoka et al. (1997) were able to resolve inter- and intraspecies relationships between 89 streptomycete type strains, representing several clusters of Williams et al. (1983a) by sequencing the *c* region. Forty-two of the strains were found to have unique sequences; the remaining strains were assigned to 15 groups. In a more extensive study, Kataoka and his colleagues (1997) deposited the sequences of the *c* region of 485 *Streptomyces* strains in GenBank. At present, this is the largest publicly available set of streptomycete 16S rRNA gene sequence data.

A phylogenetic tree based on comparison of the *c* regions of representatives of the major cluster-groups defined by Williams et al. (1983a) was published by Anderson and Wellington (2001). They were able to confirm the taxonomic status of the phenotypic groups, apart from the *Streptomyces olivaceoviridis* and *Streptomyces griseoruber* strains which had identical *c* regions; these strains were found in clusters 20 and 21 of Williams et al. (1983a), respectively, but formed cluster 9 in the study of Kämpfer et al. (1991). The sequence data also showed that the 60 strains assigned to three species-groups in the *Streptomyces albidoflavus* group (Williams et al., 1983a) could be divided into six groups (Kataoka et al., 1997); the three phenotypic subgroups of Williams et al. (1983a) were maintained, but did not cluster together.

Hain et al. (1997) designed 16S rRNA oligonucleotide probes to determine intraspecific relationships within the *Streptomyces albidoflavus* group and found that the resultant sequences were useful for species delineation, but not for strain differentiation. The intergenic 16S–23S rRNA spacer regions were found to be more suitable for the delineation of intraspecific relationships within that cluster. Genus-specific probes have also been developed based on 23S rRNA gene (Mehling et al., 1995) and 5S rRNA gene (Park et al., 1991) sequences. The reclassification of the genera *Chainia*, *Elytrosporangium*, *Kitasatoa*, *Microellobosporia*, and *Streptoverticillium* into the genus *Streptomyces* was confirmed using 5S rRNA gene sequence data (Park et al., 1991).

At present, complete 16S rRNA gene sequences for almost all *Streptomyces* type strains are available from public databases. However, the variation in the 16S rRNA genes – even in the variable regions – is too limited to help resolve problems of species differentiation and to establish taxonomic structure within the genus (Anderson and Wellington, 2001; Stackebrandt et al., 1991a, 1991b, 1992; Witt and Stackebrandt, 1990). The situation is complicated by the fact that *Streptomyces* species may harbor different 16S rRNA gene sets, for example, *Streptomyces coelicolor* A3(2), "*Streptomyces lividans*", and several other *Streptomyces* species contain six ribosomal rRNA gene sets. Each set of rRNA genes comprises one gene copy for 16S, 23S, and 5S rRNA (van Wezel et al., 1991) and lacks tRNA genes.

Other genes can be used to establish inter- and intraspecies level relationships within the genus. Hatano et al. (2003) examined the partial sequences of the *gyrB* gene of 64 whorl-forming streptomycetes. This gene represents the structural gene of the B subunit of DNA gyrase. Most members of the 46 species, eight subspecies, and 13 species with names that have not been validly published (including 10 strains examined by the ISP) were assigned to two major groups. The larger group, which consisted of typical whorl-forming species (59 strains), was subdivided into six major clusters of three or more species, seven minor clusters of two species, and five single-member clusters at the 97% *gyrB* sequence similarity level. The major clusters contained *Streptomyces abikoensis*, *Streptomyces cinnamoneus*, *Streptomyces distallicus*, *Streptomyces griseocarneus*, *Streptomyces hiroshimensis*, and *Streptomyces netropsis* strains, results that were in line with phenotypic data.

With the exception of the *Streptomyces distallicus* cluster (which was divided phenotypically into the *Streptomyces distallicus* and *Streptomyces stramineus* subclusters) and the *Streptomyces netropsis* cluster (which was divided into the *Streptomyces netropsis* and *Streptomyces eurocidicus* subclusters), members of each of the clusters resembled one another closely, as did members of the minor clusters. Hatano and his colleagues classified 59 strains of typical whorl-forming *Streptomyces* species into the following 18 species [including subjective synonym(s)]: *Streptomyces abikoensis*, *Streptomyces ardus*, *Streptomyces blastmyceticus*, *Streptomyces cinnamoneus*, *Streptomyces eurocidicus*, *Streptomyces griseocarneus*, *Streptomyces hiroshimensis*, *Streptomyces lilacinus*, "*Streptomyces luteoreticuli*", *Streptomyces luteosporeus*, *Streptomyces mashuensis*, *Streptomyces mobaraensis*, *Streptomyces morookaense*, *Streptomyces netropsis*, *Streptomyces orinoci*, *Streptomyces stramineus*, *Streptomyces thioluteus*, and *Streptomyces viridiflavus* (Table 272). In addition, all of the strains, which showed 98.5–100% *gyrB* sequence similarities, had high DNA–DNA similarities (70–100%), indicating that *gyrB* sequences give a better resolution than corresponding 16S rRNA gene sequences.

Other conserved genes that are prime targets for taxonomic studies include the housekeeping genes (e.g. elongation factors and ATPase subunits; Ludwig and Schleifer, 1994) and tryptophan synthase genes (Huddleston et al., 1997), which were used to determine the phylogeny of streptomycin-producing streptomycetes and provide evidence for horizontal transfer of antibiotic resistance genes. Guo et al. (2008) sequenced five housekeeping genes (*atpD*, *gyrB*, *recA*, *rpoB*, *trpB*) and the corresponding 16S rRNA genes of 55 *Streptomyces* strains classified in the *Streptomyces griseus* clade. The strains were assigned to four clusters that contained strains with identical 16S rRNA gene sequences, but these results were not congruent with those based on the sequences of the individual or from corresponding concatenated gene sequences. Some of the strains with identical 16S rRNA gene sequences were assigned to different clusters in the housekeeping gene sequence trees. The trees based on individual gene sequences gave a higher resolution between strains than the concatenated tree. The authors concluded that phylogenetic trees generated on more than one gene sequence are more reliable and give a higher resolution power and topological stability.

Kim et al. (2004) analyzed the sequences of 16S rRNA and RNA polymerase β-subunit genes (*rpoB*) of 57 *Streptomyces* strains, five *Kitasatospora* strains, and a single *Micromonospora* strain and found that the resultant phylogenetic trees had similar topologies. They also found good congruence between the *rpoB* sequence and corresponding numerical phenetic data of Williams et al. (1983a). The five *Kitasatospora* strains were clearly separated from the *Streptomyces* strains in the *rpoB* gene tree. Such results show that sequence analysis of additional genes (i.e. other housekeeping genes) will help to give a better insight into the intraspecific structure of the genus *Streptomyces* (Stackebrandt et al., 2002).

Rapid methods for gene analysis in streptomycete taxonomy. Several alternative methods for gene analysis have been described which do not involve sequencing. These methods are based on either restriction analysis (Clarke et al., 1993; Fulton et al., 1995) or specialized gel electrophoresis techniques which are used to monitor the mobility of products (Hain et al., 1997; Heuer et al., 1997). Clarke et al. (1993) used a combination of *Bgl*I, *Eco*RI, *Pst*I, and *Pvu*II to obtain restriction fragment length polymorphism (RFLP) patterns of purified rRNA extracted from members of the *Streptomyces albidoflavus* cluster [subgroups 1A and 1B of Williams et al. (1983a)] and were able to differentiate between phenotypically similar strains, although profiles varied considerably between *Streptomyces albidoflavus* species-groups. Ribosomal restriction analysis of 98 named streptomycete strains, including members of cluster-groups A (comprising clusters 1–41) and F (comprising clusters 55–67) of Williams et al. (1983a) and some other strains, was performed by Fulton et al. (1995) using *Mse*I fingerprints of rRNA operons (RiDiTS) and 11 pattern types with varying degrees of similarity to the Williams subclusters were highlighted. Cluster-groups A and F were differentiated, albeit at a low resolution (70% similarity), but individual clusters could not be distinguished.

Further methods used to compare genotypic variation amongst streptomycetes include denaturing gradient gel electrophoresis (DGGE; Muyzer et al., 1993) with or without DNA-binding agents (Hain et al., 1997). Anderson and Wellington (2001) recommended DGGE in combination with other techniques. This method uses variable 16S rRNA regions and enables the delimitation of genus and species-groups. Isolates ASB33, ASB37, and ASSF22 were allocated to *Streptomyces albidoflavus*, *Streptomyces griseoruber*, and *Streptomyces albidoflavus*, respectively, by using a combination of techniques, including numerical taxonomy, PFGE, and sequence comparisons (Huddleston et al., 1997; Huddleston et al., 1995).

Additional comments. Nowadays, often the first step in characterization of an isolate is the determination of the 16S rRNA gene sequence. Those isolates that may represent novel species are then further characterized in order to find additional markers which are different from those reported for already established taxa. In several cases, only a very restricted set of phenotypic differences are found, especially within the genus *Streptomyces*, and hence the classification of novel taxa is based largely on the 16S rRNA gene sequence differences. More and more, additional (housekeeping) gene sequence differences are also reported, which are sometimes regarded as sufficient for the delineation of novel species. There is clearly a current trend to delineate species more and more on the basis of the genotype.

However, recent analyses have shown that, although the 16S rRNA gene sequence has been widely accepted as the "backbone" of bacterial systematics as part of the often called "tree of life", in the light of genome data the concept of a single universal tree of life appears increasingly obsolete, especially given the impact of lateral or horizontal gene transfer events (see, e.g. Bapteste et al., 2009; Bapteste and Boucher, 2008; Boucher and Bapteste, 2009; Dagan et al., 2008; Fournier and Gogarten, 2010; Fournier et al., 2009; Kreimer et al., 2008; Wolf et al., 2002; and others for a more detailed discussion). It is methodologically difficult to infer horizontal or lateral gene transfer unless one has an *a priori* hypothesis of relationship that indicates that the presumptive transferred genes are not homologs. But there is no doubt that horizontal gene transfer (HGT) is widespread in the microbial world. HGT can result in either acquisition of new genetic material or homologous replacement of existing genes. The evolutionary significance of homologous recombination

in a population can be quantified by examining the relative rates at which polymorphisms are introduced from recombination (rho) and mutation [theta(w)]. In the study of Doroghazi and Buckley (2010), multilocus sequence analysis (MLSA) was used to quantify both intraspecies and interspecies homologous recombination among streptomycetes and some other Gram-stain-positive bacteria. These authors found that intraspecies recombination in *Streptomyces flavogriseus* isolated from soils at five locations spanning 1000 km showed a >99.8% nucleotide identity across the loci examined. The authors found remarkable levels of gene exchange within *Streptomyces flavogriseus* and that the population was in linkage equilibrium (standardized index of association=0.0018), providing evidence for a freely recombining sexual population structure. As an even more interesting result, extensive interspecies homologous recombination was found among different *Streptomyces* species summing up to 40% of housekeeping-genes that had been acquired through HGT. This recombination rate found for these named species exceeded by far that observed within many species of bacteria. Hence, it was concluded that this pattern of gene exchange and recombination clearly shaped the evolution of streptomycetes and this has also a tremendous effect on classification based on sequence data of housekeeping genes.

Molecular data can provide an enormous amount of information. However, at this point, we are far from able to interpret these data, especially the information behind them, well enough to draw decisive conclusions. There are numerous open questions, e.g. which genes belong to the conserved genome core and are considered probably useful to define a taxon and which belong to accessory dispensible genetic elements? The "overall" impact of processes such as lateral gene transfer, gene duplication, recombination, and rearrangements of genes in the genome is not clear and may vary considerably in different lineages (see Bapteste et al., 2009; Dagan et al., 2008; and other publications). In addition, the presence of genes and gene clusters (whether expressed or "silent") can have a totally different biological meaning and the roles of structural elements (some of them phenotypically recognizable by the so-called "chemotaxonomic" methods) and biochemical pathways (also recognized by studying the phenotype at different levels) should be consistent with the underlying genetic data, which is essentially the aim of a "polyphasic taxonomic" study.

Despite the advantages of molecular methods (including the generation and analyses of whole genome sequence data), it is often impossible to deduce phenotypic properties from the presence or absence of genes and gene clusters, because genes do not exist for their own sake. This is especially true for seemingly simple, but nevertheless "complex" phenotypic properties, like temperature, NaCl, or pH tolerances, and some complex chemotaxonomic features (just to name a few), which may be affected by very different and complex regulatory biochemical networks and are based on the underlying genetic potential and expression network. It should be emphasized again, as also pointed out by Tindall et al. (2010), that experience gained over the past six decades has continued to demonstrate the value of comparing different datasets and also of basing the description and delineation of taxa on as wide a dataset as possible. Only a combination of data acquired from DNA-based methods (DNA–DNA hybridization, gene sequences, genomic fingerprints) and phenotyping (chemotaxonomic, physiological, and morphological traits) provides a sound basis for the taxonomy of the prokaryotes in general, including streptomycetes (Tindall et al., 2010).

Differentiation and characteristics of the species of the genus *Streptomyces*

It is difficult to identify *Streptomyces* species, partly due to the high number of species with validly published names (Table 272). Most of these species are named on the basis of a single strain description and, hence, at present, it is not possible to recommend a single method or even a set of methods for identification at the species level. Species allocations on the basis of the results of one or few methods should be regarded with care. The ICSP Subcommittee on the Systematics of *Streptomycetaceae* (Kämpfer and Labeda, 2003) has recommended that more genomic information should be evaluated before a species concept is formulated for the genus *Streptomyces.* It was agreed "that the proposal of new species should only be accepted on the basis of very careful studies done with sufficient practice and considering all other species".

Sequence analysis of the rRNA gene has revolutionized our insight into phylogenetic lineages of major taxonomic groups, but this method does not have sufficient resolving power to delimit *Streptomyces* species. It is clearly not possible to assign new strains to existing species solely on the basis of this molecule, though trees are a useful visual aid for placing members of putative novel species next to their nearest relatives. Even if results from the application of different treeing algorithms differ, the judgment of what is a novel species and which are its closest relatives will be decided on the basis of individual sequence homologies, and on complementary genetic (e.g. DNA–DNA hybridization) data.

Given the difficulties of interpreting the 16S rRNA, the descriptions of all species with validly published names are presented in the species descriptions in alphabetical order. The species descriptions include details of 16S rRNA similarities of related species based on pairwise comparisons. A grouping of the species was performed on the basis of a phylogenetic analysis using the 16S rRNA gene sequences. Groups which were relatively stable on the basis of different treeing methods are given in Figure 339. A table with all *Streptomyces* species, grouped according to the clusters, lists further information to all *Streptomyces* species, including type strain numbers, their inclusion in the numerical taxonomic studies of Williams et al. (1983a) and Kämpfer et al. (1991), 16S rRNA gene sequence accession numbers, DNA–DNA hybridization, and numerous other studies (Table 272). Additional genotypic and phenotypic data can be obtained from these studies.

Acknowledgements

I thank Dr Jean Euzéby for his advice on numerous nomenclatural issues and for permission to include some information given on his website (http://www.bacterio.cict.fr/) in some of the species descriptions. I am indebted to Dr Wolfgang Ludwig for preparing the phylogenetic tree (Figure 339) and his help in interpreting sequence data, and to Professor Michael Goodfellow for his excellent and very helpful comments to this chapter. Many thanks go to my coworkers Dr Nicole Lodders, Dr Kathrin Thummes, Kerstin Fallschissel, and Corina Lang for support in writing and proofreading this chapter.

List of species of the genus *Streptomyces*

1a. **Streptomyces albus subsp. albus** (Rossi Doria 1891) Waksman and Henrici 1943, 339^AL^ ["*Streptotrix alba*" (*sic*) Rossi Doria 1891, 421]

al′bus. L. masc. adj. *albus* white.

Spore chains in Section *Spirales*. Spirals are most abundant on oatmeal agar and may be poorly developed on yeast-malt agar, salts-starch agar, and glycerol-asparagine agar. Mature spore chains generally have 10–50 spores per chain, but shorter chains may be common on some media. Spore surface is smooth.

Color of colony: aerial mass color in the White color series on oatmeal agar and salts-starch agar; White or Yellow color series on yeast-malt agar and glycerol-asparagine agar. Reverse side of colony with no distinctive pigments (colorless to pale yellow) on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar.

Color in medium: melanoid pigments are not formed in peptone-yeast-iron agar, tyrosine agar, or tryptone-yeast broth. No pigment is found in the medium in yeast-malt agar, oatmeal agar, salts-starch agar, or glycerol-asparagine agar.

D-Glucose, D-xylose, D-mannitol, and D-fructose are utilized for growth. Reports vary on utilization of L-arabinose and raffinose. No growth or only traces of growth with iso-inositol, rhamnose, and sucrose.

Type strain shows the highest sequence similarity to: *S. almquistii*, AB184258, 100%; *S. rangoonensis*, AB184295, 100%; *S. gibsonii*, AB184663, 100%; *S. flocculus*, AB184272, 99.8%.

Source: not known.

DNA G+C content (mol%): not known.

Type strain: AS 4.164, ATCC 25426, ATCC 3004, IMRU 3004, CBS 410.63, CBS 924.69, BCRC (formerly CCRC) 10802, CCUG 33990, CECT 3077, CIP 104432, DSM 40313, HUT 6613, IFM 1119, IFO (now NBRC) 13014, NBRC 3710, IMET 40241, JCM 4450, JCM 4177, KCTC 1082, NCIMB 9558, NRRL B-1811, NRRL B-2208, NRRL-ISP 5313, RIA 1206, VKM Ac-35.

Sequence accession no. (16S rRNA gene): AJ621602.

1b. **Streptomyces albus subsp. pathocidicus** Nagatsu, Anzai and Suzuki 1962, 105^AL^

pa.tho.ci′di.cus. Gr. n. *pathos* disease; L. suff. -*cida* (from L. v. *caedo*, to cut or kill) murderer, killer; L. masc. suff. -*icus* suffix used with the sense of belonging to; N.L. masc. adj. *pathocidicus* belonging to pathocide, but obviously referring to the antibiotic pathocidin.

Aerial mycelium has a closed spiral or an open loop on glucose-asparagine agar and starch agar. Conidia are elliptical, 1.0 × 1.3–1.4 × 1.8 μm in size, and show a chain-like growth. Usually grows at 25–30°C on various media. At first, it has no color, then it changes from white to pale yellow, with white aerial mycelium. Hydrolyzes starch, does not liquefy gelatin, and forms small amounts of nitrite.

Type strain shows no sequence similarity over 99%.

Source: not known.

DNA G+C content (mol%): not known.

Type strain: AS 4.1633, ATCC 14510, BCRC 12331, CIP 104431, DSM 40799, NBRC 13812, JCM 4166, KCTC 9671, VKM Ac-598.

Sequence accession no. (16S rRNA gene): AB184501.

2. **Streptomyces abikoensis** (Umezawa, Tazaki and Fukuyama 1951) Witt and Stackebrandt 1991, 456^VP^ (Effective publication: Witt and Stackebrandt 1990, 370.) ("*Streptomyces abokobensis*" Umezawa, Tazaki and Fukuyama 1951, 333; *Streptoverticillium abikoense* Locci, Baldacci and Petrolini Baldan 1969, 59; "*Verticillomyces abikobensis*" Shinobu 1965, 96)

a.bi.ko.en′sis. N.L. masc. adj. *abikoensis* of or belonging to Abiko (named after Abiko, Japan).

Spore chains are straight. Reverse colors tend to appear darker. Aerial mycelium can be beige, pale pink to pinkish white. Soluble pigments are present on Bacto Emerson agar. Utilizes starch, casein, and, more readily, gelatin. Growth at 37°C is the same as that at 27°C; however, aerial mycelium is less abundant and lighter in color. Brown soluble pigments are produced at 37°C on potato-glucose agar (Baldacci et al., 1954). The type strain produces abikoviromycin and exhibits polyenic anti-fungal activity.

Type strain shows the highest sequence similarity to: *S. lilacinus*, AB184819, 99.7%; *S. hygroscopius* subsp. *angustmyceticus*, DQ442509, 99.7%%; *S. ehimensis*, AY999834, 99.6%; *S. sapporonensis*, AB184508, 99.5%; *S. hiroshimensis*, AB184789, 99.5%; *S. caeruleus*, EF178675, 99.5%; *S. luteireticuli* AB249969, 99.3%; *S. thioluteus*, AB184753, 99.3%; *S. varsoviensis*, DQ026653, 99.2%; *S. morookaense* AJ781349, 99.1%; *S. lavenduligriseus*, DQ442515, 99.1%; *S. cinnamoneus*, AB184850, 99.1%; *S. blastmyceticus*, AY999802, 99%; *S. pseudoechinosporeus*, AB184100, 99%; *S. olivoverticillatus*, AB184636, 99%.

Source: soil.

DNA G+C content (mol%): not known.

Type strain: AS 4.1162, ATCC 12766, CBS 487.62, BCRC 12461, DSM 40831, NBRC 13860, JCM 4002, KCTC 9662, KCTC 9741, NRRL B-1518, NRRL B-2113, PCM 2364, RIA 497.

Sequence accession no. (16S rRNA gene): AB184537.

Further comments: in violation of Rule 33c of the *Bacteriological Code* (1990 Revision), in Validation List no. 38, *Streptomyces abikoensis* is proposed as a *nomen revictum* (basonym: "*Streptomyces abikoensis*" Umezawa, Tazaki and Fukuyama (1951).

According to Hatano et al. (2003), *Streptomyces abikoensis* (Umezawa et al. 1951) Witt and Stackebrandt 1991 is an earlier heterotypic synonym of *Streptomyces ehimensis* corrig. (Shibata et al. 1954) Witt and Stackebrandt 1991, of *Streptomyces luteoverticillatus* (Shinobu 1956) Witt and Stackebrandt 1991, of *Streptomyces olivoreticuli* (Arai et al. 1957) Witt and Stackebrandt 1991, and of *Streptomyces parvisporogenes* (Locci et al. 1969) Witt and Stackebrandt 1991. Hatano et al. (2003) also propose that *Streptomyces abikoensis* (Umezawa et al. 1951) Witt and Stackebrandt 1991 be a heterotypic synonym of "*Streptomyces olivoreticuli* subsp. *cellulophilus*" (NBRC 15929), of "*Streptomyces paucisporogenes*" (NBRC 13070), of "*Streptomyces takataensis*" (NBRC 13470), of "*Streptoverticillium rubrireticuli*" (NBRC 13082), and of "*Streptoverticillium rubroverticillatum*" (NBRC 15818). In the paper by Hatano et al. (2003), "*Streptomyces olivoreticuli* subsp. *cellulophilus*" is not in quotes. However, this name has no standing in bacterial nomenclature.

3. **Streptomyces aburaviensis** Nishimura, Kimura, Tawara, Sasaki, Nakajima, Shimaoka, Okamoto, Shimohira and Isono 1957, 206[AL].

a.bu.ra.vi.en′sis. N.L. masc. adj. *aburaviensis* of or belonging to Aburabi, Shiga Prefecture, Japan, the source of the soil from which the organism was isolated.

Spore chains in Section *Rectiflexibiles*. Mature spore chains are moderately long with 10–50, or sometimes more than 50, spores per chain. This morphology is seen on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar. Spore surface is smooth.

Color of colony: aerial mass color in the Gray color series on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar. Reverse side of colony is yellowish brown on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar (this pigment is not a pH indicator).

Color in medium: melanoid pigments are not formed in peptone-yeast-iron agar, tyrosine agar, or tryptone-yeast broth. No pigment other than trace of yellow is found in medium in yeast-malt agar, oatmeal agar, salts-starch agar, or glycerol-asparagine agar.

Growth on carbon utilization media is generally poor. D-Glucose, D-fructose, and D-xylose are utilized for growth. Reports vary from no growth to traces of growth on L-arabinose, sucrose, iso-inositol, D-mannitol, rhamnose, and raffinose.

Type strain shows the highest sequence similarity to: *S. herbaricolor*, AB184801, 99.6%; *S. indigoferus*, AB184214, 99.6%; *S. purpureus*, AJ781324, 99.5%; *S. chrysomallus* subsp. *fumigatus*, AB184645, 99.5%; *S. xanthocidicus*, AY999858, 99.4%; *S. psammoticus*, AY999862, 99.1%; *S. purpeofuscus*, AJ781364, 99.1%; *S. avellaneus*, AB184413, 99%. Type strain shows the highest sequence similarity to following *Kitasatospora* species: *Kitasatospora kifunensis*, AB022874, 99.3%; *Kitasatospora gansuensis*, AY442265, 99%.

Source: soil from Japan.

DNA G+C content (mol%): not known.

Type strain: AS 4.1469, ATCC 23869, CBS 280.60, CBS 608.68, BCRC 11617, CECT 3315, DSM 40033, IFM 1083, NBRC 12830, IMET 43081, JCM 4613, JCM 4170, KCTC 9663, LMG 19305, NRRL B-2218, NRRL-ISP 5033, RIA 1107, RIA 732, VKM Ac-1868.

Sequence accession no. (16S rRNA gene): AY999779.

4a. **Streptomyces achromogenes subsp. achromogenes** (*sic*) Okami and Umezawa *in* Umezawa, Takeuchi, Okami and Tazaki 1953, 268[AL]

a.chro.mo′ge.nes. Gr. pref. *a-* not; Gr. n. *chroma* color; N.L. suff. *-genes* (from Gr. v. *gennaô* to produce), producing; N.L. adj. *achromogenes* not producing color.

Spore chains in Section *Rectiflexibiles*. Mature spore chains generally comprise 10–50 spores per chain. This morphology is seen on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar. One observer recorded some loops or hooks on salts-starch agar. Spore surface is smooth.

Color of colony: aerial mass color in the Gray color series on yeast-malt agar, oatmeal agar, and glycerol-asparagine agar. Reverse side of colony with no distinctive pigments on yeast-malt agar, oatmeal agar, salts-starch agar, or glycerol-asparagine agar. Substrate pigment is not a pH indicator.

Color in medium: melanoid pigments formed in peptone-yeast-iron agar, tyrosine agar, and other organic media. Pigments other than melanoids are not formed in yeast-malt agar, oatmeal agar, salt-starch agar, or glycerol-asparagine agar.

D-Glucose, L-arabinose, D-mannitol, and D-fructose are utilized for growth. No growth or only traces of growth on sucrose, D-xylose, and raffinose. Variable reports of slight growth with rhamnose and iso-inositol.

Type strain shows the highest sequence similarity to: *S. cellostaticus*, AB184192, 99%; *S. niveoruber*, DQ445796, 99%.

Source: not known.

DNA G+C content (mol%): not known.

Type strain: ATCC 12767, ATCC 19719, CBS 458.68, BCRC 11618, CECT 3074, DSM 40028, HAMBI 1002, IFM 1173, NBRC 12735, IMET 43080, JCM 4561, JCM 4121, KCTC 1740, NRRL B-2120, NRRL-ISP 5028, PCM 2365, RIA 1000, RIA 756, UNIQEM 115, VKM Ac-1258.

Sequence accession no. (16S rRNA gene): AB184109.

4b. **Streptomyces achromogenes subsp. rubradiris** Bhuyan, Owen and Dietz 1965, 95[AL]

rub.ra.di′ris. L. adj. *ruber -bra -brum* red; L. n. *iris* the rainbow; N.L. n. *rubradiris* (*sic*) reddish rainbow.

S. achromogenes and its variants are melanin-positive and have gray aerial growth containing chains of oval and oblong spores. These chains contain more than 10 spores born in sporophores characterized as open loops. The cultures grow well at temperatures ranging from 18 to 37°C, but do not grow at 55°C. The effect of several nitrogen sources on antibiotic production was investigated in a medium containing starch as the carbohydrate source. A medium with Soludri, cornsteep liquor, $NaNO_3$, and starch gave the highest yields. Good growth and the proper pH level for antibiotic production were obtained in all cases, except when Wilson peptone liquor or Pharmamedia were used.

The effect of several carbohydrate sources was studied in a medium containing cornsteep liquor, Soludri, and $NaNO_3$. Cerelose or starch at a level of 1% were the preferred carbon sources. Equivalent growth was obtained in all cases, except for very heavy growth in glycerol-containing medium. Fermentations incubated at 25°C gave higher titers that those at 28 or 32°C. Incubation temperature during the first 2 d was critical for antibiotic production. Thus, the fermentation transferred to 28°C after 2 d of growth at 25°C gave the same yields as the fermentation at 25°C (21 µg/ml). However, when the fermentation was transferred to 25°C after 2 d growth at 28°C, lower yields (4 µg/ml) were obtained. Maximal yields were obtained when the pH was maintained near 8 during the production phase. Thus, when the pH was maintained at 7.2 until the third day by raising the starch concentration in the medium to 4% or by intermittent addition of acid, only 3 µg/ml antibiotic was produced as compared with 21 µg/ml when 1% starch was used.

During fermentation obtained with a medium containing starch, cornsteep liquor, Soludri, and $NaNO_3$, most of

the carbohydrate was used during the first 24 h, after which little sugar was utilized. Free ammoniacal nitrogen utilized during the first 24 h resulted in a decrease in pH. Release of NH_3 after this period raised the pH on the second day to above 8, where it remained during the rest of the fermentation. Rubradirin was produced mainly during the later phase of the fermentation to give a maximal titer of 21 µg/ml after 4 d.

Rubradirin was active *in vitro* mainly against Gram-stain-positive organisms. It was also active *in vitro* against two clinical strains of *Staphylococcus aureus* which were resistant to several antibiotics. It was inactive at 100 µg/ml against *Escherichia coli*, *Salmonella paratyphi*, *Proteus vulgaris*, *Salmonella typhi*, *Klebsiella pneumoniae*, and *Pseudomonas aeruginosa*. The antibiotic inhibited *Nocardia asteroides* at 1 µg/ml, but was inactive against a group of fungi at 1 mg/ml. Rubradirin was not cross-resistant with penicillin, streptomycin, erythromycin, chloramphenicol, or albamycin.

In vivo, mice infected with *Staphylococcus aureus* and *Streptococcus pyogenes* were protected by non-toxic doses of rubradirin. The antibiotic was active both by the oral and subcutaneous routes. It was inactive against *Pasteurella multocida in vivo* and *in vitro*. Although the antibiotic was active against *Mycobacterium tuberculosis* H 37-RV *in vitro*, it was inactive against it *in vivo*.

The type strain shows the highest sequence similarities to: *S. bangladeshensis*, AY750056, 99%; *S. glomeratus*, AB249917, 99%.

Source: not known.

DNA G+C content (mol%): not known.

Type strain: AS 4.1601, CBS 566.70, CECT 3075, DSM 40789, NBRC 14000, JCM 4955, KCTC 9742, NCIMB 9516, NRRL 3061.

Sequence accession no. (16S rRNA gene): AY999846.

5. **Streptomyces acidiscabies** Lambert and Loria 1989a, 395[VP]

a.ci.di.sca′bi.es. L. adj. *acidus* sour, acid; L. n. *scabies* scab, mange; N.L. n. *acidiscabies* acid scab, referring to the ability of the organism to cause acid scab of potatoes.

Spores are 0.4–0.5 × 0.6 or 0.9–1.1 µm, smooth, and white (reddish on certain high pH media) and are borne in mature flexuous chains containing 20 or more spores. A diffusible pigment is produced which is red above pH 8.3 and golden-yellow below pH 8.3. Melanin is not produced. L-Arabinose, D-fructose, D-glucose, D-mannitol, rhamnose, sucrose, and D-xylose are used as carbon sources, but not raffinose. *S. acidiscabies* differs from all other streptomycetes that cause typical raised or pitted symptoms and have white spores, red pigment, and acid tolerance. It differs in at least one major characteristic from all other strains described in the ISP having red pigments or spores. *Streptomyces acidiscabies* is placed in the genus *Streptomyces* as it possesses typical morphology and cell walls with the LL-A_2pm isomer. To date, *Streptomyces acidiscabies* has been isolated only from potatoes (*Solanum tuberosum*), and its pathogenicity towards other species has not been determined. The ability of this species to persist in soil is relatively poor and it is usually transmitted by infected seed tubers.

The type strain shows no sequence similarity over 99%.

Source: potatoes.

DNA G+C content (mol%): 71.0.

Type strain: RL-110, ATCC 49003, ICMP 12536, DSM 41668, JCM 7913, KCTC 9736, LMG 19856, NRRL B-16524.

Sequence accession no. (16S rRNA gene): D63865.

6. **Streptomyces acrimycini** (Preobrazhenskaya, Blinov and Ryabova *in* Gauze, Preobrazhenskaya, Kudrina, Blinov, Ryabova and Sveshnikova 1957) Pridham, Hesseltine and Benedict 1958, 65[AL] ("*Actinomyces acrimycini*" Preobrazhenskaya, Blinov and Ryabova *in* Gauze, Preobrazhenskaya, Kudrina, Blinov, Ryabova and Sveshnikova 1957, 140).

a.cri.my.ci′ni. L. adj. *acer-cris-cre* sharp, keen, pungent; N.L. suff. *-mycinum*, -mycin (antibiotics produced by *Streptomyces* strains); N.L. gen. n. *acrimycini* of the sharp antibiotic.

Spore chains in Section *Spirales*, with many loose spirals, but spore chains representative of Section *Retinaculiaperti* and some flexuous chains are also observed. Mature spore chains generally have 10–50 spores per chain; longer chains are sometimes observed. This morphology is seen on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar. Spore surface is hairy.

Color of colony: aerial mass color in the Green color series on yeast-malt agar and salts-starch agar; Green or Gray series on oatmeal agar and glycerol-asparagine agar. Reverse side of colony with no distinctive pigments on yeast-malt agar, oatmeal agar, salts-starch agar, or glycerol-asparagine agar. Substrate pigment is not a pH indicator (one observer reports a slight change from green to light green with addition of 0.05 M NaOH).

Color in medium: melanoid pigments are not formed in peptone-yeast-iron agar or tyrosine agar. No pigment is found in yeast-malt agar, oatmeal agar, salts-starch agar, or glycerol-asparagine agar.

D-Glucose, L-arabinose, D-xylose, iso-inositol, D-mannitol, D-fructose, and rhamnose are utilized for growth. No growth or only traces of growth on sucrose and raffinose.

Type strain shows the highest sequence similarity to: *S. griseoplanus*, AY999894, 100%; *S. anulatus*, DQ026637, 100%; *S. praecox*, AB184293, 100%; *S. flavofuscus*, AB249935, 100%; *S. fimicarius*, AY999784, 100%; *S. badius*, AY999783, 99.9%; *S. lavendulae* subsp. *lavendulae*, AB184080, 99.9%; *S. griseinus*, AB184205, 99.9%; *S. rubiginosohelvolus*, AB184240, 99.9%; *S. sindenensis*, AB184759, 99.9%; *S. mediolani*, AB184674, 99.9%; *S. cavourensis* subsp. *washingtonensis*, DQ026671, 99.9%; *S. pluricolorescens*, DQ442540, 99.9%; *S. fulvorobeus*, AB184711, 99.8%; *S. microflavus*, DQ445795, 99.8%; *S. albovinaceus*, AB249958, 99.8%; *S. cyaneofuscatus*, AB184860, 99.8%; *S. lipmanii*, AB184148, 99.8%; *S. baarnensis*, EF178688, 99.8%; *S. cinereorectus*, AB184646, 99.8%; *S. globisporus* subsp. *globisporus*, EF178686, 99.8%; *S. alboviridis*, AB184256, 99.8%; *S. californicus*, AB184755, 99.7%; *S. parvus*, DQ442537, 99.7%; *S. argenteolus*, AB045872, 99.7%; *S. floridae*, AB184656, 99.6%; *S. halstedii*, EF178695, 99.6%; *S. griseus* subsp. *griseus*, AY207604, 99.6%; *S. griseolus*, AB184768, 99.6%; *S. luridiscabiei*, AF361784, 99.6%; *S. flavovirens*, DQ026635, 99.6%; *S. flavogriseus*, AJ494864, 99.6%; *S. pulveraceus*, AB184806, 99.4%; *S. yanii*, AB006159, 99.4%; *S. olivoviridis*, AB184227, 99.4%; *S. bacillaris*, AB184439, 99.4%; *S. mutomycini*, AB249951, 99.4%; *S. nitrosporeus*, EF178680, 99.4%; *S. finlayi*, AY999788, 99.4%; *S. clavifer*,

DQ026670, 99.4%; *S. atroolivaceus*, AJ781320, 99.4%; *S. albolongus*, AB184425, 99.2%; *S. sanglieri*, AB249945, 99.2%; *S. griseobrunneus*, AB249912, 99.2%; *S. celluloflavus*, AB184476, 99.2%; *S. atratus*, DQ026638, 99.1%; *S. cavourensis* subsp. *cavourensis*, DQ445791, 99.1%; *S. spiroverticillatus*, AB184814, 99.1%; *S. gelaticus*, DQ026636, 99.1%; *S. candidus*, DQ026663, 99%.

Source: not known.

DNA G+C content (mol%): not known.

Type strain: AS 4.1673, ATCC 19720, ATCC 19885, CBS 459.68, BCRC 12220, CECT 3122, DSM 40135, NBRC 12736, INA 7699, JCM 4339, KCTC 9679, NRRL B-2565, NRRL-ISP 5135, RIA 1001, UNIQEM 117, VKM Ac-769.

Sequence accession no. (16S rRNA gene): AY999889.

7. **Streptomyces aculeolatus** Shomura, Gomi, Ito, Yoshida, Tanaka, Amano, Watabe, Ohuchi, Itoh, Sezaki, Takebe and Uotani 1988, 136[VP] (Effective publication: Shomura, Gomi, Ito, Yoshida, Tanaka, Amano, Watabe, Ohuchi, Itoh, Sezaki, Takebe and Uotani 1987, 738.)

acu.le.o.la′tus. N.L. masc. adj. *aculeolatus* (probably used in the place of the Latin adjective *aculeatus*) somewhat spiny, referring to the spore surfaces.

Mature spore chains have 10 or more spores per chain. This morphology is observed in sucrose-nitrate agar, glycerol-asparagine agar, and inorganic salts-starch agar. Spores are ellipsoidal in shape, 0.8–1.2 × 1.0–1.6 µm in size. Surface irregularities on spores are intermediate between warts and spines. Sporangia, flagellated spores, and sclerotic granules are not observed. Aerial mass color is in the White, Yellow, or Red color series. Vegetative mycelium is well developed and branched. The hyphae do not fragment into coccoid or bacillary elements. Aerial mycelium is simply branched and terminates in open or closed coils. The reverse side of colonies varies from pale yellow to orange depending on the medium. This orange color is somewhat pH-sensitive, changing from orange to reddish with addition of 0.05 M NaOH and from orange to yellowish with addition of 0.05 M HCl. Light brownish orange, water-soluble pigment is occasionally produced. On ISP 9 medium, utilizes D-glucose, D-fructose, D-xylose, L-arabinose, D-mannitol, raffinose, and rhamnose, but not iso-inositol or sucrose. Grows within the temperature range 15–37°C, with optimum growth at 26–30°C. Positive for hydrolysis of starch and liquefaction of gelatin. Reduction of nitrate, peptonization and coagulation of milk, and formation of melanoid pigment are all negative. Tolerates 3% NaCl, but no growth occurs on more than 4% NaCl. LL-A_2pm is detected in whole-cell hydrolysates of the culture.

The type strain shows no sequence similarity over 99%.

Source: not known.

DNA G+C content (mol%): not known.

Type strain: SF2415, DSM 41644, NBRC 14824, JCM 6055, KCTC 9680.

Sequence accession no. (16S rRNA gene): AB184624.

8. **Streptomyces afghaniensis** Shimo, Shiga, Tomosugi and Kamoi 1959, 1[AL]

af.gha.ni.en′sis. N.L. masc. adj. *afghaniensis* of or belonging to Afghanistan, the source of the soil from which the organism was isolated.

Spore chains in Section *Spirales*, but short spore chains usually result in hooks, incomplete spirals, or spirals with only one or two turns. Short, straight, and flexuous chains of only a few spores are common, but hooks and loops of wide diameter as found in *Retinaculum-Apertum* type cultures are not found. Spore chains are short, often with only 3–10 spores per chain, but more than 10 spores may be found on some chains. This morphology is best developed on oatmeal agar and salts-starch agar. Spore surface is spiny.

Color of colony: aerial mass color in the Blue color series on oatmeal agar. Immature aerial mycelium or mycelium producing few spores is in the Yellow color series. Observers have reported both Blue and Yellow color series for yeast-malt agar and salts-starch agar. Mature spores are not usually found on glycerol-asparagine agar. Reverse side of colony is grayed yellow on oatmeal agar, modified to orange or reddish brown on yeast-malt agar, salts-starch agar, and glycerol-asparagine agar; this pigment is not a pH indicator.

Color in medium: melanoid pigments are formed in peptone-yeast-iron agar, tyrosine agar, and peptone-yeast broth. Orange to red or brown pigments are found in the medium in yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar; these pigments are not pH-sensitive when tested with 0.05 M HCl or NaOH.

D-Glucose, L-arabinose, sucrose, D-xylose, iso-inositol, D-mannitol, D-fructose, rhamnose, and raffinose are utilized for growth.

Type strain shows the highest sequence similarity to: *S. africanus*, AY208912, 99.8%; *S. levis*, AB184670, 99.2%; *S. brasiliensis*, AB249981, 99.1%; *S. tuirus*, AB184690, 99.1%; *S. albosporeus* subsp. *albosporeus*, AJ781327, 99%; *S. cellulosae*, DQ442495, 99%; *S. azureus*, EF178674, 99%; *S. capillispiralis*, AB184577, 99%; *S. gancidicus*, AB184660, 99%; *S. pseudogriseolus*, DQ442541, 99%; *S. roseoviolaceus*, AJ399484, 99%; *S. violaceus*, AB184315, 99%; *S. janthinus*, AB184851, 99%; *S. hawaiiensis*, AB184143, 99%.

Source: soil from Afghanistan.

DNA G+C content (mol%): not known.

Type strain: ATCC 23871, CBS 610.68, DSM 40228, NBRC 12831, JCM 4340, NRRL B-5621, NRRL-ISP 5228, RIA 1169.

Sequence accession no. (16S rRNA gene): AJ399483.

9. **Streptomyces africanus** Meyers, Goodwin, Bennett, Aken, Price and Van Rooyen 2004, 1534[VP]

af.ri.ca′nus. L. masc. adj. *africanus* of Africa.

Spirales-type spore chains with spiny spore sheaths are produced. Aerial mycelium is blue and substrate mycelium is yellow. The color of the substrate mycelium is not pH-sensitive. Verticils are not present. The mycelium does not fragment. No diffusible pigments are produced on glycerol-asparagine agar or on any other medium tested. Melanin pigment is not produced on peptone-yeast extract-iron agar or on tyrosine agar. The cell wall contains LL-A_2pm (cell-wall type I); there are no diagnostic sugars. Excellent growth occurs on inorganic salts-starch agar. Very good growth occurs on yeast extract-malt extract agar, oatmeal agar, and Czapek's solution agar. Growth is good on Bennett's agar. Growth on glycerol-asparagine agar is moderate. Grows in the presence of 2-phenylethanol (0.3%), 7% NaCl (but not 10%), carbenicillin (100 µg/ml) cefataxime (100 µg/ml),

D-cycloserine (50 μg/ml), nalidixic acid (25 μg/ml), oleandomycin (100 μg/ml), and penicillin G (10 IU/ml). Grows at pH 4.3 and 45°C, but not at 4°C or in the presence of sodium azide, capreomycin (20 μg/ml), cephaloridine (100 μg/ml), chloramphenicol (50 μg/ml), erythromycin (50 μg/ml), gentamicin (100 μg/ml), kanamycin (10 μg/ml), lincomycin (100 μg/ml), neomycin (50 μg/ml), phenol (0.1%), rifampin (50 μg/ml), spectinomycin (20 μg/ml), streptomycin (100 μg/ml), tobramycin (50 μg/ml), or vancomycin (50 μg/ml). The organism degrades adenine, esculin, arbutin, casein, DNA, gelatin, hypoxanthine, starch, Tween 80, and L-tyrosine, but not allantoin, cellulose, guanine, urea, xanthine, or xylan. Utilizes adonitol, (+)-L-arabinose, (+)-D-cellobiose, (−)-D-fructose, (+)-D-galactose, glycerol, *myo*-inositol, inulin, lactose, maltose, D-mannitol, (+)-D-mannose, (+)-D-melibiose, methyl α-D-glucoside, raffinose, (+)-L-rhamnose, (−)-D-ribose, salicin, sodium acetate, sodium butyrate, sodium citrate, sodium DL-malate, sodium malonate, sodium propionate, sodium pyruvate, sodium salicylate, sodium succinate, sucrose, trehalose and (+)-D-xylose as sole carbon sources, but not *meso*-erythritol, (+)-D-melezitose, sodium benzoate, sodium formate, sodium maleate, sodium oxalate, sodium (+)-L-tartrate, (−)-L-sorbose, or xylitol. Utilizes 4-amino-n-butyric acid, DL-α-amino-n-butyric acid (weak growth), L-arginine, L-cysteine, L-histidine, L-hydroxyproline (weak growth), L-methionine, DL-ornithine, L-phenylalanine, potassium nitrate, L-serine, L-threonine, and L-valine as sole nitrogen sources. Tests for nitrate reductase and H_2S production are positive. Pectin is hydrolyzed, but hippurate is not. Protease, lipase, and lecithinase activities are produced on egg-yolk agar (the proteolytic reaction is weak). Weak antibiotic activity is exhibited against *Enterococcus faecium*, but no antibiotic activity is observed against *Escherichia coli* ATCC 25922 or *Pseudomonas aeruginosa* ATCC 27853.

Type strain shows the highest sequence similarity to: *S. afghaniensis*, AJ399483, 99.8%; *S. janthinus*, AB184851, 99.2%; *S. roseoviolaceus*, AJ399484, 99.2%; *S. albosporeus* subsp. *albosporeus*, AJ781327, 99.2%; *S. levis*, AB184670, 99.2%; *S. violaceus*, AB184315, 99.2%; *S. brasiliensis*, AB249981, 99.2%; *S. azureus*, EF178674, 99.1%; *S. tuirus*, AB184690, 99.1%; *S. mutabilis*, EF178679, 99%; *S. hawaiiensis*, AB184143, 99%; *S. pseudogriseolus*, DQ442541, 99%; *S. capillispiralis*, AB184577, 99%; *S. gancidicus*, AB184660, 99%; *S. cellulosae*, DQ442495, 99%.

Type strain shows DNA–DNA similarity to: *S. afghaniensis* NRRL B-5621^T, 46.2 ± 0.9%; "*S. steffisburgensis*" NRRL ISP 5547, 38.4 ± 0.5%.

Source: not known.

DNA G+C content (mol%): 73.2.

Type strain: CPJVR-H, DSM 41829, JCM 13243, NBRC 101005.

Sequence accession no. (16S rRNA gene): AY208912.

10. **Streptomyces alanosinicus** Thiemann and Beretta 1966, 158[AL]

a.la.no.si′ni.cus. N.L. n. *alanosinum* alanosine (name of an antibiotic); L. masc. suff. *-icus* suffix used with the sense of belonging to; N.L. masc. adj. *alanosinicus* belonging to alanosine.

Spore chains in Section *Spirales*. Mature spore chains are generally long, often with more than 50 spores per chain. This morphology is seen on yeast-malt agar, oatmeal agar, and salts-starch agar, but sporulating aerial mycelium is thin or absent on glycerol-asparagine agar. Spore surface is spiny.

Color of colony: aerial mass color in the Red color series (4ec, grayish yellowish pink, or 3ca, pale orange yellow) on yeast-malt agar, oatmeal agar, and salts-starch agar; sporulating aerial mycelium is thin or absent on glycerol-asparagine agar. Reverse side of colony has no distinctive pigments (moderate reddish brown to strong brown on yeast-malt agar; pale grayish yellow to light yellowish brown or olive brown on oatmeal agar, salts-starch agar, and glycerol-asparagine agar).

Color in medium: melanoid pigments are formed in peptone-yeast-iron agar, tyrosine agar, and tryptone-yeast broth, but reaction may be delayed or weak in tyrosine agar. No pigment is found in medium in salts-starch agar or glycerol-asparagine agar. Some yellow pigment may or may not be seen in yeast-malt agar and oatmeal agar.

D-Glucose, L-arabinose, D-xylose, iso-inositol, D-mannitol, D-fructose, and raffinose are utilized for growth. Reports vary on utilization of sucrose; no growth or only traces of growth with rhamnose.

Type strain shows no sequence similarity over 99%.

Source: not known.

DNA G+C content (mol%): not known.

Type strain: V119, AS 4.1634, ATCC 15710, CBS 348.69, CBS 794.72, DSM 40606, HAMBI 983, NBRC 13493, JCM 4714, KCTC 9683, NRRL B-3627, NRRL-ISP 5606, RIA 1454, VKM Ac-1752.

Sequence accession no. (16S rRNA gene): AB184442.

11. **Streptomyces albaduncus** Tsukiura, Okanishi, Ohmori, Koshiyama, Miyaki, Kitazima and Kawaguchi 1964b, 41[AL]

al.ba.dun′cus. L. adj. *albus* white; L. adj. *uncus* hooked, crooked; N.L. masc. adj. *albaduncus* (*sic*) white, hooked, probably referring to color of aerial mycelium and nature of spore chains of the organism.

Spore chains in Section *Retinaculiaperti* to *Spirales*. Short spore chains are flexuous or form hooks, loops, and incomplete or imperfect spirals. True spirals are not reported. Mature spore chains generally contain more than 10 spores per chain but are shorter than those found in *Retinaculum-Apertum* type cultures. This morphology is seen on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar. Spore surface is spiny with numerous long spines.

Color of colony: aerial mass color in the White or Yellow or Gray color series on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar. The nearest matching color tab in the Yellow series is 2ba, pale yellow, and in the Gray color series is d, light gray, 2fe, medium gray, or 3fe, light brownish gray. Reverse side of colony has no distinctive pigments (grayish yellow to yellowish brown, olive brown, or strong brown) on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar.

Color in medium: melanoid pigments are not formed in peptone-yeast-iron agar, tyrosine agar, or tryptone-yeast broth. Yellow pigment is found in the medium in yeast-malt

agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar. This pigment is not pH-sensitive when tested with 0.05 M NaOH or HCl.

D-Glucose, L-arabinose, D-xylose, iso-inositol, D-mannitol, D-fructose, and rhamnose are utilized for growth. No growth or only traces of growth with sucrose or raffinose.

Type strain shows the highest sequence similarity to: *S. griseoloalbus*, AB184275, 100%; *S. matensis*, AB184221, 99.3%; *S. paradoxus*, AB184628, 99.2%; *S. pseudogriseolus*, DQ442541, 99.2%; *S. gancidicus*, AB184660, 99.2%; *S. capillispiralis*, AB184577, 99.1%; *S. heliomycini*, AB184712, 99%; *S. malachitofuscus*, AB184282, 99%; *S. griseoflavus*, AJ781322, 99%; *S. ambofaciens*, M27245, 99%; *S. cellulosae*, DQ442495, 99%; *S. lusitanus*, AB184424, 99%.

Source: not known.

DNA G+C content (mol%): not known.

Type strain: AS 4.158, ATCC 14698, CBS 698.72, CECT 3226, DSM 40478, NBRC 13397, JCM 4715, KCTC 1741, NRRL B-3605, NRRL-ISP 5478, RIA 1358, VKM Ac-1753, ISP 5478.

Sequence accession no. (16S rRNA gene): AY999757.

12. **Streptomyces albiaxialis** Kuznetsov, Zajtseva, Vakulenko and Flippova 1993, 398[VP] (Effective publication: Kuznetsov, Zajtseva, Vakulenko and Flippova 1992, 90.)

al.bi.a.xi.a'lis. L. adj. *albus -a -um* white; L. n. *axis* axle; N.L. adj. *axialis -is -e* axial; N.L. masc. adj. *albiaxialis* white axial.

Forms spiral sporophores with 3–8 extended spirals; sporophores are distributed on a long axis as pseudowhorls. Spores are oblong with a smooth envelope. On solid growth media, the culture forms a white aerial mycelium and dark cream-colored substrate mycelium. The population of *S. albiaxialis* consists of three spontaneous variants: basic, faded, and oligosporous. Basic variant: colonies are slightly bulging, aerial mycelium is white, substrate mycelium is dark cream-colored. Faded variant: colonies are flattened, aerial mycelium is whitish, substrate mycelium is pale yellow. Oligosporous variant: colonies are slightly prominent, whitish aerial mycelium develops only in the center of the colony, substrate mycelium is beige-colored. Soluble pigment is not formed by any variant. It is interesting to note that a population of newly isolated culture originally was absolutely homogeneous and the above-mentioned variants were identified in the population of *Streptomyces albiaxialis* only after cultivation for 7–8 months under laboratory conditions. Probably, this is due to the specific and stable inhabitance conditions of the organism in highly mineralized abyssal (2000 m) stratal water.

The organism liquefies gelatin, coagulates and peptonizes milk, and weakly hydrolyzes starch. It assimilates glucose, sucrose, rhamnose, arabinose, and xylose as a sole carbon source, but rather weakly utilizes raffinose and does not assimilate mannitol or inositol. It assimilates oil hydrocarbons, wax, and Vaseline oil. It is halotolerant and thermotolerant and is able to develop in media containing from 3 to 30% NaCl. The temperature optimum for development is between 28 and 33°C; maximum at 48–50°C. The cell wall contains LL-A_2pm and no differentiating sugars (Type I cell wall). The organism inhibits growth of Gram-stain-positive bacteria, acid-fast mycobacteria, and actinomycetes. It has a slight effect on the growth of some mycelial fungi (*Helminthosporium sativum*), but does not inhibit growth of Gram-stain-negative bacteria and yeasts. The culture is sensitive to monomycin, streptomycin, kanamycin, neomycin, sisomycin, gentamicin, lincomycin, erythromycin, oleandomycin, ristomycin, levomycin, and fusidin, but is resistant to penicillin, carbenicillin, methicillin, polymyxin, tetracycline, and oxytetracycline.

Type strain shows no sequence similarity over 99%.

Source: not known.

DNA G+C content (mol%): not known.

Type strain: DSM 41799, NBRC 101002, VKM A-691, NRRL B-24327.

Sequence accession no. (16S rRNA gene): AY999901.

13. **Streptomyces albidochromogenes** Preobrazhenskaya 1986, 573[VP] (Effective publication: Preobrazhenskaya *in* Gause, Preobrazhenskaya, Sveshnikova, Terekhova and Maximova 1983.)

al.bi.do.chro.mo'ge.nes. L. adj. *albidus -a -um*, white; Gr. n. *chroma* color; N.L. suff. *-genes* (from Gr. v. *gennaô* to produce), producing; N.L. part. adj. *albidochromogenes* producing white color.

Spore chains are spiral. Spore surface is smooth. On mineral agar 1, glycerol-nitrate agar: aerial mycelium is whitish, creamy, yellow, grayish yellow; substrate mycelium is gray brownish yellow, yellow-gray-brown; no diffusible pigment. On starch-ammonia agar, glycerol-asparagine agar, oatmeal agar: aerial mycelium is white-yellow, yellow; substrate mycelium is colorless or yellow; no diffusible pigment. On organic agar 2: substrate mycelium is absent or whitish, grayish yellow; substrate mycelium and diffusible pigment are gray-brown. Melanoid pigment is found. Grows on glucose, fructose, xylose, arabinose, mannitol, and sucrose; no growth on rhamnose, raffinose, or inositol. Antibiotic is not produced.

Type strain shows the highest sequence similarity to: *S. flavidovirens*, AB184270, 100%; *S. chryseus*, AY999787, 99.9%; *S. helvaticus*, AB184367, 99.9%; *S. enissocaesilis*, AB249930, 99.1%.

Source: not known.

DNA G+C content (mol%): not known.

Type strain: DSM 41800, INA 11792, JCM 13858, NBRC 101003.

Sequence accession no. (16S rRNA gene): AB249953.

Further comments: the name of the individual to be credited for *Streptomyces albidochromogenes* proposed in reference Gause et al. (1983) was supplied by T.P. Preobrazhenskaya in a personal communication to the Associate Editor, IJSB.

Culture was originally described as *Actinomyces albidus invertens* Kudrina 1957.

14. **Streptomyces albidoflavus** (Rossi Doria 1891) Waksman and Henrici *in* Breed, Murray and Hitchens 1948, 949[AL] ["*Streptotrix albidoflava*" (*sic*) Rossi Doria 1891, 407; "*Streptothrix albidoflava*" Rossi Doria 1891, 407; "*Actinomyces albidoflavus*" Gasperini 1894, 87; "*Cladothrix albido-flava*" (*sic*) Macé 1901, 1095]

al.bi.do.fla'vus. L. adj. *albidus* white; L. adj. *flavus* yellow; N.L. masc. adj. *albidoflavus* whitish yellow.

Spore chains in Section *Rectiflexibiles*. Two of three observers were unable to find sporulating aerial mycelium. Mature spore chains, when formed, are generally short with 3–10 spores per chain. Sporulating aerial mycelium is sometimes found on yeast-malt agar and glycerol-asparagine agar, but is not seen on oatmeal agar or salts-starch agar. Spore surface is smooth. Special morphological characteristics are as follows: substrate mycelium fragments, forming conidia-like or irregular spores; and unusually large spores may sometimes be formed by fragmentation of aerial hyphae.

Color of colony: aerial mass color in the White or Gray color series on yeast-malt agar. Sporulating aerial mycelium is not produced on other ISP media. Reverse side of colony with no distinctive pigments (light yellow to grayish yellow or orange yellow) on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar.

Color in medium: melanoid pigments are not formed in peptone-yeast-iron agar, tyrosine agar, or tryptone-yeast broth. No pigment is found in the medium in yeast-malt agar, oatmeal agar, salts-starch agar, or glycerol-asparagine agar.

D-Glucose, L-arabinose, D-xylose, D-mannitol, and D-fructose are utilized for growth. No growth or only traces of growth with sucrose, iso-inositol, rhamnose, and raffinose.

Type strain shows the highest sequence similarity to: *S. limosus*, AB184147, 100%; *S. felleus*, AB184129, 100%; *S. daghestanicus*, DQ442497, 100%; *S. odorifer*, Z76682, 100%; *S. violascens*, AY999737, 100%; *S. hydrogenans*, AB184868, 100%; *S. griseus* subsp. *solvifaciens*, AB249915, 100%; *S. champavatii*, DQ026642, 100%; *S. canescens*, AB184117, 100%; *S. sampsonii*, D63871, 99.9%; *S. koyangensis*, AY079156, 99.7%.

Source: not known.

DNA G+C content (mol%): not known.

Type strain: CBS 416.34, ATCC 25422, CBS 416.34, CBS 920.69, BCRC 13699, CIP 105122, DSM 40455, ICMP 12537, NBRC 13010, JCM 4446, KCTC 9202, LMG 19300, NCIMB 10043, NRRL B-1271, NRRL B-2663, NRRL-ISP 5455, RIA 1202, VKM Ac-746.

Sequence accession no. (16S rRNA gene): AB184255.

15. **Streptomyces albiflaviniger** Goodfellow, Kumar, Labeda and Sembiring 2008, 1[VP] (Effective publication: Goodfellow, Kumar, Labeda and Sembiring 2007, 197.)

al.bi.fla.vi.ni′ger. L. adj. *albus* white; L. adj. *flavus* yellow; L. adj. *niger* black; N.L. masc. adj. *albiflaviniger* white, yellow, and black colors.

Spore chains in Section *Spirales*; spore surface is rugose. On oatmeal agar, the aerial spore mass color is white, becoming black and moist when mature; the reverse side of colonial growth is yellow. Brown, orange, and yellow diffusible pigments are formed, but not melanin pigments.

Type strain shows the highest sequence similarity to: *S. violaceusniger*, AJ391823, 99.7%; *S. yogyakartensis*, AB249942, 99.7%; *S. demainii*, DQ334782, 99.3%; *S. endus*, AY999911, 99.3%; *S. sporocinereus*, AB249933, 99.3%; *S. hygroscopius* subsp. *hygroscopius*, AB184428, 99.3%. Type strain shows DNA–DNA similarity to: *S. geldanamycinus* NRRL 3602^T, 99.1%; *S. griseiniger* NRRL B1865^T, 99.1%.

Source: not known.

DNA G+C content (mol%): 70.5.

Type strain: DSM 41598, NRRL B-1356.

Sequence accession no. (16S rRNA gene): AJ391812.

16. **Streptomyces albireticuli** (Nakazawa 1955) Witt and Stackebrandt 1991, 456[VP] (Effective publication: Witt and Stackebrandt 1990, 370.) ("*Streptomyces albireticuli*" Nakazawa 1955, 248; "*Verticillomyces albireticuli*" Shinobu 1965; *Streptoverticillium albireticuli* Locci, Baldacci and Petrolini Baldan 1969, 59).

al.bi.re.ti′cu.li. L. adj. *albus* white; L. n. *reticulum* a small net; N.L. gen. n. *albireticuli* of a small white net.

Spore chains in Section *Verticillati*. Both monoverticillate and umbellate moverticillate (biverticillate) sporophores are found. Mature spore chains generally have 10–50 spores per chain on suitable media. This morphology is seen on yeast-malt agar, oatmeal agar, and salts-starch agar after 2–3 weeks; poor growth of aerial mycelium is seen on glycerol-asparagine agar and variable aerial growth is observed on oatmeal agar and salts-starch agar. Spore surface is smooth.

Color of colony: aerial mass color in the Yellow or White color series on yeast-malt agar, oatmeal agar, and salts-starch agar. Reverse side of colony with no distinctive pigments on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar; substrate pigment is not a pH indicator.

Color in medium: melanoid pigments are formed in peptone-yeast-iron agar and tyrosine agar. Pigments other than melanoids not formed in yeast-malt agar, oatmeal agar, salts-starch agar, or glycerol-asparagine agar.

D-Glucose and iso-inositol are utilized for growth. No growth or only traces of growth on L-arabinose, sucrose, D-xylose, D-mannitol, rhamnose, and raffinose. Variable reports on growth with D-fructose.

Type strain shows the highest sequence similarity to: *S. eurocidicus*, AY999790, 99.8%; *S. biverticillatus*, AJ781381, 99.5%; *S. werraensis*, DQ442558, 99.5%; *S. blastmyceticus*, AY999802, 99.3%; *S. stramineus*, AB184720, 99%; *S. netropsis*, AB184848, 99%; *S. hiroshimensis*, AB184789, 99%.

Source: not known.

DNA G+C content (mol%): not known.

Type strain: AS 4.1649, ATCC 19721, CBS 460.68, BCRC 12427, CECT 3253, DSM 40051, HUT 6040, IFM 1068, NBRC 12737, NBRC 3400, JCM 4562, JCM 4116, KCTC 9685, NCIMB 9600, NRRL B-1670, NRRL B-5493, NRRL-ISP 5051, RIA 1002, UNIQEM 209.

Sequence accession no. (16S rRNA gene): AB184881.

Further comments: in violation of Rule 33c, in Validation List no. 38, *Streptomyces albireticuli* is proposed as a *nomen revictum* (basonym: "*Streptomyces albireticuli*" Nakazawa 1955).

According to Hatano et al. (2003), *Streptomyces albireticuli* (Nakazawa 1955) Witt and Stackebrandt 1991 is a later heterotypic synonym of *Streptomyces eurocidicus* (Okami et al. 1954) Witt and Stackebrandt 1991.

17. **Streptomyces albofaciens** Thirumalachar and Bhatt 1960, 63[AL]

al.bo.fa′ci.ens. L. adj. *albus* white; L. v. *facio* make; N.L. part adj. *albofaciens* making white.

Spore chain morphology in Section *Spirales*. Open irregular spirals sometimes appear to arise from an axial

hypha, but true whorls typical of verticillate cultures are not formed. Mature spore chains generally have 10–50, or sometimes more than 50, spores per chain. This morphology is seen on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar. Spore surface is smooth.

Color of colony: aerial mass color in the White color series on yeast-malt agar and glycerol-asparagine agar; White or Gray color series on oatmeal agar and inorganic salts-starch agar. Reverse side of colony with no distinctive pigments on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar.

Color in medium: melanoid pigments are not formed in peptone-yeast-iron agar, tyrosine agar, or tryptone-yeast broth. No pigment or only trace of yellow pigment found in medium in yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar.

D-Glucose, L-arabinose, iso-inositol, D-mannitol, D-fructose, and raffinose are utilized for growth. No growth or only traces of growth on rhamnose. Utilization of sucrose and D-xylose is doubtful.

Type strain shows the highest sequence similarity to: *S. rimosus* subsp. *paromomycinus*, AJ621610, 99.7%; *S. chrestomyceticus*, AJ621609, 99.7%; *S. erumpens*, AJ621603, 99.1%.

Source: not known.

DNA G+C content (mol%): not known.

Type strain: AS 4.1655, ATCC 23873, ATCC 25184, CBS 612.68, BCRC 12072, CIP 104425, DSM 40268, NBRC 12833, IMET 43518, JCM 4342, KCTC 9686, NCIMB 10975, NRRL B-12172, NRRL-ISP 5268, RIA 1189, VKM Ac-724.

Sequence accession no. (16S rRNA gene): AB045880.

18. **Streptomyces alboflavus** (Waksman and Curtis 1916) Waksman and Henrici *in* Breed, Murray and Hitchens 1948, 954[AL] ("*Actinomyces alboflavus*" Waksman and Curtis 1916, 120)

al.bo.fla′vus. L. adj. *albus* white; L. adj. *flavus* yellow; N.L. masc. adj. *alboflavus* whitish yellow.

Typical aerial mycelium is not formed on yeast-malt agar, oatmeal agar, salts-starch agar, or glycerol-asparagine agar. Spore chain morphology, spore surface, and aerial mass color of colony cannot be observed on ISP media. Loss of ability to produce sporulating aerial mycelium was noted in an early description of this culture (Waksman 1919, 90). Early descriptions of the culture describe white or yellowish white aerial mycelium on synthetic agar or Czapek's agar only.

Special morphological characteristics: coremia formation on salts-starch agar and glycerol-asparagine agar was recorded by two observers. This same phenomenon was recorded in the original description on Czapek's agar (Waksman 1916, 120): "…aerial mycelium was found to have a tendency to produce…a mass of hyphae massed together into a rope, and from this rope fine filaments coming out in the shape of side branches. The structure looks like the root of a tree and fine rootlets coming out on the side". One ISP observer found straight spore chains within the coremia on glycerol-asparagine agar. Reverse side of colony with no distinctive pigment (grayed yellow or grayed greenish yellow) on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar.

Color in medium: melanoid pigments form weakly in peptone-yeast-iron agar and tryptone-yeast broth, but not on tyrosine agar. Pigments other than melanoids are not formed in yeast-malt agar, oatmeal agar, salts-starch agar, or glycerol-asparagine agar.

D-Glucose, L-arabinose, D-xylose, iso-inositol, D-mannitol, D-fructose, and raffinose are utilized for growth. No growth or only traces of growth on rhamnose. Utilization of sucrose is doubtful.

Type strain shows the highest sequence similarity to: *S. fulvissimus*, AB184434, 99.3%; *S. flavofungini*, AB184359, 99%; *S. variegatus*, AJ781371, 99%.

Source: not known.

DNA G+C content (mol%): not known.

Type strain: IMRU 3008, AS 4.1461, ATCC 12626, ATCC 23874, CBS 613.68, BCRC 13664, CIP 104427, DSM 40045, NBRC 13196, IMET 42936, JCM 4615, KCTC 9674, NRRL B-1273, NRRL B-2373, NRRL-ISP 5045, RIA 1112, VKM Ac-972.

Sequence accession no. (16S rRNA gene): EF178699.

19. **Streptomyces albogriseolus** Benedict, Shotwell, Pridham, Lindenfelser and Haynes 1954, 653[AL]

al.bo.gri.se.o′lus. L. adj. *albus* white; N.L. adj. *griseus* gray; L. masc. suff. *-olus* diminutive ending; N.L. dim. masc. adj. *albogriseolus* white grayish.

Spore chains in Section *Spirales*. Spirals are open. Flexuous or *Retinaculum-Apertum* type spore chains are also common. Mature spore chains generally have 10–50 spores per chain. This morphology is seen on yeast-malt agar, oatmeal agar, and salts-starch agar, but not on glycerol-asparagine agar. Spore surface is warty. Warts are not prominent or regular and some smooth spores may be found.

Color of colony: aerial mass color in the Gray color series on yeast-malt agar, oatmeal agar, and salts-starch agar. Reverse side of colony with no distinctive pigments (colorless or yellowish gray on oatmeal agar, salts-starch agar, and glycerol-asparagine agar; grayed yellowish brown or olive brown on yeast-malt agar).

Color in medium: melanoid pigments are not formed in peptone-yeast-iron agar, tyrosine agar, or tryptone-yeast broth. No pigment is found in medium in yeast-malt agar, oatmeal agar, salts-starch agar, or glycerol-asparagine agar.

D-Glucose, sucrose, D-xylose, iso-inositol, D-mannitol, D-fructose, and rhamnose are utilized for growth, but growth on sucrose is less abundant than on other carbon sources. No growth or only traces of growth on raffinose.

Type strain shows the highest sequence similarity to: *S. viridodiastaticus*, AY999852, 99.8%; *S. coeruleorubidus*, AY999719, 99.4%; *S. bellus*, AB184849, 99.2%; *S. coerulescens*, AY999720, 99.2%; *S. griseorubens*, AB184139, 99.1%; *S. longispororuber*, AB184440, 99.1%; *S. atrovirens*, DQ026672, 99.1%; *S. griseoincarnatus*, AJ781328, 99%; *S. labedae*, AB184704, 99%; *S. ambofaciens*, M27245, 99%; *S. erythrogriseus*, AJ781328, 99%; *S. iakyrus*, AB184877, 99%; *S. lusitanus*, AB184424, 99%.

Source: not known.

DNA G+C content (mol%): not known.

Type strain: ATCC 23875, CBS 614.68, BCRC 12230, CIP 104424, CIP 104428, DSM 40003, HUT 6045, NBRC 12834, NBRC 3413, NBRC 3709, JCM 4616, JCM 4004, KCTC 9675, NCIMB 9604, NRRL B-1305, NRRL-ISP 5003, RIA 1101, VKM Ac-1200.

Sequence accession no. (16S rRNA gene): AJ494865.

20. **Streptomyces albolongus** Tsukiura, Okanishi, Koshiyama, Ohmori, Miyaki and Kawaguchi 1964a, 225[AL]

al.bo.lon′gus. L. adj. *albus* white; L. adj. *longus* long; N.L. masc. adj. *albolongus* white and long.

Color of colony: aerial mass color in the White color series on yeast-malt agar and salts-starch agar; White or Yellow color series (2ba, pale yellow) on oatmeal agar and glycerol-asparagine agar. Reverse side of colony with no distinctive pigments (grayish yellow, orange yellow, or yellowish brown) on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar.

Color in medium: melanoid pigments are formed in peptone-yeast-iron agar, tyrosine agar, and tryptone-yeast broth. No pigment is found in medium in yeast-malt agar, oatmeal agar, salts-starch agar, or glycerol-asparagine agar.

D-Glucose, L-arabinose, and D-xylose are utilized for growth. Only traces of growth are found on iso-inositol, D-mannitol, D-fructose, rhamnose, sucrose, or raffinose.

Type strain shows the highest sequence similarity to: *S. cavourensis* subsp. *cavourensis*, DQ445791, 100%; *S. griseobrunneus*, AB249912, 100%; *S. celluloflavus*, AB184476, 100%; *S. californicus*, AB184755, 99.5%; *S. bacillaris*, AB184439, 99.5%; *S. floridae*, AB184656, 99.5%; *S. globisporus* subsp. *globisporus*, EF178686, 99.5%; *S. griseinus*, AB184205, 99.4%; *S. fulvorobeus*, AB184711, 99.4%; *S. pluricolorescens*, DQ442540, 99.4%; *S. candidus*, DQ026663, 99.4%; *S. spiroverticillatus*, AB184814, 99.4%; *S. sindenensis*, AB184759, 99.4%; *S. badius*, AY999783, 99.4%; *S. rubiginosohelvolus*, AB184240, 99.4%; *S. mediolani*, AB184674, 99.4%; *S. praecox*, AB184293, 99.3%; *S. albovinaceus*, AB249958, 99.3%; *S. microflavus*, DQ445795, 99.3%; *S. fimicarius*, AY999784, 99.3%; *S. flavofuscus*, AB249935, 99.5%; *S. alboviridis*, AB184256, 99.3%; *S. lipmanii*, AB184148, 99.3%; *S. cremeus*, AB184124, 99.3%; *S. griseoplanus*, AY999894, 99.3%; *S. anulatus*, DQ026637, 99.3%; *S. cavourensis* subsp. *washingtonensis*, DQ026671, 99.3%; *S. lavendulae* subsp. *lavendulae*, AB184080, 99.3%; *S. parvus*, DQ442537, 99.3%; *S. luridiscabiei*, AF361784, 99.2%; *S. acrimycini*, AY999889, 99.2%; *S. baarnensis*, EF178688, 99.2%; *S. cyaneofuscatus*, AB184860, 99.2%; *S. cinereorectus*, AB184646, 99.2%; *S. flavovirens*, DQ026635, 99.2%; *S. nitrosporeus*, EF178680, 99.1%; *S. argenteolus*, AB045872, 99.1%; *S. finlayi*, AY999788, 99.1%; *S. flavogriseus*, AJ494864, 99.1%; *S. griseolus*, AB184768, 99.1%; *S. pulveraceus*, AB184806, 99%; *S. clavifer*, DQ026670, 99%; *S. griseus* subsp. *griseus*, AY207604, 99%; *S. halstedii*, EF178695, 99%; *S. cinnamonensis*, AB184707, 99%.

Source: not known.

DNA G+C content (mol%): not known.

Type strain: AS 4.1661, ATCC 27414, CBS 766.72, DSM 40570, NBRC 13465, JCM 4716, KCTC 9676, NRRL B-3604, NRRL-ISP 5570, RIA 1426, VKM Ac-704.

Sequence accession no. (16S rRNA gene): AB184425.

21. **Streptomyces alboniger** (*sic*) Porter, Hewitt, Hesseltine, Krupka, Lowery, Wallace, Bohonos and Williams 1952, 409[AL]

al.bo.ni′ger. L. adj. *albus* white; L. adj. *niger* black; N.L. adj. *alboniger* whitish black.

Spore chains in Section *Rectiflexibiles.* Mature spore chains generally have 10 to 50 or more spores per chain. This morphology is seen on oatmeal agar, salts-starch agar, and glycerol-asparagine agar. Spore surface is smooth.

Color of colony: aerial mass color in the White color series on oatmeal agar, salts-starch agar, and glycerol-asparagine agar; aerial mycelium is poorly developed or absent on yeast-malt agar. Reverse side of colony with no distinctive pigments on yeast-malt agar, oatmeal agar, salts-starch agar, or glycerol-asparagine agar, except that transient dark reverse colors ranging from very dark brown to nearly black are sometimes reported on salts-starch agar and glycerol-asparagine agar. Reverse pigment is not a pH indicator.

Color in medium: melanoid pigments not formed (or only a faint brown) in peptone-yeast-iron agar in 2–4 d, but transient dark pigments are reported on various media for older cultures. All observers report dark pigment in salts-starch agar in 7 d. This pigment is not pH-sensitive.

D-Glucose, L-arabinose, iso-inositol, and D-mannitol are utilized for growth. No growth or only trace of growth on sucrose and rhamnose. Variable reports on growth with D-fructose, D-xylose, and raffinose.

Type strain shows the highest sequence similarity to: *S. resistomycificus*, AB184166, 99%.

Source: not known.

DNA G+C content (mol%): not known.

Type strain: P-638, AS 4.1695, ATCC 12461, ATCC 19722, CBS 461.68, BCRC 11606, CECT 3270, DSM 40043, HAMBI 53, NBRC 12738, IMET 43691, JCM 4563, JCM 4309, KCTC 9014, NCIMB 13007, NRRL B-1832, NRRL B-2403, NRRL-ISP 5043, RIA 1003, UNIQEM 118, VKM Ac-838.

Sequence accession no. (16S rRNA gene): AY845349.

22. **Streptomyces albospinus** (*sic*) Wang, Hamada, Okami and Umezawa 1966, 217[AL]

al.bo.spi′nus. L. adj. *albus* white; L. adj. *spineus* spiny; N.L. masc. adj. *albospinus* (*sic*) white, spiny, referring to the color of the aerial mycelium and nature of spore wall ornamentation.

Produces spinamycin, a non-polyenic anti-fungal antibiotic. Type strain shows the highest sequence similarity to: *S. sioyaensis*, DQ026654, 99%.

Source: not known.

DNA G+C content (mol%): not known.

Type strain: M750-G1 (single isolate), AS 4.1628, ATCC 29808, DSM 41674, NBRC 13846, JCM 3399, KCTC 9664, NRRL B-16926.

Sequence accession no. (16S rRNA gene): AB184527.

23a. **Streptomyces albosporeus subsp. albosporeus** (Krainsky 1914) Waksman and Henrici *in* Breed, Murray and Hitchens 1948, 954[AL] ("*Actinomyces albosporeus*" Krainsky 1914, 649; "*Nocardia albosporea*" Chalmers and Christopherson 1916, 268)

al.bo.spo′re.us. L. adj. *albus* white; N.L. n. *spora* (from Gr. n. *spora* a seed) a spore; N.L. masc. adj. *albosporeus* white spored.

Very sparse formation of aerial mycelium; forms yellow, red, red-brown, violet, or orange-colored vegetative mycelium on some media. Excellent growth on Czapek's solution agar. Tolerates 10% NaCl, but not 13% or more NaCl. No antimicrobial activity detected; inhibited by streptomycin.

Type strain shows the highest sequence similarity to: *S. violaceus*, AB184315, 100%; *S. roseoviolaceus*, AJ399484, 100%; *S. janthinus*, AB184851, 100%; *S. luteogriseus*, AB184379, 99.5%; *S. lomondensis*, AB184673, 99.3%; *S. hawaiiensis*, AB184143, 99.3%; *S. flavoviridis*, AB184842, 99.3%; *S. pilosus*, AB184161, 99.2%; *S. arenae*, AB249977, 99.2%; *S. africanus*, AY208912, 99.2%; *S. tuirus*, AB184690, 99.2%; *S. bellus*, AB184849, 99.1%; *S. mutabilis*, EF178679, 99.1%; *S. massasporeus*, AB184152, 99%; *S. afghaniensis*, AJ399483, 99%; *S. levis*, AB184670, 99%; *S. coerulescens*, AY999720, 99%; *S. parvulus*, AB184326, 99%; *S. coeruleorubidus*, AY999719, 99%.

Source: not known.

DNA G+C content (mol%): not known.

Type strain: AS 4.1599, ATCC 15394, ATCC 3003, CCM 3157, DSM 40795, IFM 1163, NBRC 15386, JCM 4135, KCTC 9665, NRRL B-1239, NRRL B-2372, RIA 482, VKM Ac-1818.

Sequence accession no. (16S rRNA gene): AJ781327.

Further comment: Lanoot et al. (2002) are of the opinion that *Streptomyces albosporeus* subsp. *albosporeus* (Krainsky 1914) Waksman and Henrici 1948^AL^ is a later heterotypic synonym of *Streptomyces aurantiacus* (Rossi Doria 1891) Waksman 1953^AL^ emend. Lanoot et al. 2002. The type of the subspecies *Streptomyces albosporeus* subsp. *albosporeus* is automatically the type of *Streptomyces albosporeus* (Krainsky 1914) Waksman and Henrici 1948^AL^. Consequently, if an author agrees with Lanoot et al., *Streptomyces albosporeus* must be considered as a later heterotypic synonym of *Streptomyces aurantiacus.* In expressing that opinion Lanoot et al. have placed the type of *Streptomyces albosporeus* in a different species. In this case, Rule 37a of the *Bacteriological Code* (1990 Revision) applies and the authors should have dealt with the nomenclature and taxonomic position of *Streptomyces albosporeus* subsp. *labilomyceticus* Okami et al. 1963^AL^. Authors who follow the proposal to treat the types of *Streptomyces aurantiacus* and *Streptomyces albosporeus* (including the subspecies *Streptomyces albosporeus* subsp. *albosporeus*) as synonyms are not at liberty to use the name *Streptomyces albosporeus* subsp. *labilomyceticus* and must make a taxonomic proposal for placing this subspecies in another species or subspecies.

23b. **Streptomyces albosporeus subsp. labilomyceticus** Okami, Suzuki and Umezawa 1963, 154^AL^ ["*Streptomyces albosporeus* subsp. *labiolomyceticus*" (*sic*, *lapsus calami*) Okami, Suzuki and Umezawa 1963, 154]

la.bi.lo.my.ce′ti.cus. N.L. n. *labilomycinum* labomycin (name of an unstable antibiotic); L. masc. suff. -*ticus* suffix used with the sense of belonging to; N.L. masc. adj. *labilomyceticus* belonging to labilomycin.

Vegetative mycelium is red, yellow, violet, brown, or orange. Produces labilomycin. Type strain shows no sequence similarity over 99%.

Source: not known.

DNA G+C content (mol%): not known.

Type strain: A955-Y3, NIHJ 425 (single isolate), AS 4.1618, DSM 41672, NBRC 15387, JCM 3383, KCTC 9666.

Sequence accession no. (16S rRNA gene): AB184638.

Further comment: Lanoot et al. (2002) are of the opinion that *Streptomyces albosporeus* subsp. *albosporeus* (Krainsky 1914) Waksman and Henrici 1948^AL^ is a later heterotypic synonym of *Streptomyces aurantiacus* (Rossi Doria 1891) Waksman 1953^AL^ emend. Lanoot et al. 2002. The type of the subspecies *Streptomyces albosporeus* subsp. *albosporeus* is automatically the type of *Streptomyces albosporeus* (Krainsky 1914) Waksman and Henrici 1948^AL^. In expressing that opinion Lanoot et al. have placed the type of *Streptomyces albosporeus* in a different species. In this case Rule 37a (1) applies and the authors should have dealt with the nomenclature and taxonomic position of *Streptomyces albosporeus* subsp. *labilomyceticus* Okami et al. 1963^AL^. Authors who follow the proposal to treat the types of *Streptomyces aurantiacus* and *Streptomyces albosporeus* (including the subspecies *Streptomyces albosporeus* subsp. *albosporeus*) as synonyms are not at liberty to use the name *Streptomyces albosporeus* subsp. *labilomyceticus* and must make a taxonomic proposal for placing this subspecies in another species or subspecies.

24. **Streptomyces alboverticillatus** (Locci and Schofield 1989) Witt and Stackebrandt 1996, 836^VP^ (Effective publication: Witt and Stackebrandt 1990, 370.) ["*Streptoverticillium alboverticillatus*" (*sic*) Arai 1976; "*Streptoverticillium alboverticillatum*" Locci 1985; *Streptoverticillium alboverticillatum* Locci and Schofield 1989, 2504]

al.bo.ver.ti.cil′la.tus. L. adj. *albus* white; N.L. adj. *verticillatus* forming whorls; N.L. adj. *alboverticillatus* white and forming whorls.

Off-white spore mass. Melanin pigment is not produced. Acid is not produced from ribose. Resistant to cephaloxidine, colistin, and cephamandole. *Aspergillus niger* is not inhibited, whereas *Bacillus subtilis* is inhibited. Type strain shows no sequence similarity over 99%.

Source: not known.

DNA G+C content (mol%): not known.

Type strain: AS 4.1638, ATCC 29818, DSM 41678, NBRC 13861, JCM 5010.

Sequence accession no. (16S rRNA gene): AY999766.

Further comment: according to Hatano et al. (2003), *Streptomyces alboverticillatus* (Locci and Schofield 1989) Witt and Stackebrandt 1996 is a later heterotypic synonym of *Streptomyces griseocarneus* (Benedict et al. 1950) Witt and Stackebrandt 1996.

25. **Streptomyces albovinaceus** (Kudrina *in* Gauze, Preobrazhenskaya, Kudrina, Blinov, Ryabova and Sveshnikova 1957) Pridham, Hesseltine and Benedict 1958, 57^AL^ ("*Actinomyces albovinaceus*" Kudrina *in* Gauze, Preobrazhenskaya, Kudrina, Blinov, Ryabova and Sveshnikova 1957, 118)

al.bo.vi.na′ce.us. L. adj. *albus* white; L. adj. *vinaceus* of or belonging to wine or grape: N.L. *albovinaceus* white wine or white grape, but probably refers to pink or red-tinged aerial mycelium, vegetative mycelium and diffusible pigments.

Spore chains in Section *Rectiflexibiles.* Forms short spore chains of more than three spores per chain. This morphology with sporulation aerial mycelium is found on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar. Spore surface is smooth.

Color of colony: aerial mass color in the White color series on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar. Reverse side of colony with no distinctive pigments on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar; substrate pigment is not a pH indicator.

Color in medium: melanoid pigments not formed in peptone-yeast-iron agar and tyrosine agar. No pigment found in medium in yeast-malt agar, oatmeal agar, salts-starch agar, or glycerol-asparagine agar.

D-Glucose, L-arabinose, D-xylose, D-mannitol, D-fructose, and rhamnose are utilized for growth. No growth or only traces of growth on sucrose, iso-inositol, and raffinose.

Type strain shows the highest sequence similarity to: *S. sindenensis*, AB184759, 100%; *S. pluricolorescens*, DQ442540, 100%; *S. mediolani*, AB184674, 100%; *S. badius*, AY999783, 100%; *S. griseinus*, AB184205, 100%; *S. rubiginosohelvolus*, AB184240, 100%; *S. fimicarius*, AY999784, 99.9%; *S. flavofuscus*, AB249935, 99.9%; *S. globisporus* subsp. *globisporus*, EF178686, 99.9%; *S. griseoplanus*, AY999894, 99.9%; *S. praecox*, AB184293, 99.9%; *S. anulatus*, DQ026637, 99.9%; *S. parvus*, DQ442537, 99.8%; *S. acrimycini*, AY999889, 99.8%; *S. californicus*, AB184755, 99.8%; *S. cavourensis* subsp. *washingtonensis*, DQ026671, 99.8%; *S. lavendulae* subsp. *lavendulae*, AB184080, 99.8%; *S. cinereorectus*, AB184646, 99.7%; *S. fulvorobeus*, AB184711, 99.7%; *S. microflavus*, DQ445795, 99.7%; *S. cyaneofuscatus*, AB184860, 99.7%; *S. luridiscabiei*, AF361784, 99.7%; *S. baarnensis*, EF178688, 99.7%; *S. lipmanii*, AB184148, 99.7%; *S. alboviridis*, AB184256, 99.7%; *S. argenteolus*, AB045872, 99.7%; *S. floridae*, AB184656, 99.7%; *S. griseus* subsp. *griseus*, AY207604, 99.6%; *S. flavogriseus*, AJ494864, 99.6%; *S. flavovirens*, DQ026635, 99.6%; *S. griseolus*, AB184768, 99.6%; *S. nitrosporeus*, EF178680, 99.5%; *S. halstedii*, EF178695, 99.5%; *S. pulveraceus*, AB184806, 99.5%; *S. bacillaris*, AB184439, 99.5%; *S. atroolivaceus*, AJ781320, 99.4%; *S. olivoviridis*, AB184227, 99.4%; *S. finlayi*, AY999788, 99.4%; *S. atratus*, DQ026638, 99.3%; *S. griseobrunneus*, AB249912, 99.3%; *S. celluloflavus*, AB184476, 99.3%; *S. yanii*, AB006159, 99.3%; *S. gelaticus*, DQ026636, 99.3%; *S. sanglieri*, AB249945, 99.3%; *S. albolongus*, AB184425, 99.3%; *S. clavifer*, DQ026670, 99.3%; *S. cavourensis* subsp. *cavourensis*, DQ445791, 99.2%; *S. spiroverticillatus*, AB184814, 99.1%; *S. mutomycini*, AB249951, 99.1%; *S. candidus*, DQ026663, 99.1%; *S. cremeus*, AB184124, 99%.

Source: not known.

DNA G+C content (mol%): not known.

Type strain: AS 4.1631, ATCC 15823, ATCC 19723, ATCC 23613, CBS 256.66, CBS 462.68, CCM 3005, BCRC 13757, DSM 40136, NBRC 12739, INA 273/53, JCM 4343, NCIMB 13010, NRRL B-2566, NRRL-ISP 5136, RIA 1004, UNIQEM 119, VKM Ac-572.

Sequence accession no. (16S rRNA gene): AB249958.

26. **Streptomyces alboviridis** (Duché 1934) Pridham, Hesseltine and Benedict 1958, 74[AL] ("*Actinomyces alboviridis*" Duché 1934, 317)

al.bo.vi'ri.dis. L. adj. *albus* white; L. adj. *viridis* green; N.L. masc. adj. *alboviridis* whitish green.

Spore chains in Section *Rectiflexibiles*. Mature spore chains are generally long with 10 to 50 or more spores per chain on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar. Spore surface is smooth.

Color of colony: aerial mass color in the Yellow color series on yeast-malt agar and salts-starch agar; Yellow or white color series on oatmeal agar and glycerol-asparagine agar. Reverse side of colony with no distinctive pigments (pale yellow or grayish yellow) on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar.

Color in medium: melanoid pigments are not formed in peptone-yeast-iron agar, tyrosine agar, or tryptone-yeast broth. No pigment is found in the medium in yeast-malt agar, oatmeal agar, salts-starch agar, or glycerol-asparagine agar.

D-Glucose, D-xylose, D-mannitol, D-fructose, and rhamnose are utilized for growth. Reports vary on utilization of L-arabinose. No growth or only traces of growth with sucrose, iso-inositol, and raffinose.

Type strain shows the highest sequence similarity to: *S. cavourensis* subsp. *washingtonensis*, DQ026671, 100%; *S. fulvorobeus*, AB184711, 100%; *S. microflavus*, DQ445795, 100%; *S. lipmanii*, AB184148, 100%; *S. lavendulae* subsp. *lavendulae*, AB184080, 100%; *S. luridiscabiei*, AF361784, 99.9%; *S. flavofuscus*, AB249935, 99.9%; *S. cinereorectus*, AB184646, 99.9%; *S. fimicarius*, AY999784, 99.9%; *S. griseoplanus*, AY999894, 99.9%; *S. cyaneofuscatus*, AB184860, 99.9%; *S. floridae*, AB184656, 99.9%; *S. praecox*, AB184293, 99.9%; *S. anulatus*, DQ026637, 99.9%; *S. pluricolorescens*, DQ442540, 99.8%; *S. acrimycini*, AY999889, 99.8%; *S. argenteolus*, AB045872, 99.8%; *S. griseinus*, AB184205, 99.8%; *S. badius*, AY999783, 99.8%; *S. rubiginosohelvolus*, AB184240, 99.8%; *S. sindenensis*, AB184759, 99.8%; *S. mediolani*, AB184674, 99.8%; *S. californicus*, AB184755, 99.8%; *S. parvus*, DQ442537, 99.7%; *S. halstedii*, EF178695, 99.7%; *S. baarnensis*, EF178688, 99.7%; *S. griseolus*, AB184768, 99.7%; *S. griseus* subsp. *griseus*, AY207604, 99.7%; *S. globisporus* subsp. *globisporus*, EF178686, 99.7%; *S. albovinaceus*, AB249958, 99.7%; *S. flavovirens*, DQ026635, 99.6%; *S. finlayi*, AY999788, 99.5%; *S. flavogriseus*, AJ494864, 99.5%; *S. yanii*, AB006159, 99.4%; *S. atroolivaceus*, AJ781320, 99.4%; *S. olivoviridis*, AB184227, 99.4%; *S. bacillaris*, AB184439, 99.4%; *S. pulveraceus*, AB184806, 99.4%; *S. celluloflavus*, AB184476, 99.3%; *S. griseobrunneus*, AB249912, 99.3%; *S. albolongus*, AB184425, 99.3%; *S. clavifer*, DQ026670, 99.3%; *S. nitrosporeus*, EF178680, 99.3%; *S. spiroverticillatus*, AB184814, 99.2%; *S. sanglieri*, AB249945, 99.2%; *S. cavourensis* subsp. *cavourensis*, DQ445791, 99.2%; *S. atratus*, DQ026638, 99.1%; *S. mutomycini*, AB249951, 99.1%; *S. candidus*, DQ026663, 99.1%; *S. gelaticus*, DQ026636, 99.1%; *S. cremeus*, AB184124, 99%.

Source: not known.

DNA G+C content (mol%): not known.

Type strain: AS 4.1627, ATCC 25425, CBS 923.69, BCRC 12054, DSM 40326, NBRC 13013, JCM 4449, KCTC 9667, NRRL B-3633, NRRL-ISP 5326, RIA 1205, VKM Ac-736.

Sequence accession no. (16S rRNA gene): AB184256.

27. **Streptomyces albulus** Routien *in* Pridham and Lyons 1969, 194[AL]

al.bu'lus. L. masc. adj. *albulus* whitish.

Spore chains in Section *Spirales*. Mature spore chains are moderately long with 10 to 50 or more spores per chain.

This morphology is seen on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar. Spore surface is spiny.

Color of colony: aerial mass color in the Gray color series (5fe, light grayish reddish brown; 4li, brownish gray; 4ig, light grayish brown) on yeast-malt agar and oatmeal agar; Gray or Red color series on salts-starch agar and glycerol-asparagine agar (nearest tab in the Red color series is 5dc, grayish yellowish pink). Reverse side of colony with no distinctive pigments (pale or grayish yellow) on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar.

Color in medium: melanoid pigments are not formed in peptone-yeast-iron agar, tyrosine agar, and tryptone-yeast broth. No pigment is found in medium in yeast-malt agar, oatmeal agar, salts-starch agar, or glycerol-asparagine agar.

D-Glucose, iso-inositol, D-mannitol, and D-fructose are utilized for growth. No growth or only traces of growth with L-arabinose, D-xylose, rhamnose, sucrose, and raffinose.

Type strain shows the highest sequence similarity to: *S. noursei*, AB184678, 100%; *S. yunnanensis*, AF346818, 99.6%.

Source: not known.

DNA G+C content (mol%): not known.

Type strain: AS 4.1585, ATCC 12757, CBS 711.72, BCRC 11819, DSM 40492, NBRC 13410, JCM 4718, KCTC 9668, NRRL B-5386, NRRL-ISP 5492, RIA 1371, IMC S-0802.

Sequence accession no. (16S rRNA gene): AB024440.

28. **Streptomyces almquistii** (Duché 1934) Pridham, Hesseltine and Benedict 1958, 74[AL] ["*Actinomyces almquisti*" (*sic*) Duché 1934, 278]

alm.quis'ti.i. N.L. gen. masc. n. *almquistii* of Ernst Bernhard Almquist (1852–1946), named for an early investigator of *Actinomycetales.*

Spore chains in Section *Spirales*; many straight to flexuous hyphae or immature spore chains may also be present. Mature spore chains generally have 10 to 50 or more spores per chain. This morphology is seen on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar. Spore surface is smooth.

Color of colony: aerial mass color in the White color series on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar. Reverse side of colony with no distinctive pigments (colorless to pale grayish yellow) on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar.

Color in medium: melanoid pigments are not formed in peptone-yeast-iron agar, tyrosine agar, or tryptone-yeast broth. No pigment is found in the medium in yeast-malt agar, oatmeal agar, salts-starch agar, or glycerol-asparagine agar.

D-Glucose, D-xylose, D-mannitol, and D-fructose are utilized for growth. Reports vary on utilization of L-arabinose and iso-inositol. No growth or only trace of growth with rhamnose, sucrose, and raffinose.

Type strain shows the highest sequence similarity to: *S. rangoonensis*, AB184295, 100%; *S. gibsonii*, AB184663, 100%; *S. albus* subsp. *albus*, AJ621602, 100%; *S. flocculus*, AB184272, 99.9%.

Source: not known.

DNA G+C content (mol%): not known.

Type strain: AS 4.1685, ATCC 25427, ATCC 618, CBS 925.69, BCRC 12098, DSM 40447, HAMBI 50, HUT 6614, NBRC 13015, IMET 43380, JCM 4451, KCTC 9672, NRRL B-1685, NRRL-ISP 5447, RIA 1207.

Sequence accession no. (16S rRNA gene): AB184258.

29. **Streptomyces althioticus** Yamaguchi, Nakayama, Takeda, Tawara, Maeda, Takeuchi and Umezawa 1957, 196[AL]

al.thi.o'ti.cus. N.L. n. *althiomycinum* althiomycin, name of a sulfur-containing antibiotic; L. masc. suff. -*ticus* suffix used with the sense of belonging to; N.L. masc. adj. *althioticus* belonging to althiomycin.

Spore chains in Section *Spirales*, but flexuous sporophores are also common, especially on yeast-malt agar. Mature spore chains generally have 10–50 spores per chain. This morphology is observed on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar. Spore surface is spiny, with only minor surface irregularities suggestive of spines or warts.

Color of colony: aerial mass color in the Gray color series on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar. Reverse side of colony is grayed yellow, modified to blue-violet or red on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar. Reverse pigment is pH indicator; changes from red or reddish brown to blue or blue-violet by addition of 0.05 M NaOH.

Color in medium: melanoid pigments are not formed or occur only in trace amounts in peptone-yeast-iron agar and tyrosine agar; blue-violet or red pigment (depending upon pH) occurs in yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar. This pigment is pH-sensitive, becoming blue or blue-violet when tested with 0.05 M NaOH and red when tested with 0.05 M HCl.

D-Glucose, L-arabinose, D-xylose, iso-inositol, D-mannitol, D-fructose, and rhamnose are utilized for growth. No growth or only traces of growth on raffinose. Variable reports on growth with sucrose.

Type strain shows the highest sequence similarity to: *S. matensis*, AB184221, 99.8%; *S. griseorubens*, AB184139, 99.4%; *S. labedae*, AB184704, 99.3%; *S. erythrogriseus*, AJ781328, 99.2%; *S. griseoflavus*, AJ781322, 99.2%; *S. variabilis*, DQ442551, 99.2%; *S. griseoincarnatus*, AJ781328, 99.2%; *S. paradoxus*, AB184628, 99.1%.

Source: not known.

DNA G+C content (mol%): not known.

Type strain: AS 4.1608, ATCC 19724, CBS 463.68, BCRC 13686, DSM 40092, NBRC 12740, NBRC 15956, JCM 4344, KCTC 9752, NRRL B-3981, NRRL-ISP 5092, RIA 1005, UNIQEM 120, VKM Ac-705.

Sequence accession no. (16S rRNA gene): AY999808.

30. **Streptomyces amakusaensis** Nagatsu, Anzai, Ohkuma and Suzuki 1963, 209[AL]

a.ma.ku.sa.en'sis. N.L. masc. adj. *amakusaensis* of or belonging to Amakusa Island, Japan, the source of the soil from which the organism was isolated.

Spore chains in Section *Spirales.* Mature spore chains have 10 to 50 or more spores per chain on yeast-malt agar and salts-starch agar. Sporulating aerial mycelium is

poorly developed or absent on oatmeal agar and glycerol-asparagine agar. Spore surface is smooth.

Color of colony: aerial mass color of mature aerial mycelium is in the Blue color series on salts-starch agar and the Green or Blue color series on yeast-malt agar; younger mycelium may be in the Gray color series. Mature aerial mycelium is usually not formed on oatmeal agar or glycerol-asparagine agar. Reverse side of colony with distinctive pigments (colorless or pale yellow) on yeast-malt agar, oatmeal agar, salts-starch agar, or glycerol-asparagine agar.

This culture does not show good growth with any of the carbon sources tested on Pridham and Gottlieb carbon utilization medium. D-Glucose is utilized for growth. No significant growth occurs on D-xylose, iso-inositol, D-mannitol, D-fructose, rhamnose, or raffinose and only doubtful traces are seen on L-arabinose and sucrose.

Type strain shows the highest sequence similarity to: *S. inusitatus*, AB184445, 99.8%.

Source: not known.

DNA G+C content (mol%): not known.

Type strain: AS 4.1462, ATCC 23876, CBS 615.68, DSM 40219, NBRC 12835, JCM 4617, JCM 4167, KCTC 9753, LMG 19350, NRRL B-3351, NRRL-ISP 5219, RIA 1163, VKM Ac-995.

Sequence accession no. (16S rRNA gene): AY999781.

31. **Streptomyces ambofaciens** Pinnert-Sindico 1954, 702[AL]

am.bo.fa′ci.ens. L. adj. *ambo* both; L. part. adj. *faciens* producing; N.L. part. adj. *ambofaciens* producing both, referring to the production of two different antibiotics by the organism.

Spore chains in Section *Spirales*. Open terminal spirals on long spore chains also suggest *Retinaculum-Apertum* type morphology. Mature spore chains generally have 10–50 spores per chain. This morphology is seen on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar, although sporulating aerial mycelium is not abundant on glycerol-asparagine agar. Spore surface is smooth to warty; surface irregularities suggesting warts are small.

Color of colony: aerial mass color in the Gray color series on yeast-malt agar, oatmeal agar, and salts-starch agar. Reverse side of colony with no distinctive pigments (colorless to grayed yellow) on yeast-malt agar, oatmeal agar, and glycerol-asparagine agar. Dark brown, dark blue, or almost black substrate mycelium pigments are found on salts-starch agar. This pigment is not pH-sensitive.

Color in medium: melanoid pigments are not formed in peptone-yeast-iron agar, tyrosine agar, or tryptone-yeast broth. Yellow pigments may be found in medium in salts-starch agar; no pigment is found in the medium in yeast-malt agar, oatmeal agar, or glycerol-asparagine agar.

D-Glucose, L-arabinose, D-xylose, iso-inositol, D-mannitol, D-fructose, and rhamnose are utilized for growth. No growth or only trace of growth on raffinose. Utilization of sucrose is doubtful.

Type strain shows the highest sequence similarity to: *S. collinus*, AB184123, 99.2%; *S. paradoxus*, AB184628, 99.2%; *S. griseoflavus*, AJ781322, 99.2%; *S. lienomycini*, AJ781353, 99.1%; *S. flaveolus*, AB184764, 99.1%; *S. heliomycini*, AB184712, 99.1%; *S. matensis*, AB184221, 99%; *S. griseorubens*, AB184139, 99%; *S. albaduncus*, AY999757, 99%; *S. albogriseolus*, AJ494865, 99%; *S. coelescens*, AF503496, 99%; *S. rubrogriseus*, AB184681, 99%; *S. violaceoruber*, AF503492, 99%.

Source: not known.

DNA G+C content (mol%): not known.

Type strain: ATCC 23877, CBS 616.68, BCRC 11857, CECT 3101, DSM 40053, NBRC 12836, JCM 4618, JCM 4204, KCTC 9111, NRRL 2420, NRRL B-2516, NRRL-ISP 5053, RIA 1115.

Sequence accession no. (16S rRNA gene): M27245.

32. **Streptomyces aminophilus** Foster *in* Oswald, Reedy and Randall *in* Hütter 1961, 370[AL]

a.mi.no′phi.lus. N.L. n. *aminum* amine; N.L. masc. adj. *philus* (from Gr. masc. adj. *philos*) loving; N.L. masc. adj. *aminophilus* amine-nitrogen loving.

Spore chains in Section *Spirales*. Mature spore chains have 3–10, or sometimes more than 10 spores per chain. This morphology is seen on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar, but formation of sporulation aerial mycelium is poor on oatmeal agar. Spore surface is smooth.

Color of colony: aerial mass color in the Yellow color series on yeast-malt agar, salts-starch agar, and glycerol-asparagine agar; aerial mycelium poorly developed on oatmeal agar. Reverse side of colony with no distinctive pigments (yellow or grayed yellow) on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar.

Color in medium: melanoid pigments not formed in peptone-yeast-iron agar, tyrosine agar, or tryptone-yeast broth. No pigments, or only traces of yellow pigment, are found in medium in yeast-malt agar, oatmeal agar, salts-starch agar, or glycerol-asparagine agar.

D-Glucose, D-xylose, D-mannitol, and D-fructose are utilized for growth. No growth or only traces of growth on iso-inositol and rhamnose. Reports vary on utilization of sucrose and raffinose.

For sequence similarity, see type strain of *S. cacaoi* subsp. *cacaoi*.

Source: not known.

DNA G+C content (mol%): not known.

Type strain: AS 4.1416, ATCC 13558, ATCC 14961, ATCC 23878, CBS 617.68, BCRC 11858, DSM 40186, NBRC 12837, JCM 4619, JCM 4275, KCTC 9673, LMG 19319, NCIMB 9827, NRRL 2390, NRRL-ISP 5186, RIA 1140, VKM Ac-706.

Sequence accession no. (16S rRNA gene): AB184183.

Further comments: according to Lanoot et al. (2002), *Streptomyces aminophilus* Foster 1961[AL] is a later heterotypic synonym of *Streptomyces cacaoi* subsp. *cacaoi* (Waksman 1932) Waksman and Henrici 1948[AL] emend. Lanoot et al. 2002.

33. **Streptomyces anandii** Batra and Bajaj 1965, 242[AL]

a.nan.di′i. N.L. gen. n. *anandii* of Anand, Gujarat, India, the source of the soil from which the organism was isolated.

Spore chains in Section *Spirales*, but open loops and flexuous chains are also seen. Mature spore chains are moderately long with 10–50 spores per chain. This morphology is seen on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar. Spore surface is smooth. One of

three observers found globose bodies in the aerial mycelium on yeast-malt agar, and coremia formation on oatmeal agar and salts-starch agar.

Color of colony: aerial mass color in the White or Gray (2dc, yellowish gray or 3ge, light grayish yellowish brown) color series on yeast-malt agar and glycerol-asparagine agar; Gray or Red color series (2dc, yellowish gray to 4ec or 5cb, grayish yellowish pink) on oatmeal agar and salts-starch agar. Reverse side of colony with no distinctive pigments (pale or grayish yellow to olive brown or strong brown) on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar.

Color in medium: melanoid pigments are formed in peptone-yeast-iron agar, tyrosine agar, tryptone-yeast broth, and Gause's medium no. 2. No pigment or only a trace of yellow is found in the medium in yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar.

D-Glucose, L-arabinose, D-xylose, iso-inositol, D-mannitol, D-fructose, sucrose, and raffinose are utilized for growth. No growth or only traces of growth with rhamnose.

Type strain shows the highest sequence similarity to: *S. geysiriensis*, DQ442501, 99.3%; *S. minutiscleroticus*, EF178696, 99.3%; *S. rochei*, AB184237, 99.2%; *S. ghanaensis*, AY999851, 99.2%; *S. plicatus*, AB184291, 99.2%; *S. vinaceusdrappus*, AY999929, 99.2%; *S. mutabilis*, EF178679, 99.1%; *S. djakartensis*, AB184657, 99.1%; *S. calvus*, AB184329, 99.1%; *S. asterosporus*, AB184706, 99%; *S. tuirus*, AB184690, 99%; *S. virens*, DQ442554, 99%; *S. aureorectus*, AB184710, 99%.

Source: isolated from soil from Anand, Gujarat, India.

DNA G+C content (mol%): not known.

Type strain: ATCC 19388, CBS 739.72, BCRC 11825, DSM 40535, NBRC 13438, JCM 4720, KCTC 9687, NRRL B-12487, NRRL B-3590, NRRL-ISP 5535, RIA 1399, VKM Ac-1920.

Sequence accession no. (16S rRNA gene): AB184402.

34. **Streptomyces anthocyanicus** (Krasil'nikov et al. 1965) Pridham 1970, 7[AL] ("*Actinomyces anthocyanicus*" Krasil'nikov, Sorokina, Alferova and Bezzubenkova 1965, 118)

an.tho.cya'ni.cus. N.L. n. *anthocyaninum* anthocyanin (a water-soluble vacuolar pigments that may appear red, purple, or blue according to pH), L. masc. suff. *-icus* suffix used with the sense of belonging to; N.L. masc. adj. *anthocyanicus* belonging to anthocyanin, presumably referring to dark blue color.

Spore chains are of typical *Retinaculum-Apertum* type. Blue-colored vegetative mycelium and diffusible pigments are formed on some media. Exhibits slight anti-bacterial activity.

Type strain shows the highest sequence similarity to: *S. tricolor*, AB184687, 100%; *S. violaceoruber*, AF503492, 99.9%; *S. coelescens*, AF503496, 99.9%; *S. rubrogriseus*, AB184681, 99.9%; *S. violaceolatus* AF503497, 99.8%; *S. humiferus*, AF503491, 99.8%; *S. lienomycini*, AJ781353, 99.7%; *S. tendae*, D63873, 99.5%; *S. violaceorubidus*, AJ781374, 99.5%; *S. coelicoflavus*, AB184650, 99.5%; *S. olivaceus*, AB184743, 99.1%; *S. pactum*, AB184398, 99.1%.

Source: not known.

DNA G+C content (mol%): not known.

Type strain: AS 4.1683, ATCC 19821, DSM 41422, NBRC 14892, JCM 5058, KCTC 9755, NRRL B-12341.

Sequence accession no. (16S rRNA gene): AB184631.

35. **Streptomyces antibioticus** (Waksman and Woodruff 1941) Waksman and Henrici *in* Breed, Murray and Hitchens 1948, 942[AL] ("*Actinomyces antibioticus*" Waksman and Woodruff 1941, 246)

an.ti.bio'ti.cus. N.L. masc. adj. *antibioticus* (from Gr. prep. *anti* against; Gr. n. *bios* life; L. suff. *-ticus -a -um* suffix of various meanings, but signifying in general made of or belonging to) against life, antibiotic.

Spore chains in Section *Rectiflexibiles*. Mature spore chains are short, often with only 3–10 spores per chain; longer chains are also found. This morphology is observed on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar. Spore surface is smooth.

Color of colony: aerial mass color in the Gray color series on yeast-malt agar, salts-starch agar, and glycerol-asparagine agar; mature aerial mycelium poorly developed on oatmeal agar. Reverse side of colony with no distinctive pigments (grayed yellow-brown on yeast-malt agar, grayed yellow or dark grayish greenish yellow on oatmeal agar, salts-starch agar, and glycerol-asparagine agar); substrate mycelium pigment is not a pH indicator.

Color in medium: melanoid pigments are produced in peptone-yeast-iron agar, tyrosine agar, and peptone-yeast broth. No pigment or only trace of yellow in medium in yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar.

D-Glucose, L-arabinose, iso-inositol, D-mannitol, D-fructose, and rhamnose are utilized for growth. No growth or only trace of growth on sucrose and raffinose. Utilization of D-xylose is doubtful.

Type strain shows the highest sequence similarity to: *S. griseoruber*, AB184209, 99.3%.

Source: not known.

DNA G+C content (mol%): not known.

Type strain: ATCC 23879, ATCC 8663, CBS 478.48, CBS 659.68, CCM 3159, BCRC 12164, CECT 3225, DSM 40234, NBRC 12838, IMET 40227, JCM 4620, KCTC 9688, LMG 5966, NCIMB 8504, NRRL B-1701, NRRL B-2770, NRRL-ISP 5234, RIA 1174, VKM Ac-964.

Sequence accession no. (16S rRNA gene): AY999776.

36. **Streptomyces antimycoticus** Waksman *in* Breed, Murray and Smith 1957, 799[AL]

an.ti.my.co'ti.cus. N.L. masc. adj. *antimycoticus* (from Gr. prep. *anti* against; Gr. n. *mukês -etis* fungus; L. suff. *-icus -a -um* suffix of various meanings, but signifying in general made of or belonging to), against fungal, antimycotic.

Spore chains in Section *Spirales*; compact clusters of closed spirals on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar; mature spore chains of generally 10–50 spores per chain are found on these media. Spore surface is spiny to warty. Surface irregularities on spores are intermediate between very short, thick spines, and warts.

Color of colony: aerial mass color in the Gray color series on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar. Reverse side of colony with no distinct pigment (pale grayed yellow or grayed greenish yellow) on yeast-malt agar, oatmeal agar, salts-starch agar, or glycerol-asparagine agar.

Color in medium: melanoid pigments are not produced

on peptone-yeast-iron agar, tyrosine agar, or tryptone-yeast broth. No pigment is found in medium in yeast-malt agar, oatmeal agar, salts-starch agar, or glycerol-asparagine agar.

D-Glucose, L-arabinose, sucrose, D-xylose, iso-inositol, D-mannitol, D-fructose, rhamnose, and raffinose are utilized for growth. Growth on L-arabinose and D-xylose is generally less abundant than on the other carbon sources.

Type strain shows the highest sequence similarity to: *S. sporoclivatus*, AB249934, 100%; *S. rutgersensis* subsp. *castelarensis*, AY508511, 99.8%; *S. geldanamycininus*, DQ334781, 99.8%; *S. melanosporofaciens*, AJ271887, 99.6%; *S. rhizosphaericus*, AB249941, 99.1%; *S. asiaticus*, AB249947, 99.1%.

Source: not known.

DNA G+C content (mol%): not known.

Type strain: AS 4.1591, ATCC 23880, CBS 660.68, DSM 40284, NBRC 12839, JCM 4621, JCM 4228, KCTC 9694, NRRL 2421, NRRL-ISP 5284, RIA 1198, VKM Ac-1824.

Sequence accession no. (16S rRNA gene): AB184185.

37. **Streptomyces anulatus** (Beijerinck 1912) Waksman *in* Waksman and Lechevalier 1953, 40[AL] ["*Actinomyces Streptothrix annulatus*" (*sic*) Beijerinck 1912, 7; "*Actinomyces annulatus*" (*sic*) Beijerinck 1912, 4; "*Streptomyces annulatus*" (*sic*) Waksman *in* Waksman and Lechevalier 1953, 40]

a.nu.la′tus. L. masc. adj. *anulatus* furnished with a ring.

Spore chains in Section *Rectiflexibiles* on oatmeal agar, salts-starch agar, and glycerol-asparagine agar, but open spirals of two to several turns, hooks and loops of small diameter, as well as straight and flexuous chains are found on yeast-malt agar. The strain as observed on yeast-malt agar can therefore be placed in Section *Spirales* or *Retinaculum-Apertum* type. Spore surface is smooth.

Color of colony: aerial mass color in the Yellow or White color series on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar. Observers selected color tab 2ba (pale yellow) from the Yellow color series and tab a (white) from the White color series; both yellow and white may be observed on the same medium. Reverse side of colony with no distinctive pigments (colorless to pale yellow; grayish yellow or yellowish brown) on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar.

Color in medium: melanoid pigments are not formed in peptone-yeast-iron agar, tyrosine agar, or tryptone-yeast broth. No pigment (or only a trace of yellow) is found in the medium in yeast-malt agar, oatmeal agar, salts-starch agar, or glycerol-asparagine agar.

D-Glucose, L-arabinose, D-xylose, D-mannitol, D-fructose, and rhamnose are utilized for growth. No growth or only traces of growth with iso-inositol, sucrose, and raffinose.

Type strain shows the highest sequence similarity to: *S. cavourensis* subsp. *washingtonensis*, DQ026671, 100%; *S. fimicarius*, AY999784, 100%; *S. badius*, AY999783, 100%; *S. sindenensis*, AB184759, 100%; *S. rubiginosohelvolus*, AB184240, 100%; *S. pluricolorescens*, DQ442540, 100%; *S. griseinus*, AB184205, 100%; *S. acrimycini*, AY999889, 100%; *S. flavofuscus*, AB249935, 100%; *S. mediolani*, AB184674, 100%; *S. praecox*, AB184293, 100%; *S. lavendulae* subsp. *lavendulae*, AB184080, 100%; *S. griseoplanus*, AY999894, 100%; *S. alboviridis*, AB184256, 99.9%; *S. lipmanii*, AB184148, 99.9%; *S. albovinaceus*, AB249958, 99.9%; *S. globisporus* subsp. *globisporus*, EF178686, 99.9%; *S. cyaneofuscatus*, AB184860, 99.9%; *S. cinereorectus*, AB184646, 99.9%; *S. fulvorobeus*, AB184711, 99.9%; *S. microflavus*, DQ445795, 99.8%; *S. argenteolus*, AB045872, 99.8%; *S. parvus*, DQ442537, 99.8%; *S. californicus*, AB184755, 99.8%; *S. baarnensis*, EF178688, 99.8%; *S. halstedii*, EF178695, 99.7%; *S. luridiscabiei*, AF361784, 99.7%; *S. flavovirens*, DQ026635, 99.7%; *S. floridae*, AB184656, 99.7%; *S. flavogriseus*, AJ494864, 99.7%; *S. griseolus*, AB184768, 99.7%; *S. griseus* subsp. *griseus*, AY207604, 99.7%; *S. atroolivaceus*, AJ781320, 99.5%; *S. nitrosporeus*, EF178680, 99.5%; *S. olivoviridis*, AB184227, 99.5%; *S. finlayi*, AY999788, 99.5%; *S. bacillaris*, AB184439, 99.5%; *S. clavifer*, DQ026670, 99.5%; *S. pulveraceus*, AB184806, 99.5%; *S. yanii*, AB006159, 99.4%; *S. gelaticus*, DQ026636, 99.3%; *S. mutomycini*, AB249951, 99.3%; *S. albolongus*, AB184425, 99.3%; *S. sanglieri*, AB249945, 99.3%; *S. atratus*, DQ026638, 99.3%; *S. griseobrunneus*, AB249912, 99.3%; *S. celluloflavus*, AB184476, 99.3%; *S. spiroverticillatus*, AB184814, 99.2%; *S. candidus*, DQ026663, 99.1%; *S. cavourensis* subsp. *cavourensis*, DQ445791, 99.1%; *S. mauvecolor*, AB184532, 99%; *S. cremeus*, AB184124, 99%.

Source: not known.

DNA G+C content (mol%): not known.

Type strain: AS 4.1421, ATCC 27416, CBS 100.18, CBS 670.72, DSM 40361, NBRC 13369, IMET 43334, JCM 4721, KCTC 9756, LMG 19301, NRRL B-2000, NRRL-ISP 5361, RIA 1330, VKM Ac-728.

Sequence accession no. (16S rRNA gene): DQ026637.

Further comments: according to Lanoot et al. (2005b), *Streptomyces anulatus* (Beijerinck 1912) Waksman 1953[AL] emend. Lanoot et al. 2005b is an earlier heterotypic synonym of *Streptomyces chrysomallus* subsp. *chrysomallus* Lindenbein 1952[AL], an earlier heterotypic synonym of *Streptomyces citreofluorescens* (Korenyako et al. 1960) Pridham 1970[AL], and an earlier heterotypic synonym of *Streptomyces fluorescens* (Krasil'nikov 1958) Pridham 1970[AL].

38. **Streptomyces arabicus** Shibata, Nakazawa, Miyake, Inoue and Okabori 1957, 36[AL]

a.ra′bi.cus. L. masc. adj. *arabicus* of or belonging to Arabia, the source of the soil from which the organism was isolated.

Spore chains in Section *Spirales* on oatmeal agar and salts-starch agar. Short spore chains on yeast-malt agar and glycerol-asparagine agar may appear to be *Rectiflexibiles* morphology or may show only incomplete spirals or hooks. Mature spore chains on oatmeal agar and salts-starch agar generally have 10 to 50 or more spores per chain; shorter spore chains are found on yeast-malt agar and glycerol-asparagine agar. Spore surface is warty to spiny. Surface irregularities are short and blunt; smooth spores may also be found.

Color of colony: aerial mass color in the Gray color series on yeast-malt agar, oatmeal agar, and salts-starch agar; Red color series on glycerol-asparagine agar. Observers selected color tabs representing neutral grays to light grayish reddish brown (5fe) for the Gray color series and grayish yellowish pink (5dc) from the Red color series for glycerol-asparagine agar. Reverse side of colony is grayish yellowish brown on

yeast-malt agar; colorless to pale yellowish gray or brown on oatmeal agar, salts-starch agar, and glycerol-asparagine agar. Substrate mycelium pigment is not a pH indicator.

Color in medium: melanoid pigment is not produced in peptone-yeast-iron agar, tyrosine agar, or tryptone-yeast broth. Trace of yellow pigment may be found in the medium in oatmeal agar and salts-starch agar or may be absent. This pigment is not pH-sensitive.

D-Glucose, L-arabinose, D-xylose, iso-inositol, D-mannitol, D-fructose, and rhamnose are utilized for growth. No growth or only trace of growth on raffinose. Utilization of sucrose is doubtful.

No detailed sequence information is available (only partial sequence).

Source: soil from Arabia.

DNA G+C content (mol%): not known.

Type strain: ATCC 23881, CBS 661.68, DSM 40252, HAMBI 995, HUT 6041, IFM 1118, NBRC 12840, NBRC 14035, NBRC 3406, JCM 4622, JCM 4161, NRRL B-1733, NRRL-ISP 5252, RIA 1178, RIA 512, VKM Ac-1754.

Sequence accession no. (16S rRNA gene): D44271.

Further comments: according to Lanoot et al. (2004), *Streptomyces arabicus* Shibata et al. 1957[AL] is a later heterotypic synonym of *Streptomyces vinaceus* Jones 1952[AL].

39. **Streptomyces ardus** (DeBoer et al. 1961) Witt and Stackebrandt 1996, 836[VP] (Effective publication: Witt and Stackebrandt 1990, 370.) ("*Streptomyces ardus*" DeBoer, Dietz, Lummis and Savage 1961; *Streptoverticillium ardum* Locci, Baldacci and Petrolini Baldan 1969, 59)

ar′dus. L. masc. adj. *ardus* dry, withered, shrunk up, shrivelled, meagre.

Sporulating aerial mycelium is usually absent or very poorly developed on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar. Sterile aerial hyphae are straight or flexuous. Coremia may be produced on yeast-malt agar and oatmeal agar in 7–14 d. The original description reported that this strain sporulates with difficulty, producing only a few monoverticillate and biverticillate sporophores.

Color of colony: aerial mass color of sporulating aerial mycelium not observed. Sterile hyphae or scant aerial mycelia appear to be white to light gray when produced on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar. Reverse side of colony with no distinctive pigments (pale grayish yellow to light yellowish brown) on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar.

Color in medium: melanoid pigments are formed in peptone-yeast-iron agar and tryptone-yeast broth, but not on tyrosine agar or Gause's medium no. 2. No pigment is found in the medium in oatmeal agar, salts-starch agar, or glycerol-asparagine agar. One observer reports traces of red pigment in yeast-malt agar. This pigment is not pH-sensitive when tested with 0.05 M NaOH or HCl.

D-Glucose, iso-Inositol, and D-fructose are utilized for growth. Reports vary on utilization of L-arabinose. No growth or only traces of growth with D-xylose, D-mannitol, rhamnose, sucrose, and raffinose.

Type strain shows the highest sequence similarity to: *S. blastmyceticus*, AY999802, 99.4%; *S. hiroshimensis*, AB184789, 99.2%; *S. cinnamoneus*, AB184850, 99.1%; *S. caeruleus*, EF178675, 99%.

Source: not known.

DNA G+C content (mol%): not known.

Type strain: AS 4.167, ATCC 27417, CBS 731.72, BCRC 12319, CECT 3254, DSM 40527, NBRC 13430, JCM 4722, JCM 4543, RIA 1391, NRRL 2817, NRRL-ISP 5527, VKM Ac-930.

Sequence accession no. (16S rRNA gene): AB184864.

Further comments: according to Hatano et al. (2003), *Streptomyces ardus* (DeBoer et al. 1961) Witt and Stackebrandt 1991 is a heterotypic synonym of "*Streptomyces caespitosus*" (NBRC 13490).

40. **Streptomyces arenae** Pridham, Hesseltine and Benedict 1958, 67[AL]

a.re′na.e. L. n. *arena* sand; L. gen. n. *arenae* of sand, referring to a sandy area near Zion, Illinois, USA, from which the organism was isolated.

Spore chains in Section *Spirales*. Mature spore chains are moderately long with 10 to 50 or more spores per chain. This morphology is seen on yeast-malt agar, salts-starch agar, and glycerol-asparagine agar. Spore surface is spiny.

Color of colony: aerial mass color in the Red or Gray color series on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar. Observers selected color tabs 5fe (light grayish reddish brown) from the Gray color wheel and 5cb or 5dc (grayish yellowish pink) from the red color series. One observer selected tab 11ec from the Violet color series as nearest matching color for sporulating aerial growth on salts-starch agar, oatmeal agar, and glycerol-asparagine agar. Reverse side of colony with no distinctive pigments (pale yellow to yellowish brown) on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar.

Color in medium: melanoid pigments are formed in peptone-yeast-iron agar, tyrosine agar, or tryptone-yeast broth. Yellow pigment may be found in yeast-malt agar, oatmeal agar, and salts-starch agar. This pigment is not pH-sensitive when tested with 0.05 M NaOH or HCl.

D-Glucose, L-arabinose, sucrose, D-xylose, iso-inositol, D-mannitol, D-fructose, rhamnose, and raffinose are utilized for growth.

Type strain shows the highest sequence similarity to: *S. hawaiiensis*, AB184143, 99.7%; *S. luteogriseus*, AB184379, 99.4%; *S. massasporeus*, AB184152, 99.4%; *S. purpurascens*, AJ399486, 99.3%; *S. violaceus*, AB184315, 99.2%; *S. albosporeus* subsp. *albosporeus*, AJ781327, 99.2%; *S. janthinus*, AB184851, 99.2%; *S. roseoviolaceus*, AJ399484, 99.2%; *S. flavoviridis*, AB184842, 99%; *S. bellus*, AB184849, 99%; *S. indiaensis*, AB184553, 99%; *S. pilosus*, AB184161, 99%.

Source: the type strain was isolated from a sandy area near Zion, Illinois, USA.

DNA G+C content (mol%): not known.

Type strain: AS 4.1610, ATCC 25428, CBS 926.69, BCRC 11827, DSM 40293, NBRC 13016, JCM 4452, NRRL 2377, NRRL-ISP 5293, RIA 1208, VKM Ac-1201.

Sequence accession no. (16S rRNA gene): AB249977.

41. **Streptomyces argenteolus** Tresner, Davies and Backus 1961, 74^{AL}

ar.gen.te′o.lus. L. masc. adj. *argenteolus* of silver.

Spore chains in Section *Spirales*. This culture is described as "not forming loops or spirals" in the original descriptions appearing in the patents cited above. However, the type strain was observed to produce spirals in subsequent studies by Pridham et al. (1958) and Tresner et al. (1961). Mature spore chains generally have 10 to 50 or more spores per chain on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar. Spore surface is smooth.

Color of colony: aerial mass color in the Gray color series on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar. Reverse side of colony is colorless to grayish yellowish green or gray on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar; substrate mycelium pigment is not a pH indicator.

Color in medium: melanoid pigments are not formed on peptone-yeast-iron agar, tyrosine agar, or tryptone-yeast broth. No pigment is found in medium in yeast-malt agar, oatmeal agar, salts-starch agar, or glycerol-asparagine agar.

D-Glucose, L-arabinose, D-xylose, D-mannitol, D-fructose, and rhamnose are utilized for growth. No growth or only trace of growth on sucrose, iso-inositol, and raffinose.

Type strain shows the highest sequence similarity to: *S. cinereorectus*, AB184646, 100%; *S. lavendulae* subsp. *lavendulae*, AB184080, 99.9%; *S. cavourensis* subsp. *washingtonensis*, DQ026671, 99.9%; *S. microflavus*, DQ445795, 99.8%; *S. griseoplanus*, AY999894, 99.8%; *S. cyaneofuscatus*, AB184860, 99.8%; *S. praecox*, AB184293, 99.8%; *S. anulatus*, DQ026637, 99.8%; *S. fimicarius*, AY999784, 99.8%; *S. flavofuscus*, AB249935, 99.8%; *S. griseolus*, AB184768, 99.8%; *S. alboviridis*, AB184256, 99.8%; *S. lipmanii*, AB184148, 99.8%; *S. fulvorobeus*, AB184711, 99.8%; *S. griseus* subsp. *griseus*, AY207604, 99.7%; *S. halstedii*, EF178695, 99.7%; *S. albovinaceus*, AB249958, 99.7%; *S. sindenensis*, AB184759, 99.7%; *S. floridae*, AB184656, 99.7%; *S. globisporus* subsp. *globisporus*, EF178686, 99.7%; *S. acrimycini*, AY999889, 99.7%; *S. pluricolorescens*, DQ442540, 99.7%; *S. mediolani*, AB184674, 99.7%; *S. luridiscabiei*, AF361784, 99.7%; *S. griseinus*, AB184205, 99.7%; *S. flavovirens*, DQ026635, 99.7%; *S. baarnensis*, EF178688, 99.7%; *S. badius*, AY999783, 99.7%; *S. rubiginosohelvolus*, AB184240, 99.7%; *S. californicus*, AB184755, 99.6%; *S. flavogriseus*, AJ494864, 99.6%; *S. parvus*, DQ442537, 99.6%; *S. pulveraceus*, AB184806, 99.5%; *S. yanii*, AB006159, 99.4%; *S. nitrosporeus*, EF178680, 99.4%; *S. olivoviridis*, AB184227, 99.3%; *S. sanglieri*, AB249945, 99.3%; *S. atroolivaceus*, AJ781320, 99.3%; *S. bacillaris*, AB184439, 99.3%; *S. finlayi*, AY999788, 99.3%; *S. clavifer*, DQ026670, 99.3%; *S. gelaticus*, DQ026636, 99.2%; *S. atratus*, DQ026638, 99.2%; *S. celluloflavus*, AB184476, 99.1%; *S. cremeus*, AB184124, 99.1%; *S. spiroverticillatus*, AB184814, 99.1%; *S. griseobrunneus*, AB249912, 99.1%; *S. albolongus*, AB184425, 99.1%; *S. cavourensis* subsp. *cavourensis*, DQ445791, 99%; *S. mutomycini*, AB249951, 99%.

Source: not known.

DNA G+C content (mol%): not known.

Type strain: AS 4.1693, ATCC 11009, ATCC 23882, CBS 662.68, BCRC 11815, DSM 40226, NBRC 12841, IMET 43659, JCM 4623, JCM 4229, KCTC 1742, LMG 5967, NCIMB 9625, NRRL B-1806, NRRL-ISP 5226, RIA 1168, VKM Ac-747.

Sequence accession no. (16S rRNA gene): AB045872.

Further comments: according to Liu et al. (2005b), *Streptomyces argenteolus* Tresner et al. 1961^{AL} is a later heterotypic synonym of *Streptomyces griseus* (Krainsky 1914) Waksman and Henrici 1948^{AL} emend. Liu et al. 2005b.

According to Guo et al. (2008), *Streptomyces argenteolus* Tresner et al. 1961^{AL} is not a later heterotypic synonym of *Streptomyces griseus* (Krainsky 1914) Waksman and Henrici 1948^{AL}.

42. **Streptomyces armeniacus** (Kalakoutskii and Kusnetsov 1964) Wellington and Williams 1981a, 80^{VP} (*Actinoplanes armeniacus* Kalakoutskii and Kusnetsov 1964, 613)

ar.me.ni′a.cus. L. fem. n. *armeniaca* apricot-tree, N.L. masc. adj. *armeniacus* apricot-colored.

Three-week-old cultures show white aerial mycelium and development of spiral spore chains on chitin medium, nutrient agar, oatmeal agar, and ISP 4. On all media, the substrate mycelium is stable and cream-colored to brown. No distinctive pigments are produced in the substrate mycelium or in the medium. According to the original description of *A. armeniacus*, milk is not coagulated or peptonized, gelatin is liquefied, and nitrate is not reduced. The following carbon sources are utilized for growth: L-rhamnose, sorbitol, galactose, D-fructose, D-arabinose, trehalose, cellobiose, esculin, glycerin, and inulin. Growth is weak on glucose, sucrose, lactose, maltose, raffinose, sorbose, xylose, salicin, and dulcitol. No growth occurs on mannitol. Starch is hydrolyzed. Whole-cell hydrolysates contain LL-A_2pm, which is characteristic of cell-wall chemotype I. Susceptible to penicillin, streptomycin, chlortetracycline, erythromycin, and polymyxin; resistant to kanamycin and neomycin.

Type strain shows no sequence similarity over 99%.

Source: not known.

DNA G+C content (mol%): not known.

Type strain: 26 A-32, RIA 807, AS 4.1684, ATCC 15676, CBS 559.75, DSM 43125, IFM 1166, IFM 1244, NBRC 12555, IMET 9250, JCM 3070, KCTC 9120, NCIMB 10179, VKM Ac-905.

Sequence accession no. (16S rRNA gene): AB018092.

43. **Streptomyces asiaticus** Sembiring, Ward and Goodfellow 2001, 1619^{VP} (Effective publication: Sembiring, Ward and Goodfellow 2000, 365.)

a.si.a′ti.cus. L. masc. adj. *asiaticus* Asian.

Spore chains are *Spirales*; the spore surface is rugose. On oatmeal agar, the spore mass is gray, the substrate mycelium is grayish-yellow, and the diffusible pigment is yellow. Melanin pigments are not produced. The strain degrades pectin and grows at 45°C.

Type strain shows the highest sequence similarity to: *Streptomyces* sp., AJ391828, 99.5%; *Streptomyces* sp., AJ391831, 99.5%; *S. cangkringensis*, AJ391831, 99.5%; *Streptomyces* sp., AJ391825, 99.4%; *Streptomyces* sp., AJ391826, 99.4%; *Streptomyces* sp., AJ391832, 99.4%; *Streptomyces* sp., A33R1, DSM 41763, AJ391832, 99.4%; *Streptomyces* sp., B23P1, DSM 41765, AJ391825, 99.4%; *Streptomyces* sp., DSM 41768, AJ391826, 99.4%; *Streptomyces* sp., AJ391818, 99.3%; *Streptomyces* sp.,

AJ391827, 99.3%; *Streptomyces* sp., AJ391829, 99.3%; *Streptomyces* sp., AJ391836, 99.3%; *S. griseiniger*, AJ391818, 99.3%; *S. yogyakartensis*, DSM 41766^{T}, 99.3%; *Streptomyces* sp., AJ391836, 99.3%; *S. violaceusniger*, AJ391822, 99.1%; *S. violaceusniger*, AJ391823, 99.1%; *S. antimycoticus*, DSM 40284^{T}, 99.1%; *S. violaceusniger*, NRRL-ISP 5562^{T}, 99.1%; *Streptomyces* sp., AJ391834, 99%; *S. albiflaviniger*, AJ391812, 99%; *S. albiaxialis*, DSM 41799^{T}, 99%; *S. rhizosphaericus*, DSM 41760^{T}, 99%; *Streptomyces* sp., DSM 40602, 99%.

Type strain shows DNA–DNA similarity to: *S. rhizosphaericus*, AB249941, 100%; *S. cangkringensis*, AJ391831, 99.8%; *S. griseinger*, AJ391818, 99.8%; *S. indonesiensis*, DQ334783, 99.7%; *S. antimycoticus*, AB184185, 99.1%; *S. sporoclivatus*, AB249934, 99.1%; *S. rutgersensis* subsp. *castelarensis*, AY508511, 99.1%; *S. geldanamycininus*, DQ334781, 99%.

Source: isolated from the rhizosphere of the tropical legume *Paraserianthes falcataria*.

DNA G+C content (mol%): not known.

Type strain: A14P1, DSM 41761, JCM 11443, NBRC 100774, NCIMB 13675.

Sequence accession no. (16S rRNA gene): AB249947.

44. **Streptomyces asterosporus** (*ex* Krasil'nikov 1970b) Preobrazhenskaya 1986, 573^{VP} (Effective publication: Preobrazhenskaya *in* Gause, Preobrazhenskaya, Sveshnikova, Terekhova and Maximova 1983.) ("*Actinomyces asterosporus*" Krasil'nikov (1970b)

as.te.ro.spo′rus. L. n. *aster*-eris a star; N.L. n. *spora* (from. Gr. n. *spora* seed) spore; N.L. masc. adj. *asterosporus* star-shaped spore.

The type strain, INMI 16^{T}, easily loses its ability to construct aerial mycelium. Spore chains *Spirales*; spores are spiny, of medium-size, and with wide bodies. On mineral agar 1: aerial mycelium is gray, sometimes black, depending on moisture and release; substrate mycelium is colorless, sometimes gray; no diffusible pigment. On glycerol-nitrate agar and oatmeal agar: aerial mycelium is gray; substrate mycelium is colorless or gray; no diffusible pigment. On starch-ammonia agar: aerial mycelium is gray; substrate mycelium is colorless to creamy yellowish; no diffusible pigment. On glycerol-asparagine agar: aerial mycelium is poor, gray; substrate mycelium is colorless; no diffusible pigment. On organic agar 2: aerial mycelium is absent or gray; substrate mycelium is colorless or gray; no diffusible pigment. Melanoid pigments are not formed. Grows on glucose, fructose, sucrose, arabinose, galactose, rhamnose, and mannitol; no growth on raffinose or xylose.

Type strain shows the highest sequence similarity to: *S. aureorectus*, AB184710, 100%; *S. calvus*, AB184329, 100%; *S. virens*, DQ442554, 99.8%; *S. minutiscleroticus*, EF178696, 99.1%; *S. geysiriensis*, DQ442501, 99.1%; *S. djakartensis*, AB184657, 99.1%; *S. mutabilis*, EF178679, 99%; *S. rochei*, AB184237, 99%; *S. plicatus*, AB184291, 99%; *S. anandii*, AB184402, 99%; *S. vinaceusdrappus*, AY999929, 99%; *S. tuirus*, AB184690, 99%; *S. ghanaensis*, AY999851, 99%.

Source: not known.

DNA G+C content (mol%): not known.

Type strain: AS 4.1605, DSM 41452, NBRC 15872, INMI 16, JCM 6912, VKM Ac-40.

Sequence accession no. (16S rRNA gene): AB184706.

45. **Streptomyces atratus** Shibata, Higashide, Yamamoto and Nakazawa 1962, 232^{AL}

a.tra′tus. L. masc. adj. *atratus* clothed in black.

Spore chains of atypical *Retinaculum-Apertum* type. Forms gray to black vegetative mycelium on some media. Probably grows poorly on Czapek's solution agar. Produces rufomycin A, rufomycin B, and other anti-bacterial activity.

Type strain shows the highest sequence similarity to: *S. sanglieri*, AB249945, 100%; *S. yanii*, AB006159, 99.8%; *S. pulveraceus*, AB184806, 99.7%; *S. gelaticus*, DQ026636, 99.6%; *S. badius*, AY999783, 99.3%; *S. pluricolorescens*, DQ442540, 99.3%; *S. sindenensis*, AB184759, 99.3%; *S. albovinaceus*, AB249958, 99.3%; *S. globisporus* subsp. *globisporus*, EF178686, 99.3%; *S. griseinus*, AB184205, 99.3%; *S. flavofuscus*, AB249935, 99.3%; *S. mediolani*, AB184674, 99.3%; *S. fimicarius*, AY999784, 99.3%; *S. anulatus*, DQ026637, 99.3%; *S. rubiginosohelvolus*, AB184240, 99.3%; *S. praecox*, AB184293, 99.3%; *S. lavendulae* subsp. *lavendulae*, AB184080, 99.2%; *S. argenteolus*, AB045872, 99.2%; *S. parvus*, DQ442537, 99.2%; *S. cinereorectus*, AB184646, 99.2%; *S. griseoplanus*, AY999894, 99.2%; *S. californicus*, AB184755, 99.2%; *S. cavourensis* subsp. *washingtonensis*, DQ026671, 99.2%; *S. alboviridis*, AB184256, 99.1%; *S. griseolus*, AB184768, 99.1%; *S. baarnensis*, EF178688, 99.1%; *S. griseus* subsp. *griseus*, AY207604, 99.1%; *S. lipmanii*, AB184148, 99.1%; *S. cyaneofuscatus*, AB184860, 99.1%; *S. fulvorobeus*, AB184711, 99.1%; *S. halstedii*, EF178695, 99.1%; *S. acrimycini*, AY999889, 99.1%; *S. flavogriseus*, AJ494864, 99.1%; *S. flavovirens*, DQ026635, 99.1%; *S. microflavus*, DQ445795, 99.1%; *S. floridae*, AB184656, 99.1%; *S. luridiscabiei*, AF361784, 99%; *S. nitrosporeus*, EF178680, 99%.

Source: not known.

DNA G+C content (mol%): not known.

Type strain: AS 4.1632, ATCC 14046, DSM 41673, NBRC 3897, JCM 3386, NRRL B-16927.

Sequence accession no. (16S rRNA gene): DQ026638.

46. **Streptomyces atroaurantiacus** Nakagaito, Shimazu, Yokota and Hasegawa 1993a, 624^{VP} (Effective publication: Nakagaito, Shimazu, Yokota and Hasegawa 1992a, 632.)

a.tro.au.ran.ti.a′cus. L. adj. *ater -tra -trum* black, dark; N.L. adj. *aurantiacus -a -um* orange; N.L. masc. adj. *atroaurantiacus* dark orange.

Mature spore chains are long and straight to slightly flexuous. Spores are cylindrical with a smooth surface. Melanoid pigments are produced on tyrosine agar. Brown soluble pigments are produced on yeast extract-malt extract agar and yellow pigments are produced on oatmeal agar. The color of vegetative mycelia is dark orange to slightly orange. The color of aerial mycelia is white. Aerial mycelia are formed slightly on yeast extract-malt extract agar, oatmeal agar, inorganic salts-starch agar, tyrosine agar, Bennett's agar, and water agar. The temperature range for growth is 10–37°C. The concentration of NaCl at which growth occurs is less than 2%. Nitrate is not reduced, starch is hydrolyzed, gelatin is not liquefied, and milk is peptonized and coagulated. D-Glucose, D-arabinose, D-fructose, sucrose, and D-xylose are utilized, but D-mannitol, rhamnose, raffinose, and isoinositol are not utilized or are poorly utilized. The cell wall contains both LL- and *meso*-A_2pm and glycine. Galactose and

a trace of madurose are detected as whole-cell sugars. The phospholipid pattern is type II. MK-9(H_6) and MK-9(H_8) are detected.

Type strain shows no sequence similarity over 99% to other *Streptomyces* species. Type strain shows the highest sequence similarity to following *Kitasatospora*: *Kitasatospora gansuensis*, AY442265, 99.4%.

Source: not known.

DNA G+C content (mol%): 70.2.

Type strain: ATCC 51343, DSM 41649, NBRC 14327, JCM 3337, NRRL B-24282.

Sequence accession no. (16S rRNA gene): DQ026645.

47. **Streptomyces atroolivaceus** (Preobrazhenskaya, Blinov and Ryabova *in* Gauze, Preobrazhenskaya, Kudrina, Blinov, Ryabova and Sveshnikova 1957) Pridham, Hesseltine and Benedict 1958, 68[AL] ("*Actinomyces atroolivaceus*" Preobrazhenskaya, Blinov and Ryabova *in* Gauze, Preobrazhenskaya, Kudrina, Blinov, Ryabova and Sveshnikova 1957, 143)

at.ro.o.li.va′ce.us. L. adj. *ater -tra -trum* black, dark; N.L. adj. *olivaceus* olive colored; N.L. masc. adj. *atroolivaceus* of a dark olive color, referring to the pigment on an organic medium.

Spore chains in Section *Rectiflexibiles.* Mature spore chains generally have 10–50 spores per chain. This morphology is seen on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar. Spore surface is smooth.

Color of colony: aerial mass color in the Gray color series on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar. Reverse side of colony with no distinctive pigments on yeast-malt agar, oatmeal agar, salts-starch agar, or glycerol-asparagine agar; substrate pigment is not a pH indicator.

Color in medium: melanoid pigments not formed in peptone-yeast-iron agar or tyrosine agar. No pigment found in medium in yeast-malt agar, oatmeal agar, salts-starch agar, or glycerol-asparagine agar.

D-Glucose, L-arabinose, D-xylose, D-fructose, and rhamnose are utilized for growth. Variable reports on growth with sucrose, iso-inositol, D-mannitol, and raffinose. Two collaborators reported difficulty in observing results because of poor growth on all carbon sources with Pridham and Gottlieb basal medium.

Type strain shows the highest sequence similarity to: *S. clavifer*, DQ026670, 100%; *S. olivoviridis*, AB184227, 100%; *S. finlayi*, AY999788, 99.7%; *S. mutomycini*, AB249951, 99.7%; *S. pluricolorescens*, DQ442540, 99.5%; *S. sindenensis*, AB184759, 99.5%; *S. cavourensis* subsp. *washingtonensis*, DQ026671, 99.5%; *S. mediolani*, AB184674, 99.5%; *S. lavendulae* subsp. *lavendulae*, AB184080, 99.5%; *S. flavofuscus*, AB249935, 99.5%; *S. griseoplanus*, AY999894, 99.5%; *S. anulatus*, DQ026637, 99.5%; *S. praecox*, AB184293, 99.5%; *S. fimicarius*, AY999784, 99.5%; *S. rubiginosohelvolus*, AB184240, 99.5%; *S. badius*, AY999783, 99.5%; *S. griseinus*, AB184205, 99.5%; *S. acrimycini*, AY999889, 99.4%; *S. albovinaceus*, AB249958, 99.4%; *S. globisporus* subsp. *globisporus*, EF178686, 99.4%; *S. alboviridis*, AB184256, 99.4%; *S. cinereorectus*, AB184646, 99.4%; *S. baarnensis*, EF178688, 99.4%; *S. microflavus*, DQ445795, 99.4%; *S. cyaneofuscatus*, AB184860, 99.4%; *S. lipmanii*, AB184148, 99.4%; *S. fulvorobeus*, AB184711, 99.4%; *S. parvus*, DQ442537, 99.3%; *S. floridae*, AB184656, 99.3%; *S. luridiscabiei*, AF361784, 99.3%; *S. griseolus*, AB184768, 99.3%; *S. flavovirens*, DQ026635, 99.3%; *S. argenteolus*, AB045872, 99.3%; *S. californicus*, AB184755, 99.3%; *S. halstedii*, EF178695, 99.2%; *S. griseus* subsp. *griseus*, AY207604, 99.2%; *S. flavogriseus*, AJ494864, 99.2%; *S. candidus*, DQ026663, 99.1%; *S. pulveraceus*, AB184806, 99%; *S. nitrosporeus*, EF178680, 99%; *S. bacillaris*, AB184439, 99%.

Source: not known.

DNA G+C content (mol%): not known.

Type strain: AS 4.1405, ATCC 19725, CBS 464.68, BCRC 12073, CCUG 11112, CECT 3316, DSM 40137, NBRC 12741, IMET 43088, INA 4776, JCM 4345, KCTC 9017, LMG 19306, NRRL-ISP 5137, RIA 1006, UNIQEM 121, VKM Ac-970.

Sequence accession no. (16S rRNA gene): AJ781320.

48. **Streptomyces atrovirens** (*ex* Preobrazhenskaya et al. 1971) Preobrazhenskaya and Terekhova 1986, 573[VP] (Effective publication: Preobrazhenskaya and Terekhova *in* Gause, Preobrazhenskaya, Sveshnikova, Terekhova and Maximova 1983.) ("*Actinomyces atrovirens*" Preobrazhenskaya et al. 1971)

a.tro.vi′rens. L. adj. *ater -tra -trum* black, dark; L. part. adj. *virens* being green; N.L. part. adj. *atrovirens* dark green.

Spore chains in *Spirales*; spore surface is covered with long hair. On mineral agar 1, glycerol-nitrate agar, oatmeal agar, starch ammonia agar, and glycerol-asparagine agar: aerial mycelium is gray; substrate mycelium is grayish green, blue green to dark green; diffusible pigment is pale blue green, grayish green. For several strains, colorless substrate mycelium on some media is possible. On organic agar 2: aerial mycelium is gray; substrate mycelium and diffusible pigment are dark green blue to dark green, black green. The strain builds blue-green color at low pH and red-violet to gray-brownish color at high pH. Melanoid pigments are not formed. Sucrose, mannitol, rhamnose, xylose, and inositol are utilized for growth; weak growth is seen on fructose and raffinose; no growth on arabinose. Produces antibiotic no. 300 (antimetabolite Leycin).

Type strain shows the highest sequence similarity to: *S. heliomycini*, AB184712, 99.2%; *S. viridodiastaticus*, AY999852, 99.1%; *S. flavoviridis*, AB184842, 99.1%; *S. albogriseolus*, AJ494865, 99.1%; *S. coeruleorubidus*, AY999719, 99%; *S. pilosus*, AB184161, 99%.

Source: not known.

DNA G+C content (mol%): not known.

Type strain: AS 4.1595, DSM 41467, NBRC 15388, INA 1551, JCM 6913, NRRL B-16357, VKM Ac-1213.

Sequence accession no. (16S rRNA gene): DQ026672.

49. **Streptomyces aurantiacus** (Rossi Doria 1891) Waksman *in* Waksman and Lechevalier 1953, 53[AL] ["*Streptotrix aurantiaca*" (*sic*) Rossi Doria 1891, 417; "*Actinomyces aurantiacus*" (*sic*) Gasperini 1892, 222; "*Actinomyces aurantiacus*" Gasperini 1894, 84; "*Cladothrix aurantiaca*" Macé 1897, 1033; "*Nocardia aurantiaca*" Chalmers and Christopherson 1916, 268]

au.ran.ti.a′cus. N.L. n. *aurantium* generic name of the orange; N.L. masc. adj. *aurantiacus* orange colored.

Spore chains in Section *Spirales.* Short spore chains of 3 to 10 or more spores per chain form irregular hooks and

loops of small diameter and imperfect spirals of one to three turns. Sporulating aerial mycelium is often absent or poorly developed on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar. Spore surface is smooth. Fragmenting substrate mycelium may be seen after 12–14 d on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar. One observer also notes irregular terminal swellings on some substrate hyphae as well as the presence of subglobose to clavate bodies 5–8 µm in diameter.

Color of colony: aerial mycelium is generally poorly developed or absent on ISP media. When adequate sporulating aerial mycelium is produced, it is in the Red color series (5cb or 6ec, grayish yellowish pink) in yeast-malt agar, oatmeal agar, and salts-starch agar. Reverse side of colony is yellow to yellow brown and modified by red to reddish brown on yeast-malt agar and salts-starch agar and to grayish yellowish pink or reddish orange on oatmeal agar and glycerol-asparagine agar. The substrate pigment is not a pH indicator.

Color in medium: melanoid pigments are not formed in peptone-yeast-iron agar, tyrosine agar, or tryptone-yeast broth. No pigment is found in medium in yeast-malt agar, oatmeal agar, salts-starch agar, or glycerol-asparagine agar.

D-Glucose, L-arabinose, iso-inositol, D-mannitol, D-fructose, and rhamnose are utilized for growth. Two of three observers also record utilization of D-xylose, sucrose, and raffinose.

Type strain shows the highest sequence similarity to: *S. glomeroaurantiacus*, AB249983, 100%; *S. tauricus*, AB045879, 99%.

Source: not known.

DNA G+C content (mol%): not known.

Type strain: AS 4.1429, ATCC 19822, ATCC 25429, CBS 927.69, DSM 40412, NBRC 13017, INMI 1373, JCM 4453, LMG 19358, NRRL-ISP 5412, RIA 1209, VKM Ac-44.

Sequence accession no. (16S rRNA gene): AJ781383.

Further comment: Streptomyces aurantiacus (Rossi Doria 1891) Waksman 1953[AL] emend. Lanoot et al. 2002 is an earlier heterotypic synonym of *Streptomyces albosporeus* subsp. *albosporeus* (Krainsky 1914) Waksman and Henrici 1948[AL].

50. **Streptomyces aurantiogriseus** (Preobrazhenskaya *in* Gauze, Preobrazhenskaya, Kudrina, Blinov, Ryabova and Sveshnikova 1957) Pridham, Hesseltine and Benedict 1958, 67[AL] ("*Actinomyces aurantiogriseus*" Preobrazhenskaya *in* Gauze, Preobrazhenskaya, Kudrina, Blinov, Ryabova and Sveshnikova 1957, 74)

au.ran.tio.gri′se.us. N.L. n. *Aurantium* generic name of the orange; N.L. adj. *griseus* gray; N.L. masc. adj. *aurantiogriseus* orange, gray.

Spore chains in Section *Retinaculiaperti* or *Spirales*. Spirals are open and often are irregular and poorly developed. Long spore chains of the *Retinaculum-Apertum* type and flexuous chains are common. Mature spore chains may be slow to develop; they are moderately long with more than 10 spores per chain on oatmeal agar, salts-starch agar, and glycerol-asparagine agar. Spore surface is smooth.

Color of colony: aerial mass color is in both the Red and the Gray color series on yeast-malt agar, salts-starch agar, and glycerol-asparagine agar; both colors may appear on the same medium. One observer noted an increase in gray aerial color between 14 and 21 d on glycerol-asparagine agar; this tendency to change from red to gray was also included in the original description. The color tabs most frequently were 2ec (yellowish gray) from the Gray color series and 5ge (light grayish reddish brown) from the Red color series. Reverse side of colony with no distinctive pigment (grayed yellow on oatmeal agar and glycerol-asparagine agar to orange-yellow or brown on yeast-malt and salts-starch agar). Substrate mycelium pigment is not a pH indicator.

Color in medium: melanoid pigments are produced in peptone-yeast-iron agar, tyrosine agar, and tryptone-yeast broth. Pigments other than melanoids are not found in yeast-malt agar, oatmeal agar, salts-starch agar, or glycerol-asparagine agar.

D-Glucose, L-arabinose, sucrose, D-xylose, iso-inositol, D-mannitol, D-fructose, rhamnose, and raffinose are utilized for growth.

Type strain shows the highest sequence similarity to: *S. coelicolor*, DQ442496, 99.9%; *S. griseoviridis*, AY999807, 99.3%.

Source: not known.

DNA G+C content (mol%): not known.

Type strain: AS 4.1450, ATCC 19887, ATCC 23883, CBS 663.68, BCRC 13758, DSM 40138, NBRC 12842, INA 10369/58, JCM 4346, LMG 19298, NCIMB 9849, NRRL B-5416, NRRL-ISP 5138, RIA 1130, VKM Ac-1093.

Sequence accession no. (16S rRNA gene): AY999793.

51. **Streptomyces auratus** Goodfellow, Kumar, Labeda and Sembiring 2008, 1[VP] (Effective publication: Goodfellow, Kumar, Labeda and Sembiring 2007, 198.)

au.ra′tus. L. masc. adj. *auratus* gold colored.

Spore chains are *Spirales*; spore surface is smooth. On oatmeal agar, the aerial spore mass color is gray and the substrate mycelium grayish-yellow; an orange diffusible pigment is produced, but not melanin pigments. The organism produces hydrogen sulfide and degrades adenine, arbutin, chitin, hypoxanthine, Tweens 40, 60 and 80, uric acid, and xanthine, but not casein, guanine, tyrosine, or xylan. It does not reduce nitrate or hydrolyze esculin or urea. Butan-1,4-diol, cellobiose, citric acid, dextrin, D-fructose, L-fucose, D-galactose, D-glucose, *myo*-inositol, maltose, D-mannitol, D-mannose, melezitose, methanol, propanol, pyruvic acid, raffinose, L-rhamnose, D-ribose, L-salicin, and sucrose are used as sole carbon sources, but not α-lactose. α- and L-Alanine, L-aminobutyric acid, L-glutamic acid, L-glycine, L-histidine, L-proline, L-serine, and L-threonine are used as sole carbon and nitrogen sources, but not aspartic acid, L-leucine, DL-methionine, DL-norleucine, L-phenylalanine, L-tryptophan, or L-valine. Grows from pH 5 to 10, at 25 and 37°C, but not at 10 or 40°C. Growth occurs in the presence of 13% (w/v) NaCl. Resistant to carbenicillin, cefoxitin, cephaloridine, chlortetracycline hydrochloride, doxycycline hydrochloride, rifampin, and novobiocin, but sensitive to cefoxitin, cephaloridine, doxycycline hydrochloride, fusidic acid, lincomycin hydrochloride, and oleandomycin phosphate.

Type strain shows the highest sequence similarity to: *S. sioyaensis*, DQ026654, 99%.

Source: not known.
DNA G+C content (mol%): 65.6.
Type strain: DSM 41897, NRRL 8097.
Sequence accession no. (16S rRNA gene): AJ391816.

52. **Streptomyces aureocirculatus** (Krasil'nikov and Yuan *in* Krasil'nikov 1965) Pridham 1970, 8[AL] ("*Actinomyces aureocirculatus*" Krasil'nikov and Yuan *in* Krasil'nikov 1965, 33)

au.re.o.cir.cu.la'tus. L. adj. *aureus* golden; L. part. adj. *circulatus* made circular or round; N.L. masc. adj. *aureocirculatus* golden-curled.

Spore chains in Section *Rectiflexibiles* with some irregular hooks and loops or imperfect spirals. Mature spore chains, when present, are generally long, sometimes with more than 50 spores per chain. Aerial mycelium is poorly developed on all ISP media. Mature spore chains may be found on yeast-malt agar, oatmeal agar, and salts-starch agar. One observer records fragmentation of substrate mycelium on glycerol-asparagine agar at 21 d. Another observer notes the presence of globular bodies and sclerotia on yeast-malt agar, oatmeal agar, and salts-starch agar and conidia-like spores on the substrate mycelium on salts-starch agar at 7 d. Spore surface is smooth.

Color of colony: sporulating aerial mycelium is inadequate for determination of aerial mass color on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar. The scant aerial mycelium that develops on yeast-malt agar, oatmeal agar, and salts-starch agar is in the White color series. Reverse side of colony with no distinctive pigments (pale yellow to light grayish yellow) on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar.

Color in medium: melanoid pigments are not formed in peptone-yeast-iron agar, tyrosine agar, or tryptone-yeast broth. Yellow pigment is found in the medium in yeast-malt agar, oatmeal agar, and salts-starch agar. This pigment is not pH-sensitive when tested with 0.05 M NaOH or HCl.

D-Glucose, D-xylose, iso-inositol, D-mannitol, and D-fructose are utilized for growth. Reports vary on utilization of L-arabinose, rhamnose, sucrose, and raffinose. Only one of three observers records utilization of rhamnose and raffinose.

Type strain shows the highest sequence similarity to: *S. novaecaesareae*, AB184357, 99.5%; *S. galilaeus*, AB045878, 99.5%; *S. bobili*, AB249925, 99.4%; *S. pseudovenezuelae*, AB184233, 99.1%; *S. resistomycificus*, AB184166, 99.1%; *S. phaeoluteigriseus*, AJ391815, 99%; *S. chartreusis*, AB184839, 99%.

Source: not known.
DNA G+C content (mol%): not known.
Type strain: AS 4.1609, ATCC 15851, ATCC 19823, ATCC 25430, CBS 928.69, DSM 40386, NBRC 13018, INMI 735, JCM 4454, NRRL B-3324, NRRL-ISP 5386, RIA 1210, RIA 682.
Sequence accession no. (16S rRNA gene): AB184260.

53. **Streptomyces aureofaciens** Duggar 1948, 177[AL]

au.re.o.fa'ci.ens. L. adj. *aureus* golden; L. part. adj. *faciens* producing; N.L. part adj. *aureofaciens* golden-producing, referring pigment produced.

Spore chains in Section *Retinaculiaperti* but chains representative of Section *Rectiflexibiles* are also common. Mature spore chains generally have 10–50 spores per chain. This morphology is seen on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar. Spore surface is smooth.

Color of colony: aerial mass color in the Gray color series on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar. Reverse side of colony with no distinctive pigments (grayed yellow, orange-yellow, or brown on yeast-malt agar, and grayed yellow or greenish yellow on oatmeal agar, salts-starch agar, and glycerol-asparagine agar). Substrate mycelium pigment is not a pH indicator.

Color in medium: melanoid pigments are not formed in peptone-yeast-iron agar, tyrosine agar, or tryptone-yeast broth. No pigment is found in medium in yeast-malt agar, oatmeal agar, salts-starch agar, or glycerol-asparagine agar.

D-Glucose, L-arabinose, sucrose, D-xylose, and D-fructose are utilized for growth. No growth or only trace of growth on iso-inositol, D-mannitol, rhamnose, and raffinose.

Type strain shows the highest sequence similarity to: *S. psammoticus*, AY999862, 99.6%; *S. avellaneus*, AB184413, 99.5%; *S. xanthocidicus*, AY999858, 99.1%.

Source: not known.
DNA G+C content (mol%): not known.
Type strain: ATCC 10762, ATCC 23884, CBS 434.51, CBS 664.68, CCM 3239, BCRC 11610, CECT 3206, CIP 57.11, DSM 40127, HAMBI 313, HAMBI 1072, HUT 6048, HUT 6097, ICMP 499, IFM 1042, IFM 1218, IFM 1219, NBRC 12594, NBRC 12843, IMET 43577, JCM 4624, JCM 4008, KACC 20180, LMG 5968, NCAIM B.01479, NCIMB 8234, NRRL 2209, NRRL B-2183, NRRL B-2657, NRRL B-5404, NRRL-ISP 5127, RIA 1129, RIA 57, VKM Ac-771.
Sequence accession no. (16S rRNA gene): AY207608.

54. **Streptomyces aureorectus** (*ex* Taig, Solovieva and Braginskaya 1969) Taig and Solovieva 1986, 573[VP] (Effective publication: Taig and Solovieva *in* Gause, Preobrazhenskaya, Sveshnikova, Terekhova and Maximova 1983.) ("*Actinomyces aureorectus*" Taig, Solovieva and Braginskaya 1969)

au.re.o.rec'tus. L. adj. *aureus* golden; L. adj. *rectus* straight; N.L. masc. adj. *aureorectus* golden, straight.

Spore chains are straight, spores are smooth. On mineral agar 1 and glycerol-nitrate agar: aerial mycelium is white gray, substrate mycelium is lemon yellow, yellow, dark yellow; diffusible pigment is light yellow, yellow. On starch-ammonia agar (ISP 4): no aerial mycelium; colorless substrate mycelium; no diffusible pigment. On organic agar 2: no aerial mycelium; colorless substrate mycelium; no diffusible pigment. On glycerol-asparagine agar (ISP 5): white aerial mycelium; substrate mycelium and diffusible pigment are yellow. Melanoid pigments are not formed. Good digestion of xylose, galactose, maltose, and raffinose. Antibiotic aurenin is formed.

Type strain shows the highest sequence similarity to: *S. asterosporus*, AB184706, 100%; *S. calvus*, AB184329, 99.9%; *S. virens*, DQ442554, 99.9%; *S. minutiscleroticus*, EF178696, 99%; *S. geysiriensis*, DQ442501, 99%; *S. djakartensis*, AB184657, 99%; *S. anandii*, AB184402, 99%; *S. tuirus*, AB184690, 99%.

Source: not known.
DNA G+C content (mol%): not known.

Type strain: 2843-10, DSM 41692, NBRC 15896, INA A-78, JCM 9947, RIA 553, VKM Ac-1828.

Sequence accession no. (16S rRNA gene): AB184710.

55. **Streptomyces aureoversilis** corrig. (Locci, Baldacci and Petrolini Baldan 1969) Witt and Stackebrandt 1996, 836[VP] (Effective publication: Witt and Stackebrandt 1990, 370.) (*Streptoverticillium aureoversile* corrig. Locci, Baldacci and Petrolini Baldan 1969, 59)

au.re.o.ver′si.lis. L. adj. *aureus* golden; L. adj. *versilis* that may be turned, movable; N.L. masc. adj. *aureoversilis* golden, movable.

On potato-glucose agar (Baldacci et al., 1954) and Oxoid nutrient agar, reverse colors are brown; traces of whitish aerial mycelium on potato-glucose agar (Baldacci et al., 1954), more abundant on Oxoid nutrient agar. Growth is fair on Bacto Czapek agar and Casamino acids Czapek agar (1 g/l Difco vitamin-free Casamino acids, replacing sodium nitrate), the reverse being dirty pinkish.

On Casamino acids Czapek agar (1 g/l Difco vitamin-free Casamino acids, replacing sodium nitrate), glucose-asparagine agar (ISP medium 5 with 1% glucose replacing glycerol), glycerol-asparagine agar (ISP medium 5), inorganic salts-starch agar (ISP medium 4), and yeast extract-malt extract agar (ISP medium 2), aerial mycelium is pinkish. On inorganic salts-starch agar (ISP medium 4) and glucose-asparagine agar (ISP medium 5 with 1% glucose replacing glycerol), reverse color is red.

Grows equally well at 27 and 37°C; aerial mycelium production slightly less at 37°C and slow in appearing. The reference strain produces tetraene 380 and pentaene 380 and exhibits anti-bacterial activity.

Type strain shows the highest sequence similarity to: *S. hiroshimensis*, AB184789, 99.5%; *S. cinnamoneus*, AB184850, 99.2%; *S. pseudoechinosporeus*, AB184100, 99.1%; *S. caeruleus*, EF178675, 99.1%.

Source: not known.

DNA G+C content (mol%): not known.

Type strain: AS 4.1641, ATCC 15853, ATCC 25433, CBS 664.69, BCRC 12451, DSM 40387, NBRC 13021, INMI 380, JCM 4457, NRRL B-3325, NRRL-ISP 5387, RIA 1213, RIA 681, VKM Ac-884.

Sequence accession no. (16S rRNA gene): AB184855.

Further comments: according to Hatano et al. (2003), *Streptomyces aureoversilis* corrig. (Locci et al. 1969) Witt and Stackebrandt 1991 is a later heterotypic synonym of *Streptomyces hiroshimensis* (Shinobu 1955) Witt and Stackebrandt 1991.

56. **Streptomyces aureoverticillatus** (Krasil′nikov and Yuan 1960) Pridham 1970, 54[AL] ("*Actinomyces aureoverticillatus*" Krasil′nikov and Yuan 1960, 487)

au.re.o.ver.ti.cil.la′tus. L. adj. *aureus* golden; N.L. adj. *verticillatus* whorled; N.L. adj. *aureoverticillatus* golden, whorled (referring to color of the vegetative mycelium of the organism and nature of its morphology).

Spore chains in Section *Retinaculiaperti* but spore chains representative of Section *Rectiflexibiles* are common. Pseudoverticillate sporophores (suggesting Section *Verticillati*) are found, especially on salts-starch agar. Verticils are not evenly spaced on an enlarged axial hypha as in true verticillate forms. Mature spore chains generally have10–50 spores per chain. This morphology is seen on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar, but aerial mycelium is not abundant on yeast-malt agar or oatmeal agar. Spore surface is smooth.

Color of colony: aerial mass color in the Red color series on salts-starch agar and glycerol-asparagine agar (White is also found on these media and poorly sporulated surface on yeast-malt agar). Reverse side of colony is grayed yellow and modified to red on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar. Substrate pigment is a pH indicator; it changes from orange to yellow by addition of 0.05 M NaOH, and from orange to red with HCl.

Color in medium: melanoid pigments not formed in peptone-yeast-iron agar and tyrosine agar. No pigment found in medium in yeast-malt agar, oatmeal agar, salts-starch agar, or glycerol-asparagine agar.

D-Glucose, L-arabinose, D-xylose, iso-inositol, D-mannitol, D-fructose, and raffinose are utilized for growth. No growth or only trace of growth on rhamnose. Variable reports on growth with sucrose.

Type strain shows the highest sequence similarity to: *S. diastaticus* subsp. *diastaticus*, AB184785, 99%; *S. gougerotii*, AB184742, 99%; *S. intermedius*, AB184277, 99%; *S. rutgersensis* subsp. *rutgersensis*, AB184170, 99%.

Source: not known.

DNA G+C content (mol%): not known.

Type strain: AS 4.1666, ATCC 15854, ATCC 19726, CBS 465.68, BCRC 12185, DSM 40080, NBRC 12742, INMI 1007, JCM 4347, NRRL B-3326, NRRL-ISP 5080, RIA 1007, RIA 679, VKM Ac-48.

Sequence accession no. (16S rRNA gene): AY999774.

57. **Streptomyces aureus** Manfio, Atalan, Zakrzewska-Czerwinska, Mordarski, Rodriguez, Collins and Goodfellow 2003b, 1219[VP] (Effective publication: Manfio, Atalan, Zakrzewska-Czerwinska, Mordarski, Rodriguez, Collins and Goodfellow 2003a, 254.)

au′re.us. L. masc. adj. *aureus* golden, referring to the color of the diffusible pigment on oatmeal agar.

Forms extensively branched substrate and aerial hyphae. Open looped chains of smooth-surfaced spores are evident on aerial hyphae. Aerial spore mass is gray; a reddish-orange colored substrate mycelium is formed on Bennett's, glycerol-asparagine, inorganic salts-starch, oatmeal, and yeast extract-malt extract agars. A golden colored diffusible pigment is produced on oatmeal agar. Hippurate is hydrolyzed and hypoxanthine and xanthine are degraded. Cleaves L-citrulline-7-amino-methylcoumarin (7AMC), L-phenylalanine-7AMC (endopeptidase substrates), L-cysteine-7AMC, L-glutamine-7AMC, L-ornithine-7AMC, L-proline-7AMC (exopeptidase substrates), and 4-methylumbelliferone (4MU)-elaidate (organic ester), but not methoxysuccinyl-L-alanine (endopeptidase substrate), L-pyroglutamate-7AMC (exopeptidase substrate), 4MU-β-D-galactopyranoside, 4MU-*N*-acetyl-β-D-glucosaminide, 4MU-α-D-glucoside, 4MU-2-deoxy-β-D-glucopyranoside, 4MU-β-D-xyloside (glycosides), or 4MU-pyrophosphate (inorganic ester). *myo*-Inositol, inulin, D(+)mannitol, citrate, and pyruvate are used as sole carbon sources for energy and growth but not dextran. L-Hydroxyproline is used as a sole carbon and nitrogen source, but not histidine. Grows between 10 and 35°C and in the

presence of thallous acetate (0.001%, w/v), but does not show antimicrobial activity against *Bacillus subtilis* NCIMB 3610, *Candida albicans* CBS 562, *Micrococcus luteus* NCIMB 196, or *Streptomyces murinus* ISP 5091.

Type strain shows the highest sequence similarity to: *S. durmitorensis*, DQ067287, 99.6%; *S. kanamyceticus*, DQ442511, 99.5%.

Source: not known.

DNA G+C content (mol%): 67.0–73.0.

Type strain: B7319, DSM 41785, JCM 12605, NBRC 100912, NCIMB 13927.

Sequence accession no. (16S rRNA gene): AB249976.

58. **Streptomyces avellaneus** Baldacci and Grein 1966, 195[AL]

a.vel.la′ne.us. N.L. masc. adj. *avellaneus* (from L. n. *avellana* hazel) hazel colored, referring to color of the aerial mycelium of the organism.

Spore chains in Section *Rectiflexibiles*. Mature spore chains are moderately long with 10 to 50 or more spores per chain. This morphology is seen on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar. Spore surface is smooth.

Color of colony: aerial mass color in the Gray color series (2dc, yellowish gray; 3fe, light brownish gray; or 5fe, light grayish reddish brown) on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar. Reverse side of colony with no distinctive pigment (strong yellowish brown to dark brown on yeast-malt agar; grayish yellow to orange yellow, light brown, or strong brown on oatmeal agar, salts-starch agar, and glycerol-asparagine agar).

Color in medium: melanoid pigments are not formed in peptone-yeast-iron agar, tyrosine agar, or tryptone-yeast broth. No pigment or only a trace of yellow is found in medium in yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar.

D-Glucose, D-fructose, and sucrose are utilized for growth. No growth or only traces of growth with L-arabinose, D-xylose, iso-inositol, D-mannitol, rhamnose, and raffinose.

Type strain shows the highest sequence similarity to: *S. psammoticus*, AY999862, 100%; *S. aureofaciens*, AY207608, 99.5%; *S. chrysomallus* subsp. *fumigatus*, AB184645, 99.2%; *S. purpureus*, AJ781324, 99.2%; *S. aburaviensis*, AY999779, 99%.

Source: not known.

DNA G+C content (mol%): not known.

Type strain: AS 4.1687, ATCC 23730, CBS 752.72, BCRC 12219, CMI 126840, DSM 40554, NBRC 13451, JCM 4725, JCM 4321, NCIMB 11000, NRRL B-3447, NRRL-ISP 5554, RIA 1412, VKM Ac-1720.

Sequence accession no. (16S rRNA gene): AB184413.

59. **Streptomyces avermectinius** Takahashi, Matsumoto, Seino, Ueno, Iwai and Ōmura 2002, 2167[VP]

a.ver.mec.ti′ni.us. N.L. adj. *avermectinius* pertaining to avermectin, an antibiotic produced by the organism.

Forms spiral spore chains. Spores are oval in shape (0.8 × 1.2 µm) and have a smooth surface. Soluble pigment is produced. Vegetative mycelia are brown. Aerial mass color is gray. Melanin and H_2S production, hydrolysis of starch and casein, liquefaction of gelatin, and peptonization of milk are positive. Adenine, casein, hypoxanthine and xanthine are decomposed but not cellulose. Grows in the presence of 5% (w/v) NaCl, but is sensitive to streptomycin (20 µg/ml) and novobiocin (20 µg/ml). Arabinose, fructose, glucose, inositol, lactose, maltose, mannitol, mannose, raffinose, rhamnose, sucrose, and xylose are decomposed.

For sequence similarity, see type strain of *Streptomyces avermitilis*. Type strain shows DNA–DNA similarity to: *S. bottropensis* DSM 40262^T, 15%; *S. cinnabarinus* DSM 40467^T, 21%; *S. galbus* DSM 40089^T, 33%; *S. griseochromogenes* DSM 40499^T, 7%; *S. luteogriseus* DSM 40483^T, 22%; *S. mirabilis* DSM 40553^T, 16%; *S. olivochromogenes* DSM 40451^T, 26%; *S. phaeochromogenes* DSM 40073^T, 20%.

Source: not known.

DNA G+C content (mol%): 70.3.

Type strain: MA-4680, ATCC 31267, JCM 5070, NCIMB 12804, NRRL 8165.

Sequence accession no. (16S rRNA gene): AB078897.

Further comments: Streptomyces avermectinius Takahashi et al. 2002 is a later homotypic synonym of *Streptomyces avermitilis* (*ex* Burg et al. 1979) Kim and Goodfellow 2002.

60. **Streptomyces avermitilis** (*ex* Burg, Miller, Baker, Birnbaum, Curri, Hartman, Kong, Monaghan, Olson, Putter, Tunac, Wallick, Stapley, Oiwa and Ōmura 1979) Kim and Goodfellow 2002, 2013[VP] ("*Streptomyces avermitilis*" Burg, Miller, Baker, Birnbaum, Curri, Hartman, Kong, Monaghan, Olson, Putter, Tunac, Wallick, Stapley, Oiwa and Ōmura 1979, 363)

a.ver.mi′ti.lis. N.L. masc. adj. *avermitilis* intended to mean avermectin producer.

The spore chains consist of 15 or more spherical to oval-shaped spores with smooth surfaces. Sporulation occurs on standard media such as egg albumin, glycerol-asparagine, inorganic salts-starch, and oatmeal agars. A gray aerial spore mass is formed on oatmeal agar; the colony reverse is dark brown to tan. Melanin pigments are produced on peptone-yeast extract-iron agar and brown diffusible pigments are produced on a range of standard media. Forms an extensively branched substrate mycelium and aerial hyphae that differentiate into long, compact spiral chains which become more open as the culture ages. The culture grows well at 28 and 37°C, but does not grow at 50°C. It metabolizes casein, but not tyrosine. It liquefies gelatin and uses glucose, maltose, mannose, and rhamnose, but not cellulose as sole carbon sources for energy and growth. Avermectins, a family of 16-membered antiparasitic macromolecules, are produced.

Type strain shows no sequence similarity over 99%. Type strain shows DNA–DNA similarity to: *S. cinnabarinus* DSM 40467^T, 98±7%; *S. griseochromogenes* DSM 40499^T, 99±0%.

Source: isolated from a soil sample collected at Kawana, Ito City, Shizuoka Prefecture, Japan.

DNA G+C content (mol%): not known.

Type strain: MA-4680, ATCC 31267, JCM 5070, NBRC 14893, NCIMB 12804, NRRL 8165.

Sequence accession no. (16S rRNA gene): BA000030.

Further comments: Streptomyces avermitilis (*ex* Burg et al. 1979) Kim and Goodfellow 2002 is an earlier homotypic synonym of *Streptomyces avermectinius* Takahashi et al. 2002.

61. **Streptomyces avidinii** Stapley, Mata, Miller, Demny and Woodruff 1964, 20[AL]

a.vi.di′ni.i. N.L. n. *avidinum* avidin the name of a biotin-binding protein; N.L. gen. n. *avidinii* of avidin.

Produces the antibiotic MSD-235 complex, streptavidin, and antibiotic MSD-235S, a synergistic anti-bacterial complex; poor growth on Czapek's solution agar.

Type strain shows the highest sequence similarity to: *S. nojiriensis*, AJ781355, 99.7%; *S. subrutilus*, X80825, 99.7%; *S. sporoaveus*, AJ781370, 99.7%; *S. xanthophaeus*, DQ442560, 99.7%; *S. goshikiensis*, EF178693, 99.6%; *S. cirratus*, AY999794, 99.6%; *S. vinaceus*, AB184394, 99.6%; *S. sporoverrucosus*, DQ442544, 99.5%; *S. cinnamonensis*, AB184707, 99.5%; *S. colombiensis*, DQ026646, 99.5%; *S. virginiae*, D85119, 99.3%.

Source: not known.

DNA G+C content (mol%): not known.

Type strain: MA-833, AS 4.1583, ATCC 27419, CBS 730.72, BCRC 13384, DSM 40526, NBRC 13429, JCM 4726, IMET 43538, KCTC 9757, NCIMB 11996, NRRL 3077, NRRL-ISP 5526, PCM 2342, RIA 1390, VKM Ac-1074.

Sequence accession no. (16S rRNA gene): AB184395.

62. **Streptomyces azureus** Kelly, Kutscher and Tuoti 1959, 1334[AL]

a.zur′e.us. N.L. masc. adj. *azureus* azure-blue, referring to the color of the aerial mycelium of the organism.

Spore chains in Section *Spirales*. Some sporophores supporting spiral spore chains appear to arise singly, in pairs, or in simple or branched whorls along an axial hypha (suggesting verticillate morphology) especially on salts-starch agar and glycerol-asparagine agar. Mature spore chains generally have 10–50 spores per chain. This morphology is seen on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar. Spore surface is smooth.

Color of colony: aerial mass color in the Blue color series on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar. Reverse side of colony with no distinctive pigments on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar; substrate pigment is not a pH indicator.

Color in medium: melanoid pigments formed in peptone-yeast-iron agar, tyrosine agar and tryptone-yeast extract broth. Pigments (other than melanoids) not formed in yeast-malt agar, oatmeal agar, salts-starch agar, or glycerol-asparagine agar.

D-Glucose, L-arabinose, sucrose, D-xylose, iso-inositol, D-mannitol, D-fructose, rhamnose, and raffinose are utilized for growth.

Type strain shows the highest sequence similarity to: *S. capillispiralis*, AB184577, 99.5%; *S. gancidicus*, AB184660, 99.5%; *S. pseudogriseolus*, DQ442541, 99.5%; *S. caelestis*, X80824, 99.5%; *S. cellulosae*, DQ442495, 99.4%; *S. levis*, AB184670, 99.4%; *S. paradoxus*, AB184628, 99.3%; *S. djakartensis*, AB184657, 99.2%; *S. coerulescens*, AY999720, 99.2%; *S. tuirus*, AB184690, 99.2%; *S. bellus*, AB184849, 99.2%; *S. carpinensis*, AB184574, 99.1%; *S. minutiscleroticus*, EF178696, 99.1%; *S. africanus*, AY208912, 99.1%; *S. mutabilis*, EF178679, 99.1%; *S. geysiriensis*, DQ442501, 99.1%; *S. afghaniensis*, AJ399483, 99%; *S. rochei*, AB184237, 99%; *S. plicatus*, AB184291, 99%; *S. vinaceusdrappus*, AY999929, 99%; *S. matensis*, AB184221, 99%; *S. ghanaensis*, AY999851, 99%.

Source: not known.

DNA G+C content (mol%): not known.

Type strain: AS 4.1675, ATCC 14921, ATCC 19728, CBS 467.68, BCRC 12479, DSM 40106, NBRC 12744, IMET 43765, JCM 4564, NRRL B-2655, NRRL-ISP 5106, PCM 2313, RIA 1009, UNIQEM 122, VKM Ac-719.

Sequence accession no. (16S rRNA gene): EF178674.

63. **Streptomyces baarnensis** Pridham, Hesseltine and Benedict 1958, 74[AL]

ba.arn.en′sis. N.L. masc. adj. *baarnensis* of or belonging to Baarn, a community in the Netherlands province of Utrecht.

This strain has apparently lost the ability to produce good sporulating aerial mycelium. Spore chains, when found, are straight (Section *Rectiflexibiles*) and usually contain more than 10 spores per chain. Spore surface is smooth.

Color of colony: aerial mycelium on yeast-malt agar, oatmeal agar, salts-starch agar, or glycerol-asparagine agar is inadequate for determination of aerial mass color. The original description of Duché (op. cit) describes early appearance of abundant greenish aerial mycelium on comparable media. Reverse side of colony with no distinctive pigment; yellow-brown on yeast-malt agar, light grayish yellow on oatmeal agar, salts-starch agar, and glycerol-asparagine agar.

Color in medium: melanoid pigments not formed in peptone-yeast-iron agar, tyrosine agar, or tryptone-yeast broth. No pigment found in medium in yeast-malt agar, oatmeal agar, salts-starch agar, or glycerol-asparagine agar.

D-Glucose, L-arabinose, D-xylose, iso-inositol, D-mannitol, D-fructose, and rhamnose are utilized for growth. No growth or only trace of growth on raffinose. Utilization of sucrose is doubtful.

Type strain shows the highest sequence similarity to: *S. griseoplanus*, AY999894, 99.9%; *S. fimicarius*, AY999784, 99.9%; *S. flavofuscus*, AB249935, 99.9%; *S. praecox*, AB184293, 99.9%; *S. pluricolorescens*, DQ442540, 99.8%; *S. sindenensis*, AB184759, 99.8%; *S. anulatus*, DQ026637, 99.8%; *S. griseinus*, AB184205, 99.8%; *S. acrimycini*, AY999889, 99.8%; *S. badius*, AY999783, 99.8%; *S. mediolani*, AB184674, 99.8%; *S. lavendulae* subsp. *lavendulae*, AB184080, 99.8%; *S. rubiginosohelvolus*, AB184240, 99.8%; *S. lipmanii*, AB184148, 99.7%; *S. fulvorobeus*, AB184711, 99.7%; *S. albovinaceus*, AB249958, 99.7%; *S. microflavus*, DQ445795, 99.7%; *S. cavourensis* subsp. *washingtonensis*, DQ026671, 99.7%; *S. argenteolus*, AB045872, 99.7%; *S. parvus*, DQ442537, 99.7%; *S. globisporus* subsp. *globisporus*, EF178686, 99.7%; *S. cinereorectus*, AB184646, 99.7%; *S. cyaneofuscatus*, AB184860, 99.7%; *S. alboviridis*, AB184256, 99.7%; *S. californicus*, AB184755, 99.7%; *S. griseolus*, AB184768, 99.6%; *S. griseus* subsp. *griseus*, AY207604, 99.6%; *S. floridae*, AB184656, 99.6%; *S. flavovirens*, DQ026635, 99.5%; *S. halstedii*, EF178695, 99.5%; *S. atroolivaceus*, AJ781320, 99.4%; *S. finlayi*, AY999788, 99.4%; *S. flavogriseus*, AJ494864, 99.4%; *S. olivoviridis*, AB184227, 99.4%; *S. pulveraceus*, AB184806, 99.4%; *S. bacillaris*, AB184439, 99.4%; *S. clavifer*, DQ026670, 99.3%; *S. yanii*, AB006159, 99.3%; *S. nitrosporeus*, EF178680, 99.3%; *S. albolongus*,

AB184425, 99.2%; *S. griseobrunneus*, AB249912, 99.2%; *S. celluloflavus*, AB184476, 99.2%; *S. sanglieri*, AB249945, 99.2%; *S. atratus*, DQ026638, 99.1%; *S. cavourensis* subsp. *cavourensis*, DQ445791, 99.1%; *S. luridiscabiei*, AF361784, 99.1%; *S. spiroverticillatus*, AB184814, 99.1%; *S. mutomycini*, AB249951, 99.1%; *S. gelaticus*, DQ026636, 99.1%; *S. candidus*, DQ026663, 99%.

Source: not known.

DNA G+C content (mol%): not known.

Type strain: AS 4.1607, ATCC 23885, CBS 306.55, CBS 665.68, DSM 40232, HAMBI 1044, NBRC 14727, JCM 4349, IMET 43091, NRRL B-1902, NRRL-ISP 5232, RIA 1172, VKM Ac-1774.

Sequence accession no. (16S rRNA gene): EF178688.

64. **Streptomyces bacillaris** (Krasil'nikov 1958) Pridham 1970, 9[AL] ("*Actinomyces bacillaris*" Krasil'nikov 1958, 258)

ba.cil.lar′is.L. n. *bacillus* a rodlet; L. masc. suff. *-aris* suffix used with the sense of pertaining to; N.L. masc. adj. *bacillaris* pertaining to a rodlet.

Spore chains in Section *Rectiflexibiles.* Mature spore chains are generally long with 10 to more than 50 spores per chain. This morphology is seen on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar. Spore surface is smooth.

Color of colony: aerial mass color in the Yellow color series (2ba, 1ba, pale yellow) on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar; some parts of the aerial mycelium on yeast-malt agar may also be in the Gray color series (3ge, light grayish yellow brown) and white aerial mycelium is also observed. Reverse side of colony with no distinctive pigments (orange-yellow to yellowish brown on yeast-malt agar; light grayish yellow on oatmeal agar; yellowish or orange-yellow on salts-starch agar or glycerol-asparagine agar).

Color in medium: melanoid pigments are formed in peptone-yeast-iron agar and tryptone-yeast broth, but not on tyrosine agar. No pigment or only a trace of yellow is found in the medium in yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar.

D-Glucose, D-mannitol, and D-fructose are utilized for growth. Utilization of D-xylose is doubtful. Only traces of growth are seen with L-arabinose, iso-inositol, rhamnose, sucrose, and raffinose.

Type strain shows the highest sequence similarity to: *S. globisporus* subsp. *globisporus*, EF178686, 99.7%; *S. pluricolorescens*, DQ442540, 99.6%; *S. griseinus*, AB184205, 99.6%; *S. rubiginosohelvolus*, AB184240, 99.6%; *S. sindenensis*, AB184759, 99.6%; *S. badius*, AY999783, 99.6%; *S. mediolani*, AB184674, 99.6%; *S. californicus*, AB184755, 99.6%; *S. griseoplanus*, AY999894, 99.5%; *S. fulvorobeus*, AB184711, 99.5%; *S. albolongus*, AB184425, 99.5%; *S. albovinaceus*, AB249958, 99.5%; *S. parvus*, DQ442537, 99.5%; *S. floridae*, AB184656, 99.5%; *S. cavourensis* subsp. *washingtonensis*, DQ026671, 99.5%; *S. anulatus*, DQ026637, 99.5%; *S. flavofuscus*, AB249935, 99.5%; *S. fimicarius*, AY999784, 99.5%; *S. lavendulae* subsp. *lavendulae*, AB184080, 99.5%; *S. praecox*, AB184293, 99.5%; *S. baarnensis*, EF178688, 99.4%; *S. acrimycini*, AY999889, 99.4%; *S. cavourensis* subsp. *cavourensis*, DQ445791, 99.4%; *S. celluloflavus*, AB184476, 99.4%; *S. lipmanii*, AB184148, 99.4%; *S. griseobrunneus*, AB249912, 99.4%; *S. nitrosporeus*, EF178680, 99.4%; *S. cinereorectus*, AB184646, 99.4%; *S. cyaneofuscatus*, AB184860, 99.4%; *S. flavovirens*, DQ026635, 99.4%; *S. microflavus*, DQ445795, 99.4%; *S. alboviridis*, AB184256, 99.4%; *S. argenteolus*, AB045872, 99.3%; *S. flavogriseus*, AJ494864, 99.3%; *S. halstedii*, EF178695, 99.2%; *S. griseus* subsp. *griseus*, AY207604, 99.2%; *S. finlayi*, AY999788, 99.2%; *S. griseolus*, AB184768, 99.2%; *S. pulveraceus*, AB184806, 99.2%; *S. luridiscabiei*, AF361784, 99.2%; *S. clavifer*, DQ026670, 99.1%; *S. sanglieri*, AB249945, 99%; *S. olivoviridis*, AB184227, 99%; *S. atroolivaceus*, AJ781320, 99%; *S. mutomycini*, AB249951, 99%; *S. yanii*, AB006159, 99%.

Source: not known.

DNA G+C content (mol%): not known.

Type strain: AS 4.1548, ATCC 15855, CBS 788.72, DSM 40598, NBRC 13487, INMI 445, JCM 4727, KCTC 9018, NRRL B-3038, NRRL-ISP 5598, RIA 1448, RIA 336, VKM Ac-58.

Sequence accession no. (16S rRNA gene): AB184439.

65. **Streptomyces badius** (Kudrina *in* Gauze, Preobrazhenskaya, Kudrina, Blinov, Ryabova and Sveshnikova 1957) Pridham, Hesseltine and Benedict 1958, 58[AL] ("*Actinomyces badius*" Kudrina *in* Gauze, Preobrazhenskaya, Kudrina, Blinov, Ryabova and Sveshnikova 1957, 87)

ba′di.us. L. masc. adj. *badius* brown.

Spore chains in Section *Rectiflexibiles.* Mature spore chains generally have 10–50 spores per chain. This morphology is found on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar. Spore surface is smooth.

Color of colony: aerial mass color in the Yellow color series on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar (or intermediate between Gray series and Yellow series – one observer). Reverse side of colony with no distinctive pigments on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar; substrate pigment is not a pH indicator.

Color in medium: melanoid pigments not formed in peptone-yeast-iron agar, but some variable darkening is observed in tyrosine agar and tryptone-yeast broth; other pigments are not formed.

D-Glucose, L-arabinose, D-xylose, D-mannitol, and D-fructose are utilized for growth. No growth or only trace of growth on sucrose, iso-inositol, rhamnose, and raffinose.

Type strain shows the highest sequence similarity to: *S. sindenensis*, AB184759, 100%; *S. albovinaceus*, AB249958, 100%; *S. griseoplanus*, AY999894, 100%; *S. anulatus*, DQ026637, 100%; *S. globisporus* subsp. *globisporus*, EF178686, 100%; *S. pluricolorescens*, DQ442540, 100%; *S. praecox*, AB184293, 100%; *S. flavofuscus*, AB249935, 100%; *S. fimicarius*, AY999784, 100%; *S. mediolani*, AB184674, 100%; *S. griseinus*, AB184205, 100%; *S. rubiginosohelvolus*, AB184240, 100%; *S. californicus*, AB184755, 99.9%; *S. lavendulae* subsp. *lavendulae*, AB184080, 99.9%; *S. cavourensis* subsp. *washingtonensis*, DQ026671, 99.9%; *S. acrimycini*, AY999889, 99.9%; *S. parvus*, DQ442537, 99.9%; *S. fulvorobeus*, AB184711, 99.8%; *S. lipmanii*, AB184148, 99.8%; *S. microflavus*, DQ445795, 99.8%; *S. cyaneofuscatus*, AB184860, 99.8%; *S. floridae*, AB184656, 99.8%; *S. alboviridis*, AB184256, 99.8%;

S. baarnensis, EF178688, 99.8%; *S. cinereorectus*, AB184646, 99.8%; *S. griseus* subsp. *griseus*, AY207604, 99.7%; *S. griseolus*, AB184768, 99.7%; *S. luridiscabiei*, AF361784, 99.7%; *S. flavovirens*, DQ026635, 99.7%; *S. argenteolus*, AB045872, 99.7%; *S. bacillaris*, AB184439, 99.6%; *S. pulveraceus*, AB184806, 99.6%; *S. halstedii*, EF178695, 99.6%; *S. flavogriseus*, AJ494864, 99.6%; *S. atroolivaceus*, AJ781320, 99.5%; *S. olivoviridis*, AB184227, 99.5%; *S. finlayi*, AY999788, 99.5%; *S. nitrosporeus*, EF178680, 99.5%; *S. albolongus*, AB184425, 99.4%; *S. clavifer*, DQ026670, 99.4%; *S. griseobrunneus*, AB249912, 99.4%; *S. sanglieri*, AB249945, 99.4%; *S. celluloflavus*, AB184476, 99.4%; *S. yanii*, AB006159, 99.4%; *S. gelaticus*, DQ026636, 99.3%; *S. atratus*, DQ026638, 99.3%; *S. cavourensis* subsp. *cavourensis*, DQ445791, 99.3%; *S. mutomycini*, AB249951, 99.2%; *S. candidus*, DQ026663, 99.2%; *S. spiroverticillatus*, AB184814, 99.1%; *S. cremeus*, AB184124, 99.1%.

Source: not known.

DNA G+C content (mol%): not known.

Type strain: AS 4.1406, ATCC 19729, ATCC 19888, CBS 468.68, BCRC 13759, DSM 40139, HAMBI 1008, NBRC 12745, IMET 43089, INA 1203/53, JCM 4350, LMG 19353, NCIMB 13011, NRRL B-2567, NRRL-ISP 5139, RIA 1010, VKM Ac-735.

Sequence accession no. (16S rRNA gene): AY999783.

66. **Streptomyces baldaccii** corrig. (Farina and Locci 1966) Witt and Stackebrandt 1991, 456[VP] (Effective publication: Witt and Stackebrandt 1990, 370.) (*Streptoverticillium baldaccii* Farina and Locci 1966, 48)

bal.dac′ci.i. N.L. gen. masc. n. *baldacii* of Baldacci (named for Elio Baldacci (1909–1987), who introduced the genus *Streptoverticillium*).

On potato-glucose agar (Baldacci et al., 1954): abundant growth; color, reverse is pink to salmon pink and aerial mycelium is pale pink to salmon pink with whitish tufts. On Bacto Czapek agar: limited growth; color, reverse is colorless to pinkish and aerial mycelium is traces of pink. On Casamino acids Czapek agar (1 g/l Difco vitamin-free Casamino acids replacing sodium nitrate): growth slightly better than on Bacto Czapek agar; colors similar. On glucose-asparagine agar (ISP medium 5 with 1% glucose replacing glycerol): good growth; color, reverse is red and aerial mycelium is pale pink with whitish overgrowth. On glycerol-asparagine agar (ISP medium 5): good growth; color, reverse is red and aerial mycelium is pale pink. On inorganic salts-starch agar (ISP medium 4): good growth; color, reverse is red to cherry red and aerial mycelium is pale pink. On yeast extract-malt extract agar (ISP medium 2): good growth; color, reverse is orange-red and aerial mycelium is pink with whitish patches. On Bacto Emerson agar and Bennett agar (1% glucose, 0.1% Bacto beef agar, 0.1% yeast extract, 0.2% peptone, 1.5% agar): good growth; color, reverse is orange-red and aerial mycelium is pink. On Oxoid nutrient agar: good growth; color, reverse is initially orange-red turning to brown-red and aerial mycelium (poor) is pink with violet shades; brown soluble pigment. Optimal growth at 27°C; only substrate mycelium is formed at 37°C; no growth at 45°C. Type strain exhibits anti-bacterial and anti-fungal activity.

No detailed sequence information is available (only partial sequence).

Source: not known.

DNA G+C content (mol%): not known.

Type strain: ATCC 23654, DSM 40845, HUT 6222, IPV 1339, IPV 174, NBRC 14693, JCM 4272, NRRL B-3500.

Sequence accession no. (16S rRNA gene): X53164.

Further comments: D.P. Labeda proposes *Streptomyces biverticillatus* (Preobrazhenskaya and Ryabova 1957) Witt and Stackebrandt 1991, *Streptomyces fervens* (DeBoer et al. 1959–1960) Witt and Stackebrandt 1991, and *Streptomyces roseoverticillatus* (Shinobu 1956) Witt and Stackebrandt 1991 as later synonyms of *Streptomyces baldaccii* corrig. (Farina and Locci 1966) Witt and Stackebrandt 1991. This proposal is in violation of Rule 24b(1) because the senior epithet is *roseoverticillatus*. *Streptomyces baldaccii*, *Streptomyces biverticillatus*, and *Streptomyces fervens* are therefore to be regarded as later synonyms of *Streptomyces roseoverticillatus*.

Hatano et al. (1997) propose *Streptomyces baldaccii* corrig. (Farina and Locci 1966) Witt and Stackebrandt 1991 as an earlier heterotypic synonym of *Streptomyces spitsbergensis* Wieczorek et al. (1993).

According to Hatano et al. (2003), *Streptomyces baldaccii* corrig. (Farina and Locci 1966) Witt and Stackebrandt 1991 is a later heterotypic synonym of *Streptomyces hiroshimensis* (Shinobu 1955) Witt and Stackebrandt 1991.

67. **Streptomyces bambergiensis** Wallhäusser, Nesemann, Präve and Steigler 1966, 734[AL]

bam.ber.gi.en′sis. N.L. masc. adj. *bambergiensis* of or belonging to Bamberg, Germany, the source of the soil from which the organism was isolated.

Spore chains in Section *Retinaculiaperti* to *Rectiflexibiles*. Strongly flexuous chains with open or primitive spirals and terminal loops or turns. Mature spore chains are moderately long, usually with more than 10 spores per chain. This morphology is seen on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar. Spore surface is hairy.

Color of colony: aerial mass color in the Green color series (1½li-ig, light grayish olive or olive gray to 24½ih-li, dark greenish gray or grayish olive green) on yeast-malt agar, oatmeal agar, and salts-starch agar. White, Yellow or Green color series on glycerol-asparagine agar. Reverse side of colony is strong brown on yeast-malt agar; light yellow on oatmeal agar; light grayish olive to olive brown on salts-starch agar; and yellowish pink, orange-yellow, or reddish brown on glycerol-asparagine agar. Reverse mycelium pigment is not a pH indicator.

Color in medium: melanoid pigments are not formed in peptone-yeast-iron agar, tyrosine agar, or tryptone-yeast broth. Greenish to reddish pigment is reported in the medium in yeast-malt agar, salts-starch agar, and glycerol-asparagine agar. One observer reports no pigments in these media. The pigment, when present, is not pH-sensitive.

D-Glucose, D-xylose, iso-inositol, D-mannitol, D-fructose, and rhamnose are utilized for growth. Reports vary on the utilization of L-arabinose. No growth or only traces of growth with sucrose and raffinose.

Type strain shows the highest sequence similarity to: *S. prasinus*, DQ026658, 99.9%; *S. hirsutus*, AB184844, 99.1%; *S. cyanoalbus*, AB184882, 99%.

Source: soil, Bamberg, Germany.

DNA G+C content (mol%): not known.

Type strain: AS 4.1439, ATCC 13879, CBS 780.72, CECT 3211, DSM 40590, NBRC 13479, JCM 4728, KCTC 9019, LMG 19299, NRRL B-12101, NRRL B-12521, NRRL-ISP 5590, RIA 1440, VKM Ac-975.

Sequence accession no. (16S rRNA gene): AB184869.

68. **Streptomyces bangladeshensis** Al-Bari, Bhuiyan, Flores, Petrosyan, García-Varela and Ul Islam 2005, 1976[VP]

ban.gla.desh.en′sis. N.L. masc. adj. *bangladeshensis* of or belonging to Bangladesh, the source of the soil from which the organism was isolated.

Forms highly branched substrate mycelium and aerial hyphae that differentiate into long *Rectiflexibiles* chains of 8–10 spores. Aerial spore mass color is yellow-green. Substrate mycelium is beige on standard media. Yellowish diffusible pigments are formed on Czapek–Dox agar. Melanin pigments are not produced on peptone-iron or tyrosine agars. Positive for H_2S production. Utilizes glucose, sucrose, *myo*-inositol, mannitol, mannose, maltose, fructose, L-arabinose, rhamnose, glycerol, raffinose, and trehalose as sole carbon sources. Growth occurs at 20–50°C, at pH 6.0–11.0, and in the presence of 2% (w/v) NaCl, neomycin sulfate (50 µg/ml), and penicillin (10 IU/ml). Produces bis-(2-ethylhexyl)-phthalate, an antimicrobial agent.

Type strain shows the highest sequence similarity to: *S. rameus*, AY999821, 99.8%; *S. glomeratus*, AB249917, 99.2%; *S. achromogenes* subsp. *rubradiris*, AY999846, 99%.

Source: isolated from soil from Bangladesh.

DNA G+C content (mol%): not known.

Type strain: AAB-4, LMG 22738, NRRL B-24326.

Sequence accession no. (16S rRNA gene): AY750056.

69. **Streptomyces beijiangensis** Li, Zhang, Xu, Cui, Lu, Xu and Jiang 2002b, 1698[VP]

bei.ji.ang.en′sis. N.L. masc. adj. *beijiangensis* of or pertaining to Beijiang, a place in Yinjiang province in western China.

Aerial mycelium at maturity forms long chains of spores that are straight to flexuous or occasionally *Retinaculiaperti* and are nonmotile. Aerial mycelium and substrate mycelium are well developed. Good growth on most media. Optimum growth temperature is between 8 and 20°C. Diffusible pigment is not produced. The color of the colonies is medium-dependent. Glucose, galactose, and glycerol are utilized; lactose, mannose, inulin, acetate, and oxalate are not utilized. Positive for nitrate reduction and urease. Diagnostic amino acid of peptidoglycan is *meso*-A_2pm. Whole-cell hydrolysates contain glucose and small quantities of xylose, galactose, and arabinose. The predominant menaquinones are MK-9(H_6) and MK-9(H_8) and phosphatidylethanolamine is the diagnostic phospholipid. Predominant cellular fatty acids are $C_{15:0}$ anteiso, $C_{16:0}$ iso, and $C_{17:0}$ cyclo.

Type strain shows no sequence similarity over 99%.

Source: isolated from soil in low-temperature habitats collected from Beijiang, western China.

DNA G+C content (mol%): not known.

Type strain: YIM6, AS 4.1718, CCTCC 99005, DSM 41794, JCM 11882, NBRC 100044.

Sequence accession no. (16S rRNA gene): AF385681.

70. **Streptomyces bellus** Margalith and Beretta 1960, 193[AL]

bel′lus. L. masc. adj. *bellus* pretty, handsome.

Spore chains in Section *Spirales*. Short spore chains may form incomplete spirals, hooks, and loops of small diameter or flexuous chains. Spirals are best developed on salts-starch agar. Hooks and loops are of small diameter and therefore are not typical of *Retinaculum-Apertum* cultures. Mature spore chains with 3–10, or sometimes more than 10, spores per chain are produced on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar. Spore surface is spiny.

Color of colony: aerial mass color in the Blue color series on oatmeal agar and salts-starch agar; White or Blue color series on yeast-malt agar and glycerol-asparagine agar. Reverse side of colony: substrate color is modified by red (orange) pigment on yeast-malt agar and glycerol-asparagine agar and by red or blue pigments on oatmeal agar and salts-starch agar. These pigments are not pH indicators.

Color in medium: melanoid pigments are formed in peptone-yeast-iron agar, tyrosine agar, and tryptone-yeast broth. Trace of red pigment may be found in the medium in glycerol-asparagine agar; it is not a pH indicator. Pigments are not found in the medium in yeast-malt agar, oatmeal agar, or salts-starch agar.

D-Glucose, L-arabinose, sucrose, D-xylose, iso-inositol, D-mannitol, D-fructose, rhamnose, and raffinose are utilized for growth.

Type strain shows the highest sequence similarity to: *S. coerulescens*, AY999720, 100%; *S. coeruleorubidus*, AY999719, 99.6%; *S. lomondensis*, AB184673, 99.4%; *S. purpurascens*, AJ399486, 99.4%; *S. lusitanus*, AB184424, 99.4%; *S. iakyrus*, AB184877, 99.3%; *S. paradoxus*, AB184628, 99.2%; *S. viridodiastaticus*, AY999852, 99.2%; *S. parvulus*, AB184326, 99.2%; *S. matensis*, AB184221, 99.2%; *S. azureus*, EF178674, 99.2%; *S. indiaensis*, AB184553, 99.2%; *S. albogriseolus*, AJ494865, 99.2%; *S. longispororuber*, AB184440, 99.2%; *S. hawaiiensis*, AB184143, 99.2%; *S. violaceus*, AB184315, 99.1%; *S. albosporeus* subsp. *albosporeus*, AJ781327, 99.1%; *S. janthinus*, AB184851, 99.1%; *S. thermocarboxydus*, U94490, 99.1%; *S. spinoverrucosus*, AB184578, 99.1%; *S. griseorubens*, AB184139, 99.1%; *S. erythrogriseus*, AJ781328, 99%; *S. griseoincarnatus*, AJ781328, 99%; *S. labedae*, AB184704, 99%; *S. variabilis*, DQ442551, 99%; *S. massasporeus*, AB184152, 99%; *S. luteogriseus*, AB184379, 99%; *S. roseoviolaceus*, AJ399484, 99%; *S. arenae*, AB249977, 99%.

Source: not known.

DNA G+C content (mol%): not known.

Type strain: A/870, ATCC 14925, ATCC 23886, CBS 666.68, DSM 40185, NBRC 12844, JCM 4292, JCM 4625, NRRL B-2575, NRRL-ISP 5185, RIA 1139.

Sequence accession no. (16S rRNA gene): AB184849.

71. **Streptomyces bikiniensis** Johnstone and Waksman 1947, 294[AL] ("*Actinomyces bikiniensis*" Krasil'nikov 1949, 100)

bi.ki.ni.en′sis. N.L. masc. adj. *bikiniensis* of or pertaining to Bikini Atoll.

Spore chains in Section *Rectiflexibiles* with long, straight to slightly flexuous spore chains of more than 50 spores per chain. This morphology is seen on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar. Spore surface is smooth.

Color of colony: aerial mass color in the Gray color series on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar. Nearest matching color tabs are 3ih, dark gray; 2fe, medium gray; 3fe, light brownish gray; 5fe, light grayish reddish brown; and 2dc, yellowish gray. Reverse side of colony with no distinctive pigments (grayish yellow to yellowish brown or olive brown on yeast-malt agar; pale to grayish yellow or light grayish yellowish brown on oatmeal agar, salts-starch agar, and glycerol-asparagine agar).

Color in medium: melanoid pigments are formed in peptone-yeast-iron agar, tyrosine agar, and tryptone-yeast broth, but the reaction is weak in tyrosine agar. No pigment or only a trace of yellow is found in the medium in yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar.

D-Glucose, D-xylose, and D-fructose are utilized for growth. Reports vary on utilization of L-arabinose; utilization of D-mannitol, rhamnose, and raffinose is doubtful and no significant growth is seen with iso-inositol or sucrose.

Type strain shows the highest sequence similarity to: *S. violaceorectus*, AB184314, 99.7%; *S. vietnamensis*, DQ311081, 99.5%; *S. cinereoruber* subsp. *cinereoruber*, AB184121, 99.3%; *S. tanashiensis*, AJ781362, 99.1%; *S. nashvillensis*, AB184286, 99%; *S. viridobrunneus*, AJ781372, 99%; *S. laurentii*, AJ781342, 99%; *S. showdoensis*, AB184389, 99%.

Source: not known.

DNA G+C content (mol%): not known.

Type strain: AS 4.569, ATCC 11062, CBS 412.54, DSM 40581, HUT 6084, IFM 1057, NBRC 14598, IMET 41362, JCM 4011, KCTC 9172, NRRL B-1049, NRRL B-2690, NRRL-ISP 5581, RIA 471, RIA 74, VKM Ac-999.

Sequence accession no. (16S rRNA gene): X79851.

72. **Streptomyces biverticillatus** (Preobrazhenskaya *in* Gauze, Preobrazhenskaya, Kudrina, Blinov, Ryabova and Sveshnikova 1957) Witt and Stackebrandt 1991, 456[VP] (Effective publication: Witt and Stackebrandt 1990, 370.) ["*Actinomyces biverticillatus*" Preobrazhenskaya *in* Gauze, Preobrazhenskaya, Kudrina, Blinov, Ryabova and Sveshnikova 1957, 75; "*Streptomyces biverticillatus*" Pridham, Hesseltine and Benedict 1958, 72; "*Streptoverticillium biverticillatus*" (*sic*) Baldacci 1958, 25; *Streptoverticillium biverticillatum* Farina and Locci 1966, 49]

bi.ver.ti.cil′la.tus. L. adv. num. *bi*s twice; L. masc. n. *verticillus* whorl; N.L. adj. *verticillatus* whorled; N.L. masc. adj. *biverticillatus* whorled twice.

Spore chains in Section *Verticillati* with umbellate-monoverticillate (biverticillate) spore chains. Mature spore chains generally contain 10 or more spores per chain. This morphology is seen on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar. Spore surface is smooth.

Color of colony: aerial mass color in the Red color series on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar (one observer, only, placed this culture in the Violet color series on yeast-malt agar and salts-starch agar). Reverse side of colony is red on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar; this pigment is pH-sensitive, changing from red to orange-red with addition of 0.05 M NaOH and from red to violet-red or blue with addition of 0.05 M HCl.

Color in medium: melanoid pigments are formed in less than 2 d in peptone-yeast-iron agar and tryptone-yeast broth, but more slowly in tyrosine agar. Pigments other than melanoids are not formed in yeast-malt agar, oatmeal agar, salts-starch agar, or glycerol-asparagine agar.

D-Glucose and possibly iso-inositol and D-fructose are utilized for growth; growth on iso-inositol and D-fructose is much less abundant than on D-glucose. No growth or only trace of growth on L-arabinose, sucrose, D-xylose, D-mannitol, rhamnose, and raffinose. Type strain shows the highest sequence similarity to: *S. werraensis*, DQ442558, 100%; *S. albireticuli*, AB184881, 99.5%; *S. netropsis*, AB184848, 99.3%; *S. eurocidicus*, AY999790, 99.3%; *S. cinnamoneus*, AB184850, 99%; *S. hiroshimensis*, AB184789, 99%.

Source: not known.

DNA G+C content (mol%): not known.

Type strain: ATCC 23615, CBS 668.68, NBRC 12845, JCM 4431, NRRL-ISP 5272, RIA 1190.

Sequence accession no. (16S rRNA gene): AJ781381.

Further comments: in violation of Rule 33c of the *Bacteriological Code* (1990 Revision), in Validation List no. 38, *Streptomyces biverticillatus* is proposed as a *nomen revictum* [basonym: "*Streptomyces biverticillatus*" (Preobrazhenskaya and Ryabova 1957) Pridham et al. 1958].

Labeda (1996) proposes *Streptomyces biverticillatus* (Preobrazhenskaya and Ryabova 1957) Witt and Stackebrandt 1991, *Streptomyces fervens* (DeBoer et al. 1959–1960) Witt and Stackebrandt 1991, and *Streptomyces roseoverticillatus* (Shinobu 1956) Witt and Stackebrandt 1991 as later synonyms of *Streptomyces baldaccii* corrig. (Farina and Locci 1966) Witt and Stackebrandt 1991. This proposal is in violation of Rule 24b of the *Bacteriological Code* (1990 Revision) because the senior epithet is *roseoverticillatus. Streptomyces baldaccii*, *Streptomyces biverticillatus*, and *Streptomyces fervens* are therefore to be regarded as later synonyms of *Streptomyces roseoverticillatus*.

According to Hatano et al. (2003), *Streptomyces biverticillatus* (Preobrazhenskaya and Ryabova 1957) Witt and Stackebrandt 1991 is a later heterotypic synonym of *Streptomyces hiroshimensis* (Shinobu 1955) Witt and Stackebrandt 1991.

73. **Streptomyces blastmyceticus** (Watanabe et al. 1957) Witt and Stackebrandt 1991, 456[VP] (Effective publication: Witt and Stackebrandt 1990, 370.) ("*Streptomyces blastmyceticus*" Watanabe, Tanaka, Fukuhara, Miyairi, Yonehara and Umezawa 1957, 39; *Streptoverticillium blastmyceticum* Locci, Baldacci and Petrolini Baldan 1969, 43)

blast.my.ce′ti.cus. N.L. n. *blastomycinum* blastomycin; L. masc. suff. *-icus* suffix used with the sense of belonging to; N.L. masc. adj. *blastmyceticus* belonging to blastomycin.

Spore chains in Section *Verticillati*. Both monoverticillate and umbellate monoverticillate (biverticillate) spore chains are found. Spore chains are short; usually only 3–10

spores per chain. This morphology is seen on oatmeal agar; mature spore chains may not develop on other ISP media. Spore surface is smooth.

Color of colony: aerial mass color in the Gray color series on oatmeal agar. Better sporulation and color is produced on tomato paste-oatmeal agar or on Hickey and Tresner's agar. On these media, an olive-gray color (Gray color series) is produced. Immature and poorly sporulated aerial growth on other media may appear to place this species in the Yellow or Red color series. Reverse side of colony is characteristic grayed yellow on yeast-malt agar and grayed greenish yellow to olive-brown on oatmeal agar, salts-starch agar, and glycerol-asparagine agar; substrate pigment is not a pH indicator.

Color in medium: melanoid pigments are formed in peptone-yeast-iron agar and tryptone-yeast-broth. Pigments other than melanoids are not formed in yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar.

D-Glucose, L-arabinose, iso-inositol, and D-fructose are utilized for growth. No growth or only trace of growth on D-xylose, rhamnose, and raffinose. Utilization of sucrose and D-mannitol is doubtful.

Type strain shows the highest sequence similarity to: *S. hiroshimensis*, AB184789, 99.5%; *S. ardus*, AB184864, 99.4%; *S. cinnamoneus*, AB184850, 99.3%; *S. albireticuli*, AB184881, 99.3%; *S. eurocidicus*, AY999790, 99.2%; *S. caeruleus*, EF178675, 99.1%; *S. pseudoechinosporeus*, AB184100, 99.1%; *S. abikoensis*, AB184537, 99%.

Source: not known.

DNA G+C content (mol%): not known.

Type strain: AS 4.1647, ATCC 19731, CBS 470.68, BCRC 13387, CECT 3257, DSM 40029, NBRC 12747, JCM 4184, JCM 4565, NCIMB 9800, NRRL B-5480, NRRL-ISP 5029, RIA 1012.

Sequence accession no. (16S rRNA gene): AY999802.

Further comments: in violation of Rule 33c of the *Bacteriological Code* (1990 Revision), in Validation List no. 38, *Streptomyces blastmyceticus* is proposed as a *nomen revictum* (basonym: "*Streptomyces blastmyceticus*" Watanabe et al. 1957).

74. **Streptomyces bluensis** Mason, Dietz and Hanka 1963b, 608[AL]

blu.en'sis. N.L. masc. adj. *bluensis* (from French adj. *bleu*) belonging to blue, referring to the blue color of the aerial mycelium.

Spore chains in Section *Spirales*. Spirals may be poorly developed on some media and open loops, imperfect spirals, or flexuous spore chains may be common. Observers do not agree on the best medium for spiral formation. Some spirals are reported by different observers on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar. Spore chains are short to long, usually with more than 10 spores per chain. Spore surface is spiny.

Color of colony: aerial mass color in the Blue or White color series on yeast-malt agar; White color series on oatmeal agar, salts-starch agar, and glycerol-asparagine agar. Nearest matching color tab in the Blue color series is 19dc, pale blue. Reverse side of colony with no distinctive pigments (light grayish yellow to pale yellow) on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar.

Color in medium: melanoid pigments are not formed in peptone-yeast-iron agar, tyrosine agar, or tryptone-yeast broth. No pigment is found in the medium in yeast-malt agar, oatmeal agar, salts-starch agar, or glycerol-asparagine agar.

D-Glucose, L-arabinose, D-xylose, iso-inositol, D-mannitol, D-fructose, rhamnose, sucrose, and raffinose are all utilized for growth.

Type strain shows no sequence similarity over 99%.

Source: not known.

DNA G+C content (mol%): not known.

Type strain: AS 4.1463, ATCC 27420, CBS 239.69, CBS 761.72, DSM 40564, NBRC 13460, JCM 4729, LMG 5969, NCIMB 9754, NRRL 2876, NRRL-ISP 5564, RIA 1421.

Sequence accession no. (16S rRNA gene): X79324.

75. **Streptomyces bobili** (Waksman and Curtis 1916) Waksman and Henrici *in* Breed, Murray and Hitchens 1948, 937[AL] ["*Actinomyces bobili*" Waksman and Curtis 1916, 121; "*Streptomyces bobiliae*" (*sic*) Waksman and Henrici *in* Breed, Murray and Hitchens 1948, 937]

bo.bi'li. N.L. gen. n. *bobili* named for Bobili, the nickname of an individual.

Spore chains in Section *Spirales*. Sporulating aerial mycelium is not produced at 27 or 37°C on yeast-malt agar, oatmeal agar, salts-starch agar, or glycerol-asparagine agar, or on supplementary media (Czapek's agar and potato-glucose agar) used by one observer. Two observers found some spots of sporulating aerial mycelium on salts-starch agar. One collaborator (J.B. Routien) found spiral spore chains with 10 to 30 or more spores per chain on Pridham and Gottlieb's basal salts medium for carbon utilization enriched with xylose, raffinose, or glucose. Incubation was at 37°C. Spirals were most abundant when xylose was carbon source. The first description by Waksman (1916, op. cit) notes the absence of true aerial mycelium or spores on Czapek's medium. The description published in 1919 (Waksman, (1919), op. cit) notes spiral formation on scant white aerial mycelium on glycerin-synthetic agar and traces of aerial mycelium in spots only on starch-agar plates and potato plugs. Aerial mycelium was not produced on the seven other solid media used by Waksman. The present culture seems to conform well to the early descriptions. Spore surface is smooth.

Color of colony: aerial mass color cannot be observed on yeast-malt agar, oatmeal agar, salts-starch agar, or glycerol-asparagine agar. Sparse aerial mycelium, when produced, is white. On the reverse side of the colony, substrate mycelium color may be grayish yellow, or may be modified with red to reddish gray or pink, reddish brown, or reddish orange. The substrate mycelium color is dependent on pH, changing from yellowish pink to violet pink with addition of 0.05 M NaOH and from yellowish pink to yellow orange with 0.05 M HCl.

Color in medium: melanoid pigments are not produced in peptone-yeast-iron agar, tyrosine agar, or tryptone-yeast broth. No pigments are found in medium in yeast-malt agar, oatmeal agar, salts-starch agar, or glycerol-asparagine agar.

D-Glucose, L-arabinose, sucrose, D-xylose, iso-inositol, D-fructose, rhamnose, and raffinose are utilized for growth. No growth or only trace of growth on D-mannitol.

Type strain shows the highest sequence similarity to: *S. galilaeus*, AB045878, 100%; *S. phaeoluteigriseus*, AJ391815, 99.5%; *S. resistomycificus*, AB184166, 99.5%; *S. aureocirculatus*, AB184260, 99.4%; *S. novaecaesareae*, AB184357, 99.3%; *S. chartreusis*, AB184839, 99.3%; *S. pseudovenezuelae*, AB184233, 99.2%; *S. prunicolor*, DQ026659, 99%.

Source: not known.

DNA G+C content (mol%): not known.

Type strain: AS 4.1624, ATCC 23889, ATCC 3310, CBS 419.34, CBS 675.68, BCRC 13671, DSM 40056, HAMBI 1059, NBRC 13199, NBRC 16166, IMET 41372, JCM 4012, JCM 4627, NRRL B-1338, NRRL B-2097, NRRL-ISP 5056, RIA 1116, VKM Ac-1756.

Sequence accession no. (16S rRNA gene): AB249925.

76. **Streptomyces bottropensis** Waksman 1961 182[AL]

bot.trop.en′sis. N.L. masc. adj. *bottropensis* of or pertaining to the German town Bottrop.

Spore chains in Section *Spirales*. Hooks, primitive spirals, and terminal spirals of only one or two turns are common together with open spirals of several turns. Mature spore chains generally have 10–50 spores per chain. This morphology is seen on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar, but spirals are best developed on salts-starch agar. Spore surface is smooth.

Color of colony: aerial mass color in the Gray color series on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar. Reverse side of colony is yellow to strong brown, reddish brown, or sometimes very dark brown.

Color in medium: melanoid pigments are formed in peptone-yeast-iron agar, tyrosine agar, and tryptone-yeast broth. Brown pigment is found in the medium in yeast-malt agar and glycerol-asparagine agar; this pigment is not pH-sensitive.

D-Glucose, L-arabinose, D-xylose, iso-inositol, D-mannitol, D-fructose, rhamnose, sucrose, and raffinose are utilized for growth, but growth on raffinose is less abundant than on other carbon sources.

Type strain shows no sequence similarity over 99%.

Source: not known.

DNA G+C content (mol%): not known.

Type strain: AS 4.1669, ATCC 25435, CBS 163.64, CBS 667.69, BCRC 12063, CIP 105278, DSM 40262, NBRC 13023, JCM 4459, NRRL-ISP 5262, RIA 1215, VKM Ac-1755.

Sequence accession no. (16S rRNA gene): AB026217.

77. **Streptomyces brasiliensis** (Falcão de Morais, Chaves Batista and Massa et al. 1966) Goodfellow, Williams and Alderson 1986a, 573[VP] (Effective publication: Goodfellow, Williams and Alderson 1986c, 53.) (*Elytrosporangium brasiliense* Falcão de Morais, Chaves Batista and Massa 1966, 170)

bra.si.li.en′sis. N.L. masc. adj. *brasiliensis* of or pertaining to Brazil.

Forms extensively branched substrate and aerial mycelium. The latter bears long spiral spore chains, the former bears occasional short chains of spores (0.8–1.0 µm diameter). The spore surface is smooth. The aerial spore mass is gray to greenish gray; the reverse color is colorless to light cream. Does not form melanin pigments. Esculin, casein, guanine, starch, testosterone, and tyrosine are degraded, but allantoin, arbutin, chitin, elastin, hypoxanthine, lecithin, pectin, urea, and xanthine are not. Nitrate is reduced to nitrite and hydrogen sulfide is produced. Adonitol, L-arabinose, cellobiose, D-fructose, D-galactose, D-glucose, *myo*-inositol, inulin, D-lactose, mannitol, D-mannose, melezitose, melibiose, raffinose, L-rhamnose, salicin, sucrose, trehalose, and D-xylose are used as sole carbon sources, but xylitol is not. Grows on L-arginine, L-cysteine, L-histidine, L-methionine, L-phenylalanine, L-serine, L-threonine, and L-valine, but not on DL-amino-n-butyric acid, L-hydroxyproline, or potassium nitrate, as sole nitrogen sources. Growth occurs at 25–37°C, but not at 10 or 45°C. Tolerant to phenol (0.1%, w/v), sodium azide (0.02%, w/v), and sodium chloride (4%, w/v). Sensitive to rifampin and 7% (w/v) sodium chloride. Does not show antimicrobial activity against *Aspergillus niger* LIV 131, *Bacillus subtilis* NCIB 3610, *Candida albicans* CBS 562, *Escherichia coli* NCIB 9132, *Micrococcus luteus* NCIB 196, *Pseudomonas fluorescens* NCIB 9046[T], *Saccharomyces cerevisiae* CBS 1171[T], or *Streptomyces murinus* ISP 5091. The peptidoglycan contains LL-A_2pm as major diamino acid and is of the A3γ type (Stackebrandt et al., 1981). Contains octahydrogenated menaquinones with nine isoprene units as the predominant isoprenolog.

Type strain shows the highest sequence similarity to: *S. africanus*, AY208912, 99.2%; *S. afghaniensis*, AJ399483, 99.1%.

Source: isolated from soil at Alianca, north of Pernambuco, Brazil.

DNA G+C content (mol%): not known.

Type strain: ATCC 23727, CBS 520.68, DSM 43159, IFM 1210, NBRC 12596, KCC A-0086, KCTC 9071, JCM 3086, NBRC 101283, RIA 911, VKM Ac-1310, VKM Ac-656.

Sequence accession no. (16S rRNA gene): AB249981.

78. **Streptomyces bungoensis** Eguchi, Takada, Nakamura, Tanaka, Makino and Oshima 1993, 797[VP]

bun.go.en′sis. N.L. masc. adj. *bungoensis* of or belonging to Bungo, a region of Japan.

Mature spore chains are spiral, with 20 or more spores per chain. Spores are ellipsoidal, 0.5–0.7 µm in diameter. Spore surface is spiny. Mycelia do not fragment into coccoid or bacilliary structures. Aerial mass on glucose-asparagine agar, glycerol-asparagine agar, inorganic salts-starch agar, and oatmeal agar is grayish brown or gray. Reverse sides of colonies are brownish yellow on glucose-asparagine agar and glycerol-asparagine agar, gray on inorganic salts-starch agar and oatmeal agar, yellow on tyrosine agar, and reddish brown on yeast extract-malt extract agar. The melanoid pigment produced is pale brown on tyrosine agar and brownish black on peptone-yeast extract-iron agar and in tryptone-yeast extract broth. A reddish brown soluble pigment is produced on nutrient agar and a brownish black pigment is produced on yeast extract-malt extract agar. No soluble pigment is produced on sucrose-nitrate agar, glucose-asparagine agar, glycerol-asparagine agar, organic salts-starch agar, oatmeal agar, or Czapek's solution agar. Starch is not hydrolyzed, gelatin is liquefied, and milk is peptonized, but not coagulated. D-Glucose, D-fructose, D-galactose, D-xylose, L-arabinose, and D-mannitol are utilized for growth, but rhamnose, iso-inositol, and salicin are not. Little, if any, growth is observed with raffinose and scant growth is observed with sucrose. The cell-wall composition is chemotype I containing LL-A_2pm and glycine, but no galactose, arabinose, or *meso*-A_2pm.

Type strain shows the highest sequence similarity to: *S. longwoodensis*, AB184580, 99.7%; *S. galbus*, X79852, 99.7%; *S. capoamus*, AB045877, 99.5%; *S. corchorusii*, AB184267, 99.4%; *S. olivaceoviridis*, AB184288, 99.3%; *S. canarius*, AB184396, 99.2%.

Source: not known.

DNA G+C content (mol%): 70.3.

Type strain: AS 4.1653, DSM 41781, FERM 8432, NBRC 15711, JCM 9925, MS16-10G.

Sequence accession no. (16S rRNA gene): AB184696.

79a. **Streptomyces cacaoi subsp. cacaoi** (Waksman *in* Bunting 1932) Waksman and Henrici *in* Breed, Murray and Hitchens 1948, 951[AL] emend. Lanoot, Vancanneyt, Cleenwerck,Wang, Li, Liu and Swings 2002, 828 ("*Actinomyces cacaoi*" Waksman *in* Bunting 1932, 516)

ca.ca′o.i. Mexican Spanish *cacao* the cacao; N.L. gen. n. *cacaoi* of cacao.

Spore chains in Section *Spirales* and poorly developed on all ISP media. Spirals, when formed, are open. Incomplete spirals, loops, hooks, or even flexuous chains may be common when spore chains are short. These are not typical of the large hooks and loops of *Retinaculum-Apertum* cultures. Mature spore chains may contain 10–50 spores per chain on yeast-malt agar, salts-starch agar, and glycerol-asparagine agar, but long chains are not common. Oatmeal agar is not suitable for observation of morphology or aerial mass color. Spore surface is smooth.

Color of colony: aerial mass color in the White color series on yeast-malt agar, salts-starch agar, and glycerol-asparagine agar. Reverse side of colony with no distinctive pigments on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar; substrate pigment is not a pH indicator.

Color in medium: melanoid pigments are not formed on peptone-yeast-iron agar, tyrosine agar, or tryptone-yeast extract broth. No pigment is found in medium in yeast-malt agar, oatmeal agar, salts-starch agar, or glycerol-asparagine agar. D-Glucose, L-arabinose, D-xylose, D-mannitol, and D-fructose are utilized for growth. No growth or only trace of growth on iso-inositol and rhamnose. Utilization of sucrose and raffinose is doubtful.

Type strain shows no sequence similarity over 99%.

Source: not known.

DNA G+C content (mol%): 73.0.

Type strain: AS 4.1466, ATCC 19732, ATCC 3082, CBS 471.68, BCRC 12103, DSM 40057, NBRC 12748, IMET 40260, IMRU 3082, JCM 4352, KCTC 9758, LMG 19320, NCIMB 9626, NRRL B-1220, NRRL B-2686, NRRL-ISP 5057, RIA 1013, UNIQEM 123, VKM Ac-733.

Sequence accession no. (16S rRNA gene): AB184115.

Further comments: Streptomyces cacaoi subsp. *cacaoi* (Waksman 1932) Waksman and Henrici 1948 emend. Lanoot et al. 2002 is an earlier heterotypic synonym of *Streptomyces aminophilus* Foster 1961.

79b. **Streptomyces cacaoi subsp. asoensis** Isono, Nagatsu, Kawashima and Suzuki 1965, 853[AL]

aso.en′sis. N.L. masc. adj. *asoensis* probably of or pertaining to Aso, a geographical area in Japan.

Produces the polyoxin complex of selectively specific anti-fungal antibiotics comprised of polyoxins A to L. Polyoxin C apparently exhibits no biological activity.

Type strain shows the highest sequence similarity to: *S. humidus*, DQ442508, 99.5%; *S. rishiriensis*, EF178691, 99.1%; *S. flavovariabilis*, EF178691, 99.1%.

Source: not known.

DNA G+C content (mol%): not known.

Type strain: AS 4.1602, ATCC 19093, CBS 378.69, DSM 41440, NBRC 13813, JCM 4185, KCTC 9700, NCIMB 12769, NRRL B-16592.

Sequence accession no. (16S rRNA gene): DQ026644.

80. **Streptomyces caelestis** DeBoer, Dietz, Wilkins, Lewis and Savage 1955a, 831[AL]

ca.e.les′tis. L. masc. adj. *caelestis* of the sky, heavenly (referring to the blue color of the aerial mycelium and spores).

Spore chains in Section *Spirales* or *Retinaculiaperti*. Spirals are usually poorly developed and show only a few turns; loops and hooks are of small diameter and therefore are not representative of typical *Retinaculum-Apertum* cultures. Short spore chains; usually only 3–10 spores per chain on yeast-malt agar, oatmeal agar, and salts-starch agar. Spore surface is smooth.

Color of colony: aerial mass color in the Blue color series on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar. Reverse side of colony with no distinctive pigments on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar; substrate pigment is not a pH indicator.

Color in medium: melanoid pigments are formed in peptone-yeast-iron agar and tyrosine agar. Pigments other than melanoids are not formed in yeast-malt agar, oatmeal agar, salts-starch agar, or glycerol-asparagine agar. D-Glucose, L-arabinose, sucrose, D-xylose, iso-inositol, D-fructose, rhamnose, and raffinose are utilized for growth. No growth or only trace of growth on D-mannitol. Type strain shows the highest sequence similarity to: *S. azureus*, EF178674, 99.5%; *S. gancidicus*, AB184660, 99.2%; *S. levis*, AB184670, 99.1%; *S. cellulosae*, DQ442495, 99.1%; *S. capillispiralis*, AB184577, 99.1%; *S. pseudogriseolus*, DQ442541, 99.1%.

Source: not known.

DNA G+C content (mol%): not known.

Type strain: AS 4.1688, ATCC 14924, ATCC 15084, ATCC 19733, CBS 472.68, CBS 967.70, BCRC 13685, DSM 40084, NBRC 12749, IMET 43502, SP 5084, JCM 4218, JCM 4566, LMG 5970, NCIMB 9751, NRRL 2418, RIA 1014, VKM Ac-1822.

Sequence accession no. (16S rRNA gene): X80824.

81. **Streptomyces caeruleus** (Baldacci 1944) Pridham, Hesseltine and Benedict 1958, 60[AL] emend. Lanoot, Vancanneyt, Cleenwerck,Wang, Li, Liu and Swings 2002, 828 ("*Actinomyces caeruleus*" Baldacci 1944, 180)

ca.e.ru′le.us. L. masc. adj. *caeruleus* dark blue, azure.

Spore chains in Section *Rectiflexibiles*. Two observers report poor growth or no sporulating aerial mycelium on yeast-malt agar and oatmeal agar. Mature spore chains with more than 10 spores per chain are usually produced on salts-starch agar, glycerol-asparagine agar, and Hickey and

Tresner medium. Typical spores are not observed; atypical spores are smooth. One observer reports fragmentation of substrate mycelium on Hickey and Tresner agar in 14 d.

Color of colony: aerial mass color in the Gray color series on salts-starch agar, glycerol-asparagine agar, and Hickey and Tresner agar. One observer also records gray aerial mycelium on yeast-malt agar and oatmeal agar, but sporulating growth on these media may be inadequate for color determination. Reverse side of colony with a distinct blackish blue to very dark grayish purple pigment on salts-starch agar, glycerol-asparagine agar, and Hickey and Tresner agar. A dark blue violet to blackish pigment may also be seen in yeast-malt agar or glycerol-asparagine agar when adequate growth occurs. Reverse mycelium pigment is a pH indicator, changing from dark violet or blue to deep green with addition of 0.05 M NaOH.

Color in medium: melanoid pigments probably are not formed in peptone-yeast-iron agar, tyrosine agar, or tryptone-yeast broth; observation may be obscured by dark violet or blue pigments. Brown violet, blue, or blue black pigment is found in the medium in yeast-malt agar, oatmeal agar, salts-starch agar, glycerol-asparagine agar, and Hickey and Tresner agar. This pigment may not be pH-sensitive when tested with 0.05 M NaOH or HCl, or may be somewhat sensitive to 0.05 M NaOH, changing from violet to very deep green.

Growth is generally very poor or absent on Pridham and Gottlieb carbon-utilization medium containing D-glucose or other carbon sources. Carbon utilization cannot be determined accurately on this medium. One observer notes that traces of growth on sucrose and rhamnose are probably equal to growth on D-glucose, but this observation is of doubtful significance.

Type strain shows the highest sequence similarity to: *S. hiroshimensis*, AB184789, 99.7%; *S. abikoensis*, AB184537, 99.5%; *S. lilacinus*, AB184819, 99.3%; *S. cinnamoneus*, AB184850, 99.3%; *S. pseudoechinosporeus*, AB184100, 99.2%; *S. sapporonensis*, AB184508, 99.1%; *S. aureoversilis*, AB184855, 99.1%; *S. blastmyceticus*, AY999802, 99.1%; *S. ardus*, AB184864, 99%.

Source: not known.

DNA G+C content (mol%): not known.

Type strain: ATCC 27421, CBS 645.72, BCRC 13659, DSM 40103, NBRC 13344, IMET 40622, IPV 930, ISP 5103, JCM 4014, JCM 4730, LMG 19399, NRRL B-1623, NRRL B-2194, NRRL-ISP 5103, RIA 1305, RIA 755, VKM Ac-1918.

Sequence accession no. (16S rRNA gene): EF178675.

Further comments: Streptomyces caeruleus (Baldacci 1944) Pridham et al. 1958 emend. Lanoot et al. 2002 is an earlier heterotypic synonym of *Streptomyces niveus* Smith et al. 1956 and an earlier heterotypic synonym of *Streptomyces spheroides* Wallick et al. 1956.

82. **Streptomyces californicus** (Waksman and Curtis 1916) Waksman and Henrici *in* Breed, Murray and Hitchens 1948, 936[AL] ("*Actinomyces californicus*" Waksman and Curtis 1916, 122)

ca.li.for'ni.cus. N.L. masc. adj. *californicus* of or belonging to California, the source of the soil from which the organism was isolated.

Spore chains in Section *Rectiflexibiles*. Mature spore chains generally have 10 to 50 or more spores per chain. This morphology is seen on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar. Spore surface is smooth.

Color of colony: aerial mass color in the Red color series on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar. One observer recorded both Gray series and Violet series for aerial color on oatmeal agar, salts-starch agar, and glycerol-asparagine agar. This difference may be due to pH. Reverse side of colony is modified by Violet or Red on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar; substrate pigment is a pH indicator and is changed from blue-violet to red by addition of 0.05 M HCl, or from red to blue-violet by addition of NaOH.

Color in medium: melanoid pigments not formed in peptone-yeast-iron agar and tyrosine agar; pigment is found in medium in oatmeal agar and tyrosine-asparagine agar. It is pH-sensitive when tested with 0.05 M NaOH or HCl, showing same change as noted for reverse color.

D-Glucose, D-xylose, D-mannitol, and D-fructose are utilized for growth. No growth or only traces of growth on L-arabinose, sucrose, iso-inositol, rhamnose, and raffinose.

Type strain shows the highest sequence similarity to: *S. floridae*, AB184656, 100%; *S. griseinus*, AB184205, 99.9%; *S. badius*, AY999783, 99.9%; *S. pluricolorescens*, DQ442540, 99.9%; *S. sindenensis*, AB184759, 99.9%; *S. mediolani*, AB184674, 99.9%; *S. rubiginosohelvolus*, AB184240, 99.9%; *S. fulvorobeus*, AB184711, 99.8%; *S. alboviridis*, AB184256, 99.8%; *S. praecox*, AB184293, 99.8%; *S. flavofuscus*, AB249935, 99.8%; *S. griseoplanus*, AY999894, 99.8%; *S. lipmanii*, AB184148, 99.8%; *S. albovinaceus*, AB249958, 99.8%; *S. fimicarius*, AY999784, 99.8%; *S. globisporus* subsp. *globisporus*, EF178686, 99.8%; *S. anulatus*, DQ026637, 99.8%; *S. microflavus*, DQ445795, 99.8%; *S. cinereorectus*, AB184646, 99.7%; *S. baarnensis*, EF178688, 99.7%; *S. parvus*, DQ442537, 99.7%; *S. luridiscabiei*, AF361784, 99.7%; *S. acrimycini*, AY999889, 99.7%; *S. cyaneofuscatus*, AB184860, 99.7%; *S. lavendulae* subsp. *lavendulae*, AB184080, 99.7%; *S. cavourensis* subsp. *washingtonensis*, DQ026671, 99.7%; *S. argenteolus*, AB045872, 99.6%; *S. finlayi*, AY999788, 99.6%; *S. bacillaris*, AB184439, 99.6%; *S. halstedii*, EF178695, 99.5%; *S. albolongus*, AB184425, 99.5%; *S. griseobrunneus*, AB249912, 99.5%; *S. celluloflavus*, AB184476, 99.5%; *S. pulveraceus*, AB184806, 99.5%; *S. flavogriseus*, AJ494864, 99.5%; *S. flavovirens*, DQ026635, 99.5%; *S. griseolus*, AB184768, 99.5%; *S. griseus* subsp. *griseus*, AY207604, 99.5%; *S. cavourensis* subsp. *cavourensis*, DQ445791, 99.4%; *S. nitrosporeus*, EF178680, 99.4%; *S. clavifer*, DQ026670, 99.4%; *S. sanglieri*, AB249945, 99.3%; *S. atroolivaceus*, AJ781320, 99.3%; *S. candidus*, DQ026663, 99.3%; *S. olivoviridis*, AB184227, 99.3%; *S. mutomycini*, AB249951, 99.2%; *S. gelaticus*, DQ026636, 99.2%; *S. yanii*, AB006159, 99.2%; *S. atratus*, DQ026638, 99.2%; *S. spiroverticillatus*, AB184814, 99.1%; *S. cremeus*, AB184124, 99%.

Source: isolated from soil from California.

DNA G+C content (mol%): not known.

Type strain: ATCC 19734, ATCC 3312, CBS 125.20, CBS 354.53, CBS 473.68, BCRC 13688, DSM 40058, HUT 6049, IFM 1070, NBRC 12750, NBRC 3386, IMET 40261, JCM 4015, JCM 4567, LMG 19309, NCAIM B.01475, NRRL B-1221, NRRL B-2098, NRRL-ISP 5058, RIA 1015, VKM Ac-575.

Sequence accession no. (16S rRNA gene): AB184755.

83. **Streptomyces calvus** Backus, Tresner and Campbell 1957, 533[AL]

cal′vus. L. masc. adj. *calvus* bald, referring to the sparse formation of aerial mycelium of the organism.

Spore chains in Section *Spirales* on salts-starch agar. Spore chains are short and poorly developed or absent on yeast-malt agar, oatmeal agar, and glycerol-asparagine agar. The original description by Backus, Tresner and Campbell also indicates that aerial mycelium is poorly developed on most media with best sporulation and spiral development on starch containing inorganic salts media. Spore surface is spiny to hairy.

Color of colony: aerial mycelium is inadequate for color determination on most media. When mature sporulating aerial mycelium is formed on yeast-malt agar or salts-starch agar, it is in the Gray color series. Reverse side of colony with no distinctive pigments; colorless or grayish yellowish brown on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar.

Color in medium: melanoid pigments are not found in peptone-yeast-iron agar, tyrosine agar, or tryptone-yeast broth. No pigment is found in medium in yeast-malt agar, oatmeal agar, salts-starch agar, or glycerol-asparagine agar.

D-Glucose, L-arabinose, sucrose, D-xylose, iso-inositol, D-mannitol, D-fructose, rhamnose, and raffinose are utilized for growth.

Type strain shows the highest sequence similarity to: *S. asterosporus*, AB184706, 100%; *S. virens*, DQ442554, 99.9%; *S. aureorectus*, AB184710, 99.9%; *S. djakartensis*, AB184657, 99.2%; *S. minutiscleroticus*, EF178696, 99.1%; *S. tuirus*, AB184690, 99.1%; *S. ghanaensis*, AY999851, 99.1%; *S. geysiriensis*, DQ442501, 99.1%; *S. anandii*, AB184402, 99.1%; *S. plicatus*, AB184291, 99%; *S. pilosus*, AB184161, 99%; *S. vinaceusdrappus*, AY999929, 99%; *S. mutabilis*, EF178679, 99%; *S. graminearus* AJ781333, 99%; *S. griseomycini*, AB184137, 99%; *S. griseostramineus*, AB184140, 99%; *S. flavoviridis*, AB184842, 99%; *S. rochei*, AB184237, 99%.

Source: not known.

DNA G+C content (mol%): not known.

Type strain: AS 4.1691, ATCC 13382, ATCC 23890, CBS 350.62, CBS 676.68, BCRC 11859, CECT 3271, DSM 40010, IFM 1093, NBRC 13200, JCM 4326, JCM 4628, NCIMB 12240, NRRL B-2399, NRRL-ISP 5010, RIA 1103, VKM Ac-1185.

Sequence accession no. (16S rRNA gene): AB184329.

84. **Streptomyces canarius** Vavra and Dietz 1965, 76[AL]

ca.na′ri.us. L. masc. adj. *canarius* of or belonging to the Canary Islands; intended to refer to production of a bright canary yellow pigment on a variety of media.

Spore chains in Section *Spirales*; straight to flexuous chains are also seen. Mature spore chains are moderately long with more than 10 spores per chain. This morphology is seen on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar. Spore surface is smooth.

Color of colony: aerial mass color in the White to Gray color series (b, white, to 2ge, light olive brown) on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar when adequate sporulating aerial mycelium is produced. Aerial mycelium may be inadequate for color determination on some of these media. Reverse side of colony is distinctive greenish yellow to moderate yellow, orange-yellow or strong yellowish brown on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar. Reverse mycelium pigment is not a pH indicator.

Color in medium: melanoid pigments are not formed in peptone-yeast-iron agar, tyrosine agar, or tryptone-yeast broth. Yellow to yellow-green pigment may be found in the medium in oatmeal agar and glycerol-asparagine agar. This pigment is not pH-sensitive.

D-Glucose, L-arabinose, D-xylose, iso-inositol, D-mannitol, D-fructose, rhamnose, sucrose, and raffinose are utilized for growth.

Type strain shows the highest sequence similarity to: *S. olivaceoviridis*, AB184288, 100%; *S. corchorusii*, AB184267, 99.8%; *S. capoamus*, AB045877, 99.5%; *S. longwoodensis*, AB184580, 99.3%; *S. bungoensis*, AB184696, 99.2%; *S. galbus*, X79852, 99.1%.

Source: not known.

DNA G+C content (mol%): not known.

Type strain: AS 4.1581, ATCC 27423, CBS 732.72, BCRC 11621, DSM 40528, HAMBI 1014, NBRC 13431, IMET 43539, JCM 4549, JCM 4733, NCIMB 9468, NRRL 2976, NRRL-ISP 5528, RIA 1392.

Sequence accession no. (16S rRNA gene): AB184396.

85. **Streptomyces candidus** (*ex* Krasil'nikov 1941) Sveshnikova 1986, 574[VP] (Effective publication: Sveshnikova *in* Gause, Preobrazhenskaya, Sveshnikova, Terekhova and Maximova 1983.) ("*Actinomyces candidus*" Krasil'nikov 1941)

can′di.dus. L. masc. adj. *candidus* shining white, clear, bright.

Spore chains in Section *Rectiflexibiles*. Mature spore chains are generally long and flexuous, often with more than 50 spores per chain. This morphology is seen on salts-starch agar and glycerol-asparagine agar. Sporulation may be poor on yeast-malt agar and oatmeal agar. Spore surface is smooth.

Color of colony: aerial mass color in the White color series on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar, although aerial mycelium may be poorly developed on yeast-malt agar and oatmeal agar. Reverse side of colony with no distinctive pigments (colorless or very pale grayed yellow) on yeast-malt agar, oatmeal agar, salts-starch agar, or glycerol-asparagine agar.

Color in medium: melanoid pigments are not formed in peptone-yeast-iron agar, tyrosine agar, or tryptone-yeast broth. Pigments are not formed in medium in yeast-malt agar, oatmeal agar, salts-starch agar, or glycerol-asparagine agar.

D-Glucose, L-arabinose, D-xylose, D-mannitol, and rhamnose are utilized for growth. No growth or only traces of growth on sucrose, iso-inositol, and raffinose.

Type strain shows the highest sequence similarity to: *S. albolongus*, AB184425, 99.4%; *S. celluloflavus*, AB184476, 99.4%; *S. griseobrunneus*, AB249912, 99.4%; *S. cavourensis* subsp. *cavourensis*, DQ445791, 99.3%; *S. finlayi*, AY999788, 99.3%; *S. spiroverticillatus*, AB184814, 99.3%; *S. floridae*, AB184656, 99.3%; *S. californicus*, AB184755, 99.3%; *S. badius*, AY999783, 99.2%; *S. cremeus*, AB184124, 99.2%;

S. sindenensis, AB184759, 99.2%; *S. pluricolorescens*, DQ442540, 99.2%; *S. mediolani*, AB184674, 99.2%; *S. griseinus*, AB184205, 99.2%; *S. rubiginosohelvolus*, AB184240, 99.2%; *S. clavifer*, DQ026670, 99.2%; *S. anulatus*, DQ026637, 99.1%; *S. parvus*, DQ442537, 99.1%; *S. fimicarius*, AY999784, 99.1%; *S. lipmanii*, AB184148, 99.1%; *S. fulvorobeus*, AB184711, 99.1%; *S. atroolivaceus*, AJ781320, 99.1%; *S. olivoviridis*, AB184227, 99.1%; *S. globisporus* subsp. *globisporus*, EF178686, 99.1%; *S. albovinaceus*, AB249958, 99.1%; *S. alboviridis*, AB184256, 99.1%; *S. praecox*, AB184293, 99.1%; *S. griseoplanus*, AY999894, 99.1%; *S. microflavus*, DQ445795, 99.1%; *S. flavofuscus*, AB249935, 99.1%; *S. cavourensis* subsp. *washingtonensis*, DQ026671, 99.1%; *S. pulveraceus*, AB184806, 99%; *S. cyaneofuscatus*, AB184860, 99%; *S. luridiscabiei*, AF361784, 99%; *S. acrimycini*, AY999889, 99%; *S. lavendulae* subsp. *lavendulae*, AB184080, 99%; *S. cinereorectus*, AB184646, 99%; *S. baarnensis*, EF178688, 99%; *S. mutomycini*, AB249951, 99%.

Source: not known.

DNA G+C content (mol%): not known.

Type strain: AS 4.1664, ATCC 19735, ATCC 19891, ATCC 23891, CBS 677.68, BCRC 13760, DSM 40141, ICMP 12538, NBRC 12846, INA 5855/54, JCM 4629, KCTC 9020, NCIMB 12827, NRRL-ISP 5141, RIA 1131, VKM Ac-1091.

Sequence accession no. (16S rRNA gene): DQ026663.

86. **Streptomyces canescens** Waksman *in* Breed, Murray and Smith 1957, 768[AL] ["*Streptomyces canescus*" (*sic*) Hickey, Corum, Hidy, Cohen, Nager and Kropp 1952, 473]

ca.nes′cens. L. part. adj. *canescens* growing white, whiten.

Spore chains in Section *Rectiflexibiles*. Mature spore chains generally have 10–50 spores per chain. This morphology is observed on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar. Spore surface is smooth.

Color of colony: aerial mass color in the Yellow color series on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar. Reverse side of colony with no distinctive pigments on yeast-malt agar, oatmeal agar, or glycerol-asparagine agar; grayed greenish yellow on salts-starch agar; substrate is not a pH indicator.

Color in medium: melanoid pigments are not formed in peptone-yeast-iron agar or tyrosine agar. No pigment is found in medium in yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar.

D-Glucose, D-xylose, and D-mannitol are utilized for growth. No growth or only traces of growth on sucrose, iso-inositol, rhamnose and raffinose. Variable reports on growth with D-fructose and L-arabinose.

Type strain shows the highest sequence similarity to: *S. limosus*, AB184147, 100%; *S. felleus*, AB184129, 100%; *S. daghestanicus*, DQ442497, 100%; *S. albidoflavus*, AB184255, 100%; *S. hydrogenans*, AB184868, 100%; *S. odorifer*, Z76682, 100%; *S. violascens*, AY999737, 100%; *S. griseus* subsp. *solvifaciens*, AB249915, 99.9%; *S. champavatii*, DQ026642, 99.9%; *S. sampsonii*, D63871, 99.7%; *S. koyangensis*, AY079156, 99.6%.

Source: not known.

DNA G+C content (mol%): not known.

Type strain: AS 4.1681, ATCC 19736, CBS 474.68, BCRC 12206, DSM 40001, NBRC 12751, IMET 43077, JCM 4196, JCM 4568, NRRL 2419, NRRL-ISP 5001, RIA 1016, UNIQEM 124, VKM Ac-732.

Sequence accession no. (16S rRNA gene): AB184117.

87. **Streptomyces cangkringensis** Sembiring, Ward and Goodfellow 2001, 1619[VP] (Effective publication: Sembiring, Ward and Goodfellow 2000, 365.)

cang.krin.gen′sis. N.L. masc. adj. *cangkringensis* of or pertaining to Cangkringan, a place in Java, Indonesia.

Spore chains are *Spirales*; spore surface is rugose. On oatmeal agar, the spore mass is gray, the substrate mycelium is grayish-yellow, and the diffusible pigment is yellow. Melanin pigments are not produced. The organism degrades pectin but not adenine and grows at 45°C.

Type strain shows the highest sequence similarity to: *S. rhizosphaericus*, AB249941, 99.8%; *S. asiaticus*, AB249947, 99.8%; *S. indonesiensis*, DQ334783, 99.7%; *S. griseinger*, AJ391818, 99.5%. Type strain shows DNA–DNA similarity to: *S. albiflaviniger* NRRL B-1356^T, 98.4%; *S. geldanamycinus* NRRL 3602^T, 98.9%; *S. griseiniger* NRRL B1865^T, 99.3%; *S. rhizosphaericus* DSM 41760^T, 99.0%; *S. asiaticus* DSM 41761^T, 99.2%; *S. indonesiensis* DSM 41759^T, 98.4%; *S. javensis* DSM 41764^T, 98.4%; *S. yogyakartensis* DSM 41766^T, 98.2%.

Source: isolated from non-rhizosphere soil adjacent to a stand of the tropical legume *Paraserianthes falcataria*.

DNA G+C content (mol%): not known.

Type strain: D13P3, DSM 41769, JCM 11444, NBRC 100775, NCIMB 13684.

Sequence accession no. (16S rRNA gene): AJ391831.

88. **Streptomyces caniferus** (*ex* Krasil'nikov 1970b) Preobrazhenskaya 1986, 574[VP] (Effective publication: Preobrazhenskaya *in* Gause, Preobrazhenskaya, Sveshnikova, Terekhova and Maximova 1983.) ("*Actinomyces caniferus*" Krasil'nikov 1970b)

ca.ni′fe.rus. L. adj. *canus* white; L. adj. *ferus* (from L. v. *fero* to bear) bearing; N.L. masc. adj. *caniferus* bearing (producing) white.

Spore chains are spiral (*Spirales*); spores are smooth. On mineral agar 1 and oatmeal agar: aerial mycelium is gray; substrate mycelium is yellow, dark yellow; no diffusible pigment. On starch-ammonia agar: poor growth; aerial mycelium is gray; substrate mycelium is colorless to yellowish beige; no diffusible pigment. On glycerol-nitrate agar and glycerol-asparagine agar: aerial mycelium is white, whitish gray to gray; substrate mycelium is yellow, dark-yellow; diffusible pigment is yellow, but sometimes absent. On organic agar 2: aerial mycelium is white, later gray; substrate mycelium is colorless, yellow, or dark yellow; no diffusible pigment. Melanoid pigments are not formed. Grows on starch, inositol, mannitol, rhamnose, maltose, sucrose, and glucose; no growth on fructose, arabinose, galactose, xylose, lactose, or raffinose.

Type strain shows the highest sequence similarity to: *S. libani* subsp. *rufus*, AJ781351, 100%; *S. hygroscopius* subsp. *glebosus*, AB184479, 100%; *S. platensis*, AB045882, 99.8%; *S. libani* subsp. *libani*, AB184414, 99.6%; *S. hygroscopius* subsp. *decoyicus*, AY999883, 99.6%; *S. tubercidicus*, AJ621612, 99.5%; *S. nigrescens*, DQ442530, 99.5%; *S. catenulae*, AJ621613, 99.4%; *S. misakiensis*, AB217605, 99.4%; *S. ramulosus*, DQ026662, 99.3%; *S. sioyaensis*, DQ026654, 99.2%; *S. monomycini*, DQ445790, 99%.

Source: not known.

DNA G+C content (mol%): not known.

Type strain: VKM Ac-68, INMI 377, JCM 6914, AS 4.1588, ATCC 43699, DSM 41453, NBRC 15389, NRRL B-16358.

Sequence accession no. (16S rRNA gene): AB184640.

89. **Streptomyces canus** Heinemann, Kaplan, Muir and Hooper 1953, 1239[AL]

ca′nus. L. masc. adj. *canus* white, hoary.

Spore chains in Section *Spirales*, but some spore chains representative of Section *Rectiflexibiles* and *Retinaculiaperti* are also found. Mature spore chains are long, generally 10 to 50 or more spores per chain. This morphology is seen on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar. Spore surface is spiny.

Color of colony: aerial mass color in the Gray color series on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar. Reverse side of colony with no distinctive pigments on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar, except for dark brown on glycerol-asparagine agar in 14–21 d. Substrate is not a pH indicator.

Color in medium: true melanoid pigments are probably not formed in peptone-yeast-iron agar and tyrosine agar. Reports differ on production of melanoid pigments. Each of two observers reported dark pigment, but on different test media; a third collaborator saw no melanoid pigment in peptone-yeast-iron agar or tyrosine agar, but dark brown pigment in the substrate mycelium on glycerol-asparagine agar. An additional test showed no characteristic melanoid pigment. Pigments other than dark pigments noted above are not formed on yeast-malt agar, oatmeal agar, or salts-starch agar.

D-Glucose, L-arabinose, sucrose, D-xylose, iso-inositol, D-mannitol, D-fructose, and rhamnose are utilized for growth. Variable reports on growth with raffinose.

Type strain shows the highest sequence similarity to: *S. ciscaucasicus*, AY508512, 99.8%; *S. pseudovenezuelae*, AB184233, 99.4%; *S. resistomycificus*, AB184166, 99.3%; *S. novaecaesareae*, AB184357, 99%.

Source: not known.

DNA G+C content (mol%): not known.

Type strain: AS 4.1468, ATCC 12237, ATCC 19737, CBS 475.68, BCRC 13652, DSM 40017, IFM 1092, NBRC 12752, JCM 4212, JCM 4569, LMG 19329, NCIMB 9627, NRRL B-1989, NRRL B-3980, NRRL-ISP 5017, RIA 1017, UNIQEM 125, VKM Ac-1011.

Sequence accession no. (16S rRNA gene): AY999775.

90. **Streptomyces capillispiralis** Mertz and Higgens 1982, 123[VP]

ca.pil.li.spi.ra′lis. L. n. *capillus* hair; N.L. adj. *spiralis* (from L. n. *spira* a coil) spiral or spiraled; N.L. masc. adj. *capillispiralis* hairy spiraled.

Spore chains in Section *Spirales.* This morphology is readily observed on all media which support formation of aerial mycelia. Oatmeal agar (ISP 3), ISP 7, tomato paste oatmeal agar, and tap-water agar provide excellent observation of spiral morphology. The spirals are simple, open, loose coils of two to three turns. The sporophores bear chains of 10–50 spores. Spore shape is oval to globose. The spore size ranges from 0.96 to 1.19 µm by 0.54 to 0.71 µm. The mean size is 1.05 by 0.62 µm. Spore surface ornamentation is hairy. Coremia are observed on yeast-malt extract agar (ISP 2). Aerial mycelium is in the Gray color series of Tresner and Backus; light brownish-gray is the predominant shade. Produces a non-fragmenting substrate or primary mycelium which varies from yellow-brown to brownish-black, depending on the medium. A brown, water-soluble pigment is occasionally produced. Does not produce melanoid pigments.

Positive for catalase, phosphatase, and urease; decomposes casein, hypoxanthine, tyrosine, and xanthine. Liquefaction of gelatin, hydrolysis of starch, reduction of nitrate, peptonization of milk, resistance to lysozyme, and decomposition of esculin are negative. Acetate, D-arabinose, melibiose, raffinose, salicin, and sucrose are not utilized. Growth occurs at 10–45°C, with optimum growth at 30°C. Tolerates a pH range of 6–9.5, levels of NaCl up to 6%, and sucrose concentrations up to 35%. Cell analysis demonstrated the presence of LL-A_2pm; no *meso* isomer was detected. Sugar determinations indicated that only glucose and ribose were present. This information indicates a type I cell wall and a type C sugar pattern.

Type strain shows the highest sequence similarity to: *S. pseudogriseolus*, DQ442541, 100%; *S. gancidicus*, AB184660, 100%; *S. cellulosae*, DQ442495, 99.8%; *S. azureus*, EF178674, 99.5%; *S. levis*, AB184670, 99.5%; *S. lusitanus*, AB184424, 99.3%; *S. rubiginosus*, AY999810, 99.3%; *S. carpinensis*, AB184574, 99.3%; *S. matensis*, AB184221, 99.2%; *S. tuirus*, AB184690, 99.2%; *S. albaduncus*, AY999757, 99.1%; *S. caelestis*, X80824, 99.1%; *S. paradoxus*, AB184628, 99.1%; *S. djakartensis*, AB184657, 99%; *S. griseoloalbus*, AB184275, 99%; *S. afghaniensis*, AJ399483, 99%; *S. geysiriensis*, DQ442501, 99%; *S. africanus*, AY208912, 99%; *S. minutiscleroticus*, EF178696, 99%; *S. rochei*, AB184237, 99%; *S. vinaceusdrappus*, AY999929, 99%; *S. mutabilis*, EF178679, 99%; *S. ghanaensis*, AY999851, 99%; *S. plicatus*, AB184291, 99%.

Source: not known.

DNA G+C content (mol%): not known.

Type strain: A49492, DSM 41695, NBRC 14222, JCM 5075, KCTC 1719, NCIMB 12832, NRRL 12279.

Sequence accession no. (16S rRNA gene): AB184577.

91. **Streptomyces capoamus** Gonçalves de Lima, Albert and Gonçalves de Lima 1964, 317[AL]

ca.po.a′mus. Nheêngatû Amazonian dialect *capoama* island; N.L. n. *capoamus* island, referring to Ascension Island, the source of the soil from which the organism was isolated.

Spore chains in Section *Retinaculiaperti.* Terminal spirals, loops, and hooks may be found on moderately long chains of 10–50 spores. Straight to flexuous chains are also common. This morphology is seen on yeast-malt agar, oatmeal agar, and salts-starch agar, but not on glycerol-asparagine agar. Spore surface is smooth.

Color of colony: aerial mass color in the Gray or Red color series on yeast-malt agar and salts-starch agar; Red color series on oatmeal agar. Aerial mycelium is poorly developed or absent on glycerol-asparagine agar. Nearest matching color tabs from the Gray color series are e, medium gray, and 5fe, light grayish reddish brown. Nearest matching tabs from the Red color series are 6ec, grayish yellowish pink, and 7ca, light yellowish pink. Reverse side of colony is red; light reddish brown to grayish yellowish pink on yeast-malt

agar, oatmeal agar and salts-starch agar. Reverse mycelium pigment is a pH indicator, changing from red or reddish brown to blue or violet with the addition of 0.05 M NaOH.

Color in medium: melanoid pigments are formed in peptone-yeast-iron agar, tyrosine agar, and tryptone-yeast broth, but the reaction may be weak in tyrosine agar. Yellow, red, or orange may or may not be found in the medium in yeast-malt agar, oatmeal agar, salts-starch agar, or glycerol-asparagine agar. One of three observers reports a pH-sensitive pigment showing essentially the same reactions noted for the reverse mycelium pigments in yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar.

D-Glucose, L-arabinose, D-xylose, D-mannitol, and D-fructose are utilized for growth. Utilization of raffinose is doubtful. No growth or only traces of growth with iso-inositol, rhamnose, and sucrose.

Type strain shows the highest sequence similarity to: *S. longwoodensis*, AB184580, 99.6%; *S. canarius*, AB184396, 99.5%; *S. corchorusii*, AB184267, 99.5%; *S. olivaceoviridis*, AB184288, 99.5%; *S. galbus*, X79852, 99.5%; *S. bungoensis*, AB184696, 99.5%; *S. regensis*, DQ026649, 99.1%; *S. curacoi*, EF626595, 99%.

Source: isolated from soil from Ascension Island.

DNA G+C content (mol%): not known.

Type strain: AS 4.1696, ATCC 19006, CBS 712.72, BCRC 11860, DSM 40494, NBRC 13411, JCM 4253, JCM 4734, NRRL B-3632, NRRL-ISP 5494, RIA 1372.

Sequence accession no. (16S rRNA gene): AB045877.

92. **Streptomyces carpaticus** Maximova and Terekova 1986, 574[VP] (Effective publication: Maximova and Terekova *in* Gause, Preobrazhenskaya, Sveshnikova, Terekhova and Maximova 1983.)

car.pa′ti.cus. N.L. masc. adj. *carpaticus* of or pertaining to the Carpathians.

Spore chains are spiral (*Spirales*); spores are smooth. On mineral agar 1, glycerol-asparagine agar, oatmeal agar, and glycerol-nitrate agar: aerial mycelium is grayish gray-brown; substrate mycelium and diffusible pigment are dark brown, nearly black with reddish or olive shadow. On starch-ammonia agar: aerial mycelium is light gray; substrate mycelium is colorless; no diffusible pigment. Sometimes substrate mycelium and diffusible pigment are light brown. On organic agar 2 and agar 79: aerial mycelium is grayish gray-brown; substrate mycelium and diffusible pigment are greenish gray-brown, reddish gray-brown; melanoid pigments are not formed.

Rhamnose is utilized for growth; weak growth is observed with sucrose, fructose, glucose, xylose, mannitol, inositol, raffinose, and arabinose. Antibiotic is not produced.

Type strain shows no sequence similarity over 99%.

Source: not known.

DNA G+C content (mol%): not known.

Type strain: AS 4.1621, ATCC 43678, DSM 41468, NBRC 15390, INA 8851, JCM 6915, NRRL B-16359, VKM Ac-1211.

Sequence accession no. (16S rRNA gene): DQ442494.

93. **Streptomyces carpinensis** (Falcão de Morais, Oliveira Da Silva and Machado 1971) Goodfellow, Williams and Alderson 1986a, 574[VP] (Effective publication: Goodfellow, Williams and Alderson 1986c, 53.) (*Elytrosporangium carpinense* Falcão de Morais, Oliveira Da Silva and Machado 1971, 205)

car.pin.en′sis. Gr. n. *karpos* fruit, seed with seed-vessel; N.L. masc. adj. *carpinensis* pertaining to spores within a sporangium.

Forms extensively branched substrate and aerial mycelium. The latter bears long spiral chains and the former bears single spores or short chain of up to seven spores of various sizes (1.0–3.0 µm diameter). The spore surface is smooth. Aerial spore mass is dark gray; the reverse color is black. Does not form melanin pigments. Adenine, esculin, casein, guanine, hypoxanthine, starch, testosterone, and tyrosine are degraded, but allantoin, arbutin, chitin, elastin, lecithin, pectin, urea, and xanthine are not. Hydrogen sulfide is produced, but nitrate is not reduced. L-Arabinose, cellobiose, D-fructose, D-galactose, D-glucose, mannitol, D-mannose, melezitose, melibiose, raffinose, L-rhamnose, salicin, trehalose, and xylitol are used as sole carbon sources, but adonitol, *myo*-inositol, inulin, D-lactose, sucrose, and D-xylose are not. Grows on DL-amino-n-butyric acid, L-arginine, L-cysteine, L-histidine, L-phenylalanine, potassium nitrate, L-serine, L-threonine, and L-valine, but not on L-hydroxyproline or L-methionine, as sole nitrogen source. Growth occurs at 10, 37 and 45°C, but not at 4°C. Tolerant to phenol (0.1%, w/v), sodium azide (0.02%, w/v), and sodium chloride (7%, w/v). Resistant to rifampin but sensitive to sodium chloride at 10% (w/v). Does not show antimicrobial activity against *Aspergillus niger* LIV 131, *Bacillus subtilis* NCIB 3610, *Candida albicans* CBS 562, *Escherichia coli* NCIB 9132, *Micrococcus luteus* NCIB 196, *Saccharomyces cerevisiae* CBS 1171[T], or *Streptomyces murinus* ISP 5091. Contains major amounts of hexa- and octa-hydrogenated menaquinones with nine isoprene units.

Type strain shows the highest sequence similarity to: *S. levis*, AB184670, 99.5%; *S. cellulosae*, DQ442495, 99.4%; *S. pseudogriseolus*, DQ442541, 99.4%; *S. gancidicus*, AB184660, 99.4%; *S. capillispiralis*, AB184577, 99.3%; *S. azureus*, EF178674, 99.1%; *S. spinoverrucosus*, AB184578, 99%; *S. djakartensis*, AB184657, 99%.

Source: isolated from soil in Pernambuco, Brazil.

DNA G+C content (mol%): not known.

Type strain: 70-6-2 (Lab. Microbiol. Inst. Biociencias Univer. Federal Pernambuco, Recife, Brazil), ATCC 27116, DSM 43835, NBRC 14214, IMET 43558, JCM 3301, KCC A-0301, KCTC 9128, NRRL B 16921, RIA 982, VKM Ac-1300, VKM Ac-657.

Sequence accession no. (16S rRNA gene): AB184574.

94. **Streptomyces catenulae** Davisson and Finlay *in* Waksman 1961, 190[AL]

ca.te.nu′la.e. L. dim. n. *catenula* a small chain; L. gen. dim. n. *catenulae* of a small chain.

Spore chains in Section *Rectiflexibiles*. Very short spore chains of 3–10 spores occur in dense clusters so that morphology is difficult to determine. Short chains often form hooks or incomplete spirals. Tight spirals may occur in the dense clusters, but most chains are too short to form true spirals. Short chains are also not typical of *Retinaculiaperti*

cultures. This morphology is seen on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar. It is representative of morphology described by Davisson and Finlay (op. cit). Spore surface is smooth.

Color of colony: aerial mass color in the Green color series on yeast-malt agar, oatmeal agar, and glycerol-asparagine agar; Gray or Green color series on salts-starch agar. The color on all media is medium gray to olive gray or grayish olive (see tabs 2ih or 2ge in Gray color series and 1½ge in Green color series). Reverse side of colony is colorless or grayish yellow to light grayish olive or olive brown on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar. Substrate mycelium pigment is not pH-sensitive.

Color in medium: melanoid pigments are not found in peptone-yeast-iron agar, tyrosine agar, or tryptone-yeast broth; no pigment or only trace of yellow pigment in medium in yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar.

D-Glucose, D-mannitol, D-fructose, and raffinose are utilized for growth. No growth or only traces of growth on L-arabinose, sucrose, D-xylose, iso-inositol, and rhamnose.

Type strain shows the highest sequence similarity to: *S. misakiensis*, AB217605, 100%; *S. libani* subsp. *libani*, AB184414, 99.6%; *S. nigrescens*, DQ442530, 99.5%; *S. tubercidicus*, AJ621612, 99.5%; *S. caniferus*, AB184640, 99.4%; *S. libani* subsp. *rufus*, AJ781351, 99.3%; *S. hygroscopius* subsp. *glebosus*, AB184479, 99.3%; *S. platensis*, AB045882, 99.2%; *S. sioyaensis*, DQ026654, 99.2%; *S. ramulosus*, DQ026662, 99.1%.

Source: not known.

DNA G+C content (mol%): not known.

Type strain: AS 4.1701, ATCC 12476, ATCC 23893, CBS 679.68, BCRC 12092, DSM 40258, HAMBI 986, NBRC 12848, IMET 42944, JCM 4353, KCTC 9223, NRRL B-2342, NRRL-ISP 5258, RIA 1183, VKM Ac-758.

Sequence accession no. (16S rRNA gene): AJ621613.

95. **Streptomyces caviscabies** Goyer, Faucher and Beaulieu 1996, 638[VP]

ca.vi.sca'bies. L. adj. *cavus* hollow, excavated; L. n. *scabies* scab, mange; N.L. n. *caviscabies* excavated scab, referring to the ability of the organism to cause deep-pitted scab of potatoes.

The cylindrical, smooth spores are 0.87–1.08 × 0.5–0.63 µm. Strains are characterized by a gold mycelium on yeast-malt extract medium and a white mass of spores borne in flexuous chains. Does not produce melanin. All strains grow on proline or methionine as sole nitrogen source. Utilizes raffinose as a sole carbon source. All strains grow in the presence of 4% NaCl, 0.1% phenol, 10 IU/ml penicillin, 10 µg/ml thallous acetate, and 20 µg/ml streptomycin sulfate. Growth is inhibited at pH 4.5. The cell walls contain LL-A_2pm.

For sequence similarity, see type strain of *Streptomyces griseus.*

Source: not known.

DNA G+C content (mol%): 71.0.

Type strain: EF-87, ATCC 51928, CIP 104962, DSM 41811.

Sequence accession no. (16S rRNA gene): AF112160.

Further comments: according to Liu et al. (2005b), *Streptomyces caviscabies* Goyer et al. 1996 is a later heterotypic synonym of *Streptomyces griseus* (Krainsky 1914) Waksman and Henrici 1948 emend. Liu et al. 2005b.

According to Guo et al. (2008), *Streptomyces caviscabies* Goyer et al. (1996) is not a later heterotypic synonym of *Streptomyces griseus* (Krainsky 1914) Waksman and Henrici 1948.

96a. **Streptomyces cavourensis subsp. cavourensis** Skarbek and Brady 1978, 52[AL]

ca.vour.en'sis. N.L. masc. adj. *cavourensis* of or pertaining to Cavour, named for Conti de Cavour (1810–1861), an Italian statesman and hero.

Spore chains in Section *Rectiflexibiles.* Mature spore chains are generally flexuous and very long, with more than 50 spores per chain. This morphology is seen on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar. Spore surface is smooth, sometimes with minor surface irregularities.

Color of colony: aerial mass color in the Yellow color series (2db or 2ba, pale yellow) on yeast-malt agar, oatmeal agar, and salts-starch agar; Yellow or White color series on glycerol-asparagine agar. Reverse side of colony with no distinctive pigments (moderate to strong brown on yeast-malt agar; yellow, orange yellow, or olive brown on oatmeal agar, salts-starch agar, and glycerol-asparagine agar).

Color in medium: melanoid pigments are formed in peptone-yeast-iron agar and tryptone-yeast broth, but not in tyrosine agar. No pigment (other than brown) is found in the medium in yeast-malt agar, oatmeal agar, salts-starch agar, or glycerol-asparagine agar.

D-Glucose, L-arabinose, D-xylose, D-mannitol, and D-fructose are utilized for growth. No growth or only traces of growth with iso-inositol, rhamnose, sucrose, and raffinose. Type strain shows the highest sequence similarity to: *S. celluloflavus*, AB184476, 100%; *S. albolongus*, AB184425, 100%; *S. griseobrunneus*, AB249912, 99.9%; *S. bacillaris*, AB184439, 99.4%; *S. californicus*, AB184755, 99.4%; *S. fulvorobeus*, AB184711, 99.3%; *S. floridae*, AB184656, 99.3%; *S. griseinus*, AB184205, 99.3%; *S. parvus*, DQ442537, 99.3%; *S. pluricolorescens*, DQ442540, 99.3%; *S. candidus*, DQ026663, 99.3%; *S. spiroverticillatus*, AB184814, 99.3%; *S. globisporus* subsp. *globisporus*, EF178686, 99.3%; *S. sindenensis*, AB184759, 99.3%; *S. badius*, AY999783, 99.3%; *S. praecox*, AB184293, 99.2%; *S. albovinaceus*, AB249958, 99.2%; *S. microflavus*, DQ445795, 99.2%; *S. fimicarius*, AY999784, 99.2%; *S. flavofuscus*, AB249935, 99.2%; *S. alboviridis*, AB184256, 99.2%; *S. rubiginosohelvolus*, AB184240, 99.2%; *S. lipmanii*, AB184148, 99.2%; *S. mediolani*, AB184674, 99.2%; *S. cremeus*, AB184124, 99.2%; *S. nitrosporeus*, EF178680, 99.1%; *S. anulatus*, DQ026637, 99.1%; *S. acrimycini*, AY999889, 99.1%; *S. griseoplanus*, AY999894, 99.1%; *S. baarnensis*, EF178688, 99.1%; *S. lavendulae* subsp. *lavendulae*, AB184080, 99.1%; *S. cavourensis* subsp. *washingtonensis*, DQ026671, 99.1%; *S. argenteolus*, AB045872, 99%; *S. flavovirens*, DQ026635, 99%; *S. cinereorectus*, AB184646, 99%; *S. cyaneofuscatus*, AB184860, 99%; *S. finlayi*, AY999788, 99%; *S. luridiscabiei*, AF361784, 99%.

Source: not known.

DNA G+C content (mol%): not known.

Type strain: AS 4.1692, ATCC 14889, ATCC 25438, CBS 669.69, DSM 40300, NBRC 13026, JCM 4249, JCM 4298, JCM 4555, NCIMB 8918, NRRL 2740, NRRL-ISP 5300, RIA 1218, VKM Ac-731.

Sequence accession no. (16S rRNA gene): DQ445791.

96b. **Streptomyces cavourensis subsp. washingtonensis** Skarbek and Brady 1978, 52[AL]

wash.ing.ton.en′sis. N.L. masc. adj. *washingtonensis* of or pertaining to Washington.

Strain exhibits straight to flexuous chains of spores on all standard media and thus belongs to the section *Rectiflexibiles*. The spore type is smooth. Malate is moderately solubilized by all strains tested, except one, which gave a weak response. No distinctive pigments other than yellow-brown are found in the reverse mycelium (i.e. reverse side of colony) of cultures. Diffusible, soluble pigments other than brown or black are found only with one strain grown on the standard media. This yellow, soluble pigment is not a pH indicator. All cultures, except that of strain Illinois 205-2M, are found to produce melanoid pigments on at least three of the four media which were utilized. Strain Illinois 205-2M failed to produce melanoid pigments on any of the melanin formation media employed.

Tolerance to heavy metals is as follows. Strain AUW-83 differs from all the other strains in that it produces a soluble, dark brown pigment, modified by gray-green, after 10 d of incubation on the basal agar supplemented with zinc; little soluble pigment, except for a slight yellow-brown exhibited by strain 689, was observed with the remaining strains on the zinc agar.

Type strain shows the highest sequence similarity to: *S. cyaneofuscatus*, AB184860, 100%; *S. lavendulae* subsp. *lavendulae*, AB184080, 100%; *S. alboviridis*, AB184256, 100%; *S. fulvorobeus*, AB184711, 100%; *S. praecox*, AB184293, 100%; *S. anulatus*, DQ026637, 100%; *S. flavofuscus*, AB249935, 100%; *S. cinereorectus*, AB184646, 100%; *S. griseoplanus*, AY999894, 100%; *S. lipmanii*, AB184148, 100%; *S. fimicarius*, AY999784, 100%; *S. microflavus*, DQ445795, 99.9%; *S. sindenensis*, AB184759, 99.9%; *S. rubiginosohelvolus*, AB184240, 99.9%; *S. mediolani*, AB184674, 99.9%; *S. griseinus*, AB184205, 99.9%; *S. acrimycini*, AY999889, 99.9%; *S. pluricolorescens*, DQ442540, 99.9%; *S. argenteolus*, AB045872, 99.9%; *S. badius*, AY999783, 99.9%; *S. floridae*, AB184656, 99.8%; *S. albovinaceus*, AB249958, 99.8%; *S. globisporus* subsp. *globisporus*, EF178686, 99.8%; *S. griseus* subsp. *griseus*, AY207604, 99.8%; *S. griseolus*, AB184768, 99.8%; *S. luridiscabiei*, AF361784, 99.8%; *S. baarnensis*, EF178688, 99.7%; *S. flavovirens*, DQ026635, 99.7%; *S. californicus*, AB184755, 99.7%; *S. parvus*, DQ442537, 99.7%; *S. halstedii*, EF178695, 99.7%; *S. flavogriseus*, AJ494864, 99.6%; *S. olivoviridis*, AB184227, 99.5%; *S. bacillaris*, AB184439, 99.5%; *S. finlayi*, AY999788, 99.5%; *S. pulveraceus*, AB184806, 99.5%; *S. atroolivaceus*, AJ781320, 99.5%; *S. clavifer*, DQ026670, 99.4%; *S. yanii*, AB006159, 99.4%; *S. nitrosporeus*, EF178680, 99.4%; *S. albolongus*, AB184425, 99.3%; *S. sanglieri*, AB249945, 99.3%; *S. mutomycini*, AB249951, 99.2%; *S. atratus*, DQ026638, 99.2%; *S. celluloflavus*, AB184476, 99.2%; *S. gelaticus*, DQ026636, 99.2%; *S. griseobrunneus*, AB249912, 99.2%; *S. spiroverticillatus*, AB184814, 99.1%; *S. cavourensis* subsp. *cavourensis*, DQ445791, 99.1%; *S. cremeus*, AB184124, 99.1%; *S. candidus*, DQ026663, 99.1%.

Source: not known.

DNA G+C content (mol%): not known.

Type strain: AS 4.1635, ATCC 27732, DSM 41423, NBRC 15391, JCM 4967, NRRL B-8030.

Sequence accession no. (16S rRNA gene): DQ026671.

97. **Streptomyces cellostaticus** Hamada 1958, 178[AL]

Etymology of specific epithet is unknown.

Spore chains in Section *Spirales*. Mature spore chains generally have 10–50 spores per chain; longer chains are often observed. This morphology is seen on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar. Spore surface is spiny.

Color of colony: aerial mass color in the Red (or Gray) color series on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar. Characteristic color is usually between 3ge (light grayish yellowish brown) and 5ge or 5dc (grayish yellowish pink) color tabs of Tresner–Backus color wheels. Reverse side of colony with no distinctive pigment (characteristic grayed yellow or light yellow brown) on yeast-malt agar, oatmeal agar, salts-starch agar, or glycerol-asparagine agar.

Color in medium: melanoid pigments are formed in peptone-yeast-iron agar, tyrosine agar, and tryptone-yeast broth. Pigments other than melanoids or faint traces of yellow pigment not formed in yeast-malt agar, oatmeal agar, salts-starch agar, or glycerol-asparagine agar.

D-Glucose, L-arabinose, sucrose, D-xylose, iso-inositol, D-mannitol, D-fructose, rhamnose, and raffinose are utilized for growth.

Type strain shows the highest sequence similarity to: *S. griseochromogenes*, AB184387, 99.7%; *S. yokosukanensis*, DQ026652, 99.5%; *S. achromogenes* subsp. *achromogenes*, AB184109, 99%; *S. griseoruber*, AB184209, 99%; *S. olivaceoviridis*, AB184288, 99%; *S. corchorusii*, AB184267, 99%.

Source: not known.

DNA G+C content (mol%): not known.

Type strain: AS 4.1637, ATCC 23894, CBS 680.68, DSM 40189, NBRC 12849, IMET 41374, JCM 4183, JCM 4631, NCIMB 9830, NRRL-ISP 5189, RIA 1143, VKM Ac-1222.

Sequence accession no. (16S rRNA gene): AB184192.

98. **Streptomyces celluloflavus** Nishimura, Kimura and Kuroya 1953, 64[AL]

cel.lu.lo.fla′vus. N.L. n. *cellulosum* cellulose; L. adj. *flavus* yellow; N.L. masc. adj. *celluloflavus* cellulose, yellow (intended to refer to the yellow streptomycete that attacks cellulose).

Sparse formation of aerial mycelium. Poor growth on Czapek's solution agar. Produces aureothricin; inhibited by streptomycin.

Type strain shows the highest sequence similarity to: *S. cavourensis* subsp. *cavourensis*, DQ445791, 100%; *S. albolongus*, AB184425, 100%; *S. griseobrunneus*, AB249912, 100%; *S. californicus*, AB184755, 99.5%; *S. bacillaris*, AB184439, 99.4%; *S. fulvorobeus*, AB184711, 99.4%; *S. floridae*,

AB184656, 99.4%; *S. griseinus*, AB184205, 99.4%; *S. pluricolorescens*, DQ442540, 99.4%; *S. candidus*, DQ026663, 99.4%; *S. spiroverticillatus*, AB184814, 99.4%; *S. globisporus* subsp. *globisporus*, EF178686, 99.4%; *S. sindenensis*, AB184759, 99.4%; *S. badius*, AY999783, 99.4%; *S. rubiginosohelvolus*, AB184240, 99.4%; *S. mediolani*, AB184674, 99.4%; *S. praecox*, AB184293, 99.3%; *S. albovinaceus*, AB249958, 99.3%; *S. microflavus*, DQ445795, 99.3%; *S. fimicarius*, AY999784, 99.3%; *S. flavofuscus*, AB249935, 99.5%; *S. alboviridis*, AB184256, 99.3%; *S. lipmanii*, AB184148, 99.3%; *S. cremeus*, AB184124, 99.3%; *S. griseoplanus*, AY999894, 99.3%; *S. anulatus*, DQ026637, 99.3%; *S. parvus*, DQ442537, 99.2%; *S. luridiscabiei*, AF361784, 99.2%; *S. acrimycini*, AY999889, 99.2%; *S. baarnensis*, EF178688, 99.2%; *S. cyaneofuscatus*, AB184860, 99.2%; *S. cavourensis* subsp. *washingtonensis*, DQ026671, 99.2%; *S. cinereorectus*, AB184646, 99.2%; *S. flavovirens*, DQ026635, 99.2%; *S. lavendulae* subsp. *lavendulae*, AB184080, 99.2%; *S. nitrosporeus*, EF178680, 99.1%; *S. argenteolus*, AB045872, 99.1%; *S. finlayi*, AY999788, 99.1%; *S. flavogriseus*, AJ494864, 99.1%; *S. griseolus*, AB184768, 99%; *S. pulveraceus*, AB184806, 99%; *S. clavifer*, DQ026670, 99%; *S. griseus* subsp. *griseus*, AY207604, 99%; *S. halstedii*, EF178695, 99%; *S. cinnamonensis*, AB184707, 99%.

Source: not known.

DNA G+C content (mol%): not known.

Type strain: AS 4.1659, ATCC 29806, CECT 3242, DSM 40839, NBRC 13780, JCM 4126, KCTC 9702, NRRL B-2493.

Sequence accession no. (16S rRNA gene): AB184476.

99. **Streptomyces cellulolyticus** Li 1997, 444[VP]

cel.lu.lo.ly′ti.cus. N.L. n. *cellulosum* cellulose; N.L. masc. adj. *lyticus* (from Gr. masc. adj. *lutikos*) able to dissolve; N.L. masc. adj. *cellulolyticus* decomposing cellulose.

Spore chains are *Rectiflexibiles*, with 20 or more spores per chain. Spores are oval and are 2.1–2.3 × 2.5–2.7 µm. Spore surface is warty. Mycelia do not fragment into coccoid or bacillary structures. The branching substrate mycelium is: yellow on yeast extract-malt extract agar, inorganic salts-starch agar, glucose-asparagine agar, and nutrient agar; brown on glycerol-asparagine agar, tyrosine agar, and Czapek's solution agar; and colorless on oatmeal agar. Aerial spore mass is white to pink. Soluble pigments, including melanin, are not produced. Cellulose is decomposed. D-Glucose, D-fructose, L-arabinose, sucrose, D-xylose, raffinose, and iso-inositol are utilized for growth, but rhamnose is not utilized. L-Asparagine, L-cysteine, and L-threonine can be used as nitrogen sources. Positive for catalase activity and production of H_2S and negative for oxidase activity and indole production. Nitrate is reduced. Starch, casein, and esculin are hydrolyzed. Gelatin is not liquefied. Good growth occurs at pH 7.2 and the optimum temperature is 30°C. Growth occurs in the presence of up to 10% NaCl. Isolate LX[T] is susceptible to penicillin G, but not to dimethylchlortetracycline, vancomycin, kanamycin, rifampin, or aminobenzylpenicillin. The cell-wall chemotype is chemotype I and the cell wall contains LL-A_2pm and glycine; no characteristic sugars are detected as whole-cell sugars.

Source: not known.

DNA G+C content (mol%): not known.

Type strain: LX, AS 4.1332.

Sequence accession no. (16S rRNA gene): no sequence available.

100. **Streptomyces cellulosae** (Krainsky 1914) Waksman and Henrici *in* Breed, Murray and Hitchens 1948, 938[AL] ["*Actinomyces cellulosae*" (*sic*) Krainsky 1914, 683]

cel.lu.lo′sa.e. N.L. n. *cellulosum* cellulose; N.L. gen. n. *cellulosae* (*sic*) of cellulose (probably intended to mean the species that degrades cellulose).

Spore chains are typically flexuous. Excellent growth on Czapek's solution agar; exhibits slight anti-bacterial activity.

Type strain shows the highest sequence similarity to: *S. pseudogriseolus*, DQ442541, 99.9%; *S. gancidicus*, AB184660, 99.9%; *S. capillispiralis*, AB184577, 99.8%; *S. carpinensis*, AB184574, 99.4%; *S. azureus*, EF178674, 99.4%; *S. levis*, AB184670, 99.3%; *S. rubiginosus*, AY999810, 99.2%; *S. lusitanus*, AB184424, 99.2%; *S. lavendulocolor*, DQ442516, 99.1%; *S. thermocarboxydus*, U94490, 99.1%; *S. caelestis*, X80824, 99.1%; *S. matensis*, AB184221, 99.1%; *S. djakartensis*, AB184657, 99%; *S. africanus*, AY208912, 99%; *S. viridiviolaceus*, AY999854, 99%; *S. afghaniensis*, AJ399483, 99%; *S. griseoloalbus*, AB184275, 99%; *S. tuirus*, AB184690, 99%; *S. albaduncus*, AY999757, 99%.

Source: not known.

DNA G+C content (mol%): not known.

Type strain: AS 4.1411, ATCC 25439, CBS 122.18, CBS 670.69, BCRC 12087, DSM 40362, NBRC 13027, JCM 4462, KCTC 9703, LMG 19315, NRRL B-2889, NRRL-ISP 5362, RIA 1219, VKM Ac-829.

Sequence accession no. (16S rRNA gene): DQ442495.

101. **Streptomyces champavatii** Uma and Narasimha Rao 1959, 133[AL]

cham.pa.va′ti.i. N.L. gen. n. *champavatii* of Champavathi, named after the Champavathi River in Andhra Pradesh, India.

Forms green vegetative mycelium and diffusible pigment on some media. Poor growth on Czapek's solution agar; produces champamycins A and B (heptaenic antifungal antibiotics) and champavatin, a non-polyenic antifungal antibiotic; produces vitamin B_{12}.

Type strain shows the highest sequence similarity to: *S. limosus*, AB184147, 100%; *S. felleus*, AB184129, 100%; *S. daghestanicus*, DQ442497, 100%; *S. albidoflavus*, AB184255, 100%; *S. odorifer*, Z76682, 100%; *S. violascens*, AY999737, 100%; *S. hydrogenans*, AB184868, 100%; *S. griseus* subsp. *solvifaciens*, AB249915, 99.9%; *S. canescens*, AB184117, 99.9%; *S. koyangensis*, AY079156, 99.5%; *S. sampsonii*, D63871, 99.3%.

Source: not known.

DNA G+C content (mol%): not known.

Type strain: AS 4.1615, BCRC 12231, DSM 40841, NBRC 15392, JCM 5066, NCIMB 12859, NRRL B-5682.

Sequence accession no. (16S rRNA gene): DQ026642.

102. **Streptomyces chartreusis** Leach, Calhoun, Johnson, Teeters and Jackson 1953, 4011[AL] ["*Actinomyces chartreusis*" Preobrazhenskaya 1966, 852]

char.treu′sis. N.L. n. *chartreusum* (from French n. *chartreuse*), a Carthusian monastery famed for a sweet yellow liqueur, hence the color "chartreuse"; N.L. masc. adj. *chartreusis* yellow, referring to color of the diffusible pigment formed by the organism.

Spore chains in Section *Spirales*. Clusters of spiral spore chains sometimes resemble whorls of verticils. Pseudoverticils are not uniformly distributed and do not arise from an acial hypha characteristic of true verticillate forms. Mature spore chains generally have 10–50 spores per chain. This morphology is seen on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar. Spore surface is spiny.

Color of colony: aerial mass color in the Blue color series on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar (Gray series also reported on oatmeal agar). Reverse side of colony with no distinctive pigments on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar; substrate is not a pH indicator.

Color in medium: melanoid pigments formed in peptone-yeast-iron agar and tryptone-yeast broth, but not in tyrosine agar. Pigments other than melanoids are not formed in yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar.

D-Glucose, L-arabinose, sucrose, D-xylose, iso-inositol, D-mannitol, D-fructose, rhamnose, and raffinose are utilized for growth.

Type strain shows the highest sequence similarity to: *S. resistomycificus*, AB184166, 99.5%; *S. galilaeus*, AB045878, 99.3%; *S. bobili*, AB249925, 99.3%; *S. novaecaesareae*, AB184357, 99.1%; *S. phaeoluteigriseus*, AJ391815, 99.1%; *S. pseudovenezuelae*, AB184233, 99.1%; *S. prunicolor*, DQ026659, 99%; *S. aureocirculatus*, AB184260, 99%.

Source: not known.

DNA G+C content (mol%): not known.

Type strain: AS 4.1639, ATCC 14922, ATCC 19738, CBS 476.68, BCRC 13673, CCT 5005, DSM 40085, NBRC 12753, JCM 4570, KCTC 9704, NRRL 2287, NRRL-ISP 5085, RIA 1018, UNIQEM 126, VKM Ac-1721.

Sequence accession no. (16S rRNA gene): AB184839.

103. **Streptomyces chattanoogensis** Burns and Holtman 1959, 398[AL]

chat.ta.no.o.gen′sis. N.L. masc. adj. *chattanoogensis* of or belonging to Chattanooga, Tennessee, the source of the soil from which the organism was isolated.

Spore chains Section *Spirales*, but spore chains representative of Section *Rectiflexibiles* and Section *Retinaculiaperti* are also found. Mature spore chains generally have 10–50 spores per chain on suitable media. This morphology is seen on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar. Spore surface is spiny.

Color of colony: aerial mass color in the Yellow or White color series on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar. Yellow, when present, is a very pale yellow. Reverse side of colony with no distinctive pigments on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar; substrate is not a pH indicator.

Color in medium: melanoid pigments are not formed in peptone-yeast-iron agar and tyrosine agar. Yellow pigment is found in medium in yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar; this pigment is not pH-sensitive when tested with 0.05 M NaOH or HCl.

D-Glucose, iso-inositol, D-mannitol, D-fructose, raffinose, and sucrose are utilized for growth. No growth or only traces of growth on D-xylose, rhamnose, and L-arabinose.

Type strain shows the highest sequence similarity to: *S. lydicus*, Y15507, 99.8%; *S. sioyaensis*, DQ026654, 99.3%; *S. rimosus* subsp. *paromomycinus*, AJ621610, 99.2%; *S. chrestomyceticus*, AJ621609, 99.2%; *S. tubercidicus*, AJ621612, 99%; *S. libani* subsp. *libani*, AB184414, 99%; *S. nigrescens*, DQ442530, 99%.

Source: isolated from soil from Chattanooga, Tennessee, USA.

DNA G+C content (mol%): not known.

Type strain: AS 4.1415, ATCC 13358, ATCC 19739, CBS 477.68, BCRC 13655, CECT 3321, DSM 40002, NBRC 12754, JCM 4299, JCM 4571, KCTC 1087, LMG 19339, NCIMB 9809, NRRL B-2255, NRRL-ISP 5002, RIA 1019, VKM Ac-1775.

Sequence accession no. (16S rRNA gene): AJ621611.

104. **Streptomyces cheonanensis** Kim, Lee and Hwang 2006, 474[AL]

che.on.an.en′sis. N.L. masc. adj. *cheonanensis* of or pertaining to Cheonan, Republic of Korea, the geographical origin of the type strain.

Forms extensively branched aerial and substrate hyphae. Short or long, straight to flexuous chains of smooth-surfaced spores are evident on the aerial hyphae. Aerial mycelium is gray to white and the substrate mycelium appears light yellow when grown on ISP 4 agar. Aerial and substrate mycelia grow abundantly on both ISP 3 agar and Bennett's agar. Soluble pigments are generated on ISP3, ISP 3, ISP 5 (glycerol-asparagine agar), and ISP 7. The cell wall contains LL-A_2pm. Predominant cellular fatty acids are 14-methylpentadecanoic acid ($C_{16:0}$ iso; 47.82%), hexadecanoic acid ($C_{16:0}$; 14.44%), and *cis*-9-hexadecenoic acid ($C_{16:1}$ *cis9*; 10.24%). Optimum growth occurs at 29°C. Grows well in yeast extract-malt extract broth adjusted to pH 6.5–8.0. Tolerates NaCl concentrations up to 7%. Capable of utilizing several carbon sources, including adonitol, arabinose, dextran, fructose, *myo*-inositol, mannitol, melezitose, melibiose, raffinose, L-rhamnose, sucrose, xylitol, and xylose. Can also use several nitrogen sources: DL-α-amino-n-butyric acid, L-cysteine, L-histidine, L-hydroxyproline, L-phenylalanine, and L-valine. Resistant to penicillin G, but sensitive to neomycin, oleandomycin, and rifampin. Secretes compounds the inhibit mycelial growth of plant-pathogenic fungi including *Alternaria mali*, *Collectotrichum orbiculare*, *Magnaporthe grisea*, *Fusarium oxysporum* f. sp. *lycopersici*, and *Rhizotonia solani* and the oomycete *Phytophthora capsici*.

Type strain shows no sequence similarity over 99%. Type strain shows DNA–DNA similarity to: *S. thermolineatus* DSM 41451ᵀ, 21.5%; "*S. cattleya*" JCM 4925, 39.8%; *S. macrosporus* DSM 41449ᵀ, 19.8%; *S. acidiscabies* ATCC 49003ᵀ, 60.6%.

Source: not known.

DNA G+C content (mol%): 75.5.

Type strain: VC-A46, KCCM 42119, NBRC 100940.

Sequence accession no. (16S rRNA gene): AY822606.

105. **Streptomyces chibaensis** Suzuki, Nakamura, Okama and Tomiyama 1958, 81[AL]

chi.ba.en′sis. N.L. masc. adj. *chibaensis* of or belonging to Chiba City, Japan, the source of the soil from which the organism was isolated.

Spore chains in Section *Spirales* or *Rectiflexibiles*. Spirals, when formed, are open and poorly developed. Some spore chains are straight and many are flexuous or curved to form hooks or partial spirals. Most spore chains are too short (3 to 10 or 20 spores per chain) for this culture to be placed in Section *Retinaculiaperti*. This morphology is seen on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar. Spore surface is smooth.

Color of colony: mature aerial mass color is usually in the Gray color series on yeast-malt agar, oatmeal agar, and salts-starch agar; Gray or Yellow color series on glycerol-asparagine agar. Color tabs selected from the Gray color series were yellowish gray or grayish yellowish brown. Reverse side of colony with no distinctive pigments (grayed greenish yellow, yellow, or rarely orange-yellow) on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar; substrate mycelium pigment is not a pH indicator.

Color in medium: melanoid pigments are not found in peptone-yeast-iron agar, tyrosine agar, or tryptone-yeast broth. Yellow pigment may or may not be found in the medium in yeast-malt agar, oatmeal agar, and glycerol-asparagine agar; this pigment is not pH-sensitive.

D-Glucose, L-arabinose, sucrose, D-xylose, iso-inositol, D-mannitol, D-fructose, rhamnose, and raffinose are all utilized for growth.

For sequence similarity, see type strain of *Streptomyces corchorusii*.

Source: not known.

DNA G+C content (mol%): not known.

Type strain: AS 4.1654, ATCC 23895, CBS 681.68, DSM 40220, IFM 1085, NBRC 12850, JCM 4017, JCM 4632, KCTC 9786, LMG 20456, NRRL B-2904, NRRL-ISP 5220, RIA 1164, VKM Ac-1893.

Sequence accession no. (16S rRNA gene): AB184193.

Further comments: according to Lanoot et al. (2005b), *Streptomyces chibaensis* Suzuki et al. 1958 is a later heterotypic synonym of *Streptomyces corchorusii* Ahmad and Bhuiyan 1958 emend. Lanoot et al. 2005b.

According to Rule 24b of the *Bacteriological Code* (1990 Revision), if two names compete for priority and if both names are listed on the Approved Lists of Bacterial Names, the priority shall be determined by the date of the effective publication of the name before 1 January 1980. *Streptomyces chibaensis* and *Streptomyces corchorusii* are cited in the Approved Lists. The dates of effective publications are 1958. Consequently, to determine priority it is necessary to know the month (and perhaps the day) of the effective publications. *Streptomyces chibaensis* was effectively published in the *Journal of Antibiotics* (*Tokyo*) *Series A*, 1958, vol. 11, pp. 81–83, and *Streptomyces corchorusii* in the *Pakistan Journal of Biological and Agricultural Sciences*, 1958, vol. 1, pp. 137–143. It is certainly not easy to know the exact dates of publication of these articles. However, according to Rule 42 of the *Bacteriological Code* (1990 Revision), if the epithets are of the same date, the author who first unites the taxa has the right to choose one of them, and his choice must be followed. Lanoot et al. chose the epithet *corchorusii*.

106. **Streptomyces chrestomyceticus** Canevazzi and Scotti 1959, 248[AL]

chres.to.my.ce′ti.cus. N.L. n. *chrestomycinum* chrestomycin, name of an antibiotic; L. masc. suff. *-icus* suffix used with the sense of belonging to; N.L. masc. adj. *chrestomyceticus* belonging to chrestomycin.

Spore chains in Section *Spirales*. Spirals are often irregular and may become entangled. Mature spore chains generally are long with 10 to 50 or often more than 50 spores per chain. This morphology is seen on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar. Spore surface is smooth. Special morphological characteristics are moist droplets (hygroscopic droplets are sometimes found on oatmeal agar; one observer describes these as similar to sporangia of *Actinosporangium*). Coremia may also be formed on salts-starch agar or glycerol-asparagine agar.

Color of colony: aerial mass color in the Yellow or White color series on yeast-malt agar and glycerol-asparagine agar; White color series on salts-starch agar; Green or White color series on oatmeal agar. Nearest matching color tabs in the Yellow color series are ½ec to 1dc, pale yellow green; 2ba, pale yellow; and 2fb, light yellow. Nearest matching color tab in the Green color series is 1½ge, light grayish olive. Reverse side of colony with no distinctive pigments (colorless to pale yellow or light yellowish brown) on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar.

Color in medium: melanoid pigments are not formed in peptone-yeast-iron agar, tyrosine agar, tryptone-yeast broth, or Gause's medium no. 2. No pigment is found in medium in yeast-malt agar, oatmeal agar, salts-starch agar, or glycerol-asparagine agar.

D-Glucose, L-arabinose, D-mannitol, and D-fructose are utilized for growth. Utilization of sucrose, D-xylose, iso-inositol, and raffinose is doubtful. No growth or only traces of growth with rhamnose.

Type strain shows the highest sequence similarity to: *S. rimosus* subsp. *paromomycinus*, AJ621610, 100%; *S. albofaciens*, AB045880, 99.7%; *S. lydicus*, Y15507, 99.3%; *S. erumpens*, AJ621603, 99.3%; *S. chattanoogensis*, AJ621611, 99.2%; *S. sclerotialus*, AJ621608, 99.1%; *S. sioyaensis*, DQ026654, 99%.

Source: not known.

DNA G+C content (mol%): not known.

Type strain: AS 4.1657, ATCC 14947, CBS 745.72, BCRC 12173, DSM 40545, NBRC 13444, JCM 4735, NCAIM B.01478, NCIMB 8995, NRRL B-3293, NRRL B-3310, NRRL B-3672, NRRL-ISP 5545, RIA 1405.

Sequence accession no. (16S rRNA gene): AJ621609.

Further comments: the ISP description of this strain differs from the original description with respect to spiral spore chains versus very short *Rectiflexibiles* chains, Yellow or Green aerial mycelium versus White, and in growth on L-arabinose.

107. **Streptomyces chromofuscus** (Preobrazhenskaya, Blinov and Ryabova *in* Gauze, Preobrazhenskaya, Kudrina, Blinov, Ryabova and Sveshnikova 1957) Pridham, Hesseltine and Benedict 1958, 68[AL] ("*Actinomyces chromofuscus*" Preobrazhenskaya, Blinov and Ryabova *in* Gauze, Preobrazhenskaya, Kudrina, Blinov, Ryabova and Sveshnikova 1957, 176)

chro.mo.fus′cus. Gr. n. *chroma* color; L. adj. *fuscus* dark, tawny; N.L. masc. adj. *chromofuscus* dark or tawny colored.

Spore chains in Section *Spirales*. Mature spore chains are moderately long with 10–50, or often more than 50, spores per chain. This morphology is seen on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar. Spore surface is spiny.

Color of colony: aerial mass color in the Gray color series on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar. Reverse side of colony with no distinctive pigment (grayed yellow to olive brown) on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar.

Color in medium: melanoid pigments are formed in peptone-yeast-iron agar and tryptone-yeast broth, but not in tyrosine agar. Pigments other than melanoids or faint traces of yellow are not found in the medium in yeast-malt agar, oatmeal agar, salts-starch agar, or glycerol-asparagine agar.

D-Glucose, L-arabinose, D-xylose, iso-inositol, D-mannitol, D-fructose, and rhamnose are utilized for growth. Reports vary on utilization of raffinose and iso-inositol. No growth or only traces of growth on sucrose.

Type strain shows the highest sequence similarity to: *S. cinereospinus*, AB184648, 99.1%; *S. coeruleofuscus*, DQ026668, 99.1%.

Source: not known.

DNA G+C content (mol%): not known.

Type strain: AS 4.1451, ATCC 23896, CBS 682.68, DSM 40273, NBRC 12851, INA 13638/58, JCM 4354, LMG 19317, NRRL B-12175, NRRL-ISP 5273, RIA 1191, VKM Ac-974.

Sequence accession no. (16S rRNA gene): AB184194.

108. **Streptomyces chryseus** (Krasil'nikov, Korenyako and Nikitina *in* Krasil'nikov 1965) Pridham 1970, 10[AL] ("*Actinomyces chryseus*" Krasil'nikov, Korenyako and Nikitina *in* Krasil'nikov 1965, 224)

chry′se.us. N.L. masc. adj. *chryseus* (from Gr. masc. adj. *khruseos*) golden.

Spore chains in Section *Spirales* on oatmeal agar or salts-starch agar, but flexuous spore chains suggesting *Rectiflexibiles* morphology may be common on these media and are the predominant morphology on glycerol-asparagine agar. Sporulating aerial mycelium is poorly developed or absent on yeast-malt agar. Mature spore chains are moderately long with 10 to 50 or more spores per chain. Spore surface is smooth.

Color of colony: aerial mass color in the Red color series (5ca, light yellowish pink, or 3ca, pale orange-yellow) on oatmeal agar, salts-starch agar, and glycerol-asparagine agar in 14–21 d. White aerial mycelium may also be seen on these media. Reverse side of colony is pale yellow to light yellowish brown or orange-yellow on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar.

Color in medium: melanoid pigments are not formed in peptone-yeast-iron agar, tyrosine agar, and tryptone-yeast broth. Yellow pigment may be found in the medium in yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar. This pigment is not pH-sensitive when tested with 0.05 M NaOH or HCl.

D-Glucose, L-arabinose, and D-fructose are utilized for growth. Reports vary on utilization of D-xylose and iso-inositol. No growth or only traces of growth with D-mannitol, rhamnose, sucrose, and raffinose.

Type strain shows the highest sequence similarity to: *S. flavidovirens*, AB184270, 100%; *S. helvaticus*, AB184367, 100%; *S. albidochromogenes*, AB249953, 99.9%; *S. enissocaesilis*, AB249930, 99.1%.

Source: not known.

DNA G+C content (mol%): not known.

Type strain: AS 4.1694, ATCC 19829, CBS 678.72, DSM 40420, NBRC 13377, JCM 4737, NCIMB 10041, NRRL B-12347, NRRL-ISP 5420, RIA 1338, VKM Ac-200.

Sequence accession no. (16S rRNA gene): AY999787.

109a. **Streptomyces chrysomallus subsp. chrysomallus** Lindenbein 1952, 369[AL]

chry.so′mal.lus. N.L. masc. adj. *chrysomallus* (from Gr. masc. adj. *khrusomallos*) with golden wool.

Spore chains in Section *Rectiflexibiles*. Mature spore chains generally have 10–50 spores per chain. Typical morphology on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar. Spore surface is smooth (some surface irregularities are present, but are less distinct than on characteristic warty spores).

Color of colony: aerial mass color in the Yellow color series on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar. Reverse side of colony with no distinctive pigments (yellow to grayed-yellow) on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar; substrate pigment is not a pH indicator.

Color in medium: melanoid pigments are not formed in peptone-yeast-iron agar or tyrosine agar. Yellow pigment found in medium in yeast-malt agar and oatmeal agar; traces of yellow pigment may be formed in salts-starch agar and glycerol-asparagine agar. This pigment is not a pH indicator.

D-Glucose, L-arabinose, D-xylose, D-mannitol, D-fructose, and rhamnose are utilized for growth. No growth or only trace of growth on sucrose, iso-inositol, and raffinose.

For sequence similarity, see type strain of *Streptomyces anulatus*.

Source: not known.

DNA G+C content (mol%): not known.

Type strain: AS 4.1676, ATCC 11523, BCRC 11511, DSM 40128, NBRC 15393, IMET 41360, JCM 4296, LMG 20459, NRRL 2250, NRRL 2280, UNIQEM 127.

Sequence accession no. (16S rRNA gene): AB184644.

Further comments: Lanoot et al. (2005b) are of the opinion that *Streptomyces chrysomallus* subsp. *chrysomallus* Lindenbein 1952 is a later heterotypic synonym of *Streptomyces anulatus* (Beijerinck 1912) Waksman 1953. The type of the subspecies *Streptomyces chrysomallus* subsp. *chrysomallus* is automatically the type of *Streptomyces chrysomallus* Lindenbein 1952[AL]. Consequently, if an author agrees with Lanoot et al., *Streptomyces chrysomallus* must be considered as a later heterotypic synonym of *Streptomyces anulatus*. In expressing that opinion, Lanoot et al. (2005b) have placed the type of *Streptomyces chrysomallus* in a different species. In this case, Rule 37a of the *Bacteriological Code* (1990 Revision) applies and the authors should have dealt with the nomenclature and taxonomic position of *Streptomyces chrysomallus* subsp. *fumigatus* Frommer 1959. Authors who follow the proposal to treat the types of *Streptomyces anulatus* and *Streptomyces chrysomallus* (including the subspecies *Streptomyces chrysomallus* subsp. *chrysomallus*) as synonyms are not at liberty to use the name *Streptomyces chrysomallus* subsp. *fumigatus* and must make a taxonomic proposal for placing this subspecies in another species or subspecies.

109b. **Streptomyces chrysomallus subsp. fumigatus** Frommer 1959, 202[AL]

fu.mi.ga′tus. L. masc. part. adj. *fumigatus* smoked.

Produces the actinomycin C complex; inhibited by streptomycin; poor growth on Czapek's solution agar.

Type strain shows the highest sequence similarity to: *S. purpureus*, AJ781324, 99.9%; *S. herbaricolor*, AB184801, 99.5%; *S. indigoferus*, AB184214, 99.5%; *S. aburaviensis*, AY999779, 99.5%; *S. xanthocidicus*, AY999858, 99.4%; *S. psammoticus*, AY999862, 99.3%; *S. avellaneus*, AB184413, 99.2%. Type strain shows the highest sequence similarity to following *Kitasatospora*: *Kitasatospora kifunensis*, AB022874, 99.2%.

Source: not known.

DNA G+C content (mol%): not known.

Type strain: AS 4.1589, DSM 41424, NBRC 15394, JCM 3371, KCTC 9705, NRRL B-2289.

Sequence accession no. (16S rRNA gene): AB184645.

Further comments: Lanoot et al. (2005b) are of the opinion that *Streptomyces chrysomallus* subsp. *chrysomallus* Lindenbein 1952 is a later heterotypic synonym of *Streptomyces anulatus* (Beijerinck 1912) Waksman 1953. The type of the subspecies *Streptomyces chrysomallus* subsp. *chrysomallus* is automatically the type of *Streptomyces chrysomallus* Lindenbein 1952[AL]. So, in expressing that opinion Lanoot et al. have placed the type of *Streptomyces chrysomallus* in a different species. In this case, Rule 37a of the *Bacteriological Code* (1990 Revision) applies and the authors should have dealt with the nomenclature and taxonomic position of *Streptomyces chrysomallus* subsp. *fumigatus* Frommer 1959. Authors who follow the proposal to treat the types of *Streptomyces anulatus* and *Streptomyces chrysomallus* (including the subspecies *Streptomyces chrysomallus* subsp. *chrysomallus*) as synonyms are not at liberty to use the name *Streptomyces chrysomallus* subsp. *fumigatus* and must make a taxonomic proposal for placing this subspecies in another species or subspecies.

110. **Streptomyces cinereorectus** Terekhova and Preobrazhenskaya 1986, 574[VP] (Effective publication: Terekhova and Preobrazhenskaya *in* Gause, Preobrazhenskaya, Sveshnikova, Terekhova and Maximova 1983.) emend. Lanoot, Vancanneyt, Dawyndt, Cnockaert, Zhang, Huang, Liu and Swings 2005a, 8 (Effective publication: Lanoot, Vancanneyt, Dawyndt, Cnockaert, Zhang, Huang, Liu and Swings 2004, 88.)

ci.ne.re.o.rec′tus. L. adj. *cinereus* similar to ashes, ash-colored; L. adj. *rectus* straight; N.L. masc. adj. *cinereorectus* ash-colored, straight.

Spore chains are straight, short, up to 10 spores per chain (*Rectiflexibiles*); spores are smooth. On mineral agar 1, oatmeal agar, and starch-ammonia agar: moderate or poor growth; aerial mycelium is poor, light gray; substrate mycelium colorless; no diffusible pigment. Glycerol-nitrate agar: abundant growth; aerial mycelium is gray; substrate mycelium grayish yellowish; no diffusible pigment. On glycerol-asparagine agar: moderate growth; aerial mycelium is gray; substrate mycelium colorless; no diffusible pigment. On organic agar 2: no aerial mycelium; substrate mycelium is colorless to yellowish; no diffusible pigment. Melanoid pigments are not formed. Good growth on glucose; moderate growth on fructose and mannitol; poor growth on raffinose; no growth on rhamnose or xylose. Forms antibiotic penicillin N. Contains LL-A_2pm and no diagnostic sugars in whole-cell hydrolysates.

Type strain shows the highest sequence similarity to: *S. argenteolus*, AB045872, 100%; *S. cavourensis* subsp. *washingtonensis*, DQ026671, 100%; *S. lavendulae* subsp. *lavendulae*, AB184080, 100%; *S. cyaneofuscatus*, AB184860, 99.9%; *S. fulvorobeus*, AB184711, 99.9%; *S. lipmanii*, AB184148, 99.9%; *S. griseoplanus*, AY999894, 99.9%; *S. alboviridis*, AB184256, 99.9%; *S. flavofuscus*, AB249935, 99.9%; *S. fimicarius*, AY999784, 99.9%; *S. praecox*, AB184293, 99.9%; *S. griseolus*, AB184768, 99.9%; *S. anulatus*, DQ026637, 99.9%; *S. microflavus*, DQ445795, 99.9%; *S. halstedii*, EF178695, 99.8%; *S. pluricolorescens*, DQ442540, 99.8%; *S. sindenensis*, AB184759, 99.8%; *S. griseinus*, AB184205, 99.8%; *S. badius*, AY999783, 99.8%; *S. mediolani*, AB184674, 99.8%; *S. acrimycini*, AY999889, 99.8%; *S. rubiginosohelvolus*, AB184240, 99.8%; *S. albovinaceus*, AB249958, 99.7%; *S. flavogriseus*, AJ494864, 99.7%; *S. parvus*, DQ442537, 99.7%; *S. griseus* subsp. *griseus*, AY207604, 99.7%; *S. floridae*, AB184656, 99.7%; *S. baarnensis*, EF178688, 99.7%; *S. luridiscabiei*, AF361784, 99.7%; *S. flavovirens*, DQ026635, 99.7%; *S. californicus*, AB184755, 99.7%; *S. globisporus* subsp. *globisporus*, EF178686, 99.7%; *S. yanii*, AB006159, 99.5%; *S. pulveraceus*, AB184806, 99.5%; *S. nitrosporeus*, EF178680, 99.5%; *S. finlayi*, AY999788, 99.4%; *S. olivoviridis*, AB184227, 99.4%; *S. atroolivaceus*, AJ781320, 99.4%; *S. bacillaris*, AB184439, 99.4%; *S. sanglieri*, AB249945, 99.3%; *S. clavifer*, DQ026670, 99.3%; *S. albolongus*, AB184425, 99.2%; *S. gelaticus*, DQ026636, 99.2%; *S. celluloflavus*, AB184476, 99.2%; *S. mutomycini*,

AB249951, 99.2%; *S. griseobrunneus*, AB249912, 99.2%; *S. atratus*, DQ026638, 99.2%; *S. cremeus*, AB184124, 99%; *S. candidus*, DQ026663, 99%; *S. cavourensis* subsp. *cavourensis*, DQ445791, 99%; *S. mauvecolor*, AB184532, 99%; *S. spiroverticillatus*, AB184814, 99%.

Source: not known.

DNA G+C content (mol%): not known.

Type strain: AS 4.1622, ATCC 43679, DSM 41469, NBRC 15395, INA 5202, JCM 6916.

Sequence accession no. (16S rRNA gene): AB184646.

Further comments: according to Lanoot et al. (2004), *Streptomyces cinereorectus* Terekhova and Preobrazhenskaya 1986 is an earlier heterotypic synonym of *Streptomyces cochleatus* Nakagaito et al. 1993b.

111a. **Streptomyces cinereoruber subsp. cinereoruber** Corbaz, Ettlinger, Keller-Schierlein and Zähner 1957b, 331[AL]

ci.ne.re.o.ru′ber. L. adj. *cinereus* similar to ashes, ash-colored; L. adj. *ruber* red; N.L. masc. adj. *cinereoruber* ashy red.

Spore chains in Section *Rectiflexibiles*. Mature spore chains are long, often more than 50 spores per chain. This morphology is found on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar. Spore surface is smooth.

Color of colony: aerial mass color in the Gray color series on oatmeal agar; Red series or Gray series on yeast-malt agar, salts-starch agar, and glycerol-asparagine agar. Nearly all color tabs selected by collaborators are near-gray containing some pink or red. Reverse side of colony is the characteristic grayed yellow modified by red on yeast-malt agar or red to violet on oatmeal agar, salts-starch agar, and glycerol-asparagine agar. A change from red to blue reverse color by addition of 0.05 M NaOH is reported by one observer only.

Color in medium: melanoid pigments are formed in peptone-yeast-iron agar and tryptone-yeast broth. Pigments other than melanoids are probably not formed in yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar.

D-Glucose, L-arabinose, and D-xylose are utilized for growth. No growth or only traces of growth on sucrose, iso-inositol, D-mannitol, rhamnose, and raffinose. Growth on D-fructose is doubtful.

Type strain shows the highest sequence similarity to: *S. violaceorectus*, AB184314, 99.7%; *S. showdoensis*, AB184389, 99.5%; *S. viridobrunneus*, AJ781372, 99.4%; *S. bikiniensis*, X79851, 99.3%; *S. tanashiensis*, AJ781362, 99.1%; *S. nashvillensis*, AB184286, 99.1%; *S. vietnamensis*, DQ311081, 99%; *S. litmocidini*, AB184149, 99%.

Source: not known.

DNA G+C content (mol%): not known.

Type strain: AS 4.1698, ATCC 19740, CBS 479.68, BCRC 11816, DSM 40012, NBRC 12756, JCM 4205, JCM 4572, KCTC 9706, NCIMB 9797, NRRL 2589, NRRL-ISP 5012, RIA 1021, RIA 535, UNIQEM 116, VKM Ac-1860.

Sequence accession no. (16S rRNA gene): AB184121.

111b. **Streptomyces cinereoruber subsp. fructofermentans** Corbaz, Ettlinger, Keller-Schierlein and Zähner 1957b, 331[AL]

fruc.to.fer.men′tans. L. n. *fructus* fruit; L. part. adj. *fermentans* fermenting; N.L. part. adj. *fructofermentans* fruit fermenting (but pertaining to ability of the organism to utilize L-rhamnose, D-fructose, and D-sorbitol).

Moderate growth on Czapek's solution agar; vegetative growth and diffusible pigment in tints and shades of red on some media. Produces cinerobin A and cinerobin B; inhibited by streptomycin.

Type strain shows no sequence similarity over 99%.

Source: not known.

DNA G+C content (mol%): not known.

Type strain: AS 4.1593, DSM 40692, NBRC 15396, JCM 4956, KCTC 9707, NRRL 2588.

Sequence accession no. (16S rRNA gene): AY999758.

112. **Streptomyces cinereospinus** Terekhova, Preobrazhenskaya and Gause 1986, 574[VP] (Effective publication: Terekhova, Preobrazhenskaya and Gause *in* Gause, Preobrazhenskaya, Sveshnikova, Terekhova and Maximova 1983.)

ci.ne.re.o.spi′nus. L. adj. *cinereus* similar to ashes, ash-colored; L. adj. *spineus* spiny; N.L. masc. adj. *cinereospinus (sic)* ash-colored, spiny.

Spore chains are spirals (*Spirales*); spores are spiny, spines are short. On mineral agar 1: aerial mycelium is gray or greenish gray; substrate mycelium is colorless or, after several days, light yellow; no diffusible pigment. On glycerol-nitrate agar: no aerial mycelium; substrate mycelium and diffusible pigment are blue-green to dark gray-green. On oatmeal agar: aerial mycelium is greenish gray; substrate mycelium is colorless or, after several days, spotted green; no diffusible pigment. On starch-ammonia agar: aerial mycelium is gray, greenish gray; substrate mycelium is colorless; no diffusible pigment. On glycerol-asparagine agar: no aerial mycelium; substrate mycelium and diffusible pigment are colorless or light pink to blue-green. On glucose-asparagine agar: no aerial mycelium, substrate mycelium is colorless; diffusible pigment is light pink. On organic agar 2: no aerial mycelium; substrate mycelium colorless; no diffusible pigment. Melanoid pigments are not formed. Moderate growth on glycerin and glucose; poor growth on mannitol, rhamnose, and fructose; no growth on raffinose, inositol, arabinose, xylose, or sugar. Forms antibiotic 1719 from group azotomycin.

Type strain shows the highest sequence similarity to: *S. coeruleofuscus*, DQ026668, 99.2%; *S. chromofuscus*, AB184194, 99.1%.

Source: not known.

DNA G+C content (mol%): not known.

Type strain: AS 4.163, ATCC 43680, DSM 41470, NBRC 15397, INA 1719, JCM 6917, VKM Ac-1215.

Sequence accession no. (16S rRNA gene): AB184648.

113. **Streptomyces cinereus** (Cross, Lechevalier and Lechevalier 1963) Goodfellow, Williams and Alderson 1986a, 574[VP] (Effective publication: Goodfellow, Williams and Alderson 1986a, 53.) (*Microellobosporia cinerea* Cross, Lechevalier and Lechevalier 1963, 428)

ci.ne′re.us. L. masc. adj. *cinereus* ash-colored.

Short straight spore chains (2–5); spore surface is smooth; spores are round to oval and borne in both the

substrate and aerial mycelium. Spores sizes vary from 1.5 to 3.5 μm (mean diameter 2.5 μm). Extensively branched substrate and aerial mycelium. The aerial spore mass is white; reverse color is red-orange and the pigment is sensitive to pH. Does not form melanin pigments. Adenine, esculin, allantoin, arbutin, casein, hypoxanthine, starch, testosterone, tyrosine, and urea are degraded, but chitin, elastin, guanine, lecithin, pectin, xanthine, and xylan are not. Hydrogen sulfide is produced but nitrate is not reduced. L-Arabinose, cellobiose, D-fructose, D-galactose, D-glucose, *myo*-inositol, inulin, D-lactose, mannitol, D-mannose, melibiose, melezitose, raffinose, L-rhamnose, salicin, sucrose, trehalose, and D-xylose are used as sole carbon sources, but adonitol and xylitol are not. Grows on L-arginine, L-histidine, potassium nitrate, L-threonine, and L-valine, but not on DL-α-amino-n-butyric acid, L-cysteine, L-hydroxyproline, L-methionine, and L-phenylalanine, as sole nitrogen source. Growth occurs at 10–37°C but not at 4 or 45°C. Tolerant to phenol (0.1%, w/v) and sodium chloride (7%, w/v) but not to sodium azide (0.01%, w/v). Resistant to rifampin but sensitive to sodium chloride (10%, w/v). Antimicrobial activity is shown against *Bacillus subtilis* NCIB 3610, but not against *Aspergillus niger* LIV 131, *Candida albicans* CBS 562, *Escherichia coli* NCIB 9132, *Micrococcus luteus* NCIB 196, *Pseudomonas fluorescens* NCIB 9046[T], *Saccharomyces cerevisiae* CBS 1171[T], or *Streptomyces murinus* ISP 5091. The peptidoglycan contains LL-A_2pm as the major diamino acid and is of the A3γ type (Stackebrandt et al., 1981). Contains major amounts of hexa- and octahydrogenated menaquinones with nine isoprene units (Collins et al., 1984).

Type strain shows no sequence similarity over 99%.

Source: not known.

DNA G+C content (mol%): 67.6.

Type strain: AS 4.1672, ATCC 15840, CBS 356.67, BCRC 11616, DSM 43033, IFM 1137, IFM 1237, NBRC 12247, IMET 43557, JCM 3040, KCC A-0040, KCTC 9066, NCIMB 9586, NRRL B-2909, VKM Ac-812.

Sequence accession no. (16S rRNA gene): AB184072.

114. **Streptomyces cinerochromogenes** Miyairi, Tajashima, Shimizu and Sakai 1966, 58[AL]

ci.ne.ro.chro.mo′ge.nes. L. adj. *cinereus* similar to ashes, ash-colored; Gr. n. *chroma* color; N.L. suff. *-genes* (from Gr. v. *gennaô* to produce) producing; N.L. part. adj. *cinerochromogenes* producing ashy color.

Produces the anti-bacterial antibiotics cineromycin A and cineromycin B. Type strain shows no sequence similarity over 99%.

Source: not known.

DNA G+C content (mol%): not known.

Type strain: Fuji 50, AS 4.162, ATCC 33339, DSM 41651, NBRC 13822, JCM 3385, NRRL B-16928.

Sequence accession no. (16S rRNA gene): AB184507.

115. **Streptomyces cinnabarinus** (Ryabova and Preobrazhenskaya *in* Gauze, Preobrazhenskaya, Kudrina, Blinov, Ryabova and Sveshnikova 1957) Pridham, Hesseltine and Benedict 1958, 62[AL] ("*Actinomyces cinnabarinus*" Ryabova and Preobrazhenskaya *in* Gauze, Preobrazhenskaya, Kudrina, Blinov, Ryabova and Sveshnikova 1957, 196)

cin.na.ba′ri.nus. N.L. masc. adj. *cinnabarinus* of cinnabar, referring to the vermilion color of vegetative mycelium and diffusible pigment.

Spore chains in Section *Rectiflexibiles* but a very small proportion of strongly flexuous spore chains may suggest *Retinaculiaperti* or *Spiral* morphology on yeast-malt agar or oatmeal agar. Mature spore chains are generally long, often with more than 50 spores per chain. This morphology is seen on yeast-malt agar, oatmeal agar, and salts-starch agar. Sporulating aerial mycelium is poorly developed or absent on glycerol-asparagine agar and yeast-malt agar. Spore surface is smooth; spores of phalangeal type are common.

Color of colony: aerial mass color in the Red color series (3ca, pale orange yellow) on oatmeal agar and salts-starch agar and also on yeast-malt agar when adequate sporulation occurs on this medium. White aerial mycelium may also be seen on these media. Mature sporulating mycelium is inadequate for aerial mass color determination on glycerol-asparagine agar. Reverse side of colony is pale yellow or grayish on yeast-malt agar; yellow is modified by red to yellowish pink or reddish gray on oatmeal agar and to reddish orange, grayish red or reddish brown on salts-starch agar and glycerol-asparagine agar. Substrate pigment is not a pH indicator or is changed only slightly by addition of 0.05 M NaOH or HCl.

Color in medium: melanoid pigments are formed in peptone-yeast-iron agar and tryptone-yeast broth. Red or lavender pigment is found in the medium in oatmeal agar and salts-starch agar. One observer only found this pigment to be pH-sensitive when tested with 0.05 M NaOH and recorded a change from light violet to yellow-colorless. Two observers recorded no change.

D-Glucose, L-arabinose, D-xylose, iso-inositol, D-mannitol, D-fructose, rhamnose, sucrose, and raffinose are utilized for growth.

Type strain shows the highest sequence similarity to: *Streptomyces griseoruber*, AB184209, 99%.

Source: not known.

DNA G+C content (mol%): not known.

Type strain: AS 4.1590, ATCC 23617, ATCC 25440, CBS 671.69, DSM 40467, NBRC 13028, INA 1242, JCM 4463, NRRL B-12382, NRRL-ISP 5467, PCM 2311, RIA 1220, VKM Ac-1904.

Sequence accession no. (16S rRNA gene): AB184266.

116. **Streptomyces cinnamonensis** Okami *in* Maeda, Okami, Kosaka, Taya and Umezawa 1952, 572[AL]

cin.na.mo.nen′sis. L. n. *cinnamum* cinnamon; N.L. masc. adj. *cinnamonensis* belonging to cinnamon, referring to the color of the aerial mycelium.

Spore chains of typical *Retinaculum-Apertum* type; spores phalangiform. NaCl tolerance >4%, but <7%. Produces actihiazic acid, a biotin antagonist and anti-mycobacterial antibiotic; exhibits anti-fungal activity.

Type strain shows the highest sequence similarity to: *S. pseudoechinosporeus*, AB184100, 99.9%; *S. hiroshimensis*, AB184789, 99.7%; *S. blastmyceticus*, AY999802, 99.3%; *S. caeruleus*, EF178675, 99.3%; *S. aureoversilis*, AB184855, 99.2%; *S. werraensis*, DQ442558, 99.1%; *S. lilacinus*, AB184819, 99.1%; *S. abikoensis*, AB184537, 99.1%; *S. ardus*,

AB184864, 99%; *S. biverticillatus*, AJ781381, 99%.

Source: not known.

DNA G+C content (mol%): not known.

Type strain: 154-T4, NIHJ 35, AS 4.1619, ATCC 12308, CBS 411.63, CECT 3198, DSM 40803, HUT 6050, NBRC 15873, JCM 4019, KCTC 9708, NCIMB 12604, NRRL B-1588, VKM Ac-1912.

Sequence accession no. (16S rRNA gene): AB184707.

117. **Streptomyces cinnamoneus** (Benedict, Dvonch, Shotwell, Pridham and Lindenfelser 1952) Witt and Stackebrandt 1991, 456[VP] (Effective publication: Witt and Stackebrandt 1990, 370.) ["*Streptomyces cinnamoneus*" Benedict, Dvonch, Shotwell, Pridham and Lindenfelser 1952, 591; "*Streptomyces cinnamomeus* forma *cinnamomeus*" (*sic*) Pridham, Shotwell, Stodola, Lindenfelser, Benedict and Jackson 1956, 576; "*Streptoverticillium cinnamomeus* forma *cinnamomeus*" (*sic*) Baldacci 1958, 25; "*Verticillomyces cinnamomeus* forma *cinnamomeus*" (*sic*) Shinobu 1965, 104; *Streptoverticillium cinnamoneum* Baldacci, Farina and Locci 1966, 158]

cin.na.mo′ne.us. L. n. *cinnamum* cinnamon; N.L. adj. *cinnamoneus* cinnamon-colored (after the color of the aerial mycelium).

Spore chains in *Umballate Monoverticillate* (= *Streptomyces* section *Verticillati*, biverticillate). Mature spore chains generally have 3–10, often more than 10, spores per chain. This morphology is seen on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar. Spore surface is smooth.

Color of colony: aerial mass color in the Red color series (grayish yellowish pink) on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar. Reverse side of colony with no distinctive pigment (grayish yellow to yellow-brown or brown) on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar.

Color in medium: melanoid pigments are not formed in peptone-yeast-iron agar, tyrosine agar, or tryptone-yeast broth. No pigment is found in the medium in yeast-malt agar, oatmeal agar, salts-starch agar, or glycerol-asparagine agar. Vegetative growth is generally poor on Pridham and Gottlieb's carbon utilization medium plus D-glucose. Reports form collaborators vary from no growth to slight growth with other carbon sources, but good growth is not observed on any of the following: L-arabinose, sucrose, D-xylose, iso-inositol, D-mannitol, D-fructose, rhamnose, or raffinose. Poor growth on all carbon sources including D-glucose may indicate a requirement for a growth factor not present in the basal medium.

Type strain shows the highest sequence similarity to: *S. cinnamoneus*, X53171, 99.8%; *S. cinnamoneus*, X53165, 99.6%; *S. olivoreticuli*, X53166, 99.6%; *S. cinnamoneus* subsp. *albosporus* DSM 40897^T, 99.6%; *S. cinnamoneus* subsp. *lanosus* DSM 40898^T, 99.6%; *S. cinnamoneus* subsp. *sparsus* DSM 40899^T, 99.6%; *S. roseoverticillatus* DSM 40845^T, 99.6%; *S. olivoreticuli* subsp. *cellulophilus*, X53166, 99.5%; *S. lavendofoliae* DSM 40217^T, 99.5%; *S. parvisporogenes*, DSM 40473^T, 99.5%; *S. baldacii*, X53164, 99.4%; *S. albireticuli*, DSM 40051^T, 99.4%; *S. alboverticillatus*, DSM 41678^T, 99.4%; *S. hiroshimensis*, DSM 40037^T, 99.4%; *S. lilacinus*, DSM 40254^T, 99.4%; *S. blastmyceticus*, DSM 40029^T, 99.3%; *S. gobitricini*, DSM 41701^T, 99.3%; *S. lavendulocolor*, DSM 40216^T, 99.3%; *S. septatus*, DSM 40577^T, 99.3%; *S. eurocidicus*, DSM 40604^T, 99.2%; *S. kashmirensis*, DSM 40336^T, 99.2%; *S. luridus*, DSM 40081^T, 99.2%; *S. kasugaensis*, AB024441, 99.1%; *S. kasugaensis*, AB024442, 99.1%; *Streptomyces* sp., AF012739, 99.1%; *S. salmonis*, X53169, 99.1%; *S. baldacii*, X53164, 99.1%; *S. roseoverticillatus*, X53164, 99.1%; *S. griseoruber*, DSM 40181^T, 99.1%; *S. luteoverticillatus*, DSM 40038^T, 99.1%; *S. celluloflavus*, DSM 40839^T, 99.1%; *S. ehimensis*, DSM 40253^T, 99.1%; *S. mauvecolor*, DSM 41702^T, 99.1%; *S. melanogenes*, DSM 40192^T, 99.1%; *S. michiganensis*, DSM 40015^T, 99.1%; *S. noboritoensis*, DSM 40223^T, 99.1%; *S. roseofulvus*, DSM 40172^T, 99.1%; *S. roseolus*, DSM 40174^T, 99.1%; *S. sapporonensis*, DSM 41675^T, 99.1%; *S. viridoflavum*, DSM 40237^T, 99.1%; *S. xanthochromogenes*, DSM 40111^T, 99.1%; *S. alanosinicus*, DSM 40606^T, 99%; *S. ardus*, DSM 40527^T, 99%; *S. aureoversile*, DSM 40387^T, 99%; *S. filamentosus*, DSM 40022^T, 99%; *S. griseoverticillatus*, DSM 40507^T, 99%; *S. hachijoensis*, DSM 40114^T, 99%; *S. netropsis*, DSM 40259^T, 99%; *S. roseosporus*, DSM 40122^T, 99%; *S. thioluteus*, DSM 40027^T, 99%; *S. varsoviensis*, DSM 40346^T, 99%.

Source: not known.

DNA G+C content (mol%): not known.

Type strain: ATCC 11874, AS 4.1084, AS 4.1706, ATCC 23897, CBS 293.64, CBS 683.68, BCRC 12169, CCUG 11122, CECT 3258, DSM 40005, HAMBI 1067, NBRC 12852, IMET 41381, JCM 4152, JCM 4633, LMG 5971, NCIMB 8851, NRRL B-1285, NRRL-ISP 5005, RIA 1102, RIA 360, VKM Ac-876.

Sequence accession no. (16S rRNA gene): AB184850.

Further comments: in violation of Rule 33c of the *Bacteriological Code* (1990 Revision), on Validation List no. 38, *Streptomyces cinnamoneus* is proposed as a *nomen revictum* (basonym: "*Streptomyces cinnamoneus*" Benedict et al. 1952).

Witt and Stackebrandt proposed to transfer *Streptoverticillium cinnamoneum* (Benedict et al. 1952) Baldacci et al. 1966 to the genus *Streptomyces* as *Streptomyces cinnamoneus* (Benedict et al. 1952) Witt and Stackebrandt 1991. However, Validation List no. 38 does not include formal propositions about *Streptoverticillium cinnamoneum* subsp. *albosporum* Thirumalachar 1968, *Streptoverticillium cinnamoneum* subsp. *cinnamoneum* (Benedict et al. 1952) Baldacci et al. 1966, *Streptoverticillium cinnamoneum* subsp. *lanosum* Thirumalachar 1968 and *Streptoverticillium cinnamoneum* subsp. *sparsum* Thirumalachar 1968.

According to Hatano et al. (2003), *Streptomyces cinnamoneus* (Benedict et al. 1952) Witt and Stackebrandt 1991 is an earlier heterotypic synonym of *Streptomyces griseoverticillatus* (Shinobu and Shimada 1962) Witt and Stackebrandt 1991, of *Streptomyces hachijoensis* (Hosoya et al. 1952) Witt and Stackebrandt 1991, and of *Streptomyces sapporonensis* (Locci and Schofield 1989) Witt and Stackebrandt 1991.

118. **Streptomyces cirratus** Koshiyama, Okanishi, Ohmori, Miyake, Tsukiura, Matsuzaki and Kawaguchi 1963, 65[AL]

cir.ra′tus. L. masc. adj. *cirratus* curled, having ringlets.

Spore chains in Section *Retinaculiaperti*. Spore chains often with terminal loops, primitive spirals, or sometimes well-defined coils of several turns. Mature spore chains are

generally long, often with more than 50 spores per chain. This morphology is seen on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar. Spore surface is smooth. Special morphological characteristics: knots and moist, nest-like tangles may be seen in long aerial hyphae or moist droplets may form around terminal coils.

Color of colony: aerial mass color usually in the Red color series (5dc or 5cb, grayish yellowish pink, or 5ge, light grayish reddish brown) on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar; aerial mycelium sometimes is in the Gray color series (5fe, light grayish reddish brown) on these media. Reverse side of colony with no distinctive pigments (grayish yellow to light yellowish brown on yeast-malt agar; nearly colorless, pale grayish yellow, or light olive brown on oatmeal agar, salts-starch agar, and glycerol-asparagine agar).

Color in medium: melanoid pigments are usually formed in peptone-yeast-iron agar and tryptone-yeast broth, but are formed weakly or not at all in tyrosine agar. Yellow pigment is usually found in the medium in yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar. This pigment is not pH-sensitive when tested with 0.05 M NaOH or HCl.

D-Glucose, L-arabinose, D-xylose, and D-fructose are utilized for growth. No growth or only traces of growth with iso-inositol, D-mannitol, rhamnose, sucrose, or raffinose.

Type strain shows the highest sequence similarity to: *S. vinaceus*, AB184394, 100%; *S. spororaveus*, AJ781370, 99.9%; *S. nojiriensis*, AJ781355, 99.9%; *S. xanthophaeus*, DQ442560, 99.8%; *S. sporoverrucosus*, DQ442544, 99.7%; *S. goshikiensis*, EF178693, 99.7%; *S. cinnamonensis*, AB184707, 99.6%; *S. avidinii*, AB184395, 99.6%; *S. colombiensis*, DQ026646, 99.6%; *S. subrutilus*, X80825, 99.5%; *S. virginiae*, D85119, 99.4%.

Source: not known.

DNA G+C content (mol%): not known.

Type strain: AS 4.1679, ATCC 14699, CBS 699.72, DSM 40479, NBRC 13398, JCM 4738, KCTC 9709, NRRL B-3250, NRRL-ISP 5479, RIA 1359, VKM Ac-620.

Sequence accession no. (16S rRNA gene): AY999794.

119. **Streptomyces ciscaucasicus** Sveshnikova 1986, 574[VP] (Effective publication: Sveshnikova *in* Gause, Preobrazhenskaya, Sveshnikova, Terekhova and Maximova 1983.)

cis.cau.ca.si′cus. N.L. masc. adj. *ciscaucasicus* of or pertaining to Ciscaucasus.

Spore chains are spiral (*Spirales*); spores are spiny, spines are medium sized. On mineral agar 1, glycerol-nitrate agar, and oatmeal agar: aerial mycelium is light gray, gray; substrate mycelium is yellowish red to red; no diffusible pigment. On starch-ammonia agar: aerial mycelium is gray, sometimes with brown shadow; substrate mycelium is red with yellowish or brownish shadow; no diffusible pigment. On glycerol-asparagine agar: aerial mycelium is light gray to gray; substrate mycelium is brownish red, raspberry red; no diffusible pigment. On organic agar 2: aerial mycelium is white to light gray; substrate mycelium is yellowish, reddish, yellowish reddish, light gray-brownish red; no diffusible pigment. Melanoid pigments are not formed. Good growth on glucose, arabinose, sucrose, xylose, fructose, rhamnose, raffinose, and mannitol. Produces antibiotic pigment of the prodigiosin group. Pigment of substrate mycelium can act as an indicator: yellow under alkaline reaction; dark pink to pinkish red under acidic reaction.

Type strain shows the highest sequence similarity to: *S. canus*, AY999775, 99.8%; *S. pseudovenezuelae*, AB184233, 99.2%; *S. resistomycificus*, AB184166, 99.1%.

Source: not known.

DNA G+C content (mol%): not known.

Type strain: AS 4.1603, ATCC 23626, ATCC 23918, CBS 839.68, DSM 40275, NBRC 12872, IMET 42945, INA 2022/55, JCM 4384, NRRL B-16362, NRRL-ISP 5275, RIA 1193, VKM Ac-1184, VKM Ac-998.

Sequence accession no. (16S rRNA gene): AY508512.

120. **Streptomyces citreofluorescens** (Korenyako, Krasil'nikov, Nikitina and Sokolova 1960) Pridham 1970, 10[AL] ("*Actinomyces citreofluorescens*" Korenyako, Krasil'nikov, Nikitina and Sokolova *in* Rautenshtein 1960, 156)

cit.re.o.flu.o.res′cens. L. n. *citrus* the citrus, the citrontree; N.L. v. *fluoresco* fluoresce; N.L. part. adj. *citreofluorescens* with a yellow fluorescence.

Spore chains in Section *Rectiflexibiles*. Mature spore chains are long, often with more than 50 spores per chain. This morphology is seen on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar. Spore surface is smooth.

Color of colony: aerial mass color in the Yellow color series on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar. Reverse side of colony with no distinctive pigments (light yellow or grayish yellow to light olive brown) on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar.

Color in medium: melanoid pigments are not produced in peptone-yeast-iron agar, tyrosine agar, and tryptone-yeast broth. Yellow pigment is found in medium in glycerol-asparagine agar and yellow or green pigment is found in medium in yeast-malt agar, oatmeal agar, and salts-starch agar. This pigment is not a pH indicator.

D-Glucose, L-arabinose, D-xylose, D-mannitol, D-fructose, and rhamnose are utilized for growth. No growth or only traces of growth on sucrose, iso-inositol, or raffinose.

For sequence similarity, see type strain of *Streptomyces anulatus*.

Source: not known.

DNA G+C content (mol%): not known.

Type strain: AS 4.1652, ATCC 15858, ATCC 23898, CBS 684.68, BCRC 11820, DSM 40265, NBRC 12853, INMI 2292, JCM 4356, KCTC 9710, LMG 20475, NCIMB 9806, NRRL B-3362, NRRL-ISP 5265, RIA 1187, RIA 648, VKM Ac-96.

Sequence accession no. (16S rRNA gene): AB184195.

Further comments: according to Lanoot et al. (2005b), *Streptomyces citreofluorescens* (Korenyako et al. 1960) Pridham 1970 is a later heterotypic synonym of *Streptomyces anulatus* (Beijerinck 1912) Waksman 1953 emend. Lanoot et al. 2005b.

121. **Streptomyces clavifer** (Millard and Burr 1926) Waksman *in* Waksman and Lechevalier 1953, 103[AL] ("*Actinomyces clavifer*" Millard and Burr 1926, 630)

cla′vi.fer. L. n. *clava* club; L. suff. *-fer* carrying, bearing; N.L. masc. adj. *calvifer* club-bearing.

Poor to fair growth on Czapek's solution agar; NaCl tolerance >10%, but <13%.

Type strain shows the highest sequence similarity to: *S. olivoviridis*, AB184227, 100%; *S. atroolivaceus*, AJ781320, 100%; *S. mutomycini*, AB249951, 99.8%; *S. finlayi*, AY999788, 99.8%; *S. fimicarius*, AY999784, 99.5%; *S. praecox*, AB184293, 99.5%; *S. flavofuscus*, AB249935, 99.5%; *S. anulatus*, DQ026637, 99.5%; *S. badius*, AY999783, 99.4%; *S. griseoplanus*, AY999894, 99.4%; *S. cavourensis* subsp. *washingtonensis*, DQ026671, 99.4%; *S. acrimycini*, AY999889, 99.4%; *S. lavendulae* subsp. *lavendulae*, AB184080, 99.4%; *S. sindenensis*, AB184759, 99.4%; *S. pluricolorescens*, DQ442540, 99.4%; *S. rubiginosohelvolus*, AB184240, 99.4%; *S. griseinus*, AB184205, 99.4%; *S. californicus*, AB184755, 99.4%; *S. mediolani*, AB184674, 99.4%; *S. globisporus* subsp. *globisporus*, EF178686, 99.3%; *S. fulvorobeus*, AB184711, 99.3%; *S. albovinaceus*, AB249958, 99.3%; *S. argenteolus*, AB045872, 99.3%; *S. parvus*, DQ442537, 99.3%; *S. floridae*, AB184656, 99.3%; *S. lipmanii*, AB184148, 99.3%; *S. alboviridis*, AB184256, 99.3%; *S. cinereorectus*, AB184646, 99.3%; *S. baarnensis*, EF178688, 99.3%; *S. cyaneofuscatus*, AB184860, 99.3%; *S. microflavus*, DQ445795, 99.3%; *S. luridiscabiei*, AF361784, 99.2%; *S. candidus*, DQ026663, 99.2%; *S. flavovirens*, DQ026635, 99.2%; *S. griseolus*, AB184768, 99.2%; *S. griseus* subsp. *griseus*, AY207604, 99.2%; *S. flavogriseus*, AJ494864, 99.1%; *S. bacillaris*, AB184439, 99.1%; *S. halstedii*, EF178695, 99.1%; *S. albolongus*, AB184425, 99%; *S. griseobrunneus*, AB249912, 99%; *S. pulveraceus*, AB184806, 99%; *S. celluloflavus*, AB184476, 99%.

Source: not known.

DNA G+C content (mol%): not known.

Type strain: AS 4.1604, CBS 101.27, DSM 40843, NBRC 15398, JCM 5059, NRRL B-2557.

Sequence accession no. (16S rRNA gene): DQ026670.

122. **Streptomyces clavuligerus** Higgens and Kastner 1971, 330[AL]

cla.vu.li.ge′rus. L. fem. n. *clavula* little club; N.L. suff. *-gerus* bearing; N.L. masc. adj. *clavuligerus* bearing little clubs.

Produces aerial mycelium which is composed of a network of sympodially branched, aerial hyphae that eventually segment into spores. Short, clavate, side branches are formed that usually produce from one to four spores each. Spore chain morphology is classified in the *Rectus-flexibilis* section. Spores are oblong to short-cylindrical averaging 0.64 by 1.53 μm in size, with smooth spore surfaces. Neither fragmentation of hyphae nor formation of spores occurs in the substrate mycelium.

Aerial mycelium is dark grayish green on media with abundant sporulation. The color ranges from white to gray, to light grayish white on other media. Substrate mycelia vary from pale yellow to grayish yellow. No soluble pigment is produced on any of the 11 media used. The culture is assigned to the Gray (GY) and Green (GN) series of Tresner and Backus. The Maerz and Paul (1950) color block most similar to the spore color en masse is 21-B1, and the light grayish olive color of the ISCC-NBS designation method corresponds to this color block. The culture grows over the pH range 5.0–8.5. Growth does not occur at pH 4.0 or 9.0. Sporulation occurs from pH 5.0 to 6.5 and is most abundant at pH 6.0. Whole-cell hydrolysates contain LL-A_2pm, glycine, aspartic acid, alanine, glutamic acid and leucine as major constituents.

Type strain shows no sequence similarity over 99%.

Source: soil.

DNA G+C content (mol%): not known.

Type strain: AS 4.1611, ATCC 27064, CBS 226.75, BCRC 11518, CECT 3125, DSM 40751, DSM 738, NBRC 13307, IMET 43657, JCM 4710, KCTC 9095, NCIMB 12785, NRRL 3585, VKM Ac-602.

Sequence accession no. (16S rRNA gene): AY 999718.

123. **Streptomyces coelescens** (Krasil'nikov, Sorokina, Alferova and Bezzubenkova *in* Krasil'nikov 1965) Pridham 1970, 21[AL] ("*Actinomyces coelescens*" Krasil'nikov, Sorokina, Alferova and Bezzubenkova *in* Krasil'nikov 1965, 110)

co.e.les′cens. N.L. adj. *coelescens* slightly blue.

Spore chains in Section *Spirales*; spirals are best developed on salts-starch agar or oatmeal agar. Flexuous chains and imperfect spirals suggesting *Retinaculiaperti* morphology are also common on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar. Mature spore chains are moderately long with 10–50 spores per chain. Spore surface is smooth.

Color of colony: aerial mass color in the Gray color series (3fe, light brownish gray; 2dc, yellowish gray or 3ge light grayish yellowish brown) on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar. Reverse side of colony is dark grayish purple or blackish purple on salts-starch agar and glycerol-asparagine agar; grayish brown to dark brown or reddish black on yeast-malt agar and oatmeal agar. Reverse mycelium pigment is a pH indicator, changing from reddish violet to bluish violet or blue with the addition of 0.05 M NaOH and from bluish violet to reddish violet, brown, or red with the addition of 0.05 M HCl.

Color in medium: melanoid pigments are not formed in peptone-yeast-iron agar, tyrosine agar, or tryptone-yeast broth. Red, violet, or blue pigment, depending on pH, may be found in the medium in yeast-malt agar, oatmeal agar, salts-starch agar, or glycerol-asparagine agar, but pigment is not always found in these media. This pigment is pH-sensitive, showing essentially the same changes noted for the reverse mycelium pigment.

D-Glucose, L-arabinose, D-xylose, iso-inositol, D-mannitol, D-fructose, and rhamnose are utilized for growth. Utilization of sucrose or raffinose is doubtful.

Type strain shows the highest sequence similarity to: *S. humiferus*, AF503491, 100%; *S. violaceolatus*, AF503497, 100%; *S. violaceoruber*, AF503492, 100%; *S. tricolor*, AB184687, 99.9%; *S. anthocyanicus*, AB184631, 99.9%; *S. rubrogriseus*, AB184681, 99.7%; *S. tendae*, D63873, 99.6%; *S. lienomycini*, AJ781353, 99.6%; *S. violaceorubidus*, AJ781374, 99.4%; *S. coelicoflavus*, AB184650, 99.3%; *S. ambofaciens*, M27245, 99%; *S. pactum*, AB184398, 99%.

Source: not known.
DNA G+C content (mol%): not known.
Type strain: AS 4.1594, ATCC 19830, CBS 679.72, DSM 40421, ICSSB 1021, NBRC 13378, INMI 20-41, JCM 4739, NCIMB 10042, NRRL B-12348, NRRL-ISP 5421, RIA 1339, VKM Ac-98.
Sequence accession no. (16S rRNA gene): AF503496.

124. **Streptomyces coelicoflavus** (*ex* Ryabova and Preobrazhenskaya) Terekhova 1986, 574[VP] (Effective publication: Terekhova *in* Gause, Preobrazhenskaya, Sveshnikova, Terekhova and Maximova 1983.) ["*Actinomyces coelicoflavus*" (Ryabova and Preobrazhenskaya) Krasil'nikov 1970b]

co.e.li.co.fla′vus. L. n. *caelum* the sky, heaven; L. adj. *flavus* yellow; N.L. masc. adj. *coelicoflavus* (*sic*) azure, yellow.

Spore chains are spiral (*Spirales*); spores are smooth. On mineral agar 1: aerial mycelium is light gray to gray; substrate mycelium is yellowish to dark blue, dark grayish blue; no diffusible pigment. On starch-ammonia agar: aerial mycelium is absent; substrate mycelium is gray-brownish-blue; no diffusible pigment. On glycerol-nitrate agar: aerial mycelium is light gray to light gray-brownish gray; substrate mycelium is pink to red, later blue, dark blue; no diffusible pigment. On glycerol-asparagine agar: aerial mycelium is light beige; substrate mycelium is yellow, gray; no diffusible pigment. On oatmeal agar: aerial mycelium is light gray, gray; substrate mycelium is colorless to light yellow; no diffusible pigment. On organic agar 2: aerial mycelium is white to light gray; substrate mycelium is first pink to red, later yellow-gray-brown; diffusible pigment is gray-brown or gray-brownish yellow. Melanoid pigments are not formed. Good growth on glucose, sucrose, fructose, rhamnose, arabinose, xylose, mannitol, and inositol.

Type strain shows the highest sequence similarity to: *S. anthocyanicus*, AB184631, 99.5%; *S. tricolor*, AB184687, 99.4%; *S. fragilis*, AY999917, 99.4%; *S. humiferus*, AF503491, 99.3%; *S. violaceolatus* AF503497, 99.3%; *S. violaceoruber*, AF503492, 99.3%; *S. coelescens*, AF503496, 99.3%; *S. rubrogriseus*, AB184681, 99.3%; *S. lienomycini*, AJ781353, 99.2%; *S. diastaticus*, subsp. *ardesiacus*, DQ026631, 99.2%; *S. flaveolus*, AB184764, 99.1%; *S. violaceorubidus*, AJ781374, 99%.
Source: not known.
DNA G+C content (mol%): not known.
Type strain: AS 4.1596, DSM 41471, NBRC 15399, INA 9630, JCM 6918, NRRL B-16363, VKM Ac-1221.
Sequence accession no. (16S rRNA gene): AB184650.

125. **Streptomyces coelicolor** (Müller 1908) Waksman and Henrici *in* Breed, Murray and Hitchens 1948, 935[AL] ("*Streptothrix coelicolor*" Müller 1908, 197; "*Cladothrix coelicolor*" Macé 1913, 758; "*Nocardia coelicolor*" Chalmers and Christopherson 1916, 271; "*Actinomyces coelicolor*" Lieske 1921, 28; "*Corynebacterium coelicolor*" Müller 1950, 274)

co.e.li.co′lor. L. n. *caelum* heaven, sky (blue); L. n. *color* color; N.L. n. *coelicolor* (*sic*) sky (blue) color.

Spore chains in Section *Rectiflexibliles*. Mature spore chains usually have 10–50, or often more than 50, spores per chain. This morphology is seen on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar. Spore surface is smooth. Special morphological characteristics: one observer reports substrate conidia on oatmeal agar, Emerson's potato glucose agar, and potato carrot agar. Sclerotia formation on yeast-malt agar and oatmeal agar was reported by one observer.

Color in colony: aerial mass color in the Yellow color series on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar. Reverse side of colony is light olive brown to strong brown or very dark brown on yeast-malt agar, salts-starch agar, and glycerol-asparagine agar; brown substrate mycelium pigment on oatmeal agar is pH-sensitive changing from brown to green with addition of 0.05 M NaOH. Potato plug is pigmented dark blue in 4–7 d.

Color in medium: melanoid pigments are not formed in peptone-yeast-iron agar, tyrosine agar, or tryptone-yeast broth. Reddish yellowish brown or greenish pigments are found in the medium in yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar; yellowish brown pigment is changed to green with addition of 0.05 M NaOH. Liquid surrounding dark blue potato plug is pigmented greenish brown in 4–7 d.

D-Glucose, L-arabinose, D-xylose, D-mannitol, and D-fructose are utilized for growth. No growth or only traces of growth on sucrose, iso-inositol, rhamnose, and raffinose.

Type strain shows the highest sequence similarity to: *S. aurantiogriseus*, AY999793, 99.9%; *S. griseoviridis*, AY999807, 99.3%.
Source: not known.
DNA G+C content (mol%): not known.
Type strain: AS 4.1658, ATCC 23899, CBS 210.27, CBS 795.68, BCRC 12067, CCUG 11110, DSM 40233, NBRC 12854, JCM 4357, NCIMB 9798, NRRL B-2812, NRRL-ISP 5233, PCM 2324, RIA 1173, VKM Ac-738.
Sequence accession no. (16S rRNA gene): DQ442496.

126. **Streptomyces coeruleoflavus** Preobrazhenskaya and Maximova 1986, 574[VP] (Effective publication: Preobrazhenskaya and Maximova *in* Gause, Preobrazhenskaya, Sveshnikova, Terekhova and Maximova 1983.)

co.e.ru.le.o.fla′vus. L. adj. *caeruleus* dark blue, azure; L. adj. *flavus* yellow; N.L. masc. adj. *coeruleoflavus* (*sic*) dark blue, yellow.

Spore chains are spiral (*Spirales*); spores are spiny; spines are medium-sized. On mineral agar 1: aerial mycelium is smooth, color is gray to light blue; substrate mycelium is yellow, dark orange, or brown-yellow; no diffusible pigment. On glycerol-nitrate agar: aerial mycelium is light blue; substrate mycelium and diffusible pigment are yellow-brown to brown. On starch-ammonia agar: aerial mycelium is light blue; substrate mycelium is brown-yellow to brown; diffusible pigment is yellow-gray. On glycerol-asparagine agar: aerial mycelium and diffusible pigment are not found; substrate mycelium is colorless. On oatmeal agar: aerial mycelium is light blue; substrate mycelium and diffusible pigment are dark brown. Melanoid pigments are not formed. Good growth on rhamnose, glucose, mannitol, sucrose, arabinose, xylose, fructose, inositol, and raffinose. Antibiotic: actinomycin D.
Source: not known.
DNA G+C content (mol%): not known.

Type strain: INA 2206.

Sequence accession no. (16S rRNA gene): no sequence available.

127. **Streptomyces coeruleofuscus** (Preobrazhenskaya *in* Gauze, Preobrazhenskaya, Kudrina, Blinov, Ryabova and Sveshnikova 1957) Pridham, Hesseltine and Benedict 1958, 67[AL] ("*Actinomyces coeruleofuscus*" Preobrazhenskaya *in* Gauze, Preobrazhenskaya, Kudrina, Blinov, Ryabova and Sveshnikova 1957, 128)

co.e.ru.le.o.fus′cus. L. adj. *caeruleus* dark blue, azure; L. adj. *fuscus* dark, tawny; N.L. masc. adj. *coeruleofuscus* (*sic*) dark blue, tawny (referring to the bluish aerial mycelium and brownish vegetative mycelium of the organism).

Spore chains in Section *Spirales*. Mature spore chains are long, often more than 50 spores per chain. This morphology is found on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar. Spore surface is spiny.

Color of colony: aerial mass color in the Blue color series on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar. Reverse side of colony is variable in color; it is yellow-brown modified by red on yeast-malt agar and characteristic grayed yellow or grayed greenish yellow (center of growth) modified by green, red, and brown (borders) on oatmeal agar, salts-starch agar, and glycerol-asparagine agar. Substrate is not a pH indicator; however, one observer reports that on glycerol-asparagine agar, the reverse color is changed from olive green to bluish green by addition of 0.05 M NaOH, and from olive green to very pale red with HCl.

Color in medium: melanoid pigments are formed in peptone-yeast-iron agar, tryptone-yeast broth, and some other media. Pigments other than melanoids are not formed in yeast-malt agar, oatmeal agar, salts-starch agar, or glycerol-asparagine agar.

D-Glucose, L-arabinose, sucrose, D-xylose, iso-inositol, D-mannitol, D-fructose, rhamnose, and raffinose are utilized for growth.

Type strain shows the highest sequence similarity to: *S. cinereospinus*, AB184648, 99.2%; *S chromofuscus*, AB184194, 99.1%.

Source: not known.

DNA G+C content (mol%): not known.

Type strain: AS 4.1667, ATCC 19741, ATCC 23618, CBS 480.68, BCRC 12186, DSM 40144, NBRC 12757, IMET 43574, INA 2922/57, JCM 4358, NRRL B-5417, NRRL-ISP 5144, RIA 1022, UNIQEM 128, VKM Ac-619.

Sequence accession no. (16S rRNA gene): DQ026668.

128. **Streptomyces coeruleoprunus** Preobrazhenskaya 1986, 574[VP] (Effective publication: Preobrazhenskaya *in* Gause, Preobrazhenskaya, Sveshnikova, Terekhova and Maximova 1983.)

co.e.ru.le.o.pru′nus. L. adj. *caeruleus* dark blue, azure; L. n. *prunum* plum; N.L. masc. adj. *coeruleoprunus* (*sic*) dark blue plum.

Spore chains are straight (*Rectiflexibiles*); spores are smooth. On mineral agar 1: aerial mycelium is smooth, color is gray to light blue; substrate mycelium is brown-violet to blue-black or brown-yellow; diffusible pigment is brown-pink to brown. On oatmeal agar: aerial mycelium is light blue; substrate mycelium and diffusible pigment are pink to gray-violet. On starch-ammonia agar: aerial mycelium is light blue; substrate mycelium is colorless to brown; no diffusible pigment. On glycerol-asparagine agar: aerial mycelium color is light to pale blue; substrate mycelium is colorless; no diffusible pigment. On organic agar 2: white to light blue aerial mycelium; substrate mycelium and diffusible pigment are dark gray-brown or brown. Melanoid pigments are not formed. Good growth on glucose, sucrose, rhamnose, mannitol, arabinose, xylose, fructose, inositol, and raffinose. Antibiotic: neomycin.

Type strain shows the highest sequence similarity to: *S. fradiae*, DQ026630, 99.2%; *S. somaliensis*, AJ007403, 99%.

Source: not known.

DNA G+C content (mol%): not known.

Type strain: AS 4.1648, ATCC 43681, DSM 41472, NBRC 15400, INA 1655, JCM 6919, NRRL B-16364, VKM Ac-1208.

Sequence accession no. (16S rRNA gene): AB184651.

129. **Streptomyces coeruleorubidus** (Preobrazhenskaya *in* Gauze, Preobrazhenskaya, Kudrina, Blinov, Ryabova and Sveshnikova 1957) Pridham, Hesseltine and Benedict 1958, 67[AL] ("*Actinomyces coeruleorubidus*" Preobrazhenskaya *in* Gauze, Preobrazhenskaya, Kudrina, Blinov, Ryabova and Sveshnikova 1957, 125)

co.e.ru.le.o.ru′bi.dus. L. adj. *caeruleus* dark blue, azure; L. adj. *rubidus* dark red; N.L. masc. adj. *coeruleorubidus* (*sic*) dark blue, dark red (referring to the bluish aerial mycelium and red vegetative mycelium of the organism on chemically defined media).

Spore chains in Section *Spirales*. Salts-starch agar shows best spiral morphology; incomplete spirals and hooks may also be common, especially on yeast-malt agar, oatmeal agar, and glycerol-asparagine agar. Mature spore chains generally have 10–50 spores per chain. Spore surface is spiny.

Color of colony: aerial mass color is in the Blue color series on oatmeal agar and salts-starch agar; Blue or White color series on yeast-malt agar and glycerol asparagine agar. Reverse side of colony is grayish yellow to yellowish brown on yeast-malt agar; grayed yellow to yellowish green on oatmeal agar and salts-starch agar; both grayed yellowish green and grayed red or orange on glycerol-asparagine agar. These substrate mycelium pigments are not pH indicators when tested with 0.05 M NaOH or HCl.

Color in medium: melanoid pigments are formed in peptone-yeast-iron agar, tyrosine agar, and tryptone-yeast broth. Red pigment may be found in medium in yeast-malt agar, salts-starch agar, and glycerol-asparagine agar after 14 d; it is not pH-sensitive.

D-Glucose, L-arabinose, sucrose, D-xylose, iso-inositol, D-mannitol, D-fructose, rhamnose, and raffinose are utilized for growth.

Type strain shows the highest sequence similarity to: *S. coerulescens*, AY999720, 99.6%; *S. bellus*, AB184849, 99.6%; *S. albogriseolus*, AJ494865, 99.4%; *S. viridodiastaticus*, AY999852, 99.4%; *S. lomondensis*, AB184673, 99.3%; *S. iakyrus*, AB184877, 99.3%; *S. lusitanus*, AB184424, 99.2%;

S. longispororuber, AB184440, 99.2%; *S. olivaceus*, AB184743, 99.2%; *S. purpurascens*, AJ399486, 99.2%; *S. parvulus*, AB184326, 99.2%; *S. pactum*, AB184398, 99.2%; *S. indiaensis*, AB184553, 99%; *S. roseoviolaceus*, AJ399484, 99%; *S. janthinus*, AB184851, 99%; *S. violaceus*, AB184315, 99%; *S. spinoverrucosus*, AB184578, 99%; *S. hawaiiensis*, AB184143, 99%; *S. albosporeus* subsp. *albosporeus*, AJ781327, 99%; *S. atrovirens*, DQ026672, 99%.

Source: not known.

DNA G+C content (mol%): not known.

Type strain: AS 4.1678, ATCC 13740, ATCC 23900, CBS 796.68, BCRC 11463, DSM 40145, NBRC 12855, IMET 42060, INA 12531/54, JCM 4359, KCTC 1922, NCIMB 9620, NRRL B-2569, NRRL-ISP 5145, RIA 1132, VKM Ac-576.

Sequence accession no. (16S rRNA gene): AY999719.

130. **Streptomyces coerulescens** (Preobrazhenskaya *in* Gauze, Preobrazhenskaya, Kudrina, Blinov, Ryabova and Sveshnikova 1957) Pridham, Hesseltine and Benedict 1958, 67[AL] ("*Actinomyces coerulescens*" Preobrazhenskaya *in* Gauze, Preobrazhenskaya, Kudrina, Blinov, Ryabova and Sveshnikova 1957, 120)

co.e.ru.les′cens. L. adj. *caerulus* dark blue, azure; N.L. part. adj. *coerulescens* (*sic*) becoming blue, slightly blue (referring to the color of the aerial mycelium on a chemically defined medium).

Spore chains in Section *Spirales*. Mature spore chains generally have 10–50 spores per chain. Typical morphology is observed on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar. Spore surface is spiny.

Color of colony: aerial mass color in the Blue color series on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar. Reverse side of colony with no distinctive pigments on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar; substrate is not a pH indicator.

Color in medium: melanoid pigments are formed in peptone-yeast-iron agar, and tryptone-yeast broth. Pigments other than melanoids are not formed in yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar.

D-Glucose, L-arabinose, sucrose, D-xylose, iso-inositol, D-mannitol, D-fructose, rhamnose, and raffinose are utilized for growth.

Type strain shows the highest sequence similarity to: *S. bellus*, AB184849, 100%; *S. coeruleorubidus*, AY999719, 99.6%; *S. lomondensis*, AB184673, 99.4%; *S. purpurascens*, AJ399486, 99.4%; *S. lusitanus*, AB184424, 99.4%; *S. iakyrus*, AB184877, 99.3%; *S. paradoxus*, AB184628, 99.2%; *S. viridodiastaticus*, AY999852, 99.2%; *S. parvulus*, AB184326, 99.2%; *S. matensis*, AB184221, 99.2%; *S. azureus*, EF178674, 99.2%; *S. indiaensis*, AB184553, 99.2%; *S. albogriseolus*, AJ494865, 99.2%; *S. longispororuber*, AB184440, 99.2%; *S. hawaiiensis*, AB184143, 99.2%; *S. thermocarboxydus*, U94490, 99.1%; *S. spinoverrucosus*, AB184578, 99.1%; *S. griseorubens*, AB184139, 99.1%; *S. erythrogriseus*, AJ781328, 99%; *S. griseoincarnatus*, AJ781328, 99%; *S. labedae*, AB184704, 99%; *S. variabilis*, DQ442551, 99%; *S. massasporeus*, AB184152, 99%; *S. roseoviolaceus*, AJ399484, 99%; *S. violaceus*, AB184315, 99%; *S. albosporeus* subsp. *albosporeus*, AJ781327, 99%; *S. janthinus*, AB184851, 99%.

Source: not known.

DNA G+C content (mol%): not known.

Type strain: AS 4.1597, ATCC 19742, ATCC 19896, CBS 481.68, BCRC 11464, DSM 40146, NBRC 12758, IMET 43578, INA 4562, JCM 4360, NCIMB 9615, NRRL B-2701, NRRL-ISP 5146, PCM 2312, RIA 1023, UNIQEM 129, VKM Ac-1843.

Sequence accession no. (16S rRNA gene): AY999720.

131. **Streptomyces collinus** Lindenbein 1952, 380[AL]

col.li′nus. L. masc. adj. *collinus* hilly, mounded.

Spore chains in Section *Spirales* or *Retinaculiaperti*. Spore chains are short resulting in poorly developed spirals of only a few turns; loops and hooks are of small diameter and therefore are not representative of true *Retinaculiaperti* cultures. Distinct spore chains are relatively short, often with only 3–10 spores per chain; longer chains occur, but often coalesce as masses of spores. This morphology is seen on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar. Spore surface is smooth. After 14 d, subglobose bodies, composed primarily of masses of spores, can be seen. They appear to originate at the hooked or spiral ends of *Retinaculiaperti* spore chains and to be held together by fluid.

Color of colony: aerial mass color in the Gray color series on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar; or (by one observer) in the Red series on salts-starch agar. Reverse side of colony with no distinctive pigments on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar.

Color in medium: melanoid pigments formed in peptone-yeast-iron agar and sometimes in tryptone-yeast broth, but not on tyrosine agar. Pigments other than melanoids are not formed in yeast-malt agar, oatmeal agar, salts-starch agar, or glycerol-asparagine agar.

D-Glucose, L-arabinose, sucrose, iso-inositol, D-mannitol, D-fructose, rhamnose, and raffinose are utilized for growth. Two of three observers found good growth with D-xylose.

Type strain shows the highest sequence similarity to: *S. violaceochromogenes*, AY999867, 99.6%; *S. paradoxus*, AB184628, 99.5%; *S. griseoflavus*, AJ781322, 99.5%; *S. viridochromogenes*, DQ442555, 99.3%; *S. iakyrus*, AB184877, 99.3%; *S. matensis*, AB184221, 99.2%; *S. ambofaciens*, M27245, 99.2%; *S. flaveolus*, AB184764, 99.2%; *S. griseorubens*, AB184139, 99.2%; *S. erythrogriseus*, AJ781328, 99.1%; *S. griseoincarnatus*, AJ781328, 99.1%; *S. variabilis*, DQ442551, 99.1%; *S. labedae*, AB184704, 99.1%; *S. violaceorubidus*, AJ781374, 99%.

Source: not known.

DNA G+C content (mol%): not known.

Type strain: AS 4.1623, ATCC 19743, CBS 482.68, BCRC 11465, DSM 2012, DSM 40129, ICMP 12539, NBRC 12759, JCM 4361, KCTC 9713, NRRL B-5412, NRRL-ISP 5129, RIA 1024, UNIQEM 130, VKM Ac-710.

Sequence accession no. (16S rRNA gene): AB184123.

132. **Streptomyces colombiensis** Pridham, Hesseltine and Benedict 1958, 76[AL] emend. Lanoot, Vancanneyt, Dawyndt, Cnockaert, Zhang, Huang, Liu and Swings 2005a, 8 (Effective publication: Lanoot, Vancanneyt, Dawyndt, Cnockaert, Zhang, Huang, Liu and Swings 2004, 88.)

co.lom.bi.en′sis. N.L. masc. adj. *colombiensis* of or belonging to Columbia.

Spore chains in Section *Spirales.* Flexuous spore chains and open loops resembling *Retinaculiaperti* morphology are also present. Mature spore chains are generally long with more than 50 spores per chain. This morphology is seen on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar. Spore surface is smooth.

Color of colony: aerial mass color in the Red color series (5dc, 5ec, 5cb, 6ec, grayish yellowish pink; or 5ge, light grayish reddish brown) on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar. Reverse side of colony is moderate reddish orange to strong brown on yeast-malt agar; grayish yellow, orange yellow, or yellowish brown on oatmeal agar, salts-starch agar, and glycerol-asparagine agar. Reverse mycelium pigment is not a pH indicator.

Color in medium: melanoid pigments are not formed in tyrosine agar; reports vary on production of melanoid pigments in peptone-yeast-iron agar and tryptone-yeast broth. No pigment is found in medium in yeast-malt agar, oatmeal agar, salts-starch agar, or glycerol-asparagine agar.

D-Glucose and D-fructose are utilized for growth. No growth or only traces of growth with L-arabinose, D-xylose, iso-inositol, D-mannitol, rhamnose, sucrose, and raffinose.

Type strain shows the highest sequence similarity to: *S. goshikiensis*, EF178693, 100%; *S. sporoverrucosus*, DQ442544, 99.9%; *S. nojiriensis*, AJ781355, 99.8%; *S. spororaveus*, AJ781370, 99.8%; *S. xanthophaeus*, DQ442560, 99.7%; *S. vinaceus*, AB184394, 99.7%; *S. cirratus*, AY999794, 99.6%; *S. cinnamonensis*, AB184707, 99.5%; *S. avidinii*, AB184395, 99.5%; *S. subrutilus*, X80825, 99.3%; *S. virginiae*, D85119, 99.1%.

Source: not known.

DNA G+C content (mol%): not known.

Type strain: ATCC 27425, CBS 755.72, DSM 40558, NBRC 13454, JCM 4675, JCM 4740, NRRL B-1990, NRRL-ISP-5558, RIA 739, RIA 1415.

Sequence accession no. (16S rRNA gene): DQ026646.

Further comments: according to Lanoot et al. (2004), *Streptomyces colombiensis* Pridham et al. 1958 is an earlier heterotypic synonym of *Streptomyces distallicus* (Locci et al. 1969) Witt and Stackebrandt 1991.

133. **Streptomyces corchorusii** Ahmad and Bhuiyan 1958, 143[AL] emend. Lanoot, Vancanneyt, Van Shoor, Liu and Swings 2005b, 731

cor.cho.ru′si.i. L. n. *Corchorus* name of a plant and a scientific generic name; L. gen. n. *corchorusii* (*sic*) of Corchorus, because the organism was isolated from soil of a field of jute, *Corchorus capsulatus.*

Spore chains in Section *Spirales* to *Rectiflexibiles.* Incomplete spirals (hooks) and flexuous or straight spore chains are common; spirals usually have only 1–3 turns. Hooks and primitive spirals are on relatively short chains and are therefore not representative of typical *Retinaculiaperti* or *Rectiflexibiles* morphology. Mature spore chains generally have 10–20 spores per chain. This morphology is seen on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar. Spore surface is smooth. Electron micrographs show spores of irregular size and shape; some surface irregularities may be present.

Color of colony: aerial mass color in the Gray color series (color tabs 3ge, light grayish yellowish brown and 3fe or 4li, brownish gray) on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar. Reverse side of colony with no distinctive pigments (yellow to yellowish brown or olive brown) on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar.

Color in medium: melanoid pigments are not formed in peptone-yeast-iron agar, tyrosine agar, or tryptone-yeast broth. No pigment (or only a trace of yellow) is found in the medium in yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar.

D-Glucose, L-arabinose, D-xylose, iso-inositol, D-mannitol, D-fructose, rhamnose, sucrose, and raffinose are all utilized for growth.

Type strain shows the highest sequence similarity to: *S. olivaceoviridis*, AB184288, 99.9%; *S. canarius*, AB184396, 99.8%; *S. capoamus*, AB045877, 99.5%; *S. longwoodensis*, AB184580, 99.4%; *S. bungoensis*, AB184696, 99.4%; *S. galbus*, X79852, 99.3%; *S. griseochromogenes*, AB184387, 99%; *S. cellostaticus*, AB184192, 99%.

Source: isolated from soil from a field of jute.

DNA G+C content (mol%): not known.

Type strain: AS 4.1592, ATCC 25444, CBS 677.69, BCRC 11821, DSM 40340, NBRC 13032, JCM 4286, JCM 4467, KCTC 9715, LMG 20488, NCIMB 9476, NCIMB 9979, NRRL B-12289, NRRL-ISP 5340, RIA 1224, VKM Ac-1906.

Sequence accession no. (16S rRNA gene): AB184267.

Further comments: according to Lanoot et al. (2005b), *Streptomyces corchorusii* Ahmad and Bhuiyan 1958 emend. Lanoot et al. 2005b is an earlier heterotypic synonym of *Streptomyces chibaensis* Suzuki et al. 1958.

According to Rule 24b of the *Bacteriological Code* (1990 Revision), if two names compete for priority and if both names are listed on the Approved Lists of Bacterial Names, the priority shall be determined by the date of the effective publication of the name before 1 January 1980. S*treptomyces chibaensis* and *Streptomyces corchorusii* are cited in the Approved Lists. The dates of effective publications are 1958. Consequently, to determine priority it is necessary to know the month (and perhaps the day) of the effective publications. *Streptomyces chibaensis* was effectively published in the *Journal of Antibiotics* (*Tokyo*) *Series A*, 1958, vol. 11, pp. 81–83, and *Streptomyces corchorusii* in the *Pakistan Journal of Biological and Agricultural Sciences*, 1958, vol. 1, pp. 137–143. It is certainly not easy to know the exact dates of publication of these articles. However, according to Rule 42 of the *Bacteriological Code* (1990 Revision), if the epithets are of the same date, the author who first unites the taxa has the right to choose one of them, and his choice must be followed. Lanoot et al. chose the epithet *corchorusii.*

134. **Streptomyces costaricanus** Esnard, Potter and Zuckerman 1995, 778[VP]

cos.ta.ri.can′us. N.L. adj. masc. *costaricanus* of or belonging to Costa Rica, the geographic origin of the organism.

Mature spore chains are tightly coiled spirals with 10–50 spores per chain. Spores are smooth and gray-brown in mature colonies. Aerial mycelial mass is grayish brown on yeast extract-malt extract agar, oatmeal agar, inorganic salts-starch agar, and glycerol-asparagine agar and yellow on NZamine medium containing soluble starch and glucose (ATCC medium 172). The substrate mycelium is light yellow on yeast extract-malt extract agar and glycerol-asparagine agar, golden on ATCC medium 172, brown on oatmeal agar, and yellow on inorganic salts-starch agar. A yellow pH-insensitive diffusible pigment is produced on yeast extract-malt extract agar and glycerol-asparagine agar. The pigment color is yellow-orange on ATCC medium 172. No melanoid pigment is produced on peptone-yeast extract-iron agar or tyrosine agar. Color of the reverse side of the colonies is also not sensitive to pH.

D-Fructose, D-glucose, D-mannitol, D-xylose, salicin, and galactose are utilized for growth, but L-arabinose, raffinose, rhamnose, and sucrose are not utilized. Acid is produced from cellobiose, D-glucose, glycerol, maltose, galactose, D-mannitol, and D-xylose but not from L-arabinose, D-fructose, lactose, or sucrose. No growth occurs in the presence of ribitol, galactitol, erythritol, or iso-inositol; 7% NaCl is inhibitory. Cell walls contain LL-A_2pm. The most abundant hydrolyzable fatty acids are $C_{15:0}$ anteiso, $C_{16:0}$, $C_{17:0}$ anteiso, $C_{15:0}$ iso, $C_{16:0}$ iso, and $C_{17:0}$ iso in cells grown on ISP medium 2 agar. The concentration of octadecanoic acid is ninefold higher in ISP medium 2 broth.

Exhibits anti-nematodal activity against *Caenorhabditis elegans* and antibiotic activity against *Rhizoctonia solani* and *Phytium aphanidermatum.*

Type strain shows the highest sequence similarity to: *S. griseofuscus*, AB184206, 100%; *S. murinus*, AB184155, 100%; *S. phaeogriseichromatogenes*, AJ391813, 99.6%.

Source: isolated from a tropical soil.

DNA G+C content (mol%): not known.

Type strain: CR-43, ATCC 55274, DSM 41827, NRRL B-16897, JCM 11306, NBRC 100773, NCIMB 13455.

Sequence accession no. (16S rRNA gene): AB249939.

135. **Streptomyces cremeus** (Kudrina *in* Gauze, Preobrazhenskaya, Kudrina, Blinov, Ryabova and Sveshnikova 1957) Pridham, Hesseltine and Benedict 1958, 66[AL] (“*Actinomyces cremeus*” Kudrina *in* Gauze, Preobrazhenskaya, Kudrina, Blinov, Ryabova and Sveshnikova 1957, 93)

cre′me.us. N.L. masc. adj. *cremeus* cream-white.

Spore chains in Section *Retinaculiaperti*, but with many straight to flexuous spore chains. Open coils and primitive spirals are common. In the original description (Kudrina, ibid), this is characterized as a spiral culture. Mature spore chains are long, often with more than 50 spores per chain. This morphology is observed on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar. Spore surface is smooth.

Color of colony: aerial mass color in the Red color series on yeast-malt and salts-starch agar; Yellow series on glycerol-asparagine agar; Yellow or Red series on oatmeal agar. Reverse side of colony with no distinctive pigments on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar; substrate is not a pH indicator (one observer reports slight change from pale yellow to pale pink with 0.05 M NaOH).

Color in medium: melanoid pigments are not formed in peptone-yeast-iron agar and tyrosine agar. No pigments or only traces of yellow pigments in yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar.

D-Glucose, L-arabinose, D-xylose, and D-fructose are utilized for growth. No growth or only traces of growth on sucrose, iso-inositol, D-mannitol, rhamnose, and raffinose.

Type strain shows the highest sequence similarity to: *S. spiroverticillatus*, AB184814, 99.7%; *S. celluloflavus*, AB184476, 99.3%; *S. griseobrunneus*, AB249912, 99.3%; *S. albolongus*, AB184425, 99.3%; *S. cavourensis* subsp. *cavourensis*, DQ445791, 99.2%; *S. candidus*, DQ026663, 99.2%; *S. sindenensis*, AB184759, 99.1%; *S. pluricolorescens*, DQ442540, 99.1%; *S. lavendulae* subsp. *lavendulae*, AB184080, 99.1%; *S. floridae*, AB184656, 99.1%; *S. cavourensis* subsp. *washingtonensis*, DQ026671, 99.1%; *S. mediolani*, AB184674, 99.1%; *S. rubiginosohelvolus*, AB184240, 99.1%; *S. argenteolus*, AB045872, 99.1%; *S. badius*, AY999783, 99.1%; *S. griseinus*, AB184205, 99.1%; *S. anulatus*, DQ026637, 99%; *S. flavogriseus*, AJ494864, 99%; *S. halstedii*, EF178695, 99%; *S. cyaneofuscatus*, AB184860, 99%; *S. parvus*, DQ442537, 99%; *S. albovinaceus*, AB249958, 99%; *S. californicus*, AB184755, 99%; *S. graminofaciens*, AJ781329, 99%; *S. alboviridis*, AB184256, 99%; *S. globisporus* subsp. *globisporus*, EF178686, 99%; *S. lipmanii*, AB184148, 99%; *S. microflavus*, DQ445795, 99%; *S. fulvorobeus*, AB184711, 99%; *S. griseoplanus*, AY999894, 99%; *S. flavovirens*, DQ026635, 99%; *S. praecox*, AB184293, 99%; *S. flavofuscus*, AB249935, 99%; *S. pulveraceus*, AB184806, 99%; *S. griseolus*, AB184768, 99%; *S. fimicarius*, AY999784, 99%; *S. cinereorectus*, AB184646, 99%.

Source: not known.

DNA G+C content (mol%): not known.

Type strain: AS 4.1625, ATCC 19744, ATCC 19897, CBS 483.68, BCRC 11466, DSM 40147, NBRC 12760, IMET 43743, INA 815/54, JCM 4362, NCIMB 10030, NCIMB 9596, NRRL 3241, NRRL B-2583, NRRL-ISP 5147, RIA 1025, UNIQEM 131, VKM Ac-1844.

Sequence accession no. (16S rRNA gene): AB184124.

136. **Streptomyces crystallinus** Tresner, Davies and Backus 1961, 74[AL]

crys.tal.lin′us. L. masc. adj. *crystallinus* made of crystal, crystalline, referring to crystals formed by the organism in some media.

Spore chains are straight to long and flexuous; forms light to dark brown vegetative mycelium and diffusible pigment on many media. Poor growth on Czapek’s solution agar; produces hygromycin A and other antibiotics; exhibits anti-fungal activity; inhibited by streptomycin.

Type strain shows the highest sequence similarity to: *S. melanogenes*, AB184222, 99.2%; *S. noboritoensis*, AB184287, 99.2%.

Source: not known.

DNA G+C content (mol%): not known.

Type strain: AS 4.16, DSM 40945, NBRC 15401, JCM 5067, KCTC 9717, NCIMB 12860, NRRL B-3629.

Sequence accession no. (16S rRNA gene): AB184652.

137. **Streptomyces curacoi** Cataldi *in* Trejo and Bennett 1963, 683[AL]

cu.ra′co.i. N.L. gen. n. *curacoi* of Cura-Co in the province of La Pampa, Argentina.

Spore chains in Section *Spirales.* Mature spore chains generally have 10–50 spores per chain; long chains are not common. This morphology is seen on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar, but formation of sporulating aerial mycelium is not uniformly good in different laboratories with this strain. Spore surface is spiny.

Color of colony: aerial mass color in the Blue color series on salts-starch agar and on other media (yeast-malt agar, oatmeal agar, glycerol-asparagine agar) when mature spores occur; immature spore chains or non-sporulation aerial mycelium appears white. Reverse side of colony with no distinctive pigments on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar; substrate is not a pH indicator.

Color in medium: melanoid pigments are formed in peptone-yeast-iron agar, tyrosine agar, and tryptone-yeast broth. Pigments other than melanoids are not formed in yeast-malt agar, oatmeal agar, salts-starch agar, or glycerol-asparagine agar.

D-Glucose, L-arabinose, sucrose, D-xylose, iso-inositol, D-mannitol, and D-fructose are utilized for growth. Trace of growth is seen on rhamnose or raffinose.

Type strain shows the highest sequence similarity to: *S. longwoodensis*, AB184580, 99%; *S. capoamus*, AB045877, 99%.

Source: not known.

DNA G+C content (mol%): not known.

Type strain: 5828, ATCC 13385, ATCC 19745, CBS 484.68, NBRC 12761, JCM 4219, JCM 4573, NRRL B-2901, NRRL-ISP 5107, RIA 1026, SC 3604, UNIQEM 132.

Sequence accession no. (16S rRNA gene): EF626595.

138. **Streptomyces cuspidosporus** Higashide, Hasegawa, Shibata, Mizumo and Akaike 1966, 2[AL]

cu.spi.do.spo′rus. L. n. *cuspis -idis* point; N.L. n. *spora* a spore; N.L. masc. adj. *cuspidosporus* spore with points or spines.

Forms green to blue to yellowish green diffusible pigment on some media. Excellent growth on Czapek's solution agar; produces sparsomycin, tubercidin, and several other antibiotics.

Type strain shows the highest sequence similarity to: *S. sparsogenes*, AB184301, 99.9%.

Source: not known.

DNA G+C content (mol%): not known.

Type strain: AS 4.1886, ATCC 33340, CBS 192.78, DSM 41425, DSM 41653, NBRC 12378, JCM 4316, KCTC 9718, NRRL B-5620, VKM Ac-599.

Sequence accession no. (16S rRNA gene): AB184090.

139. **Streptomyces cyaneofuscatus** (Kudrina *in* Gauze, Preobrazhenskaya, Kudrina, Blinov, Ryabova and Sveshnikova 1957) Pridham, Hesseltine and Benedict 1958, 58[AL] ("*Actinomyces cyaneofuscatus*" Kudrina *in* Gauze, Preobrazhenskaya, Kudrina, Blinov, Ryabova and Sveshnikova 1957, 85)

cy.a.ne.o.fus.ca′tus. L. adj. *cyaneus* dark blue; L. adj. *fuscus* dark, tawny; L. masc. suff. *-atus* suffix used with the sense of provided with; N.L. masc. adj. *cyaneofuscatus* provided with dark blue, tawny, referring to different pigments formed.

Spore chains are in Section *Rectiflexibiles.* Mature spore chains generally have 10–50 spores per chain. Typical morphology is observed on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar. Spore surface is smooth. Coremia may be formed on oatmeal agar, salts-starch agar, and tyrosine agar.

Color of colony: aerial mass color in the Yellow color series on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar. Reverse side of colony with no distinctive pigments on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar. Reverse color is changed from pale yellowish green to pink by addition of 0.05 M HCl or from pale yellow to yellowish green with NaOH.

Color in medium: melanoid pigments are formed in peptone-yeast-iron agar. Pigments other than a weak yellow pigment are not formed in yeast-malt agar, oatmeal agar, salts-starch agar, or glycerol-asparagine agar. On glucose-mineral salts medium (CPI Kuznetsov: glucose, 2%; KNO_3, 0.1%; $MgSO_4$, 0.05%; NaCl, 0.05%; K_2HPO_4, 0.05%; $CaCO_3$, 0.3%; agar, 2.0%), blue pigment is formed in the medium. Blue pigment is pH-sensitive, changing from blue to pink with 0.05 M HCl.

D-Glucose, D-xylose, D-mannitol, D-fructose, and rhamnose are utilized for growth. No growth or only traces of growth on L-arabinose, sucrose, iso-inositol, and raffinose.

Type strain shows the highest sequence similarity to: *S. cavourensis* subsp. *washingtonensis*, DQ026671, 100%; *S. lavendulae* subsp. *lavendulae*, AB184080, 100%; *S. alboviridis*, AB184256, 99.9%; *S. fulvorobeus*, AB184711, 99.9%; *S. praecox*, AB184293, 99.9%; *S. anulatus*, DQ026637, 99.9%; *S. flavofuscus*, AB249935, 99.9%; *S. cinereorectus*, AB184646, 99.9%; *S. griseoplanus*, AY999894, 99.9%; *S. lipmanii*, AB184148, 99.9%; *S. fimicarius*, AY999784, 99.9%; *S. microflavus*, DQ445795, 99.9%; *S. sindenensis*, AB184759, 99.8%; *S. rubiginosohelvolus*, AB184240, 99.8%; *S. mediolani*, AB184674, 99.8%; *S. griseinus*, AB184205, 99.8%; *S. acrimycini*, AY999889, 99.8%; *S. pluricolorescens*, DQ442540, 99.8%; *S. argenteolus*, AB045872, 99.8%; *S. badius*, AY999783, 99.8%; *S. floridae*, AB184656, 99.7%; *S. albovinaceus*, AB249958, 99.7%; *S. globisporus* subsp. *globisporus*, EF178686, 99.7%; *S. griseus* subsp. *griseus*, AY207604, 99.7%; *S. griseolus*, AB184768, 99.7%; *S. luridiscabiei*, AF361784, 99.7%; *S. baarnensis*, EF178688, 99.7%; *S. californicus*, AB184755, 99.7%; *S. parvus*, DQ442537, 99.7%; *S. halstedii*, EF178695, 99.7%; *S. flavovirens*, DQ026635, 99.6%; *S. flavogriseus*, AJ494864, 99.5%; *S. olivoviridis*, AB184227, 99.4%; *S. bacillaris*, AB184439, 99.4%; *S. finlayi*, AY999788, 99.4%; *S. pulveraceus*, AB184806, 99.4%; *S. atroolivaceus*, AJ781320, 99.4%; *S. yanii*, AB006159, 99.4%; *S. clavifer*,

DQ026670, 99.3%; *S. nitrosporeus*, EF178680, 99.3%; *S. albolongus*, AB184425, 99.2%; *S. sanglieri*, AB249945, 99.2%; *S. mutomycini*, AB249951, 99.2%; *S. gelaticus*, DQ026636, 99.2%; *S. griseobrunneus*, AB249912, 99.2%; *S. celluloflavus*, AB184476, 99.2%; *S. atratus*, DQ026638, 99.1%; *S. spiroverticillatus*, AB184814, 99%; *S. cavourensis* subsp. *cavourensis*, DQ445791, 99%; *S. cremeus*, AB184124, 99%; *S. mauvecolor*, AB184532, 99%; *S. candidus*, DQ026663, 99%.

Source: not known.

DNA G+C content (mol%): not known.

Type strain: AS 4.1612, ATCC 19746, ATCC 23619, CBS 485.68, BCRC 11467, DSM 40148, NBRC 13190, IMET 41583, INA 99/54, JCM 4364, NCIMB 13021, NRRL B-2570, NRRL-ISP 5148, RIA 1027, UNIQEM 133, VKM Ac-752.

Sequence accession no. (16S rRNA gene): AB184860.

140. **Streptomyces cyaneus** (Krasil'nikov 1941) Waksman *in* Waksman and Lechevalier 1953, 42[AL] ("*Actinomyces cyaneus*" Krasil'nikov 1941, 14)

cy'a.ne.us. L. masc. adj. *cyaneus* dark blue.

Spore chains in Section *Spirales.* Compact spirals of 2–6 turns are common, but open spirals, imperfect spirals or flexuous spore chains may also be found. Mature spore chains are moderately long with 10–50 spores per chain. This morphology is seen on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar. Spore surface is spiny.

Color of colony: aerial mass color in the Blue or Gray color series on yeast-malt agar, salts-starch agar, and glycerol-asparagine agar; and Gray color series on oatmeal agar. Aerial mycelium may be poorly developed and atypical on glycerol-asparagine agar. The most representative color tabs from the Blue color series are 19fe or dc, pale blue, and the most representative color from the Gray color series is tab e, medium gray. Reverse side of colony is dark grayish blue to dark grayish purple on yeast-malt agar, oatmeal agar, and salts-starch agar; this pigmentation sometimes fails to develop on glycerol-asparagine agar. Reverse mycelium pigment is a pH indicator, changing from violet or purple to blue with the addition of 0.05 M NaOH and from blue to violet or purple with the addition of 0.05 M HCl.

Color in medium: melanoid pigments are formed in peptone-yeast-iron agar, tyrosine agar, and tryptone-yeast broth. Blue or violet pigment is found in the medium in yeast-malt agar, oatmeal agar, and salts-starch agar; it is often not formed in glycerol-asparagine agar. This pigment is pH-sensitive, showing the same changes noted for the reverse mycelium pigment.

D-Glucose, L-arabinose, D-xylose, iso-inositol, D-mannitol, D-fructose, rhamnose, sucrose, and raffinose are all utilized for growth.

Type strain shows the highest sequence similarity to: *S. pseudovenezuelae*, AB184233, 99.1%.

Source: not known.

DNA G+C content (mol%): not known.

Type strain: AS 4.1671, ATCC 14923, CBS 647.72, BCRC 13767, DSM 40108, NBRC 13346, JCM 4220, JCM 4743, KCTC 9719, NRRL B-16305, NRRL B-2296, NRRL-ISP 5108, PCM 2297, RIA 1307, VKM Ac-1712.

Sequence accession no. (16S rRNA gene): AF346475.

141. **Streptomyces cyanoalbus** (Krasil'nikov and Agre in Rautenshtein 1960) Pridham 1970, 13[AL] ("*Actinomyces cyanoalbus*" Krasil'nikov and Agre *in* Rautenshtein 1960, 273)

cy.a.no.al'bus. L. adj. *cyaneus* dark blue; L. adj. *albus* white; N.L. masc. adj. *cyanoalbus* blue, white, referring to formation of colorless (white) or blue vegetative mycelium.

Spore chains in Section *Spirales* or *Rectiflexibiles.* Distinct spirals are rare. Moderately short chains, often with only 10–20 spores, may form strongly wavy chains to open spirals of 1–4 turns. This morphology is seen on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar. Spore surface is spiny.

Color of colony: mature aerial mass color (14–21 d) is in the Green color series on yeast-malt agar and salts-starch agar; Green, Gray, or White color series on oatmeal agar and glycerol-asparagine agar. Aerial mycelium is usually poorly developed on oatmeal agar. Reverse side of colony is grayed yellow to grayed yellow-green on yeast-malt and oatmeal agar; grayed yellow-green to blue on salts-starch agar and glycerol-asparagine agar. Substrate mycelium pigment is not a pH indicator.

Color in medium: melanoid pigments are not formed in peptone-yeast-iron agar, tyrosine agar, or tryptone-yeast broth. No pigment is found in medium in yeast-malt agar, oatmeal agar, salts-starch agar, or glycerol-asparagine agar.

D-Glucose, L-arabinose, sucrose, D-xylose, iso-inositol, D-mannitol, D-fructose, and rhamnose are utilized for growth. No growth or only traces of growth on raffinose.

Type strain shows the highest sequence similarity to: *S. hirsutus*, AB184844, 100%; *S. bambergiensis*, AB184869, 99%; *S. prasinus*, DQ026658, 99%.

Source: not known.

DNA G+C content (mol%): not known.

Type strain: AS 4.1426, ATCC 15859, ATCC 23902, CBS 798.68, DSM 40198, HAMBI 1045, NBRC 12857, INMI 414, JCM 4363, LMG 19343, NCIMB 9831, NRRL B-3040, NRRL-ISP 5198, RIA 1150, RIA 662, VKM Ac-585.

Sequence accession no. (16S rRNA gene): AB184882.

142. **Streptomyces daghestanicus** (Sveshnikova *in* Gauze, Preobrazhenskaya, Kudrina, Blinov, Ryabova and Sveshnikova 1957) Pridham, Hesseltine and Benedict 1958, 67[AL] ("*Actinomyces daghestanicus*" Sveshnikova *in* Gauze, Preobrazhenskaya, Kudrina, Blinov, Ryabova and Sveshnikova 1957, 59)

da.ghes.ta'ni.cus. N.L. masc. adj. *daghestanicus* of or belonging to Daghestan, A.S.S.R., the source of the soil from which the organism was isolated.

Spore chains in Section *Retinaculiaperti* but with some well defined spiral spore chains. Mature chains generally have 10–50 spores per chain. Typical morphology is seen on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar. Spore surface is smooth with some minor irregularities suggesting wartiness.

Color of colony: aerial mass color in the Red color series on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar. Reverse side of colony is grayed yellow to yellow-brown on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar; the color is changed only slightly or not at all by addition of 0.05 M NaOH or HCl.

Color in medium: melanoid pigments not formed in peptone-yeast-iron agar or tyrosine agar. No pigment found in medium in yeast-malt agar, oatmeal agar, salts-starch agar, or glycerol-asparagine agar.

D-Glucose, L-arabinose, D-xylose, D-mannitol, D-fructose, and rhamnose are utilized for growth. No growth or only traces of growth on sucrose, iso-inositol, and raffinose.

Type strain shows the highest sequence similarity to: *S. canescens*, AB184117, 100%; *S. felleus*, AB184129, 100%; *S. limosus*, AB184147, 100%; *S. albidoflavus*, AB184255, 100%; *S. hydrogenans*, AB184868, 100%; *S. odorifer*, Z76682, 100%; *S. violascens*, AY999737, 100%; *S. griseus* subsp. *solvifaciens*, AB249915, 100%; *S. champavatii*, DQ026642, 100%; *S. sampsonii*, D63871, 99.8%; *S. koyangensis*, AY079156, 99.7%.

Source: isolated from soil from Daghestan, A.S.S.R.

DNA G+C content (mol%): not known.

Type strain: AS 4.169, ATCC 19747, ATCC 23620, CBS 486.68, BCRC 11468, DSM 40149, NBRC 12762, INA 2656/55, JCM 4365, NRRL B-5418, NRRL-ISP 5149, RIA 1028, UNIQEM 134, VKM Ac-1722, VKM Ac-1862.

Sequence accession no. (16S rRNA gene): DQ442497.

143. **Streptomyces demainii** Goodfellow, Kumar, Labeda and Sembiring 2008, 1[VP] (Effective publication: Goodfellow, Kumar, Labeda and Sembiring 2007, 198.)

de.main′i.i. N.L. gen. masc. n. *demainii* of Demain, named in honor of Arnold Demain, a celebrated actinomycete biologist.

Spore chains in *Spirales*; spore surface is rugose. On oatmeal agar, the aerial spore mass color is gray, becoming black and moist when mature; the reverse side of colony growth is grayish-yellow. Melanin pigments are not formed.

Type strain shows the highest sequence similarity to: *S. sporocinereus*, AB249933, 99.9%; *S. hygroscopius* subsp. *hygroscopius*, AB184428, 99.9%; *S. endus*, AY999911, 99.9%; *S. violaceusniger*, AJ391823, 99.5%; *S. yogyakartensis*, AB249942, 99.5%; *S. albiflaviniger*, AJ391812, 99.3%.

Source: not known.

DNA G+C content (mol%): 71.2.

Type strain: DSM 41600, NRRL B-1478.

Sequence accession no. (16S rRNA gene): DQ334782.

144a. **Streptomyces diastaticus subsp. diastaticus** (Krainsky 1914) Waksman and Henrici *in* Breed, Murray and Hitchens 1948, 939[AL] ("*Actinomyces diastaticus*" Krainsky 1914, 687)

di.a.sta′ti.cus. N.L. masc. adj. *diastaticus* diastatic.

Spore chains in Section *Rectiflexibiles* to *Spirales*. Good spirals are not formed on yeast-malt agar, oatmeal agar, salts-starch agar, or glycerol-asparagine agar, but highly flexuous or crooked spore chains, some of which suggest irregular spirals, are common on these media. Hooks, loops, or irregular spirals are of small diameter and are therefore not representative of true *Retinaculiaperti* morphology. Spore chains are often short, with 3–10 spores per chain, but longer chains are found. Spore surface is smooth.

Color of colony: sporulating aerial mycelium is often poorly developed on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar so that aerial mass color is difficult to determine. When sporulating aerial mycelium is formed on these media, it may be in the Gray color series (2dc, yellowish gray) or the aerial color may appear to be in the Yellow color series (2ba, pale yellow; 1cb, pale yellowish green). Reverse side of colony with no distinctive pigments (grayish yellow or pale yellowish green) on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar.

Color in medium: melanoid pigments are not formed in peptone-yeast-iron agar, tyrosine agar, or tryptone-yeast broth. No pigment is found in medium in yeast-malt agar, oatmeal agar, salts-starch agar, or glycerol-asparagine agar.

D-Glucose, L-arabinose, D-xylose, D-fructose, sucrose, and D-mannitol are utilized for growth. No growth or only traces of growth with iso-inositol, rhamnose, and raffinose.

Type strain shows the highest sequence similarity to: *S. gougerotii*, AB184742, 100%; *S. rutgersensis* subsp. *rutgersensis*, AB184170, 100%; *S. intermedius*, AB184277, 99.8%; *S. misionensis*, EF178678, 99.2%; *S. phaeoluteichromatogenes*, AJ391814, 99.1%; *S. matensis*, AB184221, 99%; *S. aureoverticillatus*, AY999774, 99%.

Source: not known.

DNA G+C content (mol%): not known.

Type strain: AS 4.1420, ATCC 3315, CBS 126.20, CBS 713.72, CCUG 11116, DSM 40496, NBRC 13412, NBRC 3714, IMET 40274, JCM 4128, JCM 4745, LMG 19322, NRRL B-1241, NRRL B-1270, NRRL-ISP 5496, RIA 104, RIA 1373, VKM Ac-723.

Sequence accession no. (16S rRNA gene): AB184785.

144b. **Streptomyces diastaticus subsp. ardesiacus** (Baldacci, Grein and Spalla 1955) Pridham, Hesseltine and Benedict 1958, 78[AL] ("*Actinomyces diastaticus* subsp. *ardesiacus*" Baldacci, Grein and Spalla 1955, 136)

ar.de.si′a.cus. N.L. masc. adj. *ardesiacus* (from Italian n. *ardesia* slate) intended to mean slate colored.

Excellent growth on Czapek's solution agar. Inhibited by streptomycin. Type strain shows the highest sequence similarity to: *S. coelicoflavus*, AB184650, 99.2%.

Source: not known.

DNA G+C content (mol%): not known.

Type strain: AS 4.1682, CBS 100.56, DSM 40934, NBRC 15402, JCM 5815, NRRL B-1773.

Sequence accession no. (16S rRNA gene): DQ026631.

145. **Streptomyces diastatochromogenes** (Krainsky 1914) Waksman and Henrici *in* Breed, Murray and Hitchens 1948, 941[AL] ("*Actinomyces diastatochromogenes*" Krainsky 1914, 687)

di.a.sta.to.chro.mo′gen.es. Gr. adj. *diastatus* split, divided; Gr. n. *chroma* color; N.L. suff. *-genes* (from Gr. v. *gennaô* to produce) producing; N.L. part. adj. *diastatochromogenes* producing diastatic color, presumably intended to mean producing diastase and color.

Spore chains in Section *Spirales* (or *Retinaculiaperti*). Spirals are open with 4–6 turns, and strongly flexuous to straight spore chains are also common. Mature spore chains are moderately long with 10–50 spores per chain. This morphology is seen on yeast-malt agar, oatmeal agar, and salts-starch agar, but aerial mycelium may be poorly developed or absent on glycerol-asparagine agar. Spore surface is smooth.

Color of colony: aerial mass color in the Gray color series on yeast-malt agar, oatmeal agar, and salts-starch agar; aerial mycelium may be absent on glycerol-asparagine agar, or white aerial mycelium sometimes may be found on this medium, oatmeal agar, and salts-starch agar. Reverse side of colony with no distinctive pigments (light brown or olive brown on yeast-malt agar; pale yellow or grayish yellow on oatmeal agar, salts-starch agar, and glycerol-asparagine agar).

Color in medium: melanoid pigments are formed in peptone-yeast-iron agar, tyrosine agar, tryptone-yeast broth, and Gause's medium no. 2. No pigment is found in medium in yeast-malt agar, oatmeal agar, salts-starch agar, or glycerol-asparagine agar.

D-Glucose, L-arabinose, D-xylose, iso-inositol, D-mannitol, D-fructose, rhamnose, sucrose, and raffinose are all utilized for growth.

Type strain shows no sequence similarity over 99%.

Source: not known.

DNA G+C content (mol%): not known.

Type strain: AS 4.1606, ATCC 12309, CBS 370.58, CBS 690.72, BCRC 13668, CFBP 4540, CIP 105123, DSM 40449, NBRC 13389, NBRC 3337, JCM 4119, JCM 4746, NRRL B-1698, NRRL-ISP 5449, RIA 1350, VKM Ac-1760.

Sequence accession no. (16S rRNA gene): D63867.

146. **Streptomyces distallicus** (Locci, Baldacci and Petrolini Baldan 1969) Witt and Stackebrandt 1991, 456[VP] (Effective publication: Witt and Stackebrandt 1990, 370.) (*Streptoverticillium distallicum* Locci, Baldacci and Petrolini Baldan 1969, 42).

Etymology of specific epithet is unknown.

Spore chains are straight to flexuous and ending in hooks. Reverse colors are darker, after 15 d, and then tend to equal those of *S. kentuckensis.* No great differences in aerial mycelium colors. Aerial mycelium is poorer on Bacto Emerson agar and absent on liquid media. Growth at 37°C is inferior to that at 27°C. Type strain produces distamycins A, B and C, and mycolutein.

For sequence similarity, see type strain of *Streptomyces netropsis.*

Source: not known.

DNA G+C content (mol%): not known.

Type strain: DSM 40846, IMI 72676, JCM 4544, NBRC 15815, NCIB (now NCIMB) 8936, NRRL 2886.

Sequence accession no. (16S rRNA gene): AB184703.

Further comments: in violation of Rule 33c of the *Bacteriological Code* (1990 Revision), on Validation List no. 38, *Streptomyces distallicus* is proposed as a *nomen revictum* (basonym: "*Streptomyces distallicus*" Arcamone et al. 1959.

According to Labeda (1996) and Hatano et al. (2003), *Streptomyces distallicus* (Locci et al. 1969) Witt and Stackebrandt 1991 is a later heterotypic synonym of *Streptomyces netropsis* (Finlay et al. 1951) Witt and Stackebrandt 1991.

According to Lanoot et al. (2004), *Streptomyces distallicus* (Locci et al. 1969) Witt and Stackebrandt 1991 is a later heterotypic synonym of *Streptomyces colombiensis* Pridham et al. (1958).

147. **Streptomyces djakartensis** Huber, Wallhäusser, Fries, Steigler and Weidenmüller 1962, 1191[AL]

dja.kart.en′sis. N.L. masc. adj. *djakartensis* of or belonging to Djakarta, Indonesia, the source of the soil from which the organism was isolated.

Good growth on Czapek's solution agar. Produces niddamycin (3-desacetyl carbomycin B), an anti-bacterial macrolide.

Type strain shows the highest sequence similarity to: *S. ghanaensis*, AY999851, 99.5%; *S. geysiriensis*, DQ442501, 99.5%; *S. minutiscleroticus*, EF178696, 99.5%; *S. mutabilis*, EF178679, 99.4%; *S. rochei*, AB184237, 99.4%; *S. vinaceusdrappus*, AY999929, 99.4%; *S. tuirus*, AB184690, 99.4%; *S. plicatus*, AB184291, 99.4%; *S. calvus*, AB184329, 99.2%; *S. levis*, AB184670, 99.2%; *S. azureus*, EF178674, 99.2%; *S. anandii*, AB184402, 99.1%; *S. asterosporus*, AB184706, 99.1%; *S. flavoviridis*, AB184842, 99.1%; *S. pseudogriseolus*, DQ442541, 99.1%; *S. gancidicus*, AB184660, 99.1%; *S. pilosus*, AB184161, 99.1%; *S. malachitofuscus*, AB184282, 99%; *S. cellulosae*, DQ442495, 99%; *S. aureorectus*, AB184710, 99%; *S. virens*, DQ442554, 99%; *S. carpinensis*, AB184574, 99%; *S. capillispiralis*, AB184577, 99%; *S. brasiliensis*, AB249981, 99%.

Source: isolated from soil from Djakarta, Indonesia.

DNA G+C content (mol%): not known.

Type strain: AS 4.1674, ATCC 13441, DSM 40743, NBRC 15409, JCM 4957, KCTC 9722, NRRL B-12103.

Sequence accession no. (16S rRNA gene): AB184657.

148. **Streptomyces drozdowiczii** Semêdo, Gomes, Linhares, Duarte, Nascimento, Rosado, Margis-Pinheiro, Margis, Silva, Alviano, Manfio, Soares, Linhares and Coelho 2004, 1327[VP]

droz.do.wic′zi.i. N.L. gen. masc. n. *drozdowiczii* of Drozdowicz, named after Adam Drozdowicz, a soil microbiologist who worked in Brazil.

Spore chains in Section *Rectiflexibiles.* Oval spores, borne in chains on the tip of the aerial mycelium. Spore surface is smooth. Branched, gray colored aerial mycelium is produced on inorganic salts-starch agar. The substrate mycelium has no distinctive pigment, but a diffusible yellow-brown pigment is produced. Melanin is produced on tyrosine agar and peptone-yeast extract-iron agar. Cell-wall hydrolysates contain LL-A_2pm. The predominant amino acids in the cell-wall hydrolysate are alanine (major), glycine, glutamic acid, and leucine. The predominant fatty acids found in whole cell methanolysates are $C_{16:0}$ iso (22%), $C_{15:0}$ iso (19%), $C_{15:0}$ anteiso (18%), $C_{17:0}$ iso (10%), $C_{17:0}$ anteiso (7%), $C_{16:0}$ (6%), and $C_{14:0}$ iso (6%). The species description is based on a single strain and hence serves as the type strain description.

Type strain shows no sequence similarity over 99%.

Source: isolated from the Brazilian Atlantic forest soil

(Mendanha Forest. Rio de Janeiro, RJ, Brazil).

DNA G+C content (mol%): not known.

Type strain: M7a, CBMAI 0498, CIP 107837, JCM 13580, NRRL B-24297.

Sequence accession no. (16S rRNA gene): EF654097.

149. **Streptomyces durhamensis** Gordon and Lapa 1966, 754[AL]

dur.ham.en′sis. N.L. masc. adj. *durhamensis* of or belonging to Durham, named after Durham, North Carolina, USA.

Spore chains in Section *Spirales*. Mature spore chains generally contain 10–50 spores per chain. This morphology is seen on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar. Spore surface is spiny, but smooth spores may also be seen.

Color of colony: aerial mass color in the Gray or Red color series on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar. Nearest matching color tabs in the Gray color series are 3ge, light grayish yellowish brown; 3fe, light brownish gray; and 2dc, yellowish gray. Nearest tab in the Red color series is 5dc, grayish yellowish pink. Reverse side of colony with no distinctive pigments (pale or grayish yellow to yellowish brown) on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar.

Color in medium: melanoid pigments are formed in peptone-yeast-iron agar, tyrosine agar, and tryptone-yeast broth. No pigment is found in medium in yeast-malt agar, oatmeal agar, salts-starch agar, or glycerol-asparagine agar.

D-Glucose, L-arabinose, D-xylose, iso-inositol, D-mannitol, D-fructose, sucrose, and raffinose are all utilized for growth. No growth or only traces of growth with rhamnose.

Type strain shows the highest sequence similarity to: *S. filipinensis*, AB184198, 99.8%; *S. puniciscabiei*, AF361785, 99.1%.

Source: not known.

DNA G+C content (mol%): not known.

Type strain: AS 4.1699, ATCC 23194, CBS 742.72, DSM 40539, HAMBI 1064, NBRC 13441, IMET 43359, JCM 4291, JCM 4747, KCTC 9723, NRRL B-3309, NRRL-ISP 5539, RIA 1402, VKM Ac-763.

Sequence accession no. (16S rRNA gene): AY999785.

150. **Streptomyces durmitorensis** Savic, Bratic and Vasiljevic 2007, 2123[VP]

dur.mi.tor.en′sis. N.L. masc. adj. *durmitorensis* of or pertaining to Durmitor, Serbia and Montenegro, where the type strain was isolated.

Spore chains are *Rectiflexibiles*, with 10 or more rod-shaped smooth-surfaced spores (0.5–0.9 × 1–1.5 µm) per chain. Produces a yellowish gray and greenish gray substrate mycelium and a greenish yellow aerial spore-mass on yeast extract-malt extract and glycerol-asparagine agars. Soluble pigments are not formed on oatmeal, yeast extract-malt extract, or glycerol-asparagine agars, while dark gray pigment is formed on inorganic salts-starch agar. Melanoid pigments are not formed on peptone-yeast extract-iron or tyrosine agars. Nitrate is reduced to nitrite, gelatin is liquefied, starch is not hydrolyzed, esculin, and DNA are not degraded. Cellobiose, D(−)-fructose, D(+)-galactose, D(+)-glucose, D(+)-mannose, α-melibiose, D(+)-raffinose, L(+)-rhamnose, D(−)-sucrose, D(+)-xylose, α-trehalose, glycerol, and D(+)-mannitol are utilized for growth, but α-lactose, β-lactose, D(−)-maltose, L(+)-arabinose, D-sorbitol, and *myo*-inositol are not utilized. Acid is produced from glucose. L-Alanine, L-arginine, L-cysteine, L-glycine, L-histidine, L-methionine, L-proline, and L-valine are utilized as sole nitrogen sources, but L-asparagine, L-lysine, L-phenylalanine, ornithine hydrochloride, and thiamine are not utilized. Positive for nitrate reduction, catalase, extracellular protease, haemolysin (β-hemolysis), and urease; lecithinase-negative. H_2S and indole are not produced. Temperature range for growth is 10–37°C, with an optimum between 28 and 32°C. The cell wall contains LL-A_2pm. Grows in the presence of NaCl (9%, w/v) and thallous acetate (0.001%, w/v), but not in sodium-azide (0.01%, w/v), phenol (0.1%, w/v), or potassium tellurite (0.001%, w/v). Susceptible to: apramycin (10 µg/ml), kanamycin (5 µg/ml), gentamicin (5 µg/ml), tetracycline (10 µg/ml), thiostrepton (10 µg/ml), chloramphenicol (35 µg/ml), and spectinomycin(90 µg/ml). Resistant to: ampicillin (100 µg/ml), erythromycin (100 µg/ml), and FK506 (100 µg/ml). Shows antimicrobial activity against *Micrococcus luteus* NCIMB 196 and *Saccharomyces cerevisiae* FAV20, but not against *Bacillus subtilis* NCIMB 3610, *Candida albicans* CBS 562, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, *Saccharomyces cerevisiae* FAS20, or *Staphylococcus aureus* ATCC 25923.

Type strain shows the highest sequence similarity to: *S. aureus*, AB249976, 99.6%; *S. kanamyceticus*, DQ442511, 99.2%. Type strain shows DNA–DNA similarity to: *S. aureus* DSM 41785[T], 15.5%; *S. kanamyceticus* DSM 40500[T], 13.3%.

Source: not known.

DNA G+C content (mol%): 72.0.

Type strain: MS405, CIP 108995, DSM 41863.

Sequence accession no. (16S rRNA gene): DQ067287.

151. **Streptomyces echinatus** Corbaz, Ettlinger, Gäumann, Keller-Schierlein, Kradolfer, Neipp, Prelog, Reusser and Zähner 1957a, 203[AL]

e.chi.na′tus. L. masc. adj. *echinatus* set with prickles, prickly.

Spore chain morphology in Section *Retinaculiaperti* or *Spirales*. Tight spirals sometimes occur at tips of sporophores. Mature spore chains generally have 10–50 spores per chain. This morphology is observed on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar. Spore surface is spiny.

Color of colony: aerial mass color in the Gray color series on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar. Reverse side of colony is grayed yellow modified by green on oatmeal agar, salts-starch agar, and glycerol-asparagine agar. Substrate pigment is not a pH indicator.

Color in medium: melanoid pigments are formed in peptone-yeast-iron agar, tyrosine agar, and tryptone-yeast broth. Pigments other than melanoids are not formed in yeast-malt agar, oatmeal agar, salts-starch agar, or glycerol-asparagine agar.

D-Glucose, L-arabinose, D-xylose, iso-inositol, D-mannitol, D-fructose, rhamnose, and raffinose are utilized for growth (growth on L-arabinose and D-xylose is less than on other sugars). No growth or only traces of growth on sucrose.

Type strain shows no sequence similarity over 99%.

Source: not known.

DNA G+C content (mol%): not known.

Type strain: AS 4.1642, ATCC 19748, ATCC 21133, CBS 409.59, CBS 487.68, BCRC 13656, CECT 3313, DSM 40013, HUT 6090, IFM 1076, NBRC 12763, IMET 40461, JCM 4144, JCM 4574, KCTC 9724, LMG 5972, NCIMB 9598, NCIMB 9799, NRRL 2587, NRRL-ISP 5013, RIA 1029, UNIQEM 135, VKM Ac-762.

Sequence accession no. (16S rRNA gene): AJ399465.

152. **Streptomyces echinoruber** Palleroni, Reichelt, Müller, Epps, Tabenkin, Bull, Schüep and Berger 1981, 382[VP] (Effective publication: Palleroni, Reichelt, Müller, Epps, Tabenkin, Bull, Schüep and Berger 1978, 1224.)

e.chi.no.ru′ber. L. n. *echinus* a hedgehog, urchin; L. adj. *ruber* red; N.L. masc. adj. *echinoruber* spiny red.

Strain shows gray spore mass color, spiral spore chains, and spiny spore surface. Colonies are raised and coarse, with well-defined edges. The most striking character of strain X-14077[T] is the production of the deep cherry-red pigment which freely diffuses into the medium. This pigment is produced in most media tested and it becomes evident after 1 or 2 d of incubation. For a more detailed description of morphological properties, see Palleroni et al. (1978).

Source: not known.

DNA G+C content (mol%): not known.

Type strain: X-14077, AC 4.1707, DSM 41696, NBRC 14238, JCM 5016, KCTC 9725, NCIMB 12831, NRRL 8144.

Sequence accession no. (16S rRNA gene): no sequence available.

153. **Streptomyces ederensis** Wallhäusser, Nesemann, Präve and Steigler 1966, 734[AL]

e.der.en′sis. N.L. masc. adj. *ederensis* of or pertaining to Eder, named for the Eder valley in Germany.

Produces the moenomycin complex of anti-bacterial antibiotics comprised of moenomycins A, B_1, B_2, and C.

Type strain shows the highest sequence similarity to: *S. umbrinus*, AB184305, 100%; *S. phaeochromogenes*, AB184738, 99.6%.

Source: not known.

DNA G+C content (mol%): not known.

Type strain: AS 4.1665, ATCC 15304, CBS 545.70, BCRC 11896, CECT 3212, DSM 40741, NBRC 15410, JCM 4958, KCTC 9726, NRRL B-8146, VKM Ac-845.

Sequence accession no. (16S rRNA gene): AB184658.

154. **Streptomyces ehimensis** corrig. (Shibata, Honso, Tokui and Nakazawa 1954) Witt and Stackebrandt 1991, 456[VP] (Effective publication: Witt and Stackebrandt 1990, 370.) ("*Streptomyces ehimensis*" Shibata, Honso, Tokui and Nakazawa 1954, 168; "*Verticillomyces ehimensis*" Shinobu 1965, 109; *Streptoverticillium ehimense* Locci, Baldacci and Petrolini Baldan 1969, 40).

Etymology of specific epithet is unknown.

Spore chain morphology in Section *Verticillati*. Mature aerial mycelium is usually umbellate-monoverticillate (biverticillate), but sporulating aerial mycelium is very sparse or absent on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar. Biverticillate spore chains can be seen on Pridham and Gottlieb carbon utilization medium plus D-xylose or L-arabinose and sometimes can be found on oatmeal agar after 21 d. Spore chains are short, usually with 3–10 spores per chain. Spore surface is smooth.

Color of colony: aerial mass color for mature sporulating aerial mycelium cannot be determined accurately because of poor sporulation on yeast-malt agar, salts-starch agar, and glycerol-asparagine agar. When sporulating aerial mycelium occurs on oatmeal agar, it is in the Gray color series. The spore surface of poorly sporulating growth is generally in the Yellow or Red color series on yeast-malt agar, salts-starch agar, and glycerol-asparagine agar or sparse aerial mycelium may be white. Reverse side of colony is brown to dark brown on yeast-malt agar, but grayish yellow, orange yellow, or yellowish brown on oatmeal agar, salts-starch agar, and glycerol-asparagine agar. Substrate mycelium pigment is not a pH indicator.

Color in medium: melanoid pigments are produced in peptone-yeast-iron agar and tryptone-yeast broth, but only weakly or not at all on tyrosine agar. Yellow or grayish yellowish brown pigment may be found in the medium in yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar. This pigment is not pH-sensitive.

D-Glucose, iso-inositol, D-mannitol, and D-fructose are utilized for growth. Traces of growth occur on the carbon-free control and on other ISP carbon sources.

Type strain shows the highest sequence similarity to: *S. hygroscopius* subsp. *angustmyceticus*, DQ442509, 99.8%; *S. abikoensis*, AB184537, 99.6%; *S. sapporonensis*, AB184508, 99.4%; *S. lilacinus*, AB184819, 99.3%; *S. luteireticuli*, AB249969, 99.2%; *S. varsoviensis*, DQ026653, 99%.

Source: not known.

DNA G+C content (mol%): not known.

Type strain: AS 4.1668, ATCC 23903, CBS 799.68, BCRC 13319, DSM 40253, HAMBI 1042, NBRC 12858, NBRC 3398, JCM 4162, JCM 4635, KCTC 9727, NRRL B-1967, NRRL-ISP 5253, RIA 1179, VKM Ac-945.

Sequence accession no. (16S rRNA gene): AY999834.

Further comments: the original spelling, *Streptomyces ehimensis* (*sic*), has been corrected in accordance with Rule 61 of the *Bacteriological Code* (1990 Revision).

In violation of Rule 33c of the *Bacteriological Code* (1990 Revision), in Validation List no. 38, *Streptomyces ehimensis* is proposed as a *nomen revictum* (basonym: "*Streptomyces ehimensis*" Shibata et al. 1954.

According to Hatano et al. (2003), *Streptomyces ehimensis* corrig. (Shibata et al. 1954) Witt and Stackebrandt 1991 is a later heterotypic synonym of *Streptomyces abikoensis* (Umezawa et al. 1951) Witt and Stackebrandt 1991.

155. **Streptomyces emeiensis** Sun, Huang, Zhang and Liu 2007, 1638[VP]

e.me.i.en′sis. N.L. masc. adj. *emeiensis* of or pertaining to Emei, a famous mountain in Sichuan Province, southern China, where the sample yielding the type strain was collected.

Aerobic, mesophilic, Gram-stain-positive actinomycete that develops well-branched substrate and aerial mycelium. *Rectiflexibiles* and hooked spore chains of elliptical, spiny-surfaced spores are frequently arranged in a verticillate structure. Diffusible pigments are not formed, nor are melanin pigments produced on peptone-yeast extract-iron or tyrosine agars. Nitrate is reduced. Amylase and gelatinase are not produced. Growth occurs between 15 and 40°C and at pH values from 5.5 to 9.5, but not at pH 4.5 or 10.5. Growth occurs in the presence of 0.1% (w/v) phenol, 5% (w/v) NaCl, and 0.01 (w/v) NaN_3, but not in the presence of 7% (w/v) NaCl. Shows weak antimicrobial activity against strains of *Bacillus subtilis* and *Mycobacterium smegmatis*, but not against strains of *Staphylococcus epidermis*, *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, or *Candida albicans*. Sensitive to filter-paper discs soaked in the following (µg/ml): novobiocin (5), streptomycin (10), oxacillin (1), chloramphenicol (30), ciprofloxacin (5), and erythromycin (15). The cell-wall is of type I. Type II phospholipids and menaquinones MK-9(H_6, H_8, H_4) are detected. The fatty acid profile is composed of $C_{15:0}$ anteiso (14.6%), $C_{16:0}$ iso (13.3%), $C_{17:0}$ anteiso (12.6%), $C_{16:0}$ (9.4%), $C_{16:1}\omega7c$ (8.2%), $C_{17:1}$ anteiso$\omega9c$ (7.0%), $C_{18:0}$ (3.7%), $C_{17:1}\omega8c$ (3.6%), iso H-$C_{16:1}$ (3.6%), $C_{15:0}$ (3.1%), $C_{15:0}$ iso (3.0%), $C_{17:1}$ iso$\omega9c$ (3.0%), $C_{15:0}$ iso (3.0%), $C_{14:0}$ (2.8%), $C_{18:1}\omega9c$ (2.4%), $C_{18:1}\omega7c$ (1.6%), $C_{17:0}$ iso (1.3%), $C_{17:0}$ (1.1%), $C_{14:0}$ iso (1.1%), and iso I-$C_{15:1}$ (1.1%).

Type strain shows the highest sequence similarity to: *S. prasinopilosus*, EF626597, 99.4%. Type strain shows DNA–DNA similarity to: *S. prasinopilosus* DSM 40098^T, 62.7%; *S. prasinus* JCM 4603^T, 55.5%; *S. hirsutus* DSM 40095^T, 46.4%; *S. bambergiensis* DSM 40590^T, 31.7%; *S. cyanoalbus* DSM 40198^T, 26.1%.

Source: the type strain was isolated from a soil sample collected from Emei Mountain, Sichuan Province, China.

DNA G+C content (mol%): 70.8.

Type strain: 4776, CGMCC 4.3504, DSM 41884.

Sequence accession no. (16S rRNA gene): DQ462649.

156. **Streptomyces endus** Anderson and Gottlieb 1952, 302[AL]

en′dus. L. praep. *endo* in; N.L. masc. adj. *endus* referring to the site (inside the hyphae) of formation of the antibiotic endomycin.

Spore chains in Section *Spirales* with tightly closed spirals. Individual spores are not easily detached from the spiral chain and compact coils may become hydroscopic (coalesce in black moist droplets). Spore surface is warty.

Color of colony: aerial mass color in the Gray color series after 21 d on yeast-malt agar, oatmeal agar, and salts-starch agar. Aerial mycelium on glycerol-asparagine agar and young aerial mycelium on other media may be white. Older colonies sometimes become moist and black. Reverse side of colony with no distinctive pigments (grayed yellow to yellowish brown or gray) on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar. In older cultures, the substrate mycelium sometimes becomes moist and black. Substrate color is not affected by pH change.

Color in medium: melanoid pigments are not formed in peptone-yeast-iron agar, tyrosine agar, or tryptone-yeast broth. No pigments are found in the medium in yeast-malt agar, oatmeal agar, salts-starch agar, or glycerol-asparagine agar.

D-Glucose, L-arabinose, D-xylose, D-mannitol, D-fructose, and rhamnose are utilized for growth. No growth or only traces of growth on sucrose, iso-inositol, and raffinose.

Source: not known.

DNA G+C content (mol%): not known.

Type strain: 9-20, ATCC 23904, CBS 800.68, DSM 40187, NBRC 12859, JCM 4213, JCM 4636, NRRL 2339, NRRL-ISP 5187, RIA 1141.

Sequence accession no. (16S rRNA gene): AY999911.

Type strain shows the highest sequence similarity to: *S. sporocinereus*, AB249933, 100%; *S. hygroscopius* subsp. *hygroscopius*, AB184428, 100%; *S. demainii*, DQ334782, 99.9%; *S. yogyakartensis*, AB249942, 99.5%; *S. violaceusniger*, AJ391823, 99.5%; *S. albiflaviniger*, AJ391812, 99.3%.

157. **Streptomyces enissocaesilis** (*ex* Krasil'nikov 1970b) Sveshnikova 1986, 574[VP] (Effective publication: Sveshnikova *in* Gause, Preobrazhenskaya, Sveshnikova, Terekhova and Maximova 1983.) ("*Actinomyces enissocaesilis*" Krasil'nikov 1970b).

Etymology of specific epithet is unknown.

Spore chains are spiral (*Spirales*); spores are smooth. On mineral agar 1: aerial mycelium is light pink-yellowish-grayish; substrate mycelium is brown; diffusible pigment is brown with grayish violet shadows. On starch-ammonia agar: aerial mycelium is absent or poorly developed, whitish; substrate mycelium is colorless to pale yellowish, growth is poor; no diffusible pigment. On glycerol-nitrate agar: aerial mycelium is white to brownish pink; substrate mycelium and diffusible pigment are brown to dark brown. On glycerol-asparagine agar: aerial mycelium is absent or poorly developed, whitish; substrate mycelium is black-grayish brown; no diffusible pigment. On oatmeal agar: aerial mycelium is poorly developed; substrate mycelium is brown; diffusible pigment is brownish, weak. On organic agar 2: aerial mycelium is light pink-yellowish-grayish; substrate mycelium and diffusible pigment are brown. Melanoid pigment: not extant or poorly developed. Grows on glucose, arabinose, xylose, and mannitol; no growth seen on sucrose, rhamnose, or raffinose.

Type strain shows the highest sequence similarity to: *S. flavidovirens*, AB184270, 99.2%; *S. chryseus*, AY999787, 99.1%; *S. helvaticus*, AB184367, 99.1%; *S. albidochromogenes*, AB249953, 99.1%.

Source: not known.

DNA G+C content (mol%): not known.

Type strain: AS 4.1586, ATCC 43682, DSM 41454, INMI 40-31, JCM 9088, NBRC 100763, NRRL B-16365, VKM Ac-130.

Sequence accession no. (16S rRNA gene): AB249930.

158. **Streptomyces erumpens** Calot and Cercós 1963, 159[AL]

e.rum′pens. L. masc. part. adj. *erumpens* bursting forth.

Poor growth on Czapek's solution agar. Exhibits antibacterial activity; inhibited by streptomycin; produces the tetraenic anti-fungal antibiotic 17732 (tetrins A and B and another polyene).

Type strain shows the highest sequence similarity to: *S. rimosus* subsp. *rimosus*, AB045883, 99.7%; *S. monomycini*, DQ445790, 99.4%; *S. chrestomyceticus*, AJ621609, 99.3%; *S. rimosus* subsp. *paromomycinus*, AJ621610, 99.3%; *S. ochraceiscleroticus*, AB184094, 99.3%; *S. sioyaensis*, DQ026654, 99.2%; *S. hygroscopius* subsp. *decoyicus*, AY999883, 99.2%; *S. purpurogeneiscleroticus*, AJ621604, 99.1%; *S. albofaciens*, AB045880, 99.1%; *S. violens*, AJ621605, 99.1%; *S. sclerotialus*, AJ621608, 99.1%.

Source: not known.

DNA G+C content (mol%): not known.

Type strain: AS 4.1626, ATCC 23266, CBS 252.65, DSM 40941, NBRC 15403, JCM 5060, KCTC 9729, NRRL B-3163.

Sequence accession no. (16S rRNA gene): AJ621603.

Further comments: according to Guo et al. (2008), *Streptomyces erumpens* Calot and Cercós 1963 is a later heterotypic synonym of *Streptomyces griseus* (Krainsky 1914) Waksman and Henrici 1948.

159. **Streptomyces erythraeus** (Waksman *in* Bergey, Harrison, Breed, Hammer and Huntoon 1923) Waksman and Henrici *in* Breed, Murray and Hitchens 1948, 938[AL] ["*Actinomyces erythreus*" (*sic*) Waksman *in* Bergey, Harrison, Breed, Hammer and Huntoon 1923, 370; "*Streptomyces erythreus*" (*sic*) Waksman and Henrici *in* Breed, Murray and Hitchens 1948, 938]

e.ry.thra′e.us. L. masc. adj. *erythraeus* reddish, referring to colony color.

Spore chains in Section *Spirales* or *Retinaculiaperti*. Spore chains are usually short so that imperfect spirals, hooks, or loops of only one turn, and short, straight to flexuous chains are common. Hooks and loops are usually of small diameter and are not representative of true *Retinaculiaperti* morphology. This morphology is seen on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar, but aerial mycelium may be poorly developed on glycerol-asparagine agar. Spore surface is spiny.

Color of colony: aerial mass color in the Red color series (4ec, 5cb, 5db, grayish yellowish pink; or 3ca, pale orange yellow) on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar. White aerial mycelium may also be seen. Reverse side of colony is brown to grayish reddish brown on yeast-malt agar; orange-yellow to light yellowish brown or grayish yellow on oatmeal agar and salts-starch agar; yellowish brown to strong brown on glycerol-asparagine agar. Reverse mycelium pigment is usually not a pH indicator, but, when pink pigment is present, it may change to pale yellow with the addition of 0.05 M HCl.

Color in medium: melanoid pigments are not formed in peptone-yeast-iron agar, tyrosine agar, or tryptone-yeast broth. Pink or yellow pigment may be found in the medium in yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar, but pigment is not always present on these media. Pink pigment, when present, becomes colorless with the addition of either 0.05 M HCl or NaOH.

D-Glucose, D-fructose, sucrose, raffinose, and D-mannitol are utilized for growth. Reports vary on utilization of L-arabinose, D-xylose, iso-inositol, and rhamnose. Significant growth is often observed on control plates of carbon-utilization medium without added carbon source.

Source: not known.

DNA G+C content (mol%): not known.

Type strain: ATCC 11635, CBS 727.72, DSM 40517, HUT 6087, IAM 0045, NBRC 13426, JCM 4748, IMRU 3737, NCIB (now NCIMB) 8594, NRRL 2338, NRRL-ISP 5517, RIA 1387, VKM Ac-1189.

Sequence accession no. (16S rRNA gene): no sequence available.

Further comments: the type strain of *Streptomyces erythraeus* (Waksman 1923) Waksman and Henrici 1948 is not a representative of the genus *Streptomyces* Waksman and Henrici 1943 and a new species, *Saccharopolyspora erythraea* Labeda 1987, is created for this strain and other strains having type IV cell walls. Labeda (1987) also proposed an emendation of *Streptomyces erythraeus* [*Streptomyces erythraeus* (Waksman 1923) Waksman and Henrici 1948 emend. Labeda 1987] with a neotype strain NRRL B-5616^T (NRRL-ISP 5059^T, Sanchez-Marroquin A-24^T). According to Rules 18c and 18g of the *Bacteriological Code* (1990 Revision), only the Judicial Commission may decide to take action leading to replacement of the type strain and, according to Rule 37a(2) of the *Bacteriological Code* (1990 Revision), retention of a name in a sense which excludes the type can only be effected by conservation and only by the Judicial Commission. Therefore, *Streptomyces erythraeus* (Waksman 1923) Waksman and Henrici 1948 emend. Labeda 1987 is illegitimate and may not be used [Rule 51a of the *Bacteriological Code* (1990 Revision)] and a new species *Streptomyces labedae* Lacey 1987 is provided for this taxon (Lacey, 1987).

Rule 17 of the *Bacteriological Code* (1990 Revision) states that the type determines the application of a taxon if the taxon is subsequently divided or united with another taxon, and Rule 40b of the *Bacteriological Code* (1990 Revision) that the specific epithet must be retained for the species which includes the type strain. Since *Saccharopolyspora erythraea* (Waksman 1923) Labeda 1987 contains the type strain of *Streptomyces erythraeus* (Waksman 1923) Waksman and Henrici 1948, the new taxon must be regarded as a new combination.

160. **Streptomyces erythrogriseus** Falcão de Morais and Dália Maia 1959, 64[AL]

e.ry.thro.gri′se.us. Gr. adj. *euruthros* red; N.L. adj. *griseus* gray; N.L. masc. adj. *erythrogriseus* red, gray (referring to change in color of aerial mycelium from gray to red).

Spore chains in Section *Spirales*, but short spore chains may form imperfect or incomplete spirals or crooked hooks or loops. Spirals are most numerous on salts-starch agar after 14 d and are rare on yeast-malt agar

and glycerol-asparagine agar. Mature spore chains are generally short, with 3 to 10 or more spores per chain, but long chains with more than 50 spores are also reported. Spore surface is spiny with short spines. Smooth spores are also found.

Color of colony: aerial mass color usually in the Gray color series on yeast-malt agar and salts-starch agar; Gray, Red, or White color series on glycerol-asparagine agar. Aerial mycelium suitable for color determination is usually not formed on oatmeal agar, but when present it is in the Gray color series. Nearest matching color tabs in the Gray color series are d, light gray, and 5fe, light grayish reddish brown. Nearest matching tabs in the Red color series are 5ge, light grayish reddish brown and 5dc, light yellowish pink. Reverse side of colony is strong brown on yeast-malt agar; orange yellow to yellowish brown on oatmeal agar and salts-starch agar; reddish orange to reddish brown on glycerol-asparagine agar. Reverse mycelium pigment is a pH indicator, changing from yellow to orange with the addition of 0.05 M NaOH and from yellow brown to yellow with the addition of 0.05 M HCl.

Color in medium: melanoid pigments are not formed in peptone-yeast-iron agar, tyrosine agar, or tryptone-yeast broth. Yellow to orange pigment is sometimes found in the medium in yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar. When this pigment is present, it is pH-sensitive, showing the same changes observed in the reverse mycelium pigment.

D-Glucose, L-arabinose, D-xylose, iso-inositol, D-mannitol, D-fructose, and rhamnose are utilized for growth. No growth or only traces of growth with sucrose and raffinose.

Type strain shows the highest sequence similarity to: *S. griseoincarnatus*, AJ781328, 100%; *S. labedae*, AB184704, 100%; *S. variabilis*, DQ442551, 100%; *S. griseorubens*, AB184139, 99.9%; *S. griseoflavus*, AJ781322, 99.6%; *S. matensis*, AB184221, 99.6%; *S. althioticus*, AY999808, 99.2%; *S. paradoxus*, AB184628, 99.2%; *S. heliomycini*, AB184712, 99.1%; *S. flaveolus*, AB184764, 99.1%; *S. collinus*, AB184123, 99.1%; *S. viridochromogenes*, DQ442555, 99.1%; *S. bellus*, AB184849, 99%; *S. albogriseolus*, AJ494865, 99%; *S. viridodiastaticus*, AY999852, 99%; *S. violaceochromogenes*, AY999867, 99%; *S. malachitofuscus*, AB184282, 99%; *S. violaceorubidus*, AJ781374, 99%; *S. coerulescens*, AY999720, 99%.

Source: not known.

DNA G+C content (mol%): not known.

Type strain: ATCC 27427, CBS 485.74, BCRC 13770, DSM 40116, NBRC 14601, JCM 9650, NRRL B-3808, NRRL-ISP 5116.

Sequence accession no. (16S rRNA gene): AJ781328.

161. **Streptomyces eurocidicus** (Okami, Utahara, Nakamura and Umezawa 1954) Witt and Stackebrandt 1991, 456[VP] (Effective publication: Witt and Stackebrandt 1990, 370.) ("*Streptomyces eurocidicus*" Okami, Utahara, Nakamura and Umezawa 1954, 102; "*Verticillomyces eurocidicus*" Shinobu 1965, 111; *Streptoverticillium eurocidicum* Locci, Baldacci and Petrolini Baldan 1969, 36)

eu.ro.ci′di.cus. N.L. n. *eurocidinum* eurodicin; L. masc. suff. *-icus* suffix used with the sense of belonging to; N.L. masc. adj. *eurocidicus* belonging to eurodicin.

Spore chains in Section Umbellate Monoverticillate (=*Streptomyces* Section Verticillati biverticillate). Mature spore chains contain 3 to 10 or more spores per chain. This morphology is seen on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar. Spore surface is smooth.

Color of colony: aerial mass color in the Yellow color series (1ba or 2ba, pale yellow; 1½db, pale greenish yellow; 1db, pale yellow green; 1½fb, light yellow) on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar. Reverse side of colony with no distinctive pigments (olive brown to reddish brown on yeast-malt agar; pale grayish yellow to yellowish brown or olive brown on oatmeal agar, salts-starch agar, and glycerol-asparagine agar).

Color in medium: melanoid pigments are formed in peptone-yeast-iron agar and tryptone-yeast broth, but not in tyrosine agar. No pigment is found in the medium in yeast-malt agar, oatmeal agar, salts-starch agar, or glycerol-asparagine agar.

D-Glucose, iso-inositol, and D-fructose are utilized for growth. No growth or only traces of growth with L-arabinose, D-xylose, D-mannitol, rhamnose, sucrose, and raffinose.

Type strain shows the highest sequence similarity to: *S. albireticuli*, AB184881, 99.8%; *S. werraensis*, DQ442558, 99.3%; *S. biverticillatus*, AJ781381, 99.3%; *S. blastmyceticus*, AY999802, 99.2%; *S. stramineus*, AB184720, 99.2%; *S. netropsis*, AB184848, 99.1%.

Source: not known.

DNA G+C content (mol%): not known.

Type strain: AS 4.1086, ATCC 27428, CBS 792.72, BCRC 12424, CECT 3259, DSM 40604, NBRC 13491, IMET 43412, JCM 4029, JCM 4749, NRRL B-1676, NRRL-ISP 5604, RIA 1452, RIA 733, VKM Ac-903.

Sequence accession no. (16S rRNA gene): AY999790.

Further comments: in violation of Rule 33c of the *Bacteriological Code* (1990 Revision), in Validation List no. 38, *Streptomyces eurocidicus* is proposed as a *nomen revictum* (basonym: "*Streptomyces eurocidicus*" Okami et al. 1954.

According to Hatano et al. (2003), *Streptomyces eurocidicus* (Okami et al. 1954) Witt and Stackebrandt 1991 is an earlier heterotypic synonym of *Streptomyces albireticuli* (Nakazawa 1955) Witt and Stackebrandt 1991.

162. **Streptomyces europaeiscabiei** Bouchek-Mechiche, Gardan, Normand and Jouan 2000, 98[VP]

eu.ro.pa.ei.sca′bi.ei. L. adj. *europaeus* european; L. n. *scabies* scab, mange; N.L. gen. n. *europaeiscabiei* of European scab, referring to the European origin of the strains.

Spores are gray and are borne in mature spiral chains. Melanin is produced on tyrosine agar. L-Arabinose, D-fructose, D-glucose, D-mannitol, inositol, raffinose, rhamnose, sucrose, and D-xylose are utilized for growth. Degradation of xanthine differs between the strains studied. All strains are susceptible to 20 μg/ml streptomycin and 0.5 μg/ml crystal violet. They are not susceptible to 25 μg/ml oleandomycin or 10 IU/ml penicillin G. They utilize *trans*-aconitate, D(+)trehalose, ONPG, melibiose, and 5-keto-D-gluconate; most (about 78%) of the strains assimilate gentisate. They do not use betaine, mucate, D-saccharate, DL-lactate, or turanose.

Type strain shows the highest sequence similarity to: *S. scabies*, D63862, 99.8%. Type strain shows DNA–DNA similarity to: *S. stelliscabiei* DSM 41803^T, 42%; *S. reticuliscabiei* DSM 41804^T, 20%.

Source: these strains were isolated from common scab lesions, mostly on potato, but also on carrot and beet, and have been confirmed to be pathogenic on potato cvs Bintje and Urgenta, on carrot cv. Premia and on radish cv. Polka.

DNA G+C content (mol%): 71.3.

Type strain: CFBP 4497, CIP 107062, DSM 41802, ICMP 13714, KACC 20186, NCPPB 4039.

Sequence accession no. (16S rRNA gene): AY207598.

163. **Streptomyces eurythermus** Corbaz, Ettlinger, Gäumann, Keller-Schierlein, Kradolfer, Kyburz, Neipp, Prelog, Reusser and Zähner 1955, 1202^{AL}

eu.ry.ther′mus. Gr. adj. *eurus* wide; Gr. adj. *thermos* hot; N.L. masc. adj. *eurythermus* wide, hot.

Spore chains in Section *Retinaculiaperti* (including many straight and flexuous spore chains and occasional spirals). Mature spore chains generally have 10–50 spores per chain. This morphology is observed on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar. Spore surface is smooth.

Color of colony: aerial mass color in the Gray color series on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar. Reverse side of colony with no distinctive pigments on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar; substrate pigment is not a pH indicator. One observer reports slight change from yellowish brown to reddish brown with addition of 0.05 M NaOH.

Color in medium: melanoid pigments are formed in peptone-yeast-iron agar, tyrosine agar, and tryptone-yeast broth. Pigments other than melanoids are not formed in yeast-malt agar, oatmeal agar, salts-starch agar, or glycerol-asparagine agar.

D-Glucose, L-arabinose, sucrose, D-xylose, D-mannitol, and D-fructose are utilized for growth. Variable reports on growth with iso-inositol, rhamnose, and raffinose.

Type strain shows the highest sequence similarity to: *S. nogalater*, AB045886, 99.2%; *S. fragilis*, AY999917, 99%; *S. tendae*, D63873, 99%.

Source: not known.

DNA G+C content (mol%): not known.

Type strain: AS 4.1697, ATCC 14975, ATCC 19749, CBS 488.68, BCRC 13650, DSM 40014, NBRC 12764, IMET 43078, JCM 4206, JCM 4575, KCTC 9731, NRRL 2539, NRRL-ISP 5014, RIA 1030, VKM Ac-1729.

Sequence accession no. (16S rRNA gene): D63870.

164. **Streptomyces exfoliatus** (Waksman and Curtis 1916) Waksman and Henrici *in* Breed, Murray and Hitchens 1948, 951^{AL} ("*Actinomyces exfoliatus*" Waksman and Curtis 1916, 116)

ex.fo.li.a′tus. L. masc. part. adj. *exfoliatus* stripped of leaves.

Spore chains in Section *Rectiflexibiles*. Mature spore chains generally have 10–50 spores per chain. Typical morphology on oatmeal agar. Some sporulation aerial mycelium with typical morphology on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar. Spore surface is smooth. One observer reported fragmentation and spore formation in substrate-mycelium on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar in 14 d; another observer reported coremia formation on glycerol-asparagine agar in 14 d.

Color of colony: aerial mass color in the Red color series on oatmeal agar; sporulation aerial mycelium is also in the Red series on yeast-malt agar and salts-starch agar when formed on these media. Reverse side of colony with no distinctive pigments on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar; substrate pigment is not a pH indicator.

Color in medium: melanoid pigments are not formed in peptone-yeast-iron agar and tyrosine agar. No pigment found in medium in yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar.

D-Glucose, L-arabinose, sucrose, D-xylose, D-fructose, rhamnose, and raffinose are utilized for growth. No growth on iso-inositol or D-mannitol.

Type strain shows the highest sequence similarity to: *S. zaomyceticus*, EF178685, 99.9%; *S. venezuelae*, AB045890, 99.9%; *S. lateritius*, AJ781326, 99.7%; *S. wedmorensis*, DQ442557, 99.7%; *S. litmocidini*, AB184149, 99.6%; *S. omiyaensis*, EF178697, 99.6%; *S. yereyanensis*, EF178684, 99.4%; *S. narbonensis*, DQ445794, 99.3%.

Source: not known.

DNA G+C content (mol%): not known.

Type strain: AS 4.1407, ATCC 12627, ATCC 19750, CBS 489.68, BCRC 11469, CCUG 11113, DSM 40060, NBRC 13191, JCM 4366, LMG 19307, NCIMB 12599, NRRL B-1237, NRRL B-2924, NRRL-ISP 5060, PCM 2367, RIA 1031, UNIQEM 137, VKM Ac-767.

Sequence accession no. (16S rRNA gene): AB184324.

165. **Streptomyces felleus** Lindenbein 1952, 374^{AL}

fel′le.us. L. masc. adj. *felleus* of gall, like gall (pertaining to the bitter taste of proactinomycin A).

Spore chains in Section *Rectiflexibiles*. Mature spore chains generally have 10–50 spores per chain. This morphology is observed on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar. Spore surface is smooth.

Color of colony: aerial mass color in the Yellow color series on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar. Reverse side of colony with no distinctive pigments on yeast-malt agar, oatmeal agar, salts-starch agar, or glycerol-asparagine agar; substrate pigment is not a pH indicator.

Color in medium: melanoid pigments not formed in peptone-yeast-iron agar and tyrosine agar. No pigment in medium or faint yellow to yellowish green color in yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar.

D-Glucose, L-arabinose, D-xylose, D-mannitol, and D-fructose are utilized for growth. No growth or only traces of growth on sucrose, iso-inositol, rhamnose, and raffinose.

Type strain shows the highest sequence similarity to: *S. limosus*, AB184147, 100%; *S. canescens*, AB184117, 100%; *S. daghestanicus*, DQ442497, 100%; *S. albidoflavus*, AB184255, 100%; *S. hydrogenans*, AB184868, 100%; *S. odorifer*, Z76682, 100%; *S. violascens*, AY999737, 100%; *S. griseus* subsp. *solvifaciens*, AB249915, 100%; *S. champavatii*, DQ026642, 100%; *S. sampsonii*, D63871, 99.8%; *S. koyangensis*, AY079156, 99.7%.

Source: not known.

DNA G+C content (mol%): not known.

Type strain: AS 4.1677, ATCC 19752, CBS 491.68, BCRC 11471, DSM 40130, NBRC 12766, JCM 4368, NCIMB 12974, NRRL-ISP 5130, RIA 1033, UNIQEM 139, VKM Ac-722.

Sequence accession no. (16S rRNA gene): AB184129.

166. **Streptomyces ferralitis** Saintpierre-Bonaccio, Amir, Pineau, Lemriss and Goodfellow 2004, 2064[VP]

fer.ra'li.tis. N.L. gen. n. *ferralitis* of ferralite, denoting the type of soil from which the type strain was isolated.

Forms an extensively branched substrate mycelium and aerial hyphae that differentiate into looped or spiral chains of spores. The spore chains consist of up to 15 barrel-shaped spores with smooth surface. A brown substrate mycelium and a white aerial spore mass are formed on modified Bennett's agar (Jones, 1949). Melanin pigments are not produced on peptone-yeast extract-iron agar. The culture grows well at 20 and 45°C, but does not grow at 10°C. Metabolizes casein, hypoxanthine, L-tyrosine, urea, and xanthine, but not adenine, elastin, gelatin, guanine, starch, Tween 80, or xanthine. D(+)-Galactose, D(+)-glucose, D(+)-mannitol, D(+)-mannose, and D(+)-trehalose are used as sole carbon sources for energy and growth, but adonitol, D-arabinose, D(+)-cellobiose, D(+)-melibiose, and sodium citrate are not. Resistant to penicillin (25 µg/ml), but does not grow in the presence of erythromycin (4 µg/ml), gentamicin sulfate (10 µg/ml), rifampin (6 µg/ml), streptomycin sulfate (5 µg/ml), tetracycline hydrochloride (30 µg/ml), vancomycin hydrochloride (10 µg/ml), crystal violet (0.0002%, w/v), phenol (0.01%, w/v), potassium tellurite (0.005%, w/v), or sodium chloride (5%, w/v). It shows activity against clinical isolates of *Candida albicans*, *Staphylococcus aureus*, *Staphylococcus epidermidis* and a *Corynebacterium* strain, but not against *Fusarium oxysporum*, *Bacillus*, *Erwinia*, *Escherichia coli*, *Klebsiella pneumoniae*, or *Pseudomonas aeruginosa* strains. The species description is based upon a single strain and hence serves as the description of the type strain.

Type strain shows no sequence similarity over 99%.

Source: the type strain was isolated from a ferralitic, oxidic ultramafic soil collected at the southern end of the main island of New Caledonia.

DNA G+C content (mol%): not known.

Type strain: SFOp68, DSM 41836, NCIMB 13954.

Sequence accession no. (16S rRNA gene): AY262826.

167. **Streptomyces fervens** (DeBoer et al. 1959–1960) Witt and Stackebrandt 1991, 456[VP] (Effective publication: Witt and Stackebrandt 1990, 370.) ("*Streptomyces fervens*" DeBoer Dietz, Evans and Michaels 1959–1960), 220; (*Streptoverticillium fervens* Locci, Baldacci and Petrolini Baldan 1969, 23)

fer'vens. L. part. adj. *fervens* boiling hot, referring to its high growth temperature.

Spore chain morphology: Section not determined. Sporulating aerial mycelium is not produced on yeast-malt agar, oatmeal agar, salts-starch agar, or glycerol-asparagine agar. Note that the original descriptions of DeBoer et al. and Locci et al. (op. cit) call attention to the limited sporulation; they report verticillate morphology. Spore surface is smooth.

Color of colony: the thin aerial mycelium, which generally lacks sporophores and spore chains, is in the Red color series on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar. Two observers report that an appropriate color tab cannot be found in the red color wheel of Tresner and Backus; one observer identifies nonsporulating aerial mycelium color as 7 ca, light yellowish pink. Reverse side of colony with distinctive red color reported as dark red or eosin red on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar. Reverse mycelium pigment is pH-sensitive, changing from deep red to reddish brown with the addition of 0.05 M NaOH and from red to violet red with the addition of 0.05 M HCl.

Color in medium: melanoid pigments are formed in peptone-yeast-iron agar and tryptone-yeast broth, but not in tyrosine agar. No pigment other than melanoids is found in the medium in yeast-malt agar, oatmeal agar, salts-starch agar, or glycerol-asparagine agar.

D-Glucose and iso-inositol are utilized for growth. A trace of growth is seen on L-arabinose, D-xylose, D-mannitol, D-fructose, rhamnose, sucrose and raffinose; utilization of these carbon sources is doubtful.

For sequence similarity, see type strain of *Streptomyces hiroshimensis*.

Source: not known.

DNA G+C content (mol%): not known.

Type strain: ATCC 27429, JCM 4310, JCM 4750, NBRC 13343, NRRL 2755.

Sequence accession no. (16S rRNA gene): AB184871.

Further comments: in violation of Rule 33c of the *Bacteriological Code* (1990 Revision), in Validation List no. 38, *Streptomyces fervens* is proposed as a *nomen revictum* (basonym: "*Streptomyces fervens*" DeBoer et al. 1959–1960).

Witt and Stackebrandt proposed to transfer *Streptoverticillium fervens* (DeBoer et al. 1959–1960) Locci et al. 1969 to the genus. However, Validation List no. 38 does not include formal propositions about *Streptoverticillium fervens* subsp. *fervens* Baldacci and Locci 1974 and *Streptoverticillium fervens* subsp. *melrosporus* Mason et al. 1965.

Labeda (1996) proposes *Streptomyces biverticillatus* (Preobrazhenskaya and Ryabova 1957) Witt and Stackebrandt 1991, *Streptomyces fervens* (DeBoer et al. 1959–1960) Witt and Stackebrandt 1991, and *Streptomyces roseoverticillatus* (Shinobu 1956) Witt and Stackebrandt 1991 as later synonyms of *Streptomyces baldaccii* corrig. (Farina and Locci 1966) Witt and Stackebrandt 1991. This proposal is in violation of Rule 24b(1) of the *Bacteriological Code* (1990 Revision) because the senior epithet is *roseoverticillatus*. *Streptomyces baldaccii*, *Streptomyces biverticillatus*, and *Streptomyces fervens* are therefore to be regarded as later synonyms of *Streptomyces roseoverticillatus*.

According to Hatano et al. (2003), *Streptomyces fervens* (DeBoer et al. 1959–1960) Witt and Stackebrandt 1991 is a later heterotypic synonym of *Streptomyces hiroshimensis* (Shinobu 1955) Witt and Stackebrandt 1991.

168. **Streptomyces filamentosus** Okami and Umezawa *in* Okami, Okuda, Takeuchi, Nitta and Umezawa 1953, 153[AL] emend. Lanoot, Vancanneyt, Dawyndt, Cnockaert, Zhang, Huang, Liu and Swings 2005a, 8 (Effective publication: Lanoot, Vancanneyt, Dawyndt, Cnockaert, Zhang, Huang, Liu and Swings 2004, 88.)

fi.la.men.to′sus. L. n. *filamentum* assembly of threads; L. masc. suff. *-osus* suffix used with the sense of full of, prone to; N.L. masc. adj. *filamentosus* full of threads or filaments.

Spore chains in Section *Rectiflexibiles.* Mature spore chains generally have 10–50 spores per chain. This morphology is found on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar. Spore surface is smooth.

Color of colony: aerial mass color in the Red color series on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar. Reverse side of colony with no distinctive pigments on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar; substrate pigment is not a pH indicator.

Color in medium: melanoid pigments not formed in peptone-yeast-iron agar and tyrosine agar. No pigment found in medium in yeast-malt agar, oatmeal agar, salts-starch agar, or glycerol-asparagine agar.

D-Glucose, L-arabinose, sucrose, D-xylose, and rhamnose are utilized for growth. No growth or only trace of growth on iso-inositol, D-mannitol, and raffinose. Variable reports on utilization of D-fructose.

Type strain shows the highest sequence similarity to: *S. roseolus*, AB184168, 99.4%; *S. roseoviridis*, AB184239, 99%.

Source: not known.

DNA G+C content (mol%): not known.

Type strain: AS 4.1656, ATCC 19753, CBS 492.68, BCRC 13644, DSM 40022, HAMBI 1010, IFM 1180, NBRC 12767, IMET 43562, JCM 4122, JCM 4576, NCIMB 13018, NRRL B-2114, NRRL-ISP 5022, RIA 1034, UNIQEM 140, VKM Ac-1266.

Sequence accession no. (16S rRNA gene): AB184130.

Further comments: according to Lanoot et al. (2004), *Streptomyces filamentosus* Okami and Umezawa 1953 is an earlier heterotypic synonym of *Streptomyces roseosporus* Falcão de Morais and Dália Maia 1961.

169. **Streptomyces filipinensis** Ammann, Gottlieb, Brock, Carter and Whitfield 1955, 559[AL]

fi.li.pi.nen′sis. N.L. masc. adj. *filipinensis* of or belonging to the Philippines, the source of the soil from which the organism was isolated.

Spore chains in Section *Spirales.* Tight spirals or open coils, usually of several turns, occur at the end of moderately long spore chains or 10 to 50 or more spores per chain on yeast-malt agar, oatmeal agar, and salts-starch agar. Spirals may be replaced by *Retinaculiaperti* morphology on glycerol-asparagine agar. Spore surface is spiny.

Color of colony: aerial mass color in the Gray color series on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar. Reverse side of colony with no distinctive pigments (colorless to grayish yellow) on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar.

Color in medium: melanoid pigments are produced in peptone-yeast-iron agar, tyrosine agar, and peptone-yeast broth. Pigments other than melanoids are not found in the medium in yeast-malt agar, oatmeal agar, salts-starch agar, or glycerol-asparagine agar.

Carbon utilization: D-glucose, L-arabinose, sucrose, D-xylose, iso-inositol, D-mannitol, D-fructose and raffinose are utilized for growth. No growth or only trace of growth on rhamnose.

Type strain shows the highest sequence similarity to: *S. durhamensis*, AY999785, 99.8%.

Source: not known.

DNA G+C content (mol%): not known.

Type strain: AS 4.1452, ATCC 23905, CBS 309.56, CBS 801.68, BCRC 11472, DSM 40112, NBRC 12860, JCM 4369, LMG 19333, NRRL 2437, NRRL-ISP 5112, RIA 1124, VKM Ac-966.

Sequence accession no. (16S rRNA gene): AB184198.

170. **Streptomyces fimbriatus** (Millard and Burr 1926) Waksman *in* Waksman and Lechevalier 1953, 104[AL] ("*Actinomyces fimbriatus*" Millard and Burr 1926, 639)

fim.bri.a′tus. L. masc. adj. *fimbriatus* fibrous, fringed.

Excellent growth on Czapek's solution agar. Produces septacidin, an anti-tumor and anti-fungal purine antibiotic; not inhibited by streptomycin.

Type strain shows no sequence similarity over 99%.

Source: Millard and Burr's original single isolate (no longer extant) was obtained from a case of common potato scab.

DNA G+C content (mol%): not known.

Type strain: AS 4.1598, ATCC 15051, CBS 453.65, DSM 40942, NBRC 15411, JCM 5080, NCIMB 13039, NRRL B-3175, VKM Ac-761.

Sequence accession no. (16S rRNA gene): AY999844.

171. **Streptomyces fimicarius** (Duché 1934) Waksman and Henrici *in* Breed, Murray and Hitchens 1948, 940[AL] ("*Actinomyces fimicarius*" Duché 1934, 346)

fi.mi.ca′ri.us. L. n. *fimus* dung, manure; L. adj. *carus* dear, loving; N.L. masc. adj. *fimicarius* dung-loving.

Spore chains Section *Rectiflexibiles.* Mature spore chains are generally long with 10 to 50 or more spores per chain. This morphology is seen on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar. Spore surface is smooth. Fragmentation of the substrate mycelium may be seen on glycerol-asparagine agar in 16 d.

Color of colony: aerial mass color in the Yellow or White color series on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar. Reverse side of colony with no distinctive pigments (pale or grayish yellow to yellowish brown) on oatmeal agar, salts-starch agar, and glycerol-asparagine agar; yellow is modified by red (to orange or reddish brown) on yeast-malt agar. This pigment changes from reddish brown to pale brown with addition of 0.05 M HCl.

Color in medium: melanoid pigments are not formed in peptone-yeast-iron agar, tyrosine agar, or tryptone-yeast

broth (but according to one observer, some brown pigment is formed in the medium in Gause's medium no. 2). Red (pink to light reddish brown) pigment is found in the medium in yeast-malt agar and oatmeal agar; it may or may not be seen in salts-starch agar and glycerol-asparagine agar. This pigment is pH-sensitive, changing from pink or reddish brown to yellow when tested with 0.05 M HCl.

D-Glucose, L-arabinose, D-xylose, D-mannitol, D-fructose, and rhamnose are utilized for growth. Only traces of growth is seen with iso-inositol, sucrose, and raffinose.

Type strain shows the highest sequence similarity to: *S. cavourensis* subsp. *washingtonensis*, DQ026671, 100%; *S. anulatus*, DQ026637, 100%; *S. badius*, AY999783, 100%; *S. sindenensis*, AB184759, 100%; *S. rubiginosohelvolus*, AB184240, 100%; *S. pluricolorescens*, DQ442540, 100%; *S. griseinus*, AB184205, 100%; *S. acrimycini*, AY999889, 100%; *S. flavofuscus*, AB249935, 100%; *S. mediolani*, AB184674, 100%; *S. praecox*, AB184293, 100%; *S. lavendulae* subsp. *lavendulae*, AB184080, 100%; *S. griseoplanus*, AY999894, 100%; *S. microflavus*, DQ445795, 99.9%; *S. cinereorectus*, AB184646, 99.9%; *S. fulvorobeus*, AB184711, 99.9%; *S. lipmanii*, AB184148, 99.9%; *S. cyaneofuscatus*, AB184860, 99.9%; *S. baarnensis*, EF178688, 99.9%; *S. globisporus* subsp. *globisporus*, EF178686, 99.9%; *S. albovinaceus*, AB249958, 99.9%; *S. alboviridis*, AB184256, 99.9%; *S. californicus*, AB184755, 99.8%; *S. parvus*, DQ442537, 99.8%; *S. argenteolus*, AB045872, 99.8%; *S. halstedii*, EF178695, 99.7%; *S. luridiscabiei*, AF361784, 99.7%; *S. flavogriseus*, AJ494864, 99.7%; *S. griseus* subsp. *griseus*, AY207604, 99.7%; *S. griseolus*, AB184768, 99.7%; *S. floridae*, AB184656, 99.7%; *S. flavovirens*, DQ026635, 99.7%;%; *S. nitrosporeus*, EF178680, 99.5%; *S. pulveraceus*, AB184806, 99.5%; *S. olivoviridis*, AB184227, 99.5%; *S. bacillaris*, AB184439, 99.5%; *S. atroolivaceus*, AJ781320, 99.5%; *S. finlayi*, AY999788, 99.5%; *S. clavifer*, DQ026670, 99.5%; *S. yanii*, AB006159, 99.4%; *S. gelaticus*, DQ026636, 99.3%; *S. atratus*, DQ026638, 99.3%; *S. albolongus*, AB184425, 99.3%; *S. mutomycini*, AB249951, 99.3%; *S. celluloflavus*, AB184476, 99.3%; *S. griseobrunneus*, AB249912, 99.3%; *S. sanglieri*, AB249945, 99.3%; *S. spiroverticillatus*, AB184814, 99.2%; *S. cavourensis* subsp. *cavourensis*, DQ445791, 99.2%; *S. candidus*, DQ026663, 99.1%; *S. mauvecolor*, AB184532, 99%; *S. cremeus*, AB184124, 99%.

Source: not known.

DNA G+C content (mol%): not known.

Type strain: AS 4.1629, ATCC 25449, CBS 420.34, CBS 682.69, BCRC 12245, DSM 40322, NBRC 13037, JCM 4472, NRRL-ISP 5322, RIA 1229, VKM Ac-1724.

Sequence accession no. (16S rRNA gene): AY999784.

172. **Streptomyces finlayi** (Szabó, Marton, Buti and Pártai 1963) Pridham 1970, 35[AL] ("*Actinomyces finlayi*" Szabó, Marton, Buti and Pártai 1963, 209)

fin.lay′i. N.L. gen. masc. n. *finlayi* of Finlay, named for Alexander C. Finlay, discoverer of oxytetracycline.

Spore chains in Section *Rectiflexibiles* to *Spirales*. Short spore chains of 10 or more spores per chain are generally flexuous, crooked, hooked, or in imperfect spirals of only one or two turns; longer chains may form open spirals of three or four turns. Hooks and loops on short spore chains are not characteristic of *Retinaculiaperti* morphology. This morphology is seen on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar; spirals are best developed on yeast-malt agar and oatmeal agar. Spore surface is hairy.

Color of colony: aerial mass color in the Gray color series on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar. Reverse side of colony is grayed yellow to yellow-brown modified by green (grayish yellow to olive brown on yeast-malt agar and oatmeal agar; pale greenish yellow or yellow-green to moderate or dark olive green on salts-starch agar or glycerol-asparagine agar). Substrate mycelium pigment is not a pH indicator.

Color in medium: melanoid pigments are not produced in peptone-yeast-iron agar, tyrosine agar, or tryptone-yeast broth. No pigments are found in the medium in yeast-malt agar, oatmeal agar, salts-starch agar, or glycerol-asparagine agar.

D-Glucose is utilized for growth. Significant growth also occurs on L-arabinose, D-xylose, and rhamnose, although growth on these carbon sources is less than on D-glucose. Utilization of sucrose is doubtful. No growth or only trace of growth on iso-inositol, D-mannitol, D-fructose, and raffinose.

Type strain shows the highest sequence similarity to: *S. clavifer*, DQ026670, 99.8%; *S. olivoviridis*, AB184227, 99.7%; *S. atroolivaceus*, AJ781320, 99.7%; *S. californicus*, AB184755, 99.6%; *S. mutomycini*, AB249951, 99.6%; *S. pluricolorescens*, DQ442540, 99.5%; *S. rubiginosohelvolus*, AB184240, 99.5%; *S. fulvorobeus*, AB184711, 99.5%; *S. lipmanii*, AB184148, 99.5%; *S. fimicarius*, AY999784, 99.5%; *S. sindenensis*, AB184759, 99.5%; *S. praecox*, AB184293, 99.5%; *S. flavofuscus*, AB249935, 99.5%; *S. badius*, AY999783, 99.5%; *S. mediolani*, AB184674, 99.5%; *S. cavourensis* subsp. *washingtonensis*, DQ026671, 99.5%; *S. anulatus*, DQ026637, 99.5%; *S. griseoplanus*, AY999894, 99.5%; *S. floridae*, AB184656, 99.5%; *S. lavendulae* subsp. *lavendulae*, AB184080, 99.5%; *S. alboviridis*, AB184256, 99.5%; *S. microflavus*, DQ445795, 99.5%; *S. griseinus*, AB184205, 99.5%; *S. cyaneofuscatus*, AB184860, 99.4%; *S. albovinaceus*, AB249958, 99.4%; *S. cinereorectus*, AB184646, 99.4%; *S. globisporus* subsp. *globisporus*, EF178686, 99.4%; *S. baarnensis*, EF178688, 99.4%; *S. acrimycini*, AY999889, 99.4%; *S. luridiscabiei*, AF361784, 99.4%; *S. griseolus*, AB184768, 99.3%; *S. candidus*, DQ026663, 99.3%; *S. griseus* subsp. *griseus*, AY207604, 99.3%; *S. argenteolus*, AB045872, 99.3%; *S. flavovirens*, DQ026635, 99.3%; *S. parvus*, DQ442537, 99.3%; *S. bacillaris*, AB184439, 99.2%; *S. flavogriseus*, AJ494864, 99.2%; *S. halstedii*, EF178695, 99.2%; *S. griseobrunneus*, AB249912, 99.1%; *S. albolongus*, AB184425, 99.1%; *S. celluloflavus*, AB184476, 99.1%; *S. pulveraceus*, AB184806, 99%; *S. nitrosporeus*, EF178680, 99%; *S. cavourensis* subsp. *cavourensis*, DQ445791, 99%.

Source: not known.

DNA G+C content (mol%): not known.

Type strain: AS 4.1436, ATCC 23340, ATCC 23906, CBS 802.68, DSM 40218, HAMBI 1071, NBRC 13201, JCM 4216, JCM 4637, NCIMB 9834, NRRL B-12114, NRRL-ISP 5218, RIA 1162, VKM Ac-967.

Sequence accession no. (16S rRNA gene): AY999788.

173. **Streptomyces flaveolus** (Waksman *in* Bergey, Harrison, Breed, Hammer and Huntoon 1923) Waksman and Henrici *in* Breed, Murray and Hitchens 1948, 936^{AL} ("*Actinomyces flaveolus*" Waksman *in* Bergey, Harrison, Breed, Hammer and Huntoon 1923, 368; "*Streptomyces flaveolus* subsp. *flaveolus*" Waksman *in* Pridham, Lyons and Seckinger 1965, 220)

fla.ve′o.lus. L. adj. *flavus* yellow; N.L. dim. masc. adj. *flaveolus* somewhat yellow.

Spore chains in Section *Spirales*, but short spore chains representative of Section *Retinaculiaperti* are also common. Mature spore chains generally have 10–50 spores per chain; shorter chains (3–10 spores) may be found. This morphology is seen on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar (although sporulating aerial mycelium may be poorly developed on oatmeal agar or salts-starch agar). Spore surface is hairy with some tendency toward spines; carbon replica method suggests spiny spore surface. Coremia may form on salts-starch agar.

Color of colony: aerial mass color in the Gray color series on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar (White series also reported by one observer on salts-starch agar and glycerol-asparagine agar). Reverse side of colony with no distinctive pigments on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar; substrate pigment is not a pH indicator.

Color in medium: melanoid pigments not formed in peptone-yeast-iron agar and tyrosine agar. Yellow pigment found in medium in yeast-malt agar, glycerol-asparagine agar, and sometimes in other media. This pigment is not pH-sensitive when tested with 0.05 M NaOH or HCl.

D-Glucose, L-arabinose, sucrose, D-xylose, iso-inositol, D-mannitol, D-fructose, and rhamnose are utilized for growth. No growth or only trace of growth on raffinose.

Type strain shows the highest sequence similarity to: *S. griseoflavus*, AJ781322, 99.5%; *S. fragilis*, AY999917, 99.3%; *S. collinus*, AB184123, 99.2%; *S. viridochromogenes*, DQ442555, 99.2%; *S. griseorubens*, AB184139, 99.2%; *S. ambofaciens*, M27245, 99.1%; *S. glaucescens*, AB184843, 99.1%; *S. paradoxus*, AB184628, 99.1%; *S. matensis*, AB184221, 99.1%; *S. variabilis*, DQ442551, 99.1%; *S. coelicoflavus*, AB184650, 99.1%; *S. griseoincarnatus*, AJ781328, 99.1%; *S. erythrogriseus*, AJ781328, 99.1%; *S. malachitofuscus*, AB184282, 99%; *S. labedae*, AB184704, 99%.

Source: not known.

DNA G+C content (mol%): not known.

Type strain: AS 4.1432, ATCC 19754, ATCC 3319, CBS 128.20, CBS 493.68, CCM 3171, BCRC 12489, CECT 3181, DSM 40061, HAMBI 893, NBRC 12768, NBRC 3408, NBRC 3715, IMET 40233, JCM 4032, JCM 4577, KCTC 9022, LMG 19328, NRRL B-1334, NRRL B-2688, NRRL-ISP 5061, RIA 1035, RIA 485, UNIQEM 141, VKM Ac-965.

Sequence accession no. (16S rRNA gene): AB184764.

174. **Streptomyces flaveus** (Cross, Lechevalier and Lechevalier 1963) Goodfellow, Williams and Alderson 1986a, 574^{VP} (Effective publication: Goodfellow, Williams and Alderson 1986a, 53.) (*Microellobosporia flavea* Cross, Lechevalier and Lechevalier 1963, 428)

fla.ve′us. N.L. masc. adj. *flaveus* presumably from *flavus* yellow.

Short straight spore chains (2–5). Spore surface is smooth. Spores borne on both the substrate and aerial mycelium. Forms extensively branched substrate and aerial mycelium. The aerial spore mass is gray; the reverse color is yellow brown. Does not form melanin pigments. Adenine, esculin, casein, hypoxanthine, starch, testosterone, tyrosine, and urea are degraded, but allantoin, arbutin, chitin, elastin, guanine, lecithin, pectin, xanthine, and xylan are not. Hydrogen sulfide is produced but nitrate is not reduced. L-Arabinose, cellobiose, D-fructose, D-galactose, D-glucose, *myo*-inositol, inulin, D-lactose, mannitol, D-mannose, melibiose, raffinose, L-rhamnose, salicin, sucrose, trehalose, and D-xylose are used as sole carbon sources, but adonitol, D-melezitose, and xylitol are not. Grows on DL-α-aminobutyric acid, L-arginine, L-cysteine, L-histidine, L-phenylalanine, potassium nitrate, L-serine, L-threonine, and L-valine, but not on L-hydroxyproline or L-methionine, as sole nitrogen source. Growth occurs at 10 and 37°C, but not at 4 or 45°C. Tolerant to phenol (0.1%, w/v) and sodium chloride (7%, w/v), but not to sodium azide (0.01%, w/v). Sensitive to rifampin and sodium chloride (10%, w/v). Does not show antimicrobial activity against *Aspergillus niger* LIV 131, *Bacillus subtilis* NCIB 3610, *Candida albicans* CBS 562, *Escherichia coli* NCIB 9132, *Micrococcus luteus* NCIB 196, *Pseudomonas fluorescens* NCIB 9046^{T}, *Saccharomyces cerevisiae* CBS 1171^{T}, or *Streptomyces murinus* ISP 5091. Wall peptidoglycan contains LL-A_2pm as the major diamino acid. The organism has a type II phospholipid pattern (*sensu* Lechevalier et al., 1977) and contains major amounts of hexa- and octahydrogenated menaquinones with nine isoprene units (Collins et al., 1984).

Type strain shows no sequence similarity over 99%.

Source: not known.

DNA G+C content (mol%): 68–71.

Type strain: ATCC 15332, CBS 355.67, DSM 43153, IFM 1234, NBRC 12190, IMET 43554, LMG 19323, NCIMB 9587, NRRL B-16074, RIA 896, VKM Ac-1295, VKM Ac-633, KCC A-0035, JCM 3035.

Sequence accession no. (16S rRNA gene): DQ026643.

175. **Streptomyces flavidofuscus** Preobrazhenskaya 1986, 574^{VP} (Effective publication: Preobrazhenskaya *in* Gause, Preobrazhenskaya, Sveshnikova, Terekhova and Maximova 1983.)

fla.vi.do.fus′cus. L. adj. *flavidus* yellowish; L. adj. *fuscus* dark, tawny; N.L. masc. adj. *flavidofuscus* yellowish, tawny.

Spore chains are spiral (*Spirales*); spores are smooth. On mineral agar 1: starch-ammonia agar: aerial mycelium is velvety, creamy, yellow, sometimes poor; substrate mycelium is colorless; no diffusible pigment. On glycerol-nitrate agar, glycerol-asparagine agar: aerial mycelium is yellow; substrate mycelium is colorless or yellow; no diffusible pigment. On oatmeal agar: aerial mycelium is yellow; substrate mycelium is colorless or pink; no diffusible pigment. On organic agar 2: aerial mycelium is creamy, yellow; substrate mycelium and diffusible pigment are gray-brown. Melanoid pigments are found. Grows on fructose, sucrose, rhamnose, glucose, arabinose, raffinose,

mannitol, and inositol. Strains of this species produce the antibiotic echinomycin.

Type strain shows no sequence similarity over 99%.

Source: not known.

DNA G+C content (mol%): 68–71.

Type strain: AS 4.1617, ATCC 43683, DSM 41473, NBRC 15404, INA 15719, JCM 6920, NRRL B-16366, VKM Ac-1209.

Sequence accession no. (16S rRNA gene): AY999914.

Further comments: according to Guo et al. (2008), *Streptomyces flavidofuscus* Preobrazhenskaya 1986 should be transferred to the genus *Nocardiopsis*. However, no formal proposition is made in the paper by Guo et al. (2008).

176. **Streptomyces flavidovirens** (Kudrina *in* Gauze, Preobrazhenskaya, Kudrina, Blinov, Ryabova and Sveshnikova 1957) Pridham, Hesseltine and Benedict 1958, 66[AL] ("*Actinomyces flavidovirens*" Kudrina *in* Gauze, Preobrazhenskaya, Kudrina, Blinov, Ryabova and Sveshnikova 1957, 90)

fla.vi.do.vi′rens. L. adj. *flavidus* yellowish; L. part. adj. *virens* being green; N.L. part. adj. *flavidovirens* being yellowish green.

Spore chains in Section *Retinaculiaperti* or *Rectiflexibiles*. Preponderance of very flexuous spore chains, some of which appear as imperfect or open spirals together with some straight or slightly flexuous chains makes this strain difficult to categorize in respect to spore chain morphology. The illustration accompanying the original description would place this species in Section *Spirales*, but regular spirals were not found by ISP observers. Mature spore chains are moderately long with 10 to 50 or more spores per chain. This morphology is found on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar. Spore surface is smooth.

Color of colony: aerial mass color in the Yellow or White color series on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar. The nearest matching color tab in the Yellow series is 2ba, pale yellow. Reverse side of colony with no distinctive pigments (colorless to pale yellow or light grayish yellow) on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar.

Color in medium: melanoid pigments are not formed in peptone-yeast-iron agar, tyrosine agar, or tryptone-yeast broth. Traces of yellow pigment may or may not be found in the medium in yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar. This pigment, when present, is not pH-sensitive when tested with 0.05 M NaOH or HCl.

D-Glucose, L-arabinose, and D-xylose are utilized for growth. Reports vary on utilization of iso-inositol, D-fructose, rhamnose, and sucrose. No growth or only trace of growth with D-mannitol and raffinose.

Type strain shows the highest sequence similarity to: *S. helvaticus*, AB184367, 100%; *S. albidochromogenes*, AB249953, 100%; *S. chryseus*, AY999787, 100%; *S. enissocaesilis*, AB249930, 99.2%.

Source: not known.

DNA G+C content (mol%): not known.

Type strain: ATCC 19900, ATCC 25451, CBS 684.69, BCRC 13761, DSM 40150, NBRC 13039, IMET 43744, INA 12287, JCM 4474, NRRL B-2708, NRRL-ISP 5150, RIA 1231, VKM Ac-1771.

Sequence accession no. (16S rRNA gene): AB184270.

177. **Streptomyces flaviscleroticus** (*ex* Pridham 1970) Goodfellow, Williams and Alderson 1986a, 574[VP] (Effective publication: Goodfellow, Williams and Alderson 1986d, 59.)

fla.vi.scle.ro′ti.cus. L. adj. *flavus* yellow; N.L. neut. n. *sclerotium* sclerotium; N.L. masc. adj. *flaviscleroticus* yellow sclerotium, referring to yellow and ability to form sclerotia.

Spore chain morphology: Section not determined. Aerial mycelium is poorly developed or absent on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar. Rare spore chains on yeast-malt agar or on glycerol-asparagine agar are reported to be straight or flexuous. The original description by Thirumalachar reports irregular spirals. Spore surface is not determined.

One of three observers reports "globula sporangia" on yeast-malt agar, oatmeal agar, and salts-starch agar, and sclerotia on yeast-malt agar. Fragmentation of substrate mycelium into spore-like bodies is also recorded by this observer.

Color of colony is not determined. Aerial mycelium is not produced on yeast-malt agar, oatmeal agar, salts-starch agar, or glycerol-asparagine agar. Reverse side of colony is olive brown to strong brown on yeast-malt agar; greenish yellow on oatmeal agar; grayish yellow, orange yellow, or moderate yellow on salts-starch agar and glycerol-asparagine agar.

Color in medium: melanoid pigments are not formed in peptone-yeast-iron agar, tyrosine agar, or tryptone-yeast broth. No pigment or only a trace of yellow is found in the medium in yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar.

D-Glucose, L-arabinose, D-xylose, D-mannitol, D-fructose, and rhamnose are utilized for growth. Utilization of sucrose is doubtful; no growth or only traces of growth with iso-inositol and raffinose.

For sequence similarity, see type strain of *Streptomyces minutiscleroticus*.

Source: not known.

DNA G+C content (mol%): not known.

Type strain: ATCC 19347, AS 4.1071, CBS 658.72, BCRC 12108, CMI 117723, DSM 40270, DSM 43152, NBRC 12998, NBRC 13357, NBRC 14019, NBRC 15148, IMET 43617, JCM 3100, JCM 4751, KCC A-0100, LMG 19886, NCIB (now NCIMB) 11008, NRRL B-12173, NRRL-ISP 5270, PCM 2303, RIA 1318, RIA 883.

Sequence accession no. (16S rRNA gene): AB184634.

Further comments: according to Goodfellow et al. (1986a), the species *Streptomyces flaviscleroticus* (*ex* Pridham 1970) Goodfellow et al. 1986a is a synonym of the species *Chainia flava* Thirumalachar and Sukapure 1964. However, according to Rule 51b (2) of the *Bacteriological Code* (1990 Revision), the transfer of *Chainia flava* Thirumalachar and Sukapure 1964 in the genus *Streptomyces* Waksman and Henrici 1943 as *Streptomyces flaviscleroticus* (*ex* Pridham 1970) Goodfellow et al. 1986a sp. nov., nom. rev. is illegitimate.

According to Lanoot et al. (2005b), *Streptomyces flaviscleroticus* (*ex* Pridham 1970) Goodfellow et al. 1986a is a later heterotypic synonym of *Streptomyces minutiscleroticus* (Thirumalachar 1965) Pridham 1970 emend. Lanoot et al. 2005b.

178. **Streptomyces flavofungini** (*ex* Uri and Békési 1958) Szabó and Preobrazhenskaya 1986, 574[VP] (Effective publication: Szabó and Preobrazhenskaya *in* Gause, Preobrazhenskaya, Sveshnikova, Terekhova and Maximova 1983.) ["*Actinomyces flavofungini*" (Uri and Békési 1958) Szabó and Preobrazhenskaya 1962]

fla.vo.fun.gi′ni. L. adj. *flavus* golden yellow, reddish yellow; L. adj. *funginus* of a mushroom, pertaining to a mushroom; N.L. adj. *flavofungini* yellow and from a mushroom.

Spore chains in Section *Rectiflexibiles*, but sporulation is poor or absent on most media. Absence of sporulation is also mentioned in the original description for this strain. ISP observers record short to long spore chains on oatmeal agar and salts-starch agar but not on yeast-malt agar or glycerol-asparagine agar. Spore chains may also be seen on carbon-utilization media. Spore surface is smooth.

Color of colony: aerial mass color in the White or Yellow color series on salts-starch agar. Nearest matching color tab in the Yellow color series is 1cb, pale yellow green. Reverse side of colony is yellow to greenish yellow or light olive or light olive brown on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar.

Color in medium: two of three observers report dark pigments in peptone-yeast-iron agar. No melanoid pigments are seen in tyrosine agar or tryptone-yeast broth. Yellow or pale greenish yellow pigment is sometimes found in the medium in oatmeal agar, salts-starch agar, or glycerol-asparagine agar, or pigment may be absent in these media.

D-Glucose, L-arabinose, D-xylose, iso-inositol, D-mannitol, D-fructose, and raffinose are utilized for growth. Utilization of rhamnose is doubtful. No growth or only traces of growth with sucrose.

Type strain shows the highest sequence similarity to: *S. fulvissimus*, AB184434, 99.7%; *S. alboflavus*, EF178699, 99%.

Source: not known.

DNA G+C content (mol%): not known.

Type strain: SA-IX-3, ATCC 27430, CBS 411.59, CBS 672.72, DSM 40366, NBRC 13371, JCM 4753, NRRL B-12307, NRRL-ISP 5366, RIA 1332, VKM Ac-1179.

Sequence accession no. (16S rRNA gene): AB184359.

179. **Streptomyces flavofuscus** (Kudrina *in* Gauze, Preobrazhenskaya, Kudrina, Blinov, Ryabova and Sveshnikova 1957) Preobrazhenskaya 1986, 574[VP] (Effective publication: Preobrazhenskaya *in* Gause, Preobrazhenskaya, Sveshnikova, Terekhova and Maximova 1983.) ("*Actinomyces globisporus* var. *flavofuscus*" Kudrina *in* Gauze, Preobrazhenskaya, Kudrina, Blinov, Ryabova and Sveshnikova 1957, 81; *Streptomyces globisporus* subsp. *flavofuscus* Pridham, Hesseltine and Benedict 1958, 59)

fla.vo.fus′cus. L. adj. *flavus* yellow; L. adj. *fuscus* dark, tawny; N.L. masc. adj. *flavofuscus* yellow, tawny.

Spore chains are straight (*Rectiflexibiles*); spores are smooth. On mineral agar 1, glycerol-nitrate agar: aerial mycelium is mealy, yellow, green-yellow; substrate mycelium and diffusible pigment are gray-brownish, gray-brown. On oatmeal agar: aerial mycelium is yellow; substrate mycelium is colorless; no diffusible pigment. On synthetic agar Korenjako (Kuchaeva et al., 1960): aerial mycelium is yellow; substrate mycelium is dark green; no diffusible pigment. On organic agar 2: aerial mycelium is yellow; substrate mycelium and diffusible pigment are yellow-gray-brown, gray-brown. Melanoid pigments are not formed. Antibiotic: not produced.

Type strain shows the highest sequence similarity to: *S. rubiginosohelvolus*, AB184240, 100%; *S. praecox*, AB184293, 100%; *S. griseinus*, AB184205, 100%; *S. cavourensis* subsp. *washingtonensis*, DQ026671, 100%; *S. mediolani*, AB184674, 100%; *S. badius*, AY999783, 100%; *S. acrimycini*, AY999889, 100%; *S. griseoplanus*, AY999894, 100%; *S. anulatus*, DQ026637, 100%; *S. flavofuscus*, AB249935, 100%; *S. sindenensis*, AB184759, 100%; *S. fimicarius*, AY999784, 100%; *S. pluricolorescens*, DQ442540, 100%; *S. lavendulae* subsp. *lavendulae*, AB184080, 100%; *S. microflavus*, DQ445795, 99.9%; *S. alboviridis*, AB184256, 99.9%; *S. cyaneofuscatus*, AB184860, 99.9%; *S. globisporus* subsp. *globisporus*, EF178686, 99.9%; *S. baarnensis*, EF178688, 99.9%; *S. lipmanii*, AB184148, 99.9%; *S. fulvorobeus*, AB184711, 99.9%; *S. cinereorectus*, AB184646, 99.9%; *S. albovinaceus*, AB249958, 99.9%; *S. parvus*, DQ442537, 99.8%; *S. californicus*, AB184755, 99.8%; *S. argenteolus*, AB045872, 99.8%; *S. luridiscabiei*, AF361784, 99.7%; *S. halstedii*, EF178695, 99.7%; *S. flavogriseus*, AJ494864, 99.7%; *S. griseus* subsp. *griseus*, AY207604, 99.7%; *S. floridae*, AB184656, 99.7%; *S. flavovirens*, DQ026635, 99.7%; *S. griseolus*, AB184768, 99.7%; *S. bacillaris*, AB184439, 99.5%; *S. olivoviridis*, AB184227, 99.5%; *S. atroolivaceus*, AJ781320, 99.5%; *S. clavifer*, DQ026670, 99.5%; *S. finlayi*, AY999788, 99.5%; *S. pulveraceus*, AB184806, 99.5%; *S. nitrosporeus*, EF178680, 99.5%; *S. yanii*, AB006159, 99.4%; *S. atratus*, DQ026638, 99.3%; *S. gelaticus*, DQ026636, 99.3%; *S. griseobrunneus*, AB249912, 99.3%; *S. celluloflavus*, AB184476, 99.3%; *S. albolongus*, AB184425, 99.3%; *S. mutomycini*, AB249951, 99.3%; *S. sanglieri*, AB249945, 99.3%; *S. cavourensis* subsp. *cavourensis*, DQ445791, 99.2%; *S. spiroverticillatus*, AB184814, 99.2%; *S. candidus*, DQ026663, 99.1%; *S. mauvecolor*, AB184532, 99%; *S. cremeus*, AB184124, 99%.

Source: not known.

DNA G+C content (mol%): not known.

Type strain: ATCC 19908, CBS 121.60, DSM 41426, INA 1565/53, JCM 9766, KCTC 9737, NBRC 100768, NRRL B-2594, RIA 310, VKM Ac-1841.

Sequence accession no. (16S rRNA gene): AB249935.

180. **Streptomyces flavogriseus** (Duché 1934) Waksman *in* Waksman and Lechevalier 1953, 55[AL] ("*Actinomyces flavogriseus*" Duché 1934, 341)

fla.vo.gri′se.us. L. adj. *flavus* yellow; N.L. adj. *griseus* gray; N.L. masc. adj. *flavogriseus* yellowish gray.

Spore chains in Section *Rectiflexibiles*. Mature spore chains have 3–10, or often more than 10 spores per chain; long chains are not common. This morphology is seen on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar. Spore surface is smooth.

Color of colony: aerial mass color in the Gray color series on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar. Reverse side of colony is

strong yellow or orange-yellow on yeast-malt agar, grayish yellow to olive brown on oatmeal agar, greenish yellow on salts-starch agar; reverse mycelium pigment is not a pH indicator.

Color in medium: melanoid pigments are not formed in peptone-yeast-iron agar, tyrosine agar, or tryptone-yeast broth. No pigment, or only a trace of yellow, is found in the medium in yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar.

D-Glucose, L-arabinose, D-xylose, D-mannitol, D-fructose, and rhamnose are utilized for growth. No growth or only traces of growth with iso-inositol, sucrose, and raffinose.

Type strain shows the highest sequence similarity to: *S. flavovirens*, DQ026635, 100%; *S. nitrosporeus*, EF178680, 99.7%; *S. fimicarius*, AY999784, 99.7%; *S. praecox*, AB184293, 99.7%; *S. cinereorectus*, AB184646, 99.7%; *S. griseolus*, AB184768, 99.7%; *S. flavofuscus*, AB249935, 99.7%; *S. anulatus*, DQ026637, 99.7%; *S. halstedii*, EF178695, 99.6%; *S. pluricolorescens*, DQ442540, 99.6%; *S. sindenensis*, AB184759, 99.6%; *S. mediolani*, AB184674, 99.7%; *S. lavendulae* subsp. *lavendulae*, AB184080, 99.6%; *S. albovinaceus*, AB249958, 99.6%; *S. rubiginosohelvolus*, AB184240, 99.6%; *S. cavourensis* subsp. *washingtonensis*, DQ026671, 99.6%; *S. griseinus*, AB184205, 99.6%; *S. badius*, AY999783, 99.6%; *S. argenteolus*, AB045872, 99.6%; *S. acrimycini*, AY999889, 99.6%; *S. griseoplanus*, AY999894, 99.6%; *S. luridiscabiei*, AF361784, 99.5%; *S. microflavus*, DQ445795, 99.5%; *S. cyaneofuscatus*, AB184860, 99.5%; *S. parvus*, DQ442537, 99.5%; *S. californicus*, AB184755, 99.5%; *S. lipmanii*, AB184148, 99.5%; *S. fulvorobeus*, AB184711, 99.5%; *S. alboviridis*, AB184256, 99.5%; *S. floridae*, AB184656, 99.4%; *S. globisporus* subsp. *globisporus*, EF178686, 99.4%; *S. griseus* subsp. *griseus*, AY207604, 99.4%; *S. baarnensis*, EF178688, 99.4%; *S. pulveraceus*, AB184806, 99.3%; *S. bacillaris*, AB184439, 99.3%; *S. olivoviridis*, AB184227, 99.2%; *S. atroolivaceus*, AJ781320, 99.2%; *S. finlayi*, AY999788, 99.2%; *S. yanii*, AB006159, 99.2%; *S. celluloflavus*, AB184476, 99.1%; *S. griseobrunneus*, AB249912, 99.1%; *S. gelaticus*, DQ026636, 99.1%; *S. spiroverticillatus*, AB184814, 99.1%; *S. sanglieri*, AB249945, 99.1%; *S. clavifer*, DQ026670, 99.1%; *S. albolongus*, AB184425, 99.1%; *S. atratus*, DQ026638, 99.1%; *S. cremeus*, AB184124, 99%.

Source: not known.

DNA G+C content (mol%): not known.

Type strain: ATCC 25452, CBS 101.34, CBS 685.69, BCRC 13440, CECT 3327, DSM 40323, NBRC 13040, IMET 43576, JCM 4475, KCTC 9778, NRRL B-1671, NRRL-ISP 5323, RIA 1232, VKM Ac-1325.

Sequence accession no. (16S rRNA gene): AJ494864.

181. **Streptomyces flavopersicus** (Oliver, Goldstein, Bower, Holper and Otto 1961) Witt and Stackebrandt 1991, 456[VP] (Effective publication: Witt and Stackebrandt 1990, 370.) ("*Streptomyces flavopersicus*" Oliver, Goldstein, Bower, Holper and Otto 1961, 495; *Streptoverticillium flavopersicum* Locci, Baldacci and Petrolini Baldan 1969, 41)

fla.vo.per'si.cus. L. adj. *flavus* yellow; L. n. *persicus* peach; N.L. masc. adj. *flavopersicus* of yellow peach.

Spore chains in Section *Verticillati*. Umbellate monoverticillate (biverticillate); individual spore chains may form hooks, loops, or primitive spirals. Mature spore chains generally have 10–50 spores per chain; but shorter chains of 3–10 spores are also found. This morphology is seen on yeast-malt agar, oatmeal agar, and salts-starch agar. Spore surface is smooth.

Color of colony: aerial mass color in the Red color series on yeast-malt agar, oatmeal agar, and salts-starch agar. Reverse side of colony with no distinctive pigments on yeast-malt agar, oatmeal agar, salts-starch agar, or glycerol-asparagine agar; substrate pigment is not a pH indicator.

Color in medium: melanoid pigments formed weakly after prolonged cultivation in peptone-yeast-iron agar. No pigment found in medium, or very faint color in yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar.

D-Glucose and iso-inositol are utilized for growth. No growth or only trace of growth on L-arabinose, sucrose, D-xylose, D-mannitol, rhamnose, and raffinose. Utilization of D-fructose is doubtful.

For sequence similarity, see type strain of *Streptomyces netropsis.*

Source: not known.

DNA G+C content (mol%): not known.

Type strain: ATCC 19756, JCM 4307, JCM 4370, NBRC 12769, NRRL 2820, UNIQEM 142.

Sequence accession no. (16S rRNA gene): AB249911.

Further comments: in violation of Rule 33c of the *Bacteriological Code* (1990 Revision), in Validation List no. 38, *Streptomyces flavopersicus* is proposed as a *nomen revictum* (basonym: "*Streptomyces flavopersicus*" Oliver et al. 1961).

According to Labeda (1996) and Hatano et al. (2003), *Streptomyces flavopersicus* (Oliver et al. 1961) Witt and Stackebrandt 1991 is a later heterotypic synonym of *Streptomyces netropsis* (Finlay et al. 1951) Witt and Stackebrandt 1991.

182. **Streptomyces flavotricini** (Preobrazhenskaya and Sveshnikova *in* Gauze, Preobrazhenskaya, Kudrina, Blinov, Ryabova and Sveshnikova 1957) Pridham, Hesseltine and Benedict 1958, 60[AL] ("*Actinomyces flavotricini*" Preobrazhenskaya and Sveshnikova *in* Gauze, Preobrazhenskaya, Kudrina, Blinov, Ryabova and Sveshnikova 1957, 49)

fla.vo.tri.ci'ni. L. adj. *flavus* yellow; Gr. n. *thrix* the hair; N.L. *flavotricini* (*sic*) of yellow hair, probably referring to yellow diffusible pigment and formation of a streptothricin-like antibiotic.

Spore chains in Section *Rectiflexibiles.* Mature spore chains are generally long, often with more than 50 spores per chain. This morphology is seen on yeast-malt agar, oatmeal agar, and salts-starch agar, but not on glycerol-asparagine agar. Spore surface is smooth.

Color of colony: aerial mass color in the Red color series on yeast-malt agar, oatmeal agar, and salts-starch agar. Reverse side of colony with no distinctive pigments on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar; substrate pigment is not a pH indicator.

Color in medium: melanoid pigments formed in peptone-yeast-iron agar and tryptone-yeast broth. Pigments other than melanoids not formed in yeast-malt agar, oatmeal agar, salts-starch agar, or glycerol-asparagine agar.

D-Glucose is utilized for growth; doubtful utilization of D-fructose. No growth or only trace of growth on

L-arabinose, sucrose, D-xylose, iso-inositol, D-mannitol, rhamnose, and raffinose.

Type strain shows the highest sequence similarity to: *S. polychromogenes*, AB184292, 99.7%; *S. racemochromogenes*, DQ026656, 99.7%; *S. globosus*, AJ781330, 99.4%; *S. toxytricini*, DQ442548, 99.3%; *S. katrae*, AB184409, 99.2%; *S. cinnamonensis*, AB184707, 99.2%; *S. virginiae*, D85119, 99%; *S. tanashiensis*, AJ781362, 99%.

Source: not known.

DNA G+C content (mol%): not known.

Type strain: ATCC 19757, ATCC 23621, CBS 495.68, BCRC 13762, DSM 40152, NBRC 12770, IMET 42057, INA 11669/58, JCM 4371, NRRL B-5419, NRRL-ISP 5152, RIA 1037, UNIQEM 143, VKM Ac-1277.

Sequence accession no. (16S rRNA gene): AB184132.

183. **Streptomyces flavovariabilis** (*ex* Korenyako and Nikitina *in* Krasil'nikov 1965) Sveshnikova 1986, 574[VP] (Effective publication: Sveshnikova *in* Gause, Preobrazhenskaya, Sveshnikova, Terekhova and Maximova 1983.) ("*Actinomyces flavovariabilis*" Korenyako and Nikitina *in* Krasil'nikov 1965; "*Streptomyces flavovariabilis*" Pridham 1970)

fla.vo.va.ri.a'bi.lis L. adj. *flavus* yellow; L. adj. *variabilis* variable; N.L. masc. adj. *flavovariabilis* yellow, variable.

Spore chains are spiral (*Spirales*); spore surface is spiny or smooth. On mineral agar 1, oatmeal agar: aerial mycelium is whitish, creamy to pale pink; substrate mycelium is grayish green or yellowish green; diffusible pigment weak or not extant. On starch-ammonia agar: aerial mycelium is poor, whitish; substrate mycelium is pale olive; no diffusible pigment. On glycerol-nitrate agar: aerial mycelium is whitish to creamy or grayish; substrate mycelium is greenish gray; diffusible pigment not extant or weak, greenish-grayish. On glycerol-asparagine agar: aerial mycelium is poor, white; substrate mycelium is colorless to pale olive; no diffusible pigment. On organic agar 2: aerial mycelium is pale to creamy; substrate mycelium is dark olive or brown; diffusible pigment is brown. Melanoid pigment is formed.

Good growth on glucose, sucrose, arabinose, rhamnose, xylose, fructose, raffinose, and mannitol. Color of substrate mycelium changes to red under acidic conditions. Blue pigment is very unstable and degenerated quickly, forming dirty yellow, dirty green and other similar pigments which were also found on mycelia of *Streptomyces iakyrus* (Blinov et al., 1975; Machenko et al., 1970). Exhibits anti-bacterial and anti-tumor activity; grows on Czapek's solution agar.

Type strain shows the highest sequence similarity to: *S. pseudovenezuelae*, AB184233, 99.2%; *S. novaecaesareae*, AB184357, 99.1%; *S. cacaoi* subsp. *asoensis*, DQ026644, 99.1%.

Source: not known.

DNA G+C content (mol%): not known.

Type strain: ATCC 43684, DSM 41479, INMI 702, JCM 9089, NBRC 100764, NRRL B-16367, VKM Ac-141.

Sequence accession no. (16S rRNA gene): EF178691.

184. **Streptomyces flavovirens** (Waksman *in* Bergey, Harrison, Breed, Hammer and Huntoon 1923) Waksman and Henrici *in* Breed, Murray and Hitchens 1948, 940[AL] ("*Actinomyces flavovirens*" Waksman *in* Bergey, Harrison, Breed, Hammer and Huntoon 1923, 352)

fla.vo.vi'rens. L. adj. *flavus* golden yellow, reddish yellow; L. v. *vireo* to be green; N.L. part adj. *flavovirens* yellow and becoming green.

Spore chains in Section *Rectiflexibiles*. Mature spore chains are generally long, often with more than 50 spores per chain. This morphology is seen on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar. Spore surface is smooth.

Color of colony: aerial mass color in the Gray color series on yeast-malt agar, oatmeal agar, and salts-starch agar. Reverse side of colony is colorless or characteristic grayed yellow modified by green on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar; substrate pigment is not a pH indicator.

Color in medium: melanoid pigments not formed in peptone-yeast-iron agar or tyrosine agar. Pigments other than melanoids not formed, or some yellow pigment may be produced on oatmeal agar and glycerol-asparagine agar. This pigment is not pH-sensitive when tested with 0.05 M NaOH or HCl.

D-Glucose, L-arabinose, D-xylose, D-mannitol, and rhamnose are utilized for growth. No growth on sucrose, iso-inositol, or raffinose; trace of growth on D-fructose.

Type strain shows the highest sequence similarity to: *S. flavogriseus*, AJ494864, 100%; *S. sindenensis*, AB184759, 99.7%; *S. pluricolorescens*, DQ442540, 99.7%; *S. fimicarius*, AY999784, 99.7%; *S. cinereorectus*, AB184646, 99.7%; *S. griseinus*, AB184205, 99.7%; *S. badius*, AY999783, 99.7%; *S. halstedii*, EF178695, 99.7%; *S. griseolus*, AB184768, 99.7%; *S. argenteolus*, AB045872, 99.7%; *S. praecox*, AB184293, 99.7%; *S. flavofuscus*, AB249935, 99.7%; *S. nitrosporeus*, EF178680, 99.7%; *S. mediolani*, AB184674, 99.7%; *S. cavourensis* subsp. *washingtonensis*, DQ026671, 99.7%; *S. griseoplanus*, AY999894, 99.7%; *S. rubiginosohelvolus*, AB184240, 99.7%; *S. lavendulae* subsp. *lavendulae*, AB184080, 99.7%; *S. anulatus*, DQ026637, 99.7%; *S. acrimycini*, AY999889, 99.6%; *S. globisporus* subsp. *globisporus*, EF178686, 99.6%; *S. albovinaceus*, AB249958, 99.6%; *S. cyaneofuscatus*, AB184860, 99.6%; *S. fulvorobeus*, AB184711, 99.6%; *S. fulvorobeus*, AB184711, 99.6%; *S. alboviridis*, AB184256, 99.9%; *S. lipmanii*, AB184148, 99.6%; *S. luridiscabiei*, AF361784, 99.5%; *S. baarnensis*, EF178688, 99.5%; *S. griseus* subsp. *griseus*, AY207604, 99.5%; *S. microflavus*, DQ445795, 99.5%; *S. floridae*, AB184656, 99.5%; *S. californicus*, AB184755, 99.5%; *S. parvus*, DQ442537, 99.5%; *S. pulveraceus*, AB184806, 99.4%; *S. bacillaris*, AB184439, 99.4%; *S. yanii*, AB006159, 99.3%; *S. olivoviridis*, AB184227, 99.3%; *S. atroolivaceus*, AJ781320, 99.3%; *S. finlayi*, AY999788, 99.3%; *S. sanglieri*, AB249945, 99.2%; *S. albolongus*, AB184425, 99.2%; *S. spiroverticillatus*, AB184814, 99.2%; *S. griseobrunneus*, AB249912, 99.2%; *S. clavifer*, DQ026670, 99.2%; *S. celluloflavus*, AB184476, 99.2%; *S. atratus*, DQ026638, 99.1%; *S. gelaticus*, DQ026636, 99.1%; *S. cavourensis* subsp. *cavourensis*, DQ445791, 99%; *S. mutomycini*, AB249951, 99%; *S. cremeus*, AB184124, 99%.

Source: not known.

DNA G+C content (mol%): not known.

Type strain: AS 4.575, ATCC 19758, ATCC 3320, CBS 129.20, CBS 279.30, CBS 496.68, CCM 3243, BCRC 13689, DSM 40062, HAMBI 1007, HUT 6019, HUT 6053, NBRC 12771, NBRC 3412, NBRC 3716, IMET 40280, JCM 4035, JCM 4578, LMG 20516, NRRL B-1329, NRRL B-2685, NRRL-ISP 5062, RIA 1038, RIA 635, UNIQEM 144, VKM Ac-1723.

Sequence accession no. (16S rRNA gene): DQ026635.

Further comments: according to Lanoot et al. (2005b), *Streptomyces flavovirens* (Waksman 1923) Waksman and Henrici 1948 emend. Lanoot et al. 2005b is an earlier heterotypic synonym of *Streptomyces nigrifaciens* Waksman (1961).

185. **Streptomyces flavoviridis** (*ex* Preobrazhenskaya *in* Gauze, Preobrazhenskaya, Kudrina, Blinov, Ryabova and Sveshnikova 1957) Preobrazhenskaya 1986, 574[VP] (Effective publication: Preobrazhenskaya *in* Gause, Preobrazhenskaya, Sveshnikova, Terekhova and Maximova 1983.) ("*Actinomyces flavoviridis*" Preobrazhenskaya *in* Gauze, Preobrazhenskaya, Kudrina, Blinov, Ryabova and Sveshnikova 1957)

fla.vo.vi′ri.dis. L. adj. *flavus* yellow; L. adj. *viridis* green; N.L. masc. adj. *flavoviridis* yellow-green.

Spore chains in Section *Spirales* (young spore chains may resemble *Retinaculiaperti*). Mature spore chains generally have 10–50 spores per chain; longer chains are sometimes observed. This morphology is found on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar. Spore surface is hairy.

Color of colony: aerial mass color in the Gray or Green series on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar; color tabs selected by observers are all light olive gray or light olive brown. Reverse side of colony with no distinctive pigments (grayed yellow to yellow-brown) on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar; substrate pigment is not a pH indicator.

Color in medium: melanoid pigments formed in peptone-yeast-iron agar and tryptone-yeast broth. No other pigment in medium in yeast-malt agar, oatmeal agar, salts-starch agar, or glycerol-asparagine agar.

D-Glucose, L-arabinose, D-xylose, iso-inositol, D-mannitol, D-fructose, and rhamnose are utilized for growth. No growth or only traces of growth on sucrose or raffinose.

Type strain shows the highest sequence similarity to: *S. pilosus*, AB184161, 100%; *S. violaceus*, AB184315, 99.3%; *S. albosporeus* subsp. *albosporeus*, AJ781327, 99.3%; *S. janthinus*, AB184851, 99.3%; *S. lomondensis*, AB184673, 99.2%; *S. geysiriensis*, DQ442501, 99.2%; *S. minutiscleroticus*, EF178696, 99.2%; *S. roseoviolaceus*, AJ399484, 99.2%; *S. ghanaensis*, AY999851, 99.2%; *S. luteogriseus*, AB184379, 99.2%; *S. djakartensis*, AB184657, 99.1%; *S. plicatus*, AB184291, 99.1%; *S. rochei*, AB184237, 99.1%; *S. vinaceusdrappus*, AY999929, 99.1%; *S. mutabilis*, EF178679, 99.1%; *S. hawaiiensis*, AB184143, 99.1%; *S. atrovirens*, DQ026672, 99.1%; *S. arenae*, AB249977, 99%; *S. calvus*, AB184329, 99%; *S. tuirus*, AB184690, 99%.

Source: not known.

DNA G+C content (mol%): not known.

Type strain: ATCC 19759, ATCC 19903, CBS 497.68, BCRC 11474, DSM 40153, NBRC 12772, IMET 42058, INA 2314/53, JCM 4372, NRRL-ISP 5153, RIA 1039, UNIQEM 145, VKM Ac-754.

Sequence accession no. (16S rRNA gene): AB184842.

186. **Streptomyces flocculus** (Duché 1934) Waksman and Henrici *in* Breed, Murray and Hitchens 1948, 955[AL] ("*Actinomyces flocculus*" Duché 1934, 300)

floc′cu.lus. L. n. *floccus* a flock of wool; N.L. dim. masc. adj. *flocculus* like a small flock of wool.

Spore chains in Section *Spirales*. Spiral spore chains are usually formed on oatmeal agar; spirals of two or more turns may be formed or short chains of only 3–10 spores may form loops, partial spirals, or hooks. Sporulating aerial mycelium is usually poorly developed on yeast-malt agar, salts-starch agar, and glycerol-asparagine agar so that spirals and hooks may be sparse or absent. The weak growth of this strain on most media was noted by Duché in his original description. Spore surface is smooth.

Color of colony: aerial mass color in the White or Yellow color series on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar. The most representative color tab in the Yellow color series is 2ba (pale yellow). Reverse side of colony with no distinctive pigments (colorless or pale grayish yellow) on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar.

Color in medium: melanoid pigments are not formed in peptone-yeast-iron agar, tyrosine agar, or tryptone-yeast broth. No pigment found in medium in yeast-malt agar, oatmeal agar, salts-starch agar, or glycerol-asparagine agar.

D-Glucose, L-arabinose, D-xylose, iso-inositol, D-mannitol, D-fructose, and sucrose are utilized for growth. Utilization of rhamnose and raffinose is doubtful.

Type strain shows the highest sequence similarity to: *S. rangoonensis*, AB184295, 99.9%; *S. gibsonii*, AB184663, 99.9%; *S. almquistii*, AB184258, 99.9%; *S. albus* subsp. *albus*, AJ621602, 99.8%.

Source: not known.

DNA G+C content (mol%): not known.

Type strain: ATCC 25453, CBS 686.69, BCRC 12068, DSM 40327, HUT 6615, NBRC 13041, IMET 43522, JCM 4476, NRRL 2960, NRRL B-2465, NRRL B-2843, NRRL-ISP 5327, RIA 1233.

Sequence accession no. (16S rRNA gene): AB184272.

187. **Streptomyces floridae** Bartz, Ehrlich, Mold, Penner and Smith 1951, 4[AL]

flo.ri′da.e. N.L. gen. n. *floridae* of Florida, the source of the soil from which the organism was isolated.

Spore chains are flexuous. Forms dull violet to red-brown vegetative mycelium and diffusible pigment on some media. Poor growth on Czapek's solution agar; produces the viomycin complex; inhibited by streptomycin.

Type strain shows the highest sequence similarity to: *S. californicus*, AB184755, 100%; *S. lipmanii*, AB184148, 99.9%; *S. microflavus*, DQ445795, 99.9%; *S. fulvorobeus*, AB184711, 99.9%; *S. alboviridis*, AB184256, 99.9%; *S.*

cavourensis subsp. *washingtonensis*, DQ026671, 99.8%; *S. lavendulae* subsp. *lavendulae*, AB184080, 99.8%; *S. griseinus*, AB184205, 99.8%; *S. badius*, AY999783, 99.8%; *S. pluricolorescens*, DQ442540, 99.8%; *S. sindenensis*, AB184759, 99.8%; *S. mediolani*, AB184674, 99.8%; *S. rubiginosohelvolus*, AB184240, 99.8%; *S. praecox*, AB184293, 99.7%; *S. flavofuscus*, AB249935, 99.7%; *S. griseoplanus*, AY999894, 99.7%; *S. albovinaceus*, AB249958, 99.7%; *S. fimicarius*, AY999784, 99.7%; *S. globisporus* subsp. *globisporus*, EF178686, 99.7%; *S. anulatus*, DQ026637, 99.7%; *S. cinereorectus*, AB184646, 99.7%; *S. argenteolus*, AB045872, 99.7%; *S. luridiscabiei*, AF361784, 99.7%; *S. cyaneofuscatus*, AB184860, 99.7%; *S. parvus*, DQ442537, 99.7%; *S. griseolus*, AB184768, 99.6%; *S. griseus* subsp. *griseus*, AY207604, 99.6%; *S. baarnensis*, EF178688, 99.6%; *S. acrimycini*, AY999889, 99.6%; *S. finlayi*, AY999788, 99.5%; *S. bacillaris*, AB184439, 99.5%; *S. halstedii*, EF178695, 99.5%; *S. albolongus*, AB184425, 99.5%; *S. flavovirens*, DQ026635, 99.5%; *S. griseobrunneus*, AB249912, 99.4%; *S. celluloflavus*, AB184476, 99.4%; *S. pulveraceus*, AB184806, 99.4%; *S. flavogriseus*, AJ494864, 99.4%; *S. cavourensis* subsp. *cavourensis*, DQ445791, 99.3%; *S. nitrosporeus*, EF178680, 99.3%; *S. clavifer*, DQ026670, 99.3%; *S. atroolivaceus*, AJ781320, 99.3%; *S. candidus*, DQ026663, 99.3%; *S. olivoviridis*, AB184227, 99.3%; *S. sanglieri*, AB249945, 99.2%; *S. yanii*, AB006159, 99.2%; *S. mutomycini*, AB249951, 99.1%; *S. gelaticus*, DQ026636, 99.1%; *S. atratus*, DQ026638, 99.1%; *S. spiroverticillatus*, AB184814, 99.1%; *S. cremeus*, AB184124, 99.1%.

Source: isolated from soil from Florida.

DNA G+C content (mol%): not known.

Type strain: DSM 40938, NBRC 15405, JCM 5068, NCIMB 12830, NRRL 2423.

Sequence accession no. (16S rRNA gene): AB184656.

188. **Streptomyces fluorescens** (Krasil'nikov 1958) Pridham 1970, 15[AL] ("*Actinomyces fluorescens*" Krasil'nikov 1958, 258)

flu.o.res′cens. N.L. v. *fluoresco* to fluoresce; N.L. part. adj. *fluorescens* fluorescing, referring to the yellow fluorescent pigment produced by the organism.

Spore chains in Section *Rectiflexibiles*. Mature spore chains generally have 10–50 spores per chain. This morphology is seen on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar. Spore surface is smooth.

Color of colony: aerial mass color in the Yellow color series on yeast-malt agar, oatmeal agar, and salts-starch agar; White or Yellow color series on glycerol-asparagine agar. Reverse side of colony with no distinctive pigments (colorless to grayish yellow on oatmeal agar, salts-starch agar, and glycerol-asparagine agar; grayed orange-yellow to olive brown on yeast-malt agar). Reverse pigment is not pH-sensitive or is changed only slightly from yellow-brown to yellow-green, with addition of 0.05 M NaOH.

Color in medium: melanoid pigments are not produced in peptone-yeast-iron agar, tyrosine agar, or tryptone-yeast broth. A small amount of yellow pigment may be found in the medium in yeast-malt agar, oatmeal agar, salts-starch agar, and tryptone-yeast broth. This pigment, when present, is only slightly pH-sensitive, changing from yellow-brown to yellow-green with addition of 0.05 NaOH.

D-Glucose, L-arabinose, D-xylose, D-mannitol, D-fructose, and rhamnose are utilized for growth. No growth or only traces of growth on sucrose, iso-inositol, and raffinose. The original description for this species states that rhamnose is not utilized, noting that this is one difference between *A. fluorescens* and other species in the fluorescent group. However, all of the three ISP observers recorded good growth on rhamnose, equivalent to growth on D-glucose.

For sequence similarity, see type strain of *Streptomyces anulatus*.

Source: not known.

DNA G+C content (mol%): not known.

Type strain: ATCC 15860, ATCC 23907, CBS 803.68, BCRC 11475, CECT 3130, DSM 40203, NBRC 12861, INMI 592, JCM 4373, LMG 8579, NCIMB 9851, NRRL B-2873, NRRL-ISP 5203, RIA 1154, RIA 647, VKM Ac-147.

Sequence accession no. (16S rRNA gene): AB184199.

Further comments: according to Lanoot et al. (2005b), *Streptomyces fluorescens* (Krasil'nikov 1958) Pridham 1970 is a later heterotypic synonym of *Streptomyces anulatus* (Beijerinck 1912) Waksman 1953 emend. Lanoot et al. 2005b.

189. **Streptomyces fradiae** (Waksman and Curtis 1916) Waksman and Henrici *in* Breed, Murray and Hitchens 1948, 954[AL] emend. Lanoot, Vancanneyt, Dawyndt, Cnockaert, Zhang, Huang, Liu and Swings 2005a, 8 (Effective publication: Lanoot, Vancanneyt, Dawyndt, Cnockaert, Zhang, Huang, Liu and Swings 2004, 88.) ["*Actinomyces fradii*" (*sic*) Waksman and Curtis 1916, 125; "*Streptomyces fradii*" (*sic*) Waksman and Henrici *in* Breed, Murray and Hitchens 1948, 954; "*Streptomyces fradiae* subsp. *fradiae*" Waksman and Curtis *in* Pridham, Lyons and Seckinger 1965, 222]

fra.di′a.e. N.L. gen. n. *fradiae* of Fradia, a patronymic.

Spore chains in Section *Retinaculiaperti* with characteristic range from straight to spiral spore chains. Straight or flexuous spore chains are most common on yeast-malt agar; *Retinaculiaperti* morphology, including open spirals, best developed on oatmeal agar and salts-starch agar. Mature spore chains generally have 10–50 spores per chain; longer chains are sometimes observed. This morphology is seen on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar. Spore surface is smooth.

Color of colony: aerial mass color in the Red color series on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar. Reverse side of colony with no distinctive pigments on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar; substrate pigment is not a pH indicator.

Color in medium: melanoid pigments not formed in peptone-yeast-iron agar and tyrosine agar. No pigment found in medium in yeast-malt agar, oatmeal agar, salts-starch agar, or glycerol-asparagine agar.

D-Glucose, L-arabinose, and D-fructose are utilized for growth. No growth or only traces of growth on sucrose, iso-inositol, D-mannitol, rhamnose, and raffinose. Variable reports on growth with D-xylose.

Type strain shows the highest sequence similarity to: *S. coeruleoprunus*, AB184651, 99.2%.

Source: not known.

DNA G+C content (mol%): not known.

Type strain: ATCC 10745, ATCC 19760, CBS 498.68, CCM 3174, BCRC 12196, CECT 3197, DSM 40063, HAMBI 965, HUT 6095, IFM 1030, NBRC 12773, NBRC 3718, IMET 42051, IMI 061202, JCM 4133, JCM 4579, KCTC 9760, NCIMB 11005, NCIMB 8233, NRRL B-1195, NRRL-ISP 5063, PCM 2330, RIA 1040, RIA 97, UNIQEM 146, VKM Ac-150, VKM Ac-151, VKM Ac-152, VKM Ac-764.

Sequence accession no. (16S rRNA gene): DQ026630.

Further comments: according to Lanoot et al. (2004), *Streptomyces fradiae* (Waksman and Curtis 1916) Waksman and Henrici 1948 is an earlier heterotypic synonym of *Streptomyces roseoflavus* Arai 1951.

190. **Streptomyces fragilis** Anderson, Ehrlich, Sun and Burkholder 1956, 105[AL]

fra′gi.lis. L. masc. adj. *fragilis* fragile.

Spore chains of atypical *Retinaculiaperti* type. Poor growth on Czapek's solution agar; produces *O*-diazoacetyl-L-serine (azaserine), an anti-bacterial, anti-fungal, anti-protozoal, and anti-tumor antibiotic; inhibited by streptomycin; NaCl tolerance: >4%, but <7%.

Type strain shows the highest sequence similarity to: *S. coelicoflavus*, AB184650, 99.4%; *S. flaveolus*, AB184764, 99.3%; *S. matensis*, AB184221, 99%; *S. eurythermus*, D63870, 99%.

Source: not known.

DNA G+C content (mol%): not known.

Type strain: ATCC 23908, CBS 804.68, BCRC 13654, DSM 40044, HAMBI 1083, HAMBI 1090, NBRC 12862, IMET 43575, JCM 4187, JCM 4638, NCIMB 9795, NRRL 2424, NRRL-ISP 5044, RIA 1111, VKM Ac-1773.

Sequence accession no. (16S rRNA gene): AY999917.

191. **Streptomyces fulvissimus** (Jensen 1930) Waksman and Henrici *in* Breed, Murray and Hitchens 1948, 946[AL] ("*Actinomyces fulvissimus*" Jensen 1930, 66)

ful.vis′si.mus. L. sup. masc. adj. *fulvissimus* very yellow.

Spore chains in Section *Rectiflexibiles*. Mature spore chains generally contain 10 to 50 or more spores per chain. This morphology is seen on yeast-malt agar, salts-starch agar, and glycerol-asparagine agar, but sporulating aerial mycelium may be poorly developed or absent on oatmeal agar. Spore surface is smooth.

Color of colony: aerial mass color in the Red color series on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar; white aerial mycelium may also be present. Nearest matching color tabs in the Red color series are 3ea, light orange yellow; 5ca and 7ca, light yellowish pink; and 4ea, moderate yellowish pink. Reverse side of colony with distinctive reddish orange pigments on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar. Reverse mycelium pigment is not a pH indicator.

Color in medium: melanoid pigments are formed in peptone-yeast-iron agar or tryptone-yeast broth, but not in tyrosine agar. No pigment is found in the medium in yeast-malt agar, oatmeal agar, salts-starch agar, or glycerol-asparagine agar.

Carbon utilization: D-glucose, L-arabinose, iso-inositol, D-mannitol, D-fructose, and raffinose are utilized for growth. Reports vary on the utilization of D-xylose, and the utilization of sucrose and rhamnose is doubtful.

Type strain shows the highest sequence similarity to: *S. flavofungini*, AB184359, 99.7%; *S. alboflavus*, EF178699, 99.3%; *S. variegatus*, AJ781371, 99.1%.

Source: not known.

DNA G+C content (mol%): not known.

Type strain: ATCC 27431, CBS 783.72, BCRC 12172, CCUG 11114, CIP 105783, DSM 40593, NBRC 13482, JCM 4129, JCM 4754, KCTC 9779, LMG 19310, NCIMB 10505, NCIMB 9609, NRRL B-1453, NRRL-ISP 5593, RIA 1443, VKM Ac-994.

Sequence accession no. (16S rRNA gene): AB184434.

192. **Streptomyces fulvorobeus** Vinogradova and Preobrazhenskaya 1986, 574[VP] (Effective publication: Vinogradova and Preobrazhenskaya *in* Gause, Preobrazhenskaya, Sveshnikova, Terekhova and Maximova 1983.)

ful.vo.ro′be.us. L. adj. *fulvus* reddish yellow; L. adj. *robeus* reddish brown; N.L. masc. adj. *fulvorobeus* reddish yellow, reddish brown.

Spore chains are spiral (*Spirales*); spores are smooth. On mineral agar 1, glycerol-nitrate agar: aerial mycelium is creamy, yellow, grayish yellow; substrate mycelium is gray-brownish yellow, yellow-gray-brown. On starch ammonia agar, glycerol-asparagine agar, oatmeal agar: aerial mycelium is white-yellow, yellow; substrate mycelium is colorless; no diffusible pigment. On organic agar 2: aerial mycelium is whitish or absent; substrate mycelium is colorless; no diffusible pigment. Melanoid pigments are not formed. Grows on glucose, maltose, sucrose, and arabinose; no growth on mannitol, xylose, inositol, rhamnose, or galactose.

Type strain shows the highest sequence similarity to: *S. cavourensis* subsp. *washingtonensis*, DQ026671, 100%; *S. microflavus*, DQ445795, 100%; *S. lipmanii*, AB184148, 100%; *S. lavendulae* subsp. *lavendulae*, AB184080, 100%; *S. alboviridis*, AB184256, 100%; *S. luridiscabiei*, AF361784, 99.9%; *S. flavofuscus*, AB249935, 99.9%; *S. cinereorectus*, AB184646, 99.9%; *S. fimicarius*, AY999784, 99.9%; *S. griseoplanus*, AY999894, 99.9%; *S. cyaneofuscatus*, AB184860, 99.9%; *S. floridae*, AB184656, 99.9%; *S. praecox*, AB184293, 99.9%; *S. anulatus*, DQ026637, 99.9%; *S. pluricolorescens*, DQ442540, 99.8%; *S. acrimycini*, AY999889, 99.8%; *S. argenteolus*, AB045872, 99.8%; *S. griseinus*, AB184205, 99.8%; *S. badius*, AY999783, 99.8%; *S. rubiginosohelvolus*, AB184240, 99.8%; *S. sindenensis*, AB184759, 99.8%; *S. mediolani*, AB184674, 99.8%; *S. californicus*, AB184755, 99.8%; *S. globisporus* subsp. *globisporus*, EF178686, 99.8%; *S. parvus*, DQ442537, 99.7%; *S. halstedii*, EF178695, 99.7%; *S. baarnensis*, EF178688, 99.7%; *S. griseolus*, AB184768, 99.7%; *S. griseus* subsp. *griseus*, AY207604, 99.7%; *S. albovinaceus*, AB249958, 99.7%; *S. flavovirens*, DQ026635, 99.6%; *S. finlayi*, AY999788, 99.5%; *S. flavogriseus*, AJ494864, 99.5%; *S. bacillaris*, AB184439, 99.5%; *S. yanii*, AB006159, 99.4%; *S. atroolivaceus*, AJ781320, 99.4%; *S. olivoviridis*, AB184227, 99.4%; *S. pulveraceus*, AB184806, 99.4%; *S. celluloflavus*, AB184476, 99.4%; *S. griseobrunneus*, AB249912, 99.4%; *S. albolongus*, AB184425, 99.4%; *S. clavifer*, DQ026670, 99.3%; *S. nitrosporeus*, EF178680, 99.3%; *S. cavourensis*

subsp. *cavourensis*, DQ445791, 99.3%; *S. spiroverticillatus*, AB184814, 99.2%; *S. sanglieri*, AB249945, 99.2%; *S. atratus*, DQ026638, 99.1%; *S. mutomycini*, AB249951, 99.1%; *S. candidus*, DQ026663, 99.1%; *S. gelaticus*, DQ026636, 99.1%; *S. cremeus*, AB184124, 99%.

Source: not known.

DNA G+C content (mol%): not known.

Type strain: DSM 41455, NBRC 15897, INMI 34-280, JCM 9090, VKM Ac-158.

Sequence accession no. (16S rRNA gene): AB184711.

193. **Streptomyces fumanus** (Sveshnikova 1957) *in* Gauze, Preobrazhenskaya, Kudrina, Blinov, Ryabova and Sveshnikova 1957 Pridham, Hesseltine and Benedict 1958, 67[AL] ["*Actinomyces fumanus*" Sveshnikova *in* Gauze, Preobrazhenskaya, Kudrina, Blinov, Ryabova and Sveshnikova 1957, 61; "*Actinomyces fumarius*" (*sic*) Danga and Gottlieb 1959, 43]

fu.ma′nus. N.L. masc. adj. *fumanus* smoky, probably referring to the color of the vegetative mycelium of the organism.

Spore chains in Section *Spirales*. Mature spore chains are moderately long with 10 to 50 or more spores per chain. This morphology is found on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar. Spore surface is smooth.

Color of colony: aerial mass color in the Red color series on yeast-malt agar and glycerol-asparagine agar; Red or sometimes Yellow color series on oatmeal agar and salts-starch agar. Reverse side of colony is light grayish yellow may or may not change to dark brown on salts-starch agar and glycerol-asparagine agar or to orange-yellow or strong brown on yeast-malt agar and oatmeal agar.

Color in medium: melanoid pigments are not formed in peptone-yeast-iron agar, tyrosine agar, or tryptone-yeast broth. No pigment (or only a trace of yellow or greenish yellow) is found in the medium in yeast-malt agar, oatmeal agar, salts-starch agar, or glycerol-asparagine agar.

D-Glucose, L-arabinose, D-xylose, D-mannitol, D-fructose, rhamnose, and raffinose are utilized for growth. No growth or only traces of growth on iso-inositol and sucrose.

Type strain shows no sequence similarity over 99%.

Source: not known.

DNA G+C content (mol%): not known.

Type strain: ATCC 19904, ATCC 25454, CBS 687.69, BCRC 12058, DSM 40154, NBRC 13042, INA 10256/54, JCM 4477, NRRL B-3898, NRRL B-5420, NRRL-ISP 5154, RIA 1234, VKM Ac-1845.

Sequence accession no. (16S rRNA gene): AB184273.

194. **Streptomyces fumigatiscleroticus** (*ex* Pridham 1970) Goodfellow, Williams and Alderson 1986a, 59[VP] (Effective publication: Goodfellow, Williams and Alderson 1986d, 59.)

fu.mi.ga.ti.scle.ro′ti.cus. L. part. adj. *fumigatus* smoked; N.L. neut. n. *sclerotium* sclerotium; N.L. masc. adj. *fumigatiscleroticus* smoked sclerotium, referring to smoke color and ability to form sclerotia.

Produces spiral spore chains and shows brownish-black crusty growth; extensively branched substrate and aerial mycelium. Produces sclerotia. Melanin pigments are not formed. Degrades gelatin, starch, urea, and xanthine. Nitrate is reduced but hydrogen sulfide is not produced. L-Arabinose, glucose, lactose, maltose, D-mannitol, mannose, sorbitol, sucrose, and D-xylose are used as sole carbon sources but dulcitol, galactose, glycerol, inulin, *myo*-inositol, L-rhamnose, salicin, and sucrose are not. Acid is produced from D-lactose, D-mannitol, melibiose, methyl α-D-glucoside, raffinose, L-rhamnose, D-sorbitol, and D-xylose but not from adonitol, dulcitol, *meso*-erythritol, *myo*-inositol, or sucrose. Grows at 42°C but not at 10°C. Shows antimicrobial activity against *Bacillus subtilis*, *Escherichia coli*, and *Staphylococcus aureus* but not against *Candida albicans*, *Cryptococcus neoformans*, or *Saccharomyces cerevisiae*.

Type strain shows the highest sequence similarity to: *S. poonensis*, DQ445792, 99.3%; *S spiralis*, EF178683, 99%.

Source: not known.

DNA G+C content (mol%): not known.

Type strain: ATCC 19345, CBS 639.66, BCRC 12344, CMI 117720, DSM 43154, NBRC 12999, KCC A-0101, JCM 3101, NCIMB 11004, NRRL B-3856, RIA 884.

Sequence accession no. (16S rRNA gene): DQ442499.

Further comments: according to Goodfellow et al. (1986d), the species *Streptomyces fumigatiscleroticus* (*ex* Pridham 1970) Goodfellow et al. 1986a is a synonym of the species *Chainia fumigata* Thirumalachar et al. 1966. However, according to Rule 51b(2) of the *Bacteriological Code* (1990 Revision) the transfer of *Chainia fumigata* Thirumalachar et al. 1966 in the genus *Streptomyces* Waksman and Henrici 1943 as *Streptomyces fumigatiscleroticus* (*ex* Pridham 1970) Goodfellow et al. 1986a sp. nov., nom. rev. is illegitimate.

195. **Streptomyces galbus** Frommer 1959, 195[AL]

gal′bus. L. masc. adj. *galbus* greenish yellow.

Spore chains in Section *Spirales* or *Retinaculiaperti*. Spirals may be open coils of several turns, tight spirals at the ends of flexuous spore chains or imperfect spirals and loops at the ends of spore chains suggesting *Retinaculiaperti* morphology. Spore chains are moderately long with 10–50, or often more than 50, spores per chain. Spore surface is smooth to warty. Surface irregularities suggesting very small warts are characteristic.

Color of colony: aerial mass color in the Gray color series on yeast-malt agar and oatmeal agar and in the Yellow color series or Gray color series on salts-starch agar and glycerol-asparagine agar. Gray color is represented by tabs 2fe (medium gray) or 3fe (light brownish gray) on all media; and 1ba or 2ba (pale yellow) or 1½db (pale grayish yellow) on salts-starch agar and glycerol-asparagine agar. Reverse side of colony with no distinctive pigments (yellow to yellow-brown or olive brown) on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar.

Color in medium: melanoid pigments are produced in peptone-yeast-iron agar and tryptone-yeast broth, but not in tyrosine agar. Yellow soluble pigment is found in the medium in yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar. This pigment is not a pH indicator.

D-Glucose, L-arabinose, D-xylose, iso-inositol, D-mannitol, and D-fructose are utilized for growth. Only traces of growth comparable to growth on carbon-free control is found on sucrose, rhamnose, and raffinose.

Type strain shows the highest sequence similarity to: *S. longwoodensis*, AB184580, 99.9%; *S. bungoensis*, AB184696, 99.7%; S. *capoamus*, AB045877, 99.5%; *S. corchorusii*, AB184267, 99.3%; *S. olivaceoviridis*, AB184288, 99.2%; *S. canarius*, AB184396, 99.1%.

Source: not known.

DNA G+C content (mol%): not known.

Type strain: ATCC 23910, CBS 831.68, BCRC 12166, DSM 40089, NBRC 12864, IMET 42937, JCM 4222, JCM 4639, NCIMB 13005, NRRL B-2283, NRRL-ISP 5089, RIA 1121, VKM Ac-165.

Sequence accession no. (16S rRNA gene): X79852.

196. **Streptomyces galilaeus** Ettlinger, Corbaz and Hütter 1958a, 356[AL]

ga.li.la′e.us. L. masc. adj. *galilaeus* of or belonging to Galilee, a Province in Palestine, apparently the source of the soil (Newi Yusha, Israel) from which the organism was isolated.

Spore chains in Section *Spirales*. Open spirals with moderately long chains of 10 to 50 or more spores are found on oatmeal agar, salts-starch agar, and glycerol-asparagine agar; shorter flexuous spore chains are usually found on yeast-malt agar. Spore surface is smooth.

Color of colony: aerial mass color in the Gray color series on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar. Nearest matching color tabs for 14- to 21-d-old cultures are 7ih, grayish pink, and 7fe, pale purple. Observers do not agree on the nearest matching color tab from the Gray color series for the aerial mass on salts-starch agar or glycerol-asparagine agar. Reverse side of colony with no distinctive pigments (pale or grayish yellow to yellowish brown or olive brown) on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar. Two of three observers note that the yellowish color of the reverse side of the mycelium is changed to yellowish pink or faint pink by the addition of 0.05 M NaOH.

Color in medium: melanoid pigments are formed in peptone-yeast-iron agar, tyrosine agar, and tryptone-yeast broth. No pigment or only a trace of yellow is found in the medium in yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar. When a faint yellow pigment is found, it shows the same color changes noted for the reverse mycelium pigment when 0.05 M NaOH is added.

D-Glucose, L-arabinose, D-xylose, iso-inositol, D-fructose, rhamnose, sucrose, and raffinose are utilized for growth. No growth or only traces of growth with D-mannitol.

Type strain shows the highest sequence similarity to: *S. bobili*, AB249925, 100%; *S. aureocirculatus*, AB184260, 99.5%; *S. phaeoluteigriseus*, AJ391815, 99.5%; *S. resistomycificus*, AB184166, 99.5%; *S. novaecaesareae*, AB184357, 99.4%; *S. chartreusis*, AB184839, 99.3%; *S. pseudovenezuelae*, AB184233, 99.3%; *S. prunicolor*, DQ026659, 99%.

Source: soil from Palestine.

DNA G+C content (mol%): not known.

Type strain: ATCC 14969, CBS 701.72, BCRC 11828, CCT 4839, DSM 40481, NBRC 13400, JCM 4231, JCM 4757, KCTC 1921, NRRL 2722, NRRL-ISP 5481, RIA 1361, VKM Ac-729.

Sequence accession no. (16S rRNA gene): AB045878.

197. **Streptomyces gancidicus** Suzuki 1957, 538[AL]

gan.ci′di.cus. N.L. n. *gancidinum* gancidin name of an antibiotic; L. masc. suff. *-icus* suffix used with the sense of belonging to; N.L. masc. adj. *gancidicus* belonging to gancidin.

Moderate growth on Czapek's solution agar; produces the gancidin complex (components A and W) effective against Gram-stain-positive bacteria and tumors; inhibited by streptomycin.

Type strain shows the highest sequence similarity to: *S. pseudogriseolus*, DQ442541, 100%; *S. capillispiralis*, AB184577, 100%; *S. cellulosae*, DQ442495, 99.9%; *S. azureus*, EF178674, 99.5%; *S. levis*, AB184670, 99.4%; *S. lusitanus*, AB184424, 99.4%; *S. rubiginosus*, AY999810, 99.4%; *S. carpinensis*, AB184574, 99.4%; *S. albaduncus*, AY999757, 99.2%; *S. matensis*, AB184221, 99.2%; *S. griseoloalbus*, AB184275, 99.2%; *S. caelestis*, X80824, 99.2%; *S. djakartensis*, AB184657, 99.1%; *S. tuirus*, AB184690, 99.1%; *S. paradoxus*, AB184628, 99.1%; *S. afghaniensis*, AJ399483, 99%; *S. geysiriensis*, DQ442501, 99%; *S. viridiviolaceus*, AY999854, 99%; *S. africanus*, AY208912, 99%; *S. lavendulocolor*, DQ442516, 99%; *S. minutiscleroticus*, EF178696, 99%; *S. spinoverrucosus*, AB184578, 99%; *S. thermocarboxydus*, U94490, 99%; *S. malachitofuscus*, AB184282, 99%.

Source: not known.

DNA G+C content (mol%): not known.

Type strain: DSM 40935, IFM 1024, NBRC 15412, JCM 4171, NCIMB 12858, NRRL B-1872.

Sequence accession no. (16S rRNA gene): AB184660.

198. **Streptomyces gardneri** (Waksman *in* Waksman, Horning, Welsch and Woodruff 1942) Waksman 1961, 215[AL] ("*Proactinomyces gardneri*" Waksman *in* Waksman, Horning, Welsch and Woodruff 1942, 289; "*Nocardia gardneri*" Waksman and Henrici *in* Breed, Murray and Hitchens 1948, 914)

gard′ne.ri. N.L. gen. masc. n. *gardneri* of Gardner, named for Professor A.D. Gardner, one of the two who first isolated the organism.

Spore chains in Section *Rectiflexibiles*. Spore chains are moderately long, usually 10–50, or often more than 50, spores per chain. This morphology is seen on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar, although aerial mycelium may be poorly developed on oatmeal agar and glycerol-asparagine agar. Spore surface is smooth.

Color of colony: aerial mass color in the Gray color series on salts-starch agar in 14–21 d. Mature aerial mycelium may be inadequate for determination of aerial mass color on yeast-malt agar, oatmeal agar, and glycerol-asparagine agar. One observer obtained good sporulating aerial growth in the Gray color series on potato agar (potato, 200 g; agar, 10 g; tap water, 1 l; pH 6.9–7.1). Reverse side of colony with no distinctive pigment (colorless to grayish yellow or grayed yellow-brown) on yeast-malt agar, oatmeal agar, salts-starch agar, glycerol-asparagine agar, and potato agar.

Color in medium: melanoid pigments are produced in peptone-yeast-iron agar and tryptone-yeast broth, but not in tyrosine agar (or only in trace amounts) in 2–4 d. No

pigments are found in the medium in yeast-malt agar, oatmeal agar, salts-starch agar, or glycerol-asparagine agar.

D-Glucose, L-arabinose, sucrose, D-xylose, D-fructose, rhamnose, and raffinose are utilized for growth. No growth or only traces of growth on iso-inositol and D-mannitol.

Type strain shows no sequence similarity over 99%.

Source: not known.

DNA G+C content (mol%): not known.

Type strain: ATCC 23911, ATCC 9604, CBS 832.68, BCRC 12346, BCRC 13687, BCRC 13731, DSM 40064, NBRC 12865, NBRC 13974, NBRC 3385, IMET 7182, JCM 3004, JCM 4375, NCTC 6531, NRRL B-5615, NRRL-ISP 5064, RIA 1117, VKM Ac-1829.

Sequence accession no. (16S rRNA gene): AB184757.

199. **Streptomyces gelaticus** (Waksman *in* Bergey, Harrison, Breed, Hammer and Huntoon 1923) Waksman and Henrici *in* Breed, Murray and Hitchens 1948, 979[AL] ("*Actinomyces gelaticus*" Waksman *in* Bergey, Harrison, Breed, Hammer and Huntoon 1923, 356)

ge.la'ti.cus. L. part adj. *gelatus* congealed, jellied; N.L. masc. adj. *gelaticus* intended to mean resembling hardened gelatin.

Spore chains in Section *Rectiflexibiles.* Aerial mycelium is not produced on most media. Absence of aerial mycelium on most media was also noted in the original description (Waksman and Curtis, 1916) which, however, records open spirals on the scant growth on synthetic agar. ISP observers found wavy spore chains, but no spirals in the thin aerial mycelium on yeast-malt agar and glycerol-asparagine agar. The absence of spirals has also been noted by Anderson et al. (1956), by Waksman in a later description (Waksman, 1961), and by Hütter (1967a). Spore surface is smooth.

Color of colony: aerial mycelium is poorly developed on most media. When an adequate spore mass is produced it is in the Gray color series. This was observed on yeast-malt agar and glycerol-asparagine agar. Additional media used unsuccessfully by collaborators in an effort to get good sporulating growth including Bennett's agar, Anderson's agar, blood agar, Lemko agar, and milk agar. Reverse side of colony with no distinctive pigments (colorless to grayish yellow) on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar.

Color in medium: melanoid pigments are not produced in peptone-yeast-iron agar, tyrosine agar, or tryptone-yeast broth. No pigment is found in medium in yeast-malt agar, oatmeal agar, salts-starch agar, or glycerol-asparagine agar.

D-Glucose, sucrose, D-xylose, and rhamnose are utilized for growth. No growth or only traces of growth on L-arabinose, iso-inositol, D-mannitol, and raffinose.

Type strain shows the highest sequence similarity to: *S. pulveraceus*, AB184806, 99.7%; *S. atratus*, DQ026638, 99.6%; *S. sanglieri*, AB249945, 99.5%; *S. yanii*, AB006159, 99.4%; *S. globisporus* subsp. *globisporus*, EF178686, 99.3%; *S. sindenensis*, AB184759, 99.3%; *S. pluricolorescens*, DQ442540, 99.3%; *S. albovinaceus*, AB249958, 99.3%; *S. flavofuscus*, AB249935, 99.3%; *S. griseinus*, AB184205, 99.3%; *S. fimicarius*, AY999784, 99.3%; *S. anulatus*, DQ026637, 99.3%; *S. mediolani*, AB184674, 99.3%; *S. praecox*, AB184293, 99.3%; *S. badius*, AY999783, 99.3%; *S. rubiginosohelvolus*, AB184240, 99.3%; *S. lavendulae* subsp. *lavendulae*, AB184080, 99.2%; *S. californicus*, AB184755, 99.2%; *S. parvus*, DQ442537, 99.2%; *S. cavourensis* subsp. *washingtonensis*, DQ026671, 99.2%; *S. argenteolus*, AB045872, 99.2%; *S. cinereorectus*, AB184646, 99.2%; *S. cyaneofuscatus*, AB184860, 99.2%; *S. griseoplanus*, AY999894, 99.2%; *S. fulvorobeus*, AB184711, 99.1%; *S. acrimycini*, AY999889, 99.1%; *S. microflavus*, DQ445795, 99.1%; *S. griseolus*, AB184768, 99.1%; *S. flavovirens*, DQ026635, 99.1%; *S. halstedii*, EF178695, 99.1%; *S. lipmanii*, AB184148, 99.1%; *S. alboviridis*, AB184256, 99.1%; *S. baarnensis*, EF178688, 99.1%; *S. floridae*, AB184656, 99.1%; *S. griseus* subsp. *griseus*, AY207604, 99.1%; *S. flavogriseus*, AJ494864, 99.1%; *S. luridiscabiei*, AF361784, 99%; *S. nitrosporeus*, EF178680, 99%.

Source: not known.

DNA G+C content (mol%): not known.

Type strain: ATCC 23912, ATCC 3323, CBS 131.20, CBS 369.39, CBS 833.68, BCRC 11477, DSM 40065, NBRC 12866, IMET 40285, JCM 4376, NCIMB 9848, NRRL B-1252, NRRL B-2928, NRRL-ISP 5065, RIA 1118, RIA 89, VKM Ac-1704.

Sequence accession no. (16S rRNA gene): DQ026636.

200. **Streptomyces geldanamycininus** Goodfellow, Kumar, Labeda and Sembiring 2008, 1[VP] (Effective publication: Goodfellow, Kumar, Labeda and Sembiring 2007, 198.)

gel.da.na.my.ci'ni.nus. N.L. neut. n. *geldanamycinum* geldanamycin; L. suff. *-inus* adjectival suffix used with the sense of belonging to or related to; N.L. masc. adj. *geldanamycininus* related to geldanamycin, producing the antibiotic geldanamycin.

Spore chains are *Spirales*; the spore surface is rugose. On oatmeal agar, the aerial spore mass color is grayish-brown and the substrate mycelium grayish-yellow. Melanin pigments are not produced.

Type strain shows the highest sequence similarity to: *S. antimycoticus*, AB184185, 99.8%; *S. sporoclivatus*, AB249934, 99.8%; *S. rutgersensis* subsp. *castelarensis*, AY508511, 99.6%; *S. asiaticus*, AB249947, 99%; *S. melanosporofaciens*, AJ271887, 99%; *S. rhizosphaericus*, AB249941, 99%.

Source: not known.

DNA G+C content (mol%): 70.2.

Type strain: DSM 41894, NRRL B-3602.

Sequence accession no. (16S rRNA gene): DQ334781.

201. **Strept.omyces geysiriensis** Wallhäusser, Nesemann, Präve and Steigler 1966, 734[AL]

gey.si.ri.en'sis. N.L. masc. adj. *geysiriensis* (from Icel. n. *geysir* a geyser) of or belonging to a geyser, referring to the source of the organism (an Iceland geyser).

Moderate growth on Czapek's solution (synthetic) agar; produces the moenomycin complex of anti-bacterial antibiotics (moenomycins A, B_1, B_2, and C).

Type strain shows the highest sequence similarity to: *S. vinaceusdrappus*, AY999929, 100%; *S. ghanaensis*, AY999851, 100%; *S. plicatus*, AB184291, 100%; *S. minutiscleroticus*, EF178696, 100%; *S. rochei*, AB184237, 100%; *S. mutabilis*, EF178679, 99.8%; *S. tuirus*, AB184690, 99.5%; *S. djakartensis*, AB184657, 99.5%; *S. anandii*, AB184402, 99.3%; *S. viola-*

ceorubidus, AJ781374, 99.3%; *S. pilosus*, AB184161, 99.2%; *S. flavoviridis*, AB184842, 99.2%; *S. tendae*, D63873, 99.1%; *S. calvus*, AB184329, 99.1%; *S. azureus*, EF178674, 99.1%; *S. asterosporus*, AB184706, 99.1%; *S. levis*, AB184670, 99%; *S. luteogriseus*, AB184379, 99%; *S. capillispiralis*, AB184577, 99%; *S. pseudogriseolus*, DQ442541, 99%; *S. aureorectus*, AB184710, 99%; *S. virens*, DQ442554, 99%; *S. gancidicus*, AB184660, 99%; *S. naganishii*, DQ442529, 99%.

Source: not known.

DNA G+C content (mol%): not known.

Type strain: ATCC 15303, CBS 546.70, CECT 3209, DSM 40742, NBRC 15413, JCM 4962, NRRL B-12102, VKM Ac-844.

Sequence accession no. (16S rRNA gene): DQ442501.

202. **Streptomyces ghanaensis** Wallhäusser, Nesemann, Präve and Steigler 1966, 734[AL]

gha.na.en′sis. N.L. masc. adj. *ghanaensis* of or belonging to Ghana, the source of the soil from which the organism was isolated.

Moderate growth on synthetic agar; produces the moenomycin complex of anti-bacterial antibiotics (components A, B, B_1, and C).

Type strain shows the highest sequence similarity to: *S. vinaceusdrappus*, AY999929, 100%; *S. geysiriensis*, DQ442501, 100%; *S. plicatus*, AB184291, 100%; *S. minutiscleroticus*, EF178696, 100%; *S. rochei*, AB184237, 100%; *S. mutabilis*, EF178679, 99.8%; *S. tuirus*, AB184690, 99.5%; *S. djakartensis*, AB184657, 99.5%; *S. pilosus*, AB184161, 99.2%; *S. violaceorubidus*, AJ781374, 99.2%; *S. flavoviridis*, AB184842, 99.2%; *S. anandii*, AB184402, 99.2%; *S. calvus*, AB184329, 99.1%; *S. levis*, AB184670, 99%; *S. asterosporus*, AB184706, 99%; *S. tendae*, D63873, 99%; *S. luteogriseus*, AB184379, 99%; *S. capillispiralis*, AB184577, 99%; *S. azureus*, EF178674, 99%.

Source: isolated from soil from Ghana.

DNA G+C content (mol%): not known.

Type strain: ATCC 14672, CBS 544.70, CECT 3210, DSM 40746, NBRC 15414, JCM 4963, KCTC 9882, NRRL B-12104.

Sequence accession no. (16S rRNA gene): AY999851.

203. **Streptomyces gibsonii** (Erikson 1935) Waksman and Henrici *in* Breed, Murray and Hitchens 1948, 963[AL] ["*Actinomyces gibsoni*" (*sic*) Dodge 1935, 722; "*Actinomyces gibsonii*" (*sic*) Erikson 1935, 36; "*Nocardia gibsonii*" Waksman *in* Waksman and Lechevalier 1953, 155]

gib.so′ni.i. N.L. gen. masc. n. *gibsonii* of Gibson, named for A.G. Gibson who first isolated the organism.

Poor growth on Czapek's solution agar; exhibits slight anti-bacterial activity.

Type strain shows the highest sequence similarity to: *S. almquistii*, AB184258, 100%; *S. rangoonensis*, AB184295, 100%; *S. albus* subsp. *albus*, AJ621602, 100%; *S. flocculus*, AB184272, 99.9%.

Source: isolated from a monkey injected with strain NCTC 450 subsequently named *Actinomyces upcottii* Erikson 1935, 36. Originally obtained from the spleen on a case of acholuric jaundice by Dr A.G. Gibson in 1920.

DNA G+C content (mol%): not known.

Type strain: ATCC 6852, CBS 118.60, CBS 119.60, DSM 43284, HUT 6617, NBRC 15415, IMET 7023, JCM 5061, NCTC 4575, NRRL B-1335.

Sequence accession no. (16S rRNA gene): AB184663.

204. **Streptomyces glaucescens** (Preobrazhenskaya *in* Gauze, Preobrazhenskaya, Kudrina, Blinov, Ryabova and Sveshnikova 1957) Pridham, Hesseltine and Benedict 1958, 67[AL] ("*Actinomyces glaucescesens*" Preobrazhenskaya *in* Gauze, Preobrazhenskaya, Kudrina, Blinov, Ryabova and Sveshnikova 1957, 122)

glau.ces′cens. L. adj. *glaucus* bluish gray; N.L. part. adj. *glaucescens* becoming slightly bluish gray, referring to the bluish green color of the aerial mycelium on a chemically defined medium.

Spore chains in Section *Spirales*. Mature spore chains are short with 3–10 spores per chain. Typical morphology on yeast-malt agar, oatmeal agar, and salts-starch agar, but not typical on glycerol-asparagine agar. Spore surface is hairy. Hairs are coarse, showing some tendency towards spines.

Color of colony: aerial mass color in the Blue or Green color series on yeast-malt agar, oatmeal agar, and salts-starch agar. Color tabs selected by observers fall in both series, but all tabs selected are grayish green. Reverse side of colony is grayed yellow modified by red on yeast-malt agar and glycerol-asparagine agar; modified by green on oatmeal agar and salts-starch agar. Substrate pigment is not a pH indicator.

Color in medium: melanoid pigments formed in peptone-yeast-iron agar, tyrosine agar, and tryptone-yeast broth. Pigments other than melanoids are not formed in yeast-malt agar, oatmeal agar, salts-starch agar, or glycerol-asparagine agar.

D-Glucose, L-arabinose, D-xylose, iso-inositol, D-mannitol, D-fructose, and rhamnose are utilized for growth. No growth or only traces of growth on sucrose and raffinose.

Type strain shows the highest sequence similarity to: *S. pharetrae*, AY699792, 99.3%; *S. matensis*, AB184221, 99.2%; *S. paradoxus*, AB184628, 99.2%; *S. flaveolus*, AB184764, 99.1%; *S. misionensis*, EF178678, 99%; *S. viridochromogenes*, DQ442555, 99%; *S. malachitofuscus*, AB184282, 99%.

Source: not known.

DNA G+C content (mol%): not known.

Type strain: AS 4.1408, ATCC 19761, ATCC 23622, CBS 499.68, BCRC 11478, CECT 3133, DSM 40155, NBRC 12774, IMET 43584, INA 8731, JCM 4377, LMG 19330, NCIMB 9619, NCIMB 9844, NRRL B-2706, NRRL-ISP 5155, RIA 1041, UNIQEM 147, VKM Ac-617.

Sequence accession no. (16S rRNA gene): AB184843.

205. **Streptomyces glauciniger** Huang, Li, Wang, Lanoot, Vancanneyt, Rodriguez, Liu, Swings and Goodfellow 2004b, 2088[VP]

glau.ci.ni′ger. L. adj. *glaucus* greenish gray; L. adj. *niger* black; N.L. masc. adj. *glauciniger* greenish black, referring to the color of colony reverse on modified Bennett's agar.

Forms an extensively branched substrate mycelium and aerial hyphae that differentiate into long spiral spore

chains with 15–20 cylindrical spores per chain. Spore surface is smooth. Aerial spore mass on oatmeal agar is grayish brown. Soluble pigments are not produced, nor are melanin pigments formed on peptone-yeast extract-iron or tyrosine agars. The organism degrades adenine, casein, hypoxanthine, starch, and xanthine, but not cellulose or elastin. Nitrate is reduced. Gelatin is not liquefied. It uses dextrin, D-galactose, D-glucose (all at 1%, w/v), and sodium acetate, and sodium citrate (both at 0.1%, w/v), but not maltose (1%, w/v), as sole carbon sources for energy and growth. Growth occurs at 10–35°C and pH 5.0–10.0, but not at 40°C or at pH 4.0 or 11.0. Growth also occurs in the presence of phenol (0.1%, w/v) but not in the presence of NaCl (5%, w/v), novobiocin (5 μg/ml), or streptomycin (10 μg/ml). It shows antimicrobial activity against strains of *Bacillus subtilis* and *Candida albicans*, but not against strains of *Aspergillus niger*, *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, or *Staphylococcus aureus*. Cell wall is of type I, phospholipid type II and menaquinone MK-9(H_6, H_8). The fatty acid profile is composed of $C_{16:0}$ iso (25.4%), $C_{17:0}$ anteiso (17.4%), $C_{15:0}$ anteiso (16.7%), $C_{15:0}$ iso (9.5%), $C_{16:0}$ (7.7%), $C_{17:0}$ iso (3.8%), $C_{14:0}$ iso (3.5%), $C_{17:1}$ iso ω9*c* (3.3%), $C_{17:1}$ anteiso ω9*c* (3.0%), $C_{16:1}$ iso (2.9%), $C_{15:0}$ (2.8%), and $C_{17:0}$cyclo (2.7%).

Type strain shows no sequence similarity over 99%.

Source: the type strain was isolated from soil in a willow wood collected in Nanning City, Guangxi Province, China.

DNA G+C content (mol%): 67.0.

Type strain: FXJ14, AS 4.1858, JCM 12278, LMG 22082, NBRC 100913.

Sequence accession no. (16S rRNA gene): AB249964.

206. **Streptomyces glaucosporus** (*ex* Krasil'nikov, Agre, Dorokhova and Sokolov 1968) Agre 1986, 574[VP] (Effective publication: Agre *in* Gause, Preobrazhenskaya, Sveshnikova, Terekhova and Maximova 1983.) ("*Actinomyces glaucosporus*" Krasil'nikov, Agre, Dorokhova and Sokolov 1968)

glau.co.spo′rus. L. adj. *glaucus* bluish gray; N.L. n. *spora* a spore; N.L. masc. adj. *glaucosporus* bluish gray spored.

Spore chains are spiral (*Spirales*); spores are warty. On mineral agar 1, oatmeal agar: aerial mycelium poorly or well developed, green-gray; substrate mycelium is dark yellow; no diffusible pigment. On starch-ammonia agar: aerial mycelium is green-gray; substrate mycelium is yellowish; no diffusible pigment. On glycerol-nitrate agar: aerial mycelium is poorly developed, green-gray; substrate mycelium is colorless; no diffusible pigment. On glycerol-asparagine agar: aerial mycelium poorly developed, gray; substrate mycelium is colorless; no diffusible pigment. On organic agar 2: aerial mycelium is green-gray; substrate mycelium and diffusible pigment are dark yellow, brown-yellow. Melanoid pigments are not formed. Good utilization of glucose, maltose, and xylose; weak utilization of sucrose, rhamnose, and inositol.

Type strain shows no sequence similarity over 99%.

Source: not known.

DNA G+C content (mol%): not known.

Type strain: ATCC 25183, DSM 41689, NBRC 15416, INMI 2979, INA G-72, JCM 6921, VKM Ac-1763.

Sequence accession no. (16S rRNA gene): AB184664.

207. **Streptomyces glaucus** (*ex* Lehmann and Schütze 1912) Agre and Preobrazhenskaya 1986, 574[VP] (Effective publication: Agre and Preobrazhenskaya *in* Gause, Preobrazhenskaya, Sveshnikova, Terekhova and Maximova 1983.) ("*Actinomyces glaucus*" Lehmann and Schütze 1912; "*Streptomyces glaucus*" Waksman 1953)

glau′cus. L. masc. adj. *glaucus* bluish gray.

Spore chains are spiral (*Spirales*); spore surface is hairy and rough. On mineral agar 1 and oatmeal agar: aerial mycelium mealy, greenish-bluish; substrate mycelium and diffusible pigment are brown. On glycerol-nitrate agar: aerial mycelium poorly developed, white; substrate mycelium dark brown; diffusible pigment brown. On starch-ammonia agar: aerial mycelium is bluish-greenish; substrate mycelium colorless; diffusible pigment brown. On glycerol-asparagine agar: aerial mycelium is bluish; substrate mycelium is colorless to brownish; diffusible pigment is brownish or absent. On organic agar 2: aerial mycelium poorly developed, white; substrate mycelium dark grayish brown. Melanoid pigments are not formed. Good growth on starch, glucose, lactose, xylose, maltose, rhamnose, glycerol, and mannitol; moderate growth on arabinose and laevulose; poor growth on galactose and inositol; no growth on dulcitol, sorbitol, or sucrose. Cultures belonging to this species are produce antibiotic RA 166 (PA 166-russ).

Type strain shows the highest sequence similarity to: *S. griseomycini*, AB184137, 99.1%; *S. gramineárus* AJ781333, 99.1%; *S. griseostramineus*, AB184140, 99%.

Source: not known.

DNA G+C content (mol%): not known.

Type strain: ATCC 43685, DSM 41456, NBRC 15417, INMI 2965, INA G-86, JCM 6922, NRRL B-16368, VKM Ac-803.

Sequence accession no. (16S rRNA gene): AB184665.

208a. **Streptomyces globisporus subsp. globisporus** (Krasil'nikov 1941) Waksman *in* Waksman and Lechevalier 1953, 39[AL] ("*Actinomyces globisporus*" Krasil'nikov 1941, 48)

glo.bi.spo′rus. L. n. *globus* a round body; N.L. n. *spora* a spore; N.L. masc. adj. *globisporus* round spored (as determined by light microscopy).

Spore chains in Section *Rectiflexibiles*. Mature spore chains are predominantly flexuous and moderately long with 10–50, or often more than 50, spores per chain. This morphology is seen on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar. Spore surface is smooth.

Color of colony: aerial mass color in the Yellow color series on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar. Reverse side of colony with no distinctive pigments (pale yellow to light olive brown) on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar.

Color in medium: melanoid pigments are not produced in peptone-yeast-iron agar, tyrosine agar, or tryptone-yeast broth. Yellow to greenish yellow pigment may be found in medium in yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar. This pigment is not sensitive when tested with 0.05 M HCl or NaOH.

D-Glucose, L-arabinose, D-xylose, D-mannitol, D-fructose, and rhamnose are utilized for growth. No growth or only traces of growth on sucrose, iso-inositol, and raffinose.

Type strain shows the highest sequence similarity to: *S. sindenensis*, AB184759, 100%; *S. pluricolorescens*, DQ442540, 100%; *S. mediolani*, AB184674, 100%; *S. badius*, AY999783, 100%; *S. griseinus*, AB184205, 100%; *S. rubiginosohelvolus*, AB184240, 100%; *S. praecox*, AB184293, 99.9%; *S. flavofuscus*, AB249935, 99.9%; *S. fimicarius*, AY999784, 99.9%; *S. albovinaceus*, AB249958, 99.9%; *S. anulatus*, DQ026637, 99.9%; *S. griseoplanus*, AY999894, 99.9%; *S. californicus*, AB184755, 99.8%; *S. fulvorobeus*, AB184711, 99.8%; *S. parvus*, DQ442537, 99.8%; *S. cavourensis* subsp. *washingtonensis*, DQ026671, 99.8%; *S. acrimycini*, AY999889, 99.8%; *S. lavendulae* subsp. *lavendulae*, AB184080, 99.8%; *S. argenteolus*, AB045872, 99.7%; *S. baarnensis*, EF178688, 99.7%; *S. lipmanii*, AB184148, 99.7%; *S. microflavus*, DQ445795, 99.7%; *S. cyaneofuscatus*, AB184860, 99.7%; *S. floridae*, AB184656, 99.7%; *S. bacillaris*, AB184439, 99.7%; *S. cinereorectus*, AB184646, 99.7%; *S. alboviridis*, AB184256, 99.7%; *S. griseolus*, AB184768, 99.6%; *S. flavovirens*, DQ026635, 99.6%; *S. albolongus*, AB184425, 99.5%; *S. pulveraceus*, AB184806, 99.5%; *S. halstedii*, EF178695, 99.5%; *S. nitrosporeus*, EF178680, 99.5%; *S. atroolivaceus*, AJ781320, 99.4%; *S. olivoviridis*, AB184227, 99.4%; *S. celluloflavus*, AB184476, 99.4%; *S. yanii*, AB006159, 99.4%; *S. finlayi*, AY999788, 99.4%; *S. griseobrunneus*, AB249912, 99.4%; *S. flavogriseus*, AJ494864, 99.4%; *S. cavourensis* subsp. *cavourensis*, DQ445791, 99.3%; *S. atratus*, DQ026638, 99.3%; *S. griseus* subsp. *griseus*, AY207604, 99.3%; *S. luridiscabiei*, AF361784, 99.3%; *S. clavifer*, DQ026670, 99.3%; *S. sanglieri*, AB249945, 99.3%; *S. gelaticus*, DQ026636, 99.3%; *S. mutomycini*, AB249951, 99.1%; *S. candidus*, DQ026663, 99.1%; *S. spiroverticillatus*, AB184814, 99.1%; *S. cremeus*, AB184124, 99%.

Source: not known.

DNA G+C content (mol%): not known.

Type strain: ATCC 15864, ATCC 23913, CBS 834.68, BCRC 11479, CCUG 11107, DSM 40199, NBRC 12867, INMI 2302, JCM 4378, KCTC 9026, NCAIM B.01476, NCIMB 9796, NRRL B-2872, NRRL-ISP 5199, RIA 1151, RIA 335, VKM Ac-179.

Sequence accession no. (16S rRNA gene): EF178686.

208b. **Streptomyces globisporus subsp. caucasicus** (Kudrina *in* Gauze, Preobrazhenskaya, Kudrina, Blinov, Ryabova and Sveshnikova 1957) Pridham, Hesseltine and Benedict 1958, 59^AL^ ("*Actinomyces globisporus* var. *caucasicus*" Kudrina *in* Gauze, Preobrazhenskaya, Kudrina, Blinov, Ryabova and Sveshnikova 1957, 79)

cau.ca′si.cus. L. n. *Caucasius* region of the Caucasus; N.L. masc. adj. *caucasicus* belonging to the Caucasus.

Spore chains are typically flexuous; poor growth on Czapek's solution agar. Exhibits anti-bacterial and anti-fungal activity; inhibited by streptomycin; NaCl tolerance >10%, but <13%.

Type strain shows no sequence similarity over 99%.

Source: not known.

DNA G+C content (mol%): not known.

Type strain: ATCC 19907, CBS 120.60, DSM 40814, INA 13195/54, JCM 9867, NBRC 100770, NRRL B-2593, NRRL-ISP 5157, RIA 319, VKM Ac-1846.

Sequence accession no. (16S rRNA gene): EF178676.

208c. **Streptomyces globisporus subsp. flavofuscus** (Kudrina *in* Gauze, Preobrazhenskaya, Kudrina, Blinov, Ryabova and Sveshnikova 1957) Pridham, Hesseltine and Benedict 1958, 59^AL^ ("*Actinomyces globisporus* var. *flavofuscus*" Kudrina *in* Gauze, Preobrazhenskaya, Kudrina, Blinov, Ryabova and Sveshnikova 1957, 81)

fla.vo.fus′cus. L. adj. *flavus* yellow; L. adj. *fuscus* dark, tawny; N.L. masc. adj. *flavofuscus* dark yellow.

Spore chains are typically flexuous; poor growth observed on Czapek's solution agar. Exhibits anti-bacterial and anti-fungal activity; inhibited by streptomycin; NaCl tolerance >10%, but <13%.

For sequence similarity, see type strain of *Streptomyces flavofuscus*.

Source: not known.

DNA G+C content (mol%): not known.

Type strain: ATCC 19908, INA 1565/53, JCM 9766.

Sequence accession no. (16S rRNA gene): DQ026648.

Further comments: according to Preobrazhenskaya (1986), *Streptomyces globisporus* subsp. *flavofuscus* (Kudrina 1957) Pridham, Hesseltine and Benedict 1958, is a later heterotypic synonym of *Streptomyces flavofuscus* (Kudrina 1957) Preobrazhenskaya 1986.

209. **Streptomyces globosus** (Krasil'nikov 1941) Waksman *in* Waksman and Lechevalier 1953, 68^AL^ ("*Actinomyces globosus*" Krasil'nikov 1941, 58)

glo.bo′sus. L. masc. adj. *globosus* spherical (referring to the shape of the spores when examined with the light microscope).

Forms straight and short chains of spores; excellent growth on Czapek's solution agar; melanin-like chromogenicity is not expressed to great degree with strain IMRU 3763. Exhibits anti-bacterial and anti-fungal activity; inhibited by streptomycin.

Type strain shows the highest sequence similarity to: *S. toxytricini*, DQ442548, 100%; *S. flavotricini*, AB184132, 99.4%; *S. racemochromogenes*, DQ026656, 99.1%; *S. polychromogenes*, AB184292, 99.1%.

Source: not known.

DNA G+C content (mol%): not known.

Type strain: DI-15, ATCC 14979, DSM 40815, IMRU 3736, JCM 13859, NRRL B-2292.

Sequence accession no. (16S rRNA gene): AJ781330.

210. **Streptomyces glomeratus** (*ex* Gause and Sveshnikova 1978) Gause and Preobrazhenskaya 1986a, 574^VP^ (Effective publication: Gause and Preobrazhenskaya *in* Gause, Preobrazhenskaya, Sveshnikova, Terekhova and Maximova 1983.) ("*Streptomyces glomeratus*" Gause and Sveshnikova *in* Gause et al. 1978)

glo.me.ra′tus. L. masc. part. adj. *glomeratus*, formed into a ball, conglobated, glomerated.

Spore chains are hooks, loops, or spiral with 1 or 2 turns; spores look like quasi-sporangia. Spores are smooth. On mineral agar 1: aerial mycelium is whitish gray, light gray to greenish gray; substrate mycelium is grayish blue to grayish brown-blue; diffusible pigment is absent or sometimes pale reddish violet colored. On starch-ammonia agar: aerial mycelium is white to gray brownish gray, greenish gray; substrate mycelium is colorless to gray brown and grayish brown-blue; no diffusible pigment. On glycerol-nitrate agar: aerial mycelium poorly developed, whitish, sometimes greenish gray; substrate mycelium is gray brownish; no diffusible pigment. On glycerol-asparagine agar: aerial mycelium is white to gray brownish gray, sometimes with greenish shadow; substrate mycelium is grayish gray-brown, violet gray-brown; no diffusible pigment. On glucose-asparagine agar: aerial mycelium is grayish white, light greenish gray; substrate mycelium is colorless to brown and grayish brown-blue; no diffusible pigment. Oatmeal agar: aerial mycelium is gray-green; substrate mycelium is gray-brown, gray- brown-blue, gray- blue, sometimes with olive shadow; no diffusible pigment. On organic agar: aerial mycelium is grayish green, sometimes absent; substrate mycelium is grayish gray-brown; diffusible pigment is gray brown. Melanoid pigment is formed. Antibiotic: beromycin, nogalamycin (indicator pigment changing color from yellow in acidic medium to red-violet in alkaline medium). Moderate growth on xylose, rhamnose, fructose, and mannitol; poor growth on arabinose, raffinose, and sucrose; no growth on cellulose.

Type strain shows the highest sequence similarity to: *S. bangladeshensis*, AY750056, 99.2%; *S. rameus*, AY999821, 99.1%; *S. achromogenes* subsp. *rubradiris*, AY999846, 99%.

Source: not known.

DNA G+C content (mol%): not known.

Type strain: DSM 41457, NBRC 15898, INA 3980, JCM 9091, VKM Ac-834.

Sequence accession no. (16S rRNA gene): AB249917.

211. **Streptomyces glomeroaurantiacus** (Krasil'nikov and Yuan *in* Krasil'nikov 1965) Pridham 1970, 17[AL] ("*Actinomyces glomeroaurantiacus*" Krasil'nikov and Yuan *in* Krasil'nikov 1965, 50)

glo.me.ro.au.ran.ti′a.cus. L. v. *glomero* form into a ball; N.L. adj. *aurantiacus* orange colored; N.L. masc. adj. *glomeroaurantiacus* (*sic*) orange-colored ball.

Spore chains in Section *Spirales*, but aerial mycelium is poorly developed and typical spore chains are difficult to find on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar. Mature spore chains on these media are generally very short or absent. Spore surface: Smooth. Conidia-like spores or fragmentation of the substrate mycelium may be seen on yeast-malt agar, oatmeal agar, salts-starch agar, or glycerol-asparagine agar.

Color of colony: growth of aerial mycelium is not adequate for determination of aerial mass color on yeast-malt agar, oatmeal agar, salts-starch agar, or glycerol-asparagine agar. Reverse side of colony is yellowish pink, orange or reddish orange on yeast-malt agar and glycerol-asparagine agar; no distinctive pigments (grayish yellow, orange yellow or yellowish brown) on oatmeal agar or salts-starch agar. Reverse mycelium pigment is not a pH indicator.

Color in medium: melanoid pigments are not formed in peptone-yeast-iron agar, tyrosine agar, or tryptone-yeast broth. One of three observers records orange to pink or red pigment in the medium in yeast-malt agar, oatmeal agar, and glycerol-asparagine agar. This pigment is not pH-sensitive when tested with 0.05 M NaOH or HCl.

D-Glucose, L-arabinose, D-mannitol, D-fructose, and rhamnose are utilized for growth. Reports vary from doubtful growth to positive growth with D-xylose, iso-inositol, sucrose, and raffinose.

Type strain shows the highest sequence similarity to: *S. aurantiacus*, AJ781383, 100%; *S. tauricus*, AB045879, 99.1%.

Source: not known.

DNA G+C content (mol%): not known.

Type strain: ATCC 15866, DSM 41782, JCM 4677, NBRC 15418, INMI 1464, NRRL B-3375, RIA 683.

Sequence accession no. (16S rRNA gene): AB249983.

212. **Streptomyces gobitricini** (Preobrazhenskaya and Sveshnikova *in* Gauze, Preobrazhenskaya, Kudrina, Blinov, Ryabova and Sveshnikova 1957) Pridham, Hesseltine and Benedict 1958, 67[AL] ("*Actinomyces gobitricini*" Preobrazhenskaya and Sveshnikova *in* Gauze, Preobrazhenskaya, Kudrina, Blinov, Ryabova and Sveshnikova 1957, 34)

go.bi.tri.ci′ni. English n. *Gobi* the Gobi Desert in Mongolia; Gr. n. *thrix* the hair; N.L. *gobitricini* (*sic*) of gobi hair, referring to the Gobi desert, the first source of the soil from which the organism was isolated, and probably to formation of a streptothricin-like antibiotic.

Some spore chains are of atypical *Retinaculum-Apertum* type; moderate growth on Czapek's solution agar. Exhibits anti-bacterial and anti-fungal activity; inhibited by streptomycin; NaCl tolerance >7%, but <10%.

Type strain shows the highest sequence similarity to: *S. lavendofoliae*, AJ781336, 99.8%; *S. luridus*, DQ442523, 99.5%.

Source: isolated from soil from the Gobi desert.

DNA G+C content (mol%): not known.

Type strain: CBS 123.60, DSM 41701, NBRC 15419, JCM 5062, NRRL B-2596.

Sequence accession no. (16S rRNA gene): AB184666.

213. **Streptomyces goshikiensis** Niida *in* Shirling and Gottlieb 1966, 324[AL]

gos.hi.ki.en′sis. etymology unknown.

Spore chains in Section *Spirales*, with open spirals of 4–10 convolutions at the end of long spore chains. Mature spore chains have 10–50 spores per chain; longer chains are often observed. This morphology is seen on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar. Spore surface is smooth with minor surface irregularities, but no true spines.

Color of colony: aerial mass color in the Red (or Gray) color series on yeast-malt agar, oatmeal agar, and salts-starch agar. Characteristic color on these media in between 3ge (light grayish yellowish brown) and 5dc or 6ec (grayish yellowish pink) color tabs of Tresner–Backus

Color Wheels. Aerial mycelium may be poorly developed on glycerol-asparagine agar. Reverse side of colony with no distinctive pigments (characteristic grayed yellow on oatmeal agar, salts-starch agar, and glycerol-asparagine agar to light orange yellow or light brown on yeast-malt agar).

Color in medium: melanoid pigments are formed in peptone-yeast-iron agar, tyrosine agar, and tryptone-yeast broth. Pigments other than melanoids or faint traces of yellow are not produced in yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar.

D-Glucose and possibly D-fructose are utilized for growth. Only traces of growth comparable to growth on carbon-free basal medium seen on L-arabinose, sucrose, D-xylose, iso-inositol, D-mannitol, rhamnose, and raffinose.

Type strain shows the highest sequence similarity to: *S. colombiensis*, DQ026646, 100%; *S. sporoverrucosus*, DQ442544, 100%; *S. nojiriensis*, AJ781355, 99.9%; *S. spororaveus*, AJ781370, 99.9%; *S. xanthophaeus*, DQ442560, 99.8%; *S. vinaceus*, AB184394, 99.7%; *S. cirratus*, AY999794, 99.7%; *S. cinnamonensis*, AB184707, 99.6%; *S. avidinii*, AB184395, 99.6%; *S. subrutilus*, X80825, 99.5%; *S. virginiae*, D85119, 99.4%.

Source: not known.

DNA G+C content (mol%): not known.

Type strain: ATCC 23914, CBS 835.68, BCRC 12330, CCUG 11121, DSM 40190, NBRC 12868, IMET 42067, JCM 4294, JCM 4640, NCIMB 9828, NRRL B-5428, NRRL-ISP 5190, RIA 1144, VKM Ac-1212.

Sequence accession no. (16S rRNA gene): EF178693.

214. **Streptomyces gougerotii** (Duché 1934) Waksman and Henrici *in* Breed, Murray and Hitchens 1948, 947[AL] [“*Actinomyces gougeroti*” (*sic*) Duché 1934, 272; “*Streptomyces gougerotii*” Waksman and Henrici *in* Hütter 1967b, 75]

gou.ge.ro′ti.i. N.L. gen. masc. n. *gougerotii* of Gougerot; named for Professor Gougerot from whom the original culture was obtained.

Spore chains in Section *Rectiflexibiles* on yeast-malt agar; aerial mycelium is very thin on this medium and is usually not produced on oatmeal agar, salts-starch agar, or glycerol-asparagine agar. Spore chains, when produced, are moderately short with 3 to 10 or more spores per chain. Two observers recorded fragmentation of the substrate mycelium and this characteristic is also mentioned in Duché’s original description (op. cit). Spore surface is smooth.

Color of colony: aerial mass color in the White or Yellow (2ba, pale yellow) color series on yeast-malt agar. Aerial mycelium is usually thin on yeast-malt agar and is often inadequate for color observation on all other ISP media. Reverse side of colony with no distinctive pigments (colorless to grayish yellow) on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar.

Color in medium: melanoid pigments are not formed in tyrosine agar or tryptone-yeast broth; reports vary on production of dark pigment in peptone-yeast-iron agar. No pigment found in medium in yeast-malt agar, oatmeal agar, salts-starch agar, or glycerol-asparagine agar.

D-Glucose, D-xylose, D-mannitol, and D-fructose are utilized for growth. Reports vary on utilization of L-arabinose, iso-inositol, and rhamnose. No growth or only traces of growth on sucrose and raffinose.

Type strain shows the highest sequence similarity to: *S. diastaticus* subsp. *diastaticus*, AB184785, 100%; *S. rutgersensis* subsp. *rutgersensis*, AB184170, 100%; *S. intermedius*, AB184277, 99.8%; *S. misionensis*, EF178678, 99.2%; *S. phaeoluteichromatogenes*, AJ391814, 99.1%%; *S. matensis*, AB184221, 99%%; *S. aureoverticillatus*, AY999774, 99%.

Source: not known.

DNA G+C content (mol%): not known.

Type strain: ATCC 10975, ATCC 25455, CBS 422.34, CBS 688.69, BCRC 12105, DSM 40324, NBRC 13043, NBRC 3198, IMET 40289, JCM 4136, JCM 4478, NRRL B-1344, NRRL B-1903, NRRL-ISP 5324, RIA 1235, VKM Ac-713.

Sequence accession no. (16S rRNA gene): AB184742.

215. **Streptomyces graminearus** Preobrazhenskaya 1986, 574[VP] (Effective publication: Preobrazhenskaya *in* Gause, Preobrazhenskaya, Sveshnikova, Terekhova and Maximova 1983.)

gra.mi.ne.a′rus. N.L. masc. adj. *graminearus* related to grain, isolated from grain.

Spore chains are spiral (*Spirales*); spores are smooth. On mineral agar 1: aerial mycelium is gray or green-gray; substrate mycelium is light yellow; no diffusible pigment. On glycerol-nitrate agar: aerial mycelium is poor, white; substrate mycelium is yellow, citreous; no diffusible pigment. On oatmeal agar: aerial mycelium is gray; substrate mycelium is colorless or light citreous; no diffusible pigment. On starch-ammonia agar: aerial mycelium is grayish gray; substrate mycelium is yellow; no diffusible pigment. On glycerol-asparagine agar: aerial mycelium is poor, white; substrate mycelium is yellow; no diffusible pigment. On organic agar 2: aerial mycelium is white; substrate mycelium is colorless or yellow; no diffusible pigment. Melanoid pigments are not formed. Grows on glucose, fructose, starch, mannitol, sucrose, and arabinose; weak growth on raffinose, rhamnose, and inositol.

Type strain shows the highest sequence similarity to: *S. griseostramineus*, AB184140, 100%; *S. griseomycini*, AB184137, 100%; *S. glaucus*, AB184665, 99.1%; *S. calvus*, AB184329, 99%.

Source: not known.

DNA G+C content (mol%): not known.

Type strain: DSM 41474, NBRC 15420, INA 13982, JCM 6923, NRRL B-16369, VKM Ac-1847.

Sequence accession no. (16S rRNA gene): AJ781333.

216. **Streptomyces graminofaciens** Charney, Fisher, Curran, Machlowitz and Tytell 1953, 1283[AL]

gra.mi.no.fa′ci.ens. L. n. *gramen* grass; L. part. adj. *faciens* producing; N.L. part. adj. *graminofaciens* grass producing, probably refers to production of (strepto)gramin.

Spore chains in Section *Spirales*. Open spirals of four or more turns, irregular spirals and hooks, or tight terminal spirals may be found on various media. Mature spore chains are generally long, sometimes with more than 50 spores per chain. This morphology is seen on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar. Spore surface is warty.

Color of colony: aerial mass color in the Gray or White color series on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar. Only one of three observers records aerial mycelium in the Red color series (5dc, grayish yellowish pink) on yeast-malt agar in 21 d. Nearest matching color tabs in the Gray color series are d, light gray; 2dc, yellow gray; and 5fe, light grayish reddish brown. Reverse side of colony with no distinctive pigments (grayish yellow to brown on yeast-malt agar; pale grayish or greenish yellow to light yellowish brown on oatmeal agar, salts-starch agar, and glycerol-asparagine agar).

Color in medium: melanoid pigments are not formed in peptone-yeast-iron agar, tyrosine agar, or tryptone-yeast broth. No pigment is found in the medium in yeast-malt agar, oatmeal agar, salts-starch agar, or glycerol-asparagine agar.

D-Glucose, L-arabinose, D-xylose, iso-inositol, D-mannitol, D-fructose, rhamnose, sucrose, and raffinose are all utilized for growth.

Type strain shows the highest sequence similarity to: *S. kurssanovii*, AB184325, 100%; *S. xantholiticus*, AB184349, 99.8%; *S. peucetius*, AB045887, 99.7%; *S. cremeus*, AB184124, 99%.

Source: not known.

DNA G+C content (mol%): not known.

Type strain: ATCC 12705, CBS 756.72, BCRC 12352, CECT 3217, DSM 40559, HAMBI 982, NBRC 13455, IMET 43540, JCM 4157, JCM 4762, NRRL B-2609, NRRL-ISP 5559, RIA 1416, VKM Ac-973.

Sequence accession no. (16S rRNA gene): AJ781329.

217. **Streptomyces griseiniger** Goodfellow, Kumar, Labeda and Sembiring 2008, 1[VP] (Effective publication: Goodfellow, Kumar, Labeda and Sembiring 2007, 198.)

gri.se.i.ni′ger. N.L. adj. *griseus* gray; L. adj. *niger* black; N.L. masc. adj. *griseiniger* gray-black.

Spore chains are *Spriales*; spore surface is rugose. On oatmeal agar, the aerial spore mass color is gray, becoming black and moist when mature; the reverse side of colonial growth is grayish-yellow. Melanin pigments are not formed.

Type strain shows the highest sequence similarity to: *S. rhizosphaericus*, AB249941, 99.8%; *S. asiaticus*, AB249947, 99.8%; *S. cangkringensis*, AJ391831, 99.5%; *S. indonesiensis*, DQ334783, 99.4%. Type strain shows DNA–DNA similarity to: *S. cangkringensis* DSM 41769^T, 59%; *S. hygroscopicus* subsp. *geldanus* NRRL 3602^T, 56%; *S. indonesiensis* DSM 41759^T, 62%; *S. melanosporofaciens* NRRL B-12234^T, 57%.

Source: not known.

DNA G+C content (mol%): 70.2.

Type strain: DSM 41895, NRRL B-1865.

Sequence accession no. (16S rRNA gene): AJ391818.

218. **Streptomyces griseinus** Waksman 1959, 1045[AL]

gri.se.i′nus.N.L. n. *griseinum* grisein, name of an antibiotic; L. masc. suff. *-inus* suffix used with the sense of belonging to; N.L. masc. adj. *griseinus* belonging to grisein, the antibiotic produced by the organism.

Spore chains in Section *Rectiflexibiles*. Mature spore chains are predominantly flexuous and moderately long with 10–50, or often more than 50, spores per chain on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar. Spore surface is smooth.

Color of colony: aerial mass color in the Yellow color series on salts-starch agar and glycerol-asparagine agar and in the Yellow or Gray color series on oatmeal agar and yeast-malt agar. Nearest matching color tab from the Gray color series is 2dc, yellowish gray. Reverse side of colony: no distinctive pigments (grayish yellow to yellowish brown or light olive brown) on yeast-malt agar, oatmeal agar, salts-starch agar, or glycerol-asparagine agar. The substrate mycelium pigment may be changed slightly from pale yellow to pale yellow-brown or pale violet with addition of 0.05 M NaOH, or from yellowish brown to pale yellow with 0.05 M HCl.

Color in medium: melanoid pigments are not produced in peptone-yeast-iron agar, tyrosine agar, or tryptone-yeast broth. A trace of yellow to reddish brown pigment may be found in the medium in oatmeal agar. This pigment, when present, is slightly pH-sensitive changing from pale yellow to pale violet with addition of 0.05 M NaOH.

D-Glucose, L-arabinose, D-xylose, D-mannitol, D-fructose, and rhamnose are utilized for growth. No growth or only traces of growth on sucrose, iso-inositol, and raffinose.

Type strain shows the highest sequence similarity to: *S. sindenensis*, AB184759, 100%; *S. albovinaceus*, AB249958, 100%; *S. griseoplanus*, AY999894, 100%; *S. anulatus*, DQ026637, 100%; *S. globisporus* subsp. *globisporus*, EF178686, 100%; *S. pluricolorescens*, DQ442540, 100%; *S. praecox*, AB184293, 100%; *S. flavofuscus*, AB249935, 100%; *S. fimicarius*, AY999784, 100%; *S. mediolani*, AB184674, 100%; *S. badius*, AY999783, 100%; *S. rubiginosohelvolus*, AB184240, 100%; *S. californicus*, AB184755, 99.9%; *S. lavendulae* subsp. *lavendulae*, AB184080, 99.9%; *S. cavourensis* subsp. *washingtonensis*, DQ026671, 99.9%; *S. acrimycini*, AY999889, 99.9%; *S. parvus*, DQ442537, 99.9%; *S. fulvorobeus*, AB184711, 99.8%; *S. lipmanii*, AB184148, 99.8%; *S. microflavus*, DQ445795, 99.8%; *S. cyaneofuscatus*, AB184860, 99.8%; *S. floridae*, AB184656, 99.8%; *S. alboviridis*, AB184256, 99.8%; *S. baarnensis*, EF178688, 99.8%; *S. cinereorectus*, AB184646, 99.8%; *S. griseus* subsp. *griseus*, AY207604, 99.7%; *S. griseolus*, AB184768, 99.7%; *S. luridiscabiei*, AF361784, 99.7%; *S. flavovirens*, DQ026635, 99.7%; *S. argenteolus*, AB045872, 99.7%; *S. bacillaris*, AB184439, 99.6%; *S. pulveraceus*, AB184806, 99.6%; *S. halstedii*, EF178695, 99.6%; *S. flavogriseus*, AJ494864, 99.6%; *S. atroolivaceus*, AJ781320, 99.5%; *S. olivoviridis*, AB184227, 99.5%; *S. finlayi*, AY999788, 99.5%; *S. nitrosporeus*, EF178680, 99.5%; *S. albolongus*, AB184425, 99.4%; *S. clavifer*, DQ026670, 99.4%; *S. griseobrunneus*, AB249912, 99.4%; *S. sanglieri*, AB249945, 99.4%; *S. celluloflavus*, AB184476, 99.4%; *S. yanii*, AB006159, 99.4%; *S. gelaticus*, DQ026636, 99.3%; *S. atratus*, DQ026638, 99.3%; *S. cavourensis* subsp. *cavourensis*, DQ445791, 99.3%; *S. mutomycini*, AB249951, 99.2%; *S. candidus*, DQ026663, 99.2%; *S. spiroverticillatus*, AB184814, 99.1%; *S. cremeus*, AB184124, 99.1%.

Source: not known.

DNA G+C content (mol%): not known.

Type strain: ATCC 23915, CBS 836.68, BCRC 11480, CCUG 11106, DSM 40047, NBRC 12869, JCM 4379, NRRL B-1076, NRRL-ISP 5047, RIA 1113.

Sequence accession no. (16S rRNA gene): AB184205.

219. **Streptomyces griseoaurantiacus** (Krasil'nikov and Yuan *in* Krasil'nikov 1965) Pridham 1970, 17[AL] ("*Actinomyces griseoaurantiacus*" Krasil'nikov and Yuan *in* Krasil'nikov 1965, 52)

gri.se.o.au.ran.ti.a′cus N.L. adj. *griseus* gray; N.L. adj. *aurantiacus* orange colored; N.L. masc. adj. *griseoaurantiacus* orange colored with gray.

Spore chains in Section *Spirales*. Hooks, loops, and primitive spirals suggesting *Retinaculiaperti* morphology are very common. Mature spore chains have 10 to 50 or more spores per chain. This morphology is seen on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar. Spore surface is smooth.

Color of colony: aerial mass color in the Gray color series (2dc, yellowish gray; 2fe, medium gray; 3fe, light brownish gray; or 5fe, light grayish reddish brown) on yeast-malt agar, oatmeal agar and salts-starch agar. Gray or Red color series (5dc, grayish yellowish pink) on glycerol-asparagine agar. Reverse side of colony is grayish yellow to orange yellow, yellowish pink, or dark reddish orange on yeast-malt agar; yellowish pink or reddish gray to reddish orange on oatmeal agar, salts-starch agar, and glycerol-asparagine agar. Reverse mycelium pigment on yeast-malt agar, salts-starch agar, and glycerol-asparagine agar is a pH indicator, changing from orange to red with the addition of 0.05 M HCl and from orange to brown with the addition of 0.05 M NaOH.

Color in medium: melanoid pigments are not formed in peptone-yeast-iron agar, tyrosine agar, or tryptone-yeast broth. Yellow orange or pinkish red may be found in the medium in yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar in 21 d. This pigment, when formed in sufficient quantity, is pH-sensitive, showing the same changes observed for reverse mycelium pigment.

D-Glucose, L-arabinose, D-xylose, iso-inositol, D-mannitol, D-fructose, and rhamnose are utilized for growth. Utilization of raffinose is doubtful. No growth or only traces of growth with sucrose.

Type strain shows the highest sequence similarity to: *S. jietaisiensis*, AY314783, 99.7%.

Source: not known.

DNA G+C content (mol%): not known.

Type strain: ATCC 19840, CBS 682.72, DSM 40430, NBRC 13381, NBRC 15440, JCM 4763, NRRL-ISP 5430, RIA 1342, VKM Ac-1728.

Sequence accession no. (16S rRNA gene): AB184676.

220. **Streptomyces griseobrunneus** Waksman 1961, 220[AL]

gri.se.o.brun′ne.us. N.L. adj. *griseus* gray; N.L. adj. *brunneus* dark brown; N.L. masc. adj. *griseobrunneus* grayish dark-brown colored.

Spore chains in Section *Rectiflexibiles*. Mature spore chains are generally long, often with more than 50 spores per chain. This morphology is seen on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar. Spore surface is smooth. Special morphological characteristics: substrate conidia noted by two observers.

Color of colony: aerial mass color in the Yellow color series on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar. Reverse side of colony with no distinctive pigments on yeast-malt agar, oatmeal agar, salts-starch agar, or glycerol-asparagine agar; substrate pigment is not a pH indicator.

Color in medium: melanoid pigments formed in peptone-yeast-iron agar. Pigments other than melanoid not formed in yeast-malt agar, oatmeal agar, salts-starch agar, or glycerol-asparagine agar.

D-Glucose, D-xylose, D-mannitol, and D-fructose are utilized for growth. No growth or only trace of growth on L-arabinose, sucrose, iso-inositol, rhamnose, and raffinose.

Type strain shows the highest sequence similarity to: *S. celluloflavus*, AB184476, 100%; *S. albolongus*, AB184425, 100%; *S. cavourensis* subsp. *cavourensis*, DQ445791, 99.9%; *S. californicus*, AB184755, 99.5%; *S. bacillaris*, AB184439, 99.4%; *S. floridae*, AB184656, 99.4%; *S. globisporus* subsp. *globisporus*, EF178686, 99.4%; *S. griseinus*, AB184205, 99.4%; *S. fulvorobeus*, AB184711, 99.4%; *S. pluricolorescens*, DQ442540, 99.4%; *S. candidus*, DQ026663, 99.4%; *S. spiroverticillatus*, AB184814, 99.4%; *S. sindenensis*, AB184759, 99.4%; *S. badius*, AY999783, 99.4%; *S. rubiginosohelvolus*, AB184240, 99.4%; *S. mediolani*, AB184674, 99.4%; *S. praecox*, AB184293, 99.3%; *S. albovinaceus*, AB249958, 99.3%; *S. microflavus*, DQ445795, 99.3%; *S. fimicarius*, AY999784, 99.3%; *S. flavofuscus*, AB249935, 99.5%; *S. alboviridis*, AB184256, 99.3%; *S. lipmanii*, AB184148, 99.3%; *S. cremeus*, AB184124, 99.3%; *S. griseoplanus*, AY999894, 99.3%; *S. anulatus*, DQ026637, 99.3%; *S. cavourensis* subsp. *washingtonensis*, DQ026671, 99.2%; *S. lavendulae* subsp. *lavendulae*, AB184080, 99.2%; *S. parvus*, DQ442537, 99.2%; *S. luridiscabiei*, AF361784, 99.2%; *S. acrimycini*, AY999889, 99.2%; *S. baarnensis*, EF178688, 99.2%; *S. cyaneofuscatus*, AB184860, 99.2%; *S. cinereorectus*, AB184646, 99.2%; *S. flavovirens*, DQ026635, 99.2%; *S. argenteolus*, AB045872, 99.1%; *S. finlayi*, AY999788, 99.1%; *S. flavogriseus*, AJ494864, 99.1%; *S. nitrosporeus*, EF178680, 99%; *S. griseolus*, AB184768, 99%; *S. pulveraceus*, AB184806, 99%; *S. clavifer*, DQ026670, 99%; *S. griseus* subsp. *griseus*, AY207604, 99%; *S. halstedii*, EF178695, 99%; *S. cinnamonensis*, AB184707, 99%.

Source: not known.

DNA G+C content (mol%): not known.

Type strain: ATCC 19762, CBS 500.68, BCRC 13674, CCUG 11105, DSM 40066, HAMBI 1015, NBRC 12775, IMET 42052, JCM 4380, NCIMB 12975, NRRL B-2095, NRRL-ISP 5066, RIA 1042, UNIQEM 148, VKM Ac-753.

Sequence accession no. (16S rRNA gene): AB249912.

221. **Streptomyces griseocarneus** (Benedict, Lindenfelser, Stodola and Traufler 1950) Witt and Stackebrandt 1991, 456[VP] (Effective publication: Witt and Stackebrandt 1990, 370.) ["*Streptomyces griseocarneus*" Benedict, Lindenfelser, Stodola and Traufler 1950; "*Streptoverticillium griseocarneus*" (*sic*) Baldacci 1958; "*Verticillomyces griseocarneus*" Shinobu 1965; *Streptoverticillium griseocarneum* Baldacci, Farina and Locci 1966, 170]

gri.se.o.car′ne.us. L. adj. *griseus* gray; L. adj. *carneus* pertaining to flesh; N.L. masc. adj. *griseocarneus* gray flesh-colored.

Spore chains in Umbellate monoverticillate (=*Streptomyces* Section *Verticillati*, biverticillate). Mature spore chains are short, generally 3–10 spores per chain. This morphology is seen on yeast-malt agar, salts-starch agar, and glycerol-asparagine agar. Mature spores often not produced until 14–21 d. Spore surface is smooth.

Color of colony: aerial mass color in the Red color series on yeast-malt agar, salts-starch agar, and glycerol-asparagine agar; good spore mass not produced on oatmeal agar. Reverse side of colony with no distinctive pigments (grayed yellow to yellow-brown) on yeast-malt agar, salts-starch agar, and glycerol-asparagine agar; substrate pigment is not a pH indicator.

Color in medium: melanoid pigments formed in peptone-yeast-iron agar. Pigments other than melanoids not formed in yeast-malt agar, oatmeal agar, salts-starch agar, or glycerol-asparagine agar.

D-Glucose is utilized for growth. No growth or only traces of growth on sucrose, rhamnose, and raffinose. Variable reports on growth with L-arabinose, D-xylose, iso-inositol, D-mannitol, and D-fructose (but if growth occurs with these carbon sources it is less than with D-glucose).

Type strain shows no sequence similarity over 99%.

Source: not known.

DNA G+C content (mol%): not known.

Type strain: AS 4.1088, ATCC 12628, ATCC 19763, CBS 501.68, CCM 3228, BCRC 13304, CCUG 11123, CECT 3250, DSM 40004, NBRC 12776, NBRC 3387, JCM 4095, JCM 4580, LMG 5973, NCIMB 9623, NRRL B-1068, NRRL B-1350, NRRL-ISP 5004, PCM 2326, PCM 2345, RIA 1043, RIA 132, UNIQEM 149, VKM Ac-881.

Sequence accession no. (16S rRNA gene): X99943.

Further comments: in violation of Rule 33c of the *Bacteriological Code* (1990 Revision), in Validation List no. 38, *Streptomyces griseocarneus* is proposed as a *nomen revictum* (basonym: "*Streptomyces griseocarneus*" Benedict et al. (1950).

According to Hatano et al. (2003), *Streptomyces griseocarneus* (Benedict et al. 1950) Witt and Stackebrandt 1991 is an earlier heterotypic synonym of *Streptomyces alboverticillatus* (Locci and Schofield 1989) Witt and Stackebrandt 1991 and of *Streptomyces septatus* (Locci et al. 1969) Witt and Stackebrandt 1991. Hatano et al. (2003) also propose that *Streptomyces griseocarneus* (Benedict et al. 1950) Witt and Stackebrandt 1991 be a heterotypic synonym of "*Streptomyces tropicalensis*" Gupta 1965b.

222. **Streptomyces griseochromogenes** Fukunaga *in* Fukunaga, Misato, Ishii and Asakawa 1955, 181[AL]

gri.se.o.chro.mo′ge.nes. N.L. adj. *griseus* gray; Gr. n. *chroma* color; N.L. suff. -*genes* (from Gr. v. *gennaô* to produce) producing; N.L. part. adj. *griseochromogenes* producing gray color.

Spore chains in Section *Spirales* to *Retinaculiaperti*. Open loops and hooks as well as well-developed spirals are common. Mature spore chains generally contain 10–50 spores per chain. This morphology is seen on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar. Spore surface is spiny.

Color of colony: aerial mass color in the Gray color series (2fe, medium gray, or 3ge, light grayish yellowish brown) on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar; white aerial mycelium may also be seen on young cultures or on 21-d-old cultures on glycerol-asparagine agar. Reverse side of colony with no distinctive pigments (grayish yellow to orange yellow on yeast-malt agar; grayish yellow to light olive brown or grayish greenish yellow on oatmeal agar, salts-starch agar, or glycerol-asparagine agar).

Color in medium: melanoid pigments are formed in peptone-yeast-iron agar, tyrosine agar, and tryptone-yeast broth. No pigment is found in the medium in yeast-malt agar, oatmeal agar, salts-starch agar, or glycerol-asparagine agar.

D-Glucose, L-arabinose, D-xylose, iso-inositol, D-mannitol, D-fructose, sucrose, and raffinose are utilized for growth. No growth or only traces of growth with rhamnose.

Type strain shows the highest sequence similarity to: *S. cellostaticus*, AB184192, 99.7%; *S. yokosukanensis*, DQ026652, 99.3%; *S. corchorusii*, AB184267, 99%; *S. olivaceoviridis*, AB184288, 99%.

Source: not known.

DNA G+C content (mol%): not known.

Type strain: ATCC 14511, CBS 714.72, BCRC 11818, DSM 40499, IFM 1229, NBRC 13413, JCM 4039, JCM 4764, KCTC 9027, NRRL B-12423, NRRL-ISP 5499, RIA 1374.

Sequence accession no. (16S rRNA gene): AB184387.

223. **Streptomyces griseoflavus** (Krainsky 1914) Waksman and Henrici *in* Breed, Murray and Hitchens 1948, 948[AL] ("*Actinomyces griseoflavus*" Krainsky 1914, 694)

gri.se.o.fla′vus. N.L. adj. *griseus* gray; L. adj. *flavus* yellow; N.L. masc. adj. *griseoflavus* grayish yellow.

Spore chains in Section *Spirales*. Mature spore chains generally have 10–50 spores per chain, although shorter chains may also be common. This morphology is seen on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar. Spore surface is spiny.

Color of colony: aerial mass color in the Gray color series on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar (immature cultures may appear to be in the Yellow color series on glycerol-asparagine agar). Reverse side of colony is yellow to orange-yellow on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar. Reverse mycelium pigment is not a pH indicator.

Color in medium: melanoid pigments are not formed in peptone-yeast-iron agar, tyrosine agar, or tryptone-yeast broth. No pigment found in medium in yeast-malt agar, oatmeal agar, salts-starch agar, or glycerol-asparagine agar.

D-Glucose, L-arabinose, D-xylose, iso-inositol, D-mannitol, D-fructose, and rhamnose are utilized for growth. No growth or only traces of growth with sucrose and raffinose.

Type strain shows the highest sequence similarity to: *S. griseorubens*, AB184139, 99.7%; *S. griseoincarnatus*, AJ781328, 99.6%; *S. erythrogriseus*, AJ781328, 99.6%; *S. labedae*, AB184704, 99.6%; *S. variabilis*, DQ442551, 99.6%; *S. flaveolus*, AB184764, 99.5%; *S. collinus*, AB184123, 99.5%;

S. matensis, AB184221, 99.5%; *S. heliomycini*, AB184712, 99.3%; *S. paradoxus*, AB184628, 99.3%; *S. malachitofuscus*, AB184282, 99.3%; *S. althioticus*, AY999808, 99.2%; *S. ambofaciens*, M27245, 99.2%; *S. viridochromogenes*, DQ442555, 99.2%; *S. violaceochromogenes*, AY999867, 99.1%; *S. albaduncus*, AY999757, 99%; *S. speibonae*, AF452714, 99%; *S. violaceorubidus*, AJ781374, 99%; *S. longispororuber*, AB184440, 99%; *S. iakyrus*, AB184877, 99%.

Source: not known.

DNA G+C content (mol%): not known.

Type strain: AS 4.1454, ATCC 25456, CBS 409.52, CBS 689.69, BCRC 12232, DSM 40456, NBRC 13044, IMET 43530, JCM 4479, LMG 19344, NRRL B-5312, NRRL-ISP 5456, RIA 1236, VKM Ac-993.

Sequence accession no. (16S rRNA gene): AJ781322.

224. **Streptomyces griseofuscus** Sakamoto, Kondo, Yumoto and Arishima 1962, 98[AL]

gri.se.o.fus′cus. N.L. adj. *griseus* gray; L. adj. *fuscus* dark, tawny; N.L. masc. adj. *griseofuscus* gray, tawny.

Spore chains in Section *Spirales*. Mature spore chains generally have 10–50 spores per chain. This morphology is observed on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar. Spore surface is smooth.

Color of colony: aerial mass color in the Red or Gray color series on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar. Characteristic color is between 4ge or 5ge (light grayish reddish brown) and 4ig (light grayish brown) color tabs of Tresner–Backus color wheels. Reverse side of colony with no distinctive pigments on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar.

Color in medium: melanoid pigments not formed in peptone-yeast-iron agar, tyrosine agar, or tryptone-yeast broth. No pigment found in medium in yeast-malt agar, oatmeal agar, salts-starch agar, or glycerol-asparagine agar.

D-Glucose, L-arabinose, D-xylose, iso-inositol, D-mannitol, and D-fructose are utilized for growth. No growth or only traces of growth on sucrose, iso-inositol, rhamnose, and raffinose.

Type strain shows the highest sequence similarity to: *S. murinus*, AB184155, 100%; *S. costaricanus*, AB249939, 100%; *S. phaeogriseichromatogenes*, AJ391813, 99.6%.

Source: not known.

DNA G+C content (mol%): not known.

Type strain: ATCC 23916, CBS 837.68, BCRC 10483, CECT 3307, DSM 40191, NBRC 12870, IMET 42068, JCM 4276, JCM 4641, NCIMB 9821, NRRL B-5429, NRRL-ISP 5191, RIA 1145, VKM Ac-1707.

Sequence accession no. (16S rRNA gene): AB184206.

225. **Streptomyces griseoincarnatus** (Preobrazhenskaya, Ryabova and Blinov *in* Gauze, Preobrazhenskaya, Kudrina, Blinov, Ryabova and Sveshnikova 1957) Pridham, Hesseltine and Benedict 1958, 69[AL] ("*Actinomyces griseoincarnatus*" Preobrazhenskaya, Ryabova and Blinov *in* Gauze, Preobrazhenskaya, Kudrina, Blinov, Ryabova and Sveshnikova 1957, 169)

gri.se.o.in.car.na′tus. N.L. adj. *griseus* gray; L. part. adj. *incarnatus* flesh-colored; N.L. masc. part. adj. *griseoincarnatus* grayish flesh-colored, referring to changes in color of the aerial mycelium.

Spore chains in Section *Spirales*. Spirals are generally open with 3 to 7 or more turns; flexuous spore chains and imperfect spirals suggesting *Retinaculiaperti* morphology are also common. Spirals are best developed on oatmeal agar and salts-starch agar. Spore chains are moderately long, especially on oatmeal agar and salts-starch agar with 10–50, or often more than 50, spores per chain. Spore surface is spiny.

Color of colony: aerial mass color in the Gray color series on salts-starch agar ater 14–21 d; younger aerial mycelium may be in the Red color series. Aerial mass color in the Gray or Red color series yeast-malt agar, oatmeal agar, and salts-starch agar. Observers did not agree on the selection of individual color tabs representing the aerial color, but all chose tabs ranging form grayish yellowish pink or grayish reddish brown to medium gray. Reverse side of colony is yellow to yellow brown plus red (grayish yellow on salts-starch agar; orange-yellow to reddish brown on yeast-malt agar, oatmeal agar). Substrate mycelium pigment is not a pH indicator.

Color in medium: melanoid pigments are not produced in peptone-yeast-iron agar, tyrosine agar, or tryptone-yeast broth. Red or orange pigment is found in the medium in yeast-malt agar in 7 d and in oatmeal agar in 14–21 d. Red or orange pigment may or may not be found in glycerol-asparagine agar. This pigment is not a pH indicator.

D-Glucose, L-arabinose, sucrose, D-xylose, D-mannitol, D-fructose, and rhamnose are utilized for growth. Reports vary on utilization of iso-inositol and only traces of growth are found on raffinose.

Type strain shows the highest sequence similarity to: *S. erythrogriseus*, AJ781328, 100%; *S. labedae*, AB184704, 100%; *S. variabilis*, DQ442551, 100%; *S. griseorubens*, AB184139, 99.9%; *S. griseoflavus*, AJ781322, 99.6%; *S. matensis*, AB184221, 99.6%; *S. althioticus*, AY999808, 99.2%; *S. paradoxus*, AB184628, 99.2%; *S. heliomycini*, AB184712, 99.1%; *S. collinus*, AB184123, 99.1%; *S. viridochromogenes*, DQ442555, 99.1%; *S. flaveolus*, AB184764, 99.1%; *S. bellus*, AB184849, 99%; *S. albogriseolus*, AJ494865, 99%; *S. viridodiastaticus*, AY999852, 99%; *S. violaceochromogenes*, AY999867, 99%; *S. malachitofuscus*, AB184282, 99%; *S. violaceorubidus*, AJ781374, 99%; *S. coerulescens*, AY999720, 99%.

Source: not known.

DNA G+C content (mol%): not known.

Type strain: AS 4.1409, ATCC 23623, ATCC 23917, CBS 838.68, BCRC 11481, DSM 40274, NBRC 12871, INA 9673/55, JCM 4381, LMG 19316, NCIMB 9825, NRRL B-5313, NRRL-ISP 5274, RIA 1192.

Sequence accession no. (16S rRNA gene): AJ781321.

226. **Streptomyces griseoloalbus** (Kudrina *in* Gauze, Preobrazhenskaya, Kudrina, Blinov, Ryabova and Sveshnikova 1957) Pridham, Hesseltine and Benedict 1958, 58[AL] ("*Actinomyces griseoloalbus*" Kudrina *in* Gauze, Preobrazhenskaya, Kudrina, Blinov, Ryabova and Sveshnikova 1957, 112)

gri.se.o.lo.al′bus. N.L. dim. adj. *griseolus* somewhat gray; L. adj. *albus* white; N.L. masc. adj. *griseoloalbus* somewhat grayish white.

Spore chain in Section *Rectiflexibiles*. Aerial hyphae may be sterile or spores may be poorly defined. Spore surface is smooth. Terminal swellings of two types are sometimes seen on aerial hyphae. Aerial mycelium is best developed on yeast-malt agar and salts-starch agar, but good sporulating aerial mycelium is not found on any of the ISP media. In addition to the terminal swellings noted above, one observer records fragmentation of the substrate mycelium on yeast-malt agar and salts-starch agar.

Color of colony: aerial mass color in the White or Yellow (2ba, pale yellow) color series on yeast-malt agar and salts-starch agar; a white aerial mycelium may or may not be formed on oatmeal agar and glycerol-asparagine agar, but this mycelium is usually sterile. Reverse side of colony is light yellow or grayish yellow to orange-yellow on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar.

Color in medium: melanoid pigments are not formed in peptone-yeast-iron agar, tyrosine agar, or tryptone-yeast broth. A trace of yellow to orange-yellow pigment may be found in the medium in yeast-malt agar, oatmeal agar, salts-starch agar or glycerol-asparagine agar. This pigment is not pH-sensitive when tested with 0.05 M NaOH or HCl.

D-Glucose, L-arabinose, D-xylose, iso-inositol, D-mannitol, D-fructose, rhamnose, and sucrose are utilized for growth. Reports vary on the utilization of raffinose.

Type strain shows the highest sequence similarity to: *S. albaduncus*, AY999757, 100%; *S. matensis*, AB184221, 99.2%; *S. gancidicus*, AB184660, 99.2%; *S. paradoxus*, AB184628, 99.%; *S. pseudogriseolus*, DQ442541, 99.1%; *S. capillispiralis*, AB184577, 99%; *S. heliomycini*, AB184712, 99%; *S. malachitofuscus*, AB184282, 99%; *S. cellulosae*, DQ442495, 99%; *S. lusitanus*, AB184424, 99%.

Source: not known.

DNA G+C content (mol%): not known.

Type strain: ATCC 23624, ATCC 25458, CBS 691.69, DSM 40468, NBRC 13046, INA 1875/54, JCM 4480, NRRL B-12383, NRRL-ISP 5468, RIA 1238, VKM Ac-1739.

Sequence accession no. (16S rRNA gene): AB184275.

227. **Streptomyces griseolus** (Waksman 1923) Waksman and Henrici *in* Breed, Murray and Hitchens 1948, 938[AL] ("*Actinomyces griseolus*" Waksman *in* Bergey, Harrison, Breed, Hammer and Huntoon 1923, 369)

gri.se′o.lus. N.L. adj. *griseus* gray; N.L. dim. masc. adj. *griseolus* somewhat gray.

Spore chain in Section *Rectiflexibiles*. Mature spore chains are moderately short with 3–10, or sometimes more than 10, spores per chain (one observer reports 10–50 spores per chain). This morphology is seen on yeast-malt agar; sporulation on oatmeal agar, salts-starch agar, or glycerol-asparagine agar may be poor. Spore surface is smooth.

Color of colony: aerial mass color in the Gray color series on yeast-malt agar; sporulation usually inadequate for color determination on oatmeal agar, salts-starch agar, and glycerol-asparagine agar. Reverse side of colony is yellow-brown to brown on yeast-malt agar and colorless or characteristic grayed yellow to grayed yellow-brown on oatmeal agar, salts-starch agar, and glycerol-asparagine agar. Substrate pigment is not a pH indicator.

Color in medium: melanoid pigments are not formed in peptone-yeast-iron agar and tyrosine agar. No pigment is found in medium in yeast-malt agar, oatmeal agar, salts-starch agar, or glycerol-asparagine agar.

D-Glucose, L-arabinose, D-xylose, and D-fructose are utilized for growth. No growth or only traces of growth with iso-inositol and raffinose. Variable reports on growth with sucrose, D-mannitol, and rhamnose; interpretation is difficult because of significant growth on the carbon-free basal medium.

Type strain shows the highest sequence similarity to: *S. halstedii*, EF178695, 100%; *S. cinereorectus*, AB184646, 99.9%; *S. cavourensis* subsp. *washingtonensis*, DQ026671, 99.8%; *S. lavendulae* subsp. *lavendulae*, AB184080, 99.8%; *S. argenteolus*, AB045872, 99.8%; *S. mediolani*, AB184674, 99.7%; *S. pluricolorescens*, DQ442540, 99.7%; *S. alboviridis*, AB184256, 99.7%; *S. flavofuscus*, AB249935, 99.7%; *S. sindenensis*, AB184759, 99.7%; *S. lipmanii*, AB184148, 99.7%; *S. praecox*, AB184293, 99.7%; *S. microflavus*, DQ445795, 99.7%; *S. griseinus*, AB184205, 99.7%; *S. rubiginosohelvolus*, AB184240, 99.7%; *S. anulatus*, DQ026637, 99.7%; *S. fimicarius*, AY999784, 99.7%; *S. flavovirens*, DQ026635, 99.7%; *S. badius*, AY999783, 99.7%; *S. fulvorobeus*, AB184711, 99.7%; *S. flavogriseus*, AJ494864, 99.7%; *S. griseoplanus*, AY999894, 99.7%; *S. cyaneofuscatus*, AB184860, 99.7%; *S. baarnensis*, EF178688, 99.6%; *S. acrimycini*, AY999889, 99.6%; *S. globisporus* subsp. *globisporus*, EF178686, 99.6%; *S. floridae*, AB184656, 99.6%; *S. griseus* subsp. *griseus*, AY207604, 99.6%; *S. albovinaceus*, AB249958, 99.6%; *S. luridiscabiei*, AF361784, 99.6%; *S. parvus*, DQ442537, 99.5%; *S. californicus*, AB184755, 99.5%; *S. nitrosporeus*, EF178680, 99.5%; *S. pulveraceus*, AB184806, 99.4%; *S. yanii*, AB006159, 99.4%; *S. olivoviridis*, AB184227, 99.3%; *S. finlayi*, AY999788, 99.3%; *S. atroolivaceus*, AJ781320, 99.3%; *S. bacillaris*, AB184439, 99.2%; *S. sanglieri*, AB249945, 99.2%; *S. clavifer*, DQ026670, 99.2%; *S. spiroverticillatus*, AB184814, 99.1%; *S. albolongus*, AB184425, 99.1%; *S. gelaticus*, DQ026636, 99.1%; *S. atratus*, DQ026638, 99.1%; *S. celluloflavus*, AB184476, 99%; *S. cremeus*, AB184124, 99%; *S. mutomycini*, AB249951, 99%; *S. griseobrunneus*, AB249912, 99%.

Source: not known.

DNA G+C content (mol%): not known.

Type strain: ATCC 19764, ATCC 3325, CBS 502.68, BCRC 13677, DSM 40067, HAMBI 1000, HUT 6056, HUT 6099, NBRC 12777, NBRC 3415, NBRC 3719, IMET 42053, JCM 4042, JCM 4043, JCM 4581, KCTC 9028, NCIMB 9606, NRRL B-1062, NRRL B-2925, NRRL-ISP 5067, RIA 1044, RIA 88, UNIQEM 150, VKM Ac-1726.

Sequence accession no. (16S rRNA gene): AB184768.

228. **Streptomyces griseoluteus** Umezawa, Hayano, Maeda, Ogata and Okami 1950, 112[AL]

gri.se.o.lu′te.us. N.L. adj. *griseus* gray; L. adj. *luteus* yellow; N.L. masc. adj. *griseoluteus* grayish yellow.

Spore chains in Section *Rectiflexibiles*. Crooked or hooked spore chains may also be seen. Mature spore chains are generally short, sometimes with less than 10 spores per chain. This morphology is seen on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar. Spore surface is smooth. One observer reports

sclerotia-like bodies on the substrate mycelium of salts-starch agar and unusual morphology in the sporulating aerial mycelium. Another observer reports short chains of conidia-like spores on the substrate hyphae on yeast-malt agar and salts-starch agar.

Color of colony: aerial mass color in the Gray color series on yeast-malt agar, oatmeal agar, and salts-starch agar. Aerial mycelium may be poorly developed or absent on glycerol-asparagine agar. Reverse side of colony with no distinctive pigments (pale or grayish yellow to yellowish brown or olive brown) on yeast-malt agar, oatmeal agar, and glycerol-asparagine agar. Yellow to yellow brown may or may not be modified by red (orange) on salts-starch agar. Reverse mycelium pigment on salts-starch agar is a pH indicator, changing from red or reddish brown to gray with the addition of 0.05 M NaOH.

Color in medium: melanoid pigments are formed in peptone-yeast-iron agar, tyrosine agar, and tryptone-yeast broth. Red to bluish gray pigment is found in the medium in salts-starch agar. This pigment may show the same change observed for reverse mycelium pigment with the addition of 0.05 M NaOH.

D-Glucose, L-arabinose, D-xylose, D-mannitol, and D-fructose are utilized for growth. No growth or only traces of growth with iso-inositol, rhamnose, sucrose, and raffinose.

Type strain shows the highest sequence similarity to: *S. recifensis*, AB184165, 99.9%; *S. seoulensis*, AB249970, 99.7%.

Source: not known.

DNA G+C content (mol%): not known.

Type strain: AS 4.1440, ATCC 12768, CBS 676.72, DSM 40392, HUT 6058, IFM 1055, NBRC 13375, JCM 4041, JCM 4765, LMG 19356, NRRL B-1315, NRRL-ISP 5392, RIA 1336, VKM Ac-976.

Sequence accession no. (16S rRNA gene): AY999751.

229. **Streptomyces griseomycini** (Preobrazhenskaya, Blinov and Ryabova *in* Gauze, Preobrazhenskaya, Kudrina, Blinov, Ryabova and Sveshnikova 1957) Pridham, Hesseltine and Benedict 1958, 69[AL] ("*Actinomyces griseomycini*" Preobrazhenskaya, Blinov and Ryabova *in* Gauze, Preobrazhenskaya, Kudrina, Blinov, Ryabova and Sveshnikova 1957, 136)

gri.se.o.my.ci′ni. N.L. adj. *griseus* gray; N.L. suff. *-mycinum*, -mycin (antibiotics produced by *Streptomyces* strains); N.L. gen. adj. *griseomycini* of gray, antibiotic (referring to gray aerial mycelium and antibiotic activity).

Spore chains in Section *Spirales* or *Retinaculiaperti*. Sporophores are short and poorly developed but are frequently coiled at the tips; true spirals are rare. Hooks and loops are of small diameter and therefore are not representative of typical *Retinaculiaperti* cultures. Mature spore chains are short, generally 3–10 spores per chain. This morphology is found on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar, but sporulation may be poor on oatmeal agar and glycerol-asparagine agar. Spore surface is hairy. Some smooth spores may be observed.

Color of colony: aerial mass color in the Green color series on yeast-malt and salts-starch agar (one observer placed the culture in the Gray series on these two media). Reverse side of colony is grayed yellow modified by green on yeast-malt agar and salts-starch agar; it is colorless, grayed yellow, or grayed greenish yellow on oatmeal agar and glycerol-asparagine agar. Substrate pigment is not a pH indicator.

Color in medium: melanoid pigments formed in peptone-yeast-iron agar. Pigments other than melanoids not formed in yeast-malt agar, oatmeal agar, salts-starch agar, or glycerol-asparagine agar.

D-Glucose, L-arabinose, D-xylose, iso-inositol, D-mannitol, D-fructose, and rhamnose are utilized for growth. No growth or only traces of growth on sucrose and raffinose.

Type strain shows the highest sequence similarity to: *S. graminearus* AJ781333, 100%; *S. griseostramineus*, AB184140, 100%; *S. glaucus*, AB184665, 99.1%; *S. calvus*, AB184329, 99%.

Source: not known.

DNA G+C content (mol%): not known.

Type strain: ATCC 19765, ATCC 23625, CBS 503.68, BCRC 13763, DSM 40159, NBRC 12778, INA 13984, JCM 4382, NCIMB 9845, NRRL B-5421, NRRL-ISP 5159, RIA 1045, UNIQEM 151.

Sequence accession no. (16S rRNA gene): AB184137.

230. **Streptomyces griseoplanus** Backus, Tresner and Campbell 1957, 536[AL]

gri.se.o.pla′nus. N.L. adj. *griseus* gray; L. adj. *planus* flat, level; N.L. masc. adj. *griseoplanus* flat, gray (referring to the restricted, flat, plane growth and grayish spore color *en masse* of the organism).

Spore chains in Section *Spirales*. Spore chains range from flexuous to spiral. Spirals are generally open: Mature spore chains generally have 10–50 spores per chain. This morphology is seen on yeast-malt agar and oatmeal agar; poor growth on salts-starch agar and glycerol-asparagine agar. Spore surface is warty.

Color of colony: aerial mass color in the Gray color series on yeast-malt agar and oatmeal agar. Reverse side of colony with no distinctive pigments (grayed yellow to yellow-brown on yeast-malt agar and colorless or grayed yellow on oatmeal agar, salts-starch agar, and glycerol-asparagine agar). Substrate pigment is not a pH indicator.

Color in medium: melanoid pigments not formed in peptone-yeast-iron agar, tyrosine agar, or tryptone-yeast broth. No pigment found in medium in yeast-malt agar, oatmeal agar, salts-starch agar, or glycerol-asparagine agar.

D-Glucose, L-arabinose, D-xylose, raffinose, and D-fructose are utilized for growth. No growth or only traces of growth on sucrose, iso-inositol, D-mannitol, and rhamnose.

Type strain shows the highest sequence similarity to: *S. fimicarius*, AY999784, 100%; *S. sindenensis*, AB184759, 100%; *S. praecox*, AB184293, 100%; *S. cavourensis* subsp. *washingtonensis*, DQ026671, 100%; *S. rubiginosohelvolus*, AB184240, 100%; *S. lavendulae* subsp. *lavendulae*, AB184080, 100%; *S. anulatus*, DQ026637, 100%; *S. griseinus*, AB184205, 100%; *S. acrimycini*, AY999889, 100%; *S. badius*, AY999783, 100%; *S. pluricolorescens*, DQ442540, 100%; *S. mediolani*, AB184674, 100%; *S. flavofuscus*, AB249935, 100%; *S. albo-*

viridis, AB184256, 99.9%; *S. lipmanii*, AB184148, 99.9%; *S. cinereorectus*, AB184646, 99.9%; *S. baarnensis*, EF178688, 99.9%; *S. albovinaceus*, AB249958, 99.9%; *S. cyaneofuscatus*, AB184860, 99.9%; *S. microflavus*, DQ445795, 99.9%; *S. fulvorobeus*, AB184711, 99.9%; *S. globisporus* subsp. *globisporus*, EF178686, 99.9%; *S. argenteolus*, AB045872, 99.8%; *S. californicus*, AB184755, 99.8%; *S. parvus*, DQ442537, 99.8%; *S. flavovirens*, DQ026635, 99.7%; *S. griseolus*, AB184768, 99.7%; *S. floridae*, AB184656, 99.7%; *S. griseus* subsp. *griseus*, AY207604, 99.7%; *S. luridiscabiei*, AF361784, 99.7%; *S. flavogriseus*, AJ494864, 99.6%; *S. halstedii*, EF178695, 99.6%; *S. bacillaris*, AB184439, 99.5%; *S. pulveraceus*, AB184806, 99.5%; *S. olivoviridis*, AB184227, 99.5%; *S. atroolivaceus*, AJ781320, 99.5%; *S. finlayi*, AY999788, 99.5%; *S. nitrosporeus*, EF178680, 99.4%; *S. clavifer*, DQ026670, 99.4%; *S. yanii*, AB006159, 99.4%; *S. mutomycini*, AB249951, 99.4%; *S. celluloflavus*, AB184476, 99.3%; *S. griseobrunneus*, AB249912, 99.3%; *S. sanglieri*, AB249945, 99.3%; *S. albolongus*, AB184425, 99.3%; *S. gelaticus*, DQ026636, 99.2%; *S. atratus*, DQ026638, 99.2%; *S. spiroverticillatus*, AB184814, 99.1%; *S. candidus*, DQ026663, 99.1%; *S. cavourensis* subsp. *cavourensis*, DQ445791, 99.1%; *S. cremeus*, AB184124, 99%.

Source: not known.

DNA G+C content (mol%): not known.

Type strain: AS 4.1868, ATCC 19766, CBS 504.68, BCRC 13649, DSM 40009, NBRC 12779, JCM 4300, JCM 4582, NCIMB 9811, NRRL B-3064, NRRL-ISP 5009, RIA 1046, UNIQEM 152, VKM Ac-1727.

Sequence accession no. (16S rRNA gene): AY999894.

231. **Streptomyces griseorubens** (Preobrazhenskaya, Blinov and Ryabova *in* Gauze, Preobrazhenskaya, Kudrina, Blinov, Ryabova and Sveshnikova 1957) Pridham, Hesseltine and Benedict 1958, 65[AL] ("*Actinomyces griseorubens*" Preobrazhenskaya, Blinov and Ryabova *in* Gauze, Preobrazhenskaya, Kudrina, Blinov, Ryabova and Sveshnikova 1957, 144)

gri.se.o.ru′bens. N.L. adj. *griseus* gray; L. part. adj. *rubens* blushing, reddening; N.L. part. adj. *griseorubens* gray-reddening, referring to color of the aerial mycelium, vegetative mycelium and diffusible pigment.

Spore chains in Section *Spirales* or *Rectiflexibiles.* Flexuous sporophores are the dominant forms; hooks, loops, and some open spirals are present. On ISP media, this culture does not produce the long spore chains with wide diameter hooks and loops characteristic of typical *Retinaculiaperti* cultures. Mature spore chains are moderately short with 3–10, or sometimes more than 10, spores per chain. This morphology is observed on yeast-malt agar. Sporulation is poor on oatmeal agar, salts-starch agar, and glycerol-asparagine agar. Spore surface is spiny with very short spines or sometimes with smooth spores.

Color of colony: aerial mass color in the Gray color series on yeast-malt and salts-starch agar. Reverse side of colony with no distinctive pigments on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar; substrate pigment is not a pH indicator.

Color in medium: melanoid pigments not formed in peptone-yeast-iron agar, tyrosine agar, or tryptone-yeast broth. No pigment found in medium in yeast-malt agar, oatmeal agar, salts-starch agar, or glycerol-asparagine agar.

D-Glucose, D-xylose, D-mannitol, D-fructose, and rhamnose are utilized for growth. No growth or only traces of growth on sucrose and raffinose. Utilization of L-arabinose and iso-inositol is doubtful.

Type strain shows the highest sequence similarity to: *S. labedae*, AB184704, 99.9%; *S. variabilis*, DQ442551, 99.9%; *S. erythrogriseus*, AJ781328, 99.9%; *S. griseoincarnatus*, AJ781328, 99.9%; *S. griseoflavus*, AJ781322, 99.7%; *S. matensis*, AB184221, 99.7%; *S. althioticus*, AY999808, 99.4%; *S. heliomycini*, AB184712, 99.3%; *S. paradoxus*, AB184628, 99.3%; *S. collinus*, AB184123, 99.2%; *S. viridodiastaticus*, AY999852, 99.2%; *S. flaveolus*, AB184764, 99.2%; *S. viridochromogenes*, DQ442555, 99.2%; *S. bellus*, AB184849, 99.1%; *S. violaceochromogenes*, AY999867, 99.1%; *S. violaceorubidus*, AJ781374, 99.1%; *S. malachitofuscus*, AB184282, 99.1%; *S. tendae*, D63873, 99.1%; *S. albogriseolus*, AJ494865, 99.1%; *S. coerulescens*, AY999720, 99.1%; *S. ambofaciens*, M27245, 99%; *S. speibonae*, AF452714, 99%; *S. longispororuber*, AB184440, 99%; *S. iakyrus*, AB184877, 99%.

Source: not known.

DNA G+C content (mol%): not known.

Type strain: ATCC 19767, ATCC 19909, CBS 505.68, BCRC 12104, DSM 40160, NBRC 12780, INA 6124/54, JCM 4383, NCIMB 9846, NRRL B-3982, NRRL-ISP 5160, RIA 1047, UNIQEM 153, VKM Ac-1894.

Sequence accession no. (16S rRNA gene): AB184139.

232. **Streptomyces griseoruber** Yamaguchi and Saburi 1955, 220[AL]

gri.se.o.ru′ber. N.L. adj. *griseus* gray; L. adj. *ruber* red; N.L. masc. adj. *griseoruber* grayish red.

Spore chains in Section *Spirales* or *Retinaculiaperti.* Open or tight spirals are produced at the ends of moderately long spore chains of 10 to 50 or more spores per chain. Spirals are often incomplete, forming only 1 or 2 turns or a hook at the end of the spore chain. Straight to flexuous chains without hooks or spirals are also present. This morphology is usually best on glycerol-asparagine agar, but may also be observed on yeast-malt agar, oatmeal agar, and salts-starch agar. Spore surface is smooth.

Color of colony: aerial mass color in the Red or Gray color series on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar. The color is represented by tab 5fe (light grayish reddish brown) from the Gray color series and tab 5ge (also light grayish reddish brown) from the Red color series. Reverse side of colony is yellow to yellow-brown plus red (reddish orange or reddish brown on yeast-malt agar, oatmeal agar, and salts-starch agar; light yellowish pink on glycerol-asparagine agar). Substrate mycelium pigment is a pH indicator changing from reddish orange to purple with addition of 0.05 M NaOH and from reddish orange to yellowish orange with addition of 0.05 M HCl.

Color in medium: melanoid pigments are produced in peptone-yeast-iron agar, tyrosine agar, and tryptone-yeast broth. Yellow to red pigment (depending upon pH) may be found in the medium in yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar. This pigment is pH-sensitive, changing from orange to purple with addition of 0.05 M NaOH and from orange to yellow with

addition of 0.05 M HCl.

D-Glucose, L-arabinose, D-xylose, iso-inositol, D-fructose, and rhamnose are utilized for growth. No growth or only traces of growth on sucrose, D-mannitol, and raffinose.

Type strain shows the highest sequence similarity to: *S. antibioticus*, AY999776, 99.3%; *S. cinnabarinus*, AB184266, 99%; *S. cellostaticus*, AB184192, 99%.

Source: not known.

DNA G+C content (mol%): not known.

Type strain: AS 4.1417, ATCC 23919, CBS 903.68, BCRC 11826, CCUG 11117, DSM 40281, HAMBI 1051, NBRC 12873, JCM 4200, JCM 4642, LMG 19325, NRRL B-1818, NRRL-ISP 5281, RIA 1195, VKM Ac-1900.

Sequence accession no. (16S rRNA gene): AB184209.

233. **Streptomyces griseorubiginosus** (Ryabova and Preobrazhenskaya *in* Gauze, Preobrazhenskaya, Kudrina, Blinov, Ryabova and Sveshnikova 1957) Pridham, Hesseltine and Benedict 1958, 62[AL] ("*Actinomyces griseorubiginosus*" Ryabova and Preobrazhenskaya *in* Gauze, Preobrazhenskaya, Kudrina, Blinov, Ryabova and Sveshnikova 1957, 193)

gri.se.o.ru.bi.gin.o′sus. N.L. adj. *griseus* gray; N.L. adj. *robiginosus* (*sic*) rusty; N.L. masc. adj. *griseorubiginosus* gray, rusty (referring to the gray aerial mycelium and rosy reddish vegetative mycelium and diffusible pigment on a chemically defined medium).

Spore chains in Section *Rectiflexibiles*. The very long spore chains with more than 50 spores per chain may also form a few open loops and terminal hooks suggestive of *Retinaculiaperti* morphology. This morphology is seen on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar. Spore surface is smooth; two observers found only smooth spores, a third observer found both smooth and spiny spores. Long aerial hyphae may be entangled, forming knots and sclerotia-like bodies on yeast-malt agar, oatmeal agar, glycerol-asparagine agar, or Czapek's sucrose agar. One observer records *in situ* germination of spores in 7 d on Czapek's sucrose agar.

Color of colony: aerial mass color in the Gray color series (2dc, yellowish gray to 3fe, light brownish gray or 5fe, light grayish reddish brown) on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar. Reverse side of colony is brown, grayish reddish brown or dark brown on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar. Reverse mycelium pigment is a pH indicator changing from yellowish brown to gray with addition of 0.05 M HCl.

Color in medium: melanoid pigments are formed in peptone-yeast-iron agar, tyrosine agar, or tryptone-yeast broth. Red or reddish brown pigment is found in the medium in yeast-malt agar, oatmeal agar, and glycerol-asparagine agar and yellow or yellowish brown pigment is found in the medium in salts-starch agar. This pigment is pH-sensitive, showing the same changes noted for reverse mycelium pigment.

D-Glucose, L-arabinose, D-xylose, iso-inositol, D-mannitol, D-fructose, rhamnose, sucrose, and raffinose are all utilized for growth.

Type strain shows the highest sequence similarity to: *S. phaeopurpureus*, DQ026666, 100%.

Source: not known.

DNA G+C content (mol%): not known.

Type strain: ATCC 23627, ATCC 25459, CBS 692.69, BCRC 12124, DSM 40469, NBRC 13047, INA 7712, JCM 4481, NRRL B-12384, NRRL-ISP 5469, RIA 1239, VKM Ac-1203.

Sequence accession no. (16S rRNA gene): AJ781339.

234. **Streptomyces griseosporeus** Niida and Ogasawara 1960, 23[AL]

gri.se.o.spo′re.us. N.L. adj. *griseus* gray; N.L. n. *spora* a spore; N.L. masc. adj. *griseosporeus* gray spored.

Spore chains in Section *Retinaculiaperti*. Spore chains may be flexuous or in imperfect spirals, hooks and loops. Flexuous chains are common. Mature spore chains are moderately long with 10–50 or more spores per chain. This morphology is seen on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar. Spore surface is smooth.

Color of colony: aerial mass color in the Gray color series (2fe or e, moderate gray; 3fe, light brownish gray; or 5fe, light grayish reddish brown) on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar. Reverse side of colony with no distinctive pigments (pale or grayish yellow or sometimes light yellowish brown on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar).

Color in medium: melanoid pigments are formed in peptone-yeast-iron agar, tyrosine agar, and tryptone-yeast broth. No pigment is found in the medium in yeast-malt agar, oatmeal agar, salts-starch agar, or glycerol-asparagine agar.

D-Glucose, L-arabinose, D-xylose, iso-inositol, D-mannitol, D-fructose, rhamnose, sucrose, and raffinose are all utilized for growth.

Type strain shows no sequence similarity over 99%.

Source: not known.

DNA G+C content (mol%): not known.

Type strain: ATCC 27435, CBS 137.72, CBS 759.72, DSM 40562, HAMBI 1009, NBRC 13458, IMET 43543, JCM 4766, NRRL B-12498, NRRL-ISP 5562, RIA 1419, VKM Ac-1731.

Sequence accession no. (16S rRNA gene): AB184419.

235. **Streptomyces griseostramineus** (Preobrazhenskaya, Kudrina, Blinov and Ryabova *in* Gauze, Preobrazhenskaya, Kudrina, Blinov, Ryabova and Sveshnikova 1957) Pridham, Hesseltine and Benedict 1958, 65[AL] ("*Actinomyces griseostramineus*" Preobrazhenskaya, Kudrina, Blinov and Ryabova *in* Gauze, Preobrazhenskaya, Kudrina, Blinov, Ryabova and Sveshnikova 1957, 155)

gri.se.o.stra.mi′ne.us. N.L. adj. *griseus* gray; L. adj. *stramineus* of straw, here straw-colored; N.L. masc. adj. *griseostramineus* gray, straw-colored (referring to the gray aerial mycelium and straw-yellow vegetative mycelium on a chemically defined medium).

Spore chains in Section *Spirales*. Short spore chains may form tight spirals of only a few turns on yeast-malt agar and salts-starch agar. Hooks and loops suggestive of Section *Retinaculiaperti* may also be found on these media and

on oatmeal agar. Mature spore chains are short, generally 3–10 spores per chain. This morphology is seen on yeast-malt agar and salts-starch agar; sporulation may be poor on oatmeal agar and glycerol-asparagine agar. Spore surface is hairy to spiny: appendages are shorter than characteristic hairs, but longer and more flexuous than typical spines.

Color of colony: aerial mass color in the Green color series on yeast-malt agar and salts-starch agar. Reverse side of colony with no distinctive pigment (grayed yellow or grayed yellow modified by green) on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar; substrate pigment is not a pH indicator.

Color in medium: melanoid pigments formed in peptone-yeast-iron agar. Pigments other than melanoids not formed in yeast-malt agar, oatmeal agar, salts-starch agar, or glycerol-asparagine agar.

D-Glucose, L-arabinose, D-xylose, iso-inositol, D-mannitol, D-fructose, and rhamnose are utilized for growth. No growth or only trace of growth on sucrose. Variable reports on growth with raffinose.

Type strain shows the highest sequence similarity to: *S. griseomycini*, AB184137, 100%; *S. graminearus* AJ781333, 100%; *S. calvus*, AB184329, 99%; *S. glaucus*, AB184665, 99%.

Source: not known.

DNA G+C content (mol%): not known.

Type strain: ATCC 19768, ATCC 23628, CBS 506.68, BCRC 12075, CECT 3273, DSM 40161, NBRC 12781, INA 10381, JCM 4385, NRRL B-5422, NRRL-ISP 5161, RIA 1048, UNIQEM 154, VKM Ac-968.

Sequence accession no. (16S rRNA gene): AB184140.

236. **Streptomyces griseoverticillatus** (Shinobu and Shimada 1962) Witt and Stackebrandt 1991, 456[VP] (Effective publication: Witt and Stackebrandt 1990, 370.) ("*Streptomyces griseoverticillatus*" Shinobu and Shimada 1962, 174; "*Verticillomyces griseoverticillatus*" Shinobu 1965; *Streptoverticillium griseoverticillatum* Locci, Baldacci and Petrolini Baldan 1969, 59)

gri.se.o.ver.ti.cil.la′tus. N.L. adj. *griseus* gray; L. masc. n. *verticillus* whorl; N.L. adj. *verticillatus* whorled; N.L. masc. adj. *griseoverticillatus* gray and whorled.

Spore chains in Section *Verticillati*, umbellate monoverticillate (biverticillate). Mature spore chains are short with 3 to 10 or more spores per chain. This morphology is seen on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar. Spore surface is smooth.

Color of colony: aerial mass color in the Red color series (usually 4ec, grayish yellowish pink; sometimes 4ge, light grayish reddish brown; 4ie, light brown; or 3ca, pale orange-yellow) on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar. Reverse side of colony is yellowish brown or orange-yellow on yeast-malt agar; grayish yellowish pink to light olive brown on oatmeal agar; yellowish brown to strong brown on salts-starch agar; pale or grayish yellow on glycerol-asparagine agar. Reverse mycelium pigment is not a pH indicator.

Color in medium: melanoid pigments are not formed in peptone-yeast-iron agar, tyrosine agar, or tryptone-yeast broth. No pigment is found in the medium in yeast-malt agar, oatmeal agar, salts-starch agar, or glycerol-asparagine agar.

D-Glucose and iso-inositol are utilized for growth. Utilization of D-fructose is doubtful. No growth or only traces of growth with L-arabinose, D-xylose, rhamnose, sucrose, raffinose, and D-mannitol.

For sequence similarity, see type strain of *Streptomyces cinnamoneus*.

Source: not known.

DNA G+C content (mol%): not known.

Type strain: ATCC 27436, CBS 721.72, BCRC 12430, DSM 40507, NBRC 13420, JCM 4202, JCM 4767, NRRL B-12432, NRRL-ISP 5507, PCM 2351, RIA 1381, VKM Ac-883.

Sequence accession no. (16S rRNA gene): AB184862.

Further comments: in violation of Rule 33c of the *Bacteriological Code* (1990 Revision), in Validation List no. 38, *Streptomyces griseoverticillatus* is proposed as a *nomen revictum* (basonym: "*Streptomyces griseoverticillatus*" Shinobu and Shimada (1962).

According to Hatano et al. (2003), *Streptomyces griseoverticillatus* (Shinobu and Shimada 1962) Witt and Stackebrandt 1991 is a later heterotypic synonym of *Streptomyces cinnamoneus* (Benedict et al. 1952) Witt and Stackebrandt 1991.

237. **Streptomyces griseoviridis** Anderson, Ehrlich, Sun and Burkholder 1956, 114[AL]

gri.se.o.vi′ri.dis. N.L. adj. *griseus* gray; L. adj. *viridis* green; N.L. masc. adj. *griseoviridis* gray-green.

Spore chains in Section *Spirales*. Some open spirals have only one or two turns or are poorly developed suggesting *Retinaculiaperti* morphology. Spore chains are moderately long with 10–50 spores per chain. Spore surface is smooth.

Color of colony: aerial mass color in the Red color series (grayish yellowish pink to pale orange yellow) on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar. One observer placed this culture in the Yellow color series. The original description (Anderson et al., 1956) included the following statement: "Aerial mycelium is white to pink-tan, ping-gray, or brown or occasionally greenish". The greenish color was not observed on ISP media. Reverse side of colony is grayed yellow to olive brown or dark brown on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar. Substrate mycelium pigment is not a pH indicator or is changed only slightly by 0.05 M NaOH or HCl.

Color in medium: melanoid pigments are not found in peptone-yeast-iron agar, tyrosine agar, or tryptone-yeast broth. No pigment in medium, or only trace of yellow, in yeast-malt agar, oatmeal agar, salts-starch agar, or glycerol-asparagine agar.

D-Glucose, L-arabinose, D-xylose, D-mannitol, and rhamnose are utilized for growth. Utilization of D-fructose is doubtful. No growth or only traces of growth on sucrose, iso-inositol, and raffinose.

Type strain shows the highest sequence similarity to: *S. aurantiogriseus*, AY999793, 99.4%; *S. coelicolor*, DQ442496, 99.3%.

Source: not known.

DNA G+C content (mol%): not known.

Type strain: AS 4.1418, ATCC 23920, CBS 904.68, DSM 40229, HAMBI 1086, NBRC 12874, JCM 4250, JCM 4643, KCTC 9780, LMG 19321, NCIMB 9853, NRRL 2427, NRRL-ISP 5229, RIA 1170, VKM Ac-622.

Sequence accession no. (16S rRNA gene): AY999807.

238a. **Streptomyces griseus subsp. griseus** (Krainsky 1914) Waksman and Henrici *in* Breed, Murray and Hitchens 1948, 948[AL] ("*Actinomyces griseus*" Krainsky 1914, 662)

gri′se.us. N.L. masc. adj. *griseus* gray.

Spore chains in Section *Rectiflexibiles*. Mature spore chains are moderately long with 10–50 spores per chain. This morphology is seen on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar. Spore surface is smooth.

Color of colony: aerial mass color in the Yellow color series (2db, pale yellow, or 1½db–1½ec, pale greenish yellow) on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar. Reverse side of colony with no distinctive pigments (grayed yellow to olive brown or light yellowish brown) on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar.

Color in medium: melanoid pigments are formed in tyrosine agar, but not in peptone-yeast-iron agar or tryptone-yeast broth. No pigments other than traces of yellow are found in the medium in yeast-malt agar, oatmeal agar, salts-starch agar, or glycerol-asparagine agar.

D-Glucose, D-xylose, D-mannitol, and D-fructose are utilized for growth. No growth or only traces of growth on L-arabinose, sucrose, iso-inositol, rhamnose, or raffinose.

Type strain shows the highest sequence similarity to: *S. cavourensis* subsp. *washingtonensis*, DQ026671, 99.8%; *S. lavendulae* subsp. *lavendulae*, AB184080, 99.8%; *S. cinereorectus*, AB184646, 99.7%; *S. alboviridis*, AB184256, 99.7%; *S. sindenensis*, AB184759, 99.7%; *S. mediolani*, AB184674, 99.7%; *S. fimicarius*, AY999784, 99.7%; *S. lipmanii*, AB184148, 99.7%; *S. fulvorobeus*, AB184711, 99.7%; *S. anulatus*, DQ026637, 99.7%; *S. praecox*, AB184293, 99.7%; *S. flavofuscus*, AB249935, 99.7%; *S. cyaneofuscatus*, AB184860, 99.7%; *S. pluricolorescens*, DQ442540, 99.7%; *S. griseinus*, AB184205, 99.7%; *S. microflavus*, DQ445795, 99.7%; *S. griseoplanus*, AY999894, 99.7%; *S. rubiginosohelvolus*, AB184240, 99.7%; *S. argenteolus*, AB045872, 99.7%; *S. badius*, AY999783, 99.7%; *S. griseolus*, AB184768, 99.6%; *S. luridiscabiei*, AF361784, 99.6%; *S. floridae*, AB184656, 99.6%; *S. albovinaceus*, AB249958, 99.6%; *S. acrimycini*, AY999889, 99.6%; *S. baarnensis*, EF178688, 99.6%; *S. flavovirens*, DQ026635, 99.5%; *S. halstedii*, EF178695, 99.5%; *S. californicus*, AB184755, 99.5%; *S. parvus*, DQ442537, 99.5%; *S. flavogriseus*, AJ494864, 99.4%; *S. pulveraceus*, AB184806, 99.4%; *S. yanii*, AB006159, 99.4%; *S. finlayi*, AY999788, 99.3%; *S. globisporus* subsp. *globisporus*, EF178686, 99.3%; *S. bacillaris*, AB184439, 99.2%; *S. nitrosporeus*, EF178680, 99.2%; *S. olivoviridis*, AB184227, 99.2%; *S. atroolivaceus*, AJ781320, 99.2%; *S. clavifer*, DQ026670, 99.2%; *S. gelaticus*, DQ026636, 99.1%; *S. atratus*, DQ026638, 99.1%; *S. mutomycini*, AB249951, 99%; *S. sanglieri*, AB249945, 99%; *S. albolongus*, AB184425, 99%; *S. celluloflavus*, AB184476, 99%; *S. griseobrunneus*, AB249912, 99%.

Source: not known.

DNA G+C content (mol%): not known.

Type strain: AS 4.1419, ATCC 23345, ATCC 23921, CBS 905.68, BCRC 13478, CCT 4836, CCUG 11104, CECT 3330, CFBP 4546, CIP 105124, DSM 40236, HAMBI 2315, NBRC 12875, NBRC 15744, JCM 4047, JCM 4644, KACC 20084, KCTC 9080, KCTC 9135, LMG 19302, NCIMB 13023, NCTC 13033, NRRL B-2682, NRRL-ISP 5236, PCM 2331, RIA 1176, VKM Ac-800.

Sequence accession no. (16S rRNA gene): AY207604.

Further comments: according to Liu et al. (2005b), *Streptomyces griseus* (Krainsky 1914) Waksman and Henrici 1948 emend. Liu et al. 2005b is an earlier heterotypic synonym of *Streptomyces argenteolus* Tresner et al. 1961, an earlier heterotypic synonym of *Streptomyces caviscabies* Goyer et al. 1996, and an earlier heterotypic synonym of *Streptomyces setonii* (Millard and Burr 1926) Waksman 1953.

According to Guo et al. (2008), *Streptomyces griseus* (Krainsky 1914) Waksman and Henrici 1948 is an earlier heterotypic synonym of *Streptomyces erumpens* Calot and Cercós 1963.

According to Guo et al. (2008), *Streptomyces argenteolus* Tresner et al. 1961 is not a later heterotypic synonym of *Streptomyces griseus* (Krainsky 1914) Waksman and Henrici 1948.

According to Guo et al. (2008), *Streptomyces caviscabies* Goyer et al. 1996 is not a later heterotypic synonym of *Streptomyces griseus* (Krainsky 1914) Waksman and Henrici 1948.

238b. **Streptomyces griseus subsp. alpha** (Ciferri 1927) Pridham 1970, 37[AL] ["*Actinomyces albus* subsp. alpha" (*sic*) Ciferri 1927, 83]

al′pha. L. n. *alpha*, alpha, first letter of the Greek alphabet.

Spore chains are typically flexuous; grows poorly on Czapek's solution agar. Exhibits slight anti-bacterial activity; inhibited by streptomycin. Reported as a cause of musty odor in cacao beans (*Theobroma cacao* L.).

For sequence similarity, see type strain of *Streptomyces microflavus.*

Source: not known.

DNA G+C content (mol%): not known.

Type strain: CBS 219.25, DSM 40937, NBRC 15421, JCM 5078, LMG 19953, NRRL B-2249.

Sequence accession no. (16S rRNA gene): AB184668.

Further comments: according to Lanoot et al. (2005b), *Streptomyces griseus* subsp. *alpha* (Ciferri 1927) Pridham 1970 is a later heterotypic synonym of *Streptomyces microflavus* (Krainsky 1914) Waksman and Henrici 1948 emend. Lanoot et al. 2005b.

238c. **Streptomyces griseus subsp. cretosus** Pridham 1970, 37[AL] ("*Oospora cretacea*" Krüger 1905, 286; "*Actinomyces cretaceus*" Krasil'nikov 1941, 34; "*Streptomyces cretaceus*" Waksman 1950, 143)

cre.tos′us. L. masc. adj. *cretosus* chalky.

Exhibits slight anti-microbial activity; inhibited by streptomycin; moderate growth observed on Czapek's solution agar.

For sequence similarity, see type strain of *Streptomyces microflavus*.

Source: not known.

DNA G+C content (mol%): not known.

Type strain: ATCC 27903, CBS 137.21, CBS 758.72, DSM 40561, NBRC 13457, JCM 4742, KCTC 9079, LMG 19946, NRRL B-2252, NRRL-ISP 5561, RIA 1418, VKM Ac-712.

Sequence accession no. (16S rRNA gene): AB184418.

Further comments: according to Lanoot et al. (2005b), *Streptomyces griseus* subsp. *cretosus* Pridham 1970 is a later heterotypic synonym of *Streptomyces microflavus* (Krainsky 1914) Waksman and Henrici 1948 emend. Lanoot et al. 2005b.

238d. **Streptomyces griseus subsp. solvifaciens** Pridham 1970, 38[AL]

sol.vi.fa′ci.ens. L. v. *solvo* to loosen; L. v. *facio* to make; N.L. part. adj. *solvifaciens* making loose, dissolving, referring to the lytic activity of actinomycetin.

Spore chains are typically flexuous; poor growth is seen on Czapek's solution agar. Exhibits anti-bacterial and anti-fungal activity; produces actinomycetin, now considered a general term for a number of different lytic enzymic anti-bacterial and anti-viral factors; inhibited by streptomycin.

Type strain shows the highest sequence similarity to: *S. limosus*, AB184147, 100%; *S. felleus*, AB184129, 100%; *S. daghestanicus*, DQ442497, 100%; *S. albidoflavus*, AB184255, 100%; *S. hydrogenans*, AB184868, 100%; *S. odorifer*, Z76682, 100%; *S. violascens*, AY999737, 100%; *S. champavatii*, DQ026642, 99.9%; *S. canescens*, AB184117, 99.9%; *S. sampsonii*, D63871, 99.7%; *S. koyangensis*, AY079156, 99.6%.

Source: not known.

DNA G+C content (mol%): not known.

Type strain: JCM 5079, DSM 40933, NBRC 13689, NRRL B-1561.

Sequence accession no. (16S rRNA gene): AB249915.

Further comments: according to Guo et al. (2008), *Streptomyces griseus* subsp. *solvifaciens* Pridham 1970 should be removed from *Streptomyces griseus*. However, no formal proposition is made in the paper by Guo et al. (2008).

239. **Streptomyces guanduensis** Xu, Wang, Cui, Huang, Liu, Zheng and Goodfellow 2006, 1114[VP]

gu.an.du.en′sis. N.L. masc. adj. *guanduensis* of or belonging to Guandu, the source of the soil from which the type strain was isolated.

Acidophilic streptomycete; forms branched substrate and aerial hyphae. Smooth-surfaced spores are borne in flexuous spore chains. Deep-brown colonies that carry a white to gray aerial spore mass are formed on oatmeal agar, ISP medium 9 supplemented with glucose (1%, w/v), and on yeast extract-malt extract agar. Diffusible pigments are not formed and melanin pigments are not produced on peptone-yeast extract-iron agar or tyrosine agar. Degrades Tween 80, but not adenine, guanine, starch, or xanthine. Cellobiose, D-galactose, D-glucose, D-inulin, D-lactose, D-mannitol, and D-salicin (each at 1%, w/v), and adipic acid and L-phenylalanine (each at 0.1%, w/v) are used as sole carbon sources for energy and growth, but adonitol and D-sorbitol (each at 1%, w/v), and L-alanine, DL-aminobutyric acid, L-arginine, α-L-aspartic acid, L-cysteine, L-valine, sodium acetate, sodium citrate, and sodium oxalate (each at 0.1%, w/v) are not. Growth occurs at temperatures between 20 and 37°C, but not at 15°C, and at pH values from 4.5–7.0, but not at pH 3.5. Does not grow in the presence of 5% (w/v) NaCl. The organism is sensitive to filter-paper discs soaked in the following (μg/ml unless indicated): acetylspiramycin (15), carbenicillin (10), cephalothin (30), ciprofloxacin (5), doxycycline hydrochloride (30), erythromycin (15), josamycin (15), kanamycin sulfate (30), minocyclin hydrochloride (30), and tobramycin sulfate (10), but not to amoxycillin (10), ampicillin (10), azitrhomycin (30), aztreonam (30), penicillin G (10 IU/ml), or sulfamethoxazole (25). Additional properties are shown in Table 264. Chemotaxonomic properties are typical of members of the genus *Streptomyces*.

Type strain shows no sequence similarity over 99%. Type strain shows DNA–DNA similarity to: *S. yeochonensis* NRRL B-24245^T, 18.7%; *S. paucisporeus* JCM 13276^T, 14.9%; *S. rubidus* JCM 13277^T, 21.4%; *S. yanglinensis* JCM 13275^T, 23.1%.

Source: the type strain was isolated from soil from Guandu.

DNA G+C content (mol%): 72.7.

Type strain: 701, CGMCC 4.2022, JCM 13274.

Sequence accession no. (16S rRNA gene): AY876942.

240. **Streptomyces hachijoensis** (Hosoya, Komatsu, Soeda and Sonoda 1952) Witt and Stackebrandt 1991, 456[VP] (Effective publication: Witt and Stackebrandt 1990, 370.) ("*Streptomyces hachijoensis*" Hosoya, Komatsu, Soeda and Sonoda 1952, 508; *Streptoverticillium hachijoense* Locci, Baldacci and Petrolini Baldan 1969, 59)

ha.chi.jo.en′sis. N.L. masc. adj. *hachijoensis* or or belonging to Hachijo (named for the place of origin, Hachijo Jima, a small island in the Pacific Ocean).

Spore chains in Section *Verticillati*. Both monoverticillate and umbellate monoverticillate (biverticillate) spore chains are found. Mature spore chains are short, generally 3–10 spores per chain. This morphology is seen on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar. Spore surface is smooth.

Color of colony: aerial mass color in the Red color series on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar. Reverse side of colony with no distinctive pigment (grayed yellow to yellow-brown) on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar; substrate pigment is not a pH indicator.

Color in medium: melanoid pigments not formed in peptone-yeast-iron agar and tyrosine agar. No pigment in medium, or only trace of yellow, in yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar.

D-Glucose and iso-inositol are utilized for growth. No growth or only traces of growth on L-arabinose, sucrose, D-xylose, D-mannitol, D-fructose, rhamnose, and raffinose.

For sequence similarity, see type strain of *Streptomyces cinnamoneus*.

Source: isolated from soil from Hachijo Jima, a small island in the Pacific Ocean.

DNA G+C content (mol%): not known.

Type strain: ATCC 19769, CBS 507.68, BCRC 12419, CECT 3260, DSM 2011, DSM 40114, NBRC 12782, JCM 4331, JCM 4583, NRRL B-3106, NRRL-ISP 5114, RIA 1049, UNIQEM 155, VKM Ac-191.

Sequence accession no. (16S rRNA gene): AB184141.

Further comments: in violation of Rule 33c of the *Bacteriological Code* (1990 Revision), in Validation List no. 38, *Streptomyces hachijoensis* is proposed as a *nomen revictum* (basonym: "*Streptomyces hachijoensis*" Hosoya et al. (1952).

According to Hatano et al. (2003), *Streptomyces hachijoensis* (Hosoya et al. 1952) Witt and Stackebrandt 1991 is a later heterotypic synonym of *Streptomyces cinnamoneus* (Benedict et al. 1952) Witt and Stackebrandt 1991.

241. **Streptomyces hainanensis** Jiang, Tang, Wiese, Xu, Imhoff and Jiang 2007, 2697[VP]

hai.nan.en′sis. N.L. masc. adj. *hainanensis* of or pertaining to Hainan, a province of south China, from where the type strain was isolated.

Spore chains are spiral or looped. Spores are elliptical or short rod-shaped. Spore surface is smooth. Aerial mycelia are white and pink white to pink gray. Vegetative mycelia are pale to deep orange yellow. Produces light brown to orange yellowish soluble pigments. Gelatin liquefaction, milk coagulation and peptonization, arginase, phenylalanine deaminase, DNase, and melanin production are negative. Starch hydrolysis, arginine decarboxylase, nitrate reduction, gas production from nitrate, growth on cellulose, and H_2S production are positive. Glucose, cellobiose, starch, esculin, galactoside, and urea are utilized; acids are not produced from the five carbon sources. Galactose, mannose, fructose, arabinose, xylose, ribose, rhamnose, sucrose, lactose, maltose, melibiose, raffinose, turanose, melezitose, sorbin, dextrin, mycose, salicin, adonitol, inositol, mannitol, sorbitol, xylitol, galactitol, erythritol, amygdaloside, sodium citrate, sodium acetate, gluconate, malonate, tartrate, lysine, ornithine, and acetamide are not utilized. Optimal growth occurs at pH 7.0 (range pH 6.0–9.0) and without NaCl (range 0–10% NaCl). Resistant to penicillin G (10 IU), amoxycillin/clavulanic acid (20/10 µg), novobiocin (30 µg), rifampin (5 µg), and ampicillin (10 µg), and sensitive to erythromycin (15 µg IU), gentamicin (10 µg), kanamycin (30 µg), tetracycline (30 µg), vancomycin (30 µg), midecamycin (15 µg), clindamycin (2 µg), sulfamethoxazole/trimethoprim (23.75/1.25 µg), chloramphenicol (30 µg), polymyxin B (300 IU), and norfloxacin (10 µg). Cell wall contains LL-A_2pm, trace *meso*-A_2pm, and glycine. Whole-cell hydrolysates contain galactose and xylose. Main phospholipids are phosphatidylethanolamine and diphosphatidylglycerol (phospholipid type II). The predominant menaquinones are MK-9(H_4) (45.4%), MK-9(H_6) (14.0%), MK-9(H_8) (13.6%), and MK-10(H_0) (27.0%). Fatty acid composition comprises $C_{15:0}$ iso (1.1%), $C_{16:0}$ iso (30.9%), $C_{16:1}\omega7c$/$C_{16:1}\omega6c$ (2.1%), $C_{16:0}$ (13.7%), $C_{17:1}$ iso$\omega9c$ (1.4%), $C_{17:0}$ iso (2.8%), $C_{17:0}$ anteiso (10.8%), $C_{17:1}\omega8c$ (7.1%), cyclo-$C_{17:0}$ (1.4%), $C_{17:0}$ (5.6%), $C_{18:0}$ anteiso/$C_{18:2}\omega6,9c$ (9.7%), $C_{18:1}\omega9c$ (4.9%), $C_{18:0}$ (1.2%), and $C_{17:1}$ iso$\omega9c$/$C_{16:0}$ 10-methyl (1.4%).

Type strain shows no sequence similarity over 99%.

Source: the type strain was isolated from a soil sample collected from evergreen broadleaf forest in Wuzhi Mountain, Hainan Province, China.

DNA G+C content (mol%): 73.4.

Type strain: YIM 47672, CCTCC AA 205017, DSM 41900.

Sequence accession no. (16S rRNA gene): AM398645.

242. **Streptomyces halstedii** (Waksman and Curtis 1916) Waksman and Henrici *in* Breed, Murray and Hitchens 1948, 953[AL] ("*Actinomyces halstedii*" Waksman and Curtis 1916, 124)

hal.ste′di.i. N.L. gen. masc. n. *halstedii* of Halsted, named for Byron David Halsted (1852–1918) of Rutgers University.

Spore chains Section *Rectiflexibiles*. Spore chains are predominantly flexuous, but many hooks and some irregular coils similar to *Rectiflexibiles* morphology are found on yeast-malt agar and glycerol-asparagine agar. Spore chains are short, 3–10 spores per chain, on yeast-malt agar, salts-starch agar, and glycerol-asparagine agar. Sporulation may be poor, especially on oatmeal agar. Since the original characterization by Waksman (1916) describes closed spirals 7–10 µm in diameter, the short flexuous or hooked chains may be atypical. Spore surface is smooth. Fragmentation of substrate mycelium is noted by one observer.

Color of colony: aerial mass color in the Gray color series on yeast-malt agar, salts-starch agar, and glycerol-asparagine agar. Reverse side of colony has no distinctive pigment (grayed yellow to yellow-brown) on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar; substrate pigment is not a pH indicator.

Color in medium: melanoid pigments not formed in peptone-yeast-iron agar, tyrosine agar, or tryptone-yeast broth. No pigment found in medium in yeast-malt agar, oatmeal agar, salts-starch agar, or glycerol-asparagine agar.

D-Glucose, L-arabinose, D-xylose, and D-fructose are utilized for growth. No growth or only traces of growth on sucrose, iso-inositol, D-mannitol, rhamnose, and raffinose.

Type strain shows the highest sequence similarity to: *S. griseolus*, AB184768, 100%; *S. cinereorectus*, AB184646, 99.8%; *S. lavendulae* subsp. *lavendulae*, AB184080, 99.7%; *S. argenteolus*, AB045872, 99.7%; *S. cyaneofuscatus*, AB184860, 99.7%; *S. cavourensis* subsp. *washingtonensis*, DQ026671, 99.7%; *S. praecox*, AB184293, 99.7%; *S. anulatus*, DQ026637, 99.7%; *S. flavovirens*, DQ026635, 99.7%; *S. fimicarius*, AY999784, 99.7%; *S. lipmanii*, AB184148, 99.7%; *S. flavofuscus*, AB249935, 99.7%; *S. microflavus*, DQ445795, 99.7%; *S. alboviridis*, AB184256, 99.7%; *S. fulvorobeus*, AB184711, 99.7%; *S. mediolani*, AB184674, 99.6%; *S. badius*, AY999783, 99.6%; *S. acrimycini*, AY999889, 99.6%;

S. griseoplanus, AY999894, 99.6%; *S. sindenensis*, AB184759, 99.6%; *S. pluricolorescens*, DQ442540, 99.6%; *S. rubiginosohelvolus*, AB184240, 99.6%; *S. griseinus*, AB184205, 99.6%; *S. flavogriseus*, AJ494864, 99.6%; *S. baarnensis*, EF178688, 99.5%; *S. luridiscabiei*, AF361784, 99.5%; *S. albovinaceus*, AB249958, 99.5%; *S. parvus*, DQ442537, 99.5%; *S. floridae*, AB184656, 99.5%; *S. californicus*, AB184755, 99.5%; *S. griseus* subsp. *griseus*, AY207604, 99.5%; *S. globisporus* subsp. *globisporus*, EF178686, 99.5%; *S. nitrosporeus*, EF178680, 99.4%; *S. yanii*, AB006159, 99.3%; *S. pulveraceus*, AB184806, 99.3%; *S. finlayi*, AY999788, 99.2%; *S. atroolivaceus*, AJ781320, 99.2%; *S. bacillaris*, AB184439, 99.2%; *S. olivoviridis*, AB184227, 99.2%; *S. gelaticus*, DQ026636, 99.1%; *S. sanglieri*, AB249945, 99.1%; *S. clavifer*, DQ026670, 99.1%; *S. atratus*, DQ026638, 99.1%; *S. albolongus*, AB184425, 99%; *S. spiroverticillatus*, AB184814, 99%; *S. cremeus*, AB184124, 99%; *S. celluloflavus*, AB184476, 99%; *S. griseobrunneus*, AB249912, 99%.

Source: not known.

DNA G+C content (mol%): not known.

Type strain: ATCC 10897, ATCC 19770, CBS 508.68, BCRC 13680, CECT 3328, DSM 40068, HAMBI 993, NBRC 12783, IMET 40322, JCM 4584, NCIMB 9839, NRRL B-1238, NRRL-ISP 5068, RIA 1050, UNIQEM 156, VKM Ac-1768.

Sequence accession no. (16S rRNA gene): EF178695.

243. **Streptomyces hawaiiensis** Cron, Whitehead, Hooper, Heinemann and Lein 1956, 63[AL]

ha.wai.i.en′sis. N.L. masc. adj. *hawaiiensis* of or belonging to Hawaii, the source of the soil from which the organism was isolated.

Spore chains in Section *Spirales*, but on some media short or poorly developed spore chains are flexuous or have hooks, loops or open spirals resembling *Retinaculiaperti* morphology. Mature spore chains are moderately short with 3–10, or sometimes more than 10 spores per chain. This morphology is seen on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar. Spore surface is spiny.

Color of colony: aerial mass color in the White color series on salts-starch agar and glycerol-asparagine agar; Yellow or White series on yeast-malt agar and oatmeal agar. Reverse side of colony with no distinctive pigment (grayed yellow to yellow-brown) on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar; substrate pigment is not a pH indicator.

Color in medium: melanoid pigments formed in peptone-yeast-iron agar and tryptone-yeast broth; pigments other than melanoids not formed in yeast-malt agar, oatmeal agar, salts-starch agar, or glycerol-asparagine agar.

D-Glucose, L-arabinose, sucrose, iso-inositol, D-mannitol, D-fructose, rhamnose, and raffinose are utilized for growth; utilization of D-xylose is doubtful.

Type strain shows the highest sequence similarity to: *S. massasporeus*, AB184152, 99.7%; *S. arenae*, AB249977, 99.7%; *S. purpurascens*, AJ399486, 99.5%; *S. luteogriseus*, AB184379, 99.4%; *S. indiaensis*, AB184553, 99.3%; *S. janthinus*, AB184851, 99.3%; *S. violaceus*, AB184315, 99.3%; *S. albosporeus* subsp. *albosporeus*, AJ781327, 99.3%; *S. coerulescens*, AY999720, 99.2%; *S. bellus*, AB184849, 99.2%; *S. roseoviolaceus*, AJ399484, 99.2%; *S. flavoviridis*, AB184842, 99.1%; *S. pilosus*, AB184161, 99%; *S. afghaniensis*, AJ399483, 99%; *S. africanus*, AY208912, 99%; *S. lomondensis*, AB184673, 99%; *S. levis*, AB184670, 99%; *S. coeruleorubidus*, AY999719, 99%.

Source: isolated from soil from Hawaii.

DNA G+C content (mol%): not known.

Type strain: ATCC 12236, ATCC 19771, CBS 509.68, BCRC 13653, DSM 40042, IFM 1071, NBRC 12784, IMET 43082, JCM 4172, JCM 4585, LMG 5975, NCIMB 9410, NRRL B-1988, NRRL-ISP 5042, PCM 2315, RIA 1051, UNIQEM 157, VKM Ac-1761.

Sequence accession no. (16S rRNA gene): AB184143.

244. **Streptomyces hebeiensis** Xu, Li, Wu, Wang, Xu and Jiang 2004a, 730[VP]

he.bei.en′sis. N.L. masc. adj. *hebeiensis* of or pertaining to Hebei, a province in northern China where the sample yielding the type strain was collected.

Aerial mycelium and substrate mycelium are well developed. Aerial mycelium at maturity forms long, straight to *Rectiflexibiles* spore chains composed of nonmotile and coccoid spores with a warty surface. Diffusible pigments are produced on several media. The pigment is not a pH indicator or is changed only slightly with addition of 0.05 M HCl in ISP 5 and ISP 6. Colony color is medium-dependent. Casein and xanthine can be metabolized, but adenine and pectin cannot. Tests for gelatin, nitrate reduction, and melanin production are positive and tests for H_2S production and peptonization of milk are negative. Galactose, lactose, mannose, maltose, xylose, sorbitol, sodium citrate, sodium acetate, oxalate, starch, and glycerol are utilized as sole carbon and energy sources, but cellulose and xylan are not. Acid is formed from mannose and starch, but not from arabinose, fructose, galactose, glucose, inositol, lactose, mannitol, maltose, rhamnose, raffinose, sucrose, sorbitol, xylose, sodium citrate, sodium acetate, oxalate, or glycerol. L-Histidine and L-hydroxyproline can be used as sole carbon and nitrogen sources. Grows well at 27, 30, and 37°C but does not grow at 45 or 10°C. Grows in the presence of 4 or 7% NaCl and 0.1% phenol. Diagnostic amino acid of peptidoglycan is LL-A_2pm with trace amounts of *meso*-A_2pm. Whole-cell hydrolysates contain glucose and small quantities of xylose, galactose, and arabinose. The menaquinones are MK9(H_4) (4.6%), MK-9(H_6) (60%), MK-9(H_8) (30.7%), and MK-9(H_{10}) (4.7%) and phosphatidylethanolamine is the diagnostic phospholipid.

Type strain shows no sequence similarity over 99%.

Source: not known.

DNA G+C content (mol%): 71.4.

Type strain: YIM 001, CCTCC AA 203005, CIP 107974, DSM 41837, JCM 12696. *Sequence accession no. (16S rRNA gene):* AY277529.

245. **Streptomyces heliomycini** (*ex* Braznikova, Uspenskaya, Sokolova, Preobrazhenskaya, Gause, Ukholina, Shorin, Rossolimo and Vertogradova 1958) Preobrazhenskaya 1986, 574[VP] (Effective publication: Preobrazhenskaya *in* Gause, Preobrazhenskaya, Sveshnikova, Terekhova and

Maximova 1983.) ("*Actinomyces flavochromogenes* subsp. *heliomycini*" Braznikova, Uspenskaya, Sokolova, Preobrazhenskaya, Gause, Ukholina, Shorin, Rossolimo and Vertogradova 1958)

he.li.o.my.ci′ni. N.L. n. *heliomycinum* heliomycin; N.L. gen. n. *heliomycini* of heliomycin, intended to mean heliomycin producing.

Spore chains are spiral (*Spirales*); spore surface is warty, hairy, and spiny. On mineral agar 1, starch-ammonia agar: aerial mycelium is green-gray; substrate mycelium is yellow, brown-orange; no diffusible pigment. On glycerol-nitrate agar, glycerol-asparagine agar: aerial mycelium is gray; substrate mycelium is dark yellow, brown yellowish, dark gray; no diffusible pigment. On oatmeal agar: aerial mycelium is gray; substrate mycelium is dark yellow, yellow brown; no diffusible pigment. On organic agar 2: aerial mycelium is gray, dark gray, green gray; substrate mycelium is brownish yellow, yellow brown; sometimes no diffusible pigment. Melanoid pigments are not formed. Utilization of glucose, sucrose, xylose, inositol, mannitol, fructose, and rhamnose. Antibiotic: geliomycin.

Type strain shows the highest sequence similarity to: *S. griseorubens*, AB184139, 99.3%; *S. griseoflavus*, AJ781322, 99.3%; *S. atrovirens*, DQ026672, 99.2%; *S. matensis*, AB184221, 99.2%; *S. griseoincarnatus*, AJ781328, 99.1%; *S. erythrogriseus*, AJ781328, 99.1%; *S. labedae*, AB184704, 99.1%; *S. variabilis*, DQ442551, 99.1%; *S. ambofaciens*, M27245, 99.1%; *S. albaduncus*, AY999757, 99%; *S. griseoloalbus*, AB184275, 99%; *S. lienomycini*, AJ781353, 99%.

Source: not known.

DNA G+C content (mol%): not known.

Type strain: DSM 41690, NBRC 15899, INA 2915, JCM 9767, VKM Ac-1778.

Sequence accession no. (16S rRNA gene): AB184712.

246. **Streptomyces helvaticus** (Krasil'nikov, Korenyako and Nikitina *in* Krasil'nikov 1965) Pridham 1970, 18[AL] ("*Actinomyces helvaticus*" Krasil'nikov, Korenyako and Nikitina *in* Krasil'nikov 1965, 224)

helv.va′ti.cus. N.L. n. *Helvetia* Switzerland; N.L. masc. adj. orth. var. *helvaticus* of or belonging to Switzerland.

Spore chains in Section *Retinaculiaperti* to *Spirales*. Straight to flexuous spore chains may also be common, especially when short spore chains are produced. This morphology is seen on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar. Sporulating aerial mycelium may be poorly developed or absent on yeast-malt agar. Spore surface is smooth.

Color of colony: aerial mass color probably in the Red color series (3ca, pale orange-yellow to 4ca, light yellowish pink) on oatmeal agar and salts-starch agar when optimum sporulation occurs. Aerial mass color may also appear to be white or yellow (2ba or 2db, pale yellow) on oatmeal agar and salts-starch agar. Aerial mycelium is poorly developed on yeast-malt agar and glycerol-asparagine agar. Reverse side of colony with no distinctive pigment (light yellow or pale yellow) on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar.

Color in medium: melanoid pigments are not formed in peptone-yeast-iron agar, tyrosine agar, or tryptone-yeast broth. No pigment or only a trace of yellow is found in the medium in yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar.

D-Glucose, L-arabinose, D-xylose and iso-inositol are utilized for growth. Utilization of D-fructose is doubtful. No growth or only traces of growth with rhamnose, sucrose, raffinose, and D-mannitol.

Type strain shows the highest sequence similarity to: *S. flavidovirens*, AB184270, 100%; *S. chryseus*, AY999787, 100%; *S. albidochromogenes*, AB249953, 99.9%; *S. enissocaesilis*, AB249930, 99.1%.

Source: not known.

DNA G+C content (mol%): not known.

Type strain: ATCC 19841, CBS 683.72, DSM 40431, NBRC 13382, INMI 1013-B, JCM 4768, NRRL B-12365, NRRL-ISP 5431, RIA 1343, VKM Ac-192.

Sequence accession no. (16S rRNA gene): AB184367.

247. **Streptomyces herbaricolor** Kawato and Shinobu 1959, 114[AL]

her.ba.ri.co′lor. L. n. *herbarius* one skilled in plants, a botanist; L. n. *color* color; N.L. adj. *herbaricolor* grass colored, green (referring to the grass green diffusible pigment produced by the organism on chemically defined media).

Spore chains in Section *Rectiflexibiles*. Mature spore chains generally have 10–50 spores per chain on yeast-malt agar and glycerol-asparagine agar. Sporulation aerial mycelium is poorly developed or absent on oatmeal agar and salts-starch agar. Spore surface is smooth.

Color of colony: aerial mass color in the Gray color series on glycerol-asparagine agar and yeast-malt agar. Aerial mycelium is poorly developed on all ISP media; mass color cannot be determined on oatmeal agar or salts-starch agar. Reverse side of colony: substrate mycelium may be grayish yellow to olive brown or brown or it may contain an additional pH-sensitive pigment with a color range from pale purple or pale purplish pink to green or greenish blue. When this pigment is present, addition of 0.05 M NaOH changes color from violet-red to green or blue and this change is reversed by addition of 0.05 M HCl. The pH-sensitive mycelial pigment was found by one observer in yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar.

Color in medium: melanoid pigments are not formed or occur only in trace amounts in peptone-yeast-iron agar and tyrosine agar. Pigment may or may not be formed in the medium in yeast-malt agar, oatmeal agar, salts-starch agar, or glycerol-asparagine agar. When formed, this pigment shows the same pH range found in the transient substrate pigment.

D-Glucose, L-arabinose, sucrose, D-xylose, D-fructose, and raffinose are utilized for growth. No growth or only trace of growth on iso-inositol, D-mannitol, and rhamnose.

Type strain shows the highest sequence similarity to: *S. indigoferus*, AB184214, 100%; *S. aburaviensis*, AY999779, 99.6,%; *S. purpureus*, AJ781324, 99.6%; *S. chrysomallus* subsp. *fumigatus*, AB184645, 99.5%; *S. purpeofuscus*, AJ781364, 99.2%; *S. xanthocidicus*, AY999858, 99.2%. Type strain shows the highest sequence similarity to following

Kitasatospora species: *Kitasatospora kifunensis*, AB022874, 99.3%; *Kitasatospora nipponensis*, AY442263, 99%; *Kitasatospora gansuensis*, AY442265, 99%.

Source: not known.

DNA G+C content (mol%): not known.

Type strain: ATCC 23922, CBS 424.61, CBS 906.68, BCRC 13772, DSM 40123, NBRC 12876, NBRC 3932, JCM 4138, JCM 4645, NBRC 3838, NCIMB 9837, NRRL B-3299, NRRL-ISP 5123, RIA 1126, RIA 654, VKM Ac-793.

Sequence accession no. (16S rRNA gene): AB184801.

248. **Streptomyces hiroshimensis** (Shinobu 1955) Witt and Stackebrandt 1991, 456[VP] (Effective publication: Witt and Stackebrandt 1990, 370.) ("*Streptomyces hiroshimensis*" Shinobu 1955; "*Verticillomyces hiroshimensis*" Shinobu 1965; *Streptoverticillium hiroshimense* Farina and Locci 1966, 51)

hi.ro.shim.en′sis. N.L. masc. adj. *hiroshimensis* of or pertaining to Hiroshima.

Spore chains in Umbellate Monoverticillate (=*Streptomyces* Section *Verticillati*, biverticillate). Mature spore chains are short, generally 3–10 spores per chain. This morphology is seen on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar in 14–21 d. Spore surface is smooth.

Color of colony: aerial mass color in the Red color series on yeast-malt agar, oatmeal agar, and salts-starch agar. Reverse side of colony is grayed yellow, modified by red on oatmeal agar, salts-starch agar, and glycerol-asparagine agar; or yellow-brown is modified by red on yeast-malt agar. Substrate pigment is not a pH indicator (one observer reports slight change from pink to brown with NaOH on oatmeal agar).

Color in medium: melanoid pigments formed in peptone-yeast-iron agar and tryptone-yeast broth; pigments other than melanoids not formed in yeast-malt agar, oatmeal agar, salts-starch agar, or glycerol-asparagine agar.

D-Glucose, iso-inositol, and D-fructose are utilized for growth. No growth or only traces of growth on L-arabinose, sucrose, D-xylose, D-mannitol, rhamnose, and raffinose.

Type strain shows the highest sequence similarity to: *S. caeruleus*, EF178675, 99.7%; *S. cinnamoneus*, AB184850, 99.7%; *S. abikoensis*, AB184537, 99.5%; *S. blastmyceticus*, AY999802, 99.5%; *S. aureoversilis*, AB184855, 99.5%; *S. pseudoechinosporeus*, AB184100, 99.5%; *S. lilacinus*, AB184819, 99.4%; *S. ardus*, AB184864, 99.2%; *S. biverticillatus*, AJ781381, 99%; *S. albireticuli*, AB184881, 99%; *S. sapporonensis*, AB184508, 99%; *S. werraensis*, DQ442558, 99%.

Source: not known.

DNA G+C content (mol%): not known.

Type strain: ATCC 19772, CBS 510.68, BCRC 13375, CECT 3261, DSM 40037, HUT 6033, NBRC 12785, NBRC 3839, IMET 43546, JCM 4098, JCM 4586, KCTC 9781, NBRC 3720, NCIMB 9838, NRRL B-1823, NRRL B-5484, NRRL-ISP 5037, RIA 1052, RIA 592, UNIQEM 158, VKM Ac-902.

Sequence accession no. (16S rRNA gene): AB184789.

Further comments: according to Hatano et al. (2003), *Streptomyces hiroshimensis* (Shinobu 1955) Witt and Stackebrandt 1991 is an earlier heterotypic synonym of *Streptomyces aureoversilis* corrig. (Locci et al. 1969) Witt and Stackebrandt 1991, of *Streptomyces baldaccii* corrig. (Farina and Locci 1966) Witt and Stackebrandt 1991, of *Streptomyces biverticillatus* (Preobrazhenskaya and Ryabova 1957) Witt and Stackebrandt 1991, of *Streptomyces fervens* (DeBoer et al. 1959–1960) Witt and Stackebrandt 1991, of *Streptomyces rectiverticillatus* (Krasil'nikov and Yuan 1965) Witt and Stackebrandt 1991, of *Streptomyces roseoverticillatus* (Shinobu 1956) Witt and Stackebrandt 1991, of *Streptomyces salmonis* (Baldacci et al. 1966) Witt and Stackebrandt 1991, and of *Streptomyces spitsbergensis* Wieczorek et al. 1993. Hatano et al. (2003) also propose that *Streptomyces hiroshimensis* (Shinobu 1955) Witt and Stackebrandt 1991 be a heterotypic synonym of "*Streptomyces fervens* subsp. *melrosporus*" (NBRC 15920), and of "*Streptoverticillium rubrochlorinum*" (NBRC 14694). In the paper by Hatano et al. (2003), "*Streptomyces fervens* subsp. *melrosporus*" is not in quotes. However, this name has no standing in bacterial nomenclature.

249. **Streptomyces hirsutus** Ettlinger, Corbaz and Hütter 1958a, 344[AL]

hir.su′tus. L. masc. adj. *hirsutus* shaggy, bristly, with stiff hairs.

Spore chains in Section *Retinaculiaperti* or *Spirales*. Flexuous spore chains and chains terminating in hooks and loops are common; well developed spirals of more than 1 or 2 turns are rare. Mature spore chains generally have 10–30 spores per chain. This morphology is seen on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar. Spore surface is spiny.

Color of colony: aerial mass color in the Green color series on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar. Reverse side of colony with no distinctive pigment (grayed yellow to grayed greenish yellow) on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar; substrate pigment is not a pH indicator.

Color in medium: melanoid pigments not formed in peptone-yeast-iron agar or tyrosine agar. No pigment found in medium in yeast-malt agar, oatmeal agar, salts-starch agar, or glycerol-asparagine agar.

D-Glucose, L-arabinose, sucrose, D-xylose, iso-inositol, D-mannitol, D-fructose, rhamnose, and raffinose are utilized for growth.

Type strain shows the highest sequence similarity to: *S. cyanoalbus*, AB184882, 100%; S. *prasinus*, DQ026658, 99.1%; *S. bambergiensis*, AB184869, 99.1%.

Source: not known.

DNA G+C content (mol%): not known.

Type strain: ATCC 19773, CBS 511.68, BCRC 13676, DSM 40095, HAMBI 1003, NBRC 12786, IMET 42054, JCM 4191, JCM 4587, NRRL B-2713, NRRL-ISP 5095, RIA 1053, UNIQEM 159, VKM Ac-623.

Sequence accession no. (16S rRNA gene): AB184844.

250. **Streptomyces humidus** Nakazawa and Shibata *in* Imamura, Hori, Nakazawa, Shibata, Tatsuoka and Miyake 1956, 648[AL]

hu′mi.dus. L. masc. adj. *humidus* wet, damp, moist.

Spore chains in Section *Spirales* with many incomplete spirals resembling *Retinaculiaperti* morphology. Spore chains short to moderately long with 10–50 spores per chain. This morphology is seen on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar.

Spore surface is smooth. Although the original description reports production of black, moist (hygroscopic) areas on mature aerial mycelium, this was not recorded on ISP reports.

Color of colony: aerial mass color in the Gray or Red color series (3fe or 3li, brownish gray from the Gray color series to 5dc or 5ch, grayish yellowish pink, in the Red color series). Reverse side of colony with no distinctive pigments (colorless, pale or grayish yellow or light olive gray) on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar.

Color in medium: melanoid pigments not produced in peptone-yeast-iron agar, tyrosine agar, or tryptone-yeast broth. No pigment, or only trace of yellow pigment, is found in the medium in yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar.

D-Glucose, L-arabinose, D-xylose, iso-inositol, D-mannitol, D-fructose, and rhamnose are utilized for growth. No growth or only traces of growth on sucrose and raffinose.

Type strain shows the highest sequence similarity to: *S. cacaoi* subsp. *asoensis*, DQ026644, 99.5%; *S. rishiriensis*, EF178691, 99.3%; *S. novaecaesareae*, AB184357, 99.1%; *S. pseudovenezuelae*, AB184233, 99%.

Source: not known.

DNA G+C content (mol%): not known.

Type strain: ATCC 12760, ATCC 23923, CBS 907.68, BCRC 13707, DSM 40263, NBRC 12877, JCM 4386, NRRL B-3172, NRRL-ISP 5263, RIA 1186, VKM Ac-1703.

Sequence accession no. (16S rRNA gene): DQ442508.

251. **Streptomyces humiferus** Goodfellow, Williams and Alderson 1986a, 574^{VP} (Effective publication: Goodfellow, Williams and Alderson 1986e, 63.) (*Actinopycnidium caeruleum* Krasil'nikov 1962, 250)

hu.mi'fer.us. L. n. *humus* ground; L. v. *fero* to bear; N.L. masc. adj. *humiferus* borne of the ground, i.e. soil-borne.

Spore chains are *Spirales*; the spore surface is smooth. Forms extensively branched substrate and aerial mycelium. The aerial spore mass is gray; the reverse is red-orange and the pigment is pH-sensitive; red-orange diffusible pigments which are also pH-sensitive are produced. Does not form melanin pigments. Allantoin, adenine, esculin, arbutin, elastin, gelatin, guanine, hypoxanthine, pectin, starch, testosterone, tyrosine, urea, and xanthine are degraded but chitin and lecithin are not. Hydrogen sulfide is produced and nitrate is reduced. L-Arabinose, cellobiose, D-fructose, D-galactose, D-glucose, *myo*-inositol, D-lactose, mannitol, D-mannose, melibiose, raffinose, L-rhamnose, trehalose, and D-xylose are used as sole carbon sources but adonitol, inulin, melezitose, sucrose, and xylitol are not. Grows on L-arginine, L-cysteine, L-histidine, L-phenylalanine, potassium nitrate, L-threonine, and L-valine but not on DL-amino-n-butyric acid, L-hydroxyproline, L-methionine, or L-serine as sole nitrogen sources. Grows at 10–37°C, but not at 4 or 45°C. Tolerant to phenol (0.01%, w/v) and sodium chloride (10%, w/v) but not to sodium azide (0.01%, w/v). Resistant to rifampin. Antimicrobial activity shown against *Streptomyces murinus* ISP 5091 but not towards *Aspergillus niger* LIV 131, *Bacillus subtilis* NCIB 3610, *Candida albicans* CBS 562, *Escherichia coli* NCIB 9132, *Micrococcus luteus* NCIB 196, *Pseudomonas fluorescens* NCIB 9046^T, and *Saccharomyces cerevisiae* CBS 1171^T. The peptidoglycan contains LL-A_2pm as the major diamino acid.

Type strain shows the highest sequence similarity to: *S. coelescens*, AF503496, 100%; *S. violaceolatus* AF503497, 100%; *S. violaceoruber*, AF503492, 100%; *S. tricolor*, AB184687, 99.8%; *S. anthocyanicus*, AB184631, 99.8%; *S. rubrogriseus*, AB184681, 99.7%; *S. tendae*, D63873, 99.5%; *S. lienomycini*, AJ781353, 99.5%; *S. violaceorubidus*, AJ781374, 99.3%; *S. coelicoflavus*, AB184650, 99.3%.

Source: not known.

DNA G+C content (mol%): 70.4.

Type strain: AS 4.1070, ATCC 15719, ATCC 15812, DSM 43030, IFM 1139, NBRC 12244, IMET 43409, JCM 3037, KCC A-0037, KCTC 9116, NCIMB 10164, RIA 729, VKM Ac-644.

Sequence accession no. (16S rRNA gene): AF503491.

Further comments: the genus *Actinopycnidium* Krasil'nikov 1962 is reduced to synonymy with *Streptomyces* Waksman and Henrici 1943. For *Actinopycnidium caeruleum* Krasil'nikov 1962, it is necessary to substitute a new specific epithet, because there is a senior homonym, *Streptomyces caeruleus* (Baldacci 1944) Pridham et al. 1958, cited on the Approved Lists of Bacterial Names (Rules 34a and 41a of the *Bacteriological Code* (1990 Revision).

252. **Streptomyces hydrogenans** Lindner, Junk, Nesemann and Schmidt-Thomé 1958, 117^{AL}

hy.dro'gen.ans. Gr. n. *hudôr* water; Gr. v. *gennaô* to produce; N.L. part. adj. *hydrogenans* water producing.

Spore chains in Section *Rectiflexibiles*. Mature spore chains are generally long with more than 50 spores per chain. This morphology is seen on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar. Spore surface is smooth.

Color of colony: aerial mass color in the Gray color series on yeast-malt agar; White or Yellow color series on oatmeal agar, salts-starch agar, and glycerol-asparagine agar. Nearest matching color tabs in the Gray color series are d, light gray, and 5fe, light grayish reddish brown. Nearest matching tab in the Yellow color series is 2ba, pale yellow. Reverse side of colony with no distinctive pigments (pale or grayish yellow) on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar.

Color in medium: melanoid pigments are not formed in peptone-yeast-iron agar, tyrosine agar, or tryptone-yeast broth. No pigment is found in the medium in yeast-malt agar, oatmeal agar, salts-starch agar, or glycerol-asparagine agar.

D-Glucose, L-arabinose, D-xylose, and rhamnose are utilized for growth. No growth or only traces of growth with iso-inositol, D-mannitol, D-fructose, sucrose, and raffinose.

Type strain shows the highest sequence similarity to: *S. limosus*, AB184147, 100%; *S. felleus*, AB184129, 100%; *S. daghestanicus*, DQ442497, 100%; *S. albidoflavus*, AB184255, 100%; *S. violascens*, AY999737, 100%; *S. odorifer*, Z76682, 100%; *S. griseus* subsp. *solvifaciens*, AB249915, 100%; *S. champavatii*, DQ026642, 100%; *S. canescens*, AB184117, 100%; *S. sampsonii*, D63871, 99.8%; *S. koyangensis*, AY079156, 99.7%.

Source: not known.
DNA G+C content (mol%): not known.
Type strain: ATCC 19631, CBS 776.72, BCRC 11855, DSM 40586, HAMBI 405, NBRC 13475, JCM 4771, NRRL B-12091, NRRL-ISP 5586, RIA 1436, VKM Ac-1919.
Sequence accession no. (16S rRNA gene): AB184868.

253a. **Streptomyces hygroscopicus subsp. hygroscopius** (Jensen 1931) Waksman and Henrici *in* Breed, Murray and Hitchens 1948, 953[AL] ("*Actinomyces hygroscopicus*" Jensen 1931, 357)

hy.gro.sco′pi.cus. Gr. adj. *hugros* moist; Gr. n. *skopos* one that watches, watcher; N.L. masc. adj. *hygroscopicus* detecting moisture, covered with moisture, hygroscopic.

Spore chains in Section *Spirales*; tight spirals in dense clusters. Spirals may coalesce as dark, moist masses of spores. Mature spore chains generally contain 10–50 spores per chain. This morphology is seen on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar. Spore surface is warty; individual spores are poorly delineated. Moist, black, liquefied (hygroscopic) areas are found in the aerial mycelium in 14–21 d. These are especially common on oatmeal agar and salts-starch agar.

Color of colony: aerial mass color in the Gray color series (3fe, 3li or 5ih, brownish gray; or 5fe, light grayish reddish brown) on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar. Dark or medium gray areas are also reported on oatmeal agar and salts-starch agar, and moist black (hygroscopic) areas may be seen on older cultures. Reverse side of colony with no distinctive pigments (colorless to grayish yellow, pale yellow or light olive brown or gray) on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar, except that dark discoloration may be seen beneath hygroscopic areas.

Color in medium: melanoid pigments are not formed in peptone-yeast-iron agar, tyrosine agar, or tryptone-yeast broth. No pigment or only a trace of yellow is found in the medium in yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar.

D-Glucose, D-mannitol, D-fructose, and rhamnose are utilized for growth. Good growth is reported on carbon-free control medium as well as on L-arabinose, D-xylose, and iso-inositol, so that utilization of these carbon sources is doubtful. Sucrose and raffinose are probably not utilized.

Type strain shows the highest sequence similarity to: *S. endus*, AY999911, 100%; *S. sporocinereus*, AB249933, 100%; *S. demainii*, DQ334782, 99.9%; *S. yogyakartensis*, AB249942, 99.5%; *S. violaceusniger*, AJ391823, 99.5%; *S. albiflaviniger*, AJ391812, 99.3%.
Source: not known.
DNA G+C content (mol%): not known.
Type strain: ATCC 27438, CBS 773.72, BCRC 11611, CIP 106840, DSM 40578, NBRC 13472, JCM 4772, LMG 19335, NRRL 2387, NRRL-ISP 5578, RIA 1433, VKM Ac-831.
Sequence accession no. (16S rRNA gene): AB184428.

253b. **Streptomyces hygroscopicus subsp. angustmyceticus** Yüntsen, Ohkuma, Ishii and Yonehara 1956, 200[AL]

an.gust.my.ce′ti.cus. L. adj. *angustus* narrow; Gr. n. *mukês* fungus; N.L. adj. *myceticus* fungus-like; N.L. masc. adj. *angustmyceticus* like a narrow fungus, but referring to the narrow spectrum of anti-bacterial activity of the organism, hence angustmycin.

Excellent growth on Czapek's solution agar; hygroscopic; NaCl tolerance >10%, but <13%. Produces angustmycin A, angustmycin B (adenine) and angustmycin C-anti-mycobacterial antibiotics; exhibits anti-fungal activity; inhibited by streptomycin.

Type strain shows the highest sequence similarity to: *S. ehimensis*, AY999834, 99.8%; *S. abikoensis*, AB184537, 99.7%; *S. sapporonensis*, AB184508, 99.5%; *S. lilacinus*, AB184819, 99.4%; *S. luteireticuli*, AB249969, 99.1%; *S. varsoviensis*, DQ026653, 99.1%; *S. thioluteus*, AB184753, 99%; *S. morookaense*, AJ781349, 99%; *S. mobaraensis*, DQ442528, 99%.
Source: not known.
DNA G+C content (mol%): not known.
Type strain: ATCC 15484, DSM 41683, NBRC 3934, JCM 4053, KCTC 1089, NRRL B-2347, NRRL B-3306.
Sequence accession no. (16S rRNA gene): DQ442509.

253c. **Streptomyces hygroscopicus subsp. decoyicus** Vavra, Dietz, Churchill, Siminoff and Koepsell 1959, 427[AL]

de.co′yi.cus. N.L. n. *decoyininum* decoyinine, name of an antibiotic; L. masc. suff. *-icus* suffix used with the sense of belonging to; N.L. masc. adj. *decoyicus* intended to mean possessing decoyinine.

Excellent growth on Czapek's solution agar; hygroscopic; NaCl tolerance >10%, but <13%. Produces angustmycins A and C; exhibits anti-fungal activity; inhibited by streptomycin.

Type strain shows the highest sequence similarity to: *S. caniferus*, AB184640, 99.6%; *S. libani* subsp. *rufus*, AJ781351, 99.6%; *S. sioyaensis*, DQ026654, 99.6%; *S. hygroscopius* subsp. *glebosus*, AB184479, 99.6%; *S. platensis*, AB045882, 99.4%; *S. monomycini*, DQ445790, 99.4%; *S. erumpens*, AJ621603, 99.2%; *S. libani* subsp. *libani*, AB184414, 99.2%; *S. tubercidicus*, AJ621612, 99.2%; *S. nigrescens*, DQ442530, 99.2%; *S. lydicus*, Y15507, 99%.
Source: not known.
DNA G+C content (mol%): not known.
Type strain: AS 4.1861, CIP 106836, DSM 41427, NBRC 13977, JCM 4550, NCIMB 10502, NCIMB 9752, NRRL 2666.
Sequence accession no. (16S rRNA gene): AY999883.

253d. **Streptomyces hygroscopicus subsp. glebosus** Ohmori, Okanishi and Kawaguchi 1962, 26[AL]

gle.bo′sus. L. masc. adj. *glebosus* full of clods, cloddy.

Ridged spores; excellent growth on Czapek's solution agar; hygroscopic; NaCl tolerance >10%, but <13%. Produces glebomycin; inhibited by streptomycin.

Type strain shows the highest sequence similarity to: *S. caniferus*, AB184640, 100%; *S. libani* subsp. *rufus*, AJ781351, 100%; *S. platensis*, AB045882, 99.9%; *S. libani*

subsp. *libani*, AB184414, 99.7%; *S. tubercidicus*, AJ621612, 99.6%; *S. nigrescens*, DQ442530, 99.6%; *S. hygroscopius* subsp. *decoyicus*, AY999883, 99.6%; *S. catenulae*, AJ621613, 99.3%; *S. ramulosus*, DQ026662, 99.3%; *S. misakiensis*, AB217605, 99.3%; *S. sioyaensis*, DQ026654, 99.2%; *S. monomycini*, DQ445790, 99%.

Source: not known.

DNA G+C content (mol%): not known.

Type strain: ATCC 14607, CIP 106832, DSM 40823, NBRC 13786, NBRC 13982, JCM 4954, KCTC 9782, NRRL B-3248.

Sequence accession no. (16S rRNA gene): AB184479.

253e. **Streptomyces hygroscopicus subsp. ossamyceticus** Schmitz, Jubinski, Hooper, Crook, Price and Lein 1965, 87[AL]

os.sa.my.ce′ti.cus. N.L. n. *ossamycinum* ossamycin, name of an antibiotic named for Mount Ossa of Greek mythology; L. masc. suff. *-icus* suffix used with the sense of belonging to; N.L. masc. adj. *ossamyceticus* belonging to ossamycin.

Petri dish cultures grown at 28°C on inorganic salts-starch agar show following morphology. Vegetative mycelium: branched, approx. 0.75–1 µm in diameter, no evidence of fragmentation. Aerial mycelium: branched, approx. 0.75–1 µm in diameter. Sporophore morphology: short side branches located along the main axial hyphae terminate in tight spiral spore chains of two to many turns; sporophores arranged singly, in pairs, or in clusters along the axial hyphae; no evidence of whorl formation. Conidia: catenulate, subglobose to elongated ovoid, most conidia ovoid measuring approx. 0.75 × 1–1.5 µm, smooth walls. For detailed cultural characteristics, see Schmitz et al. (1965). Produces ossamycin.

Type strain shows the highest sequence similarity to: *S. torulosus*, AJ781367, 100%; *S. neyagawaensis*, D63869, 99.4%; *S. ipomoeae*, AB184857, 99.3%.

Source: isolated from a soil sample collected in South America.

DNA G+C content (mol%): not known.

Type strain: ATCC 15420, CIP 106834, DSM 40824, NBRC 13983, JCM 4965, NRRL B-3822.

Sequence accession no. (16S rRNA gene): AB184560.

254. **Streptomyces iakyrus** de Querioz and Albert 1962, 33[AL]

i.a.ky′rus. N.L. masc. adj. *iakryus* [from Amazonian oral aboriginal language (Nheêngatû) adj. *iakryus*] green, referring to the color of the vegetative mycelium and diffusible pigment on some media.

Spore chains in Section *Spirales*. Mature spore chains are generally long with 10 to 50 or more spores per chain. This morphology is seen on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar. Spore surface is spiny.

Color of colony: aerial mass color in the Gray or Red color series on oatmeal agar and salts-starch agar; nearest matching color tab in the Gray color series is 5fe, light grayish reddish brown, and in the Red color series 5dc, grayish yellowish pink. These colors as well as white aerial mycelium are reported for yeast-malt agar and glycerol-asparagine agar (a variety of colors reported on yeast-malt agar also includes tabs from the Yellow color series and the Blue color series). Reverse side of colony is greenish yellow, olive gray, olive, or olive brown on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar.

Color in medium: melanoid pigments are formed in peptone-yeast-iron agar and tryptone-yeast broth, but only slightly or not at all on tyrosine agar. Yellow to yellow-green pigment is found in the medium in yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar. This pigment is not pH-sensitive when tested with 0.05 M NaOH or HCl.

D-Glucose, L-arabinose, D-xylose, iso-inositol, D-mannitol, D-fructose, rhamnose, sucrose, and raffinose are all utilized for growth.

Type strain shows the highest sequence similarity to: *S. violaceochromogenes*, AY999867, 99.5%; *S. longispororuber*, AB184440, 99.4%; *S. coerulescens*, AY999720, 99.3%; *S. collinus*, AB184123, 99.3%; *S. coeruleorubidus*, AY999719, 99.3%; *S. bellus*, AB184849, 99.3%; *S. speibonae*, AF452714, 99.3%; *S. lomondensis*, AB184673, 99.1%; *S. purpurascens*, AJ399486, 99.1%; *S. viridodiastaticus*, AY999852, 99.1%; *S. griseorubens*, AB184139, 99%; *S. albogriseolus*, AJ494865, 99%; *S. lusitanus*, AB184424, 99%; *S. griseoflavus*, AJ781322, 99%.

Source: not known.

DNA G+C content (mol%): not known.

Type strain: ATCC 15375, CBS 702.72, BCRC 11930, DSM 40482, NBRC 13401, INMI 15375, JCM 4254, JCM 4773, NRRL B-3317, NRRL B-3634, NRRL-ISP 5482, RIA 1362, VKM Ac-201.

Sequence accession no. (16S rRNA gene): AB184877.

255. **Streptomyces indiaensis** (Gupta 1965a) Kudo and Seino 1987, 243[VP] ("*Streptosporangium indianense*" Gupta 1965a)

in.di.a.en′sis. L. n. *india* India; L. suff. *ensis* indicating origin; N.L. masc. adj. *indiaensis* of or belonging to India.

Spore chains are *Spirales*; spore surface is smooth. Branching substrate and aerial mycelia are formed. Pseudosporangia on aerial mycelia are 2–10 µm in diameter and globular to oval or irregular. Aerial mass color is gray to grayish-violet; the reverse side of the colony is red to violet. Reverse color is changed from violet to red by addition of 0.05 M NaOH. Soluble pigment is red or violet. Melanoid pigment is formed in peptone-yeast extract iron agar, but not in tyrosine agar or tryptone-yeast extract agar. L-Arabinose, D-fructose, D-glucose, iso-inositol, lactose, D-mannitol, sucrose, and D-xylose are utilized for growth, but raffinose, L-rhamnose and salicin are not.

Type strain shows the highest sequence similarity to: *S. purpurascens*, AJ399486, 99.6%; *S. massasporeus*, AB184152, 99.6%; *S. thermocarboxydus*, U94490, 99.3%; *S. hawaiiensis*, AB184143, 99.3%; *S. coerulescens*, AY999720, 99.2%; *S. bellus*, AB184849, 99.2%; *S. lomondensis*, AB184673, 99.1%; *S. coeruleorubidus*, AY999719, 99%; *S. lusitanus*, AB184424, 99%; *S. arenae*, AB249977, 99%.

Source: not known.

DNA G+C content (mol%): 73.7.

Type strain: ATCC 33330, CBS 560.75, DSM 43803, NBRC 13964, JCM 3053, KCC A-0053, KCTC 9489, NCIB (now NCIMB) 9794.

Sequence accession no. (16S rRNA gene): AB184553.

256. **Streptomyces indigoferus** Shinobu and Kawato 1960, 49[AL]

in.di.go.fer′us. N.L. n. *indigo* (from Fr. n. *indigo*, derived from L. n. *indicum*, indigo) the dye indigo; L. suff. *-fer -fera -ferum* (from L. v. *fero*, to bear), bearing; N.L. masc. adj. indigoferus (*sic*) bearing (producing) indigo and referring to production of blue to green diffusible pigments on chemically defined media.

Spore chains in Section *Rectiflexibiles*. Mature spore chains are moderately long with 10–50, or sometimes more than 50, spores per chain. Aerial mycelium is poorly developed on all ISP media and is obscured by extensive coremia formation on yeast-malt agar, salts-starch agar, and glycerol-asparagine agar. Spore surface is smooth.

Color of colony: aerial mass color often cannot be determined because of inadequate aerial growth on oatmeal agar or because of extensive coremia formation on yeast-malt agar, salts-starch agar, and glycerol-asparagine agar. When adequate aerial mycelium is formed, it is in the Gray color series on yeast-malt agar and oatmeal agar. Aerial mass color in the Gray color series was also found on glycerol-asparagine agar and soil extract agar by one observer. Reverse side of colony: substrate mycelium is usually nearly colorless. grayish yellow or dark brown on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar. A transient blue to red pigment (depending on pH) may also be found in the mycelium on these media. This pigment changes from blue or colorless to red with addition of 0.05 M HCl.

Color in medium: melanoid pigments are formed in peptone-yeast-iron agar and tryptone-yeast broth, but are not formed in tyrosine agar. Pigments other than melanoids are not found in the medium in yeast-malt agar, oatmeal agar, salts-starch agar, or glycerol-asparagine agar.

D-Glucose and L-arabinose are utilized for growth. Utilization of xylose is doubtful. No growth or only traces of growth on sucrose, iso-inositol, D-mannitol, D-fructose, rhamnose, and raffinose.

Type strain shows the highest sequence similarity to: *S. herbaricolor*, AB184801, 100%; *S. aburaviensis*, AY999779, 99.6%; *S. purpureus*, AJ781324, 99.6%; *S. chrysomallus* subsp. *fumigatus*, AB184645, 99.5%; *S. purpeofuscus*, AJ781364, 99.2%; *S. xanthocidicus*, AY999858, 99.2%. Type strain shows the highest sequence similarity to following *Kitasatospora* species: *Kitasatospora kifunensis*, AB022874, 99.3%; *Kitasatospora nipponensis*, AY442263, 99%; *Kitasatospora gansuensis*, AY442265, 99%.

Source: not known.

DNA G+C content (mol%): not known.

Type strain: ATCC 23924, CBS 908.68, BCRC 13773, DSM 40124, NBRC 12878, NBRC 3868, IMET 42938, JCM 4646, NCIMB 9718, NRRL B-3301, NRRL-ISP 5124, RIA 1127.

Sequence accession no. (16S rRNA gene): AB184214.

257. **Streptomyces indonesiensis** Sembiring, Ward and Goodfellow 2001, 1619[VP] (Effective publication: Sembiring, Ward and Goodfellow2000, 365.)

in.do.ne.si.en′sis. N.L. masc. adj. *indonesiensis* of or pertaining to Indonesia.

Spore chains are *Spirales*; spore surface is rugose. On oatmeal agar, the spore mass is gray, the substrate mycelium grayish-yellow and the diffusible pigment yellow. Melanin pigments are not produced. The strain degrades pectin but not adenine.

Type strain shows the highest sequence similarity to: *S. asiaticus*, AB249947, 99.1%; *S. cangkringensis*, AJ391831, 99.7%; *S. rhizosphaericus*, AB249941, 99.7%; *S. griseinger*, AJ391818, 99.4%. Type strain shows DNA–DNA similarity to: *S. albiflaviniger* NRRL B-1356[T] 98.3%; *S. geldanamycinus* NRRL 3602[T], 98.2%; *S. griseiniger* NRRL B1865[T], 98.7%; *S. rhizosphaericus* DSM 41760[T], 98.3%; *S. asiaticus* DSM 41761[T], 99.1%; *S. javensis* DSM 41764[T], 98.2%; *S. yogyakartensis* DSM 41766[T], 98.1%; *S. cangkringensis* DSM 41769[T], 99.2%.

Source: isolated from the rhizosphere of the tropical legume *Paraserianthes falcataria*.

DNA G+C content (mol%): not known.

Type strain: A4R2, DSM 41759, JCM 11445, NBRC 100776, NCIMB 13673.

Sequence accession no. (16S rRNA gene): DQ334783.

258. **Streptomyces intermedius** (Krüger 1904) Waksman *in* Waksman and Lechevalier 1953, 116[AL] ("*Oospora intermedia*" Krüger 1904, 289; "*Actinomyces intermedius*" Wollenweber 1920, 13)

in.ter.me′di.us. L. masc. adj. *intermedius* intermediate.

Spore chains in Section *Rectiflexibiles*. Flexuous chains of 10–50 spores occur as tufts or clusters on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar, although production of sporulating aerial mycelium is not uniformly good in different laboratories with this strain. Spore surface is smooth.

Color of colony: sporulating aerial mycelium is usually inadequate for determination of aerial mass color. When aerial mycelium is formed, it is in the Yellow color series (1bc, pale yellow-green; 2ba, pale yellow) on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar (aerial mycelium is described as light green or light gray in the descriptions of Krasil'nikov, 1941, and Waksman, 1953). Reverse side of colony with no distinctive pigments (colorless to pale grayish yellow or light yellowish brown) on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar.

Color in medium: melanoid pigments are not formed in peptone-yeast-iron agar, tyrosine agar, or tryptone-yeast broth. No pigment or only a trace of yellow is found in the medium in yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar.

D-Glucose, L-arabinose, D-xylose, D-mannitol, and D-fructose are utilized for growth. No growth or only traces of growth with iso-inositol, rhamnose, sucrose, and raffinose.

Type strain shows the highest sequence similarity to: *S. gougerotii*, AB184742, 99.8%; *S. diastaticus* subsp. *diastaticus*, AB184785, 99.8%; *S. rutgersensis* subsp. *rutgersensis*, AB184170, 99.8%; *S. koyangensis*, AY079156, 99.1%; *S. misionensis*, EF178678, 99%; *S. phaeoluteichromatogenes*, AJ391814, 99%; *S. aureoverticillatus*, AY999774, 99%; *S. matensis*, AB184221, 99%.

Source: not known.

DNA G+C content (mol%): not known.

Type strain: ATCC 3329, ICMP 12540, AS 4.1467, ATCC 25461, CBS 101.21, CBS 694.69, BCRC 13706, DSM 40372,

ICMP 12540, NBRC 13049, IMET 41384, JCM 4197, JCM 4483, LMG 19304, NRRL B-1327, NRRL B-2670, NRRL-ISP 5372, RIA 1241.

Sequence accession no. (16S rRNA gene): AB184277.

259. **Streptomyces inusitatus** Hasegawa, Yamano and Yoneda 1978, 409[AL]

i.nu.si.ta′tus. L. masc. adj. *inusitatus* unusual.

Spore-bearing hyphae are simply branched and possess open or closed coils of spores. Spores (0.5–0.7 × 0.9–1.4 μm) are ellipsoidal to cylindrical, have smooth spore surfaces, and are arranged in coiled chains. The color of the aerial mycelium is blue-gray to gray-blue on Czapek agar, glucose-asparagine agar, yeast extract-malt extract agar, inorganic salts-starch agar, and tyrosine agar. Reverse side of colony with no distinctive pigments on either chemically defined or organic media. Color in medium: no pigment or only a trace of ivory color is formed on oatmeal agar. Starch is hydrolyzed, gelatin is weakly liquefied, and milk is peptonized. Carbon sources such as maltose, lactose, glycerol, starch, and sodium citrate are utilized for growth. Poor growth is obtained with D-galactose and D-glucose. L-A_2pm acid, glycine, aspartic acid, alanine, leucine, and glutamic acid, but not arabinose and galactose, are contained in cell-wall preparations. Produces oxamicetin.

Type strain shows the highest sequence similarity to: *S. amakusaensis*, AY999781, 99.8%.

Source: not known.

DNA G+C content (mol%): not known.

Type strain: ATCC 33341, CBS 196.78, DSM 41441, NBRC 13601, JCM 4988, NRRL B-16929.

Sequence accession no. (16S rRNA gene): AB184445.

260. **Streptomyces ipomoeae** (Person and Martin 1940) Waksman and Henrici *in* Breed, Murray and Hitchens 1948, 958[AL] ["*Actinomyces ipomoea*" (*sic*) Person and Martin 1940, 923; "*Streptomyces ipomoea*" (*sic*) Waksman and Henrici *in* Breed, Murray and Hitchens 1948, 958]

i.po.mo.e′a.e. N.L. n. *Ipomoea* generic name of the sweet potato; N.L. gen. n. *ipomoeae* of *Ipomoea*, referring to the source of the organism.

Spore chains in Section *Spirales*. Spore chains are usually short with 3–10 spores per chain and may give rise to hooks, loops, incomplete spirals, or spirals of only 1 or 2 turns. This morphology is seen on yeast-malt agar, oatmeal agar, and salts-starch agar, but typical aerial mycelium is usually not found on glycerol-asparagine agar. Spore surface is smooth; wrinkles or folds may be present.

Color of colony: aerial mass color in the Blue color series on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar. Reverse side of colony is pale yellow or grayish yellow on yeast-malt agar and glycerol-asparagine agar; yellow may be modified by blue (very pale blue or pale green) on reverse side of mature growth on oatmeal agar and salts-starch agar. This pigment is not a pH indicator.

Color in medium: melanoid pigments are not formed in peptone-yeast-iron agar, tyrosine agar, or tryptone-yeast broth. Traces of yellow or green pigment may or may not be found in the medium in yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar.

D-Glucose, L-arabinose, D-xylose, iso-inositol, D-mannitol, D-fructose, rhamnose, sucrose, and raffinose are all utilized for growth.

Type strain shows the highest sequence similarity to: *S. hygroscopicus* subsp. *ossamyceticus*, AB184560, 99.3%; *S. torulosus*, AJ781367, 99.2%.

Source: not known.

DNA G+C content (mol%): not known.

Type strain: 9820, ATCC 25462, CBS 695.69, DSM 40383, ICMP 12541, NBRC 13050, KCC S-0484, NRRL-ISP 5383, RIA 1242.

Sequence accession no. (16S rRNA gene): AB184857.

261. **Streptomyces janthinus** (Artamonova and Krasil'nikov 1960) Pridham 1970, 19[AL] ("*Actinomyces janthinus*" Artamonova and Krasil'nikov *in* Rautenshtein 1960, 334)

jan′thi.nus. L. masc. adj. *janthinus* violet-colored, referring to color of vegetative mycelium and diffusible pigment.

Spore chains in Section *Spirales*. Spore chains are moderately long with 10–50, or sometimes more than 50, spores per chain. This morphology is seen on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar. Spore surface is spiny.

Color of colony: aerial mass color in the Red or White color series on yeast-malt agar and salts-starch agar; White color series on oatmeal agar, and White or Gray color series on glycerol-asparagine agar. The nearest color tabs in the Red color series are 5dc, 5ca, 5cb, and 6ec (all grayish yellowish pink). Reverse side of colony with no distinctive pigments (colorless to grayed yellow or pale yellow) on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar.

Color in medium: melanoid pigments are formed in peptone-yeast-iron agar and tryptone-yeast broth, but not in tyrosine agar. Yellow pigment is found in the medium in salts-starch agar. This pigment is not pH-sensitive when tested with 0.05 M NaOH or HCl.

D-Glucose, L-arabinose, sucrose, D-xylose, iso-inositol, D-mannitol, D-fructose, rhamnose, and raffinose are utilized for growth.

Type strain shows the highest sequence similarity to: *S. roseoviolaceus*, AJ399484, 100%; *S. violaceus*, AB184315, 100%; *S. albosporeus* subsp. *albosporeus*, AJ781327, 100%; *S. luteogriseus*, AB184379, 99.5%; *S. lomondensis*, AB184673, 99.3%; *S. hawaiiensis*, AB184143, 99.3%; *S. flavoviridis*, AB184842, 99.3%; *S. pilosus*, AB184161, 99.2%; *S. arenae*, AB249977, 99.2%; *S. africanus*, AY208912, 99.2%; *S. tuirus*, AB184690, 99.2%; *S. bellus*, AB184849, 99.1%; *S. mutabilis*, EF178679, 99.1%; *S. massasporeus*, AB184152, 99%; *S. afghaniensis*, AJ399483, 99%; *S. levis*, AB184670, 99%; *S. coerulescens*, AY999720, 99%; *S. parvulus*, AB184326, 99%; *S. coeruleorubidus*, AY999719, 99%.

Source: not known.

DNA G+C content (mol%): not known.

Type strain: ATCC 15870, ATCC 23925, CBS 909.68, DSM 40206, NBRC 12879, JCM 4387, INMI 117, KCC S-0387, NRRL B-3365, NRRL-ISP 5206, RIA 659.

Sequence accession no. (16S rRNA gene): AB184851.

262. **Streptomyces javensis** Sembiring, Ward and Goodfellow 2001, 1619[VP] (Sembiring, Ward and Goodfellow 2000, 365)

ja.ven′sis. N.L. masc. adj. *javensis* of or pertaining to Java, Indonesia.

Spore chains are *Spirales*; spore surface is rugose. On oatmeal agar, the spore mass is gray, the substrate mycelium grayish-yellow and the diffusible pigment yellow. Melanin pigments are not produced. The organism degrades xylan and does not grow at 45°C.

Type strain shows the highest sequence similarity to: *S. yogyakartensis*, AB249942, 99.2%; *S. violaceusniger*, AJ391823, 99.1%. Type strain shows DNA–DNA similarity to: *S. albiflaviniger* NRRL B-1356^T, 98.9%; *S. geldanamycinus* NRRL 3602^T, 98.9%; *S. griseiniger* NRRL B-1865^T, 98.6%; *S. rhizosphaericus* DSM 41760^T, 98.3%; *S. asiaticus* DSM 41761^T, 98.2%; *S. indonesiensis* DSM 41759^T, 98.6%; *S. yogyakartensis* DSM 41766^T, 98.6%; *S. cangkringensis* DSM 41769^T, 98.4%.

Source: isolated from non-rhizosphere soil adjacent to a stand of the tropical legume *Paraserianthes falcataria*.

DNA G+C content (mol%): not known.

Type strain: B22P3, DSM 41764, JCM 11446, NBRC 100777, NCIMB 13679.

S*equence accession no. (16S rRNA gene):* AJ391833.

263. **Streptomyces jietaisiensis** He, Li, Huang, Wang, Liu, Lanoot, Vancanneyt and Swings 2005, 1943^{VP}

jie.tai.si.en′sis. N.L. masc. adj. *jietaisiensis* of or pertaining to Jietaisi, a place in a suburb of Beijing, where the type strain was isolated.

Spore chains with 10–20 cylindrical spores are *Rectiflexibiles*. The spore surface is smooth. Diffusible pigments are not produced, nor are melanin pigments formed on peptone-yeast extract-iron agar, or tyrosine agars. Adonitol, cellobiose, dextrin, D-galactose, D-glucose, inulin, glycogen, maltose, D-mannitol, D-mannose, melezitose, salicin, trehalose, and D-xylose (all at 1%, w/v), and L-alanine, L-arginine, L-aspartic acid, L-cysteine, L-glutamic acid, L-histidine, L-isoleucine, L-phenylalanine, sodium oxalate, sodium pyruvate, L-threonine, and L-valine (all at 0.1%, w/v) are used as sole carbon sources for energy and growth, but not glycerol, glycine, and xylitol (all at 1%, w/v) or DL-aminobutyric acid (at 0.1%, w/v). L-Alanine, L-arginine, L-aspartic acid, L-glutamic acid, and L-phenylalanine (all at 0.1%, w/v) are metabolized as sole carbon and nitrogen sources, but not L-isoleucine (at 0.1%, w/v). Growth occurs between 10 and 40°C, and between pH 5.0 and 10.0, but not at pH 4.0 or 11.0 or in the presence of streptomycin (10 μg/ml) or novobiocin (5 μg/ml). Cell-wall type I, phospholipid type II, and menaquinone MK-9(H_6,H_8,H_4). The fatty acid profile is composed of $C_{15:0}$ anteiso (35.7%), $C_{17:0}$ anteiso (18.9%), $C_{16:0}$ iso (14.8%), $C_{17:1}$ anteiso ω9*c* (8.1%), $C_{16:0}$ (6.2%), $C_{16:1}$ iso (4.5%), $C_{15:0}$ iso (4.3%), $C_{16:1}$ ω7*c* (2.33%), $C_{17:1}$ iso ω9*c* (1.8%), $C_{17:0}$ iso (1.7%), and $C_{14:0}$ iso (1.7%).

Type strain shows the highest sequence similarity to: *S. griseoaurantiacus*, AB184676, 99.7%. Type strain shows DNA–DNA similarity to: *S. griseoaurantiacus* DSM 40430^T, 48.8%.

Source: not known.

DNA G+C content (mol%): 72.3.

Type strain: FXJ46, AS 4.1859, JCM 12279.

Sequence accession no. (16S rRNA gene): AY314783.

264. **Streptomyces kanamyceticus** Okami and Umezawa *in* Umezawa, Ueda, Maeda, Yagishita, Kondō, Okami, Utahara, Ōsato, Nitta and Takeuchi 1957, 183^{AL}

ka.na.my.ce′ti.cus. N.L. n. *kanamycinum* kanamycin, name of an antibiotic; L. masc. suff. *-icus* suffix used with the sense of belonging to; N.L. masc. adj. *kanamyceticus* belonging to kanamycin.

Spore chains in Section *Rectiflexibiles*. Sporulating aerial mycelium is usually not abundant or may be absent on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar, although one observer reports long spore chains with 50 or more spores per chain on all of these media. Spore surface is smooth. Special morphological characteristics: terminal droplets showing wide variation in size are often found on aerial hyphae. These may contain coalesced masses of spores but they do not appear to be sporangia.

Color of colony: aerial mass color in the Yellow or White color series when sporulating aerial mycelium is present on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar. Nearest matching color tabs from the Yellow color series are 1½fb, light yellow; 2db, pale yellow; and 1db, pale yellow-green. Reverse side of colony with no distinctive pigments (colorless to pale yellow or grayish yellow) on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar.

Color in medium: melanoid pigments are not formed in peptone-yeast-iron agar, tyrosine agar, or tryptone-yeast broth. No pigment is found in the medium in yeast-malt agar, oatmeal agar, salts-starch agar, or glycerol-asparagine agar.

D-Glucose, L-arabinose, D-xylose, D-fructose, raffinose, and D-mannitol are utilized for growth. No growth or only traces of growth with iso-inositol, rhamnose, or sucrose.

Type strain shows the highest sequence similarity to: *S. aureus*, AB249976, 99.5%; *S. durmitorensis*, DQ067287, 99.2%.

Source: not known.

DNA G+C content (mol%): not known.

Type strain: AS 4.1441, ATCC 12853, CBS 715.72, BCRC 11515, DSM 40500, NBRC 13414, JCM 4433, JCM 4775, KCTC 9225, LMG 5976, LMG 19351, NCIMB 9343, NRRL B-2535, NRRL-ISP 5500, RIA 1375, RIA 690, VKM Ac-837.

Sequence accession no. (16S rRNA gene): DQ442511.

265. **Streptomyces kashmirensis** (*sic*) (Gupta and Chopra 1963b) Witt and Stackebrandt 1991, 456^{VP} (Effective publication: Witt and Stackebrandt 1990, 370.) ("*Streptomyces kashmirensis*" Gupta and Chopra 1963a, 112; *Streptoverticillium kashmirense* Locci, Baldacci and Petrolini Baldan 1969, 59)

kash.mir.en′sis. N.L. masc. adj. *kashimirensis* (*sic*) of or pertaining to Kashmir.

Spore chains in Section *Verticillati*, umbellate monoverticillate (biverticillate). Mature spore chains are generally short with 3 to 10 or more spores per chain. This morphology is usually seen on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar, although sporulation is not always good on these media. Spore surface is smooth.

Color of colony: aerial mass color in the Red color series (5cb or 5dc, grayish yellowish pink) on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar. When aerial mycelium is poorly developed, the surface color may appear to be in the Gray (d, light gray, or 2dc, yellowish gray) or White color series. The original description of Gupta and Chopra (1963b) reports blue aerial mycelium on Emmerson's medium. Reverse side of colony with distinctive red pigments (grayish red or moderate reddish brown on yeast-malt agar and oatmeal agar; light brown to dark brown on salts-starch agar and glycerol-asparagine agar). The reverse mycelium pigment is not a pH indicator.

Color in medium: melanoid pigments are formed in peptone-yeast-iron agar, tyrosine agar, and tryptone-yeast broth in 4 d; reaction may be weak on tyrosine agar. Yellow to orange or reddish pigment is found in the medium in yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar. This pigment is not pH-sensitive when tested with 0.05 M NaOH or HCl.

D-Glucose and iso-inositol are utilized for growth. Only traces of growth are seen with L-arabinose, D-xylose, D-mannitol, D-fructose, rhamnose, sucrose, and raffinose.

For sequence similarity, see type strain of *Streptomyces lilacinus*.

Source: not known.

DNA G+C content (mol%): not known.

Type strain: ATCC 27439, CBS 665.72, DSM 40336, NBRC 13906, JCM 4776, NRRL B-3103, NRRL-ISP 5336, RIA 1325, VKM Ac-885.

Sequence accession no. (16S rRNA gene): AB184546.

Further comments: in violation of Rule 33c of the *Bacteriological Code* (1990 Revision), in Validation List no. 38, *Streptomyces kashmirensis* is proposed as a *nomen revictum* (basonym: "*Streptomyces kashmirensis*" Gupta and Chopra 1963b).

According to Hatano et al. (2003), *Streptomyces kashmirensis* (sic) (Gupta and Chopra 1963b) Witt and Stackebrandt 1991 is a later heterotypic synonym of *Streptomyces lilacinus* (Nakazawa et al. 1956) Witt and Stackebrandt 1991.

266. **Streptomyces kasugaensis** Hamada, Kinoshita, Hattori, Yoshida, Okami, Higashide, Sakata and Hori 1995b, 879[VP] (Effective publication: Hamada, Kinoshita, Hattori, Yoshida, Okami, Higashide, Sakata and Hori 1995a, 35.)

ka.su.ga.en′sis. N.L. masc. adj. *kasugaensis* of or pertaining to Kasuga, Japan.

Substrate mycelia are well-branched. Aerial mycelia form complete spiral chains of spores. Mature spore chains consist of 10 or more spores. The spores are oval (0.6–0.7 × 0.6–0.8 μm) and the spore surface is smooth. The spores are not motile. No synnemata, sclerotia, or sporangia are observed. Aerial mycelia are light olive gray to light brownish gray, and vegetative mycelia are colorless to pale yellowish brown on various media. Yellow to brownish soluble pigments are produced on various media. Melanoid pigments are not produced on peptone-yeast extract iron agar (ISP 6), tyrosine agar (ISP 7), or in tryptone-yeast extract broth (ISP 1). Starch is not hydrolyzed, gelatin is liquefied, milk is peptonized and coagulated, and nitrate is reduced. D-Glucose, D-fructose, raffinose, and *myo*-inositol are utilized for growth, but L-arabinose, D-xylose, rhamnose, sucrose, and D-mannitol are not utilized. Permissive temperature range for growth is 20–37°C. Whole-cell hydrolysates contain LL-A_2pm, but no detectable arabinose, galactose, or *meso*-A_2pm. Phospholipid pattern type is PII. Mycolic acids are absent. Major menaquinones are MK-9(H_6), MK-9(H_8), and MK-9(H_4). Major components of cellular fatty acids are $C_{15:0}$ anteiso, $C_{16:0}$, $C_{17:0}$ anteiso, and $C_{16:0}$ iso.

Type strain shows no sequence similarity over 99%.

Source: not known.

DNA G+C content (mol%): 70.4–70.9.

Type strain: M338-M1, ATCC 15714, BCRC 12349, DSM 40819, NBRC 13851, JCM 4208, KCTC 1078, KCTC 2113, NCIMB 12239, NCIMB 12718.

Sequence accession no. (16S rRNA gene): AB024441.

267. **Streptomyces katrae** Gupta and Chopra 1963b, 1[AL]

kat′ra.e. N.L. gen. n. *katrae* of Katra, named for Katra, Jammu Province, India, the source of the soil from which the organism was isolated.

Spore chains in Section *Retinaculiaperti*. Long spore chains with terminal primitive spirals, hooks, or loops of wide diameter. Some well-defined spirals are also seen. Mature spore chains are long, often with more than 50 spores per chain. This morphology is seen on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar. Spore surface is smooth.

Color of colony: aerial mass color in the Red color series (5dc, 5cb or 4ca, grayish yellowish pink; 5ca, light yellowish pink; or 3ca, pale orange-yellow) on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar. Reverse side of colony with no distinctive pigments (yellowish brown to olive brown on yeast-malt agar; grayish yellow to yellowish brown on salts-starch agar, oatmeal agar, and glycerol-asparagine agar).

Color in medium: melanoid pigments are formed in peptone-yeast-iron agar and tryptone-yeast broth but not in tyrosine agar. No pigment is found in the medium in yeast-malt agar, oatmeal agar, salts-starch agar, or glycerol-asparagine agar.

D-Glucose, L-arabinose, D-xylose, D-fructose, and sucrose are utilized for growth. No growth or only traces of growth with iso-inositol, D-mannitol, rhamnose, or raffinose.

Type strain shows the highest sequence similarity to: *S. polychromogenes*, AB184292, 99.5%; *S. racemochromogenes*, DQ026656, 99.5%; *S. flavotricini*, AB184132, 99.2%.

Source: isolated from soil from Katra, Jammu Province, India.

DNA G+C content (mol%): not known.

Type strain: ATCC 27440, CBS 748.72, DSM 40550, NBRC 13447, IMET 43361, JCM 4777, NRRL B-3093, NRRL-ISP 5550, RIA 1408, RIA 794, VKM Ac-1220.

Sequence accession no. (16S rRNA gene): AB184409.

268. **Streptomyces kentuckensis** (Barr and Carman 1956) Witt and Stackebrandt 1991, 456[VP] (Effective publication: Witt and Stackebrandt 1990, 370.) ("*Streptomyces kentuckensis*"

Barr and Carman 1956; "*Verticillomyces kentuckensis*" Shinobu 1965; *Streptoverticillium kentuckense* Baldacci, Farina and Locci 1966, 170)

ken.tuck.en′sis. N.L. masc. adj. *kentuckensis* of or pertaining to Kentucky.

Spore chains in Umbellate Monoverticillate (=*Streptomyces* Section *Verticillati*, biverticillate). Mature spore chains moderately long with 10–50 spores per chain. This morphology is seen on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar. Spore surface is smooth.

Color of colony: aerial mass color in the Red color series on yeast-malt agar, oatmeal agar, and salts-starch agar; Red or White color series on glycerol-asparagine agar. Reverse side of colony with no distinct pigments (grayed yellow on oatmeal agar, salts-starch agar, and glycerol-asparagine agar to brown on yeast-malt agar).

Color in medium: melanoid pigments are produced in peptone-yeast-iron agar and tryptone-yeast broth, but not in tyrosine agar. No pigment is found in the medium in yeast-malt agar, oatmeal agar, salts-starch agar, or glycerol-asparagine agar.

D-Glucose, iso-inositol, and fructose are utilized for growth; utilization of D-xylose is doubtful. No growth or only trace of growth on L-arabinose, sucrose, D-mannitol, rhamnose, and raffinose.

For sequence similarity, see type strain of *Streptomyces netropsis*.

Source: not known.

DNA G+C content (mol%): not known.

Type strain: ATCC 12691, ATCC 23926, CBS 910.68, DSM 40052, HAMBI 52, NBRC 12880, IPV 940, IPV 1780, IPV1958, JCM 4153, JCM 4647, KCC S-153, LMG 5977, NRRL B-1831, NRRL-ISP 5052, RIA 1114.

Sequence accession no. (16S rRNA gene): AB184215.

Further comments: in violation of Rule 33c of the *Bacteriological Code* (1990 Revision), in Validation List no. 38, *Streptomyces kentuckensis* is proposed as a *nomen revictum* (basonym: "*Streptomyces kentuckensis*" Barr and Carman 1956).

According to Labeda (1996) and to Hatano et al. (2003), *Streptomyces kentuckensis* (Barr and Carman 1956) Witt and Stackebrandt 1991 is a later heterotypic synonym of *Streptomyces netropsis* (Finlay et al. 1951) Witt and Stackebrandt 1991.

269. **Streptomyces kishiwadensis** (Shinobu and Kayamura 1964) Witt and Stackebrandt 1991, 456[VP] (Effective publication: Witt and Stackebrandt 1990, 370.) ("*Streptomyces kishiwadensis*" Shinobu and Kayamura 1964; "*Verticillomyces kishiwadenis*" Shinobu 1965; *Streptoverticillium kishiwadense* Locci, Baldacci and Petrolini Baldan 1969, 59)

ki.shi.wad.en′sis. N.L. masc. adj. *kishiwadensis* of or belonging to Kishiwada (named after the place of origin, Kishiwada City, Japan).

Spore chains in Section *Verticillati*. Both monoverticillate and umbellate monoverticillate (biverticillate) sporophores are found. Mature sporophores are usually umbellate monoverticillate. Spore chains are short with 3–10 spores per chain. This morphology is seen on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar. Spore surface is smooth.

Color of colony: aerial mass color in the Red color series (5ca, light yellowish pink to 5cb, grayish yellowish pink) on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar; white to pale yellow or pale orange-yellow (3ca) aerial mass color may also be seen on these media. Reverse side of colony with no distinctive pigments (pale grayish yellow to orange-yellow or yellowish brown) on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar.

Color in medium: melanoid pigments are formed in peptone-yeast-iron agar and tryptone-yeast broth, but only weakly or not at all in tyrosine agar. No pigment found in medium with yeast-malt agar, oatmeal agar, salts-starch agar, or glycerol-asparagine agar.

D-Glucose, iso-inositol, D-fructose, and sucrose are utilized for growth. Utilization of D-xylose is doubtful and there is no growth or only traces of growth with L-arabinose, D-mannitol, rhamnose, and raffinose.

For sequence similarity, see type strain of *Streptomyces mashuensis*.

Source: not known.

DNA G+C content (mol%): not known.

Type strain: ATCC 25464, CBS 697.69, DSM 40397, NBRC 13052, JCM 4486, NRRL B-12326, NRRL-ISP 5397, RIA 1244, VKM Ac-931.

Sequence accession no. (16S rRNA gene): AB184858.

Further comments: in violation of Rule 33c of the *Bacteriological Code* (1990 Revision), in Validation List no. 38, *Streptomyces kishiwadensis* is proposed as a *nomen revictum* (basonym: "*Streptomyces kishiwadensis*" Shinobu and Kayamura 1964).

According to Hatano et al. (2003), *Streptomyces kishiwadensis* (Shinobu and Kayamura 1964) Witt and Stackebrandt 1991 is a later heterotypic synonym of *Streptomyces mashuensis* (Sawazaki et al. 1955) Witt and Stackebrandt 1991.

270. **Streptomyces koyangensis** Lee, Lee, Jung and Hwang 2005, 261[VP]

ko.yang.en′sis. N.L. masc. adj. *koyangensis* of or pertaining to Koyang, Republic of Korea, the geographical origin of the type strain.

Spore chains containing 10 or more spores per chain are *Rectiflexibiles*. Spores are spherical (1.2 m in diameter) with a smooth surface. The spore mass is white to gray and the reverse sides of colonies are brown on most agar media. Grows well on yeast extract-malt extract (ISP 2), oatmeal agar (ISP 3), inorganic salts-starch agar (ISP 4), peptone-yeast extract-iron agar (ISP 6), and tyrosine agar (ISP 7). Does not grow well on ISP 5 medium. Aerial mycelia are abundant on most of these media. The color of substrate mycelium is pale brown to dark brown. Production of spores on ISP 4 is prolific. Melanin pigments are produced on ISP 6 and ISP 7. Degrades casein, elastin, esculin, gelatin, starch, tyrosine, and xanthine, but not cellulose. As sole carbon sources, utilizes L-arabinose, D-fructose, mannitol, and xylose for growth, but not adon-

itol, dextran, *myo*-inositol, melezitose, melibiose, raffinose, L-rhamnose, sucrose, or xylitol. As nitrogen sources, utilizes L-cysteine, L-histidine, L-phenylalanine, and L-valine. It cannot utilize DL-α-amino-n-butyric acid or L-hydroxyproline. Pectin hydrolysis, nitrate reduction, and H_2S production are positive, whereas lecithinase, lipolysis, and hippurate hydrolysis are negative. Grows in the presence of 4, 7, and 10% sodium chloride, but not in 13%. It grows in 0.02% NaN_3 and 0.001% thallous acetate, but not in 0.1% phenol or 0.001% potassium tellurite. Whole-cell hydrolysates contain LL-A_2pm. The predominant cellular fatty acids are $C_{15:0}$ anteiso (16.54%), $C_{16:0}$ iso (28.77%), and $C_{16:0}$ (11.60%). In addition, $C_{17:0}$ anteiso (9.01%), $C_{14:0}$ iso (8.84%), $C_{15:0}$ iso (7.02%), $C_{17:0}$ cyclo (4.54%), $C_{17:1}$ anteiso (3.23%), $C_{17:0}$ iso (1.94%), $C_{14:0}$ (1.33%), $C_{16:1}$ iso (1.86%), and $C_{16:1}$ *cis*9 (2.57) are detected. Resistant to penicillin G, but sensitive to neomycin, rifampin, and oleandomycin. Produces 4-phenyl-3-buteonic acid, which inhibits the mycelial growth of several plant-pathogenic fungi, such as *Alternaria mali*, *Cladosporium cucumerinum*, *Colletotrichum gloeosporiuides*, *Colletotrichum orbiculare*, *Magnaporthe grisea*, and *Fusarium oxysporum* f. sp. *cucumerinum*.

Type strain shows the highest sequence similarity to: *S. odorifer*, Z76682, 99.7%; *S. hydrogenans*, AB184868, 99.7%; *S. daghestanicus*, DQ442497, 99.7%; *S. limosus*, AB184147, 99.7%; *S. albidoflavus*, AB184255, 99.7%; *S. felleus*, AB184129, 99.7%; *S. violascens*, AY999737, 99.7%; *S. griseus* subsp. *solvifaciens*, AB249915, 99.6%; *S. canescens*, AB184117, 99.6%; *S. champavatii*, DQ026642, 99.5%; *S. sampsonii*, D63871, 99.5%; *S. intermedius*, AB184277, 99.1%. Type strain shows DNA–DNA similarity to: *S. griseus* IFO 12875^T, 68.5%; *S. canescens* DSM 40001^T, 55.2%; *S. coelicolor* DSM 40233^T, 20.8%; *S. sampsonii* ATCC 25495^T, 64.0%; *S. odorifer* DSM 40347^T 34.4%; *S. limosus* DSM 40131^T, 66.8%; *S. felleus* DSM 40130^T, 25.6%; *S. somaliensis* DSM 40267, 57.5%.

Source: not known.

DNA G+C content (mol%): 67.8.

Type strain: VK-A60, KCCM 10555, NBRC 100598.

Sequence accession no. (16S rRNA gene): AY079156.

271. **Streptomyces kunmingensis** (Ruan, Lechevalier, Jiang and Lechevalier 1985) Goodfellow, Williams and Alderson 1986a, 574[VP] (Effective publication: Goodfellow, Williams and Alderson 1986d, 59.) (*Chainia kunmingensis* Ruan, Lechevalier, Jiang and Lechevalier 1985, 167)

kun.ming.en′sis. N.L. masc. adj. *kunmingensis* of or pertaining to Kunming, a province of South China.

Forms extensively branched substrate and aerial mycelium. Chains of conidia in loose spirals are occasionally borne on the aerial hyphae. Colonies are usually yellowish tan to dark orange yellow, depending on the medium, with or without yellowish white aerial mycelium. Produces sclerotia (5–25 μm) in and on the surface of agar media. Melanin pigments are not formed. Adenine, esculin, casein, hypoxanthine, starch, tyrosine, and xanthine are degraded, but gelatin and urea are not. Phosphatase and nitrate reductase are produced. L-Arabinose, D-mannitol, raffinose, L-rhamnose, and D-xylose are used as sole carbon sources, but *myo*-inositol and sucrose are not.

Acid is formed from L-arabinose, cellobiose, fructose, galactose, glucose, glycerol, *myo*-inositol, D-lactose, maltose, D-mannitol, mannose, melibiose, methyl α-D-glucoside, methyl α-D-xyloside, raffinose, L-rhamnose, salicin, sucrose, trehalose, and D-xylose but not from adonitol, dulcitol, *meso*-erythritol, or D-sorbitol. Grows at 10 and 37°C, but not at 42°C. The wall peptidoglycan contains LL-A_2pm as the major diamino acid. The organism has a type II phospholipid pattern (*sensu* Lechevalier et al., 1977).

Type strain shows no sequence similarity over 99%.

Source: isolated from soil at Dagyanlow's Lake in Kunming, Peoples' Republic of China.

DNA G+C content (mol%): 71.3.

Type strain: 80-3024, ATCC 35682, DSM 41681, NBRC 14463, JCM 7473, NRRL B-16240, VKM Ac-895.

Sequence accession no. (16S rRNA gene): DQ442513.

272. **Streptomyces kurssanovii** (Preobrazhenskaya, Kudrina, Ryabova and Blinov *in* Gauze, Preobrazhenskaya, Kudrina, Blinov, Ryabova and Sveshnikova 1957) Pridham, Hesseltine and Benedict 1958, 69[AL] ("*Actinomyces kurssanovii*" Preobrazhenskaya, Kudrina, Ryabova and Blinov *in* Gauze, Preobrazhenskaya, Kudrina, Blinov, Ryabova and Sveshnikova 1957, 156)

kurs.sa.nov′i.i. N.L. gen. masc. n. *kurssanovii* of Kursanov, possibly named after L.I. Kursanov, a Russian microbiologist.

Spore chains in Section *Retinaculiaperti*. Spirals are not observed on ISP media. Hooks and loops in the short spore chains are of small diameter and therefore are not truly representative of *Retinaculiaperti* morphology. The original description (Preobrazhenskaya and Ryabova, 1957) characterizes this culture as spiral. Mature spore chains are moderately short with 3–10, or sometimes more than 10, spores per chain. This morphology is seen on yeast-malt agar, oatmeal agar, and salts-starch agar; sporulation may be unsatisfactory on glycerol-asparagine agar. Spore surface is smooth.

Color of colony: aerial mass color in the Gray color series on yeast-malt agar and salts-starch agar. Reverse side of colony with no distinctive pigment (grayed yellow to yellow-brown) on oatmeal agar, salts-starch agar, and glycerol-asparagine agar; yellow-brown is modified by red on yeast-malt agar. Substrate pigment is not a pH indicator.

Color in medium: melanoid pigments formed in peptone-yeast-iron agar; pigments other than melanoids not formed in yeast-malt agar, oatmeal agar, salts-starch agar, or glycerol-asparagine agar.

D-Glucose, L-arabinose, sucrose, D-xylose, D-fructose, and raffinose are utilized for growth. No growth or only traces of growth on iso-inositol, D-mannitol, and rhamnose.

Type strain shows the highest sequence similarity to: *S. graminofaciens*, AJ781329, 100%; *S. xantholiticus*, AB184349, 99.9%; *S. peucetius*, AB045887, 99.8%.

Source: not known.

DNA G+C content (mol%): not known.

Type strain: ATCC 15824, ATCC 19774, ATCC 23629, CBS 512.68, BCRC 12133, CECT 3274, DSM 40162, NBRC

13192, INA 10294, JCM 4388, NCIMB 12788, NRRL B-3366, NRRL-ISP 5162, RIA 1054, UNIQEM 160.

Sequence accession no. (16S rRNA gene): AB184325.

273. **Streptomyces labedae** Lacey 1987, 458[VP]

la.be′da.e. N.L. gen. masc. n. *labedae* of Labeda, named after David P. Labeda, who first recorded the difference between the type strain and second reference strain of *Saccharopolyspora erythraeus* designated by Waksman.

Spore chains are in long, open spirals (*Spirales*). The spore surface is spiny. Spores are moderate gray on most media and reddish gray on glycerol-asparagine or Czapek's agars. Substrate mycelium olive gray to dark reddish brown. Aerial mycelium white to pinkish, especially on glycerol-asparagine or Czapek's agars. Melanin pigments are not produced on Czapek's agar. Glucose, xylose, rhamnose, fructose, galactose, mannitol, and inositol are assimilated as sole carbon sources; arabinose, salicin, and sucrose are assimilated moderately well; raffinose is poorly assimilated. Wall chemotype I, with LL-A_2pm and glycine.

Type strain shows the highest sequence similarity to: *S. griseoincarnatus*, AJ781328, 100%; *S. erythrogriseus*, AJ781328, 100%; *S. variabilis*, DQ442551, 100%; *S. griseorubens*, AB184139, 99.9%; *S. griseoflavus*, AJ781322, 99.6%; *S. matensis*, AB184221, 99.6%; *S. althioticus*, AY999808, 99.3%; *S. paradoxus*, AB184628, 99.2%; *S. heliomycini*, AB184712, 99.1%; *S. collinus*, AB184123, 99.1%; *S. viridochromogenes*, DQ442555, 99.1%; *S. flaveolus*, AB184764, 99%; *S. bellus*, AB184849, 99%; *S. albogriseolus*, AJ494865, 99%; *S. viridodiastaticus*, AY999852, 99%; *S. violaceochromogenes*, AY999867, 99%; *S. malachitofuscus*, AB184282, 99%; *S. violaceorubidus*, AJ781374, 99%; *S. coerulescens*, AY999720, 99%.

Source: isolated from soil.

DNA G+C content (mol%): not known.

Type strain: Sanchez-Marroquin A-24, DSM 41446, NBRC 15864, IMRU 3737, JCM 9381, NRRL B-5616, NRRL-ISP 5059.

Sequence accession no. (16S rRNA gene): AB184704.

Further comments: Saccharopolyspora erythraeus (Waksman 1923) Waksman and Henrici 1948 emend. Labeda 1987 is illegitimate [Rules 37a, 51a and 53 of the *Bacteriological Code* (1990 Revision)] and the name *Streptomyces labedae* Lacey 1987 is provided for this taxon.

274. **Streptomyces laceyi** Manfio, Atalan, Zakrzewska-Czerwinska, Mordarski, Rodriguez, Collins and Goodfellow 2003b, 1219[VP] (Effective publication: Manfio, Atalan, Zakrzewska-Czerwinska, Mordarski, Rodriguez, Collins and Goodfellow 2003a, 254.)

la′cey.i. N.L. gen. masc. n. *laceyi* of Lacey, named in honor of John Lacey, an English microbiologist.

Forms extensively branched substrate and aerial hyphae. Spiral chains of smooth-surfaced spores are evident on aerial hyphae. Aerial spore mass varies from gray to yellow pink depending on the medium; similarly, the substrate mycelium color varies from yellow to yellow pink. Diffusible pigments are not produced. Degrades xanthine, but shows no activity against hippurate or nitrate. Cleaves methoxysuccinyl-L-alanine-L-lysine-7AMC, L-phenylalanine-7AMC, *N*-acetyl-L-phenylalanine-L-arginine-7AMC (endopeptidase substrates), benzylocarbonyl-L-arginine-7AMC, L-proline-7AMC, L-pyroglutamate-7AMC (exopeptidase substrates), 4MU-β-D-galactopyranoside, 4MU-*N*-acetyl-β-D-glucosaminide, 4MU-α-D-mannopyranoside (glycosides), and 4MU-α-D-pyrophosphate (inorganic ester) but not L-citrulline-7AMC, glutaryl-glycine-L-phenylalanine-7AMC, glutaryl-L-phenylananine-7AMC (endopeptidases), L-glutamine-7AMC, L-iso-leucine-7AMC, L-ornithine-7AMC (exopeptidases), 4MU-α-D-glucoside, 2-deoxy-β-D-glucopyranoside (glucosides), 4MU-elaidate, or 4MU-oleate (organic esters). Dextran, inulin, D(+)mannitol, citrate, and pyruvate are used as sole sources of carbon for energy and growth, but not *myo*-inositol. L-Histidine, potassium nitrate, and L-valine are used as sole carbon and nitrogen sources, but not L-hydroxyproline. Grows from 10–30°C and in the presence of sodium azide (0.01%, w/v), thallous acetate (0.001%, w/v), and neomycin sulfate (50 µg/ml), but shows no activity against *Bacillus subtilis* NCIMB 3610, *Candida albicans* CBS 562, *Micrococcus luteus* NCIMB 196, or *Streptomyces murinus* ISP 5091.

Type strain shows no sequence similarity over 99%.

Source: isolated from soil taken from meadow hay plots.

DNA G+C content (mol%): 69–74.

Type strain: C7654, DSM 41788, JCM 12606, NBRC 100783, NCIMB 13928.

Sequence accession no. (16S rRNA gene): AB249944.

275. **Streptomyces ladakanum** (Hanka, Evans, Mason and Dietz 1966) Witt and Stackebrandt 1991, 456[VP] (Effective publication: Witt and Stackebrandt 1990, 370.) (*Streptoverticillium ladakanus* Hanka, Evans, Mason and Dietz 1966, 620)

la.da.ka′num. N.L. adj. *ladakanum* (*sic*) from Ladislav J. Hanka, who isolated the strain.

Spore chains in Section *Verticillati*, umbellate monoverticillate (biverticillate). Mature spore chains are generally short with 3 to 10 or more spores per chain. This morphology can be seen on yeast-malt agar, oatmeal agar, salts-starch agar, or glycerol-asparagine agar, but sporulation may be poor on these media. Spore surface is smooth.

Color of colony: aerial mass color in the White or Yellow color series on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar. Abundant aerial mycelium is usually in the White color series; shorter or poorly developed aerial mycelium may be 2ba or 2db, pale yellow. Reverse side of colony is moderate orange to strong brown on yeast-malt agar; light or pale yellow on oatmeal agar; yellowish pink to grayish reddish orange on salts-starch agar, and grayish yellow to reddish brown or strong brown on glycerol-asparagine agar. Reverse mycelium pigment is not a pH indicator.

Color in medium: melanoid pigments are not formed in peptone-yeast-iron agar, tyrosine agar, or tryptone-yeast broth. No pigment or only a trace of yellow is found in the medium in yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar.

D-Glucose and D-fructose are utilized for growth. No growth or only traces of growth with L-arabinose, D-xylose, iso-inositol, D-mannitol, rhamnose, sucrose, and raffinose.

For sequence similarity, see type strain of *Streptomyces mobaraensis*.

Source: not known.

DNA G+C content (mol%): not known.

Type strain: ATCC 27441, JCM 4778, NBRC 13476, NRRL 3191.

Sequence accession no. (16S rRNA gene): AB184430.

Further comments: according to Labeda (1996), *Streptomyces ladakanum* (Hanka et al. 1966) Witt and Stackebrandt 1991 is a later heterotypic synonym of *Streptomyces mobaraensis* (Nagatsu and Suzuki 1963) Witt and Stackebrandt 1991.

According to Hatano et al. (2003), *Streptomyces ladakanum* (Hanka et al. 1966) Witt and Stackebrandt 1991 is a later heterotypic synonym of *Streptomyces mobaraensis* (Nagatsu and Suzuki 1963) Witt and Stackebrandt 1991.

276. **Streptomyces lanatus** Frommer 1959, 204[AL]

la.na′tus. L. masc. adj. *lanatus* woolly, referring to the nature of the aerial mycelium of the organism.

Spore chains in Section *Spirales*. Mature spore chains generally have 10–50 spores per chain. This morphology is found on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar. Spore surface is spiny.

Color of colony: aerial mass color in the Blue color series on yeast-malt agar, oatmeal agar, and salts-starch agar. Reverse side of colony with no distinctive pigment on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar; substrate pigment is not a pH indicator.

Color in medium: melanoid pigments formed in peptone-yeast-iron agar, tyrosine agar, and tryptone-yeast broth. Pigments other than yellow or brown not formed in yeast-malt agar, oatmeal agar, salts-starch agar, or glycerol-asparagine agar.

D-Glucose, L-arabinose, sucrose, D-xylose, iso-inositol, D-mannitol, D-fructose, rhamnose, and raffinose are utilized for growth.

Type strain shows no sequence similarity over 99%.

Source: not known.

DNA G+C content (mol%): not known.

Type strain: ATCC 19775, CBS 513.68, BCRC 12060, DSM 40090, NBRC 12787, JCM 4332, JCM 4588, NRRL B-2291, NRRL-ISP 5090, RIA 1055, UNIQEM 161.

Sequence accession no. (16S rRNA gene): AB184845.

277. **Streptomyces lateritius** (Sveshnikova *in* Gauze, Preobrazhenskaya, Kudrina, Blinov, Ryabova and Sveshnikova 1957) Pridham, Hesseltine and Benedict 1958, 67[AL] ("*Actinomyces lateritius*" Sveshnikova *in* Gauze, Preobrazhenskaya, Kudrina, Blinov, Ryabova and Sveshnikova 1957, 70)

la.te.ri′ti.us. L. masc. adj. *lateritius* made or consisting of bricks, intended to mean brick red (referring to the color of the aerial mycelium).

Spore chains in Section *Retinaculiaperti*, but spore chains representative of Section *Rectiflexibiles* are also common. Mature spore chains generally have 10–50 spores per chain; longer chains are sometimes observed. This morphology is seen on salts-starch agar. Aerial mycelium is not produced on glycerol-asparagine agar and may be poorly developed on yeast-malt agar and oatmeal agar. Spore surface is warty.

Color of colony: aerial mass color in the Red color series on salts-starch agar. When sporulating aerial mycelium is formed on yeast-malt agar or oatmeal agar, it is in the Red color series. Reverse side of colony is grayed yellow is modified by violet or blue (see soluble pigment) on oatmeal agar, salts-starch agar, and glycerol-asparagine agar. Reverse color is changed from violet to blue by addition of 0.05 M NaOH, and from violet or blue to red with 0.05 M HCl.

Color in medium: melanoid pigments formed in peptone-yeast-iron agar. Blue or violet pigments found in medium in oatmeal agar, salts-starch agar, and glycerol-asparagine agar. This pigment is pH-sensitive when tested with 0.05 M NaOH or HCl. Violet pigment is changed to blue by 0.05 M NaOH and to red by 0.05 M HCl.

D-Glucose, L-arabinose, D-xylose, D-fructose, and rhamnose are utilized for growth. No growth or only traces of growth on sucrose, D-mannitol, and raffinose. Utilization of iso-inositol is doubtful.

Type strain shows the highest sequence similarity to: *S. venezuelae*, AB045890, 99.9%; *S. zaomyceticus*, EF178685, 99.7%; *S. exfoliatus*, AB184324, 99.7%; *S. wedmorensis*, DQ442557, 99.6%; *S. omiyaensis*, EF178697, 99.5%; *S. litmocidini*, AB184149, 99.5%; *S. yereyanensis*, EF178684, 99.3%; *S. narbonensis*, DQ445794, 99.2%.

Source: not known.

DNA G+C content (mol%): not known.

Type strain: AS 4.1427, ATCC 19776, ATCC 19913, CBS 514.68, BCRC 13774, DSM 40163, NBRC 12788, INA 6993, JCM 4389, NRRL B-5349, NRRL B-5423, NRRL-ISP 5163, RIA 1056, UNIQEM 162, VKM Ac-1849, VKM Ac-577.

Sequence accession no. (16S rRNA gene): AJ781326.

278. **Streptomyces laurentii** Trejo, Dean, Pluscec, Meyers and Brown 1977, 642[AL]

lau.ren′ti.i. N.L. gen. masc. n. *laurentii* derived from latinization of Lawrence, after Lawrence Township, New Jersey, the origin of the soil isolate.

On oatmeal agar, the aerial mycelium is predominantly straight (Rectus) with rare primitive spirals of a single turn; however, on starch-casein agar, hooks and primitive spirals predominate. The spores are smooth as seen by electron microscopy. In shaken culture [18 h at 25°C in tryptone-yeast extract broth (ISP medium 1)], a dusty pink soluble pigment is produced and the whole mycelium fragments into arthrospores and rods of varying length.

On yeast extract malt agar (ISP medium 2): sporulation is scant as a faint pinkish blush on white aerial mycelium; reverse: burnt orange; soluble pigment: light rose. On oatmeal agar (ISP medium 3): sporulation is good and occurs rapidly within 4 d; aerial mycelium is grayish yellow pink; CHM no. 5ec; dusty pink; there is no distinctive reverse color or soluble pigment. On inorganic salts starch agar (ISP medium 4): sporulation is good; CHM no. 4ec; light

rose beige; reverse is reddish-orange; no soluble pigment. No melanin is produced on sodium caseinate-tyrosine agar (25 g sodium caseinate, 10 g $NaNO_3$, 1 g L-tyrosine, 1 liter tap water). Carbohydrate utilization was determined on the basal medium of Pridham and Gottlieb (1948) supplemented with individual carbon sources at 1% (w/v). The following carbohydrates, as the sole carbon source, support growth: glucose, xylose, galactose, melibiose, sucrose, and lactose. No growth on mannitol, inositol, sorbitol, arabinose, rhamnose, fructose, raffinose, and trehalose. Cell-wall hydrolysates contain LL-A_2pm.

Type strain shows the highest sequence similarity to: *S. roseofulvus*, AB184327, 99.2%; *S. bikiniensis*, X79851, 99%.

Source: isolated from soil from Lawrence Township, New Jersey, USA.

DNA G+C content (mol%): not known.

Type strain: ATCC 31255, DSM 41684, NBRC 15422, JCM 5063, PCM 2368.

Sequence accession no. (16S rRNA gene): AJ781342.

279. **Streptomyces lavendofoliae** (Kuchaeva, Krasil'nikov, Taptykova and Gesheva 1961) Pridham 1970, 19[AL] ("*Actinomyces lavendofoliae*" Kuchaeva, Krasil'nikov, Taptykova and Gesheva 1961, 120)

la.ven.do.fo.li'a.e. N.L. n. *lavendula* lavender; L. n. *folium* a leaf; N.L. gen. n. *lavendofoliae* (*sic*) of lavender leaf, referring to the color of the aerial mycelium.

Spore chains in Section *Retinaculiaperti*. Long, flexuous spore chains often terminate in spirals or hooks and loops of wide diameter. Mature spore chains have 10–50, or often more than 50, spores per chain. This morphology is seen on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar. Spore surface is smooth.

Color of colony: aerial mass color in the Red color series (grayish yellowish pink or yellowish pink) on yeast-malt agar, oatmeal agar, and salts-starch agar. Reverse side of colony with no distinctive pigment (pale yellow to light yellowish brown) on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar.

Color in medium: melanoid pigments are formed in peptone-yeast-iron agar and usually in tyrosine agar and tryptone-yeast broth. Pigments other than melanoids are not formed in the medium in yeast-malt agar, oatmeal agar, salts-starch agar, or glycerol-asparagine agar.

D-Glucose, L-arabinose, D-xylose, and iso-inositol are utilized for growth. Utilization of fructose is doubtful. No growth or only traces of growth on sucrose, D-mannitol, rhamnose, and raffinose.

Type strain shows the highest sequence similarity to: *S. gobitricini*, AB184666, 99.8%; *S. luridus*, DQ442523, 99.4%.

Source: not known.

DNA G+C content (mol%): not known.

Type strain: ATCC 15872, ATCC 23928, CBS 912.68, DSM 40217, NBRC 12882, INA 3613, JCM 4391, NCIMB 9823, NRRL B-3371, NRRL-ISP 5217, RIA 1161, RIA 750, VKM Ac-272.

Sequence accession no. (16S rRNA gene): AJ781336.

280a. **Streptomyces lavendulae subsp. lavendulae** (Waksman and Curtis 1916) Waksman and Henrici *in* Breed, Murray and Hitchens 1948, 944[AL] ("*Actinomyces lavendulae*" Waksman and Curtis 1916, 126)

la.ven.du'la.e. N.L. n. *lavendula* lavender; N.L. gen. n. *lavendulae* of lavender color.

Spore chains in Section *Retinaculiaperti* with many *Rectiflexibiles* spore chains as well as coils of wide diameter and some spirals. Mature spore chains generally have 10–50 spores per chain; longer chains are often observed. This morphology is seen on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar. Spore surface is smooth.

Color of colony: aerial mass color in the Red (or Gray) color series on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar. Characteristic color is between 3ge (light grayish yellowish brown) and 4ec or 5ec (grayish yellowish pink) color tabs of Tresner–Backus Color Wheels. Reverse side of colony with no distinctive pigment on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar; substrate pigment is not a pH indicator (one observer reports slight change from yellowish brown to greenish brown by addition of 0.05 M NaOH).

Color in medium: melanoid pigments formed in peptone-yeast-iron agar and usually in tyrosine agar and tryptone-yeast broth. Pigments other than melanoids not formed in yeast-malt agar, oatmeal agar, salts-starch agar, or glycerol-asparagine agar.

D-Glucose is utilized for growth. No growth or only traces of growth on L-arabinose, sucrose, D-xylose, iso-inositol, D-mannitol, rhamnose, and raffinose. Limited growth with D-fructose.

Type strain shows the highest sequence similarity to: *S. cyaneofuscatus*, AB184860, 100%; *S. cavourensis* subsp. *washingtonensis*, DQ026671, 100%; *S. alboviridis*, AB184256, 100%; *S. fulvorobeus*, AB184711, 100%; *S. microflavus*, DQ445795, 100%; *S. praecox*, AB184293, 100%; *S. anulatus*, DQ026637, 100%; *S. flavofuscus*, AB249935, 100%; *S. cinereorectus*, AB184646, 100%; *S. griseoplanus*, AY999894, 100%; *S. lipmanii*, AB184148, 100%; *S. fimicarius*, AY999784, 100%; *S. sindenensis*, AB184759, 99.9%; *S. rubiginosohelvolus*, AB184240, 99.9%; *S. mediolani*, AB184674, 99.9%; *S. griseinus*, AB184205, 99.9%; *S. acrimycini*, AY999889, 99.9%; *S. pluricolorescens*, DQ442540, 99.9%; *S. argenteolus*, AB045872, 99.9%; *S. badius*, AY999783, 99.9%; *S. floridae*, AB184656, 99.8%; *S. albovinaceus*, AB249958, 99.8%; *S. globisporus* subsp. *globisporus*, EF178686, 99.8%; *S. griseus* subsp. *griseus*, AY207604, 99.8%; *S. griseolus*, AB184768, 99.8%; *S. luridiscabiei*, AF361784, 99.8%; *S. baarnensis*, EF178688, 99.8%; *S. flavovirens*, DQ026635, 99.7%; *S. californicus*, AB184755, 99.7%; *S. parvus*, DQ442537, 99.7%; *S. halstedii*, EF178695, 99.7%; *S. flavogriseus*, AJ494864, 99.6%; *S. olivoviridis*, AB184227, 99.5%; *S. bacillaris*, AB184439, 99.5%; *S. finlayi*, AY999788, 99.5%; *S. pulveraceus*, AB184806, 99.5%; *S. atroolivaceus*, AJ781320, 99.5%; *S. clavifer*, DQ026670, 99.4%; *S. yanii*, AB006159, 99.4%; *S. nitrosporeus*, EF178680, 99.4%; *S. albolongus*, AB184425,

99.3%; *S. sanglieri*, AB249945, 99.3%; *S. mutomycini*, AB249951, 99.2%; *S. atratus*, DQ026638, 99.2%; *S. celluloflavus*, AB184476, 99.2%; *S. gelaticus*, DQ026636, 99.2%; *S. griseobrunneus*, AB249912, 99.2%; *S. spiroverticillatus*, AB184814, 99.1%; *S. cavourensis* subsp. *cavourensis*, DQ445791, 99.1%; *S. cremeus*, AB184124, 99.1%; *S. candidus*, DQ026663, 99%.

Source: not known.

DNA G+C content (mol%): not known.

Type strain: ATCC 19777, ATCC 23950, ATCC 8664, CBS 515.68, CCM 3010, CCUG 11120, DSM 2014, DSM 40069, DSM 40213, HUT 6006, IFM 1031, NBRC 12789, NBRC 12903, NBRC 3177, NRRL-ISP 5213, NRRL-ISP 5069, JCM 4055, JCM 4589, JCM 4664, KCTC 1398, NBRC 12343, NCIMB 9840, NRRL B-1230, NRRL B-5617, RIA 1057, RIA 1159, RIA 144, RIA 531, RIA 744, UNIQEM 163, VKM Ac-1278, VKM Ac-624.

Sequence accession no. (16S rRNA gene): D85116.

280b. **Streptomyces lavendulae subsp. grasserius** (Kuchaeva, Krasil'nikov, Taptykova and Gesheva 1961) Pridham 1970, 54[AL] ("*Actinomyces lavendulae grasserius*" Kuchaeva, Krasil'nikov, Taptykova and Gesheva 1961, 119)

gras.se′ri.us. Fr. n. *grasserié* a disease of silkworms; N.L. adj. *grasserius* grasserial (referring to the activity of the organism against silkworm jaundice virus, grasserié).

Spore chains of typical *Retinaculum-Apertum* type; poor growth on Czapek's solution agar. Produces the grasseriomycin complex (a streptothricin complex); inhibited by streptomycin.

Type strain shows no sequence similarity over 99%.

Source: not known.

DNA G+C content (mol%): not known.

Type strain: ATCC 15875, ATCC 25457, CBS 690.69, DSM 40385, NBRC 13045, JCM 4056, JCM 4556, NRRL B-3072, NRRL B-3372, NRRL-ISP 5385, RIA 1237, RIA 746, VKM Ac-1178.

Sequence accession no. (16S rRNA gene): AY999841.

281. **Streptomyces lavenduligriseus** (Locci, Baldacci and Petrolini Baldan 1969) Witt and Stackebrandt 1991, 456[VP] (Effective publication: Witt and Stackebrandt 1990, 370.) (*Streptoverticillium lavenduligriseum* Locci, Baldacci and Petrolini Baldan 1969, 59)

la.ven.du.li.gri′se.us. N.L. n. *lavendula* lavender; N.L. adj. *griseus* gray; N.L. masc. adj. *lavenduligriseus* gray and lavender colored.

Spore chains in Section *Rectiflexibiles* to *Retinaculiaperti* (one observer records monoverticillate sporophores). Clusters of short, flexuous spore chains include hooks, loops, and primitive spirals. Straight spore chains are also seen, and spore chains or clusters of sporophores sometimes seem to be opposite or in whorls. These do not appear to have the uniformity or even spacing characteristic of verticillate cultures. This morphology is seen on yeast-malt agar, oatmeal agar, and salts-starch agar; sporulation is poor or absent on glycerol-asparagine agar. Spore surface is smooth.

Color of colony: aerial mass color in the Gray color series (3fe, light brownish gray; 3ge, light grayish yellowish brown; 3ig, grayish yellowish brown; or 2fe, medium gray) on yeast-malt agar, oatmeal agar, and salts-starch agar. Aerial mycelium is poorly developed or absent on glycerol-asparagine agar. Reverse side of colony with no distinctive pigments (brown to grayish yellow on yeast-malt agar and oatmeal agar; grayish yellow or pale yellow on salts-starch agar and glycerol-asparagine agar).

Color in medium: melanoid pigments are formed in peptone-yeast-iron agar, tyrosine agar, and tryptone-yeast broth. One of three observers reports reddish to orange pigment in the medium in yeast-malt agar and oatmeal agar, and yellow pigment in glycerol-asparagine agar. Two observers report no pigment in ISP media. When soluble pigment is produced, it is reported to be pH-sensitive, changing from red, orange, or yellow to violet with the addition of 0.05 M NaOH.

D-Glucose, L-arabinose, D-xylose, D-fructose, and D-mannitol are utilized for growth. Reports vary on utilization of iso-inositol. Utilization of rhamnose or sucrose is doubtful. No growth or only traces of growth with raffinose.

Type strain shows the highest sequence similarity to: *S. morookaense* AJ781349, 99.6%; *S. thioluteus*, AB184753, 99.2%; *S. abikoensis*, AB184537, 99.1%; *S. luteireticuli* AB249969, 99%; *S. sapporonensis*, AB184508, 99%.

Source: not known.

DNA G+C content (mol%): not known.

Type strain: ATCC 13306, ATCC 29661, CBS 706.72, DSM 40487, NBRC 13405, JCM 4545, JCM 4779, NRRL B-3173, NRRL-ISP 5487, RIA 1366, VKM Ac-1159.

Sequence accession no. (16S rRNA gene): DQ442515.

282. **Streptomyces lavendulocolor** (Kuchaeva, Krasil'nikov, Taptykova and Gesheva 1961) Pridham 1970, 20[AL] ("*Actinomyces lavendulocolor*" Kuchaeva, Krasil'nikov, Taptykova and Gesheva 1961, 120)

la.ven.du.lo.co′lor. N.L. n. *lavendula* the lavender; L. n. *color* color; N.L. *lavendulocolor* intended to mean lavender colored.

Spore chains of typical *Retinaculum-Apertum* type; poor growth on Czapek's solution agar. Said to produce the streptothricin complex; inhibited by streptomycin; said to inhibit some other streptothricin complex-producing strains.

Type strain shows the highest sequence similarity to: *S. cellulosae*, DQ442495, 99.1; *S. gancidicus*, AB184660, 99; *S. pseudogriseolus*, DQ442541, 99%.

Source: not known.

DNA G+C content (mol%): not known.

Type strain: ATCC 15871, ATCC 23927, CBS 911.68, BCRC 12057, DSM 40216, NBRC 12881, INA 4518, JCM 4390, NCIMB 9829, NRRL B-3367, NRRL-ISP 5216, RIA 1160, RIA 749, VKM Ac-215.

Sequence accession no. (16S rRNA gene): DQ442516.

283. **Streptomyces levis** Sveshnikova 1986, 574[VP] (Effective publication: Sveshnikova *in* Gause, Preobrazhenskaya, Sveshnikova, Terekhova and Maximova 1983.)

le′vis. L. masc. adj. *levis* smooth.

Spore chains are spiral (*Spirales*); spores are smooth. On mineral agar 1, oatmeal agar: aerial mycelium is pale pink to pale violet; substrate mycelium is brown; diffusible pigment is brown with grayish violet shadow. On starch-ammonia agar: aerial mycelium is not extant or poor, whitish; substrate mycelium is violet, gray-brown violet or plum black; diffusible pigment is pale violet, weak, or not extant. On glycerol-nitrate agar: aerial mycelium is white, poor; substrate mycelium is colorless to violet; no diffusible pigment. On glycerol-asparagine agar: aerial mycelium is light violet to light pink-violet; substrate mycelium is grayish-brownish yellow later grayish-brownish violet, plum black; no diffusible pigment. On organic agar 2: aerial mycelium is white to pale pink; substrate mycelium is brown to yellow brown; diffusible pigment is brown. Melanoid pigments are not formed. Grows on glucose, arabinose, xylose, raffinose, fructose, and sucrose; moderate growth on mannitol. Antibiotic pigment of group ribromycin-griseorodin having indicator character: alkaline reaction, violet; acidic reaction, red.

Type strain shows the highest sequence similarity to: *S. pseudogriseolus*, DQ442541, 99.5%; *S. capillispiralis*, AB184577, 99.5%; *S. carpinensis*, AB184574, 99.5%; *S. tuirus*, AB184690, 99.4%; *S. gancidicus*, AB184660, 99.4%; *S. azureus*, EF178674, 99.4%; *S. cellulosae*, DQ442495, 99.3%; *S. djakartensis*, AB184657, 99.2%; *S. afghaniensis*, AJ399483, 99.2%; *S. viridiviolaceus*, AY999854, 99.2%; *S. africanus*, AY208912, 99.2%; *S. caelestis*, X80824, 99.1%; *S. spinoverrucosus*, AB184578, 99.1%; *S. mutabilis*, EF178679, 99.1%; *S. misionensis*, EF178678, 99.1%; *S. paradoxus*, AB184628, 99%; *S. ghanaensis*, AY999851, 99%; *S. violaceus*, AB184315, 99%; *S. hawaiiensis*, AB184143, 99%; *S. vinaceusdrappus*, AY999929, 99%; *S. luteogriseus*, AB184379, 99%; *S. albosporeus* subsp. *albosporeus*, AJ781327, 99%; *S. geysiriensis*, DQ442501, 99%; *S. plicatus*, AB184291, 99%; *S. janthinus*, AB184851, 99%; *S. rochei*, AB184237, 99%; *S. minutiscleroticus*, EF178696, 99%; *S. roseoviolaceus*, AJ399484, 99%.

Source: not known.

DNA G+C content (mol%): not known.

Type strain: ATCC 43686, DSM 41458, NBRC 15423, INA 9020, JCM 6924, NRRL B-16370, VKM Ac-835.

Sequence accession no. (16S rRNA gene): AB184670.

284a. **Streptomyces libani subsp. libani** Baldacci and Grein 1966, 196[AL]

li′ba.ni. L. gen. n. *libani* of Lebanon, the source of the soil from which the organism was isolated.

Spore chains in Section *Spirales*. Tight terminal spirals are usually seen on moderately short chains of 10 or more spores. This morphology is found on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar. Spore surface is smooth.

Color of colony: aerial mass color in the Gray color series on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar. Nearest matching color tabs reported for aerial mass color on yeast-malt agar, oatmeal agar, and glycerol-asparagine agar are d, light gray; e, medium gray; and 2dc and 2bc, yellowish gray. Nearest matching tabs reported for salts-starch agar are 3fe, light brownish gray; 4li, brownish gray; and 3ge, light yellowish brown. Reverse side of colony is gray or pale yellow to orange yellow on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar.

Color in medium: melanoid pigments are not formed in peptone-yeast-iron agar, tyrosine agar, or tryptone-yeast broth. No pigment or only a trace of yellow is found in the medium in yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar.

D-Glucose, D-xylose, iso-inositol, D-mannitol, D-fructose, rhamnose, sucrose, and raffinose are utilized for growth. Utilization of L-arabinose is doubtful.

Type strain shows the highest sequence similarity to: *S. nigrescens*, DQ442530, 100%; *S. tubercidicus*, AJ621612, 100%; *S. hygroscopius* subsp. *glebosus*, AB184479, 99.7%; *S. libani* subsp. *rufus*, AJ781351, 99.7%; *S. caniferus*, AB184640, 99.6%; *S. catenulae*, AJ621613, 99.6%; *S. misakiensis*, AB217605, 99.6%; *S. sioyaensis*, DQ026654, 99.5%; *S. platensis*, AB045882, 99.5%; *S. hygroscopius* subsp. *decoyicus*, AY999883, 99.2%; *S. lydicus*, Y15507, 99.1%; *S. chattanoogensis*, AJ621611, 99%.

Source: isolated from soil from Lebanon.

DNA G+C content (mol%): not known.

Type strain: ATCC 23732, CBS 753.72, DSM 40555, NBRC 13452, JCM 4322, JCM 4781, KCTC 9113, NCAIM B.01474, NCIMB 11012, NRRL B-3446, NRRL-ISP 5555, RIA 1413, VKM Ac-1905.

Sequence accession no. (16S rRNA gene): AB184414.

284b. **Streptomyces libani subsp. rufus** Baldacci and Grein 1966, 197[AL]

ru′fus. L. masc. adj. *rufus* red, reddish.

Moderate growth on Czapek's solution agar; aerial mycelium typically colored in shades of hazel-nut brown (*avellaneus*); hygroscopic; forms reddish violet diffusible pigment with some media. Produces libanomycin; exhibits anti-fungal activity; inhibited by streptomycin.

Type strain shows the highest sequence similarity to: *S. caniferus*, AB184640, 100%; *S. hygroscopius* subsp. *glebosus*, AB184479, 100%; *S. platensis*, AB045882, 99.9%; *S. libani* subsp. *libani*, AB184414, 99.7%; *S. tubercidicus*, AJ621612, 99.6%; *S. nigrescens*, DQ442530, 99.6%; *S. hygroscopius* subsp. *decoyicus*, AY999883, 99.6%; *S. catenulae*, AJ621613, 99.3%; *S. ramulosus*, DQ026662, 99.3%; *S. misakiensis*, AB217605, 99.3%; *S. sioyaensis*, DQ026654, 99.2%; *S. monomycini*, DQ445790, 99%.

Source: not known.

DNA G+C content (mol%): not known.

Type strain: ATCC 23731, CMI 130779, DSM 41230, NBRC 15424, JCM 4325, NCIMB 10976, NRRL B-3445, VKM Ac-600.

Sequence accession no. (16S rRNA gene): AJ781351.

285. **Streptomyces lienomycini** Gause and Maximova 1986a, 574[VP] (Effective publication: Gause and Maximova *in* Gause, Preobrazhenskaya, Sveshnikova, Terekhova and Maximova 1983.)

li.e.no.my.ci′ni. N.L. n. *lienomycinum* lienomycin; N.L. gen. n. *lienomycini* of lienomycin, intended to mean lienomycin producing.

Spore chains are spiral (*Spirales*); spores are smooth. On mineral agar 1: aerial mycelium is white, later gray; substrate mycelium is yellowish or gray brownish yellow; no diffusible pigment. On glycerol-nitrate agar: aerial mycelium is absent; substrate mycelium colorless; no diffusible pigment. On oatmeal agar: aerial mycelium is light gray; substrate mycelium colorless; no diffusible pigment. On starch-ammonia agar: aerial mycelium is grayish white; substrate mycelium yellowish; no diffusible pigment. On glycerol-asparagine agar: aerial mycelium is absent; substrate mycelium yellowish; no diffusible pigment. On organic agar 2: aerial mycelium is absent or whitish; substrate mycelium and diffusible pigment are gray brown. Melanoid pigments are formed. Good growth on xylose, fructose, inositol, sucrose, arabinose, mannitol, rhamnose, and raffinose. Antibiotic: lienomycin.

Type strain shows the highest sequence similarity to: *S. rubrogriseus*, AB184681, 99.9%; *S. violaceorubidus*, AJ781374, 99.7%; *S. anthocyanicus*, AB184631, 99.7%; *S. tricolor*, AB184687, 99.7%; *S. violaceoruber*, AF503492, 99.6%; *S. coelescens*, AF503496, 99.6%; *S. violaceolatus* AF503497, 99.5%; *S. humiferus*, AF503491, 99.5%; *S. tendae*, D63873, 99.5%; *S. coelicoflavus*, AB184650, 99.2%; *S. olivaceus*, AB184743, 99.2%; *S. pactum*, AB184398, 99.2%; *S. ambofaciens*, M27245, 99.1%; *S. matensis*, AB184221, 99.1%; *S. heliomycini*, AB184712, 99%.

Source: not known.

DNA G+C content (mol%): not known.

Type strain: ATCC 43687, DSM 41475, NBRC 15425, INA 478, JCM 6925, VKM Ac-1767.

Sequence accession no. (16S rRNA gene): AJ781353.

286. **Streptomyces lilacinus** (Nakazawa, Tanabe, Shibata, Miyake and Takewaka 1956) Witt and Stackebrandt 1991, 456[VP] (Effective publication: Witt and Stackebrandt 1990, 370.) ("*Streptomyces lilacinus*" Nakazawa, Tanabe, Shibata, Miyake and Takewaka 1956, 81; "*Verticillomyces lilacinus*" Shinobu 1965; *Streptoverticillium lilacinum* Locci, Baldacci and Petrolini Baldan 1969, 59)

li.la′ci.nus. N.L. masc. adj. *lilacinus* lilac colored.

Spore chains in Section *Verticillati*, umbellate monoverticillate (biverticillate). Spore chains are short, usually with 3–10 spores per chain. This morphology is seen on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar. Spore surface is smooth.

Color of colony: aerial mass color in the Red color series (grayish yellowish pink) on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar. Reverse side of colony is yellow-brown plus red (grayish reddish brown to strong brown) on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar; this pigment is somewhat pH-sensitive changing from brown to reddish brown with addition of 0.05 M NaOH and from brown to yellowish brown with addition of 0.05 M HCl.

Color in medium: melanoid pigments are formed in peptone-yeast-iron agar, tyrosine agar, and tryptone-yeast broth. Red pigment is found in the medium in yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar. This pigment is pH-sensitive when tested with 0.05 M NaOH or HCl, showing the same change noted for substrate color.

D-Glucose is utilized for growth; reports vary on positive utilization of iso-inositol. No growth or only trace of growth on L-arabinose, sucrose, D-xylose, D-mannitol, D-fructose, rhamnose, and raffinose.

Type strain shows the highest sequence similarity to: *S. abikoensis*, AB184537, 99.7%; *S. hygroscopius* subsp. *angustmyceticus*, DQ442509, 99.4%; *S. hiroshimensis*, AB184789, 99.4%; *S. caeruleus*, EF178675, 99.3%; *S. sapporonensis*, AB184508, 99.3%; *S. ehimensis*, AY999834, 99.3%; *S. cinnamoneus*, AB184850, 99.1%; *S. pseudoechinosporeus*, AB184100, 99%.

Source: not known.

DNA G+C content (mol%): not known.

Type strain: ATCC 23930, CBS 914.68, BCRC 12421, CECT 3264, DSM 40254, NBRC 12884, NBRC 3944, JCM 4188, JCM 4648, NRRL B-1968, NRRL-ISP 5254, RIA 1180.

Sequence accession no. (16S rRNA gene): AB184819.

Further comments: in violation of Rule 33c of the *Bacteriological Code* (1990 Revision), in Validation List no. 38, *Streptomyces lilacinus* is proposed as a *nomen revictum* (basonym: "*Streptomyces lilacinus*" Nakazawa et al. 1956).

According to Hatano et al. (2003), *Streptomyces lilacinus* (Nakazawa et al. 1956) Witt and Stackebrandt 1991 is an earlier heterotypic synonym of *Streptomyces kashmirensis* (*sic*) (Gupta and Chopra 1963b) Witt and Stackebrandt 1991.

287. **Streptomyces limosus** Lindenbein 1952, 379[AL]

li.mo′sus. L. masc. adj. *limosus* slimy, referring to the river bank slime from which the organism was isolated.

Spore chains in Section *Rectiflexibiles*. Mature spore chains on suitable media generally 10–50 spores per chain; longer chains are sometimes observed. This morphology is seen on yeast-malt agar, salts-starch agar, and glycerol-asparagine agar. Spore surface is smooth.

Color of colony: aerial mass color in the Yellow color series on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar. Reverse side of colony with no distinctive pigment on yeast-malt agar, oatmeal agar, salts-starch agar, or glycerol-asparagine agar; substrate pigment is not a pH indicator.

Color in medium: melanoid pigments not formed in peptone-yeast-iron agar or tyrosine agar; no pigment found in medium in yeast-malt agar, oatmeal agar, salts-starch agar, or glycerol-asparagine agar.

D-Glucose, L-arabinose, D-xylose, D-mannitol, and D-fructose are utilized for growth. No growth or only trace of growth on sucrose, iso-inositol, rhamnose, and raffinose.

Type strain shows the highest sequence similarity to: *S. canescens*, AB184117, 100%; *S. felleus*, AB184129, 100%; *S. daghestanicus*, DQ442497, 100%; *S. albidoflavus*, AB184255, 100%; *S. hydrogenans*, AB184868, 100%; *S. odorifer*, Z76682, 100%; *S. violascens*, AY999737, 100%; *S. griseus* subsp. *solvifaciens*, AB249915, 100%; *S. champavatii*, DQ026642, 100%; *S. sampsonii*, D63871, 99.8%; *S. koyangensis*, AY079156, 99.7%.

Source: isolated from river bank slime.

DNA G+C content (mol%): not known.

Type strain: ATCC 19778, CBS 531.68, BCRC 13700, DSM 40131, NBRC 12790, JCM 4393, KCTC 9033, NCIMB 12976, NRRL B-5413, NRRL-ISP 5131, RIA 1058, UNIQEM 165, VKM Ac-850.

Sequence accession no. (16S rRNA gene): AB184147.

288. **Streptomyces lincolnensis** Mason, Dietz and DeBoer 1963a, 555[AL]

lin.coln.en′sis. N.L. masc. adj. *lincolnensis* of or belonging to Lincoln, referring to the source of the soil from which the organism was isolated, *viz.* Gehring, near Lincoln, Nebraska, USA.

Spore chains in Section *Rectiflexibiles.* Mature spore chains are long and flexuous, often with more than 50 spores per chain. This morphology is seen on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar. Spore surface is smooth.

Color of colony: aerial mass color in the Yellow or Red color series on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar. Representative color tabs are 2ba (pale yellow) from the Yellow color series; 2ca (pale yellow) and 3ca (pale orange yellow) from the Red color series. Reverse side of colony with no distinctive pigments (pale grayish yellow to orange yellow) on oatmeal agar and salts-starch agar; yellowish brown to brown on yeast-malt agar and olive brown to dark grayish brown on glycerol-asparagine agar.

Color in medium: melanoid pigments are formed in peptone-yeast-iron agar, tyrosine agar, and tryptone-yeast broth. A trace of yellow to greenish yellow pigment is found in the medium in oatmeal agar and glycerol-asparagine agar. This pigment is not pH-sensitive when tested with 0.05 M NaOH or HCl.

D-Glucose, L-arabinose, D-xylose, iso-inositol, D-mannitol, D-fructose, rhamnose, sucrose, and raffinose are all utilized for growth.

Type strain shows no sequence similarity over 99%.

Source: isolated from soil in Lincoln, Nebraska.

DNA G+C content (mol%): not known.

Type strain: ATCC 25466, CBS 630.70, CBS 699.69, BCRC 11173, DSM 2013, DSM 40355, NBRC 13054, JCM 4287, JCM 4488, KCTC 1868, KCTC 9088, KCTC 9089, NCIMB 9413, NRRL 2936, NRRL-ISP 5355, RIA 1246, VKM Ac-727.

Sequence accession no. (16S rRNA gene): AB184279.

289. **Streptomyces lipmanii** (Waksman and Curtis 1916) Waksman and Henrici *in* Breed, Murray and Hitchens 1948, 952[AL] ("*Actinomyces lipmanii*" Waksman and Curtis 1916, 123)

lip.man′i.i. N.L. gen. masc. n. *lipmanii* of Lipman, named for Jacob Goodale Lipman (1874–1939) of the New Jersey Agricultural Experiment Station.

Spore chains in Section *Rectiflexibiles.* Mature spore chains generally have 10–50 spores per chain; longer chains are sometimes observed on suitable media. This morphology is seen on yeast-malt agar and glycerol-asparagine agar. Sporulation may be poor on oatmeal agar and salts-starch agar. Spore surface is smooth.

Color of colony: aerial mass color in the Yellow color series on yeast-malt agar, salts-starch agar and glycerol-asparagine agar. Reverse side of colony with no distinctive pigment on oatmeal agar, salts-starch agar and glycerol-asparagine agar; yellow to yellow-brown on yeast-malt agar. Substrate pigment is not a pH indicator.

Color in medium: melanoid pigments not formed in peptone-yeast-iron agar or tyrosine agar. No pigment found in medium in yeast-malt agar, oatmeal agar, salts-starch agar, or glycerol-asparagine agar.

D-Glucose, D-xylose, D-mannitol, D-fructose, and rhamnose are utilized for growth. No growth or only traces of growth on L-arabinose, iso-inositol, and raffinose. Utilization of sucrose is doubtful.

Type strain shows the highest sequence similarity to: *S. cavourensis* subsp. *washingtonensis*, DQ026671, 100%; *S. fulvorobeus*, AB184711, 100%; *S. microflavus*, DQ445795, 100%; *S. lavendulae* subsp. *lavendulae*, AB184080, 100%; *S. alboviridis*, AB184256, 100%; *S. luridiscabiei*, AF361784, 99.9%; *S. flavofuscus*, AB249935, 99.9%; *S. cinereorectus*, AB184646, 99.9%; *S. fimicarius*, AY999784, 99.9%; *S. griseoplanus*, AY999894, 99.9%; *S. cyaneofuscatus*, AB184860, 99.9%; *S. floridae*, AB184656, 99.9%; *S. praecox*, AB184293, 99.9%; *S. anulatus*, DQ026637, 99.9%; *S. pluricolorescens*, DQ442540, 99.8%; *S. acrimycini*, AY999889, 99.8%; *S. argenteolus*, AB045872, 99.8%; *S. griseinus*, AB184205, 99.8%; *S. badius*, AY999783, 99.8%; *S. rubiginosohelvolus*, AB184240, 99.8%; *S. sindenensis*, AB184759, 99.8%; *S. mediolani*, AB184674, 99.8%; *S. californicus*, AB184755, 99.8%; *S. parvus*, DQ442537, 99.7%; *S. halstedii*, EF178695, 99.7%; *S. baarnensis*, EF178688, 99.7%; *S. griseolus*, AB184768, 99.7%; *S. griseus* subsp. *griseus*, AY207604, 99.7%; *S. globisporus* subsp. *globisporus*, EF178686, 99.7%; *S. albovinaceus*, AB249958, 99.7%; *S. flavovirens*, DQ026635, 99.6%; *S. finlayi*, AY999788, 99.5%; *S. flavogriseus*, AJ494864, 99.5%; *S. yanii*, AB006159, 99.4%; *S. atroolivaceus*, AJ781320, 99.4%; *S. olivoviridis*, AB184227, 99.4%; *S. bacillaris*, AB184439, 99.4%; *S. pulveraceus*, AB184806, 99.4%; *S. celluloflavus*, AB184476, 99.3%; *S. griseobrunneus*, AB249912, 99.3%; *S. albolongus*, AB184425, 99.3%; *S. clavifer*, DQ026670, 99.3%; *S. nitrosporeus*, EF178680, 99.3%; *S. spiroverticillatus*, AB184814, 99.2%; *S. sanglieri*, AB249945, 99.2%; *S. cavourensis* subsp. *cavourensis*, DQ445791, 99.2%; *S. mutomycini*, AB249951, 99.2%; *S. atratus*, DQ026638, 99.1%; *S. candidus*, DQ026663, 99.1%; *S. gelaticus*, DQ026636, 99.1%; *S. cremeus*, AB184124, 99%.

Source: not known.

DNA G+C content (mol%): not known.

Type strain: ATCC 19779, ATCC 3331, CBS 532.68, DSM 40070, HAMBI 1075, HUT 6059, NBRC 12791, NBRC 3410, NBRC 3721, IMET 40336, JCM 4058, JCM 4590, LMG 20047, NCIMB 9841, NRRL B-1229, NRRL B-2100, NRRL-ISP 5070, RIA 1059, RIA 85, UNIQEM 166.

Sequence accession no. (16S rRNA gene): AB184148.

Further comments: according to Lanoot et al. (2005b), *Streptomyces lipmanii* (Waksman and Curtis 1916) Waksman and Henrici 1948 is a later heterotypic synonym of *Streptomyces microflavus* (Krainsky 1914) Waksman and Henrici 1948 emend. Lanoot et al. 2005b.

290. **Streptomyces litmocidini** (Ryabova and Preobrazhenskaya *in* Gauze, Preobrazhenskaya, Kudrina, Blinov, Ryabova and Sveshnikova 1957) Pridham, Hesseltine and Benedict 1958, 65[AL] ("*Actinomyces litmocidini*" Ryabova and Preobrazhenskaya *in* Gauze, Preobrazhenskaya, Kudrina, Blinov, Ryabova and Sveshnikova 1957, 187)

lit.mo.ci.di′ni. N.L. gen. n. *litmocidini* of litmocidin.

Spore chains in Section *Rectiflexibiles* with some straight spore chains and occasional hooks or primitive spirals suggestive of Section *Retinaculiaperti*. Mature spore chains generally have 10–50 spores per chain; longer chains are often observed. This morphology is seen on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar. Spore surface is smooth.

Color of colony: aerial mass color in the Gray color series on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar. Reverse side of colony is colorless or characteristic grayed yellow on oatmeal agar, but the grayed yellow is modified by violet or red (depending on pH) on salts-starch agar, glycerol-asparagine agar, and sometimes on yeast-malt agar. Reverse color is changed from violet to blue by addition of 0.05 M NaOH, and from violet to red with 0.05 M HCl.

Color in medium: melanoid pigments formed in peptone-yeast-iron agar. Violet, blue, or red pigments found in medium in yeast-malt agar, salts-starch agar, and glycerol-asparagine agar; violet pigment is changed to blue by 0.05 M NaOH and to red by addition of 0.05 M HCl.

D-Glucose and L-arabinose are utilized for growth. No growth or only trace of growth on sucrose, iso-inositol, D-mannitol, rhamnose, and raffinose. Variable reports on growth with D-xylose and D-fructose.

Type strain shows the highest sequence similarity to: *S. yereyanensis* EF178684, 99.8%; *S. exfoliatus*, AB184324, 99.6%; *S. venezuelae*, AB045890, 99.6%; *S. zaomyceticus*, EF178685, 99.6%; *S. omiyaensis*, EF178697, 99.5%; *S. wedmorensis*, DQ442557, 99.5%; *S. lateritius*, AB184324, 99.5%; *S. narbonensis*, DQ445794, 99.1%; *S. cinereoruber* subsp. *cinereoruber*, AB184121, 99%.

Source: not known.

DNA G+C content (mol%): not known.

Type strain: ATCC 19780, ATCC 19914, CBS 533.68, BCRC 11866, DSM 40164, NBRC 12792, INA 1823/55, JCM 4394, NRRL B-3635, NRRL-ISP 5164, RIA 1060, UNIQEM 167, VKM Ac-1887.

Sequence accession no. (16S rRNA gene): AB184149.

291. **Streptomyces lomondensis** Johnson and Dietz 1969, 758[AL]

lo.mond.en′sis. N.L. masc. adj. *lomondensis* possibly pertaining to Loch Lomond, Scotland.

Warty to spiny spores borne on straight to open spiral to spiral sporophores (*Rectiflexibiles*, *Retinaculiaperti*, *Spirales*). Spores are poorly differentiated by carbon repligraphy. Blue aerial mycelium. Melanin-positive. The culture may be placed in the Red (R) and Blue (B) color series of Tresner and Backus (1963). Growth of the culture on carbon compounds in a synthetic medium was determined according to the procedure of Pridham and Gottlieb (1948). Growth is good on D-xylose, L-arabinose, rhamnose, D-fructose, D-galactose, D-glucose, D-mannose, maltose, sucrose, lactose, cellobiose, raffinose, dextrin, inulin, soluble starch, glycerol, D-mannitol, inositol, sodium acetate, and sodium succinate; slight on dulcitol and D-sorbitol. No growth on the control, salicin, phenol, cresol, sodium formate, sodium oxalate, sodium tartrate, or sodium salicylate. Grows at temperatures of 18–37°C. It does not grow at 55°C. The optimal temperature is 37°C. Produces the antibiotic lomofungin (lomondomycin, U-24792).

Type strain shows the highest sequence similarity to: *S. lusitanus*, AB184424, 99.4%; *S. bellus*, AB184849, 99.4%; *S. coerulescens*, AY999720, 99.4%; *S. purpurascens*, AJ399486, 99.4%; *S. janthinus*, AB184851, 99.3%; *S. coeruleorubidus*, AY999719, 99.3%; *S. parvulus*, AB184326, 99.3%; *S. roseoviolaceus*, AJ399484, 99.3%; *S. albosporeus* subsp. *albosporeus*, AJ781327, 99.3%; *S. violaceus*, AB184315, 99.3%; *S. luteogriseus*, AB184379, 99.3%; *S. flavoviridis*, AB184842, 99.2%; *S. pilosus*, AB184161, 99.2%; *S. malachitospinus*, AB249954, 99.2%; *S. iakyrus*, AB184877, 99.1%; *S. indiaensis*, AB184553, 99.1%; *S. violaceorubidus*, AJ781374, 99.1%; *S. matensis*, AB184221, 99%; *S. spinoverrucosus*, AB184578, 99%; *S. hawaiiensis*, AB184143, 99%; *S. thermocarboxydus*, U94490, 99%; *S. tuirus*, AB184690, 99%.

Source: soil.

DNA G+C content (mol%): not known.

Type strain: ATCC 25299, BCRC 12208, DSM 41428, NBRC 15426, JCM 4866, NCIMB 10094, NRRL 3252.

Sequence accession no. (16S rRNA gene): AB184673.

292. **Streptomyces longisporoflavus** Waksman *in* Waksman and Lechevalier 1953, 94[AL]

lon.gi.spo.ro.fla′vus. L. adj. *longus* long; N.L. n. *spora* a spore; L. adj. *flavus* yellow; N.L. masc. adj. *longisporoflavus* long-spored, yellow.

Spore chains in Section *Spirales*. Open spirals; spore chains resembling *Retinaculiaperti* morphology are also common. Mature spore chains are long with 10–50 spores per chain; longer chains are often observed. This morphology may be observed on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar. Spore surface is smooth.

Color of colony: aerial mass color in the Yellow color series on salts-starch agar. Aerial mycelium is poorly developed or absent on other ISP media. Reverse side of colony with no distinct pigments (colorless, grayish yellow, or light grayish yellowish brown) on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar.

Color in medium: melanoid pigments are not formed in peptone-yeast-iron agar, tyrosine agar, or tryptone-yeast broth. No pigment found in medium in yeast-malt agar, oatmeal agar, salts-starch agar, or glycerol-asparagine agar.

D-Glucose, L-arabinose, D-xylose, iso-inositol, D-fructose, and rhamnose are utilized for growth. No growth or only trace of growth on sucrose and raffinose. Utilization of iso-inositol and D-mannitol is doubtful.

No detailed sequence information available.

Source: not known.

DNA G+C content (mol%): not known.

Type strain: AS 4.1453, ATCC 19781, ATCC 19915, ATCC 23932, CBS 915.68, BCRC 13775, DSM 40165, NBRC 12886, IMET 43506, INA 81/53, JCM 4396, LMG 19347, NRRL-ISP 5165, RIA 1133, RIA 312, VKM Ac-1003.

Sequence accession no. (16S rRNA gene): AB184220.

293. **Streptomyces longispororuber** Waksman *in* Waksman and Lechevalier 1953, 99[AL]

lon.gi.spo.ro.ru′ber L. adj. *longus* long; Gr. n. *spora* a seed; N.L. n. *spora* a spore; L. adj. *ruber* red; N.L. adj. *longispororuber* long-spored, red.

Spore chains in Section *Spirales*. Spirals may be found on oatmeal agar or salts-starch agar but are rarely found on yeast-malt agar or glycerol-asparagine agar. Sporulation is generally poor on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar. One observer reports only sterile aerial mycelium on these media. Spore surface was not determined. Spores are poorly defined in electron micrographs submitted by ISP observers.

Color of colony: aerial mass color in the Red color series (5ca or 7ca, light yellowish pink; 4ea, moderate yellowish pink; or 5cb or 6ec, grayish yellowish pink) on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar. One observer also reports 10ec, grayish purplish pink, in the Violet color series on salts-starch agar. Reverse side of colony with distinctive red pigments (strong or deep reddish orange or purplish pink). Substrate pigment is a pH indicator, changing from yellow orange or orange to pink with the addition of 0.05 M HCl and orange or pink to yellow orange or yellow with the addition of 0.05 M NaOH.

Color in medium: melanoid pigments are formed in peptone-yeast-iron agar and tryptone-yeast broth, but not in tyrosine agar. No pigment is found in the medium in yeast-malt agar, oatmeal agar, salts-starch agar, or glycerol-asparagine agar.

D-Glucose, L-arabinose, iso-inositol, D-mannitol, and D-fructose are utilized for growth. Reports vary on the utilization of D-xylose. No growth or only traces of growth with sucrose, rhamnose, and raffinose.

Type strain shows the highest sequence similarity to: *S. iakyrus*, AB184877, 99.4%; *S. coeruleorubidus*, AY999719, 99.2%; *S. lusitanus*, AB184424, 99.2%; *S. bellus*, AB184849, 99.2%; *S. coerulescens*, AY999720, 99.2%; *S. thermocarboxydus*, U94490, 99.1%; *S. viridodiastaticus*, AY999852, 99.1%; *S. albogriseolus*, AJ494865, 99.1%; *S. speibonae*, AF452714, 99.1%; *S. matensis*, AB184221, 99%; *S. griseorubens*, AB184139, 99%; *S. griseoflavus*, AJ781322, 99%.

Source: not known.

DNA G+C content (mol%): not known.

Type strain: ATCC 27443, CBS 789.72, DSM 40599, NBRC 13488, INA 11668/54, JCM 4784, NCIMB 9629, NRRL B-3736, NRRL B-5761, NRRL-ISP 5599, PCM 2396, RIA 1449, VKM Ac-1735.

Sequence accession no. (16S rRNA gene): AB184440.

294. **Streptomyces longisporus** (Krasil'nikov 1941) Waksman *in* Waksman and Lechevalier 1953, 39[AL] ("*Actinomyces longisporus*" Krasil'nikov 1941, 47)

lon.gi'spo.rus. L. adj. *longus* long; Gr. n. *spora* a seed; N.L. n. *spora* a spore; N.L. adj. *longisporus* long-spored.

Spore chains in Section *Spirales*. Mature spore chains are moderately short, usually 10–50 spores per chain. Spore surface is spiny (one observer found smooth spores).

Color of colony: aerial mass color in the White color series on yeast-malt agar, oatmeal agar, and glycerol-asparagine agar; White or Red (5cb grayish yellowish pink) color series on salts-starch agar. Reverse side of colony with no distinctive pigments (light or grayish yellow on oatmeal agar, salts-starch agar, and glycerol-asparagine agar to olive brown or brown on yeast-malt agar).

Color in medium: melanoid pigments are produced in peptone-yeast-iron agar, tyrosine agar, and tryptone-yeast broth. Pigments other than melanoid are not found in the medium in yeast-malt agar, oatmeal agar, salts-starch agar, or glycerol-asparagine agar.

D-Glucose, L-arabinose, sucrose, D-xylose, iso-inositol, D-mannitol, D-fructose, rhamnose, and raffinose are utilized for growth.

Type strain shows no sequence similarity over 99%.

Source: not known.

DNA G+C content (mol%): not known.

Type strain: ATCC 23931, CBS 916.68, BCRC 13776, CCT 5006, DSM 40166, NBRC 12885, IMET 43090, INA 4417/56, JCM 4395, NRRL B-5336, NRRL-ISP 5166, PCM 2394, RIA 1134, VKM Ac-1896.

Sequence accession no. (16S rRNA gene): AJ399475.

295. **Streptomyces longwoodensis** Prosser and Palleroni 1981, 382[VP] (Effective publication: Prosser and Palleroni 1976, 321.)

long.wood.en'sis. N.L. masc. adj. *longwoodensis* of or belonging to Longwood, named after Longwood Gardens in Kennett Square, Pennsylvania, USA.

Strain produces a substrate mycelium, which does not fragment into spores, and an aerial mycelium, which later forms spore chains. After 14 d of incubation at 28°C, the spore chains appear spiral in form with greater than 10 spores per chain (range: 5 to >50). Spores are smooth and range in size from 1.2 × 0.32 μm to 1.8 × 0.74 μm. Does not hydrolyze hippurate; decomposes adenine, hypoxanthine, and tyrosine but not xanthine; and it slowly peptonizes skim milk. The cell wall of this organism contains LL-A_2pm.

Type strain shows the highest sequence similarity to: *S. galbus*, X79852, 99.9%; *S. bungoensis*, AB184696, 99.7%; *S. capoamus*, AB045877, 99.6%; *S. corchorusii*, AB184267, 99.4%; *S. canarius*, AB184396, 99.3%; *S. olivaceoviridis*, AB184288, 99.2%; *S. curacoi*, EF626595, 99%.

Source: not known.

DNA G+C content (mol%): not known.

Type strain: Roche X-14537, ATCC 29251, BCRC 12034, DSM 41677, NBRC 14251, JCM 4976, KCTC 9783, NRRL B-16923.

Sequence accession no. (16S rRNA gene): AB184580.

296. **Streptomyces lucensis** Arcamone, Bertazzoli, Canevazzi, DiMarco, Ghione and Grein 1957, 119[AL]

lu.cen'sis. L. masc. adj. *lucensis* of or belonging to Lucca, a city in Italy, the source of the soil from which the organism was isolated.

Spore chains in Section *Spirales*. Flexuous spore chains and imperfect spirals are common together with some regular open spirals of 4 or more turns; mature spore chains have 10–50 spores per chain. This morphology is seen on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar. Spore surface is hairy to spiny.

Color of colony: aerial mass color in the in the Gray color series (brownish gray) on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar. Reverse side of colony with no distinctive pigments

(colorless to pale grayish yellow or light yellowish brown) on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar.

Color in medium: melanoid pigments are formed in peptone-yeast-iron agar and tryptone-yeast broth, but not in tyrosine agar. No pigment is found in medium in yeast-malt agar, oatmeal agar, salts-starch agar, or glycerol-asparagine agar.

D-Glucose, L-arabinose, D-xylose, D-mannitol, D-fructose, and sucrose are utilized for growth. No growth or only trace of growth with iso-inositol, rhamnose, and raffinose.

Type strain shows the highest sequence similarity to: *S. niveoruber*, DQ445796, 99.5%.

Source: not known.

DNA G+C content (mol%): not known.

Type strain: ATCC 17804, ATCC 25468, CBS 701.69, DSM 40317, NBRC 13056, JCM 4490, NCIMB 12679, NRRL B-16066, NRRL B-5626, NRRL-ISP 5317, RIA 1248, VKM Ac-1737.

Sequence accession no. (16S rRNA gene): DQ442522.

297. **Streptomyces luridiscabiei** Park, Kim, Kwon, Wilson, Yu, Hur and Lim 2003, 2053[VP]

lu.ri.di.sca'bi.ei. L. adj. *luridus* pale yellow; L. n. *scabies* roughness, scabbiness; N.L. gen. n. *luridiscabiei* pale yellow, scab-causing bacteria.

Spores are yellow-white, smooth, and borne in monoverticillus flexuous spore chains. Melanin is produced on tyrosine and peptone agars. L-Arabinose, D-fructose, D-glucose, D-mannitol, raffinose, rhamnose, sucrose, D-xylose, and iso-inositol are utilized for growth. Minimum pH for growth is 4.5. Sensitive to 5, 6, and 7% (w/v) NaCl, 100 µg/ml thallium acetate, 0.1% (w/v) phenol, 25 and 100 µg/ml oleandomycin, and 20 µg/ml streptomycin, but not to 10 µg/ml thallium acetate, 0.5 µg/ml crystal violet, or 10 IU/ml penicillin.

Type strain shows the highest sequence similarity to: *S. fulvorobeus*, AB184711, 99.9%; *S. lipmanii*, AB184148, 99.9%; *S. alboviridis*, AB184256, 99.9%; *S. lavendulae* subsp. *lavendulae*, AB184080, 99.8%; *S. cavourensis* subsp. *washingtonensis*, DQ026671, 99.8%; *S. microflavus*, DQ445795, 99.8%; *S. cinereorectus*, AB184646, 99.7%; *S. fimicarius*, AY999784, 99.7%; *S. mediolani*, AB184674, 99.7%; *S. rubiginosohelvolus*, AB184240, 99.7%; *S. griseinus*, AB184205, 99.7%; *S. badius*, AY999783, 99.7%; *S. anulatus*, DQ026637, 99.7%; *S. praecox*, AB184293, 99.7%; *S. flavofuscus*, AB249935, 99.7%; *S. argenteolus*, AB045872, 99.7%; *S. californicus*, AB184755, 99.7%; *S. pluricolorescens*, DQ442540, 99.7%; *S. albovinaceus*, AB249958, 99.7%; *S. cyaneofuscatus*, AB184860, 99.7%; *S. griseoplanus*, AY999894, 99.7%; *S. sindenensis*, AB184759, 99.7%; *S. floridae*, AB184656, 99.7%; *S. acrimycini*, AY999889, 99.6%; *S. griseus* subsp. *griseus*, AY207604, 99.6%; *S. griseolus*, AB184768, 99.6%; *S. flavogriseus*, AJ494864, 99.5%; *S. parvus*, DQ442537, 99.5%; *S. halstedii*, EF178695, 99.5%; *S. flavovirens*, DQ026635, 99.5%; *S. finlayi*, AY999788, 99.4%; *S. pulveraceus*, AB184806, 99.3%; *S. atroolivaceus*, AJ781320, 99.3%; *S. globisporus* subsp. *globisporus*, EF178686, 99.3%; *S. olivoviridis*, AB184227, 99.3%; *S. griseobrunneus*, AB249912, 99.2%; *S. nitrosporeus*, EF178680, 99.2%; *S. yanii*, AB006159, 99.2%; *S. celluloflavus*, AB184476, 99.2%; *S. bacillaris*, AB184439, 99.2%; *S. clavifer*, DQ026670, 99.2%; *S. albolongus*, AB184425, 99.2%; *S. spiroverticillatus*, AB184814, 99.1%; *S. sanglieri*, AB249945, 99.1%; *S. baarnensis*, EF178688, 99.1%; *S. gelaticus*, DQ026636, 99%; *S. mutomycini*, AB249951, 99%; *S. atratus*, DQ026638, 99%; *S. cavourensis* subsp. *cavourensis*, DQ445791, 99%; *S. candidus*, DQ026663, 99%. Type strain shows DNA–DNA similarity to: *S. scabies* ATCC 49173^T, 16%; *S. turgidiscabies* ATCC 700248^T, 15%; *S. acidiscabies* ATCC 49003^T, 10%; *S. bottropensis* DSM 40262^T, 15%; *S. neyagawaensis* DSM 40588^T, 7%; *S. diastatochromogenes* DSM 40449^T, 13%; *S. setonii* DSM 40395^T, 10%; *S. griseus* subsp. *griseus* DSM 40236^T, 7%; *S. sampsonii* DSM 40394^T, 18%; *S. eurythermus* DSM 40014^T, 13%; *S. tendae* DSM 40101^T, 12%; *S. coelicolor* DSM 40233^T 9%; "*S. lividans*" DSM 40434, 10%; *S. ambofaciens* DSM 40053^T, 13%; *S. puniciscabiei* LMG 21391^T, 11%; *S. niveiscabiei* LMG 21392^T, 9%.

Source: isolated from raised corky lesions on potato cv. Atlantic and pathogenic on potato cv. Daeji-ma.

DNA G+C content (mol%): 70.3.

Type strain: S63, KACC 20252, LMG 21390.

Sequence accession no. (16S rRNA gene): AF361784.

298. **Streptomyces luridus** (Krasil'nikov, Korenyako, Meksina, Valedinskaya and Veselov 1957) Waksman 1961, 237[AL] ("*Actinomyces luridus*" Krasil'nikov, Korenyako, Meksina, Valedinskaya and Veselov 1957, 563)

lu'ri.dus. L. adj. *luridus* pale yellow, ghostly pallid (probably referring to the color of the vegetative mycelium).

Spore chains in Section *Retinaculiaperti* with characteristic range of flexuous spore chains, hooks, loops, and occasional spirals (spirals rare). Mature spore chains generally have 10–50 spores per chain; longer chains are often observed. This morphology is seen on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar. Spore surface is smooth.

Color of colony: aerial mass color in the Red color series on yeast-malt agar in 14 d. Sporulation aerial mycelium in the Red color series usually develops on oatmeal agar, salts-starch agar, and glycerol-asparagine agar in 21 d. Reverse side of colony with no distinctive pigment on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar; substrate pigment is not a pH indicator.

Color in medium: melanoid pigments formed in peptone-yeast-iron agar, tyrosine agar, and tryptone yeast broth. Pigments other than melanoids are not formed in yeast-malt agar, oatmeal agar, salts-starch agar, or glycerol-asparagine agar.

D-Glucose, L-arabinose, D-xylose, and iso-inositol are utilized for growth. No growth or only traces of growth on sucrose, D-mannitol, rhamnose, and raffinose. Utilization of D-fructose is doubtful.

Type strain shows the highest sequence similarity to: *S. gobitricini*, AB184666, 99.5%; *S. lavendofoliae*, AJ781336, 99.4%.

Source: not known.

DNA G+C content (mol%): not known.

Type strain: AS 4.1458, ATCC 19782, CBS 534.68, BCRC 13684, DSM 40081, NBRC 12793, INMI 111, JCM 4591, LMG 19365, NRRL B-5409, NRRL-ISP 5081, RIA 1061, UNIQEM 168, VKM Ac-245.

Sequence accession no. (16S rRNA gene): DQ442523.

299. **Streptomyces lusitanus** Villax 1963, 661[AL]

lu.si.ta′nus. L. masc. adj. *lusitanus* of or belonging to *Lusitania*, the Roman name of Portugal.

Spore chains in Section *Spirales*. Spirals are best developed on salts-starch agar and glycerol-asparagine agar; flexuous chains as well as open spirals and imperfect spirals are common on yeast-malt agar and oatmeal agar. Mature spore chains generally are long with 10 to 50 or more spores per chain. Spore surface is spiny with short spines. Some spores may appear smooth.

Color of colony: aerial mass color in the Gray or White color series on yeast-malt agar, oatmeal agar, salts-starch agar and glycerol-asparagine agar. Nearest matching color tabs in the Gray color series are 3fe, light brownish gray, and 5fe, light grayish reddish brown. Reverse side of colony with no distinctive pigments (pale or grayish yellow on oatmeal agar and glycerol-asparagine agar; olive brown or yellowish brown on yeast-malt agar and salts-starch agar).

Color in medium: melanoid pigments are not formed in peptone-yeast-iron agar, tyrosine agar, or tryptone-yeast broth. No pigment (or only a trace of yellow or pale olive) is found in the medium in yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar.

D-Glucose, L-arabinose, D-xylose, iso-inositol, D-mannitol, D-fructose, rhamnose, and sucrose are utilized for growth. No growth or only traces of growth with raffinose.

Type strain shows the highest sequence similarity to: *S. thermocarboxydus*, U94490, 99.5%; *S. coerulescens*, AY999720, 99.4%; *S. bellus*, AB184849, 99.4%; *S. lomondensis*, AB184673, 99.4%; *S. gancidicus*, AB184660, 99.4%; *S. pseudogriseolus*, DQ442541, 99.4%; *S. capillispiralis*, AB184577, 99.3%; *S. matensis*, AB184221, 99.3%; *S. longispororuber*, AB184440, 99.2%; *S. coeruleorubidus*, AY999719, 99.2%; *S. purpurascens*, AJ399486, 99.2%; *S. spinoverrucosus*, AB184578, 99.2%; *S. cellulosae*, DQ442495, 99.2%; *S. griseoloalbus*, AB184275, 99.1%; *S. parvulus*, AB184326, 99%; *S. speibonae*, AF452714, 99%; *S. iakyrus*, AB184877, 99%; *S. indiaensis*, AB184553, 99%; *S. viridodiastaticus*, AY999852, 99%; *S. albaduncus*, AY999757, 99%; *S. albogriseolus*, AJ494865, 99%.

Source: not known.

DNA G+C content (mol%): not known.

Type strain: ATCC 15842, ATCC 27444, CBS 765.72, BCRC 11552, DSM 40568, NBRC 13464, JCM 4785, NCIMB 9585, NRRL B-12501, NRRL B-5637, NRRL-ISP 5568, RIA 1425, VKM Ac-1194.

Sequence accession no. (16S rRNA gene): AB184424.

300. **Streptomyces luteireticuli** (*ex* Katoh and Arai 1957) Hatano, Nishii and Kasai 2003, 1528[VP] ("*Streptoverticillium luteoreticuli*" Katoh and Arai 1957)

lu.te.i.re.ti′cu.li. L. adj. *luteus* yellow; L. n. *reticulum* net; N.L. gen. n. *luteireticuli* of a yellow net.

Spore chains in Section Umbellate Monoverticillate (=*Streptomyces Verticillati*, biverticillate). Mature spore chains are generally short with 3 to 10 or more spores per chain. This morphology is seen on oatmeal agar and sometimes on salts-starch agar. Sporulating aerial mycelium is usually thin or absent on yeast-malt agar or glycerol-asparagine agar. Spore surface is smooth.

Color of colony: aerial mass color in the Yellow or Gray color series on oatmeal agar. Nearest matching color tabs in the Yellow color series are 1dc and 1½ec, pale yellow green, and 2db, pale yellow. Nearest matching tabs in the Gray color series are 2dc, yellowish gray, and 2ge, light olive brown. Reverse side of colony with no distinctive pigments (olive brown to dark brown on yeast-malt agar, grayish yellow or yellowish brown to olive brown on oatmeal agar, salts-starch agar, and glycerol-asparagine agar).

Color in medium: melanoid pigments are formed in peptone-yeast-iron agar, tyrosine agar, and tryptone-yeast broth. Yellowish to greenish yellow pigment is found in the medium in yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar. This pigment is not pH-sensitive when tested with 0.05 M NaOH or HCl.

D-Glucose and iso-inositol are utilized for growth. Utilization of L-arabinose, D-xylose, D-mannitol, D-fructose, rhamnose, sucrose, and raffinose is doubtful since only a small amount of growth is found on these carbon sources.

Type strain shows the highest sequence similarity to: *S. abikoensis*, AB184537, 99.3%; *S. sapporonensis*, AB184508, 99.2%; *S. ehimensis*, AY999834, 99.2%; *S. hygroscopius* subsp. *angustmyceticus*, DQ442509, 99.1%; *S. varsoviensis*, DQ026653, 99.1%; *S. lavenduligriseus*, DQ442515, 99%; *S. thioluteus*, AB184753, 99%.

Source: not known.

DNA G+C content (mol%): not known.

Type strain: ATCC 27446, CBS 723.72, DSM 40509, ISP 5509, JCM 4788, NBRC 13422, RIA 1383.

Sequence accession no. (16S rRNA gene): AB249969.

301. **Streptomyces luteogriseus** Schmitz, Deak, Crook and Hooper 1964, 89[AL]

lu.te.o.gri′se.us. L. adj. *luteus* golden yellow; N.L. adj. *griseus* gray; N.L. masc. adj. *luteogriseus* grayish, golden-yellow, referring to the yellowish gray color of sporulating aerial mycelium on certain media.

Spore chains in Section *Spirales*. Mature spore chains generally contain 10 to 50 or more spores per chain. This morphology is seen on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar. Spore surface is smooth.

Color of colony: aerial mass color in the Gray color series on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar. Nearest matching color tabs are 5fe, light grayish reddish brown, and 2dc, yellowish gray. One observer also reports aerial mycelium in the Red color series (5dc, grayish yellowish pink) on oatmeal agar and salts-starch agar. Reverse side of colony is strong yellowish brown to dark brown on yeast-malt agar; grayish yellow to olive brown on oatmeal agar, salts-starch agar, and glycerol-asparagine agar.

Color in medium: melanoid pigments are formed in peptone-yeast-iron agar, tyrosine agar, and tryptone-yeast broth, but the reaction may be weak in tyrosine agar. Yellow to brown pigment is found in the medium in yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar. This pigment is not pH-sensitive when tested with 0.05 M NaOH or HCl.

D-Glucose, L-arabinose, D-xylose, iso-inositol, D-mannitol, D-fructose, rhamnose, sucrose, and raffinose are all utilized for growth.

Type strain shows the highest sequence similarity to: *S. janthinus*, AB184851, 99.5%; *S. albosporeus* subsp. *albosporeus*, AJ781327, 99.5%; *S. roseoviolaceus*, AJ399484, 99.5%; *S. violaceus*, AB184315, 99.5%; *S. hawaiiensis*, AB184143, 99.4%; *S. tuirus*, AB184690, 99.4%; *S. arenae*, AB249977, 99.4%; *S. lomondensis*, AB184673, 99.3%; *S. flavoviridis*, AB184842, 99.2%; *S. parvulus*, AB184326, 99.2%; *S. pilosus*, AB184161, 99.2%; *S. massasporeus*, AB184152, 99.2%; *S. purpurascens*, AJ399486, 99.1%; *S. mutabilis*, EF178679, 99.1%; *S. malachitospinus*, AB249954, 99.1%; *S. rochei*, AB184237, 99%; *S. minutiscleroticus*, EF178696, 99%; *S. geysiriensis*, DQ442501, 99%; *S. bellus*, AB184849, 99%; *S. plicatus*, AB184291, 99%; *S. ghanaensis*, AY999851, 99%; *S. levis*, AB184670, 99%; *S. vinaceusdrappus*, AY999929, 99%.

Source: not known.

DNA G+C content (mol%): not known.

Type strain: ATCC 15072, CBS 703.72, DSM 40483, IFM 1203, NBRC 13402, JCM 4786, NRRL B-12422, NRRL-ISP 5483, RIA 1363, VKM Ac-1913.

Sequence accession no. (16S rRNA gene): AB184379.

302. **Streptomyces luteosporeus** Witt and Stackebrandt 1991, 456[VP] (Effective publication: Witt and Stackebrandt 1990, 370.) (*Streptoverticillium album* Locci, Baldacci and Petrolini Baldan 1969, 59)

lu.te.o.spo′re.us. L. adj. *luteus* yellow; Gr. n. *spora* a seed and in biology a spore; N.L. masc. adj. *luteosporeus* yellow spored.

Spore chains are usually shorter. Reverse colors varying from yellow to hazel-nut yellow. Aerial mycelium colors are lighter, tending toward pinkish beige on glucose-asparagine agar, glycerol-asparagine agar, inorganic salts-starch agar, and yeast extract-malt extract agar. Melanin pigment is not produced. L-Methionine, L-proline, and shikimic acid are utilized, but not coumarin, mannitol, melibiose, raffinose, sorbitol, or DL-α-aminobutyric acid. Esculin and L-tyrosine are degraded, but not citrate. H_2S is not produced. Acid is produced from D-ribose and trehalose, but not from D-galactose, D-fructose, or *myo*-inositol. *Aspergillus niger* is not inhibited. Growth is as good at 37°C as at 27°C. Aerial mycelium is better at 37°C. The type strain produces acetopyrrothine (thiolutin).

Type strain shows no sequence similarity over 99%.

Source: not known.

DNA G+C content (mol%): not known.

Type strain: ATCC 33049, JCM 4542, DSM 40833, NBRC 14657, NRRL 2401, VKM Ac-927.

Sequence accession no. (16S rRNA gene): DQ442525.

Further comments: for the transfer of *Streptoverticillium album* Locci et al. 1969 to the genus *Streptomyces* Waksman and Henrici 1943, it is necessary to substitute a new specific epithet to produce *Streptomyces luteosporeus* because there is a senior homonym, *Streptomyces albus* (Rossi Doria 1891) Waksman and Henrici 1943 included on the Approved Lists of Bacterial Names [Rules 34a and 41a of the *Bacteriological Code* (1990 Revision)].

303. **Streptomyces luteoverticillatus** (Shinobu 1956) Witt and Stackebrandt 1991, 456[VP] (Effective publication: Witt and Stackebrandt 1990, 370.) ("*Streptomyces luteoverticillatus*" Shinobu 1956; "*Verticillomyces luteoverticillatus*" Shinobu 1965; *Streptoverticillium luteoverticillatum* Locci, Baldacci and Petrolini Baldan 1969, 59)

lu.te.o.ver.ti.cil.la′tus. L. adj. *luteus* yellow; L. n. *verticillus* a whorl; N.L. adj. *verticillatus* whorled; N.L. masc. adj. *luteoverticillatus* yellow, whorled.

Spore chains in Section *Verticillati*, umbellate monoverticillate (biverticillate). Mature spore chains generally 10 to 20 or more spores per chain after 21 d on salts-starch agar or yeast-malt agar. Aerial mycelium is poorly developed or absent on yeast-malt agar, oatmeal agar, and glycerol-asparagine agar. Absence of good aerial mycelium on various media was also noted by Shinobu in his original description (op. cit). Spore surface is smooth.

Color of colony: mature aerial mycelium in the Red or Yellow color series on salts-starch agar; it is absent or poorly developed (white or yellow) on other ISP media. Reverse side of colony with no distinctive pigments (light brown to grayed yellow) on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar.

Color in medium: melanoid pigments are formed in peptone-yeast-iron agar, tyrosine agar, and tryptone-yeast broth. Yellow pigment is found in medium in yeast-malt agar, oatmeal agar, and salts-starch agar after 14–21 d; it is not a pH indicator.

D-Glucose, iso-inositol, and D-fructose are utilized for growth. No growth or only traces of growth on L-arabinose, sucrose, D-xylose, D-mannitol, rhamnose, and raffinose.

For sequence similarity, see type strain of *Streptomyces abikoensis*.

Source: not known.

DNA G+C content (mol%): not known.

Type strain: ATCC 23933, CBS 917.68, BCRC 13323, DSM 40038, NBRC 12887, NBRC 3840, JCM 4099, JCM 4649, NCIMB 9720, NRRL B-1995, NRRL-ISP 5038, RIA 1109, VKM Ac-889.

Sequence accession no. (16S rRNA gene): AB184803.

Further comments: in violation of Rule 33c of the *Bacteriological Code* (1990 Revision), in Validation List no. 38, *Streptomyces luteoverticillatus* is proposed as a *nomen revictum* (basonym: "*Streptomyces luteoverticillatus*" Shinobu 1956).

According to Hatano et al. (2003), *Streptomyces luteoverticillatus* (Shinobu 1956) Witt and Stackebrandt 1991 is a later heterotypic synonym of *Streptomyces abikoensis* (Umezawa et al. 1951) Witt and Stackebrandt 1991.

304. **Streptomyces lydicus** DeBoer, Dietz, Silver and Savage 1955b, 886[AL]

ly′di.cus. L. n. *Lydia* an ancient state in Asia Minor; N.L. masc. adj. *lydicus* of or belonging to Lydia.

Spore chains in Section *Spirales*. Spirals are sometimes at the tips of long spore chains suggesting *Retinaculiaperti* morphology. Mature spore chains generally have 10–50 or sometimes more than 50 spores per chain. This morphology is seen on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar. Spore surface is smooth.

Color of colony: aerial mass color in the Gray color series (3fe, light brownish gray, to 3ih, dark gray) on yeast-malt agar and oatmeal agar; Gray or Yellow (2ba, pale yellow) or White color series on salts-starch agar and glycerol-asparagine agar. Reverse side of colony with no distinctive pigments (colorless, pale grayish-yellow or orange-yellow) on yeast No distinctive pigments (colorless, pale grayish-yellow or orange-yellow) on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar.

Color in medium: melanoid pigments are not formed in peptone-yeast-iron agar, tyrosine agar, or tryptone-yeast broth, according to two of three observers. The third observer records some darkening of peptone-yeast-iron agar and a trace of dark pigment on tyrosine agar, but not darkening of tryptone-yeast broth. No pigment (or only a trace of yellow) is found in the medium in yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar.

D-Glucose, L-arabinose, D-xylose, iso-inositol, D-mannitol, D-fructose, sucrose, and raffinose are utilized for growth. Utilization of rhamnose is doubtful.

Type strain shows the highest sequence similarity to: *S. chattanoogensis*, AJ621611, 99.8%; *S. sioyaensis*, DQ026654, 99.3%; *S. rimosus* subsp. *paromomycinus*, AJ621610, 99.3%; *S. chrestomyceticus*, AJ621609, 99.3%; *S. tubercidicus*, AJ621612, 99.1%; *S. libani* subsp. *libani*, AB184414, 99.1%; *S. nigrescens*, DQ442530, 99%; *S. hygroscopius* subsp. *decoyicus*, AY999883, 99%.

Source: not known.

DNA G+C content (mol%): not known.

Type strain: AS 4.1412, ATCC 25470, CBS 703.69, BCRC 11919, CECT 3163, DSM 40461, HAMBI 1063, NBRC 13058, IMET 43531, JCM 4492, LMG 19331, NCIMB 12977, NRRL 2433, NRRL-ISP 5461, RIA 1250, VKM Ac-1869.

Sequence accession no. (16S rRNA gene): Y15507.

305. **Streptomyces macrosporus** (*ex* Krasil'nikov, Agre, Dorokhova and Sokolov 1968) Goodfellow, Lacey and Todd 1988, 328[VP] (Effective publication: Goodfellow, Lacey and Todd 1987a, 3148.) ("*Streptomyces macrosporus*" Krasil'nikov, Agre, Dorokhova and Sokolov 1968)

ma.cro.spo′rus. Gr. adj. *makros* long, large, Gr. n. *spora* seed; N.L. masc. adj. *macrosporus* large spored.

Spores mostly in tight spirals of up to six turns and 50 spores but sometimes in short straight chains of only five spores. Spores appear warty in transmission electron micrographs but are characteristically wrinkled in scanning electron micrographs, 0.7–1.8 × 0.7–1.6 µm, sometimes broader than long, mean 1.12 × 0.96 µm. Growth at 40°C on half-strength nutrient and V-8 juice agars is good; aerial mycelium is in the Green color series near 24ih (Tresner and Backus, 1963), with white flecks, although may be thin and white on half-strength nutrient agar. At 25°C, small colonies only are formed, sometimes with sparse white aerial mycelium. Substrate mycelium is colorless to dark brown with no distinctive pigments but often crystalline deposits are found in the agar. Melanoid pigments are not produced on peptone iron agar. No soluble pigments. Degrades esculin, arbutin, casein, DNA, gelatin, RNA, starch, and L-tyrosine; utilizes D-fructose, *myo*-inositol, D-mannose, L-rhamnose, trehalose, and D-xylose as sole carbon sources, and L-arginine, L-phenylalanine, potassium nitrate, and L-threonine as sole nitrogen sources. Proteolysis and lipolysis evident on egg yolk agar; pectin hydrolyzed; hydrogen sulfide produced but nitrate not reduced.

Type strain shows no sequence similarity over 99%.

Source: isolated from sewage compost and soil.

DNA G+C content (mol%): not known.

Type strain: K44, A1201, ATCC 51533, DSM 41449, HUT 6608, NBRC 14748, INMI 2892, JCM 6305, NCIMB 12473, VKM Ac-777.

Sequence accession no. (16S rRNA gene): Z68099.

306. **Streptomyces malachitofuscus** (*ex* Preobrazhenskaya et al. 1964) Preobrazhenskaya and Terekhova 1986, 574[VP] (Effective publication: Preobrazhenskaya and Terekhova *in* Gause, Preobrazhenskaya, Sveshnikova, Terekhova and Maximova 1983.) ("*Actinomyces malachitofuscus*" Preobrazhenskaya et al. 1964; "*Streptomyces malachitofuscus*" Pridham 1970)

ma.la.chi.to.fus′cus. L. n. *malache* the mallow; L. adj. *fuscus* dark or tawny; N.L. masc. adj. *malachitofuscus* mallow, dark (dark green).

Spore chains in Section *Spirales*. Mature spore chains generally have 10–50 spores per chain on yeast-malt agar, oatmeal agar, and salts-starch agar. Shorter spore chains, especially on glycerol-asparagine agar, may form irregular or incomplete spirals, loops, and hooks. Spore surface is spiny to hairy.

Color of colony: aerial mass color in the Gray color series (3fe, light brownish gray to 5fe, light reddish grayish brown) on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar. Reverse side of colony is nearly colorless to yellow or yellowish gray on oatmeal agar and salts-starch agar; grayish olive to olive brown on glycerol-asparagine agar.

Color in medium: melanoid pigments are formed in peptone-yeast-iron agar, tyrosine agar, and tryptone-yeast broth. No pigment is found in medium in salts-starch agar and glycerol-asparagine agar; a trace of yellow or yellow-brown pigment may or may not be found in yeast-malt agar and oatmeal agar.

D-Glucose, L-arabinose, D-xylose, iso-inositol, D-mannitol, D-fructose, rhamnose, and sucrose are utilized for growth. No growth or only traces of growth with raffinose.

Type strain shows the highest sequence similarity to: *S. griseoflavus*, AJ781322, 99.3%; *S. matensis*, AB184221, 99.3%; *S. griseorubens*, AB184139, 99.1%; *S. labedae*, AB184704, 99%; *S. griseoincarnatus*, AJ781328, 99%; *S. erythrogriseus*, AJ781328, 99%; *S. variabilis*, DQ442551, 99%; *S. flaveolus*, AB184764, 99%; *S. violaceorubidus*, AJ781374, 99%; *S. paradoxus*, AB184628, 99%; *S. glaucescens*, AB184843, 99%; *S. albaduncus*, AY999757, 99%; *S. gancidicus*, AB184660, 99%; *S. griseoloalbus*, AB184275, 99%; *S. djakartensis*, AB184657, 99%.

Source: not known.

DNA G+C content (mol%): not known.

Type strain: ATCC 25471, CBS 881.69, DSM 40332, NBRC 13059, INA 739, JCM 4493, KCC S-0493, NRRL B-12273, NRRL-ISP 5332, RIA 1251, VKM Ac-1850.

Sequence accession no. (16S rRNA gene): AB184282.

307. **Streptomyces malachitospinus** (*ex* Preobrazhenskaya et al. 1957) Preobrazhenskaya and Terekhova 1986, 574[VP] (Effective publication: Preobrazhenskaya and Terekhova *in* Gause, Preobrazhenskaya, Sveshnikova, Terekhova and Maximova 1983.) ("*Actinomyces malachitospinus*" Preobrazhenskaya et al. 1957)

ma.la.chi.to.spi′nus. L. n. *malache* the mallow the mallow; L. adj. *spineus* spiny; N.L. masc. adj. *malachitospinus* spiny and mallow green.

Spore chains are spiral (*Spirales*); spores are spiny, spines are long. On mineral agar 1, glycerol-nitrate agar, oatmeal agar: aerial mycelium is light gray; substrate mycelium is green; no diffusible pigment. On starch-ammonia agar: aerial mycelium is absent or light gray; substrate mycelium is green; no diffusible pigment. On glycerol-asparagine agar: aerial mycelium is white to light gray; substrate mycelium is yellowish green; no diffusible pigment. On organic agar 2: aerial mycelium is white or light gray; substrate mycelium is yellowish to greenish; no diffusible pigment. Color of substrate mycelium is a result of green pigment ferroverdin. Melanoid pigments are not formed. Glucose, arabinose, galactose, glycerol, and starch are utilized for growth; no growth with sucrose. Antibiotic: not isolated. Strain INMI 217^T builds a physiologically active substance which stimulates the building of zygotes in Mucorales.

Type strain shows the highest sequence similarity to: *S. parvulus*, AB184326, 99.3%; *S. violaceorubidus*, AJ781374, 99.2%; *S. lomondensis*, AB184673, 99.2%; *S. pactum*, AB184398, 99.2%; *S. luteogriseus*, AB184379, 99.1%; *S. rubrogriseus*, AB184681, 99%; *S. olivaceus*, AB184743, 99%; *S. tendae*, D63873, 99%.

Source: not known.

DNA G+C content (mol%): not known.

Type strain: INMI 217, INA 316, NBRC 101004.

Sequence accession no. (16S rRNA gene): AB249954.

308. **Streptomyces malaysiensis** Al-Tai, Kim, Kim, Manfio and Goodfellow 1999, 1401[VP]

mal.ay.si.en′sis. N.L. masc. adj. *malaysiensis* of or belonging/pertaining to Malaysia, the source of the soil from which the organism was isolated.

Forms extensively branched substrate hyphae (0.3–0.5 µm in diameter) and aerial hyphae which differentiate into tight, spiral spore chains. The appearance of the spores ranges from cylindrical to barrel-shaped (1.3–1.0 × 1.5 µm) and the spore surface is rugose. On inorganic salts-starch agar, the aerial spore-mass color is gray and a yellow diffusible pigment is formed. Xanthine is not degraded. Growth does not occur at 10 or 45°C.

Type strain shows no sequence similarity over 99%.

Source: isolated from a soil sample collected at Tasek Bera, Malaysia's first Ramsar Site.

DNA G+C content (mol%): 72.2.

Type strain: ATB-11, ATCC BAA-13, DSM 41697, NBRC 16446, JCM 10672.

Sequence accession no. (16S rRNA gene): AB249918.

309. **Streptomyces mashuensis** (Sawazaki, Suzuki, Nakamura, Kawasaki, Yamashita, Isono, Anzai, Serizawa and Sekiyama 1955) Witt and Stackebrandt 1991, 456[VP] (Effective publication: Witt and Stackebrandt 1990, 370.) ("*Streptomyces mashuensis*" Sawazaki, Suzuki, Nakamura, Kawasaki, Yamashita, Isono, Anzai, Serizawa and Sekiyama 1955, 46; "*Verticillomyces mashuensis*" Shinobu 1965; *Streptoverticillium mashuense* Locci, Baldacci and Petrolini Baldan 1969, 59)

ma.shu.en′sis. N.L. masc. adj. *mashuensis* of or pertaining to Lake Mashu, Japan.

Spore chains in Section *Verticillati*, umbellate monoverticillate (biverticillate). Spore chains are short with 3–10, or sometimes more than 10, spores per chain. Spore surface is smooth.

Color of colony: aerial mass color in the Red color series on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar. Mature aerial mycelium may be poorly developed on glycerol-asparagine agar. Reverse side of colony with no distinctive pigments (grayish yellow to orange yellow on oatmeal agar, salts-starch agar, and glycerol-asparagine agar; olive brown to brown on yeast-malt agar).

Color in medium: melanoid pigments are produced in peptone-yeast-iron agar and tryptone-yeast broth, but not in tyrosine agar. No pigment found in medium in yeast-malt agar, oatmeal agar, salts-starch agar, or glycerol-asparagine agar.

D-Glucose, sucrose, and D-fructose are utilized for growth. Utilization of iso-inositol is doubtful. No growth or only traces of growth on L-arabinose, D-xylose, D-mannitol, rhamnose, and raffinose.

Type strain shows no sequence similarity over 99%.

Source: not known.

DNA G+C content (mol%): not known.

Type strain: ATCC 23934, CBS 279.65, CBS 918.68, BCRC 12420, DSM 40221, IFM 1082, NBRC 12888, IMET 42941, JCM 4059, JCM 4650, NRRL B-3352, NRRL B-8164, NRRL-ISP 5221, RIA 1165, VKM Ac-949.

Sequence accession no. (16S rRNA gene): X79323.

Further comments: in violation of Rule 33c of the *Bacteriological Code* (1990 Revision), in Validation List no. 38, *Streptomyces mashuensis* is proposed as a *nomen revictum* (basonym: "*Streptomyces mashuensis*" Sawazaki et al. 1955).

According to Hatano et al. (2003), *Streptomyces mashuensis* (Sawazaki et al. 1955) Witt and Stackebrandt 1991 is an earlier heterotypic synonym of *Streptomyces kishiwadensis* (Shinobu and Kayamura 1964) Witt and Stackebrandt 1991.

310. **Streptomyces massasporeus** Shinobu and Kawato 1959, 283[AL]

mas.sa.spo′re.us. L. n. *massa* mass, lump; N.L. n. *spora* a spore; N.L. masc. adj. *massasporeus* mass, spore, referring to the coalescence of spores into moist masses.

Spore chains in Section *Spirales*, but spore chains representative of Section *Retinaculiaperti* are also present. Mature spore chains generally have 10–50 spores per chain. This morphology is seen on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar. Spore surface is smooth. Special morphological characteristics: spores frequently coalesce to form moist masses of spores. Small masses may form from single spiral spore chains of large masses may form by the coalescence of many spirals.

Color of colony: aerial mass color in the Red color series on yeast-malt agar, salts-starch agar, and glycerol-asparagine agar. Red or White series on oatmeal agar. Reverse side of colony is grayed yellow modified by red or violet (see pH) on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar. Reverse color is changed from violet to red by addition of 0.05 M HCl and from red to reddish violet to blue violet by addition of 0.05 M NaOH.

Color in medium: melanoid pigments formed in peptone-yeast-iron agar. Red pigments found in medium in oatmeal agar and glycerol-asparagine agar. One observer detected red pigment in yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar in 14 d and violet pigment in these media at 21 d; changes in color from violet to blue by addition of 0.05 M NaOH and from violet to red by addition of 0.05 M HCl were also noted.

D-Glucose, L-arabinose, sucrose, D-xylose, iso-inositol, D-mannitol, D-fructose, rhamnose, and raffinose are utilized for growth.

Type strain shows the highest sequence similarity to: *S. hawaiiensis*, AB184143, 99.7%; *S. indiaensis*, AB184553, 99.6%; *S. arenae*, AB249977, 99.4%; *S. purpurascens*, AJ399486, 99.3%; *S. luteogriseus*, AB184379, 99.2%; *S. coerulescens*, AY999720, 99%; *S. albosporeus* subsp. *albosporeus*, AJ781327, 99%; *S. janthinus*, AB184851, 99%; *S. roseoviolaceus*, AJ399484, 99%; *S. violaceus*, AB184315, 99%; *S. bellus*, AB184849, 99%.

Source: not known.

DNA G+C content (mol%): not known.

Type strain: AS 4.1433, ATCC 19785, CBS 537.68, BCRC 13647, DSM 40035, NBRC 12796, NBRC 3841, JCM 4139, JCM 4593, LMG 19362, NRRL B-3300, NRRL-ISP 5035, RIA 1064, RIA 652, UNIQEM 171, VKM Ac-578.

Sequence accession no. (16S rRNA gene): AB184152.

311. **Streptomyces matensis** Margalith, Beretta and Timbal 1959, 71[AL]

mat.en′sis. N.L. masc. adj. *matensis* of or belonging to mat (of uncertain derivation).

Spore chains in Section *Spirales*. Open spirals are the dominant forms; spore chains suggesting primitive spirals of *Retinaculiaperti* cultures or flexuous chains of *Rectiflexibiles* are also present. Mature spore chains are moderately long with 10–50 spores per chain. This morphology is seen on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar. Spore surface is spiny. Spines are short and inconspicuous; some smooth spores may be seen.

Color of colony: mature aerial mass color in the Gray color series (color tab 3ge, light grayish yellowish brown; to 5fe light grayish reddish brown) on salts-starch agar; Gray or White color series on yeast-malt agar and glycerol-asparagine agar. One observer reports tab 5dc (grayish yellowish pink) from the Red color series for growth on yeast-malt agar, oatmeal agar, and salts-starch agar. This trace of red (or violet) pigment in aerial color is also noted in the original description (Margalith et al., 1959). Reverse side of colony with red pigments in mycelium which modify characteristic substrate colors to dark reddish brown on yeast-malt agar and salts-starch agar; light grayish brown or yellowish pink on oatmeal agar and glycerol-asparagine agar. The substrate mycelium pigment is not a strong pH indicator (one observer reports change from violet to pink with addition of 0.05 M HCl).

Color in medium: melanoid pigments are not found in peptone-yeast-iron agar, tyrosine agar, or tryptone-yeast broth. Red or violet pigments are found in yeast-malt agar, oatmeal agar, and glycerol-asparagine agar. One observer reports a change from violet to red by addition of 0.05 M HCl.

D-Glucose, L-arabinose, D-xylose, iso-inositol, D-mannitol, D-fructose, and rhamnose are utilized for growth. No growth or only traces of growth on raffinose. Utilization of sucrose is doubtful.

Type strain shows the highest sequence similarity to: *S. althioticus*, AY999808, 99.8%; *S. griseorubens*, AB184139, 99.7%; *S. labedae*, AB184704, 99.6%; *S. variabilis*, DQ442551, 99.6%; *S. griseoincarnatus*, AJ781328, 99.6%; *S. paradoxus*, AB184628, 99.6%; *S. erythrogriseus*, AJ781328, 99.6%; *S. griseoflavus*, AJ781322, 99.5%; *S. violaceorubidus*, AJ781374, 99.4%; *S. viridochromogenes*, DQ442555, 99.3%; *S. albaduncus*, AY999757, 99.3%; *S. lusitanus*, AB184424, 99.3%; *S. malachitofuscus*, AB184282, 99.3%; *S. coerulescens*, AY999720, 99.2%; *S. heliomycini*, AB184712, 99.2%; *S. glaucescens*, AB184843, 99.2%; *S. tendae*, D63873, 99.2%; *S. capillispiralis*, AB184577, 99.2%; *S. gancidicus*, AB184660, 99.2%; *S. pseudogriseolus*, DQ442541, 99.2%; *S. griseoloalbus*, AB184275, 99.2%; *S. bellus*, AB184849, 99.2%; *S. collinus*, AB184123, 99.2%; *S. lienomycini*, AJ781353, 99.1%; *S. violaceochromogenes*, AY999867, 99.1%; *S. flaveolus*, AB184764, 99.1%; *S. cellulosae*, DQ442495, 99.1%; *S. longispororuber*, AB184440, 99%; *S. rubrogriseus*, AB184681, 99%; *S. ambofaciens*, M27245, 99%; *S. diastaticus* subsp. *diastaticus*, AB184785, 99%; *S. gougerotii*, AB184742, 99%; *S. rutgersensis* subsp. *rutgersensis*, AB184170, 99%; *S. azureus*, EF178674, 99%; *S. lomondensis*, AB184673, 99%; *S. fragilis*, AY999917, 99%; *S. intermedius*, AB184277, 99%; *S. misionensis*, EF178678, 99%.

Source: not known.

DNA G+C content (mol%): not known.

Type strain: ATCC 23935, CBS 919.68, DSM 40188, HAMBI 1048, NBRC 12889, IMET 42065, JCM 4277, JCM 4268, JCM 4651, NCIMB 9826, NRRL B-2576, NRRL-ISP 5188, RIA 1142, RIA 570.

Sequence accession no. (16S rRNA gene): AB184221.

312. **Streptomyces mauvecolor** Okami and Umezawa *in* Murase, Hikiji, Nitta, Okami, Takeuchi and Umezawa 1961, 117[AL]

mau.ve.co′lor. L. n. *malva* mallow, a plant with violet-colored petals, hence French *mauve*; L. n. *color* color; N.L. adj. *mauvecolor* mauve colored.

Probably grows poorly on Czapek's solution agar. Produces peptimycin, a peptidic anti-tumor antibiotic.

Type strain shows the highest sequence similarity to: *S. xanthochromogenes*, DQ442559, 99.4%; *S. michiganensis*, AB184153, 99.4%; *S. cyaneofuscatus*, AB184860, 99%; *S. flavofuscus*, AB249935, 99%; *S. cinereorectus*, AB184646, 99%; *S. sannanensis*, AB184579, 99%; *S. fimicarius*, AY999784, 99%; *S. anulatus*, DQ026637, 99%; *S. praecox*, AB184293, 99%.

Source: not known.

DNA G+C content (mol%): not known.

Type strain: ATCC 29835, DSM 41702, NBRC 13854, JCM 5002.

Sequence accession no. (16S rRNA gene): AB184532.

313. **Streptomyces mediolani** Bianchi, Grein, Julita, Marnati and Spalla 1970, 243[AL]

me.di.o.la′ni. L. n. *Mediolanum* Milan; L. gen. adj. *mediolani* of Milan.

Sporophores are long and straight; from these, through monopodial branching, the spore bearing hyphae originate as long, straight to slightly flexuous filaments. Spores are cylindrical in shape, and devoid of any ornamentation. Growth is always very good on most common agar media; the substrate mycelium grows as a compact, smooth patina, showing a yolk-yellow to orange-yellow color. The aerial mycelium is very abundant showing a velvety to dusty aspect; its color varies from yellow-vanilla to yellow-rose or yellow-beige, according to the different substrates on which it is grown.

The spore suspension prepared from a culture of *S. mediolani* grown on a solid medium containing yeast extract, glucose, and inorganic salts, was used to inoculate 300 ml Erlenmeyer flasks each containing 60 ml liquid medium having dextrin, casein, corn-steep liquor, calcium carbonate and other inorganic salts as ingredients. After 27 h of incubation at 28°C on a rotary shaker, aliquots of the culture were transferred to 300 ml Erlenmeyer flasks each containing 30 ml of the following production medium: soluble starch, 37.5 g; morsuit (a maltose-rich syrup manufactured by F.R.A.G.D., Italy), 33 g; soybean meal 40 g; NaCl, 1.25 g; KH_2PO_2, 3 g; $MgSO_4{\cdot}7H_2O$, 0.5 g; $CaCO_3$, 3.5 g; $(NH_4)_2SO_4$, 1.5 g; $ZnSO_4{\cdot}7H_2O$, 0.005 g; $FeSO_4{\cdot}7H_2O$, 0.005 g; $CuSO_4{\cdot}5H_2O$, 0.001 g; tap water up to 1000 ml. The flasks were incubated for 8 d on a rotary shaker at 29°C.

The extracts prepared from the mycelium of *S. mediolani* with the aid of different organic solvents contained three main carotenoids, designated A, B, and C, together with other minor pigments. Compounds A and B were epiphasic, while pigment C precipitated at the interfaces in the biphasic system petroleum ether (b.p. 40–60°C)/90% aqueous methanol. Separation of the epiphasic pigments by chromatography on aluminium oxide, using petroleum ether containing increasing amounts of acetone as developing agent, allowed isolation of pigment A [$C_{40}H_{48}$, red crystals m.p. 200–201°C, λ_{max} (petroleum ether) 278, 428 (inflexion), 448, 478 nm] and pigment B [amorphous red power, m.p. 180–185°C, λ_{max} (petroleum ether) 278, 340 (*cis*-peak), 425 (inflexion), 446, 472 nm]. Pigment C was purified by chromatography on silicic acid using 10% acetone in benzene as developing solvent and isolated as a dark-red amorphous solid [m.p. 200°C (dec), λ_{max} (petroleum ether) 278, 340 (*cis*-peak), 425 (inflexion), 445, 475 nm].

Pigment A proved to be identical to isorenieratene (i) by direct comparison with an authentic sample. Pigments C and B were soluble in ethanolic potassium hydroxide and showed a hydroxyl band in the IR at 3590 cm^{-1} (methylene chloride solution). On treatment with acetic anhydride and pyridine, pigments B and C were converted to a monoacetate ($C_{42}H_{50}O_2$, m.p. 156–158°C) and a diacetate ($C_{44}H_{52}O_4$, m.p. 182–184°C), respectively, both derivatives showing UV and visible spectra identical to those displayed by I (no *cis*-peak), and a phenolic acetate band at 1758 cm^{-1} in the IR (KBr pellets). Treatment of the acetyl derivative of pigment B with chromic anhydride in benzene-acetic acid gave crocetindial, 2,3,6-trimethylbenzaldehyde, and 2,3,6-trimethyl-4-acetoxybenzaldehyde, as main breakdown products. All compounds were identified by direct comparison (UV, visible, and IR-spectra, mixed m.p., chromatographic behavior) with authentic samples prepared by synthesis. Similarly, oxidation of the diacetyl derivative of pigment C gave crocetindial and 2,3,6-trimethyl-4-acetoxybenzaldehyde as major reaction products. Structures II and III could therefore be written for pigments B and C.

The new carotenoids B (3-hydroxyisorenieratene) and C (3,3′-dihydroxyisorenieratene) have been subsequently prepared by chemical synthesis. The Wittig reaction of crocetindial with triphenyl-2,3,6-trimethylbenzylphosphonium bromide and triphenyl-[2,3,6-trimethyl-4-(2′-tetrahydropyranyloxy)]-benzylphosphonium bromide as reagents was used. For the preparation of the latter reagent, 2,3,6-trimethyl-4-hydroxybenzaldehyde was converted to the 4-(2′-tetrahydopyranyloxy) derivative, then reduced with lithium aluminium hydride to 2,3,6-trimethyl-4-(2′-tetrahydropyranyloxy)-benzylalcohol (m.p. 85°C), which was in turn converted to the phosphonium bromide by the usual procedure. When crocetindial was allowed to react with triphenyl-2,3,6-trimethyl-4-(2′-tetrahydropyranyloxy)-benzylphosphonium bromide in the presence of *n*-butyl lithium, symmetric 3,3′-ditetrahydropyranyloxyisorenieratene, $C_{50}H_{64}O_4$ (m.p. 185–187°C), was obtained. Removal of the protecting group by acid treatment afforded III (overall yield 60%). When the Wittig reaction was repeated starting with a mixture of both phosphonium bromides, a product was obtained from which, after removal of the tetrahydropyranyl group, 3-hydoxy-isorenieratene (II) was isolated in 30% yield, together with lower amounts of I and III. The synthetic compounds II and II displayed the same visible and IR-spectra, and identical chromatographic behavior, also after acetylation, as natural pigments B and C.

The peptide antibiotic histidomycin was isolated from the filtered broth by absorption-elution on ion exchange resins followed by molecular sieves fractionation. The identification of the antibiotic was performed on the basis of its chemical and biological properties, and of direct comparison with an authentic sample. The substance was responsible for the anti-bacterial activity displayed by culture liquids of *S. mediolani.*

Type strain shows the highest sequence similarity to: *S. sindenensis*, AB184759, 100%; *S. rubiginosohelvolus*, AB184240, 100%; *S. albovinaceus*, AB249958, 100%; *S. griseoplanus*, AY999894, 100%; *S. anulatus*, DQ026637, 100%; *S. globisporus* subsp. *globisporus*, EF178686, 100%; *S. pluricolorescens*, DQ442540, 100%; *S. praecox*, AB184293, 100%; *S. flavofuscus*, AB249935, 100%; *S. fimicarius*, AY999784, 100%; *S. badius*, AY999783, 100%; *S. griseinus*,

AB184205, 100%; *S. californicus*, AB184755, 99.9%; *S. lavendulae* subsp. *lavendulae*, AB184080, 99.9%; *S. cavourensis* subsp. *washingtonensis*, DQ026671, 99.9%; *S. acrimycini*, AY999889, 99.9%; *S. parvus*, DQ442537, 99.9%; *S. fulvorobeus*, AB184711, 99.8%; *S. lipmanii*, AB184148, 99.8%; *S. microflavus*, DQ445795, 99.8%; *S. cyaneofuscatus*, AB184860, 99.8%; *S. floridae*, AB184656, 99.8%; *S. alboviridis*, AB184256, 99.8%; *S. baarnensis*, EF178688, 99.8%; *S. cinereorectus*, AB184646, 99.8%; *S. griseus* subsp. *griseus*, AY207604, 99.7%; *S. griseolus*, AB184768, 99.7%; *S. luridiscabiei*, AF361784, 99.7%; *S. flavovirens*, DQ026635, 99.7%; *S. argenteolus*, AB045872, 99.7%; *S. bacillaris*, AB184439, 99.6%; *S. pulveraceus*, AB184806, 99.6%; *S. halstedii*, EF178695, 99.6%; *S. flavogriseus*, AJ494864, 99.6%; *S. atroolivaceus*, AJ781320, 99.5%; *S. olivoviridis*, AB184227, 99.5%; *S. finlayi*, AY999788, 99.5%; *S. nitrosporeus*, EF178680, 99.5%; *S. albolongus*, AB184425, 99.4%; *S. clavifer*, DQ026670, 99.4%; *S. griseobrunneus*, AB249912, 99.4%; *S. sanglieri*, AB249945, 99.4%; *S. celluloflavus*, AB184476, 99.4%; *S. yanii*, AB006159, 99.4%; *S. gelaticus*, DQ026636, 99.3%; *S. atratus*, DQ026638, 99.3%; *S. mutomycini*, AB249951, 99.3%; *S. candidus*, DQ026663, 99.2%; *S. cavourensis* subsp. *cavourensis*, DQ445791, 99.2%; *S. spiroverticillatus*, AB184814, 99.1%; *S. cremeus*, AB184124, 99.1%.

Source: not known.

DNA G+C content (mol%): not known.

Type strain: ATCC 33021, BCRC 12035, CMI 134886, DSM 41058, DSM 41647, NBRC 15427, JCM 5076, NCIMB 10969, VKM Ac-1917.

Sequence accession no. (16S rRNA gene): AB184674.

314. **Streptomyces megasporus** (*ex* Krasil'nikov, Agre, Dorokhova and Sokolov 1968) Agre 1986, 575[VP] (Effective publication: Agre *in* Gause, Preobrazhenskaya, Sveshnikova, Terekhova and Maximova 1983.) ("*Actinomyces megasporus*" Krasil'nikov, Agre, Dorokhova and Sokolov 1968)

me.ga.spo′rus. Gr. adj. *megas* big; Gr. n. *spora* seed; N.L. masc. adj. *megasporus* large spored.

Spore chains are spirals (*Spirales*); spores have spines and warts. On mineral agar 1: aerial mycelium is pale green-gray; substrate mycelium is colorless; no diffusible pigment. On glycerol-nitrate agar and oatmeal agar: aerial mycelium is white or absent; substrate mycelium is colorless; no diffusible pigment. On starch-ammonia agar: poor growth; aerial mycelium is poor, grayish; substrate mycelium is colorless; no diffusible pigment. On glycerol-asparagine agar: poor growth; aerial mycelium is white; substrate mycelium is colorless; no diffusible pigment. On organic agar 2: aerial mycelium absent; substrate mycelium colorless or yellow; no diffusible pigment. Melanoid pigments are not formed. Growth on glucose, maltose, xylose, rhamnose, and arabinose.

Type strain shows no sequence similarity over 99%.

Source: not known.

DNA G+C content (mol%): not known.

Type strain: ATCC 43688, DSM 41476, HUT 6610, NBRC 14749, INA M-22, INMI 2869, JCM 6926, NRRL B-16372, VKM Ac-1776.

Sequence accession no. (16S rRNA gene): AB184617.

315. **Streptomyces melanogenes** Sugawara and Onuma 1957, 141[AL]

me.la.no′ge.nes. Gr. adj. *melas* black; N.L. suff. -*genes* (from Gr. v. *gennaô* to produce) producing; N.L. part. adj. *melanogenes* producing black.

Spore chains in Section *Rectiflexibiles*. Mature spore chains are long with 10–50, or often more than 50, spores per chain. This morphology is seen on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar. Spore surface is smooth.

Color of colony: aerial mass color in the Red color series (5cb, grayish yellowish pink) on oatmeal agar and salts-starch agar; White or Gray color series on yeast-malt agar and salts-starch agar. Reverse side of colony is grayed yellow to yellow-brown is usually modified by red (becoming dark brown to reddish brown) on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar; this pigment is not a pH indicator.

Color in medium: melanoid pigments are formed in peptone-yeast-iron agar, tyrosine agar, and tryptone-yeast broth. This pigment is not pH-sensitive when tested with 0.05 M NaOH or HCl.

D-Glucose, L-arabinose, D-xylose, iso-inositol, D-mannitol, D-fructose, and raffinose are utilized for growth. Reports vary on utilization of sucrose. No growth or only traces of growth rhamnose.

Type strain shows the highest sequence similarity to: *S. noboritoensis*, AB184287, 100%; *S. crystallinus*, AB184652, 99.2%.

Source: not known.

DNA G+C content (mol%): not known.

Type strain: ATCC 23937, CBS 921.68, BCRC 12053, DSM 40192, NBRC 12890, JCM 4398, NCIMB 9835, NRRL B-2072, NRRL-ISP 5192, RIA 1146.

Sequence accession no. (16S rRNA gene): AB184222.

316. **Streptomyces melanosporofaciens** Arcamone, Bertazzoli, Ghione and Scotti 1959, 215[AL]

me.la.no.spo.ro.fa′ci.ens. Gr. adj. *melas* black; N.L. n. *spora* a spore; L. part. adj. *faciens* producing; N.L. part. adj. *melanosporofaciens* black spore producing.

Spore chains in Section *Spirales*, but spore chain morphology is sometimes difficult to observe because of accumulations of moist exudate ("hygroscopic" masses) on the sporulating surfaces. Identifiable spore chains are often short with only 3–10 spores per chain and may form loops or incomplete spirals as well as true spirals. This morphology is seen on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar. Spore surface is smooth, or with unevenly ridged surface suggesting warty spores on some preparations. In 7–21 d, sporulating surfaces form globose accumulations of liquid exudate. These coalesce and may eventually give rise to a moist black surface containing loose spores.

Color of colony: aerial mass color in the Gray color series on yeast-malt agar and oatmeal agar; poorly sporulating cultures on salts-starch agar and glycerol-asparagine agar may appear to be in the Yellow color series. Areas of sporulation are usually gray at first, but may become

moist-black as spore masses coalesce in a liquid exudate on the aerial mycelium. Reverse side of colony is dark grayish yellow to orange-yellow on yeast-malt agar; light yellow to light grayish olive on oatmeal agar; nearly colorless to strong yellow on salts-starch agar and glycerol-asparagine agar. Substrate pigment is not a pH indicator.

Color in medium: melanoid pigments are not formed in peptone-yeast-iron agar, tyrosine agar, or tryptone-yeast broth, but a reddish brown, rose, or yellow non-melanoid pigment may be found in tyrosine agar. No pigment is found in medium in yeast-malt agar, oatmeal agar, salts-starch agar, or glycerol-asparagine agar.

D-Glucose, L-arabinose, D-xylose, iso-inositol, D-mannitol, D-fructose, rhamnose, and raffinose are utilized for growth. Only traces of growth are found with sucrose.

Type strain shows the highest sequence similarity to: *S. sporoclivatus*, AB249934, 99.6%; *S. antimycoticus*, AB184185, 99.6%; *S. rutgersensis* subsp. *castelarensis*, AY508511, 99.4%; *S. geldanamycininus*, DQ334781, 99%.

Source: not known.

DNA G+C content (mol%): not known.

Type strain: ATCC 25473, CBS 883.69, BCRC 12064, DSM 40318, NBRC 13061, JCM 4495, NCIMB 12978, NRRL B-12234, NRRL-ISP 5318, RIA 1253, VKM Ac-1864.

Sequence accession no. (16S rRNA gene): AJ271887.

317. **Streptomyces mexicanus** Petrosyan, García-Varela, Luz-Madrigal, Huitrón and Flores 2003, 272[VP]

mex.i.ca'nus. N.L. masc. adj. *mexicanus* of or belonging to Mexico, the source of the soil from which the organism was isolated.

Forms highly branched substrate mycelium and aerial hyphae which differentiate into long *Rectiflexibiles* chains of 10 or more, green, smooth spores. Spores are spherical, about 0.88 μm in diameter, or slightly oval. Aerial spore mass color is gray with a slight green tint. Substrate mycelium is beige on standard media. Yellowish diffusible pigments are formed on yeast-extract, malt-extract, and oatmeal agar. Melanin pigments are not produced on peptone-iron or tyrosine agars. Positive for H_2S production. Degrades arbutin, starch, xylan, adenine, casein, hypoxanthine, and L-tyrosine, but not guanine or testosterone. Utilizes L-arabinose, fructose, glucose, raffinose, mannitol, mannose, xylose, galactose, maltose, glycerol, lactose, cellobiose, trehalose, and sodium acetate as sole carbon sources, but not *myo*-inositol, melezitose, L-rhamnose, sorbitol, or sucrose. Growth occurs between 20 and 55°C, from pH 4.3–8.0, and in the presence of 6% (w/v) NaCl and ampicillin (100 μg/ml). Growth is inhibited in the presence of chloramphenicol, erythromycin, gentamicin, nalidixic acid sodium salt, kanamycin sulfate, rifampin, tetracycline hydrochloride, and thiostrepton, and in the presence of 7% (w/v) NaCl. No antimicrobial activity is shown against *Escherichia coli* JM 109 or against representative strains of *Aspergillus flavipes*, *Aureobasidium* sp., *Bacillus subtilis*, *Pichia pastoris*, "*Sarcina lutea*", or *Trichoderma viridae*.

Type strain shows the highest sequence similarity to: *S. thermoviolaceus* subsp. *apingens*, Z68095, 99%.

Source: isolated from a soil sample obtained at a sugarcane field in the State of Morelos, Mexico.

DNA G+C content (mol%): not known.

Type strain: CH-M-1035, BM-B-384, DSM 41796, JCM 12681, NBRC 100915, NRRL B-24196.

Sequence accession no. (16S rRNA gene): AB249966.

318. **Streptomyces michiganensis** Corbaz, Ettlinger, Keller-Schierlein and Zähner 1957b, 205[AL]

mi.chi.gan.en'sis. N.L. masc. adj. *michiganensis* of or belonging to Michigan, the source of the soil from which the organism was isolated.

Spore chain morphology in Section *Rectiflexibiles*. Mature spore chains are generally long, often with more than 50 spores per chain. This morphology is seen on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar. Spore surface is smooth.

Color of colony: aerial mass color in the Yellow color series on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar. Reverse side of colony with no distinctive pigment (colorless or characteristic grayed yellow on oatmeal agar, salts-starch agar, and glycerol-asparagine agar).

Color in medium: melanoid pigments formed in peptone-yeast-iron agar and tryptone-yeast broth. Yellow pigment found in medium in yeast-malt agar and oatmeal agar. This pigment is not pH-sensitive when tested with 0.05 M NaOH of HCl.

D-Glucose, D-xylose, iso-inositol, D-mannitol, and D-fructose are utilized for growth. No growth or only trace of growth on L-arabinose, sucrose, and rhamnose. Variable reports on growth with raffinose.

Type strain shows the highest sequence similarity to: *S. xanthochromogenes*, DQ442559, 100%; *S. mauvecolor*, AB184532, 99.4%.

Source: isolated from soil from Michigan.

DNA G+C content (mol%): not known.

Type strain: ATCC 14970, ATCC 19786, CBS 538.68, BCRC 11613, DSM 40015, NBRC 12797, JCM 4594, NRRL B-1940, NRRL-ISP 5015, RIA 1065, UNIQEM 172, VKM Ac-862.

Sequence accession no. (16S rRNA gene): AB184153.

319. **Streptomyces microflavus** (Krainsky 1914) Waksman and Henrici *in* Breed, Murray and Hitchens 1948, 950[AL] ("*Actinomyces microflavus*" Krainsky 1914, 686; "*Micromonospora microflava*" Duché 1934, 29)

mic.ro.fla'vus. Gr. adj. *mikros* small; L. adj. *flavus* yellow; N.L. masc. adj. *microflavus* small, yellow.

Spore chains in Section *Rectiflexibiles*. Mature spore chains generally have 10–50 spores per chain. This morphology is seen on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar. Spore surface is smooth.

Color of colony: aerial mass color in the Gray or Yellow color series (2dc, yellowish gray; 2db, pale yellow) on yeast-malt agar and oatmeal agar; Yellow color series (2ba or 2db, pale yellow) on salts-starch agar and glycerol-asparagine agar. Reverse side of colony with no distinctive pigments (grayish yellow to yellowish brown, olive brown or

strong brown) on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar.

Color in medium: melanoid pigments are not formed in peptone-yeast-iron agar, tyrosine agar, or tryptone-yeast broth. No pigment found in medium in yeast-malt agar, oatmeal agar, salts-starch agar, or glycerol-asparagine agar.

D-Glucose, D-xylose, D-mannitol, D-fructose, and rhamnose are utilized for growth. Reports vary on the utilization of sucrose. Utilization of L-arabinose is doubtful. No growth or only traces of growth with iso-inositol and raffinose.

Type strain shows the highest sequence similarity to: *S. fulvorobeus*, AB184711, 100%; *S. lipmanii*, AB184148, 100%; *S. lavendulae* subsp. *lavendulae*, AB184080, 100%; *S. alboviridis*, AB184256, 100%; *S. cavourensis* subsp. *washingtonensis*, DQ026671, 99.9%; *S. flavofuscus*, AB249935, 99.9%; *S. cinereorectus*, AB184646, 99.9%; *S. fimicarius*, AY999784, 99.9%; *S. griseoplanus*, AY999894, 99.9%; *S. cyaneofuscatus*, AB184860, 99.9%; *S. floridae*, AB184656, 99.9%; *S. praecox*, AB184293, 99.9%; *S. anulatus*, DQ026637, 99.8%; *S. luridiscabiei*, AF361784, 99.8%; *S. pluricolorescens*, DQ442540, 99.8%; *S. acrimycini*, AY999889, 99.8%; *S. argenteolus*, AB045872, 99.8%; *S. griseinus*, AB184205, 99.8%; *S. badius*, AY999783, 99.8%; *S. rubiginosohelvolus*, AB184240, 99.8%; *S. sindenensis*, AB184759, 99.8%; *S. mediolani*, AB184674, 99.8%; *S. californicus*, AB184755, 99.8%; *S. parvus*, DQ442537, 99.7%; *S. halstedii*, EF178695, 99.7%; *S. baarnensis*, EF178688, 99.7%; *S. griseolus*, AB184768, 99.7%; *S. griseus* subsp. *griseus*, AY207604, 99.7%; *S. globisporus* subsp. *globisporus*, EF178686, 99.7%; *S. albovinaceus*, AB249958, 99.7%; *S. flavovirens*, DQ026635, 99.5%; *S. finlayi*, AY999788, 99.5%; *S. flavogriseus*, AJ494864, 99.5%; *S. yanii*, AB006159, 99.4%; *S. atroolivaceus*, AJ781320, 99.4%; *S. olivoviridis*, AB184227, 99.4%; *S. bacillaris*, AB184439, 99.4%; *S. pulveraceus*, AB184806, 99.4%; *S. celluloflavus*, AB184476, 99.3%; *S. griseobrunneus*, AB249912, 99.3%; *S. albolongus*, AB184425, 99.3%; *S. clavifer*, DQ026670, 99.3%; *S. nitrosporeus*, EF178680, 99.3%; *S. spiroverticillatus*, AB184814, 99.2%; *S. sanglieri*, AB249945, 99.2%; *S. cavourensis* subsp. *cavourensis*, DQ445791, 99.2%; *S. atratus*, DQ026638, 99.1%; *S. mutomycini*, AB249951, 99.1%; *S. candidus*, DQ026663, 99.1%; *S. gelaticus*, DQ026636, 99.1%; *S. cremeus*, AB184124, 99%.

Source: not known.

DNA G+C content (mol%): not known.

Type strain: AS 4.1428, ATCC 13231, ATCC 25474, CBS 124.18, CBS 884.69, BCRC 12084, DSM 40331, HAMBI 1019, NBRC 13062, JCM 4496, LMG 19327, NRRL B-2156, NRRL B-2888, NRRL-ISP 5331, RIA 1254, VKM Ac-971.

Sequence accession no. (16S rRNA gene): DQ445795.

Further comments: according to Lanoot et al. (2005b), *Streptomyces microflavus* (Krainsky 1914) Waksman and Henrici 1948 emend. Lanoot et al. 2005b is an earlier heterotypic synonym of *Streptomyces griseus* subsp. *alpha* (Ciferri 1927) Pridham 1970, an earlier heterotypic synonym of *Streptomyces griseus* subsp. *cretosus* Pridham 1970[AL], an earlier heterotypic synonym of *Streptomyces lipmanii* (Waksman and Curtis 1916) Waksman and Henrici 1948, and an earlier heterotypic synonym of *Streptomyces willmorei* (Erikson 1935) Waksman and Henrici 1948.

320. **Streptomyces minutiscleroticus** (Thirumalachar *in* Thirumalachar, Rahlkar, Desmukh and Sukapure 1965) Pridham 1970, 41[AL] emend. Lanoot, Vancanneyt, van Schoor, Liu and Swings 2005b, 731 (*Chainia minutisclerotica* Thirumalachar *in* Thirumalachar, Rahlkar, Desmukh and Sukapure 1965, 7)

mi.nu.ti.scle.ro′ti.cus. L. masc. adj. *minutus* little, small, minute; N.L. n. *sclerotium* sclerotium; L. masc. suff. *-icus* suffix used in adjectives with the sense of belonging to; N.L. masc. adj. *minutiscleroticus* belonging to a small sclerotium.

Spore chains in Section *Spirales*. Mature spore chains generally contain 10 to 50 or more spores per chain. This morphology is seen on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar. Spore surface is smooth. Special morphological characteristics: sclerotic granules are produced. Minute sclerotic granules are produced on broad hyphae, often on the agar surface or vegetative substrate. One observer compares these small sclerotia to actinosporangium-like formations, on oatmeal agar and salts-starch agar. Sclerotic granules are reported on yeast-malt agar, salts-starch agar, and glycerol-asparagine agar in 7–21 d.

Color of colony: aerial mass color in the Gray or Yellow color series (2dc, yellowish gray; 24½dc, pale yellow green; or 2ba, pale yellow) on yeast-malt agar and salts-starch agar; Yellow color series (2ba, pale yellow) on oatmeal agar and glycerol-asparagine agar. Reverse side of colony is strong brown on yeast-malt agar; grayish yellow to orange yellow or brown on oatmeal agar and salts-starch agar; yellowish brown, orange yellow or reddish brown on glycerol-asparagine agar. Reverse mycelium pigment is not a pH indicator.

Color in medium: melanoid pigments are not formed in peptone-yeast-iron agar, tyrosine agar, or tryptone-yeast broth. Some yellow pigment may or may not be found in the medium in yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar. When present, this pigment is not pH-sensitive.

D-Glucose, L-arabinose, D-xylose, iso-inositol, D-mannitol, D-fructose, and rhamnose are utilized for growth. No growth or only traces of growth with sucrose or raffinose.

Type strain shows the highest sequence similarity to: *S. vinaceusdrappus*, AY999929, 100%; *S. geysiriensis*, DQ442501, 100%; *S. plicatus*, AB184291, 100%; *S. ghanaensis*, AY999851, 100%; *S. rochei*, AB184237, 100%; *S. mutabilis*, EF178679, 99.8%; *S. tuirus*, AB184690, 99.5%; *S. djakartensis*, AB184657, 99.5%; *S. anandii*, AB184402, 99.3%; *S. violaceorubidus*, AJ781374, 99.3%; *S. pilosus*, AB184161, 99.2%; *S. flavoviridis*, AB184842, 99.2%; *S. calvus*, AB184329, 99.1%; *S. tendae*, D63873, 99.1%; *S. azureus*, EF178674, 99.1%; *S. asterosporus*, AB184706, 99.1%; *S. levis*, AB184670, 99%; *S. luteogriseus*, AB184379, 99%; *S. aureorectus*, AB184710, 99%; *S. capillispiralis*, AB184577, 99%; *S. pseudogriseolus*, DQ442541, 99%; *S. naganishii*, DQ442529, 99%; *S. virens*, DQ442554, 99%; *S. gancidicus*, AB184660, 99%.

Source: not known.

DNA G+C content (mol%): not known.

Type strain: ATCC 17757, ATCC 19346, CBS 231.65, CBS 662.72, BCRC 12544, CMI 112786, DSM 40301, NBRC

13000, NBRC 13361, JCM 3102, JCM 4790, KCTC 9123, LMG 20062, NCIMB 10996, NRRL B-12202, NRRL-ISP 5301, PCM 2304, RIA 1322, RIA 885.

Sequence accession no. (16S rRNA gene): EF178696.

Further comments: Streptomyces minutiscleroticus (Thirumalachar 1965) Pridham 1970 and *Chainia minutisclerotica* Thirumalachar et al. (1965) have the same type strain and therefore are homotypic synonyms [Rules 24a and 24b (1) of the Bacteriological Code].

According to Lanoot et al. (2005b), *Streptomyces minutiscleroticus* (Thirumalachar 1965) Pridham 1970 emend. Lanoot et al. 2005b is an earlier heterotypic synonym of *Streptomyces flaviscleroticus* (*ex* Pridham 1970) Goodfellow et al. 1986a.

321. **Streptomyces mirabilis** (Kudrina *in* Gauze, Preobrazhenskaya, Kudrina, Blinov, Ryabova and Sveshnikova 1957) Ruschmann 1952, 543[AL] ("*Actinomyces mirabilis*" Kudrina *in* Gauze, Preobrazhenskaya, Kudrina, Blinov, Ryabova and Sveshnikova 1957, 107)

mi.ra'bi.lis. L. masc. adj. *mirabilis* marvellous.

Spore chains in Section *Spirales*. Sterile hyphae may be abundant; these are often flexuous or hooked. Mature spore chains are moderately long, sometimes with more than 10 spores per chain. This morphology is seen on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar, but sporulation may be poor, especially on yeast-malt agar and glycerol-asparagine agar. Spore surface is smooth.

Color of colony: aerial mass color in the Gray color series (2fe, medium gray; 3fe, light brownish gray; also 3ig, 4ig, 3li, grayish brown and brownish gray) on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar. Reverse side of colony is grayish yellow becoming moderate olive brown to dark olive or dark brown on oatmeal agar, salts-starch agar, and glycerol-asparagine agar; grayish blue or grayish green and moderate yellowish brown on yeast-malt agar. Substrate pigment is not a pH indicator or is modified only slightly from olive brown to dark brown by 0.05 M NaOH on oatmeal agar and glycerol-asparagine agar.

Color in medium: melanoid pigments are formed in peptone-yeast-iron agar and in trace amounts in tryptone-yeast broth but not in tyrosine agar. No pigment is found in the medium in yeast-malt agar, oatmeal agar, salts-starch agar, or glycerol-asparagine agar.

D-Glucose, L-arabinose, iso-inositol, D-mannitol, D-fructose, and rhamnose are utilized for growth. Reports vary on utilization of D-xylose. No growth or only traces of growth with sucrose or raffinose.

Type strain shows the highest sequence similarity to: *S. olivochromogenes*, AY094370, 99.4%.

Source: not known.

DNA G+C content (mol%): not known.

Type strain: ATCC 27447, CBS 751.72, DSM 40553, NBRC 13450, JCM 4551, JCM 4791, NRRL B-2400, NRRL-ISP 5553, RIA 1411.

Sequence accession no. (16S rRNA gene): AB184412.

322. **Streptomyces misakiensis** Nakamura 1961, 86[AL]

mi.sa.ki.en'sis. N.L. masc. adj. *misakiensis* of or belonging to Misaki, referring to Misakicho, Kanagawa Prefecture, Japan, the source of the soil from which the organism was isolated.

Spore chains in Section *Rectiflexibiles*. Mature spore chains are generally long with 10–50, or sometimes more than 50, spores per chain. This morphology is seen on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar. Spore surface is smooth.

Color of colony: aerial mass color in the Gray color series on salts-starch agar and glycerol-asparagine agar; Gray or Red color series on yeast-malt agar and oatmeal agar. The most representative color tab from the Gray color-wheel is 2dc (yellowish gray) for all ISP media. One observer selected tab 5dc (grayish yellowish pink) from the Red color-wheel as the most representative color on yeast-malt agar and oatmeal agar. Reverse side of colony is yellow to yellow-brown is modified by red, becoming brown to strong brown on yeast-malt agar, salts-starch agar and glycerol-asparagine agar and orange to light brown on oatmeal agar; this pigment is not a pH indicator.

Color in medium: melanoid pigments are not formed in peptone-yeast-iron agar, tyrosine agar, or tryptone-yeast broth. Red pigment is found in the medium in yeast-malt agar, oatmeal agar, and salts-starch agar. This pigment is not pH-sensitive when tested with 0.05 NaOH or HCl.

D-Glucose, sucrose, D-fructose, and raffinose are utilized for growth. No growth or only trace of growth with L-arabinose, D-xylose, iso-inositol, D-mannitol and rhamnose. In the original description (Nakamura, 1961), L-arabinose and D-mannitol were also reported as supporting growth with Czapek's agar as basal medium.

Type strain shows the highest sequence similarity to: *S. catenulae*, AJ621613, 100%; *S. libani* subsp. *libani*, AB184414, 99.6%; *S. nigrescens*, DQ442530, 99.5%; *S. tubercidicus*, AJ621612, 99.5%; *S. caniferus*, AB184640, 99.4%; *S. libani* subsp. *rufus*, AJ781351, 99.3%; *S. hygroscopius* subsp. *glebosus*, AB184479, 99.3%; *S. platensis*, AB045882, 99.2%; *S. sioyaensis*, DQ026654, 99.2%; *S. ramulosus*, DQ026662, 99.1%.

Source: isolated from soil from Misakicho, Kanagawa Prefecture, Japan.

DNA G+C content (mol%): not known.

Type strain: AS 4.1437, ATCC 23938, CBS 278.65, CBS 922.68, DSM 40222, IFM 1195, NBRC 12891, JCM 4062, JCM 4653, NCIMB 9852, NRRL B-2923, NRRL-ISP 5222, RIA 1166, VKM Ac-625.

Sequence accession no. (16S rRNA gene): AB217605.

323. **Streptomyces misionensis** Cercós, Eilberg, Goyena, Souto, Vautier and Widuczynski 1962, 22[AL]

mi.si.on.en'sis. N.L. masc. adj. *misionensis* of or belonging to Misiones, a province in Argentina, South America, the source of the soil from which the organism was isolated.

Spore chains in Section *Spirales*. Mature spore chains are moderately long, often with more than 50 spores per chain. This morphology is seen on yeast-malt agar, oatmeal

agar, salts-starch agar, and glycerol-asparagine agar. Spore surface is smooth or slightly warty.

Color of colony: aerial mass color in the Gray or Red color series (4ig, light grayish brown in Gray series or 4ge, light grayish reddish brown in the Red series) on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar. Reverse side of colony is olive brown to moderate orange on yeast-malt agar; grayish yellow, yellowish brown, or olive brown on oatmeal agar, salts-starch agar, and glycerol-asparagine agar.

Color in medium: melanoid pigments not formed in peptone-yeast-iron agar and tyrosine agar. No pigment is found in medium in yeast-malt agar, oatmeal agar, salts-starch agar, or glycerol-asparagine agar.

D-Glucose, L-arabinose, D-xylose, D-mannitol, and D-fructose are utilized for growth. Utilization of iso-inositol, rhamnose, sucrose and raffinose is doubtful.

Type strain shows the highest sequence similarity to: *S. phaeoluteichromatogenes*, AJ391814, 99.8%; *S. rutgersensis* subsp. *rutgersensis*, AB184170, 99.2%; *S. gougerotii*, AB184742, 99.2%; *S. diastaticus* subsp. *diastaticus*, AB184785, 99.2%; *S. levis*, AB184670, 99.1%; *S. paradoxus*, AB184628, 99.1%; *S. glaucescens*, AB184843, 99%; *S. matensis*, AB184221, 99%; *S. intermedius*, AB184277, 99%.

Source: isolated from soil from Misiones, a province in Argentina, South America.

DNA G+C content (mol%): not known.

Type strain: ATCC 14991, ATCC 25475, CBS 885.69, BCRC 12094, DSM 40306, NBRC 13063, JCM 4497, NRRL B-3230, NRRL-ISP 5306, RIA 1255, VKM Ac-626.

Sequence accession no. (16S rRNA gene): EF178678.

324. **Streptomyces mobaraensis** (Nagatsu and Suzuki 1963) Witt and Stackebrandt 1991, 456[VP] (Effective publication: Witt and Stackebrandt 1990, 370.) (*Streptomyces mobaraensis* Nagatsu and Suzuki 1963, 47; *Streptoverticillium mobaraense* Locci, Baldacci and Petrolini Baldan 1969, 42)

mo.ba.ra.en′sis. N.L. masc. adj. *mobaraensis* of or belonging to Mobara (named after the place of origin, Mobara City, Chiba Prefecture, Japan).

Good growth on potato-glucose agar (Baldacci et al., 1954). Color: reverse light beige to greenish yellow; aerial mycelium dirty white with greenish shades to greenish gray. On Bacto Czapek agar and Casamino acids-Czapek agar (1 g/l Difco vitamin-free Casamino acids, replacing sodium nitrate): very poor growth; colorless; traces only of off-white aerial mycelium. On glucose-asparagine agar (ISP medium 5 with 1% glucose replacing glycerol): good growth; color, reverse yellowish; aerial mycelium whitish. On glycerol-asparagine agar: good growth; color, reverse beige to light brown to yellowish brown; aerial mycelium white to off-white beige. On inorganic salts-starch agar: good growth; color, reverse brown-yellowish to dirty greenish yellow; aerial mycelium beige to dirty greenish beige. On yeast extract-malt extract agar: good growth; color: reverse brown-yellow; aerial mycelium white to dirty beige. On Bacto Emerson agar: good growth; color: reverse yellowish; aerial mycelium off-white. On Bennett agar (1% glucose, 0.1% Bacto beef agar, 0.1% yeast extract, 0.2% peptone, 1.5% agar): good growth; color, reverse yellow; aerial mycelium white with pale pink shades in patches. On Oxoid nutrient agar: good growth; color, reverse yellowish; poor, white aerial mycelium.

Grows at 27°C as well as at 37°C. There are also no differences in the amount of aerial mycelium, which is greener in color at 37°C. Greenish shades of the reverse are also more accentuated. No growth at 45°C. The type strain produces piericidin A, piericidin B, and detoxin and exhibits anti-bacterial activity.

Type strain shows the highest sequence similarity to: *S. sapporonensis*, AB184508, 99.1%; *S. hygroscopius* subsp. *angustmyceticus*, DQ442509, 99%.

Source: not known.

DNA G+C content (mol%): not known.

Type strain: ATCC 29032, CBS 199.75, BCRC 12165, DSM 40847, NBRC 13819, JCM 4168, NCIMB 11159, NRRL B-3729, RIA 1627, VKM Ac-928.

Sequence accession no. (16S rRNA gene): DQ442528.

Further comments: in violation of Rule 33c of the *Bacteriological Code* (1990 Revision), in Validation List no. 38, *Streptomyces mobaraensis* is proposed as a *nomen revictum* (basonym: "*Streptomyces mobaraensis*" Kubo et al. (1964).

According to Labeda (1996), *Streptomyces mobaraensis* (Nagatsu and Suzuki 1963) Witt and Stackebrandt 1991 is an earlier synonym of *Streptomyces ladakanum* (Hanka et al. 1966) Witt and Stackebrandt 1991.

According to Hatano et al. (2003), *Streptomyces mobaraensis* (Nagatsu and Suzuki 1963) Witt and Stackebrandt 1991 is an earlier heterotypic synonym of *Streptomyces ladakanum* (Hanka et al. 1966) Witt and Stackebrandt 1991.

325. **Streptomyces monomycini** Gause and Terekhova 1986a, 575[VP] (Effective publication: Gause and Terekhova *in* Gause, Preobrazhenskaya, Sveshnikova, Terekhova and Maximova 1983.)

mo.no.my.ci′ni. N.L. n. *monomycinum* monomycin; N.L. gen. n. *monomycini* of monomycin, intended to mean monomycin producing.

Spore chains are spiral (*Spirales*); spores are smooth. On mineral agar 1: aerial mycelium is white, poor; substrate mycelium is colorless to greenish-gray-brownish; no diffusible pigment. On starch-ammonia agar: no aerial mycelium; colorless substrate mycelium; no diffusible pigment. On glycerol-nitrate agar: aerial mycelium is white; substrate mycelium is gray-brownish-yellow to green-gray-brownish; no diffusible pigment. On glycerol-asparagine agar: aerial mycelium is light gray; substrate mycelium is colorless; no diffusible pigment. On oatmeal agar: aerial mycelium is poorly developed, white; substrate mycelium is greenish yellow; no diffusible pigment. On organic agar 2: aerial mycelium is poorly developed, white; substrate mycelium is gray-brownish yellow to green-gray-brownish; diffusible pigment is absent or weak, colors are similar to substrate mycelium. Melanoid pigments are not formed. Growth on fructose, glucose, xylose, and mannitol, no growth on sucrose, rhamnose, inositol, raffinose, and arabinose. Antibiotic: monomycin.

Type strain shows the highest sequence similarity to: *S. ochraceiscleroticus*, AB184094, 99.5%; *S. hygroscopius* subsp. *decoyicus*, AY999883, 99.4%; *S. niger*, AJ621607,

99.4%; *S. erumpens*, AJ621603, 99.4%; *S. olivaceiscleroticus*, AJ621606, 99.4%; S. *violens*, AJ621605, 99.3%; *S. purpurogeneiscleroticus*, AJ621604, 99.3%; *S. rimosus* subsp. *rimosus*, AB045883, 99.1%; *S. platensis*, AB045882, 99%; *S. sioyaensis*, DQ026654, 99%; *S. libani* subsp. *rufus*, AJ781351, 99%; *S. caniferus*, AB184640, 99%; *S. hygroscopius* subsp. *glebosus*, AB184479, 99%.

Source: not known.

DNA G+C content (mol%): not known.

Type strain: DSM 41801, INA 1465, JCM 9768, NBRC 100769, NRRL B-24309.

Sequence accession no. (16S rRNA gene): DQ445790.

326. **Streptomyces morookaense** (Locci 1985) Witt and Stackebrandt 1991, 456[VP] (Effective publication: Witt and Stackebrandt 1990, 370.) ("*Streptomyces moro-okaensis*" Niida, Hamamoto, Tsuruoka and Hara 1963; "*Streptoverticillium moro-okaense*" Arai 1976; "*Streptoverticillium morookaense*" Locci 1985, 232)

mo.ro.ok.a.en′se. N.L. adj. *morookaense* (*sic*) of or belonging to Moro-oka (possibly the isolation place).

Spore chains in Section *Verticillati*; mature spore chains are predominantly umbellate monoverticillate (biverticillate). Mature spore chains are moderately long, often with more than 10 spores per chain. This morphology is seen on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar. Spore surface is smooth.

Color of colony: aerial mass color in the Yellow color series (1dc, 1cb, 1½ec, pale yellow green) on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar. Reverse side of colony with no distinctive pigments (pale yellow to pale greenish yellow) on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar.

Color in medium: melanoid pigments are not formed in peptone-yeast-iron agar, tyrosine agar, or tryptone-yeast broth. No pigment is found in the medium in yeast-malt agar, oatmeal agar, salts-starch agar, or glycerol-asparagine agar.

D-Glucose, iso-inositol, D-mannitol, D-fructose, and raffinose are utilized for growth. Utilization of rhamnose is doubtful. No growth or only traces of growth with L-arabinose, sucrose, or D-xylose.

Type strain shows the highest sequence similarity to: *S. lavenduligriseus*, DQ442515, 99.6%; *S. thioluteus*, AB184753, 99.5%; *S. abikoensis*, AB184537, 99.1%; *S. hygroscopius* subsp. *angustmyceticus*, DQ442509, 99%.

Source: not known.

DNA G+C content (mol%): not known.

Type strain: ATCC 19166, CBS 717.72, DSM 40503, NBRC 13416, JCM 4673, JCM 4793, NRRL B-12429, NRRL-ISP 55036, RIA 1377, VKM Ac-1916.

Sequence accession no. (16S rRNA gene): AJ781349.

Further comments: in violation of Rule 33c of the *Bacteriological Code* (1990 Revision), in Validation List no. 38, *Streptomyces morookaense* is proposed as a *nomen revictum* (basonym: "*Streptomyces morookaense*" Niida et al. 1963).

According to Hatano et al. (2003), *Streptomyces morookaense* (Locci and Schofield 1989) Witt and Stackebrandt 1991 is a heterotypic synonym of "*Streptomyces aspergilloides*" (NBRC 13461).

327. **Streptomyces murinus** Frommer 1959, 198[AL]

mu.ri′nus. L. masc. adj. *murinus* of mice, mouse-gray; referred to as reddish-gray in original description.

Spore chains in Section *Spirales*. Mature spore chains generally 10–50 spores per chain. This morphology is seen on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar. Spore surface is smooth. Air-dried preparation for electron microscopy may show internal contractions that suggest a warty appearance.

Color of colony: aerial mass color in the Red color series on glycerol-asparagine agar; Gray series on salts-starch agar; Gray or Red color series on oatmeal agar and glycerol-asparagine agar. Reverse side of colony is grayed yellow on oatmeal agar and glycerol-asparagine agar; grayed yellow or yellow modified by red on yeast-malt and salts-starch agar. Substrate pigment is not a pH indicator.

D-Glucose, D-xylose, D-mannitol, and D-fructose are utilized for growth. No growth or only trace of growth on L-arabinose, sucrose, rhamnose, and raffinose. Variable reports on growth with iso-inositol.

Type strain shows the highest sequence similarity to: *S. griseofuscus*, AB184206, 100%; *S. costaricanus*, AB249939, 100%; *S. phaeogriseichromatogenes*, AJ391813, 99.6%.

Source: not known.

DNA G+C content (mol%): not known.

Type strain: ATCC 19788, CBS 540.68, BCRC 12061, CECT 3309, DSM 40091, NBRC 12799, NBRC 14802, JCM 4333, JCM 4595, KCTC 9492, LMG 10475, NCIMB 12701, NRRL B-2286, NRRL-ISP 5091, PCM 2369, RIA 1067, UNIQEM 174, VKM Ac-1190.

Sequence accession no. (16S rRNA gene): AB184155.

328. **Streptomyces mutabilis** (Preobrazhenskaya and Ryabova *in* Gauze, Preobrazhenskaya, Kudrina, Blinov, Ryabova and Sveshnikova 1957) Pridham, Hesseltine and Benedict 1958, 69[AL] ("*Actinomyces mutabilis*" Preobrazhenskaya and Ryabova *in* Gauze, Preobrazhenskaya, Kudrina, Blinov, Ryabova and Sveshnikova 1957, 166)

mu.ta′bi.lis. L. masc. adj. *mutabilis* changeable, so named because the organism could not be assigned to any of the species known in the literature.

Spore chains in Section *Spirales* or *Retinaculiaperti*. Spore chains are poorly developed so that hooks, loops, or partial spirals are shorter and of smaller diameter than on typical *Retinaculiaperti* cultures. Spirals, when found, are aberrant. Mature spore chains generally contain only 3–10 spores per chain on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar. Spore surface is smooth.

Color of colony: aerial mass color in the Gray or White color series on yeast-malt agar and oatmeal agar; White series on salts-starch agar and glycerol-asparagine agar. Reverse side of colony with no distinctive pigment on yeast-malt agar, oatmeal agar, salts-starch agar, or glycerol-asparagine agar; substrate pigment is not a pH indicator.

Color in medium: melanoid pigments not formed in peptone-yeast-iron agar, tyrosine agar, or tryptone-yeast broth. No pigments found in medium, except for traces of yellow or greenish yellow pigments in yeast-malt agar.

This pigment is not pH-sensitive when tested with 0.05 M NaOH or HCl.

D-Glucose, L-arabinose, D-xylose, iso-inositol, D-mannitol, D-fructose, and rhamnose are utilized for growth. Some growth occurs with sucrose as the carbon source; raffinose is not utilized.

Type strain shows the highest sequence similarity to: *S. rochei*, AB184237, 99.9%; *S. vinaceusdrappus*, AY999929, 99.9%; *S. plicatus*, AB184291, 99.9%; *S. geysiriensis*, DQ442501, 99.8%; *S. ghanaensis*, AY999851, 99.8%; *S. minutiscleroticus*, EF178696, 99.8%; *S. tuirus*, AB184690, 99.6%; *S. djakartensis*, AB184657, 99.4%; *S. violaceorubidus*, AJ781374, 99.2%; *S. flavoviridis*, AB184842, 99.1%; *S. anandii*, AB184402, 99.1%; *S. levis*, AB184670, 99.1%; *S. tendae*, D63873, 99.1%; *S. violaceus*, AB184315, 99.1%; *S. pilosus*, AB184161, 99.1%; *S. luteogriseus*, AB184379, 99.1%; *S. azureus*, EF178674, 99.1%; *S. janthinus*, AB184851, 99.1%; *S. albosporeus* subsp. *albosporeus*, AJ781327, 99.1%; *S. capillispiralis*, AB184577, 99%; *S. roseoviolaceus*, AJ399484, 99%; *S. rubrogriseus*, AB184681, 99%; *S. asterosporus*, AB184706, 99%; *S. africanus*, AY208912, 99%; *S. calvus*, AB184329, 99%.

Source: not known.

DNA G+C content (mol%): not known.

Type strain: ATCC 19789, ATCC 19919, CBS 541.68, DSM 40169, HAMBI 1069, NBRC 12800, IMET 43509, JCM 4400, NRRL-ISP 5169, RIA 1068, UNIQEM 175, VKM Ac-1851.

Sequence accession no. (16S rRNA gene): EF178679.

329. **Streptomyces mutomycini** Gause and Maximova 1986a, 575[VP] (Effective publication: Gause and Maximova *in* Gause, Preobrazhenskaya, Sveshnikova, Terekhova and Maximova 1983.)

mu.to.my.ci'ni. N.L. n. *mutomycinum* mutomycin; N.L. gen. n. *mutomycini* of mutomycin, intended to mean mutomycin producing.

Spore chains are spiral (*Spirales*); spores are spiny, spines are short. On mineral agar 1 and oatmeal agar: aerial mycelium is gray to dark gray; substrate mycelium is colorless, sometimes with gray spots; no diffusible pigment. On glycerol-nitrate agar: aerial mycelium is poor, gray; substrate mycelium and diffusible pigment are yellow-brown. On starch-ammonia agar: poor, gray aerial mycelium; colorless substrate mycelium; no diffusible pigment. On glycerol-asparagine agar: aerial mycelium and diffusible pigment are gray, poor; substrate mycelium is colorless or pinkish gray to yellow-brown. On organic agar 2: no aerial mycelium: substrate mycelium and diffusible pigment are dark yellow or olive-gray. On organic agar 79: no aerial mycelium; substrate mycelium and diffusible pigment are brown. Melanoid pigments are not formed. Moderate growth on sugar and sucrose, fructose, glucose, and mannitol; poor growth on arabinose and xylose. Antibiotic: mutomycin.

Type strain shows the highest sequence similarity to: *S. clavifer*, DQ026670, 99.8%; *S. olivoviridis*, AB184227, 99.7%; *S. atroolivaceus*, AJ781320, 99.7%; *S. finlayi*, AY999788, 99.6%; *S. acrimycini*, AY999889, 99.4%; *S. griseoplanus*, AY999894, 99.4%; *S. fimicarius*, AY999784, 99.3%; *S. flavofuscus*, AB249935, 99.3%; *S. praecox*, AB184293, 99.3%; *S. mediolani*, AB184674, 99.3%; *S. anulatus*, DQ026637, 99.3%; *S. sindenensis*, AB184759, 99.2%; *S. griseinus*, AB184205, 99.2%; *S. rubiginosohelvolus*, AB184240, 99.2%; *S. badius*, AY999783, 99.2%; *S. cavourensis* subsp. *washingtonensis*, DQ026671, 99.2%; *S. cyaneofuscatus*, AB184860, 99.2%; *S. pluricolorescens*, DQ442540, 99.2%; *S. cinereorectus*, AB184646, 99.2%; *S. californicus*, AB184755, 99.2%; *S. lipmanii*, AB184148, 99.2%; *S. lavendulae* subsp. *lavendulae*, AB184080, 99.2%; *S. baarnensis*, EF178688, 99.1%; *S. floridae*, AB184656, 99.1%; *S. albovinaceus*, AB249958, 99.1%; *S. fulvorobeus*, AB184711, 99.1%; *S. microflavus*, DQ445795, 99.1%; *S. alboviridis*, AB184256, 99.1%; *S. globisporus* subsp. *globisporus*, EF178686, 99.1%; *S. griseus* subsp. *griseus*, AY207604, 99%; *S. argenteolus*, AB045872, 99%; *S. griseolus*, AB184768, 99%; *S. candidus*, DQ026663, 99%; *S. bacillaris*, AB184439, 99%; *S. luridiscabiei*, AF361784, 99%; *S. flavovirens*, DQ026635, 99%; *S. parvus*, DQ442537, 99%.

Source: not known.

DNA G+C content (mol%): not known.

Type strain: AS 4.1747, ATCC 43689, DSM 41691, INA 4305, NBRC 100999.

Sequence accession no. (16S rRNA gene): AB249951.

330. **Streptomyces naganishii** Yamaguchi and Saburi 1955, 219[AL]

na.ga.ni'shi.i. N.L. gen. masc. n. *naganishii* of Naganishi, named for Professor H. Naganishi of the University of Hiroshima, Japan.

Spore chains in Section *Spirales*. Mature spore chains are short to moderately long, usually with more than 10 spores per chain. This morphology is best developed on yeast-malt agar. Short spore chains on salts-starch agar and glycerol-asparagine agar may form incomplete spirals or hooks. Sporulation on oatmeal agar is especially poor. Aerial hyphae may terminate in atypical club-like swellings instead of spores. Spore surface is smooth.

Color of colony: aerial mass color in the Gray or Red color series on yeast-malt agar and on carbon utilization medium plus D-glucose. The sporulated aerial growth on oatmeal agar, salts-starch agar, and glycerol-asparagine agar is usually white. Reverse side of colony is pale or grayish yellow to yellow-brown modified in spots or at margins by red (dark pink, purplish pink, or grayish reddish brown). Substrate pigment is not a pH indicator.

Color in medium: melanoid pigments are formed in peptone-yeast-iron agar and tryptone-yeast broth in 2 d, but are produced slowly or not at all in tyrosine agar. A transient red pigment is found in the medium in oatmeal agar and salts-starch agar. This pigment is not pH-sensitive when tested with 0.05 M NaOH or HCl.

D-Glucose, L-arabinose, D-xylose, iso-inositol, D-mannitol, D-fructose, and rhamnose are utilized for growth. Utilization of raffinose is doubtful. No growth or only traces of growth with sucrose.

Type strain shows the highest sequence similarity to: *Streptomyces geysiriensis*, DQ442501, 99; *Streptomyces minutiscleroticus*, EF178696, 99%.

Source: not known.

DNA G+C content (mol%): not known.

Type strain: ATCC 23939, CBS 923.68, DSM 40282, NBRC 12892, JCM 4190, JCM 4654, NRRL B-1816, NRRL-ISP 5282, RIA 1196.

Sequence accession no. (16S rRNA gene): DQ442529.

331. **Streptomyces narbonensis** Corbaz, Ettlinger, Gäumann, Keller-Schierlein, Kradolfer, Kyburz, Neipp, Prelog, Reusser and Zähner 1955, 935[AL] ["*Streptomyces narboensis*" (*sic*) Corbaz, Ettlinger, Gäumann, Keller-Schierlein, Kradolfer, Kyburz, Neipp, Prelog, Reusser and Zähner 1955, 941]

nar.bo.nen′sis. L. masc. adj. *narbonensis* of or belonging to Narbonne, France, the source of the soil from which the organism was isolated.

Spore chains in Section *Rectiflexibiles*. Mature spore chains generally have 10–50 spores per chain; longer chains are sometimes observed. This morphology is seen on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar. Spore surface is smooth. Fragmentation of the substrate mycelium was noted by one observer only; unusual fragmentation of the aerial mycelium was recorded by another observer.

Color of colony: aerial mass color in the Gray color series on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar. Reverse side of colony with no distinctive pigment (grayed yellow to grayed greenish yellow) on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar; substrate pigment is not a pH indicator.

Color in medium: melanoid pigments formed in peptone-yeast-iron agar. Pigments other than melanoids not formed in yeast-malt agar, oatmeal agar, salts-starch agar, or glycerol-asparagine agar.

D-Glucose, L-arabinose, sucrose, D-xylose, D-fructose, rhamnose, and raffinose are utilized for growth. No growth or only traces of growth on iso-inositol and D-mannitol.

Type strain shows the highest sequence similarity to: *S. zaomyceticus*, EF178685, 99.3%; *S. exfoliatus*, AB184324, 99.3%; *S. venezuelae*, AB045890, 99.3%; *S. lateritius*, AB184324, 99.2%; *S. wedmorensis*, DQ442557, 99.2%; *S. litmocidini*, AB184149, 99.1%; *S. omiyaensis*, EF178697, 99.1%.

Source: isolated from soil from Narbonne, a small community near Cannes on the Côte d'Azur, France.

DNA G+C content (mol%): not known.

Type strain: ATCC 19790, CBS 310.55, CBS 542.68, BCRC 13651, DSM 40016, NBRC 12801, JCM 4147, JCM 4596, NRRL B-1680, NRRL-ISP 5016, RIA 1069, RIA 529, UNIQEM 176, VKM Ac-1891.

Sequence accession no. (16S rRNA gene): DQ445794.

332. **Streptomyces nashvillensis** McVeigh and Reyes 1961, 312[AL]

nash.vil.len′sis. N.L. masc. adj. *nashvillensis* of or belonging to Nashville, a city in Tennessee, the source of the soil from which the organism was isolated.

Spore chains in Section *Rectiflexibiles*. Long chains, often with more than 50 spores, are predominantly straight but a very small number of these chains may show *Retinaculiaperti* morphology including some spirals. This morphology is seen on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar. Spore surface is smooth.

Color of colony: aerial mass color in the Gray color series (3fe, light brownish gray to 5fe, light grayish reddish brown or 5ih, brownish gray) on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar. Reverse side of colony is strong brown to light grayish brown on yeast-malt agar; grayish yellow, light olive gray, or yellowish brown on oatmeal agar, salts-starch agar, and glycerol-asparagine agar. Reverse mycelium pigment is somewhat pH-sensitive changing from yellowish brown to reddish brown with addition of 0.05 M NaOH or from yellowish brown to yellow with addition of 0.05 M HCl.

Color in medium: melanoid pigments are formed in peptone-yeast-iron agar, tyrosine agar, or tryptone-yeast broth, but melanin reaction may be delayed or weak in tyrosine agar. Yellow (to brown) pigment is found in the medium in yeast-malt agar and oatmeal agar and usually salts-starch agar and glycerol-asparagine agar. This pigment is somewhat pH-sensitive when tested with 0.05 M NaOH or HCl, showing the same color change noted for the reverse mycelium pigment.

D-Glucose, L-arabinose, D-xylose, and raffinose are utilized for growth. Utilization of D-fructose is doubtful and there is no growth or only traces of growth with iso-inositol, D-mannitol, rhamnose, or sucrose.

Type strain shows the highest sequence similarity to: *S. tanashiensis*, AJ781362, 100%; *S. violaceorectus*, AB184314, 99.2%; *S. showdoensis*, AB184389, 99.1%; *S. cinereoruber* subsp. *cinereoruber*, AB184121, 99.1%; *S. roseoviridis*, AB184239, 99.1%; *S. racemochromogenes*, DQ026656, 99.1%; *S. viridobrunneus*, AJ781372, 99.1%; *S. roseolus*, AB184168, 99%; *S. polychromogenes*, AB184292, 99%; *S. bikiniensis*, X79851, 99%.

Source: isolated from soil from Nashville, a city in Tennessee.

DNA G+C content (mol%): not known.

Type strain: ATCC 25476, CBS 886.69, BCRC 13625, DSM 40314, NBRC 13064, JCM 4498, NRRL B-2606, NRRL-ISP 5314, RIA 1256, VKM Ac-1766.

Sequence accession no. (16S rRNA gene): AB184286.

333. **Streptomyces netropsis** (Finlay et al. 1951) Witt and Stackebrandt 1991, 456[VP] (Effective publication: Witt and Stackebrandt 1990, 370.) [*Streptoverticillium netropsis* (Finlay et al. 1951) Baldacci, Farina and Locci 1966, 161]

ne.trop′sis. Gr. n. *netron* spindle; Gr. fem. n. *opsis* aspect, appearance; N.L. masc. adj. *netropsis* spindle-like.

Spore chains in Umbellate monoverticillate (=*Streptomyces* Section Verticillati, biverticillate). Whorls may be composed of straight (BIV), flexuous, or spiral (BIV-S) spore chains. Mature spore chains generally have 10–20 spores per chain. This morphology is seen on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar. Spore surface is smooth.

Color of colony: aerial mass color in the Red color series on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar. Reverse side of colony with no distinctive pigments (grayish yellow to olive brown or brown) on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar.

Color in medium: melanoid pigments are formed in

peptone-yeast-iron agar, but not in tyrosine agar. No pigment or only a trace of yellow or brown pigment is found in the medium in yeast-malt agar, oatmeal agar, and salts-starch agar. Pigment, if present, may be slightly pH-sensitive changing from pale yellow to pale pink when tested with 0.05 M NaOH.

D-Glucose, iso-inositol, and D-fructose are utilized for growth. No growth or only trace of growth with L-arabinose, sucrose, D-xylose, D-mannitol, rhamnose, and raffinose.

Type strain shows the highest sequence similarity to: *S. werraensis*, DQ442558, 99.3%; *S. biverticillatus*, AJ781381, 99.3%; *S. stramineus*, AB184720, 99.2%; *S. eurocidicus*, AY999790, 99.1%; *S. albireticuli*, AB184881, 99%.

Source: not known.

DNA G+C content (mol%): not known.

Type strain: ATCC 23940, CBS 924.68, BCRC 13374, CECT 3265, DSM 40259, HUT 6086, IFM 1035, NBRC 12893, NBRC 3723, JCM 4063, JCM 4655, LMG 5979, NCIMB 9592, NRRL 2268, NRRL-ISP 5259, PCM 2251, RIA 1184, RIA 605, VKM Ac-820.

Sequence accession no. (16S rRNA gene): AB184848.

Further comments: in violation of Rule 33c of the *Bacteriological Code* (1990 Revision), in Validation List no. 38, *Streptomyces netropsis* is proposed as a *nomen revictum* (basonym: "*Streptomyces netropsis*" Finlay et al. (1951).

According to Labeda (1996), *Streptomyces netropsis* (Finlay et al. 1951) Witt and Stackebrandt 1991 is an earlier heterotypic synonym of *Streptomyces distallicus* (Locci et al. 1969) Witt and Stackebrandt 1991, an earlier synonym of *Streptomyces flavopersicus* (Oliver et al. 1961) Witt and Stackebrandt 1991, and an earlier synonym of *Streptomyces kentuckensis* (Barr and Carman 1956) Witt and Stackebrandt 1991.

According to Hatano et al. (2003), *Streptomyces netropsis* (Finlay et al. 1951) Witt and Stackebrandt 1991 is an earlier heterotypic synonym of *Streptomyces distallicus* (Locci et al. 1969) Witt and Stackebrandt 1991, of *Streptomyces flavopersicus* (Oliver et al. 1961) Witt and Stackebrandt 1991, of *Streptomyces kentuckensis* (Barr and Carman 1956) Witt and Stackebrandt 1991, and of *Streptomyces syringium* (Konev 1986) Witt and Stackebrandt 1991.

334. **Streptomyces neyagawaensis** Yamamoto, Nakazawa, Horii and Miyake 1960, 286[AL]

ne.ya.ga.wa.en′sis. N.L. masc. adj. *neyagawaensis* of or belonging to Neyagawa City, Japan, near which the soil was obtained from which the organism was isolated.

Spore chains in Section *Spirales* on oatmeal agar and salts-starch agar. Short spore chains may form incomplete spirals (hooks) or flexuous to straight chains on yeast-malt agar and glycerol-asparagine agar. Spore chains are short on some media but long chains of 10–50 or more spores per chain may also be found. Spore chain morphology is often difficult to observe when spore chains and aerial hyphae coalesce in black moist (hygroscopic) masses. Spore surface is smooth. Surface of growth may become moist and black (hygroscopic) on older cultures.

Color of colony: aerial mass color in the Gray color series (3fe, light brownish gray and 5fe, light grayish reddish brown on yeast-malt agar, oatmeal agar, and salts-starch agar; 2dc, yellowish gray or 5ih, brownish gray on glycerol-asparagine agar). Reverse side of colony is dark olive on yeast-malt agar; yellowish gray to light olive gray or brown on oatmeal agar, salts-starch agar, and glycerol-asparagine agar.

Color in medium: melanoid pigments are formed in peptone-yeast-iron agar, tyrosine agar, and tryptone-yeast broth. No pigment, or a trace of gray or olive brown pigment, is found in the medium in yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar.

D-Glucose, L-arabinose, D-xylose, iso-inositol, D-mannitol, D-fructose, rhamnose, sucrose, and raffinose are all utilized for growth.

Type strain shows the highest sequence similarity to: *S. hygroscopicus* subsp. *ossamyceticus*, AB184560, 99.4%; *S. torulosus*, AJ781367, 99.3%.

Source: isolated from soil near Neyagawa City, Japan.

DNA G+C content (mol%): not known.

Type strain: ATCC 27449, CBS 778.72, DSM 40588, NBRC 13477, NBRC 3784, NRRL B-3092, NRRL-ISP 5588, RIA 1438, VKM Ac-1915, JCM 4796.

Sequence accession no. (16S rRNA gene): D63869.

335. **Streptomyces niger** (Thirumalachar 1955) Goodfellow, Williams and Alderson 1986a, 575[VP] (Effective publication: Goodfellow, Williams and Alderson 1986d, 59.) (*Chainia nigra* Thirumalachar 1955, 935)

ni′ger. L. masc. adj. *niger* black.

Forms extensively branched substrate and aerial mycelium. Spiral spore chains of smooth-surfaced spores are borne on the aerial mycelium. Aerial spore mass is white and the substrate mycelium is green. A green soluble pigment is produced. Sclerotia are formed in 7–14 d on agar media. Melanin pigments are not produced. Adenine, esculin, allantoin, arbutin, casein, elastin, guanine, hypoxanthine, pectin, starch, testosterone, L-tyrosine, xanthine, and xylan are degraded but chitin, lecithin, and urea are not. Nitrate is reduced but hydrogen sulfide is not produced. Adonitol, L-arabinose, cellobiose, D-fructose, D-galactose, D-glucose, *myo*-inositol, inulin, D-lactose, D-mannitol, D-mannose, melibiose, melezitose, raffinose, L-rhamnose, salicin, sucrose, trehalose, xylitol, and D-xylose are all used as sole carbon sources. Acid is formed from adonitol, *meso*-erythritol, *myo*-inositol, D-lactose, D-mannitol, melibiose, methyl α-D-glucoside, raffinose, L-rhamnose, sucrose, and D-xylose but not from dulcitol or D-sorbitol. Grows on DL-α-amino-n-butyric acid, L-arginine, L-histidine, L-phenylalanine, potassium nitrate, L-serine, L-threonine, and L-valine, but not on L-cysteine or L-methionine as sole nitrogen source. Grows at 10, 37, and 45°C but not at 4°C. Tolerant to phenol (0.1%, w/v) and sodium azide (0.02%, w/v) and sodium chloride (10%, w/v). Resistant to rifampin but not to sodium chloride (13%, w/v). Antibiotic activity shown against *Bacillus subtilis* NCIB 3610 and *Saccharomyces cerevisiae* CBS 1171[T], but not against *Aspergillus niger* LIV 131, *Escherichia coli* NCIB 9132, *Micrococcus luteus* NCIB 196, *Pseudomonas fluorescens* NCIB 9046[T], or *Streptomyces murinus* ISP 5091. The organism has a type II phospholipid pattern (*sensu* Lechevalier et al., 1977) and contains major amounts of

hexahydrogenated menaquinones with nine isoprene units (Collins et al., 1984).

Type strain shows the highest sequence similarity to: *S. olivaceiscleroticus*, AJ621606, 100%; *S. monomycini*, DQ445790, 99.4%; *S. rimosus* subsp. *rimosus*, AB045883, 99.3%; *S. sclerotialus*, AJ621608, 99.2%; *S. purpurogeneiscleroticus*, AJ621604, 99.1%; *S. ochraceiscleroticus*, AB184094, 99.1%.

Source: not known.

DNA G+C content (mol%): not known.

Type strain: ATCC 17756, CBS 230.65, CBS 663.72, BCRC 11877, CMI 112787, DSM 43049, KCC A-0158, NBRC 13362, NBRC 13902, JCM 3158, NCIMB 10992, NRRL B-3857, NRRL-ISP 5302, PCM 2305, RIA 1323, VKM Ac-1736.

Sequence accession no. (16S rRNA gene): AJ621607.

336. **Streptomyces nigrescens** (Sveshnikova *in* Gauze, Preobrazhenskaya, Kudrina, Blinov, Ryabova and Sveshnikova 1957) Pridham, Hesseltine and Benedict 1958, 70[AL] ("*Actinomyces nigrescens*" Sveshnikova *in* Gauze, Preobrazhenskaya, Kudrina, Blinov, Ryabova and Sveshnikova 1957, 146)

ni.gres'cens. L. part. adj. *nigrescens* becoming black.

Spore chains in Section *Spirales*. Mature spore chains are moderately long with 10–50, or sometimes more than 50, spores per chain. This morphology is seen on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar. Spore surface is smooth to warty.

Color of colony: aerial mass color in the Gray color series on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar. In the original description (Gauze et al., 1957), emphasis is placed on an autolytic change of gray aerial mycelium to a black shiny mass in ageing cultures. This phenomenon was not observed by ISP cooperators. Reverse side of colony with no distinctive pigments (colorless to grayish yellow) on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar.

Color in medium: melanoid pigments are not formed in peptone-yeast-iron agar, tyrosine agar, or tryptone-yeast broth. No pigment is found in the medium in yeast-malt agar, oatmeal agar, salts-starch agar, or glycerol-asparagine agar.

D-Glucose, sucrose, D-xylose, iso-inositol, D-mannitol, D-fructose, and raffinose are utilized for growth. No growth or only trace of growth with L-arabinose and rhamnose.

Type strain shows the highest sequence similarity to: *S. libani* subsp. *libani*, AB184414, 100%; *S. tubercidicus*, AJ621612, 99.9%; *S. hygroscopius* subsp. *glebosus*, AB184479, 99.6%; *S. libani* subsp. *rufus*, AJ781351, 99.6%; *S. caniferus*, AB184640, 99.5%; *S. catenulae*, AJ621613, 99.5%; *S. misakiensis*, AB217605, 99.5%; *S. sioyaensis*, DQ026654, 99.5%; *S. platensis*, AB045882, 99.5%; *S. hygroscopius* subsp. *decoyicus*, AY999883, 99.2%; *S. lydicus*, Y15507, 99%; *S. chattanoogensis*, AJ621611, 99%.

Source: not known.

DNA G+C content (mol%): not known.

Type strain: AS 4.1410, ATCC 23941, CBS 925.68, DSM 40276, NBRC 12894, INA 1800/54, JCM 4401, LMG 19332, NCIMB 9856, NRRL B-12176, NRRL-ISP 5276, RIA 1194, VKM Ac-1705.

Sequence accession no. (16S rRNA gene): DQ442530.

337. **Streptomyces nigrifaciens** Waksman 1961, 247[AL]

ni.gri.fa'ci.ens. L. adj. *niger -gra -grum* black; L. part adj. *faciens* producing; N.L. part adj. *nigrifaciens* producing black pigment.

Spore chains in Section *Rectiflexibiles*. Mature spore chains generally have 10–50 spores per chain; longer chains are sometimes observed. This morphology is seen on yeast-malt agar, oatmeal agar, and salts-starch agar. Spore surface is smooth.

Color of colony: aerial mass color in the Gray color series on yeast-malt agar, oatmeal agar, and salts-starch agar. Reverse side of colony with no distinctive pigment (grayed yellow to yellow-brown on yeast-malt agar, salts-starch agar, glycerol-asparagine agar, and grayed greenish yellow on oatmeal agar). Substrate pigment is not a pH indicator.

Color in medium: melanoid pigments not formed in peptone-yeast-iron agar and tyrosine agar. Some orange to yellow pigment found in medium in yeast-malt agar. In oatmeal agar, salts-starch agar, and glycerol-asparagine agar, yellow pigment is very faint or absent; it is not pH-sensitive.

D-Glucose, L-arabinose, D-xylose, D-mannitol, and rhamnose are utilized for growth. No growth or only trace of growth on sucrose, iso-inositol, and raffinose. Variable reports on growth with D-fructose.

For sequence similarity, see type strain of *Streptomyces flavovirens*.

Source: not known.

DNA G+C content (mol%): not known.

Type strain: ATCC 19791, CBS 543.68, BCRC 13672, DSM 40071, NBRC 12802, JCM 4223, JCM 4597, LMG 20048, NCIMB 13019, NRRL B-2094, NRRL-ISP 5071, RIA 1070, UNIQEM 177, VKM Ac-1888.

Sequence accession no. (16S rRNA gene): AB184158.

Further comments: according to Lanoot et al. (2005b), *Streptomyces nigrifaciens* Waksman 1961 is a later heterotypic synonym of *Streptomyces flavovirens* (Waksman 1923) Waksman and Henrici 1948 emend. Lanoot et al. 2005b.

338. **Streptomyces nitrosporeus** Okami 1952, 477[AL]

ni.tro.spo're.us. N.L. n. *nitras* nitrate; Gr. n. *spora* a seed; N.L. n. *spora* a spore; N.L. masc. adj. *nitrosporeus* nitrate spored, name is based on rapid spore formation accompanied by vigorous reduction of nitrate.

Spore chains in Section *Rectiflexibiles*. Mature spore chains generally have 10–50 spores per chain. This morphology is seen on yeast-malt agar, oatmeal agar, and salts-starch agar. Spore surface is smooth.

Color of colony: aerial mass color in the Gray color series on yeast-malt agar, oatmeal agar, and salts-starch agar. Reverse side of colony with no distinctive pigment (grayed yellow to yellow-brown) on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar; substrate pigment is not a pH indicator.

Color in medium: melanoid pigments not formed in peptone-yeast-iron agar and tyrosine agar. Indistinct yellow, gray, or brownish gray pigment found in medium in yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar; it is not pH-sensitive.

D-Glucose, L-arabinose, D-xylose, and rhamnose utilized for growth. No growth or only trace of growth on sucrose, iso-inositol, D-mannitol, and raffinose. Variable reports with D-fructose.

Type strain shows the highest sequence similarity to: *S. flavogriseus*, AJ494864, 99.7%; *S. flavovirens*, DQ026635, 99.7%; *S. mediolani*, AB184674, 99.5%; *S. rubiginosohelvolus*, AB184240, 99.5%; *S. badius*, AY999783, 99.5%; *S. cinereorectus*, AB184646, 99.5%; *S. albovinaceus*, AB249958, 99.5%; *S. praecox*, AB184293, 99.5%; *S. flavofuscus*, AB249935, 99.5%; *S. globisporus* subsp. *globisporus*, EF178686, 99.5%; *S. pluricolorescens*, DQ442540, 99.5%; *S. sindenensis*, AB184759, 99.5%; *S. griseinus*, AB184205, 99.5%; *S. fimicarius*, AY999784, 99.5%; *S. anulatus*, DQ026637, 99.5%; *S. griseolus*, AB184768, 99.5%; *S. californicus*, AB184755, 99.4%; *S. parvus*, DQ442537, 99.4%; *S. bacillaris*, AB184439, 99.4%; *S. cavourensis* subsp. *washingtonensis*, DQ026671, 99.4%; *S. argenteolus*, AB045872, 99.4%; *S. griseoplanus*, AY999894, 99.4%; *S. lavendulae* subsp. *lavendulae*, AB184080, 99.4%; *S. acrimycini*, AY999889, 99.4%; *S. halstedii*, EF178695, 99.4%; *S. alboviridis*, AB184256, 99.3%; *S. baarnensis*, EF178688, 99.3%; *S. pulveraceus*, AB184806, 99.3%; *S. fulvorobeus*, AB184711, 99.3%; *S. lipmanii*, AB184148, 99.3%; *S. microflavus*, DQ445795, 99.3%; *S. cyaneofuscatus*, AB184860, 99.3%; *S. floridae*, AB184656, 99.3%; *S. griseus* subsp. *griseus*, AY207604, 99.2%; *S. luridiscabiei*, AF361784, 99.2%; *S. albolongus*, AB184425, 99.1%; *S. celluloflavus*, AB184476, 99.1%; *S. sanglieri*, AB249945, 99.1%; *S. cavourensis* subsp. *cavourensis*, DQ445791, 99.1%; *S. olivoviridis*, AB184227, 99%; *S. griseobrunneus*, AB249912, 99%; *S. atratus*, DQ026638, 99%; *S. finlayi*, AY999788, 99%; *S. atroolivaceus*, AJ781320, 99%; *S. yanii*, AB006159, 99%; *S. gelaticus*, DQ026636, 99%.

Source: not known.

DNA G+C content (mol%): not known.

Type strain: ATCC 12769, ATCC 19792, CBS 544.68, BCRC 13645, DSM 40023, HUT 6032, NBRC 12803, NBRC 3362, JCM 4064, JCM 4598, KCTC 9761, NCIMB 9717, NRRL B-1316, NRRL-ISP 5023, RIA 1071, RIA 503, UNIQEM 178, VKM Ac-1191, VKM Ac-1202.

Sequence accession no. (16S rRNA gene): EF178680.

339. **Streptomyces niveiscabiei** Park, Kim, Kwon, Wilson, Yu, Hur and Lim 2003, 2053[VP]

ni.ve.i.sca'bi.ei. L. adj. *niveus* snow-white; L. n. *scabies* roughness, scabbiness; N.L. gen. n. *niveiscabiei* of white scab, intended to mean white and scab-causing bacteria.

Spores are white, smooth, and borne in simple rectus flexuous spore chains. Melanin is not produced on tyrosine or peptone agars. L-Arabinose, D-fructose, D-glucose, D-mannitol, raffinose, rhamnose, sucrose, D-xylose, and iso-inositol are utilized for growth. Minimum pH for growth is 3.5. Sensitive to 5, 6, and 7% (w/v) NaCl, 20 and 100 μg/ml thallium acetate, 0.5 μg/ml crystal violet, 0.1% (w/v) phenol, 25 and 100 μg/ml oleandomycin, and 20 μg/ml streptomycin, but not to 10 IU/ml penicillin.

Type strain shows no sequence similarity over 99%. Type strain shows DNA–DNA similarity to: *S. scabies* ATCC 49173^T, 16%; *S. turgidiscabies* ATCC 700248^T, 15%; *S. acidiscabies* ATCC 49003^T, 17%; *S. bottropensis* DSM 40262^T, 15%; *S. neyagawaensis* DSM 40588^T, 18%; *S. diastatochromogenes* DSM 40449^T, 19%; *S. setonii* DSM 40395^T, 15%; *S. griseus* subsp. *griseus* DSM 40236^T, 22%; *S. sampsonii* DSM 40394^T, 19%; *S. eurythermus* DSM 40014^T, 17%; *S. tendae* DSM 40101^T, 16%; *S. coelicolor* DSM 40233^T, 12%; " *S. lividans*" DSM 40434, 19%; *S. ambofaciens* DSM 40053^T, 15%; *S. luridiscabiei* LMG 21390^T, 17%; *S. puniciscabiei* LMG 21391^T, 13%.

Source: isolated from raised corky lesions on potato cv. Daeji-ma and pathogenic on potato cv. Daeji-ma.

DNA G+C content (mol%): 70.1.

Type strain: S78, KACC 20254, LMG 21392.

Sequence accession no. (16S rRNA gene): AF361786.

340. **Streptomyces niveoruber** Ettlinger, Corbaz and Hütter 1958a, 350[AL]

ni.ve.o.ru'ber. L. adj. *niveus* snow-white; L. adj. *ruber* red; N.L. masc. adj. *niveoruber* snow-white-red (referring to the white color of the aerial mycelium and red color of the vegetative mycelium).

Excellent growth on Czapek's solution agar; forms red vegetative mycelium on some media. This taxon might also be placed in the White color series. Produces cinerubins A and B.

Type strain shows the highest sequence similarity to: *S. lucensis*, DQ442522, 99.5%; *S. achromogenes* subsp. *achromogenes*, AB184109, 99%.

Source: not known.

DNA G+C content (mol%): not known.

Type strain: ATCC 14971, DSM 40638, NBRC 15428, IMET 43354, JCM 4234, NRRL B-2724.

Sequence accession no. (16S rRNA gene): DQ445796.

341. **Streptomyces niveus** Smith, Dietz, Sokolski and Savage 1956, 135[AL]

ni've.us. L. masc. adj. *niveus* snow-white, referring to the color of the aerial mycelium of the organism.

Spore chains in Section *Spirales*, with as many as 10 or more well-formed volutions of narrow diameter at the ends of long sporophores. Mature spore chains generally have 10–50 spores per chain; longer chains are sometimes observed. This morphology is seen on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar. Spore surface is smooth.

Color of colony: aerial mass color in the Yellow color series on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar. Reverse side of colony with no distinctive pigment on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar; substrate pigment is not a pH indicator.

Color in medium: melanoid pigments are not formed in peptone-yeast-iron agar and tyrosine agar. Pigment, other than traces of yellow, are not formed in yeast-malt agar, oatmeal agar, salts-starch agar, or glycerol-asparagine agar.

D-Glucose, D-xylose, and D-fructose are utilized for growth. No growth or only trace of growth on sucrose, iso-inositol, and raffinose. Variable reports on growth with L-arabinose, D-mannitol, and rhamnose. However, reports from four cooperating laboratories indicate unusual disagreement regarding carbon utilization by this strain. The original description includes sucrose, rhamnose, and raffinose among carbon sources utilized for growth.

For sequence similarity, see type strain of *Streptomyces caeruleus*.

Source: not known.

DNA G+C content (mol%): not known.

Type strain: ATCC 19793, CBS 545.68, BCRC 11514, CCUG 11108, DSM 40088, IFM 1181, NBRC 12804, IMET 43503, JCM 4251, JCM 4599, LMG 5980, LMG 19395, NCIMB 9219, NRRL 2466, NRRL-ISP 5088, RIA 1072, UNIQEM 179.

Sequence accession no. (16S rRNA gene): AB184160.

Further comments: according to Lanoot et al. (2002), *Streptomyces niveus* (Smith et al. 1956) is a later heterotypic synonym of *Streptomyces caeruleus* (Baldacci 1944) Pridham et al. 1958 emend. Lanoot et al. 2002.

342. **Streptomyces noboritoensis** Isono, Yamashita, Tomiyama, Suzuki and Sakai 1957, 21[AL]

no.bo.ri.to.en′sis. N.L. masc. adj. *noboritoensis* of or belonging to noborito, referring to Inada-noborito, Kawasaki City, Kanagawa Prefecture, Japan, the source of the soil from which the organism was isolated.

Spore chains in Section *Rectiflexibiles*. Mature spore chains are moderately long, often with more than 50 spores per chain. This morphology is seen on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar, although the aerial mycelium may be thin or poorly developed on some of these media. Spore surface is smooth.

Color of colony: aerial mass color in the Gray color series on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar when mature sporulating mycelium is produced in adequate amount. Development of aerial mycelium may not be adequate for spore mass color determination on some or these media. Reverse side of colony is yellow to yellow brown modified to dark reddish brown or very dark brown on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar; substrate pigment is not a pH indicator.

Color in medium: melanoid pigments are formed in peptone-yeast-iron agar and tryptone-yeast broth, but may be absent or present only in trace amount in tyrosine agar. Brown pigment is sometimes found in the medium in yeast-malt agar, oatmeal agar, and salts-starch agar or pigment may be absent from these media. The brown pigment is not pH-sensitive when tested with 0.05 M NaOH or HCl.

D-Glucose, L-arabinose, D-xylose, D-mannitol, D-fructose, and raffinose are utilized for growth. Reports vary on the utilization of iso-inositol and utilization of sucrose is doubtful. No growth or only trace of growth with rhamnose.

Type strain shows the highest sequence similarity to: *S. melanogenes*, AB184222, 100%; *S. crystallinus*, AB184652, 99.2%.

Source: isolated from soil from Inada-noborito, Kawasaki City, Kanagawa Prefecture, Japan.

DNA G+C content (mol%): not known.

Type strain: AS 4.1457, ATCC 25477, CBS 887.69, BCRC 11553, DSM 40223, NBRC 13065, JCM 4065, JCM 4557, KCTC 9060, LMG 19337, NRRL B-12152, NRRL-ISP 5223, RIA 1257, VKM Ac-1012.

Sequence accession no. (16S rRNA gene): AB184287.

343. **Streptomyces nodosus** Trejo *in* Waksman 1961, 250[AL]

no.do′sus. L. masc. adj. *nodosus* knotty.

Spore chains in Section *Spirales*. Spirals on short spore chains may be poorly developed showing only a few turns or hooks of small diameter; or tightly knotted spirals may be formed. Spore chains are short, often with only 3–10 spores per chain. This morphology is seen on yeast-malt agar, salts-starch agar, and glycerol-asparagine agar. Spore surface is smooth, but some wrinkles or folds are found on the spore surface.

Color of colony: aerial mass color in the Gray color series on yeast-malt agar, salts-starch agar, and glycerol-asparagine agar. Reverse side of colony is grayed yellow or grayed greenish yellow to dark olive brown or near black on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar; substrate mycelium pigment is not a pH indicator.

Color in medium: melanoid pigments not formed in peptone-yeast-iron agar, tyrosine agar, and tryptone-yeast extract broth. Traces of red or yellow orange pigment may be found in the medium in yeast-malt agar, oatmeal agar, and glycerol-asparagine agar. One observer reports this pigment is pH-sensitive changing from red to green by addition of 0.05 M NaOH and from green to blue-red by 0.05 M HCl.

D-Glucose, D-xylose, iso-inositol, D-mannitol, D-fructose, and rhamnose are utilized for growth. No growth or only trace of growth on sucrose and raffinose. Utilization of L-arabinose is doubtful.

Type strain shows no sequence similarity over 99%.

Source: not known.

DNA G+C content (mol%): not known.

Type strain: AS 4.1459, ATCC 14899, ATCC 23942, CBS 926.68, BCRC 13768, DSM 40109, NBRC 12895, JCM 4297, JCM 4656, KCTC 9035, LMG 19340, NCIMB 12816, NRRL B-2371, NRRL-ISP 5109, RIA 1123, RIA 831, VKM Ac-1224.

Sequence accession no. (16S rRNA gene): AF114033.

344. **Streptomyces nogalater** Bhuyan and Dietz 1966, 838[AL]

no.gal.at′er. Spanish n. *nogal* walnut; L. adj. *ater* black; N.L. masc. adj. *nogalater* black walnut, referring to the production (by the organism) on most media of an odor like that of black walnuts.

Spore chains in Section *Rectiflexibiles* to *Retinaculiaperti* or possibly *Spirales*. Spore chains may be straight, strongly flexuous, or in irregular spirals. Straight to flexuous chains are usually seen on yeast-malt agar and glycerol-asparagine agar; strongly flexuous, *Retinaculiaperti* or *Spirales* spore chains may be found on oatmeal agar and salts-starch agar. Spore surface is smooth.

Color of colony: aerial mass color in the Gray color series (3fe or 4ig, light brownish gray; 4li, brownish gray; 3ig, grayish yellowish brown; or 2ih, light olive gray) on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar. Reverse side of colony is orange-yellow to light yellowish pink on yeast-malt agar; light yellow to orange yellow on oatmeal agar; pale or grayish yellow on salts-starch agar and glycerol-asparagine agar. One observer

reports that the reverse mycelium pigment is a pH indicator changing from orange to dark red or dark violet with the addition of 0.05 M NaOH and from orange to yellow orange with the addition of 0.05 M HCl.

Color in medium: melanoid pigments are not formed in peptone-yeast-iron agar, tyrosine agar, or tryptone-yeast broth. Red or yellow pigment may be found in the medium in yeast-malt agar, oatmeal agar, and glycerol-asparagine agar. One observer reports that this pigment is pH-sensitive, showing the same changes observed with the reverse mycelium pigments when 0.05 M NaOH or HCl is added.

D-Glucose, L-arabinose, D-xylose, D-mannitol, D-fructose, rhamnose, and raffinose are probably utilized for growth, but reports vary for L-arabinose, D-fructose, and rhamnose. No growth or only traces of growth is seen with sucrose or iso-inositol.

Type strain shows the highest sequence similarity to: *Streptomyces eurythermus*, D63870, 99.2%.

Source: not known.

DNA G+C content (mol%): not known.

Type strain: AS 4.1442, ATCC 27451, CBS 238.69, CBS 746.72, BCRC 12316, DSM 40546, HAMBI 951, NBRC 13445, IMET 43360, JCM 4553, JCM 4799, LMG 5981, LMG 19338, NCIMB 9489, NRRL 3035, NRRL-ISP 5546, RIA 1406, VKM Ac-1290.

Sequence accession no. (16S rRNA gene): AB045886.

345. **Streptomyces nojiriensis** Ishida, Kumagai, Niida, Hamamoto and Shomura 1967, 64[AL]

no.ji.ri.en′sis. N.L. masc. adj. *nojiriensis* of or belonging to Nojiri, named for Lake Nojiri at Nagano, Japan, the source of the soil from which the organism was isolated.

Poor growth on Czapek's solution agar. Produces nojirimycin, an anti-bacterial antibiotic.

Type strain shows the highest sequence similarity to: *S. xanthophaeus*, DQ442560, 100%; *S. spororaveus*, AJ781370, 100%; *S. goshikiensis*, EF178693, 99.9%; *S. cirratus*, AY999794, 99.9%; *S. vinaceus*, AB184394, 99.9%; *S. colombiensis*, DQ026646, 99.8%; *S. sporoverrucosus*, DQ442544, 99.8%; *S. cinnamonensis*, AB184707, 99.7%; *S. avidinii*, AB184395, 99.7%; *S. subrutilus*, X80825, 99.7%; *S. virginiae*, D85119, 99.5%; *S. racemochromogenes*, DQ026656, 99%; *S. polychromogenes*, AB184292, 99%.

Source: isolated from soil from Lake Nojiri at Nagano, Japan.

DNA G+C content (mol%): not known.

Type strain: ATCC 29781, DSM 41655, NBRC 13794, JCM 3382, KCTC 9784, NRRL B-16930.

Sequence accession no. (16S rRNA gene): AJ781355.

346. **Streptomyces noursei** Brown, Hazen and Mason 1953, 609[AL]

nour′sei. N.L. gen. masc. n. *noursei* of Nourse, referring to the owner of the farm where soil was obtained from which the organism was isolated.

Poor growth on Czapek's solution agar; NaCl tolerance >7%, but <10%. Exhibits anti-bacterial activity; produces nystatin and cycloheximide.

Type strain shows the highest sequence similarity to: *S. albulus*, AB024440, 100%; *S. yunnanensis*, AF346818, 99.7%.

Source: isolated from soil.

DNA G+C content (mol%): not known.

Type strain: ATCC 11455, CBS 240.57, BCRC 12044, CECT 3240, DSM 40635, NBRC 15452, JCM 4922, KCTC 1083, LMG 5982, NCIMB 8593, NRRL B-1714.

Sequence accession no. (16S rRNA gene): AB184678.

347. **Streptomyces novaecaesareae** Waksman and Henrici *in* Breed, Murray and Hitchens 1948, 951[AL]

no.va.e.ca.e.sa.re′a.e. N.L. gen. n. *novaecaesareae* intended to mean of New Jersey, USA.

Spore chain section undetermined. Aerial mycelium is not found on any of the ISP media. The original description for *Actinomyces violaceus-caeseri* Waksman and Curtis (1916) states that on Czapek agar: "Aerial mycelium produced very late; … Conidia could not be demonstrated", and little or no aerial mycelium was reported for other media included in the original description. Spore surface undetermined. Color of colony: not determined. Reverse side of colony with distinctive grayish blue or blue green to grayish violet or red violet pigment is produced on oatmeal agar, salts-starch agar, and glycerol-asparagine agar, but the pigment is not always present on these media. Reverse mycelium is usually grayish yellow to grayish olive on yeast-malt agar. Reverse mycelium pigment is a pH indicator changing from pale violet to pale grayish blue with the addition of 0.05 M NaOH and from pale violet to reddish violet or pale grayish red with the addition of 0.05 M HCl.

Color in medium: melanoid pigments are not formed in peptone-yeast-iron agar, tyrosine agar, or tryptone-yeast broth. Blue or violet pigment is sometimes found in the medium in yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar; it is most consistently present in oatmeal agar. This pigment is pH-sensitive showing the same changes reported for the reverse mycelium pigment.

D-Glucose, L-arabinose, D-xylose, iso-inositol, D-mannitol, D-fructose, rhamnose, sucrose, and raffinose are all utilized for growth.

Type strain shows the highest sequence similarity to: *S. aureocirculatus*, AB184260, 99.5%; *S. pseudovenezuelae*, AB184233, 99.5%; *S. phaeoluteigriseus*, AJ391815, 99.5%; *S. galilaeus*, AB045878, 99.4%; *S. resistomycificus*, AB184166, 99.3%; *S. bobili*, AB249925, 99.3%; *S. humidus*, DQ442508, 99.1%; *S. chartreusis*, AB184839, 99.1%; *S. flavovariabilis*, EF178691, 99.1%; *S. canus*, AY999775, 99%; *S. prunicolor*, DQ026659, 99%.

Source: not known.

DNA G+C content (mol%): not known.

Type strain: AS 4.1471, ATCC 27452, CBS 134.20, CBS 669.72, DSM 40358, NBRC 13368, JCM 4800, NRRL B-1267, NRRL B-3011, NRRL-ISP 5358, RIA 1329, VKM Ac-963.

Sequence accession no. (16S rRNA gene): AB184357.

348. **Streptomyces ochraceiscleroticus** Pridham 1970, 22[AL]

o.chra.ce.i.scle.ro′ti.cus. L. n. *ochra* ochre; N.L. adj. *ochraceus* like-ochre, rust colored; N.L. n. *sclerotium* sclerotium; N.L. masc. adj. *ochraceiscleroticus* sclerotium with rust color.

Spore chains in Section *Spirales*. Mature spore chains are moderately long with 10 to 50 or more spores per chain.

This morphology is seen on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar. Spore surface is smooth. Sclerotia-like granules are formed on glycerol-asparagine agar and other media.

Color of colony: aerial mass color in the Yellow or Red color series on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar. White or gray aerial mycelium may also be seen on these media. Nearest matching color tab recorded for the Yellow color series is 2ba, pale yellow; nearest tabs in the Red color series are 4ie, light brown, and 3ea, light orange-yellow. Reverse side of colony is strong brown, moderate orange, or orange-yellow on yeast-malt agar and glycerol-asparagine agar; light yellow, yellow or light yellowish brown on oatmeal agar and salts-starch agar. Reverse mycelium pigment is not a pH indicator.

Color in medium: melanoid pigments are not formed in peptone-yeast-iron agar, tyrosine agar, or tryptone-yeast broth. No pigment is found in the medium in yeast-malt agar, oatmeal agar, salts-starch agar, or glycerol-asparagine agar.

D-Glucose, L-arabinose, D-xylose, iso-inositol, D-mannitol, D-fructose, rhamnose, sucrose, and raffinose are all utilized for growth.

Type strain shows the highest sequence similarity to: *S. purpurogeneiscleroticus*, AJ621604, 99.9%; S. *violens*, AJ621605, 99.8%; *S. monomycini*, DQ445790, 99.5%; *S. erumpens*, AJ621603, 99.3%; *S. sioyaensis*, DQ026654, 99.1%; *S. olivaceiscleroticus*, AJ621606, 99.1%; *S. niger*, AJ621607, 99.1%.

Source: not known.

DNA G+C content (mol%): not known.

Type strain: AS 4.1096, ATCC 15814, CBS 168.62, CBS 784.72, BCRC 13310, DSM 40594, DSM 43155, NBRC 12394, NBRC 13483, IMET 43492, JCM 3048, JCM 4801, LMG 19349, NRRL B-3041, NRRL-ISP 5594, PCM 2307, RIA 1444, RIA 710, VKM Ac-651.

Sequence accession no. (16S rRNA gene): AB184094.

Further comments: Streptomyces ochraceiscleroticus Pridham 1970 and *Chainia ochracea* Kuznetsov 1962 have the same type strain and therefore are homotypic synonyms [Rules 24a and 24b (1) of the *Bacteriological Code* (1990 Revision)].

349. **Streptomyces odorifer** (Rullman 1895) Waksman *in* Waksman and Lechevalier 1953, 79[AL] ["*Cladothrix odorifera*" Rullmann 1895, 44; "*Oospora odorifera*" Lehmann and Neumann 1896, 392; "*Actinomyces odoriferus rullmanni I*" (*sic*) Berestnev 1897, 167; "*Cladothrix odoriferus rullmanni I*" (*sic*) Berestnev 1897, 167; "*Actinomyces odorifer*" Lachner-Sandoval 1898, 65; "*Streptothrix odorifera*" Foulerton and Price-Jones 1902, 112; "*Nocardia odorifera*" Castellani and Chalmers 1913, 818]

o.do'ri.fer. L. masc. adj. *odorifer* fragrant.

Spore chains in Section *Rectiflexibiles*. Flexuous chains are common. Mature spore chains generally contain 10 to 50 or more spores per chain. This morphology is seen on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar, but aerial mycelium may be poorly developed on salts-starch agar. Spore surface is smooth.

Color of colony: aerial mass color in the Yellow color series (1ba, 2ba, or 2db, pale yellow, or 1dc, pale yellow green) on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar. Reverse side of colony with no distinctive pigments (strong brown to olive brown on yeast-malt agar; light yellow to greenish yellow, grayish yellow, or light brown on oatmeal agar, salts-starch agar, and glycerol-asparagine agar).

Color in medium: melanoid pigments are not formed in peptone-yeast-iron agar, tyrosine agar, or tryptone-yeast broth. Yellow pigment is found in the medium in yeast-malt agar and salts-starch agar. One observer also reports yellow pigment on oatmeal agar and glycerol-asparagine agar. This pigment is not pH-sensitive when tested with 0.05 M NaOH or HCl.

D-Glucose, L-arabinose, D-xylose, D-mannitol, D-fructose, and sucrose are utilized for growth. No growth or only traces of growth with iso-inositol, rhamnose, and raffinose.

Type strain shows the highest sequence similarity to: *S. limosus*, AB184147, 100%; *S. felleus*, AB184129, 100%; *S. daghestanicus*, DQ442497, 100%; *S. albidoflavus*, AB184255, 100%; *S. violascens*, AY999737, 100%; *S. hydrogenans*, AB184868, 100%; *S. griseus* subsp. *solvifaciens*, AB249915, 100%; *S. champavatii*, DQ026642, 100%; *S. canescens*, AB184117, 100%; *S. sampsonii*, D63871, 99.9%; *S. koyangensis*, AY079156, 99.7%.

Source: not known.

DNA G+C content (mol%): not known.

Type strain: ATCC 6246, CBS 666.72, BCRC 13704, CECT 3178, DSM 40347, NBRC 13365, IMET 41377, JCM 4198, JCM 4803, NRRL B-1328, NRRL-ISP 5347, RIA 1326, VKM Ac-748.

Sequence accession no. (16S rRNA gene): Z76682.

350. **Streptomyces olivaceiscleroticus** (Thirumalachar and Sukapure 1964) Pridham 1970, 41[AL] (*Chainia olivacea* Thirumalachar and Sukapure 1964, 160)

o.li.va.ce.i.scle.ro'ti.cus. N.L. adj. *olivaceus* olive colored; N.L. n. *sclerotium* sclerotium; N.L. masc. adj. *olivaceiscleroticus* sclerotium with olive color.

Spore chains in Section *Spirales*. Mature spore chains generally contain 10–50 spores per chain. This morphology may be seen on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar, or sporulating aerial mycelium may be poorly developed on various media. Spore surface is smooth. Tight spirals may coalesce. One of three observers reports sclerotia formation.

Color of colony: aerial mass color in the Gray color series (2dc, yellowish gray; 3ge light grayish yellowish brown; 3–4li, brownish gray; or 2ge, light olive brown) on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar. White or yellow (2ba, pale yellow) aerial mycelium is also reported. Reverse side of colony with no distinctive pigments (grayish yellow to olive brown) on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar.

Color in medium: melanoid pigments are not formed in peptone-yeast-iron agar, tyrosine agar, or tryptone-yeast

broth. Reddish pigment is sometimes found in the medium in yeast-malt agar, oatmeal agar, and salts-starch agar. Yellowish green pigment may be present in yeast-malt agar and glycerol-asparagine agar, or pigment may be absent in these media; pigments are not pH-sensitive when tested with 0.05 M NaOH or HCl.

D-Glucose, L-arabinose, D-xylose, iso-inositol, D-mannitol, D-fructose, rhamnose, sucrose, and raffinose are all utilized for growth.

Type strain shows the highest sequence similarity to: *S. niger*, AJ621607, 100%; *S. monomycini*, DQ445790, 99.4%; *S. rimosus* subsp. *rimosus*, AB045883, 99.3%; *S. sclerotialus*, AJ621608, 99.2%; *S. purpurogeneiscleroticus*, AJ621604, 99.1%; *S. ochraceiscleroticus*, AB184094, 99.1%.

Source: not known.

DNA G+C content (mol%): not known.

Type strain: ATCC 15722, CBS 296.66, CBS 785.72, BCRC 11608, DSM 40595, NBRC 13484, JCM 3045, JCM 4805, NRRL B-2318, NRRL-ISP 5595, RIA 1445.

Sequence accession no. (16S rRNA gene): AJ621606.

Further comments: Streptomyces olivaceiscleroticus Pridham 1970 and *Chainia olivacea* Thirumalachar and Sukapure 1964 have the same type strain and therefore are homotypic synonyms [Rules 24a and 24b (1) of the *Bacteriological Code* (1990 Revision)].

351. **Streptomyces olivaceoviridis** (Preobrazhenskaya and Ryabova *in* Gauze, Preobrazhenskaya, Kudrina, Blinov, Ryabova and Sveshnikova 1957) Pridham, Hesseltine and Benedict 1958, 65[AL] ("*Actinomyces olivaceoviridis*" Preobrazhenskaya and Ryabova *in* Gauze, Preobrazhenskaya, Kudrina, Blinov, Ryabova and Sveshnikova 1957, 163)

o.li.va.ce.o.vi′ri.dis. N.L. adj. *olivaceus* olive colored; L. adj. *viridis* green; N.L. masc. adj. *olivaceoviridis* olive-green colored, referring to the gray-olive colored aerial mycelium and greenish gray-brown vegetative mycelium on a chemically defined medium.

Spore chains in Section *Retinaculiaperti* or *Spirales*. Hooks, loops, and terminal spirals of only one or two turns are common; flexuous chains and primitive spirals may also be found. Mature spore chains generally contain 10 to 50 or more spores per chain. This morphology is seen on oatmeal agar, salts-starch agar, and glycerol-asparagine agar; spore chains may be short or poorly developed on yeast-malt agar. Spore surface is smooth.

Color of colony: aerial mass color usually in the Gray or Yellow color series on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar. The aerial mass color is usually pale yellowish green to grayish yellow or even light grayish olive (tabs 1cb or 1½ec from the Yellow color series; 2dc or 2ge from the Gray color series or 3ec from the Red color series; 1½ge from the Green color series). Immature aerial mycelium may also appear to be in the White color series. Reverse side of colony with no distinctive pigments (pale yellow to orange-yellow on yeast-malt agar; pale yellow to grayish yellow on oatmeal agar, salts-starch agar, and glycerol-asparagine agar).

Color in medium: melanoid pigments are not formed in peptone-yeast-iron agar, tyrosine agar, or tryptone-yeast broth. No pigment, or only a trace of yellow, is found in the medium in yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar.

D-Glucose, L-arabinose, D-xylose, D-mannitol, D-fructose, rhamnose, sucrose, and raffinose are utilized for growth. Some growth is also reported on iso-inositol, but less than with the other carbon sources noted above.

Type strain shows the highest sequence similarity to: *S. canarius*, AB184396, 100%; *S. corchorusii*, AB184267, 99.9%; *S. capoamus*, AB045877, 99.5%; *S. bungoensis*, AB184696, 99.3%; *S. galbus*, X79852, 99.2%; *S. longwoodensis*, AB184580, 99.2%; *S. cellostaticus*, AB184192, 99%; *S. griseochromogenes*, AB184387, 99%.

Source: not known.

DNA G+C content (mol%): not known.

Type strain: AS 4.1430, ATCC 23630, ATCC 25478, CBS 888.69, DSM 40334, NBRC 13066, IMET 43128, INA 11584, JCM 4499, KCTC 9132, LMG 19324, NCIMB 9982, NRRL B-12280, NRRL-ISP 5334, RIA 1258, VKM Ac-1852.

Sequence accession no. (16S rRNA gene): AB184288.

352. **Streptomyces olivaceus** (Waksman 1923) Waksman and Henrici *in* Breed, Murray and Hitchens 1948, 950[AL] ("*Actinomyces olivaceus*" Waksman 1923, 354)

o.li.va′ce.us. N.L. masc. adj. *olivaceus* olive colored, apparently referring to the color of vegetative mycelium.

Spore chains in Section *Spirales*, with open spirals intergrading through flexuous spore chains suggestive of Section *Rectiflexibiles*. Mature spore chains are generally long, often with more than 50 spores per chain. This morphology is seen on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar. Spore surface is smooth.

Color of colony: aerial mass color in the Gray color series on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar. Reverse side of colony is usually grayed yellow on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar. Production of dark reverse color is variable on these media; substrate pigment is not a pH indicator.

Color in medium: melanoid pigments not formed in peptone-yeast-iron agar and tyrosine agar. No pigment found in medium in yeast-malt agar, oatmeal agar, salts-starch agar, or glycerol-asparagine agar.

D-Glucose, L-arabinose, D-xylose, iso-inositol, D-mannitol, D-fructose, and rhamnose are utilized for growth. No growth or only trace of growth on sucrose and raffinose.

Type strain shows the highest sequence similarity to: *S. pactum*, AB184398, 100%; *S. parvulus*, AB184326, 99.3%; *S. coeruleorubidus*, AY999719, 99.2%; *S. rubrogriseus*, AB184681, 99.2%; *S. lienomycini*, AJ781353, 99.2%; *S. anthocyanicus*, AB184631, 99.1%; *S. tricolor*, AB184687, 99%; *S. malachitospinus*, AB249954, 99%.

Source: not known.

DNA G+C content (mol%): not known.

Type strain: ATCC 19794, ATCC 3335, CBS 546.68, CCM 3188, BCRC 11485, CCUG 11111, DSM 40072, NBRC 12805, INA 3200, JCM 4402, NRRL B-1224, NRRL B-3009, NRRL-ISP 5072, RIA 1073, RIA 481, UNIQEM 180, VKM Ac-254.

Sequence accession no. (16S rRNA gene): AB184743.

353. **Streptomyces olivochromogenes** (Waksman 1923) Waksman and Henrici *in* Breed, Murray and Hitchens 1948, 941[AL] ["*Actinomyces olivochromogenus*" (*sic*) Waksman 1923; "*Streptomyces olivochromogenus*" (*sic*) Waksman and Henrici 1948]

o.li.vo.chro.mo′ge.nes. L. n. *oliva* olive; Gr. n. *chroma* color; N.L. suff. *-genes* (from Gr. v. *gennaô* to produce) producing; N.L. adj. *olivochromogenes* producing an olive color.

Spore chains in Section *Spirales*, but sporulation aerial mycelium is poorly developed and spore chains are generally short (3–10 spores per chain) so that flexuous chains, hooks, loops, and imperfect or irregular spirals are common. This morphology is seen on yeast-malt agar, oatmeal agar, salts-starch agar, glycerol-asparagine agar, Czapek's sucrose agar, and soil agar. The abundant cottony aerial mycelium mentioned in the original description (op. cit) is not seen on ISP media. Spore surface is smooth.

Color of colony: sporulation on yeast-malt agar, oatmeal agar, salts-starch agar and glycerol-asparagine agar is inadequate for accurate spore mass color determination. Reverse side of colony with no distinctive pigments (nearly colorless or grayish yellow to grayish greenish yellow or moderate yellow) on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar.

Color in medium: melanoid pigments are formed in peptone-yeast-iron agar, and tryptone-yeast broth; a faint brown pigment may be also produced in tyrosine agar in 4 d. A trace of yellow or greenish pigment may or may not be found in the medium in yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar.

D-Glucose, L-arabinose, D-xylose, iso-inositol, D-mannitol, D-fructose, rhamnose, and sucrose are utilized for growth. No growth or only trace of growth with raffinose.

Type strain shows the highest sequence similarity to: *S. mirabilis*, AB184412, 99.4%.

Source: not known.

DNA G+C content (mol%): not known.

Type strain: ATCC 25479, ATCC 3336, CBS 889.69, DSM 40451, NBRC 13067, NBRC 3178, IMET 40352, JCM 4163, JCM 4500, KCTC 9064, NRRL B-1341, NRRL-ISP 5451, RIA 1259.

Sequence accession no. (16S rRNA gene): AY094370.

354. **Streptomyces olivomycini** (Gause and Sveshnikova 1986b) Witt and Stackebrandt 1991, 456[VP] (Effective publication: Witt and Stackebrandt 1990, 370.) (*Streptoverticillium olivomycini* Gause and Sveshnikova (Gause and Sveshnikova 1986b)

o.li.vo.my.ci′ni. N.L. n. *olivomycinum* olivomycin; N.L. gen. n. *olivomycini* of olivomycin, intended to mean olivomycin producing.

Spore chains are straight, spores are smooth. On mineral agar 1: aerial mycelium is creamy, pale lilac with a brownish note; substrate mycelium is brownish-greenish, brownish-olive, grayish-greenish; no diffusible pigment. On starch-ammonia agar (ISP 4): aerial mycelium is creamy, poor; substrate mycelium is brownish-gray, weak growth; no diffusible pigment. On glycerol-nitrate agar: aerial mycelium is creamy; substrate mycelium is brownish-greenish, olive green; diffusible pigment is greenish, weak. On glycerol-asparagine agar (ISP 5): aerial mycelium is pale lilac; substrate mycelium is brown-gray greenish; no diffusible pigment. On oatmeal agar: aerial mycelium is creamy, pale lilac; substrate mycelium is olive to brownish; diffusible pigment is light greenish. On organic agar 2: aerial mycelium yellowish to light pink; substrate mycelium is yellowish-brownish to greenish; no diffusible pigment.

Melanoid pigments are not formed. Carbon utilization: weak digestion of sucrose, arabinose, rhamnose, xylose, fructose, raffinose, and mannitol. Antibiotic: olivomycin.

Source: not known.

DNA G+C content (mol%): not known.

Type strain: INA 16749.

Sequence accession no. (16S rRNA gene): no sequence available.

355. **Streptomyces olivoreticuli** (Arai, Nakada and Suzuki 1957) Witt and Stackebrandt 1996, 456[VP] (Effective publication: Witt and Stackebrandt 1990, 370.) ("*Streptomyces olivoreticuli*" Arai, Nakada and Suzuki 1957, 441; *Streptoverticillium olivoreticuli* Baldacci, Farina and Locci 1966, 162)

o.li.vo.re.ti′cu.li. L. n. *oliva* olive; L. n. *reticulum* small net; N.L. gen. n. *olivoreticuli* of olive-colored net, itended to mean having the appearance of olive-colored nets.

Spore chains in Umbellate monoverticillate (=*Streptomyces* Section Verticillati, biverticillate). Mature spore chains are generally short with 3–10 spores per chain. This morphology may be observed on Pridham and Gottlieb carbon utilization medium with glucose or inositol, but good sporulation is usually not seen on yeast-malt agar, oatmeal agar, salts-starch agar, or glycerol-asparagine agar. Spore surface is smooth.

Color of colony: aerial mass color in the White color series on media supporting sporulating aerial growth. In the absence of sporulation, the surface is pale yellow on yeast-malt agar, oatmeal agar, and glycerol-asparagine agar and white on salts-starch agar. Reverse side of colony with no distinctive pigments (colorless to grayish yellowish brown) on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar.

Color in medium: melanoid pigments are formed in peptone-yeast-iron agar and tryptone-yeast broth, but not in tyrosine agar. No pigment found in medium in yeast-malt agar, oatmeal agar, salts-starch agar, or glycerol-asparagine agar. Carbon utilization appears to be unusually variable. Reports from three observers differ on utilization of each carbon source tested and results vary on duplicate dishes in the same laboratory. The original description (op. cit.) directs attention to fluctuation of carbon utilization by ultra-violet irridiated strains. This fluctuation appears also to be a characteristic of the type strain.

For sequence similarity, see type strain of *Streptomyces abikoensis*.

Source: not known.

DNA G+C content (mol%): not known.

Type strain: ATCC 23943, CBS 927.68, BCRC 13765, DSM 40105, IFM 1018, NBRC 12896, IMET 43690, JCM 4176, JCM 4657, NRRL B-2091, NRRL-ISP 5105, RIA 1122, VKM Ac-839.

Sequence accession no. (16S rRNA gene): AB184853.

Further comments: in violation of Rule 33c of the *Bacteriological Code* (1990 Revision), in Validation List no. 38, *Streptomyces olivoreticuli* is proposed as a *nomen revictum* (basonym: "*Streptomyces olivoreticuli*" Arai et al. (1957).

Witt and Stackebrandt proposed to transfer *Streptoverticillium olivoreticuli* corrig. (Arai et al. 1957) Baldacci et al. (1966) to the genus *Streptomyces* as *Streptomyces olivoreticuli* (Arai et al. 1957) Witt and Stackebrandt 1991. However, Validation List no. 38 does not include formal propositions about *Streptoverticillium olivoreticuli* subsp. *cellulophilum* corrig. Locci and Schofield 1989 and *Streptoverticillium olivoreticuli* subsp. *olivoreticuli* corrig. (Arai et al. 1957) Baldacci et al. 1966.

According to Hatano et al. (2003), *Streptomyces olivoreticuli* (Arai et al. 1957) Witt and Stackebrandt 1991 is a later heterotypic synonym of *Streptomyces abikoensis* (Umezawa et al. 1951) Witt and Stackebrandt 1991.

356. **Streptomyces olivoverticillatus** (Shinobu 1956) Witt and Stackebrandt 1991, 456[VP] (Effective publication: Witt and Stackebrandt 1990, 370.) ("*Streptomyces olivoverticillatus*" Shinobu 1956, 92; "*Verticillomyces olivoverticillatus*" Shinobu 1965; *Streptoverticillium olivoverticillatum* Baldacci, Farina and Locci 1966, 163)

o.li.vo.ver.ti.cil.la′tus. L. n. *oliva* olive; L. n. *verticillus* a whorl; N.L. adj. *verticillatus* whorled; N.L. masc. adj. *olivoverticillatus* olive, whorled.

Spore chain Section not determined. The original description of Shinobu ((1956), op. cit) states: "The formation of aerial mycelium of this strain was generally not good, even on a suitable media for growth". ISP observers found some aerial mycelium with straight, flexuous, and abortive spiral spore chains on oatmeal agar. Electron microscope preparations from yeast-malt agar, oatmeal agar, and salts-starch agar contained some flexuous to spiral chains of 13 to 20 or more spores. "Whirls" as described by Shinobu ((1956), op. cit) were not found by ISP observers. Spore surface is spiny.

Color of colony: aerial mass color probably in the Yellow or Gray color series (1db, pale yellow green; 3fe light brownish gray) when mature spores occur on yeast-malt agar, oatmeal agar, and salts-starch agar. Thin or immature aerial mycelium is white. Reverse side of colony with no distinctive pigments (nearly colorless, yellow, greenish yellow or olive brown) on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar.

Color in medium: melanoid pigments are not formed in peptone-yeast-iron agar, tyrosine agar, or tryptone-yeast broth. Brown or yellow-brown pigment may be found in the medium in yeast-malt agar, oatmeal agar, and salts-starch agar or the medium may remain unpigmented.

D-Glucose and iso-inositol are utilized for growth. Utilization of L-arabinose, D-xylose, D-mannitol, D-fructose, rhamnose, sucrose, and raffinose is doubtful.

Type strain shows the highest sequence similarity to: *S. thioluteus*, AB184753, 99.1%; *S. abikoensis*, AB184537, 99%.

Source: not known.

DNA G+C content (mol%): not known.

Type strain: ATCC 25480, CBS 890.69, BCRC 13610, CECT 3266, DSM 40250, NBRC 13068, NBRC 15273, NBRC 3842, NBRC 3929, JCM 4100, JCM 4501, NCIMB 97148, NRRL B-1994, NRRL-ISP 5250, RIA 1260, RIA 551, VKM Ac-890.

Sequence accession no. (16S rRNA gene): AB184636.

Further comments: in violation of Rule 33c of the *Bacteriological Code* (1990 Revision), in Validation List no. 38, *Streptomyces olivoverticillatus* is proposed as a *nomen revictum* (basonym: "*Streptomyces olivoverticillatus*" Shinobu 1956).

According to Hatano et al. (2003), *Streptomyces olivoverticillatus* (Shinobu 1956) Witt and Stackebrandt 1991 is an earlier heterotypic synonym of *Streptomyces viridiflavus* corrig. (Locci and Schofield 1989) Witt and Stackebrandt 1991.

357. **Streptomyces olivoviridis** (Kuchaeva, Krasil'nikov, Skryabin and Taptykova *in* Rautenshtein 1960) Pridham 1970, 23[AL] ["*Actinomyces olivoviridis*" Kuchaeva, Krasil'nikov, Skryabin and Taptykova *in* Rautenshtein 1960; "*Actinomyces olivovirilis*" (*sic*) Kuchaeva, Krasil'nikov, Skryabin and Taptykova *in* Rautenshtein 1960]

o.li.vo.vi′ri.dis. L. n. *oliva* olive; L. adj. *viridis* green; N.L. masc. adj. *olivoviridis* olive-green.

Spore chains in Section *Rectiflexibiles.* The predominantly flexuous or wavy spore chains may also form occasional hooks and open spirals (rare) resembling *Retinaculiaperti* or Spiral morphology. Mature spore chains are generally long with 10–50 spores per chain. This morphology is seen on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar. Spore surface is spiny.

Color of colony: aerial mass color in the Gray color series on yeast-malt agar, oatmeal agar, and salts-starch agar; aerial mycelium is poorly developed or white on glycerol-asparagine agar. Reverse side of colony is yellow to yellow brown is usually modified by green (olive brown to yellow green or grayish green) on yeast-malt agar, oatmeal agar, and salts-starch agar; no distinctive substrate mycelium pigment on glycerol-asparagine agar. Substrate mycelium pigment is not a pH indicator.

Color in medium: melanoid pigments are not formed in peptone-yeast-iron agar or tyrosine agar. Some coloration may appear in medium in tryptone-yeast broth. Yellow or green pigment is found in the medium in yeast-malt agar, oatmeal agar, and salts-starch agar. This pigment is not pH-sensitive when tested with 0.05 M NaOH or HCl.

D-Glucose, L-arabinose, D-xylose, D-mannitol, D-fructose, and rhamnose are utilized for growth. No growth or only trace of growth with sucrose, iso-inositol, and raffinose.

Type strain shows the highest sequence similarity to: *S. clavifer*, DQ026670, 100%; *S. atroolivaceus*, AJ781320, 100%; *S. mutomycini*, AB249951, 99.7%; *S. finlayi*, AY999788, 99.7%; *S. sindenensis*, AB184759, 99.5%; *S. fimicarius*, AY999784, 99.5%; *S. pluricolorescens*, DQ442540, 99.5%; *S. griseoplanus*, AY999894, 99.5%; *S. griseinus*, AB184205, 99.5%; *S. lavendulae* subsp. *lavendulae*, AB184080, 99.5%; *S. mediolani*, AB184674, 99.5%; *S. cavourensis* subsp. *washingtonensis*, DQ026671, 99.5%; *S. flavofuscus*, AB249935, 99.5%; *S. badius*, AY999783, 99.5%; *S. anulatus*, DQ026637, 99.5%; *S. rubiginosohelvolus*, AB184240, 99.5%; *S. praecox*, AB184293, 99.5%; *S. albovinaceus*, AB249958, 99.4%; *S. cinereorectus*, AB184646, 99.4%; *S. lipmanii*, AB184148, 99.4%; *S. baarnensis*, EF178688, 99.4%; *S. fulvorobeus*,

AB184711, 99.4%; *S. acrimycini*, AY999889, 99.4%; *S. microflavus*, DQ445795, 99.4%; *S. alboviridis*, AB184256, 99.4%; *S. cyaneofuscatus*, AB184860, 99.4%; *S. globisporus* subsp. *globisporus*, EF178686, 99.4%; *S. parvus*, DQ442537, 99.3%; *S. griseolus*, AB184768, 99.3%; *S. argenteolus*, AB045872, 99.3%; *S. luridiscabiei*, AF361784, 99.3%; *S. floridae*, AB184656, 99.3%; *S. californicus*, AB184755, 99.3%; *S. flavovirens*, DQ026635, 99.3%; *S. flavogriseus*, AJ494864, 99.2%; *S. halstedii*, EF178695, 99.2%; *S. griseus* subsp. *griseus*, AY207604, 99.2%; *S. candidus*, DQ026663, 99.1%; *S. pulveraceus*, AB184806, 99%; *S. nitrosporeus*, EF178680, 99%; *S. bacillaris*, AB184439, 99%.

Source: not known.

DNA G+C content (mol%): not known.

Type strain: ATCC 15882, ATCC 23944, CBS 928.68, DSM 40211, NBRC 12897, INMI 1475, JCM 4432, JCM 4658, NRRL B-3374, NRRL-ISP 5211, RIA 1157, RIA 661, VKM Ac-259.

Sequence accession no. (16S rRNA gene): AB184227.

358. **Streptomyces omiyaensis** Umezawa and Okami *in* Umezawa,Tazaki, Okami and Fukuyama 1950, 293[AL]

o.mi.ya.en′sis. N.L. masc. adj. *omiyaensis* of or belonging to Omiya City near Tokyo, Japan, the source of the soil from which the organism was isolated.

Spore chains in Section *Rectiflexibiles*. Mature spore chains are generally long with 10 to 50 or more spores per chain. This morphology is seen on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar. Spore surface is smooth.

Color of colony: aerial mass color in the Gray color series (d. light gray, or 2dc, yellowish gray) on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar. Reverse side of colony with no distinctive pigments (light yellow, pale yellow, or grayish yellow) on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar.

Color in medium: melanoid pigments are not formed in peptone-yeast-iron agar, tyrosine agar or tryptone-yeast broth. No pigment is found in the medium in yeast-malt agar, oatmeal agar, salts-starch agar or glycerol-asparagine agar.

D-Glucose, D-xylose, and rhamnose are utilized for growth. Only traces of growth are seen on L-arabinose or D-fructose; iso-inositol, D-mannitol, raffinose, and sucrose are not utilized.

Type strain shows the highest sequence similarity to: *S. exfoliatus*, AB184324, 99.6%; *S. wedmorensis*, DQ442557, 99.6%; *S. venezuelae*, AB045890, 99.6%; *S. zaomyceticus*, EF178685, 99.6%; *S. lateritius*, AB184324, 99.5%; *S. litmocidini*, AB184149, 99.5%; *S. yereyanensis* EF178684, 99.3%; *S. narbonensis*, DQ445794, 99.1%.

Source: isolated from soil from Omiya City near Tokyo, Japan.

DNA G+C content (mol%): not known.

Type strain: ATCC 27454, CBS 750.72, BCRC 11897, DSM 40552, NBRC 13449, IMET 43362, JCM 4806, NRRL B-1587, NRRL-ISP 5552, RIA 1410, VKM Ac-1903.

Sequence accession no. (16S rRNA gene): EF178697.

359. **Streptomyces orinoci** (Cassinelli, Grein, Orezzi, Pennella and Sanfilippo 1967) Witt and Stackebrandt 1991, 456[VP] (Effective publication: Witt and Stackebrandt 1990, 370.) (*Streptoverticillium orinoci* Cassinelli, Grein, Orezzi, Pennella and Sanfilippo 1967, 367)

o.ri.no′ci. N.L. gen. n. *orinoci* of Orinoco, named after the Orinoco River, South America, from whose banks it was isolated.

Spore chains in Section Umbellate Monoverticillate (=*Streptomyces* Section Verticillati, biverticillate). Mature spore chains generally contain more than 10 spores per chain. This morphology is usually seen on ISP yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar, but sporulating aerial mycelium may be observed in the scant growth on carbon-utilization medium enriched with raffinose, D-fructose, rhamnose, or iso-inositol or on half-strength Emerson potato-glucose agar. Spore surface is smooth.

Color of colony: sporulating aerial mycelium is not produced on yeast-malt agar, oatmeal agar, salts-starch agar, or glycerol-asparagine agar. Aerial mass color on carbon-utilization medium plus fructose is in the Gray color series. Reverse side of colony is strong yellow or deep yellow on yeast-malt agar, glycerol-asparagine agar, and half-strength Emerson potato-glucose agar. Colorless to pale or grayish yellow on salts-starch agar and glycerol-asparagine agar.

Color in medium: melanoid pigments are not formed in peptone-yeast-iron agar, tyrosine agar, or tryptone-yeast broth. No pigment is found in the medium in yeast-malt agar, oatmeal agar, salts-starch agar, or glycerol-asparagine agar.

D-Glucose is utilized for growth. No growth or only traces of growth with L-arabinose, D-xylose, iso-inositol, D-mannitol, D-fructose, rhamnose, sucrose, and raffinose.

Type strain shows no sequence similarity over 99%.

Source: isolated from the banks of the Orinoco River, South America.

DNA G+C content (mol%): not known.

Type strain: ATCC 23202, CBS 767.72, CECT 3267, DSM 40571, IFM 1226, NBRC 13466, JCM 4546, JCM 4807, NRRL B-3379, NRRL-ISP 5571, RIA 1427, VKM Ac-929.

Sequence accession no. (16S rRNA gene): AB184866.

360. **Streptomyces pactum** (*sic*) Bhuyan, Dietz and Smith 1962, 185[AL]

pac′tum. L. adj. *pactum* settled (referring to the compactness of the coiled chains of spores).

Spore chains in Section *Spirales*. Mature spore chains contain 10–50 spores per chain. This morphology is seen on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar, but sporulation is usually poor on glycerol-asparagine agar. Spore surface is hairy.

Color of colony: aerial mass color in the Gray color series (d, light gray, or e, medium gray) on oatmeal agar and salts-starch agar; Gray or Blue color series on oatmeal agar. Nearest matching color tab in the Blue color series is 19fe, pale blue. On glycerol-asparagine agar, aerial mycelium is in the White color series. Reverse side of colony

with no distinctive pigments (light yellowish brown, grayish yellow or grayish greenish yellow) on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar.

Color in medium: melanoid pigments are not formed in peptone-yeast-iron agar, tyrosine agar, or tryptone-yeast broth. Yellow pigment may be found in the medium in yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar. This pigment is not pH-sensitive when tested with 0.05 M NaOH or HCl.

D-Glucose is utilized for growth and only traces of growth are reported with D-fructose. No growth with L-arabinose, D-xylose, iso-inositol, D-mannitol, rhamnose, sucrose, or raffinose.

Type strain shows the highest sequence similarity to: *S. olivaceus*, AB184743, 100%; *S. parvulus*, AB184326, 99.4%; *S. coeruleorubidus*, AY999719, 99.2%; *S. rubrogriseus*, AB184681, 99.2%; *S. lienomycini*, AJ781353, 99.2%; *S. malachitospinus*, AB249954, 99.2%; *S. anthocyanicus*, AB184631, 99.1%; *S. tricolor*, AB184687, 99.1%; *S. coelescens*, AF503496, 99%; *S. violaceoruber*, AF503492, 99%; *S. violaceorubidus*, AJ781374, 99%.

Source: not known.

DNA G+C content (mol%): not known.

Type strain: AS 4.1443, ATCC 27456, CBS 461.69, CBS 734.72, BCRC 12076, DSM 40530, NBRC 13433, IMET 43357, JCM 4288, JCM 4809, KCTC 9165, LMG 19357, NCIMB 9445, NRRL 2939, NRRL-ISP 5530, RIA 1394, VKM Ac-1911.

Sequence accession no. (16S rRNA gene): AB184398.

361. **Streptomyces paradoxus** (Krasil'nikov and Yuan 1961) Goodfellow, Williams and Alderson 1986a, 575[VP] (Effective publication: Goodfellow, Williams and Alderson 1986e, 62.) (*Actinosporangium violaceum* Krasil'nikov and Yuan 1961, 115)

pa.ra′do.xus. L. masc. adj. *paradoxus* contrary to all expectation.

Spore chains in Section *Retinaculiaperti*. Spore surface is smooth. Forms extensively branched substrate and aerial mycelium. The aerial spore mass is gray; sclerotia are formed. Produces melanin pigments. Allantoin, adenine, esculin, arbutin, gelatin, guanine, hypoxanthine, testosterone, tyrosine, urea, and xanthine are degraded, but chitin, elastin, lecithin, pectin, and starch are not. Hydrogen sulfide is produced but nitrate is not reduced. L-Arabinose, cellobiose, D-fructose, D-galactose, D-glucose, *myo*-inositol, inulin, D-lactose, mannitol, D-mannose, melezitose, melibiose, raffinose, L-rhamnose, sucrose, trehalose, and D-xylose are used as sole carbon sources but adonitol and xylitol are not. Grows on L-arginine, L-cysteine, L-histidine, L-hydroxyproline, L-methionine, potassium nitrate, L-serine, L-threonine, and L-valine, but not on DL-amino-n-butyric acid or L-phenylalanine, as sole nitrogen sources. Grows at 10 and 37°C, but not at 4 or 45°C.Tolerant to phenol (0.1%, w/v) and sodium chloride (4%, w/v), but not sodium azide (0.01%, w/v). Sensitive to rifampin and inhibited by sodium chloride at 7% (w/v). Antimicrobial activity not shown against *Aspergillus niger* LIV 131, *Bacillus subtilis* NCIB 3610, *Candida albicans* CBS 562, *Escherichia coli* NCIB 9132, *Micrococcus luteus* NCIB 196, *Pseudomonas fluorescens* NCIB 9046[T], *Saccharomyces cerevisiae* CBS 171, or *Streptomyces murinus* ISP 5091. The peptidoglycan contains LL-A_2pm as the major diamino acid and is of the A3γ type (Stackebrandt et al., 1981).

Type strain shows the highest sequence similarity to: *S. matensis*, AB184221, 99.6%; *S. collinus*, AB184123, 99.5%; *S. viridochromogenes*, DQ442555, 99.4%; *S. azureus*, EF178674, 99.3%; *S. griseoflavus*, AJ781322, 99.3%; *S. griseorubens*, AB184139, 99.3%; *S. bellus*, AB184849, 99.2%; *S. ambofaciens*, M27245, 99.2%; *S. glaucescens*, AB184843, 99.2%; *S. albaduncus*, AY999757, 99.2%; *S. erythrogriseus*, AJ781328, 99.2%; *S. labedae*, AB184704, 99.2%; *S. griseoincarnatus*, AJ781328, 99.2%; *S. variabilis*, DQ442551, 99.2%; *S. coerulescens*, AY999720, 99.2%; *S. violaceorubidus*, AJ781374, 99.1%; *S. flaveolus*, AB184764, 99.1%; *S. griseoloalbus*, AB184275, 99.1%; *S. capillispiralis*, AB184577, 99.1%; *S. althioticus*, AY999808, 99.1%; *S. pseudogriseolus*, DQ442541, 99%; *S. malachitofuscus*, AB184282, 99%; *S. levis*, AB184670, 99%.

Source: isolated from soil.

DNA G+C content (mol%): 70.4–71.6.

Type strain: ATCC 15813, BCRC 12521, DSM 43350, IFM 1160, NBRC 14887, IMET 43491, INMI 3180, JCM 3052, KCC A-0052, KCTC 9118, NRRL B-3457, NRRL B-3483, PCM 2310, RIA 655, VKM Ac-645.

Sequence accession no. (16S rRNA gene): AB184628.

362. **Streptomyces parvisporogenes** (Locci, Baldacci and Petrolini Baldan 1969) Witt and Stackebrandt 1991, 456[VP] (Effective publication: Witt and Stackebrandt 1990, 370.) (*Streptoverticillium parvisporogenes* Locci, Baldacci and Petrolini Baldan 1969, 37)

par.vi.spo.ro′ge.nes. L. adj. *parvus* little; Gr. n. *spora* a seed and in biology a spore; N.L. suff. *-genes* (from Gr. v. *gennaô* to produce) producing; N.L. part. adj. *parvisporogenes* producing a little number of spores.

Spore chains in Section Verticillate, umbellate monoverticillate (biverticillate). This morphology is seen on yeast-malt agar and oatmeal agar; a sporulating aerial mycelium is usually not produced on salts-starch agar and may also be absent on glycerol-asparagine agar. Spore surface is smooth or with some surface irregularities.

Color of colony: aerial mass color in the Red, White or Yellow color series on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar. The yellow color (1ba, 2ba, or 2db, pale yellow) is representative of aerial color in the absence of sporulation. Aerial mass color should be determined on a sporulating surface. Mature (21 d) sporulating aerial mycelium on oatmeal agar and on carbon-utilization medium plus fructose is in the Red color series (3ca, pale orange yellow). Reverse side of colony with no distinctive pigments (pale or grayish yellow on oatmeal agar, salts-starch agar, and glycerol-asparagine agar; yellowish brown or olive brown on yeast-malt agar).

Color in medium: melanoid pigments are formed in peptone-yeast-iron agar and tryptone-yeast broth; weakly or not at all in tyrosine agar. No pigment, or only a trace of yellow, is found in the medium in yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar.

D-Glucose and iso-inositol are utilized for growth. Reports vary on utilization of D-mannitol, and utilization of D-fructose is doubtful. Only traces of growth are seen on L-arabinose, D-xylose, rhamnose, sucrose, and raffinose.

For sequence similarity, see type strain of *Streptomyces abikoensis*.

Source: not known.

DNA G+C content (mol%): not known.

Type strain: ATCC 12568, CBS 695.72, DSM 40473, NBRC 13907, JCM 4694, JCM 4812, NRRL B-12386, NRRL B-5464, NRRL-ISP 5473, RIA 1355, VKM Ac-878.

Sequence accession no. (16S rRNA gene): AB249913.

Further comments: in violation of Rule 33c of the *Bacteriological Code* (1990 Revision), in Validation List no. 38, *Streptomyces parvisporogenes* is proposed as a *nomen revictum* (basonym: "*Streptomyces parvisporogenes*" *ignotus* 1960).

According to Hatano et al. (2003), *Streptomyces parvisporogenes* (Locci et al. 1969) Witt and Stackebrandt 1991 is a later heterotypic synonym of *Streptomyces abikoensis* (Umezawa et al. 1951) Witt and Stackebrandt 1991.

363. **Streptomyces parvulus** corrig. Waksman and Gregory 1954, 1055[AL]

par'vu.lus. L. masc. adj. *parvulus* very small.

Spore chains in Section *Spirales*. Mature spore chains are generally long, often with more than 50 spores per chain. This morphology is seen on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar. Spore surface is smooth.

Color of colony: aerial mass color in the Gray color series on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar. Reverse side of colony with no distinctive pigment (grayed yellow to grayed greenish yellow) on yeast-malt agar, oatmeal agar, salts-starch agar, and or glycerol-asparagine agar; substrate pigment is not a pH indicator.

Color in medium: melanoid pigments not formed in peptone-yeast-iron agar and tyrosine agar. No pigment found in medium in yeast-malt agar, oatmeal agar, salts-starch agar, or glycerol-asparagine agar.

D-Glucose, L-arabinose, sucrose, D-xylose, iso-inositol, D-mannitol, D-fructose, and rhamnose are utilized for growth. Variable reports on growth with raffinose.

Type strain shows the highest sequence similarity to: *S. pactum*, AB184398, 99.4%; *S. lomondensis*, AB184673, 99.3%; *S. olivaceus*, AB184743, 99.3%; *S. malachitospinus*, AB249954, 99.3%; *S. coeruleorubidus*, AY999719, 99.2%; *S. bellus*, AB184849, 99.2%; *S. coerulescens*, AY999720, 99.2%; *S. luteogriseus*, AB184379, 99.2%; *S. violaceorubidus*, AJ781374, 99.1%; *S. roseoviolaceus*, AJ399484, 99%; *S. lusitanus*, AB184424, 99%; *S. albosporeus* subsp. *albosporeus*, AJ781327, 99%; *S. spinoverrucosus*, AB184578, 99%; *S. janthinus*, AB184851, 99%; *S. violaceus*, AB184315, 99%; *S. tendae*, D63873, 99%; *S. purpurascens*, AJ399486, 99%.

Source: not known.

DNA G+C content (mol%): not known.

Type strain: ATCC 12434, ATCC 19796, CBS 418.59, CBS 548.68, BCRC 12046, DSM 40048, HUT 6081, ICMP 156, NBRC 13193, IMET 41380, JCM 4068, JCM 4601, LMG 19312, NCIMB 11240, NRRL B-1628, NRRL-ISP 5048, RIA 1075, RIA 507, UNIQEM 182, VKM Ac-1063.

Sequence accession no. (16S rRNA gene): AB184326.

Further comments: the original spelling, *Streptomyces parvullus* (*sic*), was corrected by Hill et al. (1984).

364. **Streptomyces parvus** (Krainsky 1914) Waksman and Henrici *in* Breed, Murray and Hitchens 1948, 939[AL] ("*Actinomyces parvus*" Krainsky 1914, 685; "*Nocardia parva*" Chalmers and Christopherson 1916, 268)

par'vus. L. masc. adj. *parvus* small.

Spore chains in Section *Rectiflexibiles*. Mature spore chains generally contain 10–50 or sometimes more than 50 spores per chain. This morphology is seen on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar. Spore surface is smooth.

Color of colony: aerial mass color in the Yellow color series (1ba, 2ba, pale yellow) on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar. Reverse side of colony with no distinctive pigments (light yellow to light greenish yellow) on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar.

Color in medium: melanoid pigments are not formed in peptone-yeast-iron agar, tyrosine agar, or tryptone-yeast broth. Yellow pigment is found in the medium in yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar. This pigment is not pH-sensitive when tested with 0.05 M NaOH or HCl.

D-Glucose, L-arabinose, D-xylose, D-mannitol, D-fructose, and rhamnose are utilized for growth. No growth or only traces of growth on sucrose, iso-inositol, and raffinose.

Type strain shows the highest sequence similarity to: *S. rubiginosohelvolus*, AB184240, 99.9%; *S. mediolani*, AB184674, 99.9%; *S. badius*, AY999783, 99.9%; *S. pluricolorescens*, DQ442540, 99.9%; *S. griseinus*, AB184205, 99.9%; *S. sindenensis*, AB184759, 99.9%; *S. flavofuscus*, AB249935, 99.8%; *S. globisporus* subsp. *globisporus*, EF178686, 99.8%; *S. griseoplanus*, AY999894, 99.8%; *S. praecox*, AB184293, 99.8%; *S. fimicarius*, AY999784, 99.8%; *S. albovinaceus*, AB249958, 99.8%; *S. anulatus*, DQ026637, 99.8%; *S. microflavus*, DQ445795, 99.7%; *S. cinereorectus*, AB184646, 99.7%; *S. lipmanii*, AB184148, 99.7%; *S. alboviridis*, AB184256, 99.7%; *S. floridae*, AB184656, 99.7%; *S. lavendulae* subsp. *lavendulae*, AB184080, 99.7%; *S. cyaneofuscatus*, AB184860, 99.7%; *S. cavourensis* subsp. *washingtonensis*, DQ026671, 99.7%; *S. fulvorobeus*, AB184711, 99.7%; *S. acrimycini*, AY999889, 99.7%; *S. californicus*, AB184755, 99.7%; *S. baarnensis*, EF178688, 99.7%; *S. argenteolus*, AB045872, 99.6%; *S. luridiscabiei*, AF361784, 99.5%; *S. flavogriseus*, AJ494864, 99.5%; *S. griseus* subsp. *griseus*, AY207604, 99.5%; *S. halstedii*, EF178695, 99.5%; *S. bacillaris*, AB184439, 99.5%; *S. griseolus*, AB184768, 99.5%; *S. pulveraceus*, AB184806, 99.5%; *S. flavovirens*, DQ026635, 99.5%; *S. nitrosporeus*, EF178680, 99.4%; *S. cavourensis* subsp. *cavourensis*, DQ445791, 99.3%; *S. finlayi*, AY999788, 99.3%; *S. yanii*, AB006159, 99.3%; *S. atroolivaceus*, AJ781320, 99.3%; *S. sanglieri*, AB249945, 99.3%; *S. olivoviridis*, AB184227, 99.3%; *S. clavifer*, DQ026670, 99.3%; *S. albolongus*, AB184425, 99.3%; *S. atratus*, DQ026638, 99.2%; *S. griseobrunneus*, AB249912, 99.2%; *S. gelaticus*, DQ026636, 99.2%; *S. celluloflavus*, AB184476, 99.2%; *S. candidus*, DQ026663, 99.1%; *S. cremeus*, AB184124, 99%; *S. mutomycini*, AB249951, 99%; *S. spiroverticillatus*, AB184814, 99%.

Source: not known.

DNA G+C content (mol%): not known.

Type strain: ATCC 12433, CBS 427.61, DSM 40348, HAMBI 1018, HUT 6062, NBRC 14599, JCM 4069, NCIMB 9608, NRRL B-1255, NRRL B-1455, NRRL-ISP 5348, RIA 610, VKM Ac-725.

Sequence accession no. (16S rRNA gene): DQ442537.

365. **Streptomyces paucisporeus** Xu, Wang, Cui, Huang, Liu, Zheng and Goodfellow 2006, 1113[VP]

pau.ci.spo′re.us. L. adj. *paucus* few; N.L. adj. *sporeus* spored; N.L. masc. adj. *paucisporeus* few spored, forming few spores.

Few spores with smooth surfaces are borne in short flexuous spore chains. Forms branched substrate and aerial hyphae. Khaki-colored colonies that carry a white to gray aerial spore mass are formed on acidified oatmeal agar, yeast extract-starch agar, and yeast extract-malt extract agar. Diffusible pigments are not formed, though melanin pigments are produced on tyrosine agar but not on peptone-yeast extract-iron agar. Degrades Tween 80, but not adenine, guanine, starch, or xanthine. Cellobiose, D-galactose, D-glucose, D-mannitol, D-sorbitol (each at 1%, w/v), L-alanine, L-arginine, α-L-aspartic acid, L-cysteine, L-glutamic acid, L-phenylalanine, and L-valine (each at 0.1%, w/v) are used as sole carbon sources for energy and growth, but adonitol, inulin, D-lactose (each at 1%, w/v), adipic acid, DL-aminobutyric acid, sodium acetate, sodium citrate, and sodium oxalate (each at 0.1%, w/v) are not. Does not use L-alanine, L-arginine, L-aspartic acid, L-glutamic acid, L-isoleucine, or L-phenylalanine (each at 0.1%, w/v) as sole carbon and nitrogen sources. Growth occurs at temperatures between 20 and 37°C, but not at 15°C, and at pH values from 4.5–7.5, but not at pH 3.5. Does not grow in the presence of 5% (w/v) NaCl. Sensitive to filter-paper discs soaked in the following (μg/ml unless indicated): azithromycin (30), doxycycline hydrochloride (30), erythromycin (15), josamycin (15), kanamycin sulfate (30), minocycline hydrochloride (30), neomycin sulfate (30), streptomycin sulfate (10), tetracycline hydrochloride (30), and tobramycin sulfate (10), but not to filter-paper discs soaked in acetylspiramycin (15), amoxycillin (10), ampicillin (10), azetreonam (30), carbenicillin (10), penicillin G (10 IU/ml), rifampin (5), or sulfamethoxazole (25). Chemotaxonomic properties are typical of the genus *Streptomyces.*

Type strain shows no sequence similarity over 99%. Type strain shows DNA–DNA similarity to: *S. yeochonensis* NRRL B-24245^T, 35.0%; *S. guanduensis* JCM 13274^T, 14.9%; *S. rubidus* JCM 13277^T, 19.9%; *S. yanglinensis* JCM 13275^T, 25.9%.

Source: the type strain was isolated from Dahao pine-forest in Guandu, Yunnan Province, People's Republic of China.

DNA G+C content (mol%): 74.8.

Type strain: 1413, CGMCC 4.2025, JCM 13276.

Sequence accession no. (16S rRNA gene): AY876943.

366. **Streptomyces peucetius** Grein, Spalla, DiMarco and Canevazzi 1963, 109[AL]

peu.ce′ti.us. L. masc. adj. *peucetius* of or pertaining to Peucetia, an ancient name for Central Puglia in Italy, the source of the soil from which the organism was isolated.

Spore chains of atypical *Retinaculiaperti* type. Excellent growth on Czapek's solution agar; reported to form coremia; forms pinkish vegetative mycelium on some media. Produces daunomycin, an anti-bacterial and anti-tumor antibiotic; produces polyenic anti-fungal activity.

Type strain shows the highest sequence similarity to: *S. xantholiticus*, AB184349, 100%; *S. kurssanovii*, AB184325, 99.8%; *S. graminofaciens*, AJ781329, 99.7%.

Source: isolated from soil from Central Puglia, Italy.

DNA G+C content (mol%): not known.

Type strain: ATCC 29050, CCT 4840, CMI 101335, DSM 40754, JCM 9920, KCTC 9199, NBRC 100596, NCIMB 10972.

Sequence accession no. (16S rRNA gene): AB045887.

367. **Streptomyces phaeochromogenes** (Conn 1917) Waksman *in* Breed, Murray and Smith 1957, 778[AL] ["*Actinomyces pheochromogenus*" (*sic*) Conn 1917, 16; "*Streptomyces phaeochromogenus*" (*sic*) Waksman and Henrici 1948, 943; "*Streptomyces pheochromogenus*" Pridham, Hesseltine and Benedict 1958, 74]

pha.e.o.chro.mo′ge.nes. Gr. adj. *phaios* brown; Gr. n. *chroma* color; N.L. suff. *-genes* (from Gr. v. *gennaô* to produce) producing; N.L. part. adj. *phaeochromogenes* producing brown color.

Spore chains in Section *Rectiflexibiles.* Mature spore chains are generally straight chains of 10–50, or sometimes more than 50, spores per chain. This morphology is seen on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar. Spirals were reported in Waksman's (1919) description, but not in Conn's original description (Conn, 1917; op. cit). Spore surface is smooth.

Color of colony: aerial mass color usually in the Red color series on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar. Most representative color-tabs from the Red color series were 3ca (pale orange yellow) and 5ca or 5cb (light yellowish pink to grayish yellowish pink). Aerial mass color may also be white or pale yellow on oatmeal agar and pale yellow on salts-starch agar and glycerol-asparagine agar. Reverse side of colony with no distinctive pigments (pale yellow or grayish yellow on oatmeal agar and salts-starch agar; light brown to strong brown on glycerol-asparagine agar and yeast-malt agar).

Color in medium: melanoid pigments are formed in peptone-yeast-iron agar, tyrosine agar, and tryptone-yeast broth. No pigment other than trace of yellow is found in the medium in yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar.

D-Glucose, L-arabinose, sucrose, D-xylose, iso-inositol, D-mannitol, D-fructose, and rhamnose are utilized for growth. Reports vary on utilization of raffinose.

Type strain shows the highest sequence similarity to: *S. ederensis*, AB184658, 99.6%; *S. umbrinus*, AB184305, 99.4%.

Source: not known.

DNA G+C content (mol%): not known.

Type strain: ATCC 23945, ATCC 3338, CBS 282.30, CBS 288.60, CBS 929.68, BCRC 12484, CECT 3070, DSM 40073, IFM 1051, NBRC 12898, NBRC 3180, IMET 40355, JCM 4070, JCM 4659, KCTC 9763, LMG 19348, NCIMB

8505, NRRL B-1248, NRRL B-3010, NRRL-ISP 5073, RIA 1119, RIA 61, VKM Ac-1002.

Sequence accession no. (16S rRNA gene): AB184738.

368. **Streptomyces phaeofaciens** Maeda, Okami, Kosaka, Taya and Umezawa 1952, 327[AL]

pha.e.o.fa'ci.ens. Gr. adj. *phaios* brown; L. part adj. *faciens* producing; N.L. part adj. *phaeofaciens* producing brown, referring to production of brown diffusible pigment.

Spore chains in Section *Spirales*. Mature spore chains are long with 10 to 50 or more spores per chain. This morphology is seen on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar. Spore surface is smooth.

Color of colony: aerial mass color usually in the Gray color series (3ih, dark gray; 3ig, grayish yellowish brown; 3ge, light grayish yellowish brown; or 5fe, light grayish reddish brown) on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar; the yellow to reddish brown aerial mycelium may also fall in the Red color series as represented by color tab 4ge, light grayish reddish brown. Reverse side of colony with no distinctive pigments (greenish yellow to light olive brown on yeast-malt agar; pale yellow to light olive gray on oatmeal agar, salts-starch agar, and glycerol-asparagine agar).

Color in medium: melanoid pigments are formed in peptone-yeast-iron agar and tryptone-yeast broth, but are formed only weakly or as trace amounts in tyrosine agar. No pigment is found in the medium in yeast-malt agar, oatmeal agar, salts-starch agar, or glycerol-asparagine agar.

D-Glucose, L-arabinose, D-xylose, iso-inositol, D-mannitol, D-fructose, and rhamnose are utilized for growth. No growth or only traces of growth with sucrose or raffinose.

Type strain shows the highest sequence similarity to: *S. phaeofaciens*, AB184360, 100%; *S. rishiriensis*, EF178691, 99.2%.

Source: not known.

DNA G+C content (mol%): not known.

Type strain: ATCC 15034, CBS 426.64, CBS 673.72, DSM 40367, IFM 1177, NBRC 13372, JCM 4125, JCM 4814, NRRL B-1516, NRRL-ISP 5367, RIA 1333, VKM Ac-1865.

Sequence accession no. (16S rRNA gene): AB184360.

369. **Streptomyces phaeogriseichromatogenes** Goodfellow, Kumar, Labeda and Sembiring 2008, 1[VP] (Effective publication: Goodfellow, Kumar, Labeda and Sembiring 2007, 194.)

pha.e.o.gri.se.i.chro.ma.to'ge.nes. Gr. adj. *phaios* brown; N.L. adj. *griseus* gray; Gr. n. *chroma -atos* color; N.L. suff. *-genes* (from Gr. v. *gennaô* to produce) producing; N.L. part. adj. *phaeogriseichromatogenes* producing brown and gray colors.

Spore chains are *Rectiflexibiles*; spore surface is ridged. Aerial spore mass colors range from grayish-white to grayish-brown and substrate mycelium is yellow to yellowish-brown, a yellow diffusible pigment is formed on yeast extract-malt extract agar, but melanin pigments are not produced. Hydrogen sulfide is produced, esculin is hydrolyzed, but not urea. Nitrate is not reduced. Casein, chitin, hypoxanthine, tyrosine, Tween 60, uric acid, and xanthine are degraded, but not adenine, arbutin, guanine, or xylan. L-Arabinose, D-arabitol, butan-1,4-diol, cellobiose, citric acid, dextrin, D-fructose, D-galactose, D-glucose, α-lactose, maltose, D-mannitol, D-mannose, D-ribose, and D-sucrose are used as sole carbon sources, but not L-arabitol, L-fucose, *myo*-inositol, melezitose, methanol, propanol, pyruvic acid, raffinose, L-rhamnose, or salicin. L-Alanine, L-arginine, L-glutamic acid, L-histidine, L-leucine, DL-methionine, DL-norleucine, L-proline, and L-valine are used as sole carbon and nitrogen sources, but not α-alanine, L-aminobutyric acid, L-glycine, L-phenylalanine, L-threonine, or L-tryptophan. Grows from pH 5 to pH 10, at 25 and 37°C, but not at 10 or 40°C. Does not grow in the presence of 13% (w/v) NaCl. Resistant (μg/ml) to cephalosporin (32), cefoxitin (32), cephaloridine (32, 64), doxycycline hydrochloride (4), and rifampin (64), but not to cefoxitin (64), carbenicillin (32, 64), erythromycin (32, 64), oleandomycin phosphate (32, 64), chlortetracycline hydrochloride (4, 8), doxycycline hydrochloride (8), rifampin (32, 64), fusidic acid (8, 16), lincomycin hydrochloride (32, 64), or novobiocin (4, 8).

Type strain shows the highest sequence similarity to: *S. costaricanus*, AB249939, 99.6%; *S. griseofuscus*, AB184206, 99.6%; *S. murinus*, AB184155, 99.6%.

Source: not known.

DNA G+C content (mol%): 71.2.

Type strain: DSM 40710, NRRL 2834.

Sequence accession no. (16S rRNA gene): AJ391813.

370. **Streptomyces phaeoluteichromatogenes** Goodfellow, Kumar, Labeda and Sembiring 2008, 1[VP] (Effective publication: Goodfellow, Kumar, Labeda and Sembiring 2007, 195.)

pha.e.o.lu.te.i.chro.ma.to'ge.nes. Gr. adj. *phaios* brown, dark; L. adj. *luteus* yellow; Gr. n. *chroma -atos* color; N.L. suff. *-genes* (from Gr. v. *gennaô* to produce) producing; N.L. part. adj. *phaeoluteichromatogenes* producing brown and yellow colors.

Spore chains are *Rectiflexibiles*; spore surface is smooth. On oatmeal agar the aerial spore mass color is brown and the substrate mycelium is yellow; a yellow diffusible pigment is produced. Melanin pigments are not formed. Esculin and urea are hydrolyzed. Does not produce hydrogen sulfide or reduce nitrate. Adenine, casein, chitin, hypoxanthine, tyrosine, and Tween 80 are degraded, but not casein, guanine, uric acid, xanthine, or xylan. Uses L-arabinose, D- and L-arabitol. butan-1,4-diol, cellobiose, citric acid, D-fructose, D-galactose, D-glucose, *myo*-inositol, D-mannitol, D-mannose, melezitose, methanol, propanol, and pyruvic acid as sole carbon sources, but not dextrin, L-fucose, α-lactose, maltose, raffinose, L-rhamnose, D-ribose, D-salicin, or sucrose. α-Alanine, L-glutamic acid, L-glycine, L-leucine, L-proline, L-serine, and L-threonine are used as sole carbon and nitrogen sources, but not L-alanine, L-aminobutyric acid, L-histidine, DL-methionine, DL-norleucine, L-phenylalanine, L-tryptophan, or L-valine. Grows from pH 4 to pH 9 and at 25°C and 40°C, but not at 10°C. Growth occurs in the presence of 13% (w/v) NaCl. Resistant (μg/ml) to carbenicillin (32, 64), cephalosporin (32), cefoxitin (32, 64), cephaloridine (32, 64),

chlortetracycline hydrochloride (4, 8), doxycycline hydrochloride (4, 8), tetracycline hydrochloride (64), rifampin (32, 64), and novobiocin (4), but sensitive to erythromycin (32, 64), oleandomycin phosphate (32, 64), fusidic acid (8, 16), and lincomycin hydrochloride (32, 64).

Type strain shows the highest sequence similarity to: *S. misionensis*, EF178678, 99.8%; *S. diastaticus* subsp. *diastaticus*, AB184785, 99.1%; *S. rutgersensis* subsp. *rutgersensis*, AB184170, 99.1%; *S. gougerotii*, AB184742, 99.1%; *S. intermedius*, AB184277, 99%.

Source: not known.

DNA G+C content (mol%): 69.8.

Type strain: DSM 41898, NRRL B-5799.

Sequence accession no. (16S rRNA gene): AJ391814.

371. **Streptomyces phaeoluteigriseus** Goodfellow, Kumar, Labeda and Sembiring 2008, 1[VP] (Effective publication: Goodfellow, Kumar, Labeda and Sembiring 2007, 196.)

pha.e.o.lu.te.o.gri′se.us. Gr. adj. *phaios* brown; L. adj. *luteus* yellow; N.L. adj. *griseus* gray; N.L. masc. adj. *phaeoluteigriseus* brown, yellow, and gray-colored.

Spore chains are *Rectiflexibiles*; spore surface is smooth. On oatmeal agar the aerial spore mass color is gray and the substrate mycelium is yellowish-brown; a yellow diffusible pigment is produced, as are melanin pigments. Esculin is hydrolyzed but not urea. Hydrogen sulfide is produced, but nitrate is not reduced. Adenine, arbutin, chitin, hypoxanthine, Tween 80, and xanthine are degraded, but not arbutin, guanine, tyrosine, uric acid, or xylan. Adonitol, L-arabitol, citric acid, and dextrin are used as sole carbon sources, but not L-arabitol, butan-1,4-diol, cellobiose, D-fructose, L-fucose, D-galactose, D-glucose, *myo*-inositol, α-lactose, D-mannitol, D-mannose, melezitose, methanol, propanol, pyruvic acid, raffinose, L-rhamnose, D-ribose, salicin, or sucrose. α-Alanine, L-histidine, and L-threonine are used as sole carbon and nitrogen sources, but not L-alanine, L-aminobutyric acid, L-arginine, L-glutamic acid, L-leucine, DL-methionine, DL-norleucine, L-phenylalanine, L-serine, L-threonine, L-tryptophan, or L-valine. Grows from pH 4 to pH 10 and at 25 and 37°C, but not at 10 or 40°C. Growth occurs in the presence of 13% (w/v) NaCl. Resistant (μg/ml) to carbenicillin (32, 64), cefocitin (32, 64), cephaloridine (32), oleandomycin phosphate (32), chlortetracycline hydrochloride (4), tetracycline hydrochloride (64), fusidic acid (8, 16), and novobiocin (4), but sensitive to cephalosporin (32), cephaloridine (64), erythromycin (32, 64), oleandomycin phosphate (64), chlortetracycline hydrochloride (8), doxycycline hydrochloride (4, 8), rifampin (32, 64), lincomycin hydrochloride (32, 64), and novobiocin (8).

Type strain shows the highest sequence similarity to: *S. bobili*, AB249925, 99.5%; *S. novaecaesareae*, AB184357, 99.5%; *S. galilaeus*, AB045878, 99.5%; *S. pseudovenezuelae*, AB184233, 99.4%; *S. resistomycificus*, AB184166, 99.4%; *S. chartreusis*, AB184839, 99.1%; *S. prunicolor*, DQ026659, 99%.

Source: not known.

DNA G+C content (mol%): 72.2.

Type strain: DSM 41896, ISP 5182, NRRL ISP-5182.

Sequence accession no. (16S rRNA gene): AJ391815.

372. **Streptomyces phaeopurpureus** Shinobu 1957, 63[AL] emend. Lanoot, Vancanneyt, Dawyndt, Cnockaert, Zhang, Huang, Liu and Swings 2005a, 8 (Effective publication: Lanoot, Vancanneyt, Dawyndt, Cnockaert, Zhang, Huang, Liu and Swings 2004, 88.)

pha.e.o.pur.pu′re.us. Gr. adj. *phaios* brown; L. adj. *purpureus* purple colored; N.L. masc. adj. *phaeopurpureus* brown, purple colored.

Spore chains in Section *Rectiflexibiles*. Aerial mycelium is usually poorly developed or absent on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar. Mature spore chains may be observed on other media including Gause's medium no. 1 where chains of 10 to 50 or more spores may be formed. Spore surface is smooth.

Color of colony: aerial mass color probably in the Gray or Red color series, but aerial sporulation on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar is usually inadequate for accurate spore mass color determination. Reverse side of colony is yellow to yellow brown modified by red (light brown, strong brown, or grayish reddish brown on oatmeal agar, salts-starch agar, and glycerol-asparagine agar; moderate to strong reddish brown on yeast-malt agar). Substrate mycelium pigment is not a pH indicator (or is changed only slightly from brown to yellow brown by 0.05 M HCl).

Color in medium: melanoid pigments are formed in peptone-yeast-iron agar, tyrosine agar, and tryptone-yeast broth. Yellow, orange, or red pigment is found in the medium in yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar. This pigment is slightly pH-sensitive changing from reddish orange or brown to yellow brown with 0.05 M HCl.

D-Glucose, L-arabinose, sucrose, D-xylose, iso-inositol, D-mannitol, D-fructose, rhamnose, and raffinose are utilized for growth.

Type strain shows the highest sequence similarity to: *S. griseorubiginosus*, AJ781339, 100%.

Source: not known.

DNA G+C content (mol%): not known.

Type strain: ATCC 23946, CBS 930.68, BCRC 13754, DSM 40125, HAMBI 950, NBRC 12899, NBRC 3930, JCM 4101, JCM 4660, KCTC 9764, NRRL B-2260, NRRL-ISP 5125, RIA 1128.

Sequence accession no. (16S rRNA gene): DQ026666.

Further comments: according to Lanoot et al. (2004), *Streptomyces phaeopurpureus* Shinobu 1957 is an earlier heterotypic synonym of *Streptomyces phaeoviridis* Shinobu 1957.

373. **Streptomyces phaeoviridis** Shinobu 1957, 63[AL]

pha.e.o.vi′ri.dis. Gr. adj. *phaios* brown; L. adj. *viridis* green; N.L. masc. adj. *phaeoviridis* brown-green.

Spore chains in Section *Rectiflexibiles*. Mature spore chains generally 10–50 spores per chain. This morphology is seen on yeast-malt agar, oatmeal agar and salts-starch agar, but aerial mycelium with mature spore chains is not formed on glycerol-asparagine agar. Spore surface is smooth.

Color of colony: aerial mass color in the Gray color series on yeast-malt agar, oatmeal agar and salts-starch

agar. (White also may be seen on these media and on the poorly sporulated surface of glycerol-asparagine agar) Reverse side of colony is yellow to yellow brown is modified slightly by red or orange (light yellowish brown to strong brown, yellowish pink, or grayish reddish orange). Substrate mycelium pigment is not a pH indicator.

Color in medium: melanoid pigments are formed in peptone-yeast-iron agar, tyrosine agar and tryptone-yeast broth. Orange or reddish pigment is found in the medium in yeast-malt agar, oatmeal agar and glycerol-asparagine agar. This pigment is not pH-sensitive when tested with 0.05 M NaOH or HCl. D-Glucose, L-arabinose, sucrose, D-xylose, iso-inositol, D-mannitol, D-fructose, rhamnose and raffinose are utilized for growth.

For sequence similarity, see type strain of *Streptomyces phaeopurpureus*.

Source: not known.

DNA G+C content (mol%): not known.

Type strain: ATCC 23947, CBS 931.68, DSM 40285, NBRC 12900, JCM 4102, JCM 4661, NCIMB 9832, NRRL B-2258, NRRL-ISP 5285, RIA 1199.

Sequence accession no. (16S rRNA gene): AB184230.

Further comments: according to Lanoot et al. (2004), *Streptomyces phaeoviridis* Shinobu 1957 is a later heterotypic synonym of *Streptomyces phaeopurpureus* Shinobu 1957.

374. **Streptomyces pharetrae** Le Roes and Meyers 2005a, 2236[VP] (Effective publication: Le Roes and Meyers 2005b, 489.)

pha.re′tra.e. L. gen. n. *pharetrae* of a quiver for holding arrows, isolated from soil taken from the base of a Giant quiver tree (*Aloe pillansii*).

Aerobic, Gram-stain-positive actinomycete which forms green aerial mycelium and yellow-blue substrate mycelium on ISP 4 (inorganic salts-starch agar). The color of the substrate mycelium is not pH-sensitive. No fragmentation occurs and no verticils are present. Mature spore chains are of the *Spirales*-type with hairy spore ornamentation. Spores are nonmotile. Good growth is observed on Czapek's solution agar, yeast extract-malt extract agar (ISP 2), and inorganic salts-starch agar (ISP 4). Substrate mycelium color is medium-dependent, but aerial mycelium color is constant. No diffusible pigment is produced on glycerol-asparagine agar (ISP 5). Melanin is produced on peptone-yeast extract-iron agar (ISP 6), but not on tyrosine agar (ISP 7). H_2S production occurs and nitrate is reduced (weak reaction after 14 d). Lecithinase, protease, and lipase activities are observed on egg-yolk agar. Pectin is hydrolyzed but not hippurate. Degrades adenine, esculin, arbutin, casein, cellulose, gelatin, hypoxanthine, L-tyrosine, Tween 80, and urea. Starch hydrolysis is negative at 7 d, but weakly positive when tested at 14 and 21 d. Allantoin, guanine, xanthine, and xylan are not degraded. Uses β-alanine, DL-alanine, ammonium phosphate, L-arginine, L-asparagine, L-cysteine, L-glutamic acid, L-glutamine, L-histidine, L-hydroxyproline, L-isoleucine, L-leucine, L-methionine, L-phenylalanine, potassium nitrate, L-proline, L-serine, urea, and L-valine, but not DL-α-amino-*n*-butyric acid as sole nitrogen sources. Uses dextran, D(−) fructose, D(+)glucose, methyl α-D-glucoside, glycerol, glycogen, D(−)ribose, sodium butyrate, and sodium sorbate as sole carbon sources, but not adonitol, *meso*-erythritol, *myo*-inositol, inulin, maltose, D(+)melibiose, D(+)melezitose, raffinose, salicin, sodium benzoate, sodium formate, sodium lactate, sodium maleate, sodium oxalate, sodium salicylate, sodium L(+)tartrate, trehalose, or xylitol. Weak growth is observed after 21 d on L(+)arabinose, D(−)lactose, D(−)mannitol, D(+)mannose, L(+)rhamnose, sodium acetate, sodium citrate, sodium gluconate, sodium DL-malate, sodium succinate, sucrose, and D(+)xylose. Grows in the presence of 0.3% 2-phenylethanol, 0.0001% crystal violet, 7% NaCl (but not 10%), and 0.1% phenol, but not in the presence of sodium azide (0.01%). Growth is also observed in the presence of cephaloridine (100 μg/ml), lincomycin (100 μg/ml), penicillin G (10 IU/ml), and rifampin (50 μg/ml), but not in the presence of neomycin (50 μg/ml), and streptomycin (100 μg/ml). Growth is observed at 30, 37, and 45°C, but not at 4°C or at pH 4.3. Weak antibiosis is exhibited against *Bacillus coagulans* ATCC 7050[T], *Mycobacterium aurum* A+, and *Acinetobacter colcoaceticus* C91, but there is no antibiosis against *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, and *Staphylococcus aureus* ATCC 25923.

Type strain shows the highest sequence similarity to: *S. glaucescens*, AB184843, 99.3%.

Source: isolated from soil taken from the base of a Giant quiver tree (*Aloe pillansii*).

DNA G+C content (mol%): 76.0 (±1.4).

Type strain: CZA14, DSM 41856, JCM 13860, NRRL B-24333.

Sequence accession no. (16S rRNA gene): AY699792.

375. **Streptomyces pilosus** Ettlinger, Corbaz and Hütter 1958a, 347[AL]

pi.lo′sus. L. masc. adj. *pilosus* hairy, shaggy.

Spore chains in Section *Rectiflexibiles* to *Spirales*. Short spore chains include straight or flexuous chains, hooks, loops, and poorly developed spirals. Hooks and loops of small diameter on short sporophores are not typical of the broad hooks and loops of *Retinaculiaperti* cultures. In the original description (Ettlinger et al., 1958a), spore chains are described as open, regular spirals. Mature spore chains are moderately short with 3–10 or sometimes more than 10 spores per chain. This morphology is seen on yeast-malt agar, oatmeal agar, and salts-starch agar; sporulation may be poor on glycerol-asparagine agar. Spore surface is hairy, with a tendency toward spines on some spores.

Color of colony: aerial mass color in the Gray color series on yeast-malt agar, oatmeal agar, and salts-starch agar. Reverse side of colony is grayed yellow to yellow-brown on glycerol-asparagine agar and dark grayed yellow-brown to near black on yeast-malt agar, oatmeal agar, and salts-starch agar; substrate pigment is not a pH indicator.

Color in medium: melanoid pigments formed in peptone-yeast-iron agar and tryptone-yeast extract broth. Pigments other than melanoids are not formed in yeast-malt agar, oatmeal agar, salts-starch agar, or glycerol-asparagine agar.

D-Glucose, L-arabinose, D-xylose, iso-inositol, D-mannitol, D-fructose, and rhamnose are utilized for growth. No growth or only trace of growth on sucrose and raffinose.

Type strain shows the highest sequence similarity to: *S. flavoviridis*, AB184842, 100%; *S. violaceus*, AB184315, 99.2%; *S. albosporeus* subsp. *albosporeus*, AJ781327, 99.2%; *S. janthinus*, AB184851, 99.2%; *S. lomondensis*, AB184673, 99.2%; *S. geysiriensis*, DQ442501, 99.2%; *S. minutiscleroticus*, EF178696, 99.2%; *S. roseoviolaceus*, AJ399484, 99.2%; *S. ghanaensis*, AY999851, 99.2%; *S. luteogriseus*, AB184379, 99.2%; *S. djakartensis*, AB184657, 99.1%; *S. plicatus*, AB184291, 99.1%; *S. rochei*, AB184237, 99.1%; *S. vinaceusdrappus*, AY999929, 99.1%; *S. mutabilis*, EF178679, 99.1%; *S. hawaiiensis*, AB184143, 99%; *S. atrovirens*, DQ026672, 99%; *S. arenae*, AB249977, 99%; *S. calvus*, AB184329, 99%; *S. tuirus*, AB184690, 99%.

Source: not known.

DNA G+C content (mol%): not known.

Type strain: ATCC 19797, CBS 549.68, BCRC 12091, DSM 40097, NBRC 12807, JCM 4403, NRRL 2721, NRRL-ISP 5097, RIA 1076, UNIQEM 183, VKM Ac-1765.

Sequence accession no. (16S rRNA gene): AB184161.

376. **Streptomyces platensis** Tresner and Backus 1956, 244[AL]

plat.en′sis. Gr. adj. *platus* flat, broad, wide; N.L. masc. adj. *platensis* belonging to flat.

Spore chains in Section *Spirales*. Mature spore chains generally have 10–50, or sometimes more than 50, spores per chain. This morphology is seen on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar. Spore surface is smooth.

Color of colony: aerial mass color in the Gray color series on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar. Spores frequently coalesce to form black, moist (hygroscopic) masses of spores. Sometimes much of the aerial growth will be converted to a black, moist surface. Reverse side of colony is grayish yellow on oatmeal agar and salts-starch agar; sometimes modified by red to grayish yellowish pink or light reddish brown on yeast-malt agar and glycerol-asparagine agar. Reddish substrate mycelium pigment may be changed to colorless or pale yellow by 0.05 M HCl.

Color in medium: melanoid pigments are not formed in peptone-yeast-iron agar, tyrosine agar, or tryptone-yeast broth. Traces of red or yellow pigments are found in the medium in yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar. The reddish pigment is somewhat pH-sensitive, changing from grayed red to nearly colorless with 0.05 M HCl.

D-Glucose, sucrose, iso-inositol, D-mannitol, D-fructose, and raffinose are utilized for growth. Utilization of L-arabinose and D-xylose is doubtful. No growth or only trace of growth with rhamnose.

Type strain shows the highest sequence similarity to: *S. libani* subsp. *rufus*, AJ781351, 99.9%; *S. hygroscopius* subsp. *glebosus*, AB184479, 99.9%; *S. caniferus*, AB184640, 99.8%; *S. libani* subsp. *libani*, AB184414, 99.5%; *S. nigrescens*, DQ442530, 99.5%; *S. tubercidicus*, AJ621612, 99.5%; *S. hygroscopius* subsp. *decoyicus*, AY999883, 99.4%; *S. misakiensis*, AB217605, 99.2%; *S. catenulae*, AJ621613, 99.2%; *S. ramulosus*, DQ026662, 99.1%; *S. sioyaensis*, DQ026654, 99.1%; *S. monomycini*, DQ445790, 99%.

Source: not known.

DNA G+C content (mol%): not known.

Type strain: ATCC 13865, ATCC 23948, CBS 310.56, CBS 932.68, BCRC 11898, CCUG 11118, DSM 40041, NBRC 12901, JCM 4189, JCM 4662, KCTC 1088, NCAIM B.01481, NCIMB 9607, NRRL 2364, NRRL B-5486, NRRL-ISP 5041, RIA 1110, VKM Ac-1288.

Sequence accession no. (16S rRNA gene): AB045882.

377. **Streptomyces plicatus** Pridham, Hesseltine and Benedict 1958, 65[AL]

pli.ca′tus. L. masc. part. adj. *plicatus* folded, coiled.

Spore chains in Section *Spirales*. Spirals are characteristically open. Wavy spore chains or long flexuous chains terminating in hooks, loops, or incomplete spirals may also suggest *Retinaculiaperti* morphology. Mature spore chains are generally long, often with more than 50 spores per chain. This morphology is seen on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar. Spore surface is smooth.

Color of colony: aerial mass color usually in the Gray color series (3fe, light brownish gray or 5fe, light grayish reddish brown) on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar, but color similar to 5dc (grayish yellowish pink) from the Red color series may also be seen on yeast-malt agar and salts-starch agar. Reverse side of colony with no distinctive pigments (pale grayish yellow, yellowish brown or occasionally dark grayish brown) on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar.

Color in medium: melanoid pigments are not formed in peptone-yeast-iron agar, tyrosine agar, or tryptone-yeast broth. No pigment is found in medium in yeast-malt agar, oatmeal agar, salts-starch agar, or glycerol-asparagine agar.

D-Glucose, L-arabinose, D-xylose, iso-inositol, D-mannitol, D-fructose, and rhamnose are utilized for growth. No growth or only trace of growth with sucrose and raffinose.

Type strain shows the highest sequence similarity to: *S. vinaceusdrappus*, AY999929, 100%; *S. ghanaensis*, AY999851, 100%; *S. geysiriensis*, DQ442501, 100%; *S. minutiscleroticus*, EF178696, 100%; *S. rochei*, AB184237, 100%; *S. mutabilis*, EF178679, 99.9%; *S. tuirus*, AB184690, 99.5%; *S. djakartensis*, AB184657, 99.4%; *S. anandii*, AB184402, 99.2%; *S. violaceorubidus*, AJ781374, 99.2%; *S. pilosus*, AB184161, 99.1%; *S. flavoviridis*, AB184842, 99.1%; *S. tendae*, D63873, 99%; *S. calvus*, AB184329, 99%; *S. azureus*, EF178674, 99%; *S. asterosporus*, AB184706, 99%; *S. levis*, AB184670, 99%; *S. luteogriseus*, AB184379, 99%; *S. capillispiralis*, AB184577, 99%.

Source: not known.

DNA G+C content (mol%): not known.

Type strain: ATCC 25483, CBS 911.69, BCRC 12279, DSM 40319, NBRC 13071, JCM 4504, NCAIM B.01841, NRRL 2428, NRRL-ISP 5319, RIA 1263, VKM Ac-627.

Sequence accession no. (16S rRNA gene): AB184291.

378. **Streptomyces pluricolorescens** Okami and Umezawa *in* Waksman 1961, 259[AL]

plu.ri.co.lor.es′cens. L. comp. adj. *plus, pluris* more, many; L. n. *color* color; N.L. part. adj. *pluricolorescens* becoming many-colored or variegated.

Spore chains in Section *Rectiflexibiles*. Mature spore chains are moderately short with 3–10, or sometimes more than 10 spores per chain. This morphology is observed on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar. Spore surface is smooth.

Color of colony: aerial mass color in the Yellow or Red color series on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar (usually pale yellow in 7 d becoming pale orange-yellow or grayish yellowish pink in 14–21 d). Reverse side of colony is grayed yellow to yellow-brown (modified slightly by red in 14–21 d) on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar; substrate pigment is not a pH indicator.

Color in medium: melanoid pigments not formed in peptone-yeast-iron agar and tyrosine agar. No pigment found in medium in yeast-malt agar, oatmeal agar, salts-starch agar, or glycerol-asparagine agar.

D-Glucose, D-xylose, D-mannitol, D-fructose, and rhamnose are utilized for growth. No growth or only trace of growth on sucrose, iso-inositol, and raffinose. Utilization of L-arabinose is doubtful.

Type strain shows the highest sequence similarity to: *S. sindenensis*, AB184759, 100%; *S. albovinaceus*, AB249958, 100%; *S. griseoplanus*, AY999894, 100%; *S. anulatus*, DQ026637, 100%; *S. globisporus* subsp. *globisporus*, EF178686, 100%; *S. praecox*, AB184293, 100%; *S. flavofuscus*, AB249935, 100%; *S. fimicarius*, AY999784, 100%; *S. mediolani*, AB184674, 100%; *S. badius*, AY999783, 100%; *S. griseinus*, AB184205, 100%; *S. rubiginosohelvolus*, AB184240, 100%; *S. californicus*, AB184755, 99.9%; *S. lavendulae* subsp. *lavendulae*, AB184080, 99.9%; *S. cavourensis* subsp. *washingtonensis*, DQ026671, 99.9%; *S. acrimycini*, AY999889, 99.9%; *S. parvus*, DQ442537, 99.9%; *S. fulvorobeus*, AB184711, 99.8%; *S. lipmanii*, AB184148, 99.8%; *S. microflavus*, DQ445795, 99.8%; *S. cyaneofuscatus*, AB184860, 99.8%; *S. floridae*, AB184656, 99.8%; *S. alboviridis*, AB184256, 99.8%; *S. baarnensis*, EF178688, 99.8%; *S. cinereorectus*, AB184646, 99.8%; *S. griseus* subsp. *griseus*, AY207604, 99.7%; *S. griseolus*, AB184768, 99.7%; *S. luridiscabiei*, AF361784, 99.7%; *S. flavovirens*, DQ026635, 99.7%; *S. argenteolus*, AB045872, 99.7%; *S. bacillaris*, AB184439, 99.6%; *S. pulveraceus*, AB184806, 99.6%; *S. halstedii*, EF178695, 99.6%; *S. flavogriseus*, AJ494864, 99.6%; *S. atroolivaceus*, AJ781320, 99.5%; *S. olivoviridis*, AB184227, 99.5%; *S. finlayi*, AY999788, 99.5%; *S. nitrosporeus*, EF178680, 99.5%; *S. albolongus*, AB184425, 99.4%; *S. clavifer*, DQ026670, 99.4%; *S. griseobrunneus*, AB249912, 99.4%; *S. sanglieri*, AB249945, 99.4%; *S. celluloflavus*, AB184476, 99.4%; *S. yanii*, AB006159, 99.4%; *S. gelaticus*, DQ026636, 99.3%; *S. atratus*, DQ026638, 99.3%; *S. cavourensis* subsp. *cavourensis*, DQ445791, 99.3%; *S. mutomycini*, AB249951, 99.2%; *S. candidus*, DQ026663, 99.2%; *S. spiroverticillatus*, AB184814, 99.1%; *S. cremeus*, AB184124, 99.1%.

Source: not known.

DNA G+C content (mol%): not known.

Type strain: ATCC 19798, CBS 550.68, BCRC 13657, DSM 40019, IFM 1101, NBRC 12808, JCM 4302, JCM 4602, NCIMB 9813, NRRL B-2121, NRRL-ISP 5019, RIA 1077, UNIQEM 184, VKM Ac-765.

Sequence accession no. (16S rRNA gene): DQ442540.

379. **Streptomyces polychromogenes** Hagemann, Pénasse and Teillon *in* Hütter 1964, 615[AL]

po.ly.chro.mo′ge.nes. Gr. adj. *polu* many; Gr. n. *chroma* color; N.L. suff. *-genes* (from Gr. v. *gennaô* to produce) producing; N.L. part. adj. *polychromogenes* producing many colors, referring to the characteristic variation of pigmentation.

Spore chains characteristically in Section *Rectiflexibiles* with long, straight spore chains of more than 50 spores per chain on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar. A few spore chains may terminate in spirals. Spore surface is smooth. Knots and nest-like tangles may be seen in the aerial mycelium. Some of these tangles fragment into spore-like bodies and one observer reports that spores may also be seen on the substrate mycelium.

Color of colony: aerial mass color in the Red color series (grayish yellowish pink) on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar. Reverse side of colony with no distinctive pigments (nearly colorless to grayish yellow or light yellow on oatmeal agar, salts-starch agar, and glycerol-asparagine agar; light grayish reddish brown to strong brown on yeast-malt agar).

Color in medium: melanoid pigments are formed in peptone-yeast-iron agar and tryptone-yeast broth, but only weakly or not at all in tyrosine agar. No pigment, or only a trace of yellow, is found in the medium in yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar.

D-Glucose, L-arabinose, D-xylose, D-fructose, and sucrose are utilized for growth. Only traces of growth is seen with iso-inositol, D-mannitol, rhamnose, and raffinose.

Type strain shows the highest sequence similarity to: *S. racemochromogenes*, DQ026656, 100. *S. flavotricini*, AB184132, 99.7. *S. katrae*, AB184409, 99.5. *S. cinnamonensis*, AB184707, 99.1. *S. tanashiensis*, AJ781362, 99.1. *S. globosus*, AJ781330, 99.1. *S. nojiriensis*, AJ781355, 99. *S. spororaveus*, AJ781370, 99. *S. toxytricini*, DQ442548, 99. *S. nashvillensis*, AB184286, 99%.

Source: not known.

DNA G+C content (mol%): not known.

Type strain: ATCC 12595, ATCC 25484, CBS 311.56, CBS 912.69, BCRC 11899, DSM 40316, NBRC 13072, JCM 4236, JCM 4505, KCTC 9765, NCIMB 8791, NRRL B-12233, NRRL B-2656, NRRL B-3032, NRRL B-5697, NRRL-ISP 5316, RIA 1264, RIA 362, VKM Ac-1207.

Sequence accession no. (16S rRNA gene): AB184292.

380. **Streptomyces poonensis** (Thirumalachar *in* Kalakoutskii and Krasil'nikov *in* Rautenshtein 1960) Pridham 1970, 42[AL] ("*Chainia poonensis*" Thirumalachar *in* Kalakoutskii and Krasil'nikov *in* Rautenshtein 1960, 45)

po.on.en′sis. N.L. masc. adj. *poonensis* of or belonging to Poona, India, the source of the soil from which the organism was isolated.

Spore chains in Section *Spirales*. Mature spore chains generally contain 3 to 10 or more spores per chain. Aerial mycelium may be absent or spiral spore chains may be seen on yeast-malt agar and salts-starch agar. Straight spore chains are reported on oatmeal agar. Spore surface is smooth. Sclerotia are abundant on yeast-malt agar, oat-

meal agar, salts-starch agar, and glycerol-asparagine agar in 14 d. One observer reports LL-A_2pm and no arabinose in the cell wall, placing this strain in cell-wall type I.

Color of colony: aerial mass color in the White color series on yeast-malt agar, oatmeal agar, and salts-starch agar when aerial mycelium is produced. Aerial mycelium is usually not seen on glycerol-asparagine agar and may be absent on oatmeal agar or other ISP media. Reverse side of colony is colorless on glycerol-asparagine agar; yellow to yellow brown is modified by red becoming reddish brown, brownish pink, or purplish pink on yeast-malt agar, oatmeal agar, and salts-starch agar within 21 d. Only one of three observers reports reverse mycelium pigment is pH-sensitive, changing from pink to violet with the addition of 0.05 M NaOH.

Color in medium: melanoid pigments are not formed in peptone-yeast-iron agar, tyrosine agar, or tryptone-yeast broth. Two observers report no pigment in the medium in yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar. One observer reports pinkish pigment in yeast-malt agar, oatmeal agar, and salts-starch agar; it is reported that this pigment is pH-sensitive, changing from pink to violet with the addition of 0.05 M NaOH.

D-Glucose, L-arabinose, D-xylose, iso-inositol, D-mannitol, and D-fructose are utilized for growth. Reports vary on utilization of rhamnose; no growth or only traces of growth with sucrose or raffinose.

Type strain shows the highest sequence similarity to: *S. fumigatiscleroticus*, DQ442499, 99.3%; *S. ruber*, AB184604, 99.1%.

Source: isolated from soil from Poona, India.

DNA G+C content (mol%): not known.

Type strain: AS 4.1097, ATCC 15723, CBS 295.66, CBS 786.72, BCRC 13311, DSM 40596, HAMBI 987, NBRC 12556, NBRC 13485, IMET 43406, JCM 3071, JCM 3079, JCM 4815, LMG 19326, NRRL B-2319, NRRL B-2951, NRRL-ISP 5596, PCM 2246, RIA 1446, RIA 569, VKM Ac-1715.

Sequence accession no. (16S rRNA gene): DQ445792.

Further comments: Streptomyces poonensis (Thirumalachar 1960) Pridham 1970 and *Chainia poonensis* Thirumalachar 1960 have the same type strain and therefore are homotypic synonyms [Rules 24a and 24b (1) of the *Bacteriological Code* (1990 Revision)].

381. **Streptomyces praecox** (Millard and Burr 1926) Waksman *in* Waksman and Lechevalier 1953, 107[AL] ("*Actinomyces praecox*" Millard and Burr 1926, 633)

pra.e′cox. L. masc. adj. *praecox* premature, precocious.

Spore chains predominantly in Section *Rectiflexibiles* on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar. One of three ISP observers records strongly flexuous chains on yeast-malt agar to open spirals on oatmeal agar in 14 d. The original work of Millard and Burr (op. cit) describes "widely open spirals". Spore chains are moderately long with 10–50 or sometimes more than 50 spores per chain. Spore surface is smooth.

Color of colony: mature aerial mass color in the Yellow color series (pale yellow or pale yellow-green) on yeast-malt agar, oatmeal agar, and salts-starch agar; White color series on glycerol-asparagine agar. Reverse side of colony with no distinctive pigments (pale or grayish yellow to light olive-brown) on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar.

Color in medium: melanoid pigments are not formed in peptone-yeast-iron agar, tyrosine agar, or tryptone-yeast broth. No pigment, or only a trace of yellow, is found in the medium in yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar.

D-Glucose, L-arabinose, D-xylose, D-mannitol, D-fructose, and rhamnose are utilized for growth. Utilization of iso-inositol, sucrose, and raffinose is doubtful.

Type strain shows the highest sequence similarity to: *S. flavofuscus*, AB249935, 100%; *S. lavendulae* subsp. *lavendulae*, AB184080, 100%; *S. cavourensis* subsp. *washingtonensis*, DQ026671, 100%; *S. anulatus*, DQ026637, 100%; *S. acrimycini*, AY999889, 100%; *S. griseinus*, AB184205, 100%; *S. fimicarius*, AY999784, 100%; *S. badius*, AY999783, 100%; *S. griseoplanus*, AY999894, 100%; *S. mediolani*, AB184674, 100%; *S. sindenensis*, AB184759, 100%; *S. rubiginosohelvolus*, AB184240, 100%; *S. pluricolorescens*, DQ442540, 100%; *S. globisporus* subsp. *globisporus*, EF178686, 99.9%; *S. alboviridis*, AB184256, 99.9%; *S. lipmanii*, AB184148, 99.9%; *S. fulvorobeus*, AB184711, 99.9%; *S. cyaneofuscatus*, AB184860, 99.9%; *S. albovinaceus*, AB249958, 99.9%; *S. cinereorectus*, AB184646, 99.9%; *S. microflavus*, DQ445795, 99.9%; *S. baarnensis*, EF178688, 99.9%; *S. californicus*, AB184755, 99.8%; *S. parvus*, DQ442537, 99.8%; *S. argenteolus*, AB045872, 99.8%; *S. flavovirens*, DQ026635, 99.7%; *S. griseolus*, AB184768, 99.7%; *S. luridiscabiei*, AF361784, 99.7%; *S. griseus* subsp. *griseus*, AY207604, 99.7%; *S. flavogriseus*, AJ494864, 99.7%; *S. halstedii*, EF178695, 99.7%; *S. floridae*, AB184656, 99.7%; *S. pulveraceus*, AB184806, 99.5%; *S. clavifer*, DQ026670, 99.5%; *S. atroolivaceus*, AJ781320, 99.5%; *S. olivoviridis*, AB184227, 99.5%; *S. nitrosporeus*, EF178680, 99.5%; *S. finlayi*, AY999788, 99.5%; *S. bacillaris*, AB184439, 99.5%; *S. yanii*, AB006159, 99.4%; *S. celluloflavus*, AB184476, 99.3%; *S. griseobrunneus*, AB249912, 99.3%; *S. sanglieri*, AB249945, 99.3%; *S. albolongus*, AB184425, 99.3%; *S. mutomycini*, AB249951, 99.3%; *S. atratus*, DQ026638, 99.3%; *S. gelaticus*, DQ026636, 99.3%; *S. spiroverticillatus*, AB184814, 99.2%; *S. cavourensis* subsp. *cavourensis*, DQ445791, 99.2%; *S. candidus*, DQ026663, 99.1%; *S. mauvecolor*, AB184532, 99%; *S. cremeus*, AB184124, 99%.

Source: not known.

DNA G+C content (mol%): not known.

Type strain: ATCC 25485, ATCC 3374, CBS 104.27, CBS 913.69, DSM 40393, NBRC 13073, IMET 40356, JCM 4506, NRRL B-1586, NRRL B-2551, NRRL-ISP 5393, RIA 1265, RIA 66, VKM Ac-1873.

Sequence accession no. (16S rRNA gene): AB184293.

382. **Streptomyces prasinopilosus** Ettlinger, Corbaz and Hütter 1958a, 345[AL]

pra.si.no.pi.lo′sus. L. adj. *prasinus* green; L. adj. *pilosus* hairy; N.L. masc. adj. *prasinopilosus* green-hairy.

Spore chains in Section *Retinaculiaperti*. Mature spore chains generally have 10–50 spores per chain. This morphology is seen on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar. Spore surface is hairy.

Color of colony: aerial mass color in the Green color series on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar. Reverse side of colony is grayed yellow or grayed greenish yellow on oatmeal agar, salts-starch agar, and glycerol-asparagine agar, and grayed yellow modified by orange on yeast-malt agar. Substrate pigment is not a pH indicator (one observer reports slight change form orange to brown with NaOH).

Color in medium: melanoid pigments not formed in peptone-yeast-iron agar or tyrosine agar. No pigment found in medium in yeast-malt agar, oatmeal agar, salts-starch agar, or glycerol-asparagine agar.

D-Glucose, L-arabinose, iso-inositol, D-mannitol, D-fructose, and rhamnose are utilized for growth. No growth or only trace of growth on sucrose and raffinose. Variable reports on growth with D-xylose and D-mannitol.

Type strain shows the highest sequence similarity to: *S. emeiensis*, DQ462649, 99.4%.

Source: not known.

DNA G+C content (mol%): not known.

Type strain: ATCC 19799, CBS 551.68, BCRC 13678, DSM 40098, NBRC 12809, JCM 4207, JCM 4404, LMG 19345, NCIMB 9842, NRRL B-2711, NRRL-ISP 5098, RIA 1078, UNIQEM 185, VKM Ac-1740.

Sequence accession no. (16S rRNA gene): EF626597.

383. **Streptomyces prasinosporus** Tresner, Hayes and Backus 1966, 162[AL]

pra.si.no′spo.rus. L. adj. *prasinus* green; N.L. n. *spora* a spore; N.L. masc. adj. *prasinosporus* green-spored.

Spore chains in Section *Spirales* with irregular spirals of a few turns and loops and hooks of small diameter. Spirals are often clumped or tangled. Mature spore chains are generally short with 3 to 10 or more spores per chain. This morphology is seen on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar. Spore surface is hairy, with short hairs.

Color of colony: aerial mass color usually in the Green color series when good sporulation occurs on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar (1li, olive gray; 1ig, light grayish olive, or 24½ih, dark greenish gray). Aerial mass color may also be in the Gray color series (2ih, light olive gray or, if sporulating aerial mycelium is less developed, g, medium gray or 2dc, yellowish gray). Reverse side of colony with no distinctive pigments (pale yellow to light olive gray on oatmeal agar, salts-starch agar, and glycerol-asparagine agar; grayish greenish yellow on yeast-malt agar).

Color in medium: melanoid pigments are formed in peptone-yeast-iron agar and tryptone-yeast broth, but not in tyrosine agar. No pigment is found in the medium in yeast-malt agar, oatmeal agar, salts-starch agar, or glycerol-asparagine agar.

D-Glucose, L-arabinose, D-xylose, iso-inositol, D-mannitol, D-fructose, and rhamnose are utilized for growth. Utilization of sucrose and raffinose is doubtful.

Type strain shows no sequence similarity over 99%.

Source: not known.

DNA G+C content (mol%): not known.

Type strain: AS 4.1422, ATCC 17918, CBS 720.72, DSM 40506, NBRC 13419, JCM 4816, LMG 19346, NRRL B-12431, NRRL-ISP 5506, RIA 1380, VKM Ac-979.

Sequence accession no. (16S rRNA gene): AB184390.

384. **Streptomyces prasinus** Ettlinger, Corbaz and Hütter 1958a, 343[AL]

pra′si.nus. L. masc. adj. *prasinus* green.

Spore chains in Section *Retinaculiaperti* or *Spirales*. Spirals are poorly developed and show only one to three turns; loops and hooks are of small diameter on short spore chains and therefore are not typical of well developed *Retinaculiaperti* spore chains. Mature spore chains are moderately short with 3–10, or sometimes more than 10 spores per chain. This morphology is seen on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar. Spore surface is spiny.

Color of colony: aerial mass color in the Green color series on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar. Reverse side of colony with no distinctive pigment (grayed yellow to yellow-brown on yeast-malt agar and oatmeal agar, and grayed yellow-brown modified slightly by green on salts-starch agar and glycerol-asparagine agar). Substrate pigment is not a pH indicator.

Color in medium: melanoid pigments not formed in peptone-yeast-iron agar and tyrosine agar. No pigment found in medium in yeast-malt agar, oatmeal agar, salts-starch agar, or glycerol-asparagine agar.

D-Glucose, sucrose, D-xylose, iso-inositol, D-mannitol, D-fructose, and rhamnose are utilized for growth. Variable reports on growth with L-arabinose and raffinose.

Type strain shows the highest sequence similarity to: *S. bambergiensis*, AB184869, 99.9%; *S. hirsutus*, AB184844, 99.1%; *S. cyanoalbus*, AB184882, 99%.

Source: not known.

DNA G+C content (mol%): not known.

Type strain: ATCC 19800, CBS 552.68, BCRC 13681, DSM 40099, NBRC 12810, JCM 4192, JCM 4603, NRRL B-2712, NRRL-ISP 5099, RIA 1079, UNIQEM 186, VKM Ac-1725.

Sequence accession no. (16S rRNA gene): DQ026658.

385. **Streptomyces prunicolor** (Ryabova and Preobrazhenskaya *in* Gauze, Preobrazhenskaya, Kudrina, Blinov, Ryabova and Sveshnikova 1957) Pridham, Hesseltine and Benedict 1958, 63[AL] ("*Actinomyces prunicolor*" Ryabova and Preobrazhenskaya *in* Gauze, Preobrazhenskaya, Kudrina, Blinov, Ryabova and Sveshnikova 1957, 184)

pru.ni.co′lor. L. n. *prunum* plum; L. n. *color* color; N.L. *prunicolor* plum colored, referring to the color of the vegetative mycelium of the organism.

Spore chains in Section *Rectiflexibiles*. Straight to flexuous spore chains are generally long with 10–50, or sometimes more than 50 spores per chain. This morphology is seen on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar. Spore surface is smooth. Long aerial hyphae may become entangled into knots or nest-like bodies.

Color of colony: mature aerial mass color in the Red color series (5cb, grayish yellowish pink) on yeast-malt agar, salts-starch agar, and glycerol-asparagine agar; immature aerial mycelium may be white to yellow on these media and sporulation on oatmeal agar is usually inadequate for accurate spore mass color determination. Reverse side of colony is light brown to reddish brown on yeast-malt agar; grayish yellow, reddish gray, reddish purple, or dark reddish purple on oatmeal agar, salts-starch agar, and glycerol-asparagine agar. Reports vary on the production of the reddish purple reverse pigment. Reverse mycelium pigment is not a pH indicator, or is changed only slightly with addition of 0.05 M HCl.

Color in medium: melanoid pigments are not formed in peptone-yeast-iron agar, tyrosine agar, or tryptone-yeast broth. No pigment, or only a trace of pale brown, is found in the medium in yeast-malt agar, oatmeal agar, salts-starch agar or glycerol-asparagine agar.

D-Glucose, L-arabinose, D-xylose, iso-inositol, D-mannitol, D-fructose, rhamnose, and raffinose are utilized for growth. Reports vary on utilization of sucrose.

Type strain shows the highest sequence similarity to: *S. resistomycificus*, AB184166, 99.1%; *S. galilaeus*, AB045878, 99%; *S. phaeoluteigriseus*, AJ391815, 99%; *S. novaecaesareae*, AB184357, 99%; *S. chartreusis*, AB184839, 99%; *S. bobili*, AB249925, 99%.

Source: not known.

DNA G+C content (mol%): not known.

Type strain: AS 4.1413, ATCC 25487, CBS 915.69, DSM 40335, NBRC 13075, IMET 43129, INA 8805/64, JCM 4508, LMG 19311, NCIMB 9978, NRRL B-12281, NRRL-ISP 5335, PCM 2370, RIA 1267, VKM Ac-992.

Sequence accession no. (16S rRNA gene): DQ026659.

386. **Streptomyces psammoticus** Virgilio and Hengeller 1960, 167[AL]

psam.mo′ti.cus. Gr. n. *psammos* sand; N.L. masc. adj. *psammoticus* sandy.

Spore chains in Section *Rectiflexibiles* to *Retinaculiaperti* with 10 to 50 or more spores per chain on oatmeal agar and glycerol-asparagine agar. Spore chains may be flexuous or irregularly turned in various forms and coremia or sclerotia-like bodies may be formed. Yeast-malt agar and salts-starch agar are not suitable media for observation of spore chain morphology. Spore surface is smooth; spores are irregular in size and shape.

Color of colony: aerial mass color in the Green color series (1½ge, light grayish olive) on oatmeal agar and salts-starch agar, and sometimes in marginal areas on yeast-malt agar or glycerol-asparagine agar. According to one observer the aerial mass color is in the Yellow color series (color tab 1dc, pale yellow-green). Reverse side of colony with no distinctive pigments (nearly colorless to pale yellow or grayish yellow on oatmeal agar, salts-starch agar, and glycerol-asparagine agar; orange-yellow to yellow-brown on yeast-malt agar).

Color in medium: melanoid pigments are not formed in peptone-yeast-iron agar, tyrosine agar, or tryptone-yeast broth. No pigment or a trace of yellow pigment is found in the medium in yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar.

D-Glucose, sucrose, and D-fructose are utilized for growth. Utilization of L-arabinose is doubtful. No growth or only trace of growth with D-xylose, iso-inositol, D-mannitol, rhamnose, and raffinose.

Type strain shows the highest sequence similarity to: *S. avellaneus*, AB184413, 100%; *S. aureofaciens*, AY207608, 99.6%; *S. chrysomallus* subsp. *fumigatus*, AB184645, 99.3%; *S. purpureus*, AJ781324, 99.3%; *S. aburaviensis*, AY999779, 99.1%; *S. xanthocidicus*, AY999858, 99%.

Source: not known.

DNA G+C content (mol%): not known.

Type strain: AS 4.1465, ATCC 14125, ATCC 25488, CBS 175.61, CBS 299.65, CBS 916.69, BCRC 12241, DSM 40341, NBRC 13877, NBRC 13971, JCM 4434, NRRL B-3291, NRRL B-5753, NRRL-ISP 5341, PCM 2371, RIA 1268, RIA 832, VKM Ac-996.

Sequence accession no. (16S rRNA gene): AY999862.

387. **Streptomyces pseudoechinosporeus** (Konev, Tsyganov, Minbaev and Morogov 1967) Goodfellow, Williams and Alderson 1986a, 575[VP] (Effective publication: Goodfellow, Williams and Alderson 1986a, 52.) ("*Microechinospora grisea*" Konev, Tsyganov, Minbaev and Morogov 1967, 309; *Microellobosporia grisea* Pridham 1970, 17)

pseu.do.e.chi.no.spo′re.us. Gr. adj. *pseudes* false; Gr. n. *ekhinos* hedgehog, sea-urchin; Gr. n. *spora* seed; N.L. masc. adj. *pseudoechinosporeus* false spiny spored.

Forms extensively branched substrate and aerial mycelium. Single or chains of up to three spores are formed on both the substrate and aerial mycelium. Spores have smooth surfaces and are unequal in size (1.8–3.5 µm diameter). They were originally thought to be spiny but are now known to be heavily encrusted in needle-like crystals. The aerial spore mass is white to glaucous gray; the reverse color is pink to light violet pink. Does not form melanin pigments. Gelatin is degraded but cellulose is not. Does not reduce nitrate or produce hydrogen sulfide. L-Arabinose, D-fructose, D-galactose, D-glucose, inulin, D-lactose, maltose, mannitol, raffinose, L-rhamnose, starch, sucrose, and D-xylose are used as sole carbon sources but cellulose and sorbitol are not. Grow at 20, 26, and 37°C, but not at 50°C. The wall peptidoglycan contains LL-A_2pm as the major diamino acid.

Type strain shows the highest sequence similarity to: *S. cinnamoneus*, AB184850, 99.9%; *S. hiroshimensis*, AB184789, 99.5%; *S. caeruleus*, EF178675, 99.2%; *S. aureoversilis*, AB184855, 99.1%; *S. blastmyceticus*, AY999802, 99.1%; *S. lilacinus*, AB184819, 99%; *S. abikoensis*, AB184537, 99%.

Source: isolated from sand collected from the south-west part of the Kyzyl-Kum desert, USSR.

DNA G+C content (mol%): 69.0.

Type strain: ATCC 19618, DSM 43035, IFM 1243, NBRC 12518, IMET 43494, KCC A-0066, KCTC 9178, JCM 3066, NCIMB 9918, RIA 554, RIA 897, VKM Ac-1226.

Sequence accession no. (16S rRNA gene): AB184100.

Further comments: for the transfer of *Microellobosporia grisea* (Konev et al. 1967) Pridham 1970 in the genus *Streptomyces* Waksman and Henrici 1943 it is necessary to substitute a new specific epithet to produce *Streptomyces pseudoechinosporeus* because there is a senior homonym, *Streptomyces*

griseus (Krainsky 1914) Waksman and Henrici 1948, included on the Approved Lists of Bacterial Names [Rules 34a and 41a of the *Bacteriological Code* (1990 Revision)].

388. **Streptomyces pseudogriseolus** Okami and Umezawa *in* Okami, Utahara, Ōyagi, Nakamura, Umezawa, Yanagisawa and Tunematsu 1955, 128[AL]

pseu.do.gri.se.o′lus. Gr. adj. *pseudes* false; N.L. dim. adj. *griseolus* specific epithet; N.L. dim. adj. *pseudogriseolus* the false *griseolus* referring to resemblance to *Streptomyces griseolus*.

Spore chains in Section *Spirales*. Spore chains suggesting *Retinaculiaperti* morphology may also be common. Mature spore chains are moderately short, often with 3–10 spores per chain, but longer chains are formed on suitable media. This morphology is seen on yeast-malt agar and salts-starch agar, but aerial mycelium is poorly developed on oatmeal agar and glycerol-asparagine agar. Spore surface is smooth.

Color of colony: aerial mass color in the White color series on yeast-malt agar and salts-starch agar; poor sporulation on oatmeal agar and glycerol-asparagine agar. The aerial mycelium is described as grayish buff in the original description by Okami and Umezawa (1955). Reverse side of colony with no distinctive pigments (colorless to grayish yellow or yellowish brown) on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar.

Color in medium: melanoid pigments are not formed in peptone-yeast-iron agar, tyrosine agar, or tryptone-yeast broth. No pigment, or only trace of yellow, is found in the medium in yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar.

D-Glucose, L-arabinose, D-xylose, iso-inositol, D-mannitol, D-fructose, and rhamnose are utilized for growth. No growth or only trace of growth with sucrose and raffinose.

Type strain shows the highest sequence similarity to: *S. gancidicus*, AB184660, 100%; *S. capillispiralis*, AB184577, 100%; *S. cellulosae*, DQ442495, 99.9%; *S. levis*, AB184670, 99.5%; *S. azureus*, EF178674, 99.5%; *S. lusitanus*, AB184424, 99.4%; *S. rubiginosus*, AY999810, 99.4%; *S. carpinensis*, AB184574, 99.4%; *S. albaduncus*, AY999757, 99.2%; *S. matensis*, AB184221, 99.2%; *S. griseoloalbus*, AB184275, 99.1%; *S. djakartensis*, AB184657, 99.1%; *S. tuirus*, AB184690, 99.1%; *S. caelestis*, X80824, 99.1%; *S. paradoxus*, AB184628, 99%; *S. afghaniensis*, AJ399483, 99%; *S. geysiriensis*, DQ442501, 99%; *S. viridiviolaceus*, AY999854, 99%; *S. africanus*, AY208912, 99%; *S. lavendulocolor*, DQ442516, 99%; *S. minutiscleroticus*, EF178696, 99%.

Source: not known.

DNA G+C content (mol%): not known.

Type strain: ATCC 12770, ATCC 23949, CBS 933.68, BCRC 12132, DSM 40026, NBRC 12902, JCM 4071, JCM 4663, NCIMB 9411, NCIMB 9814, NRRL B-3288, NRRL-ISP 5026, RIA 1106, VKM Ac-1859.

Sequence accession no. (16S rRNA gene): DQ442541.

389. **Streptomyces pseudovenezuelae** (Kuchaeva, Krasil'nikov, Taptykova and Gesheva 1961) Pridham 1970, 24[AL] ("*Actinomyces pseudovenezuelae*" Kuchaeva, Krasil'nikov, Taptykova and Gesheva 1961, 114)

pseu.do.ve.ne.zu.e′la.e. Gr. adj. *pseudes* false; N.L. gen. n. *venezuelae* a specific epithet; N.L. gen. n. *pseudovenezuelae* the false venezuelae, referring to resemblance to *Streptomyces venezuelae.*

Spore chains in Section *Rectiflexibiles.* Mature spore chains are generally long, often with more than 50 spores per chain. This morphology is seen on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar. Spore surface is smooth.

Color of colony: aerial mass color in the Red color series (5dc, grayish yellowish pink) on yeast-malt agar, oatmeal agar, and salts-starch agar; Gray or White color series on glycerol-asparagine agar (Gray or White is also sometimes reported for yeast-malt agar, oatmeal agar, and salts-starch agar). Reverse side of colony with no distinctive pigments (light grayish yellow to orange yellow or light yellowish brown) on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar.

Color in medium: melanoid pigments are formed in peptone-yeast-iron agar, tyrosine agar, and tryptone-yeast broth. Trace of grayish yellow pigment may be found in the medium in yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar. This pigment is not pH-sensitive when tested with 0.05 M NaOH or HCl.

D-Glucose, L-arabinose, sucrose, D-xylose, iso-inositol, D-mannitol, D-fructose, rhamnose, and raffinose are utilized for growth.

Type strain shows the highest sequence similarity to: *S. resistomycificus*, AB184166, 99.5%; *S. novaecaesareae*, AB184357, 99.5%; *S. phaeoluteigriseus*, AJ391815, 99.4%; *S. canus*, AY999775, 99.4%; *S. galilaeus*, AB045878, 99.3%; *S. bobili*, AB249925, 99.2%; *S. ciscaucasicus*, AY508512, 99.2%; *S. flavovariabilis*, EF178691, 99.2%; *S. chartreusis*, AB184839, 99.1%; *S. cyaneus*, AF346475, 99.1%; *S. aureocirculatus*, AB184260, 99.1%; *S. humidus*, DQ442508, 99%.

Source: not known.

DNA G+C content (mol%): not known.

Type strain: ATCC 23951, CBS 934.68, BCRC 11487, DSM 40212, NBRC 12904, IMET 43512, JCM 4405, JCM 11516, NRRL B-3623, NRRL-ISP 5212, RIA 1158, RIA 742, VKM Ac-1199.

Sequence accession no. (16S rRNA gene): AB184233.

390. **Streptomyces pulveraceus** Shibata, Higashide, Kanzaki, Yamamoto and Nakazawa 1961, 172[AL]

pul.ve.ra′ce.us. L. n. *pulvis, pulveris* powder; N.L. masc. adj. *pulveraceus* powdery.

Probably grows poorly on Czapek's solution agar. Produces neomycins E and F (paromomycin and paromomycin II), zygomycin B, cycloheximide, and naramycin B.

Type strain shows the highest sequence similarity to: *S. atratus*, DQ026638, 99.7%; *S. gelaticus*, DQ026636, 99.7%; *S. sanglieri*, AB249945, 99.7%; *S. griseinus*, AB184205, 99.6%; *S. rubiginosohelvolus*, AB184240, 99.6%; *S. pluricolorescens*, DQ442540, 99.6%; *S. sindenensis*, AB184759, 99.6%; *S. mediolani*, AB184674, 99.6%; *S. badius*, AY999783, 99.6%; *S. albovinaceus*, AB249958, 99.5%; *S. lavendulae* subsp. *lavendulae*, AB184080, 99.5%; *S. californicus*, AB184755, 99.5%; *S. yanii*, AB006159, 99.5%; *S. praecox*, AB184293, 99.5%; *S. griseoplanus*, AY999894, 99.5%; *S. parvus*,

DQ442537, 99.5%; *S. argenteolus*, AB045872, 99.5%; *S. flavofuscus*, AB249935, 99.5%; *S. globisporus* subsp. *globisporus*, EF178686, 99.5%; *S. fimicarius*, AY999784, 99.5%; *S. cavourensis* subsp. *washingtonensis*, DQ026671, 99.5%; *S. anulatus*, DQ026637, 99.5%; *S. cinereorectus*, AB184646, 99.5%; *S. cyaneofuscatus*, AB184860, 99.4%; *S. griseus* subsp. *griseus*, AY207604, 99.4%; *S. baarnensis*, EF178688, 99.4%; *S. griseolus*, AB184768, 99.4%; *S. acrimycini*, AY999889, 99.4%; *S. flavovirens*, DQ026635, 99.4%; *S. alboviridis*, AB184256, 99.4%; *S. microflavus*, DQ445795, 99.4%; *S. lipmanii*, AB184148, 99.4%; *S. floridae*, AB184656, 99.4%; *S. fulvorobeus*, AB184711, 99.4%; *S. flavogriseus*, AJ494864, 99.3%; *S. luridiscabiei*, AF361784, 99.3%; *S. nitrosporeus*, EF178680, 99.3%; *S. halstedii*, EF178695, 99.3%; *S. bacillaris*, AB184439, 99.2%; *S. finlayi*, AY999788, 99%; *S. celluloflavus*, AB184476, 99%; *S. griseobrunneus*, AB249912, 99%; *S. albolongus*, AB184425, 99.%; *S. spiroverticillatus*, AB184814, 99%; *S. olivoviridis*, AB184227, 99%; *S. candidus*, DQ026663, 99%; *S. clavifer*, DQ026670, 99%; *S. atroolivaceus*, AJ781320, 99%; *S. cremeus*, AB184124, 99%.

Source: not known.

DNA G+C content (mol%): not known.

Type strain: ATCC 13875, DSM 41657, NBRC 3855, KCTC 9766, JCM 7545.

Sequence accession no. (16S rRNA gene): AB184806.

391. **Streptomyces puniceus** Patelski *in* Routien and Hofmann 1951, 387[AL]

pu.ni′ce.us. L. adj. *puniceus* reddish, purple.

Spore chains in Section *Rectiflexibiles*. Mature spore chains are generally long, often with more than 50 spores per chain. This morphology is seen on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar. Spore surface is smooth.

Color of colony: aerial mass color in the Yellow color series on yeast-malt agar, oatmeal agar, salts-starch agar and glycerol-asparagine agar. One observer places this culture in the Gray series (tabs 2dc, yellowish gray and 3fe, light grayish brown) on these media. Reverse side of colony is violet on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar. Reverse color is changed from violet to blue-violet or blue by addition of 0.05 M NaOH and from violet to pink or red with 0.05 M HCl.

Color in medium: melanoid pigments not formed in peptone-yeast-iron agar or tyrosine agar. Traces of blue or violet pigment found in medium in yeast-malt agar and glycerol-asparagine agar after 14–21 d. Pigment, when present, is changed from blue or violet to red-violet by 0.05 M HCl.

D-Glucose, D-xylose, D-mannitol, and D-fructose are utilized for growth. No growth or only trace of growth on L-arabinose, iso-inositol, and rhamnose. Utilization of sucrose is doubtful and reports are variable for growth with raffinose.

Type strain shows the highest sequence similarity to: *S. phaeofaciens*, AB184360, 100%; *S. rishiriensis*, EF178691, 99.1%.

Source: not known.

DNA G+C content (mol%): not known.

Type strain: ATCC 19801, CBS 308.55, CBS 553.68, BCRC 12097, DSM 40083, NBRC 12811, JCM 4406, NRRL B-2895, NRRL-ISP 5083, RIA 1080, UNIQEM 187, VKM Ac-579.

Sequence accession no. (16S rRNA gene): DQ442542.

392. **Streptomyces puniciscabiei** Park, Kim, Kwon, Wilson, Yu, Hur and Lim 2003, 2053[VP]

pu.ni.ci.sca′bi.ei. L. adj. *puniceus* purple or red; L. n. *scabies* roughness, scabbiness; N.L. gen. n. *puniciscabiei* of purple roughness, intended to mean purple or red, scab-causing bacteria.

Spores are pale orange, spiny, and borne in simple rectus flexuous spore chains. Melanin is produced on tyrosine agar, but not on peptone agar. L-Arabinose, D-fructose, D-glucose, D-mannitol, raffinose, rhamnose, sucrose, D-xylose, and iso-inositol are utilized for growth. Minimum pH for growth is 3.5. Sensitive to 10 and 100 μg/ml thallium acetate and 20 μg/ml streptomycin, but not to 5, 6, and 7% (w/v) NaCl, 0.5 μg/ml crystal violet, 0.1% (w/v) phenol, 10 IU/ml penicillin, or 25 or 100 μg/ml oleandomycin.

Type strain shows the highest sequence similarity to: *S. durhamensis*, AY999785, 99.1%. Type strain shows DNA–DNA similarity to: *S. scabies* ATCC 49173[T], 13%; *S. turgidiscabies* ATCC 700248[T], 13%; *S. acidiscabies* ATCC 49003[T], 11%; *S. bottropensis* DSM 40262[T], 12%; *S. neyagawaensis* DSM 40588[T], 6%; *S. diastatochromogenes* DSM 40449[T], 17%; *S. setonii* DSM 40395[T], 13%; *S. griseus* subsp. *griseus* DSM 40236[T], 16%; *S. sampsonii* DSM 40394[T], 42%; *S. eurythermus* DSM 40014[T], 14%; *S. tendae* DSM 40101[T], 15%; *S. coelicolor* DSM 40233[T], 8%; " *S. lividans*" DSM 40434, 29%; *S. ambofaciens* DSM 40053[T], 12%; *S. luridiscabiei* LMG 21390[T], 14%; *S. niveiscabiei* LMG 21392[T], 13%.

Source: isolated from raised corky lesions on potato cv. Daeji-ma and pathogenic on potato cv. Daeji-ma.

DNA G+C content (mol%): 68.3.

Type strain: S77, KACC 20253, LMG 21391.

Sequence accession no. (16S rRNA gene): AF361785.

393. **Streptomyces purpeofuscus** Yamaguchi and Saburi 1955, 207[AL]

pur.pe.o.fus′cus. L. adj. *purpureus* purple; L. adj. *fuscus* dark, tawny; N.L. masc. adj. *purpeofuscus* dark purple, referring to color of vegetative mycelium.

Spore chains in Section *Rectiflexibiles*. Mature spore chains are generally long, often with more than 50 spores per chain. This morphology is seen on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar. Spore surface is smooth.

Color of colony: aerial mass color in the Gray color series on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar. Reverse side of colony with no distinctive pigments (grayish yellow to olive brown) on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar, but dark brown to reddish brown substrate mycelium pigment may be produced. The original description (Yamaguchi and Saburi, 1955) describes purple to brown reverse mycelium color but notes that this coloration may be lost on repeated transfer.

Color in medium: melanoid pigments are formed in peptone-yeast-iron agar, but only traces of brownish black color in tyrosine agar or tryptone-yeast broth. Traces of red, violet, or yellow pigment may be found in the medium in yeast-malt agar, oatmeal agar, and salts-starch agar. This pigment is not pH-sensitive when tested with 0.05 M NaOH or HCl.

D-Glucose, L-arabinose, and D-xylose are utilized for growth. Utilization of fructose is doubtful. No growth or only trace of growth with sucrose, iso-inositol, D-mannitol, rhamnose, and raffinose.

Type strain shows the highest sequence similarity to: *S. indigoferus*, AB184214, 99.2%; *S. herbaricolor*, AB184801, 99.2%; *S. aburaviensis*, AY999779, 99.1%. Type strain shows the highest sequence similarity to following *Kitasatospora* species: *Kitasatospora azatica*, U93312, 99.1%.

Source: not known.

DNA G+C content (mol%): not known.

Type strain: ATCC 23952, CBS 935.68, BCRC 12093, DSM 40283, NBRC 12905, JCM 4156, JCM 4665, NCIMB 9822, NRRL B-1817, NRRL-ISP 5283, RIA 1197.

Sequence accession no. (16S rRNA gene): AJ781364.

394. **Streptomyces purpurascens** Lindenbein 1952, 371[AL]

pur.pur.as′cens. L. part. adj. *purpurascens* making purple.

Spore chains in Section *Spirales*. Mature spore chains are generally long with 10–50 or often more than 50 spores per chain. This morphology is seen on oatmeal agar, salts-starch agar, and glycerol-asparagine agar, but spiral spore chains may be absent on yeast-malt agar. Spore surface is spiny.

Color of colony: aerial mass color in the Red color series (grayish yellowish pink) or White color series on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar (aerial mycelium may be thin and white or absent on yeast-malt agar or on glycerol-asparagine agar). Reverse side of colony is grayish yellowish pink to reddish brown on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar. Reverse mycelium pigment is a pH indicator changing from red or purple to blue with addition of 0.05 M NaOH and to red or orange with addition of 0.05 M HCl.

Color in medium: melanoid pigments are not formed in peptone-yeast-iron agar and tryptone-yeast broth and may or may not develop after 4 d in tyrosine agar. Red to violet pigment is sometimes found in the medium in yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar. When present, this pigment is pH-sensitive showing the same color changes recorded for reverse mycelium pigments.

D-Glucose, L-arabinose, D-xylose, iso-inositol, D-mannitol, D-fructose, rhamnose, sucrose, and raffinose are all utilized for growth.

Type strain shows the highest sequence similarity to: *S. indiaensis*, AB184553, 99.6%; *S. hawaiiensis*, AB184143, 99.5%; *S. bellus*, AB184849, 99.4%; *S. coerulescens*, AY999720, 99.4%; *S. arenae*, AB249977, 99.3%; *S. lomondensis*, AB184673, 99.3%; *S. massasporeus*, AB184152, 99.3%; *S. coeruleorubidus*, AY999719, 99.2%; *S. lusitanus*, AB184424, 99.2%; *S. luteogriseus*, AB184379, 99.1%; *S. iakyrus*, AB184877, 99.1%; *S. parvulus*, AB184326, 99%.

Source: not known.

DNA G+C content (mol%): not known.

Type strain: ATCC 25489, CBS 917.69, BCRC 11872, DSM 40310, NBRC 13077, JCM 4509, NRRL B-12230, NRRL-ISP 5310, PCM 2299, RIA 1269, VKM Ac-755.

Sequence accession no. (16S rRNA gene): AB045888.

395. **Streptomyces purpureus** (Matsumae and Hata 1968) Goodfellow, Williams and Alderson 1986a, 575[VP] (Effective publication: Goodfellow, Williams and Alderson 1986b, 65.) (*Kitasatoa purpurea* Matsumae and Hata *in* Matsumae, Ohtani, Takeshima and Hata 1968, 617.)

pur.pur′e.us. L. masc. adj. *purpureus* purple colored.

Forms extensively branched substrate and aerial mycelium. Spore chains in Section *Rectiflexibiles*. Spore surface is smooth. Hyphae have been reported to carry club-shaped sporangia enclosing chains of zoospores with a single polar flagellum. The aerial spore mass is either gray or red and a yellow-brown diffusible pigment is produced. Melanin pigments are formed. Adenine, esculin, allantoin, arbutin, casein, elastin, guanine, hypoxanthine, starch, testosterone, tyrosine, urea, and xanthine are degraded but pectin and xylan are not. Nitrate is reduced to nitrite and hydrogen sulfide is produced. L-Arabinose, cellobiose, D-fructose, D-glucose, glycerol, *myo*-inositol, maltose, D-mannose, melezitose, salicin, and trehalose are used as sole carbon sources but adonitol, dulcitol, inulin, D-lactose, mannitol, melibiose, raffinose, L-rhamnose, sucrose, xylitol, and D-xylose are not. Grows on L-arginine and potassium nitrate but not on DL-amino-n-butyric acid, L-cysteine, L-histidine, L-hydroxyproline, L-methionine, L-phenylalanine, L-serine, L-threonine, or L-valine as sole nitrogen source. Growth occurs at 37°C but not at 10 or 45°C. Tolerant to sodium chloride (4%, w/v) but not to phenol (0.1%, w/v) or sodium azide (0.01%, w/v). Resistant to rifampin but sensitive to sodium chloride at 7% (w/v). Some strains show antimicrobial activity against *Aspergillus niger* LIV 131, *Bacillus subtilis* NCIB 3610, *Micrococcus luteus* NCIB 196, and *Streptomyces murinus* ISP 5091, but such activity is not shown against *Candida albicans* CBS 562, *Escherichia coli* NCIB 9132, *Pseudomonas fluorescens* NCIB 9046[T], or *Saccharomyces cerevisiae* CBS 1171[T]. The peptidoglycan contains LL-A_2pm as the major diamino acid and is of the A3γ type (Stackebrandt et al., 1981). Have a type II phospholipid pattern (*sensu* Lechevalier et al., 1977) and contain octahydrogenated menaquinones with nine isoprene units as the major isoprenolog. Produces the antibiotic chloramphenicol.

Type strain shows the highest sequence similarity to: *S. chrysomallus* subsp. *fumigatus*, AB184645, 99.9%; *S. herbaricolor*, AB184801, 99.6%; *S. indigoferus*, AB184214, 99.6%; *S. aburaviensis*, AY999779, 99.5,%; *S. xanthocidicus*, AY999858, 99.5%; *S. psammoticus*, AY999862, 99.3%; *S. avellaneus*, AB184413, 99.2%. Type strain shows the highest sequence similarity to following *Kitasatospora* species: *Kitasatospora kifunensis*, AB022874, 99.2%.

Source: isolated from soil in Hawaii and Japan.

DNA G+C content (mol%): not known.

Type strain: ATCC 27787, BCRC 12101, DSM 43362, NBRC 13927, IMET 9041, JCM 3172, KCC A-0172, KCTC

9187, LMG 19368, NCIMB 11311, NRRL B-5403, VKM Ac-1298.

Sequence accession no. (16S rRNA gene): AJ781324.

Further comments: Goodfellow et al. (1986a) proposed that *Kitasatoa diplospora* Matsumae et al. 1968, *Kitasatoa kauaiensis* Matsumae et al. 1968, and *Kitasatoa nagasakiensis* Matsumae and Hata 1968 become later synonyms of *Kitasatoa purpurea* Matsumae and Hata 1968, and that the latter be transferred to the genus *Streptomyces* as *Streptomyces purpureus* (Matsumae and Hata 1968) Goodfellow et al. 1986b, comb. nov.

396. **Streptomyces purpurogeneiscleroticus** (Thirumalachar *in* Thirumalachar and Sukapure 1964) Pridham 1970, 43[AL] ("*Chainia purpurogena*" Thirumalachar *in* Thirumalachar and Sukapure 1964, 160)

pur.pur.o.ge.ni.scle.ro′ti.cus. L. adj. *purpureus* purple colored; N.L. suff. *-genes* (from Gr. v. *gennaô* to produce) producing; N.L. n. *sclerotium* sclerotium; N.L. part. adj. *purpurogeneiscleroticus* sclerotium along with producing purple color.

Spore chains in Section *Spirales* on yeast-malt agar and salts-starch agar. Sporulation may be thin on these media and is generally absent on oatmeal agar and glycerol-asparagine agar. Mature spore chains generally contain 10 or more spores per chain. Spore surface is smooth. Sclerotia are produced on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar in 14 d.

Color of colony: aerial mass color in the White color series on yeast-malt agar and salts-starch agar; aerial mycelium is not produced on oatmeal agar or glycerol-asparagine agar. Reverse side of colony is grayish yellow on salts-starch agar; brown or reddish brown on yeast-malt agar, oatmeal agar, and glycerol-asparagine agar. One of three observers reports the reddish brown reverse mycelium pigment is changed from dark brown to light brown with the addition of 0.05 M HCl. The other observers report no change.

Color in medium: melanoid pigments are not formed in peptone-yeast-iron agar, tyrosine agar, or tryptone-yeast broth. Reddish or yellowish pigment is sometimes found in the medium in yeast-malt agar, oatmeal agar, salts-starch agar, or glycerol-asparagine agar. This pigment is pH-sensitive, changing from brown to reddish brown or red with the addition of 0.05 M NaOH and from brown to yellowish brown or yellow with the addition of 0.05 M HCl.

D-Glucose, L-arabinose, D-xylose, iso-inositol, D-mannitol, D-fructose, rhamnose, sucrose, and raffinose are all utilized for growth.

Type strain shows the highest sequence similarity to: *S. ochraceiscleroticus*, AB184094, 99.9%; S. *violens*, AJ621605, 99.7%; *S. monomycini*, DQ445790, 99.3%; *S. olivaceiscleroticus*, AJ621606, 99.1%; *S. sioyaensis*, DQ026654, 99.1%; *S. erumpens*, AJ621603, 99.1%; *S. niger*, AJ621607, 99.1%.

Source: not known.

DNA G+C content (mol%): not known.

Type strain: ATCC 19348, CBS 409.66, CBS 659.72, BCRC 13317, CM 3103, CMI 112722, DSM 43156, HAMBI 1061, NBRC 13001, NBRC 13903, JCM 3080, JCM 4818, NCIMB 10981, NRRL B-2952, NRRL-ISP 5271, PCM 2306, RIA 1319, RIA 886.

Sequence accession no. (16S rRNA gene): AJ621604.

Further comments: Streptomyces purpurogeneiscleroticus Pridham 1970 and *Chainia purpurogena* Thirumalachar and Sukapure 1964 have the same type strain and therefore are homotypic synonyms [Rules 24a and 24b (1) of the *Bacteriological Code* (1990 Revision)].

397. **Streptomyces racemochromogenes** Sugai 1956, 171[AL]

ra.ce.mo.chro.mo′ge.nes. L. n. *racemus* a raceme or cluster of berries; N.L. part. adj. *chromogenes* producing color; N.L. part. adj. *racemochromogenes* raceme, producing color (probably referring to morphology of spore chains and to chromogenicity).

Spore chains in Section *Retinaculiaperti* including many *Rectiflexibiles* spore chains, and some long chains with terminal hooks or primitive spirals of wide diameter. Mature spore chains are generally long with 10–50, or often more than 50, spores per chain. This morphology is seen on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar. Spore surface is smooth.

Color of colony: aerial mass color in the Red color series (5ec and 5cb, grayish yellowish pink) on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar. Areas of gray or white sporulation may also be seen. Reverse side of colony with no distinctive pigments on oatmeal agar, but yellow to yellow brown substrate mycelium color may be modified in some areas by blue pigment (dark olive green, grayish green or grayish blue) on yeast-malt agar, salts-starch agar, and glycerol-asparagine agar. The blue pigment is not always present. When present, it can be intensified from grayish green to reddish gray by 0.05 M HCl.

Color in medium: melanoid pigments are produced in peptone-yeast-iron agar, tyrosine agar, and tryptone-yeast broth. No pigment other than a trace of yellow is found in the medium in yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar.

D-Glucose, L-arabinose, and sucrose are utilized for growth. Only traces of growth are found on D-xylose, iso-inositol, D-mannitol, D-fructose, rhamnose, and raffinose.

Type strain shows the highest sequence similarity to: *S. polychromogenes*, AB184292, 100%; *S. flavotricini*, AB184132, 99.7%; *S. katrae*, AB184409, 99.5%; *S. cinnamonensis*, AB184707, 99.1%; *S. tanashiensis*, AJ781362, 99.1%; *S. globosus*, AJ781330, 99.1%; *S. toxytricini*, DQ442548, 99.1%; *S. nashvillensis*, AB184286, 99.1%; *S. nojiriensis*, AJ781355, 99%; *S. sporoaveus*, AJ781370, 99%.

Source: not known.

DNA G+C content (mol%): not known.

Type strain: ATCC 23954, CBS 937.68, BCRC 12318, DSM 40194, NBRC 12906, JCM 4407, NRRL B-5430, NRRL-ISP 5194, RIA 1147, VKM Ac-1206.

Sequence accession no. (16S rRNA gene): DQ026656.

398. **Streptomyces radiopugnans** Mao, Tang, Zhang, Wang, Wei, Huang, Liu, Shi and Goodfellow 2007, 2581[VP]

ra.di.o.pug′nans. L. n. *radius* a beam or ray; N.L. pref. *radio-* pertaining to radiation; L. part. adj. *pugnans* fighting or resisting; N.L. part. adj. *radiopugnans* radiation-resisting.

Radiation-resistant actinomycete that forms an extensively branched substrate mycelium which carries aerial

hyphae that differentiate into spiral chains of spores with rough to warty surfaces. Moderate to abundant, white to pale gray aerial spore mass is formed on modified Bennett's, Gause's synthetic medium no. 1, inorganic salts-starch, yeast extract-malt extract, and yeast-starch agars. Substrate mycelium is yellowish brown on modified Bennett's, Gause's synthetic medium no. 1, and yeast-starch agars, and light pinkish yellow on inorganic salts-starch and yeast extract-malt extract agars. Diffusible pigments are not formed on any of the media nor are melanin pigments formed on peptone-yeast extract iron or tyrosine agars. L-Arabinose, melezitose, and ribose are used as sole carbon sources for energy and growth, but not cellobiose, lactose, lactulose, melibiose, raffinose, or trehalose (all at 1%, w/v). Similarly, L-cysteine, L-glycine, D-glutamate, sodium azelate, sodium isobutyrate, and sodium malonate are used as sole carbon sources for energy and growth, but not D-glutamic acid, L-hydroxyproline, DL-isoleucine, L-leucine, methyl-D-glucopyranoside, methyl α-D-mannopyranoside, L-phenylalanine, sodium propionate, sodium pyruvate, sodium suberate, or spermidine (all at 0.1%, w/v).

The fatty acid profile consists of $C_{16:0}$ iso (34.5%), $C_{15:0}$ anteiso (15.4%), $C_{16:1}$ iso-H (14.2%), $C_{17:0}$ anteiso (9.1%), $C_{14:0}$ iso (5.6%), and $C_{17:1}$ anteiso ω9*c* (5.2%).

Type strain shows no sequence similarity over 99%.

Source: strain R97^{T} was isolated from a radiation-contaminated soil sample collected from Yinjiang Province, Northwestern China.

DNA G+C content (mol%): 72.7.

Type strain: R97, CGMCC 4.3519, DSM 41901.

Sequence accession no. (16S rRNA gene): DQ912930.

399. **Streptomyces rameus** Shibata 1959, 398^{AL}

ra′me.us. L. masc. adj. *rameus* pertaining to branches.

Produces streptomycin.

Type strain shows the highest sequence similarity to: *S. bangladeshensis*, AY750056, 99.8%; *S. glomeratus*, AB249917, 99.1%.

Source: not known.

DNA G+C content (mol%): not known.

Type strain: ATCC 21273, DSM 41685, NBRC 16196, JCM 5064, KCTC 9767.

Sequence accession no. (16S rRNA gene): AY999821.

Further comments: in a Request for an Opinion, Hatano (1999) proposed that the type strain of *Streptomyces rameus* Shibata 1959 is strain 43797, NBRC 3782, which was the originally designated type strain. The Judicial Commission supported this request and decided that strain 43797 [NBRC (previously IFO) 3782, ATCC 700861, JCM 11574] has to replace ATCC 21273 (DSM 41685, NBRC 16196, JCM 5064) as given in the Approved Lists.

400. **Streptomyces ramulosus** Ettlinger, Gäumann, Hütter, Keller-Schierlein, Kradolfer, Neipp, Prelog and Zähner 1958b, 217^{AL}

ra.mu.lo′sus. L. masc. adj. *ramulosus* much branched.

Spore chains in Section *Retiflexibiles*. Short dichotomous spore chains often occur in dense clumps of clusters. Short spore chains of 3–10 spores per chain. This morphology is seen on yeast-malt agar, salts-starch agar, and glycerol-asparagine agar. Spore surface is smooth.

Color of colony: aerial mass color in the Gray color series on yeast-malt agar, salts-starch agar, and glycerol-asparagine agar; poor sporulation on oatmeal agar. Reverse side of colony: substrate is colorless on oatmeal agar and grayed reddish brown to dark brown on yeast-malt agar, salts-starch agar, and glycerol-asparagine agar (one observer, only, reports reverse color as changed from grayed red to grayed yellow by 0.05 M NaOH and from grayed red to grayed purplish color by 0.05 M HCl).

Color in medium: melanoid pigments probably not formed in peptone-yeast-iron agar and tyrosine agar (a light orange pigment is formed in peptone-yeast-iron agar). Red pigment found in medium in yeast-malt agar and glycerol-asparagine agar; red to violet pigment found in oatmeal agar and salts-starch agar. One observer, only, reports pigment changed to yellow by 0.05 M NaOH and to violet by 0.05 M HCl (two observers reported no change).

D-Glucose, D-mannitol, and raffinose are utilized for growth. Doubtful growth on D-fructose. No growth or only trace of growth on L-arabinose, sucrose, D-xylose, iso-inositol, and rhamnose.

Type strain shows the highest sequence similarity to: *S. hygroscopius* subsp. *glebosus*, AB184479, 99.3%; *S. libani* subsp. *rufus*, AJ781351, 99.3%; *S. caniferus*, AB184640, 99.3%; *S. catenulae*, AJ621613, 99.1%; *S. misakiensis*, AB217605, 99.1%; *S. platensis*, AB045882, 99.1%; *S. rimosus* subsp. *rimosus*, AB045883, 99%.

Source: not known.

DNA G+C content (mol%): not known.

Type strain: AS 4.1434, ATCC 19802, CBS 554.68, BCRC 12343, DSM 40100, HAMBI 981, NBRC 12812, NBRC 15798, JCM 4193, JCM 4604, KCTC 9768, LMG 19354, NRRL B-2714, NRRL-ISP 5100, RIA 1081, UNIQEM 188, VKM Ac-1001.

Sequence accession no. (16S rRNA gene): DQ026662.

401. **Streptomyces rangoonensis** corrig. (Erikson 1935) Pridham, Hesseltine and Benedict 1958, 61^{AL} ["*Actinomyces rangoon*" (*sic*) Erikson 1935, 37; "*Nocardia rangoonensis*" (*sic*) Waksman and Henrici 1948, 911]

ran.goon.en′sis. N.L. masc. adj. *rangoonensis* of or belonging to Rangoon, Burma.

Spore chains in Section *Spirales*. Flexuous chains and irregular spirals are also common. Mature spore chains generally have 3 to 10 or more spores per chain. This morphology is seen on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar. Spore surface is smooth.

Color of colony: aerial mass color in the White color series on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar. Reverse side of colony with no distinctive pigments (colorless to pale yellow on oatmeal agar, salts-starch agar, and glycerol-asparagine agar; yellowish brown to light olive brown on yeast-malt agar).

Color in medium: melanoid pigments are not formed in peptone-yeast-iron agar, tyrosine agar, or tryptone-yeast broth. No pigment is found in medium in yeast-malt agar, oatmeal agar, salts-starch agar, or glycerol-asparagine agar.

D-Glucose, D-xylose, D-mannitol, and D-fructose are utilized for growth. Utilization of L-arabinose is doubtful. Only traces of growth or no growth is found on iso-inositol, rhamnose, sucrose, and raffinose.

Type strain shows the highest sequence similarity to: *S. almquistii*, AB184258, 100%; *S. gibsonii*, AB184663, 100%; *S. albus* subsp. *albus*, AJ621602, 100%; *S. flocculus*, AB184272, 99.9%.

Source: not known.

DNA G+C content (mol%): not known.

Type strain: ATCC 25490, ATCC 6860, CBS 918.69, DSM 40452, HUT 6616, NBRC 13078, IMET 41357, JCM 4510, NRRL B-12378, NRRL B-16595, NRRL-ISP 5452, RIA 1270, VKM Ac-1899.

Sequence accession no. (16S rRNA gene): AB184295.

Further comments: the original spelling of the specific epithet, *rangoon* (*sic*), has been corrected by Trüper and De'Clari (1997).

In the opinion of Kilian (1998), the change of the well-established name *Streptomyces rangoon* may be a source of confusion. So, with reference to the first Principle of the *Bacteriological Code* (1990 Revision), Kilian (2001) requested that the original name *Streptomyces rangoon* be conserved. The Judicial Commission 2005 denied this request and no opinion will be issued upon this request.

402. **Streptomyces recifensis** (Gonçalves de Lima, Machado, Araujo, Falcão de Morais and Biermann 1955) Falcão de Morais, Gonçalves de Lima and Maia 1957, 249^{AL} ("*Nocardia recifei*" Gonçalves de Lima, Machado, Araujo, Falcão de Morais and Biermann 1955, 26)

re.cif.en'sis. N.L. masc. adj. *recifensis* of or belonging to Recife, Brazil, the source of the soil from which the organism was isolated.

Spore chains in Section *Retinaculiaperti*; spore chains representative of Section *Rectiflexibiles* are also present. Mature spore chains generally have 10–50 spores per chain. This morphology is found on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar. Spore surface is smooth.

Color of colony: aerial mass color in the Gray color series on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar. Reverse side of colony with no distinctive pigment on oatmeal agar, salts-starch agar, and glycerol-asparagine agar, grayed yellow modified by dark grayish green on yeast-malt agar. Substrate pigment is not a pH indicator.

Color in medium: Melanoid pigments are not formed in peptone-yeast-iron agar and tyrosine agar. No pigment found in medium in yeast-malt agar, oatmeal agar, salts-starch agar, or glycerol-asparagine agar.

D-Glucose, L-arabinose, sucrose, D-xylose, D-mannitol, D-fructose, and raffinose are utilized for growth. No growth or only traces of growth on iso-inositol and rhamnose.

Type strain shows the highest sequence similarity to: *S. griseoluteus*, AY999751, 99.9%; *S. seoulensis*, AB249970, 99.8%.

Source: isolated from soil from Recife, Brazil.

DNA G+C content (mol%): not known.

Type strain: ATCC 19803, CBS 555.68, BCRC 12086, DSM 40115, NBRC 12813, JCM 4408, NRRL B-3811, NRRL-ISP 5115, RIA 1082, UNIQEM 189, VKM Ac-1890.

Sequence accession no. (16S rRNA gene): AB184165.

403. **Streptomyces rectiverticillatus** (Krasil'nikov and Yuan 1965) Witt and Stackebrandt 1991, 456^{VP} (Effective publication: Witt and Stackebrandt 1990, 370.) ("*Actinomyces rectiverticillatus*" Krasil'nikov and Yuan 1965, 49; *Streptoverticillium rectiverticillatum* Locci, Baldacci and Petrolini Baldan 1969, 41)

rec.ti.ver.ti.cil.la'tus. L. adj. *rectus* straight; L. masc. n. *verticillus* whorl; N.L. adj. *verticillatus* whorled; N.L. masc. adj. *rectiverticillatus* straight and whorled.

Spore chains in Section *Verticillati*, umbellate monoverticillate. Monoverticillate morphology is also common. Mature spore chains are generally short with 3–10 or sometimes more than 10 spores per chain. This morphology is seen on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar. Spore surface is smooth.

Color of colony: aerial mass color in the Red or White color series on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar. Reverse side of colony grayish yellowish pink to strong brown or reddish orange on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar. Reverse mycelium pigment is not a pH indicator.

Color in medium: melanoid pigments are formed in peptone-yeast-iron agar, tyrosine agar, or tryptone-yeast broth, but may be weak in tyrosine agar. Yellow or red pigment is found in the medium in yeast-malt agar and oatmeal agar. Traces of yellow may also be present in salts-starch agar and glycerol-asparagine agar. This pigment is not pH-sensitive when tested with 0.05 M NaOH or HCl.

D-Glucose, iso-inositol, and D-fructose are utilized for growth. Utilization of sucrose and raffinose is doubtful. No growth or only trace of growth with L-arabinose, D-xylose, D-mannitol, or rhamnose.

For sequence similarity, see type strain of *Streptomyces hiroshimensis*.

Source: not known.

DNA G+C content (mol%): not known.

Type strain: ATCC 19845, ATCC 25491, CBS 951.69, BCRC 13306, CECT 3268, DSM 40436, NBRC 13079, INMI 380, JCM 4511, NRRL B-12369, NRRL-ISP 5436, RIA 1271, VKM Ac-1503.

Sequence accession no. (16S rRNA gene): AB184296.

Further comments: in violation of Rule 33c of the *Bacteriological Code* (1990 Revision), in Validation List no. 38, *Streptomyces rectiverticillatus* is proposed as a *nomen revictum* (basonym: "*Streptomyces rectiverticillatus*" Krasil'nikov and Yuan (1965).

According to Hatano et al. (2003), *Streptomyces rectiverticillatus* (Krasil'nikov and Yuan 1965) Witt and Stackebrandt 1991 is a later heterotypic synonym of *Streptomyces hiroshimensis* (Shinobu 1955) Witt and Stackebrandt 1991.

404. **Streptomyces rectiviolaceus** (*ex* Artamonova *in* Krasil'nikov 1965) Sveshnikova 1986, 575^{VP} (Effective

publication: Sveshnikova *in* Gause, Preobrazhenskaya, Sveshnikova, Terekhova and Maximova 1983.) ("*Actinomyces rectiviolaceus*" Artamonova *in* Krasil'nikov 1965, 234; *Streptomyces rectivioleceus* Pridham 1970, 25)

rec.ti.vio.la'ce.us. L. adj. *rectus* straight; L. adj. *violaceus* violet colored; N.L. masc. adj. *rectiviolaceus* straight, violet colored.

Spore chains are straight or flexuous (*Rectiflexibiles*). Spore surface is smooth. Forms violet or red-colored vegetative mycelium on some media. On mineral agar 1: aerial mycelium is white to light pink, poor; substrate mycelium is violet; diffusible pigment is poor, light violet or not extant. Pigment is able to act as an indicator: alkaline reaction, blue; acidic reaction, red. On glycerol-nitrate agar: aerial mycelium is white, good developed; substrate mycelium is reddish violet; no diffusible pigment. On starch-ammonia agar: aerial mycelium is white, poor; substrate mycelium is colorless to light violet; no diffusible pigment. On glycerol-asparagine agar: aerial mycelium is white, good developed; substrate mycelium is pale violet; no diffusible pigment. On glucose-nitrate agar SP-1: aerial mycelium is whitish, poor; substrate mycelium violet; no diffusible pigment. On oatmeal agar: aerial mycelium is white, poor; substrate mycelium is red-violet; no diffusible pigment. On organic agar 2: no aerial mycelium; substrate mycelium is pale violet; no diffusible pigment. Melanoid pigments are not formed. Grows on glucose, arabinose, xylose, rhamnose, sucrose, raffinose, mannitol and inositol. Exhibits anti-bacterial and anti-fungal activity.

Type strain shows no sequence similarity over 99%.

Source: not known.

DNA G+C content (mol%): not known.

Type strain: ATCC 43690, DSM 41459, INMI 563, JCM 9092, NBRC 100765, NRRL B-16374, VKM Ac-282.

Sequence accession no. (16S rRNA gene): DQ026660.

405. **Streptomyces regensis** Gupta, Sobti and Chopra 1963, 15[AL]

reg.en'sis. N.L. masc. adj. *regensis* of or belonging to reg (unknown derivation, possibly referring to a place in India).

Spore chains in Section *Spirales* or *Rectiflexibiles*. Spirals are poorly developed, usually with only one or two turns or with hooks and loops of small diameter on short lateral spore chains. Flexuous or straight chains are also common. Aerial hyphae may be long, but spore chains are often moderately short with 10 or more spores per chain. This morphology is seen on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar. Spore surface is smooth or irregularly folded.

Color of colony: aerial mass color in the Gray or Yellow color series on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar. Representative color tabs from the Gray color series are 2dc, yellowish green; 2ge, light olive brown; and 3fe, light brownish gray. Representative color chips from the Yellow color series are 1½ec – 1cb, pale yellow green, and 1ba – 2ba, pale yellow. Reverse side of colony is moderate to strong or dark yellow, greenish yellow, or olive on oatmeal agar, salts-starch agar, and glycerol-asparagine agar. Strong yellow or strong brown on yeast-malt agar.

Color in medium: melanoid pigments are formed in peptone-yeast-iron agar, tyrosine agar, and tryptone-yeast broth. Yellow pigment is found in the medium in yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar. This pigment is not pH-sensitive when tested with 0.05 M NaOH or HCl.

D-Glucose, L-arabinose, D-xylose, iso-inositol, D-mannitol, D-fructose, rhamnose, sucrose, and raffinose are all utilized for growth.

Type strain shows the highest sequence similarity to: *S. capoamus*, AB045877, 99.1%.

Source: not known.

DNA G+C content (mol%): not known.

Type strain: ATCC 27461, CBS 749.72, BCRC 11890, DSM 40551, NBRC 13448, JCM 4820, NRRL B-11479, NRRL-ISP 5551, RIA 1409, VKM Ac-1289.

Sequence accession no. (16S rRNA gene): DQ026649.

406. **Streptomyces resistomycificus** Lindenbein 1952, 376[AL]

re.sis.to.my.ci'fi.cus. L. v. *restisto* to resist; Gr. n. *mukês* fungus; L. masc. suff. *-ficus* (from L. v. *facio* to make) making, producing; N.L. masc. adj. *resistomycificus* making resistant to a fungus; producing resistomycin.

Spore chains in Section *Spirales*, but with many spore chains representative of Section *Retinaculiaperti* (especially when sporophores are immature). Mature spore chains generally have 10–50 spores per chain. This morphology is seen on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar. Spore surface is smooth.

Color of colony: aerial mass color in the Gray color series on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar. Reverse side of colony dark reddish brown on yeast-malt agar; grayed brown to reddish brown on oatmeal agar and glycerol-asparagine agar; and grayed yellow to faint pinkish yellow on salts-starch agar. Substrate pigment is not a pH indicator.

Color in medium: melanoid pigments formed in peptone-yeast-iron agar, tyrosine agar, and tryptone-yeast extract broth. Pigments other than melanoids are not formed in yeast-malt agar, oatmeal agar, salts-starch agar, or glycerol-asparagine agar.

D-Glucose, L-arabinose, sucrose, D-xylose, iso-inositol, D-mannitol, D-fructose, rhamnose, and raffinose are utilized for growth.

Type strain shows the highest sequence similarity to: *S. pseudovenezuelae*, AB184233, 99.5%; *S. galilaeus*, AB045878, 99.5%; *S. chartreusis*, AB184839, 99.5%; *S. bobili*, AB249925, 99.5%; *S. phaeoluteigriseus*, AJ391815, 99.4%; *S. novaecaesareae*, AB184357, 99.3%; *S. canus*, AY999775, 99.3%; *S. aureocirculatus*, AB184260, 99.1%; *S. prunicolor*, DQ026659, 99.1%; *S. ciscaucasicus*, AY508512, 99.1%; *S. alboniger*, AY845349, 99%.

Source: not known.

DNA G+C content (mol%): not known.

Type strain: ATCC 19804, CBS 556.68, BCRC 13755, DSM 40133, NBRC 12814, JCM 4409, NCIMB 9843, NRRL

2290, NRRL-ISP 5133, PCM 2296, RIA 1083, UNIQEM 190, VKM Ac-1895.

Sequence accession no. (16S rRNA gene): AB184166.

407. **Streptomyces reticuliscabiei** Bouchek-Mechiche, Gardan, Normand and Jouan 2000, 98[VP]

re.ti.cu.li.sca'bi.ei. L. n. *reticulum* reticulum; L. n. *scabies* mange; N.L. gen. n. *reticuliscabiei* referring to the reticulum aspect of the symptoms of the disease.

Spores are light gray and borne in mature flexuous chains. Melanin is not produced on tyrosine agar. Fructose, D-glucose, D-mannitol, inositol, raffinose, rhamnose, sucrose, and D-xylose are used as carbon sources. Utilizes α-D(+)melibiose, mucate, D-saccharate, D(+)trehalose, and 5-keto-D-gluconate. Does not grow in the presence of 0.5 μg/ml crystal violet, 20 μg/ml streptomycin, 100 μg/ml oleandomycin, or 5% (w/v) NaCl. Strains are not susceptible to 10 IU/ml penicillin G and some (about 60%) of the strains are not susceptible to 25 μg/ml oleandomycin. Does not assimilate *trans*-aconitate or ONPG. Most (about 80%) of the strains utilize DL-lactate and turanose. Some strains use betaine.

Type strain shows the highest sequence similarity to: *S. turgidiscabies*, AB026221, 99.1%. Type strain shows DNA–DNA similarity to: *S. europaeiscabiei* DSM 41802[T], 47%; *S. stelliscabiei* DSM 41803[T], 24%.

Source: isolated from netted scab lesions on potato cv. Bintje and have been confirmed to be pathogenic only on potato cv. Bintje.

DNA G+C content (mol%): 69.8.

Type strain: CFBP 4531, CIP 107061, DSM 41804, ICMP 13716, NCPPB 4041.

Sequence accession no. (16S rRNA gene): AJ007428.

Further comments: a numerical analysis of phenotypic characteristics showed that *Streptomyces reticuliscabiei* Bouchek-Mechiche et al. 2000 and *Streptomyces turgidiscabies* Miyajima et al. 1998 belong to the same cluster and share almost all morphological and biochemical traits that are important in the identification of *Streptomyces* species. DNA–DNA hybridization and phylogenetic comparisons of 16S rRNA gene sequences confirm that the two species are genomically closely related. In contrast, pathological data showed that *Streptomyces turgidiscabies* and *Streptomyces reticuliscabiei* cause two distinct diseases. For the pathologist, the fusion of *Streptomyces reticuliscabiei* and *Streptomyces turgidiscabies* under a single species denomination would cause confusion of separate diseases and create a discrepancy between taxonomists and pathologists. Therefore, Bouchek-Mechiche et al. think that the two groups should continue to carry their current denominations, i.e. *Streptomyces reticuliscabiei* Bouchek-Mechiche et al. 2000 for the strains inducing netted scab and *Streptomyces turgidiscabies* Miyajima et al. 1998 for those causing common scab.

408. **Streptomyces rhizosphaericus** corrig. Sembiring, Ward and Goodfellow 2001, 1619[VP] (Effective publication: Sembiring, Ward and Goodfellow 2000, 362.)

rhi.zo.spha.e'ri.cus. Gr. n. *rhiza* root; L. adj. *sphaericus* of or belonging to a ball, spherical; N.L. masc. adj. *rhizosphaericus* belonging to the sphere of the root.

Spore chains are *Spirales*; spore surface is rugose. On oatmeal agar, the spore mass is gray, the substrate mycelium is grayish-yellow, and the diffusible pigment is yellow. Melanin pigments are not produced. Degrades adenine and pectin and grows at 10°C.

Type strain shows the highest sequence similarity to: *S. asiaticus*, AB249947, 100%; *S. cangkringensis*, AJ391831, 99.8%; *S. griseinger*, AJ391818, 99.8%; *S. indonesiensis*, DQ334783, 99.7%; *S. antimycoticus*, AB184185, 99.1%; *S. sporoclivatus*, AB249934, 99.1%; *S. rutgersensis* subsp. *castelarensis*, AY508511, 99.1%; *S. geldanamycininus*, DQ334781, 99%. Type strain shows DNA–DNA similarity to: *S. albiflaviniger* NRRL B-1356[T], 98.1%; *S. geldanamycinus* NRRL 3602[T], 98.7%; *S. griseiniger* NRRL B1865[T], 99.2%; *S. asiaticus* DSM 41761[T], 98.6%; *S. indonesiensis* DSM 41759[T], 98.3%; *S. javensis* DSM 41764[T], 98.3%; *S. yogyakartensis* DSM 41766[T], 97.9%; *S. cangkringensis* DSM 41769[T], 99.0%.

Source: isolated from the rhizosphere of the tropical legume, *Paraserianthes falcataria*.

DNA G+C content (mol%): not known.

Type strain: A10P1, DSM 41760, JCM 11447, NBRC 100778, NCIMB 13674.

Sequence accession no. (16S rRNA gene): AB249941.

Further comments: the original spelling of the specific epithet, *rhizosphaerius* (*sic*), has been corrected on validation according to Rule 61 of the *Bacteriological Code* (1990 Revision).

409a. **Streptomyces rimosus subsp. rimosus** Sobin, Finlay and Kane *in* Waksman and Lechevalier 1953, 47[AL]

ri.mo'sus. L. masc. adj. *rimosus* full of fissures.

Spore chains in Section *Spirales*, but many imperfect spirals, hooks, and loops suggesting *Retinaculiaperti* morphology are also present. Mature spore chains are short to moderately long with 3–10, or often more than 10, spores per chain. This morphology is seen on yeast-malt agar, oatmeal agar, salts-starch agar, glycerol-asparagine agar, and carbon utilization medium plus D-glucose. Spore surface is smooth.

Color of colony: aerial mass color in the Red or White color series on yeast-malt agar and in the Yellow or White color series on oatmeal agar, salts-starch agar, and glycerol-asparagine agar. Representative color tabs are 3ca (pale orange yellow), 5cb (grayish yellowish pink), and 2ca (pale yellow) from the Red color-wheel; 2db and 2ba (pale yellow) from the Yellow color-wheel; and a (white) from the White color-wheel. Reverse side of colony is grayish yellow on oatmeal agar and salts-starch agar; grayish yellow modified slightly by red (moderate to dark orange yellow) on yeast-malt agar and glycerol-asparagine agar. Substrate mycelium pigment is not a pH indicator.

Color in medium: melanoid pigments are not formed in peptone-yeast-iron agar, tyrosine agar, or tryptone-yeast broth. A trace of yellow pigment is found in the medium in yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar. This pigment is not pH-sensitive when tested with 0.05 M NaOH or HCl.

D-Glucose, L-arabinose, iso-inositol, D-mannitol, D-fructose, and raffinose are utilized for growth. Utilization of

D-xylose is doubtful. No growth or only trace of growth with sucrose and raffinose.

Type strain shows the highest sequence similarity to: *S. erumpens*, AJ621603, 99.7%; *S. sclerotialus*, AJ621608, 99.4%; *S. niger*, AJ621607, 99.3%; *S. olivaceiscleroticus*, AJ621606, 99.3%; *S. monomycini*, DQ445790, 99.1%; *S. ramulosus*, DQ026662, 99%.

Source: not known.

DNA G+C content (mol%): not known.

Type strain: AS 4.1438, ATCC 10970, ATCC 23955, CBS 437.51, CBS 938.68, BCRC 11612, CECT 3144, DSM 40260, HAMBI 1066, HUT 6064, HUT 6100, ICMP 919, IFM 1065, NBRC 12907, IMET 40364, JCM 4073, JCM 4667, KCTC 1077, LMG 5984, LMG 19352, NCIMB 8229, NRRL 2234, NRRL B-2659, NRRL-ISP 5260, RIA 1185, RIA 606, VKM Ac-849.

Sequence accession no. (16S rRNA gene): AB045883.

409b. **Streptomyces rimosus subsp. paromomycinus** Coffey, Anderson, Fisher, Galbraith, Hillegas, Kohberger, Thompson, Weston and Ehrlich 1959, 730[AL] (*Streptomyces rimosus* forma *paromomycinus* Coffey, Anderson, Fisher, Galbraith, Hillegas, Kohberger, Thompson, Weston and Ehrlich 1959, 737)

pa.r.o.mo.my.ci′nus. N.L. n. *paromomycinum* paromomycin; N.L. masc. adj. *paromomycinus* intented to mean paromomycin-producing.

Fair growth on Czapek's solution agar. Produces the aminocyclitol anti-bacterial antibiotics neomycins E and F (paromycins I and II); produces the glutarimide anti-fungal antibiotic (streptimidone); inhibited by streptomycin.

Type strain shows the highest sequence similarity to: *S. chrestomyceticus*, AJ621609, 100%; *S. albofaciens*, AB045880, 99.7%; *S. lydicus*, Y15507, 99.3%; *S. erumpens*, AJ621603, 99.3%; *S. chattanoogensis*, AJ621611, 99.2%; *S. sclerotialus*, AJ621608, 99.1%; *S. sioyaensis*, DQ026654, 99%.

Source: not known.

DNA G+C content (mol%): not known.

Type strain: ATCC 14827, DSM 41429, NBRC 15454, JCM 4541, JCM 4871, NRRL 2455, VKM Ac-605.

Sequence accession no. (16S rRNA gene): AJ621610.

410. **Streptomyces rishiriensis** Kawaguchi, Tsukiura, Okanishi, Miyaki, Ohmori, Fujisawa and Koshiyama 1965, 3[AL]

ri.shi.ri.en′sis. N.L. masc. adj. *rishiriensis* of or belonging to Rishiri, named for Rishiri Island, Hokkaido, Japan, the source of the soil from which the organism was isolated.

Spore chains may be in Section *Retinaculiaperti*. Moderate growth on Czapek's solution agar. Produces the coumermycin complex (coumermycins A_1, A_2, B, C, and D); inhibited by streptomycin.

Type strain shows the highest sequence similarity to: *S. humidus*, DQ442508, 99.3%; *S. phaeofaciens*, AB184360, 99.2%; *S. puniceus*, DQ442542, 99.1%; *S. cacaoi* subsp. *asoensis*, DQ026644, 99.1%.

Source: isolated from soil from Rishiri Island, Hokkaido, Japan.

DNA G+C content (mol%): not known.

Type strain: ATCC 14812, CBS 708.72, BCRC 12333, DSM 40489, NBRC 13407, JCM 4821, NCIMB 11890, NRRL B-3239, NRRL-ISP 5489, RIA 1368, VKM Ac-1188.

Sequence accession no. (16S rRNA gene): EF178682.

411. **Streptomyces rochei** Berger, Jampolsky and Goldberg *in* Waksman and Lechevalier 1953, 40[AL] ("*Actinomyces rochei*" Preobrazhenskaya, Blinov and Ryabova *in* Gauze, Preobrazhenskaya, Kudrina, Blinov, Ryabova and Sveshnikova 1957, 133)

ro′che.i. N.L. gen. masc. n. *rochei* of Roche.

Spore chains in Section *Spirales*. Spirals are usually open, sometimes almost flexuous. Mature spore chains are moderately long with 10–50, or often more than 50, spores per chain. This morphology is seen on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar. Spore surface is smooth to warty.

Color of colony: aerial mass color in the Gray color series on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar. Reverse side of colony with no distinctive pigments (grayish yellow to yellowish brown) on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar.

Color in medium: melanoid pigments are not formed in peptone-yeast-iron agar, tyrosine agar, and tryptone-yeast broth. No pigment is found in the medium in yeast-malt agar, oatmeal agar, salts-starch agar, or glycerol-asparagine agar.

D-Glucose, L-arabinose, D-xylose, iso-inositol, D-mannitol, D-fructose, and rhamnose are utilized for growth. No growth or only trace of growth on sucrose and raffinose.

Type strain shows the highest sequence similarity to: *S. vinaceusdrappus*, AY999929, 100%; *S. ghanaensis*, AY999851, 100%; *S. geysiriensis*, DQ442501, 100%; *S. minutiscleroticus*, EF178696, 100%; *S. plicatus*, AB184291, 100%; *S. mutabilis*, EF178679, 99.9%; *S. tuirus*, AB184690, 99.5%; *S. djakartensis*, AB184657, 99.4%; *S. anandii*, AB184402, 99.2%; *S. violaceorubidus*, AJ781374, 99.2%; *S. pilosus*, AB184161, 99.1%; *S. flavoviridis*, AB184842, 99.1%; *S. tendae*, D63873, 99%; *S. calvus*, AB184329, 99%; *S. azureus*, EF178674, 99%; *S. asterosporus*, AB184706, 99%; *S. levis*, AB184670, 99%; *S. luteogriseus*, AB184379, 99%; *S. capillispiralis*, AB184577, 99%.

Source: not known.

DNA G+C content (mol%): not known.

Type strain: AS 4.1425, ATCC 10739, ATCC 19245, ATCC 23956, CBS 224.46, CBS 939.68, CCUG 11115, CECT 3329, DSM 40231, HAMBI 2114, IFM 1188, NBRC 12908, IMET 41386, JCM 4074, JCM 4668, LMG 19313, NRRL 3533, NRRL B-1559, NRRL B-2410, NRRL-ISP 5231, RIA 1171, VKM Ac-997.

Sequence accession no. (16S rRNA gene): AB184237.

412. **Streptomyces roseiscleroticus** (Thirumalachar *in* Rautenshtein 1960) Pridham 1970, 42[AL] (*Chainia poonensis* Thirumalachar *in* Rautenshtein 1960, 45)

ro.se.i.scle′ro.ti.cus. L. adj. *roseus* rosy; N.L. n. *sclerotium* a sclerotium; N.L. masc. adj. *roseiscleroticus* rosy, belonging to sclerotium.

Spore chains in Section *Spirales*. Mature spore chains are short to long, sometimes with more then 50 spores per chain. This morphology is sometimes seen on yeast-malt

agar, oatmeal agar, salts-starch agar, glycerol-asparagine agar, and or Gause's medium no. 1. Sporulating aerial mycelium is often absent on various media. Spore surface is smooth. Special morphological characteristics: sclerotia are produced on yeast-malt agar, oatmeal agar, salts-starch agar, glycerol-asparagine agar, and Gause's medium no. 1 in 7–14 d.

Color of colony: aerial mass color in the White color series when adequate aerial mycelium is produced on yeast-malt agar, oatmeal agar, salts-starch agar, glycerol-asparagine agar, or Gause's medium no. 1. Reports vary on the media supporting good growth of aerial mycelium. Reverse side of colony is yellow to yellow brown is modified by red to strong (reddish) brown on yeast-malt agar and to reddish orange or deep orange on oatmeal agar. Reverse mycelium on salts-starch agar and glycerol-asparagine agar may be colorless, light yellow, orange, or strong brown. No change is reported in reverse mycelium color after the addition of 0.05 M NaOH or HCl.

Color in medium: melanoid pigments are not formed in peptone-yeast-iron agar, tyrosine agar, or tryptone-yeast broth. No pigment or only a trace of brown is found in the medium in yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar.

D-Glucose, L-arabinose, D-xylose, D-mannitol, D-fructose, and rhamnose are utilized for growth. Utilization of sucrose, iso-inositol, and raffinose is doubtful.

Type strain shows the highest sequence similarity to: *S. ruber*, AB184604, 100%.

Source: not known.

DNA G+C content (mol%): not known.

Type strain: ATCC 17755, CBS 226.65, CBS 664.72, BCRC 12541, CMI 112788, DSM 40303, NBRC 13002, NBRC 13363, IMET 43586, JCM 3104, JCM 4823, NCIMB 11013, NRRL B-3348, NRRL-ISP 5303, RIA 1324, RIA 887, VKM Ac-1718.

Sequence accession no. (16S rRNA gene): AB184251.

Further comments: Streptomyces roseiscleroticus Pridham 1970 and *Chainia rosea* Thirumalachar et al. 1966 have the same type strain and therefore are homotypic synonyms [Rules 24a and 24b (1) of the *Bacteriological Code* (1990 Revision)].

413. **Streptomyces roseodiastaticus** (Duché 1934) Waksman *in* Waksman and Lechevalier 1953, 27[AL] ["*Actinomyces roseodiastaticus*" Duché 1934, 329; *Streptomyces roseodiastaticus* (Wollenweber 1920) Waksman 1961]

ro.se.o.di.a.sta′ti.cus. L. adj. *roseus* rosy; N.L. adj. *diastaticus* diastatic, starch, digesting; N.L. masc. adj. *roseodiastaticus* rosy, diastatic.

Excellent growth on Czapek's solution agar. Inhibited by streptomycin.

For sequence similarity, see type strain of *Streptomyces tricolor*.

Source: not known.

DNA G+C content (mol%): not known.

Type strain: CBS 102.34, DSM 41703, NBRC 15457, JCM 4295, JCM 13861, NRRL B-1906.

Sequence accession no. (16S rRNA gene): AB184683.

Further comments: according to Lanoot et al. (2004), *Streptomyces roseodiastaticus* (Duché 1934) Waksman 1953 is a later heterotypic synonym of *Streptomyces tricolor* (Wollenweber 1920) Waksman 1961.

414. **Streptomyces roseoflavus** Arai 1951, 218[AL] [*Streptomyces fradiae* (Waksman and Curtis 1916, 125) Waksman and Henrici 1948, 954]

ro.se.o.fla′vus. L. adj. *roseus* rosy; L. adj. *flavus* yellow; N.L. masc. adj. *roseoflavus* rose-yellow.

Spore chains in Section *Retinaculiaperti* or *Spirales*. Flexuous spore chains are common; *Retinaculiaperti* and *Spirales* spore chains may not be seen on some media. Mature spore chains of more than 50 spores per chain may be seen if good sporulation occurs, but sporulation is often poor on yeast-malt agar, oatmeal agar, and salts-starch agar, and especially on glycerol-asparagine agar. Spore surface is smooth.

Color of colony: aerial mass color in the Red or Yellow color series on yeast-malt agar, salts-starch agar, and glycerol-asparagine agar. Yellow color series on oatmeal agar. Representative color tabs from the Red color series are 3ca, pale orange yellow; 4ec, grayish yellowish pink; and 5ca, light yellowish pink. Representative chips from the Yellow color series are 1 or 2ba, pale yellow; and 1½ec, pale yellowish green (yellow colors may represent poor sporulation). Reverse side of colony is moderate to deep orange on yeast-malt agar; grayish yellow to orange yellow or yellowish brown on oatmeal agar, salts-starch agar, and glycerol-asparagine agar.

Color in medium: melanoid pigments are not formed in peptone-yeast-iron agar, tyrosine agar, or tryptone-yeast broth. No pigment is found in the medium in yeast-malt agar, oatmeal agar, or glycerol-asparagine agar; a trace of yellow to orange pigment may or may not be present in salts-starch agar.

D-Glucose, L-arabinose, and D-xylose are utilized for growth. Utilization of fructose is doubtful. No growth or only traces of growth with iso-inositol, D-mannitol, rhamnose, sucrose, or raffinose.

For sequence similarity, see type strain of *Streptomyces fradiae*.

Source: not known.

DNA G+C content (mol%): not known.

Type strain: ATCC 13167, CBS 740.72, DSM 40536, NBRC 13439, JCM 4824, NRRL B-1563, NRRL B-2789, NRRL-ISP 5536, RIA 1400, VKM Ac-1907.

Sequence accession no. (16S rRNA gene): no sequence available.

Further comments: according to Lanoot et al. (2004), *Streptomyces roseoflavus* Arai 1951 is a later heterotypic synonym of *Streptomyces fradiae* (Waksman and Curtis 1916) Waksman and Henrici 1948.

415. **Streptomyces roseofulvus** (Preobrazhenskaya *in* Gauze, Preobrazhenskaya, Kudrina, Blinov, Ryabova and Sveshnikova 1957) Pridham, Hesseltine and Benedict 1958, 61[AL] ("*Actinomyces roseofulvus*" Preobrazhenskaya *in* Gauze, Preobrazhenskaya, Kudrina, Blinov, Ryabova and Sveshnikova 1957, 55)

ro.se.o.ful′vus. L. adj. *roseus* rosy: L. adj. *fulvus* deep yellow; N.L. masc. adj. *roseofulvus* rosy, deep yellow, referring to color of aerial mycelium and vegetative mycelium, respectively.

Spore chains in Section *Rectiflexibiles*. Mature spore chains are generally long and straight, often with more than 50 spores per chain. This morphology is seen on yeast-malt agar, oatmeal agar, and salts-starch agar; poor sporulation on glycerol-asparagine agar. Spore surface is smooth.

Color of colony: aerial mass color in the Red color series on yeast-malt agar, oatmeal agar, and salts-starch agar in 21 d. Younger cultures or cultures on media giving poor sporulation may show white aerial mycelium. Reverse side of colony is colorless or pale grayed yellow to pale pinkish yellow on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar. Reverse color is changed from colorless or pale yellow to pale pink by addition of 0.05 M NaOH, or from pinkish yellow to pale yellow with 0.05 M HCl.

Color in medium: melanoid pigments not formed in peptone-yeast-iron agar, tyrosine agar, or tryptone-yeast extract broth. Traces of pale grayish yellow or pale brownish gray pigment may be found in yeast-malt agar, oatmeal agar, and salts-starch agar. This faint pigment may be pH-sensitive (one observer only); the change is from pale yellow to pale pink by 0.05 M NaOH.

D-Glucose, L-arabinose, sucrose, D-xylose, D-fructose, rhamnose, and raffinose are utilized for growth. No growth or only trace of growth on iso-inositol and D-mannitol.

Type strain shows the highest sequence similarity to: *S. laurentii*, AJ781342, 99.2%; *S. roseolus*, AB184168, 99.2%.

Source: not known.

DNA G+C content (mol%): not known.

Type strain: ATCC 19805, ATCC 19921, CBS 557.68, BCRC 12051, DSM 40172, NBRC 13194, NBRC 15816, NBRC 15817, INA 14535, JCM 4334, JCM 4605, NRRL B-2729, NRRL-ISP 5172, RIA 1084, UNIQEM 191, VKM Ac-1080.

Sequence accession no. (16S rRNA gene): AB184327.

416. **Streptomyces roseolilacinus** (Preobrazhenskaya and Sveshnikova *in* Gauze, Preobrazhenskaya, Kudrina, Blinov, Ryabova and Sveshnikova 1957) Pridham, Hesseltine and Benedict 1958, 68[AL] ("*Actinomyces roseolilacinus*" Preobrazhenskaya and Sveshnikova *in* Gauze, Preobrazhenskaya, Kudrina, Blinov, Ryabova and Sveshnikova 1957, 35)

ro.se.o.li.la.ci′nus. L. adj. *roseus* rosy; N.L. adj. *lilacinus* lilac colored; N.L. masc. adj. *roseolilacinus* rose, lilac colored, referring to color of the aerial mycelium of the organism.

Spore chains in Section *Retinaculiaperti* to *Spirales*. Spirals are prominent on salts-starch agar, but spore chains representative of Section *Retinaculiaperti* are more common on yeast-malt agar and oatmeal agar; poor sporulation on glycerol-asparagine agar. Mature spore chains generally have 10–50 spores per chain. Spore surface is smooth.

Color of colony: aerial mass color in the Red color series on yeast-malt agar, oatmeal agar, and salts-starch agar. Reverse side of colony with no distinctive pigment on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar; substrate pigment is not a pH indicator (or shows only slight change with 0.05 M HCl or NaOH on salts-starch agar and glycerol-asparagine agar).

Color in medium: melanoid pigments not formed in peptone-yeast-iron agar and tyrosine agar. Pigments not formed in medium or found only as traces of pale yellowish or brownish gray, in yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar.

D-Glucose and L-arabinose are utilized for growth. No growth or only trace of growth on sucrose, D-xylose, iso-inositol, D-mannitol, rhamnose, and raffinose. Doubtful growth with D-fructose.

Type strain shows no sequence similarity over 99%.

Source: not known.

DNA G+C content (mol%): not known.

Type strain: ATCC 19806, ATCC 19922, CBS 558.68, BCRC 12329, DSM 40173, NBRC 12815, INA 14250, JCM 4335, JCM 4606, NRRL B-2699, NRRL-ISP 5173, RIA 1085, UNIQEM 192, VKM Ac-1276.

Sequence accession no. (16S rRNA gene): AB184167.

417. **Streptomyces roseolus** (Preobrazhenskaya and Sveshnikova *in* Gauze, Preobrazhenskaya, Kudrina, Blinov, Ryabova and Sveshnikova 1957) Pridham, Hesseltine and Benedict 1958, 61[AL] ("*Actinomyces roseolus*" Preobrazhenskaya and Sveshnikova *in* Gauze, Preobrazhenskaya, Kudrina, Blinov, Ryabova and Sveshnikova 1957, 37)

ro.se.o′lus. N.L. dim. masc. adj. *roseolus* somewhat rosy, referring to the color of the aerial mycelium.

Spore chains in Section *Rectiflexibiles*. Mature spore chains are generally long with 10–50 spores per chain on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar. Spore surface is smooth.

Color of colony: aerial mass color in the Red color series on yeast-malt agar and salts-starch agar, and in the Red or Yellow series on oatmeal agar. Sporulation may be poor on glycerol-asparagine agar. Reverse side of colony with no distinctive pigment on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar; substrate pigment is not a pH indicator.

Color in medium: melanoid pigments not formed in peptone-yeast-iron agar and tyrosine agar. No pigment found in medium in yeast-malt agar, oatmeal agar, salts-starch agar, or glycerol-asparagine agar.

D-Glucose, L-arabinose, D-xylose, and rhamnose are utilized for growth. No growth or only trace of growth on sucrose, iso-inositol, D-mannitol, and raffinose. Variable reports of slight growth with D-fructose.

Type strain shows the highest sequence similarity to: *S. filamentosus*, AB184130, 99.4%; *S. roseofulvus*, AB184327, 99.2%; *S. tanashiensis*, AJ781362, 99.1%; *S. nashvillensis*, AB184286, 99%.

Source: not known.

DNA G+C content (mol%): not known.

Type strain: ATCC 23210, CBS 559.68, BCRC 13778, DSM 40174, NBRC 12816, INA 5449/54, JCM 4411, NCIMB 13022, NRRL B-5424, NRRL-ISP 5174, RIA 1086, UNIQEM 193, VKM Ac-848.

Sequence accession no. (16S rRNA gene): AB184168.

418. **Streptomyces roseosporus** Falcão de Morais and Dália Maia 1961, 41[AL] [*Streptomyces venezuelae* subsp. *roseospori* (*sic*) Falcão de Morais, Dália Maia and Souto Maior Genn 1958, 102; *Streptomyces filamentosus* Okami and Umezawa *in* Okami, Okuda, Takeuchi, Nitta and Umezawa 1953, 153]

ro.se.o.spo′rus. L. adj. *roseus* rosy; N.L. n. *spora* a spore; N.L. masc. adj. *roseosporus* rosy-spored.

Spore chains in Section *Rectiflexibiles*. Mature spore chains are generally long, often with more than 50 spores per chain. This morphology is seen on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar. Spore surface is smooth.

Color of colony: aerial mass color in the Red color series on yeast-malt agar, oatmeal agar, and salts-starch agar (White, Yellow, or Red color series on glycerol-asparagine agar). Reverse side of colony with no distinctive pigments (grayish yellow to yellowish brown) on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar.

Color in medium: melanoid pigments are not formed in peptone-yeast-iron agar, tyrosine agar, or tryptone-yeast broth. No pigment is found in the medium in yeast-malt agar, oatmeal agar, salts-starch agar, or glycerol-asparagine agar.

D-Glucose, L-arabinose, D-xylose, and rhamnose are utilized for growth. Utilization of D-fructose is doubtful. No growth or only trace of growth with sucrose, iso-inositol, D-mannitol, and raffinose.

For sequence similarity, see type strain of *Streptomyces filamentosus*.

Source: not known.

DNA G+C content (mol%): not known.

Type strain: ATCC 23958, CBS 941.68, BCRC 13771, DSM 40122, NBRC 12910, JCM 4412, KCTC 9568, NCIMB 13008, NRRL B-5411, NRRL-ISP 5122, RIA 1125.

Sequence accession no. (16S rRNA gene): AB184238.

Further comments: according to Lanoot et al. (2004), *Streptomyces roseosporus* Falcão de Morais and Dália Maia 1961 is a later heterotypic synonym of *Streptomyces filamentosus* Okami and Umezawa 1953.

419. **Streptomyces roseoverticillatus** (Shinobu 1956) Witt and Stackebrandt 1991, 456[VP] (Effective publication: Witt and Stackebrandt 1990, 370.) ("*Streptomyces roseoverticillatus*" Shinobu 1956; "*Verticillomyces roseoverticillatus*" Shinobu 1965; *Streptoverticillium roseoverticillatum* Farina and Locci 1966, 49)

ro.se.o.vert.i.cil.la′tus. L. adj. *roseus* rosy; L. masc. n. *verticillus* whorl; N.L. adj. *verticillatus* whorled; N.L. masc. adj. *roseoverticillatus* rosy whorled.

Spore chains in Umbellate Monoverticillate (=*Streptomyces* Section Verticillati, biverticillate). Mature spore chains are short, generally 3–10 spores per chain. This morphology is seen on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar. Spore surface is smooth.

Color of colony: aerial mass color in the Red color series on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar. Reverse side of colony is grayed yellow is modified by red on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar. Only slight changes, if any, occur when pH is changed by addition of 0.05 NaOH or HCl.

Color in medium: melanoid pigments formed on peptone-yeast-iron agar and tryptone-yeast extract broth. Traces of yellow, orange, or red pigment may be found in yeast-malt agar and oatmeal agar. This pigment, when present, is not affected by pH or is changed slightly from orange in the presence of 0.05 M NaOH to pink in presence of 0.05 M HCl.

D-Glucose is utilized for growth. No growth or only trace of growth on L-arabinose, D-xylose, D-mannitol, rhamnose, and raffinose. Variable reports of doubtful growth to no growth with sucrose, iso-inositol, and D-fructose.

For sequence similarity, see type strain of *Streptomyces hiroshimensis*.

Source: not known.

DNA G+C content (mol%): not known.

Type strain: ATCC 19807, CBS 560.68, CECT 3269, DSM 40039, NBRC 12817, NBRC 3844, JCM 4103, JCM 4607, NRRL B-1993, NRRL-ISP 5039, PCM 2248, RIA 1087, RIA 552, UNIQEM 194, VKM Ac-880.

Sequence accession no. (16S rRNA gene): AB184169.

Further comments: Streptomyces roseoverticillatus (Shinobu 1956) Witt and Stackebrandt 1991 is an earlier synonym of *Streptomyces baldaccii* corrig. (Farina and Locci 1966) Witt and Stackebrandt 1991, an earlier synonym of *Streptomyces biverticillatus* (Preobrazhenskaya and Ryabova 1957) Witt and Stackebrandt 1991, and an earlier synonym of *Streptomyces fervens* (DeBoer et al. 1959–1960) Witt and Stackebrandt 1991.

Labeda (1996) proposes *Streptomyces biverticillatus* (Preobrazhenskaya and Ryabova 1957) Witt and Stackebrandt 1991, *Streptomyces fervens* (DeBoer et al. 1959–1960) Witt and Stackebrandt 1991, and *Streptomyces roseoverticillatus* (Shinobu 1956) Witt and Stackebrandt 1991 as later synonyms of *Streptomyces baldaccii* corrig. (Farina and Locci 1966) Witt and Stackebrandt 1991. This proposal is in violation of Rule 24b(1) of the *Bacteriological Code* (1990 Revision) because the senior epithet is *roseoverticillatus*. *Streptomyces baldaccii*, *Streptomyces biverticillatus*, and *Streptomyces fervens* are therefore to be regarded as later synonyms of *Streptomyces roseoverticillatus*.

According to Hatano et al. (2003), *Streptomyces roseoverticillatus* (Shinobu 1956) Witt and Stackebrandt 1991 is a later heterotypic synonym of *Streptomyces hiroshimensis* (Shinobu 1955) Witt and Stackebrandt 1991.

420. **Streptomyces roseoviolaceus** (Sveshnikova *in* Gauze, Preobrazhenskaya, Kudrina, Blinov, Ryabova and Sveshnikova 1957) Pridham, Hesseltine and Benedict 1958, 68[AL] ("*Actinomyces roseoviolaceus*" Sveshnikova *in* Gauze, Preobrazhenskaya, Kudrina, Blinov, Ryabova and Sveshnikova 1957, 67)

ro.se.o.vi.o.la′ce.us. L. adj. *roseus* rosy; L. adj. *violaceus* violet colored; N.L. adj. *roseoviolaceus* rosy, violet colored, referring to color of aerial mycelium, vegetative mycelium, and diffusible pigment.

Spore chains in Section *Spirales*. Mature spore chains are moderately long with 10 to 50 or more spores per chain.

This morphology is seen on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar. Spore surface is spiny.

Color of colony: aerial mass color in the Red color series on oatmeal agar and salts-starch agar; Red or Violet color series on yeast-malt agar and glycerol-asparagine agar. The most representative color-tabs in the Red color series are 5cb or 5ec, grayish yellowish pink and the most representative tab in the Violet color series is 11ca, very pale purple. Reverse side of colony is purplish pink or purplish red on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar. Reverse mycelium pigment is a pH indicator changing from red or pink to violet or blue-violet with addition of 0.05 M NaOH and violet to red or pinkish orange with addition of 0.05 M HCl.

Color in medium: melanoid pigments are formed in peptone-yeast-iron agar, tyrosine agar, and tryptone-yeast broth. Red to violet pigment is found in the medium in yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar. This pigment is pH-sensitive, showing the same changes noted for the reverse mycelium pigment.

D-Glucose, L-arabinose, D-xylose, iso-inositol, D-mannitol, D-fructose, rhamnose, sucrose, and raffinose are all utilized for growth.

Type strain shows the highest sequence similarity to: *S. janthinus*, AB184851, 100%; *S. violaceus*, AB184315, 100%; *S. albosporeus* subsp. *albosporeus*, AJ781327, 100%; *S. luteogriseus*, AB184379, 99.5%; *S. lomondensis*, AB184673, 99.3%; *S. hawaiiensis*, AB184143, 99.2%; *S. flavoviridis*, AB184842, 99.2%; *S. pilosus*, AB184161, 99.2%; *S. arenae*, AB249977, 99.2%; *S. africanus*, AY208912, 99.2%; *S. tuirus*, AB184690, 99.2%; *S. bellus*, AB184849, 99%; *S. mutabilis*, EF178679, 99%; *S. massasporeus*, AB184152, 99%; *S. afghaniensis*, AJ399483, 99%; *S. levis*, AB184670, 99%; *S. coerulescens*, AY999720, 99%; *S. parvulus*, AB184326, 99%; *S. coeruleorubidus*, AY999719, 99%.

Source: not known.

DNA G+C content (mol%): not known.

Type strain: 1020/54, ATCC 25493, CBS 953.69, NBRC 13081, JCM 4513, KCC S-0513, NRRL B-12177, NRRL-ISP 5277, RIA 1273.

Sequence accession no. (16S rRNA gene): AJ399484.

421. **Streptomyces roseoviridis** (Preobrazhenskaya *in* Gauze, Preobrazhenskaya, Kudrina, Blinov, Ryabova and Sveshnikova 1957) Pridham, Hesseltine and Benedict 1958, 61[AL] ("*Actinomyces roseoviridis*" Preobrazhenskaya *in* Gauze, Preobrazhenskaya, Kudrina, Blinov, Ryabova and Sveshnikova 1957, 57)

ro.se.o.vi′ri.dis. L. adj. *roseus* rosy; L. adj. *viridis* green; N.L. masc. adj. *roseoviridis* rosy green, referring to the rosy aerial mycelium and green vegetative mycelium and diffusible pigment.

Spore chains in Section *Rectiflexibiles*. Mature spore chains generally 10–50 spores per chain. This morphology is seen on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar. Spore surface is smooth.

Color of colony: aerial mass color in the Red or Yellow color series on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar. Reverse side of colony with no distinctive pigment on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar.

Color in medium: melanoid pigments are formed in peptone-yeast-iron agar, tyrosine agar, and tryptone-yeast broth. Pigments other than melanoids are not formed in yeast-malt agar, oatmeal agar, salts-starch agar, or glycerol-asparagine agar.

D-Glucose, L-arabinose, and D-xylose are utilized for growth. No growth or only trace of growth on sucrose, iso-inositol, D-mannitol, rhamnose, and raffinose. Utilization of D-fructose is doubtful.

Type strain shows the highest sequence similarity to: *S. viridobrunneus*, AJ781372, 99.4%; *S. showdoensis*, AB184389, 99.4%; *S. nashvillensis*, AB184286, 99.1%; *S. tanashiensis*, AJ781362, 99.1%; *S. filamentosus*, AB184130, 99%.

Source: not known.

DNA G+C content (mol%): not known.

Type strain: ATCC 23959, CBS 942.68, BCRC 13779, DSM 40175, NBRC 12911, INA 3617, JCM 4414, NCIMB 13012, NRRL B-2730, NRRL-ISP 5175, RIA 1135, VKM Ac-943.

Sequence accession no. (16S rRNA gene): AB184239.

422. **Streptomyces ruber** (Shirling and Gottlieb 1972) Goodfellow, Williams and Alderson 1986a, 575[AL] (Effective publication: Goodfellow, Willams and Alderson 1986d, 58.) (*Chainia rubra* Shirling and Gottlieb 1972, 347.)

ru′ber. L. masc. adj. *ruber* red.

Forms extensively branched substrate and aerial mycelium. Spiral spore chains containing 10 to over 50 spores are borne on the aerial mycelium. Spore surface is smooth or bears very short spines. Aerial spore mass is white or red and the substrate mycelium red. Sclerotia are formed in 14–21 d on agar medium. Melanin pigments are not produced. Xanthine is degraded but urea is not. Nitrate reductase is not formed. L-Arabinose, D-fructose, D-glucose, D-mannitol, rhamnose, and D-xylose are used as sole carbon sources, but raffinose is not utilized. Acid is formed from D-lactose, D-mannitol, melibiose, methyl α-D-glucoside, L-rhamnose, sucrose, and D-xylose but not from adonitol, dulcitol, *meso*-erythritol, *myo*-inositol, raffinose, or D-sorbitol. Grows at 42°C but not at 10°C. The organism contains major amounts of hexa- and octa-hydrogenated menaquinones with nine isoprene units (Collins et al., 1984).

Type strain shows the highest sequence similarity to: *S. roseiscleroticus*, AB184251, 100%; *S. poonensis*, DQ445792, 99.1%; *S. spiralis*, EF178683, 99%.

Source: not known.

DNA G+C content (mol%): not known.

Type strain: ATCC 17754, CBS 228.65, BCRC 12358, CMI 112789, DSM 40304, NBRC 14600, JCM 3131, KCC A-0131, NCIB (now NCIMB) 10983, NRRL B-5315, NRRL-ISP 5304.

Sequence accession no. (16S rRNA gene): AB184604.

423. **Streptomyces rubidus** Xu, Wang, Cui, Huang, Liu, Zheng and Goodfellow 2006, 1113[VP]

ru′bi.dus. L. masc. adj. *rubidus* dark red.

Neutrotolerant, acidophilic streptomycete that forms branched substrate and aerial hyphae. Smooth-surfaced

spores are borne in flexuous spore chains. Mahogany-colored substrate mycelium, sparse aerial hyphae and diffusible pigments are formed on oatmeal agar and yeast extract-malt extract agar. Melanin pigments are not produced on tyrosine agar or peptone-yeast extract-iron agar. Starch and Tween 80 are degraded, but adenine, guanine, and xanthine are not. Cellobiose, D-galactose, D-glucose, D-lactose, D-mannitol, D-salicin (each at 1%, w/v), L-arginine, and sodium citrate (0.1%, w/v) are used as sole carbon sources for energy and growth, but adonitol, inulin, D-sorbitol (each at 1%, w/v), adipic acid, L-alanine, DL-aminobutyric acid, α-L-aspartic acid, L-cysteine, L-phenylalanine, sodium acetate, sodium oxalate, and L-valine (each at 0.1%, w/v) are not. L-Alanine is used as a sole carbon and nitrogen source, but L-arginine, α-L-aspartic acid, L-glutamic acid, L-isoleucine, and L-phenylalanine (each at 0.1%, w/v) are not. Growth occurs at temperatures between 20 and 37°C, but not at 15°C, and at pH values from 4.5 to7.0, but not at pH 3.5. Does not grow in the presence of 5% (w/v) NaCl. Sensitive to filter-paper discs soaked in the following (μg/ml unless indicated): azithromycin (30), azetreonam (30), carbenicillin (10), cephalothin (30), ciprofloxacin (5), doxycycline hydrochloride (30), erythromycin (15), josamycin (15), kanamycin sulfate (30), minocycline hydrochloride (30), neomycin sulfate (30), streptomycin sulfate (10), tetracycline hydrochloride (30), and tobramycin sulfate (10), but resistant to filter-paper discs soaked in acetylspiramycin (15), amoxycillin (10), ampicillin (10),ofloxacin (5), penicillin G (10 IU/ml), rifampin (5), and sulfamethoxazole (25). Chemotaxonomic properties are typical of the genus *Streptomyces*.

Type strain shows no sequence similarity over 99%. Type strain shows DNA–DNA similarity to: *S. yeochonensis* NRRL B-24245^T, 43.0%; *S. guanduensis* JCM 13274^T, 21.4%; *S. paucisporeus* JCM 13276^T, 19.9%; *S. yanglinensis* JCM 13275^T, 23.8%.

Source: the type strain was isolated from a pine-forest Yanglin, Yunnan Province, People's Republic of China.

DNA G+C content (mol%): 70.6.

Type strain: 13c15, CGMCC 4.2026, JCM 13277.

Sequence accession no. (16S rRNA gene): AY876941.

424. **Streptomyces rubiginosohelvolus** (Kudrina *in* Gauze, Preobrazhenskaya, Kudrina, Blinov, Ryabova and Sveshnikova 1957) Pridham, Hesseltine and Benedict 1958, 59^{AL} ("*Actinomyces rubiginosohelvolus*" Kudrina *in* Gauze, Preobrazhenskaya, Kudrina, Blinov, Ryabova and Sveshnikova 1957, 89)

ru.bi.gi.no.so.hel′vo.lus. L. masc. adj. *rubiginosus* rusty; L. adj. *helvolus* pale yellow, yellowish; N.L. masc. adj. *rubiginosohelvolus* rusty, yellowish.

Spore chains in Section *Rectiflexibiles*. Aerial mycelium is sometimes poorly developed on ISP media. Mature spore chains, when formed, contain 10 to 50 or more spores per chain on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar. Spore surface is smooth.

Color of colony: aerial mass color in the Yellow color series on yeast-malt agar, salts-starch agar, and glycerol-asparagine agar. Aerial mycelium is poorly developed on oatmeal agar. One observer also recorded tabs 2ca (pale yellow) and 3ca (pale orange yellow) from the Red color series as a second color on yeast-malt agar and glycerol-asparagine agar. Reverse side of colony is colorless to pale yellow or grayed yellow on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar. However, yellowish reverse pigment is a pH indicator changing from yellow to pink with addition 0.05 M NaOH.

Color in medium: melanoid pigments not formed in peptone-yeast-iron agar, tyrosine agar, or tryptone-yeast broth. No pigments or only trace of yellow pigment found in yeast-malt agar, oatmeal agar, salts-starch agar, or glycerol-asparagine agar.

D-Glucose, L-arabinose, D-xylose, D-mannitol, D-fructose, and rhamnose are utilized for growth. No growth or only trace of growth on sucrose, iso-inositol, and raffinose.

Type strain shows the highest sequence similarity to: *S. sindenensis*, AB184759, 100%; *S. albovinaceus*, AB249958, 100%; *S. griseoplanus*, AY999894, 100%; *S. anulatus*, DQ026637, 100%; *S. globisporus* subsp. *globisporus*, EF178686, 100%; *S. pluricolorescens*, DQ442540, 100%; *S. praecox*, AB184293, 100%; *S. flavofuscus*, AB249935, 100%; *S. fimicarius*, AY999784, 100%; *S. mediolani*, AB184674, 100%; *S. badius*, AY999783, 100%; *S. griseinus*, AB184205, 100%; *S. californicus*, AB184755, 99.9%; *S. lavendulae* subsp. *lavendulae*, AB184080, 99.9%; *S. cavourensis* subsp. *washingtonensis*, DQ026671, 99.9%; *S. acrimycini*, AY999889, 99.9%; *S. parvus*, DQ442537, 99.9%; *S. fulvorobeus*, AB184711, 99.8%; *S. lipmanii*, AB184148, 99.8%; *S. microflavus*, DQ445795, 99.8%; *S. cyaneofuscatus*, AB184860, 99.8%; *S. floridae*, AB184656, 99.8%; *S. alboviridis*, AB184256, 99.8%; *S. baarnensis*, EF178688, 99.8%; *S. cinereorectus*, AB184646, 99.8%; *S. griseus* subsp. *griseus*, AY207604, 99.7%; *S. griseolus*, AB184768, 99.7%; *S. luridiscabiei*, AF361784, 99.7%; *S. flavovirens*, DQ026635, 99.7%; *S. argenteolus*, AB045872, 99.7%; *S. bacillaris*, AB184439, 99.6%; *S. pulveraceus*, AB184806, 99.6%; *S. halstedii*, EF178695, 99.6%; *S. flavogriseus*, AJ494864, 99.6%; *S. atroolivaceus*, AJ781320, 99.5%; *S. olivoviridis*, AB184227, 99.5%; *S. finlayi*, AY999788, 99.5%; *S. nitrosporeus*, EF178680, 99.5%; *S. albolongus*, AB184425, 99.4%; *S. clavifer*, DQ026670, 99.4%; *S. griseobrunneus*, AB249912, 99.4%; *S. sanglieri*, AB249945, 99.4%; *S. cellulоflavus*, AB184476, 99.4%; *S. yanii*, AB006159, 99.4%; *S. gelaticus*, DQ026636, 99.3%; *S. atratus*, DQ026638, 99.3%; *S. mutomycini*, AB249951, 99.2%; *S. candidus*, DQ026663, 99.2%; *S. cavourensis* subsp. *cavourensis*, DQ445791, 99.2%; *S. spiroverticillatus*, AB184814, 99.1%; *S. cremeus*, AB184124, 99.1%.

Source: not known.

DNA G+C content (mol%): not known.

Type strain: ATCC 19926, ATCC 23960, CBS 943.68, BCRC 13780, DSM 40176, NBRC 12912, INA 10/53, JCM 4415, NRRL B-5425, NRRL-ISP 5176, RIA 1136, VKM Ac-1072.

Sequence accession no. (16S rRNA gene): AB184240.

425. **Streptomyces rubiginosus** (Preobrazhenskaya, Blinov and Ryabova *in* Gauze, Preobrazhenskaya, Kudrina, Blinov, Ryabova and Sveshnikova 1957) Pridham, Hesseltine and Benedict 1958, 70^{AL} ("*Actinomyces rubiginosus*"

Preobrazhenskaya, Blinov and Ryabova *in* Gauze, Preobrazhenskaya, Kudrina, Blinov, Ryabova and Sveshnikova 1957, 134)

ru.bi.gi.no′sus. L. masc. adj. *rubiginosus* rusty, referring to the red-gray-brown color of vegetative mycelium.

Spore chains in Section *Spirales*. Spirals are best developed on salts-starch agar. Aerial mycelium and spore chains may be poorly developed and atypical on yeast-malt agar, oatmeal agar, and glycerol-asparagine agar. Mature spore chains generally have 10–50 spores per chain. Spore surface is spiny.

Color of colony: aerial mass color in the Gray color series on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar. Reverse side of colony with no distinctive pigment (yellowish brown, grayed orange brown or yellowish gray) on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar.

Color in medium: melanoid pigments not formed in peptone-yeast-iron agar, tyrosine agar, or tryptone-yeast broth. No pigment found in medium in yeast-malt agar, salts-starch agar, or glycerol-asparagine agar. There may be a trace of red soluble pigment in oatmeal agar; this pigment is not pH-sensitive.

D-Glucose, sucrose, D-xylose, iso-inositol, D-mannitol, D-fructose, and rhamnose are utilized for growth. Growth on sucrose is less abundant than on other carbon sources. No growth or only trace of growth on raffinose.

Type strain shows the highest sequence similarity to: *S. pseudogriseolus*, DQ442541, 99.4%; *S. gancidicus*, AB184660, 99.4%; *S. capillispiralis*, AB184577, 99.3%; *S. cellulosae*, DQ442495, 99.2%.

Source: not known.

DNA G+C content (mol%): not known.

Type strain: ATCC 19927, ATCC 23961, CBS 944.68, DSM 40177, NBRC 12913, INA 11852, JCM 4416, KCTC 9042, NRRL B-3983, NRRL-ISP 5177, RIA 1137, VKM Ac-1089.

Sequence accession no. (16S rRNA gene): AY999810.

426. **Streptomyces rubrogriseus** (*ex* Kurylowicz et al. *in* Prauser 1970) Terekhova 1986, 575[VP] (Effective publication: Terekhova *in* Gause, Preobrazhenskaya, Sveshnikova, Terekhova and Maximova 1983.) ("*Actinomyces rubrogriseus*" Kurylowicz et al. *in* Prauser 1970)

Synonym: "*Actinomyces rubrogriseus*" Kurylowicz *et al. in* Prauser (1970).

ru.bro.gri′se.us. L. adj. *ruber -bra -brum* red; N.L. adj. *griseus* gray; N.L. masc. adj. *rubrogriseus* red, gray.

Spore chains are spiral (*Spirales*); spores are smooth. On mineral agar 1, oatmeal agar: aerial mycelium is light gray, sometimes pink; substrate mycelium is red; no diffusible pigment. On glycerol-nitrate agar, starch ammonia agar, glycerol-asparagine agar: aerial mycelium is white to light gray; substrate mycelium is yellowish to red, raspberry red; no diffusible pigment. On organic agar 2: aerial mycelium is white to light gray; substrate mycelium is yellowish red to raspberry red; no diffusible pigment. Melanoid pigments are not formed. Antibiotic: prodigiosin-pigment; streptothricin.

Type strain shows the highest sequence similarity to: *S. anthocyanicus*, AB184631, 99.9%; *S. lienomycini*, AJ781353, 99.9%; *S. tricolor*, AB184687, 99.9%; *S. humiferus*, AF503491, 99.7%; *S. coelescens*, AF503496, 99.7%; *S. violaceorubidus*, AJ781374, 99.7%; *S. violaceolatus* AF503497, 99.7%; *S. violaceoruber*, AF503492, 99.7%; *S. tendae*, D63873, 99.6%; *S. coelicoflavus*, AB184650, 99.3%; *S. olivaceus*, AB184743, 99.2%; *S. pactum*, AB184398, 99.2%; *S. ambofaciens*, M27245, 99%; *S. mutabilis*, EF178679, 99%; *S. matensis*, AB184221, 99%; *S. malachitospinus*, AB249954, 99%.

Source: not known.

DNA G+C content (mol%): not known.

Type strain: ATCC 43691, DSM 41477, NBRC 15455, INA 2626, JCM 6927, VKM Ac-1216.

Sequence accession no. (16S rRNA gene): AB184681.

427a. **Streptomyces rutgersensis subsp. rutgersensis** (Waksman and Curtis 1916) Waksman and Henrici *in* Breed, Murray and Hitchens 1948, 952[AL] ("*Actinomyces rutgersensis*" Waksman and Curtis 1916, 123)

rut.gers.en′sis. N.L. masc. adj. *rutgersensis* of or belonging to Rutgers; named for Rutgers University, New Brunswick, New Jersey, USA.

Spore chains in Section *Rectiflexibiles* to *Retinaculiaperti*. Short spore chains on this strain are irregularly bent or turned and are not typical of either *Rectiflexibiles* or *Retinaculiaperti* cultures [the characterization of Waksman and Curtis (1916) describes spiral spore chains; atypical spirals are now very rare and reported by only one observer]. Usually only 3–10 spores per chain on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar. Spore surface is smooth.

Color of colony: aerial mass color in the Yellow color series on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar. Reverse side of colony is grayed yellow modified by green on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar; substrate color is not a pH indicator.

Color in medium: melanoid pigments not formed in peptone-yeast-iron agar, tyrosine agar, and tryptone-yeast extract broth. Pigments not formed in significant amounts in yeast-malt agar, oatmeal agar, salts-starch agar, or glycerol-asparagine agar.

D-Glucose, L-arabinose, D-xylose, D-mannitol, and D-fructose are utilized for growth. No growth or only trace of growth on sucrose, iso-inositol, rhamnose, and raffinose.

Type strain shows the highest sequence similarity to: *S. diastaticus* subsp. *diastaticus*, AB184785, 100%; *S. gougerotii*, AB184742, 100%; *S. intermedius*, AB184277, 99.8%; *S. misionensis*, EF178678, 99.2%; *S. phaeoluteichromatogenes*, AJ391814, 99.1%; *S. matensis*, AB184221, 99%; *S. aureoverticillatus*, AY999774, 99%.

Source: not known.

DNA G+C content (mol%): not known.

Type strain: ATCC 19809, ATCC 3350, CBS 562.68, BCRC 13670, BCRC 13701, DSM 40077, HAMBI 1038, HUT 6069, IFM 1033, NBRC 12819, NBRC 3419, NBRC 3727, IMET 43501, JCM 4082, JCM 4608, NRRL B-1256, NRRL B-2102, NRRL-ISP 5077, RIA 1089, RIA 410, UNIQEM 196, VKM Ac-1877.

Sequence accession no. (16S rRNA gene): AB184170.

427b. **Streptomyces rutgersensis subsp. castelarensis** corrig. Cercós 1954, 263[AL]

cas.te.lar.en′sis. N.L. masc. adj. *castelarensis* of or belonging to Castelar, Argentina, South America, the source of the organism (from dust).

Spores are short, cylindrical, phalangiform, and may appear roughened. Probably grows well on Czapek's solution agar. Produces camphomycin (two components); NaCl tolerance >4%, but <7%.

Type strain shows the highest sequence similarity to: *Streptomyces sporoclivatus*, AB249934, 99.8; *Streptomyces antimycoticus*, AB184185, 99.8; *Streptomyces geldanamycininus*, DQ334781, 99.6; *Streptomyces melanosporofaciens*, AJ271887, 99.4; *Streptomyces asiaticus*, AB249947, 99.1; *Streptomyces rhizosphaericus*, AB249941, 99.1%.

Source: isolated from dust from Castelar, Argentina, South America.

DNA G+C content (mol%): not known.

Type strain: ATCC 15191, CBS 309.55, BCRC 11879, DSM 40830, NBRC 15875, INA R-43, JCM 4978, RIA 851, VKM Ac-832.

Sequence accession no. (16S rRNA gene): AY508511.

Further comments: the original spelling, *Streptomyces rutgersensis* subsp. *castelarense* (*sic*), has been corrected by Hill et al. (1984).

428. **Streptomyces salmonis** (Baldacci, Farina and Locci 1966) Witt and Stackebrandt 1996, 836[VP] (Effective publication: Witt and Stackebrandt 1990, 370.) ["*Streptoverticillium salmonicida*" (Rucker 1949) Baldacci, Farina and Locci 1966, 164; *Streptoverticillium salmonis* Locci, Baldacci and Petrolini Baldan 1969, 27]

sal′mo.nis. L. gen. n. *salmonis* of a salmon.

Good growth on potato-glucose agar (Baldacci et al., 1954). Color: reverse, brick red; aerial mycelium, yellowish white; traces of soluble pigment. On Bacto Czapek agar: traces of growth only. On Casamino acids-Czapek agar (1 g/l Difco vitamin-free Casamino acids, replacing sodium nitrate): very poor, colorless growth. On glucose-asparagine agar (ISP medium 5 with 1% glucose replacing glycerol): good growth. Color: reverse, orange-yellow; aerial mycelium, white with traces of pink and yellow. On glucose-asparagine agar: good growth. Color: reverse orange-yellow; aerial mycelium, whitish with shades of yellow. On yeast extract-malt extract agar: good growth. Color: reverse, brick red; aerial mycelium, yellowish white. On Bacto Emerson agar: good growth. Color: reverse, brick red; aerial mycelium, dirty white; brown soluble pigment. On Bennett agar (1% glucose, 0.1% Bacto beef agar, 0.1% yeast extract, 0.2% peptone, 1.5% agar): good growth. Color: reverse, dirty red; aerial mycelium, dirty white. On Oxoid nutrient agar: medium growth. Color: reverse, brown; aerial mycelium, poor pink; brown soluble pigment. The strain grows also at 37°C, very poor or no aerial mycelium being formed. No growth at 45°C. The type strain exhibits anti-bacterial and anti-fungal activity.

For sequence similarity, see type strain of *Streptomyces hiroshimensis*.

Source: not known.

DNA G+C content (mol%): not known.

Type strain: BCRC 12456, DSM 40895, HUT 6085, NBRC 15865, JCM 4083, NRRL B-1472, VKM Ac-944.

Sequence accession no. (16S rRNA gene): X53169.

Further comments: according to Hatano et al. (2003), *Streptomyces salmonis* (Baldacci et al. 1966) Witt and Stackebrandt 1991 is a later heterotypic synonym of *Streptomyces hiroshimensis* (Shinobu 1955) Witt and Stackebrandt 1991.

429. **Streptomyces sampsonii** (Millard and Burr 1926) Waksman *in* Waksman and Lechevalier 1953, 155[AL] ("*Actinomyces sampsonii*" Millard and Burr 1926, 614)

samp.so′ni.i. N.L. gen. masc. n. *sampsonii* of Sampson, a patronymic.

Spore chains in Section *Rectiflexibiles*. Mature spore chains are generally long and flexuous with more than 50 spores per chain. This morphology is seen on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar. Spore surface is smooth.

Color of colony: aerial mass color in the Yellow color series (1ba-2ba, pale yellow, or 1db, pale yellowish green) on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar; or sometimes in the Gray color series (2dc, yellowish gray) on yeast-malt agar and salts-starch agar. Reverse side of colony with no distinctive pigments (pale yellow to light olive brown on salts-starch agar and glycerol-asparagine agar; strong yellow to orange-yellow or yellowish brown on yeast-malt agar and oatmeal agar).

Color in medium: melanoid pigments are not formed in peptone-yeast-iron agar, tyrosine agar, or tryptone-yeast broth. No pigment is found in medium in yeast-malt agar, oatmeal agar, salts-starch agar, or glycerol-asparagine agar.

D-Glucose, L-arabinose, D-xylose, D-mannitol, and D-fructose are utilized for growth. No growth or only trace of growth with iso-inositol, rhamnose, sucrose, and raffinose.

Type strain shows the highest sequence similarity to: *S. albidoflavus*, AB184255, 99.9%; *S. odorifer*, Z76682, 99.9%; *S. daghestanicus*, DQ442497, 99.8%; *S. felleus*, AB184129, 99.8%; *S. hydrogenans*, AB184868, 99.8%; *S. violascens*, AY999737, 99.8%; *S. limosus*, AB184147, 99.8%; *S. griseus* subsp. *solvifaciens*, AB249915, 99.7%; *S. canescens*, AB184117, 99.7%; *S. koyangensis*, AY079156, 99.5%; *S. champavatii*, DQ026642, 99.3%.

Source: not known.

DNA G+C content (mol%): not known.

Type strain: ATCC 25495, CBS 955.69, BCRC 13705, DSM 40394, NBRC 13083, JCM 4515, NRRL B-12325, NRRL-ISP 5394, RIA 1275, VKM Ac-851.

Sequence accession no. (16S rRNA gene): D63871.

430. **Streptomyces sanglieri** Manfio, Atalan, Zakrzewska-Czerwinska, Mordarski, Rodríguez, Collins and Goodfellow 2003b, 1219[VP] (Effective publication: Manfio, Atalan, Zakrzewska-Czerwinska, Mordarski, Rodríguez, Collins and Goodfellow 2003a, 252.)

san.gli.e′ri. N.L. gen. masc. n. *sanglieri* of Sanglier, named in honor of Jean-Jacques Sanglier, a Belgian biotechnologist.

Aerobe, Gram-stain-positive, mesophilic actinomycetes which form extensively branched substrate and aerial hyphae. Spiral and open loops of smooth-surfaced spores are evident on aerial hyphae. A gray aerial spore mass and a reddish-orange substrate mycelium are formed on modified Bennett's, glycerol-asparagine, oatmeal, and yeast extract-malt extract agars; a reddish-orange pigment is produced on oatmeal agar. Hippurate is hydrolyzed and nitrate is reduced. Cleaves methoxysuccinyl-L-alanine-L-lysine-7AMC, L-citrulline-7AMC, *N*-acetyl-L-phenylalanine-L-arginine-7AMC (endopeptidase substrates), L-cysteine-7AMC (exopeptidase substrates), 4MU-*N*-acetyl-β-D-glucosaminde, 4MU-2-deoxy-β-D-glucopyranoside and 4MU-β-D-xyloside (glycosides), and 4MU-oleate (organic ester), but not L-phenylalanine-7AMC (endopeptidase substrate), L-glutamine-7AMC (exopeptidase substrates), 4MU-α-D-mannopyranoside (glycoside), or 4MU-elaidate (organic ester). L-Phenylalanine is used as sole carbon and nitrogen source but not L-histidine, L-hydroxyproline, or potassium nitrate. Does not use dextran, *myo*-inositol, inulin, mannitol, or pyruvate as sole sources of carbon for energy and growth. Grows between 10 and 35°C, but not at 45°C, or in the presence of sodium azide (0.01%, w/v), sodium chloride (7.0%, w/v), or neomycin sulfate (50 μg/ml) but is sensitive to oleandomycin phosphate (100 μg/ml).

Type strain shows the highest sequence similarity to: *S. atratus*, DQ026638, 100%; *S. yanii*, AB006159, 99.7%; *S. pulveraceus*, AB184806, 99.7%; *S. gelaticus*, DQ026636, 99.5%; *S. badius*, AY999783, 99.4%; *S. pluricolorescens*, DQ442540, 99.4%; *S. sindenensis*, AB184759, 99.4%; *S. mediolani*, AB184674, 99.4%; *S. griseinus*, AB184205, 99.4%; *S. rubiginosohelvolus*, AB184240, 99.4%; *S. albovinaceus*, AB249958, 99.3%; *S. globisporus* subsp. *globisporus*, EF178686, 99.3%; *S. flavofuscus*, AB249935, 99.3%; *S. fimicarius*, AY999784, 99.3%; *S. anulatus*, DQ026637, 99.3%; *S. praecox*, AB184293, 99.3%; *S. lavendulae* subsp. *lavendulae*, AB184080, 99.3%; *S. argenteolus*, AB045872, 99.3%; *S. parvus*, DQ442537, 99.3%; *S. cinereorectus*, AB184646, 99.3%; *S. griseoplanus*, AY999894, 99.3%; *S. californicus*, AB184755, 99.3%; *S. cavourensis* subsp. *washingtonensis*, DQ026671, 99.3%; *S. alboviridis*, AB184256, 99.2%; *S. griseolus*, AB184768, 99.2%; *S. baarnensis*, EF178688, 99.2%; *S. lipmanii*, AB184148, 99.2%; *S. cyaneofuscatus*, AB184860, 99.2%; *S. fulvorobeus*, AB184711, 99.2%; *S. acrimycini*, AY999889, 99.2%; *S. flavovirens*, DQ026635, 99.2%; *S. microflavus*, DQ445795, 99.2%; *S. floridae*, AB184656, 99.2%; *S. halstedii*, EF178695, 99.1%; *S. flavogriseus*, AJ494864, 99.1%; *S. luridiscabiei*, AF361784, 99.1%; *S. nitrosporeus*, EF178680, 99.1%; *S. griseus* subsp. *griseus*, AY207604, 99%; *S. bacillaris*, AB184439, 99%.

Source: isolated from soil taken from meadow hay plots.

DNA G+C content (mol%): 70–76.

Type strain: A46R51, DSM 41791, JCM 12607, NBRC 100784, NCIMB 13929.

Sequence accession no. (16S rRNA gene): AB249945.

431. **Streptomyces sannanensis** Iwasaki, Itoh and Mori 1981, 283[VP]

san.nan.en′sis. N.L. masc. adj. *sannanensis* of or pertaining to Sannan, a town in Japan.

Spore chains in Section *Spirales*. Mature spore chains are generally moderately short, often with 20 or more spores per chain. Spore surface is smooth.

Color of colony: aerial mass color in the gray color series on sucrose-nitrate agar, glucose-asparagine agar, inorganic salts-starch agar, and oatmeal agar. Reverse side of colony with no distinctive pigments (colorless to buff in color) on all media. Diffusible pigment: melanoid pigments are not formed in peptone-yeast extract-iron agar, tyrosine agar, or tryptone-yeast extract broth. No pigment other than a trace of yellow is produced in the media used. Starch is hydrolyzed and gelatin is liquefied. Does not grow in skim milk medium. Melanoid pigment is not produced. No growth or only traces of growth with L-arabinose, D-xylose, D-glucose, D-fructose, sucrose, iso-inositol, L-rhamnose, raffinose, or D-mannitol with Pridham and Gottlieb's basal mineral salts agar. On $CuSO_4$-free Pridham and Gottlieb's basal mineral salts agar and Czapek's solution agar, D-xylose and D-glucose are utilized for growth; D-glucose gives only faint growth. Mesophilic (growth range is from 17–37°C; temperature for optimum growth is between 27 and 34°C). Cell-wall composition: LL-A_2pm occurs in whole-cell hydrolysates. The cell-wall composition is believed to be of Type I. Produces sannamycins.

Type strain shows the highest sequence similarity to: *S. mauvecolor*, AB184532, 99%.

Source: not known.

DNA G+C content (mol%): not known.

Type strain: KC-7038, ATCC 31530, DSM 41705, NBRC 14239, JCM 9651, KCTC 9770.

Sequence accession no. (16S rRNA gene): AB184579.

432. **Streptomyces sapporonensis** (Locci 1985) Witt and Stackebrandt 1996, 836[VP] (Effective publication: Witt and Stackebrandt 1990, 370.) ["*Streptomyces sapporonensis*" Sakai and Miyoshi 1972; "*Streptoverticillium sapporonense*" (*ex* Arai 1976) Locci 1985, 232]

sap.po.ro.nen′sis. N.L. masc. adj. *sapporoensis* of or pertaining to Sapporo, a town in Japan.

Reddish spore mass, aerial mycelium is cottony. Melanin pigment is not produced. Acid is produced from D-galactose and *myo*-inositol, but not from D-fructose, trehalose, or D-ribose. L-Methionine, shikimic acid, and DL-α-aminobutyric acid are utilized, but not coumarin, mannitol, melibiose, proline, raffinose, or sorbitol. Degrades citrate, but not esculin or L-tyrosine. H_2S production. No NO_3^- reduction. Resistant to carbenicillin (100 μg/ml), cephaloridine (30 μg/ml), cephalotin (30 μg/ml), and colistin (30 μg/ml). *Aspergillus niger* and *Bacillus subtilis* are inhibited, *Candida albicans* is not.

Type strain shows the highest sequence similarity to: *S. hygroscopius* subsp. *angustmyceticus*, DQ442509, 99.5%; *S. varsoviensis*, DQ026653, 99.5%; *S. abikoensis*, AB184537, 99.5%; *S. ehimensis*, AY999834, 99.4%; *S. lilacinus*, AB184819, 99.3%; *S. luteireticuli* AB249969, 99.2%; *S. mobaraensis*, DQ442528, 99.1%; *S. caeruleus*, EF178675, 99.1%; *S. lavenduligriseus*, DQ442515, 99%; *S. hiroshimensis*, AB184789, 99%; *S. thioluteus* AB184753, 99%.

Source: not known.

DNA G+C content (mol%): not known.

Type strain: ATCC 21532, DSM 41675, NBRC 13823, JCM 4934.

Sequence accession no. (16S rRNA gene): AB184508.

Further comments: according to Hatano et al. (2003), *Streptomyces sapporonensis* (Locci and Schofield 1989) Witt and Stackebrandt 1991 is a later heterotypic synonym of *Streptomyces cinnamoneus* (Benedict et al. 1952) Witt and Stackebrandt 1991.

433. **Streptomyces scabiei** corrig. (*ex* Thaxter 1891) Lambert and Loria 1989b, 391[VP] ("*Oospora scabies*" Thaxter 1891; "*Actinomyces scabies*" Güssow 1914; "*Streptomyces scabies*" Waksman and Henrici 1948)

sca′bi.ei. L. gen. n. *scabiei* of mange, in reference to the ability to cause potato scab.

Spore chains in Section *Rectiflexibiles*. Mature spore chains are moderately short with 3–10, or sometimes more than 10, spores per chain. This morphology is seen on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar. Spore surface is smooth.

Color of colony: aerial mass color in the Gray color series on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar. Reverse side of colony is yellow to yellow brown is usually modified by green to light grayish olive, olive, or olive brown on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar. Substrate mycelium pigment is not a pH indicator.

Color in medium: melanoid pigments are not formed in peptone-yeast-iron agar, tyrosine agar, or tryptone-yeast broth. Yellow or greenish yellow pigment may be found in the medium in yeast-malt agar and oatmeal agar, but not in salts-starch agar or glycerol-asparagine agar; this pigment is not pH-sensitive when tested with 0.05 M NaOH or HCl.

D-Glucose, L-arabinose, D-xylose, D-mannitol, D-fructose, and rhamnose are utilized for growth. No growth or only trace of growth with sucrose, iso-inositol, and raffinose.

Type strain shows the highest sequence similarity to: *S. europaeiscabiei*, AY207598, 99.8%.

Source: not known.

DNA G+C content (mol%): not known.

Type strain: RL-34, ATCC 49173, CIP 105438, DSM 41658, ICMP 12542, JCM 7914.

Sequence accession no. (16S rRNA gene): D63862.

Further comments: the original spelling of the specific epithet, *scabies* (*sic*), has been corrected by Trüper and De'Clari (1997).

434. **Streptomyces scabrisporus** Xu, Takahashi, Seino, Iwai and Ōmura 2004b, 580[VP]

sca.bri.spo′rus. L. adj. *scaber -bra -brum* scabby, rough; N.L. n. *spora* spore; N.L. masc. adj. *scabrisporus* referring to the rugose surface of the spores.

Mature spore chains are spiral, with more than 20 spores per chain. Spores are cylindrical in shape, 0.6–0.8 × 1.2–1.6 µm in diameter; the spore surface is rugose. Substrate and aerial mycelia are produced. The reverse sides of colonies are colorless to light ivory. The aerial mass on some agar media, such as water agar, oatmeal agar, and 1/10 V8 juice agar, is gray. Mycelia do not fragment into coccoid or bacillary elements. Melanoid or soluble pigments are not produced on any medium tested. Starch is weakly hydrolyzed, gelatin is not liquefied and milk is weakly coagulated. D-Glucose, D-fructose, D-xylose, *myo*-inositol, and rhamnose are utilized for growth, but D-mannitol, raffinose, and melibiose are not; little if any growth is observed with sucrose, L-arabinose, or salicin. Menaquinone composition is MK-9(H_4), MK-9(H_2), and MK-9(H_6) in the ratio 12:4:3.

Type strain shows no sequence similarity over 99%. Type strain shows DNA–DNA similarity to: *S. chrestomyceticus* ATCC 14947^T, 54%.

Source: the type strain was isolated from a soil sample collected from Ushiku-cho, Ibaraki Prefecture, Japan.

DNA G+C content (mol%): 70.6.

Type strain: KM-4927, JCM 11712, NBRC 100760, NRRL B-24202.

Sequence accession no. (16S rRNA gene): AB030585.

435. **Streptomyces sclerotialus** (Thirumalachar 1955) Pridham 1970, 44[AL] (*Chainia antibiotica* Thirumalachar 1955, 935)

scle.rot.i′a.lus. N.L. neut. n. *sclerotium* sclerotium; N.L. masc. adj. *sclerotialus* pertaining to sclerotia.

Spore chains in Section *Spirales*. Spirals are best developed on salts-starch agar. Aerial mycelium may be poorly developed on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar, but some spirals or chains can usually be found on these media. Spore surface is smooth. One of three observers reports sclerotia and fragmentation of substrate mycelium on glycerol-asparagine agar in 21 d. A second observer reports "globular sporangia" on yeast-malt agar, oatmeal agar, and salts-starch agar and "coremia" on yeast-malt agar in 7–14 d. Scanning electron microscopy shows coalesced masses of spores.

Color of colony: aerial mycelium is generally poorly developed on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar. Sporulating aerial mycelium adequate for color determination may be formed on salts-starch agar; it is in the White color series. One observer reports aerial mycelium in the Yellow color series (2fb, light yellowish red; 1db, pale yellow green) on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar; this may be the color of the vegetative (substrate mycelium) growth. Reverse side of colony with no distinctive pigments (orange yellow to yellowish brown on yeast-malt agar, light grayish yellow or light yellowish brown on oatmeal agar, salts-starch agar, and glycerol-asparagine agar).

Color in medium: melanoid pigments are not formed in peptone-yeast-iron agar, tyrosine agar, or tryptone-yeast broth. No pigment or only a trace of yellow is found in the medium in yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar.

D-Glucose, L-arabinose, D-xylose, iso-inositol, D-mannitol, D-fructose, rhamnose, sucrose, and raffinose are all utilized for growth.

Type strain shows the highest sequence similarity to: *S. rimosus* subsp. *rimosus*, AB045883, 99.4%; *S. olivaceiscleroticus*, AJ621606, 99.2%; *S. niger*, AJ621607, 99.2%; *S. chresto-*

myceticus, AJ621609, 99.1%; *S. rimosus* subsp. *paromomycinus*, AJ621610, 99.1%; *S. erumpens*, AJ621603, 99.1%.

Source: not known.

DNA G+C content (mol%): not known.

Type strain: ATCC 15721, CBS 167.62, BCRC 13307, DSM 43032, IFM 1141, NBRC 12246, NBRC 13356, NBRC 13904, JCM 3039, JCM 4828, KCTC 9065, NRRL B-2317, NRRL-ISP 5269, RIA 1317, VKM Ac-1909.

Sequence accession no. (16S rRNA gene): AJ621608.

Further comments: Streptomyces sclerotialus Pridham 1970 and *Chainia antibiotica* Thirumalachar 1955 have the same type strain and therefore are homotypic synonyms [Rules 24a and 24b(1) of the *Bacteriological Code* (1990 Revision)].

436. **Streptomyces scopiformis** Li, Lanoot, Zhang, Vancanneyt, Swings and Liu 2002a, 1632[VP]

sco.pi.for′mis. L. fem. n. *scopa* a broom; L. adj. suffix *-formis -is -e* (from L. n. *forma* figure, shape, appearance) -like, in the shape of; N.L. masc. adj. *scopiformis* in the form of a broom, referring to the structure of the spore chains.

Rectiflexibiles chains of roundish, spiny-surfaced spores (0.7–0.8 μm) are arranged in fastigiate form. Spore mass is gray or blue-gray, the reverse is blue to gray-blue. Substrate hyphae are extensively branching, septate, and swollen. Diffusible pigment is not formed. Tests for esculin, starch, dextrin, elastin, nitrate reduction, and gelatin are positive and tests for hippurate, cellulose, and lipolysis are negative. Utilizes L-arabinose, D-fructose, D-galactose, D-glucose, D-maltose, L-rhamnose, D-xylose, D-sucrose, dulcitol, *myo*-inositol, melibiose, trehalose, sodium acetate, and sodium citrate as sole carbon sources but not D-mannitol, D-raffinose, adonitol, methyl α-glucoside, iso-erythritol, or inulin. Grows in presence of penicillin G, biomycin, phenol, and ethanol, at 20–45°C and at pH 5–10 but not in the presence of bacteracin, lysozyme, sodium azide, or methyl violet, or at 10 or 50°C or at pH 4.0 or 11.0. Cell-wall type I, phospholipid type PII and menaquinone MK-9($H_{4,6}$) are detected. The fatty acids are type II. Mycolic acids are not present.

Type strain shows no sequence similarity over 99%. Type strain shows DNA–DNA similarity to: *S. ambofaciens* AS 41528^T, 13±1%; *S. coerulescens* JCM 4358^T, 34±3%; *S. caelestis* JCM 218^T, 23±4%; *S. nogalater* JCM 4799^T, 31±4%; *S. intermedius* JCM 4483^T, 26±3%; *S. albidoflavus* JCM 4446^T, 19±6%.

Source: not known.

DNA G+C content (mol%): 71.0.

Type strain: A25, AS 4.1331, DSM 41825, JCM 12114, LMG 20251, NBRC 100244.

Sequence accession no. (16S rRNA gene): AB249927.

437. **Streptomyces seoulensis** Chun, Youn, Yim, Lee, Kim, Hah and Kang 1997, 495[VP]

se.oul.en′sis. N.L. masc. adj. *seoulensis* of or belonging to Seoul, Republic of Korea, the geographical origin of the species.

Long-chain *Rectiflexibiles* spores with smooth surfaces are produced. Forms a yellow substrate mycelium on glycerol-asparagine agar and a gray aerial mycelium and spores on inorganic salts-starch agar. Verticils are not present. Diffusible pigments are not produced on ISP 5 medium. Melanin is not produced on peptone-yeast extract-iron agar and tyrosine agar. It utilizes L-histidine as sole nitrogen source but not DL-α-amino-*n*-butyric acid, L-cysteine, L-valine, L-phenylalanine, L-hydroxyproline, or potassium nitrate. Uses D-fructose, lactose, mannitol, D-melibiose, raffinose, L-rhamnose, salicin, sodium pyruvate, sucrose, and D-glucose as sole sources of carbon, but not adonitol, L-arabinose, dextran, *myo*-inositol, melezitose, sodium acetate, sodium citrate, sodium propionate, or xylitol. Tests for lecithinase, lipolysis, pectin hydrolysis, and H_2S production are positive but not those for hippurate hydrolysis or nitrate reduction. Degrades allantoin, arbutin, and elastin but not guanine, xanthine, or xylan. Grows in the presence of potassium tellurite, rifampin, and penicillin G but not at 45°C or in the presence of sodium chloride, sodium azide, thallous acetate, neomycin, or oleandomycin. The cell wall contains LL-A_2pm and no diagnostic sugars are present in the cell-wall fraction (chemotype I). Phosphatidylethanolamine, phosphatidylinositol, diphosphatidylglycerol, and phosphatidylinositol mannosides are present in the polar lipid fraction (phospholipid type II). The fatty acids are mainly saturated straight-chain as well as iso- and anteiso-branched fatty acids (fatty acid type 2c). Activity is not exhibited against *Aspergillus niger*, *Bacillus subtilis*, *Candida albicans*, *Micrococcus luteus*, *Saccharomyces cerevisiae*, or *Streptomyces murinus*.

Type strain shows the highest sequence similarity to: *S. recifensis*, AB184165, 99.8%; *S. griseoluteus*, AY999751, 99.7%.

Source: isolated from Seoul, Korea.

DNA G+C content (mol%): 68.0.

Type strain: IMSNU-1 (deposited in the Institute of Microbiology, Seoul National University, under accession number IMSNU 21266), CIP 105312, NBRC 16255, NBRC 16668, JCM 10116.

Sequence accession no. (16S rRNA gene): AB249970.

438. **Streptomyces septatus** (Locci, Baldacci and Petrolini Baldan 1969) Witt and Stackebrandt 1991, 456[VP] (Effective publication: Witt and Stackebrandt 1990, 370.) (*Streptoverticillium septatum* Locci, Baldacci and Petrolini Baldan 1969, 59)

sep.ta′tus. L. masc. adj. *septatus* surrounded by a bulwark, fenced.

Spore chains in Section Umbellate Monoverticillate (=*Streptomyces Verticillati*, biverticillate). Mature spore chains are generally short, with 3 to 10 or more spores per chain. This morphology is seen on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar. Spore surface is smooth.

Color of colony: aerial mass color in the Yellow or Red color series on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar (white is also present). Nearest matching color tabs in the Yellow color series are 2db and 2ba, pale yellow, and 1cb, pale yellow green. Nearest matching tabs in the Red color series are 4ec and 5cb, grayish yellowish pink, and 3ca, pale orange yellow. Red aerial mycelium may sometimes occur as scattered tufts of longer aerial hyphae in a yellow or white background.

Reverse side of colony with no distinctive pigments (olive brown to strong brown on yeast-malt agar; pale grayish yellow on oatmeal agar and salts-starch agar; grayish yellow to pale yellowish green on glycerol-asparagine agar).

Color in medium: melanoid pigments are formed in peptone-yeast-iron agar and tryptone-yeast broth, but not in tyrosine agar. No pigment is found in the medium in yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar in 14 d or a trace of yellow may sometimes be seen in yeast-malt agar, oatmeal agar, and salts-starch agar in 21 d.

D-Glucose and iso-inositol are utilized for growth. Reports vary on growth on D-fructose, but utilization of this sugar is doubtful. No growth or only traces of growth with L-arabinose, D-xylose, D-mannitol, rhamnose, sucrose, and raffinose.

For sequence similarity, see type strain of *Streptomyces griseocarneus*.

Source: not known.

DNA G+C content (mol%): not known.

Type strain: ATCC 27464, CBS 772.72, BCRC 11895, CECT 3251, DSM 40577, NBRC 13471, JCM 4547, JCM 4829, NCIMB 12982, NRRL 2974, NRRL-ISP 5577, RIA 1432, VKM Ac-888.

Sequence accession no. (16S rRNA gene): AB184883.

Further comments: according to Hatano et al. (2003), *Streptomyces septatus* (Locci et al. 1969) Witt and Stackebrandt 1991 is a later heterotypic synonym of *Streptomyces griseocarneus* (Benedict et al. 1950) Witt and Stackebrandt 1991.

439. **Streptomyces setonii** (Millard and Burr 1926) Waksman *in* Waksman and Lechevalier 1953, 107[AL] ("*Actinomyces setonii*" Millard and Burr 1926, 604)

se.to'ni.i. N.L. gen. masc. n. *setonii* of Seton, named for a person, Seton (Probably R.S. Seton of the Harris Insitute, Preston, England).

Spore chains in Section *Rectiflexibiles*. Mature spore chains are generally long, often with more than 50 spores per chain. This morphology is seen on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar. Spore surface is smooth.

Color of colony: aerial mass color in the Yellow (2ba, pale yellow) or Gray (2dc, yellowish gray) color series on yeast-malt agar and glycerol-asparagine agar; White color series on oatmeal agar and salts-starch agar. Reverse side of colony with no distinctive pigments (pale or grayish yellow to light yellowish brown) on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar.

Color in medium: melanoid pigments are not formed in peptone-yeast-iron agar, tyrosine agar, or tryptone-yeast broth. No pigment is found in medium in yeast-malt agar, oatmeal agar, salts-starch agar, or glycerol-asparagine agar.

D-Glucose, L-arabinose, D-xylose, D-mannitol, D-fructose, and rhamnose are utilized for growth. No growth or only trace of growth with iso-inositol, sucrose, and raffinose.

For sequence similarity, see type strain of *Streptomyces griseus*.

Source: not known.

DNA G+C content (mol%): not known.

Type strain: ATCC 25497, CBS 105.27, CBS 957.69, CFBP 4549, CIP 105279, DSM 40395, ICMP 12543, NBRC 13085, JCM 4226, JCM 4516, KCTC 9144, NRRL B-2555, NRRL-ISP 5395, RIA 1277.

Sequence accession no. (16S rRNA gene): D63872.

Further comments: according to Liu et al. (2005b), *Streptomyces setonii* (Millard and Burr 1926) Waksman 1953 is a later heterotypic synonym of *Streptomyces griseus* (Krainsky 1914) Waksman and Henrici 1948 emend. Liu et al. 2005b.

440. **Streptomyces showdoensis** Nishimura, Mayama, Komatsu, Kato, Shimaoka and Tanaka 1964, 150[AL]

show.do.en'sis. N.L. masc. adj. *showdoensis* of or belonging to Shodo, an island in Kagawa Prefecture, Japan, the source of the soil from which the organism was isolated.

Spore chains in Section *Rectiflexibiles*. Mature spore chains are generally long, with 10 to 50 or more spores per chain. This morphology is seen on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar. Spore surface is smooth.

Color of colony: aerial mass color in the Gray or Yellow color series on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar. White aerial mycelium may also be seen on these media. Most representative color tabs from the Gray color series are 2dc, yellowish gray, and 3ge, light grayish yellowish brown, and from the Yellow color series, 2db and 2ba, pale yellow. Reverse side of colony with no distinctive pigments (orange yellow or yellowish brown on yeast-malt agar; pale or grayish yellow on oatmeal agar, salts-starch agar, and glycerol-asparagine agar).

Color in medium: melanoid pigments are formed in peptone-yeast-iron agar and tryptone-yeast broth, and weakly in tyrosine agar. No pigment is found in the medium in yeast-malt agar, oatmeal agar, salts-starch agar, or glycerol-asparagine agar.

D-Glucose, D-xylose, and D-fructose are utilized for growth. Utilization of L-arabinose and sucrose is doubtful. No growth or only traces of growth with iso-inositol, D-mannitol, rhamnose, or raffinose.

Type strain shows the highest sequence similarity to: *S. viridobrunneus*, AJ781372, 100%; *S. cinereoruber* subsp. *cinereoruber*, AB184121, 99.5%; *S. roseoviridis*, AB184239, 99.4%; *S. tanashiensis*, AJ781362, 99.1%; *S. nashvillensis*, AB184286, 99.1%; *S. violaceorectus*, AB184314, 99.1%; *S. vietnamensis*, DQ311081, 99%; *S. bikiniensis*, X79851, 99%.

Source: isolated from soil from Shodo, an island in Kagawa Prefecture, Japan.

DNA G+C content (mol%): not known.

Type strain: ATCC 15105, CBS 718.72, BCRC 11868, DSM 40504, NBRC 13417, JCM 4830, NRRL B-12430, NRRL-ISP 5504, RIA 1378, VKM Ac-1219.

Sequence accession no. (16S rRNA gene): AB184389.

441. **Streptomyces sindenensis** Nakazawa and Fujii 1957, 109[AL]

sin.den.en'sis. N.L. masc. adj. *sindenensis* of or belonging to Sinda Village, Osaka Prefecture, Japan, the source of the soil from which the organism was isolated.

Spore chains in Section *Rectiflexibiles*. Mature spore chains are short, often with only 3–10 spores per chain (longer chains may be found on impression mounts or electron micrographs) This morphology is seen on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar. Spore surface is smooth.

Color of colony: aerial mass color is difficult to determine because sporulating aerial mycelium is poorly developed on ISP media. When sporulation occurs, it is usually in the White or Red (5cb, grayish yellowish pink) color series on yeast-malt agar and in the White or Yellow color series on oatmeal agar. When present, sporulation aerial mycelium is in the White color series on salts-starch agar and glycerol-asparagine agar. Reverse side of colony with no distinctive pigments (colorless or pale grayish yellow) on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar.

D-Glucose, L-arabinose, D-xylose, D-mannitol, and D-fructose are utilized for growth. No growth or only trace of growth with sucrose, iso-inositol, rhamnose, and raffinose.

Type strain shows the highest sequence similarity to: *S. albovinaceus*, AB249958, 100%; *S. griseoplanus*, AY999894, 100%; *S. anulatus*, DQ026637, 100%; *S. globisporus* subsp. *globisporus*, EF178686, 100%; *S. pluricolorescens*, DQ442540, 100%; *S. praecox*, AB184293, 100%; *S. flavofuscus*, AB249935, 100%; *S. fimicarius*, AY999784, 100%; *S. mediolani*, AB184674, 100%; *S. badius*, AY999783, 100%; *S. griseinus*, AB184205, 100%; *S. rubiginosohelvolus*, AB184240, 100%; *S. californicus*, AB184755, 99.9%; *S. lavendulae* subsp. *lavendulae*, AB184080, 99.9%; *S. cavourensis* subsp. *washingtonensis*, DQ026671, 99.9%; *S. acrimycini*, AY999889, 99.9%; *S. parvus*, DQ442537, 99.9%; *S. fulvorobeus*, AB184711, 99.8%; *S. lipmanii*, AB184148, 99.8%; *S. microflavus*, DQ445795, 99.8%; *S. cyaneofuscatus*, AB184860, 99.8%; *S. floridae*, AB184656, 99.8%; *S. alboviridis*, AB184256, 99.8%; *S. baarnensis*, EF178688, 99.8%; *S. cinereorectus*, AB184646, 99.8%; *S. griseus* subsp. *griseus*, AY207604, 99.7%; *S. griseolus*, AB184768, 99.7%; *S. luridiscabiei*, AF361784, 99.7%; *S. flavovirens*, DQ026635, 99.7%; *S. argenteolus*, AB045872, 99.7%; *S. bacillaris*, AB184439, 99.6%; *S. pulveraceus*, AB184806, 99.6%; *S. halstedii*, EF178695, 99.6%; *S. flavogriseus*, AJ494864, 99.6%; *S. atroolivaceus*, AJ781320, 99.5%; *S. olivoviridis*, AB184227, 99.5%; *S. finlayi*, AY999788, 99.5%; *S. nitrosporeus*, EF178680, 99.5%; *S. albolongus*, AB184425, 99.4%; *S. clavifer*, DQ026670, 99.4%; *S. griseobrunneus*, AB249912, 99.4%; *S. sanglieri*, AB249945, 99.4%; *S. celluloflavus*, AB184476, 99.4%; *S. yanii*, AB006159, 99.4%; *S. gelaticus*, DQ026636, 99.3%; *S. atratus*, DQ026638, 99.3%; *S. cavourensis* subsp. *cavourensis*, DQ445791, 99.3%; *S. mutomycini*, AB249951, 99.2%; *S. candidus*, DQ026663, 99.2%; *S. spiroverticillatus*, AB184814, 99.1%; *S. cremeus*, AB184124, 99.1%.

Source: isolated from soil from Sinda Village, Osaka Prefecture, Japan.

DNA G+C content (mol%): not known.

Type strain: ATCC 23963, CBS 946.68, BCRC 11887, DSM 40255, NBRC 12915, NBRC 3399, JCM 4164, JCM 4669, NRRL B-1866, NRRL-ISP 5255, RIA 1181.

Sequence accession no. (16S rRNA gene): AB184759.

442. **Streptomyces sioyaensis** Nishimura, Okamoto, Mayama, Ohtsuka, Nakajima, Tawara, Shimohira and Shimaoka 1961, 257[AL]

si.o.ya.en′sis. N.L. masc. adj. *sioyaensis* of or belonging to Sioya, Kobe, Japan, the source of the soil from which the organism was isolated.

Spore chains in Section *Spirales*. Mature spore chains generally have 10–50 spores per chain; longer chains are sometimes observed. Morphology can be observed on oatmeal agar and glycerol-asparagine agar in 14–21 d. Typical aerial mycelium may develop slowly or fail to develop on yeast-malt agar and salts-starch agar. Spore surface is smooth. One observer reports disintegration of spiral spore chains in dark liquid droplets.

Color of colony: aerial mass color in the Gray color series on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar. Reverse side of colony with no distinctive pigments (grayed yellow or grayed yellow modified by green) on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar; substrate pigment is not a pH indicator.

Color in medium: melanoid pigments not formed in peptone-yeast-iron agar and tyrosine agar. Trace of yellow pigment found in medium in yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar; this pigment is not pH-sensitive.

D-Glucose, sucrose, D-xylose, iso-inositol, D-mannitol, D-fructose, and raffinose are utilized for growth. No growth or only trace of growth on L-arabinose and rhamnose.

Type strain shows the highest sequence similarity to: *S. hygroscopius* subsp. *decoyicus*, AY999883, 99.6%; *S. nigrescens*, DQ442530, 99.5%; *S. libani* subsp. *libani*, AB184414, 99.5%; *S. tubercidicus*, AJ621612, 99.5%; *S. lydicus*, Y15507, 99.3%; *S. chattanoogensis*, AJ621611, 99.3%; *S. libani* subsp. *rufus*, AJ781351, 99.2%; *S. misakiensis*, AB217605, 99.2%; *S. caniferus*, AB184640, 99.2%; *S. catenulae*, AJ621613, 99.2%; *S. erumpens*, AJ621603, 99.2%; *S. hygroscopius* subsp. *glebosus*, AB184479, 99.2%; *S. ochraceiscleroticus*, AB184094, 99.1%; *S. purpurogeneiscleroticus*, AJ621604, 99.1%; *S. platensis*, AB045882, 99.1%; *S. chrestomyceticus*, AJ621609, 99%; *S. albospinus*, AB184527, 99%; *S. monomycini*, DQ445790, 99%; *S. auratus*, AJ391816, 99%; *S. rimosus* subsp. *paromomycinus*, AJ621610, 99%.

Source: isolated from soil from Sioya, Kobe, Japan.

DNA G+C content (mol%): not known.

Type strain: ATCC 13989, ATCC 19810, CBS 563.68, BCRC 11878, DSM 40032, NBRC 12820, JCM 4418, KCTC 9043, NRRL B-5408, NRRL-ISP 5032, RIA 1090, UNIQEM 197, VKM Ac-1260.

Sequence accession no. (16S rRNA gene): DQ026654.

443. **Streptomyces sodiiphilus** Li, Zhang, Zhang, Tang, Yu, Xu and Jiang 2005, 1332[AL]

so.di.i′phi.lus. N.L. n. *sodium -i* sodium; N.L. adj. *philus -a -um* (from Gr. adj. *philos -ê -on*) friend, loving; N.L. masc. adj. *sodiiphilus* sodium ion-loving, referring to the characteristic of Na^+-dependent growth.

Long or short chains of spores are straight to flexuous and spores are nonmotile. Both vegetative and aer-

ial hyphae are well-developed and not fragmented. No diffusible pigments are produced except on nutrient agar medium (pale orange-yellow). Positive for gelatin liquefaction and nitrate reduction, but negative for urease, melanin production, starch hydrolysis, H_2S production, milk coagulation, and milk peptonization. Sodium acetate and rhamnose can be used as sole carbon sources for growth, but not most other carbon sources, such as lactose, maltose, fructose, xylose, ribose, arabinose, sucrose, glucose, galactose, sodium citrate, cellobiose, raffinose, mannitol, sorbitol, glycerol, and starch. Grows optimally at 28°C and in ISP medium 2 with 3% NaCl and pH 9.0–10.0. Cell wall contains LL-A_2pm and glycine. Whole-cell hydrolysates mainly contain galactose and glucose and no diagnostic sugars. Predominant menaquinones are MK-9(H_4) (13%), MK-9(H_6) (68%), and MK-9(H_8) (19%), and the diagnostic phospholipid is phosphatidylethanolamine. Major fatty acid components are $C_{15:0}$ anteiso (16.46%), $C_{17:0}$ anteiso (13.30%), and $C_{16:0}$ iso (31.32%).

Type strain shows no sequence similarity over 99%.

Source: the type strain was isolated from soil sample collected from Chaka salt lake, Qinghai Province, China.

DNA G+C content (mol%): 70.5.

Type strain: YIM 80305, CCTCC AA 203015, CIP 107975, JCM 13581.

Sequence accession no. (16S rRNA gene): AY236339.

444. **Streptomyces somaliensis** (Brumpt 1906) Waksman and Henrici *in* Breed, Murray and Hitchens 1948, 965[AL] ("*Indiella somaliensis*" Brumpt 1906, 555)

so.ma.li.en′sis. N.L. masc. adj. *somaliensis* of or pertaining to Somalia.

Spore chains in Section *Rectiflexibiles*; flexuous chains are most common. Mature spore chains generally have 10–50, or sometimes more than 50, spores per chain. This morphology is seen on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar. Spore surface is smooth.

Color of colony: aerial mass color in the Yellow color series (2db or 2ba, pale yellow) on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar (one observer selected color tab 3ca, pale orange yellow, from the Red color-wheel as the most representative color). Aerial mycelium is often poorly developed on much of the culture surface on ISP media. Good sporulation in the Yellow color series was observed on Czapek's agar. Reverse side of colony with no distinctive pigments (grayish yellow to grayish greenish yellow and light olive brown) on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar.

Color in medium: melanoid pigments are not formed in peptone-yeast-iron agar, tyrosine agar, or tryptone-yeast broth. Yellow pigment is found in the medium in yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar; this pigment is not pH-sensitive when tested with 0.05 M NaOH or HCl.

D-Glucose, L-arabinose, sucrose, D-xylose, D-mannitol, and D-fructose are utilized for growth. No growth or only trace of growth iso-inositol, rhamnose, and raffinose.

Type strain shows the highest sequence similarity to: *S. coeruleoprunus*, AB184651, 99%.

Source: not known.

DNA G+C content (mol%): not known.

Type strain: ATCC 33201, DSM 40738, JCM 12659, IMRU 1274, NCTC 11332.

Sequence accession no. (16S rRNA gene): AJ007403.

445. **Streptomyces sparsogenes** Owen, Dietz and Camiener 1963, 772[AL] emend. Goodfellow, Kumar, Labeda and Sembiring 2008, 5 (Effective publication: Goodfellow, Kumar, Labeda and Sembiring 2007, 192.) ("*Streptomyces sparsogenes* var. *sparsogenes*" Owen, Dietz and Camiener 1963, 772)

spar.so′ge.nes. L. part. adj. *sparsus* scattered; N.L. suff. *-genes* (from Gr. v. *gennaô* to produce) producing; N.L. part. adj. *sparsogenes* scattered producing, probably referring to the sparse formation of aerial mycelium.

Spore chains in Section *Spirales*. Aerial hyphae and spirals may emerge from coremia-like structures. Mature spore chains are moderately long with 10 to 50 or more spores per chain. This morphology may be seen on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar, although sporulation is not always uniformly good on these media. One observer notes autolysis or lysis on media in 14 d. Spore surface is spiny; individual spores are indistinct.

Color of colony: aerial mass color in the Gray color series (2dc, yellowish gray or 3fe, light brownish gray to 5fe, light grayish reddish brown) on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar; aerial mycelium in the Yellow color series (2ba, pale yellow) may also be seen on yeast-malt agar and glycerol-asparagine agar. Reverse side of colony with no distinctive pigments (light or pale yellow to moderate yellow or orange-yellow) on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar.

Color in medium: melanoid pigments are not formed in peptone-yeast-iron agar, tyrosine agar, or tryptone-yeast broth. No pigment is found in medium in yeast-malt agar, oatmeal agar, salts-starch agar, or glycerol-asparagine agar.

D-Glucose, L-arabinose, D-xylose, D-mannitol, D-fructose, rhamnose, sucrose, and raffinose are utilized for growth. Utilization of iso-inositol is doubtful.

Type strain shows the highest sequence similarity to: *S. cuspidosporus*, AB184090, 99.9%.*Source:* not known.

DNA G+C content (mol%): not known.

Type strain: ATCC 25498, CBS 672.69, CBS 958.69, BCRC 12085, DSM 40356, NBRC 13086, JCM 4517, LMG 5985, NCIMB 9449, NRRL 2940, NRRL-ISP 5356, RIA 1278, VKM Ac-1744.

Sequence accession no. (16S rRNA gene): AB184301.

446. **Streptomyces specialis** Kämpfer, Huber, Buczolits, Thummes, Grün-Wollny and Busse 2008, 2605[VP]

spe.ci.a′lis. L. masc. adj. *specialis* particular, special, because of the unusual quinone type and the separate phylogenetic position.

Forms extensively branched substrate mycelium and aerial hyphae that differentiate into spiral chains of spores. Spore chains consist of up to 15 spores. Spores are elliptical or short rods. Good growth on all ISP media (ISP 1 through 5, according to Shirling and Gottlieb,

1966) after 2 weeks incubation at 28°C. On all media, a pinkish white aerial mycelium and a pale yellow substrate mycelium is produced. The isolate produces a black pigment on all tested ISP media. *N*-Acetyl-D-glucosamine, D-glucose, D-gluconate, D-ribose, D-sucrose, adonitol, D-arabitol, D-sorbitol, iso-inositol, propionate, glutarate, butyrate, isobutyrate, iso-valeric acid, L-arginine, and L-asparagine are utilized for growth. The following carbon sources are not utilized: L-arabinose, cellobiose, D-fructose, D-galactose, maltose, D-mannose, melibiose, L-rhamnose, salicin, and D-xylose. Good growth occurs on nutrient agar and medium 65 at 25–30°C; Major fatty acids are $C_{16:0}$ iso, $C_{16:0}$, $C_{17:1}$ anteiso ω9*c*, $C_{17:0}$ anteiso, and $C_{16:1}$ iso H. The quinone system is composed of the major compound MK-10(H_4) and moderate to minor amounts of MK-10(H_6), MK-9(H_4), and MK-9(H_6). In the polar lipid profile, diphosphatidylglycerol is predominant. Phosphatidylethanolamine, phosphatidylglycerol, and phosphatidylinositol are present in moderate amounts and phosphatidylinositol mannosides, an unknown aminolipid, an unknown phospholipid, and three unknown polar lipids are present in minor to trace amounts.

Type strain shows no sequence similarity over 99%.

Source: isolated from soil.

DNA G+C content (mol%): not known.

Type strain: GW41-1564, DSM 41924, CCM 7499.

Sequence accession no. (16S rRNA gene): AM934703.

447. **Streptomyces spectabilis** Mason, Dietz and Smith 1961, 118[AL]

spec.ta′bi.lis. L. masc. adj. *spectabilis* visible, notable, remarkable.

Spore chains in Section *Rectiflexibiles* with straight to flexuous spore chains. Distribution of sporophores sometimes suggests verticillate morphology, but true whorls or verticils are not produced. Mature spore chains are long with 10 to 50 or more spores per chain. This morphology is seen on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar. Spore surface is smooth.

Color of colony: aerial mass color in the Red color series (7ca or 5ca, light yellowish pink; 5gc, light reddish brown) on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar. White aerial mycelium is sometimes produced. Reverse side of colony with distinctive reddish orange pigments on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar. Reverse mycelium pigment is changed only slightly by the addition of 0.05 M NaOH or HCl.

Color in medium: melanoid pigments are formed in peptone-yeast-iron agar and sometimes in tryptone-yeast broth, but not in tyrosine agar. No pigment is found in the medium in yeast-malt agar, oatmeal agar, salts-starch agar, or glycerol-asparagine agar.

D-Glucose, L-arabinose, D-xylose, iso-inositol, D-mannitol, D-fructose, and raffinose are utilized for growth. Utilization of rhamnose is doubtful. No growth or only traces of growth with sucrose.

Type strain shows no sequence similarity over 99%.

Source: not known.

DNA G+C content (mol%): not known.

Type strain: ATCC 27465, CBS 725.72, BCRC 12648, CECT 3146, DSM 40512, NBRC 13424, NBRC 15441, JCM 4308, JCM 4832, KCTC 9218, LMG 5986, NCIMB 9733, NRRL 2494, NRRL-ISP 5512, RIA 1385.

Sequence accession no. (16S rRNA gene): AB184393.

448. **Streptomyces speibonae** Meyers, Porter, Omorogie, Pule and Kwetane 2003, 804[VP]

spei.bo′na.e. L. n. *spes -ei* hope; L. adj. *bonus* good; N.L. gen. n. *speibonae* of good hope, to indicate Cape Town, the Cape of Good Hope, South Africa, the geographical location from which the type strain was isolated.

Spirales-type spore chains with hairy spore sheaths are produced. Forms gray aerial mycelium. The color of the substrate mycelium is not pH-sensitive. Verticils are not present. The mycelium does not fragment. No diffusible pigments are produced on glycerol-asparagine agar or on any other medium. Melanin pigment is produced on both peptone-yeast extract-iron agar and tyrosine agar. Although growth on inorganic salts-starch agar is initially slow, very good growth with profuse sporulation is observed on this medium after 14 d. Very good growth occurs on yeast extract-malt extract agar. Good growth is observed on oatmeal agar and moderate growth on Czapek's solution agar (Atlas, 1993). Growth on glycerol-asparagine agar is poor. The substrate mycelium is blue on yeast extract-malt extract agar and oatmeal agar, but light gray on Czapek and glycerol-asparagine media. Degrades casein, DNA, gelatin, guanine, hypoxanthine, starch (weakly), Tween 80, L-tyrosine, xanthine, and xylan but not adenine, allantoin, or urea. Uses L(+)-arabinose, D(+)-cellobiose, D(−)-fructose, D(+)-galactose, glycerol, *myo*-inositol, lactose, maltose, D-mannitol, D(+)-mannose, L(+)-rhamnose, D(−)-ribose, sodium acetate, sodium butyrate, sodium DL-malate, sodium malonate, sodium propionate, sodium pyruvate, sodium succinate, sucrose (weak growth), trehalose, and D(+)-xylose as sole carbon sources but not adonitol, *meso*-erythritol, inulin, D(+)-melezitose, D(+)-melibiose, methyl α-D-glucoside, raffinose, salicin, sodium benzoate, sodium citrate, sodium formate, sodium maleate, sodium oxalate, sodium salicylate, sodium L(+)-tartrate, L(−)-sorbose, or xylitol. Tests for nitrate reductase and the production of H_2S are positive, but pectin is not hydrolyzed. Lipase and lecithinase are produced on egg-yolk agar, but protease activity is not seen on this medium after the recommended 2 d of incubation (there is weak activity after 6 d). The cell wall contains LL-A_2pm (cell-wall type I). The whole-cell sugar pattern contains no diagnostic sugars. No antibiosis is exhibited against *Escherichia coli* ATCC 25922 or *Pseudomonas aeruginosa* ATCC 27853. Grows in the presence of (µg/ml unless stated otherwise): cefotaxime (100), cephaloridine (100), D-cycloserine (50), lincomycin (100), oleandomycin (100), penicillin G (10 IU/ml), phenol (0.1%), 2-phenylethanol (0.1%), sodium chloride (10%), and vancomycin (50) and at 45°C but not at 4°C, pH 4.3, or in the presence of sodium azide (0.01%), capreomycin (20), gentamicin (100), kanamycin (10), neomycin (50), rifampin (50), streptomycin (100), tobramycin (50), or viomycin (8). Uses DL-α-amino-*n*-butyric acid, 4-amino-*n*-butyric acid, L-arginine, DL-citrulline, L-cysteine,

L-histidine, L-methionine, DL-ornithine, potassium nitrate, L-serine, L-threonine, and L-valine as sole nitrogen sources, but not L-hydoxyproline or L-phenylalanine.

Type strain shows the highest sequence similarity to: *S. iakyrus*, AB184877, 99.3%; *S. longispororuber*, AB184440, 99.1%; *S. griseoflavus*, AJ781322, 99%; *S. viridodiastaticus*, AY999852, 99%; *S. lusitanus*, AB184424, 99%; *S. griseorubens*, AB184139, 99%.

Source: type strain was isolated from Cape Town, the Cape of Good Hope, South Africa.

DNA G+C content (mol%): 73.4.

Type strain: PK-Blue, ATCC BAA-411, CIP 108060, DSM 41797, JCM 12682, KCTC 9973, NRRL B-24240.

Sequence accession no. (16S rRNA gene): AF452714.

449. **Streptomyces speleomycini** Preobrazhenskaya and Szabó 1986, 575[VP] (Effective publication: Preobrazhenskaya and Szabó *in* Gause, Preobrazhenskaya, Sveshnikova, Terekhova and Maximova 1983.)

spe.le.o.my.ci′ni. N.L. n. *speleomycinum* speleomycin; N.L. gen. n. *speleomycini* of speleomycin, intended to mean spelomycin producing.

Spore chains are straight (*Rectiflexibiles*); spores are smooth. On mineral agar 1: aerial mycelium is light yellowish creamy, yellow; substrate mycelium is reddish gray brownish; no diffusible pigment. On glycerol-nitrate agar, oatmeal agar, glucose-nitrate agar, glycerol-asparagine agar: aerial mycelium is mealy, whitish yellow, light yellow, yellow; substrate mycelium is colorless; no diffusible pigment. On starch-ammonia agar, starch agar (Waksman, 1961): aerial mycelium is whitish, light yellow; substrate mycelium is colorless to gray, olive gray; no diffusible pigment. On organic agar 2: aerial mycelium is whitish; substrate mycelium is gray brownish yellow; no diffusible pigment. Melanoid pigments are not formed. Growth on ribose, fructose, xylose, and galactose; no growth on rhamnose, arabinose, or inositol. Antibiotic: speleomycin.

Source: not known.

DNA G+C content (mol%): not known.

Type strain: B-23.

Sequence accession no. (16S rRNA gene): no sequence available.

Further comments: strain B-23 was first described as *Actinomyces erythreus* subsp. *speleomycini* Szabó and Preobrazhenskaya 1962.

450. **Streptomyces spheroides** (Baldacci 1944) Wallick, Harris, Reagan, Ruger and Woodruff 1956, 911[AL] (*Actinomyces caeruleus* Baldacci 1944, 180; *Streptomyces caeruleus* Pridham, Hesseltine and Benedict 1958, 60 emend. Lanoot, Vancanneyt, Cleenwerck,Wang, Li, Liu and Swings 2002, 828)

sphe.ro.i′des. Gr. n. *sphaira* a ball, a sphere; Gr. adj. suff. *eides* similar; N.L. masc. adj. *spheroides* similar to a ball referring to the characteristic compact coils of spores, resembling spheres.

Spore chains in Section *Spirales*. Mature spore chains are generally long, often with more than 50 spores per chain. This morphology is seen on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar. Spore surface is smooth.

Color of colony: aerial mass color in the Yellow color series on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar. Reverse side of colony with no distinctive pigments (colorless to pale yellow or light yellowish brown) on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar.

Color in medium: melanoid pigments are not formed in peptone-yeast-iron agar, tyrosine agar, or tryptone-yeast broth. No pigment found in medium in yeast-malt agar, oatmeal agar, salts-starch agar, or glycerol-asparagine agar.

D-Glucose, L-arabinose, sucrose, D-xylose, D-mannitol, D-fructose, and rhamnose are utilized for growth. Only traces of growth are found on iso-inositol and raffinose. A trace of growth also occurs on the carbon-free basal medium.

For sequence similarity, see type strain of *Streptomyces caeruleus*.

Source: not known.

DNA G+C content (mol%): not known.

Type strain: ATCC 23965, CBS 491.62, CBS 948.68, BCRC 11559, DSM 40292, NBRC 12917, JCM 4252, JCM 4670, LMG 19392, NCIMB 11891, NRRL 2449, NRRL-ISP 5292, RIA 1200, RIA 700.

Sequence accession no. (16S rRNA gene): EF178698.

Further comments: Streptomyces spheroides Wallick et al. 1956 is a later heterotypic synonym of *Streptomyces caeruleus* (Baldacci 1944) Pridham et al. 1958 emend. Lanoot et al. 2002.

451. **Streptomyces spinoverrucosus** Diab and Al-Gounaim 1982, 331[VP]

spi.no.ver.ru.co′sus. L. adj. *spinosus* thorny; L. adj. *verrucosus* warty; N.L. masc. adj. *spinoverrucosus* spiny and warty, referring to the spiny and warty spore surface.

Spore chains are in the form of terminal, closed, or compact spirals with two or more turns. There are 10 to 20 or more spores per chain. Spore surfaces are spiny; some spores have spiny and warty surfaces. Mature aerial mass colors in the green color series on salts starch agar, yeast malt agar, oatmeal agar, and glycerol-asparagine agar (Tresner–Backus color series tabs) were matched to the red, yellow, green, and gray color wheels. T.G. Pridham (who confirmed the color series) stated that strain 163MA represents a good example of the problem involved in use of color as a major criterion in characterization and classification of streptomycetes and streptoverticillia. The reverse sides of the colonies are colorless or faint yellow on salts starch agar and brownish red on yeast malt agar, oatmeal agar, and glycerol asparagine agar. The red color is pH-sensitive.

Color in medium: melanoid pigments are formed in peptone-yeast-iron agar, tyrosine agar, and tryptone yeast broth. The water-soluble pigments produced are brown on yeast malt agar and glycerol-asparagine agar and red or reddish brown on oatmeal agar. An orange water-soluble pigment may be formed in salts-starch agar.

D-Glucose, D-xylose, L-arabinose, iso-inositol, D-mannitol, fructose, rhamnose, sucrose, and raffinose were utilized for growth. Growth on Czapek's solution was good.

A whole-cell hydrolysate analysis revealed the presence of LL-A_2pm.

Type strain shows the highest sequence similarity to: *S. lusitanus*, AB184424, 99.2%; *S. levis*, AB184670, 99.1%; *S. bellus*, AB184849, 99.1%; *S. coerulescens*, AY999720, 99.1%; *S. coeruleorubidus*, AY999719, 99%; *S. carpinensis*, AB184574, 99%; *S. parvulus*, AB184326, 99%; *S. gancidicus*, AB184660, 99%; *S. lomondensis*, AB184673, 99%.

Source: not known.

DNA G+C content (mol%): not known.

Type strain: Diab 163MA, NCIB (now NCIMB) 11666, ATCC 33692, DSM 41648, NBRC 14228, NBRC 14250, JCM 5077, NRRL B-16932.

Sequence accession no. (16S rRNA gene): AB184578.

452. **Streptomyces spiralis** (Falcão de Morais 1970) Goodfellow, Williams and Alderson 1986a, 575[VP] (Effective publication: Goodfellow, Williams and Alderson 1986a, 50.) (*Elytrosporangium spirale* Falcão de Morais 1970, 79)

spi.ra′lis. L. n. *spira* a spiral; N.L. masc. adj. *spiralis* spiraled, referring to spiral spore chains.

Forms extensively branched substrate and aerial mycelium. The latter bears long spirals spore chains and the former occasional chains of spores. The spore surface is smooth. The aerial spore mass is yellowish gray; the reverse color is cream to yellow. Does not form melanin pigments. Adenine, esculin, casein, guanine, hypoxanthine, pectin, starch, testosterone, and tyrosine are degraded but allantoin, arbutin, chitin, elastin, lecithin, urea, and xanthine are not. Hydrogen sulfide is produced but nitrate is not reduced. L-Arabinose, cellobiose, D-fructose, D-galactose, D-glucose, *myo*-inositol, inulin, D-lactose, mannitol, D-mannose, melibiose, raffinose, L-rhamnose, salicin, sucrose, trehalose, and xylitol are used as sole carbon sources but adonitol, melezitose, and D-xylose are not. Grows on L-arginine, L-cysteine, L-histidine, potassium nitrate, L-serine, L-threonine, and L-valine, but not on DL-amino-n-butyric acid, L-hydroxyproline, L-methionine, or L-phenylalanine, as sole nitrogen source. Growth occurs at 37 and 45°C but not at 10°C. Tolerant to phenol (0.1%, w/v), sodium azide (0.02%, w/v), and sodium chloride (7%, w/v). Resistant to rifampin but sensitive to sodium chloride at 10% (w/v). Does not show antimicrobial activity against *Aspergillus niger* LIV 131, *Bacillus subtilis* NCIB 3610, *Candida albicans* CBS 562, *Escherichia coli* NCIB 9132, *Micrococcus luteus* NCIB 196, *Pseudomonas fluorescens* NCIB 9046^T, *Saccharomyces cerevisiae* CBS 1171^T, or *Streptomyces murinus* ISP 5091. Contains octahydrogenated menaquinones with nine isoprene units as the predominant isoprenolog.

Type strain shows the highest sequence similarity to: *S. ruber*, AB184604, 99%; *S. fumigatiscleroticus*, DQ442499, 99%.

Source: isolated from soil, Brazil.

DNA G+C content (mol%): not known.

Type strain: ATCC 25664, DSM 43836, NBRC 14215, KCC A-0302, JCM 3302, NRRL B-16922, VKM Ac-1311.

Sequence accession no. (16S rRNA gene): EF178683.

453. **Streptomyces spiroverticillatus** Shinobu 1958, 93[AL]

spi.ro.ver.ti.cil.la′tus. L. n. *spira* a coil, spiral; L. n. *verticillus* a whorl; N.L. adj. *verticillatus* whorled; N.L. masc. adj. *spiroverticillatus* coiled and whorled.

Spore chains in Section *Retinaculiaperti*. Shinobu's original description makes note of verticils or whorls "near the base of the aerial mycelium". Mature spore chains are generally long, often with more than 50 spores per chain. This morphology is seen on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar. Spore surface is smooth. Conidia-like fragments in substrate mycelium (one observer); sclerotia (two observers).

Color of colony: aerial mass color in the White color series on yeast-malt agar and glycerol-asparagine agar; White or Red series on oatmeal agar and salts-starch agar. Reverse side of colony is grayed yellow to yellow-brown is modified by red or orange on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar. Reverse color is changed slightly from yellow-brown to reddish brown with the addition of 0.05 M NaOH and from reddish-brown to yellow-brown with 0.05 M HCl.

Color in medium: melanoid pigments are not formed in peptone-yeast-iron agar and tyrosine agar. Pigments other than traces of yellow are not formed in yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar. One observer reports a trace of yellow pigment that may change to pink with addition of 0.05 M NaOH.

D-Glucose, L-arabinose. D-xylose, and D-fructose are utilized for growth. No growth or only trace of growth on iso-inositol, D-mannitol, rhamnose, and raffinose. Variable reports on growth with sucrose.

Type strain shows the highest sequence similarity to: *S. cremeus*, AB184124, 99.7%; *S. albolongus*, AB184425, 99.4%; *S. celluloflavus*, AB184476, 99.4%; *S. griseobrunneus*, AB249912, 99.4%; *S. candidus*, DQ026663, 99.3%; *S. cavourensis* subsp. *cavourensis*, DQ445791, 99.3%; *S. praecox*, AB184293, 99.2%; *S. fulvorobeus*, AB184711, 99.2%; *S. lipmanii*, AB184148, 99.2%; *S. fimicarius*, AY999784, 99.2%; *S. flavovirens*, DQ026635, 99.2%; *S. anulatus*, DQ026637, 99.2%; *S. flavofuscus*, AB249935, 99.2%; *S. microflavus*, DQ445795, 99.2%; *S. alboviridis*, AB184256, 99.2%; *S. pluricolorescens*, DQ442540, 99.1%; *S. albovinaceus*, AB249958, 99.1%; *S. lavendulae* subsp. *lavendulae*, AB184080, 99.1%; *S. argenteolus*, AB045872, 99.1%; *S. griseolus*, AB184768, 99.1%; *S. mediolani*, AB184674, 99.1%; *S. luridiscabiei*, AF361784, 99.1%; *S. acrimycini*, AY999889, 99.1%; *S. californicus*, AB184755, 99.1%; *S. flavogriseus*, AJ494864, 99.1%; *S. baarnensis*, EF178688, 99.1%; *S. badius*, AY999783, 99.1%; *S. rubiginosohelvolus*, AB184240, 99.1%; *S. floridae*, AB184656, 99.1%; *S. globisporus* subsp. *globisporus*, EF178686, 99.1%; *S. griseinus*, AB184205, 99.1%; *S. sindenensis*, AB184759, 99.1%; *S. cavourensis* subsp. *washingtonensis*, DQ026671, 99.1%; *S. griseoplanus*, AY999894, 99.1%; *S. pulveraceus*, AB184806, 99%; *S. parvus*, DQ442537, 99%; *S. cyaneofuscatus*, AB184860, 99%; *S. cinereorectus*, AB184646, 99%; *S. halstedii*, EF178695, 99%.

Source: not known.

DNA G+C content (mol%): not known.

Type strain: ATCC 19811, CBS 564.68, BCRC 13648, DSM 40036, NBRC 12821, IMET 42050, JCM 4104, JCM 4609, NBRC 3931, NRRL B-2259, NRRL B-5483, NRRL-ISP 5036, RIA 1091, RIA 549, UNIQEM 198, VKM Ac-751.

Sequence accession no. (16S rRNA gene): AB184814.

454. **Streptomyces spitsbergensis** Wieczorek, Mordarska, Zakrzewska-Czerwinska, Gamian and Mordarski 1993, 86[VP]

spits.ber.gen′sis. N.L. masc. adj. *spitsbergensis* of or belonging to Spitsbergen, the source of the soil sample from which the type strain was isolated.

Spore chains are *Rectiflexibiles*. Spore surface is smooth. Color of branching substrate mycelium ranges from red to reddish brown, depending on the growth medium. Aerial mycelium is pink to violet. Soluble pigments (including melanin) are not produced. Hydrolyzes gelatin and starch. Nitrate is not reduced. Grows well at 25–37°C; no growth occurs at 45°C. Type I cell wall and type PII phospholipid pattern (saturated iso and anteiso fatty acids, lack of mycolic acids and major glycolipids). The species produces some prodiginine-like metabolites with immunosuppressive activity.

For sequence similarity, see type strain of *Streptomyces hiroshimensis*.

Source: the type strain was isolated from soil from Spitsbergen.

DNA G+C content (mol%): 71.0.

Type strain: S-2, ATCC 51269, NBRC 15745, JCM 8881, PCM 2404.

Sequence accession no. (16S rRNA gene): AB184700.

Further comments: Hatano et al. (1997) propose *Streptomyces spitsbergensis* Wieczorek et al. 1993 as a later heterotypic synonym of *Streptomyces baldaccii* corrig. (Farina and Locci 1966) Witt and Stackebrandt 1991.

According to Hatano et al. (2003), *Streptomyces spitsbergensis* Wieczorek et al. 1993 is a later heterotypic synonym of *Streptomyces hiroshimensis* (Shinobu 1955) Witt and Stackebrandt 1991.

455. **Streptomyces sporocinereus** (*ex* Krasil'nikov 1970b) Preobrazhenskaya 1986, 575[VP] (Effective publication: Preobrazhenskaya *in* Gause, Preobrazhenskaya, Sveshnikova, Terekhova and Maximova 1983.) ("*Actinomyces sporocinereus*" Krasil'nikov 1970b)

spo.ro.ci.ne′re.us. Gr. n. *spora* seed; L. adj. *cinereus* ash-colored; N.L. masc. adj. *sporocinereus* ash-colored spores.

Spore chains are spiral (*Spirales*); spores are warty. On mineral agar 1 and oatmeal agar: aerial mycelium is gray, dark gray, black; substrate mycelium is colorless; no diffusible pigment. On glycerol-nitrate agar: aerial mycelium is white or beige; substrate mycelium colorless; no diffusible pigment. On starch-ammonia agar: aerial mycelium is gray; substrate mycelium is colorless to yellowish beige; no diffusible pigment. On glycerol-asparagine agar: aerial mycelium is gray; substrate mycelium colorless; no diffusible pigment. On organic agar 2: aerial mycelium is gray; substrate mycelium and diffusible pigment are yellow-brown, brown. Melanoid pigments are not formed. Utilization of glucose, galactose, and starch; no digestion of fructose.

Type strain shows the highest sequence similarity to: *S. endus*, AY999911, 100%; *S. hygroscopius* subsp. *hygroscopius*, AB184428, 100%; *S. demainii*, DQ334782, 99.9%; *S. yogyakartensis*, AB249942, 99.5%; *S. violaceusniger*, AJ391823, 99.5%; *S. albiflaviniger*, AJ391812, 99.3%.

Source: not known.

DNA G+C content (mol%): not known.

Type strain: ATCC 43692, DSM 41460, INMI 32, JCM 9093, NBRC 100766, NRRL B-16376, VKM Ac-312.

Sequence accession no. (16S rRNA gene): AB249933.

456. **Streptomyces sporoclivatus** (*ex* Krasil'nikov 1970b) Preobrazhenskaya 1986, 575[VP] (Effective publication: Preobrazhenskaya *in* Gause, Preobrazhenskaya, Sveshnikova, Terekhova and Maximova 1983.) ("*Actinomyces sporoclivatus*" Krasil'nikov 1970b).

Etymology is not provided.

Spore chains are spiral (*Spirales*); spores are warty. On mineral agar 1, oatmeal agar, starch-ammonia agar: aerial mycelium is whitish gray or gray; substrate mycelium is colorless; no diffusible pigment. On glycerol-nitrate agar: aerial mycelium is white or light yellow; substrate mycelium is dark yellow; no diffusible pigment. On glycerol-asparagine agar: aerial mycelium is white, light gray; substrate mycelium is colorless to yellow; no diffusible pigment. On organic agar 2: aerial mycelium is white; substrate mycelium is yellow; no diffusible pigment. Melanoid pigments are not formed. Grows on glucose, fructose, xylose, galactose, raffinose, and mannitol; no growth on rhamnose or arabinose.

Type strain shows the highest sequence similarity to: *S. antimycoticus*, AB184185, 100%; *S. geldanamycininus*, DQ334781, 99.8%; *S. rutgersensis* subsp. *castelarensis*, AY508511, 99.8%; *S. melanosporofaciens*, AJ271887, 99.6%; *S. rhizosphaericus*, AB249941, 99.1%; *S. asiaticus*, AB249947, 99.1%.

Source: not known.

DNA G+C content (mol%): not known.

Type strain: ATCC 43693, DSM 41461, INMI 97, JCM 9094, NBRC 100767, VKM Ac-315.

Sequence accession no. (16S rRNA gene): AB249934.

457. **Streptomyces spororaveus** (*ex* Krasil'nikov 1970b) Preobrazhenskaya 1986, 575[VP] (Effective publication: Preobrazhenskaya *in* Gause, Preobrazhenskaya, Sveshnikova, Terekhova and Maximova 1983.) ("*Actinomyces spororaveus*" Krasil'nikov 1970b)

spo.ra.ra.ve′us. Gr. n. *spora* seed; N.L. adj. *raveus* gray-yellow, gray; N.L. masc. adj. *spororaveus* gray-yellow, gray spores.

Spore chains are spiral (*Retinaculiaperti* – spore chains are hooks, loops, or spirals with one or two turns); spores are warty and smooth. On mineral agar 1, starch-ammonia agar: aerial mycelium is gray; substrate mycelium is colorless or sometimes light yellow; no diffusible pigment. On glycerol-nitrate agar: aerial mycelium is gray; substrate mycelium is brown to black; diffusible pigment is brown. On oatmeal agar: aerial mycelium is brownish gray; substrate mycelium and diffusible pigment are gray-brown. On glycerol-asparagine agar: aerial mycelium is gray;

substrate mycelium gray-brown; gray brownish or no diffusible pigment. On organic agar 2: aerial mycelium is brown; substrate mycelium and diffusible pigment are black. Melanoid pigments are formed. Grows on glucose, maltose, and starch; no growth on xylose, arabinose, galactose, fructose, raffinose, or rhamnose.

Type strain shows the highest sequence similarity to: *S. nojiriensis*, AJ781355, 100%; *S. xanthophaeus*, DQ442560, 100%; *S. goshikiensis*, EF178693, 99.9%; *S. vinaceus*, AB184394, 99.9%; *S. cirratus*, AY999794, 99.9%; *S. colombiensis*, DQ026646, 99.8%; *S. sporoverrucosus*, DQ442544, 99.8%; *S. cinnamonensis*, AB184707, 99.7%; *S. avidinii*, AB184395, 99.7%; *S. subrutilus*, X80825, 99.7%; *S. virginiae*, D85119, 99.5%; *S. racemochromogenes*, DQ026656, 99%; *S. polychromogenes*, AB184292, 99%.

Source: not known.

DNA G+C content (mol%): not known.

Type strain: ATCC 43694, DSM 41462, NBRC 15456, INMI 101, JCM 6928, NRRL B-16378, VKM Ac-318.

Sequence accession no. (16S rRNA gene): AJ781370.

458. **Streptomyces sporoverrucosus** (*ex* Krasil'nikov 1970b) Preobrazhenskaya 1986, 575[VP] (Effective publication: Preobrazhenskaya *in* Gause, Preobrazhenskaya, Sveshnikova, Terekhova and Maximova 1983.) ("*Actinomyces sporoverrucosus*" Krasil'nikov 1970b)

spo.ro.ver.ru.co'sus. Gr. n. *spora* seed; L. adj. *verrucosus* rough, rugged; N.L. masc. adj. *sporoverrucosus* having rough spores.

Spore chains are spiral; spores are warty. On mineral agar 1, oatmeal agar: aerial mycelium is gray, sometimes grayish beige; substrate mycelium is brownish yellow, yellow-brown; no diffusible pigment. On starch-ammonia agar: aerial mycelium is gray; substrate mycelium colorless; no diffusible pigment. On glycerol-nitrate agar: aerial mycelium is yellow; substrate mycelium is dark yellow; no diffusible pigment. On glycerol-asparagine agar: aerial mycelium is white-gray; substrate mycelium is yellow; no diffusible pigment. On organic agar 2: aerial mycelium is absent or light gray; substrate mycelium and diffusible pigment are brown. Melanoid pigments are formed.

Type strain shows the highest sequence similarity to: *S. goshikiensis*, EF178693, 100%; *S. colombiensis*, DQ026646, 99.9%; *S. nojiriensis*, AJ781355, 99.8%; *S. spororaveus*, AJ781370, 99.8%; *S. xanthophaeus*, DQ442560, 99.7%; *S. vinaceus*, AB184394, 99.7%; *S. cirratus*, AY999794, 99.7%; *S. cinnamonensis*, AB184707, 99.5%; *S. avidinii*, AB184395, 99.5%; *S. subrutilus*, X80825, 99.3%; *S. virginiae*, D85119, 99.2%.

Source: not known.

DNA G+C content (mol%): not known.

Type strain: ATCC 43695, DSM 41463, NBRC 15458, INMI 15, JCM 6929, NRRL B-16379, VKM Ac-321.

Sequence accession no. (16S rRNA gene): DQ442544.

459. **Streptomyces stelliscabiei** Bouchek-Mechiche, Gardan, Normand and Jouan 2000, 98[VP]

stel.li.sca'bi.ei. L. n. *stella -ae* a star; L. n. *scabies -ei* mange; N.L. gen. n. *stelliscabei* referring to lesions from which these strains were isolated, which looks like stars.

Spores are gray and are borne in mature spiral chains. Melanin is produced on tyrosine agar. L-Arabinose, D-fructose, D-glucose, D-mannitol, inositol, raffinose, rhamnose, sucrose, and D-xylose are utilized for growth. Most strains studied degrade xanthine. All strains are susceptible to 20 µg/ml streptomycin, 0.5 µg/ml crystal violet, 100 µg/ml oleandomycin, and 5% (w/v) NaCl, but not to 25 µg/ml oleandomycin or 10 IU/ml penicillin G. Utilizes *trans*-aconitate, D(+)trehalose, and α-D(+)melibiose but do not assimilate 5-keto-D-gluconate, ONPG, betaine, mucate, D-saccharate, DL-lactate, gentisate, or turanose.

Type strain shows no sequence similarity over 99%. Type strain shows DNA–DNA similarity to: *S. europaeiscabiei* DSM 41802[T], 47%; *S. reticuliscabiei* DSM 41804[T], 20%.

Source: the type strain was isolated from star-like common scab lesions on potato tubers cv. Belle de Fontenay in France.

DNA G+C content (mol%): 71.7.

Type strain: CFBP 4521, CIP 107060, CIP 107126, DSM 41803, ICMP 13715, NCPPB 4040.

Sequence accession no. (16S rRNA gene): AJ007429.

460. **Streptomyces stramineus** Labeda, Lechevalier and Testa 1997, 752[VP]

stra.mi'ne.us. L. masc. adj. *stramineus* made of straw, of straw, intended to mean straw colored, referring to the color of the aerial mycelium and spore mass.

Aerial mycelium forms verticils on which umbels consisting of straight chains of ovoid, smooth surface, straw yellow spores (0.5 × 1.0 µm) are produced. The substrate mycelium is yellow to yellowish brown. Soluble pigments that range from yellowish to shades of brown are produced on several media, such as yeast extract-malt extract. Casein, esculin, gelatin, hypoxanthine, starch, tyrosine, and xanthine are hydrolyzed; adenine and urea are not hydrolyzed. Phosphatase is produced; nitrate is not reduced. Melanin pigments are not produced from tyrosine. Growth occurs in the presence of glycerol, 5% NaCl, and lysozyme; no growth occurs in the presence of salicylate. Acetate, citrate, malate, propionate, pyruvate, and succinate are assimilated; benzoate, lactate, mucate, oxalate, and tartrate are not assimilated. Acid is produced from adonitol, dextrin, fructose, glucose, glycerol, inositol, maltose, mannitol, mannose, sorbitol, and trehalose; no acid is produced from arabinose, cellobiose, dulcitol, erythritol, galactose, melibiose, methyl α-D-glucoside, raffinose, rhamnose, salicin, sucrose, xylose, or methyl β-D-xyloside. Growth occurs on fructose, glucose, inositol, and mannitol as the sole carbon source but not on galactose, raffinose, rhamnose, salicin, or xylose. Growth occurs at 10 and 42°C, but not 52°C. Produces the antibiotics LL-BO1208α and LL-BO1208β.

Type strain shows the highest sequence similarity to: *S. eurocidicus*, AY999790, 99.2%; *S. netropsis*, AB184848, 99.2%; *S. albireticuli*, AB184881, 99.1%.

Source: isolated from a grassland soil sample from South Dakota.

DNA G+C content (mol%): 75.0.

Type strain: CIP 105398, DSM 41783, NBRC 16131, JCM 10649, NRRL 12292.

Sequence accession no. (16S rRNA gene): AB184720.

461. **Streptomyces subrutilus** Arai, Kuroda, Yamagishi and Katoh 1964, 25[AL]

sub.ru′ti.lus. L. masc. adj. *subrutilus* reddish.

Spore chains in Section *Rectiflexibiles*. Long, straight, or slightly flexuous spore chains often contain more than 50 spores per chain. This morphology is seen on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar. Spore surface is smooth.

Color of colony: aerial mass color in the Red color series (4ec, 5ec, 6ec, 5cb, grayish yellowish pink) on yeast-malt agar, oatmeal agar, and salts-starch agar; Red or White color series on glycerol-asparagine agar. Reverse side of colony with no distinctive pigments (moderate orange yellow or moderate orange brown on yeast-malt agar; pale yellow or grayish yellow on oatmeal agar, salts-starch agar, and glycerol-asparagine agar).

Color in medium: melanoid pigments are formed in peptone-yeast-iron agar and tryptone-yeast broth but are formed only weakly or not at all in tyrosine agar. No pigment or only a trace of yellow is found in the medium in yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar.

D-Glucose and D-fructose are utilized for growth. Reports vary on utilization of sucrose and D-mannitol, but growth is not abundant on these media. No growth or only traces of growth with L-arabinose, D-xylose, iso-inositol, rhamnose, and raffinose.

Type strain shows the highest sequence similarity to: *S. nojiriensis*, AJ781355, 99.7%; *S. spororaveus*, AJ781370, 99.7%; *S. avidinii*, AB184395, 99.7%; *S. xanthophaeus*, DQ442560, 99.6%; *S. vinaceus*, AB184394, 99.5%; *S. goshikiensis*, EF178693, 99.5%; *S. cirratus*, AY999794, 99.5%; *S. cinnamonensis*, AB184707, 99.4%; *S. sporoverrucosus*, DQ442544, 99.3%; *S. virginiae*, D85119, 99.3%; *S. colombiensis*, DQ026646, 99.3%.

Source: not known.

DNA G+C content (mol%): not known.

Type strain: ATCC 27467, CBS 689.72, BCRC 11921, DSM 40445, IFM 1222, NBRC 13388, JCM 4695, JCM 4834, KCTC 9045, NRRL B-12377, NRRL-ISP 5445, RIA 1349, VKM Ac-1210.

Sequence accession no. (16S rRNA gene): X80825.

462. **Streptomyces sulfonofaciens** Miyadoh, Shomura, Ito and Niida 1983, 323[VP]

sul.fon.o.fa′ci.ens N.L. n. *acidum sulfonicum* sulfonic acid; L. part. adj. *faciens* producing; N.L. part. adj. *sulfonofaciens* producing sulfonic acid.

Spores are 0.7–0.8 × 1.0–1.5 μm and oval or cylindrical; Spore surface is smooth. Spore chains are nearly straight and moderately short, bearing 10–30 spores. Fragmentation of mycelium is not observed on agar or in liquid cultures. Neither sclerotia nor sporangia are formed. Colors of the mature aerial masses produced on inorganic salts-starch agar, oatmeal agar, and yeast extract-malt extract agar belong to the Red color series (5cb, grayish yellowish pink). Undersides of colonies are colorless to pale yellowish brown. No diffusible pigments are formed, except for a light rose beige pigment formed on inorganic salts-starch agar. Frequent subculturing of strain SF-2130[T] onto fresh agar media results in the loss of the ability to produce aerial mycelium, and small aerial mycelium tends to be formed and then lysed. Positive physiological properties include gelatin liquefaction, starch hydrolysis, and milk peptonization. Negative properties include production of melanoid pigment on peptone-yeast extract-iron agar and tryptone-yeast extract broth, milk coagulation, and nitrate reduction. On ISP 9 medium, good or moderate growth is obtained with D-glucose, L-arabinose, D-xylose, L-rhamnose, and D-fructose, and poor growth is obtained with D-mannitol and sucrose. No growth is observed with iso-inositol or raffinose. The temperature range for growth is 15–42°C. Optimum growth occurs between 27 and 33°C. Cell Analysis of the cell-wall hydrolysates by paper chromatography demonstrates the presence of LL-A_2pm in addition to glycine. Thus, strain SF-2130[T] can be considered to have cell-wall type I. Produces a new β-lactam antibiotic.

Type strain shows no sequence similarity over 99%.

Source: soil.

DNA G+C content (mol%): not known.

Type strain: SF-2103, ATCC 31892, DSM 41679, NBRC 14260, JCM 5069, NRRL B-16438.

Sequence accession no. (16S rRNA gene): AB249974.

463. **Streptomyces sulphureus** (Gasperini 1894) Waksman *in* Waksman and Lechevalier 1961, 278[AL] ("*Actinomyces sulphureus*" Gasperini 1894, 78; "*Streptothrix sulphurea*" Caminiti 1907, 197; "*Nocardia sulfurea*" Vuillemin 1931, 129)

sul.phu′re.us. L. masc. adj. *sulphureus* of sulfur, sulphurous, referring to the bright sulfur-yellow color of the aerial mycelium.

Spore chains in Section *Spirales* or *Retinaculiaperti*. Short, crooked chains form irregular and imperfect spirals, hooks, or loops. These are not representative of the large hooks, loops, and primitive spirals seen in typical *Retinaculiaperti* cultures. This morphology is seen on yeast-malt agar and oatmeal agar and sometimes on glycerol-asparagine agar. Morphology may also be observed on carbon-utilization medium enriched with D-glucose. Spore surface is smooth.

Color of colony: aerial mass color in the Yellow color series (1½fb, light yellow, or 1½db, pale greenish yellow) on yeast-malt agar and oatmeal agar and sometimes on salts-starch agar or glycerol-asparagine agar if adequate aerial mycelium is developed. Reverse side of colony with no distinctive pigments (yellowish brown to olive brown on yeast-malt agar; grayish yellow, grayish greenish yellow, or olive on oatmeal agar, salts-starch agar, and glycerol-asparagine agar).

Color in medium: melanoid pigments are not formed in peptone-yeast-iron agar, tyrosine agar, or tryptone-yeast broth or are formed very weakly in peptone-yeast-iron agar and tryptone-yeast broth. No pigment is usually found in the medium in yeast-malt agar, oatmeal agar, salts-starch agar, or glycerol-asparagine agar, but one of three observers reports a pale violet pigment in oatmeal agar in 14 d. This pigment becomes pale yellowish brown with the addition of 0.05 M HCl.

Growth is poor on carbon-utilization medium enriched with D-glucose. Comparison with growth on D-glucose suggests equal or better utilization of L-arabinose, D-xylose, D-mannitol, D-fructose, and rhamnose, but utilization of all carbon sources should be verified on a basal medium supporting better growth. Sucrose apparently is not utilized, and reports vary on the utilization of raffinose and iso-inositol.

Type strain shows no sequence similarity over 99%.

Source: not known.

DNA G+C content (mol%): not known.

Type strain: ATCC 27468, CBS 646.72, BCRC 13764, DSM 40104, HUT 6080, NBRC 13345, IMET 40623, JCM 4085, JCM 4835, LMG 19355, NRRL B-1627, NRRL B-2195, NRRL-ISP 5104, RIA 1306, VKM Ac-1820.

Sequence accession no. (16S rRNA gene): DQ442546.

464. **Streptomyces synnematoformans** Hozzein and Goodfellow 2007, 2012[VP]

syn.ne.ma.to.for′mans. Gr. prep. *syn* in company with, together with; Gr. n. *nema* thread; N. Gr. n. *synnema* threads wrapping together; L. part. adj. *formans* forming; N.L. part. adj. *synnematoformans* synnemata-forming, referring to the ability of the organism to form synnemata.

Non-acid–alcohol-fast actinomycete; forms extensively branched substrate mycelium that carries aerial hyphae which differentiate into short straight to flexuous chains of smooth-surfaced spores. Forms synnemata-like structures but not sclerotia. Grayish to blackish red aerial spore mass and a dark red to reddish black substrate mycelium are formed on synthetic agar media. Deep grayish red diffusible pigment is produced on inorganic salts-starch agar. Does not hydrolyze arbutin or degrade adenine or uric acid. Cellobiose, D-fructose, D-galactose, D-glucose, maltose, D-mannose, and trehalose are used as sole carbon sources for energy and growth, but not L-arabitol, *meso*-erythritol, melezitose, D-salicin, D-sorbitol, or D-xylitol (all at 1%, w/v). Similarly, pyruvate is used as a sole carbon source, but not acetate, malonate, or succinate (all at 0.1%, w/v). Grows from 10°C to 37°C and from pH 6.5 to 9.5. Grows in the presence of 7% (w/v) sodium chloride.

Type strain shows no sequence similarity over 99%.

Source: the type strain was isolated from a sand dune soil collected at Borg El-Arab in Egypt.

DNA G+C content (mol%): not known.

Type strain: S155, CGMCC 4.2055, DSM 41902.

Sequence accession no. (16S rRNA gene): EF121313.

465. **Streptomyces syringium** (Konev 1986) Witt and Stackebrandt 1996, 836[VP] (Effective publication: Witt and Stackebrandt 1990, 370.) [*Streptoverticillium syringium* (*ex* Konev, Barashkova and Shenin 1974) Konev 1986].

Etymology is not known.

Spore chains in Section *Rectiflexibiles*. Spores are longish with a smooth surface, 0.5–0.6 × 0.8–1.4 µm in size. Aerial mycelium is well developed, white to light pink and violet color. Substrate mycelium is of a brown color. Soluble pigment is brown-greyish. No tyrosine or H_2S production. Utilizes D-glucose, rhamnose, D-fructose, and iso-inositol, but not lactate, sucrose, or raffinose. Weak growth on L-arabinose, D-xylose, and D-mannitol. Antagonistic against Gram-positive bacteria and fungi. Produces polyene.

For sequence similarity, see type strain of *Streptomyces netropsis.*

Source: not known.

DNA G+C content (mol%): not known.

Type strain: DSM 41480, DSM 41502, NBRC 15900, JCM 9948, LIA 0725, VKM Ac-1230.

Sequence accession no. (16S rRNA gene): AJ781375.

Further comments: this species was inadvertently omitted from Validation List no. 38 (Int. J. Syst. Bacteriol. 1991, 41, 456–457); the effective date of validation is that of list no. 38 (July 1991).

According to Hatano et al. (2003), *Streptomyces syringium* (Konev 1986) Witt and Stackebrandt 1991 is a later heterotypic synonym of *Streptomyces netropsis* (Finlay et al. 1951) Witt and Stackebrandt 1991.

466. **Streptomyces tanashiensis** Hata, Ohki and Higuchi 1952, 529[AL]

ta.na.shi.en′sis. N.L. masc. adj. *tanashiensis* of or belonging to Tanashi-machi, a town near Tokyo, Japan, the source of the soil from which the organism was isolated.

Spore chains in Section *Rectiflexibiles.* Mature spore chains generally have 10–50, or sometimes more than 50, spores per chain. This morphology is seen on yeast-malt agar, oatmeal agar, salts-starch agar and glycerol-asparagine agar. Spore surface is smooth.

Color of colony: aerial mass color in the Gray color series on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar. Reverse side of colony with no distinctive pigments (pale yellow to light olive brown) on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar.

Color in medium: melanoid pigments are formed in peptone-yeast-iron agar and tryptone-yeast broth, but not on tyrosine agar. No pigment is found in medium in yeast-malt agar, oatmeal agar, salts-starch agar, or glycerol-asparagine agar.

D-Glucose, L-arabinose, and D-xylose are utilized for growth. Reports vary on utilization of D-fructose. Only traces of growth are found with sucrose, iso-inositol, D-mannitol, rhamnose, and raffinose.

Type strain shows the highest sequence similarity to: *S. nashvillensis*, AB184286, 100%; *S. violaceorectus*, AB184314, 99.2%; *S. showdoensis*, AB184389, 99.1%; *S. cinereoruber* subsp. *cinereoruber*, AB184121, 99.1%; *S. roseoviridis*, AB184239, 99.1%; *S. racemochromogenes*, DQ026656, 99.1%; *S. viridobrunneus*, AJ781372, 99.1%; *S. roseolus*, AB184168, 99.1%; *S. polychromogenes*, AB184292, 99.1%; *S. bikiniensis*, X79851, 99.1%; *S. vietnamensis*, DQ311081, 99%; *S. flavotricini*, AB184132, 99%.

Source: isolated from soil from Tanashi-machi, a town near Tokyo, Japan.

DNA G+C content (mol%): not known.

Type strain: ATCC 23967, CBS 165.64, CBS 950.68, BCRC 12641, DSM 40195, HUT 6070, NBRC 12919, IMET 42939, JCM 4086, JCM 4671, NRRL B-1692, NRRL-ISP 5195, RIA 1148, VKM Ac-1892.

Sequence accession no. (16S rRNA gene): AJ781362.

467. **Streptomyces tauricus** (*ex* Ivanitskaya, Upiter, Sveshnikova and Gause 1966) Sveshnikova 1986, 575[VP] (Effective publication: Sveshnikova *in* Gause, Preobrazhenskaya, Sveshnikova, Terekhova and Maximova 1983.) ("*Actinomyces tauricus*" Ivanitskaya, Upiter, Sveshnikova and Gause 1966; "*Streptomyces tauricus*" Pridham 1970)

tau′ri.cus. L. masc. adj. *tauricus* of or belonging to the Taurians (a Thracian people, living in what is now Crimea), Albanian.

Spore chains in Section *Spirales* or *Retinaculiaperti*. Terminal spirals of several turns and some hooks or loops are found on long spore chains of 10–50 or more spores per chain. This morphology is seen on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar. Spore surface is smooth.

Color of colony: aerial mass color in the Red color series (5cb or 6ec, grayish yellowish pink; 5ca, light yellowish pink; 3ca, pale orange yellow; or 7ca, light yellowish pink) on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar. Reverse side of colony with distinctive red pigments. Grayish red, reddish brown, or yellowish pink on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar. Reverse mycelium pigment is a pH indicator, changing from orange or red to blue or violet with the addition of 0.05 M NaOH and from brownish red to yellowish red with the addition of 0.05 M HCl.

Color in medium: melanoid pigments are formed in peptone-yeast-iron agar and tryptone-yeast broth but weakly or not at all in tyrosine agar. Red (pink) pigment is sometimes found in the medium in yeast-malt agar, oatmeal agar, and glycerol-asparagine agar. When pigment is present in the medium it is pH-sensitive, showing the same changes observed with the reverse mycelium pigment.

D-Glucose, L-arabinose, D-xylose, iso-inositol, D-mannitol, D-fructose, rhamnose, sucrose, and raffinose are all utilized for growth.

Type strain shows the highest sequence similarity to: *S. glomeroaurantiacus*, AB249983, 99.1%; *S. aurantiacus*, AJ781383, 99%.

Source: not known.

DNA G+C content (mol%): not known.

Type strain: ATCC 27470, CBS 757.72, BCRC 12822, DSM 40560, NBRC 13456, IMET 43541, INA 8173, JCM 4837, NRRL B-12497, NRRL-ISP 5560, RIA 1417, VKM Ac-1853.

Sequence accession no. (16S rRNA gene): AB045879.

468. **Streptomyces tendae** Ettlinger, Corbaz and Hütter 1958a, 351[AL]

ten′da.e. N.L. gen. n. *tendae* of Tende, Germany, the source of the soil from which the organism was isolated.

Spore chains in Section *Retinaculiaperti* or *Spirales*. Straight to flexuous spore chains are most common on yeast-malt agar and spirals are best developed on salts-starch agar. Straight, flexuous, *Retinaculiaperti*, and spiral spore chains are recorded for oatmeal agar and glycerol-asparagine agar. Mature spore chains generally have 10–50 spores per chain, longer chains are sometimes observed. Spore surface is smooth.

Color of colony: aerial mass color in the Gray color series on yeast-malt agar, oatmeal agar, and salts-starch agar. Reverse side of colony is yellow or greenish yellow on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar. Reverse color is changed from yellow to orange by addition of 0.05 M HCl.

Color in medium: melanoid pigments not formed in peptone-yeast-iron agar, tyrosine agar, and tryptone-yeast extract broth. Yellow pigment found in medium in glycerol-asparagine agar; traces of yellow pigment may also diffuse into yeast-malt agar, oatmeal agar, and salts-starch agar. The yellow pigment is changed to yellowish orange or orange by 0.05 M HCl.

D-Glucose, L-arabinose, sucrose, D-xylose, iso-inositol, D-mannitol, D-fructose, and rhamnose are utilized for growth. No growth or only trace of growth on raffinose.

Type strain shows the highest sequence similarity to: *S. violaceorubidus*, AJ781374, 99.8%; *S. violaceoruber*, AF503492, 99.6%; *S. coelescens*, AF503496, 99.6%; *S. rubrogriseus*, AB184681, 99.6%; *S. anthocyanicus*, AB184631, 99.5%; *S. humiferus*, AF503491, 99.5%; *S. violaceolatus* AF503497, 99.5%; *S. lienomycini*, AJ781353, 99.5%; *S. tricolor*, AB184687, 99.4%; *S. matensis*, AB184221, 99.2%; *S. griseorubens*, AB184139, 99.1%; *S. mutabilis*, EF178679, 99.1%; *S. geysiriensis*, DQ442501, 99.1%; *S. minutiscleroticus*, EF178696, 99.1%; *S. ghanaensis*, AY999851, 99%; *S. rochei*, AB184237, 99%; *S. eurythermus*, D63870, 99%; *S. parvulus*, AB184326, 99%; *S. malachitospinus*, AB249954, 99%; *S. plicatus*, AB184291, 99%; *S. vinaceusdrappus*, AY999929, 99%.

Source: isolated from soil from Tende, Germany.

DNA G+C content (mol%): not known.

Type strain: AS 4.1460, ATCC 19812, CBS 565.68, BCRC 12167, DSM 40101, IFM 1176, NBRC 12822, IMET 40459, JCM 4149, JCM 4610, LMG 5987, LMG 19314, NCIMB 9614, NRRL B-2313, NRRL-ISP 5101, RIA 1092, RIA 534, UNIQEM 199, VKM Ac-1889.

Sequence accession no. (16S rRNA gene): D63873.

469. **Streptomyces termitum** Duché, Heim and Laboureur *in* Heim 1951, 359[AL]

ter.mi′tum. L. n. *termes -itis* woodworm, termite; L. gen. pl. n. *termitum* of termites, referring to the source of the organism.

Spore chains in Section *Rectiflexibiles*. Mature spore chains generally long and flexuous with 10–50 or often more than 50 spores per chain. This morphology is seen on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar. Spore surface is smooth.

Color of colony: aerial mass color in the Red color series (3ca, pale orange-yellow to 5cb, grayish yellowish pink) on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar. Reverse side of colony with no distinctive pigments (colorless to pale or grayish yellow) on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar.

Color in medium: melanoid pigments are not formed in peptone-yeast-iron agar, tyrosine agar, or tryptone-yeast broth. No pigment is found in medium in yeast-malt agar, oatmeal agar, salts-starch agar, or glycerol-asparagine agar.

D-Glucose and D-xylose are utilized for growth. Utilization of D-fructose is doubtful. Reports vary on utilization of sucrose and rhamnose (two of three observers find no growth on these two carbon sources). No growth or only trace of growth with L-arabinose, iso-inositol, D-mannitol, and raffinose.

Type strain shows no sequence similarity over 99%.

Source: not known.

DNA G+C content (mol%): not known.

Type strain: ATCC 25499, CBS 959.69, BCRC 12592, DSM 40329, NBRC 13087, IMET 43127, NCIMB 9980, NRRL B-3804, NRRL-ISP 5329, RIA 1279, JCM 4518.

Sequence accession no. (16S rRNA gene): AB184302.

470. **Streptomyces thermoalcalitolerans** Kim, Sahin, Minnikin, Zakrzewska-Czerwinska, Mordarski and Goodfellow 1999, 15[VP]

ther.mo.al.ca.li.to′le.rans. Gr. n. *therme* heat; N.L. n. *alcali* (from Arabic article *al* the; Arabic n. *qaliy* ashes of saltwort) alkali; L. part. adj. *tolerans* tolerating, enduring; N.L. part. adj. *thermoalcalitolerans* thermophilic alkali-tolerating.

Spiral chains of warty surfaced spores are borne on aerial hyphae. Forms extensively branched substrate and aerial hyphae. The aerial spore mass is gray; neither distinctive substrate mycelium colors nor diffusible pigments are formed. Melanin pigments are not produced on peptone-iron agar. Casein, DNA, gelatin, starch, testosterone, L-tyrosine, and xylan are degraded, but not adenine, arbutin, elastin, guanine, hypoxanthine, or xanthine. Adonitol, L-arabinose, arabitol, cellobiose, D-fructose, D-galactose, D-glucose, *myo*-inositol, α-lactose, D-mannitol, D-mannose, melezitose hydrate, melibiose, α-L-rhamnose, D-ribose, D-sorbitol, sucrose, trehalose, D-turanose, xylitol, and D-xylose are used as sole carbon sources for energy and growth, but D-raffinose is not. Growth occurs between 25 and 55°C, from pH 6.0 to 11.5 and in the presence of ampicillin (8 μg/ml), bacitracin (16 μg/ml), oleandomycin phosphate (16 μg/ml), penicillin G (15 international units), rifampin (16 μg/ml), streptomycin sulfate (4 μg/ml), tetracycline hydrochloride (16 μg/ml), and tunicamycin (10 μg/ml). In contrast, growth is inhibited in the presence of gentamicin sulfate (8 μg/ml), lincomycin hydrochloride (32 μg/ml), neomycin sulfate (8 μg/ml), novobiocin (4 μg/ml), oleandomycin phosphate (32 μg/ml), polymyxin B phosphate (32 μg/ml), rifampin (32 μg/ml), streptomycin sulfate (16 μg/ml), tetracycline hydrochloride (32 μg/ml), tobramycin sulfate (32 μg/ml), and vancomycin hydrochloride (16 μg/ml).

Type strain shows no sequence similarity over 99%.

Source: isolated from tropical garden soil collected by M. Goodfellow in 1991 from Yogyakarta, Central Java, Indonesia.

DNA G+C content (mol%): 73.0.

Type strain: TA56, DSM 41741, NBRC 16322, JCM 10673.

Sequence accession no. (16S rRNA gene): AB249909.

471. **Streptomyces thermoautotrophicus** Gadkari, Schricker, Acker, Kroppenstedt and Meyer 1991, 456[VP] (Effective publication: Gadkari, Schricker, Acker, Kroppenstedt and Meyer 1990, 3733.)

ther.mo.au.to.tro′phi.cus. Gr. adj. *thermos* hot; Gr. pref. *autos* self; Gr. adj. *throphikos* nursing, tending or feeding; N.L. masc. adj. *thermoautothrophicus* heat-loving self-nourishing, referring to the ability to grow at high temperature at the expense of CO or H_2 plus CO_2.

Forms chains of two to eight oval spores produced in a sheath residing on the substrate and scanty whitish aerial mycelium. No endospores, synnemata, sporangia, or sclerotia are found. Forms relatively stable branching vegetative hyphae with a diameter of 0.2–0.5 μm. Possesses LL-A_2pm and ribose (cell-wall type I). Mycolic acids are absent. Predominant menaquinone is MK-9(H_4); small amounts of MK-9(H_6) also occur. Phopholipid pattern is composed of phosphatidylethanolamine, diphosphatidylglycerol, phosphatidylglycerol, and phosphatidylinositol mannosides (phospholipid type 2). Iso- and anteiso-branched fatty acid patterns are found, with C_{17}, C_{17}, and C_{16} being the predominant fatty acids. Small amounts of cyclopropane fatty acids and traces of 2-hydroxy fatty acids are also present.

Source: not known.

DNA G+C content (mol%): 70.6.

Type strain: UBT1, DSM 41605.

Sequence accession no. (16S rRNA gene): no sequence available.

472. **Streptomyces thermocarboxydovorans** Kim, Falconer, Williams and Goodfellow 1998, 65[VP]

ther.mo.car.bo.xy.do.vo′rans. Gr. n. *therme* heat; N.L. n. *carboxydum* carbon monoxide; L. v. *voro* to eat greedily, devour; N.L. part. adj. *thermocarboxydovorans* thermophilic, carbon monoxide consuming.

Moderately thermophilic, facultatively chemolithotrophic actinomycete with extensively branched substrate and aerial mycelia. *Rectiflexibiles* chains of smooth-surfaced spores are borne on aerial hyphae. The aerial spore mass is gray; distinctive substrate mycelium colors are not produced. Diffusible pigments are not formed. Melanin pigments are not produced on peptone-iron agar. The organism oxidizes hydrogen and can use carbon monoxide as sole source of carbon for energy and growth. Arbutin, DNA, elastin, guanine, hypoxanthine, RNA, starch, L-tyrosine, and xanthine are degraded. Nitrate is reduced and hydrogen sulfide is produced. Neither allantoin nor urea are hydrolyzed. D-Fructose, D-lactose, sodium fumarate, and sodium pyruvate, but not adonitol or xylitol are used as sole carbon sources. L-Arginine, D-aminobutyric acid, L-cysteine, L-histidine, L-methionine, potassium nitrate, L-serine, L-threonine, and L-valine, but not L-hydroxyproline, are used as sole nitrogen sources. Positive activity is seen for chymotrypsin, cystine arylamidase, α-galactosidase and lipase but not for α-glucosidase (API ZYM). Similarly, *N*-acetyl-β-D-glucosamidase is produced but not β-D-cellobiosidase or β-D-xylosidase. Growth occurs between 20°C and 55°C and in the presence of adenine (0.5%, w/v), crystal violet (0.001%, w/v), phenyl ethanol (0.3%, w/v), potassium tellurite (0.001%, w/v), sodium azide (0.01%, w/v), chloramphenicol (2 μg/ml), chlortetracycline (4 μg/ml), gentamicin sulfate (1 μg/ml), novobiocin (0.5 μg/ml), oleandomycin phosphate (1 μg/ml), penicillin G (16 μg/ml), polymyxin B (32 μg/ml),

and tobramycin sulfate (1 μg/ml). In contrast, growth was inhibited in the presence of phenol (1%, w/v), sodium azide (0.02%, w/v), rifampin (0.5 μg/ml), and streptomycin sulfate (2 μg/ml).

Type strain shows the highest sequence similarity to: *S. thermospinosisporus*, AF333113, 99.1%.

Source: isolated from soil.

DNA G+C content (mol%): 74.7 ± 0.05.

Type strain: AT52, CIP 105544, DSM 44296, NBRC 16324, JCM 10367.

Sequence accession no. (16S rRNA gene): U94489.

473. **Streptomyces thermocarboxydus** Kim, Falconer, Williams and Goodfellow 1998, 66[VP]

ther.mo.car.bo′xy.dus. Gr. n. *therme* heat; N.L. n. *carboxydum* carbon monoxide; N.L. masc. adj. *carboxydus* pertaining to carbon monoxide.

Moderately thermophilic, facultatively chemolithotrophic actinomycete with extensively branched substrate and aerial mycelia. *Retinaculiaperti* chains of warty-surfaced spores are borne on aerial hyphae. Aerial spore mass is gray; distinctive substrate mycelium colors are not produced. Diffusible pigments are not formed. Melanin pigments are not produced on peptone-iron agar. Organism oxidizes hydrogen and can use carbon monoxide as a sole source of carbon for energy and growth. Esculin, arbutin, DNA, elastin, guanine, hypoxanthine, starch, testosterone, and xanthine are degraded. Nitrate is reduced and hydrogen sulfide is produced. Neither allantoin nor urea are hydrolyzed. D-Fructose, D-glucose, *myo*-inositol, D-lactose, mannitol, trehalose, and sodium propionate, but not adonitol, raffinose, sucrose, xylose, sodium acetate, sodium fumarate, or sodium pyruvate, are used as sole carbon sources. L-Arginine, D-aminobutyric acid, L-cysteine, L-histidine, potassium nitrate, L-serine, L-threonine, and L-valine, but not L-hydroxyproline or L-methionine, are used as sole nitrogen sources. Positive activity is seen for chymotrypsin, cystine arylamidase, α-glucosidase, lipase, and trypsin but not for α-galactosidase (API ZYM). *N*-Acetyl-β-D-galactosamidase, *N*-acetyl-β-D-glucosamidase, and α-L-arabinosidase are produced but not β-D-cellobiosidase, β-D-fucopyranosidase, α-D-mannosidase, or β-D-xylosidase. Growth occurs between 20 and 55°C and in the presence of adenine (0.5%, w/v), crystal violet (0.001%, w/v), phenyl ethanol (0.3%, v/v), potassium tellurite (0.01%, w/v), sodium azide (0.01%, w/v), chloramphenicol (32 μg/ml), chlortetracycline (4 μg/ml), gentamicin sulfate (8 μg/ml), neomycin sulfate (2 μg/ml), novobiocin (2 μg/ml), oleandomycin phosphate (32 μg/ml), penicillin G (16 μg/ml), polymyxin B (32 μg/ml), rifampin (4 μg/ml), streptomycin sulfate (2 μg/ml), tobramycin sulfate (8 μg/ml), and vancomycin (4 μg/ml).

Type strain shows the highest sequence similarity to: *S. lusitanus*, AB184424, 99.5%; *S. indiaensis*, AB184553, 99.3%; *S. longispororuber*, AB184440, 99.1%; *S. bellus*, AB184849, 99.1%; *S. cellulosae*, DQ442495, 99.1%; *S. coerulescens*, AY999720, 99.1%; *S. gancidicus*, AB184660, 99%; *S. lomondensis*, AB184673, 99%.

Source: not known.

DNA G+C content (mol%): 70.9 ± 0.4.

Type strain: AT37, CIP 105545, DSM 44293, NBRC 16323, JCM 10368.

Sequence accession no. (16S rRNA gene): U94490.

474. **Streptomyces thermocoprophilus** Kim, Al-Tai, Kim, Somasundaram and Goodfellow 2000, 506[VP]

ther.mo.co.pro.phi′lus. Gr. n. *therme* heat; Gr. n. *kopros* dung; Gr. adj. *philos* loving; N.L. masc. adj. *thermocoprophilus* dung-loving thermophile.

Moderately thermophilic actinomycete which forms a highly branched substrate mycelium and aerial hyphae which differentiate into long chains of straight spores which are cylindrical and have smooth surfaces (1.1–1.7 × 0.5 μm). Aerial spore mass color is gray. Diffusible pigments are formed on some standard media such as inorganic salts starch agar. Melanin pigments are produced on peptone-iron and tyrosine agars. Casein, starch, xanthine, and xylan are degraded but not adenine. L-Arabinose, D-fructose, D-galactose, D-glucose, *myo*-inositol, maltose, D-mannitol, D-mannose, and D-xylose are used as sole carbon sources for energy and growth but not carboxymethylcellulose, D-raffinose, starch, or sucrose. Growth occurs between 20 and 50°C and in the presence of ampicillin (10 μg/ml), erythromycin (15 μg/ml) and sodium chloride (7%, w/v) but not at 10 or 55°C. Growth is inhibited by chloramphenicol (30 μg/ml), gentamicin sulfate (15 μg/ml), kanamycin sulfate (30 μg/ml), neomycin sulfate (30 μg/ml), streptomycin sulfate (10 μg/ml), and tetracycline hydrochloride (30 μg/ml). Antimicrobial activity is shown against *Bacillus subtilis* NCIB 3610 but not towards *Escherichia coli* NCIB 9132 or *Staphylococcus aureus* ATCC 12600, or against representative strains of *Candida albicans*, *Curvularia lunata*, *Pestalotiopsis gnepini*, *Pyricularia oryzae*, and *Trichoderma viride*.

Type strain shows no sequence similarity over 99%.

Source: isolated from a sample of poultry feces collected from the poultry farm at the University of Malaya.

DNA G+C content (mol%): 68.6.

Type strain: B19, DSM 41700, JCM 10918, NBRC 100771.

Sequence accession no. (16S rRNA gene): AJ007402.

475. **Streptomyces thermodiastaticus** (Bergey, Harrison, Breed, Hammer and Huntoon 1923) Waksman 1953, 102[AL] (*Actinomyces thermodiastaticus* Bergey, Harrison, Breed, Hammer and Huntoon 1923)

ther.mo.di.a.sta′ti.cus. Gr. adj. *thermos* hot; N.L. adj. *diastaticus* starch-hydrolyzing; N.L. masc. adj. *thermodiastaticus* starch-hydrolyzing, heat-loving.

Spore chains in Section *Spirales*. Spirals are often irregular or imperfect; hooks or incomplete spirals and crooked spore chains are common. Mature spore chains are moderately short with 3 to 10 or more spores per chain. This morphology is seen on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar. Special incubation temperature: incubation temperatures used by ISP collaborators included 37, 45, 48, and 51°C; typical morphology, color reactions, and physiology are seen within this range. Spore surface is warty or spiny (very small "warts" or very blunt spines).

Color of colony: aerial mass color in the Gray color series on yeast-malt agar, oatmeal agar, salts-starch agar and glycerol-asparagine agar. Nearest matching color tabs in the Gray color series include 3fe, light brownish gray; 3ig, grayish yellowish brown; 3ge, light grayish yellowish brown; and 2ge, light olive brown. Colors are essentially the same at incubation temperatures from 37–51°C. Reverse side of colony is dark brown on yeast-malt agar; grayish yellow, yellowish brown, or olive brown on oatmeal agar, salts-starch agar, and glycerol-asparagine agar. When reddish (brown) pigment is present in the substrate mycelium, it is somewhat pH-sensitive, changing from reddish brown to olive brown or olive with the addition of 0.05 M HCl.

Color in medium: melanoid pigments are not formed in peptone-yeast-iron agar, tyrosine agar, and tryptone-yeast broth except that a grayish yellow or olive yellow pigment is found in tyrosine agar in 2–4 d. Faint reddish (brown) or yellow pigment may be found in the medium in yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar. When present, this pigment is pH-sensitive, changing from brownish to red with the addition of 0.05 M NaOH and from brownish to olive green with the addition of 0.05 M HCl.

D-Glucose, L-arabinose, D-xylose, iso-inositol, D-mannitol, D-fructose, rhamnose, and raffinose are utilized for growth. No growth or only traces of growth with sucrose. No difference in carbon utilization is seen on cultures incubated at 37 and 51°C.

Type strain shows the highest sequence similarity to: *S. thermospinosisporus*, AF333113, 99%.

Source: not known.

DNA G+C content (mol%): not known.

Type strain: ATCC 27472, CBS 769.72, BCRC 12492, BCRC 12636, DSM 40573, HUT 6606, JCM 4840, NBRC 100020, NRRL B-5316, NRRL-ISP 5573, RIA 1429.

Sequence accession no. (16S rRNA gene): AB018095.

476. **Streptomyces thermogriseus** Xu, Tiang, Zhang, Zhao and Jiang 1998, 1093[VP]

ther.mo.gri′se.us. Gr. adj. *thermos* hot; N.L. adj. *griseus* gray; N.L. masc. adj. *thermogriseus* hot, gray.

Spores are spherical, subspherical, or short rods. Spore surface is smooth. Brown or yellow vegetative hyphae are produced, becoming black after autolysis. Neither diffusible pigment nor melanin is produced. Milk is coagulated and peptonized. Hydrolyzes starch. Reduces nitrate. No H_2S production. No growth on cellulose. No degradation of urea. Utilizes rhamnose and mannitol. May or may not utilize glucose, fructose, xylose, raffinose, ribitol, and cellobiose. Does not utilize inositol. Lecithinase may or may not be produced. No resistance to neomycin or rifampin. No antimicrobial activities are detected. Grows at 65–68°C and in 0.1% phenol. Growth or no growth in 7% NaCl. The cell wall contains LL-A_2pm and glycine. The whole-cell hydrolysate contains galactose.

Type strain shows the highest sequence similarity to: *S. thermovulgaris*, Z68094, 100%.

Source: Strain Y-4027 (=CCTCC AA 97012) was isolated from soil samples of Kunming, Y-5114 (=CCTCC AA97013) was from Chenghai Lake and Y-14046^T (=CCTCC AA 97014^T) and Y-14082 (=CCTCC AA97015) were from a hot-spring in Eryuan, Yunnan, China.

DNA G+C content (mol%): not known.

Type strain: Y-14046, CCTCC AA 97014, CIP 105834, DSM 41756, JCM 11269, NBRC 100772.

Sequence accession no. (16S rRNA gene): AB249980.

477. **Streptomyces thermolineatus** Goodfellow, Lacey and Todd 1988, 329[VP] (Effective publication: Goodfellow, Lacey and Todd 1987a, 3147.)

ther.mo.lin.e.a′tus. Gr. fem. n. *therme* heat, L. part. adj. *lineatus* reduced to a straight line, to made straight; N.L. masc. adj. *thermolineatus* heat-loving linear (referring to the spore chains).

Spores in straight or flexuous chains less than 30 spores long. Spores are smooth but ends often prolonged, projecting from the oval spore body and retaining their shape under vacuum when the rest of the spore collapses, to give a phalangiform appearance (Tresner et al., 1966). Spores measure 1.0–2.1 × 0.9–1.3 μm (mean 1.43 × 1.06 μm). Good growth at 40°C on V-8 juice agar, producing abundant aerial mycelium in the green color series, 24ih to 24½ih. Reverse of colony is yellow-brown with no distinctive pigments. No melanoid pigment produced on peptone-iron agar. Degrades casein, gelatin, and starch. Uses trehalose and sometimes cellobiose and mannitol as sole carbon sources, and L-arginine, L-histidine, L-hydroxyproline, L-methionine, L-phenylalanine, potassium nitrate, L-serine, and L-valine as sole nitrogen sources. Reduces nitrate to nitrite and sometimes shows lipolytic and proteolytic activity on egg yolk.

Type strain shows no sequence similarity over 99%.

Source: isolated from sewage compost.

DNA G+C content (mol%): not known.

Type strain: K47, A1484, ATCC 51534, DSM 41451, HUT 6609, NBRC 14750, JCM 6307, NCIMB 12471.

Sequence accession no. (16S rRNA gene): Z68097.

478. **Streptomyces thermonitrificans** Desai and Dhala 1967, 137[AL]

ther.mo.ni.tri′fi.cans. Gr. n. *therme* heat; N.L. part. adj. *nitrificans* nitrifying; N.L. part. adj. *thermonitrificans* heat, nitrifying, referring to thermophily and vigorous nitrate reduction of the organism.

Spore chains in Section *Rectiflexibiles* or *Spirales*. Short spore chains often have only 3–10 spores per chain. On yeast-malt agar, salts-starch agar, and glycerol-asparagine agar, the chains are usually crooked or twisted. Longer chains may form irregular and imperfect spirals, especially on oatmeal agar and occasionally on salts-starch agar. Spore surface is smooth.

Color of colony: aerial mass color in the Gray color series when adequate sporulation occurs on yeast-malt agar, oatmeal agar, and salts-starch agar. Immature aerial mycelium is white; sporulating aerial mycelium is not found on glycerol-asparagine agar. Nearest matching color tabs are 3li and 4li, brownish gray; 3ge, light grayish yellowish brown; and 2ge, light olive brown. Reverse side of colony with no distinctive pigments (grayish olive to dark grayish brown on yeast-malt agar; grayish yellow to grayish yellow-

ish green or grayish olive on oatmeal agar, salts-starch agar, and glycerol-asparagine agar).

Color in medium: melanoid pigments are not formed in peptone-yeast-iron agar, tyrosine agar, or tryptone-yeast broth according to ISP observers [the original description by Desai and Dhala (1967) states that this strain is H_2S- and melanin-positive]. No pigment or only a trace of yellow is found in the medium in yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar.

D-Glucose, D-xylose, iso-inositol, D-mannitol, D-fructose, and sucrose are utilized for growth. No growth or only traces of growth with L-arabinose, rhamnose, and raffinose.

For sequence similarity, see type strain of *Streptomyces thermovulgaris*.

Source: not known.

DNA G+C content (mol%): not known.

Type strain: ATCC 23385, CBS 774.72, DSM 40579, NBRC 13473, NBRC 16616, IMET 43405, JCM 4841, LMG 19341, NCIM 2007, NRRL-ISP 5579, RIA 1434.

Sequence accession no. (16S rRNA gene): Z68098.

Further comments: according to Kim et al. (1999), *Streptomyces thermonitrificans* Desai and Dhala 1967 is a later heterotypic synonym of *Streptomyces thermovulgaris* Henssen 1957.

479. **Streptomyces thermospinosisporus** corrig. Kim and Goodfellow 2002, 1227[VP]

ther.mo.spi.ni.spo′rus. Gr. adj. *thermos* hot; L. adj. *spinosus* spiny; N.L. n *spora* a spore; N.L. masc. adj. *thermospinisporus* heat-loving, spiny spores.

Moderately thermophilic, facultatively carboxydotrophic actinomycete which forms extensively branched aerial and substrate hyphae. Aerial hyphae differentiate into flexuous chains of spores that show spiny ornamentation. Aerial spore mass is gray, but the substrate mycelium has no distinctive pigments. Soluble pigments are not produced, nor are melanin pigments formed on peptone-yeast extract-iron agar. Utilizes carbon monoxide and carbon dioxide as sole sources of carbon for energy and growth. Nitrate is reduced, and elastin, starch, and L-tyrosine are degraded. Growth is inhibited in the presence of phenol (0.1%, w/v), phenyl ethanol (0.3%, w/v), and sodium azide (0.02%, w/v). *myo*-Inositol, mannitol, raffinose, sucrose and sodium pyruvate are used as sole carbon sources when the organism is grown heterotrophically. Contains major amounts of LL-A_2pm, octahydrogenated menaquinones with nine isoprene units as the predominant isoprenolog, and major amounts of diphosphatidylglycerol and phosphatidylethanolamine.

Type strain shows the highest sequence similarity to: *S. thermocarboxydovorans*, U94489, 99.1%; *S. thermodiastaticus*, AB018095, 99%. Type strain shows DNA–DNA similarity to: *S. thermocarboxydovorans* DSM 44296^T, 72%; *S. thermodiastaticus* DSM 40573^T, 72%.

Source: not known.

DNA G+C content (mol%): 73.6.

Type strain: AT10, DSM 41779, JCM 11756, KCTC 9909, NBRC 100043.

Sequence accession no. (16S rRNA gene): AF333113.

Further comments: the original spelling *Streptomyces thermospinisporus* (*sic*) has been corrected by the List Editor, IJSEM.

480a. **Streptomyces thermoviolaceus subsp. thermoviolaceus** Henssen 1957, 388[AL]

ther.mo.vi.o.la′ce.us. Gr. n. *therme* heat; L. adj. *violaceus* violet colored; N.L. masc. adj. *thermoviolaceus* heat, violet colored (probably referring to the thermophilic nature of the species and the violet color of the aerial mycelium).

Spore chains in Section *Spirales*. Spirals are best developed on carbon-utilization medium plus raffinose. Imperfect spirals or loops may be common on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar. Spore surface is smooth.

Color of colony: aerial mass color in the Red color series (5dc, grayish yellowish pink) when adequate sporulating aerial mycelium is present, but sporulation is generally poor on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar at 28°C. ISP observers did not incubate at the higher temperatures (40–50°C) recommended by Henssen. Reverse side of colony with no distinctive pigments (olive brown on yeast-malt agar and oatmeal agar; grayish yellow to pale greenish yellow on salts-starch agar and glycerol-asparagine agar). The reverse mycelium pigment is pH-sensitive, changing from yellow or brown to gray or bluish gray with addition of 0.05 M NaOH and becomes reddish with the addition of 0.05 M HCl.

Color in medium: melanoid pigments are not formed in peptone-yeast-iron agar, tyrosine agar, or tryptone-yeast broth. Red or violet pigment is found in the medium in yeast-malt agar, oatmeal agar, and glycerol-asparagine agar. This pigment is pH-sensitive, changing from violet to blue with the addition of 0.05 M NaOH and from violet to red with the addition of 0.05 M HCl.

D-Glucose, D-xylose, D-mannitol, and D-fructose are utilized for growth. Utilization of L-arabinose is doubtful. No growth or only traces of growth with iso-inositol, rhamnose, sucrose, and raffinose.

Type strain shows the highest sequence similarity to: *S. thermoviolaceus* subsp. *apingens*, Z68095, 99.4%.

Source: not known.

DNA G+C content (mol%): not known.

Type strain: AS 4.1471, ATCC 19283, CBS 278.66, CBS 688.72, BCRC 12493, BCRC 12639, DSM 40443, HAMBI 1006, HUT 6604, NBRC 13905, IMET 43353, JCM 4337, JCM 4843, LMG 14943, LMG 19359, NCIMB 10076, NRRL B-12374, NRRL-ISP 5443, RIA 1348, VKM Ac-1857.

Sequence accession no. (16S rRNA gene): Z68096.

480b. **Streptomyces thermoviolaceus subsp. apingens** Henssen 1957, 390[AL]

a.pin′gens. Gr. pref. *a*- denoting negation, not; L. part. adj. *pingens* coloring; N.L. part. adj. *apingens* not coloring.

Aerial hyphae straight or branched, spore chains coiled, 20–40 μm long, spores 10–20, oval, 1.0–1.2 × 1.2–1.6 μm. Substrate mycelium is hardly branched. Colonies are yellowish to ochre brown, aerial mycelium white to violet gray. Physiological characteristics under

aerobic and anaerobic conditions at 50°C are as follows. On cellulose-dextrin agar: weak growth, aerial mycelium formed, colonies light brownish. On meat-extract agar: weak growth, aerial mycelium hardly formed, colonies yellow. On casein-glucose agar: weak growth, gray aerial mycelium. On asparagine-glycerol agar: good growth, gray aerial mycelium, colonies yellow. On Czapek's agar: weak growth, gray aerial mycelium. On yeast agar: weak growth, aerial mycelium white to gray. On yeast-glucose agar: good growth, thick, violet-gray aerial mycelium, colonies ochre brown. On potato agar I: good growth, thick, gray aerial mycelium, colonies ochre brown. On potato agar II: good growth, no aerial mycelium, colonies colorless to yellowish. On potato wedge: good growth, aerial mycelium at most marginal, colonies yellowish. Starch is quickly hydrolyzed. Grows on nitrate but does not form nitrite from nitrate. Milk is quickly coagulated and peptonized. Gelatin and nutrient-gelatin are mostly liquefied, colonies are orange yellow. This subspecies grows well at 50°C under exclusion of oxygen. Growth is weak at 28 and 60°C, aerial mycelium is not found; colonies are mostly yellow or brownish.

Type strain shows the highest sequence similarity to: *S. thermoviolaceus* subsp. *thermoviolaceus*, Z68096, 99.4%; *S. mexicanus*, AB249966, 99%.

Source: not known.

DNA G+C content (mol%): not known.

Type strain: ATCC 19994, CBS 140.67, DSM 41392, NBRC 15459, JCM 4312, NCIMB 10077.

Sequence accession no. (16S rRNA gene): Z68095.

481. **Streptomyces thermovulgaris** Henssen 1957, 391[AL]

ther.mo.vul.ga′ris. Gr. n. *therme* heat; L. adj. *vulgaris* common; N.L. masc. adj. *thermovulgaris* heat, common.

Spore chains in Section *Spirales* on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar when incubated at 40–50°C for 14 d. *Retinaculiaperti* or flexuous spore chains may also be found. At 28°C, some flexuous spore chains (only) may be found on these media, but aerial mycelium is usually absent or poorly developed at 28°C. Spore surface is smooth. Thermophilic with optimum growth at 40–50°C. Sclerotia-like masses of spore chains are common.

Color of colony: aerial mass color in the Gray color series (usually 3ig or 4ig, light grayish brown; 3fe, light brownish gray or 2dc, yellowish gray) on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar. At suboptimal temperatures, the aerial mycelium is white. Reverse side of colony with no distinctive pigments (light yellow or pale grayish yellow to olive-brown) on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar.

Color in medium: melanoid pigments are not formed in peptone-yeast-iron agar, tyrosine agar, or tryptone-yeast broth. No pigment is found in medium in yeast-malt agar, oatmeal agar, salts-starch agar, or glycerol-asparagine agar.

D-Glucose, L-arabinose, D-xylose, iso-inositol, D-mannitol, D-fructose, rhamnose, sucrose, and raffinose are all utilized for growth, but growth on rhamnose or raffinose may be less than on the other carbon sources tested.

Type strain shows the highest sequence similarity to: *S. thermogriseus*, AB249980, 100%.

Source: not known.

DNA G+C content (mol%): not known.

Type strain: AS 4.1455, ATCC 19284, ATCC 25501, CBS 276.66, CBS 643.69, BCRC 12488, BCRC 12638, DSM 40444, HUT 6605, NBRC 13089, NBRC 16615, JCM 4338, JCM 4520, NCIMB 10078, NRRL B-12375, NRRL-ISP 5444, RIA 1281, VKM Ac-1745.

Sequence accession no. (16S rRNA gene): Z68094.

Further comments: according to Kim et al. (1999), *Streptomyces thermovulgaris* Henssen 1957 is an earlier heterotypic synonym of *Streptomyces thermonitrificans* Desai and Dhala 1967.

482. **Streptomyces thioluteus** (Okami 1952) Witt and Stackebrandt 1991, 456[VP] (Effective publication: Witt and Stackebrandt 1990, 370.) (*Streptomyces thioluteus* Okami 1952, 30; *Verticillomyces thioluteus* Shinobu 1965, 161; *Streptoverticillium thioluteum* Baldacci, Farina and Locci 1966, 165)

thi.o.lu′te.us. Gr. n. *theion* (L. translit. *thium*) sulfur; L. adj. *luteus* yellow; N.L. masc. adj. *thioluteus* sulfur-yellow in color.

Good growth on potato-glucose agar (Baldacci et al., 1954). Color: reverse, orangish yellow; aerial mycelium, yellowish. On Bacto Czapek agar: very poor growth. Color: colorless; whitish tufts of aerial mycelium. On Casamino acids Czapek agar (1 g/l Difco vitamin-free Casamino acids, replacing sodium nitrate): limited growth. Color: reverse, colorless to yellowish; aerial mycelium, whitish yellow. On glucose asparagine agar (ISP medium 5 with 1% glucose replacing glycerol): good growth. Color: reverse, yellow to brown yellow; poor aerial mycelium, yellowish. On glucose-asparagine agar: good growth. Color: reverse, yellowish brown; aerial mycelium, yellowish. On inorganic salts-starch agar: good growth. Color: reverse, yellowish; aerial mycelium, yellowish. On yeast extract-malt extract agar: good growth. Color: reverse, brown; aerial mycelium, yellowish; soluble pigments present. On Bacto Emerson agar: good growth. Color: reverse, brown; aerial mycelium, greenish yellow; traces of soluble pigment. On Bennett agar (1% glucose, 0.1% Bacto beef agar, 0.1% yeast extract, 0.2% peptone, 1.5% agar): good growth. Color: reverse, yellow to brown-yellow; aerial mycelium, yellowish. On Oxoid nutrient agar: medium growth. Color: aerial mycelium, greenish yellow; reverse, brownish yellow; traces of aerial mycelium and pigment. Grows at 37°C; however, no aerial mycelium is produced in 15 d. No growth at 45°C. Some cultures form traces of H_2S and utilize rhamnose and casein. The type strain produces propiopyrrothine (aureothricin) and aureothin.

Type strain shows the highest sequence similarity to: *S. morookaense* AJ781349, 99.5%; *S. abikoensis*, AB184537, 99.3%; *S. lavenduligriseus*, DQ442515, 99.2%; *S. olivoverticillatus*, AB184636, 99.1%; *S. sapporonensis*, AB184508, 99%; *S. luteireticuli* AB249969, 99%; *S. hygroscopius* subsp. *angustmyceticus*, DQ442509, 99%.

Source: not known.

DNA G+C content (mol%): not known.

Type strain: ATCC 12310, CBS 642.72, BCRC 12428, DSM 40027, DSM 41486, HUT 6071, NBRC 13341, NBRC 3364, JCM 4087, JCM 4844, NRRL B-1667, NRRL-ISP 5027, RIA 1302, VKM Ac-1914.

Sequence accession no. (16S rRNA gene): AB184753.

Further comments: in violation of Rule 33c of the *Bacteriological Code* (1990 Revision), in Validation List no. 38, *Streptomyces thioluteus* is proposed as a *nomen revictum* (basonym: "*Streptomyces thioluteus*" Okami 1952).

483. **Streptomyces torulosus** Lyons and Pridham 1971, 192[AL]

to.ru.lo′sus. L. dim. n. *torulus* a small protuberance; L. masc. suff. *-osus* suffix used with the sense of full of, prone to; N.L. masc. adj. *torulosus* having small protuberances.

Spores are arranged in both dextrorse and sinistrorse coiled chains, with three to five volutions per coil. The color of the aerial mycelium observed with inorganic salts-starch agar after 14 d at 28°C was placed in the Gray series. A brown to black diffusible pigment is formed in peptone-iron agar. Melanin-like chromogenicity: Brown to black diffusible pigments are formed in tryptone-glucose-liver extract-yeast extract (Lyons and Pridham, 1965) broth. D-Glucose, D-xylose, L-arabinose, L-rhamnose, D-fructose, D-galactose, raffinose, D-mannitol, and iso-inositol are utilized; salicin is not. Growth is excellent on Czapek's solution-sucrose agar. The L-form of A_2pm is found in whole-cell hydrolysates. Faint spots for mannose and arabinose also can be detected. Strains are sensitive to streptomycin. The type strain is able to inhibit *Bacillus subtilis*, *Sarcina lutea*, *Escherichia coli*, and *Mucor ramannianus*. *Saccharomyces pastorianus* was very slightly inhibited, and *Candida albicans* was not inhibited.

Type strain shows the highest sequence similarity to: *S. hygroscopicus* subsp. *ossamyceticus*, AB184560, 100%; *S. neyagawaensis*, D63869, 99.3%; *S. ipomoeae*, AB184857, 99.2%.

Source: not known.

DNA G+C content (mol%): not known.

Type strain: ATCC 29340, CBS 801.71, DSM 40894, IFM 1283, NBRC 15460, JCM 4872, NRRL B-3889.

Sequence accession no. (16S rRNA gene): AJ781367.

484. **Streptomyces toxytricini** (Preobrazhenskaya and Sveshnikova *in* Gauze, Preobrazhenskaya, Kudrina, Blinov, Ryabova and Sveshnikova 1957) Pridham, Hesseltine and Benedict 1958, 68[AL] ("*Actinomyces toxytricini*" Preobrazhenskaya and Sveshnikova *in* Gauze, Preobrazhenskaya, Kudrina, Blinov, Ryabova and Sveshnikova 1957, 47)

to.xy.tri.ci′ni. N.L. n. *toxythricinum* toxythricin a suggested, but never used, antibiotic name; N.L. gen. n. *toxytricini* of toxithricin.

Spore chains in Section *Retinaculiaperti* or *Spirales*. Long spore chains of the *Retinaculiaperti* type as well as straight to flexuous sporophores are common on salts-starch agar, glycerol-asparagine agar, yeast-malt agar, and oatmeal agar. Spirals are most numerous as long chains of more than 50 spores on yeast-malt agar and oatmeal agar. Chains of 10 to 50 or more spores per chains are also found on salts-starch agar and glycerol-asparagine agar. Spore surface is smooth.

Color of colony: aerial mass color in the Red color series on yeast-malt agar, oatmeal agar, and salts-starch agar. Reverse side of colony with no distinctive pigment (grayed yellow to yellow-brown) on yeast-malt agar, oatmeal agar, salts-starch agar or glycerol-asparagine agar. Substrate pigment is not a pH indicator.

Color in medium: melanoid pigments formed in peptone-yeast-iron agar and tryptone-yeast extract broth. Pigments other than melanoids not formed in yeast-malt agar, oatmeal agar, salts-starch agar, or glycerol-asparagine agar.

D-Glucose is utilized for growth. Doubtful traces of growth or no growth on L-arabinose, sucrose, D-xylose, iso-inositol, rhamnose, and raffinose. Utilization of D-mannitol and D-fructose is also doubtful.

Type strain shows the highest sequence similarity to: *S. globosus*, AJ781330, 100%; *S. flavotricini*, AB184132, 99.3%; *S. racemochromogenes*, DQ026656, 99.1%; *S. polychromogenes*, AB184292, 99%.

Source: not known.

DNA G+C content (mol%): not known.

Type strain: ATCC 19813, CBS 566.68, BCRC 13472, DSM 40178, NBRC 12823, JCM 4421, NCIMB 9847, NRRL B-5426, NRRL-ISP 5178, RIA 1093, UNIQEM 200, VKM Ac-1279.

Sequence accession no. (16S rRNA gene): DQ442548.

485. **Streptomyces tricolor** (Wollenweber 1920) Waksman 1961, 158[AL] emend. Lanoot, Vancanneyt, Dawyndt Cnockaert, Zhang, Huang, Liu and Swings 2005a, 8 (Effective publication: Lanoot, Vancanneyt, Dawyndt Cnockaert, Zhang, Huang, Liu and Swings 2004, 90.) ("*Actinomyces tricolor*" Wollenweber 1920, 13)

tri′co.lor. L. masc. adj. *tricolor* of three colors.

Forms yellow-, red-, or blue-colored vegetative mycelium on some media; forms blue diffusible pigment on some media.

Type strain shows the highest sequence similarity to: *S. anthocyanicus*, AB184631, 100%; *S. rubrogriseus*, AB184681, 99.9%; *S. violaceoruber*, AF503492, 99.9%; *S. coelescens*, AF503496, 99.9%; *S. violaceolatus* AF503497, 99.8%; *S. humiferus*, AF503491, 99.8%; *S. lienomycini*, AJ781353, 99.7%; *S. violaceorubidus*, AJ781374, 99.5%; *S. tendae*, D63873, 99.4%; *S. coelicoflavus*, AB184650, 99.4%; *S. pactum*, AB184398, 99.1%; *S. olivaceus*, AB184743, 99%.

Source: isolated from flat scab of potato.

DNA G+C content (mol%): not known.

Type strain: CBS 103.21, DSM 41704, NBRC 15461, JCM 5065, NRRL B-16925.

Sequence accession no. (16S rRNA gene): AB184687.

Further comments: according to Lanoot et al. (2004), *Streptomyces tricolor* (Wollenweber 1920) Waksman 1961[AL] is an earlier heterotypic synonym of *Streptomyces roseodiastaticus* (Duché 1934) Waksman 1953.

486. **Streptomyces tubercidicus** Nakamura 1961, 90[AL]

tu.ber.ci′di.cus. L. n. *tuber* nodule; L. v. *caedo* to kill; N.L. masc. adj. *tubercidicus* nodule destroying, referring to antitumor activity of the antibiotic.

Spore chains in Section *Spirales*. Mature spore chains generally have 3 to 10 or more spores per chain. This morphology is seen on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar. Spore surface is smooth.

Color of colony: aerial mass color in the Gray color series (d, light gray to 3fe, light brownish gray) on yeast-malt agar, oatmeal agar, and salts-starch agar in 14 d. One observer places 21 d culture in the Red color series (5dc, grayish yellowish pink) on yeast-malt agar, oatmeal agar, and glycerol-asparagine agar. The original description notes a tendency for the aerial mycelium to become moist and black; this hygroscopic characteristic is not recorded by ISP observers. Reverse side of colony is grayish yellow to yellowish brown or olive-brown on oatmeal agar, salts-starch agar, and glycerol-asparagine agar; moderate brown to dark brown on yeast-malt agar. Reverse mycelium pigment is a pH indicator, changing from yellow or yellow-brown to pale pink with addition of 0.05 M NaOH and from pink or grayish brown to yellow with addition of 0.05 M HCl.

Color in medium: melanoid pigments are not formed in peptone-yeast-iron agar, tyrosine agar, or tryptone-yeast broth. Yellow to pink or violet-pink (depending upon pH) pigment is found in the medium in yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar. This pigment is pH-sensitive showing the same changes noted for the reverse mycelium pigment.

D-Glucose, iso-inositol, D-mannitol, D-fructose, sucrose, and raffinose are utilized for growth. No growth or only trace of growth with L-arabinose, D-xylose, and rhamnose.

Type strain shows the highest sequence similarity to: *S. libani* subsp. *libani*, AB184414, 100%; *S. nigrescens*, DQ442530, 99.9%; *S. hygroscopius* subsp. *glebosus*, AB184479, 99.6%; *S. libani* subsp. *rufus*, AJ781351, 99.6%; *S. caniferus*, AB184640, 99.5%; *S. catenulae*, AJ621613, 99.5%; *S. misakiensis*, AB217605, 99.5%; *S. sioyaensis*, DQ026654, 99.5%; *S. platensis*, AB045882, 99.5%; *S. hygroscopius* subsp. *decoyicus*, AY999883, 99.2%; *S. lydicus*, Y15507, 99.1%; *S. chattanoogensis*, AJ621611, 99%.

Source: not known.

DNA G+C content (mol%): not known.

Type strain: AS 4.1414, ATCC 25502, CBS 644.69, BCRC 11886, CECT 3272, DSM 40261, IFM 1064, NBRC 13090, IMET 43517, JCM 4054, JCM 4558, KCTC 9109, LMG 19361, NRRL B-5440, NRRL-ISP 5261, RIA 1282, VKM Ac-1073.

Sequence accession no. (16S rRNA gene): AJ621612.

487. **Streptomyces tuirus** Albert and Malaquias de Queiroz 1963, 43[AL]

tu'ir.us. Nheêngatû Amazonian dialect *tuira* violet, violet-blue; N.L. masc. adj. *tuirus* violet, violet-blue (referring to the color of vegetative mycelium and diffusible pigment).

Spore chains in Section *Spirales*. Spiral spore chains often arise in verticils from long axial hyphae, suggesting monoverticillate morphology. Spirals are usually open and wavy; hooked spore chains are also present. This morphology is seen on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar. Spore surface is smooth.

Color of colony: aerial mass color in the Gray color series (5fe, light grayish reddish brown; 2dc, yellowish gray; d-e, light to medium gray) on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar. Reverse side of colony with distinctive red pigments on yeast-malt agar, salts-starch agar, and glycerol-asparagine agar. Reverse color is reddish black on yeast-malt agar and grayish reddish brown, dark pale red, or dark grayish pink on salts-starch agar and glycerol-asparagine agar; grayish brown on oatmeal agar. One of three observers reports that the reverse mycelium pigment is a pH indicator, changing from red to violet with the addition of 0.05 M NaOH and to a more intensive red with the addition of 0.05 M HCl.

Color in medium: melanoid pigments are formed in peptone-yeast-iron agar and tryptone-yeast broth. Melanin reaction may be weak or absent in tyrosine agar. Red, reddish brown, or violet pigment is found in the medium in yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar. According to one observer, only, this pigment is pH-sensitive, showing the same changes observed in the reverse mycelium pigment.

D-Glucose, L-arabinose, D-xylose, iso-inositol, D-mannitol, D-fructose, rhamnose, sucrose, and raffinose are all utilized for growth.

Type strain shows the highest sequence similarity to: *S. mutabilis*, EF178679, 99.6%; *S. rochei*, AB184237, 99.5%; *S. vinaceusdrappus*, AY999929, 99.5%; *S. plicatus*, AB184291, 99.5%; *S. minutiscleroticus*, EF178696, 99.5%; *S. ghanaensis*, AY999851, 99.5%; *S. geysiriensis*, DQ442501, 99.5%; *S. levis*, AB184670, 99.4%; *S. djakartensis*, AB184657, 99.4%; *S. luteogriseus*, AB184379, 99.4%; *S. capillispiralis*, AB184577, 99.2%; *S. janthinus*, AB184851, 99.2%; *S. albosporeus* subsp. *albosporeus*, AJ781327, 99.2%; *S. roseoviolaceus*, AJ399484, 99.2%; *S. azureus*, EF178674, 99.2%; *S. violaceus*, AB184315, 99.2%; *S. africanus*, AY208912, 99.1%; *S. gancidicus*, AB184660, 99.1%; *S. pseudogriseolus*, DQ442541, 99.1%; *S. afghaniensis*, AJ399483, 99.1%; *S. calvus*, AB184329, 99.1%; *S. virens*, DQ442554, 99%; *S. cellulosae*, DQ442495, 99%; *S. violaceorubidus*, AJ781374, 99%; *S. anandii*, AB184402, 99%; *S. flavoviridis*, AB184842, 99%; *S. lomondensis*, AB184673, 99%; *S. pilosus*, AB184161, 99%; *S. aureorectus*, AB184710, 99%; *S. asterosporus*, AB184706, 99%.

Source: not known.

DNA G+C content (mol%): not known.

Type strain: ATCC 19007, CBS 719.72, BCRC 12217, DSM 40505, NBRC 15617, JCM 4255, JCM 4846, NRRL B-3631, NRRL-ISP 5505, RIA 1379.

Sequence accession no. (16S rRNA gene): AB184690.

488. **Streptomyces turgidiscabies** Miyajima, Tanaka, Takeuchi and Kuninaga 1998, 500[AL]

tur.gi.di.sca'bi.es. L. adj. *turgidus* swollen; L. fem. n. *scabies* scabbiness; N.L. fem. n. *turgidiscabies* a swollen scabbiness.

Spores are 0.5–0.6 × 1.0–1.2 µm, smooth, gray, and are borne in mature flexuous chains containing 8 or more spores. Diffusible pigments and melanin are not produced. Positive for peptonization but not for coagulation of milk, is not susceptible to 25 µg/ml oleandomycin or 10 IU/ml penicillin G and is not positive for utilization

of sodium acetate or sodium propionate as sole carbon sources. L-Arabinose, D-fructose, D-glucose, D-mannitol, iso-inositol, rhamnose, sucrose, D-xylose, and raffinose are used as carbon sources. None of the strains studied degrade xanthine or grow at 37°C and all are susceptible to 20 µg/ml streptomycin, 0.5 µg/ml crystal violet, 100 µg/ml potassium tellurite, and 0.1% phenol. Cell wall contains the LL-A_2pm isomer. *S. turgidiscabies* differs from other potato scab pathogens in having gray, smooth, flexuous spore chains.

Type strain shows the highest sequence similarity to: *S. reticuliscabiei*, AJ007428, 99.1%.

Source: the type strain was isolated from raised scab lesions on potato tubers in eastern Hokkaido, Japan, in 1991, and has been confirmed to be pathogenic.

DNA G+C content (mol%): 70.9–72.5.

Type strain: SY9113, ATCC 700248, CIP 105577, NBRC 16080, JCM 10429, NRRL B-24078.

Sequence accession no. (16S rRNA gene): AB026221.

Further comments: a numerical analysis of phenotypic characteristics showed that *Streptomyces reticuliscabiei* Bouchek-Mechiche et al. 2000 and *Streptomyces turgidiscabies* Miyajima et al. 1998 belong to the same cluster and share almost all morphological and biochemical traits that are important in the identification of *Streptomyces* species. DNA–DNA hybridization and phylogenetic comparisons of 16S rRNA gene sequences confirm that the two species are genomically closely related. In contrast, pathological data showed that *Streptomyces turgidiscabies* and *Streptomyces reticuliscabiei* cause two distinct diseases. For the pathologist, the fusion of *Streptomyces reticuliscabiei* and *Streptomyces turgidiscabies* under a single species denomination would cause confusion of separate diseases and create a discrepancy between taxonomists and pathologists. Therefore, Bouchek-Mechiche et al. think that the two groups should continue to carry their current denominations, i.e. *Streptomyces reticuliscabiei* Bouchek-Mechiche et al. 2000 for the strains inducing netted scab and *Streptomyces turgidiscabies* Miyajima et al. 1998 for those causing common scab.

489. **Streptomyces umbrinus** (Sveshnikova *in* Gauze, Preobrazhenskaya, Kudrina, Blinov, Ryabova and Sveshnikova 1957) Pridham, Hesseltine and Benedict 1958, 61[AL] ("*Actinomyces umbrinus*" Sveshnikova *in* Gauze, Preobrazhenskaya, Kudrina, Blinov, Ryabova and Sveshnikova 1957, 62)

um.bri′nus. N.L. masc. adj. *umbrinus* wood brown, the color of the aerial mycelium.

Spore chains in Section *Rectiflexibiles*. Mature spore chains are moderately long with 10 to 50 or more spores per chain. This morphology is seen on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar. Spore surface is smooth.

Color of colony: aerial mass color in the Red color series (3ca, pale orange-yellow to 5cb, grayish yellowish pink) on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar. Reverse side of colony is reddish brown on yeast-malt agar, light brown to strong brown on oatmeal agar and salts-starch agar, gray to reddish black on glycerol-asparagine agar. Reverse mycelium pigment is not a pH indicator.

Color in medium: melanoid pigments are formed in peptone-yeast-iron agar, tyrosine agar, and tryptone-yeast broth. Yellow pigment may be found in the medium in yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar. This pigment is not pH-sensitive when tested with 0.05 M NaOH or HCl.

D-Glucose, L-arabinose, D-xylose, iso-inositol, D-mannitol, D-fructose, rhamnose, sucrose, and raffinose are all utilized for growth.

Type strain shows the highest sequence similarity to: *S. ederensis*, AB184658, 100%; *S. phaeochromogenes*, AB184738, 99.4%.

Source: not known.

DNA G+C content (mol%): not known.

Type strain: ATCC 19929, ATCC 25503, CBS 645.69, DSM 40278, NBRC 13091, INA 1703/53, JCM 4521, NRRL B-2572, NRRL-ISP 5278, RIA 1283, VKM Ac-1747.

Sequence accession no. (16S rRNA gene): AB184305.

490. **Streptomyces variabilis** (Preobrazhenskaya, Ryabova and Blinov *in* Gauze, Preobrazhenskaya, Kudrina, Blinov, Ryabova and Sveshnikova 1957) Pridham, Hesseltine and Benedict. 1958, 70[AL] ("*Actinomyces variabilis*" Preobrazhenskaya, Ryabova and Blinov *in* Gauze, Preobrazhenskaya, Kudrina, Blinov, Ryabova and Sveshnikova 1957)

va.ri.a′bi.lis. L. masc. adj. *variabilis* variable.

Spore chains in Section *Spirales*. Open spirals are conspicuous on salts-starch agar, rare on yeast-malt agar. Flexuous spore chains, loops, hooks, and spirals suggestive of Section *Retinaculiaperti* are observed on oatmeal agar, salts-starch agar, and glycerol-asparagine agar. Spore surface is spiny. Spines are short; one observer reports warty to smooth spores.

Color of colony: aerial mass color in the Gray color series on oatmeal agar and salts-starch agar; Gray or Red series on yeast-malt agar. Aerial spore mass is not well-developed on glycerol-asparagine agar. Reverse side of colony with no distinctive pigment on oatmeal agar, salts-starch agar, and glycerol-asparagine agar; reddish brown on yeast-malt agar.

Color in medium: melanoid pigments not formed in peptone-yeast-iron agar or tyrosine agar. No pigment found in medium in yeast-malt agar, oatmeal agar, salts-starch agar, or glycerol-asparagine agar.

D-Glucose, L-arabinose, D-xylose, iso-inositol, D-mannitol, D-fructose, and rhamnose are utilized for growth. No growth or only trace of growth on sucrose and raffinose.

Type strain shows the highest sequence similarity to: *S. erythrogriseus*, AJ781328, 100%; *S. labedae*, AB184704, 100%; *S. griseoincarnatus*, AJ781328, 100%; *S. griseorubens*, AB184139, 99.9%; *S. griseoflavus*, AJ781322, 99.6%; *S. matensis*, AB184221, 99.6%; *S. althioticus*, AY999808, 99.2%; *S. paradoxus*, AB184628, 99.2%; *S. heliomycini*, AB184712, 99.1%; *S. collinus*, AB184123, 99.1%; *S. flaveolus*, AB184764, 99.1%; *S. viridochromogenes*, DQ442555, 99%; *S. bellus*, AB184849, 99%; *S. viridodiastaticus*, AY999852, 99%; *S. violaceochromogenes*, AY999867, 99%; *S. malachitofuscus*, AB184282, 99%; *S. violaceorubidus*, AJ781374, 99%; *S. coerulescens*, AY999720, 99%.

Source: not known.

DNA G+C content (mol%): not known.

Type strain: ATCC 19815, ATCC 19930, CBS 568.68, BCRC 11488, DSM 40179, NBRC 12825, IMET 42059, JCM 4422, NRRL B-3984, NRRL-ISP 5179, RIA 1095, UNIQEM 202, VKM Ac-1854.

Sequence accession no. (16S rRNA gene): DQ442551.

491. **Streptomyces variegatus** Sveshnikova and Timuk 1986, 575[VP] (Effective publication: Sveshnikova and Timuk *in* Gause, Preobrazhenskaya, Sveshnikova, Terekhova and Maximova 1983.)

va.ri.e.ga′tus. L. masc. part. adj. *variegatus* variegated, made of various colors.

Spore chains are spiral (*Spirales*); spores are smooth. On mineral agar 1: aerial mycelium is pink to loud pink; substrate mycelium is raspberry red and green, grayish brownish green. Intensity of building pigments is different, normally first red colored, later covered by green color; no diffusible pigment. Curious color of substrate mycelium is due to pigment types prodigiosin and ferroverdin. On starch-ammonia agar: aerial mycelium grows late, light pink, poor; substrate mycelium is light pink with weak violet shadow; no diffusible pigment. On glycerol-nitrate agar: aerial mycelium is white, poorly developed, sometimes absent; substrate mycelium is green with raspberry colored segments, sometimes brindled green, greenish grayish brown, light yellow, and grayish brownish pink at the same time; no diffusible pigment. On glycerol-asparagine agar: aerial mycelium is whitish to light pink and pink; substrate mycelium is brindled light yellow, orange, raspberry colored, grayish brownish red, and dark green; no diffusible pigment. On glucose-asparagine agar: aerial mycelium whitish to light pink and pink; substrate mycelium is brindled yellow, green, and light raspberry colored; no diffusible pigment. On agar Sp I Krasil'nikov: weak growth; no aerial mycelium; colorless substrate mycelium; no diffusible pigment. On oatmeal agar: aerial mycelium is whitish to light pink; substrate mycelium is yellow, pale orange, pink to raspberry colored; green pigment is weak; no diffusible pigment. On organic agar 2: aerial mycelium is whitish to pink; substrate mycelium is brindled, pale red, pale greenish; no diffusible pigment. Melanoid pigments are not formed. Grows on fructose, xylose, and arabinose; no growth on sucrose, rhamnose, or raffinose. Antibiotic: antibiotic of group α-oxi-keto-Pentaen; pigment with antibiotic character of the prodigiosin group.

Type strain shows the highest sequence similarity to: *S. fulvissimus*, AB184434, 99.1%; *S. alboflavus*, EF178699, 99%.

Source: not known.

DNA G+C content (mol%): not known.

Type strain: ATCC 43696, DSM 41464, NBRC 15462, INA T-511, JCM 6930, VKM Ac-846.

Sequence accession no. (16S rRNA gene): AJ781371.

492. **Streptomyces varsoviensis** Kurylowicz and Woźnicka 1967, 1[AL]

var.so.vi.en′sis. N.L. masc. adj. *varsoviensis* of or pertaining to Varsovia; named for Warsaw, Poland.

Spore chains in Section *Spirales*, *Retinaculiaperti*, or *Rectiflexibiles*. Spiral spore chains are abundant on yeast-malt agar; straight to flexuous chains are most common on glycerol-asparagine agar. Flexuous, spiral, or intermediate (*Retinaculiaperti*) forms may be found on oatmeal agar and salts-starch agar but sporulating aerial mycelium is usually poorly developed on these media. Spore surface is smooth.

Color of colony: aerial mass color in the White or Yellow (2ba, pale yellow) color series on oatmeal agar and salts-starch agar. Sporulation on oatmeal agar and salts-starch agar is usually inadequate for spore mass color determination. Reverse side of colony is yellow to yellow-brown and modified by red (to yellowish pink, orange, grayish reddish orange, or strong brown) on yeast-malt agar, salts-starch agar, and glycerol-asparagine agar. Substrate pigment is not a pH indicator or is modified only slightly by addition of 0.05 M NaOH or HCl.

Color in medium: reports vary on production of melanoid pigments. Some darkening of peptone-yeast-iron agar, tyrosine agar, and tryptone-yeast broth may be seen in 4 d, but usually not in 2 d. Gause's organic medium no. 2 is not darkened in 2–4 d. No pigment is found in medium in yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar.

D-Glucose and D-mannitol are utilized for growth. Utilization of fructose is doubtful. No growth or only trace of growth with L-arabinose, D-xylose, iso-inositol, rhamnose, sucrose, and raffinose.

Type strain shows the highest sequence similarity to: *S. sapporonensis*, AB184508, 99.5%; *S. abikoensis*, AB184537, 99.2%; *S. hygroscopius* subsp. *angustmyceticus*, DQ442509, 99.1%; *S. luteireticuli* AB249969, 99.1%; *S. ehimensis*, AY999834, 99%.

Source: not known.

DNA G+C content (mol%): not known.

Type strain: AS 4.1431, ATCC 14631c, ATCC 25505, CBS 357.64, CBS 647.69, BCRC 12647, DSM 40346, HAMBI 1046, NBRC 13093, IMET 43351, JCM 4303, JCM 4523, NCIMB 9522, NRRL B-3589, NRRL-ISP 5346, RIA 1285, VKM Ac-1000.

Sequence accession no. (16S rRNA gene): DQ026653.

493. **Streptomyces vastus** Szabó and Marton 1958, 245[AL]

vas′tus. L. masc. adj. *vastus* empty, unoccupied, waste, referring to the occurrence of the organism in the Hortobagy Puszta (eastern Hungary).

Spore chains in Section *Spirales*. Open, terminal spirals of only one to three turns may be seen on yeast-malt agar, oatmeal agar, and salts-starch agar, but sporulating aerial mycelium is usually poorly developed on these media. Sporulation may be absent on glycerol-asparagine agar and Gause's medium no. 1. Poor sporulation of this strain on various media is noted in Szabó and Marton's original description (op. cit.). Spore chains are often short with 3 to 10 or more spores per chain. Spore surface is smooth.

Color of colony: aerial mass color in the White or Gray color series on yeast-malt agar and oatmeal agar when mature sporulating aerial mycelium is formed. Thin,

white aerial mycelium may also be seen on salts-starch agar and glycerol-asparagine agar but is inadequate for accurate color determination. Reverse side of colony is grayish green on yeast-malt agar; colorless to pale blue or grayish blue on oatmeal agar and salts-starch agar; colorless to pale yellow on glycerol-asparagine agar. Reverse mycelium pigment is not a pH indicator.

Color in medium: melanoid pigments are not formed in peptone-yeast-iron agar, tyrosine agar, or tryptone-yeast broth. No pigment is found in medium in oatmeal agar or glycerol-asparagine agar. Traces of yellow or green pigment may or may not be found in yeast-malt agar and a trace of blue pigment may be found in salts-starch agar. This pigment, if present, is not a pH indicator when tested with 0.05 M NaOH or HCl.

D-Glucose, L-arabinose, iso-inositol, D-mannitol, D-fructose, rhamnose, sucrose, and raffinose are utilized for growth. Reports vary on utilization of D-xylose.

Type strain shows no sequence similarity over 99%.

Source: not known.

DNA G+C content (mol%): not known.

Type strain: ATCC 25506, CBS 290.60, CBS 648.69, DSM 40309, NBRC 13094, JCM 4524, NRRL B-12232, NRRL-ISP 5309, RIA 1286, VKM Ac-1871.

Sequence accession no. (16S rRNA gene): DQ442552.

494. **Streptomyces venezuelae** Ehrlich, Gottlieb, Burkholder, Anderson and Pridham 1948, 467[AL]

ve.ne.zu.e′la.e. N.L. gen. n. *venezuelae* of Venezuela.

Spore chains in Section *Rectiflexibiles.* Straight spore chains are generally long, often with more than 50 spores per chain. This morphology is seen on oatmeal agar, salts-starch agar, and glycerol-asparagine agar; sporulation may be poor on yeast-malt agar. One observer, only, records fragmentation of substrate mycelium on glycerol-asparagine agar. Spore surface is smooth.

Color of colony: aerial mass color in the Gray color series (2dc, yellowish gray to 5fe, light grayish reddish brown) on oatmeal agar, salts-starch agar, and glycerol-asparagine agar; a good spore is usually not produced on yeast-malt agar. Reverse side of colony with no distinct pigments (grayish yellow) on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar.

Color in medium: melanoid pigments are formed in peptone-yeast-iron agar and tryptone-yeast broth, but only weakly or not at all in tyrosine agar. No pigment is found in medium in yeast-malt agar, oatmeal agar, salts-starch agar, or glycerol-asparagine agar.

D-Glucose, L-arabinose, D-xylose, D-fructose, and rhamnose are utilized for growth. A trace of growth is usually found in iso-inositol, D-mannitol, sucrose, and raffinose.

Type strain shows the highest sequence similarity to: *S. zaomyceticus*, EF178685, 99.9%; *S. exfoliatus*, AB184324, 99.9%; *S. lateritius*, AB184324, 99.9%; *S. wedmorensis*, DQ442557, 99.7%; *S. litmocidini*, AB184149, 99.6%; *S. omiyaensis*, EF178697, 99.6%; *S. yereyanensis* EF178684, 99.4%; *S. narbonensis*, DQ445794, 99.3%.

Source: not known.

DNA G+C content (mol%): not known.

Type strain: ATCC 10712, ATCC 25508, CBS 650.69, BCRC 11512, DSM 40230, NBRC 12595, NBRC 13096, IMET 41356, JCM 4526, LMG 19308, NRRL 2277, NRRL-ISP 5230, RIA 1288, RIA 70, VKM Ac-589.

Sequence accession no. (16S rRNA gene): AB045890.

495. **Streptomyces vietnamensis** Zhu, Guo, Yao, Yang, Deng, Phuong, Hanh and Ryan 2007, 1773[VP]

vi.et.nam.en′sis. N.L. masc. adj. *vietnamensis* of or pertaining to Vietnam, the geographical location from where the type strain was isolated.

Straight to flexuous chains of cylindrical spores are produced. Forms a white aerial mycelium and a reddish-brown substrate mycelium. Verticils are not present. The mycelium does not fragment. Diffusible pigments are produced on ISP 2, ISP 3, ISP 4, and ISP 5 media and on Gause's synthetic agar, but not on Czapek's solution agar. Melanin is produced on ISP 7. Although growth on ISP 4 is initially slow, very good growth with profuse sporulation is observed on this medium after 14 d. Very good growth occurs on ISP 2, Gause's synthetic agar, and ISP 3. Moderate growth is observed on ISP 5 but only poor growth on Czapek agar. The substrate mycelium is reddish brown on ISP 2, ISP 5, Gause's synthetic agar, ISP 4, and ISP 3, but grayish orange on Czapek medium. Utilizes melibiose, glucose, sorbinose, sucrose, D-fructose, xylose, D-galactose, rhamnose, and arabinose. Positive for production of H_2S, but pectin is not hydrolyzed. Cell wall contains LL-A_2pm (cell-wall type I). Whole-cell sugar pattern contains diagnostic sugars: mannose, small quantities of ribose and galactose. No antibiosis is exhibited against *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 6538, *Bacillus subtilis* ATCC 6633, *Candida albicans* ATCC 10231, or *Penicillium citrinum* AS 3.2788.

Type strain shows the highest sequence similarity to: *S. bikiniensis*, X79851, 99.5%; *S. violaceorectus*, AB184314, 99.4%; *S. viridobrunneus*, AJ781372, 99.1%; *S. tanashiensis*, AJ781362, 99%; *S. showdoensis*, AB184389, 99%; *S. cinereoruber* subsp. *cinereoruber*, AB184121, 99%. Type strain shows DNA–DNA similarity to: *S. bikiniensis* ATCC 11062^T, 50.3%.

Source: the type strain was isolated from a forest soil sample in Vietnam.

DNA G+C content (mol%): 73.9.

Type strain: GIMV4.0001, CCTCC M 205143, IAM 15340, JCM 21785.

Sequence accession no. (16S rRNA gene): DQ311081.

496. **Streptomyces vinaceus** Jones 1952, 47[AL] emend. Lanoot, Vancanneyt, Dawyndt, Cnockaert, Zhang, Huang, Liu and Swings 2005a, 8 (Effective publication: Lanoot, Vancanneyt, Dawyndt Cnockaert, Zhang, Huang, Liu and Swings 2004, 90.)

vi.na′ce.us. L. masc. adj. *vinaceus* of or belonging to wine or the grape, referring to the color of the aerial mycelium.

Spore chains in Section *Retinaculiaperti* or *Spirales.* Primitive spirals or open imperfect spirals as well as hooks and loops of wide diameter are common. Mature spore chains are generally long, often with more than 50 spores

per chain. This morphology is seen on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar. Spore surface is smooth.

Color of colony: aerial mass color in the Red color series (4gc or 4ec, grayish yellowish pink; 5gc, light reddish brown; 5dc, grayish yellowish pink; 5ca, light yellowish pink) on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar; white aerial mycelium may be also present on glycerol-asparagine agar. Reverse side of colony is strong brown or orange brown on yeast-malt agar; orange yellow, yellowish brown, or grayish yellowish pink on oatmeal agar and salts-starch agar; grayish yellow to moderate yellowish pink on glycerol-asparagine agar. Reverse mycelium pigment is not a pH indicator.

Color in medium: melanoid pigments are formed in peptone-yeast-iron agar, tryptone-yeast broth, and Gause's medium no. 2, but not in tyrosine agar. No pigment (or only a trace of yellow or pink) is found in the medium in yeast-malt agar, oatmeal agar, salts-starch agar, or glycerol-asparagine agar.

D-Glucose, D-mannitol, and D-fructose are utilized for growth. Utilization of L-arabinose is doubtful or weak. No growth or only traces of growth with D-xylose, iso-inositol, rhamnose, sucrose, and raffinose.

Type strain shows the highest sequence similarity to: *S. cirratus*, AY999794, 100%; *S. sporoarveus*, AJ781370, 99.9%; *S. nojiriensis*, AJ781355, 99.9%; *S. xanthophaeus*, DQ442560, 99.8%; *S. sporoverrucosus*, DQ442544, 99.7%; *S. colombiensis*, DQ026646, 99.7%; *S. goshikiensis*, EF178693, 99.7%; *S. cinnamonensis*, AB184707, 99.6%; *S. avidinii*, AB184395, 99.6%; *S. subrutilus*, X80825, 99.5%; *S. virginiae*, D85119, 99.4%.

Source: not known.

DNA G+C content (mol%): not known.

Type strain: ATCC 27476, CBS 726.72, BCRC 11865, DSM 40515, HUT 6082, NBRC 13425, JCM 4090, JCM 4849, KCTC 9771, NRRL 2382, NRRL-ISP 5515, PCM 2366, RIA 1386, RIA 805.

Sequence accession no. (16S rRNA gene): AB184394.

Further comments: according to Lanoot et al. (2004), *Streptomyces vinaceus* Jones 1952 is an earlier heterotypic synonym of *Streptomyces arabicus* Shibata et al. 1957.

497. **Streptomyces vinaceusdrappus** Pridham, Hesseltine and Benedict 1958, 68[AL]

vi.na.ce.us.drap′pus. L. adj. *vinaceus* of or belonging to wine or the grape; L. n. *drappus* a sheet, here referring to the color "drab"; N.L. masc. adj. *vinaceusdrappus* of wine-drab, referring to the drab wine color of the aerial mycelium and spores of the organism.

Spore chains in Section *Spirales*. Mature spore chains are generally long, often with more than 50 spores per chain. This morphology is seen on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar. Spore surface is smooth.

Color of colony: aerial mass color in the Red color series (5dc, grayish yellowish pink to 4ge, light grayish reddish brown) on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar. One observer places this strain in the Gray color series (5fe, light grayish reddish brown or 3fe, light brownish gray). Reverse side of colony with no distinctive pigments (grayish yellow to yellowish or olive-brown) on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar.

Color in medium: melanoid pigments are not formed in peptone-yeast-iron agar, tyrosine agar, or tryptone-yeast broth. No pigment is found in medium in yeast-malt agar, oatmeal agar, salts-starch agar, or glycerol-asparagine agar.

D-Glucose, L-arabinose, D-xylose, iso-inositol, D-mannitol, D-fructose, rhamnose, sucrose, and raffinose are all utilized for growth.

Type strain shows the highest sequence similarity to: *S. rochei*, AB184237, 100%; *S. ghanaensis*, AY999851, 100%; *S. geysiriensis*, DQ442501, 100%; *S. minutiscleroticus*, EF178696, 100%; *S. plicatus*, AB184291, 100%; *S. mutabilis*, EF178679, 99.9%; *S. tuirus*, AB184690, 99.5%; *S. djakartensis*, AB184657, 99.4%; *S. anandii*, AB184402, 99.2%; *S. violaceorubidus*, AJ781374, 99.2%; *S. pilosus*, AB184161, 99.1%; *S. flavoviridis*, AB184842, 99.1%; *S. tendae*, D63873, 99%; *S. calvus*, AB184329, 99%; *S. azureus*, EF178674, 99%; *S. asterosporus*, AB184706, 99%; *S. levis*, AB184670, 99%; *S. luteogriseus*, AB184379, 99%; *S. capillispiralis*, AB184577, 99%.

Source: not known.

DNA G+C content (mol%): not known.

Type strain: ATCC 25511, CBS 653.69, BCRC 12170, DSM 40470, NBRC 13099, JCM 4529, NCIMB 12980, NRRL 2363, NRRL-ISP 5470, RIA 1291, VKM Ac-1902.

Sequence accession no. (16S rRNA gene): AY999929.

498. **Streptomyces violaceochromogenes** (Ryabova and Preobrazhenskaya *in* Gauze, Preobrazhenskaya, Kudrina, Blinov, Ryabova and Sveshnikova 1957) Pridham 1970, 28[AL] ["*Actinomyces violaceus chromogenes*" Krasil'nikov 1949, 55; "*Actinomyces violaceochromogenes*" Ryabova and Preobrazhenskaya *in* Gauze, Preobrazhenskaya, Kudrina, Blinov, Ryabova and Sveshnikova 1957, 183; "*Actinomyces violochromogenes*" (*sic*) Artamonova and Krasil'nikov *in* Rautenshtein 1960, 334]

vi.o.la.ce.o.chro.mo′ge.nes. L. adj. *violaceus* violet; Gr. n. *chroma* color; N.L. suff. *-genes* (from Gr. v. *gennaô* to produce) producing; N.L. part. adj. *violaceochromogenes* producing violet color.

Spore chains in Section *Retinaculiaperti* or *Spirales*. Short chains form incomplete or imperfect spirals, hooks, flexuous chains. These are neither typically spiral nor representative of the long chains with open loops and spirals on true *Retinaculiaperti* cultures. Some longer chains bear terminal spirals suggesting *Retinaculiaperti* morphology. Mature spore chains are generally short with 3–10, or sometimes more than 10, spores per chain. This morphology is seen on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar. Spore surface is smooth.

Color of colony: aerial mass color in the Red (5cb, grayish yellowish pink) or Gray (5fe, light grayish reddish brown) color series on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar. Reverse side of colony with no distinctive pigments (grayish yellow to strong brown) on yeast-malt agar and salts-starch agar; but

yellow to yellow-brown may or may not be modified by red on oatmeal agar and glycerol-asparagine agar. If red reverse, mycelium pigment is present, it is pH-sensitive changing from red to violet or purple with addition of 0.05 M NaOH or from violet to red with addition of 0.05 M HCl.

Color in medium: melanoid pigments are formed in peptone-yeast-iron agar and tryptone-yeast broth, but only weakly or not at all in tyrosine agar. Yellow or yellow-brown pigment is found in the medium in yeast-malt agar and salts-starch agar. This pigment is not pH-sensitive when tested with 0.05 M NaOH or HCl.

D-Glucose, L-arabinose, D-xylose, iso-inositol, D-mannitol, D-fructose, rhamnose, sucrose, and raffinose are all utilized for growth.

Type strain shows the highest sequence similarity to: *S. collinus*, AB184123, 99.6%; *S. iakyrus*, AB184877, 99.5%; *S. griseorubens*, AB184139, 99.1%; *S. paradoxus*, AB184628, 99.1%; *S. griseoflavus*, AJ781322, 99.1%; *S. matensis*, AB184221, 99.1%; *S. variabilis*, DQ442551, 99%; *S. griseoincarnatus*, AJ781328, 99%; *S. labedae*, AB184704, 99%; *S. erythrogriseus*, AJ781328, 99%.

Source: not known.

DNA G+C content (mol%): not known.

Type strain: ATCC 19932, ATCC 25512, CBS 654.69, DSM 40181, NBRC 13100, INA 425, JCM 4530, NRRL B-5427, NRRL-ISP 5181, RIA 1292, VKM Ac-581.

Sequence accession no. (16S rRNA gene): AY999867.

499. **Streptomyces violaceolatus** (Krasil'nikov, Sorokina, Alferova and Bezzubenkova *in* Krasil'nikov 1965) Pridham 1970, 28[AL] ("*Actinomyces violaceolatus*" Krasil'nikov, Sorokina, Alferova and Bezzubenkova *in* Krasil'nikov 1965, 113)

vi.o.la.ce.o.la′tus. L. adj. *violaceus* violet-colored; L. adj. *latus* broad; N.L. masc. adj. *violaceolatus* violet, broad.

Forms blue-, purple-, or red-colored vegetative mycelium and diffusible pigment depending upon pH.

Spore chains in Section *Spirales*. Mature spore chains are long, usually with more than 50 spores per chain. This morphology is seen on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar. Spore surface is smooth.

Color of colony: aerial mass color in the Gray color series (3fe, light brownish gray; 5fe, light grayish reddish brown; or 3ge, light grayish yellowish brown) on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar. Color may sometimes approach tab 5dc, grayish yellowish pink in the Red color series on glycerol-asparagine agar. Reverse side of colony is blue, purple, or red, depending upon pH and ranging from grayed colors to almost black, depending upon intensity of pigment. Reverse mycelium pigment is not a pH indicator, changing from red or violet to blue with addition of 0.05 M NaOH and from blue or violet to red or pink with addition of 0.05 M HCl.

Color in medium: melanoid pigments are not formed in peptone-yeast-iron agar, tyrosine agar, or tryptone-yeast broth. Blue, violet, or red pigment, depending upon pH, is found in the medium in yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar. This pigment is pH-sensitive, showing the same changes noted for the reverse mycelium pigment.

D-Glucose, L-arabinose, D-xylose, iso-inositol, D-mannitol, D-fructose, rhamnose, sucrose, and raffinose are all utilized for growth, but growth on sucrose may be somewhat less than on any other carbon sources.

Type strain shows the highest sequence similarity to: *S. coelescens*, AF503496, 100%; *S. humiferus*, AF503491, 100%; *S. violaceoruber*, AF503492, 100%; *S. tricolor*, AB184687, 99.8%; *S. anthocyanicus*, AB184631, 99.8%; *S. rubrogriseus*, AB184681, 99.7%; *S. tendae*, D63873, 99.5%; *S. lienomycini*, AJ781353, 99.5%; *S. violaceorubidus*, AJ781374, 99.3%; *S. coelicoflavus*, AB184650, 99.3%.

Source: not known.

DNA G+C content (mol%): not known.

Type strain: ATCC 19847, ATCC 25513, CBS 655.69, DSM 40438, ICSSB 1022, NBRC 13101, JCM 4531, KCTC 9772, NRRL B-12371, NRRL-ISP 5438, RIA 1293, VKM Ac-582.

Sequence accession no. (16S rRNA gene): AF503497.

500. **Streptomyces violaceorectus** (Ryabova and Preobrazhenskaya *in* Gauze, Preobrazhenskaya, Kudrina, Blinov, Ryabova and Sveshnikova 1957) Pridham, Hesseltine and Benedict 1958, 63[AL] ("*Actinomyces violaceorectus*" Ryabova and Preobrazhenskaya *in* Gauze, Preobrazhenskaya, Kudrina, Blinov, Ryabova and Sveshnikova 1957, 182)

vi.o.la.ce.o.rec′tus. L. adj. *violaceus* violet colored; L. adj. *rectus* straight; N.L. adj. *violaceorectus* violet colored, straight (referring to the color of the vegetative mycelium and diffusible pigment on some media and to the structure of sporophores).

Spore chains in Section *Rectiflexibiles*. Mature spore chains are generally long and straight, often with more than spores per chain. This morphology is seen on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar. Spore surface is smooth.

Color of colony: aerial mass color in the Gray or Red color series on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar (representative color tab from the Gray color series is 5fe, light grayish reddish brown; representative tabs from the Red color series are 5dc, 5ec, or 6ec, grayish yellowish pink). Reverse side of colony is grayish yellow to brown or grayish reddish brown on yeast-malt agar; yellowish pink or reddish brown to dark purplish pink or dark purplish red on oatmeal agar, salts-starch agar, and glycerol-asparagine agar. Reverse mycelium pigment is a pH indicator, changing from orange or pink to violet or purple with addition of 0.05 M NaOH and from violet to pink or orange with addition of 0.05 M HCl.

Color in medium: melanoid pigments are formed in peptone-yeast-iron agar and tryptone-yeast broth, but not in tyrosine agar. Traces of yellow to pinkish brown pigment may be found in the medium in yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar. Pink or pinkish brown pigment, when present, is pH-sensitive, changing from pink or brown to pale violet or gray with addition of 0.05 M NaOH.

D-Glucose, L-arabinose, and D-xylose are utilized for growth. Limited growth also may occur on D-fructose and

sucrose. No growth or only trace of growth with iso-inositol, D-mannitol, rhamnose, and raffinose.

Type strain shows the highest sequence similarity to: *S. bikiniensis*, X79851, 99.7%; *S. cinereoruber* subsp. *cinereoruber*, AB184121, 99.7%; *S. vietnamensis*, DQ311081, 99.4%; *S. tanashiensis*, AJ781362, 99.2%; *S. nashvillensis*, AB184286, 99.2%; *S. showdoensis*, AB184389, 99.1%; *S. viridobrunneus*, AJ781372, 99%.

Source: not known.

DNA G+C content (mol%): not known.

Type strain: ATCC 25514, CBS 656.69, BCRC 13626, DSM 40279, NBRC 13102, IMET 43520, INA 506, JCM 4532, NRRL B-12181, NRRL-ISP 5279, RIA 1294, VKM Ac-584.

Sequence accession no. (16S rRNA gene): AB184314.

501. **Streptomyces violaceoruber** (Waksman and Curtis 1916) Pridham 1970, 44[AL] ("*Actinomyces violaceus-ruber*" Waksman and Curtis 1916, 127; "*Streptomyces violaceoruber*" Waksman *in* Kutzner and Waksman 1959, 535)

vi.o.la.ce.o.ru′ber. L. adj. *violaceus* violet; L. adj. *ruber* red; N.L. masc. adj. *violaceoruber* violet-red.

Spore chains in Section *Spirales*, but spore chains representative of Sections *Rectiflexibiles* and *Retinaculiaperti* are also reported. Mature spore chains generally have 10–50 spores per chain. Typical morphology on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar. Spore surface is smooth.

Color of colony: aerial mass color in the Gray color series on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar. Reverse side of colony is blue or violet, depending on pH, on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar. Reverse pigment is a pH indicator changing from violet or blue-violet to blue by addition of 0.05 M NaOH and from violet to red-violet or red with 0.05 M HCl.

Color in medium: melanoid pigments not formed in peptone-yeast-iron agar, tyrosine agar, or tryptone-yeast extract broth. Blue or violet pigment found in medium in yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar. This pigment is pH-sensitive; color changes are identical to changes noted for reverse color.

D-Glucose, L-arabinose, D-xylose, iso-inositol, D-mannitol, D-fructose, and rhamnose are utilized for growth. No growth or only trace of growth on sucrose. Variable reports on growth with raffinose.

Type strain shows the highest sequence similarity to: *S. violaceolatus* AF503497, 100%; *S. coelescens*, AF503496, 100%; *S. humiferus*, AF503491, 100%; *S. tricolor*, AB184687, 99.9%; *S. anthocyanicus*, AB184631, 99.9%; *S. rubrogriseus*, AB184681, 99.7%; *S. tendae*, D63873, 99.6%; *S. lienomycini*, AJ781353, 99.6%; *S. violaceorubidus*, AJ781374, 99.4%; *S. coelicoflavus*, AB184650, 99.3%; *S. ambofaciens*, M27245, 99%; *S. pactum*, AB184398, 99%.

Source: not known.

DNA G+C content (mol%): not known.

Type strain: ATCC 14980, ATCC 19816, ATCC 3355, CBS 569.68, BCRC 11489, DSM 40049, ICSSB 1016, NBRC 12826, JCM 4423, KCTC 9787, NRRL B-12594, NRRL B-2935, NRRL B-3025, NRRL B-3319, NRRL-ISP 5049, RIA 1096, UNIQEM 203, VKM Ac-726.

Sequence accession no. (16S rRNA gene): AF503492.

502. **Streptomyces violaceorubidus** Terekhova 1986, 575[VP] (Effective publication: Terekhova *in* Gause, Preobrazhenskaya, Sveshnikova, Terekhova and Maximova 1983.)

vi.o.la.ce.o.ru′bi.dus. L. adj. *violaceus* violet; L. adj. *rubidus* dark-red; N.L. masc. adj. *violaceorubidus* violet, dark-red.

Spore chains are spiral (*Spirales*); spores are smooth. On mineral agar 1: aerial mycelium is light gray; substrate mycelium and diffusible pigment are light pink, reddish violet; diffusible pigment is weak. On starch-ammonia agar: no aerial mycelium; substrate mycelium is yellow; no diffusible pigment. On glycerol-nitrate agar: aerial mycelium is creamy, light pinkish gray; substrate mycelium and diffusible pigment are yellowish reddish to violet red. On glycerol-asparagine agar: aerial mycelium is white, light gray; substrate mycelium is dark yellow; no diffusible pigment. On oatmeal agar: aerial mycelium is light pinkish gray; substrate mycelium and diffusible pigment are yellowish red to reddish violet; diffusible pigment is weak. On organic agar 2: aerial mycelium not extant or light gray, poor; substrate mycelium is yellowish; diffusible pigment is yellowish or not extant. On organic agar 79: aerial mycelium is light gray; substrate mycelium is yellowish; diffusible pigment is yellowish or not extant. Melanoid pigments are not formed. Good growth on rhamnose, fructose, glucose, xylose, mannitol, inositol, raffinose, and arabinose; sucrose is not utilized. Antibiotic: zinerubin.

Type strain shows the highest sequence similarity to: *S. tendae*, D63873, 99.8%; *S. lienomycini*, AJ781353, 99.7%; *S. rubrogriseus*, AB184681, 99.7%; *S. tricolor*, AB184687, 99.5%; *S. anthocyanicus*, AB184631, 99.5%; *S. matensis*, AB184221, 99.4%; *S. violaceoruber*, AF503492, 99.4%; *S. coelescens*, AF503496, 99.4%; *S. geysiriensis*, DQ442501, 99.3%; *S. minutiscleroticus*, EF178696, 99.3%; *S. humiferus*, AF503491, 99.3%; *S. violaceolatus* AF503497, 99.3%; *S. rochei*, AB184237, 99.2%; *S. ghanaensis*, AY999851, 99.2%; *S. malachitospinus*, AB249954, 99.2%; *S. vinaceusdrappus*, AY999929, 99.2%; *S. mutabilis*, EF178679, 99.2%; *S. plicatus*, AB184291, 99.2%; *S. lomondensis*, AB184673, 99.1%; *S. parvulus*, AB184326, 99.1%; *S. paradoxus*, AB184628, 99.1%; *S. griseorubens*, AB184139, 99.1%; *S. pactum*, AB184398, 99%; *S. malachitofuscus*, AB184282, 99%; *S. griseoflavus*, AJ781322, 99%; *S. tuirus*, AB184690, 99%; *S. variabilis*, DQ442551, 99%; *S. labedae*, AB184704, 99%; *S. griseoincarnatus*, AJ781328, 99%; *S. erythrogriseus*, AJ781328, 99%; *S. coelicoflavus*, AB184650, 99%; *S. viridochromogenes*, DQ442555, 99%; *S. collinus*, AB184123, 99%.

Source: not known.

DNA G+C content (mol%): not known.

Type strain: ATCC 43697, DSM 41478, NBRC 15463, INA 770, JCM 6931, NRRL B-16381, VKM Ac-1292.

Sequence accession no. (16S rRNA gene): AJ781374.

503. **Streptomyces violaceus** (Rossi Doria 1891) Waksman *in* Waksman and Lechevalier 1953, 43[AL] emend. Lanoot, Vancanneyt, Cleenwerk, Wang, Li, Liu and Swings 2002, 828 ["*Streptotrix* (*sic*) *violacea*" Rossi Doria 1891, 411; "*Oospora violacea*" Sauvageau and Radais 1892, 252; "*Actinomyces violaceus*" Gasperini 1894, 84; "*Cladothrix violacea*" Macé 1897, 1032; "*Nocardia violacea*" Chalmers and Christopherson 1916, 270].

vi.o.la′ce.us. L. masc. adj. *violaceus* violet colored, referring to the color of the vegetative mycelium and diffusible pigment of the organism.

Spore chains in Section *Spirales*. Mature spore chains are moderately long with 10 to 50 or more spores per chain. This morphology is seen on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar. Spore surface is spiny.

Color of colony: aerial mass color in the Red color series (5cb, grayish yellowish pink or 7ca, light yellowish pink) on salts-starch agar and glycerol-asparagine agar; White or Red on yeast-malt agar; White color series on oatmeal agar. When aerial mycelium is thin, the red to violet color of the substrate mycelium may be evident. Reverse side of colony is reddish orange to purplish pink or pale purple, depending on pH. Reverse mycelium pigment is a pH indicator, changing from red to violet (purple) with addition of 0.05 M NaOH or from violet to red (pink) with addition of 0.05 M HCl.

Color in medium: melanoid pigments are formed in peptone-yeast-iron agar and tryptone-yeast broth, but not in tyrosine agar. Red or violet pigment, depending on pH, is found in the medium in yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar. This pigment is pH-sensitive showing the same changes noted for the reverse mycelium pigments.

D-Glucose, L-arabinose, D-xylose, iso-inositol, D-mannitol, D-fructose, rhamnose, sucrose, and raffinose are all utilized for growth.

Type strain shows the highest sequence similarity to: *S. roseoviolaceus*, AJ399484, 100%; *S. janthinus*, AB184851, 100%; *S. albosporeus* subsp. *albosporeus*, AJ781327, 100%; *S. luteogriseus*, AB184379, 99.5%; *S. lomondensis*, AB184673, 99.3%; *S. hawaiiensis*, AB184143, 99.3%; *S. flavoviridis*, AB184842, 99.3%; *S. pilosus*, AB184161, 99.2%; *S. arenae*, AB249977, 99.2%; *S. africanus*, AY208912, 99.2%; *S. tuirus*, AB184690, 99.2%; *S. bellus*, AB184849, 99.1%; *S. mutabilis*, EF178679, 99.1%; *S. massasporeus*, AB184152, 99%; *S. afghaniensis*, AJ399483, 99%; *S. levis*, AB184670, 99%; *S. coerulescens*, AY999720, 99%; *S. parvulus*, AB184326, 99%; *S. coeruleorubidus*, AY999719, 99%.

Source: not known.

DNA G+C content (mol%): not known.

Type strain: AS 4.1456, ATCC 15888, ATCC 25515, CBS 657.69, BCRC 11880, CCT 4833, CECT 3235, DSM 40082, NBRC 13103, IMET 43085, INMI 1, JCM 4533, LMG 20257, NRRL B-2869, NRRL-ISP 5082, RIA 1295, RIA 656, VKM Ac-510, VKM Ac-977.

Sequence accession no. (16S rRNA gene): AB184315.

Further comments: Streptomyces violaceus (Rossi Doria 1891) Waksman 1953 emend. Lanoot et al. 2002 is an earlier heterotypic synonym of *Streptomyces violatus* (Artamonova and Krasil'nikov 1960) Pridham 1970.

504. **Streptomyces violaceusniger** corrig. (Waksman and Curtis 1916) Pridham, Hesseltine and Benedict 1958, 63[AL] emend. Labeda and Lyons 1991b, 400 ["*Actinomyces violaceus-niger*" Waksman and Curtis 1916, 111; "*Streptomyces violaco-niger*" (*sic*) Waksman and Henrici *in* Breed, Murray and Hitchens 1948, 947]

vi.o.la.ce.us.ni′ger. L. adj. *violaceus* violet; L. adj. *niger* black; N.L. masc. adj. *violaceusniger* violet-black.

Spore chains are *Spirales*; the spore surface is smooth to rough. The spore mass is gray, becoming black and moist when it is mature. Reverse side of colonial growth is grayish yellow or light olive green to dark olive to dark grayish green. Melanoid pigments are not formed; soluble pigments are not produced. D-Glucose, L-arabinose, D-xylose, iso-inositol, D-mannitol, D-fructose, rhamnose, sucrose, and raffinose are utilized for growth. Exhibits anti-bacterial and anti-fungal activity; excellent growth on Czapek's solution agar; gray to black vegetative mycelium on some media; hygroscopic; NaCl tolerance >4%, but <7%.

Type strain shows the highest sequence similarity to: *S. yogyakartensis*, AB249942, 100%; *S. albiflaviniger*, AJ391812, 99.7%; *S. endus*, AY999911, 99.5%; *S. sporocinereus*, AB249933, 99.5%; *S. demainii*, DQ334782, 99.5%; *S. hygroscopius* subsp. *hygroscopius*, AB184428, 99.5%; *S. javensis*, AJ391833, 99.1%.

Source: not known.

DNA G+C content (mol%): 71.2.

Type strain: AS 4.1423, ATCC 27477, CBS 760.72, DSM 40563, NBRC 13459, JCM 4850, LMG 19336, NRRL B-1476, NRRL-ISP 5563, RIA 1420, VKM Ac-583.

Sequence accession no. (16S rRNA gene): AJ391823.

Further comments: the original spelling, *Streptomyces violaceoniger* (*sic*), has been corrected by Hill et al. (1984).

505. **Streptomyces violarus** (Artamonova and Krasil'nikov 1960) Pridham 1970, 30[AL] ("*Actinomyces violarus*" Artamonova and Krasil'nikov *in* Rautenshtein 1960, 334)

vi.o.la′rus. N.L. masc. adj. *violarus* (from L. adj. *violaris*) of or belonging to violets, violet, referring to the color of the vegetative mycelium.

Spore chains in Section *Spirales*. Spirals of three to four turns may be formed or poorly developed irregular spirals, hooks or loops may become entangled. Mature spore chains are moderately long with 10–50 spores per chain. This morphology is seen on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar. Spore surface is spiny.

Color of colony: aerial mass color in the Red or Violet color series on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar. Nearest matching color tabs are 5cb, 5ec or 6ec, grayish yellowish pink from the Red color series or 11ca, very pale purple, from the Violet color series. Reverse side of colony is reddish orange or reddish brown to purplish pink or purple depending upon pH. Reverse mycelium pigment is a pH indicator, changing from pink or violet to blue-violet or blue with addition of 0.05 M NaOH and from violet to pink or red with addition of 0.05 M HCl.

Color in medium: melanoid pigments are formed in peptone-yeast-iron agar and tryptone-yeast broth, but only weakly or not at all in tyrosine agar. Red or violet pigment, depending upon pH, is found in the medium in yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar. This pigment is pH-sensitive, showing the same changed noted for reverse mycelium pigment.

D-Glucose, L-arabinose, D-xylose, iso-inositol, D-mannitol, D-fructose, rhamnose, sucrose, and raffinose are all utilized for growth.

Type strain shows no sequence similarity over 99%.

Source: not known.

DNA G+C content (mol%): not known.

Type strain: ATCC 15891, ATCC 25516, CBS 658.69, CCT 5007, CECT 3237, DSM 40205, NBRC 13104, INMI 1212, JCM 4534, KCTC 9788, NRRL B-5432, NRRL-ISP 5205, RIA 1296, RIA 157, UNIQEM 204, VKM Ac-528.

Sequence accession no. (16S rRNA gene): AB184316.

506. **Streptomyces violascens** (Preobrazhenskaya and Sveshnikova *in* Gauze, Preobrazhenskaya, Kudrina, Blinov, Ryabova and Sveshnikova 1957) Pridham, Hesseltine and Benedict 1958, 68^{AL} ("*Actinomyces violascens*" Preobrazhenskaya and Sveshnikova *in* Gauze, Preobrazhenskaya, Kudrina, Blinov, Ryabova and Sveshnikova 1957, 41)

vi.o.la′scens. N.L. part. adj. *violascens* becoming violet.

Spore chains in Section *Spirales*. Mature spore chains have 10 to 50 or more spores per chain. This morphology is seen on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar. Spore surface is spiny.

Color of colony: aerial mass color in the Violet color series on salts-starch agar and glycerol-asparagine agar; Violet or White color series on oatmeal agar; Violet, White, or Red color series on yeast-malt agar. Reverse side of colony with no distinctive pigment (grayed yellow to yellow-brown) on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar.

Color in medium: melanoid pigments are formed in peptone-yeast-iron agar, tyrosine agar, and tryptone-yeast broth. Pigments other than melanoids not formed (or only traces of yellow) in yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar.

D-Glucose, L-arabinose, D-xylose, D-fructose, and raffinose are utilized for growth. No growth or only trace of growth on D-mannitol and rhamnose. Utilization of sucrose and iso-inositol is doubtful.

Type strain shows the highest sequence similarity to: *S. limosus*, AB184147, 100%; *S. felleus*, AB184129, 100%; *S. daghestanicus*, DQ442497, 100%; *S. albidoflavus*, AB184255, 100%; *S. hydrogenans*, AB184868, 100%; *S. odorifer*, Z76682, 100%; *S. griseus* subsp. *solvifaciens*, AB249915, 100%; *S. champavatii*, DQ026642, 100%; *S. canescens*, AB184117, 100%; *S. sampsonii*, D63871, 99.8%; *S. koyangensis*, AY079156, 99.7%.

Source: not known.

DNA G+C content (mol%): not known.

Type strain: ATCC 23968, CBS 266.66, CBS 951.68, BCRC 12240, CECT 3215, DSM 40183, NBRC 12920, IMET 42061, INA 3959/54, JCM 4424, KCTC 9785, NCIMB 9820, NRRL B-2700, NRRL-ISP 5183, RIA 1138, VKM Ac-1275.

Sequence accession no. (16S rRNA gene): AY999737.

507. **Streptomyces violatus** (Artamonova and Krasil'nikov 1960) Pridham 1970, 30^{AL} ("*Actinomyces violatus*" Artamonova and Krasil'nikov *in* Rautenshtein 1960, 334)

vi.o.la′tus. L. masc. adj. *violatus* flavored with violet, referring to the color of the vegetative mycelium and diffusible pigment of the organism.

Spore chains in Section *Spirales*. Mature spore chains are moderately long, with 10 to 50 or more spores per chain. This morphology is seen on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar. Spore surface is spiny.

Color of colony: aerial mass color in the White or Red color series on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar. Nearest matching color tab in the Red color series is 5cb, grayish yellowish pink. Reverse side of colony is light grayish reddish brown on yeast-malt agar; light reddish purple to dark purplish pink, or yellowish pink on oatmeal agar, salts-starch agar, and glycerol-asparagine agar. Reverse mycelium pigment is a pH indicator, changing from red or pink to violet or blue with the addition of 0.05 M NaOH and from violet to yellowish pink or orange with the addition of 0.05 M HCl.

Color in medium: melanoid pigments are formed in peptone-yeast-iron agar and tryptone-yeast broth, but appear slowly or not at all in tyrosine agar. Red to violet pigment is found in the medium in yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar. This pigment is pH-sensitive, showing the same changes recorded for the reverse mycelium pigment.

D-Glucose, L-arabinose, D-xylose, D-fructose, rhamnose, sucrose, raffinose, iso-inositol, and D-mannitol are all utilized for growth.

For sequence similarity, see type strain of *Streptomyces violaceus.*

Source: not known.

DNA G+C content (mol%): not known.

Type strain: ATCC 15892, CBS 650.72, DSM 40209, NBRC 13349, INMI 1205, JCM 4237, JCM 4851, NRRL B-2867, NRRL-ISP 5209, RIA 708.

Sequence accession no. (16S rRNA gene): AJ399480.

Further comments: Streptomyces violatus (Artamonova and Krasil'nikov 1960) Pridham 1970 is a later heterotypic synonym of *Streptomyces violaceus* (Rossi Doria 1891) Waksman 1953 emend. Lanoot et al. 2002.

508. **Streptomyces violens** (Kalakoutskii and Krasil'nikov 1960) Goodfellow, Williams and Alderson 1987c, 179^{VP} (Effective publication: Goodfellow, Williams and Alderson 1986d, 59.) (*Chainia violens* Kalakoutskii and Krasil'nikov *in* Rautenshtein 1960, 55)

vi.o.lens. L. adj. *violens* raging but probably from L. fem. n. *viola* violet, referring to pink to violet pigment produced by the organism on some media.

Spore chain Section not determined. Aerial mycelium is not produced on yeast-malt agar, oatmeal agar, salts-starch agar, or glycerol-asparagine agar. Spore surface not determined. Sclerotia are produced on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar, and the substrate (vegetative) mycelium fragments into rod-shaped or coccoid elements on these media. One observer reports L-A_2pm and the absence of arabinose in the cell wall (cell-wall type I).

Color of colony: aerial mass color not determined; aerial mycelium absent on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar. Reverse side of colony with no distinctive pigments (colorless on

glycerol-asparagine agar; pale or grayish yellow to light olive on yeast-malt agar, oatmeal agar, and salts-starch agar).

Color in medium: melanoid pigments are not formed in peptone-yeast-iron agar, tyrosine agar, or tryptone-yeast broth. One observer reports that melanin is produced on a peptone-yeast-iron agar of different composition. A pale yellow pigment in oatmeal agar is reported by one observer; and another reports orange or pinkish pigment in glycerol-asparagine agar. The latter is pH-sensitive, changing from orange or rose to violet or light purple with the addition of 0.05 M NaOH.

D-Glucose, L-arabinose, D-xylose, iso-inositol, D-mannitol, D-fructose, rhamnose, sucrose, and raffinose are all utilized for growth.

Type strain shows the highest sequence similarity to: *S. ochraceiscleroticus*, AB184094, 99.8%; *S. purpurogeneiscleroticus*, AJ621604, 99.7%; *S. monomycini*, DQ445790, 99.3%; *S. erumpens*, AJ621603, 99.1%.

Source: not known.

DNA G+C content (mol%): not known.

Type strain: ATCC 15898, CBS 451.65, CBS 787.72, BCRC 12540, DSM 40597, HAMBI 1073, NBRC 12557, NBRC 13486, INMI 1212, JCM 3072, JCM 4852, NCAIM B.01477, NRRL B-3484, NRRL-ISP 5597, PCM 2247, RIA 1447, RIA 565, VKM Ac-586, VKM Ac-653.

Sequence accession no. (16S rRNA gene): AJ621605.

509. **Streptomyces virens** Gause and Sveshnikova 1986a, 575[VP] (Effective publication: Gause and Sveshnikova *in* Gause, Preobrazhenskaya, Sveshnikova, Terekhova and Maximova 1983.)

vi′rens. L. part. adj. *virens* being green.

Spore chains are spiral (*Spirales*); spore surface has spines and warts, warts are situated between hairs and spines. On mineral agar 1: aerial mycelium is light gray to greenish, poorly developed; substrate mycelium is olive, gray brownish olive; diffusible pigment is orange red, moderate. On glycerol-nitrate agar: aerial mycelium is weak developed with white grayish appearance; substrate mycelium is olive, gray-brown olive; diffusible pigment is light brown. On starch-ammonia agar: aerial mycelium is poorly developed, whitish or grayish; substrate mycelium is colorless to light olive; no diffusible pigment. On glycerol-asparagine agar: aerial mycelium is absent or poorly developed with white appearance; substrate mycelium and diffusible pigment are olive. On oatmeal agar: aerial mycelium is absent or whitish; substrate mycelium is olive; no diffusible pigment. On organic agar 2: aerial mycelium is absent or whitish; substrate mycelium is gray brownish; diffusible pigment is reddish. Melanoid pigments are not formed. Moderate growth on arabinose, fructose, and mannitol; no growth on xylose, raffinose, rhamnose, sucrose, or cellulose. Antibiotic: virenomycin.

Type strain shows the highest sequence similarity to: *S. calvus*, AB184329, 99.9%; *S. aureorectus*, AB184710, 99.9%; *S. asterosporus*, AB184706, 99.8%; *S. djakartensis*, AB184657, 99%; *S. geysiriensis*, DQ442501, 99%; *S. tuirus*, AB184690, 99%; *S. anandii*, AB184402, 99%; *S. minutiscleroticus*, EF178696, 99%.

Source: not known.

DNA G+C content (mol%): not known.

Type strain: DSM 41465, NBRC 15901, INA 3831, JCM 9095, NRRL B-24331, VKM Ac-833.

Sequence accession no. (16S rRNA gene): DQ442554.

510. **Streptomyces virginiae** Grundy, Whitman, Rdzok, Rdzok, Hanes and Sylvester 1952, 399[AL]

vir.gi.ni′a.e. N.L. gen. n. *virginiae* of Virginia, referring to the source of the soil (near Roanoke, Virginia) from which the organism was isolated.

Spore chains in Section *Retinaculiaperti*, including flexuous, looped, and spiral forms characteristic of this section (terminal spirals on long spore chains may be common in some areas on mass cultures). Mature spore chains are moderately long with 10–50, or sometimes more than 50, spores per chain. This morphology is seen on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar. Spore surface is smooth.

Color of colony: aerial mass color in the Red or Gray color series on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar. Color tabs selected by observers: Grayish yellowish pink (5dc, 6ec, Red series), pale purple (7fe, Gray series). Reverse side of colony with no distinctive pigment (grayed yellow to yellow-brown) on yeast-malt agar, oatmeal agar, and glycerol-asparagine agar; dark grayed yellow may be modified by traces of red or violet on salts-starch agar. Substrate pigment is not a pH indicator.

Color in medium: melanoid pigments are not formed in peptone-yeast-iron agar or tryptone-yeast extract broth. Pigments other than melanoids are not formed in yeast-malt agar, oatmeal agar, salts-starch agar, or glycerol-asparagine agar.

D-Glucose and D-fructose are utilized for growth. Trace of growth on L-arabinose, sucrose, D-xylose, iso-inositol, D-mannitol, rhamnose, and raffinose is similar to, or only slight better than growth on basal medium without carbon.

Type strain shows the highest sequence similarity to: *S. cinnamonensis*, AB184707, 99.8%; *S. sporoaveus*, AJ781370, 99.5%; *S. nojiriensis*, AJ781355, 99.5%; *S. xanthophaeus*, DQ442560, 99.5%; *S. vinaceus*, AB184394, 99.4%; *S. goshikiensis*, EF178693, 99.4%; *S. cirratus*, AY999794, 99.4%; *S. avidinii*, AB184395, 99.3%; *S. subrutilus*, X80825, 99.3%; *S. sporoverrucosus*, DQ442544, 99.2%; *S. colombiensis*, DQ026646, 99.1%; *S. flavotricini*, AB184132, 99%.

Source: isolated from soil near Roanoke, Virginia, USA.

DNA G+C content (mol%): not known.

Type strain: ATCC 19817, CBS 291.60, CBS 570.68, BCRC 12069, DSM 40094, NBRC 12827, NBRC 3729, JCM 4425, KCTC 1747, NRRL B-1446, NRRL-ISP 5094, RIA 1097, UNIQEM 205, VKM Ac-1218.

Sequence accession no. (16S rRNA gene): D85119.

511. **Streptomyces viridiflavus** corrig. (Locci and Schofield 1989) Witt and Stackebrandt 1991, 457[VP] (Effective publication: Witt and Stackebrandt 1990, 370.) ["*Streptoverticillium viridoflavum*" (*ex* Waksman and Taber 1953) Locci 1985, 232; *Streptoverticillium viridoflavum* Locci and Schofield *in* Williams, Sharpe and Holt 1989, 2503]

vir.i.di.flav′us. L. adj. *viridis* green; L. adj. *flavus* yellow; N.L. masc. adj. *viridiflavus* green-yellow.

Poor off-white aerial vegetation. White spore mass. Melanin pigment is not produced. Coumarin, L-methionine, L-proline, and shikimic acid are utilized, but not mannitol, melibiose, raffinose, sorbitol, or DL-α-aminobutyric acid. Produces acid from *myo*-inositol, D-ribose, and trehalose, but not from D-galactose or D-fructose. Citrate and hypoxanthine are degraded, but not esculin or L-tyrosine. H_2S is produced. No reduction of NO_3^-. Resistance shown to cephalotin (30 μg/ml), colistin (30 μg/ml), and cephamandole (30 μg/ml). *Aspergillus niger* and *Candida albicans* are inhibited but not *Bacillus subtilis*. The strain shows antifungal activity.

For sequence similarity, see type strain of *Streptomyces olivoverticillatus*.

Source: not known.

DNA G+C content (mol%): not known.

Type strain: Y. E. Konev, ATCC 12631, CBS 652.72, DSM 40237, NBRC 13351, NBRC 15799, JCM 4221, JCM 4857, NRRL B-1548, NRRL-ISP 5237, RIA 1312.

Sequence accession no. (16S rRNA gene): AB184702.

Further comments: in violation of Rule 33c of the *Bacteriological Code* (1990 Revision), in Validation List no. 38, *Streptomyces viridiflavus* corrig. is proposed as a *nomen revictum* (basonym: "*Streptomyces viridoflavum*" Waksman and Taber 1953).

The original spelling of the specific epithet, *viridoflavum* (*sic*), has been corrected by Euzéby (1998).

According to Hatano et al. (2003), *Streptomyces viridiflavus* corrig. (Locci and Schofield 1989) Witt and Stackebrandt 1991 is a later heterotypic synonym of *Streptomyces olivoverticillatus* (Shinobu 1956) Witt and Stackebrandt 1991.

512. **Streptomyces viridiviolaceus** (Ryabova and Preobrazhenskaya *in* Gauze, Preobrazhenskaya, Kudrina, Blinov, Ryabova and Sveshnikova 1957) Pridham, Hesseltine and Benedict 1958, 70[AL] ("*Actinomyces viridiviolaceus*" Ryabova and Preobrazhenskaya *in* Gauze, Preobrazhenskaya, Kudrina, Blinov, Ryabova and Sveshnikova 1957, 188)

vi.ri.di.vi.o.la′ce.us. L. adj. *viridis* green; L. adj. *violaceus* violet-colored; N.L. masc. adj. *viridiviolaceus* green-violet, referring to the greenish color of the aerial mycelium and the violet color of diffusible pigment.

Spore chains in Section *Spirales*. Mature spore chains are moderately long, with 10–50 spores per chain. This morphology is seen on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar. Spore surface is spiny to hairy.

Color of colony: aerial mass color in the Gray color series (3fe, light brownish gray; 5fe or 5ge, light grayish reddish brown; 3ig, grayish yellowish brown) on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar. Reverse side of colony is strong brown or reddish brown on yeast-malt agar and glycerol-asparagine agar; yellowish brown or olive brown on oatmeal agar and salts-starch agar. Reverse mycelium pigment is a pH indicator, changing from yellow or orange to violet with the addition of 0.05 M NaOH and from violet or red to orange with the addition of 0.05 M HCl.

Color in medium: melanoid pigments are not formed in peptone-yeast-iron agar, tyrosine agar, or tryptone-yeast broth. Orange or red pigment is found in the medium in yeast-malt agar, salts-starch agar, and glycerol-asparagine agar but not in oatmeal agar. This pigment is pH-sensitive, showing the same changes observed with the reverse mycelium pigment.

D-Glucose, L-arabinose, D-mannitol, and D-fructose are utilized for growth. Utilization of D-xylose, iso-inositol, and sucrose is doubtful. No growth or only traces of growth with rhamnose and raffinose.

Type strain shows the highest sequence similarity to: *S. levis*, AB184670, 99.2%; *S. cellulosae*, DQ442495, 99%; *S. gancidicus*, AB184660, 99%; *S. pseudogriseolus*, DQ442541, 99%.

Source: not known.

DNA G+C content (mol%): not known.

Type strain: ATCC 27478, CBS 660.72, BCRC 12457, DSM 40280, NBRC 13359, INA 5276/56, JCM 4855, NRRL B-12182, NRRL-ISP 5280, RIA 1320, VKM Ac-587.

Sequence accession no. (16S rRNA gene): AY999854.

513. **Streptomyces viridobrunneus** (*ex* Krasil'nikov 1970b) Terekhova 1986, 575[VP] (Effective publication: Terekhova *in* Gause, Preobrazhenskaya, Sveshnikova, Terekhova and Maximova 1983.) ("*Actinomyces viridobrunneus*" Krasil'nikov 1970b)

vi.ri.do.brun′ne.us. L. adj. *viridis* green; N.L. adj. *brunneus* dark brown; N.L. masc. adj. *viridobrunneus* green dark-brown colored.

Spore chains are straight to flexuous (*Rectiflexibiles*); spores are smooth. On mineral agar 1: aerial mycelium is gray; substrate mycelium is brownish greenish olive gray; diffusible pigment is green to olive. On glycerol-nitrate agar: aerial mycelium is absent or white, poorly developed; substrate mycelium and diffusible pigment are gray brownish olive to olive gray brown. On starch-ammonia agar, glycerol-asparagine agar: aerial mycelium is absent or gray, gray brownish gray; substrate mycelium is gray brownish green; diffusible pigment is green, gray brownish green. On oatmeal agar: aerial mycelium is gray; substrate mycelium is yellowish to olive; no diffusible pigment. On organic agar 2: aerial mycelium is gray; substrate mycelium and diffusible pigment are dark gray brown. Melanoid pigments are formed. Glucose, mannose, sucrose, and raffinose are utilized for growth; no growth on mannitol, rhamnose, inositol, or arabinose. Antibiotic: pigment antibiotic viridomycin. The type strain is characterized by gray aerial mycelium, straight spore chains, building melanoid pigment and having green to olive gray brownish color of substrate mycelium and diffusible pigment under the pigment viridomycin.

Type strain shows the highest sequence similarity to: *S. showdoensis*, AB184389, 100%; *S. cinereoruber* subsp. *cinereoruber*, AB184121, 99.4%; *S. roseoviridis*, AB184239, 99.4%; *S. nashvillensis*, AB184286, 99.1%; *S. tanashiensis*, AJ781362, 99.1%; *S. vietnamensis*, DQ311081, 99.1%; *S. violaceorectus*, AB184314, 99%; *S. bikiniensis*, X79851, 99%.

Source: not known.

DNA G+C content (mol%): not known.

Type strain: ATCC 43698, DSM 41466, NBRC 15902, INMI 300, JCM 9096, VKM Ac-559.

Sequence accession no. (16S rRNA gene): AJ781372.

514. **Streptomyces viridochromogenes** (Krainsky 1914) Waksman and Henrici 1948, 942[AL] ("*Actinomyces viridochromogenes*" Krainsky 1914, 684)

vi.ri.do.chro.mo′ge.nes. L. adj. *viridis* green; Gr. n. *chroma* color; N.L. suff. *-genes* (from Gr. v. *gennaô* to produce) producing; N.L. part. adj. *viridochromogenes* producing green color.

Spore chains in Section *Spirales*. Mature spore chains are generally long, often with more than 50 spores per chain. This morphology is seen on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar. Spore surface is spiny.

Color of colony: aerial mass color in the Green color series (24ih, grayish green; 24½h, dark grayish green), Yellow color series (24½dc, pale yellow green) or Gray color series (2dc, yellowish gray) on yeast-malt agar. It is in the Yellow (1dc, pale yellow green), Green (24½li, dark greenish gray), or Blue (22fe, pale green) color series on oatmeal agar. It is in the Blue (19dc, pale blue; 22fe, pale green) color series on salts-starch agar. It is in the Blue (19dc, pale blue; 22fe, pale green) or Yellow (1dc, pale yellow green) color series on glycerol-asparagine agar. Reverse side of colony is olive brown to dark olive on yeast-malt agar; grayish green to grayish olive on oatmeal agar; grayish yellow, olive brown, or greenish yellow on salts-starch agar and glycerol-asparagine agar. Reverse mycelium pigment is a pH indicator, changing from bluish or greenish brown to reddish or purplish brown with the addition of 0.05 M HCl.

Color in medium: melanoid pigments are not formed in peptone-yeast-iron agar or tryptone-yeast broth; tyrosine agar may or may not be darkened in 2–4 d. Greenish pigment is found in the medium in oatmeal agar. This pigment is pH-sensitive, changing from blue green or greenish to pink with the addition of 0.05 M HCl.

D-Glucose, L-arabinose, D-xylose, iso-inositol, D-mannitol, D-fructose, rhamnose, and raffinose are utilized for growth. Utilization of sucrose is doubtful.

Type strain shows the highest sequence similarity to: *S. paradoxus*, AB184628, 99.4%; *S. matensis*, AB184221, 99.3%; *S. collinus*, AB184123, 99.3%; *S. griseoflavus*, AJ781322, 99.2%; *S. griseorubens*, AB184139, 99.2%; *S. flaveolus*, AB184764, 99.2%; *S. griseoincarnatus*, AJ781328, 99.1%; *S. labedae*, AB184704, 99.1%; *S. erythrogriseus*, AJ781328, 99.1%; *S. violaceorubidus*, AJ781374, 99%; *S. glaucescens*, AB184843, 99%; *S. variabilis*, DQ442551, 99%.

Source: not known.

DNA G+C content (mol%): not known.

Type strain: ATCC 14920, CBS 140.20, CBS 648.72, BCRC 13769, CECT 3216, DSM 40110, HAMBI 1023, HUT 6030, NBRC 13347, NBRC 3113, JCM 4265, JCM 4856, NCIMB 9597, NRRL B-1511, NRRL-ISP 5110, RIA 1308, VKM Ac-629.

Sequence accession no. (16S rRNA gene): DQ442555.

515. **Streptomyces viridodiastaticus** (Baldacci, Grein and Spalla 1955) Pridham, Hesseltine and Benedict 1958, 67[AL] ["*Actinomyces virido-diastaticus*" (*sic*) Baldacci, Grein and Spalla 1955, 133]

vi.ri.do.di.a.sta′ti.cus. L. adj. *viridis* green; N.L. adj. *diastaticus*, diastatic, starch, digesting; N.L. masc. adj. *diastaticus* green-diastatic.

Spore chains in Section *Spirales* but abundant open spirals and flexuous chains also suggest *Retinaculiaperti* morphology. Spirals are most abundant on salts-starch agar. Mature spore chains are moderately long with 10–50 spores per chain. This morphology is seen on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar. Spore surface is spiny, but spines may not be apparent on some spores.

Color of colony: aerial mass color in the Gray color series (2fe, medium gray to 2ih, light olive-gray or 3ig, grayish yellowish brown) on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar. Reverse side of colony with no distinctive pigments (yellowish brown to olive-brown on yeast-malt agar; pale yellow-green or pale yellow and grayish yellow on oatmeal agar, salts-starch agar, and glycerol-asparagine agar).

Color in medium: melanoid pigments are not formed in peptone-yeast-iron agar, tyrosine agar, or tryptone-yeast broth. No pigment is found in medium in yeast-malt agar, oatmeal agar, salts-starch agar, or glycerol-asparagine agar.

D-Glucose, L-arabinose, D-xylose, iso-inositol, D-mannitol, D-fructose, and rhamnose are utilized for growth. Reports vary on utilization of sucrose and raffinose.

Type strain shows the highest sequence similarity to: *S. albogriseolus*, AJ494865, 99.8%; *S. coeruleorubidus*, AY999719, 99.4%; *S. bellus*, AB184849, 99.2%; *S. griseorubens*, AB184139, 99.2%; *S. coerulescens*, AY999720, 99.2%; *S. atrovirens*, DQ026672, 99.2%; *S. iakyrus*, AB184877, 99.1%; *S. longispororuber*, AB184440, 99.1%; *S. variabilis*, DQ442551, 99%; *S. erythrogriseus*, AJ781328, 99%; *S. lusitanus*, AB184424, 99%; *S. labedae*, AB184704, 99%; *S. speibonae*, AF452714, 99%; *S. griseoincarnatus*, AJ781328, 99%.

Source: not known.

DNA G+C content (mol%): not known.

Type strain: ATCC 25518, CBS 660.69, BCRC 12458, DSM 40249, NBRC 13106, JCM 4536, NRRL B-5622, NRRL-ISP 5249, RIA 1298, VKM Ac-1749.

Sequence accession no. (16S rRNA gene): AY999852.

516. **Streptomyces viridosporus** Pridham, Hesseltine and Benedict 1958, 67[AL]

vi.ri.do.spo′rus. L. adj. *viridis* green; N.L. n. *spora* a spore; N.L. masc. adj. *viridosporus* green-spored.

Spore chains in Section *Spirales*. Spirals are sometimes irregular or imperfect when spore chains are short. Mature spore chains are generally short but contain more than 10 spores per chain. This morphology is seen on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar. Spore surface is spiny to hairy.

Color of colony: aerial mass color in the Green color series on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar. Three observers selected a variety of color tabs as the nearest matching color including: 2ih, light olive; 1½li, olive gray; 1½ge, 1½ig, 1ig, light grayish olive; and 24½ih, dark greenish gray. Reverse side of colony with no distinctive pigments (yellowish brown to olive brown on yeast-malt agar; pale grayish yellow to light yellowish brown or grayish olive on oatmeal agar, salts-starch agar, and glycerol-asparagine agar).

Color in medium: melanoid pigments are not formed in peptone-yeast-iron agar, tyrosine agar, or tryptone-yeast broth in 2–4 d. No pigment is found in the medium in yeast-malt agar, oatmeal agar, salts-starch agar, or glycerol-asparagine agar.

D-Glucose, L-arabinose, D-xylose, iso-inositol, D-mannitol, D-fructose, and rhamnose are utilized for growth. No growth or only traces of growth with sucrose or raffinose.

Type strain shows no sequence similarity over 99%.

Source: not known.

DNA G+C content (mol%): not known.

Type strain: ATCC 27479, CBS 654.72, BCRC 11870, CCUG 37512, DSM 40243, NBRC 13353, IMET 43514, JCM 4859, KCTC 9145, NCIMB 9824, NRRL 2414, NRRL-ISP 5243, RIA 1314, VKM Ac-1769, VKM Ac-618.

Sequence accession no. (16S rRNA gene): DQ442556.

517. **Streptomyces vitaminophilus** corrig. (Shomura, Amano, Yoshida, Ezaki, Ito and Niida 1983) Goodfellow, Williams and Alderson 1986a, 575[VP] (Effective publication: Goodfellow, Williams and Alderson 1986e, 63.) (*Actinosporangium vitaminophilum* Shomura, Amano, Yoshida, Ezaki, Ito and Niida 1983, 563)

vi.ta.mi.no′phi.lus. N.L. n. *vitaminum* vitamin; N.L. masc. adj. *philus* (from Gr. masc. adj. *philos*) friend, loving; N.L. masc. adj. *vitaminophilus* vitamin-loving.

Spores contained in "pseudosporangia"; the spore surface is smooth. Forms extensively branched substrate mycelium but scant aerial mycelium. The reverse side of colonies is colorless to pale tan or pale grayish yellow. Growth is often enhanced by vitamin B_{12}. Does not form melanin pigments. Gelatin and starch degraded; nitrate is reduced. D-Glucose, glycerol, L-rhamnose, and D-xylose are used as sole carbon sources but L-arabinose, D-fructose, *myo*-inositol, mannitol, raffinose, and sucrose are not. Grows at 15 and 45°C; optimal growth between 25 and 34°C. Wall peptidoglycan contains LL-A_2pm as the major diamino acid; the predominant isoprenologs are hexa- and octa-hydrogenated menaquinones with nine isoprene units. Produces antibiotics of the pyrrolomycin complex.

Type strain shows no sequence similarity over 99%.

Source: isolated from soil.

DNA G+C content (mol%): not known.

Type strain: SF 2080, ATCC 31673, DSM 41686, NBRC 14294, JCM 6054, NRRL B-16933.

Sequence accession no. (16S rRNA gene): AB184589.

Further comments: the original spelling, *Streptomyces vitaminophileus* (*sic*), has been corrected by the Associate Editor, IJSB 1986.

518. **Streptomyces wedmorensis** (*ex* Millard and Burr 1926) Preobrazhenskaya 1986, 575[VP] (Effective publication: Preobrazhenskaya *in* Gause, Preobrazhenskaya, Sveshnikova, Terekhova and Maximova 1983.) ("*Actinomyces wedmorensis*" Millard and Burr 1926)

wed.mor.en′sis. N.L. masc. adj. *wedmorensis* of or pertaining to Wedmore, a city in England.

Spore chains are straight (*Rectiflexibiles*); spores are smooth. On mineral agar 1, oatmeal agar, and starch-ammonia agar: aerial mycelium is whitish gray to gray; substrate mycelium is colorless; no diffusible pigment. On glycerol-nitrate agar: aerial mycelium is poor, white; substrate mycelium yellow; no diffusible pigment. On organic agar 2: aerial mycelium is poor, white; substrate mycelium colorless; no diffusible pigment. On glycerol-asparagine agar: aerial mycelium is poor, white; substrate mycelium light gray-brown; no diffusible pigment. Melanoid pigments are not formed. Good growth on glucose, xylose, mannitol, and fructose; poor growth on arabinose and rhamnose. Antibiotic: phosphonomycin, antibiotic 280.

Type strain shows the highest sequence similarity to: *S. exfoliatus*, AB184324, 99.7%; *S. zaomyceticus*, EF178685, 99.7%; *S. venezuelae*, AB045890, 99.7%; *S. omiyaensis*, EF178697, 99.6%; *S. lateritius*, AB184324, 99.6%; *S. litmocidini*, AB184149, 99.5%; *S. yereyanensis* EF178684, 99.3%; *S. narbonensis*, DQ445794, 99.2.

Source: not known.

DNA G+C content (mol%): not known.

Type strain: ATCC 21239, BCRC 12667, CECT 3245, DSM 41676, ICMP 12544, NBRC 14062, JCM 4937, NRRL 3426, VKM Ac-1861.

Sequence accession no. (16S rRNA gene): DQ442557.

519. **Streptomyces werraensis** Wallhäusser, Huber, Nesemann, Präve and Zepf 1964, 357[AL]

wer.ra.en′sis. N.L. masc. adj. *werraensis* of or belonging to River Werra, Germany (referring to "werramycin," the name originally assigned to the antibiotics produced).

Spore chains in Section *Spirales* or *Retinaculiaperti*. Imperfect spirals, flexuous chains, hooks, and loops are common; well-developed spirals are rare. Mature spore chains generally contain 10 to 50 or more spores per chain. This morphology is seen on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar. Spore surface is spiny.

Color of colony: aerial mass color in the Gray color series (2dc, yellowish gray; 3fe, light brownish gray; g or e, medium gray) on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar. Aerial mycelium is sometimes absent or poorly developed on oatmeal agar, salts-starch agar, and glycerol-asparagine agar. Reverse side of colony with no distinctive pigments (nearly colorless or pale yellowish gray on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar).

Color in medium: melanoid pigments are not formed in peptone-yeast-iron agar, tyrosine agar, or tryptone-yeast broth. No pigment is found in the medium in

yeast-malt agar, oatmeal agar, salts-starch agar, or glycerol-asparagine agar.

D-Glucose, L-arabinose, D-xylose, iso-inositol, D-mannitol, D-fructose, and rhamnose are utilized for growth. Reports vary on amount of growth on sucrose, but utilization is doubtful. No growth or only traces of growth with raffinose.

Type strain shows the highest sequence similarity to: *S. biverticillatus*, AJ781381, 100%; *S. albireticuli*, AB184881, 99.5%; *S. netropsis*, AB184848, 99.3%; *S. eurocidicus*, AY999790, 99.3%; *S. cinnamoneus*, AB184850, 99.1%; *S. hiroshimensis*, AB184789, 99%.

Source: not known.

DNA G+C content (mol%): not known.

Type strain: ATCC 14424, CBS 437.67, CBS 705.72, BCRC 12038, DSM 40486, NBRC 13404, JCM 4860, NRRL B-5317, NRRL-ISP 5486, RIA 1365.

Sequence accession no. (16S rRNA gene): DQ442558.

520. **Streptomyces willmorei** (Erikson 1935) Waksman and Henrici *in* Breed, Murray and Hitchens 1948, 966[AL] ["*Actinomyces willmorei*" Erikson 1935, 36; *Streptomyces microflavus* (Krainsky 1914) Waksman and Henrici 1948, 950 emend. Lanoot, Vancanneyt, Van Shoor, Liu and Swings 2005b, 731]

will.mo′re.i. N.L. gen. masc. n. *willmorei* of Willmore, named for J.G. Willmore, the surgeon who first isolated the organism.

Spore chains in Section *Rectiflexibiles*. Mature spore chains are short to moderately long, usually with more than 10 spores per chain. This morphology is seen on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar. Spore surface is smooth.

Color of colony: aerial mass color in the Gray, Yellow, or White color series on yeast-malt agar, oatmeal agar, and salts-starch agar; White or Yellow color series on glycerol-asparagine agar. Nearest matching color tabs in the Gray color series are d, medium gray, and 2dc, yellowish gray. Nearest matching color tabs in the Yellow color series are 2ba and 2db, pale yellow. Reverse side of colony with no distinctive pigments (light olive brown or yellowish brown on yeast-malt agar; colorless to pale grayish yellow or light grayish olive on oatmeal agar, salts-starch agar, and glycerol-asparagine agar).

Color in medium: melanoid pigments are not formed (or only a very weak discoloration occurs) in peptone-yeast-iron agar, tyrosine agar, or tryptone-yeast broth. No pigment or only a trace of yellow is found in the medium in yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar.

D-Glucose, D-xylose, D-mannitol, D-fructose, and rhamnose are utilized for growth. Utilization of L-arabinose is doubtful. No growth or only traces of growth with iso-inositol, sucrose, and raffinose.

For sequence similarity, see type strain of *Streptomyces microflavus*.

Source: not known.

DNA G+C content (mol%): not known.

Type strain: ATCC 6867, CBS 372.64, CBS 692.72, BCRC 12640, DSM 40459, NBRC 13391, IMET 41387, JCM 4861, 21046, NCIMB 12984, NCTC 1856, NRRL B-1332, NRRL-ISP 5459, RIA 1352, VKM Ac-1867.

Sequence accession no. (16S rRNA gene): AB184374.

Further comments: according to Lanoot et al. (2005b), *Streptomyces willmorei* (Erikson 1935) Waksman and Henrici 1948 is a later heterotypic synonym of *Streptomyces microflavus* (Krainsky 1914) Waksman and Henrici 1948 emend. Lanoot et al. 2005b.

521. **Streptomyces xanthochromogenes** Arishima, Sakamoto and Sato 1956, 469[AL]

xan.tho.chro.mo′ge.nes. Gr. adj. *xanthos* yellow; Gr. n. *chroma* color; N.L. suff. *-genes* (from Gr. v. *gennaô* to produce) producing; N.L. part. adj. *xanthochromogenes* producing yellow color.

Spore chains in Section *Rectiflexibiles*. Mature spore chains generally have 10–50 spores per chain. This morphology is seen on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar. Spore surface is smooth.

Color of colony: aerial mass color in the Yellow color series on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar. Reverse side of colony with no distinct pigment (yellow, grayed yellow, or yellow-brown) on yeast-malt agar, oatmeal agar, salts-starch agar, or glycerol-asparagine agar; substrate pigment is not a pH indicator.

Color in medium: melanoid pigments are formed in peptone-yeast-iron agar, tyrosine agar, and tryptone-yeast extract broth. Yellow pigment may be found in oatmeal agar and salts-starch agar or may be absent. Yellow pigment is not pH-sensitive.

D-Glucose, D-xylose, D-mannitol, and D-fructose are utilized for growth. Only trace of growth indicating doubtful utilization of iso-inositol, rhamnose, and raffinose. Variable reports on growth with L-arabinose and sucrose.

Type strain shows the highest sequence similarity to: *S. michiganensis*, AB184153, 100%; *S. mauvecolor*, AB184532, 99.4%.

Source: not known.

DNA G+C content (mol%): not known.

Type strain: AS 4.1435, ATCC 19818, CBS 571.68, BCRC 11876, DSM 2015, DSM 40111, NBRC 12828, JCM 4215, JCM 4612, NRRL B-5410, NRRL-ISP 5111, RIA 1098, UNIQEM 207, VKM Ac-1071.

Sequence accession no. (16S rRNA gene): DQ442559.

522. **Streptomyces xanthocidicus** Nagatsu, Asahi and Suzuki in Asahi, Nagatsu and Suzuki 1966, 196[AL]

xan.tho.ci′di.cus. Gr. adj. *xanthos* yellow; L. v. *caedo* to kill; N.L. masc. adj. *xanthocidicus* pertaining to yellow and to cut, probably referring to the name given the antibiotic produced which, in turn, was probably derived from its activity against *Xanthomonas oryzae*.

Spore chains in Section *Rectiflexibiles*. Mature spore chains generally are long and straight with more than 50 spores per chain. This morphology is seen on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar. Spore surface is smooth.

Color of colony: aerial mass color in the Gray color series (5fe, light grayish reddish brown; 3fe, light brownish

gray; d-2fe, light to moderate gray) on yeast-malt agar, oatmeal agar, salts-starch agar, glycerol-asparagine agar, and Gause's medium no. 1. Reverse side of colony with no distinctive pigments (colorless to pale grayish yellow on glycerol-asparagine agar; grayish yellow on oatmeal agar and salts-starch agar; light yellowish brown to brown on yeast-malt agar and Gause's medium no. 1).

Color in medium: melanin reaction variable in peptone-yeast-iron agar, tyrosine agar, and tryptone-yeast broth; both weakly positive and negative reactions are recorded by different observers in 2–4 d. No pigment is found in the medium in yeast-malt agar, oatmeal agar, salts-starch agar, or glycerol-asparagine agar.

D-Glucose, L-arabinose, D-xylose, D-fructose, sucrose, and raffinose are utilized for growth. No growth or only traces of growth with iso-inositol, D-mannitol, and rhamnose.

Type strain shows the highest sequence similarity to: *S. purpureus*, AJ781324, 99.5%; *S. aburaviensis*, AY999779, 99.4,%; *S. chrysomallus* subsp. *fumigatus*, AB184645, 99.4%; *S. herbaricolor*, AB184801, 99.2%; *S. indigoferus*, AB184214, 99.2%; *S. aureofaciens*, AY207608, 99.1%; *S. psammoticus*, AY999862, 99%. Type strain shows the highest sequence similarity to following *Kitasatospora* species: *Kitasatospora kifunensis*, AB022874, 99.1%.

Source: not known.

DNA G+C content (mol%): not known.

Type strain: AS 4.1424, ATCC 27480, CBS 770.72, BCRC 11874, DSM 40575, NBRC 13469, JCM 4243, JCM 4862, NRRL B-12504, NRRL-ISP 5575, RIA 1430, VKM Ac-872.

Sequence accession no. (16S rRNA gene): AY999858.

523. **Streptomyces xantholiticus** (Konev and Tsyganov 1962) Pridham 1970, 31[AL] ["*Actinomyces xantholiticus*" (*sic*) Konev and Tsyganov 1962, 1026]

xan.tho.li′ti.cus. Gr. adj. *xanthos* yellow; N.L. masc. adj. *lyticus* (from Gr. masc. adj. *lutikos*), able to loosen, able to dissolve; N.L. masc. adj. *xantholiticus* (*sic*) yellow and soluble (referring to the yellow color of the vegetative mycelium and the tendency of the organism to lyse when maintained on some solid media).

Spore chains in Section *Spirales*. Sporulating aerial mycelium is poorly developed on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar. Two observers report compact spirals on salts-starch agar; one observer reports spirals on yeast-malt agar and glycerol-asparagine agar. Spore surface is smooth.

Color of colony: aerial mass color in the Gray color series when adequate sporulating aerial mycelium is formed on salts-starch agar or glycerol-asparagine agar. Color of aerial mycelium cannot be determined on yeast-malt agar or oatmeal agar. Reverse side of colony is pale yellow green to greenish yellow on oatmeal agar, salts-starch agar, and glycerol-asparagine agar; moderate yellow to light olive brown on yeast-malt agar.

Color in medium: melanoid pigments are not formed in peptone-yeast-iron agar, tyrosine agar, or tryptone-yeast broth. Yellow pigment is found in the medium in glycerol-asparagine agar and sometimes in oatmeal agar or salts-starch agar. This pigment is not pH-sensitive when tested with 0.05 M NaOH or HCl.

D-Glucose, D-xylose, and D-fructose are utilized for growth. Utilization of iso-inositol is doubtful; no growth or only traces of growth with L-arabinose, D-mannitol, rhamnose, sucrose, and raffinose.

Type strain shows the highest sequence similarity to: *S. peucetius*, AB045887, 100%; *S. kurssanovii*, AB184325, 99.9%; *S. graminofaciens*, AJ781329, 99.8%.

Source: not known.

DNA G+C content (mol%): not known.

Type strain: ATCC 27481, CBS 655.72, BCRC 12646, DSM 40244, NBRC 13354, JCM 4282, JCM 4863, NCIMB 9857, NRRL B-12153, NRRL-ISP 5244, RIA 1315, VKM Ac-1872.

Sequence accession no. (16S rRNA gene): AB184349.

524. **Streptomyces xanthophaeus** Lindenbein 1952, 378[AL]

xan.tho.pha′e.us. N.L. masc. adj. *xanthophaeus* (from Gr. adj. *xanthopos* golden-looking) shining like gold.

Spore chains in Section *Rectiflexibiles*. Mature spore chains are very long, usually more than 50 spores per chain. This morphology is found on oatmeal agar, salts-starch agar, and glycerol-asparagine agar; poor sporulation on yeast-malt agar. Spore surface is smooth.

Color of colony: aerial mass color in the Red or Gray color series on oatmeal agar, salts-starch agar, and glycerol-asparagine agar. Nearest matching color tabs: grayish yellowish pink (5dc, Red series) to light grayish reddish brown (5fe to 4li, Gray color series). Reverse side of colony is colorless or characteristic grayed yellow to yellow-brown on yeast-malt agar, oatmeal agar, and glycerol-asparagine agar, but very dark reddish gray to near black on salts-starch agar. Reverse color is not a pH indicator.

Color in medium: melanoid pigments are formed in peptone-yeast-iron agar and tryptone-yeast extract broth. Pigments other than traces of yellow are not formed in yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar. D-Glucose is utilized for growth. No growth or only trace of growth on L-arabinose, sucrose, D-xylose, iso-inositol, D-mannitol, D-fructose, rhamnose, and raffinose.

Type strain shows the highest sequence similarity to: *S. nojiriensis*, AJ781355, 100%; *S. sporoarveus*, AJ781370, 100%; *S. vinaceus*, AB184394, 99.8%; *S. cirratus*, AY999794, 99.8%; *S. goshikiensis*, EF178693, 99.8%; *S. colombiensis*, DQ026646, 99.7%; *S. cinnamonensis*, AB184707, 99.7%; *S. avidinii*, AB184395, 99.7%; *S. sporoverrucosus*, DQ442544, 99.7%; *S. subrutilus*, X80825, 99.6%; *S. virginiae*, D85119, 99.5%.

Source: not known.

DNA G+C content (mol%): not known.

Type strain: ATCC 19819, CBS 572.68, BCRC 13756, DSM 40134, NBRC 12829, JCM 4426, KCTC 9144, KCTC 9220, NRRL B-5414, NRRL-ISP 5134, RIA 1099, UNIQEM 208, VKM Ac-1205, VKM Ac-1823.

Sequence accession no. (16S rRNA gene): DQ442560.

525. **Streptomyces yanglinensis** Xu, Wang, Cui, Huang, Liu, Zheng and Goodfellow 2006, 1114[VP]

yang.lin.en′sis. N.L. masc. adj. *yanglinensis* of or belonging to Yanglin, the source of the soil from which the type strain was isolated.

Neutrotolerant, acidophilic streptomycete that forms branched substrate and aerial hyphae. Smooth-surfaced spores are borne on flexuous spore chains. Gray-colored colonies that carry an abundant white to gray aerial spore mass are formed on acidified oatmeal agar; blackish colonies bearing an abundant white to gray aerial spore mass are formed on ISP 9 agar supplemented with fructose, mannose, or sucrose (each at 1%, w/v). Diffusible pigments are not produced, and melanin pigments are not formed on peptone-yeast extract-iron agar or tyrosine agar. Degrades starch and Tween 80, but not adenine, guanine, or xanthine. Adonitol, cellobiose, D-galactose, D-glucose, D-inulin, D-lactose, D-mannitol, D-salicin, and D-sorbitol (each at 1%, w/v) are used as sole carbon sources for energy and growth, but melezitose (at 1%, w/v), adipic acid, L-alanine, DL-aminobutyric acid, L-arginine, α-L-aspartic acid, L-cysteine, L-phenylalanine, L-valine, sodium acetate, sodium citrate, and sodium oxalate (each at 0.1%, w/v) are not. L-Glutamic acid is used as a sole carbon and nitrogen source, but L-alanine, L-arginine, L-aspartic acid, L-isoleucine, and L-phenylalanine (each at 0.1%, w/v) are not. Growth occurs at temperatures between 20 and 37°C, but not at 15°C, and at pH values between 4.5 and 7.0, but not at pH 3.5. Does not grow in the presence of 5% (w/v) NaCl. Sensitive to filter-paper discs soaked in the following (µg/ml unless indicated): cephalothin (30), doxycycline hydrochloride (30), erythromycin (15), josamycin (15), kanamycin sulfate (30), minocycline hydrochloride (30), neomycin sulfate (30), sulfamethoxazole (25), and tobramycin sulfate (10), but not to filter-paper discs soaked in acetylspiramycin (15), amoxycillin (10), ampicillin (10), azithromycin (30); azetreonam (30), carbenicillin (10), ciprofloxacin (5), ofloxacin (5), penicillin G (10 IU/ml), rifampin, streptomycin sulfate (10), or tetracycline hydrochloride (30).

Type strain shows no sequence similarity over 99%. Type strain shows DNA–DNA similarity to: *S. yeochonensis* NRRL B-24245^{T}, 21.0%; *S. guanduensis* JCM 13274^{T}, 23.1%; *S. paucisporeus* JCM 13276^{T}, 25.9%; *S. rubidus* JCM 13277^{T}, 23.8%.

Source: the type strain was isolated from a pine-forest soil in Yanglin, Yunnan Province, People's Republic of China.

DNA G+C content (mol%): 74.8.

Type strain: 1307, CGMCC 4.2023, JCM 13275.

Sequence accession no. (16S rRNA gene): AY876940.

526. **Streptomyces yanii** (Yan, Jiang and Zhang 1987) Liu, Shi, Zhang, Zhou, Lu, Li, Huang, Rodríguez and Goodfellow 2005b, 1609^{AL} ("*Microstreptospora cinerea*" Yan, Jiang and Zhang 1987)

ya'ni.i. N.L. gen. masc. n. *yanii* of Yan, named in honor of Yunchu Yan, a Chinese microbiologist.

Forms an extensively branched substrate mycelium and aerial hyphae. The substrate mycelium carries abundant spherical, smooth-surfaced spores (about 2.5 µm in diameter) singly or in short chains of 2–4 spores on short sporophores. Short chains of ellipsoidal smooth-surfaced spores (0.8 × 2.0 µm in diameter) are formed on aerial hyphae. The latter are usually formed only on inorganic media; an abundant gray aerial spore mass is apparent on oatmeal and Krass's No. 1 agars. Forms an abundant gray to black substrate mycelium on Czapek's, Krass's, and oatmeal agars. Gelatin and starch are degraded, but not cellulose or tyrosine. Nitrate is reduced. H_2S is not formed. Milk is coagulated but not peptonized. Good growth occurs at 18–35°C and pH 6–9. Whole-organism hydrolysates contain major proportions of LL-A_2pm, glycine, galactose, and mannose, and a trace of ribose. The muramic acid of the peptidoglycan is *N*-acetylated. The predominant isoprenologs are tetra- and hexa-hydrogenated menaquinones with nine isoprene units and the major phospholipids are diphosphatidylglycerol and phosphatidylinositol mannosides.

Type strain shows the highest sequence similarity to: *S. atratus*, DQ026638, 99.8%; *S. sanglieri*, AB249945, 99.7%; *S. cinereorectus*, AB184646, 99.5%; *S. pulveraceus*, AB184806, 99.5%; *S. griseinus*, AB184205, 99.4%; *S. pluricolorescens*, DQ442540, 99.4%; *S. alboviridis*, AB184256, 99.4%; *S. mediolani*, AB184674, 99.4%; *S. griseoplanus*, AY999894, 99.4%; *S. sindenensis*, AB184759, 99.4%; *S. rubiginosohelvolus*, AB184240, 99.4%; *S. gelaticus*, DQ026636, 99.4%; *S. fimicarius*, AY999784, 99.4%; *S. globisporus* subsp. *globisporus*, EF178686, 99.4%; *S. fulvorobeus*, AB184711, 99.4%; *S. lipmanii*, AB184148, 99.4%; *S. badius*, AY999783, 99.4%; *S. microflavus*, DQ445795, 99.4%; *S. argenteolus*, AB045872, 99.4%; *S. griseolus*, AB184768, 99.4%; *S. acrimycini*, AY999889, 99.4%; *S. lavendulae* subsp. *lavendulae*, AB184080, 99.4%; *S. flavofuscus*, AB249935, 99.4%; *S. anulatus*, DQ026637, 99.4%; *S. griseus* subsp. *griseus*, AY207604, 99.4%; *S. cavourensis* subsp. *washingtonensis*, DQ026671, 99.4%; *S. praecox*, AB184293, 99.4%; *S. cyaneofuscatus*, AB184860, 99.4%; *S. albovinaceus*, AB249958, 99.3%; *S. flavovirens*, DQ026635, 99.3%; *S. parvus*, DQ442537, 99.3%; *S. halstedii*, EF178695, 99.3%; *S. baarnensis*, EF178688, 99.3%; *S. luridiscabiei*, AF361784, 99.2%; *S. californicus*, AB184755, 99.2%; *S. floridae*, AB184656, 99.2%; *S. flavogriseus*, AJ494864, 99.2%; *S. nitrosporeus*, EF178680, 99%; *S. bacillaris*, AB184439, 99%. Type strain shows DNA–DNA similarity to: *S. argenteolus* AS 4.1693^{T}, 13%; *S. caviscabies* DSM 41811^{T}, 30%; *S. griseus* AS 4.1419^{T}, 31%; *S. laceyi* AS 4.1832^{T}, 14%; *S. sanglieri* AS 4.1831^{T}, 33%; *S. setonii* AS 4.1774^{T}, 25%; *S. peucetius* AS 4.1799^{T}, 14%; *S. purpureus* AS 4.1225^{T}, 11%; *S. venezuelae* AS 4.1307^{T}, 16%.

Source: the sole strain was isolated from a mud sample collected from a sewage ditch in Zhanjiang City, Guangdong Province, China.

DNA G+C content (mol%): 69.4.

Type strain: 80-133, AS 4.1146, NBRC 14669, JCM 3331.

Sequence accession no. (16S rRNA gene): AB006159.

Further comments: Streptomyces yanii Liu et al. 2005b was previously known as "*Microstreptospora cinerea*" Yan et al. 1987.

527. **Streptomyces yatensis** Saintpierre, Amir, Pineau, Sembiring and Goodfellow 2003b, 1219^{VP} (Effective publication: Saintpierre, Amir, Pineau, Sembiring and Goodfellow 2003a, 25.)

ya.ten'sis. N.L. masc. adj. *yatensis* pertaining to the Yaté region of New Caledonia, the source of the isolate.

Forms an extensively branched substrate mycelium which carries aerial hyphae that differentiate into spiral spore chains with three or four turns; there are 15–20 cylindrical to barrel-shaped spores per chain. Spore surface is rugose. On oatmeal agar, the spore mass is initially gray, then black; the substrate mycelium is grayish-yellow. Mela-

nin pigments are not produced. Growth occurs at 20, 30, and 37°C, but not at 10°C or 45°C. It also occurs in the presence of ampicillin (10 µg/ml), lysozyme, penicillin (10), and sodium chloride (3%, w/v) but not in the presence of carbenicillin (100), chloramphenicol (30), gentamicin sulfate (10), neomycin sulfate (30), streptomycin sulfate (30), tetracycline hydrochloride (30), or sodium chloride (5% w/v). The organism shows activity against some of the microbial strains isolated from clinical material (the *Bacillus*, *Corynebacterium*, *Candida albicans*, *Klebsiella pneumoniae*, and *Staphylococcus aureus* strains) and against the phytopathogen (*Fusarium oxysporum*) but not against strains of *Erwinia*, *Escherichia coli*, *Pseudomonas aeruginosa*, or *Staphylococcus epidermidis*. 70% toxicity is shown against Kb cells using a concentration of 10 µg/ml of a crude extract.

Type strain shows no sequence similarity over 99%.

Source: the organism was isolated from a ferraltic soil collected at the southern end of New Caledonia.

DNA G+C content (mol%): not known.

Type strain: SFOCin 76, DSM 41771, JCM 13244, NBRC 101000, NRRL B-24116.

Sequence accession no. (16S rRNA gene): AB249962.

528. **Streptomyces yeochonensis** Kim, Seon, Jeon, Bae and Goodfellow 2004, 213^{VP}

ye.o.chon.en′sis. N.L. masc. adj. *yeochonensis* of or belonging to Yeochon, a province in Korea, referring to the place where the organism was first isolated.

Aerobic, Gram-stain-positive, nonmotile, neutrotolerant acidophilic streptomycete that forms extensively branched substrate and aerial mycelia. Smooth-surfaced spores are borne in flexuous spore-chains. Aerial spore mass color is gray. Substrate mycelia have no distinctive color; diffusible pigments are not produced. pH range for growth is 4.3–7.3. Casein, gelatin, guanine, starch, and Tween 80 are degraded, but elastin, hypoxanthine, testosterone, Tween 20, tyrosine, and xanthine are not. Good growth occurs between 25 and 37°C, but not at 12 or 45°C. The sugars erythritol, inulin, melezitose, salicin, ribitol, and sorbitol (all at 1%, w/v) are used as sole carbon sources, as are β-hydroxybutyric acid, D-gluconic acid, hippuric acid, α-ketoglutaric acid, 2-keto-D-glucuronic acid, lactic acid, malic acid, malonic acid, oxalic acid, pyruvic acid, and succinic acid (as sodium salts) (all at 0.1%, w/v).

Type strain shows no sequence similarity over 99%.

Source: the type strain was isolated from acidic soil collected in the Yeochon area of the Republic of Korea.

DNA G+C content (mol%): not known.

Type strain: CN732, JCM 12366, KCTC 9926, IMSNU 50114, NBRC 100782, NRRL B-24245.

Sequence accession no. (16S rRNA gene): AF101415.

529. **Streptomyces yerevanensis** (Tsyganov, Zhukova and Timofeeva 1964) Goodfellow, Williams and Alderson 1986a, 575^{VP} (Effective publication: Goodfellow, Williams and Alderson 1986a, 52.) ["*Macrospora violaceus*" (*sic*) Tsyganov, Zhukova and Timofeeva 1964, 868; *Microellobosporia violacea* Pridham *in* Buchanan and Gibbons 1974, 844]

ye.re.van.en′sis. N.L. masc. adj. *yerevanensis* of or pertaining to Yerevan, Armenia.

Forms extensively branched substrate and aerial mycelium. Short chains (2–8) of smooth-surfaced spores borne on both the substrate and aerial mycelium. Aerial spore mass is light mouse gray; reverse color is violet and the pigment is pH-sensitive. Violet diffusible pigment, which is also pH-sensitive, is produced. Does not form melanin pigments. Adenine, esculin, arbutin, casein, hypoxanthine, starch, testosterone, and tyrosine are degraded but allantoin, chitin, guanine, lecithin, pectin, urea, xanthine, and xylan are not. Adonitol, L-arabinose, cellobiose, D-fructose, D-glucose, *myo*-inositol, inulin, D-lactose, mannitol, D-mannose, melibiose, melezitose, raffinose, L-rhamnose, salicin, sucrose, and D-xylose are used as sole carbon sources but D-galactose, maltose, trehalose, and xylitol are not. Grows on L-arginine, L-histidine, potassium nitrate, L-serine, L-threonine, and L-valine, but not on DL-α-amino-n-butyric acid, L-cysteine, L-hydroxyproline, L-methionine, or L-phenylalanine, as sole nitrogen source. Growth occurs at 10 and 37°C, but not at 4 or 45°C. Tolerant to phenol (0.1%, w/v) and sodium chloride (4%, w/v), but not to sodium azide (0.01%, w/v). Resistant to rifampin but sensitive to sodium chloride (7%, w/v). Does not show antimicrobial activity against *Aspergillus niger* LIV 131, *Bacillus subtilis* NCIB 3610, *Candida albicans* CBS 562, *Escherichia coli* NCIB 9132, *Micrococcus luteus* NCIB 196, *Pseudomonas fluorescens* NCIB 9046^T, *Saccharomyces cerevisiae* CBS 1171^T, or *Streptomyces murinus* ISP 5091. The peptidoglycan contains LL-A_2pm as the major diamino acid. The organism has a type II phospholipid pattern (Lechevalier et al., 1977) and contains major amounts of tetra-, hexa-, and octahydrogenated menaquinones with nine isoprene units (Alderson et al., 1985; Collins et al., 1984). Produces violacin, a pigmented antibiotic of the rhodomycin-cinerubin-mycetin type.

Type strain shows the highest sequence similarity to: *S. litmocidini*, AB184149, 99.8%; *S. exfoliatus*, AB184324, 99.4%; *S. venezuelae*, AB045890, 99.4%; *S. zaomyceticus*, EF178685, 99.4%; *S. lateritius*, AB184324, 99.3%; *S. wedmorensis*, DQ442557, 99.3%; *S. omiyaensis*, EF178697, 99.3%.

Source: isolated from soil, Armenia, USSR.

DNA G+C content (mol%): 68.0–70.0.

Type strain: AS 4.1464, ATCC 43727, BCRC 11564, DSM 43167, IFM 1151, IFM 1242, NBRC 12517, IMET 43616, JCM 3047, JCM 3065, KCC A-0047, LMG 19363, NCIB (now NCIMB) 9589, NRRL B-16943, RIA 795, VKM Ac-1234.

Sequence accession no. (16S rRNA gene): EF178684.

Further comments: for the transfer of *Microellobosporia violacea* (Tsyganov et al. 1964) Pridham 1974 to the genus *Streptomyces* Waksman and Henrici 1943 it is necessary to substitute a new specific epithet to produce *Streptomyces yerevanensis* because there is a senior homonym, *Streptomyces violaceus* (Rossi Doria 1891) Waksman 1953, included on the Approved Lists of Bacterial Names [Rules 34a and 41a of the *Bacteriological Code* (1990 Revision)].

530. **Streptomyces yogyakartensis** Sembiring, Ward and Goodfellow 2001, 1619^{VP} (Effective publication: Sembiring, Ward and Goodfellow 2000, 363.)

yog.ya.kar.ten′sis. N.L. masc. adj. *yogyakartensis* of or pertaining to Yogyakarta, Indonesia.

Spore chains are *Spirales*; spore surface is rugose. On oatmeal agar the spore mass is gray, the substrate mycelium is grayish-yellow, and the diffusible pigment is yellow. Melanin pigments are not produced. The organism grows at 45°C, degrades adenine but does not reduce nitrate.

Type strain shows the highest sequence similarity to: *S. violaceusniger*, AJ391823, 100%; *S. albiflaviniger*, AJ391812, 99.7%; *S. endus*, AY999911, 99.5%; *S. sporocinereus*, AB249933, 99.5%; *S. demainii*, DQ334782, 99.5%; *S. hygroscopius* subsp. *hygroscopius*, AB184428, 99.5%; *S. javensis*, AJ391833, 99.2%. Type strain shows DNA–DNA similarity to: *S. albiflaviniger* NRRL B-1356^T, 98.9%; *S. geldanamycinus* NRRL 3602^T, 98.7%; *S. griseiniger* NRRL B1865^T, 98.3%; *S. rhizosphaericus* DSM 41760^T, 97.9%; *S. asiaticus* DSM 41761^T, 98.1%; *S. indonesiensis* DSM 41759^T, 98.3%; *S. javensis* DSM 41764^T, 98.6%; *S. cangkringensis* DSM 41769^T, 98.2%.

Source: isolated from non-rhizosphere soil adjacent to a stand of the tropical legume, *Paraserianthes falcataria*.

DNA G+C content (mol%): not known.

Type strain: C4R3, DSM 41766, JCM 11448, NBRC 100779, NCIMB 13681.

Sequence accession no. (16S rRNA gene): AB249942.

531. **Streptomyces yokosukanensis** Nakamura 1961, 94[AL]

yo.ko.su'ka.nen'sis. N.L. masc. adj. *yokosukanensis* (*sic*) of or belonging to Yokosuka City, Kanagawa Prefecture, Japan, the source of the soil from which the organism was isolated.

Spore chains in Section *Spirales* or *Retinaculiaperti*. Tight spirals are common at the ends of long spore chains. Open spirals, hooks, and flexuous chains are also found. Spore surface is spiny according to one observer; another observer finds only smooth spores.

Color of colony: aerial mass color in the Red color series (grayish yellowish pink) on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar. Reverse side of colony with no distinctive pigments (grayish yellow, light yellowish brown, olive-brown, or strong brown) on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar.

Color in medium: melanoid pigments are formed in peptone-yeast-iron agar, tyrosine agar, and tryptone-yeast broth. No pigment or only a trace of yellow is found in medium in yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar.

D-Glucose, L-arabinose, D-xylose, iso-inositol, D-mannitol, D-fructose, rhamnose, and raffinose are utilized for growth. No growth or only trace of growth with sucrose.

Type strain shows the highest sequence similarity to: *S. cellostaticus*, AB184192, 99.5%; *S griseochromogenes*, AB184387, 99.3%.

Source: isolated from soil from Yokosuka City, Kanagawa Prefecture, Japan.

DNA G+C content (mol%): not known.

Type strain: ATCC 25520, CBS 662.69, BCRC 11875, DSM 40224, NBRC 13108, JCM 4137, JCM 4559, NRRL B-3353, NRRL-ISP 5224, RIA 1300, VKM Ac-1713.

Sequence accession no. (16S rRNA gene): DQ026652.

532. **Streptomyces yunnanensis** Zhang, Li, Cui, Li, Xu and Jiang 2003, 220[VP]

yun.nan.en'sis. N.L. masc. adj. *yunnanensis* of or pertaining to Yunnan, a province of south-west China.

Spore chains with many spores are spiral. The spores are rugose with short spines and are short pillar-shaped (0.5–1.0 μm in diameter) and nonmotile. Vegetative and aerial hyphae are abundant and well-developed. Diffusible pigments are not produced and melanin is not produced. Milk is not coagulated but peptonized, starch is hydrolyzed, and H_2S is not produced. Nitrate is not reduced and gelatin is liquefied. Does not hydrolyze cellulose. Utilizes glucose, fructose, rhamnose, inositol, mannitol, arabinose, and raffinose for growth; does not utilize sucrose or xylose. It has antimicrobial activity against *Aspergillus niger* but not against *Bacillus subtilis*. Optimum growth is at 28°C. The cell wall contains LL-A_2pm and glycine (cell-wall chemotype I). Whole-cell hydrolysates contain galactose.

Type strain shows the highest sequence similarity to: *S. albulus*, AB024440, 99.7%; *S. noursei*, AB184678, 99.7%.

Source: the type strain was isolated from red soil of suburb of Kunming in Yunnan, China.

DNA G+C content (mol%): not known.

Type strain: YIM 41004, CGMCC 4.1004, DSM 41793, JCM 12115, NBRC 100781.

Sequence accession no. (16S rRNA gene): AF346818.

533. **Streptomyces zaomyceticus** Hinuma 1954, 134[AL]

za.o.my.ce'ti.cus. English n. N.L. n. *zaomycinum* zaomycin, an antibiotic named after the Mount Zao, Japan, the source of the soil from which the organism was isolated; N.L. masc. suff. *-icus* suffix used with the sense of belonging to; N.L. masc. adj. *zaomyceticus* belonging to zaomycin.

Spore chains in Section *Rectiflexibiles*. Mature spore chains generally are long, often with more than 50 spores per chain. Long chains are flexuous, but true spirals are not formed. This morphology is seen on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar but not on salts-starch agar. Spore surface is smooth.

Color of colony: aerial mass color in the Gray or White color series on yeast-malt agar, oatmeal agar, and glycerol-asparagine agar. A good aerial mycelium is not produced on salts-starch agar. Nearest matching color tabs in the Gray color series are 2dc, yellowish gray, and d, light gray. Reverse side of colony with no distinctive pigments (olive brown to yellowish brown on yeast-malt agar; light grayish yellow to olive brown on oatmeal agar and salts-starch agar; grayish yellow on glycerol-asparagine agar).

Color in medium: melanoid pigments are formed in peptone-yeast-iron agar, tyrosine agar, and tryptone-yeast broth, but the reaction is weak in tyrosine agar. No pigment other than a melanoid pigment is found in the medium in yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar.

D-Glucose, L-arabinose, and D-xylose are utilized for growth. No growth or only traces of growth with iso-inositol, D-mannitol, D-fructose, rhamnose, sucrose, and raffinose.

Type strain shows the highest sequence similarity to: *S. exfoliatus*, AB184324, 99.9%; *S. venezuelae*, AB045890, 99.9%; *S. wedmorensis*, DQ442557, 99.7%; *S. lateritius*, AB184324, 99.7%; *S. omiyaensis*, EF178697, 99.6%; *S. litmocidini*, AB184149, 99.6%; *S. yereyanensis* EF178684, 99.4%; *S. narbonensis*, DQ445794, 99.3%.

Source: not known.

DNA G+C content (mol%): not known.

Type strain: ATCC 27482, CBS 649.72, BCRC 12317, DSM 40196, NBRC 13348, JCM 4179, JCM 4864, NCIMB 9850, NRRL B-2038, NRRL-ISP 5196, RIA 1309, VKM Ac-1192.

Sequence accession no. (16S rRNA gene): EF178685.

Genus *incertae sedis* I. **Kitasatospora** corrig. Ōmura, Takahashi, Iwai and Tanaka 1983, 672^VP (Effective publication: Ōmura Takahashi, Iwai and Tanaka 1982, 1014.) emend. Zhang, Wang and Ruan 1997, 1053

Peter Kämpfer

Ki.ta.sa.to.spo′ra. N.L. fem. n. *Kitasatoa* named for Shibasaburo Kitasato (1852–1931), a Japanese bacteriologist; N.L. fem. n. *spora* a seed and, in biology, a spore; N.L. fem. n. *Kitasatospora* Kitasato spore.

Aerobic. Gram-stain-positive, non-acid–alcohol-fast actinomycetes which form an intensively branched, non-fragmenting mycelium. A stable substrate mycelium is as well-developed as that of streptomycetes, and the aerial mycelium bears long spore chains of more than 20 spores. Does not form sporangia. The major constituents of the cell wall are glycine, galactose, and *meso*-A_2pm or LL-A_2pm, depending on the type of cells analyzed. When cells are grown on agar media, aerial spores contain LL-A_2pm, whereas the substrate mycelium contains *meso*-A_2pm. When cells are grown in liquid media, submerged spores which contain LL-A_2pm are formed while the filamentous mycelia contain *meso*-A_2pm. Whole-cell hydrolysates contain galactose, but lack arabinose, madurose, and xylose. Hexa- and octa-hydrogenated menaquinones with nine isoprene units are the predominant isoprenologs, cells are rich in saturated, iso- and anteiso-fatty acids, and have complex polar lipid patterns which contain diphosphatidylglycerol, phosphatidylethanolamine, phosphatidylinositol and phosphatidylinositol mannosides. The glycolate test is negative. The organism is chemo-organotrophic, and grows from 15 to 42°C, and within the pH range 5.5 to 9.0.

Source: not known.

DNA G+C content (mol%): 66–77.

Type species: **Kitasatospora setae** corrig. Ōmura, Takahashi, Iwai and Tanaka 1983, 672 (Effective publication: Ōmura, Takahashi, Iwai and Tanaka 1982, 1014).

Further descriptive information

The genus *Kitasatosporia* (*sic*) was proposed by Ōmura et al. (1982) for actinomycetes that were phenotypically very similar to members of the genus *Streptomyces*, but which contained major amounts of the *meso*-isomer of A_2pm and galactose in their cell walls. Zhang et al. (1997), who revived the genus, corrected the name to *Kitasatospora* albeit without a formal proposal. Some confusion has arisen as to whether the genus name *Kitasatospora* is illegitimate, because no formal proposal was made for the transfer of *Streptomyces setae* to *Kitasatospora setae*. This also has the effect of automatically changing the spelling of all species names validly published under *Kitasatosporia* (*sic*). The problem was handled by the Judicial Commission of the ICSP (De Vos et al., 2005), with the result that no further action is required by the Judicial Commission as all names listed under the genus *Kitasatospora* were validly published in accordance with the Rules of the *Bacteriological Code* (1990 Revision).

The distribution of the two isomers of A_2pm in cells of *Kitasatospora* grown at different stages of differentiation was studied by Takahashi et al. (1983), who found that *Kitasatospora* species contained LL-A_2pm in the aerial mycelium and *meso*-A_2pm in the vegetative mycelium, while *Streptomyces* species contained only LL-A_2pm in both aerial and vegetative mycelia. The relative amounts of the two isomers varied in different experiments, and some *Streptomyces* species were also found to contain various amounts of *meso*-A_2pm, though *Kitasatospora* species consistently had a much higher ratio of *meso*-A_2pm to LL-A_2pm than *Streptomyces* species (Wellington et al., 1992). Strains assigned to the genus *Kitasatospora* were, unlike most *Streptomyces* species, resistant to polyvalent *Streptomyces* phages (Wellington et al., 1992) and formed submerged spores in liquid culture (Ōmura et al., 1982; Takahashi et al., 1983). Wellington et al. (1992) also found that the 16S rRNA gene sequence of *Kitasatospora setae* showed 91.6% similarity to the 16S rRNA gene sequence of *Streptomyces baldaccii*, and that a *Streptomyces*-specific oligonucleotide probe could recognize members of all four valid *Kitasatospora* species. These observations, and the fact that many phenotypic properties are shared by *Kitasatospora* and *Streptomyces* species, led Wellington and her colleagues to propose that all *Kitasatospora* species should be reclassified as *Streptomyces* species. These findings were supported by Ochi and Hiranuma (1994) on the basis of results from an analysis of the N-terminal sequences of ribosomal protein AT-L30.

In contrast, Nakagaito et al. (1992a) had assigned *Kitasatospora* and the *Streptomyces* species to distinct clusters based on DNA–DNA reassociation and phenetic studies. In addition, Kim et al. (1996) conducted a 16S rRNA gene sequence-based analysis of members of many *Streptomyces* species and found that the three *Kitasatospora* species formed a distant lineage in the "*Streptomyces*" tree. Zhang et al. (1997) determined the nucleotide sequences of 16S rRNA genes and the 16S–23S rRNA gene spacers of 12 actinomycete strains which were either previously classified as *Kitasatospora* strains or defined as *Streptomyces* strains, but had been found to contain major amounts of *meso*-A_2pm in their whole-cell hydrolysates. On the basis of their results, Zhang and his colleagues revived the genus *Kitasatospora* Ōmura et al. (1982).

The genus *Kitasatospora* can be distinguished from the genus *Streptomyces* by the ratio of *meso*-A_2pm to LL-A_2pm in whole-cell hydrolysates. The *meso*-A_2pm content is 49–89% in *Kitasatospora* strains and 1–16% in *Streptomyces* strains. Galactose is present in whole-cell hydrolysates of *Kitasatospora* strains, but not in those of *Streptomyces* strains. At the time of writing, the genus encompassed twelve species with validly published names. Representatives of each of these species with validly published names form a distinct line in the 16S rRNA *Streptomycetaceae* gene tree (Figure 339). On the basis of 16S rRNA gene sequence data, all the species are grouped together, showing high similarities. They are very similar to members of the genera *Streptomyces* and *Streptacidiphilus*, i.e. they produce aerial mycelia with long chains of spores, contain hexa- and octa-hydrogenated menaquinones with nine isoprene units as predominant isoprenologs, are rich in saturated, iso- and anteiso-fatty acids, and have complex polar lipid patterns which contain major amounts of

diphosphatidylglycerol, phosphatidylethanolamine, phosphatidylinositol, and phosphatidylinositol mannosides. However, *Kitasatospora* and *Streptomyces* are clearly sister taxa as they share so many phenotypic properties.

Enrichment and isolation procedures

Enrichment and isolation procedures are as described for *Streptomyces* species.

Maintenance procedures

Maintenance procedures are as for *Streptomyces* species.

Differentiation of the genus *Kitasatospora* from other genera

Despite some differences, members of the family *Streptomycetaceae* are morphologically and chemically homogeneous (Table 263). Identification of most species is difficult, because in many instances only one (the type) or a few strains have been examined. *Kitasatospora* species may be distinguished from other genera in the family by slight differences in cell-wall sugars and the presence of *meso*-A_2pm in the peptidoglycan (Table 263). Table 273 lists some characters which can be used to distinguish between *Kitasatospora* species.

TABLE 273. Phenotypic properties which separate *Kitasatospora* species (modified from Groth et al., 2004)[a,b]

Test	*K. arboriphila* HKI 0189[T]	*K. azatica* DSM 41650[T]	*K. cystarginea* DSM 41680[T]	*K. gansuensis* sp. nov. HKI 0314[T]	*K. kifunensis* DSM 41654[T]	*K. kifunensis* HKI 0316	*K. mediocidica* DSM 43929[T]	*K. nipponensis* sp. nov. HKI 0315[T]	*K. paranensis* sp. nov. HKI 0190[T]	*K. phosalacinea* DSM 43860[T]	*K. putterlickiae* DSM 44665[T]	*K. terrestris* sp. nov. HKI 0186[T]
Spore chain morphology[c]	RF, RA, S	RF	S[d]	RF	RF, S	RF, S	RF, RA	RF, RA, S	RF	RF	RF	RF, RA, S
Formation of melanoid pigment	+	−	−[d]	+	+	+	+	−	+	−	+	+
Degradation of casein	+	+	nd	+	+	−	−	+	+	+	+	+
Liquefaction of gelatin	+	+	−[d]	+	−	−	−	+	+	+	+	+
Hydrolysis of potato starch	+	+	+[d]	+	+	+	+	+	+	+	−	+
Peptonization of milk	+	+	+[d]	+	−	−	−	+	+	+	+	+
Nitrate reduction	+	+	−[d]	+	−	−	−	−	+	+	+	+
Growth on sole carbon sources:												
(+)-L-Arabinose	+	+	±[d]	+	+	−	+	−	+	+[e]	−	+
(+)-D-Fructose	+	+	±[d]	+	−	+	+	(+)	−	+[e]	(+)	+
iso-Inositol	−	−	+[d]	−	−	−	−	−	−	−[e]	−	−
(+)-D-Mannitol	−	−	−[d]	−	+	+	−	−	−	−[e]	−	−
(+)-D-Raffinose	+	−	−[d]	−	−	−	−	−	−	+[e]	−	+
(+)-L-Rhamnose	−	−	−[d]	−	−	−	−	−	+	+[e]	−	(+)
(−)-D-Sucrose	−	+	−[d]	+	+	+	+	(+)	−	+[e]	(+)	(+)
(+)-D-xylose	+	+	−[d]	+	+	−	+	−	+	+[e]	−	+
Enzyme assay (API ZYM):												
N-Acetyl-β-glucosamidase	−	−	−	(+)	−	−	−	+	−	−	−	−
β-Galactosidase	(+)	+	+	+	+	+	−	+	+	+	+	+
α-Glucosidase	+	(+)	+	+	+	(+)	−	−	+	+	−	(+)
β-Glucosidase	−	−	−	+	−	−	−	−	−	−	+	−
α-Mannosidase	−	−	−	−	(+)	+	−	−	−	−	−	−
Naphthol-AS-BI-phosphohydrolase	+	+	+	+	+	+	+	+	+	+	−	+
Growth at:												
6°C	−	−	−[d]	(+)	−	−	(+)	−	−	−	−	−
10°C	−	(+)	−[d]	+	(+)	+	+	+	(+)	+	(+)	−
35°C	+	(+)	+[d]	−	−	+	−	(+)	+	+	+	+
37°C	+	−	+[d]	−	−	+	−	−	+	+	+	+
40°C	(+)	−	+[d]	−	−	−	−	−	−	+	−	+
42°C	−	−	−[d]	−	−	−	−	−	−	+[e]	−	−
Growth in the presence of NaCl (%):												
2.0	+	−	+	+	+	+	+	+	+	+	+	+
2.5	−	−	+	+	+	+	+	(+)	+	+	+	+
3.0	−	−	−	−	−	+	−	−	+	−	+	(+)
3.5	−	−	−	−	−	+	−	−	+	−	+	−

(continued)

TABLE 273. (continued)

Test	*K. arboriphila* HKI 0189^{T}	*K. azatica* DSM 41650^{T}	*K. cystarginea* DSM 41680^{T}	*K. gansuensis* sp. nov. HKI 0314^{T}	*K. kifunensis* DSM 41654^{T}	*K. kifunensis* HKI 0316	*K. mediocidica* DSM 43929^{T}	*K. nipponensis* sp. nov. HKI 0315^{T}	*K. paranensis* sp. nov. HKI 0190^{T}	*K. phosalacinea* DSM 43860^{T}	*K. putterlickiae* DSM 44665^{T}	*K. terrestris* sp. nov. HKI 0186^{T}
Growth at pH:												
8.0	+	+	+	+	+	+	−	+	+	+	+	+
9.0	−	−	+	+	+	−	−	−	+	+	+	+
9.5	−	−	−	+	−	−	−	−	−	+	+	−
Antibiotic susceptibility:												
Ampicillin (10 μg)	−	+	nd	(+)	(+)	−	−	−	(+)	+	+	−
Lincomycin hydrochloride (2 μg)	−	−	nd	−	−	−	(+)	−	(+)	−	−	−
Methicillin (5 μg)	−	+	nd	−	(+)	−	−	(+)	(+)	−	(+)	−
Norfloxacin (10 μg)	−	(+)	nd	−	−	+	(+)	−	−	−	−	(+)
Novobiocin (5 μg/ml)	+	+	nd	+	+	+	+	+	+	−	+	+
Penicillin G (10 IU)	−	+	nd	−	−	−	−	−	−	+	+	−
Polymyxin B (300 IU)	+	+	nd	−	−	−	(+)	(+)	+	(+)	(+)	+
Sulfonamide (200 μg)	−	+	nd	+	(+)	−	−	(+)	(+)	−	−	−

[a]+, Positive; −, negative; (+), weakly positive; ±, doubtful; nd, not determined.

[b]For the following properties, tests in which strain DSM 41680^{T} was not included are indicated by an asterisk. Spores of all of the tested strains are cylindrical with smooth surface. All strains were positive for the production of H_2S*, growth on (+)-D-glucose, and produced acid phosphatase, alkaline phosphatase, esterase (C4), esterase lipase (C8), and leucine arylamidase (API ZYM tests). Good growth occurred at temperatures of 15–32°C and pH 5.0–7.0. All strains were sensitive to chloramphenicol (30 μg)*, ciprofloxacin (5 μg)*, imipenem (10 μg)*, kanamycin sulfate (30 μg)*, nalidixic acid (50 μg/ml agar)*, oxytetracycline (30 μg)*, rifampin (30 μg)*, streptomycin sulfate (10 μg)*, and vancomycin (30 μg)*. They did not use cellulose* as a sole carbon source; did not produce α-chymotrypsin, cystine arylamidase, α-galactosidase, β-glucuronidase, α-fucosidase, lipase (C14), trypsin, or valine arylamidase (API ZYM tests); and did not grow in the presence of NaCl (4%, w/v), at 42°C, or at pH 4.0 or pH 10.

[c]RF, *Rectiflexibiles*; RA, *Retinaculiaperti*; S, *Spirales*.

[d]Data from Kusakabe and Isono (1988).

[e]Data from Takahashi et al. (1984).

List of species of the genus *Kitasatospora*

1. **Kitasatospora setae** corrig. Ōmura, Takahashi, Iwai and Tanaka 1983, 673^{VP} (Effective publication: Ōmura, Takahashi, Iwai and Tanaka 1982, 1014.)

se′ta.e. N.L. gen. n. *setae* of Seta, Japan, where the bacterium was isolated.

Spore chain morphology *Rectiflexibiles* type with a smooth spore surface. Color of vegetative mycelia is pale yellow to light ivory on yeast extract-malt extract and glycerol-asparagine agars (Shirling and Gottlieb, 1966). Aerial mass color is white or light gray on yeast extract-malt extract and inorganic salts-starch agars. Forms yellow diffusible pigment on inorganic salts-starch agar. Nitrate is not reduced, milk is coagulated. Raffinose, D-fructose, L-rhamnose, sucrose, melibiose, D-mannitol, iso-inositol, and cellulose are not utilized; D-glucose, L-arabinose, and D-xylose are used as sole carbon sources. Can grow in NaCl at a concentration up to 1.5%. The temperature range for growth is 15–37°C. Cell wall contains both *meso*- and LL-A_2pm. Whole-cell hydrolysates contain galactose, but lack arabinose, madurose, and xylose. Produces setamycin, an antibiotic active against trichomonads and bacteria.

The type strain shows its highest sequence similarities to the following *Kitasatospora* species: *Kitasatospora niigatensis*, AB022876, 99.1%; *Kitasatospora cineracea*, AB022875, 99.1%. It does not show any sequence similarities over 99% to any *Streptacidiphilus* or *Streptomyces* species.

Source: soil.

DNA G+C content (mol%): 73.1.

Type strain: KM-6054, ATCC 33774, DSM 43861, NBRC 14216, JCM 3304, NRRL B-16185, VKM Ac-900.

Sequence accession no. (16S rRNA gene): M55220.

2. **Kitasatospora arboriphila** Groth, Rodríguez, Schüetze, Schmitz, Leistner and Goodfellow 2004, 2125^{VP}

ar.bo.ri.phi′la. L. n. *arbor* a tree; N.L. adj. *philus -a -um* (from Gr. adj. *philos -ê -on*) friend, loving; N.L. fem. adj. *arboriphila* tree loving.

Spore chains are long, straight to spiral with hooks and loops with 20 or more cylindrical, smooth-surfaced spores (1.1–1.7 × 1.3–2.4 μm) per chain. Submerged spores are formed sparsely in liquid culture. Produces a yellowish-brown to dark brown or olive substrate mycelium and a gray to dark gray aerial spore mass on glycerol-asparagine, inorganic salts-

starch, oatmeal, and yeast extract-malt extract agars. Soluble pigments are not formed, but melanoid pigments are produced on peptone-yeast extract-iron and tyrosine agars. Temperature range for growth is 15–40°C (optimum 28–32°C); growth does not occur at 10°C or above 40°C. pH range for good growth is pH 5.0–8.0; growth does not occur at either pH 4.5 or pH 9.0. The cell wall contains *meso*- and LL-A_2pm; the muramic acid moiety is *N*-acetylated. Whole-organism hydrolysates contain galactose, mannose, glucose, and ribose. The major polar lipids are phosphatidylethanolamine, diphosphatidylglycerol, phosphatidylglycerol, phosphatidylinositol, and phosphatidylinositol mannosides. The predominant fatty acids are $C_{16:0}$ iso (25%), $C_{15:0}$ iso (17%), and $C_{15:0}$ anteiso (12%); mycolic acids are absent. The major menaquinones are MK-9(H_6) (49%) and MK-9(H_8) (28%).

The type strain does not show any sequence similarities over 99% to other *Kitasatospora* species, or to any *Streptacidiphilus* or *Streptomyces* species.

Source: the type strain was isolated from a soil sample collected from the roots of the tree *Maytenus aquifolia* in Ribeirao Preto, Brazil.

DNA G+C content (mol%): not known.

Type strain: 2291-120, HKI 0189, DSM 44785, JCM 13002, NBRC 101834, NCIMB 13973.

Sequence accession no. (16S rRNA gene): AY442267.

3. **Kitasatospora azatica** corrig. (Nakagaito, Yokota and Hasegawa 1992b) Zhang, Wang and Ruan 1997, 1053^{VP} (*Streptomyces azaticus* Nakagaito, Yokota and Hasegawa 1992b, 118)

a.za′ti.ca. N.L. fem. adj. *azatica* referring to the product azaamino acid antitumor agent.

Mature spore chains on aerial mycelia are long and straight to wavy. Spores are cylindrical with a smooth surface. Melanoid pigment is not produced. Yellow soluble pigment is produced on inorganic salts-starch agar. The color of the vegetative mycelia is pale yellow. The color of the aerial mycelia is white to grayish white. Growth is good, but aerial formation is only slight on yeast extract-malt extract agar, oatmeal agar, glycerol-asparagine agar, and Bennett's agar. The temperature range for growth is 11–34°C. The concentration of NaCl at which growth occurs is less than 3%. Nitrate is reduced, starch is hydrolyzed, gelatin is not liquefied, and milk is peptonized but not coagulated.

D-Glucose, D-fructose, D-xylose, and L-arabinose are utilized, but D-mannitol, rhamnose, raffinose, iso-inositol, and sucrose are not or poorly utilized. The cell wall contains both LL- and *meso*-A_2pm and a trace amount of glycine. Galactose is detected as whole-cell sugar. The phospholipid pattern is type II. MK-9(H_6) and MK-9(H_8) are detected.

Type strain shows the highest sequence similarity to following *Kitasatospora* species: *Kitasatospora nipponensis*, AY442263, 99.1%. The type strain shows its highest sequence similarity to *Streptomyces purpeofuscus*, AJ781364, 99.2%, but does not show any sequence similarities over 99% to any *Streptacidiphilus* species.

Source: not known.

DNA G+C content (mol%): 70.5.

Type strain: ATCC 29755, DSM 41650, NBRC 13803, JCM 8798.

Sequence accession no. (16S rRNA gene): U93312.

4. **Kitasatospora cheerisanensis** Chung, Sung, Mo, Son, Nam, Chun and Bae 1999, 757^{VP}

che.e.ri.san.en′sis. N.L. fem. adj. *cheerisanensis* of or pertaining to Cheerisan, the name of a mountain in Korea where the species was originated.

Spore chains are *Rectiflexibiles*, with 20 or more rod-shaped smooth-surfaced spores (0.75–0.90 × 1.2–1.5 μm) per chain. Submerged spores are formed in liquid culture. Produces a greenish-yellow substrate mycelium and a whitish-gray aerial mycelium on yeast extract-malt extract agar, inorganic salts-starch agar, tyrosine agar, Bennett's agar, and starch agar. Vegetative mycelia fragment into bacillary structures. Soluble pigments, including melanin, are not produced. Colonies lacking aerial mycelium during early growth stage were pasty, circular, convex, and dull milky. The strain is positive for catalase activity and reduction of nitrate. Acids are produced from arabinose, cellobiose, galactose, glucose, maltose, trehalose, and xylose. Casein, chitin, chitosan, starch, esculin, gelatin, hippurate, and Tweens 40, 60 and 80 are decomposed, but cellulose, hypoxanthine, and tyrosine are not. Growth occurs in the presence of 1% NaCl, but not in 3% NaCl. Good growth occurs at pH 7–8 and the optimum temperature range is 25–30°C. Arabinose, azelate, cellobiose, cronate, fumarate, D-glucose, galactose, iso-butyrate, malonate, maltose, rhamnose, trehalose, and xylose are utilized for growth, but not adonitol, fructose, or glycerol. Mannitol, *myo*-inositol, melezitose, raffinose, sorbitol, suberate, succinate, and sucrose are not utilized. Whole-cell hydrolysates contain glucose and mannose, but lack galactose. Phosphatidylethanolamine, phosphatidylinositol, and diphosphatidylglycerol are contained in the polar lipid fraction. The predominant fatty acids are saturated iso- and anteiso-branched as well as straight-chain fatty acids. The major menaquinone type is MK-9(H_8). This strain is susceptible to ampicillin, chloramphenicol, kanamycin, nalidixic acid, oxytetracycline, rifampin, and streptomycin. The type strain, YC75^T, produces bafilomycin-C1-like anti-fungal compounds.

The type strain does not show any sequence similarities over 99% to other *Kitasatospora* species, or to any *Streptacidiphilus* or *Streptomyces* species.

Source: isolated from Cheerisan, Korea.

DNA G+C content (mol%): 75.8.

Type strain: YC75, KCTC 2395.

Sequence accession no. (16S rRNA gene): AF050493.

5. **Kitasatospora cineracea** Tajima, Takahashi, Seino, Iwai and Ōmura 2001, 1770^{VP}

ci.ne.ra′ce.a. L. fem. adj. *cineracea* similar to ash, ash-gray, referring to the color of the aerial mycelium.

The spore chains are straight and flexuous, with 20 or more rod-shaped, smooth-surfaced spores (0.9–1.0 × 0.6 μm) per chain. Submerged spores are formed in liquid culture. Produces yellowish brown vegetative mycelium and oyster-white to silver-gray aerial mycelium on yeast extract-malt extract agar, inorganic salts-starch agar, glycerol-asparagine agar, and sucrose-nitrate agar. Soluble pigments, including melanin, are not produced. The strain is positive for peptonization of milk and hydrolysis of starch.

D-Glucose, L-arabinose, D-xylose, raffinose, melibiose, and D-rhamnose are utilized for growth, but D-mannitol, D-fructose, inositol, sucrose, and cellulose are not utilized. The cell wall contains both *meso*- and LL-A_2pm. Whole-cell hydrolysates contain galactose, mannose, and ribose but lack arabinose and xylose. Phosphatidylethanolamine and phosphatidylinositol are contained in the polar lipid fraction. MK-9(H_6) and MK-9(H_8) are predominant menaquinones. The *N*-acyl type is the acetyl type. The temperature range for growth is 15–37°C. The strain is resistant to novobiocin at 100 µg/ml.

The type strain shows its highest sequence similarities to the following *Kitasatospora* species: *Kitasatospora niigatensis*, AB022876, 100%; *Kitasatospora phosalacinea*, AB022869, 99.2%; *Kitasatospora setae*, AB022868, 99.1%; *Kitasatospora griseola*, AB022870, 99.1%. The type strain does not show any sequence similarities over 99% to any *Streptacidiphilus* or *Streptomyces* species.

Source: not known.

DNA G+C content (mol%): 73.7.

Type strain: SK-3255, NBRC 16452, JCM 10915, NRRL B-24134.

Sequence accession no. (16S rRNA gene): AB022875.

6. **Kitasatospora cochleata** corrig. (Nakagaito, Yokota and Hasegawa 1992b) Zhang, Wang and Ruan 1997, 1053^VP^ (*Streptomyces cochleatus* Nakagaito, Yokota and Hasegawa 1992b, 116)

co.chle.a′ta. L. fem. adj. *cochleata* spiral, referring to the formation of spiral aerial mycelia.

Mature spore chains are long, forming hooks and spirals on glycerol-asparagine agar, tyrosine agar, oatmeal agar, glucose-asparagine agar, and water agar. Spores are cylindrical with a smooth surface. The color of vegetative mycelia is grayish brown. The color of aerial mycelia is gray. Melanoid pigments are produced in tyrosine agar. Brown soluble pigments are produced on yeast extract-malt extract agar. Nitrate is not reduced, starch is hydrolyzed, gelatin is not liquefied, and milk is peptonized but not coagulated. D-Glucose and L-arabinose are utilized, but D-fructose, D-mannitol, D-xylose, rhamnose, iso-inositol, and sucrose are poorly utilized or not utilized. The temperature range for growth is 13–38°C. The concentration of NaCl at which growth occurs is less than 3%. The cell wall contains both LL- and *meso*-A_2pm and a small amount of glycine. Galactose and a trace of madurose are detected as whole-cell sugars. The phospholipid pattern is type II. MK-9(H_6) and MK-9(H_8) are detected.

The type strain shows its highest sequence similarity to *Kitasatospora paracochleata*, U93328, 99.5%. The type strain does not show any sequence similarities over 99% to any *Streptacidiphilus* or *Streptomyces* species.

Source: not known.

DNA G+C content (mol%): 72.4.

Type strain: M-5, ATCC 51235, DSM 41652, NBRC 14768, JCM 8799.

Sequence accession no. (16S rRNA gene): U93316.

7. **Kitasatospora cystarginea** corrig. Kusakabe and Isono 1992, 327^VP^ (Effective publication: Kusakabe and Isono 1988, 1758.)

cys.tar.gi′ne.a. N.L. n. *cystarginum* antibiotic cystargin; N.L. fem. adj. *cystarginea* referring to anti-fungal antibiotic cystargin that the organism produces.

Mature spore chains on aerial mycelium are long and spiral. The spores are cylindrical with a smooth surface. The color of vegetative mycelia is light brown. The color of aerial mycelia is gray. Neither melanoid pigment nor soluble pigment is produced. Nitrate is not reduced, starch is hydrolyzed and gelatin is not liquefied. Milk is peptonized but not coagulated. D-Glucose is utilized but D-fructose, D-xylose, L-arabinose, rhamnose, raffinose, sucrose, D-mannitol, and iso-inositol are not utilized or are poorly utilized. The temperature range for growth is 17–40°C. The cell wall contains LL- and *meso*-A_2pm and glycine. Galactose is detected as whole-cell sugar. The phospholipid pattern is type II. MK-9(H_6) and MK-9(H_8) are detected.

The type strain shows its highest sequence similarity to *Kitasatospora paracochleata*, U93328, 99%. The type strain does not show any sequence similarities over 99% to any *Streptacidiphilus* or *Streptomyces* species.

Source: not known.

DNA G+C content (mol%): 70.6.

Type strain: RK-419, ATCC 49931, DSM 41680, FERM P-8006, NBRC 14836, JCM 7356, VKM Ac-2004.

Sequence accession no. (16S rRNA gene): U93318.

8. **Kitasatospora gansuensis** Groth, Rodríguez, Schüetze, Schmitz, Leistner and Goodfellow 2004, 2127^VP^

gan.su.en′sis. N.L. fem. adj. *gansuensis* of or pertaining to Gansu, a province in China, the origin of the soil from which the type strain was isolated.

Spore chains are long, straight to flexuous, with 20 or more cylindrical, smooth-surfaced spores (0.8–1.3 × 1.6–3.0 µm) per chain. Submerged spores and irregular fragments are formed in liquid culture. Produces a yellowish- or orange-brown to dark brown substrate mycelium and a white to beige aerial spore mass on glycerol-asparagine, inorganic salts-starch, oatmeal, and yeast extract-malt extract agars; soluble pigments are not formed on these media but melanoid pigments are produced on peptone-yeast extract-iron and tyrosine agars. Temperature range for growth is 6–32°C (optimum 25–28°C); growth does not occur below 6°C or at 35°C. pH range for good growth is pH 5.0–9.5; growth does not occur at pH 4.5 or above pH 9.5. The cell wall contains *meso*- and LL-A_2pm; the muramic acid moiety is *N*-acetylated and whole organism hydrolysates contain galactose, ribose, mannose, and rhamnose. The polar lipids are phosphatidylethanolamine, diphosphatidylglycerol, phosphatidylglycerol, phosphatidylinositol, phosphatidylinositol mannosides, and an unknown glycolipid. The predominant fatty acids are $C_{15:0}$ anteiso (21%), $C_{16:0}$ (19%), $C_{15:0}$ iso (10%), and $C_{16:0}$ iso (10%); mycolic acids are absent. The major menaquinone is MK-9(H_6) (75%).

The type strain does not show any sequence similarities over 99% to other *Kitasatospora* species, or to any *Streptacidiphilus* species, but shows its highest sequence similarities to the following *Streptomyces* species: *Streptomyces atroaurantiacus*, DQ026645, 99.4%; *Streptomyces indigoferus*, AB184214, 99%; *Streptomyces aburaviensis*, AY999779, 99%; *Streptomyces herbaricolor*, AB184801, 99%.

Source: the type strain was isolated from a sample of forest soil collected in the Lianhua Shan Reservation, Gansu Province, China.

DNA G+C content (mol%): not known.

Type strain: 2050-015, HKI 0314, DSM 44786, JCM 13003, NBRC 101835, NCIMB 13974.

Sequence accession no. (16S rRNA gene): AY442265.

9. **Kitasatospora griseola** corrig. Takahashi, Iwai and Ōmura 1985, 535[VP] (Effective publication: Takahashi, Iwai and Ōmura 1984, 384.)

gri.se.o′la. N.L. dim. fem. adj. *griseola* somewhat gray.

Spore chain morphology *Rectiflexibiles* type. The aerial spores are poorly septated and the spore surface is somewhat wrinkled. Color of vegetative mycelia is golden-olive to parchment on yeast extract-malt extract and glycerol-asparagine agars. Aerial mass color is gray or silvery-gray on most media. Forms a pinkish diffusible pigment on oatmeal (Shirling and Gottlieb, 1966) and glucose-asparagine agars. Can grow in NaCl at a concentration up to 2.0%. The temperature range for growth is 15–37°C; pH range for growth is pH 5.5–9.0. Cell wall contains both *meso*- and LL-A_2pm. Whole-cell hydrolysates contain galactose, but lack arabinose, madurose, and xylose. Produces setamycin, an antibiotic active against trichomonads and bacteria.

The type strain shows its highest sequence similarity to the following *Kitasatospora* species: *Kitasatospora paracochleata*, U93328, 99.2%; *Kitasatospora cineracea*, AB022875, 99.1%; *Kitasatospora niigatensis*, AB022876, 99%. It does not show any sequence similarities over 99% to any *Streptacidiphilus* or *Streptomyces* species.

Source: not known.

DNA G+C content (mol%): 66.0.

Type strain: AM-9660, DSM 43859, NBRC 14371, JCM 3339, NRRL B-16229, VKM Ac-2002.

Sequence accession no. (16S rRNA gene): U93320.

10. **Kitasatospora kifunensis** (Nakagaito, Shimazu, Yokota and Hasegawa 1992a) Groth, Schütze, Boettcher, Pullen, Rodriguez, Leistner and Goodfellow 2003, 2037[VP] (*Streptomyces kifunensis* Nakagaito, Shimazu, Yokota and Hasegawa 1992a, 630)

ki.fu.nen′sis. N.L. fem. adj. *kifunensis* of or belonging to Mount Kifune, Kyoto Prefecture, Japan, the source of the soil from which the organism was isolated.

Spore-chains are straight, hooked to spiral (*Rectiflexibiles* to *Spirales*). Spores are short, cylindrical and smooth-surfaced (1.2–1.3 × 0.6–0.7 μm). Submerged spores are formed sparsely in liquid culture. The color of vegetative mycelia is yellowish brown. The color of aerial mycelia is gray. Produces melanoid pigments on tyrosine agar, but not on peptone-yeast extract-iron agar or in tryptone-yeast extract broth. Nitrate is not reduced to nitrite. Gelatin is not liquefied and milk is not peptonized. Casein is degraded. H_2S is produced. Starch is hydrolyzed. Alkaline phosphatase, esterase (C4), esterase lipase (C8), β-galactosidase, α-glucosidase, leucine arylamidase, naphthol-AS-BI-phosphohydrolase, α-mannosidase (weak), and acid phosphatase are produced, but *N*-acetyl-β-glucosamidase, α-chymotrypsin, cystine arylamidase, α-fucosidase, α-galactosidase, β-glucosidase, β-glucuronidase, lipase (C14), trypsin, and valine arylamidase are not. L-Arabinose, D-galactose, D-glucose, glycerol, maltose, D-mannitol, D-mannose, sucrose, trehalose, and D-xylose are used as sole sources of carbon for energy and growth, but cellulose, chitin, D-fructose, iso-inositol, inulin, D-lactose, raffinose, L-rhamnose, and salicin are not. Temperature range for growth is 10–32°C; the organism does not grow below 10°C or above 32°C. pH range for growth is 5–9; growth does not occur at pH 4.5 or 9.5. Growth is inhibited by ampicillin (10 μg/ml, weak), chloramphenicol (30 μg/ml), ciprofloxacin (5 μg/ml), imipenem (10 μg/ml), kanamycin sulfate (30 μg/ml), methicillin (5 μg/ml), nalidixic acid (50 μg/ml), novobiocin (2.5 μg/ml), oxytetracycline hydrochloride (30 μg/ml), rifampin (30 μg/ml), streptomycin sulfate (10 μg/ml), sulfonamide (200 μg/ml, weak), and vancomycin hydrochloride (30 μg/ml), but not by lincomycin hydrochloride (2 μg/ml), norfloxacin (10 μg/ml), penicillin G (10 IU), or polymyxin B (3400 IU). The strain tolerates 2.5% but not 3.5% (w/v) NaCl. Resistance is shown to the polyvalent *Streptomyces* phage S7. Whole-cell chemistry reveals the presence of both *meso*- and LL-A_2pm; the muramic acid moiety is *N*-acetylated. Whole-organism hydrolysates contain galactose, mannose, glucose, and ribose and the major polar lipids are phosphatidylethanolamine, diphosphatidylglycerol, phosphatidylglycerol, phosphatidylinositol, and phosphatidylinositol mannosides. Predominant fatty acids are $C_{16:0}$, $C_{15:0}$ anteiso, and $C_{15:0}$ iso; mycolic acids are absent. Major menaquinone is hexahydrogenated with nine isoprene units.

The type strain shows its highest sequence similarity to *Kitasatospora nipponensis*, AY442263, 99.1%. It does not show any sequence similarities over 99% to any *Streptacidiphilus* species but shares its highest sequence similarities to the following *Streptomyces* species: *S. herbaricolor*, AB184801, 99.3%; *S. indigoferus*, AB184214, 99.3%; *S. aburaviensis*, AY999779, 99.3%; *S. chrysomallus* subsp. *fumigatus*, AB184645, 99.2%; *S. purpureus*, AJ781324, 99.2%; *S. xanthocidicus*, AY999858, 99.1%.

Source: the type strain was isolated from a soil sample obtained at Mount Kifune, Kyoto Prefecture, Japan.

DNA G+C content (mol%): 71.3.

Type strain: ATCC 51379, DSM 41654, NBRC 15206, JCM 9081.

Sequence accession no. (16S rRNA gene): AY442264.

11. **Kitasatospora mediocidica** corrig. Labeda 1988, 289[VP]

med.i.o.cid′i.ca. N.L. n. *mediocidinum* mediocidin, antbiotic produced by the type strain; L. fem. suff. *-ica* suffix used with the sense of pertaining to; N.L. fem. adj. *mediocidica* posessing mediocidin.

Sporulating aerial mycelium is not produced on any ISP media or on 15 additional media tested by cooperators. Single conidia were observed on the substrate mycelium on glucose-asparagine agar and Waksman's starch agar B. Masses of crystals are formed on the substrate growth on yeast-malt agar. The original description indicates that aerial mycelium was only poorly developed or absent on most media. Color of colony: no sporulation aerial mycelium was found on any of the media tried by the cooperators. Reverse side of colony is yellow to greenish yellow on yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar. This pigment is not pH-sensitive.

Color in medium: melanoid pigments are formed in peptone-yeast-iron agar and tryptone-yeast broth, but may not be formed in tyrosine agar. Trace of yellow pigment may be found in the medium in yeast-malt agar, oatmeal agar, salts-starch agar, and glycerol-asparagine agar; this pigment is not pH-sensitive.

D-Glucose, iso-inositol, and D-fructose are utilized for growth. No growth or only trace of growth on L-arabinose, sucrose, D-xylose, D-mannitol, rhamnose, and raffinose.

The type strain does not show any sequence similarities over 99% to other *Kitasatospora* species, or to any *Streptacidiphilus* or *Streptomyces* species.

Source: not known.

DNA G+C content (mol%): not known.

Type strain: ATCC 49055, DSM 43929, NBRC 14789, JCM 9868, NRRL B-16109.

Sequence accession no. (16S rRNA gene): U93324.

12. **Kitasatospora niigatensis** Tajima, Takahashi, Seino, Iwai and Ōmura 2001, 1770[VP]

ni.i.gat.en′sis. N.L. fem. adj. *niigatensis* of or belonging to Niigata, the city in Japan where the species originated.

The spore chains are straight and flexuous, with 20 or more rod-shaped, smooth-surfaced spores (1.0–1.1 × 0.5 µm) per chain. Submerged spores are formed in liquid culture. Produces ivory to brownish-gray vegetative mycelium and brownish white to purplish gray aerial mycelium on yeast extract-malt extract agar, oatmeal agar, glycerol-asparagine agar, and sucrose-nitrate agar. Soluble pigments, including melanin, are not produced. The strain is positive for reduction of nitrate, peptonization of milk, and hydrolysis of starch. D-Glucose, L-arabinose, and D-xylose are utilized for growth, but raffinose, melibiose, D-mannitol, D-fructose, D-rhamnose, inositol, sucrose, and cellulose are not utilized. The cell wall contains both *meso*- and LL-A_2pm. Whole-cell hydrolysates contain galactose, mannose, and ribose, but lack arabinose and xylose. Phosphatidylethanolamine and phosphatidylinositol are contained in the polar lipid fraction. MK-9(H_6) and MK-9(H_8) are predominant menaquinones. The *N*-acyl type is the acetyl type. The temperature range for growth is 15–41°C. The strain is resistant to novobiocin at 100 µg/ml.

The type strain shows its highest sequence similarities to the following *Kitasatospora* species: *Kitasatospora cineracea*, AB022875, 100%; *Kitasatospora phosalacinea*, AB022869, 99.1%; *Kitasatospora setae*, AB022868, 99.1%; *Kitasatospora griseola*, AB022870, 99%. It does not show any sequence similarities over 99% to any *Streptacidiphilus* or *Streptomyces* species.

Source: isolated from soil from Niigata, Japan.

DNA G+C content (mol%): 73.5.

Type strain: SK-3406, NBRC 16453, JCM 10916, NRRL B-24135.

Sequence accession no. (16S rRNA gene): AB249960.

13. **Kitasatospora nipponensis** Groth, Rodríguez, Schüetze, Schmitz, Leistner and Goodfellow 2004, 2127[VP]

nip.pon.en′sis. N.L. fem. adj. *nipponensis* of or pertaining to Nippon, the native name for Japan, the origin of the soil from which the type strain was isolated.

Spore chains are open spirals, long straight loops and hooks with 20 or more cylindrical, smooth-surfaced spores (1.1–1.6 × 1.2–2.3 µm) per chain. Submerged spores are formed in liquid culture. Produces a yellowish- or reddish-brown substrate mycelium and a gray aerial spore mass on glycerol-asparagine, inorganic salts-starch, oatmeal, and yeast extract-malt extract agars. A purple soluble pigment is formed on oatmeal agar, but melanoid pigments are not produced on peptone-yeast extract-iron and tyrosine agars. Temperature range for growth is 10–32°C (optimum 25–28°C); growth does not occur at 6 or 37°C. Good growth is observed at pH 5.0–8.0; growth does not occur at pH 4.5 or above pH 8.5. The cell wall contains *meso*- and LL-A_2pm; the muramic acid moiety is *N*-acetylated and whole-organism hydrolysates contain galactose, mannose, ribose, and glucose. The major polar lipids are phosphatidylethanolamine, diphosphatidylglycerol, phosphatidylglycerol, phosphatidylinositol, phosphatidylinositol mannosides, and an unknown phospholipid. The predominant fatty acids are $C_{16:0}$ iso (38%) and $C_{15:0}$ iso (10%); mycolic acids are absent. The major menaquinone is MK-9(H_6) (74%).

The type strain shows its highest sequence similarity to following *Kitasatospora* species: *Kitasatospora kifunensis*, AB022874, 99.1%; *Kitasatospora azatica*, U93312, 99.1%. It does not show any sequence similarities over 99% to any *Streptacidiphilus* species, but shows its highest sequence similarities to the following *Streptomyces* species: *S. herbaricolor*, AB184801, 99%; *S. indigoferus*, AB184214, 99%.

Source: the type strain was isolated from a soil sample collected at Kumagura, Japan.

DNA G+C content (mol%): not known.

Type strain: 2148-013, HKI 0315, DSM 44787, JCM 13004, NBRC 101836, NCIMB 13975.

Sequence accession no. (16S rRNA gene): AY442263.

14. **Kitasatospora paracochleata** corrig. (Nakagaito, Yokota and Hasegawa 1992b) Zhang, Wang and Ruan 1997, 1053[VP] (*Streptomyces paracochleatus* Nakagaito, Yokota and Hasegawa 1992b, 118)

pa.ra.co.chle.a′ta. Gr. prep. *para* beside, alongside of, resembling; L. fem. adj. *cochleata* a specific epithet; N.L. fem. adj. *paracochleata* similar to *Kitasatospora cochleata*.

Mature spore chains on aerial mycelia are long, forming spirals on glycerol-asparagine agar, tyrosine agar, oatmeal agar, glucose-asparagine agar, and water agar. Spores are cylindrical with a smooth surface. The color of vegetative mycelia is grayish brown. The color of aerial mycelia is gray.

Melanoid pigments are produced on tyrosine agar. Brown soluble pigments are produced on yeast extract-malt extract agar. Nitrate is reduced, starch is hydrolyzed, and gelatin is not liquefied. Milk is peptonized but not coagulated. D-Glucose and D-fructose are utilized, but L-arabinose, D-mannitol, D-xylose, rhamnose, raffinose, iso-inositol, and sucrose are not or poorly utilized. The temperature range for growth is 11–39°C. The concentration of NaCl at which growth occurs is less than 3%. The cell wall contains both LL- and *meso*-A_2pm and glycine. Galactose, rhamnose, and a trace of madurose are detected as whole-cell sugars. The phospholipid pattern is type II. MK-9(H_6) and MK-9(H_8) are detected.

The type strain shows its highest sequence similarities to the following *Kitasatospora* species: *Kitasatospora cochleata*, U93316, 99.5%; *Kitasatospora griseola*, AB022870, 99.2%; *Kitasatospora cystarginea*, U93318, 99%. It does not show any sequence similarities over 99% to any *Streptacidiphilus* or *Streptomyces* species.

Source: not known.

DNA G+C content (mol%): 73.1.

Type strain: M-13, ATCC 51236, DSM 41656, NBRC 14769, JCM 8800.

Sequence accession no. (16S rRNA gene): U93328.

15. **Kitasatospora paranensis** Groth, Rodríguez, Schüetze, Schmitz, Leistner and Goodfellow 2004, 2128[VP]

pa.ra.nen′sis. N.L. fem. adj. *paranensis* of or pertaining to Parana, a state of Brazil, the origin of the soil from which the type strain was isolated.

Spore chains are long, straight to flexuous with 20 or more cylindrical, smooth-surfaced spores (1.1–1.4 × 1.2–2.1 μm) per chain. Submerged spores are rarely formed in liquid culture. Produces a yellowish-brown to dark brown substrate mycelium and a gray aerial spore mass on glycerol-asparagine, inorganic salts-starch, oatmeal, and yeast extract-malt extract agars. Soluble pigments are not formed, but melanoid pigments are produced on peptone-yeast extract-iron and tyrosine agars. Temperature range for growth is 10–37°C (optimum 25–28°C); growth does not occur at 6 or 40°C. Good growth is observed at pH 5.0–9.0; growth does not occur at either pH 4.0 or pH 9.5. The cell wall contains both *meso*- and LL-A_2pm; the muramic acid moiety is *N*-acetylated and whole-organism hydrolysates contain galactose, mannose, and glucose. The polar lipids are phosphatidylethanolamine, diphosphatidylglycerol, phosphatidylglycerol, phosphatidylinositol mannosides, phosphatidylserine, and an unknown phospholipid. The predominant fatty acids are $C_{16:0}$ iso (19%), $C_{15:0}$ anteiso (16%), and $C_{16:0}$ (14%); mycolic acids are absent. The major menaquinone is MK-9(H_6) (53%), with minor components MK-9(H_4) (22%) and MK-9(H_2) (14%).

The type strain shows its highest sequence similarities to the following *Kitasatospora* species: *Kitasatospora terrestris*, AY442266, 99.2%. It does not show any sequence similarities over 99% to any *Streptacidiphilus* or *Streptomyces* species.

Source: the type strain was isolated from rhizosphere soil of *Maytenus ilicifolia*, Contenda, Parana State, Brazil.

DNA G+C content (mol%): not known.

Type strain: 2292-041, HKI 0190, DSM 44788, JCM 13005, NBRC 101837, NCIMB 13976.

Sequence accession no. (16S rRNA gene): AY442268.

16. **Kitasatospora phosalacinea** corrig. Takahashi, Iwai and Ōmura 1985, 535[VP] (Effective publication: Takahashi, Iwai and Ōmura 1984, 384.)

pho.sa.la.ci′ne.a. N.L. fem. adj. *phosalacinea* pertaining to phosalacine (an antibiotic produced by the organism).

Spore chain morphology *Rectiflexibiles* type. Color of vegetative mycelia is pale yellowish-brown on most media. Aerial mass color is white or light gray on oatmeal and inorganic salts-starch agars. Forms yellowish-brown diffusible pigment on some media. Can grow in NaCl at a concentration up to 2.0%. The temperature range for growth is 15–42°C. Cell wall contains both *meso*- and LL-A_2pm. Whole-cell hydrolysates contain galactose, but lack arabinose, madurose, and xylose. Produces phosalacine, a herbicidal antibiotic; activity *in vitro* against *Bacillus subtilis* on a synthetic medium, which is reversed by glutamine.

The type strain shows its highest sequence similarities to the following *Kitasatospora* species: *Kitasatospora cineracea*, AB022875, 99.2%; *Kitasatospora niigatensis*, AB022876, 99.1%. It does not show any sequence similarities over 99% to any *Streptacidiphilus* or *Streptomyces* species.

Source: soil.

DNA G+C content (mol%): 66.6.

Type strain: KA-338, DSM 43860, NBRC 14372, JCM 3340, NRRL B-16230, VKM Ac-2006.

Sequence accession no. (16S rRNA gene): M55223.

17. **Kitasatospora putterlickiae** Groth, Schütze, Boettcher, Pullen, Rodriguez, Leistner and Goodfellow 2003, 2037[VP]

put.ter.lic′ki.ae. N.L. gen. n. *putterlickae* of the plant genus *Putterlickia*.

Spore chains are straight to flexuous (*Rectiflexibiles*), with 20 or more cylindrical, smooth-surfaced spores (1.6–2.5 × 1.0–1.5 μm) per chain. Submerged spores are formed in liquid culture. Produces a dark-brown substrate mycelium and a dark-gray aerial spore-mass on glycerol-asparagine and yeast extract-malt extract agars. Brown soluble pigments are formed on oatmeal and yeast extract-malt extract agars and melanoid pigments are formed on peptone-yeast extract-iron and tyrosine agars. Nitrate is reduced to nitrite, gelatin is liquefied, milk is peptonized, casein is degraded, and H_2S is weakly produced. Starch is not hydrolyzed. Alkaline phosphatase, esterase (C4), esterase lipase (C8), leucine arylamidase, acid phosphatase, β-galactosidase and β-glucosidase are produced, but *N*-acetyl-β-glucosamidase, α-chymotrypsin, cystine arylamidase, α-fucosidase, α-galactosidase, α-glucosidase, β-glucuronidase, lipase (C14), α-mannosidase, naphthol-AS-BI-phosphohydrolase, trypsin and valine arylamidase are not. D(+)-fructose (weak), D(+)-glucose, and D(−)-sucrose (weak) are used as sole sources of carbon for energy and growth, but L(+)-arabinose, cellulose, iso-inositol, D(−)-mannitol, D(+)-raffinose, L(+)-rhamnose, and D(+)-xylose are not. Temperature range for growth is 10–37°C (optimum is between 28 and 32°C); growth does not occur at either 6 or 40°C. Good growth occurs at pH 5–9; growth does not occur at pH 4.5 or above 9.5. Growth is inhibited by ampicillin (10 μg/ml), chloramphenicol (30 μg/ml), ciprofloxacin (5 μg/ml), imipenem (10 μg/ml), kanamycin sulfate (30 μg/ml), methicillin (5 μg/ml, weak), novobiocin (2.5 μg/ml), oxytetracycline hydrochloride (30 μg/ml), penicillin G (10 IU), polymyxin B (300 IU, weak), rifampin (30 μg/ml), streptomycin sulfate (10 μg/ml), vancomycin hydrochloride (30 μg/ml), and nalidixic acid (50 μg/ml), but not by lincomycin hydrochloride (2 μg/ml), norfloxacin (10 μg/ml), or sulfonamide (200 μg/ml). NaCl is tolerated up to a concentration of 3.5% (w/v). Resistance is shown to polyvalent *Streptomyces* phage S7. Whole cell chemistry reveals the presence of both *meso*- and LL-A_2pm; the muramic acid moiety is *N*-acetylated. Whole-organism hydrolysates contain galactose, madurose, mannose, and

rhamnose, and the major polar lipids are diphosphatidylglycerol, phosphatidylethanolamine, phosphatidylglycerol, phosphatidylinositol, and phosphatidylinositol mannosides. Predominant fatty acids are $C_{16:0}$, $C_{16:0}$, and $C_{15:0}$ iso; mycolic acids are absent. Major menaquinones are tetra- and hexahydrogenated with nine isoprene units.

The type strain does not show any sequence similarities over 99% to other *Kitasatospora* species, or to any *Streptacidiphilus* or *Streptomyces* species.

Source: isolated from the rhizosphere of *Putterlickia verrucosa.*

DNA G+C content (mol%): 66.6.

Type strain: F18-98, DSM 44665, JCM 12393, NBRC 100917, NCIMB 13932.

Sequence accession no. (16S rRNA gene): AY189976.

18. **Kitasatospora sampliensis** Mayilraj, Krishnamurthi, Saha and Saini 2006, 521[VP]

sam.pli.en′sis. N.L. fem. adj. *sampliensis* pertaining to Sampli village, Punjab State, India, where the type strain was isolated.

Spore chain morphology is *Rectiflexibiles* with 10 or more smooth-surfaced spores per chain. Produces pale-gray, dark-gray, or dark-gray-brown substrate mycelium and a pale-gray or dark-gray aerial mycelium on almost all ISP media. Dark-brown or dark-gray soluble pigments are formed on ISP 2, ISP 3, ISP 6, ISP 7, *Streptomyces* agar, actinomycetes isolation agar, and Sabouraud glucose agar. Melanin is produced on peptone-yeast extract-iron and tyrosine agar. Nitrate is reduced to nitrite, casein is not degraded, starch is not hydrolyzed, and gelatin is not liquefied. Positive for utilization of L-arabinose, L-rhamnose, D-fructose, D-mannitol, raffinose, D-sucrose, and D-xylose as sole carbon and energy sources. Can grow in NaCl at a concentration up to 2.5%, but not at 3.0% or above. Optimum growth is observed at pH 7.0–8.0 and at 30°C. Cell wall contains both *meso*- and LL-A_2pm. The *N*-acyl type of muramic acid of the peptidoglycan is acetyl. Major fatty acids are $C_{16:0}$ (16.48%), $C_{15:0}$ iso (20.07%), $C_{16.0}$ (10.94%), $C_{17:0}$ iso (12.54%), $C_{15:0}$ anteiso (9.55%), and $C_{17:0}$ anteiso (9.36%). Whole-cell sugars are galactose, glucose, mannose, and ribose. The polar lipids are phosphatidylinositol, phosphatidylethanolamine, diphosphatidylglycerol, and phosphatidylinositol mannosides. The major menaquinone is MK-9(H6).

The type strain does not show any sequence similarities over 99% to other *Kitasatospora* species, or to any *Streptacidiphilus* or *Streptomyces* species.

Source: the type strain was isolated from a sugar-cane field soil sample collected from Sampli village, Punjab state, India.

DNA G+C content (mol%): 76.5.

Type strain: VT-36, DSM 44898, JCM 13010, NBRC 102069, MTCC 6546.

Sequence accession no. (16S rRNA gene): AY260167.

19. **Kitasatospora terrestris** Groth, Rodriguez, Rodríguez, Schüetze, Leistner and Goodfellow 2004, 2128[VP]

ter.res′tris. L. fem. adj. *terrestris* of the earth, terrestrial.

Spore chains are straight, hooked and spiral with 20 or more cylindrical, smooth-surfaced spores (1.1–1.5 × 1.3–2.8 μm) per chain. Submerged spores are formed in liquid culture. Produces a yellowish-brown to dark brown substrate mycelium and a gray aerial spore mass on glycerol-asparagine, inorganic salts-starch, oatmeal, and yeast extract-malt extract agars. Soluble pigments are not formed. The formation of melanoid pigments is weak on peptone-yeast extract-iron, and tyrosine agars. Temperature range for growth is 15–40°C (optimum 28–32°C); growth does not occur at 10°C or 42°C. pH range for good growth is pH 5.0–9.0; growth does not occur at pH 4.5 or at pH 9.5. The cell wall contains *meso*- and LL-A_2pm; the muramic acid moiety is *N*-acetylated and whole-organism hydrolysates contain galactose, mannose, and glucose. The polar lipids are phosphatidylethanolamine, diphosphatidylglycerol, phosphatidylglycerol (traces), phosphatidylinositol, phosphatidylinositol mannosides, phosphatidylserine, and an unknown glycolipid. The predominant fatty acids are $C_{16:0}$ iso (21%), $C_{15:0}$ iso (16%), and $C_{15:0}$ anteiso (10%); mycolic acids are absent. The major menaquinone is MK-9(H_6) (76%).

The type strain shows its highest sequence similarity to *Kitasatospora paranensis*, AY442268, 99.2%. It does not show any sequence similarities over 99% to any *Streptacidiphilus* or *Streptomyces* species.

Source: the type strain was isolated from a soil sample of the roots of *Maytenus aquifolia*, Ribeirao Preto, Brazil.

DNA G+C content (mol%): not known.

Type strain: 2293-012, HKI 0186, DSM 44789, JCM 13006, NBRC 101838, NCIMB 13977.

Sequence accession no. (16S rRNA gene): AY442266.

20. **Kitasatospora viridis** Liu, Rodríguez, Wang, Cui, Huang, Quintana and Goodfellow 2005a, 709[VP]

vi′ri.dis. L. fem. adj. *viridis* green, referring to the production of a green aerial spore mass.

Non-acid–alcohol-fast, nonmotile actinomycete that forms an extensively branched, light-yellow substrate mycelium and a greenish aerial spore mass on acidified oatmeal agar. Aerial hyphae differentiate into long, spiral chains of smooth-surfaced, cylindrical spores (1.0–1.2 × 0.7–0.8 μm). Starch is degraded, but not adenine, guanine, hypoxanthine, xanthine, or xylan. Adonitol, cellobiose, dextran, D-galactose, D-gluconic acid, D-glucose, inulin, D-lactose, maltose, D-mannose, melezitose, melibiose, D-salicin, D-sorbitol, trehalose, and xylitol are used as sole carbon sources for energy and growth, but not glycerol, *myo*-inositol, or xylan (all at 1%, w/v). Similarly, 2-aminoethanol, α-DL-aminobutyric acid, L-alanine, L-arginine, L-cysteine, L-glutamic acid, L-histidine, L-isoleucine, L-phenylalanine, L-threonine, L-valine, sodium oxalate, and sodium pyruvate are used as sole carbon sources, but not adipic acid or L-aspartic acid (all at 0.1%, w/v). 2-Aminoethanol, L-alanine, L-arginine, L-isoleucine, and L-phenylalanine are used as sole sources of carbon and nitrogen for energy and growth. Growth occurs at 10–37°C, but not at 4 or 45°C. The pH range for growth is pH 4–7.0. Growth occurs in the presence (μg/ml) of amikacin (32), amoxycillin (32), ampicillin (32), cefalexin (32), cephaloridine (64), clindamycin (8), doxycycline hydrochloride (32), fusidic acid (16), gentamicin sulfate (16), kanamycin sulfate (16), lincomycin hydrochloride (16), midecamycin (4), neomycin sulfate

(32), penicillin G (16 IU), streptomycin sulfate (16), tetracycline hydrochloride (32), and tobramycin sulfate (16), but is inhibited by erythromycin (8) and novobiocin (8). NaCl is tolerated up to a concentration of 10%. Cell wall contains both *meso*- and LL-A_2pm and *N*-acetylated muramic acid, and whole-organism hydrolysates are rich in galactose and glucose. The major polar lipids are diphosphatidylglycerol, phosphatidylethanolamine, phosphatidylinositol, and phosphatidylinositol mannosides. The predominant isoprenologs are hexa- (76%) and octa- (17%) hydrogenated menaquinones with nine isoprene units. The major fatty acids are $C_{15:0}$ iso (19%), $C_{15:0}$ anteiso (18%), $C_{16:0}$ iso (18%), $C_{16:0}$ (22%), and $C_{17:0}$ anteiso (8%).

The type strain does not show any sequence similarities over 99% to other *Kitasatospora* species, or to any *Streptacidiphilus* or *Streptomyces* species.

Source: the type strain was isolated from a soil sample taken from the roots of *Camellia oleifera* in Jiangxi Province, China.

DNA G+C content (mol%): not known.

Type strain: 52108a, AS 4.1878, DSM 44826, JCM 14111.

Sequence accession no. (16S rRNA gene): AY613990.

Genus *incertae sedis* II. **Streptacidiphilus** Kim, Lonsdale, Seong and Goodfellow 2003a, 1219^VP (Effective publication: Kim, Lonsdale, Seong and Goodfellow 2003b, 115.)

Peter Kämpfer

Strep.ta.ci.di′phi.lus. Gr. adj. *streptos* pliant, easily twisted; N.L. neut. n. *acidum* (from L. adj. *acidus -a -um* sour) an acid; N.L. adj. *philus -a -um* (from Gr. adj. *philos -ê -on*) friend, loving; N.L. masc. n. *Streptacidiphilus* twisted, acid-loving.

Aerobic. Gram-stain-positive, non-acid–alcohol-fast actinomycetes that form an intensively branched, non-fragmenting mycelium. The aerial mycelium at maturity carries long chains of spores. Discrete leathery or lichenoid colonies are formed. Produce a range of pigments that are responsible for the color of the substrate and aerial mycelium. Colored diffusible pigments may be formed. Chemo-organotrophic, having an oxidative type of metabolism. Use a wide range of carbon compounds as sole sources of carbon for energy and growth. Most strains grow between pH 3.5 and 6.0 with an optimum around pH 5, and at temperatures between 15 and 30°C. LL-A_2pm is the major diamino acid in the peptidoglycan layer. Contains major amounts of galactose and rhamnose in whole-organism hydrolysates, hexa- and octa-hydrogenated menaquinones with nine isoprene units as predominant isoprenologs, is rich in saturated, iso- and anteiso-fatty acids, and produces complex polar lipid patterns that contain diphosphatidylglycerol, phosphatidylethanolamine, phosphatidylinositol, and phosphatidylinositol mannosides. The organism is widely distributed in acidic soil and litter.

DNA G+C content (mol%): 70–72.

Type species: **Streptacidiphilus albus** Kim, Lonsdale, Seong and Goodfellow 2003a, 1219^VP (Effective publication: Kim, Lonsdale, Seong and Goodfellow 2003b, 115.).

Further descriptive information

The genus *Streptacidiphilus* was proposed by Kim et al. (2003b) for acidophilic actinomycetes isolated from acidic soils and litter. Acidophilic actinomycetes grow at pH 3.5–6.5 with optimum growth at pH 4.5–5.5. They produce anti-fungal compounds, are involved in the turnover of organic matter at low pH values (Goodfellow and Williams, 1983; Williams et al., 1984b), and form diastases and chitinases with pH optima below those for neutrophilic streptomycetes (Williams, 1978; Williams and Robinson, 1981). In an extensive numerical taxonomic study, Lonsdale (1985) found that acidophilic actinomycetes with streptomycete-like properties were heterogeneous and formed distinct clusters, which could be clearly differentiated using several phenotypic tests. These findings were supported by Goodfellow and Simpson (1987), and Seong et al. (1993, 1995).

Representatives of clusters 2, 4, and 10 delineated in the study of Lonsdale (1985) were described as nomenspecies of the novel genus *Streptacidiphilus*, namely *Streptacidiphilus albus*, *Streptacidiphilus neutrinimicus*, and *Streptacidiphilus carbonis* by Kim et al. (2003b). A fourth species, *Streptacidiphilus jiangxiensis*, isolated from the acidic rhizosphere soil, was described by Huang et al. (2004a) and, subsequently, four additional species, *Streptacidiphilus oryzae*, *Streptacidiphilus anmyonensis*, *Streptacidiphilus rugosus*, and *Streptacidiphilus melanogenes*, were described (Cho et al., 2008; Wang et al., 2006) bringing the total number of validly published names to eight. Representatives of each of these species forms a distinct line in the 16S rRNA *Streptomycetaceae* gene tree (Kim et al., 2003b; Cho et al., 2008; Figure 339). On the basis of 16S rRNA gene sequence data, all of the species are grouped together showing high similarities. However, they are also very similar to members of the genera *Streptomyces* and *Kitasatospora*, i.e. they produce aerial mycelia with long chains of spores, they have LL-A_2pm as the major wall diamino acid, contain hexa- and octa-hydrogenated menaquinones with nine isoprene units as predominant isoprenologs, are rich in saturated, iso- and anteiso-fatty acids, and produce complex polar lipid patterns that contain major amounts of diphosphatidylglycerol, phosphatidylethanolamine, phosphatidylinositol, and phosphatidylinositol mannosides. *Streptacidiphilus* strains differ from *Kitasatospora* and *Streptomyces* strains as they contain major amounts of galactose and rhamnose in whole-organism hydrolysates. Because of the high phenotypic similarities to *Streptomyces* and *Kitasatospora* they are clearly a sister taxon of *Streptomyces*. For this reason, the genus is treated here as a genus *incertae sedis*. Phenotypic properties that distinguish between *Streptacidiphilus* species are given in Table 274.

Enrichment and isolation procedures

Kim et al. (2003b) reported the isolation of 18 strains of streptoacidiphili on acidified starch agar plates supplemented with cycloheximide and nystatin (each 50 µg/ml) using the isolation procedure described by Goodfellow and Dawson (1978). Huang et al. (2004a) isolated *Streptacidiphilus jiangxiensis* by plating acidic rhizosphere soil suspensions, prepared using a differential

TABLE 274. Phenotypic properties that distinguish *Streptacidiphilus* species (data from Cho et al., 2008)[a]

Characteristic	*S. albus* JL83^T	*S. anmyonensis* AM-11^T	*S. carbonis* JL415^T	*S. jiangxiensis* 33214^T	*S. melanogenes* SB-B34^T	*S. neutrinimicus* JL206^T	*S. oryzae* TH49^T	*S. rugosus* AM-16^T
Degradation of:								
Starch	+	+	+	+	+	+	−	+
Tween 20	−	−	−	−	−	−	+	−
Tween 40	+	+	−	−	+	−	+	−
Tween 80	−	−	+	−	+	−	+	+
Xanthine	+	+	−	+	+	−	−	−
Growth on sole carbon source at 1% (w/v):								
D-Gluconic acid	−	+	+	+	+	−	+	+
D-Glucosamine hydrochloride	+	+	+	+	+	+	+	−
myo-Inositol	−	+	+	+	+	+	+	+
Melibiose	+	+	+	+	+	+	+	−
D-Sorbitol	−	+	+	+	+	+	+	+
D-Xylose	−	+	+	−	+	+	+	−
Growth on sole carbon source at 0.1% (w/v):								
L-Arginine	+	−	+	+	+	−	+	+
L-Aspartic acid	+	+	−	+	−	+	+	−
Sodium oxalate	−	+	−	+	+	+	+	+
Growth on sole nitrogen source at 0.1% (w/v):								
L-Isoleucine	−	+	+	+	+	+	+	+
Growth at:								
pH 3	+	+	+	−	+	−	+	−

[a]+, Positive; −, negative. All taxa were positive for the utilization of glycerol and sucrose, and also for the growth at pH 4, 5, 6, 7, and 8.

centrifugation procedure (Wang et al., 2003), onto an acidified selective isolation medium containing sucrose, aspartate, and L-glutamate as carbon sources. Acidified starch-casein-nitrate agar (Küster and Williams, 1964a) can also be used (Wang et al., 2006) in combination with differential centrifugation, as described by Sembiring et al. (2000). Cho et al. (2006) reported a further procedure for the isolation of acidophilic actinobacteria from soil.

Maintenance procedures

Kim et al. (2003b) reported that short-term storage could be achieved by growing strains on acidified modified Bennett's agar (Jones, 1949). Acidified inorganic salts-starch (ISP medium 4; Shirling and Gottlieb, 1966) and acidified oatmeal (ISP medium 3; Shirling and Gottlieb, 1966) agar can also be used for this purpose. Lyophilization, storage in liquid nitrogen, and freezing in glycerol can be used for long-term preservation. Glycerol suspensions are prepared by scraping aerial growth or substrate mycelium or both from heavily inoculated plates and making heavy suspensions in 3 ml aqueous glycerol in small (e.g. bijoux) bottles, which are stored at −20°C.

Differentiation of the genus *Streptacidiphilus* from other genera

Members of the family *Streptomycetaceae* are morphologically and chemically homogeneous (Table 263). In addition, identification of most species is difficult because, in many instances, only one (the type) or a few strains have been examined. *Streptacidiphilus* may be distinguished from the other genera in the family *Streptospoangiaceae* by slight differences in cell-wall peptidoglycan composition and by growth at low pH values, as well as by 16S rRNA gene sequencing (Table 263).

List of species of the genus *Streptacidiphilus*

1. **Streptacidiphilus albus** Kim, Lonsdale, Seong and Goodfellow 2003a, 1219[VP] (Effective publication: Kim, Lonsdale, Seong and Goodfellow 2003b, 115.)

al′bus. L. masc. adj. *albus* white.

Aerial hyphae differentiate into long flexuous chains of spores (0.6 × 1.0 µm); the spore surface is smooth. Cream-colored colonies that carry moderate to abundant white aerial hyphae are formed on acidified modified Bennett's agar. The underside of colonies is either brown or cream-colored; diffusible brown pigments may be formed. Xanthine is degraded. L-Arabinose, D-galactose, D-glucose, glycerol, glycogen, D-lactose, maltose, melibiose, raffinose, D-ribose, and trehalose (all at 1%, w/v), and L-alanine, L-arginine, L-aspartic acid, L-glutamic acid, L-histidine, L-phenylalanine, sodium pyruvate, and sodium succinate (all at 0.1%, w/v) are used as sole carbon sources for energy and growth, but not acetamide, D-amygdalin, *meso*-erythritol, *myo*-inositol, inulin, melezitose, methyl α-D-glucoside, D-sorbitol (all at 1%, w/v), or *p*-hydroxybenzoic acid, sodium adipate, sodium hippurate, sodium malate, sodium oxalate, or sodium propionate (at 0.1%, w/v). L-Glutamic acid is metabolized as sole carbon and nitrogen source but not acetamide or L-isoleucine. Growth occurs between 10 and 25°C, between pH 3.5 and 6.0, and in the presence (all at µg/ml) of cephaloridine hydrochloride (2), gentamicin sulfate (32), lincomycin hydrochloride (16), neomycin sulfate (32), oleandomycin sulfate (4), penicillin G (16), streptomycin sulfate (16), tobramycin sulfate (4), bismuth citrate (10), cadmium acetate (50), cobalt chloride (10), copper sulfate (10), crystal violet (1), ferrous sulfate (50), lead acetate (100), manganese sulfate (100), potassium tellurite (50), thallous acetate (1), and zinc sulfate (100). Growth does not occur at 30°C, or in the presence of chlortetracycline hydrochloride (2), demeclocycline hydrochloride (2), vancomycin hydrochloride (2), or crystal violet (10).

The type strain does not show any sequence similarities over 99% with other *Streptacidiphilus* species, or with *Kitasatospora* or *Streptomyces* species.

Source: the type strain was isolated from litter layers in a spruce forest soil.

DNA G+C content (mol%): not known.

Type strain: JL83, DSM 41753, JCM 12363, KCTC 9910, NBRC 100918.

Sequence accession no. (16S rRNA gene): AF074415.

2. **Streptacidiphilus anmyonensis** Cho, Han, Ko and Kim 2008, 1568[VP]

an.myon.en′sis. N.L. masc. adj. *anmyonensis* of or belonging to Anmyon, where the organism was isolated.

Aerial hyphae differentiate into long flexuous chains of spores (0.6 × 0.9 µm); the spore surface is smooth. Forms cream-colored colonies that carry moderate to abundant, white to grayish-white aerial hyphae on acidified oatmeal, inorganic salt-starch, yeast extract-malt extract and modified Bennett's agar plates. The substrate mycelium is brownish gray to brown on acidified oatmeal and yeast extract-malt extract agars, but cream-colored on the other two agars. Soluble pigments are not produced on any of the above-mentioned media. Starch and Tween 80 are degraded, but xanthine, and Tweens 20 and 40 are not. Glycerol, D-gluconic acid, D(+)-glucosamine hydrochloride, *myo*-inositol, melibiose, D-sorbitol, sucrose, D(+)-xylose (all at 1%, w/v), and L-arginine (at 0.1%, w/v) are used as sole carbon sources for energy and growth, but L-aspartic acid and sodium oxalate (all at 0.1%, w/v) are not. L-Isoleucine is used as sole nitrogen source. Growth occurs at pH 3.0–8.0 and between 28 and 35°C. Chemotaxonomic properties are typical of the genus *Streptacidiphilus*. The major fatty acids are $C_{15:0}$ iso (18.4% of the total fatty acid composition), $C_{15:0}$ anteiso (11.4%), $C_{16:0}$ iso (19.1%), *n*-$C_{16:0}$ (14.7%), $C_{17:0}$ iso (7.4%), and $C_{17:0}$ anteiso (7.5%). Contains hexa- and octahydrogenated menaquinones with nine isoprene units [MK-9(H_6) and MK-9(H_8), 24.1% and 55.8% of the total composition, respectively] as predominant isoprenologs. The diamino acid of the peptidoglycan is LL-A_2pm (89% of the total A_2pm composition), although minor amounts of the *meso*-isomer (11%) are also detected.

The type strain shows its highest sequence similarities to the following: *Streptacidiphilus* species: *Streptacidiphilus jiangxiensis*, AB249948, 99.6; *Streptacidiphilus melanogenes*, DQ994689, 99.3%. It does not show any sequence similarities over 99% to any *Kitasatospora* or *Streptomyces* species.

Source: isolated from *Pinus* soils Anmyeon, near coastal areas in Tae-An, Chungnam, Republic of Korea.

DNA G+C content (mol%): not known.

Type strain: AM-11, NBRC 103185, KCTC 19278.

Sequence accession no. (16S rRNA gene): DQ904546.

3. **Streptacidiphilus carbonis** Kim, Lonsdale, Seong and Goodfellow 2003b, 1219[VP] (Effective publication: Kim, Lonsdale, Seong and Goodfellow 2003b, 114.)

car′bo.nis. L. n. *carbo -onis* coal; L. gen. n. *carbonis* of/from coal.

Aerial hyphae differentiate into long flexuous chains of spores (0.6 × 1.0 µm); the spore surface is smooth. Cream-colored colonies form sparse to abundant white aerial hyphae on acidified modified Bennett's agar. The underside of colonies is cream-colored. Elastin is not degraded. L-Arabinose, D-fructose, D-gluconic acid, glycerol, glycogen, *myo*-inositol, inulin, D-raffinose, L-rhamnose (all at 1%, w/v), sodium pyruvate, and sodium succinate (all at 0.1%, w/v) are used as sole carbon sources for energy and growth, but not D-amygdalin, melezitose, methyl α-D-glucoside, D-ribose, D-sorbitol (all at 1%, w/v), sodium adipate, L-isoleucine, or sodium oxalate (all at 0.1%, w/v). L-Alanine, L-arginine, L-aspartic acid, L-glutamic acid, and L-phenylalanine are used as sole carbon and nitrogen sources, but not 2-aminoethanol or L-isoleucine. Grows between 15 and 30°C, at pH 3.5–6.0, and in the presence (all at µg/ml) of gentamicin sulfate (4), lincomycin hydrochloride (16), neomycin sulfate (32), oleandomycin phosphate (4), tobramycin sulfate (4), bismuth citrate (1), cadmium acetate (10), cobalt chloride (10), copper sulfate (10), crystal violet (1), ferrous sulfate (10), lead acetate (50), manganese sulfate (50), thallous acetate (1), and zinc sulfate (100), but not with cephaloridine (2), demeclocycline hydrochloride (2), penicillin G (16), streptomycin sulfate (16), vancomycin hydrochloride (2), cadmium acetate (50), cobalt chloride (50), crystal violet (10), ferrous sulfate (100), lead acetate (100), potassium tellurite (50), or thallous acetate (100).

The type strain does not show any sequence similarities over 99% to other *Streptacidiphilus* species, or to any *Kitasatospora* or *Streptomyces* species.

Source: the type strain was isolated from reclaimed acid coal mine waste.

DNA G+C content (mol%): not known.

Type strain: JL415, DSM 41754, JCM 12364, KCTC 9912, NBRC 100919.

Sequence accession no. (16S rRNA gene): AF074412.

4. **Streptacidiphilus jiangxiensis** Huang, Cui, Wang, Rodriguez, Quintana, Goodfellow and Liu 2005, 1743[VP] (Effective publication: Huang, Cui, Wang, Rodriguez, Quintana, Goodfellow and Liu 2004a, 162.)

ji.ang.xi.en′sis. N.L. masc. adj. *jiangxiensis* of or pertaining to Jiangxi Province, South China, the source of the isolates.

Aerial hyphae differentiate into long *Rectiflexibiles* chains of spores (0.6 × 0.9–1.0 µm) which have smooth surfaces. The organism forms lichenoid colonies that carry moderate to abundant, white to grayish-white aerial hyphae on acidified oatmeal, inorganic salts-starch, modified Bennett's, and yeast extract-malt extract agars. The substrate mycelium is cream-colored on acidified oatmeal agar, stray yellow or Dresden brown on the other three agars. Soluble pigments are not produced on the media cited above, nor are melanin pigments formed on peptone-yeast extract-iron agar. Guanine, hypoxanthine, Tweens 40, 60, and 80, and xanthine are not degraded. Adonitol, D(+)-cellobiose, dextran, D(−)-fructose, D(+)-mannitol, D(+)-mannose, salicin, D(+)-sucrose, xylan, xylitol, D(+)-xylose (all at 1%, w/v), 2-aminoethanol, L-cystine, L-glutamic-acid, L-isoleucine, sodium oxalate, L-threonine, and L-valine (all at 0.1%, w/v) are used as sole carbon sources for energy and growth, but not adipic acid, aminobutyric acid, L-arginine, L-aspartic acid, or L-histidine (all at 0.1%, w/v). L-Alanine, 2-aminoethanol, L-arginine, L-isoleucine, and L-phenylalanine are used as sole carbon and nitrogen sources, but not aspartic acid. Growth occurs between pH 3.5–6.5 and between 15 and 35°C. Sensitive to ampicillin (10 µg/ml), carbenicillin (100), chloramphenicol (30), clarithromycin (15), gentamicin sulfate (10), kanamycin sulfate (30), midecamycin (15), minocycline hydrochloride (30), novobiocin (5), penicillin G (16), rifampin (5), streptomycin sulfate (16), and tobramycin sulfate (10). Chemotaxonomic properties are typical of the genus *Streptacidiphilus*. The predominant fatty acids are $C_{15:0}$ iso (10.8–13.2%), $C_{15:0}$ anteiso (14.7–17.5%), $C_{16:0}$ iso (18.3–22.2%), and $C_{16:0}$ (25.9–30.4%).

The type strain shows its highest sequence similarities to the following *Streptacidiphilus* species: *Streptacidiphilus anmyonensis*, DQ904546, 99.6%; *Streptacidiphilus melanogenes*, DQ994689, 99.1%. It does not show any sequence similarities over 99% to any *Kitasatospora* or *Streptomyces* species.

Source: isolated from rhizosphere soil of wild tea plants growing on the campus of Jiangxi Agricultural University, Jiangxi Province, China.

DNA G+C content (mol%): 70.8–71.7.

Type strain: 33214, AS 4.1857, JCM 12277, NBRC 100920.

Sequence accession no. (16S rRNA gene): AY314780.

5. **Streptacidiphilus melanogenes** Cho, Han, Ko and Kim 2008, 1569[VP]

me.la.no′ge.nes. Gr. adj. *melas -anos* black; N.L. suff. *-genes* (from Gr. v. *gennaô* to produce) producing; N.L. part. adj. *melanogenes* producing black (pigment).

Aerial hyphae differentiate into long flexuous chains of spores (0.6 × 1.0 µm); the spore surface is smooth. Forms cream-colored colonies that carry moderate to abundant white aerial hyphae on acidified oatmeal, inorganic salts-starch, yeast extract-malt extract, and modified Bennett's agar plates. The substrate mycelium is brownish-gray or brown on acidified inorganic salts-starch and yeast extract-malt extract agars, but cream-colored on the other two agars. Brownish gray diffusible pigments are formed on acidified oatmeal and yeast extract-malt extract agars. Soluble pigments are not produced on the two agars. Starch, Tweens 40 and 80, and xanthine are degraded, but Tween 20 is not. Glycerol, D-gluconic acid, D(+)-glucosamine hydrochloride, *myo*-inositol, melibiose, D-sorbitol, sucrose, D(+)-xylose (all at 1%, w/v), L-arginine and sodium oxalate (all at 0.1%, w/v) are used as sole carbon sources for energy and growth, but L-aspartic acid (at 0.1%, w/v) is not. L-Isoleucine is used as nitrogen source. Growth occurs at pH 3.0–8.0 and between 28 and 35°C. Chemotaxonomic properties are typical of the genus *Streptacidiphilus*. The major fatty acids are $C_{15:0}$ iso (20.4% of the total fatty acid composition), $C_{15:0}$ anteiso (6.7%), $C_{16:0}$ iso (14.7%), *n*-$C_{16:0}$ (15.7%), $C_{17:0}$ iso (7.3%), and $C_{17:0}$ anteiso (3.8%). Contains hexa- and octahydrogenated menaquinones with nine isoprene units [MK-9(H_6) and MK-9(H_8), 18.1% and 68.6% of total composition, respectively] as predominant isoprenologs. The diamino acid of the peptidoglycan is LL-A_2pm (100% of the total A_2pm composition).

The type strain shows its highest sequence similarities to the following *Streptacidiphilus* species: *Streptacidiphilus anmyonensis*, DQ904546, 99.3; *Streptacidiphilus rugosus*, DQ904547, 99.2. It does not show any sequence similarities over 99% to any *Kitasatospora* or *Streptomyces* species.

Source: isolated from *Pinus* soils, Sambong, near coastal areas in Tae-An, Chungnam, Republic of Korea.

DNA G+C content (mol%): not known.

Type strain: SB-B34, NBRC 103184, KCTC 19280.

Sequence accession no. (16S rRNA gene): DQ994690.

6. **Streptacidiphilus neutrinimicus** Kim, Lonsdale, Seong and Goodfellow 2003a, 1219[VP] (Effective publication: Kim, Lonsdale, Seong and Goodfellow 2003b, 113.)

neu.tri.ni′mi.cus. L. adj. *neuter -tra -trum* neither or both (here for neutral pH); L. masc. n. *inimicus* an enemy, foe; N.L. n. *neutrinimicus* (nominative in apposition) enemy of the neuter pH.

Aerial hyphae differentiate into long flexuous chains of spores (0.6 × 1.0 µm); the spore surface is smooth. Cream-colored colonies form sparse to abundant white aerial hyphae on acidified modified Bennett's agar. The underside of colonies is either cream-colored or brown. Neither elastin nor xanthine is degraded. L-Arabinose, D-galactose, D-glucose, D-lactose, maltose, melibiose, glycerol, glycogen, raffinose, and trehalose (all at 1%, w/v), and L-alanine, L-arginine, L-aspartic acid, L-glutamic acid, L-histidine, L-phenylalanine, sodium pyruvate, and sodium succinate (all at 0.1%, w/v) are used as sole carbon sources for energy and growth, but not D-amygdalin, *meso*-erythritol, *myo*-inositol, inulin, melezitose, methyl α-D-glucoside, D-ribose, D-sorbitol (all at 1%, w/v), sodium adipate, *p*-hydroxybenzoic acid, L-isoleucine, sodium acetate, sodium hippurate, sodium oxalate, or sodium propionate (all at 0.1%, w/v). L-Arginine, L-aspartic acid, L-glutamic acid, and L-phenylalanine are used as sole carbon and nitrogen sources but not L-isoleucine or sodium hippurate. Growth occurs between 10 and 25°C, and at pH 3.5–5.5. Growth also occurs in the presence (all at µg) of cephaloridine hydrochloride (2), gentamicin sulfate (32), lincomycin hydrochloride (16), neomycin sulfate (32), oleandomycin phosphate (4), penicillin G (16), streptomycin sulfate (16), tobramycin sulfate (32), bismuth citrate (10), cadmium acetate (50), cobalt chloride (10), copper sulfate (10), crystal violet (1), ferrous sulfate (50), lead acetate (100), manganese sulfate (100), potassium tellurite (10), thallous acetate (10), and zinc sulfate (100), but not with chlortetracycline hydrochloride (2), demeclocycline hydrochloride (2), vancomycin hydrochloride (2), bismuth citrate (100), cadmium acetate (50), cobalt chloride (100), crystal violet (10), ferrous sulfate (100), phenol (100), or potassium tellurite (50).

The type strain does not show any sequence similarities over 99% to other *Streptacidiphilus* species, or to any *Kitasatospora* or *Streptomyces* species.

Source: the type strain was isolated from litter and mineral horizons in a spruce forest soil.

DNA G+C content (mol%): not known.

Type strain: JL206, DSM 41755, JCM 12365, KCTC 9911, NBRC 100921.

Sequence accession no. (16S rRNA gene): AF074410.

7. **Streptacidiphilus oryzae** Wang, Huang, Liu, Goodfellow and Rodríguez 2006, 1260[VP]

o.ry′za.e. L. gen. n. *oryzae* of rice, denoting the isolation of the strains from a rice field.

Aerial hyphae differentiate into long flexuous chains of spores (0.7 × 1.0 µm) with smooth surfaces. Forms brown substrate mycelium and abundant grayish-white aerial hyphae on acidified modified Bennett's, inorganic salts-starch, oatmeal, and yeast extract-malt extract agars. Golden brown diffusible pigments are formed on acidified modified Bennett's, oatmeal, and yeast extract-malt extract agars, but not on inorganic salts-starch agar. Degrades adenine, casein, starch, and uric acid, but not elastin, guanine, hypoxanthine, Tween 80, L-tyrosine, xanthine, or xylan. Nitrate is reduced. Esculin, allantoin, and urea are not hydrolyzed. L-Arabinose, D-arabi-

tol, D-cellobiose, D-fructose, D-galactose, D-glucose, glycogen, D-lactose, D-mannitol, D-mannose, raffinose, L-rhamnose, D-salicin (weak), sucrose (weak), trehalose, and D-xylose (each at 1%, w/v), and L-alanine, α-aminobutyric acid, 2-aminoethanol, L-histidine (weak), L-isoleucine (weak), L-phenylalanine, sodium fumarate (weak), sodium pyruvate (weak), L-threonine, and L-valine (each at 0.1%, w/v) are used as sole carbon sources for energy and growth, but not adonitol, dextran, methyl α-D-glucoside, ethanol, and glycine (each at 1%, w/v), or adipic acid, L-arginine, L-aspartic acid, L-cysteine, potassium nitrate, and sodium oxalate (each at 0.1%, w/v). L-Alanine, 2-aminoethanol, L-aspartic acid, L-isoleucine (weak), and L-phenylalanine (each at 0.1%, w/v) are used as sole carbon and nitrogen sources. Growth occurs at pH 3.0–6.5 and at 28 and 37°C and in the presence of (µg/ml) amoxycillin (16), fusidic acid (16), gentamicin sulfate (16, weak), lincomycin hydrochloride (16), and penicillin G (8, weak), but not in the presence of amikacin (32), amoxycillin (32), ampicillin (16, 32), cephalexin (16, 32), cephaloridine hydrochloride (32, 64), clindamycin hydrochloride (4), doxycycline hydrochloride (16), gentamicin sulfate (16), neomycin sulfate (16, 32), novobiocin (8), penicillin G (16), streptomycin sulfate (8, 16), tetracycline hydrochloride (16, 32), or lead acetate (100). Weak growth occurs in the presence of 5% (w/v) NaCl.

The type strain does not show any sequence similarities over 99% to other *Streptacidiphilus* species, or to any *Kitasatospora* or *Streptomyces* species.

Source: the type strain was isolated from a rice-field soil sample collected in Nontaburi Province, Thailand.

DNA G+C content (mol%): not known.

Type strain: TH49, CGMCC 4.2012, JCM 13271.

Sequence accession no. (16S rRNA gene): DQ208700.

8. **Streptacidiphilus rugosus** Cho, Han, Ko and Kim 2008, 1568[VP]

ru.go′sus. L. masc. adj. *rugosus* wrinkled.

Aerial hyphae differentiate into long flexuous chains of spores (0.5 × 1.2 µm); the spore surface is smooth. Forms green-colored, rugose colonies that carry moderate to abundant white aerial hyphae on acidified oatmeal, inorganic salts-starch, yeast extract-malt extract, and modified Bennett's agar plates. The substrate mycelium is cream on acidified inorganic salts-starch, yeast extract-malt extract, and modified Bennett's agars, but yellowish brown or brown on oatmeal agar. Soluble pigments are not produced on any of the abovementioned media. Starch and Tween 80 are degraded, but xanthine, and Tweens 20 and 40 are not. Glycerol, D-gluconic acid, *myo*-inositol, D-sorbitol, sucrose (all at 1%, w/v), and L-arginine and sodium oxalate (at 0.1%, w/v) are used as sole carbon sources for energy and growth, but D(+)-glucosamine hydrochloride, melibiose, D(+)-xylose (all at 1%, w/v), and L-aspartic acid (at 0.1%, w/v) are not. L-Isoleucine is used as sole nitrogen source. Growth occurs at pH 3.0–8.0 and also between 28 and 35°C. Chemotaxonomic properties are typical of the genus *Streptacidiphilus*. The major fatty acids are $C_{15:0}$ iso (15% of the total fatty acid composition), $C_{15:0}$ anteiso (14.9%), $C_{16:0}$ iso (25.4%), *n*-$C_{16:0}$ (15.6%), $C_{17:0}$ iso (3.0%) and $C_{17:0}$ anteiso (4.7%). Contains hexa- and octahydrogenated menaquinones with nine isoprene units [MK-9(H_6) and MK-9(H_8), 19.8% and 59.6% of total, respectively] as predominant isoprenologs. The diamino acid of the peptidoglycan is LL-A_2pm (94% of the total A_2pm composition), although minor amounts of the *meso*-isomer (6%) are also detected.

The type strain shows highest sequence similarity to *Streptacidiphilus melanogenes*, DQ994689, 99.2%. It does not show any sequence similarities over 99% to any *Kitasatospora* or *Streptomyces* species.

Source: isolated from *Pinus* soil, Anmyeon, near coastal areas in Tea-An, Chungnam, Republic of Korea.

DNA G+C content (mol%): not known.

Type strain: AM-16, NBRC 103186, KCTC 19279.

Sequence accession no. (16S rRNA gene): DQ904547.

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Order XV. **Streptosporangiales** ord. nov.

Michael Goodfellow

Strep.to.spo.ran.gi′a.les. N.L. neut. n. *Streptosporangium* type genus of the order; suff. -*ales* ending to denote an order; N.L. fem. pl. n. *Streptosporangiales* the *Streptosporangium* order.

Aerobic, Gram-stain-positive, non-acid-fast, chemoorganotrophic actinomycete which have a wall peptidoglycan containing *meso*-diaminopimelic acid, *N*-acetylated muramic acid, and is of the A1γ type. Fatty acid profiles are complex, and mycolic acids absent. **The pattern 16S rRNA signatures consists of nucleotides at positions 127:234 (A–U), 829:857 (G–C), 830:856 (G–C), 953:1228 (U–A), 950:1231 (U–A), 955:1225 (C–G), 986:1219 (A–U) and 987:1218 (A–U)**. The order contains the families *Nocardiopsaceae*, *Streptosporangiaceae*, and *Thermomonosporaceae*.

DNA G+C content (mol%): 64–77.

Type genus: **Streptosporangium** Couch 1955, 145[AL] emend. Stackebrandt, Kroppenstedt, Jahnke, Kemmering and Gürtler 1994, 268.

Further descriptive information

The order *Streptosporangiales* was formed by elevation of the suborder *Streptosporangineae* Ward-Rainey et al. 1997 emend. Zhi et al. 2009. The families *Nocardiopsaceae*, *Thermomonosporaceae*, and *Streptosporangiaceae* form distinct clades in the 16S rRNA actinobacterial gene tree and can also be separated from one another and from other actinobacterial families by the pattern of their 16S rRNA signatures (Zhi et al., 2009). *Streptosporangiales* strains produce whole-organism hydrolysates that are rich in *meso*-diaminopimelic acid and which may or may not contain madurose, i.e. they have either a wall chemotype IIIB or IIIC *sensu* Lechevalier and Lechevalier (1970b). They contain complex mixtures of fatty acids but can be assigned to groups based on qualitative and quantitative differences in fatty acid profiles (Kroppenstedt, 1985). *Thermomonosporaceae* and *Streptosporangiaceae* strains are characterized by fatty acid patterns 3a and 3c, respectively; members of the family *Nocardiopsaceae* show greater diversity as some strains exhibit pattern 3a (*Nocardiopsis* and *Streptomonospora*) and others pattern 3e (*Thermobifida*).

Members of the three families can be distinguished on the basis of differences in menaquinone and polar lipid composition (Goodfellow et al., 1988, 1990; Kudo, 2001; Goodfellow and Quintana, 2006; Kroppenstedt and Evtushenko, 2006; Kroppenstedt and Goodfellow, 2006). *Nocardiopsaceae* strains contain menaquinones with 9, 10, or 11 isoprene units (Li et al., 2003; Zhang et al., 1998), whereas those assigned to the other two families have components with nine isoprene units (Kroppenstedt and Goodfellow, 2006; Stackebrandt et al., 1994). *Streptosporangiaceae* and *Thermomonosporaceae* strains can also be distinguished by their menaquinone profiles, as the former is characterized by the presence of major amounts of MK-9(H_2) and MK-9(H_4), saturated at sites III, and III and IV, respectively, and the latter by predominant amounts of MK-9(H_6) saturated at sites II, III, and VIII. Representatives of these taxa typically display polar lipid patterns 1 or 2 (*Thermomonosporaceae*) and IV (*Streptosporangiaceae*), according to the classification of Lechevalier et al. (1977, 1981). In contrast, *Nocardiopsaceae* strains are markedly heterogeneous as they exhibit polar lipid patterns II (*Thermobifida*), III (*Nocardiopsis*), and complex, unusual profiles (*Streptomonospora*) (Cui et al., 2001; Li et al., 2003).

Detection of chemotaxonomic markers

Members of the genera classified in the order *Streptosporangiales* can be distinguished from one another and from sporoactinomycetes assigned to other families based on the discontinuous distribution of chemotaxonomic markers, notably wall amino acids, cellular fatty acids, menaquinones, muramic acid types, sugars, and polar lipids. Standard chromatographic procedures are available for the detection of wall diamino acids (Hancock, 1994; Hasegawa et al., 1983; Staneck and Roberts, 1974), fatty acids (Kroppenstedt et al., 1990; Suzuki and Komagata, 1983) including mycolic acids (Minnikin et al., 1975), menaquinones (Collins, 1994; Kroppenstedt, 1982, 1985; Minnikin et al., 1984), muramic acid residues (Uchida and Aida, 1977; Uchida et al., 1999), and polar lipids (Minnikin et al., 1984; Suzuki et al., 1993).

Primary chemotaxonomic data can be obtained by examining whole-organism hydrolysates for the presence of the isomers of diaminopimelic acid, major diagnostic sugars, and mycolic acids using appropriate standards. The thin-layer chromatographic procedures described by Staneck and Roberts (1974) provide an easy and reliable way of detecting diagnostic amino acids and sugars. These procedures involve the application of unidimensional thin-layer chromatography to establish whether organisms contain LL-, hydroxy- or *meso*-diaminopimelic acid and major diagnostic sugars, including madurose; this sugar has almost the same R_f value as xylose but can be distinguished from the latter as it gives a yellow brown as opposed to a maroon spot. Madurose and other whole-organism sugars can also be detected by gas chromatography (Saddler et al., 1991) and high pressure liquid chromatography (Nakagaito et al., 1993; Yokota and Hasegawa, 1988). Examination of whole-organism methanolysates for the presence of mycolic acids can be achieved using the thin-layer chromatographic technique introduced by Minnikin et al. (1975).

The presence of *meso*-diaminopimelic acid and the absence of mycolic acids and diagnostic sugars other than madurose distinguishes members of the order *Streptosporangiales* from sporoactinomycetes classified in the orders *Catenulisporales*, *Corynebacteriales*, *Micromonosporales*, *Pseudonocardiales*, and *Streptomycetales*.

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Family I. **Streptosporangiaceae** Goodfellow, Stanton, Simpson and Minnikin 1990a, 321[VP] (Effective publication: Goodfellow, Stanton, Simpson and Minnikin 1990b.) emend. Ward-Rainey, Rainey and Stackebrandt 1997, 486 emend. Zhi, Li and Stackebrandt 2009, 600

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Strep.to.spo.ran.gi.a.ce′a.e. N.L. neut. n. *Streptosporangium* the type genus of the family, suff. *-aceae* ending to denote a family; N.L. fem. pl. n. *Streptosporangiaceae* the *Streptosporangium* family.

Aerobic, Gram-stain-positive, non-acid–alcohol-fast, chemoorganotrophic actinomycetes which form a branched, stable substrate mycelium. **When formed, aerial hyphae differentiate either into chains of two or more arthrospores or into spore vesicles that contain one to many spores which may be motile or nonmotile.** Spores are borne on the substrate hyphae when aerial mycelium is absent. Wall peptidoglycan contains *meso*-diaminopimelic acid and *N*-acetylmuramic acid and is of the A1γ type. **Lipid profiles typically contain straight, iso-, anteiso-, and 10-methyl branched fatty acids, major proportions of di- and tetrahydrogenated menaquinones with nine isoprene units, and phosphatidylethanolamine and glucosamine containing polar components. Mycolic acids are absent.** The pattern of 16S rRNA signatures consists of nucleotides at positions 440:497 (C–G), 485 (U), 501:544 (C–G), 502:543 (G–C), 833:853 (U–G), and 1355:1367 (A–U). Widely distributed in soil.

DNA G+C content (mol%): 64–77.

Type genus: **Streptosporangium** Couch 1955a, 145[AL] emend. Stackebrandt, Kroppenstedt, Jahnke, Kemmering and Gürtler 1994, 268.

Further descriptive information

Phylogeny. The 11 genera classified in the family *Streptosporangiaceae* form a distinct phyletic line in the 16S rRNA actinobacterial gene tree (Ara and Kudo, 2007; Goodfellow and Quintana, 2006; Goodfellow et al., 2005b). In addition to *Streptosporangium*, the type genus, the family contains the genera *Acrocarpospora* Tamura et al. 2000, *Herbidospora* Kudo et al. 1993, *Microbispora* Nonomura and Ohara 1957, *Microtetraspora* (Thiemann et al. 1968b) Zhang et al. 1998a, *Nonomuria* Zhang et al. 1998a (corrected to *Nonomuraea* by Chiba et al., 1998a), *Planobispora* Thiemann and Beretta 1968c, *Planomonospora* Thiemann et al. 1967, *Planotetraspora* Runmao et al. 1993 emend. Tamura and Sakane 2004, *Sphaerosporangium* Ara and Kudo 2007 (corrected to *Sphaerisporangium* by List Editor, IJSEM, 2007), and *Thermopolyspora* (*ex* Krasil'nikov and Agre 1964) Goodfellow et al. 2005b. Representatives of these taxa form distinct branches in the 16S rRNA *Streptosporangiaceae* gene tree (Figure 360) and share a pattern of 16S rRNA signatures (Zhi et al., 2009), as shown in the description of the family.

Cell morphology. At present, members of the genera classified in the family *Streptosporangiaceae* are distinguished mainly on the basis of morphological criteria (Table 275). Strains that carry one or more spores in chains (*Herbidospora*, *Microbispora*, *Microtetraspora*, *Nonomuraea*, and *Thermopolyspora*) are closely related to organisms that form spores in vesicles (*Acrocarpospora*, *Planobispora*, *Planomonospora*, *Planotetraspora*, *Sphaerisporangium*, and *Streptosporangium*). Individual spore vesicles contain a coiled chain of arthrospores formed by septation of an unbranched, spiral hypha within an expanded sporophore sheath (Vobis and Kothe, 1985). Spore formation is exogenous, hence the term "spore vesicle" is to be preferred to the original term "sporangium" (Cross, 1970; Sharples et al., 1974). Spores in spore vesicles and in spore chains are formed essentially in the same way, i.e. by a hypha bound by a sheath; sheaths either expand to form the vesicular envelope or remain around the spore chains (Lechevalier et al., 1966b; Sharples et al., 1974; Vobis and Kothe, 1985).

Chemotaxonomy. *Streptosporangiaceae* strains have many chemical markers in common (Kudo, 2001). They have either a wall chemotype IIIB or IIIC *sensu* Lechevalier and Lechevalier (1970, 1970), i.e. they produce hydrolysates that are rich in *meso*-diaminopimelic acid and may or may not contain madurose. They have *N*-acetylated muramic acid and an A1γ peptidoglycan type, are rich in unsaturated, saturated, iso- and anteiso, and 10-methyl branched fatty acids, lack mycolic acids, and typically have a type IV phospholipid pattern and unsaturated, di- and tetrahydrogenated menaquinones with nine isoprene units as the predominant isoprenologues [MK-9, MK-9(H_2), and MK-9(H_4)]. The tetrahydrogenation occurs at the sites of isoprene units III (the third unit from the 2-methyl–1, 4-naphthoquinone moiety) and VIII [MK-9(III, VIII-H_4)] (Ara and Kudo, 2007; Goodfellow et al., 1988; Goodfellow and Quintana, 2006; Kawamoto et al., 1981; Kroppenstedt, 1985, 1990a; Stackebrandt et al., 1994). This chemotaxonomic profile distinguishes members of the family *Streptosporangiaceae* from all of the other families classified in the order *Actinomycetales* including their nearest neighbors, the families *Nocardiopsaceae* and *Thermomonosporaceae*.

The presence of madurose in whole-organism hydrolysates is usually considered to be associated with actinomycetes that have a wall chemotype III, though this sugar has been detected in *Micromonospora rosaria* and several *Streptomyces* species (Nakagaito et al., 1993; Yokota et al., 1989). There is also an unconfirmed report of the presence of madurose in a wall chemotype I actinomycete with a streptomycete-like morphology (Weyland et al., 1982). In addition, 3-*O*-methylgalactosyl (madurosyl) units have been detected in the structure of teichoic acids of a *Nonomuraea roseoviolaceae* subsp. *carminata* strain (formerly *Actinomadura carminata*; Naumova et al., 1986).

Ecology. Members of the family *Streptosporangiaceae* are widely distributed in soil where they are presumably engaged in the turnover of organic matter. The introduction of innovative selective isolation procedures should provide an insight into the occurrence, distribution, numbers, and activities of members of the constituent genera, as exemplified by the multistage procedures introduced for the selective isolation of *Planobispora* and *Planomonospora* strains (Suzuki et al., 2001a, 2001b).

Enrichment and isolation procedures

Streptosporangiaceae strains are generally isolated by plating out suspensions of dry-heat treated, air-dried soil samples onto one or more selective synthetic media or by baiting environmental

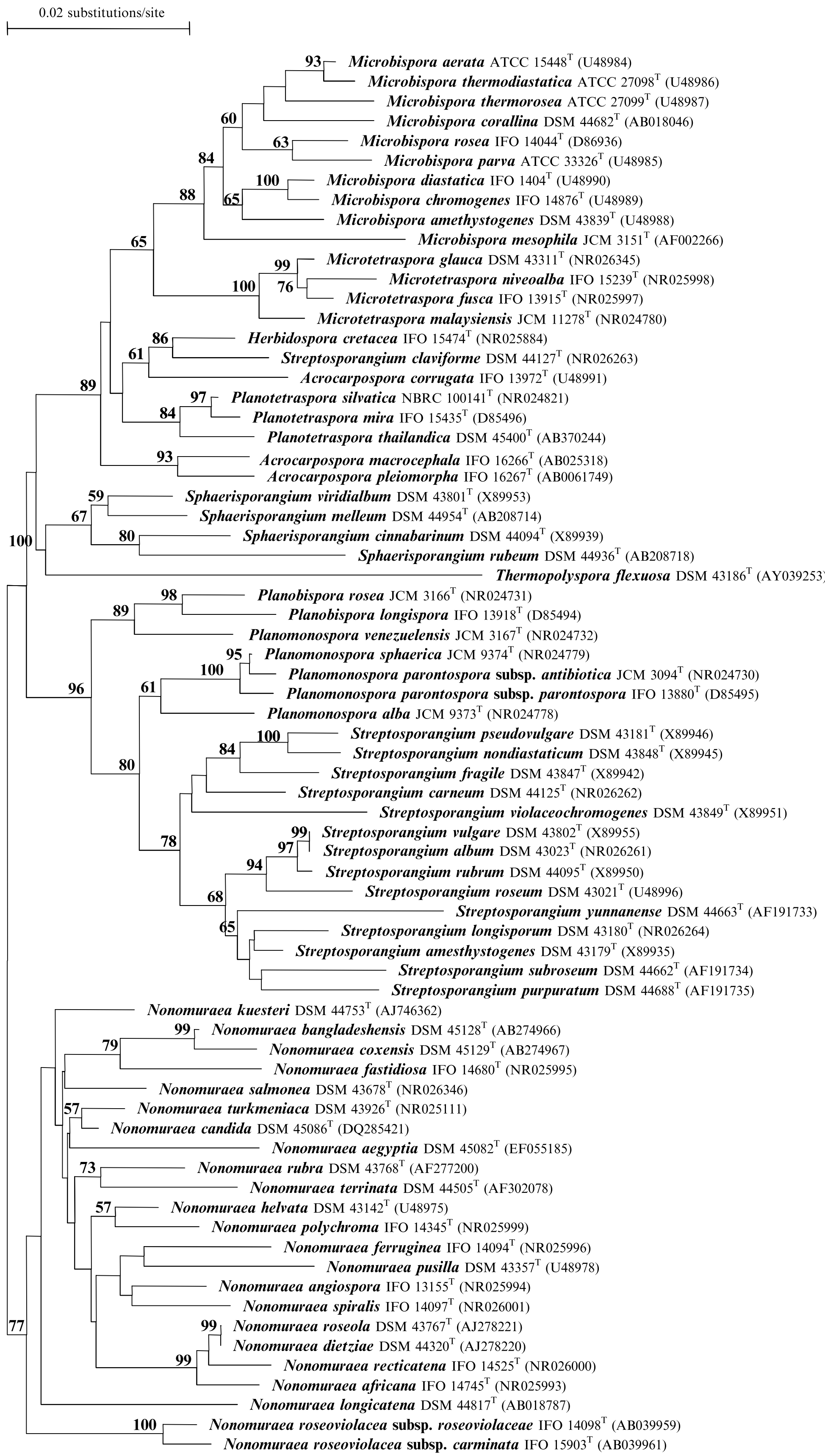

FIGURE 360. Neighbor-joining tree (Saitou and Nei, 1987) based on nearly complete 16S rRNA gene sequences (>1372 nucleotides) showing relationships between representatives of validly named species in the family *Streptosporangiaceae*. The numbers at the nodes indicate the level of bootstrap support (%) based on a neighbor-joining analysis of 1000 re-sampled datasets. ^{T}, Type strain.

TABLE 275. Morphological features and chemotaxonomic characteristics of members of the genera classified in the family *Streptosporangiaceae*[a,b]

Characteristic	*Acrocarpospora*	*Herbidospora*	*Microbispora*	*Microtetraspora*	*Nonomuraea*	*Planobispora*	*Planomonospora*	*Planotetraspora*	*Sphaerisporangium*	*Streptosporangium*	*Thermopolyspora*
Vesicle formation	Club or globose spore vesicles on aerial hyphae	Spore chains on aerial hyphae	Spores in characteristic longitudinal pairs on aerial hyphae	Spore chains containing four or more spores on short aerial hyphae	Spore chains or pseudosporangia on aerial hyphae	Cylindrical to clavate spore vesicles containing longitudinal pairs of spores on aerial hyphae	Cylindrical to clavate spore vesicles containing single spores on aerial hyphae	Spore vesicles containing four spores on aerial hyphae	Globose spore vesicles on aerial hyphae	Globose spore vesicles on aerial hyphae	Hooked or irregular spiral chains of 4–10 warty to spiny ornamented spores on aerial hyphae
Aerial mycelium	+	–	+	+	+	+	+	+	+	+	+
Motile spores	–	–	–	–	–	+	+	–		–	–
Cell-wall chemotype[c]	III	III	III	III	III	III	III	III	III	III	III
Peptidoglycan type	A1γ	A1γ	A1γ	A1γ	A1γ	A1γ	A1γ	nd	nd	A1γ	nd
Whole-organism sugar pattern[d]	B,C	B	B,C	B,C	B,C	B	B	B	B	B	C
Fatty-acid type[e]	3c	3c	3c	3c	3c	3c	3c	3d	3c	3c	nr
Menaquinones	MK-9(III, VIII-H_4), MK-9(H_6), MK-9(III-H_2)	MK-10(III, IX-H_4), MK-10(H_6), MK-10	MK-9(III, VIII-H_4), MK-9(III-H_2), MK-9(H_6)	MK-9(III, VIII-H_4), MK-9(III-H_2), MK-9(H_0)	MK-9(III, VIII-H_4), MK-9(III-H_2), MK-9(H_0)	MK-9(III, VIII-H_4), MK-9(III-H_2), MK-9(H_0)	MK-9(III-H_2), MK-9(III VIII-H_4), MK-9(H_0)	MK-9 (III, VIII-H_4), MK-9(III-H_2)	MK-9(III, VIII H_4), MK-9(H_6), MK-9(III), MK-9(H_0), MK-9(H_2)	MK-9(III, VIII-H_4), MK-9(III-H_2), MK-9(H_0)	MK-9(III, VIII-H_4), MK-9(H_0), MK-9(H_2), MK-9 (II, III, VIII-H_6)
Phospholipid type[f]	IV, II	IV	IV	IV	IV	IV	IV	IV	IV	IV	IV
DNA G+C content (mol%)	68–69	69–71	71–73	69–71	64–69	70–71	72	71	70–72	69–71	77
Growth temperature range (°C)	15–30	13–37	25–55	20–45	20–45	28–40	28–37	28–37	20–37	20–50	37–60

[a]Symbols: +, >85% positive; –, 0–15% positive; nr, not reported.

[b]Data from Kroppenstedt et al. (1990); Kudo (2001); Goodfellow et al. (2005b); and Ara and Kudo (2007).

[c]Major constituents: alanine, glutamic acid, glucosamine, and *meso*-A_2pm (Lechevalier and Lechevalier, 1970b).

[d]A, cross-linkage between positions 3 and 4 of adjacent peptide subunits; 1, peptide bridge absent; γ, *meso*-A_2pm at position 3 of the tetrapeptide subunits (Schleifer and Kandler, 1972).

[e]Saturated fatty acids, unsaturated fatty acids, iso-fatty acids (variable), and methyl-branched fatty acids (Kroppenstedt, 1985).

[f]Phospholipid patterns: PII, only phosphatidylethanolamine; PIV, phospholipids containing glucosamine (with phosphatidylmethylethanolamine variable) (Lechevalier et al., 1977, 1981).

samples with natural substrates. Various combinations of pretreatment regimes and selective media have been used to isolate members of the genera *Microbispora* and *Streptosporangium* (Nonomura and Ohara, 1960, 1969a, 1969b), *Microtetraspora* and *Nonomuraea* (Nonomura and Ohara, 1971c, 1971d), and *Nonomuraea* and *Streptosporangium* (Hayakawa and Nonomura, 1987a, 1987b; Nonomura, 1984).

Variations in the approaches mentioned above have involved treating heat-pretreated, air-dried soil suspensions with either benzethonium chloride or chloramine-T prior to plating onto HV agar and examining the incubated plates for the presence of *Streptosporangium* and related taxa (Hayakawa et al., 1991a, 1997). Similarly, *Microbispora* strains can be preferentially isolated by treating suspensions of heat-treated, air-dried soils with phenol, diluting with water, and plating onto HV agar supplemented with nalidixic acid (Nonomura and Hayakawa, 1988). *Microbispora*, *Planobispora*, *Planomonospora*, and *Streptosporangium* strains have been isolated by plating centrifuged supernatants of air-dried soils onto HV agar supplemented with streptomycin (Kizuka et al., 1997).

Members of the genera *Microbispora*, *Microtetraspora*, *Nonomuraea*, and *Streptosporangium* have been recovered from pretreated soil samples using a range of selective synthetic media supplemented with antibacterial antibiotics including ampicillin, bruneomycin, penicillin, polymixin B, rubromycin, and streptomycin (Hayakawa and Nonomura, 1987a, 1987b; Lavrova et al., 1972; Preobrazhenskaya et al., 1975b; Wang et al., 1999; Whitham et al., 1993). *Microtetraspora* and *Streptosporangium* strains have been isolated following irradiation of soil samples with microwaves, plating out soil suspensions onto Gauze agar 2 (Gauze et al., 1983) either without antibiotics or supplemented with levorin and nalidixic acid to suppress bacterial and fungal growth, respectively, and incubating at 28°C for up to six weeks (Bulina et al., 1997). Baiting environmental samples with pollen or hair has been used to good effect for the isolation of *Planobispora*, *Planomonospora*, and *Streptosporangium* strains (Couch, 1954, 1955a, 1963). Details of these and other traditional procedures recommended for the selective isolation of *Streptosporangiaceae* strains are given in the chapters dealing with the individual genera.

Taxonomic comments

The family *Streptosporangiaceae* was proposed by Goodfellow et al. (1990b) to provide a home for the genera *Microbispora*, *Microtetraspora*, *Planobispora*, *Planomonospora*, *Spirillospora*, and *Streptosporangium*. These genera had previously been assigned to higher taxa mainly based on what were seen at the time to be characteristic morphological traits. The family "*Actinosporangiaceae*", which was introduced to accommodate the genus *Streptosporangium* and "sporangiate" actinomycetes belonging to the genus *Actinoplanes* (Couch, 1955a), was subsequently renamed *Actinoplanaceae* (Couch, 1955b). In addition to *Actinoplanes*, the type genus, and *Streptosporangium*, this taxon included other actinomycetes considered to form "sporangia", namely the genera *Amorphosporangium*, *Ampullariella*, *Dactylosporangium*, *Kitasatoa*, *Pilimelia*, *Planobispora*, *Planomonospora*, and *Spirillospora* (Couch and Bland, 1974).

The original members of the family *Actinoplanaceae* were subsequently shown to belong to two distinct DNA–DNA relatedness groups. The genera *Actinoplanes*, *Ampullariella*, and *Dactylosporangium* were assigned to one group and the genera *Planobispora*, *Planomonospora*, and *Spirillospora* to the other one (Farina and Bradley, 1970). Organisms in the first group contained *meso*-and/or hydroxydiaminopimelic acid and glycine, i.e. they had a wall chemotype II *sensu* Lechevalier and Lechevalier (1970, 1970), whereas those in the second group contained *meso*-diaminopimelic acid and madurose (wall chemotype IIIB).

The realization that the genera *Actinoplanes* and *Dactylosporangium* had properties in common with the genera *Catellatospora*, *Couchioplanes*, *Catenuloplanes*, *Dactylosporangium*, *Micromonospora*, and *Pilimelia* led to these taxa being classified in the family *Micromonosporaceae* (Krasil'nikov 1938), a taxon which was emended first by Koch et al. (1996) and then by Stackebrandt et al. (1997). In the meantime, the genera *Amophosporangium* and *Ampullariella* were proposed as subjective synonyms of the genus *Actinoplanes* (Stackebrandt and Kroppenstedt, 1987). Subsequently, the genus *Kitasatoa* became a subjective synonym of the genus *Streptomyces* Goodfellow et al. 1986, and the genus *Spirillospora* became a member of the family *Thermomonosporaceae* (Stackebrandt et al. 1997) emend. Zhang et al. 2001.

Goodfellow and Cross (1984) assigned the oligosporic genera *Actinomadura*, *Excellospora*, *Microbispora*, *Microtetraspora*, and the "sporangiate" genera *Planobispora*, *Planomonospora*, *Spirillospora*, and *Streptosporangium* to what they considered to be a somewhat contrived aggregate group, the maduromycetes, a taxon that was recognized in the previous edition of *Bergey's Manual* (Goodfellow, 1989a, 1989b). However, with the exception of the genera *Actinomadura* and *Spirillospora*, these taxa were shown to form a coherent subgeneric group based on 16S rRNA gene cataloging and sequencing (Stackebrandt, 1986). The close relationships shown by the genera in this revised grouping were formalized by the proposal that *Streptosporangium* be recognized as the type genus of the new family *Streptosporangiaceae* Goodfellow et al. 1990b. In addition to the founder members, this family now includes the genera *Acrocarpospora*, *Herbidospora*, *Nonomuraea*, *Planotetraspora*, *Sphaerisporangium*, and *Thermopolyspora*. Some of these genera, notably *Nonomuraea*, *Sphaerisporangium*, and *Thermopolyspora* were based on or included taxa that had previously been misclassified with founder members of the family.

The genus *Nonomuraea* was proposed by Zhang et al. (1986a) to accommodate the *Actinomadura pusilla* group (Fischer et al., 1983; Meyer, 1989; Poschner et al., 1985) which had been assigned to the genus *Microtetraspora* (Kroppenstedt et al., 1990a) when it became apparent that the genus *Actinomadura* could be split into two distinct aggregate taxa (see corresponding section on the genus *Actinomadura*). In contrast, *Excellospora rubrobrunea* Agre and Guzeva 1975 was transferred to the revised genus *Actinomadura* as *Actinomadura rubrobrunea* (*ex* Krasil'nikov et al. 1968) Kroppenstedt et al. 1991. The genus *Thermopolyspora* was proposed to provide a taxonomic home for an organism that had initially borne the name "*Thermomonospora flexuosa*" Krasil'nikov and Agre 1964.

The genus *Streptosporangium* was shown to be markedly heterogeneous as it contained several species that had little in common either with one another or with the type species, *Streptosporangium roseum* (Kemmerling et al., 1993; Kudo et al., 1993; Ochi and Miyadoh, 1992). Stackebrandt et al. (1994) classified *Streptosporangium albidum* Furumai et al. 1968, *Streptosporangium viridogriseum* subsp. *kofuense* Okuda et al. 1966,

and *Streptosporangium viridogriseum* subsp. *viridogriseum* in a new genus, *Kutzneria*, as *Kutzneria albida*, *Kutzneria kofuensis*, and *Kutzneria viridogrisea*. Similarly, "*Streptosporangium cinnabarinum*" Celmer et al. 1977 and *Streptosporangium viridialbum* Nonomura and Ohara 1960 became initial members of the genus *Sphaerisporangium* as *Sphaerisporangium cinnabarinum* Ara and Kudo 2007 and *Sphaerisporangium viridialbum* (Nonomura and Ohara 1960) Ara and Kudo 2007.

Other changes in the composition of the genus *Streptosporangium* include the transfer of *Streptosporangium corrugatum* Williams and Sharples 1976 to the genus *Acrocarpospora* as *Acrocarpospora corrugata* (Williams and Sharples 1976) Tamura et al. 2000 and *Streptosporangium indianense* Gupta 1965 to the genus *Streptomyces* as *Streptomyces indianensis* (Gupta 1965) Kudo and Seino 1987. The type strains of *Herbidospora cretaceae* Kudo et al. 1993 and *Streptosporangium claviforme* Petrolini et al. 1992 have morphological features in common and belong to the same genomic species; these results led Kudo and his colleagues to propose that the *Streptosporangium claviforme* be seen as a subjective synonym of *Herbidospora cretacea*. However, the two strains form distinct, but related, phyletic lines in the 16S rRNA *Streptosporangiaceae* gene tree (Figure 360).

Differentiation of the genera of the family *Streptosporangiaceae*

The genera classified in the family *Streptosporangiaceae* can be delineated using genotypic and phenotypic markers. They are most readily separated by 16S rRNA gene sequence data including genus specific nucleotide signatures (Ara and Kudo, 2007; Goodfellow and Quintana, 2006), and by morphological criteria (Table 275). The genera *Acrocarpospora*, *Planobispora*, *Planomonospora*, *Planotetraspora*, *Sphaerisporangium*, and *Streptosporangium*, for instance, can be separated on the basis of the shape of spore vesicles, the number and shape of the enclosed spores, and by whether the latter are motile. Similarly, the oligosporic members of the family can be distinguished by the number of spores borne in chains on aerial hyphae and whether spores are formed on substrate hyphae. Microbisporae typically produce chains of two spores, microtetrasporae chains of four spores, whereas nonomuraea form either chains of spores or pseudovesicles. In all cases, the spores are formed on aerial hyphae. *Herbidospora* is unusual as it produces short chains of spores in clusters at the tips of sporophores that arise from substrate hyphae.

It is sometimes necessary to distinguish between genera classified in the family *Streptosporangiaceae* from morphologically similar taxa assigned to other families. Chemotaxonomic markers can be used to distinguish between *Microbispora*, *Microtetraspora* and *Nonomuraea* strains and members of the genus *Actinomadura* which also form chains of spores on aerial hyphae (Kroppenstedt et al., 1990a; Kudo et al., 1993). Actinomadurae typically contain MK-9(H_6), saturated at sites II, III, and VIII as the major isoprenologue, and diphosphatidylglycerol and phosphatidylinositol mannosides but lack nitrogenous phospholipids (phospholipid type I), whereas microbisporae, microtetrasporae, and nonomuraea have major amounts of MK-9(H_4), saturated at points III and VIII, and polar lipid patterns which contain diphosphatidylglycerol, hydroxyethanolamine, uncharacterized glycolipids, and glucosamine-containing phospholipids (phospholipid type IV) (Lechevalier et al., 1977, 1981).

Members of the family *Streptosporangiaceae* which form spore vesicles can be differentiated from morphologically similar strains belonging to the genera *Actinoplanes* and *Dactylosporangium* as the latter have a wall chemotype II and a phospholipid type I or II, whereas the former have a wall chemotype II and a phospholipid pattern IV (Goodfellow and Quintana, 2006; Koch et al., 1996; Vobis, 2006). Care also needs to be taken to distinguish between *Streptosporangium* and *Spirillospora* strains, though only the latter form motile spores (Vobis and Kothe, 1989). The vesicular hyphae within the spore vesicles of spirillosporae are branched, whereas those of streptosporangiae are not. Methods used to detect key chemical markers are cited in the section on the suborder *Streptosporangineae*.

Monciardini et al. (2002) designed a highly specific oligonucleotide primer set for the identification of *Streptosporangiaceae* strains. They also developed selective sets of oligonucleotide primers for PCR-amplification of rDNA for the recognition of members of the families *Micromonosporaceae*, *Streptomycetaceae*, and *Thermomonosporaceae*, and for *Dactylosporangium* strains. The application of these primer sets to environmental samples shows the presence of novel members of these taxa in soil samples.

Genus I. **Streptosporangium** Couch 1955a, 145[AL] emend. Stackebrandt, Kroppenstedt, Jahnke, Kemmering and Gürtler 1994, 268

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Strep.to.spo.ran′gi.um. Gr. adj. *streptos* twisted; Gr. n. *spora* a seed; Gr. n. *angium* a vessel; N.L. neut. n. *Streptosporangium* spores coiled within a sporangium.

Aerobic, Gram-stain-positive, non-acid-fast actinomycetes which form a branched, stable, non-fragmenting mycelium bearing aerial hyphae that differentiate into globose or spherical spore vesicles. Oval, spherical, or rod-shaped, nonmotile sporangiospores are formed by septation of a single, coiled, unbranched hypha within the spore vesicle. Grows on a variety of organic and synthetic media. Biochemically versatile and chemically homogeneous. **Whole-organism hydrolysates contain *meso*-diaminopimelic acid and usually madurose. The peptidoglycan is of the A1γ type. Muramic acid moieties are *N*-acetylated. Cells contain major amounts of iso-, anteiso-, saturated, unsaturated, and 10-methyl-branched fatty acids, and MK-9(H_2) and MK-9(III, VIII-H_4) as the predominant isoprenologues. Mycolic acids are absent. Major phospholipids include diphosphatidylglycerol, phosphatidylethanolamine, phosphatidylglycerol, phosphatidylinositol, and glucosamine-containing phospholipids**. The phylogenetic position of the genus *Streptosporangium*, as determined by

16S rRNA gene sequence analysis, is in the family *Streptosporangiaceae*.

DNA G+C content (mol%): 69–71.

Type species: **Streptosporangium roseum** Couch 1955a, 151[AL].

Further descriptive information

Phylogeny. The genus *Streptosporangium* forms a distinct 16S rRNA gene clade within the evolutionary radiation encompassed by genera classified in the family *Streptosporangiaceae* (Ara and Kudo, 2007; Goodfellow et al., 1990b; Kudo, 2001). The genus encompasses 13 validly published species which are most closely related to the genera *Planobispora*, *Planomonospora*, and *Planotetraspora* (Figure 361). The two most distantly related species, *Streptosporangium violaceochromogenes* and *Streptosporangium yunnanense* share a 16S rRNA gene similarity of 95.2%. In contrast, the type strains of *Streptosporangium album*, *Streptosporangium roseum*, and *Streptosporangium vulgare* have identical 16S rRNA gene sequences.

Cell morphology. *Streptosporangium* strains characteristically form nonfragmenting, extensively branched substrate hyphae which carry aerial hyphae that differentiate into spore vesicles (Figure 362) which are borne either on short or long sporangiophores (Figure 363, Figure 364, and Figure 365). The spore vesicles may be carried singly or in clusters (Figure 364, Figure 365, and Figure 366). They may be globose or spherical and are usually 4–20 µm in diameter, but may be up to 40 µm. Spore vesicles originate as sac-like structures within which single, unbranched hyphae coil repeatedly and undergo septation giving rise to “sporangiospores” (Locci, 1976; Locci and Petrolini-Baldan, 1971). “Sporangiospores” are formed in the same way as the conidia of streptomycetes and not by cytoplasmic cleavage and nuclear division as in the formation of fungal sporangiospores (Lechevalier et al., 1966b; Sharples et al., 1974; Vobis and Kothe, 1985). Consequently, the use of the term sporangium is inappropriate in this context and should be replaced by the term spore vesicle (Cross, 1970; Sharples et al., 1974). However, unlike streptomycetes, the vesicular sheath does not usually persist around released spores.

As spore vesicles increase in size, the internal arrangement of hyphae and their septation into spores can be seen by the shape of the thin vesicular walls (Figure 364, Figure 365, and Figure 366), though the vesicular membrane of *Streptosporangium fragile* cannot be detected by light microscopy (Shearer et al., 1983). Nonmotile spores are released when spores vesicles are placed in water; the surrounding sheath is ruptured when the intersporal matrices swell and exert pressure on the vesicular membrane. The spores are spherical, oval, or rod shaped and have smooth walls. *Streptosporangium roseum* also forms streptomycete-like chains of arthrospores.

Chemotaxonomy. Streptosporangiae have a wall chemotype 111B (Lechevalier and Lechevalier, 1970a, 1970b), i.e. they contain *meso*-diaminopimelic acid in the wall peptidoglycan and madurose (3-*O*-methyl-D-galactose; Lechevalier and Gerber, 1970) in whole-organism hydrolysates. They have a peptidoglycan of the A1γ type (Schleifer and Kandler, 1972) and *N*-acetylated muramic acid (Kawamoto et al., 1981) and are rich in iso-, anteiso-, saturated, and unsaturated methyl-branched fatty acids (pattern 3c *sensu* Kroppenstedt, 1985; Kudo et al, 1993; Whitham et al., 1993; Stackebrandt et al., 1994; Zhang et al., 2002, 2005), but lack mycolic acids (Goodfellow and Minnikin, 1981; Kudo et al., 1993; Whitham et al., 1993).

Most *Streptosporangium* strains contain di- and tetrahydrogenated menaquinones with nine isoprene units as predominant

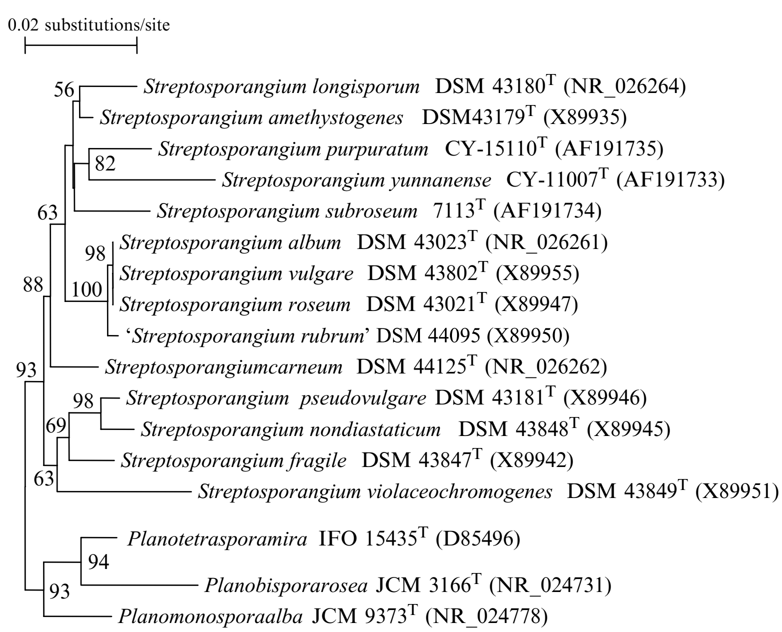

FIGURE 361. Neighbor-joining tree based on almost complete 16S rRNA gene sequences showing relationships of *Streptosporangium* species to one another and to representatives of related genera classified in the family *Streptosporangiaceae*.

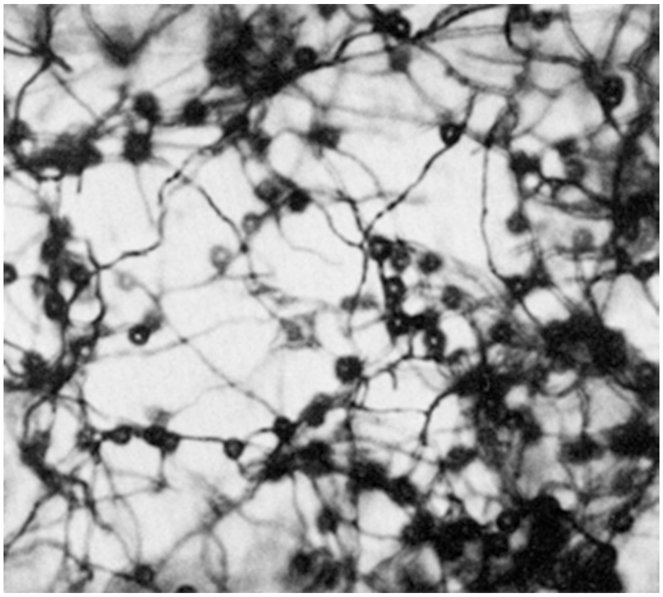

FIGURE 362. Photomicrograph of *Streptosporangium carneum* A84575^T showing profuse spore vesicle formation after growth on inorganic salts-starch agar (ISP medium 4) for 18 d at 30°C. Bright-field illumination. Magnification, 640×. (Reproduced with permission from Mertz and Yao, 1990. Int. J. Syst. Bacteriol. *40*: 247–253.)

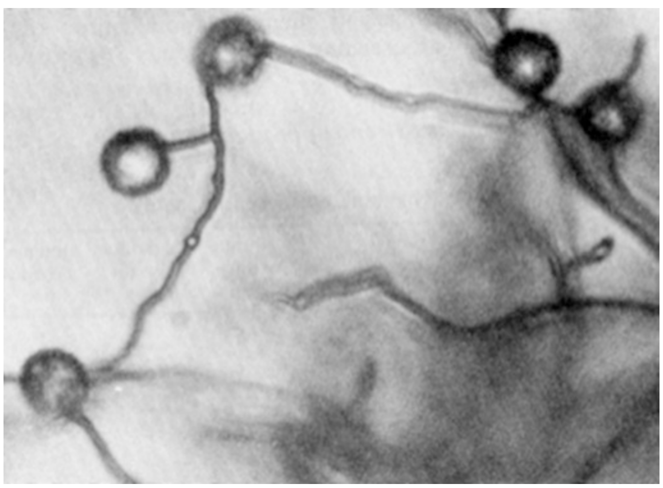

FIGURE 363. Photomicrograph of *Streptosporangium carneum* A84575^T showing sporangiophore formation after growth on inorganic salts-starch agar (ISP medium 4) for 18 d at 30°C. Bright-field illumination, oil immersion. Magnification, 1600×. (Reproduced with permission from Mertz and Yao, 1990. Int. J. Syst. Bacteriol. *40*: 247–253.)

isoprenologues (Kroppenstedt, 1985; Kudo et al., 1993; Stackebrandt et al., 1994; Whitham et al., 1993), though *Streptosporangium purpuratum*, *Streptosporangium subroseum*, and *Streptosporangium yunnanense* also contain substantial proportions of unsaturated menaquinones with nine isoprene units (Zhang et al., 2002, 2005). Streptosporangiae typically have a phospholipid type IV (Lechevalier et al., 1977, 1981); they contain glucosamine-containing lipids with diphosphatidylglycerol, phosphatidylethanolamine, and phosphatidylinositol (Kudo

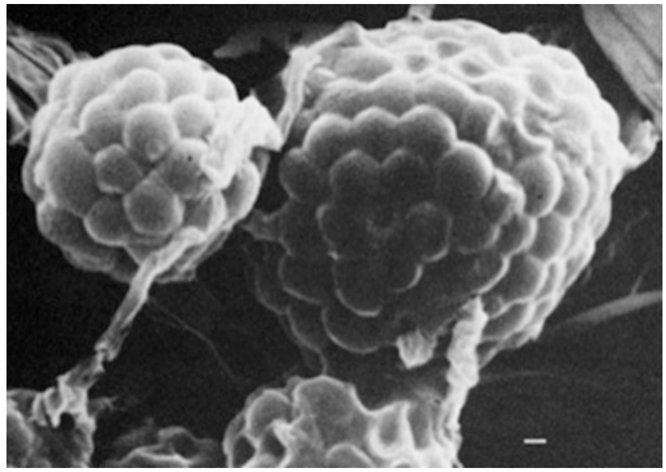

FIGURE 364. Scanning electron micrograph of spore vesicles of *Streptosporangium carneum* A84575^T after 18 d growth at 30°C on inorganic salts-starch agar (ISP medium 4). Bar = 1 µm. (Reproduced with permission from Mertz and Yao, 1990. Int. J. Syst. Bacteriol. *40*: 247–253.)

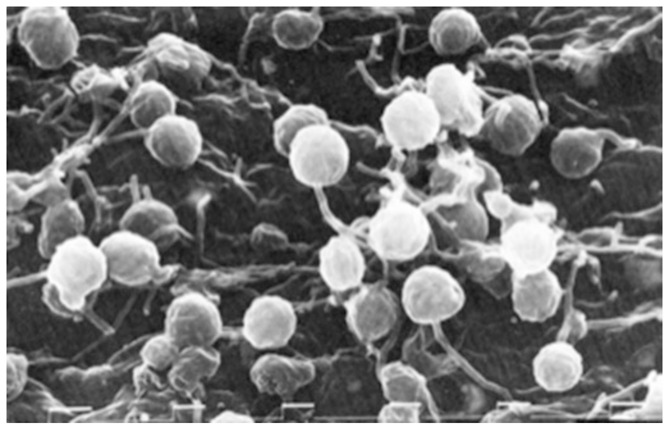

FIGURE 365. Scanning electron micrograph of *Streptosporangium album* CBS 426.61 growing on oatmeal agar. Sporangiophores are short. Bar interval = 10 µm.

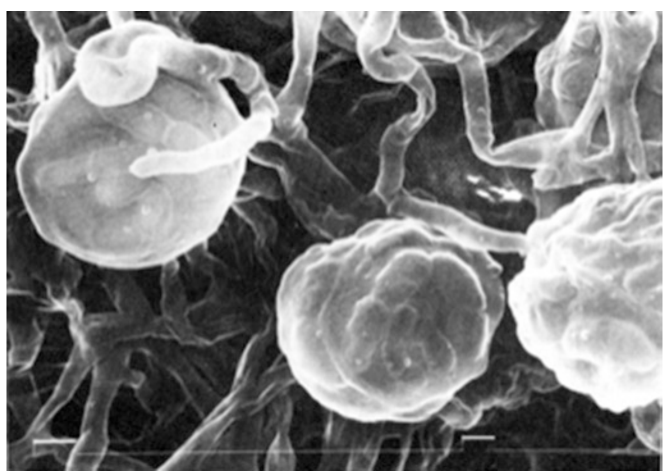

FIGURE 366. Scanning electron micrograph of *Streptosporangium album* CBS 426.61 growing on oatmeal agar. Bar interval = 10 µm. Vesicular walls are thin.

et al., 1993; Stackebrandt et al., 1994; Whitham et al., 1993). The presence of phosphatidylmethylethanolamine is variable (Zhang et al., 2005). *Streptosporangium subroseum* is unusual as it only contains phosphatidylethanolamine (Zhang et al., 2002) and hence has a wall chemotype II *sensu* Lechevalier et al. (1977, 1981). The G+C content of the DNA of streptosporangiae falls within the narrow range of 69.0–71 mol% (Farina and Bradley, 1970; Tsyganov et al., 1966; Yamaguichi, 1967; Zhang et al., 2002, 2005).

Colony morphology. *Streprosporangium* strains form well developed colonies on ISP media that are commonly used to cultivate filamentous actinomycetes (Shirling and Gottlieb, 1966). The substrate mycelium may be pink, red to dark brown, black, or deep purplish red, and the aerial mycelium pinkish-white or yellowish-pink, though the colors can vary between species and are influenced by the cultivation conditions. Some species produce colored diffusible pigments. *Streptosporangium amethystogenes* forms violet crystals of iodinin on oatmeal-YG medium (yeast extract 1 g, glucose 2 g, glycerol 2 g/l).

Nutrition and growth conditions. Streptosporangiae grow well on rich media such as arginine-vitamin agar (Nonomura and Ohara, 1969b, a), glucose-yeast extract agar (Waksman, 1950a), modified Bennett's agar (Jones, 1949), oatmeal agar (Shirling and Gottlieb, 1966), and yeast extract agar (Kudo et al., 1993). They grow particularly well and produce an abundant aerial spore mass on oatmeal-yeast extract (Nonomura and Ohara, 1960; Zhang et al., 2005). Some strains such as *Streptosporangium nondiastaticum* and *Streptosporangium pseudovulgare* require B-vitamins for growth (Zhang et al., 2005). It has also been shown that *Streptosporangium* strains do not have any particular preference for nutrient sources, either for the support of their growth or for their ability to produce anti-*Bacillus subtilis* substances (Platas et al., 1999). Streptosporangiae generally grow well at neutral pH and at 20–30°C; members of some species grow at 42°C (Nonomura, 1989a; Zhang et al., 2002, 2005).

Metabolism and genetics. Little is known about either the metabolism or genetics of *Streptosporangium* strains. In general, streptosporangiae use a broad range of compounds as sole carbon sources (Whitham et al., 1993; Zhang et al., 2002, 2005) and degrade diverse organic substrates (Whitham et al., 1993). *Streptosporangium pseudovulgare*, *Streptosporangium purpuratum*, and *Streptosporangium yunnanenis* degrade cellulose (Zhang et al., 2002, 2005). There is evidence that streptosporangiae can cleave diverse methylumbelliferone conjugated substrates (Whitham et al., 1993). Members of the genus have a chemoorganotrophic metabolism.

Streptosporangiae are a potentially rich source of novel commercially significant products, notably antibiotics (Donadio et al., 2002; Lazzarini et al., 2000), as exemplified by the production of lipopeptides from *Streptosporangium amethystogenes* (Takizawa et al., 1995); glycopeptides from *Streptosporangium carneum* (Michel and Yao, 1991); selenomycin from "*Streptosporangium brasiliense*" (Coronelli and Thiemann, 1969); anthracyclines from *Streptosporangium fragile* (Nash et al., 1981; Shearer et al., 1983); sporamycin from "*Streptosporangium koreanum*" (Celmer et al., 1978); oligopeptides from *Streptosporangium nondiastaticum* (Shokichi et al., 1988); a novel protein (Umezawa and Kamiyama, 1983) and sporamycin from *Streptosporangium pseudovulgare* (Umezawa et al., 1976); an ansa-macrolactam (Hacene et al., 1998), sporangirosomycin (Ghazal and Alb-El-Aziz, 1993), and thiosporamycin (Celmer et al., 1978) from *Streptosporangium roseum*; sibiromycin from "*Streptosporangium sibiricum*" (Brazhnikova et al., 1972; Gauze et al., 1969); platomycins A and B (Takasawa et al., 1975) and victomycin from *Streptosporangium violaceochromogenes* (Kawamoto et al., 1975); and sporacuracins A and B from *Streptosporangium vulgare* (Atsushi et al., 1975).

A new calmodulin antagonist, genistein, has been isolated from *Streptosporangium vulgare* (Goto et al., 1987), a hematopoietic inducer from *Streptosporangium amethystogenes* subsp. *fukuiensis* (Donadio et al., 2002), and endothelin converting enzyme inhibitors from *Streptosporangium roseum* (Tsurumi et al., 1994, 1995). In addition, virginiamycin type peptolides have been isolated from "*Streptosporangium cinnabarinum*" and "*Streptosporangium koreanum*" (Celmer et al., 1977), 1,6- dihydroxy-2-chlorophenazine, a novel phenazine antifungal antibiotic from a *Streptosporangium* strain (Patel et al., 1984), 1-hydroxy-4-methoxy-2-naphthoic acid, a herbicidal compound, from "*Streptosporangium cinnabarinum*" (Pfefferle et al., 1997), a thermostable glucoamylase from a streptosporangial endophyte of maize leaves (Stamford et al., 2002), and pigment-like antibiotics from a *Streptosporangium* strain isolated from an Algerian soil (Boudjella et al., 2007). Other potentially useful substances include inhibitors of lactic acid production by oral flora (Ikeda et al., 1993) and insecticidal compounds (Mishra et al., 1987).

Pfefferle et al. (2000) highlighted the importance of fermentation conditions in the production of secondary metabolites from streptosporangiae. They showed that the optimal conditions for secondary metabolite production were completely different from those of streptomycetes and identified oxygen tension as an important parameter for the optimal production of secondary metabolites from streptosporangiae in submerged culture.

Antibiotic sensitivity. The antibiotic sensitivity profiles of individual species, where known, are given in the species descriptions.

Pathogenicity. There are no proven grounds for believing that streptosporangiae have a role as clinical or veterinary pathogens, though "*Streptosporangium bovinum*" was isolated from infected bovine hooves (Batista et al., 1963).

Ecology. Streptosporangiae have been isolated infrequently from diverse sources, including coastal sediments (Bredholdt et al., 2007), cow dung (Garg et al., 2003), earthworm casts (Mba, 1997), forest soils (Potekhina, 1965; Xu et al. (1996), leaf litter (Van Brummelen and Bent, 1957), plant roots (Coombs and Franco, 2003; de Araujo et al., 2000; Sardi et al., 1992; Solans and Vobis, 2003), and lake sediments (Johnston and Cross, 1976; Lee and Hwang, 2002; Willoughby, 1969a), though organisms labeled *Streptosporangium* type 1 from freshwater streams (Willoughby, 1969b) probably belong to the genus *Actinoplanes*, given their morphological features and ability to form motile spores.

The introduction of an innovative selective isolation procedure showed that streptosporangiae are an integral part of actinomycete communities in soils (Nonomura and Ohara,

1969a) where they presumably have a role in the turnover of organic matter. The number of *Streptosporangium* strains in Japanese soils was estimated at 10^4–10^6 colony forming units per gram dry weight soil (Nonomura, 1984; Nonomura and Ohara, 1969a); lower numbers have been reported from pasture and woodland soils (Whitham et al., 1993). There is evidence that slightly acid, humus rich soils are a good source of streptosporangiae (Hayakawa et al., 1988; Lee and Hwang, 2002; Nonomura and Hayakawa, 1988; Seong et al., 2001).

The application of new selective isolation procedures designed for the recovery of rare actinomycetes have shown that streptosporangiae are present in a range of soil types including chernozem soils (Li et al., 2002), corn and paddy field soils (Nonomura and Hayakawa, 1988) (Masayuki et al., 1991), peaty soils (Zenova et al., 2008), steppe chestnut and desert pale brown soils (Norovsuren et al., 2007; Zhadambaa et al., 2007), and a Saharan soil (Boudjella et al., 2006).

Enrichment and isolation procedures

Isolation and enumeration of streptosporangiae and related actinomycetes, notably microbisporae and microtetrasporae, can be achieved by dry-heat treatment of air-dried soil samples and dilution plate culture with selective synthetic media (Hayakawa and Nonomura, 1984, 1987a, 1987b, 1989; Nonomura and Hayakawa, 1988; Nonomura and Ohara, 1969a, 1969b; Nonomura and Ohara, 1971a, 1971c, 1971d). To this end, soil samples are dried slowly at room temperature for 7 d, passed through a 2 mm sieve, gently ground in a mortar, spread on filter paper, and heated in a hot air oven at 100–120°C for an hour. This procedure greatly reduces the numbers of bacteria and streptomycetes, but leads to an increased isolation frequency of streptosporangiae. There is evidence that pretreatment of soil suspensions with sodium dodecyl sulfate (0.05%, w/v) and yeast extract (6%, w/v) at 40°C for 20 min, followed by dilution in water, activates spores of actinomycetes but kills vegetative cells of other bacteria, thereby leading to increased counts of streptosporangiae and related organisms on isolation plates.

Suspensions of pretreated soil are used to inoculate one or more of the selective synthetic media. Arginine-vitamins (AV) agar*, chitin – V agar†, humic-vitamin (HV)‡, and C-1§ and C-2** have been recommended for the selective isolation of streptosporangiae and related actinomycetes (Hayakawa and Nonomura, 1987a, 1987b; Nonomura and Ohara, 1969a). These media are supplemented with antifungal antibiotics and sometimes with penicillin and polymixin B (Whitham et al., 1993). Inoculated plates are incubated at 30°C for 4–6 weeks and colonies examined for the presence of spore vesicles by using a light microscope fitted with a long working distance objective. The highest counts and cleanest plates are usually obtained with HV-vitamin agar. Growth of streptosporangiae around soil particles can be observed when pretreated soil particles (0.5 g) are sprinkled over selective media that have been incubated for 1 month at 30°C.

Hayakawa et al. (1991a) recommended an improved method for the selective isolation of streptosporangiae from soil. This procedure was based on the ability of streptosporangial spores to withstand dry heat at 120°C for an hour and treatment with benzylthonium chloride (BC), as well as the capacity of streptosporangiae to grow in the presence of leukomycin and nalidixic acid. The dry heat and BC treatments greatly reduce the numbers of bacteria and unwanted actinomycetes, including streptomycetes, from isolation plates. In turn, nalidixic acid in HV agar suppresses the growth of BC-resistant bacteria while leukomycin increases the selectivity of HV agar for streptosporangiae by eliminating unwanted bacteria that remain after the employment of the various pretreatments. In general, streptosporangiae account for about 20% of colonies growing on the resulting isolation plates.

The improved procedure involves several steps. Initially, an air-dried soil sample is ground in a mortar and heated at 120°C for an hour prior to the preparation of a 10^{-1} dilution in water. Half a ml of the 10^{-1} preparation is added to 4.5 ml of sterile 5 mm phosphate buffer (pH 7.0) containing BC at a final concentration of 0.01%, w/v. This preparation is kept at 30°C for 30 min with occasional stirring and a 1 ml fraction diluted in sterile tap water (1:10 or 1:15). Aliquots (0.1 or 0.2 ml) of this preparation are spread over the surfaces of HV agar supplemented with leukomycin in ethanol (1 mg/l) and nalidixic acid (20 mg/l). The inoculated plates are incubated at 30°C for 3–4 weeks then examined as before.

Other procedures based on pretreatment of air-dried soil samples and plating onto selective media have been used to isolate members of genera classified in the family *Streptosporangiaceae*, notably *Herbidospora*, *Microbispora*, *Microtetraspora*, and *Streptosporangium*. Hayakawa et al. (1997) found that spores from representatives of these taxa, unlike those from *Micromonospora*, *Nocardia*, and *Streptomyces* strains, were resistant to toluene-*p*-sulfon sodiumchloroamide trihydrate (chloramine-T). Water suspensions of air-dried soil treated with chloramine-T (1%) and plated onto HV agar supported the growth of the target organisms after incubation at 30°C for 3 weeks. The chlorination treatment specifically inhibits the growth of nonfilamentous bacteria and unwanted actinomycetes, thereby facilitating the isolation of the target genera. Isolates presumptively assigned to the genus *Streptosporangium*, on the basis of

*AV agar (Nonomura and Ohara, 1969a): arginine, 0.3 g; glucose, 1 g; glycerol, 1 g, K_2HPO_4, 0.3 g; $MgSO_4 \cdot 7H_2O$, 0.2 g; NaCl, 0.3 g; agar, 15 g; distilled water, 1000 ml; B vitamins (thiamin HCl, riboflavin, niacin, pyridoxine HCl, isositol, calcium pantothenate, *p*-aminobenzoic acid, each at 0.5 mg; biotin, 0.25 mg); trace salts ($CuSO_4 \cdot 5H_2O$, 1 mg; $Fe_2(SO_4)_3$, 10 mg; $MnSO_4 \cdot 7H_2O$, 1 mg; $ZnSO_4 \cdot 7H_2O$, 1 mg); antibiotics (actidione, 50 mg; nystatin, 5 mg; penicillin G, none or 0.8 mg; polymixin B, none or 4 mg); pH 6.4.

†Chitin-V agar (Hayakawa and Nonomura, 1984): colloidal chitin, 2 g (dry w.); K_2HPO_4, 0.15 g; $MgSO_4 \cdot 7H_2O$, 0.2 g; NaCl, 0.3 g; $CaCO_3$, 0.02 g; $FeSO_4 \cdot 7H_2O$, 10 mg; $ZnSO_4 \cdot 7H_2O$, 1 mg; $MnCl_2$, 1 mg; agar, 18 g; distilled water, 1000 ml; B vitamins, as for AV agar; actidione, 50 mg; pH 7.2.

‡HV vitamin agar (Hayakawa and Nonomura, 1984; Nonomura, 1984): humic acid, 1 g (used as an alkaline solution; artificial humic acid prepared from glucose and urea may be used, as may natural humic acids from soil humus, but the pale brown humic acid designated as Rp type gives the best results); Na_2HPO_4, 0.5 g; $MgSO_4 \cdot 7H_2O$, 0.05 mg; KCl, 1.7 g; $FeSO_4 \cdot 7H_2O$, 0.01 g; $CaCO_3$, 0.02 g; agar, 18 g; distilled water, 1000 ml; B vitamins, as for AV agar; actidione, 50 mg; pH 7.2.

§C-1 medium (Nonomura and Ohara, 1969b): casamino acids, 2 g; K_2HPO_4, 0.3 g; $MgSO_4 \cdot 7H_2O$, 0.5 g; NaCl, 0.3 g; agar, 20 g; distilled water, 1000 ml; trace salts ($FeSO_4 \cdot 7H_2O$, 10 mg; $MnSO_4 \cdot 7H_2O$, 1 mg; $CuSO_4 \cdot 5H_2O$, 1 mg); B vitamins (thiamine HCl, riboflavin, niacin, pyridoxine HCl, inositol, calcium pantothenate, *p*-aminobenzoic acid, each 0.5 mg; biotin 0.25 mg); pH 7.2.

**C-2 medium (Nonomura and Ohara, 1969b): casamino acids, 0.5 g; and asparagine, 0.5 g, instead of casamino acids, 2 g, in the composition of C-1 medium. The other components are the same as for C-1 medium.

their ability to form spore vesicles, accounted for 1.8–4.1 × 10^5 c.f.u./g dry weight soil.

Pretreatment of soil suspensions with extreme high frequency (EHF) radiation prior to plating onto Gauze's agar 2 supplemented with nalidixic acid (10 μg/ml) and either levorin (20 μg/ml) or nystatin (50 μg/ml) and incubation at 28°C for 2–6 weeks led to the isolation of increased numbers of rare actinomycetes from soil including streptosporangiae (Bulina et al., 1997; Li et al., 2002). It seems likely that EHF radiation inhibits the growth of unicellular bacteria and breaks the dormancy of actinomycete spores. Streptosporangiae have also been isolated by plating out surface sterilized root material onto selective media (Cao et al., 2005; de Araujo et al., 2000).

An indirect immunomagnetic capture (IMC) method was developed by Mullins et al. (1995) for the selective recovery of *Streptosporangium fragile* from sterile and non-sterile soil. IMC recovery rates for *Streptosporangium fragile* spores from soil were usually in the range of 10–20% of the counts obtained by traditional plating. However, the capture method resulted in cleaner isolation plates and hence could be expected to enhance the successful isolation of the target organism compared with traditional plating methods.

Maintenance procedures

The most convenient method for short-term storage is by serial transfer from agar slants of appropriate media, such as oatmeal-yeast extract agar (Nonomura and Ohara, 1960), every 2 months. The tubes should be tightly closed with cotton plugs dipped in melted paraffin wax and stored at 4°C and room temperature. Longer term preservation of strains can be achieved as frozen stocks in 20% (v/v) aqueous glycerol at −80°C (mechanical freezer) to −172°C (liquid nitrogen vapor) or by using established lyophilization procedures. Strains can also be kept as mycelial suspensions in 20% aqueous glycerol at −20°C to −72°C.

Procedures for testing special characters

Iodinin production is detected after culturing on oatmeal agar supplemented with 0.1% (w/v) yeast extract for 1 month at 23–30°C. Utilization of carbon sources is determined by comparing growth on a given carbon source in basal C-1 or C-2 media with two controls, growth on the basal medium alone, and growth on the basal medium plus 0.5% (w/v) glucose. Most species of *Streptosporangium* do not grow on ISP Carbon Utilization Medium (Shirling and Gottlieb, 1966). Other useful physiological tests are hydrolysis of starch agar supplemented with yeast extract (0.5 g/l), nitrate reduction to nitrite in Bacto-nitrate broth (Difco) supplemented with yeast extract (2 g/l), and gelatin liquefaction (gelatin, 200 g; peptone, 5 g; yeast extract, 2 g; glucose, 2 g; distilled water, 1000 ml).

Differentiation of the genus *Streptosporangium* from other genera

The genus *Streptosporangium* is phylogenetically distinct from all other genera which contain organisms that produce spore vesicles. At present, morphology is the sole criterion used to distinguish between genera classified in the family *Streptosporangiaceae* (see Table 276 in the section on the family). *Streptosporangium* can be readily distinguished from the genera *Herbidospora*, *Microtetraspora*, *Microbispora*, and *Nonomuraea* by its ability to form spore vesicles and from the remaining genera by the shape of spore vesicles, the number of spores they contain, and whether or not the latter are motile. It can be difficult to distinguish between *Streptosporangium fragile*, which has a very thin vesicular membrane, and *Nonomuraea* strains which produce pseudovesicles covered by a slimy material. Similarly, the genus *Spirillospora* may be confused with species of the genus *Streptosporangium* (Vobis and Kothe, 1989). All of the strains belonging to these taxa have multispored, usually spherical, spore vesicles. However, these taxa can be distinguished on the basis of their polar lipid patterns and by the ability of spirillosporae to form motile spores.

Taxonomic comments

Couch (1955a) proposed the genus *Streptosporangium* for sporangiate actinomycetes that formed nonmotile sporangiosphores on an abundant aerial mycelium. Initially, only one species, *Streptosporangium roseum*, was recognized. Additional species were added to the genus primarily based on the capacity of strains to form spore vesicles, as exemplified by proposals for *Streptosporangium album* Nonomura and Ohara 1960, *Streptosporangium amethystogenes* Nonomura and Ohara 1960, *Streptosporangium longisporum* Schäfer 1969, *Streptosporangium nondiastaticum* Nonomura and Ohara 1969b, and *Streptosporangium viridialbum* Nonomura and Ohara 1969b. More exacting studies showed the genus to be heterogeneous, notably on the basis of spore and spore vesicular morphology detected by scanning electron microscopy (Nonomura, 1989a), electrophoretic mobility of ribosomal protein AT-L30 (Ochi and Miyadoh, 1992), partial amino acid sequencing of the N termini of AT-L30 proteins (Ochi et al., 1993), 5S and 16S rRNA gene sequences (Kemmerling et al., 1993; Kudo et al., 1993), and on the discontinuous distribution of chemotaxonomic markers (Stackebrandt et al., 1994).

Stackebrandt and his colleagues assigned members of the genus to two groups based on the discontinuous distribution of chemical markers. Most species, including *Streptosporangium roseum*, the type species, had a phospholipid type IV and predominant menaquinones of the MK-9(H_2) and MK-9(III, VIII-H_4), MK-9 and/or MK-9(H_6) type. The second group, which encompassed *Streptosporangium albidum* and the two subspecies of *Streptosporangium viridugriseum*, had a phospholipid type II and MK-9(II, III, H_4) as the predominant isoprenologues. These results were in excellent agreement with corresponding 16S rRNA gene sequencing data. Consequently, Stackebrandt and his co-workers proposed that *Streptosporangium albidum* Furumai et al. 1968, *Streptosporangium viridogriseum* subsp. *viridogriseum* Nonomura and Ohara 1969b, and *Streptosporangium viridogriseus* subsp. *kofuense* Okuda et al. (1966) be assigned to a new taxon, the genus *Kutzneria*, as *Kutzneria albida* comb. nov., *Kutzneria viridogrisea* comb. nov., and *Kutzneria kofuensis*, respectively, results supported by numerical phenetic data (Whitham et al., 1993).

The position of "*Streptosporangium cinnabarinum*" JCM 3291 and *Streptosporangium viridialbum* DSM 43801^T in the genus

TABLE 276. Characteristics differentiating between *Streptosporangium* species[a,b]

Characteristic	*S. roseum* DSM 43021[T]	*S. album* DSM 43023[T]	*S. amethystogenes* subsp. *amethystogenes* DSM 43179[T]	*S. amethystogenes* subsp. *fukuiense* DSM 44779	*S. carneum* NRRL 18437[T]	*S. fragile* DSM 43847[T]	*S. longisporum* DSM 43180[T]	*S. nondiastaticum* DSM 43848[T]	*S. pseudovulgare* DSM 43181[T]	*S. purpuratum* DSM 44668[T]	*S. subroseum* DSM 44662[T]	*S. violaceochromogenes* DSM 43849[T]	*S. vulgare* DSM 43802[T]	*S. yunnanense* DSM 44663[T]
Morphology on oatmeal agar (color of substrate mycelium):	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	
Brown-black	−	−	−	−	−	+	−	−	−	−	−	−	−	−
Red-orange	+	−	−	−	−	−	+	+	+	+	−	−	+	−
Yellowish brown to brown	+	+	+	+	+	−	−	+	−	−	+	+	+	+
Color of aerial spore mass:	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr
Greenish gray	−	−	−	−	−	−	−	−	−	−	−	−	−	−
Pink	+	−	+	−	+	+	+	+	+	+	+	+	+	+
White	−	+	−	+	−	−	−	−	−	−	−	−	−	−
Soluble pigments:	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr
Brown	−	−	−	−	−	+	−	−	−	−	−	+	−	+
Red-brown to purple brown	+	−	−	−	−	−	−	−	−	−	−	−	−	−
Violet	−	−	−	−	−	−	−	−	−	−	−	+	−	−
Yellow-brown	−	−	−	−	−	−	+	−	−	+	−	−	−	−
Spore vesicle size (µm):	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr
1–5	−	−	−	−	−	−	−	−	−	+	+	−	−	+
6–10	+	+	+	+	+	+	+	−	+	−	−	+	+	−
11–20	(+)	−	−	−	+	+	+	+	−	−	−	−	−	−
21–30	−	−	−	−	(+)	−	−	+	−	−	−	−	−	−
31–50	−	−	−	−	(+)	−	−	−	−	−	−	−	−	−
Sporophore size (µm):	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr
Short (10)	+	+	+	+	−	+	+	+	+	nd	+	+	+	nd
Long (50)	−	−	−	−	+	−	−	−	−	nd	−	−	−	nd
Spore shape:	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr
Spherical to oval	+	+	+	nr	+	+	+	+	+	+	+	+	+	+
Rods	−	−	−	nr	+	−	−	−	−	−	−	−	−	−
Iodinin production	−	−	+	+	−	−	−	−	−	+	−	−	−	−
B vitamins required	+	+	+	+	−	−	−	+	+	−	−	−	+	−
Growth at:	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr
42°C	−	−	−	−	−	+	−	+	+	+	+	−	−	+
50°C	−	−	−	−	−	−	−	−	+	+	−	−	−	−
Biochemical tests:	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr
Nitrate reduction	+	−	+	−	−	+	(+)	+	+	−	+	+	−	+
Degradation tests:	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr
Gelatin hydrolysis	+	+	−	nr	−	−	−	+	+	−	nd	(+)	d	+
Starch hydrolysis	+	−	+	+	−	+	+	−	+	+	−	+	+	+
Sole carbon source utilization (l, w/v):	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	
Adonitol	+	+	+	−	−	−	+	+	+	nd	nd	nd	+	nd
L(+)-Arabinose	+	+	nd	+	−	+	+	+	+	−	+	−	+	−
D(+)-Galactose	−	+	−	+	+	+	−	+	−	−	+	+	+	−
Glycerol	+	−	nd	−	−	−	−	−	+	nd	nd	+	+	nd
meso-Inositol	−	−	+	−	−	−	−	−	−	−	−	−	+	nd
D(+)-Mannitol	+	+	nd	+	−	+	−	+	+	−	+	−	−	−
L(+)-Rhamnose	−	−	+	+	−	+	−	−	−	−	+	−	+	−
D(+)-Turanose	+	+	nd	nd	nd	+	−	−	+	nd	nd	nd	+	nd

[a]Symbols: +, positive; (+), weak positive; −, negative; d, doubtful; nd, not determined.

[b]Data taken from Nonomura (1989a), Mertz and Yao (1990), Goodfellow (1992), Kudo (2001), Whitham et al. (1993), and Zhang (2002, 2005).

Streptosporangium was questioned by several workers, especially on the basis of 16S rRNA gene sequence data (Stackebrandt et al., 1994; Tamura et al., 2000; Ward-Rainey et al., 1996). These organisms were subsequently found to share genotypic and phenotypic features in common with five soil isolates that were assigned to the new genus *Sphaersporangium* as *Sphaersporangium melleum* and *Sphaersporangium rubeum* (Ara and Kudo, 2007). They also found that "*Streptosporangium cinnabarinum*" and *Streptosporangium viridialbum* could be distinguished from one another and from the other two taxa based on DNA–DNA relatedness and phenotypic data. Consequently, they transferred "*Streptosporangium cinnabarinum*" Celmer et al. 1977 and *Streptosporangium viridialbum* (Nonomura and Ohara 1960) to the genus *Sphaerosporangium* as *Sphaerosporangium cinnabarinum* sp. nov. and *Sphaerosporangium viridialbum* comb. nov., respectively. The genus name *Sphaerosporangium* (*sic*) was corrected on validation [Rule 61 of the *Bacteriological Code* (1990 Revision)] to *Sphaerisporangium* (Lists Editor IJSEM, 2007).

Several other species have been shown to be misplaced in the genus *Streptosporangium*. The type strain of *Streptosporangium claviforme* Petrolini et al. 1992 and *Herbidospora cretacea* Kudo et al. 1993 belong to the same genomic species and form a distinct 16S rRNA gene clade in the *Streptosporangiaceae* gene tree (Tamura et al., 2000), as can be seen in Figure 361 (see section on the family *Streptosporangiaceae*). *Streptosporangium corrugatum* Williams and Sharples 1976 was found to have properties consistent with its transfer to the genus *Acrocarpospora* as *Acrocarpospora corrugata* (Williams and Sharples 1976) Tamura et al. 2000. The proposal to transfer *Streptosporangium indianense* Gupta 1965 to the genus *Streptomyces* as *Streptomyces indianensis* (Gupta 1965) Kudo and Seino 1987 is underpinned by a wealth of phenotypic data, notably by the formation of spiral spore chains and the absence of spore vesicles (Whitham et al., 1993). "*Streptosporangium album* subsp. *thermophilum*" Manachini et al. 1965 is a thermophile, but has been shown to be a member of the genus *Thermoactinomyces* (Goodfellow and Cross, 1984).

The changes outlined above leave the genus *Streptosporangium* as a well-defined taxon that encompasses 13 validly published species. DNA–DNA relatedness studies support the taxonomic integrity of several *Streptosporangium* species (Zhang et al., 2002, 2005) though they do suggest that the two *Streptosporangium amethystogenes* subspecies represent distinct genomic species (Iinuma et al., 1996b), a result underpinned by phenotypic data (Table 276). Further comparative studies are also needed to establish relationships between *Streptosporangium album*, *Streptosporangium roseum*, and *Streptosporangium vulgare* as the type strains of these taxa have identical 16S rRNA gene sequences and share morphological properties. These organisms also have similar polar lipid patterns but can be separated using quantitative fatty acid and menaquinone data (Mertz and Yao, 1990; Stackebrandt et al., 1994). However, there is evidence that the genus is underspeciated (Wang et al., 1999; Whitham et al., 1993). Indeed, Whitham and his colleagues found that isolates from seven environmental samples presumptively assigned to the genus formed 23 centers of variation based on the numerical taxonomic data.

Strains described as "*Streptosporangium brasiliense*" (Coronelli and Thiemann, 1969), "*Streptosporangium koreanum*" (Celmer et al., 1977), and "*Streptosporangium rubrum*" (Potekhina, 1965), share very high 16S rRNA gene similarities with one another and with the type strains of *Streptosporangium album*, *Streptosporangium roseum*, and *Streptosporangium vulgare* (Tamura et al., 2000). Fatty acid date indicate that "*Streptosporangium brasiliense*" and "*Streptosporangium koreanum*" strains are closely related to one another and to the type strain of *Streptosporangium roseum*, whereas a strain labeled "*Streptosporangium sibiricum*" (Potekhina, 1965) formed a distinct single membered group when fatty acid data were expressed as Euclidian distances (Mertz and Yao, 1990). Further comparative studies are needed to determine the relationships between these invalidly named taxa and representatives of validly named *Streptosporangium* species.

Differentiation of species of the genus *Streptosporangium*

Streptosporangium species can be distinguished by using a combination of phenotypic features (Table 276), although a common set of tests has not been used in the description of all species. Most of the type strains can be distinguished on the basis of their 16S rRNA gene sequences, as seen in Figure 361. There is also evidence that these organisms can be differentiated on the basis of their sugar and polar lipid patterns and by qualitative and quantitative differences in fatty acid and menaquinone composition (Kroppenstedt, 1985; Stackebrandt et al., 1994; Whitham et al., 1993; Zhang et al., 2002), though results from individual studies on the same organism do vary, possibly reflecting differences in growth conditions and experimental protocols.

Improved phenotypic tests are needed for the identification of streptosporangiae to the species level. Whitham (1988) generated a probability matrix based on 26 phenotypic properties for the computer-assisted identification of streptosporangiae to established and putatively novel *Streptosporangium* species. Kim (1993), in a continuation of this work, was able to identify known and putatively novel streptosporangiae, notably ones isolated from a range of soil samples.

List of species of the genus *Streptosporangium*

1. **Streptosporangium roseum** Couch 1955a, 151[AL] ("*Angiococcus moliroseus*" Petersen 1959, 169)

ro′se.um. L. neut. adj. *roseum* rose-colored.

Aerial mycelium is pink and the substrate mycelium yellowish brown to orange on oatmeal-yeast extract agar; soluble pigment is reddish to purplish brown on this medium. Spore vesicles are usually 8–10 µm in diameter but larger ones, up to 20 µm, are found in some strains. Spores spherical, short or bent rods, 1.8–2 µm in diameter. Hydrolyzes esculin, but not urea. Nitrate is reduced.

Degrades arbutin, elastin, RNA, and L-tyrosine, but not guanine, hypoxanthine, keratin, testosterone, Tween 20, or xanthine.

Dextrin, inulin, mannose, sucrose, trehalose, and xylose are used as sole carbon sources, but dextran, fructose, lactose, melezitose, melibiose, raffinose, sorbitol, sorbose, stachyose, starch, and xylitol (all at 1%, w/v) are not. Does not use acetate, butyrate, citrate, hippurate, malonate, or pyruvate as sole carbon sources (all at 0.1%, w/v).

Grows in the presence of crystal violet (0.001%, w/v), phenol (0.01%, w/v), phenyl ethanol (0.2%, v/v), potassium tellurite (0.01%, w/v), sodium chloride (2%, w/v), and thallous acetate (0.001%, w/v), but is sensitive to crystal violet (0.01%, w/v), phenol (0.01%, w/v), phenyl ethanol (0.3%, v/v), sodium azide (0.001%, w/v), and sodium chloride (4%, w/v).

Resistant (μg/ml) to amoxycillin (500), azlocillin (500), bactracin (50), cefoxitin (250), cephaloridine (250), cephadrine (500), chloramphenol (25), clavulanic acid (50), flucloxicillin (50), fusidic acid (0.5), keflin (250), kufzol (500), lincomycin (5), mecillinam (500), methicillin (500), oleandomycin phosphate (250), penicillin G (50), pseudomonic acid (500), sulfomethoxazole (25), ticarcillin (50), and tyrothricin (5), but is sensitive to bacitracin (50), clavulanic acid (500), flucloxicillin (250), fusidic acid (0.5), lincomyciin (25), penicillin G (250), rifampin (0.5), tetracycline (5), ticarcillin (500), and vancomycin hydrochloride (5).

Additional phenotypic features are shown in Table 276.

Whole-organism hydrolysates contain galactose and madurose. The fatty profile includes $C_{14:0}$ iso (8.9%), $C_{15:0}$ (2.9%), $C_{16:0}$ iso (43.3%), $C_{16:0}$ (3.7%), $C_{16:0}$ 10-methyl (2.6%), $C_{17:0}$ anteiso (3.1%), $C_{17:0}$ (3.9%), $C_{17:0}$ 10-methyl (18.7%), and $C_{18:0}$ (3.0%). The cellular polar lipid pattern includes diphosphatidylglycerol, hydroxyphosphatidylethanolamine, phosphatidylethanolamine, phosphatidylinositol, and ninhydrin-positive and sugar-positive phospholipids.

Source: a vegetable garden soil.

DNA G+C content (mol%): 70 (HPLC).

Type strain: ATCC 12428, CBS 313.56, DSM 43021, NBRC 3776, JCM 3005, NCIB 10171, NRRL B-2505, VKM Ac-807.

Sequence accession no. (16S rRNA gene): U48996, X70425, X89947.

2. **Streptosporangium album** Nonomura and Ohara 1960, 407[AL]

al′bum. L. neut. adj. *album* white.

Aerial mycelium is white and the substrate mycelium pale yellow on oatmeal-yeast extract agar, but does not form a diffusible pigment on this medium. Melanin pigments are not produced. Spore vesicles are 6–8 μm in diameter and sporangiospores are 1.0–1.3 × 1.5–1.9 μm. Esculin is hydrolyzed, but hippurate and urea are not. Arbutin, L-tyrosine, and Tween 80 are degraded, but guanine, hypoxanthine, keratin, pectin, testosterone, and xanthine are not. Adonitol, dextrin, fructose, inulin, maltose, mannose, raffinose, stachyose, sucrose, turanose, and xylose are used as sole carbon sources, but lactose, melezitose, melibiose, sorbitol, starch, or xylitol (all at 1%, w/v) are not. Acetate and butyrate are used as sole carbon sources, but citrate, hippurate, malonate, propionate, or pyruvate (all at 0.1%, w/v) are not.

Grows in the presence of potassium tellurite (0.005%, w/v), sodium chloride (1%, w/v), and thallous acetate (0.005%, w/v), but is sensitive to crystal violet (0.0005%, w/v), phenol (0.005%, w/v), phenyl ethanol (0.1%, v/v), potassium tellurite (0.01%, w/v), sodium azide (0.005%, v/v), sodium chloride (2%, w/v), and thallous acetate (0.001%, w/v).

Resistant (μg/ml) to amoxycillin (50), cefoxitin (25), cephaloridine (5), chloramphenol (5), clavulanic acid (250), fusidic acid (5), kefzol (50), lincomycin (25), mecillinam (50), neomycin sulfate (0.5), oleandomycin phosphate (5), penicillin G (5), sulfomethoxazole (25), tetracycline (0.5), ticarcillin (50), and tyrothricin (5), but is sensitive to amoxycillin (500), azlocillin (25), bacitracin (25), cefoxitin (50), cephaloridine (25), cephradine (25), chloramphenicol (25), clavulanic acid (500), flucloxicillin (50), fusidic acid (25) gentamicin sulfate (5), keflin (25), kefzol (250), lincomycin (50), mecillinam (250), methicillin (250), neomycin sulfate (5), oleandomycin phosphate (25), penicillin G (25), pseudomonic acid (500), rifampin (0.5), streptomycin sulfate (5), tetracycline (5), and ticarcillin (250).

Additional phenotypic features are shown in Table 276.

Whole-organism hydrolysates contain galactose and a trace of madurose. The fatty profile includes $C_{12:0}$ (8.1%), $C_{14:0}$ iso (4.3%), $C_{15:0}$ (7.3%), $C_{16:0}$ iso (43.3%), $C_{16:0}$ iso (8.0%), $C_{16:1}$ (3.9%), $C_{16:0}$ (6.8%), $C_{16:0}$ 10-methyl (2.6%), $C_{17:0}$ anteiso (21.6%), $C_{17:0}$ (5.3%), $C_{17:0}$ 10-methyl (25.7%), $C_{18:1}$ *cis* (2.8%), and $C_{18:0}$ 10-methyl (3.7%). The cellular polar lipid pattern contains diphosphatidylglycerol, phosphatidylethanolamine, phosphatidylglycerol, phosphatidylinositol mannosides, phosphatidylmethylethanolamine, and several uncharacterized components. The predominant menaquinones are MK-9(H_2) (55.7%) and MK-9(H_4) (42.9%); also contains minor amounts of MK-9(H_0) (1.4%).

Source: a soil sample collected in Japan.

DNA G+C content (mol%): not determined.

Type strain: CBS 429.61, DSM 43023, NRBC 13900, JCM 3025, KCC A-0025, NRRL B-2635, VKM Ac-636.

Sequence accession no. (16S rRNA gene): D85469, X89934.

3. **Streptosporangium amethystogenes** Nonomura and Ohara 1960, 407[AL]

am.e.thys.to′ge.nes. L. adj. *amethystinus* amethyst colored; N.L. suff. *-genes* (from Gr. v. *gennaô* to produce) producing; N.L. part. adj. *amethystogenes* producing violet-colored (crystals).

Aerial mycelium is pink and the substrate mycelium pale brownish gray. Does not produce melanin pigments. Spore vesicles are 6–8 μm in diameter and sporangiospores are 1.0–1.3 × 1.5–1.9 μm.

Esculin is hydrolyzed, but hippurate is not. Degrades arbutin, DNA, elastin, RNA, and L-tyrosine, but not guanine, hypoxanthine, keratin, testosterone, or xanthine. Mannose, trehalose, and xylose are used as sole carbon sources, but dextran, lactose, melezitose, melibiose, raffinose, sorbitol, sorbose, stachyose, starch, and xylitol (all at 1%, w/v) are not. Does not use acetate, butyrate, citrate, hippurate, malonate, propionate, or pyruvate as sole carbon sources (all at 0.1%, w/v).

Grows in the presence of crystal violet (0.0005%, w/v), phenol (0.01%, w/v), phenyl ethanol (0.01%, v/v), potassium tellurite (0.001%, w/v), sodium chloride (1.0%, w/v), and thallous acetate (0.0005%, w/v), but is sensitive to crystal violet (0.01%, w/v), phenol (0.01%, w/v), potassium tellurite (0.01%, v/v), sodium azide (0. 01%, w/v), and sodium chloride (4%, w/v).

Resistant (μg/ml) to gentamicin sulfate (5), sulfomethoxazole (3), tetracycline (0.5), and vancomycin hydrochloride (0.5), but is sensitive to amoxycillin (25), azlocillin (25), bacitracin (25), cefoxitin (25), cephaloridine (50), cephradine (25), chloramphenicol (5), clavulanic acid (25), flucloxicillin (50), fusidic acid (0.5), gentamicin sulfate (25), keflin (25), kefzol (25), lincomycin (25), mecillinam (50), methicillin (250), neomycin sulfate (5), oleandomycin phosphate (25), penicillin G (25), pseudomonic acid (500), rifampin (5), streptomycin sulfate (5), tetracycline (5), ticarcillin (50), tyrothrecin (5), and vancomycin hydrochloride (5).

Additional phenotypic features are shown in Table 276.

Whole-organism hydrolysates contain madurose. The fatty acid profile includes $C_{16:0}$ iso (7.9%), $C_{16:0}$ (13.9%), $C_{17:0}$ anteiso (10.7%), $C_{17:0}$ (4.2%), $C_{18:1}$ *cis* (13.2%), $C_{18:1}$ *trans* (45.1%), and $C_{18:0}$ (3.4%). The cellular polar lipid pattern contains diphosphatidylglycerol, phosphatidylethanolamine, phosphatidylglycerol, phosphatidylinositol, and phosphatidylinositol mannosides. The predominant menaquinones are MK-9(H_2) (49.4%) and MK-9(III, VIII-H_4) (33.5%); also contains MK-9(H_0) (10.1%).

Source: a soil sample collected in Japan.

DNA G+C content (mol%): not determined.

Type strain: ATCC 33327, CBS 430.61, DSM 43179, NRBC 13986, JCM 3026, KCC A-0026, NRRL B-2639, RIA 767, VKM Ac-637.

Sequence accession no. (16S rRNA gene): X89935.

Additional comments: the type strain of *Streptosporangium amethystogenes* KCC A-0026 was incorrectly cited as KCC A-2006 (*sic*) on the Approved Lists of Bacterial Names 1980 (Hill et al., 1984). Subsequently, the species was divided into two subspecies (see below).

3a. **Streptosporangium amethystogenes subsp. amethystogenes** Nonomura and Ohara 1960, 407[AL]

This taxon was automatically created by the valid publication of *Streptosporangium amethystogenes* subspecies *fukuiense* Iinuma et al. (1996b) [Rule 40d of the *Bacteriological Code* (1990 Revision); formerly Rule 46]. The description of *Streptosporangium amethystogenes* subsp. *amethystogenes* is given above.

Source: a soil sample collected in Japan.

DNA G+C content (mol%): not determined.

Type strain: ATCC 33327, CBS 430.61, DSM 43179, NRBC 13986, JCM 3026, KCC A-0026, NRRL B-2639, RIA 767, VKM Ac-637.

Sequence accession no. (16S rRNA gene): X89935.

3b. **Streptosporangium amethystogenes subsp. fukuiense** Iinuma, Yokota and Kanamaru 1996a, 1189[VP] (Effective publication: Iinuma, Yokota and Kanamaru 1996b, 41.)

fu.ku.i.en′se. N.L. neut. adj. *fukuiense* of or belonging to Fukui Prefecture, Japan, source of the soil from which the organism was isolated.

Substrate mycelium is brown to dark brown on oatmeal agar, oatmeal-yeast extract agar, and yeast extract-malt extract agar; sparse white aerial hyphae are formed on oatmeal agar but not on the other two media. Spherical spore vesicles (6–8 μm in diameter) are formed. Sporangiospores are ellipsoidal (0.5–0.8 × 0.8–1.2 μm) and have smooth walls. Temperature growth range is 11–36°C. Iodinin crystals are produced on glycerol-asparagine agar, oatmeal agar, tyrosine agar, and yeast extract-malt extract agar.

Milk is neither coagulated or peptonized. Fructose, glucose, maltose, mannose, trehalose, starch, and xylose are used as sole carbon sources, but cellulose, lactose, raffinose, and sorbitol are not. Does not grow in the presence of lysozyme (0.005%, w/v) or sodium chloride (3%, w/v).

Resistant (μg/ml) to cefoxitin (100), but is sensitive to chloramphenicol (50), gentamicin sulfate (100), kanamycin (100), novobiocin (20), rifampin (50), streptomycin (100), and vancomycin hydrochloride (50).

Produces a hematopoietic cytokine inducer.

Additional phenotypic features are shown in Table 276.

Whole-organism hydrolysates contain arabinose, glucose, madurose, mannose, and xylose. Muramic acid moieties are *N*-acetylated. The fatty profile includes $C_{16:0}$ iso (4.3%), $C_{16:0}$ (18.8%), $C_{17:0}$ (12.3%), $C_{16:1}$ (13.9%), $C_{17:1}$ (11.3%), $C_{18:1}$ (12.9%), $C_{18:0}$ iso (4.3%), $C_{17:0}$ 10-methyl (10.5%), and $C_{18:0}$ 10-methyl (6.3%). The cellular polar lipid pattern contains phosphatidylethanolamine and unknown glucosamine-containing compounds. The predominant menaquinones are MK-9(H_2) (32.5%) and MK-9(H_4) (59.2%); also contains minor amounts of MK-9(H_0) and MK-9(H_2).

Source: a soil sample collected from Fukui Prefecture, Japan in 1990.

DNA G+C content (mol%): not determined.

Type strain: strain AL-23456, CIP 105397, NBRC 15365, DSM 44779, JCM 10083, NBRC 15365.

Sequence accession no. (16S rRNA gene): AB537172.

Additional comments: the 16S rRNA gene sequence of the type strain of *Streptosporangium amethystogenes* subsp. *fukuiense* has still to be determined. The DNA–DNA relatedness values between this strain and the type strains of *Streptosporangium amethystogenes* subsp. *amethystogenes*, *Streptosporangium roseum*, and *Streptosporangium vulgare* are 55, 12, and 23, respectively.

4. **Streptosporangium carneum** Mertz and Yao 1990, 252[VP]

car´ne.um. L. neut. adj. *carneum* of flesh, intended to mean flesh colored, referring to the color of the aerial spore mass.

Produces an orange substrate mycelium and a bright yellow pink aerial mycelium on Emerson agar, glycerol-asparagine agar, inorganic salts-starch agar, tyrosine agar, yeast-glucose agar, and yeast extract-malt extract agar. A reddish-brown diffusible pigment is formed on tyrosine agar and yeast-glucose agar, and a light brown diffusible pigment on Emerson agar. Aerial hyphae bear abundant spore vesicles on chitin agar, Czapek agar, inorganic salts-starch agar, oatmeal agar, tap water agar, and yeast extract-malt extract agar. Spore vesicles range from 3–9 µm in diameter. Sporangiospores are spherical and a mean of 1.3 µm in diameter.

Catalase, hydrogen sulfide, and phosphatase are produced, but does not hydrolyze allantoin, esculin, hippurate, or urea. Nitrate is not reduced. Milk is not peptonized.

Casein, testosterone, and L-tyrosine are degraded, but adenine, calcium malate, elastin, guanine, hypoxanthine, and xanthine are not.

Acid is produced from cellobiose, fructose, galactose, glucose, lactose, mannose, and trehalose, but not from adonitol, D-arabinose, cellulose, dextrin, dulcitol, ethanol, erythritol, glycerol, glycogen, inositol, inulin, maltose, mannitol, melezitose, melibiose, α-methyl-D-glucoside, raffinose, L-rhamnose, ribose, salicin, sorbitol, L-sorbose, sucrose, xylitol, or xylose.

Acetate and pyruvate are used as sole carbon sources, but benzoate, butyrate, citrate, formate, lactate, malate, mucate, oxalate, propionate, succinate, and tartrate are not.

Growth occurs in the presence of 2% (w/v) sodium chloride. Resistant to antibiotic discs containing bacitracin, cephalothin, gentamicin, lincomycin, penicillin, streptomycin, tetracycline, and tobramycin, but is susceptible to neomycin, oleandomycin, rifampin, vancomycin, and lysozyme.

Additional phenotypic features are shown in Table 276.

Whole-organism hydrolysates contain arabinose, madurose, and mannose. The major fatty acids are $C_{14:0}$ (6.9%), $C_{16:0}$ (25.2%), $C_{17:1}$ iso (19.4%), and $C_{18:1}$ iso (6.9%)-like components. The cellular polar lipid pattern contains diphosphatidylglycerol, phosphatidylethanolamine, phosphatidylglycerol, phosphatidylinositol, and unknown glucosamine-containing components. The predominant menaquinone is MK-9(H_4); also contains a minor amount of MK-9(H_2).

Source: soil collected next to the River Tana in Nairobi, Kenya.

DNA G+C content (mol%): not determined.

Type strain: strain A84575, DSM 44125, NBRC 15562, JCM 9926, NRRL 18437, VKM Ac-2007.

Sequence accession no. (16S rRNA gene): X89938.

5. **Streptosporangium fragile** Shearer, Colman and Nash 1983, 364[VP]

fra′gi.le. L. neut. adj. *fragile* fragile, easily broken (referring to vesicular membrane).

Aerial hyphae (0.5–1.0 µm in diameter) bear spore vesicles which are usually 6–12 µm in diameter. Substrate mycelium is light yellowish pink. Aerial mycelium is white to light pink and the substrate mycelium black on oatmeal agar. Soluble light brown pigment is produced. Spore vesicular membrane is so fragile that it is not detected by light microscopy. The temperature range of growth is 15–45°C.

Produces catalase, hydrogen sulfide, reduces nitrate, and peptonizes milk, but does not hydrolyze allantoin, esculin, hippurate, or urea. Arbutin, casein, and L-tyrosine are degraded, but adenine, guanine, hypoxanthine, keratin, pectin, testosterone, and xanthine are not.

Acid is produced from L-arabinose, cellobiose, dextrin, glucose, *meso*-erythritol, fructose, galactose, glycogen, lactose, maltose, mannitol, mannose, α-methyl-D-glucoside, rhamnose, ribose, salicin, starch, trehalose, and xylose, but not from adonitol, dulcitol, inulin, α-methyl-D-mannoside, melibiose, or raffinose. Citrate, lactate, malate, succinate, and pyruvate are utilized, but benzoate and tartrate are not.

Fructose, inulin, maltose, sorbitol, stachyose, starch, sucrose, and xylose are used as sole carbon sources, but dextrin, lactose, melezitose, melibiose, raffinose, and xylitol (all at 1%, w/v) are not. Acetate, butyrate, citrate, hippurate, malonate, and propionate are used as sole carbon sources (all at 0.1%, w/v).

Grows in the presence of crystal violet (0.0005, w/v), phenol (0.01%, w/v), potassium tellurite (0.005%, w/v), and sodium chloride (1%, w/v), but is inhibited by crystal violet (0.001%, w/v), phenol (0.1%, w/v), phenyl ethanol (0.1, v/v), potassium tellurite (0.01%, w/v), sodium azide (0.001%, w/v), sodium chloride (2%, w/v), and thallous acetate (0.0001%, w/v).

Resistant (µg/ml) to chloramphenicol (5), fusidic acid (0.5), gentamicin sulfate (25), lincomycin (5), neomycin sulfate (5), oleandomycin phosphate (5), rifampin (0.5), sulfomethoxazole (25) and tyrothrecin (5), but is sensitive to amoxycillin (25), azlocillin (25), bacitracin (25), cefoxitin (25), cephaloridine (5), cephradine (25), chloramphenicol (25), clavulanic acid (25), flucloxicillin (50), fusidic acid (5), gentamicin sulfate (50), keflin (25), kefzol (25), lincomycin (25), mecillinam (50), methicillin (250), neomycin sulfate (25), oleandomycin phosphate (25), penicillin G (5), pseudomonic acid (500), rifampin (5), streptomycin sulfate (5), ticacillin (50), and vancomycin hydrochloride (0.5).

Additional phenotypic features are shown in Table 276.

Whole-organism hydrolysates contain madurose. The fatty profile includes $C_{16:0}$ (17.3%), $C_{16:0}$ 10-methyl (4.7%), $C_{18:2}$ (26.0%), $C_{18:1}$ *cis* (35.5%), and $C_{18:0}$ (3.5%). The polar lipid pattern contains diphosphatidylglycerol, phosphatidylglycerol, phosphatidylethanolamine, phosphatidylinositol, phosphatidylinositol mannosides, and many uncharacterized components. The predominant menaquinones are MK-9(H_2) (15.0%), MK-9(III, VIII-H_4) (60.0%), and MK-9(H_6) (25.0%).

Source: soil taken from a cultivated field in the Northern Province of Sri Lanka.

DNA G+C content (mol%): not determined.

Type strain: SK&F-BC 2496, ATCC 31519, DSM 43847, NBRC 14311, JCM 6242, NRRL B-16437, VKM Ac-1296.

Sequence accession no. (16S rRNA gene): U48992, X89942.

6. **Streptosporangium longisporum** Schäfer 1969, 368[AL]

lon.gi.spo′rum. L. adj. *longus* long; Gr. n. *spora* a seed; N.L. neut. adj. *longisporum* long spored.

Aerial mycelium is sparse and the substrate mycelium is red on oatmeal agar. Pink aerial mycelium is formed on starch agar. Spherical spore vesicles are usually 3–7 μm in diameter, but may be up to 18 μm in diameter. The vesicular wall is relatively thick. Sporangiospores are rod-shaped (0.6–0.9 × 1.5–3.5 μm).

Esculin is hydrolyzed, but hippurate is not. Degrades arbutin, DNA, elastin, RNA, and L-tyrosine, but not guanine, hypoxanthine, keratin, testosterone, or xanthine. Mannose, trehalose, and xylose are used as sole carbon sources, but dextran, lactose, melezitose, melibiose, raffinose, sorbitol, sorbose, stachyose, starch, and xylitol (all at 1%, w/v) are not. Does not use acetate, butyrate, citrate, hippurate, malonate, propionate, or pyruvate as sole carbon sources (all at 0.1%, w/v).

Grows in the presence of crystal violet (0.0005%, w/v), phenol (0.01%, w/v), phenyl ethanol (0.01%, v/v), potassium tellurite (0.001%, w/v), sodium chloride (1.0%, w/v), and thallous acetate (0.0005%, w/v), but is sensitive to crystal violet (0.01%, w/v), phenol (0.01%, w/v), potassium tellurite (0.01%, w/v), sodium azide (0. 01%, w/v), and sodium chloride (4%, w/v).

Resistant (μg/ml) to gentamicin sulfate (5), sulfomethoxazole (3), tetracycline (0.5) and vancomycin hydrochloride (0.5), but is sensitive to amoxycillin (25), azlocillin (25), bacitracin (25), cefoxillin (25), cephaloridine (50), cephradine (25), chloramphenicol (5), clavulanic acid (25), flucloxicillin (50), fusidic acid (0.5), gentamicin sulfate (25), keflin (25), kefzol (25), lincomycin (25), mecillinam (50), methicillin (250), neomycin sulfate (5), oleandomycin phosphate (25), penicillin G (25), pseudomonic acid (500), rifampin (5), streptomycin sulfate (5), tetracycline (5), ticarcillin (50), tyrothrecin (5), and vancomycin hydrochloride (5).

Additional phenotypic features are shown in Table 276.

Whole-organism hydrolysate contains madurose. The fatty acid profile includes $C_{16:0}$ (11.5%), $C_{18:1}$ *cis* (16.0%), C_{110} *trans* (64.0%), and $C_{18:0}$ (6.0%). The cellular polar lipid pattern contains diphosphatidylglycerol, hydroxyphosphatidylethanolamine, phosphatidylinositol, and ninhydrin-positive and sugar positive phospholipids. The predominant menaquinones are MK-9(H_2) (35.1%) and MK-9(III, VIII-H_4) (45%); also contains MK-9(H_0) (12.7%) and MK-9(H_6) (7.2%).

Source: a soil sample collected in Turkey.

DNA G+C content (mol%): not determined.

Type strain: ATCC 25212, CBS 184.69, DSM 43180, NBRC 13141, JCM 3106, KCC A-0106, NRRL B-16783, VKM Ac-696.

Sequence accession no. (16S rRNA gene): U48993, X89944.

7. **Streptosporangium nondiastaticum** Nonomura and Ohara 1969b, 708[AL]

non.di.as.ta′ti.cum. L. pref. *non-* not; N.L. adj. *diastaticus, -a, -um* diastatic; N.L. neut. adj. *nondiastaticum* not starch digesting.

Aerial mycelium is pink and the substrate mycelium yellowish brown to orange on oatmeal-yeast extract agar; also produces a pale yellow-brown soluble pigment on this medium. Does not produce melanin pigments. Good growth at 42°C. Spore vesicles are 10–15 μm in diameter and sporangiospores 1.3 × 1.5 μm.

Esculin is hydrolyzed, but hippurate and urea are not. Arbutin, Tween 80, and L-tyrosine are degraded, but guanine, hypoxanthine, keratin, pectin, testosterone, and xanthine are not.

Adonitol, dextrin, fructose, inulin, maltose, mannose, surcrose, turanose, and xylose are used as sole carbon sources, but dextran, lactose, melezitose, melibiose, raffinose, sorbitol, stachyose, starch, and xylitol (all at 1%, w/v) are not. Acetate and pyruvate are used as sole carbon sources, but butyrate, citrate, hippurate, malonate, and propionate (all at 0.1%, w/v) are not.

Grows in the presence of crystal violet (0.0005%, w/v), phenol (0.01%, w/v), potassium tellurite (0.01%, w/v), and sodium chloride (2.0%, w/v), but is sensitive to crystal violet (0.001%, w/v), phenol (0. 1%, w/v), phenyl ethanol (0.1%, v/v), sodium azide (0.001%, w/v), sodium chloride (4%, w/v), and thallous acetate (0.0001%, w/v).

Resistant (μg/ml) to amoxycillin (50), azlocillin (50), bactracin (50), cefoxitin (50), cephaloridine (5), clavulanic acid (500), chloramphenicol (5), flucloxicillin (50), fusidic acid (0.5), gentamicin sulfate (5), ketlin (50), kefzol (250), lincomycin (50), mecillinam (500), methicillin (500), neomycin sulfate (5), oleandomycin phosphate (50), penicillin G (50), pseudomonic acid (500), sulfomethoxazole (25), tetracycline (5), ticarcillin (50), and vancomycin hydrochloride (0.5), but is sensitive to amoxycillin (250), azlocillin (250), cefoxitin (250), cephaloridine (25), cephradine (25), chloramphenicol (25), flucloxicillin (250), fusidic acid (5), gentamicin sulfate (25), kefzol (500), oleandomycin phosphate (250), rifampin (0.5), streptomycin sulfate (5), tetracycline (25), ticarcillin (250), and vancomycin hydrochloride (5).

Additional phenotypic features are shown in Table 276.

Whole-organism hydrolysates do not contain madurose. The fatty acid profile includes $C_{14:0}$ iso (2.7%), $C_{15:0}$ iso (1.9%), $C_{15:0}$ (11.2%), $C_{16:0}$ iso (13.2%), $C_{16:1}$ (2.0%), $C_{16:0}$ (5.2%), $C_{16:0}$ 10-methyl (3.6%), $C_{17:0}$ anteiso (19.8%), $C_{17:0}$ (4.4%), $C_{17:0}$ 10-methyl (22.1%), $C_{18:2}$ (5.5%), $C_{18:1}$ *trans* (1.7%), and $C_{18:0}$ 10-methyl (1.5%). The cellular polar lipid pattern contains diphosphatidylglycerol, phosphatidylethanolamine, phosphatidylglycerol, phosphatidylinositol, phosphatidylinositol mannosides, phosphatidylmethylethanolamine, and several uncharacterized components. The predominant menaquinones are MK-9(H_2) (55.0%) and MK-9(III, VIII-H_4) (40%); also contains MK-9(H_0) (2.0%) and MK-9(H_6) (3.0%).

Source: a soil sample collected in Japan.

DNA G+C content (mol%): not determined.

Type strain: ATCC 27101, CBS 800.70, DSM 43848, NBRC 13990, JCM 3114, VKM Ac-1299.

Sequence accession no. (16S rRNA gene): U48994, X70426, X89945.

8. **Streposporangium pseudovulgare** Nonomura and Ohara 1969b, 708[AL]

pseu.do.vul.ga′re. Gr. adj. *pseudês* false; L. neut. adj. *vulgare* a specific epithet; N.L. neut. adj. *pseudovulgare* similar in appearance to strain of *Streposporangium vulgare*.

Aerial mycelium is pink and the substrate mycelium yellowish brown to orange on oatmeal-yeast extract agar; a yellowish brown soluble pigment is also produced on this medium. Spore vesicles are 7–10 µm in diameter and sporangiospores 1.2 × 1.5 µm. Good growth at 42°C; also grows at 55°C.

Esculin is hydrolyzed, but hippurate and urea are not. Nitrate is reduced. Degrades arbutin, keratin, pectin, Tween 80, and L-tyrosine, but not guanine, hypoxanthine, testosterone, or xanthine.

Glucose, fructose, inulin, maltose, mannose, turanose, and xylose are used as sole carbon sources, but dextrin, lactose, melezitose, melibiose, raffinose, sorbitol, stachyose, starch, sucrose, and xylitol (all at 1%, w/v) are not. Similarly, pyruvate is metabolized, but acetate, butyrate, citrate, hippurate, malonate, and propionate (all at 0.1%, w/v) are not.

Grows in the presence of crystal violet (0.001, w/v), phenol (0.01%, w/v), phenyl ethanol (0.2%, v/v), potassium tellurite (0.01%, w/v), sodium azide (0.005%, w/v), sodium chloride (2%, w/v), and thallous acetate (0.001%, w/v), but is sensitive to crystal violet (0.01%, w/v), phenol (0.1%, w/v), phenyl ethanol (0.3, v/v), sodium azide (0.02%, w/v), and sodium chloride (4%, w/v).

Resistant (µg/ml) to amoxycillin (250), azlocillin (250), bacitracin (25), cefoxitin (250), cephaloridine (25), cephradine (25), chloramphenicol (5), clavulanic acid (250), flucloxicillin (50), fusidic acid (0.5), gentamicin sulfate (50), keflin (50), kefzol (500), lincomycin (5), mecillinam (500), methicillin (500), neomycin sulfate (5), oleandomycin phosphate (250), penicillin G (25), pseudomonic acid (500), rifampin (25), streptomycin sulfate (5), sulfomethoxazole (25), tetracycline (5), ticarcilliin (500), and vancomycin hydrochloride (0.5), but is sensitive to azlocillin (500), bacitracin (50), cephaloridine (50), cephradine (50), chloramphenicol (25), clavulanic acid (500), flucloxicillin (250), fusidic acid (5), lincomycin (25), penicillin G (50), streptomycin sulfate (25), tetracycline (25), and vancomycin hydrochloride (5).

Additional phenotypic features are shown in Table 276.

Whole-organism hydrolysates contain madurose. The fatty acid profile includes $C_{14:0}$ (1.3%), $C_{15:0}$ (3.5%), $C_{16:0}$ iso (5.6%), $C_{16:1}$ (3.9%), $C_{16:0}$ (25.2%), $C_{17:0}$ anteiso (9.1%), $C_{17:0}$ (4.9%), $C_{17:0}$ 10-methyl (3.2%), $C_{18:1}$ *cis* (11.9%), $C_{18:1}$ *trans* (23.5%), and $C_{18:0}$ (4.7%) The cellular polar lipid pattern contains diphosphatidylglycerol, phosphatidylethanolamine, phosphatidylglycerol, phosphatidylinositol, phosphatidylinositol mannosides, phosphatidylmethylethanolamine, and several uncharacterized components. The predominant menaquinones are MK-9(H_2) (60.4%) and MK-9(III, VIII-H_4) (28.0%); also contains MK-9(H_0) (11.6%).

Source: a soil sample collected in Japan.

DNA G+C content (mol%): not determined.

Type strain: ATCC 27100, CCRC 16308, CCTCC AA 97010, DSM 43181, NBRC 13991, JCM 3115.

Sequence accession no. (16S rRNA gene): U48995, X70428, X89946.

9. **Streptosporangium purpuratum** Zhang, Jiang and Chen 2005, 723[VP]

pur.pu.ra´tum. L. neut. adj. *purpuratum* clad in purple-violet, referring to the colony color.

Substrate mycelium is deep red to deep purplish-red on Bennett's agar, glycerol-asparagine agar, inorganic salts-starch agar, oatmeal agar, oatmeal-yeast extract agar, and yeast extract-malt extract agar; diffusible pigments are not formed on any of these media. Pinkish-white aerial mycelium is formed on most of these media. The exceptions are Bennett's agar, which does not support aerial hyphae, and yeast-extract-malt agar, where the aerial mycelium is sparse. Spherical spore vesicles, usually 2–5 µm in diameter, are formed singly or in clusters on the aerial mycelia on HV and oatmeal agars. Sporangiospores are spherical to oblate. The optimal temperature for growth is 28°C; also grows at 50°C. The optimal pH for growth is 7.2.

Hydrolyzes hippurate. Nitrate is reduced. Cellulose and uric acid are degraded. Cellobiose, fructose, glucose, sucrose, and xylose are used as sole carbon sources, but dextrin, erythrose, lactose, mannose, melibiose, α-methyl-D-glucoside, sorbitol, and sorbose are not.

Additional phenotypic features are shown in Table 276.

Whole-organism hydrolysates contain glucose and madurose. The major fatty acids are $C_{15:0}$ (23.7%), $C_{16:0}$ (42.0%), and $C_{17:0}$ (24.9%). The cellular polar lipid pattern contains diphosphatidylglycerol, phosphatidylethanolamine, phosphatidylinositol, and glucosamic-containing components. The predominant menaquinones are MK-9(H_0), MK-9(H_2), and MK-9(H_4).

Source: a soil sample collected from Yunnan Province, China.

DNA G+C content (mol%): 69.1 (T_m).

Type strain: strain CY-15110, CCRC 16308, CCTCC AA 97010, DSM 44688, JCM 14926.

Sequence accession no. (16S rRNA gene): AF191735.

Additional comments: Streptosporangium purpuratum CY-15110[T] is most closely related to the type strains of *Streptosporangium longisporum* (55.3%), *Streptosporangium pseudovulgare* (45.2%), *Streptosporangium nondiastaticum* (43.6%), and *Streptosporangium roseum* (43.3%) based on DNA–DNA relatedness data (Zhang et al., 2005).

10. **Streptosporangium subroseum** Zhang, Jiang and Chen 2002, 1237[VP]

sub.ro′se.um. N.L. neut. adj. *subroseum* pale rose colored.

Abundant white-pinkish aerial mycelium is formed on Bennett's agar, glycerol-asparagine agar, and oatmeal-yeast extract agar. The substrate mycelium is yellowish-brown. Soluble pigments are not produced. Spherical spore vesicles, commonly 4–10 µm in diameter, are formed on the aerial mycelium on HV agar and oatmeal agar. Sporan-

giospores are spherical or oval-shaped. The temperature range for growth is 10–42°C; the optimal temperature is 30°C; does not grow at 50°C.

Nitrate is reduced. Degrades cellulose. Cellobiose, fructose, glucose, mannose, raffinose, L-rhamnose, sucrose, and xylose are used as sole carbon sources.

Additional phenotypic features are shown in Table 276.

Whole-organism hydrolysates contain glucose, madurose, ribose, and rhamnose. The major fatty acids are $C_{16:0}$ (5.8%), $C_{17:0}$ (11.4%), $C_{18:0}$ (32.1%), and $C_{19:0}$ (18.6%). The diagnostic polar lipid is phosphatidylethanolamine. The predominant menaquinones are MK-9(H_6), MK-9(H_2), and MK-9(H_4).

Source: a soil sample collected in Yunnan Province, China.

DNA G+C content (mol%): 71.2 (T_m).

Type strain: strain CY-7113, ATCC 21807, BCRC 16302, CCTCC 97008, DSM 44662, JCM 11962, KCC A-0281.

Sequence accession no. (16S rRNA gene): AF191734.

Additional comments: the specific epithet *subroseum* is a N.L. neut. adj. not a L. neut adj. as cited in the paper by Zhang et al. (2002). In the abstract of the paper the type strain BCRC (formerly CCRC) 16302 is erroneously cited as CRC 16302.

11. **Streptosporangium violaceochromogenes** Kawamoto, Takasaka, Okachi, Kohakura, Tkahashi and Nara 1975, 358[AL]

vi.o.la.ce.o.chro.mo′ge.nes. L. adj. *violaceus* violet; Gr. n. *chroma* color; N.L. suff. *-genes* (from Gr. v. *gennaô* to produce) producing; N.L. part. adj. *violaceochromogenes* producing violet color.

Aerial mycelium is pink and the substrate mycelium pale yellow on oatmeal agar. Violet or rose colored pigments are produced on nutrient, Bennett's, and Emerson agars. Spherical spore vesicles (5–9 µm in diameter) with irregular surfaces are borne on long sporangiosphores on the aerial mycelium. Sporangiospores are oval to cylindrical (0.8–0.9 × 1.2–1.6 µm). Does not require biotin or thiamine for growth. Grows from 25–40°C, optimally between 30–37°C, and from pH 6.0–8.5, optimally at pH 7.3.

Reduces nitrate. Milk is coagulated and peptonized. L-Tyrosine is degraded, but cellulose is not.

Fructose, glucose, mannose, starch, sucrose, and xylose are used as sole carbon sources.

Additional phenotypic features are shown in Table 276.

Does not contain madurose in whole-organism hydrolysates. The menaquinones are MK-9(H_0) (19.0%), MK-9(H_2) (50.0%), and MK-9(III, VIII-H_4) (31.0%).

Source: a soil sample collected from a swamp in Yoshioka village, Kitaguninagum, Gunma, Japan.

DNA G+C content (mol%): not determined.

Type strain: ATCC 21807, DSM 43849, NBRC 15560, JCM 3281, KCC A-0281, NRRL B-16784.

Sequence accession no. (16S rRNA gene): U48997, X89951.

12. **Streptosporangium vulgare** Nonomura and Ohara 1960, 407[AL]

vul.ga′re. L. neut. adj. *vulgare* common.

Aerial mycelium is pink and the substrate mycelium yellowish-brown to orange on oatmeal-yeast extract agar; a pale yellow to yellow soluble pigment is also formed on this medium. Does not produce melanin pigments. Spore vesicles are 6–8 µm in diameter,- and sporangiospores are 1.0–1.2 × 1.5–1.9.

Hydrolyzes esculin, but not urea. Nitrate is reduced. Degrades arbutin, elastin, RNA, and L-tyrosine, but not guanine, hypoxanthine, keratin, testosterone, Tween 20, or xanthine. Dextrin, inulin, mannose, sucrose, trehalose, and xylose are used as sole carbon sources, but dextran, fructose, lactose, melezitose, melibiose, raffinose, sorbitol, sorbose, stachyose, starch, and xylitol (all at 1%, w/v) are not. Does not use acetate, butyrate, citrate, hippurate, malonate, or pyruvate as sole carbon sources (all at 0.1%, w/v).

Grows in the presence of crystal violet (0.001%, w/v), phenol (0.01%, w/v), phenyl ethanol (0.2%, v/v), potassium tellurite (0.01%, w/v), sodium chloride (2%, w/v), and thallous acetate (0.001%, w/v), but is sensitive to crystal violet (0.01%, w/v), phenol (0. 1%, w/v), phenyl ethanol (0.3% v/v), sodium azide (0.001%, w/v), and sodium chloride (4%, w/v).

Resistant (µg/ml) to amoxycillin (500), azlocillin (500), bacitracin (50), celoxitin (250), cephaloridine (250), cephadrine (500), chloramphenicol (25), clavulanic acid (50), flucloxicillin (50), fusidic acid (0.5), keflin (250), kefzol (500), lincomycin (5), mecillinam (500), methicillin (500), oleandomycin phosphate (250), penicillin G (50), pseudomonic acid (500), sulfomethoxazole (25), ticarcillin (50), and tyrothrecin (5), but is sensitive to bacitracin (50), clavulanic acid (500), flucloxicillin (250), fusidic acid (0.5), lincomycin (25), penicillin G (250), rifampin (0.5), tetracycline (5), ticarcillin (500), and vancomycin hydrochloride (5).

Additional phenotypic features are shown in Table 276.

Whole-organism hydrolysates contain madurose. The fatty acid profile includes $C_{12:0}$ (1.9%), $C_{14:0}$ (6.3%), $C_{15:0}$ (2.1%), $C_{16:0}$ iso (23.6%), $C_{16:0}$ (7.7%), $C_{16:0}$ 10-methyl (2.9%), $C_{17:0}$ (5.4%), $C_{17:0}$ 10-methyl (22.6%), $C_{18:1}$ cis (2.4%), $C_{18:0}$ (2.1%), and $C_{18:0}$ 10-methyl (5.6%). The cellular polar lipid pattern contains diphosphatidylglycerol, hydroxyphosphatidylethanolamine, phosphatidylethanolamine, phosphatidylinositol, and ninhydrin-positive and sugar-positive phospholipids. The predominant menaquinones are MK-9(H_0) (32.2%), MK-9(H_2) (56.7%), and MK-9(III, VIII-H_4) (15.1%).

Source: a soil sample collected from a paddy field in Anjo, Aichi Prefecture, Japan.

DNA G+C content (mol%): not determined.

Type strain: ATCC 33329, CBS 431.61, DSM 43802, NBRC 13985, JCM 3028, KCC A-0028, NRRL B-2633, RIA 765, VKM Ac-641.

Sequence accession no. (16S rRNA gene): U48999, X89955.

13. **Streptosporangium yunnanense** Zhang, Jiang and Chen 2005, 723[VP]

yun.nan.en′se. N.L. neut. adj. *yunnanense* of or pertaining to Yunnan, a province of south-west China.

Aerial mycelium is pale-pink to yellowish-pink and the substrate mycelium is brown to brownish-yellow on Bennett's agar, glycerol-asparagine agar, inorganic salts-starch agar, oatmeal agar, oatmeal-yeast extract agar, and yeast extract-malt extract agar; a pale brown diffusible pigment is formed on all of these media, except Bennett's agar and yeast extract-malt extract agar. Spherical spore vesicles (4–20 µm in diameter) are formed singly or in clusters on HV and oatmeal agars. Sporangiospores are spherical or oblate. The temperature range for growth is 10–42°C, does not grow at 50°C. Optimal pH is 7.2.

Hippurate is hydrolyzed and nitrate reduced. Degrades cellulose and uric acid. Cellobiose, glucose, and sucrose are used as sole carbon sources, but dextrin, erythrose, fructose, inositol, lactose, mannose, mannitol, melibiose, α- methyl-D-glucoside, raffinose, sorbitol, sorbose, and xylose are not.

Additional phenotypic features are shown in Table 276.

Whole-organism hydrolysates contain glucose, madurose, and rhamnose. The major fatty acids are $C_{16:0}$ (14.7%), $C_{17:0}$ (5.0%), $C_{18:0}$ (10.0%), and $C_{19:0}$ (29.6%). The cellular polar lipid pattern contains diphosphatidylglycerol, phosphatidylethanolamine, phosphatidylinositol, phosphatidylmethylethanolamine, and glucosamine-containing components. The predominant menaquinones are MK-9(H_0), MK-9(H_2), and MK-9(H_4).

Source: a soil sample collected from Yunnan Province, China.

DNA G+C content (mol%): 71.1 (T_m).

Type strain: strain CY-11007, CCRC 16307, CCTCC AA 97009, DSM 44663, JCM 14927.

Sequence accession no. (16S rRNA gene): AF191733.

Additional comments: Streptosporangium yunnanense CY-11007^T is most closely related to the type strains of *Streptosporangium nondiastaticum* (56.8%) and *Streptosporangium pseudovulgare* (53.1%) on the basis of DNA–DNA relatedness data (Zhang et al., 2005).

Species *incertae sedis*

1. **"Streptosporangium rubrum"** Potekhina 1965, 292.

ru'brum. L. neut. adj. *rubrum* red

A pale pink to white substrate mycelium and a white to pale pink aerial mycelium is formed on Czapek agar. Forms spherical spore vesicles (6.0–14 µm in diameter) and branched sporangiophores (0.5–1.2 × 1.6–27 µm). Spores are typically spherical (0.8 × 1.4 µm), but may be ovoid or rod-shaped.

Milk is peptonized and nitrates weakly reduced to nitrites. Cellulose and starch are degraded, but gelatin is not.

Source: a dark gray forest soil.

DNA G+C content (mol%): not determined.

Reference strain: DSM 44095.

Sequence accession no. (16S rRNA gene): X89950.

Genus II. **Acrocarpospora** Tamura, Suzuki and Hatano 2000, 1170^{VP}

Tomohiko Tamura

A.cro.car.po.spo'ra. Gr. adj. *akros* uttermost, topmost, highest, at the top, end; Gr. n. *karpos* fruit, harvest; Gr. fem. n. *spora* a seed; N.L. fem. n. *Acrocarpospora* an organism forming spores like fruits on the terminal mycelium

Gram-stain-positive bacterium that is not-acid-fast and forms **branching hyphae**.

Non-fragmentary substrate mycelia are present. Spherical and club shaped structures are borne on the tips of the **aerial mycelium**. These structures contain coiled or straight spore chains. Spores are oval or short rod-like (0.6–0.8 × 0.7–1.0 µm) with a smooth surface and nonmotile. **Strictly aerobic**. Good growth occurs at 20–30°C. Shows good growth on oatmeal agar. In general, the vegetative mycelia are pale yellow and aerial hyphae are white. Cell walls contain glutamate, alanine, and *meso*-diaminopimelate. Wall chemotype is III (Lechevalier and Lechevalier, 1970b), and the peptidoglycan type is presumed to be A1γ (Schleifer and Kandler, 1972). Madurose, glucose, and galactose are detected in whole cell sugars. Major cellular fatty acids are $C_{16:0}$ iso, $C_{17:0}$ 10-methyl, $C_{17:1}$, and $C_{17:0}$. The major menaquinones are MK-9(H_4) and MK-9(H_2), and small amounts of MK-9(H_6) are present. Phosphatidylethanolamine is present as a diagnostic phospholipid. Mycolic acids are absent. The acyl type of cell wall is acetyl. The type species is *Acrocarpospora pleiomorpha*.

DNA G+C content (mol%): 68–69.

Type species: **Acrocarpospora pleiomorpha** Tamura, Suzuki and Hatano 2000, 1170^{VP}.

Further descriptive information

The sporangium forms two types of structures, club-shaped and various sized spherical bodies. In 7- and 14-d-old cultures, the various sized spherical bodies are commonly found; the club-shaped structures are infrequent.

Although species of *Acrocarpospora* possess similar fatty acids, there are differences in the quantities of some of the most abundant ones (Table 277). For instance, *Acrocarpospora pleiomorpha* contains high levels of $C_{13:0}$, and *Acrocarpospora corrugate* contains low levels of $C_{16:0}$ iso. Similarly, while the physiological

TABLE 277. Cellular fatty acid compositions (%) of the members of the genus *Acrocarpospora*

Fatty acid (%)	*A. pleiomorpha* NBRC 16267^T	*A. corrugata* NBRC 13972^T	*A. macrocephala* NBRC 16266^T
$C_{12:0}$	1.7		
$C_{13:0}$	14.2	0.5	0.9
$C_{14:0}$ iso		4.4	9.8
$C_{14:0}$		0.5	
$C_{15:0}$ iso		3.3	
$C_{15:0}$ anteiso		4.3	
$C_{15:0}$	4.7	4.2	4.1
$C_{16:0}$ iso	43.4	9.2	39.6
$C_{16:1}$ 9*c*		1.2	
$C_{16:0}$	2.5	5.3	2.0
$C_{15:0}$ 2-OH		0.9	
$C_{16:0}$ 10-methyl	1.7	0.8	
$C_{17:0}$ iso		2.8	
$C_{17:0}$ anteiso		5.3	
$C_{17:1}$ 9*c*	4.2	10.6	9.2
$C_{16:0}$ iso 2-OH		1.8	
$C_{17:0}$	6.1	13.2	6.2
$C_{16:0}$ 2-OH		1.0	
$C_{17:0}$ 10-methyl	21.6	19.6	25.0
$C_{18:0}$ iso		0.9	
$C_{18:1}$ 9*c*		2.4	
$C_{17:0}$ iso 2-OH		0.4	
$C_{18:0}$		3.3	
$C_{17:0}$ 2-OH		1.1	
$C_{18:0}$ iso 10-methyl			1.9
TBSA $C_{18:0}$ 10-methyl		3.2	1.4

TABLE 278. Physiological characteristics of the members of the genus *Acrocarpospora*[a]

Characteristic	*A. pleiomorpha* NBRC 16267^T	*A. corrugata* NBRC 13972^T	*A. macrocephala* NBRC 16266^T
N-Acetyl-β-glucosaminidase	–	–	–
Acid phosphatase	+	+	+
Akaline phosphatase	+	+	+
Catalase activity	+	+	+
Chymotrypsin	+	–	–
Cystine aminopeptidase	+	w	+
Esculin hydrolysis	+	+	+
Esterase (C4)	+	w	w
Esterase lipase (C8)	+	+	+
α-Fucosidase	–	–	–
α-Galactosidase	–	w	–
β-Galactosidase	–	+	–
β-Glucosidase	w	w	+
Gelatin hydrolysis	+	+	+
Leucine aminopeptidase	+	+	+
Lipase (C14)	w	w	w
α-Mannosidase	–	–	–
Nitrate reduction	–	–	–
Phosphohydrolase	w	+	w
Pyrazinamidase	–	–	–
Pyrrolidonyl arylamidase	–	w	–
Trypsin	+	w	w
Urea hydrolysis	–	+	–
Valine aminopeptidase	+	w	+

[a]Symbols: +, >85% positive; –, 0–15% positive; w, weak reaction.

responses of the species of *Acrocarpospora* are generally similar, some differences are noted (Table 278).

Enrichment and isolation procedures

Acrocarpospora pleiomorpha strain R-31^T was isolated from soil in Louisiana, USA, and *Acrocarpospora macrocephala* strain R-55^T was isolated from soil in Saitama prefecture, Japan.

The type strain of *Acrocarpospora corrugata* was isolated from beach sand (pH 7.8) at Freshfield, Lancashire, United Kingdom, by using the dilution plate technique on starch-casein medium containing antifungal antibiotics (Williams and Sharples, 1976). In this isolation procedure, most of the actinomycetes appearing on the plates were micromonosporas, and *Streptosporangium corrugatum*-like colonies accounted for less than 1% of the total.

Maintenance procedures

Strains of the genus *Acrocarpospora* are maintained by freezing in water containing 10–30% glycerol at −70°C. Lyophilization of suspensions in 10% skim milk +1% monosodium glutamate and l-drying in 0.01 M potassium phosphate buffer (pH7.0) containing 3% monosodium glutamate are also recommended for long-term preservation.

Differentiation of the genus *Acrocarpospora* from other genera

The genus *Acrocarpospora* forms club-shaped or spherical sporangia at the heads of the aerial mycelia and produces spores by fragmentation of hyphae and the hyphal sheath, which either expands to form the sporangial envelope or remains around the spore chains. Although the morphological characteristics and spore development of isolates are similar in many respects to those of *Streptosporangium* species, there are differences in the irregular shape and variable size of spore vesicles. Under the light microscope, the isolates appear similar to *Nonomuraea pusilla*, which develops pseudosporangia, rather than to the majority of *Streptosporangium* species. The genus *Acrocarpospora* contains MK-9(H_4) as the major menaquinone and is differentiated from the genus *Herbidospora*, which has MK-10(H_4) as a predominant menaquinone.

Taxonomic comments

The phylogenetic tree based on 16S rRNA gene sequence analysis indicates that three species of the genus *Arcocarpospora* form a branch within the family *Streptosporangiaceae* of the order *Streptosporangiales* (Tamura et al., 2000). The genus *Herbidospora* is the most closely related genus to *Acrocarpospora*. *Acrocarpospora corrugata* was originally proposed as *Streptosporangium corrugatum* by Williams and Sharples (1976) and transferred to the genus *Acrocarpospora* by Tamura et al. (2000).

List of species of the genus *Acrocarpospora*

1. **Acrocarpospora pleiomorpha** Tamura, Suzuki and Hatano 2000, 1170[VP]

ple.i.o.mor′pha. Gr. adj. *pleios* full; Gr. n. *morphe* form; N.L. fem. adj. *pleiomorpha* pleiomorphic, in various shapes.

Gelatin liquefaction is negative. Hydrolyzes starch. Does not decompose calcium malate. Coagulation of milk is weak. Reduction of nitrate is positive. Glucose, raffinose, rhamnose, and mannitol are utilized, but xylose, arabinose, sucrose, fructose, and inositol are not. As diagnostic phospholipids, phosphatidylethanolamine, ninhydrin positive, and sugar positive phospholipids are present.

Source: soil.

DNA G+C content (mol%): 69 (HPLC).

Type strain: R-31, JCM 10983, NBRC 16267.

Sequence accession no. (16S rRNA gene): AB006174.

2. **Acrocarpospora corrugata** (Williams and Sharples 1976) Tamura, Suzuki and Hatano 2000, 1170[VP] (*Streptosporangium corrugatum* Williams and Sharples 1976, 45)

cor.ru.ga′ta. L. fem. part. adj. *corrugata* wrinkled.

Aerial hyphae bearing globose vesicles (1.0–5.0 µm diameter) containing coiled chains of about 20 spores and club-shaped structures (3.5–8.0 × 0.75–1.00 µm) containing three to eight spores. Spores from both structures are nonmotile, 0.8–1.0 × 0.6–0.7 µm, with prominent longitudinal ridges and terminal annular ridges. Spore walls become considerably thickened, reaching 150 nm. Aerial mycelium and spore-containing structures are white, the reverse side of the colony is pale buff, and no soluble pigment is produced.

The predominant menaquinones are MK-9(H_2) and MK-9(H_4), and the major cellular fatty acids are $C_{17:1}$ and $C_{17:0}$ (Kudo and Seino, 1987) along with $C_{17:0}$ 10-methyl. The positions of hydrogenation of menaquinone MK-9(H_4) are III and VIII, as in the case of the genus *Streptosporangium* (Collins et al., 1988; Stackebrandt et al., 1994). Galactose and trace amount of madulose are detected in the whole cell sugars (Stackebrandt et al., 1994). In addition to the diagnostic phospholipid, phosphatidylethanolamine, hydroxy-phosphatidylethanolamine, diphosphatidylglycerol, and phosphatidylinositol are present (Stackebrandt et al., 1994). Numerical analysis of the phenotypic characteristics were studied by Whitham et al. (1993).

Source: beach sand (pH 7.8).

DNA G+C content (mol%): not available.

Type strain: ATCC 29331, BCRC 12360, DSM 43316, JCM 3181, KCTC 9431, NBRC 13972, NCIMB 11120.

Sequence accession no. (16S rRNA gene): AB188150.

3. **Acrocarpospora macrocephala** Tamura, Suzuki and Hatano 2000, 1170[VP]

ma.cro.ce′pha.la. Gr. adj. *makrokephalos* long-headed; N.L. fem. n. *macrocephala* large head.

Gelatin liquefaction is negative. Hydrolyzes starch. Does not decompose calcium malate. Coagulates milk. Glucose, raffinose, rhamnose, mannitol, and sucrose are utilized, but xylose, arabinose, fructose, and inositol are not. As diagnostic phospholipids, phosphatidylethanolamine, and ninhydrin positive and sugar positive phospholipids are present.

Source: soil.

DNA G+C content (mol%): 68 (HPLC).

Type strain: R-55, JCM 10982, NBRC 16266.

Sequence accession no. (16S rRNA gene): AB025318.

Genus III. **Herbidospora** Kudo, Itoh, Miyadoh, Shomura and Seino 1993, 327[VP]

Takuji Kudo

Her.bi.do.spo′ra. L. adj. *herbidus* grassy; Gr. n. *spora* a seed; N.L. fem. n. *Herbidospora* organism forming spores like grass.

Aerobic, stains Gram-positive but non-acid-fast. **Non-fragmented branching vegetative hyphae** are produced, but distinct **aerial hyphae are not formed**. Straight and short chains of nonmotile, smooth-surfaced, oval spores (10–30 spores per chain) are borne on the tips of the sporophores which are derived from the vegetative mycelia in clusters. Cell walls contain *meso*-diaminopimelate and *N*-acetylated muramic acid, but lack a significant amount of glycine (cell-wall type III). Whole-cell hydrolysates contain trace amounts of madurose (whole cell sugar pattern B). Contains phosphatidylethanolamine and its derivatives and glucosamine-containing phospholipids as diagnostic phospholipids (phospholipid type PIV). Cellular fatty acid composition is characterized by $C_{17:0}$ 10-methyl, $C_{17:1}$ ω8c, $C_{16:0}$ iso, and $C_{17:0}$ (fatty acid type 3c). The predominant isoprenoid quinone is a tetrahydrogenated menaquinone with ten isoprene units, and the hydrogenation occurs at units III and IX [MK-10(III,IX-H_4)]. MK-10(H_2), MK-10(H_0), MK-10(H_6), and MK-9(III,VIII-H_4) are also present as minor components. Mycolic acids are absent.

DNA G+C content (mol%): 69–73.

Type species: **Herbidospora cretacea** Kudo, Itoh, Miyadoh, Shomura and Seino 1993, 327[VP].

Further descriptive information

The genus *Herbidospora* was first described in 1993 with a single species *Herbidospora cretacea*, and no additional species were proposed for 16 years. From 2009 onward, an additional four species, *Herbidospora osyris* (Li et al., 2009), *Herbidospora yilanensis*, and *Herbidospora daliensis* (Tseng et al., 2010) and *Herbidospora sakaeratensis* (Boondaeng et al., 2011) were described in succession. Based on phylogenetic analysis using 16S rRNA gene

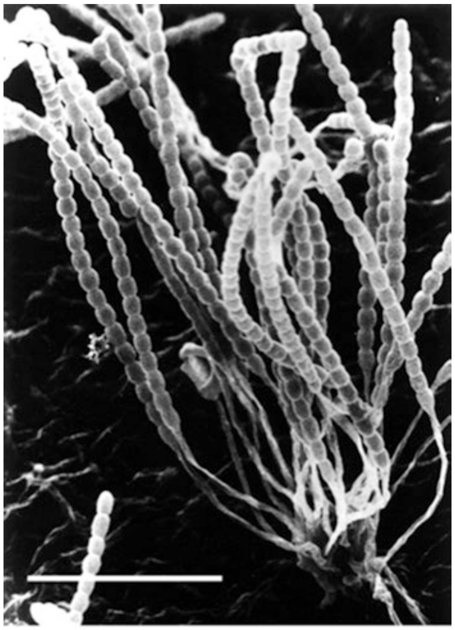

FIGURE 367. Scanning electron micrograph of *Herbidospora cretacea* JCM 8554. Bar = 10 μm.

sequences, the genus *Herbidospora* is monophyletic and within the family *Streptosporangiaceae*. In fact, the morphological and chemotaxonomic characteristics of the members are almost homogeneous and distinct from other genera belonging to the family.

Macroscopically, they produce white or brownish yellow aerial masses on some media, but microscopic observation reveals that relatively long sporophores bearing spore chains develop directly in clusters from the substrate mycelia located at the agar surface (Figure 367). This morphological characteristic is unique among other members of the family *Streptosporangiaceae*.

The chemotaxonomic characteristics of the genus, with the exception of menaquinone composition, are shared by other genera of the family *Streptosporangiaceae*, i.e. cell-wall type III, *N*-acetylated muramic acid in the cell walls, whole cell sugar pattern B, phospholipids type PIV, and cellular fatty acid type 3c. On the other hand, the members of the genus *Herbidospora* contain MK-10(H_4) as a major component, while all of the other genera in the family *Streptosporangiaceae* have menaquinones with nine isoprene units. This difference in the length of the side chain shows the apparent boundary of this genus. The tetrahydrogenation of the side chain of the *Herbidospora* menaquinone occurs at isoprene unit III and IX, while the tetrahydrogenation of those of the other genera belonging to the family *Streptosporangiaceae* occurs at units III and VIII. It is thought that these modes of tetrahydrogenation represent a common mechanism in that the third double bond from the naphthoquinone moiety and the second double bond from the ω-terminus are hydrogenated. Among actinobacteria, this hydrogenation pattern is unique to the family *Streptosporangiaceae*.

Isolation and maintenance procedures

Herbidospora strains have been isolated from soil, the sediment of a river, decayed leaves, the bark of a pine tree, the petals of flowers, and a surface-sterilized tissue of a plant. Humic acid-vitamin agar (Hayakawa and Nonomura, 1987b) was often used as an isolation medium, but there is no information on enrichment. The organisms grow relatively well on general complex agar media used for filamentous actinomycetes such as oatmeal agar (ISP 3), yeast extract-malt extract agar (ISP 2) or yeast-starch agar, but good sporulation occurs on only a few media such as ISP 3, oatmeal-nitrate agar, and ISP 2 agar. As most species of this genus require vitamin B complex, they grow poorly on defined media. They can be stored in 10% glycerol or DMSO solution at −80°C, lyophilized with 10% skim milk, or L-dried with 0.1 M phosphate buffer (pH 7.0) plus 3% monosodium glutamate and 1.5% adonitol for long-term preservation.

Differentiation of the genus *Herbidospora* from other genera

As shown in Figure 368, the genus *Herbidospora* is a member of the family *Streptosporangiaceae*, and the closest relative is the genus *Acrocarpospora*. The genus *Herbidospora* shares chemot-

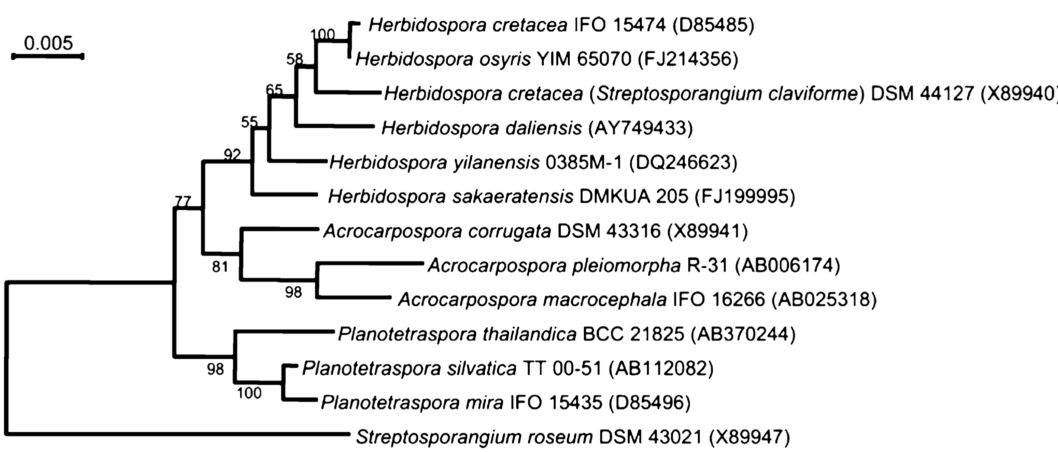

FIGURE 368. Neighbor joining tree based on 16S rRNA gene sequences showing the phylogenetic relationships among *Herbidospora* species and related taxa. Numbers represent bootstrap values for each branch (1000 replicates).

axonomic characteristics with other genera of the family *Streptosporangiaceae*, but it is readily differentiated from them by its distinctive morphology and menaquinone composition. The menaquinones of the genus *Herbidospora* are mainly composed of MK-10(H_4), but other members of the family including the closest genus, *Acrocarpospora*, contain menaquinones with nine isoprene units. Morphologically, the genus *Acrocarpospora* is characterized by formation of spherical and club-shaped sporangium-like structures, but the genus *Herbidospora* does not produce sporangium-like structures. The genus *Actinocorallia* sometimes shows similar morphology to *Herbidospora*, but this genus belongs to the family *Thermomonosporaceae* and has quite different chemotaxonomic characteristics.

Taxonomic comments

Streptosporangium claviforme was described as a sporangium-producing actinomycete (Petrolini et al., 1992), but its morphology is identical with that of the genus *Herbidospora*. In the original description, the authors thought that the chains of spores were enveloped by "sporangial" walls. However, the term "sporangium" has been defined more accurately, and even spores of streptomycetes have sheath-like structures. *Streptosporangium claviforme* also has MK-10(III, IX-H_4) as a major component. Tamura et al. (2000) indicated that the DNA–DNA relatedness between the type strains of *Streptosporangium claviforme* and *Herbidospora cretacea* was 80%, and Boondaeng et al. (2011) reclassified *Streptosporangium claviforme* as a later synonym of *Herbidospora cretacea*.

Differentiation of species of the genus *Herbidospora*

Five species are currently known, and they share morphological and chemotaxonomic characteristics. Thus, differentiation of the species depends on physiological characteristics. However, the accurate boundary of the species cannot be determined physiologically because the descriptions of the species, except for *Herbidospora cretacea*, are based on a single strain. Identification at the species level should be performed by using differential characteristics shown in Table 279 and 16S rRNA gene analysis as well as DNA–DNA hybridization.

TABLE 279. Characteristics differentiating species of the genus *Herbidospora*[a,b]

Characteristic	*H. cretacea*	*H. daliensis*	*H. osyris*	*H. sakaeratensis*	*H. yilanensis*
Decomposition of:					
Esculin	+	–	+	nd	–
Hypoxanthine	+	–	nd	nd	–
Starch	+	+	–	nd	+
Utilization as sole carbon sources:					
L-Arabinose	+	+	–	+	–
Cellobiose	+	–	+	nd	–
Glycerol	–	–	+	nd	+
myo-Inositol	–	–	+	–	+
Maltose	+	+	+	–	+
D-Mannose	+	–	+	+	+
Melezitose	–	+	nd	–	+
Melibiose	w	–	+	nd	+
Raffinose	–	+	+	–	+
L-Rhamnose	v	+	–	–	+
D-Ribose	+	–	+	–	–
Salicin	v	–	+	nd	+
Trehalose	v	–	+	nd	+
D-Xylose	+	–	+	+	–
Utilization of:					
Fumaric acid	+	–	nd	+	+
DL-Lactic acid	+	–	nd	nd	+
L-Malic acid	v	–	+	+	–
Succinic acid	+	–	nd	+	+
Requirement for:					
p-Aminobenzoic acid	–	+	nd	nd	+
myo-Inositol	–	+	nd	nd	+
Riboflavin	–	–	nd	nd	+
Thiamine	+	–	nd	nd	+
NaCl tolerance	<3%	<5%	<3%	<1.5%	<1%

[a]Symbols: +, positive; –, negative; w, weekly positive, v, variable; nd, not determined.

[b]Data from Kudo et al. (1993), Li et al. (2009), Tseng et al. (2010), and Boondaeng et al. (2011).

List of species of the genus *Herbidospora*

1. **Herbidospora cretacea** Kudo, Itoh, Miyadoh, Shomura and Seino 1993, 327[VP]

cre.ta′ce.a. L. fem. adj. *cretacea* chalk-like.

Vegetative mycelia are yellow to brown on most media. When sporulation occurs, the surface of the colony is white or brownish yellow. Good sporulation occurs on oatmeal agar (ISP 3) and oatmeal-nitrate agar. Diffusible pigment and melanoid pigments are not produced. Mesophilic (optimal growth at 28°C) and neutrophilic (optimal growth at pH 7.0–8.0). No growth occurs in the presence of 3% NaCl.

Decomposes casein, deoxyribonucleic acid, esculin, hypoxanthine, and starch, but not adenine, arbutin, guanine, keratin, tyrosine, and xanthine. Utilizes L-arabinose, cellobiose, D-fructose, D-galactose, D-glucose, maltose, D-mannitol, D-mannose, D-ribose, starch, sucrose, and D-xylose as a sole carbon source, but not adonitol, cellulose, dulcitol, *i*-erythritol, glycerol, *myo*-inositol, melezitose, and raffinose. Utilizes fumaric acid, DL-lactic acid, L-malic acid, and succinic acid, but not benzoic acid, citric acid, mucic acid, oxalic acid, propionic acid, and L-tartaric acid. Sensitive to lysozyme, gentamicin, novobiocin, rifampin, streptomycin, and vancomycin. Requires thiamine for growth.

Source: soil, decayed leaves, bark of pine tree, and petal of daisy fleabane; type strain was isolated from soil collected in Saitama, Japan.

DNA G+C content (mol%): 69–70 (HPLC).

Type strain: K-319, ATCC 51904, BCRC 16356, DSM 44071, JCM 8553, KCTC 9335, NBRC 15474, NCIMB 13372, VKM Ac-1997.

Sequence accession no. (16S rRNA gene): D85485.

2. **Herbidospora daliensis** Tseng, Yang and Yuan 2010, 1171[VP]

da.li.en′sis. N.L. fem. adj. *daliensis* of or pertaining to Dali city, Taiwan, where the type strain was isolated.

Vegetative mycelia are yellowish white to yellowish brown on most media. When sporulation occurs, the surface of the colony is white or brownish yellow. Good sporulation occurs on oatmeal agar (ISP 3). Diffusible and melanoid pigments are not produced. Neutrophilic. Growth occurs at 20–40°C. NaCl tolerance is 5%.

Decomposes starch but not adenine, hypoxanthine, tyrosine, and xanthine. L-Arabinose, fructose, galactose, glucose, maltose, mannitol, melezitose, raffinose, rhamnose, and sucrose are utilized. Adonitol, benzoic acid, cellobiose, cellulose, dulcitol, erythritol, fumaric acid, glycerol, *myo*-inositol, DL-lactic acid, L-malic acid, mannose, melibiose, mucic acid, propionic acid, D-ribose, salicin, D-sorbitol, succinic acid, L-tartaric acid, trehalose, and xylose are not utilized. Sensitive to lysozyme. Requires *p*-aminobenzoic acid and *myo*-inositol for growth. Biotin, nicotinic acid, pantothenic acid, pyridoxine, riboflavin, and thiamine are not required for growth.

Morphological and chemical characteristics are given in the description of the genus.

Source: sediment of a river in Dali city, Taiwan.

DNA G+C content (mol%): 70.7 (HPLC).

Type strain: 0385M-1, FIRDI 004, BCRC 16876, LMG 24336, NBRC 106372.

Sequence accession no. (16S rRNA gene): AY749433.

3. **Herbidospora osyris** Li, Zhao, Qin, Zhu, Xu and Li 2009, 3126[VP]

o.sy′ris. L. n. *osyris* a plant (probably the broom-like goosefoot or summer cypress), and also a botanical genus name (*Osirys*); N.L. gen. n. osyris, of *Osyris* the plant genus from which this species was isolated.

Vegetative mycelia are yellowish white to yellowish brown on most media. When sporulation occurs, the surface of the colony is white. Moderate sporulation occurs on yeast-extract-malt extract agar (ISP 2) and YIM 38 agar. A diffusible pigment is not produced. Growth occurs at 10–37°C and pH 6.0–8.0. NaCl tolerance is up to 3%.

Catalase is produced. Negative for the Voges–Proskauer and methyl red tests, for the oxidase reaction, for production of H_2S, for nitrate reduction, and for milk coagulation and peptonization. Tweens 20 and 40 and urea are hydrolyzed, but Tween 80, gelatin, starch, and cellulose are not hydrolyzed. Utilizes amygdalin, arbutin, cellobiose, esculin, D-fructose, D-galactose, glycerol, *myo*-inositol, maltose, D-mannitol, D-mannose, melibiose, raffinose, D-ribose, salicin, sodium DL-malate, D-tagatose, trehalose, turanose, and D-xylose as sole carbon sources. D-Adonitol, D-arabinose, dulcitol, erythritol, D-lactose, L-rhamnose, D-sorbitol, L-sorbose, and xylitol are not utilized. Adenine, L-alanine, L-cysteine, L-cystine, L-histidine, L-lysine, L-phenylalanine, L-proline, L-serine, L-threonine, L-tyrosine, and xanthine can be used as sole nitrogen sources, but L-arginine, L-asparagine, L-glutamic acid, glycine, hypoxanthine, DL-methionine, and L-valine cannot.

Morphological and chemical characteristics are given in the description of the genus.

Source: type strain was isolated from a surface sterilized plant sample, *Osyris wightiana* Wall. *ex* Wight, collected from Yunnan province, south-west China.

DNA G+C content (mol%): 70.4 (HPLC).

Type strain: YIM 65070, CCTCC AA 208019, DSM 45214, JCM 16900, NBRC 106571.

Sequence accession no. (16S rRNA gene): FJ214356.

4. **Herbidospora sakaeratensis** Boondaeng, Suriyachadkun, Ishida, Tamura, Tokuyama and Kitpreechavanich 2011, 779[VP]

sa.ka.e.ra.ten′sis. N.L. fem. adj. *sakaeratensis* of or pertaining to Sakaerat Biosphere Reserve, the source of soil from which the type strain was isolated.

Vegetative mycelia are yellow to yellowish brown. A melanoid pigment is not produced. Growth occurs at 20–40°C and pH 6.0–9.0. NaCl tolerance is up to 1.5%.

Utilizes L-arabinose, D-fructose, D-galactose, D-glucose, D-mannitol, D-mannose, starch, sucrose, and D-xylose as sole carbon sources, but not dulcitol, *myo*-inositol, maltose, melezitose, D-raffinose, L-rhamnose, D-ribose, and D-sorbitol. Utilizes

fumaric acid, L-malic acid, and succinic acid, but not benzoic acid and mucic acid. Cellulose degradation, gelatin liquefaction, nitrate reduction, and milk peptonization are negative.

Morphological and chemical characteristics are given in the description of the genus.

Source: a soil sample collected in Sakaerat Biosphere Reserve in Nakhonratchasima, Thailand.

DNA G+C content (mol%): 73 (HPLC).

Type strain: DMKUA 205, BCC 11662, NBRC 102641.

Sequence accession no. (16S rRNA gene): FJ199995.

5. **Herbidospora yilanensis** Tseng, Yang and Yuan 2010, 1170[VP]

yi.lan.en′sis. N.L. fem. adj. *yilanensis* of or pertaining to Yilan county, Taiwan, where the type strain was isolated.

Vegetative mycelia are yellowish white to yellow on most media. When sporulation occurs, the surface of the colony is white or brownish yellow. Moderate sporulation occurs on oatmeal agar (ISP 3), oatmeal-nitrate agar, and glycerol-asparagine agar (ISP 5). Diffusible and melanoid pigments are not produced. Neutrophilic. Growth occurs at 20–40°C. NaCl tolerance is 1%.

Decomposes starch but not adenine, esculin, hypoxanthine, tyrosine, and xanthine. Fructose, fumaric acid, galactose, glucose, glycerol, DL-lactic acid, *myo*-inositol, maltose, mannitol, mannose, melibiose, melezitose, raffinose, rhamnose, salicin, sucrose, succinic acid, and trehalose are utilized. Adonitol, L-arabinose, benzoic acid, cellobiose, cellulose, dulcitol, erythritol, L-malic acid, mucic acid, propionic acid, D-ribose, D-sorbitol, L-tartaric acid, and xylose are not utilized. Sensitive to lysozyme. *p*-Aminobenzoic acid, *myo*-inositol and thiamine are required for growth. Biotin, nicotinic acid, pantothenic acid, and pyridoxine are not required for growth.

Morphological and chemical characteristics are given in the description of the genus.

Source: sediment of a dry lake, Yilan, a county in north Taiwan.

DNA G+C content (mol%): 70.6 (HPLC).

Type strain: 0351M-12, FIRDI 003, BCRC 16875, LMG 24337, NBRC 106371.

Sequence accession no. (16S rRNA gene): DQ246623.

Genus IV. **Microbispora** Nonomura and Ohara 1957, 307[AL] emend. Zhang, Wang and Ruan 1998a, 418

Christopher M. M. Franco

Mi.cro.bi.spo′ra. Gr. adj. *mikros* small; L. adv. num. *bis* twice; Gr. n. *spora* a seed; N.L. fem. n. *Microbispora* the small two-spored (organism).

Aerobic, Gram-stain-positive, non-acid–alcohol-fast, nonmotile, mesophilic, and thermophilic actinobacteria **which form stable, highly branched substrate and aerial mycelia**. **Spore chains, typically containing two spores, are borne longitudinally on short aerial hyphae. Spores are slightly oval to cylindrical (1.0–1.4 to 1.2–1.7 µm in diameter) and have smooth surfaces**. **Mesophilic species grow from 20–37°C, with thermophilic species up to 55°C**. Most species require B vitamins, particularly thiamine, for growth. Chemoorganotrophic with an oxidative type of metabolism. **Cell walls contain *N*-acetylated muramic acid and major amounts of *meso*-diaminopimelic acid; peptidoglycan type is A1γ. Whole-cell hydrolysates contain madurose** and complex mixtures of saturated, unsaturated, iso-, anteiso-, and branched chain fatty acids. **Predominant menaquinones have nine isoprene units with hydrogenation at positions III and IV [MK-9(III, IV-H_4), MK-9(H_2), and MK-9(H_0)]. Major phospholipids are phosphatidylcholine and unknown glucosamine-containing compounds, but no phosphatidylglycerol**. Natural habitat is soil, plant litter, and as epiphytes and endophytes of plants.

DNA G+C content (mol%): 68–73.

Type species: **Microbispora rosea** Nonomura and Ohara 1957, 307[AL].

Further descriptive information

Phylogeny. The genus *Microbispora* contains 13 species including *Microbispora rosea*, *Microbispora aerata*, *Microbispora amethystogenes*, *Microbispora chromogenes*, *Microbispora corallina*, *Microbispora diastatica*, *Microbispora indica*, *Microbispora karnatakensis*, *Microbispora mesophila*, *Microbispora parva*, *Microbispora siamensis*, *Microbispora thermodiastatica*, and *Microbispora thermorosea*. The type strains of these species form a distinct phyletic line in the *Streptosporangiaceae* 16S rRNA gene tree with *Microtetraspora*, *Arcocarpospora*, *Herbidospora*, and *Nonomuraea* (Figure 369) being the most closely related genera. The 16S rRNA gene sequence similarities of the *Microbispora* type strains fall within the range 95.1–99.2% (Wang et al., 1996b). The corresponding DNA–DNA relatedness values range from 27–80% (Nakajima et al., 1999).

Cell morphology. Microbisporae form characteristic pairs of spores on the aerial hyphae or short sporophores; the latter may be short so that the spores appear sessile. Buds formed on the aerial hyphae or tips of a side branch swell and then separate by a side wall. The sporophore of *Microbispora rosea* partially encloses the basal spore giving the appearance of a ball-and-socket joint (Williams, 1970). Mature spores are easily detached from the sporophore and each other when placed in water. Spores from most species of *Microbispora* have a smooth surface and are found along the entire length of the aerial hyphae (Figures 30.11, 30.12 from *Bergey's Manual*, previous edition, Nonomura, 1989b). Single spores have been reported for *Microbispora mesophila* (Zhang et al., 1998a).

Nutrition and growth conditions. Most microbisporae require B-vitamins for growth on synthetic media (Nonomura and Ohara, 1971d) but are readily cultivated on rich media including glucose-yeast extract (Waksman, 1967), oatmeal supplemented with yeast extract (Shirling and Gottlieb, 1966), and yeast extract-malt extract (Shirling and Gottlieb, 1966) agars. Mesophilic microbisporae grow between 20 and 37°C, but the thermophilic strains grow better at 37–45°C.

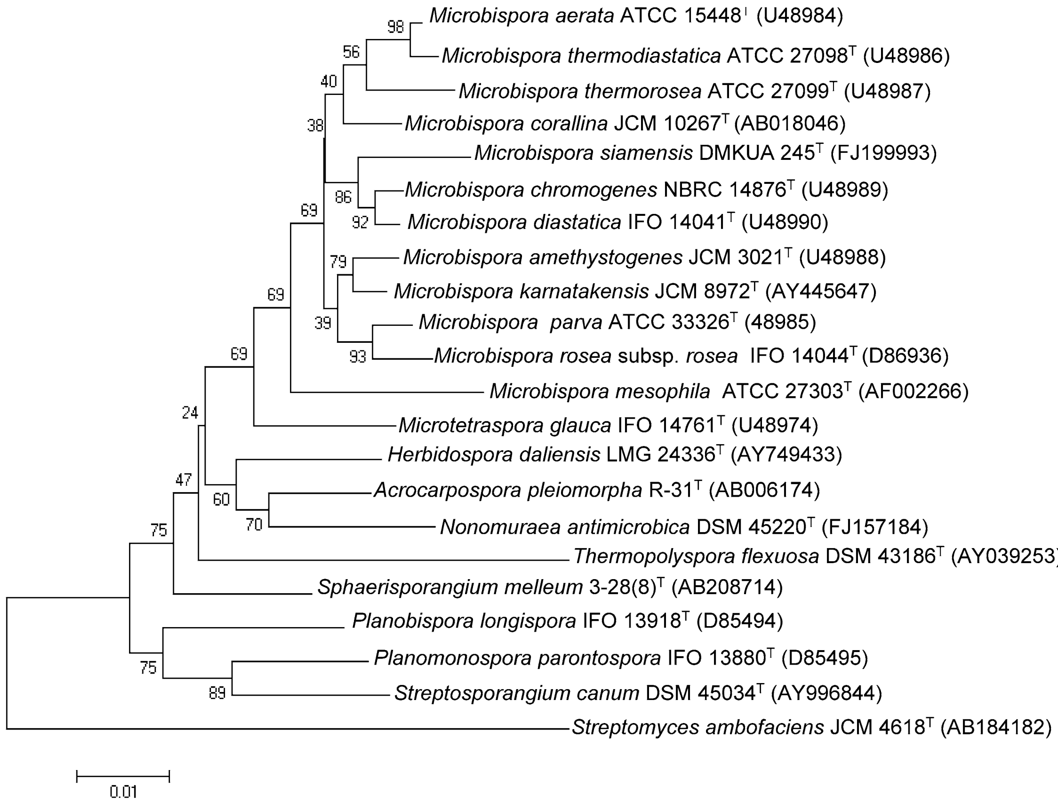

FIGURE 369. 16S rRNA gene-based neighbor-joining tree showing the phylogenetic relationships between *Microbispora* species and between selected microorganisms belonging to the family *Streptosporangiaceae*. The numbers on the branches indicate the percentage bootstrap values of 1000 replicates. *Streptomyces ambofaciens* JCM 9374^{T} was used as an outgroup.

Cell-wall composition. *Microbispora* strains contain *meso*-diaminopimelic acid as the major wall diamino acid, have a wall chemotype III, and their whole-organism hydrolysates contain madurose (Lechevalier and Lechevalier, 1970b; Nonomura and Ohara, 1971d). The wall peptidoglycan is of the type A1γ and contains *N*-acetylated muramic acid (Nakajima et al., 1999). *Microbispora* contains phosphatidylethanolamine, phosphatidylinositol, diphosphatidylglycerol, phosphatidylinositol mannosides, and ninhydrin and sugar positive phospholipids (phospholipid type IV *sensu* Lechevalier et al., 1977), major proportions of tetrahydrogenated menaquinones with nine isoprene units saturated at sites III and VIII, and substantial proportions of MK-9(H_4, H_2, and H_0) (Kroppenstedt et al., 1990; Nakajima et al., 1999). Members of the genus contain complex mixtures of fatty acids that include major proportions of hexadecanoic acid ($C_{16:0}$), heptadecanoic acid ($C_{17:0}$), 14-methylpentadecanoic ($C_{16:0}$ iso), and 10 methylheptadecanoic ($C_{17:0}$ 10-methyl) acids (Table 281; Kroppenstedt et al., 1990; Nakajima et al., 1999).

Ecology. Microbisporae are common in soils, including soils rich in leaf litter (Nonomura, 1984; Nonomura and Ohara, 1969a), and even desert soils (Takahashi et al., 1996). They have also been isolated in large numbers from surface-sterilized fallen leaves (Matsumoto et al., 1998). Okazaki et al. (1995) collected monocotyledon leaves from 170 plants to obtain 57 *Microbispora* species from the leaf surfaces. The prevalence of strains belonging to *Microbispora* from healthy plant leaves was confirmed by another study by the same group (Kizuka et al., 1998). Endophytic *Microbispora* species were isolated from surface-sterilized wheat roots (Coombs and Franco, 2003) and a range of plants (Taechowisan et al., 2003). Polyethylene-degrading thermophilic strains have been isolated in Taiwan (Hoang et al., 2007; Tseng et al., 2007). Secondary metabolites have been reported from a number of strains; these include a prolyl endopeptidase inhibitor from *Microbispora rosea* and *Microbispora indica* strains (Kimura et al., 1997), tyrosine kinase inhibitors from *Microbispora rosea* strains (Kajiura et al., 1998), antibiotics phenazins and phenoxazinones from *Microbispora aerata* (Gerber and Lechevalier, 1964), glucosylquestiomycin (Igarashi et al., 1998) and bispolides from *Microbispora* species (Okujo et al., 2007), and herbicides from a *Microbispora rosea* strain (Kizuka et al., 1998).

Enrichment and isolation procedures

Microbispora are present in low numbers in soils and constitute a minor component of the actinomycete population (Hayakawa and Nonomura, 1987b). Therefore, selective isolation media

such as AV*(Nonomura and Ohara, 1969a), Chitin-V† (Nonomura, 1984), and HV‡ agars (Hayakawa and Nonomura, 1987b) together with pretreatments of samples were developed to yield *Microbispora* species from soils. *Microbispora mesophila* was isolated as "*Thermomonospora mesophila*" using MGA-SE agar§ (Nonomura and Ohara, 1971b). In later improvements to the selective isolation procedures, soils collected from farms in Japan were air-dried, heated to 120°C for 1 h, and treated with 1.5% phenol and 0.03% chlorhexidine gluconate (Hayakawa et al., 1991b) before plating on HV containing 20 mg nalidixic acid. Matsumoto et al. (1998) used glycerol asparagine agar** or water plus 1% proline agar containing the antibiotics nystatin, benomyl, cycloserine, and nalidixic acid to obtain 34 strains of *Microbispora* from a total of 77 actinomycetes obtained from fallen leaves.

Microbispora rosea, *Microbispora amethystogenes*, *Microbispora chromogenes*, *Microbispora diastatica*, and *Microbispora parva* were isolated from soil samples collected in Japan using soil extract agar (Nonomura and Ohara, 1957, 1960). *Microbispora corallina* and *Microbispora siamensis* were isolated from forest soils in Thailand with dilution plating on HV agar (Boondaeng et al., 2009; Nakajima et al., 1999), while *Microbispora indica* and *Microbispora karnatakensis* were isolated from soil samples from India using undisclosed methods (Rao et al., 1987).

Maintenance procedures

Well-sporulated agar slant cultures can be maintained at 4°C for 3–4 months. Recommended media for mesophilic strains are oatmeal agar-YG and glycerol agar (C1 medium†† supplemented with 0.5% (w/v) glycerol) for thermophilic strains. Suspensions of spores and hyphae in glycerol (20%, v/v) can be stored in liquid nitrogen at −80°C. Lyophilization is recommended for long-term preservation by placing the spores and mycelia in skimmed milk.

Differentiation of the genus *Microbispora* from other genera

Microbispora strains can be distinguished from the genera *Microtetraspora* and *Nonomuraea* by their ability to form characteristic longitudinal pairs of spores on short aerial hyphae with smooth spore surfaces, except for *Microbispora mesophila* which is monosporic. *Microtetraspora* species have characteristic chains of four spores on distinct sporophores, whereas *Nonomuraea* species bear chains of spores.

Taxonomic comments

Eleven *Microbispora* species were included in the last edition of *Bergey's Manual of Systematic Bacteriology* (Nonomura, 1989b); these include *Microbispora aerata* Gerber and Lechevalier 1964, *Microbispora amethystogenes* Nonomura and Ohara 1960, *Microbispora bispora* Henssen 1957, *Microbispora chromogenes* Nonomura and Ohara 1960, *Microbispora diastatica* Nonomura and Ohara 1960, *Microbispora echinospora* Nonomura and Ohara 1971b, *Microbispora parva* Nonomura and Ohara 1960, *Microbispora rosea* Nonomura and Ohara 1957, *Microbispora thermodiastatica* Nonomura and Ohara 1969b, *Microbispora thermorosea* Nonomura and Ohara 1969b, and *Microbispora viridis* Miyadoh et al. 1985. Later two others were proposed as valid species, *Microbispora indica* and *Microbispora karnatakensis* (Rao et al., 1987). On the basis of differences in chemotaxonomy and DNA–DNA relatedness, "*Microbispora echinospora*" and "*Microbispora viridis*" were transferred to the genus *Actinomadura* as *Actinomadura echinospora* and *Actinomadura rugatobispora*, respectively (Kroppenstedt et al., 1990; Miyadoh et al., 1990). Miyadoh et al. (1990) further proposed the combination of the 10 extant species, other than *Microbispora bispora*, into two subspecies of *Microbispora rosea* on the basis of DNA–DNA relatedness and chemotaxonomy. "*Microbispora rosea* subsp. *rosea*" contains *Microbispora rosea*, *Microbispora amethystogenes*, *Microbispora chromogenes*, *Microbispora diastatica*, *Microbispora echinospora*, *Microbispora indica*, *Microbispora karnatakensis*, and *Microbispora parva*; whereas "*Microbispora rosea* subsp. *aerata*" contains *Microbispora aerata*, *Microbispora thermodiastatica* and *Microbispora thermorosea*. However, Wang et al. (1996b) called into question the taxonomic status of these combinations, a view supported by Ochi et al. (1991) based on electrophoretic mobility of ribosomal AT-L30 protein. The clear differences observed for *Microbispora bispora* were analyzed by Wang et al. (1996a) who found that, on the basis of 16S rRNA gene sequence dissimilarity, two strains of this species were sufficiently different from the other species of *Microbispora* as well as other members of the family *Streptosporangiaceae* to be transferred to a new genus, *Thermobispora*, as *Thermobispora bispora*. Subsequently, Zhang et al. (1986a) proposed the reclassification of the monosporic *Thermomonospora mesophila* to the genus *Microbispora*, as *Microbispora mesophila*, on the basis of 16S rRNA gene sequence similarity and reported chemotaxonomic properties. Later *Microbispora corallina* was proposed by Nakajima et al. (1999) for two new mesophilic strains isolated from deciduous dipterocarp forest soils in Thailand, and *Microbispora siamensis* was proposed for a thermotolerant strain isolated from a soil sample from the same country (Boondaeng et al., 2009).

Differentiation of species of the genus *Microbispora*

Microbispora species can be distinguished from one another by using a combination of morphological, chemotaxonomic, nutritional, and physiological characteristics (Table 280 and Table 281).

*AV agar: arginine, 0.3 g; glucose, 1 g; glycerol, 1 g; K_2HPO_4, 0.3 g; Mg $SO_4 \cdot 7H_2O$, 0.2 g; NaCl, 0.3 g; agar, 15 g; B-vitamins (0.5 mg each of *p*-aminobenzoic acid, calcium pantothenate, inositol, niacin, pyridoxine-HCl, riboflavin, thiamine HCl, and 0.25mg biotin); distilled water 1 liter; antibiotics (Actidione, 50 mg; nystatin, 50 mg; polymixin B, none or 4 mg; penicillin G, none or 0.8 mg); pH 6.4.

† Chitin-V agar: colloidal chitin, 2 g; K_2HPO_4, 0.35 g.; KH_2PO_4, 0.15 g; $MgSO_4 \cdot 7H_2O$, 0.2 g; NaCl, 0.3 g; $CaCO_3$, 0.02 g; Fe $SO_4 \cdot 7H_2O$, 0.01 g;. $ZnSO_4 \cdot 7H_2O$, 1 mg; $MnCl_2$, 1 mg;. agar, 18 g,; β-vitamins, as for AV agar; distilled water 1 liter; Actidione, 50 mg; pH 7.2.

‡ HV agar: humic acid, 1 g; $CaCO_3$, 0.02 g; Fe $SO_4 \cdot 7H_2O$, 0.01 g; KCl, 1.7g; Mg $SO_4 \cdot 7H_2O$, 0.05 g; Na_2HPO_4, 0.5 g.; agar, 15 g; β-vitamins, as for AV agar; distilled water 1 liter; Actidione, 50 mg; pH 7.2.

§ MGA-SE agar:glucose, 2 g; L-asparagine, 1 g; K_2HPO_4, 0.5g; soil extract, 200 ml; agar, 20 g; distilled water 800 ml; antibiotics (Actidione, 50 mg; nystatin, 12.5 mg; polymixin B, 4 mg; peniclllin G, 0.8 mg); pH 8.0.

**Glycerol-asparagine agar: L-asparagine, 0.1 g; glycerol, 1 g; K_2HPO_4, 1 mg; $FeSO_4 \cdot 7H_2O$, 0.5 g; $ZnSO_4 \cdot 7H_2O$, 1 mg; $MnCl_2 \cdot 4H_2O$, 1 mg;.agar, 15 g; distilled water 1 liter; antibiotics (Benlate, 20 mg; cycloserine, 50 mg; nalidixic acid, 25 mg; nystatin, 12.5 mg).

†† C1 medium (Nonomura and Ohara, 1969b): casamino acids, 2 g; K_2HPO_4, 0.3 g; $MgSO_4 \cdot 7H_2O$, 0.01 g; NaCl, 0.3 g; Fe $SO_4 \cdot 7H_2O$, 10 mg;. $ZnSO_4 \cdot 7H_2O$, 1 mg; $CuSO_4 \cdot 5H_2O$, 1 mg; B-vitamins (0.5 mg each of *p*-aminobenzoic acid, calcium pantothenate, inositol, niacin, pyridoxine-HCl, riboflavin, thiamine HCl, and 0.25 mg biotin); agar, 20 g; distilled water 1 liter, pH 7.2.

TABLE 280. Characteristics differentiating the type strains of *Microbispora* species[a,b]

Characteristic	*M. rosea*	*M. aerata*	*M. amethystogenes*	*M. chromogenes*	*M. corallina*	*M. diastatica*	*M. indica*	*M. karnatakensis*	*M. mesophila*	*M. parva*	*M. siameensis*	*M. thermodiastatica*	*M. thermorosea*
Aerial mycelium color	Pink	Off-white/ pink	Pale pink	Pink	Pink	Pink	Pinkish white	White	White	White-pale pink	Pale pink	White-pale yellow brown	Pinkish white
Substrate mycelium color	Orange	Yellow-brown	Brown	Orange	Orange-red	Yellow-brown	Violet-orange	Brown-orange	Brown	Yellow-brown	Yellow	Yellow-brown	Yellow-brown
Growth (°C)	17–35	28–55	17–35	17–35	17–35	17–35	28–50	28–50	25–40	17–35	25–50	35–55	35–55
Menaquinone MK-9(H_0) (%)	21	52/40	21	11	+	26	15	15		42	+	8/9	2/5
Menaquinone MK-9(H_2) (%)	49	36/36	54	34	+	51	43	32		45	+	44/33	14/20
Menaquinone MK-9(H_4) (%)	30	9/20	21	50	+	23	40	46		12	+	43/47	57/51
Menaquinone MK-9(H_6) (%)		0/2	2	3			1	5				3/9	22/20
Biochemical tests:													
Biotin requirement	–	+	–	+	–	–	+	+	–	–	–	+	+
Degradation of hypoxanthine	+	+	–	+	–	+	+	+	+	–	nd	+	+
Degradation of testosterone	+	+	–	+	–	+	+	+	nd	+	nd	+	+
Production of iodinin	–	+	+	–	–	–	–	–	–	+	nd	–	–
Reduction of nitrate	++	++	++	++	+	++	++	++	+	–	nd	–	–
Starch hydrolysis	–	+	–	+	+	+	+	–	+	–	nd	+	–
Thiamine requirement	+	+	+	+	+	+	+	+	nd	+	nd	+	+
Growth on sole carbon sources:													
Arabinose	+	–	+	+	nd	+	+	–	–	+	nd	+	+
Glycerol	–	+/–	–	+	++	++	–	–	–	++	–	+/–	+
Lactate	+	+/–	+	+	+	+	+	+	+	+	–	–	–
Malate	+	+	–	–	–	–	+	+	nd	+	nd	–	+
myo-Inositol	–	+/–	–	+	+	+	–	+	–	–	–	–	–
L-Rhamnose							+	+	–		+		
Sorbitol	+/–	+/–	–	+	+	+	+	+		+/–	+	+	+

[a]Symbols: + or ++, positive; –, negative; +/–, variable; nd, not determined.

[b]Data from McCarthy and Cross (1984), Rao et al. (1987), Miyadoh et al. (1990), Kroppenstedt and Goodfellow (1992), Nakajima et al. (1999), Wang et al. (1996a), and Boondaeng et al. (2009).

TABLE 281. Fatty acid profiles of the type strains of *Microbispora* species

Fatty acid	*M. rosea* JCM 3006	*M. aerata*[a] IFO 102581	*M. amethystogenes* JCM 3021	*M. chromogenes* JCM 3022	*M. corallina* JCM 10267	*M. diastatica* JCM 3023	*M. indica* ATCC 35926	*M. karnatakensis* ATCC 35927	*M. mesophila*	*M. parva* JCM 3024	*M. siamensis* BCC 14407	*M. thermodiastatica*[a] IFIO 14046	*M. thermorosea*[a] IFO 14047
Straight-chain fatty acids:													
$C_{15:0}$	2	1	4	3	10	4	3	3	nd	2		7	
$C_{16:0}$	9	7	10	6	8	7	7	8	nd	7		2	7
$C_{17:0}$	10	6	5	7	9	6	4	7	nd	2		2	1
$C_{18:0}$	3	1	3	2	1	2	2	2	nd	2		1	2
Unsaturated fatty acids:													
$C_{16:1}$	3		7	3	2	5	5	3	nd	4			
$C_{18:1}$	3		5	2	1	1	5	3	nd	5			
Branched fatty acids:													
$C_{15:0}$ iso	2		5	4	7	3	2	1	nd	4	+	1	
$C_{16:0}$ iso	43	42	18	35	19	30	32	39	nd	20	+	32	32
$C_{17:0}$ anteiso	9	8	14	6	3	10	11	9	nd	4		21	25
$C_{18:0}$ iso				1	1			4	nd			3	3
10-Methyl fatty acids:													
$C_{17:0}$ iso	2	5	7	9	4	9	8	4	nd	16		5	5
$C_{18:0}$ iso	12	16	15	14	17	17	13	10	nd	15		9	9
$C_{19:0}$ iso	1	5	6	4	2	3	3	3	nd	8		6	6

[a]Cultured at 37°C. Data from Miyadoh et al. (1990), Nakajima et al. (1999), and Boondaeng et al. (2009).

List of species of the genus *Microbispora*

1. **Microbispora rosea** Nonomura and Ohara 1957, 307[AL]

ro′se.a. L. fem. adj. *rosea* rose-colored.

Aerial hyphae white at first, becoming pale pink with the formation of spores on oatmeal yeast extract agar. Spores are oval or round (1.4–1.6 µm) (Figure 370). Good to moderate growth on Emerson's, Bennett's, C.B., nutrient, and oatmeal and soil agars. An orange substrate mycelium is formed on oatmeal agar with 0.1% peptone. B vitamins are required for growth. Grows between 17–35°C, but not at 55°C. Optimal pH range for growth is 6.0–7.0. Degrades casein, hypoxanthine, and testosterone.

Source: soil.

DNA G+C content (mol%): 69.9 (HPLC).

Type strain: ATCC 12950, DSM 43839, JCM 3006, NBRC 14044, NRRL B-2632, VKM Ac-634.

Sequence accession no. (16S rRNA gene): D86936.

2. **Microbispora aerata** (Gerber and Lechevalier 1964) Cross 1974, 859[AL] ("*Waksmania aerata*" Gerber and Lechevalier 1964, 598.)

a.e.ra′ta. L. fem. part. adj. *aerata* covered with bronze.

Poor or moderate growth on oatmeal-yeast extract agar with white to pale pink aerial mycelium, with production of iodinin. Spores are oval or round (1.3–1.5 µm). Grows between 28–55°C. Optimal pH range for growth is 6.0–7.0.

Slight production of melanoid pigments; starch hydrolyzed; nitrites produced from nitrates; slight hydrolysis of gelatin; peptonization of milk. Requires thiamine and low levels of biotin.

DNA G+C content (mol%): 72% (T_m).

Type strain: ATCC 15448, DSM 43176, JCM 3076, NBRC 12581, VKM Ac-1507.

Sequence accession no. (16S rRNA gene): U48984.

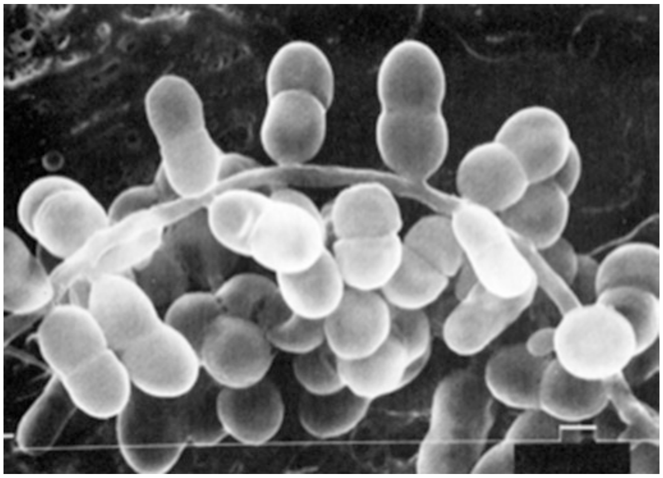

FIGURE 370. Morphology of *Microbispora rosea*: paired spores on hyphae. Scanning electron micrograph. Bar interval = 10 μm.

3. **Microbispora amethystogenes** Nonomura and Ohara 1960, 404[AL]

am.e.thys.to′gen.es. L. adj. *amethystinus* amethyst colored; N.L. suff. *-genes* (from Gr. v. *gennaô* to produce) producing; N.L. part. adj. *amethystogenes* producing violet-colored (crystals).

Aerial mycelium is pink; soluble pigment is pale yellowish brown on oatmeal-yeast extract agar. Abundant violet crystals observed after 30 d. On oatmeal-peptone agar, the substrate mycelium is light brown to brownish gray with scant aerial mycelium. Spores are oval or round (1.3–1.6 μm). Poor growth on glycerine, nutrient, and glycerol-asparagine agar-yeast extract agars with no aerial mycelium. Grows between 17–35°C, but not at 55°C. Optimal pH range for growth is 6.0–7.0.

No melanoid pigments produced; no starch hydrolysis; gelatin liquefied; slight peptonization of milk; nitrites produced from nitrates. Requires thiamine and low levels of biotin.

DNA G+C content (mol%): 70.5 (HPLC).

Type strain: DSM 43164, JCM 3021, NBRC 101907, NRRL B-2637.

Sequence accession no. (16S rRNA gene): U48988.

4. **Microbispora chromogenes** Nonomura and Ohara 1960, 404[AL]

chro.mo′ge.nes. Gr. n. *chroma* color; N.L. suff. *-genes* (from Gr. v. *gennaô* to produce) producing: N.L. part. adj. *chromogenes* producing color.

Aerial mycelium is pink; soluble pigment is dark-purple gray on oatmeal-yeast extract agar. On potato agar, the substrate mycelium is pale orange, aerial mycelium pale pink with pale yellow soluble pigment. A dark green soluble pigment is produced on glycerin agar. Spores are oval or round (1.3–1.6 μm). Good to moderate growth on glycerin, glycerol-asparagine-yeast extract, nutrient, potato, and starch-yeast extract agars. Grows between 17–35°C, but not at 55°C. No melanoid pigments produced; starch hydrolyzed; gelatin liquefied; no peptonization of milk; nitrites produced from nitrates. Requires thiamine and low levels of biotin.

Source: soil.

DNA G+C content (mol%): 70 (HPLC).

Type strain: DSM 43165, JCM 3022, NBRC 14876, NRRL B-2634.

Sequence accession no. (16S rRNA gene): U48989.

5. **Microbispora diastatica** Nonomura and Ohara 1960, 404[AL]

di.a.sta′ti.ca. N.L. fem. adj. *diastatica* starch hydrolyzing.

Aerial mycelium is pink; soluble pigment is pale yellow on oatmeal–yeast extract agar. On oatmeal–peptone agar, the substrate mycelium is pale yellowish brown covered entirely with pale pink aerial mycelium. Spores are oval or round (1.3–1.6 μm). Good growth on glycerine and nutrient agars with no aerial mycelium. Grows between 17–35°C, but not at 55°C. Optimal pH range for growth is 6.0–7.0.

No melanoid pigments produced; starch hydrolyzed; gelatin liquefied; no peptonization of milk; and nitrites not produced from nitrates. Utilizes rhamnose. Requires thiamine and low levels of biotin.

DNA G+C content (mol%): 68.9 (HPLC).

Type strain: JCM 3023, KCC A-0023, NBRC 14041, NBRC 101785, NRRL B-2630.

Sequence accession no. (16S rRNA gene): U48990.

6. **Microbispora mesophila** (Nonomura and Ohara 1971b) Zhang, Wang and Ruan 1998a, 418[VP] (*Thermomonospora mesophila* Nonomura and Ohara 1971b, 899)

me.so.phi′la. Gr. n. *mesos* middle; Gr. fem. adj. *philê* loving; N.L. fem. adj. *mesophila* middle (temperature) loving.

Colonies on agar media are small, entire, raised, with a brown reverse color and abundant white aerial mycelium, with spores either sessile or on short sporophores. Spores with smooth surfaces either oval or round (1.5–2 μm). Good to moderate growth on oatmeal, yeast extract-malt extract, nutrient, starch-yeast extract agars. Very poor growth on glycerol-asparagine agar. Optimum temperature for growth is 35–40°C. Optimum growth at pH 7.5–8.0.

No melanoid pigments produced; starch hydrolyzed; gelatin liquefied weakly; peptonization of milk; nitrites produced from nitrates. Requires thiamine and low levels of biotin.

DNA G+C content (mol%): not determined.

Type strain: ATCC 27303, CIP 105593, DSM 43048, NBRC 14179, JCM 3151, NRRL B-16986, VKM Ac-1953.

Sequence accession no. (16S rRNA gene): AB006170, AF002266.

7. **Microbispora parva** Nonomura and Ohara 1960, 403[AL]

par′va. L. fem. adj. *parva* little, small (growth).

Poor growth on oatmeal agar with a faint yellow soluble pigment; violet crystals observed after 30 d at 30°C. Aerial mycelium is pink; soluble pigment is white to pale pink on oatmeal-yeast extract agar. On oatmeal-peptone agar, the substrate mycelium is pale yellowish brown to light brown with scant aerial mycelium. Spores are oval or round (1.3–1.6 μm). Good to moderate growth on glycerine, nutrient, potato, and glycerol-asparagine agar-yeast extract agars.

Grows between 17–35°C, but not at 55°C. Optimal pH range for growth is 6.0–7.0.

No melanoid pigments produced; no starch hydrolysis; slight hydrolysis of gelatin; slight peptonization of milk. Requires thiamine and low levels of biotin.

DNA G+C content (mol%): 69.8 (HPLC).

Type strain: ATCC 33326, JCM 3024, KCC A-0024, NRRL B-2629.

Sequence accession no. (16S rRNA gene): U48985.

8. **Microbispora siamensis** Boondaeng, Ishida, Tamura, Tokuyama and Kitpreechavanich 2009, 3138[VP]

si.a.men.sis. NL. fem. adj. *siamensis* of or pertaining to Siam, the old name of Thailand, the source of the soil from which the type strain was isolated.

Substrate mycelia are colorless to yellow on most media; yellow and green soluble pigments are produced. Oval spores with smooth surfaces. Good growth on yeast-extract-malt extract agar with moderate pink spores.

Temperature growth range is 25–50°C. No growth in the presence of 3% (w/v) NaCl.

Citric acid, L-malic acid, and succinic acid are used, but benzoic acid, fumaric acid, and mucic acid are not. L-Arabinose, D-fructose, D-galactose, D-glucose, D-lactose, D-mannitol, D-mannose, and sucrose are used as sole carbon sources, but dulcitol, inositol, maltose, D-melezitose, D-raffinose, L-rhamnose, and D-sorbitol are not. Cellulose is not degraded; gelatin not liquefied; no nitrites produced from nitrates; no peptonization of milk.

Source: soil.

DNA G+C content (mol%): 68 (HPLC).

Type strain: DMKUA 245, BCC 14407, NBRC 104113.

Sequence accession no. (16S rRNA gene): FJ199993.

9. **Microbispora thermodiastatica** Nonomura and Ohara 1969b, 706[AL]

ther.mo.di.a.sta′ti.ca. Gr. adj. *thermos* hot; N.L. fem. adj. *diastatica* starch-hydrolyzing; N.L. fem. adj. *thermodiastatica* heat-loving, starch-hydrolyzing.

Poor or moderate growth on oatmeal-yeast extract agar with white to pale yellow aerial mycelium at 40°C. Good growth on yeast-starch agar with white to pale yellow brown aerial mycelium. On glucose agar, the aerial mycelium is pale pink with no soluble pigment. Spores are oval or round (1.3–1.5 µm). Grows between 35–55°C. Optimal pH range for growth is 6.0–7.0.

No melanoid pigments produced; starch hydrolyzed; no nitrites produced from nitrates; slight hydrolysis of gelatin; peptonization of milk. Requires thiamine and low levels of biotin.

DNA G+C content (mol%): 70.3 (HPLC).

Type strain: ATCC 27098, JCM 3110.

Sequence accession no. (16S rRNA gene): U48986.

10. **Microbispora thermorosea** Nonomura and Ohara 1969b, 707[AL]

ther.mo.ro′se.a. Gr. adj. *thermos* hot; L. fem. adj. *rosea* rose-colored; N.L. fem. adj. *thermorosea* heat-loving, rose-colored.

Poor or moderate growth on oatmeal agar. Good growth on yeast-starch agar; aerial mycelium is white to pale yellowish brown. Spores are oval or round (1.3–1.6 µm). Grows between 35–55°C. Optimal pH range for growth is 6.0–7.0.

No melanoid pigments produced; no starch hydrolysis; no nitrites produced from nitrates; slight hydrolysis of gelatin; slight peptonization of milk. Requires thiamine and low levels of biotin.

DNA G+C content (mol%): 70.1 (HPLC).

Type strain: ATCC 27099, JCM 3111.

Sequence accession no. (16S rRNA gene): U48987.

Species *incertae sedis*

1. **Microbispora corallina** Nakajima, Kitpreechavanich, Suzuki and Kudo 1999, 1766[VP]

co.ral′li.na. L. fem. adj. *corallina* coral colored.

Substrate mycelia pinkish to brownish red on most media and yellowish soluble pigments produced. Oval spores (0.8~1.2 µm). Good growth on glucose-asparagine agar +1% yeast extract; poor aerial mycelium pink, substrate mycelium russet, yellow diffusible pigment. Good growth on inorganic salts-starch agar +1% yeast extract with cherry pink aerial mycelium, coral substrate mycelium, no diffusible pigment. Good growth on oatmeal agar with scant pink aerial mycelium, yellow diffusible pigment. Good growth on yeast extract-starch agar with poor pink aerial mycelium, dark red substrate mycelium, yellow diffusible pigment. Good growth on yeast-extract-malt extract agar with moderate pink aerial mycelium and deep reddish brown substrate mycelium; no diffusible pigment. Temperature growth range: 17–25°C. No growth in the presence of NaCl 3% (w/v).

Casein and esculin are degraded, but adenine, elastin, guanine, hypoxanthine, keratin, testosterone, tyrosine, xanthine, xylan, and DNA are not. Amylase is produced. Amygdalin, L-arabinose, D-cellobiose, D-fructose, D-galactose, D-glucose, glycerol, *meso*-inositol, D-lactose, maltose, D-mannitol, D-mannose, D-melezitose, D-melibiose, D-ribose, salicin, D-sorbitol, sucrose, D-trehalose, and D- xylose are used as sole carbon sources, but adonitol, D-arabitol, dulcitol, iso-erythritol, inulin, D-raffinose, L-rhamnose, and xylitol are not.

DNA G+C content (mol%): 71.2–71.5 (HPLC).

Type strain: DF-32, = ATCC BAA-20, DSM 44682, NBRC 16416, JCM 10267.

Sequence accession no. (16S rRNA gene): AB018046.

2. **Microbispora indica** Rao, Prabhu, Sridhar, Venkateswarlu and Actor 1987, 184[VP]

in.di.ca. L. fem. adj. *indica* pertaining to India, the source of the soil from which the type strain was isolated.

Substrate mycelia yellow to yellowish brown on most media and orange yellowish soluble pigments produced on oatmeal agar. Oval spores (1.2~1.8 µm). Poor growth on glycerol-asparagine agar with no aerial mycelium, colorless substrate mycelium, no diffusible pigment. Good growth on glycerol-asparagine agar +1% yeast extract with light gray aerial mycelium, yellow brown substrate mycelium, no diffusible pigment. Good growth on oatmeal agar with pink aerial mycelium, violet-orange substrate mycelium, orange-yellow diffusible pigment. Good growth on yeast-extract-malt extract agar with light gray aerial mycelium, yellow brown substrate mycelium, no diffusible pigment. Temperature growth range: 28–50°C. No growth in the presence of NaCl 3% (w/v).

Casein is degraded, but amylase is not produced. L-Arabinose, D-glucose, and L-rhamnose are used as sole carbon sources, but glycerol and *meso*-inositol are not.

DNA G+C content (mol%): not reported

Type strain: SKF-I-101055, ATCC 35926, NBRC 14879, JCM 8971.

Sequence accession no. (16S rRNA gene): not available.

3. **Microbispora karnatakensis** Rao, Prabhu, Sridhar, Venkateswarlu and Actor 1987, 184[VP]

kar.na.ta.ken′sis. N.L. fem. adj. *karnatakensis* of or pertaining to the State of Karnataka, India, the source of the soil from which the type strain was isolated.

Substrate mycelia were orange to yellowish brown on most media, and yellowish orange soluble pigments were produced on most media. Oval spores (1.2–1.8 µm). Good growth on glycerol-asparagine agar, but no aerial mycelium, yellowish brown substrate mycelium, no diffusible pigment. Moderate growth on glycerol-asparagine agar +1% yeast extract with orange substrate mycelium and bright yellow diffusible pigment. Good growth on oatmeal agar with white aerial mycelium, brownish-orange substrate mycelium, deep orange-yellow diffusible pigment. Good growth on yeast-extract-malt extract agar with pale pink aerial mycelium, deep orange substrate mycelium, no diffusible pigment. Temperature growth range is 28–50°C.

Casein is degraded and amylase is produced. D-Glucose, L-rhamnose, and *meso*-inositol are used as sole carbon sources, but L-arabinose and glycerol are not.

DNA G+C content (mol%): not available.

Type strain: SKF-I-58261, ATCC 35927, IMSNU 22065, JCM 8972.

Sequence accession no. (16S rRNA gene): AY445647.

Genus V. **Microtetraspora** Thiemann, Pagani and Beretta 1968b, 296[AL] emend. Zhang, Wang and Ruan 1998a, 420[VP]

Martha E. Trujillo and Michael Goodfellow

Mi.cro.te.tra.spo′ra. Gr. adj. *mikros* small; Gr. adj. *tetra* four; Gr. n. *spora* a seed; N.L. fem. n. *Microtetraspora* the small four-spored (organism).

Aerobic, Gram-stain-positive, non-acid–alcohol-fast, nonmotile, mesophilic actinomycetes **which form stable, highly branched substrate and aerial mycelia**. **Spore chains, typically contain four spores which are borne on short aerial hyphae. Spores are spherical (1.2–1.5 µm in diameter) or slightly oval to cylindrical (1.0–1.4 to 1.2–1.7 µm in diameter) and have smooth surfaces**. **Grows from 20–37°C, but not at 40°C**. Some species require B vitamins for growth. Chemoorganotrophic with an oxidative type of metabolism. **Cell walls contain *N*-acetylated muramic acid and major amounts of *meso*-diaminopimelic acid; has a type A1γ peptidoglycan**. Contains complex mixtures of saturated, unsaturated, iso-, anteiso-, and branched chain fatty acids. **Predominant menaquinones have nine isoprene units with hydrogenation at positions III and IV [MK-9(III, IV-H_4)]. Major phospholipids are diphosphatidylglycerol, phosphatidylethanolamine, hydroxyl-phosphatidylethanolamine, and ninhydrin-positive glycophospholipids**. Common in soils.

DNA G+C content (mol%): 69–71.

Type species: **Microtetraspora glauca** Thiemann, Pagani and Beretta 1968b, 296[AL].

Further descriptive information

Phylogeny. The genus *Microtetraspora* contains four species including *Microtetraspora glauca*, *Microtetraspora fusca*, *Microtetraspora malaysiensis*, and *Microtetraspora niveoalba* (Table 282). The type strains of these species form a distinct phyletic line in the *Streptosporangiaceae* 16S rRNA gene tree that is most closely related to the genera *Microbispora* and *Nonomuraea* (Figure 371). The four *Microtetraspora* type strains share 16S rRNA gene similarities within the range 97.9–99.1%. The corresponding DNA–DNA relatedness values range from 21–52% (Nakajima et al., 2003).

Cell morphology. Microtetrasporae form short, sparsely branched aerial hyphae which typically carry chains of four spores (Figure 372, Figure 373, Figure 374, and Figure 375), though chains of two or three spores, and more rarely of five spores, have been reported (Nakajima et al., 2003; Zhang et al., 1998a). Peculiar side branches have been observed on the spore chains of *Microtetraspora niveoalba* (Figure 375). The spores of *Microtetraspora fusca* tend to fuse into a spore mass as the culture ages.

Nutrition and growth conditions. Microtetrasporae are readily cultivated on rich media including modified Bennett's (Jones, 1949), glucose-yeast extract (Gordon and Mihm, 1962), oatmeal (Shirling and Gottlieb, 1966), and yeast extract-malt extract (Shirling and Gottlieb, 1966) agars. *Microtetraspora fusca* and *Microtetraspora glauca* grow well and sporulate on Hickey–Tresner (Hickey and Tresner, 1952) agar. *Microtetraspora malaysiensis* grows well on yeast-malt extract agar (Nakajima et al., 2003). *Microtetraspora niveoalba* requires B vitamins for growth on synthetic media (Nonomura and Ohara, 1971d). Microtetrasporae grow between 20 and 37°C, but not at 40°C.

Cell-wall composition. *Microtetraspora* strains contain *meso*-diaminopimelic acid as the major wall diamino acid (wall

TABLE 282. Characteristics that differentiate the type strains of *Microtetraspora* species[a,b]

Characteristic	*M. glauca*	*M. fusca*	*M. malaysiensis*	*M. niveoalba*
Branched spore chains	−	−	−	+
Aerial spore mass color	Blue-gray/gray	Gray	White-cream	White
Substrate mycelium color	Green-blue	Purplish	Cream-yellow	Pale yellow
Biochemical tests:				
Reduction of nitrate	+	−	nd	+
Urea hydrolysis	−	+	−	+
Degradation of:				
Elastin	−	nd	−	+
Hypoxanthine	+	−	−	+
Starch	+	−	nd	+
Testosterone	+	−	+	+
Tyrosine	−	nd	+	nd
Xanthine	+	−	−	+
Xylan	+	−	−	−
Growth on sole carbon sources:				
L-Arabinose	+	+	−	+
Arbutin	+	+	−	+
D-Fructose	+	−	+	+
D-Galactose	+	−	−	−
Glycerol	+	−	−	+
Inositol	+	−	−	+
D-Mannitol	+	−	+	+
D-Mannose	+	+	−	+
L-Rhamnose	+	−	+	+
Sodium citrate	+	+	+	−
Sodium fumarate	+	−	−	+
Sodium malate	+	−	−	+
Trehalose	+	+	−	+
Xylitol	−	−	−	+
Biotin requirement	+	−	−	−

[a]Symbols:+, positive; −, negative; nd, not determined.

[b]Data from Kroppenstedt et al. (1990a), Najakima et al. (2003), and Nonomura, (1989b).

chemotype III *sensu* Lechevalier and Lechevalier, 1970b). The sugar pattern is variable and may be either B (presence of madurose) or C (no characteristic sugar). The wall peptidoglycan contains *N*-acetylated muramic acid (Kawamoto et al., 1981; Nakajima et al., 2003). Microtetrasporae contain major amounts of diphosphatidylglycerol, phosphatidylethanolamine, hydroxylphosphatidylethanolamine, and ninhydrin and sugar positive phospholipids (phospholipid type IV *sensu* Lechevalier et al., 1977), major proportions of tetrahydrogenated menaquinones with nine isoprene units saturated at sites III and VIII, and substantial proportions of MK-9(H_0) and MK-9(H_2) (Kroppenstedt et al., 1990a; Nakajima et al., 2003). Members of the genus contain complex mixtures of fatty acids that include major proportions of 14-methylpentadecanoic ($C_{16:0}$ iso) and 10-methylheptadecanoic ($C_{17:0}$ 10-methyl) acids (Table 283; Miyadoh et al., 1989; Kroppenstedt et al., 1990a; Nakajima et al., 2003).

Ecology. Microtetrasporae are common in soils including forest soils (Hayakawa et al., 1996, 1988; Lazzarini et al., 2000; Nakajima et al., 2003; Nonomura and Ohara, 1971d; Thiemann et al., 1968b). Using humic acid agar, Nonomura and Hayakawa (1988) recorded mean counts of 3.6×10^4 colony-forming units (c.f.u.) per gram dry weight of soil for soil samples collected from forests in Japan. *Microtetraspora niveoalba* strains were particularly widely distributed, albeit with counts $<10^3$ c.f.u./g dry weight soil (Nonomura and Ohara, 1971d). *Microtetraspora malaysiensis* strains have been isolated from two locations on the Malaysian Peninsula, namely from soil taken from a primary lowland dipterocarp forest at Pasok, Negeri Sembilan and from a dipterocarp forest at the Virgin Jungle Reserve, Gombak, Selangor. *Microtetraspora fusca* and *Microtetraspora glauca* have been isolated from soil samples collected in Brazil, Italy, and Thailand.

Enrichment and isolation procedures

Little is known about the activities of microtetrasporae in natural habitats, though members of the *Microtetraspora glauca* group degrade grass lignocellulose and xylan, but not cellulose (Hayakawa et al., 1996). These workers also showed that some members of the taxon show antimicrobial activity. *Microtetraspora fusca*, *Microtetraspora glauca*, and *Microtetraspora malaysiensis* were isolated from soil samples using undisclosed methods (Nakajima et al., 2003; Thiemann et al., 1968b). However, a pretreatment of dry heating air-dried soil samples, developed for the isolation of *Microbispora* and *Streptosporangium* (Nonomura and Ohara, 1969a), has proven useful for isolating most *Microtetraspora* species. Spores of microtetrasporae appear to be especially resistant to the use of dry heat at 100–120°C, a practice which significantly reduces associated actinomycetes and bacteria present in soil and allows the slow-growing *Microtetraspora* strains to develop into recognizable colonies on isolation plates. Microtetrasporae have also been isolated by plating out

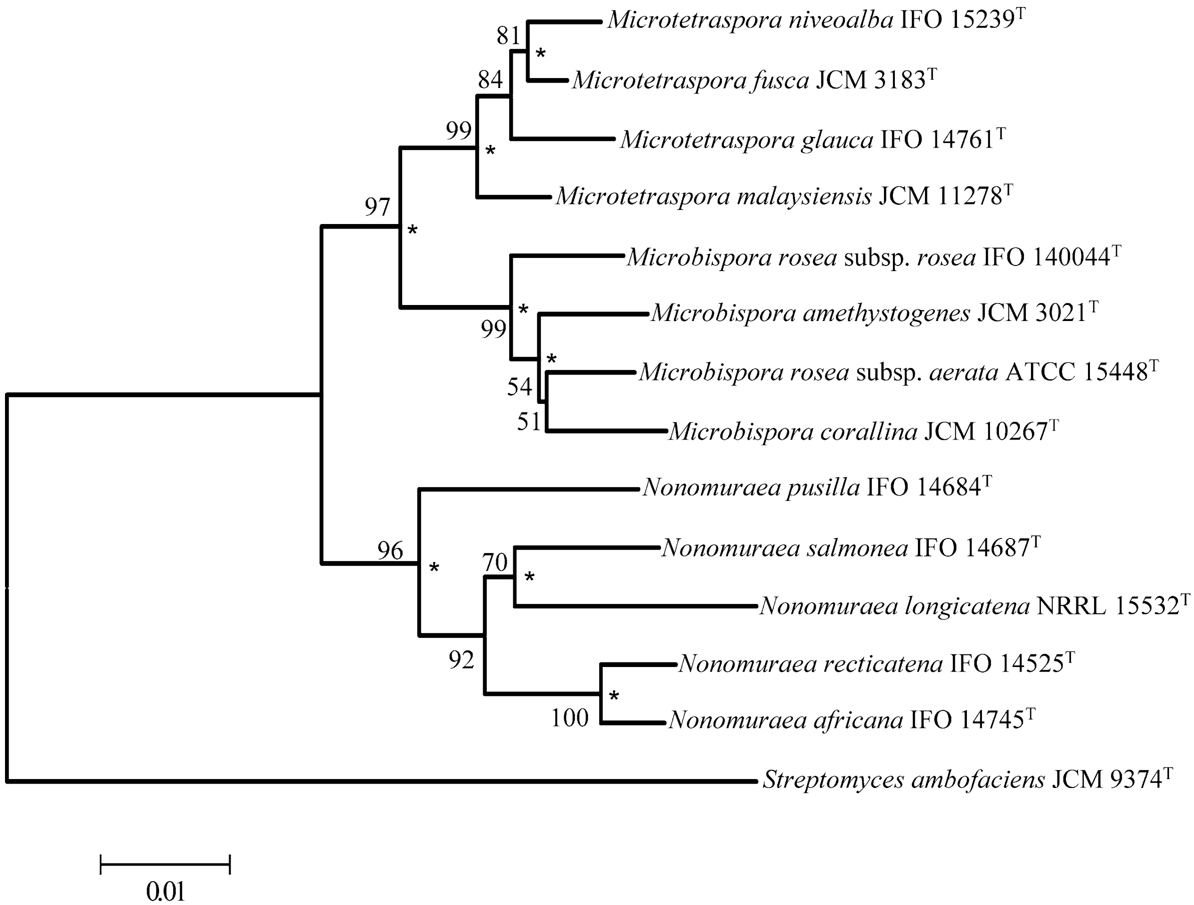

FIGURE 371. Neighbor-joining tree based on 16S rRNA gene sequences showing the phylogenetic relationships between *Microtetraspora* species and selected members of the family *Streptosporangiaceae*. Bootstrap values are shown at branching points. Asterisks indicate that the corresponding nodes were also recovered in maximum parsimony and maximum likelihood trees. *Streptomyces ambofaciens* JCM 9374^T was used as an outgroup. Bar = 1 substitution per 100 nucleotides.

FIGURE 372. Morphology of *Microtetraspora glauca* on oatmeal agar. Scanning electron micrograph. Bar = 10 µm.

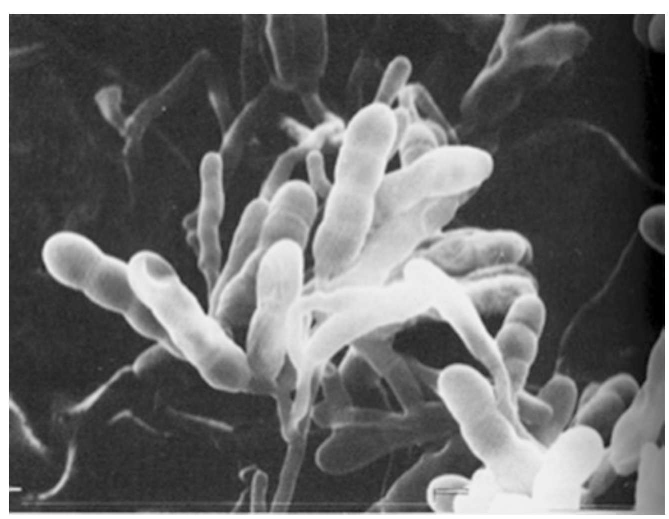

FIGURE 373. Scanning electron micrograph of *Microtetraspora glauca* grown ATCC 27645 on oatmeal agar. Bar = 10 µm.

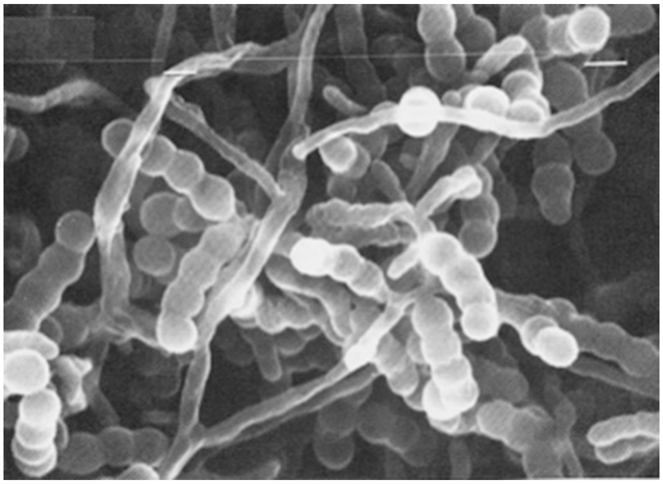

FIGURE 374. Morphology of *Microtetraspora niveoalba* ATCC 27301 on inorganic salts-starch agar. Bar = 10 μm.

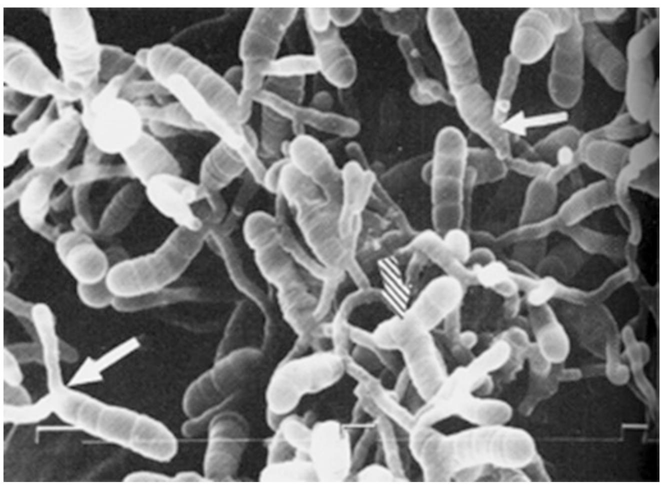

FIGURE 375. Morphology of *Microtetraspora niveoalba*. Striped arrow, branched spore chains; white arrows, branches at broad base of spore chains. Bar = 10 μm.

air-dried and heated soil onto isolation media supplemented with antibiotics such as streptomycin and rifamycin (Li, 1989). Li et al. (2002) selectively isolated actinomycetes, including microtetrasporae, from soil using high frequency radiation.

Microtetraspora niveoalba was isolated from soil which had been dry heated at 120°C for 1 h. Particles of soil were sprinkled sparingly on MGA-SE* agar plates (Nonomura, 1989b; Nonomura and Ohara, 1971d) which were incubated at 38–39°C for 1 month. To obtain pure cultures, fragments of visible colonies that appeared on the primary isolation plates were streaked onto MGA-SE agar. *Microtetraspora glauca* strains have been isolated infrequently on MGA-SE agar plates at 30°C, but *Microtetraspora fusca* was not detected, possibly because of the high pH (8.0) of the medium.

Hayakawa et al. (1996) developed a plate culture procedure for the isolation of members of the *Microtetraspora glauca* group (*Microtetraspora glauca*, *Microtetraspora fusca*, and *Microtetraspora niveoalba*) from soils and sediments. Suspensions of soil in water were treated with benzethonium chloride, plated onto LSV-SE agar† supplemented with kanamycin, norfloxacin, and naladixic acid, and incubated at 32°C for 4 weeks. Members of the *Microtetraspora glauca* group formed large, flat colonies covered with white or whitish gray aerial hyphae which differentiated into linear chains of four spores. Members of the *Microtetraspora glauca* group were isolated from 18 out of 26 environmental samples (16 from field and forest soils, 2 from freshwater sediments) and accounted for 2–27% of the total populations recovered on isolation plates.

Maintenance procedures

Sporulated slant cultures can be maintained at 4°C for 3–4 months; suitable media include glycerol-asparagine, Hickey–Tresner, and oatmeal agars. Suspensions of spores and hyphae in glycerol (20%, v/v) can be stored in liquid nitrogen at −80°C. Lyophilization is recommended for long-term preservation; this involves suspending spores and mycelia in a suitable fluid such as serum plus 7.5% (w/v) glucose or skimmed milk plus 7.5% glucose.

Differentiation of the genus *Microtetraspora* from other genera

Microtetraspora strains can be distinguished from the genera *Actinomadura*, *Microbispora*, and *Nonomuraea* by their ability to form characteristic chains of four spores on distinct sporophores.

Taxonomic comments

Four *Microtetraspora* species were included in the last edition of *Bergey's Manual of Systematic Bacteriology* (Nonomura, 1989b), namely *Microtetraspora glauca* Thiemann et al. 1968b, *Microtetraspora fusca* Thiemann et al. 1968b, *Microtetraspora niveoalba* Nonomura and Ohara 1971d, and "*Microtetraspora viridis*" Nonomura and Ohara 1971d. An additional species, "*Microtetraspora caesia*" Tomita et al. 1980 was cited as a *species incertae sedis*. Miyadoh et al. (1989) transferred "*Microtetraspora viridis*" to the genus *Actinomadura* as *Actinomadura viridis*. The genus *Microtetraspora* provided a temporary home for the *Actinomadura pusilla* group (Fischer et al., 1983; Goodfellow et al., 1988; Kroppenstedt et al., 1990a; Poschner et al., 1985) until it became clear that members of this taxon and those belonging to the *Microtetraspora glauca* group can be separated using DNA–DNA relatedness (Miyadoh et al., 1989), fatty acid (Kroppenstedt et al., 1990a; Miyadoh et al., 1989), numerical taxonomic (Athalye et al., 1985; Goodfellow and Pirouz, 1982), electrophoretic

*MGA-SE agar (Nonomura and Ohara, 1971d): asparagine, 1 g; glucose, 2 g; K_2HPO_4, 0.5 g; soil extract, 200 ml; agar, 20 g; cycloheximide, 50 mg; nystatin, 50 mg; benzyl penicillin, 0.8 mg; polymixin B, 4.0 mg: distilled water, 800 ml; pH 8.0. The soil extract was prepared by autoclaving 1000 g of soil in 1000 ml water for 30 min before decanting and filtering.

†LSV-SE agar: commercial lignin, 1 g, soybean flour, 0.2 g., soil extract, 100 ml, $CaC0_3$, 0.02 g., $FeSO_4 \cdot 7H_2O$, 0.01 g;. KCl, 1.7 g, $MgSO_4 \cdot 7H_2O$, 0.05 g; $Na_2 HPO_4$, 0.5 g., B-vitamins (0.5 mg each of *p*-aminobenzoic acid, calcium pantothenate, inositol, niacin, pyridoxine-HCl, riboflavin, thiamine HCl, and 0.25 mg biotin), cycloheximide, 50 mg, kanamycin, 20 mg, naladixic acid, 10 mg, norfloxacin, 20 mg, nystatin, 50 mg., distilled water 1 liter.

TABLE 283. Fatty acid profiles (1% of total fatty acids) of the type strains of *Microtetraspora* species[a]

Fatty acid	*M. glauca* JCM 3300	*M. fusca* JCM 3183	*M. malaysiensis* JCM 11278	*M. niveoalba* JCM 3149
Saturated fatty acids:				
$C_{13:0}$	0.2	–	0.1	–
$C_{14:0}$	0.7	0.1	0.5	0.3
$C_{15:0}$	3.8	0.9	2.9	0.5
$C_{15:0}$ 2-OH	0.5	0.3	0.4	–
$C_{16:0}$	5.5	0.3	2.3	1.9
$C_{18:0}$ 2-OH	0.5	0.1	0.2	–
$C_{17:0}$	14.9	0.3	2.8	0.5
$C_{17:0}$ 2-OH	0.3	0.3	–	–
$C_{18:0}$	4.2	–	–	–
$C_{19:0}$	0.2	–	–	–
Unsaturated fatty acids:				
$C_{15:1}$ (ω6*c*)	0.1	–	0.3	–
$C_{16:1}$ (ω9*c*)	0.3	–	0.5	–
$C_{16:1}$ 2-OH	–	0.2	–	0.2
$C_{17:1}$ ω6*c*	3.6	12.4	10.2	9.2
$C_{17:1}$ ω8*c*	3.9	0.8	1.7	0.3
$C_{17:1}$ ω9*c*	1.0	–	1.4	–
$C_{16:1}$ ω7*c*	0.4	–	0.3	–
$C_{18:1}$ ω9*c*	1.2	0.1	0.5	–
Branched fatty acids:				
$C_{14:0}$ iso	1.0	0.6	2.9	1.0
$C_{15:0}$ iso	3.3	3.0	4.1	2.7
$C_{15:0}$ anteiso	0.5	0.1	0.7	0.1
$C_{16:1}$ iso 2-OH	0.2	1.0	3.8	0.5
$C_{16:0}$ iso	28.1	56.4	44.0	65.6
$C_{17:0}$ iso	1.8	0.5	0.6	0.3
$C_{17:0}$ anteiso	1.7	0.4	0.9	0.9
$C_{18:1}$ iso 2-OH	0.1	0.3	0.3	–
$C_{18:0}$ iso	4.3	3.6	0.8	3.4
10-Methyl fatty acids:				
$C_{16:0}$ iso	1.5	1.9	1.7	3.3
$C_{17:0}$ iso	13.0	14.6	14.5	6.6
$C_{18:0}$ iso	2.2	1.5	0.8	2.8
$C_{19:0}$ iso	0.1	–	–	–

[a]Data from Kroppenstedt et al. (1990a), Miyadoh et al. (1989), and Nakijama et al. (2003). Quantitative variation between some of the values may be due to differences in cultivation conditions.

mobility of ribosomal AT-L30 protein (Ochi et al., 1991), and 16S rRNA gene sequence data (Wang et al., 1996b). The *Actinomadura pusilla* group was subsequently classified in a new taxon, the genus *Nonomuraea* Zhang et al. 1998a.

"*Microtetraspora tyrrhenii*" was proposed by Tomita et al. (1991) for an organism which formed hooked or spiral spore chains and had other properties consistent with its classification in the *Actinomadura pusilla* group. This organism probably belongs to the genus *Nonomuraea*, but this proposition cannot be tested as the type strain is no longer extant. *Microtetraspora malaysiensis* was proposed for strains isolated from dipterocarp forest soils (Nakajima et al., 2003).

Differentiation of species of the genus *Microtetraspora*

Microtetraspora species can be distinguished from one another by using a combination of morphological, nutritional and physiological characteristics (Table 282).

List of species of the genus *Microtetraspora*

1. **Microtetraspora glauca** Thiemann, Pagani and Beretta 1968b, 296[AL]

glau′ca. L. fem. adj. *glauca* grayish.

Short aerial hyphae typically contain chains of four spores; occasionally chains with two or three spores are formed and very rarely ones with five spores. Spores are smooth and spherical (1.5 μm) to slightly oval (1.4–1.7 μm) (Figure 372). Good growth on modified Bennett's, Hickey–Tresner, oatmeal, and soil agars. A blue-green to yellowish-green substrate mycelium and a blue-gray aerial spore mass are formed on Hickey–Tresner agar. B vitamins are required for growth. Grows between 20 and 37°C, but not at 40°C. Optimal pH growth range 6.0–7.0.

Produces phosphatases. Hydrogen sulfide is produced. Degrades arbutin, casein, chitin, DNA, esculin, gelatin, and Tweens 20 and 40, and xylan, but not adenine, cellulose, guanine, Tweens 60 or 80, or xanthine. Amygdalin, D-arabinose, cellobiose, glucose, glycogen, maltose, α-methyl-D-

glucoside, ribose, salicin, starch, xylose, sodium acetate, and sodium butyrate are used as sole carbon sources, but adonitol, dulcitol, *meso*-erythritol, ethanol, inulin, lactose, melezitose, raffinose, sorbitol, and sodium propionate are not.

Tolerant (%, w/v) to brilliant green (0.001), crystal violet (0.0001), sodium chloride (3%), and pyronin (0.1), but sensitive to brilliant green (0.01), crystal violet (0.001), lysozyme (0.005), and sodium chloride (5%). Resistant to (μg/ml) demethylchlortetracycline hydrochloride (500), gentamicin sulfate (50), lincomycin hydrochloride (100), novobiocin (50), rifampin (50), streptomycin sulfate (50), and vancomycin hydrochloride (50), but sensitive to cephaloridine hydrochloride (100), kanamycin sulfate (100), neomycin sulfate (50), tobramycin sulfate (50), and penicillin (10 IU) using freeze-dried filter paper discs soaked in antibiotics at appropriate concentrations.

Additional phenotypic properties are shown in Table 282.

Source: soil.

DNA G+C content (mol%): 69 (HPLC).

Type strain: ATCC 23057, DSM 43311, NBRC 14761, JCM 3300, NRRL B-3735, VKM Ac-663.

Sequence accession no. (16S rRNA gene): D85490, U48974, X97891.

2. **Microtetraspora fusca** Thiemann, Pagani and Beretta 1968b, 296[AL]

fus′ca. L. fem. adj. *fusca* dark, tawny.

Aerial spore mass is gray. Substrate mycelium brown-violet on Hickey–Tresner agar. Good sporulation on glucose-asparagine, Hickey–Tresner, glycerol-asparagine, and soil agars, but does not sporulate on oatmeal agar. Grows between 30–37°C, but not at 40°C. Optimal pH for growth range 6.0–7.0.

Arbutin is used as a sole carbon source.

Additional phenotypic properties are shown in Table 282.

Source: soil.

DNA G+C content (mol%): 70 (HPLC).

Type strain: ATCC 23058, DSM 43841, NBRC 13915, JCM 3183, NRRL B-3628, VKM Ac-662.

Sequence accession no. (16S rRNA gene): U48973.

3. **Microtetraspora malaysiensis** Nakajima, Ho and Kudo 2004, 1[VP] (Effective publication Nakajima, Ho and Kudo 2003, 188.)

mal. ay.si. en′sis. N.L. fem. adj. *malaysiensis* of or pertaining to Malaysia, the source of the soil from which the type strain was isolated.

Straight chains of four spores are formed on sporophores branching from aerial hyphae; occasionally chains with two or three spores are formed and rarely ones with five spores. Spores are oval to cylindrical and have smooth surfaces.

Hickey–Tresner agar: moderate growth; aerial mycelium pearl, substrate mycelium old gold, no diffusible pigment. Inorganic salts-starch agar: poor growth; no aerial mycelium, substrate mycelium yellow, no diffusible pigment. Oatmeal agar: moderate growth; aerial mycelium white, substrate mycelium cream, no diffusible pigment. Oatmeal-nitrate agar: moderate growth; aerial mycelium white, substrate mycelium white, no diffusible pigment. Yeast extract-malt extract agar: good growth; aerial mycelium white, substrate mycelium honey gold, no diffusible pigment. Yeast-extract-starch agar: moderate growth; aerial mycelium oyster white, substrate mycelium cream, no diffusible pigment.

Optimum temperature for growth is 25–30°C; does not grow at 40°C.

Degrades casein, DNA, esculin and testosterone, but not adenine, elastin, guanine, keratin, or xylan. Cellobiose, glucose, lactose, maltose, ribose, salicin, starch, and xylose are used as sole carbon sources, but adonitol, arabitol, arbutin, dulcitol, *meso*-erythritol, *meso*-inositol, inulin, melezitose, melibiose, α-methyl-D-glucoside, raffinose, sorbitol, sorbose, and sucrose are not. Sodium citrate is also used as a sole carbon source, but not sodium benzoate, sodium fumarate, sodium lactate, sodium malate, sodium mucate, sodium oxalate, sodium succinate, or sodium tartrate.

Does not grow in the presence of 3% (w/v) NaCl. Thiamine is needed for growth, but requirement for biotin varies. Whole-organism hydrolysates contain glucose, madurose, mannose, and ribose.

Additional phenotypic properties are shown in Table 282.

Source: dipterocarp forest soils on the Malaysian Peninsula.

DNA G+C content (mol%): 69–70 (HPLC).

Type strain: H47–7, DSM 44579, JCM 11278, NBRC 100735.

Sequence accession no. (16S rRNA gene): AB062383.

4. **Microtetraspora niveoalba** Nonomura and Ohara 1971d, 872[AL]

ni.ve.o.al′ba. L. adj. *niveus* snowy; L. adj. *albus* white; N.L. fem. adj. *niveoalba* snow white.

Mesophilic actinomycete that forms a stable, highly branched substrate mycelium. Substrate mycelium pale yellowish brown on Hickey–Tresner agar. The aerial spore mass color is white. Soluble pigments are not produced.

Good sporulation on oatmeal agar, yeast-starch agar, and on glycerol-asparagine agar supplemented with B vitamins. Branched spore chains are present. Growth occurs between 35–40°C. Optimal pH growth range 7.0–8.0. Whole-organism hydrolysates contain madurose.

Additional phenotypic properties are shown in Table 282.

DNA G+C content (mol%): 71 (HPLC).

Type strain: ATCC 27301, DSM 43174, NBRC 15239, JCM 3149.

Sequence accession no. (16S rRNA gene): U48976.

Species *incertae sedis*

1. **Microtetraspora tyrrhenii** Tomita, Oda, Hoshino, Ohkusa and Chikazawa 1992, 191[AL] (Effective publication: Tomita, Oda, Hoshino, Ohkusa and Chikazawa 1991, 945.)

tyr.re′ni-i. L. gen. n. *tyrrenii* (*sic*), of the Tyrrhenian Sea (part of the Mediterranean Sea) which faces the location from which the soil sample was taken and from which the strain was isolated.

Hooked or spiral spore chains (5–15 spores) formed monopodially on the tips of aerial hyphae. The spores (1.2 × 1.6 ~ 2.5 μm) have vertical folds on the surface.

Colonies on yeast extract-malt extract are convex and crateriform.

Glycerol-asparagine agar: moderate growth; aerial mycelium absent, substrate mycelium dark yellow, no diffusible pigment. Inorganic salts-starch agar: poor growth; scant white aerial mycelium, substrate mycelium light olive brown, no diffusible pigment. Oatmeal agar: moderate growth; no aerial mycelium, substrate mycelium light yellow, no diffusible pigment. Peptone-yeast extract-iron agar: moderate growth; no aerial mycelium, substrate mycelium deep yellowish brown, no diffusible pigment. Tyrosine agar: moderate growth; no aerial mycelium, substrate mycelium dark yellow, no diffusible pigment. Yeast extract-malt extract agar: good growth; aerial mycelium white, substrate mycelium deep yellowish brown, no diffusible pigment.

Temperature growth range: 17–40°C. Grows in the presence of NaCl 3% (w/v).

Adenine, casein, esculin, gelatin, hippuric acid, hypoxanthine, and tyrosine are degraded, but xanthine is not. Amylase is produced. Adonitol, D- and L-arabinose, cellobiose, fructose, galactose, glucose, glycerol, *meso*-inositol, lactose, mannitol, mannose, melibiose, raffinose, rhamnose, ribose, salicin, sucrose, trehalose, and xylose are used as sole carbon sources, but dulcitol, *meso*-erythritol, melezitose, sorbitol, and D-sorbose are not.

DNA G+C content (mol%): not determined.

Type species: Q464-31, ATCC 53931.

2. **"Microtetraspora caesia"** Tomita, Hoshino, Sasahira, Hasegawa and Akiyama 1980

Forms single, pairs or chains of three to eight spores on the aerial mycelium.

Aerial mass color is grayish blue-green. The cell wall contains *meso*-diaminopimelic acid and galactose.

Type strain: E864-61.

Genus VI. **Nonomuraea** corrig. Zhang, Wang and Ruan 1998b, 419[VP]

Peter Kämpfer

No.no.mu.ra′e.a. N.L. fem. n. *Nonomurea* named after Hideo Nonomura, a Japanese taxonomist who has made many contributions to the biology of actinomycetes.

Aerobic, Gram-stain-positive, non-acid-fast bacteria that form **extensively branched substrate and aerial mycelia.** Aerial mycelia bear chains of spores which are hooked, spiral, or straight. The spore surface can be folded, irregular, smooth, or warty. Growth temperature ranges from 20–45°C, in some cases up to 55°C. **Cell walls contain *meso*-diaminopimelic and whole-cell hydrolysates contain madurose as the diagnostic sugar. Contains diphosphatidylglycerol, phosphatidylethanolamine, hydroxylated phosphatidylethanolamine, and ninhydrin and sugar positive phospholipids as predominant phospholipids.. Major menaquinones are MK-9(H_4), MK-9(H_2), and MK-9(H_0).** Major types of fatty acids are C_{17} 10-methyl and C_{16} iso-branched fatty acids.

DNA G+C content (mol%): 64–73.

Type species: **Nonomuraea pusilla** corrig. (Nonomura and Ohara 1971a) Zhang, Wang and Ruan 1998b, 419 (*Actinomadura pusilla* Nonomura and Ohara 1971a, 909).

Further descriptive information

The genus *Nonomuria* (*sic*) was proposed by Zhang et al. (1998b) for species which had been classified in the genus *Actinomadura* (Athalye et al., 1985; Fischer et al., 1983; Poschner et al., 1985) prior to their reclassification to the genus *Microtetraspora* (Kroppenstedt et al., 1990a). Chiba et al. (1999), who pointed out that the name *Nonomuria* (*sic*) was a misnomer according to the *Bacteriological Code* (1990 Revision) (Rule 57a, Appendix 9), introduced the name *Nonomuraea* as the correct spelling of the genus. On the basis of 16S rRNA gene sequence data, this group belongs to the family *Streptosporangiaceae* (Goodfellow et al., 1990b; Goodfellow and Quintana, 2005; Stackebrandt et al., 1997; Zhang et al., 1998b). Interestingly, Goodfellow et al. (1988) were the first to propose the genus *Nonomuria* to accommodate species of the *Actinomadura pusilla* group, but this proposition was not formally published. The taxon encompasses 24 species and 2 subspecies with validly published names. Representatives of each of these validly named species form a distinct line in the 16S rRNA *Streptosporangiaceae* gene tree (Goodfellow and Quintana, 2005; Figure 376). The majority of the species previously assigned to the *Actinomadura pusilla* group, and now part of the genus *Nonomuraea*, originated from soil (Galatenko et al., 1981; Meyer, 1979; Nonomura and Ohara, 1971b).

Only a few reports have been published about the genetics and metabolism of members of the genus despite their potential importance, notably in the discovery of novel bioactive compounds. The gene cluster encoding the biosynthesis of glycopeptide antibiotic A40926 in *Nonomuraea* strain ATCC 39727 has been isolated and characterized by Sosio et al. (2003). This glycopeptide, which is a member of the teichoplanin family of glycopeptides, is the precursor of dalbavancin. Sosio and her colleagues also isolated the novel compound dechloromannosyl-A40926 aglycone following the construction of a *Nonomuraea* mutation by deleting the *dbr* open reading frames 8–10. Subsequently, Alduina et al. (2005) constructed a bacterial artificial chromosomal library of *Nonomuraea* ATCC 39727 using an *Escherichia coli–Streptomyces* artificial chromosome (ESAC) and screened for the presence of dbv genes known to be involved in the biosynthesis of glycopeptide A40926. The heterologous expression of *Nonomuraea* genes in *Streptomyces lividans* was successfully demonstrated by using combined RT-PCR and proteomic approaches. These

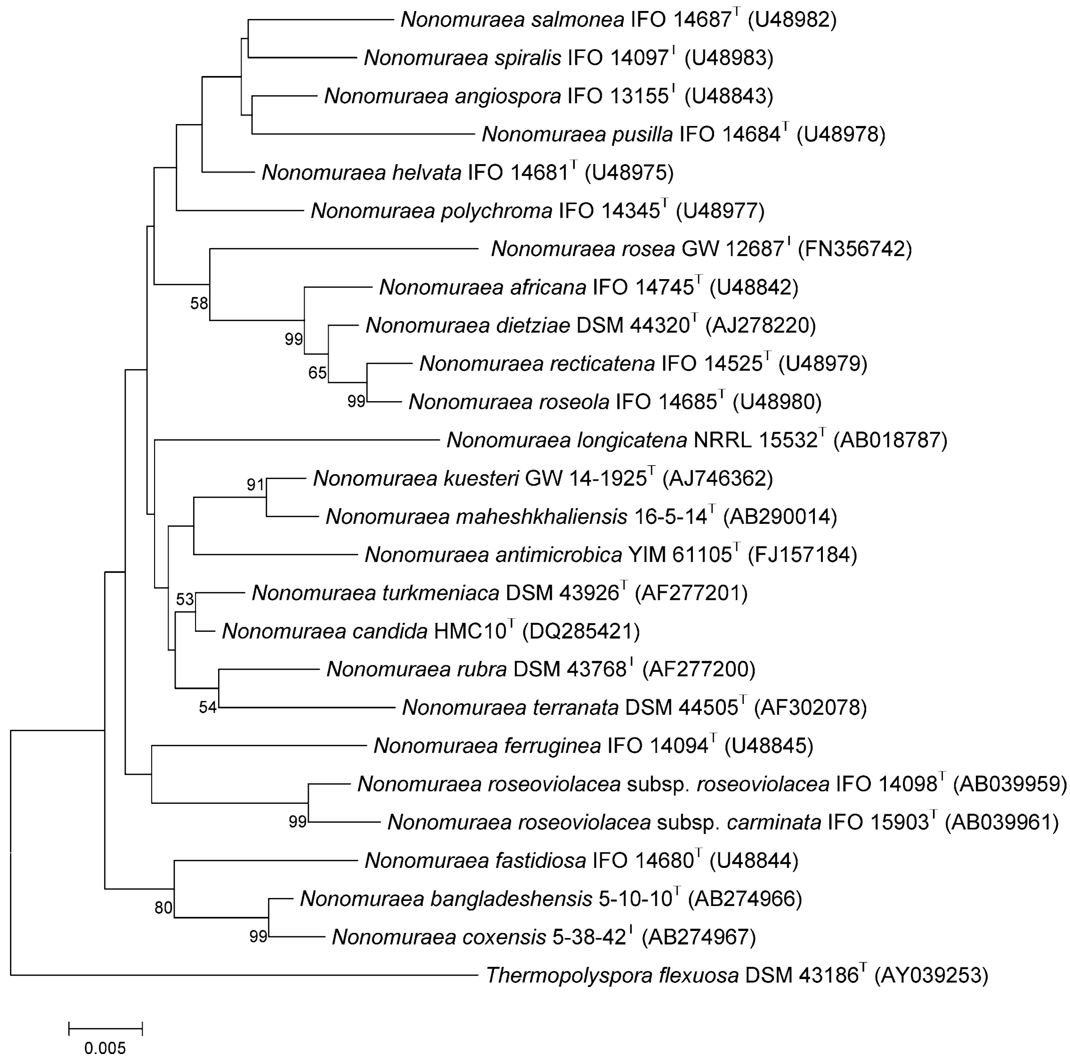

FIGURE 376. Phylogenetic analysis of *Nonomuraea* based on 16S rRNA gene sequences available from the European Molecular Biology Laboratory data library (accession numbers are given in brackets) constructed after multiple alignment of data (distance options according to the Kimura-2 model) and clustering with the neighbor joining method. Bootstrap values based on 1000 replications are listed as percentages at the branching points. Bar = 0.05 nucleotide substitutions per nucleotide position.

results indicate that *Streptomyces lividans* may be a good host for the genetic analysis of *Nonomuraea* strains. It was also demonstrated that *Nonomuraea* ATCC 39727 has a special carbon metabolism. Glucose is primarily metabolized via the Entner–Doudoroff pathway, even though the energetically more favorable Embden–Meyerhof–Parnas pathway is present in this organism (Gunnarsson et al., 2003). In addition, *Nonomuraea* utilizes a PP_i-dependent phosphofructokinase, an enzyme that has been connected with anaerobic metabolism in eukaryotes and higher plants but which has been recognized recently in several actinomycetes. Beltrametti et al. (2004, 2003) showed that the catabolism products of branched chain amino acids provide biosynthetic precursors for the formation of several lipid-containing antibiotics. The effect of valine on the production of glycopeptide antibiotic A40926 was studied in detail. The addition of valine to a minimal medium had a positive effect on the production of A40926; similar results were obtained in a rich production medium.

Members of the family *Streptosporangiaceae*, including *Nonomuraea* species, are an increasingly rich source of commercial products, notably antibiotics and enzymes (Goodfellow and Quintana, 2005). *Nonomuraea rosea* has been shown to produce deoxycephalomycin B (Okazaki and Naito, 1985), *Nonomuraea roseoviolacea* produces carminomicins (Nakagawa et al., 1983, 1989), *Nonomuraea rubra* produces maduromycin (Fleck et al., 1978), *Nonomuraea pusilla* produces actinotiocin (Tamura

et al., 1973), and *Nonomuraea spiralis* produces pyralomicin (Naganawa et al., 2002).

Enrichment and isolation procedures

Goodfellow and Quintana (2005) reported that dry heat treatment of air-dried soil samples and dilution plate culture with selective synthetic media is useful for the preferential isolation and enumeration of some members of the family *Streptosporangiaceae*, including members of the genera *Microtetraspora* and *Nonomuraea* (Nonomura and Ohara, 1971b, 1971c, 1971d). In addition, Nonomura and Hayakawa (1988) reported that pretreatment of soil suspensions with yeast extract (6%, w/v) and sodium dodecylsulfate (0.05%, w/v) at 40°C for 20 min, followed by dilution with water, activates actinomycete spores but kills vegetative cells of other soil bacteria in the suspensions, thereby leading to an increase in the counts of actinomycetes on isolation plates.

An improved procedure for the selective isolation of streptosporangiae from soil was reported by Hayakawa et al. (1991a). The method is based on the ability of streptosporangial spores to withstand dry heat and treatment with benzethonium chloride (BC) and the capacity of streptosporangiae to grow in the presence of leukomycin and nalidixic acid. Initially, an air-dried soil sample is ground in a mortar and heated in a hot-air oven for an hour; 0.5 ml of a 10^{-1} dilution in water of the heated sample is transferred to 4.5 ml of sterile 5 mM phosphate buffer (pH 7.0) containing BC at a final concentration of 0.1% (w/v). The resultant preparation is maintained at 30°C for 30 min with occasional stirring, and a portion (1 ml) is then diluted with sterile tap water (1:10 or 1:15). Inocula of 0.1 ml or 0.2 ml of the dilution is then spread over the surface of plates of HV agar supplemented with leukomycin in ethanol (1 mg/l) and nalidixic acid (20 mg/l) and the plates incubated at 30°C for 3–4 weeks. Actinomycetes which appear on the plates are examined by light microscopy (× 600) and assigned to genera on the basis of characteristic morphological properties. This pretreatment procedure has been used to isolate several *Nonomuraea* species including *Nonomuraea helvata*, *Nonomuraea pusilla*, *Nonomuraea roseoviolacea*, and *Nonomuraea spadix* (Nonomura and Ohara, 1971b).

Nonomuraea spores appear to be particularly resistant to dry heat at 100–120°C, thereby allowing the slow-growing nonomuraea to develop into recognizable colonies on dilution plates. Soil dilutions are plated onto various media including AV and MGA-SE agars, and incubated for several weeks at 28–30°C (Nonomura and Ohara, 1971d) (Methods taken from Goodfellow and Quintana, 2005.). Other *Nonomuraea* species such as *Nonomuraea salmonea* and *Nonomuraea roseola* have been isolated from soil on media supplemented with antibiotics. Lavrova et al. (1972) added rubomycin (5, 10, or 20 μg/ml) to medium no. 2 of Gauze et al. (1957); Preobrazhenskaya et al. (1975a) added bruneomycin (0.5, 1, or 2 μg/ml) or streptomycin (0.5, 1, or 2 μg/ml). The use of these antibiotics led to the growth of more *Nonomuraea* colonies on isolation plates while reducing the number of streptomycetes. In contrast, *Nonomuraea ferruginea* and *Nonomuraea spiralis* were isolated by plating soil suspensions onto oatmeal agar or Gauze's no. 1 medium without addition of selective antibiotics (Meyer, 1979).

Nonomuraea species also grow well on rich media including modified Bennett's (Jones, 1949), glucose-yeast extract (Waksman, 1950b), nutrient (BBL), oatmeal (ISP medium 3; Difco 0771), TS (BBL) and yeast extract-malt extract agars [ISP medium 2 (Difco 0770); Shirling and Gottlieb, 1966] as well as on PYES medium (Altenburgera et al., 1996). Most investigators recommend oatmeal-yeast extract agar for the growth of mesophilic strains and glycerol agar for thermophilic strains (Nonomura, 1989c).

Maintenance procedures

Goodfellow and Quintana (2005) reported that the most convenient method for short-term storage is by serial transfer from agar slants of complex media every 2 months (Meyer, 1989); tubes should be tightly closed with cotton plugs dipped in melted paraffin wax. Sporulated spore cultures can be stored at 5°C and at room temperature. Lyophilization, storage in liquid nitrogen, and freezing in glycerol can be used for long-term preservation (Goodfellow and Quintana, 2005; Meyer, 1989). *Nonomuraea* cultures may be lyophilized by procedures commonly used for bacteria. For lyophilization, the spore suspension or vegetative mycelium is suspended in a suitable fluid such as serum plus 7.5% (w/v) glucose or skimmed milk plus 7.5% (w/v) glucose. For storage in liquid nitrogen, the microorganisms are inoculated into small test tubes containing the appropriate medium and incubated until satisfactory growth is visible. Glycerol suspensions are prepared by scraping aerial growth or substrate mycelium or both from heavily inoculated plates and making heavy suspensions in 3 ml of aqueous glycerol in small (e.g. bijoux) bottles, which are stored at −20°C.

Differentiation of the genus *Nonomuraea* from other genera

Despite some differences, members of the family *Streptosporangiaceae* are chemically homogeneous but morphologically diverse (Table 284). *Nonomuraea* strains are distinguished from members of related genera by their ability to form chains of spores or pseudovesicles on aerial hyphae. The species may be distinguished by means of spore chain morphology, spore wall ornamentation, color of mature sporulated aerial mycelium, and substrate mycelium pigmentation. Nevertheless, identification of most species is difficult because, in many instances, only one (the type) or a few strains have been examined. In addition, *Nonomuraea* may be distinguished from other genera in the family *Streptosporangiaceae* by slight differences in cell-wall peptidoglycan, menaquinone type, colony pigmentation, the G+C content of the DNA, and growth at low temperatures (Table 284).

TABLE 284. Chemotaxonomic, morphological, and physiological characteristics of the genera classified in the family *Streptosporangiaceae*[a,b]

Characteristic	*Nonomuraea*	*Acrocarpospora*	*Herbidospora*	*Microbispora*	*Microtetraspora*	*Planobispora*	*Planomonospora*	*Planotetraspora*	*Streptosporangium*	*Thermopolyspora*
Morphology	Spore chains or pseudo-sporangia formed on aerial hyphae	Club or globose spore vesicles on aerial hyphae	Straight chains of smooth-surfaced spores on aerial hyphae	Smooth-surfaced spores in characteristic longitudinal pairs on aerial hyphae	Spore chains typically containing 4 smooth-surfaced spores on aerial hyphae	Cylindrical to clavate spore vesicles containing longitudinal pairs of spores on aerial hyphae	Cylindrical to clavate spore vesicles containing single spores on aerial hyphae	Spore vesicles containing four spores on aerial hyphae	Globose spore vesicles on aerial hyphae	Hooked or irregular spiral chains of 4–10 warty to spiny ornamented spores on aerial hyphae
Motile spores	–	–	–	–	–	+	+	+	–	–
Cell-wall chemotype[c]	III	III	III	III	III	III	III	III	III	III
Major menaquinones[d]	MK-9(H_2, H_4)	MK-9(H_2, H_4, H_6)	MK-10(H_4, H_6, H_8)	MK-9(H_2, H_4)	MK-9(H_2, H_4)	MK-9(H_2, H_4)	MK-9(H_2)	MK-9(H_4)	MK-9(H_2, H_4)	MK-9(H_2, H_4)
Muramic acid type	Acetylated	Acetylated	Acetylated	Acetylated	Acetylated	nd	nd	Acetylated	Acetylated	Acetylated
Phospholipid type	IV	IV, II	IV	IV	IV	IV	IV	nd	IV	IV
Whole-organism sugar pattern[e]	B,C	B,C	B	B,C	B,C	B	B	A,D	B	C
DNA G+C content (mol%)	64–69	68–69	69–71	71–73	69–71	70–71	72	71	69–71	77
Growth temperature range (°C)	20–45	15–30	nd	25–55	20–45	28–40	28–37	28–37	20–50	37–60

[a]Symbols: +, present; –, absent; nd, not determined.

[b]Data were taken from this and previous studies (Goodfellow et al., 1990b; Goodfellow and Quintana, 2005; Greiner-Mai et al., 1987; Kudo et al., 1993; Stackebrandt et al., 2001; Tamura and Sakane, 2004; Tamura et al., 2000).

[c]The major constituents of the cell wall are alanine, glutamic acid, glucosamine, and *meso*-A_2pm (Lechevalier and Lechevalier, 1970a). Diagnostic phospholipid type: II: only phosphatidylethanolamine; IV: glucosamine (with phosphatidylethanolamine and phosphatidylmethylethanolamine variable) (Lechevalier et al., 1977).

[d]Abbreviations exemplified by MK-9(H_4), menaquinone having four of the nine isoprene units hydrogenated.

[e]Whole-organism sugar patterns of actinomycetes containing *meso*-A_2pm: A, arabinose and galactose; B, madurose; C, no diagnostic sugar; D, arabinose and xylose (Lechevalier and Lechevalier, 1970b).

List of species of the genus *Nonomuraea*

1. **Nonomuraea pusilla** corrig. (Nonomura and Ohara 1971a) Zhang, Wang and Ruan 1998b, 419 (*Actinomadura pusilla* Nonomura and Ohara 1971a, 909)

pu.sil′la. L. fem. adj. *pusilla* dwarfish, referring to the aerial mycelium of the organism.

Spore chains in tightly closed spirals forming so-called pseudosporangia, i.e. spores are embedded in a slimy mass (Figure 377). Pseudosporangia are 3–6 μm in diameter. Spore surface smooth.

Glycerol-asparagine agar: good growth, aerial mycelium white, substrate mycelium grayish pink, no diffusible pigment. Inorganic salts-starch agar: scant growth, aerial mycelium microscopically visible, substrate mycelium colorless to brownish, no diffusible pigment. Inorganic salts-starch agar: scant growth, aerial mycelium microscopically visible, substrate mycelium colorless to brownish, no diffusible pigment. Oatmeal agar: abundant growth, surface farinaceous, aerial mycelium white to cream colored, substrate mycelium pale grayish brown, no diffusible pigment. Oatmeal salts-starch agar: good growth, surface granular to farcinoceus, aerial mycelium white, substrate mycelium colorless to white, no diffusible pigment. Yeast extract-malt extract agar: good growth, surface cartilaginous, aerial mycelium only microscopically visible, substrate mycelium dark brown with a red tinge, no diffusible pigment.

Cell hydrolysates contain galactose, madurose, mannose, and ribose. Diphosphatidylglycerol, phosphatidylethanolamine, ninhydrin and sugar positive phospholipids and hydroxylated phosphatidylethanolamine are present. Traces of phosphatidylinositol are found, but phosphatidylglycerol, phosphatidylinostol mannosides, and uncharacterized glycolipids are absent.

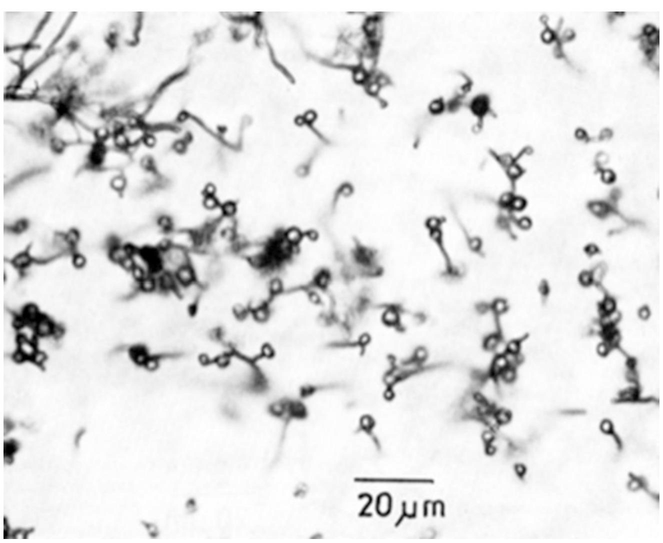

FIGURE 377. *Nonomuraea pusilla*, type strain ATCC 27296. Sporulating aerial mycelium with "pseudosporangia". Grown on yeast extract–malt agar at 28°C for 9 d.

Further chemotaxonomic data are given in Table 284, and the fatty acid pattern of the type strain is shown in Table 285.

Esculin hydrolysis and nitrate reductase tests are positive. Degrades DNA, gelatin, hypoxanthine, and tyrosine. Positive for acetoin, arginine dihydrolase, gelatinase, and urease activities. Further physiological characteristics are given in Table 286. Does not utilize L-arabinose, D-cellobiose, D-fructose, *meso*-inositol, D-mannose, L-rhamnose, or D-xylose as sole carbon sources.

Source: soil.

DNA G+C content (mol%): 68.3 (T_m).

Type strain: ATCC 27296, CBS 262.72, BCRC 11619, CECT 3284, CIP 106954, DSM 43357, NBRC 14684, IMET 9586, JCM 3144, KCTC 9278, NCIMB 11116, NRRL B-16126, VKM Ac-1508.

Sequence accession no. (16S rRNA gene): D85491, U48978.

Additional comments: Nonomuraea pusilla can be easily mistaken for a *Streptosporangium* on superficial microscopic examination; it can only be differentiated by exact proof of a sporangial wall, which indicates a *Streptosporangium* strain. Tamura et al. (1973) isolated a sulfur-containing peptide antibiotic, actinotiocin, from *Nonomuraea pusilla*.

2. **Nonomuraea africana** corrig. (Preobrazhenskaya and Sveshnikova 1974) Zhang, Wang and Ruan 1998b, 419[VP] (*Actinomadura africana* Preobrazhenskaya and Sveshnikova 1974, 865; *Nocardiopsis africana* Preobrazhenskaya and Sveshnikova 1985, 224; *Microtetraspora africana* Kroppenstedt, Stackebrandt and Goodfellow 1990a, 148)

a.fri.ca′na. L. fem. adj. *africana* referring to Africa, the source of the soil sample.

Straight spore chains with 4–10 spores per chain. Spore ornamentation smooth. Growth of aerial mycelium on oatmeal agar is grayish blue, substrate mycelium growth is yellow, and the soluble pigment color yellowish brown.

Esculin is hydrolyzed and nitrate reduced. Casein, DNA, gelatin, hypoxanthine, starch, and tyrosine are degraded. Cell hydrolysates contain madurose. Diphosphatidylglycerol, phosphatidylethanolamine, phosphatidylglycerol, phosphatidylinositol, phosphatidylinositol mannosides, ninhydrin and sugar positive phospholipids, hydroxylated phosphatidylethanolamine, and uncharacterized glycolipids are present.

Further chemotaxonomic data are given in Table 284, and the fatty acid pattern of the type strain is given in Table 285.

L-Arabinose, cellobiose, fructose, mannose, *meso*-inositol, raffinose, rhamnose, sucrose, and xylose are utilized and used as sole carbon sources. Positive for acetoin, arginine dihydrolase, citrate lyase, β-galactosidase, dihydrolase, gelatinase, lysine decarboxylase, ornithine decarboxylase, and urease activities.

Further physiological characteristics are given in Table 286.

DNA G+C content (mol%): 64.0 (T_m).

Type strain: ATCC 35107, DSM 43748, NBRC 14745, INA 1839, JCM 6240, KCTC 9260, NRRL B-16114, RIA 1839, VKM Ac-924.

Sequence accession no. (16S rRNA gene): AJ269555, U48842.

TABLE 285. Cellular fatty acid composition of the type strains of *Nonomuraea* species[a]

Fatty acid	*N. pusilla* DSM 43357	*N. africana* DSM 43748	*N. angiospora* DSM 43173	*N. antimicrobica*[b] DSM 45220	*N. bangladeshensis*[c] JCM 13930	*N. coxensis*[d] JCM 13931	*N. dietziae* DSM 44320	*N fastidiosa* DSM 43674	*N. ferruginea* DSM 43553	*N. flexuosa* DSM 43186	*N. helvata* DSM 43142	*N. kuesteri* DSM 44753	*N. maheshkhaliensis* JCM 13923	*N. longicatena* DSM44817	*N. polychroma* DSM 43925	*N. rosea*[e] DSM 45177	*N. recticatena* DSM 43937	*N. roseola* DSM 43767	*N. roseoviolacea* DSM 43144	*N. rubra* DSM 43768	*N. salmonea* DSM 43678	*N. spiralis* DSM 43555	*N. terrinata* DSM 44505	*N. turkmeniaca* DSM 43926
$C_{14:0}$ iso	1.3	2	0.6	3.9	1.5		3	0.8	0.4	0.8	0.8	0.9	0.4	1.3	0.7	1.3	1.6	1.3		0.6	1.2	3.9	0.5	0.8
$C_{14:0}$	3.6	1.4	1.4	4.2		4.6	1.1	0.7	0.9	0.5	2.3	0.9	3.3	1	0.8	0.5	0.8	2.7	1.5	0.8	0.7	1	1.3	1.6
$C_{15:0}$ iso	10.7	4.1	7.7	6.5	14.7	2.2	3.6	8.1	3	12.5	9.7	7.9	5.5	4.6	7.7	4.3	5.1	2.6	6.3	8.2	7.5	9.4	5.9	9
$C_{15:0}$ anteiso	1	0.4	0.3			2	1.2	1.2		4.3	3.4	0.3	0.5	1.4		0.4	0.7	0.6		0.2	0.6	0.8	0.4	
$C_{15:0}$	7.8	10.7	4.1		1.2	7.5	6.5	3.7	7.7	5.7	8.1	3.8	2.9	7	1.8	3.1	5.4	5.7	3.1	2.3	3.9	10.5	3.6	3.3
$C_{16:1}$ iso	2.3	0.9	4.6	12.7	7.3		4.7	1.7	0.6	0.4	0.5	8.3	3.6	2.2	2.9	6.9	0.9	1.5		4.6	2.9	0.9	2.7	6.9
$C_{16:0}$ iso	19.2	21.1	31.3	32.2	26.4	8.1	13.3	35.8	22.1	13.8	17.6	28.9	13.6	24.8	35.7	43.3	17.5	9.6	22	32.7	40.8	29.9	27.9	26.1
$C_{16:1}$ *cis*	0.7	0.8	1.2			1.3	1.8		1.2	2.8	2	1.8			1.4	0.6		4.1	1.5	2.4	1		1.7	1.4
$C_{15:0}$ iso 2-OH										1.4				1.2		0.4	0.7					2.2		
$C_{16:0}$	5.4	4.7	4.2		1.8	17.4	2.1	2.5	3.5	3.4	12.3	1.8	15.5	1.7	4.4	1.1	3.2	10.7	6.7	5.1	3.9	4.7	4.3	5
$C_{15:0}$ 2-OH	1.1	1.4	0.6				3		1.8		0.5	0.8	0.6	1.7		0.4	2.1	2.1		0.3	0.3	0.8	0.4	
$C_{16:0}$ 10-methyl	5.6	1	3.4		5.8	3.4	1	1.7	1.1	2.4	4.1	2.8	8	4.8	2.3	2.6	0.7	1.5	4.4	4.1	2.3	2.4	2.9	2.5
$C_{17:0}$ iso	2.7	1.1	2.8		10			4.1	1.5	18.7	2.4	1.8	1.8	0.6	1.2	0.5	1.6	0.7	2.9	3.6	2.9	2.2	3.1	0.8
$C_{17:0}$ anteiso	1.2	0.6	2	2.7	1.6	2.4		6.5	0.7	12.4	4.3	1	1	1.5	0.8	0.7	1.2	0.4	0.9	1.4	1.1	0.8	2.8	0.4
$C_{17:1}$ 9*c*	3.4	7	1.5		4.5	2.4	11.3	0.8	9.8	0.5	5.6	4.2		3.1	3.3	2.1	5	13.3	3.2	3.9	2.8	2	5	2.4
$C_{16:0}$ iso 2-OH	4.1	2.8	7.2			1.9	5	7.1	7.4	2.5	4	10.5		10.2	4		6.6	4.2	5.1	7	7.5	5.4	6	4
$C_{17:0}$	5.3	10.3	3.8	2.4		6.7	4.5	2.6	14.3	2	6.4	1.6	1.8	1.8	3.9	1	15.6	10	6	2.6	4.4	7.8	5.7	5.8
$C_{16:0}$ 2-OH	0.5	0.5	0.3			1.9	0.6		0.6		0.4	0.5	3.7		0.3		0.7	4.2	1	0.6	0.4		0.6	
$C_{17:0}$ 10-methyl	20.6	24.1	18.6	17.1	10.5	8.4	33.7	17.6	15.4	6.9	13	15.5	9.6	27.6	18.3	19.8	23.8	13.3	23.3	15.1	13.1	14.7	18	21.2
$C_{17:0}$ iso 2-OH	0.4									3	0.6	0.4					0.5	0.4		0.5			0.4	
$C_{18:0}$ iso	0.5	1	1.3					1.9	2		0.3	0.6	0.2		2.6		1.2		2.2	0.9	1	0.5	1.7	1.8
$C_{18:1}$ 9*c*	0.3	0.9		3	1.2	4.5	0.9	9.5	0.8		0.7	0.6	3.4		1.7		0.4	4	1	0.9	0.4		1.1	0.8
$C_{18:0}$	1.2	1.4	1.9			1.9	0.6	5.4	1.5	2.5	0.7	1.5	1.3		2.6		2.2	2.1	4.1		1.3	0.7	1.9	3.2
$C_{17:0}$ 2-OH	0.3	0.7					0.7		1.6			0.3	0.2				1.8	1.7						
$C_{18:0}$ 10-methyl	0.8	1.3	1.2	5.8	1.6	2.5	1.7	3.2	1		1.1	0.9	4.4	0.6	3.6	0.5	1.2	3.2	5.1	2.7		0.5	2.5	3.7

[a]Also contain traces of $C_{13:0}$ iso, C_{18} iso, and $C_{18:0}$; in DSM 44817 0.9% $C_{18:0}$ iso 10-methyl is found in addition.

[b]Data from Qin et al. (2009).

[c]Data from Ara et al. (2007b).

[d]Data from Ara et al. 2007a).

[e]Data from Kämpfer et al. (2010).

3. **Nonomuraea angiospora** corrig. (Zhukova, Tsyganov and Morozov 1968) Zhang, Wang and Ruan 1998b, 419^{VP} (*Micropolyspora angiospora* Zhukova, Tsyganov and Morozov 1968, 728; *Microtetraspora angiospora* Kroppenstedt, Stackebrandt and Goodfellow 1990a, 148)

an.gi.o.spo′ra. Gr. neut. n. *angeion* (L. translit. *angium*) vessel; Gr. n. *spora* a spore; N.L. n. *angiospora* spores enclosed (in capsules).

Spore chains (2–15 spores) are curved or irregularly coiled. Spores spherical to oval (1.5–2.4 × 1.2–2.2 μm); surface spines enveloped in a translucent capsule. White aerial mycelium is formed on soya flour and oatmeal agars; substrate mycelium and colonies white to deep flesh or ochre colored. Grows between 21 and 37°C, and optimally at 27°C.

Cell hydrolysates contain madurose, ribose, and traces of mannose. Diphosphatidylglycerol, phosphatidylglycerol, phosphatidylinositol mannosides, ninhydrin and sugar positive phospholipids, hydroxylated phosphatidylethanolamine, and uncharacterized glycolipids are present. Phosphatidylethanolamine and phosphatidylinositol are absent.

Further chemotaxonomic data are given in Table 284, and the fatty acid pattern of the type strain is shown in Table 285.

Esculin is hydrolyzed; nitrate is not reduced. Positive for acetoin, arginine dihydrolase, gelatinase, and urease activities. Degrades DNA, gelatin, hypoxanthine, and tyrosine. Does not use L-arabinose, cellobiose, D-fructose, *meso*-inositol, D-mannose, D-raffinose, L-rhamnose, sucrose, or D-xylose as sole carbon sources.

Further physiological characteristics are given in Table 286.

DNA G+C content (mol%): not determined.

Type strain: LIA 3479-30, AS 4.1229, BCRC 13334, DSM 43173, NBRC 13155, JCM 3109, KCTC 9191, KCTC 9261, NRRL B-3905.

Sequence accession no. (16S rRNA gene): U48843.

4. **Nonomuraea antimicrobica** Qin, Zhao, Klenk, Li, Zhu, Xu and Li 2009, 2750^{VP}

an.ti.mi.cro′bi.ca. Gr. prep. *anti* against; N.L.n. *microbium* microbe; L.adj.suff. *-icus -a -um* suffix used with various meanings; N.L.fem.adj. *antimicrobica* antimicrobial.

Gram-stain-positive, aerobic, non-acid-fast, and non-acid–alcohol-fast actinomycete that forms extensively branched, brown substrate mycelia and white-to-pink aerial mycelia on ISP 2–5 and PDA media. After 14 d of incubation at 28°C, spiral spore chains composed of smooth spores are observed on the aerial mycelium. Temperature range for growth is 15–37°C with optimal growth on 28°C. pH range for growth is 6.0–9.0 with optimal growth at 7.0. No growth is observed with 5% NaCl.

Positive for catalase. Negative for oxidase, milk coagulation, milk peptonization, gelatin liquefaction, cellulose, and starch hydrolysis, H_2S production and nitrate reduction. The diagnostic amino acid of the peptidoglycan is *meso*-diaminopimelic acid. Cell hydrolysates contain ribose, glucose, madurose, and galactose. Polar lipids include diphosphatidylglycerol, phosphatidylglycerol, phosphatidylinositol and phosphatidylinositol mannosides, and the diagnostic phospholipids of phosphatidylethanolamine and an unknown glucosamine-containing phospholipid.

The predominant menaquinone of the type strain is MK-9(H_4); MK-9(H_2), MK-9(H_6), and MK-9 are also present. Major fatty acids (>10%) are $C_{16:0}$ iso, $C_{17:0}$ 10 methyl, $C_{16:1}$ iso G, and $C_{16:0}$.

Source: a surface-sterilized leaf of *Maytenus austroyunnanensis* from the tropical rainforest of Xishuangbanna, Yunnan Province, south-west China.

DNA G+C content (mol%): 69.2 (HPLC).

Type strain: YIM 61105, DSM 45220, CCTCC AA 208016.

Sequence accession no. (16S rRNA gene): FJ157184.

5. **Nonomuraea bangladeshensis** Ara, Kudo, Matsumoto, Takahashi and Ōmura 2007b, 1506^{VP}

ban.gla.desh.en′sis. N.L. fem. adj. *bangladeshensis* of or pertaining to Bangladesh, where the strain was isolated.

Forms a well-developed, branched substrate mycelium. Abundant aerial mycelium is present.

Spore chains are spiral with 1 or 2 turns (8–12 spores). Sporangia are not detected. Spores are spherical to cylindrical, and the spore sheath surface is smooth.

Good growth occurs on Bennett's agar, glucose-yeast extract agar, Hickey–Tresner agar, oatmeal agar (ISP-3), oatmeal-nitrate agar, Seino (yeast-starch) agar, tyrosine agar (ISP-7), Waksman agar, and yeast extract-malt extract agar (ISP-2). Moderate growth is found on glycerol-asparagine agar, inorganic salts-starch agar (ISP-4), nutrient agar, and water agar. Poor growth occurs with glucose-asparagine agar and sucrose-nitrate agar. The color of the substrate mycelium is shell to cinnamon brown on various media.

Pale brown aerial mycelium and sporulation occur on ISP-7 agar, ISP-2 agar, ISP-3 agar, oatmeal-nitrate agar, and water agar media after 14 d incubation at 28°C. A soluble pigment is not produced. Aerobic, Gram-stain-positive, and able to grow at pH 5.0–9.0. The temperature range for growth is between 15 and 45°C. No production of melanoid pigments. No growth is observed at 5% NaCl.

The diagnostic diamino acid isomer of the cell-wall peptidoglycan is *meso*-DAP.

Galactose, glucose, madurose, mannose, and ribose are present in whole-cell hydrolysates.

The *N-acyl*-type of the muramic acid is acetyl. Mycolic acids are absent. The polar lipids include PE and OH-PE. Phosphatidylcholine is absent.

The major menaquinone is MK-9(H_4), with minor amounts of MK-9(H_6), MK-9(H_2), and MK-9(H_0). Major fatty acids are $C_{16:0}$ iso (26.4%), $C_{15:0}$ iso (14.7%), and $C_{17:0}$ 10 methyl (10.5%).

Source: sandy soil from Cox's Bazar, Bangladesh.

DNA G+C content (mol%): 72.7 (HPLC).

Type strain: 5-10-10, JCM 13930, MTCC 8089.

Sequence accession no. (16S rRNA gene): AB274966.

6. **Nonomuraea candida** Le Roes and Meyers 2009, 1^{VP} (Effective publication: Le Roes and Meyers 2008, 136.)

can´di.da. L. fem. adj. *candida* shining white; pertaining to the light coloring of the substrate and aerial mycelial mass when grown on oatmeal agar (IPS-3).

Gram-stain-positive, non-acid-fast, and non-acid–alcohol-fast actinomycete that forms a leathery, dark brown growth on ISP-2 agar. Tan-colored growth is observed on inorganic salts-starch agar (ISP-4); a yellow diffusible pigment is produced on glycerol-asparagine agar (ISP-5) and beige-colored substrate mycelium and white aerial mycelium is visible. No melanin production is observed on peptone-yeast extract-iron agar (ISP-6) and tyrosine agar (ISP-7). Good growth is observed on ISP-2 agar. Weak antibiosis is exhibited against *Mycobacterium aurum* A+ in agar overlays. Bioautographic analysis of organic solvent extracts of the cell mass shows weak antibiosis against *Mycobacterium aurum* A+, but no activity against *Mycobacterium tuberculosis* H37Rv^{T} (=ATCC 27294^{T}).

Growth is observed at 30°C, 37°C, and 45°C, but not at pH 4.3 and 4°C.

The type strain HMC10^{T} is catalase positive and oxidase negative. It grows in the presence of 0.3% 2-phenylethanol, 3% NaCl, and 0.1% phenol, but not in the presence of 0.01% NaN_3. Growth is observed in the presence of cephaloridine (100 μg/ml) (weak), lincomycin (100 μg/ml), oleandomycin (100 μg/ml), penicillin G (10 i.u./ml), rifampin (50 μg/ml), tobramycin (50 μg/ml) (weak), and vancomycin (50 μg/ml) (weak), but not in the presence of gentamicin (100 μg/ml), kanamycin (100 μg/ml), neomycin (50 μg/ml), and streptomycin (100 μg/ml).

Utilizes DL-α-amino-*n*-butyric acid, L-arginine, L-histidine, L-4-hydroxyproline, L-methionine, L-phenylalanine, potassium nitrate, L-serine, and L-valine as sole nitrogen sources, but not L-casteine. Adonitol, D(−) fructose, D(+) galactose, D(+) glucose, *meso*-inositol, inulin, D(−) lactose, D(−) mannose, D(+) melibiose, raffinose, L(+) rhamnose, D(−) ribose, salicin, sodium acetate (0.1%), sucrose, trehalose, and D(+) xylose are utilized as sole carbon sources. L(+) Arabinose, D(+) cellobiose, D(+) melezitose, and xylitol are weakly utilized, but sodium citrate (0.1%) is not utilized as a sole carbon source. Nitrate is not reduced, but H_2S production occurs. Lecithinase, lipase, and protease activity is observed on egg yolk agar. Hippurate and pectin are hydrolyzed. Degrades adenine, esculin, arbutin, casein, and Tween 80. Gelatin is weakly degraded, while allantoin, guanine, hypoxanthine, starch, L-tyrosine, urea, xanthine, and xylan are not degraded.

Source: South African soil.

DNA G+C content (mol%): 72.7 (HPLC).

Type strain: HMC10, DSM 45086, NRRL B-24552.

Sequence accession no. (16S rRNA gene): DQ285421.

7. **Nonomureae coxensis** Ara, Kudo, Matsumoto, Takahashi and Ōmura 2007b, 1507^{VP}

cox.en′sis. N.L. fem. adj. *coxensis* of or pertaining to Cox's Bazar, Bangladesh, the origin of the soil where the type strain was isolated.

Forms a well-developed, branched substrate mycelium. Abundant aerial mycelium is present.

Spore chains are spiral to hooked with 12–17 spores. Sporangia are not detected. Spores are spherical to oval and the spore surface is smooth.

Good growth on Bennett's agar, glucose-yeast extract agar, Hickey–Tresner agar, oatmeal agar (ISP3), oatmeal-nitrate agar, Seino (yeast-starch) agar, 1/5 yeast-starch agar, Waksman agar, and yeast extract-malt extract agar (ISP2). Moderate growth on inorganic salts-starch agar (ISP4), nutrient agar, and tyrosine agar (ISP7); poor growth on glucose-asparagine agar, glycerol-asparagine agar, and water agar.

Substrate mycelium is light melon yellow to apricot (orange-brown) on various agar media. Pale pink to white aerial mycelium and sporulation occur on ISP-2 agar, ISP-3 agar, Bennett's agar, Hickey–Tresner agar, and 1/5 yeast-starch agar media after 14 d incubation at 28°C. A soluble pigment is not produced. Aerobic; Gram-stain-positive.

Growth occurs at pH 5.0–9.0. The temperature range for growth is between 15 and 45°C.

No production of melanoid pigments. No growth on 4% NaCl. The diagnostic diamino acid isomer of the cell-wall peptidoglycan is *meso*-DAP. Galactose, glucose, madurose, mannose, and ribose are present in whole-cell hydrolysates. The *N*-acyl type of muramic acid is acetyl. Mycolic acids are absent. The polar lipids include PE and OH-PE. Phosphatidylcholine is absent. The major menaquinones are MK-9(H_6) and MK-9(H_4). The fatty acid profile is characterized by the presence of significant amounts of $C_{16:0}$ (17.4%), $C_{17:1}$ ω8c (11.5%), and $C_{17:0}$ 10 methyl (8.4%).

Source: sandy soil from Cox's Bazar, Bangladesh.

DNA G+C content (mol%): 72.3 (HPLC).

Type strain: 5-38-42, JCM 13931, MTCC 8090.

Sequence accession no. (16S rRNA gene): AB274967, AB505224.

8. **Nonomuraea dietziae** Stackebrandt, Wink, Steiner and Kroppenstedt 2001, 1439^{VP}

di.et.zi´a.e. N.L. gen. fem. n. *dietziae* of Dietz, named in honor of the American microbiologist Alma Dietz.

Long spore chains, each containing up to 30 spores, formed on aerial mycelium. Spore chains may be stalked or may form clusters emerging directly from the agar. Sporangia are not detected. Irregular spore chains with spirals of 1–2; sometimes 3–5 turns are seen.

Aerial mycelium develops different colors depending upon the ISP medium used: red-brown (yeast extract malt extract agar, ISP2); white, beige, or pink (oatmeal agar, ISP3); pink (inorganic salts starch agar, ISP4 and tyrosine agar, ISP7); antique rose (glycerol-asparagine agar, ISP5); and white (peptone-yeast extract iron agar, ISP6); after approximately 14 d. Depending upon the ISP medium used, the nonfragmenting substrate mycelium is brown (ISP2), beige (ISP3), pink (ISP3, ISP 7), or rose (ISP5, ISP6) in color. Soluble pigments not produced.

Cell hydrolysates contain galactose, glucose, and ribose, but not arabinose or xylose; madurose occurs in minor amounts. Diphosphatidylglycerol, phosphatidylethanolamine, phosphatidylinositol, ninhydrin and sugar positive phospholipids, hydroxylated phosphatidylethanolamine, and uncharacterized glycolipids are present; Phosphatidylglycerol and phosphatidylinositol mannosides are absent.

Additional chemotaxonomic data are given in Table 284, and the fatty acid pattern of the type strain is shown in Table 285.

TABLE 286. Comparison of the phenotypic properties of *Nonomuraea species*[a,b]

Characteristic	*N. pusilla*	*N. africana*	*N. angiospora*	*N. antimicrobica*	*N. bangladeshensis*	*N. candida*	*N. coxensis*	*N. dietziae*	*N. fastidosa*	*N. ferruginea*	*N. helvata*
Spore chain morphology											
Spore chains[c]	psp	str	sp	Sp	Sp	H	str, h	str, sp	s, sp	h, s	h, psp
Spore ornamentation	Smooth	Smooth	Ridged	Smooth	Smooth	Smooth	Smooth	Cross-ridged, smooth-and-rough	Irregular	Folded	Smooth
Number of spores	>01	4–10	4–15	nd	8–12	nd	12–17	Up to 30	4–10	4–10	4–10
Growth on ISP 3 medium:											
Aerial mycelium	White/ Cream	Grayish blue	White	White/ pink	Pale brown	White	Pink to white	Beige	White/ pink	White/ pink	White
Substrate mycelium	Gray/ brown	Yellow	White/ ochre	Moderate brown	Pale brown	Beige	Orange	Beige	Colorless	Pink	Yellow/ brown
Soluble pigment	None	Yellowish Brown	None	Pink	None	Yellow	None	Yellow	None	None	None
Biochemical tests:											
Esculin hydrolysis	+	+	+	–	nd	+	nd	nd	+	–	+*
Nitrate reductase	+	+	–	–	nd	–		nd	+	+	+
Degradation tests:											
Casein	–	+	+	+	nd	+	nd	nd	+	+	–
DNA	+	+	+	nd	nd	nd	nd	nd	+	+	–
Elastin	–	–	+	nd	nd	nd	nd	nd	+	+	nd
Gelatin	+	+	+	–	nd	+	nd	+	+	–	–
Hypoxanthine	+	+	+	–	nd	–	nd	nd	+	+	–
Starch	–	+	–	–	nd	–	nd	nd	–	+	–
Tyrosine	+	+	+	nd	nd	–	nd	nd	–	+	–
Xanthine	–	–	–	nd	nd	–	nd	nd	–	–	–
Utilization of:											
L-Arabinose	–	+	–	+	w	w	w	w	–	+	–
Cellobiose	–	+	–	+	+	w	+	–	–	–	–
D-Fructose	–	+	–	nd	w	+	w	+	–	+	–
m-Inositol	–	+	+	–	w	+	w	–	+	–	+
D-Mannose	–	+	–	nd	+	+	+	–	–	+	–
D-Raffinose	–	+	+	nd	+	+	w	–	+	–	+
L-Rhamnose	–	+	–	nd	+	+	+	+	–	+	–
Sucrose	–	+	–	–	w	+	+	–	–	+	+
D-Xylose	–	+	+	–	w	+	+	–	+	+	+
Production of:											
Acetoin	+	+	–	nd	nd	nd	nd	+	+	+	+
Arginine dihydrolase	+	+	+	nd	nd	nd	nd	+	+	+	+
Citrate lyase	–	+	–	nd	nd	nd	nd	+	+	+	+
β-Galactosidase	–	+	–	nd	nd	nd	nd	+	+	+	+
Gelatinase	+	+	+	nd	nd	nd	nd	+	+	+	+
Lysine decarboxylase	–	+	+	nd	nd	nd	nd	–	+	+	+
Ornithine decarboxylase	–	+	+	nd	nd	nd	nd	+	+	+	+
Urease	+	+	+	nd	nd	nd	nd	+	+	+	+

[a]Symbols: +, positive; –, negative; w, weak; nd, not determined.

[b]Data were taken from Meyer (1989), Chiba et al. (1999), Stackebrandt et al. (2001), Quintana et al. (2003), Ara et al. (2007a, 2007b), Le Roes and Meyers (2008),

[c]h, hooks, curled; psp, pseudosporingia; s, spirals of 1–2 turns; sp, spirals of 3–5 turns; str, straight.

N. kuesteri	*N. longicatena*	*N. maheshkhaliensis*	*N. polychroma*	*N. recticatena*	*N. rosea*	*N. roseola*	*N. roseoviolacea*	*N. rubra*	*N. salmonea*	*N. spiralis*	*N. terrinata*	*N. turkmeniaca*
sp	str	sp	nd	str	s	sp, str	psp	h, s, sp	h, s	sp	irregular, psp	sp
nd	Smooth	Folded and rough	nd	Smooth	nd	Folded	Smooth	Smooth	Warty	Folded	Rugose	Smooth
nd	10–30	17–20	nd	4–20	4–10	6–20	4–20	4–20	4–30	4–20	8–15	10–20
Trace	White	White	Trace	White/ cream	White	Pink	Pink/ violet	Trace	Pink	White/ yellow	White	Trace
Yellow	Ochre	Light wheat	Colorless/ brown	Dark yellow/ brown	Pink/ violet	Brown/ red	Violet	Orange/ red	Red	Yellow/ brown	White/ ochre	Violet/ red
None	None	None	None	None	None	None	Violet	Red	None	None	None	Pink/ Violet
+	+	nd	+	+	+	+	+*	–#	+#	+	+	+*
nd	–		–	+	nd	+	+	+	+	+	–	+
–	+	nd	–	–	+	–	–	–	+	–	+	+
nd	–	nd	–	–	nd	–	+	–	+	–	+	–
nd	+	nd	+	+	nd	–	–	+	+	–	–	–
–	–	nd	+	+	+	+	+	+	+	+	–	+
–	+	nd	+	+	+	+	+	+	+	–	+	+
–	+	nd	–	+	+	–	–	+	–	–	+	+
–	–	nd	–	–	+	+	–	+	+	+	–	–
–	–	nd	–	–	+	–	–	–	–	–	–	–
+	+	w	–	+	+	–	+	+	–	–	nd	+
+	–	+	–	–	+	–	–	+	–	–	nd	+
+	nd	+	–	–	+	–	+	+	–	–	nd	+
+	–	w	–	+	+	+	+	+	–	–	nd	+
+	+	+	–	–	+	–	+	+	–	+	nd	+
nd	+	nd	–	–	nd	–	+	+	–	–	nd	+
+	–	+	–	+	+	+	+	+	+	+	nd	+
+	nd	+	–	–	–	+	+	+	+	+	nd	+
+	nd	+	–	–	+	–	–	+	–	+	nd	+
nd	nd	nd	+	+	nd	+	+	+	+	+	nd	+
–	+	nd	+	–	nd	+	+	+	+	–	nd	+
nd	nd	nd	–	–	nd	+	–	+	+	–	nd	+
–	+	nd	–	–	nd	+	–	+	+	+	nd	+
–	–	nd	+	–	nd	+	+	+	+	–	nd	+
–	+	nd	+	–	nd	+	+	+	+	–	nd	+
–	+	nd	+	–	nd	+	+	+	+	–	nd	–
–	+	nd	–	+	nd	+	+	+	+	–	nd	+

Qin et al. (2009), Kämpfer et al. (2010).

Positive for acetoin, arginine dihydrolase, β-galactosidase, citrate lyase, gelatinase, ornithine decarboxylase, and urease activities. D-Fructose and L-rhamnose are utilized as sole carbon sources, but cellobiose, *meso*-inositol, D-mannose, D-rhamnose, sucrose, and D-xylose are not. Utilization of L-arabinose is weak.

Further physiological characteristics are given in Table 286.

Shows no antibacterial effect on *Micrococcus luteus*, *Streptococcus murinus*, *Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Saccharomyces cerevisiae*, *Aspergillus niger*, or *Candida albicans*.

Source: soil.

DNA G+C content (mol%): not determined.

Type strain: ATCC 35861, CIP 107127, DSM 44320, NBRC 14309, JCM 3338, NRRL 11111.

Sequence accession no. (16S rRNA gene): AB006156, AJ278220, AJ294350.

9. **Nonomuraea fastidiosa** corrig. (Soina, Sokolov and Agre 1975) Zhang, Wang and Ruan 1998b, 419[VP] (*Actinomadura fastidiosa* Soina, Sokolov and Agre 1975, 883; *Microtetraspora fastidiosa* Kroppenstedt, Stackebrandt and Goodfellow 1990a, 148)

fas.ti.di.o′sa. L. fem. adj. *fastidiosa* fastidious, referring to the difficulties in growing the organism.

Spore chains consist of irregular spirals of 1–2, more rarely 3–4, turns arranged monopodially on long aerial hyphae (Figure 378). Spore surface ornamentation smooth or slightly irregular. Temperature range: 23–55°C, optimum temperature 30–45°C.

Inorganic salts-starch agar (Gauze 1 medium): moderate growth, aerial mycelium sparse and cream colored to pinkish, substrate mycelium colorless, no diffusible pigment. Oatmeal agar: good growth, surface farinaceous, aerial mycelium white to pale pink, substrate mycelium colorless or pale brownish, no diffusible pigment. Yeast extract-malt extract agar: moderate growth, surface granular with spots of whitish aerial mycelium at the edges of colonies, substrate mycelium pale brown, no diffusible pigment.

Cell hydrolysates contain galactose, madurose, mannose, and ribose. Diphosphatidylglycerol, phosphatidylethanolamine, hydroxylated phosphatidylethanolamine, and uncharacterized glycolipids are present; traces of phosphatidylinositol and ninhydrin and sugar positive phospholipids are also found, but phosphatidylglycerol and phosphatidylinositol mannosides are absent.

Further chemotaxonomic data are given in Table 284, and the fatty acid pattern of the type strain is shown in Table 285.

Positive for acetoin, arginine dihydrolase, citrate lyase, β-galactosidase, gelatinase, lysine decarboxylase, ornithine decarboxylase, and urease activities. L-Arabinose, citrate fructose, glucose, and rhamnose are utilized as sole carbon sources, but cellobiose, mannose, *meso*-inositol, rhamnose, sucrose, and xylose are not.

Further physiological characteristics are given in Table 286.

DNA G+C content (mol%): 67 (T_m).

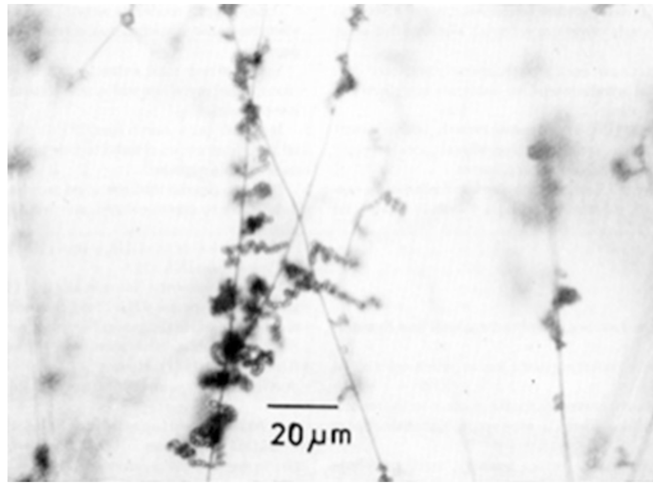

FIGURE 378. *Nonomuraea fastidiosa* strain IMET 9614. Sporulating aerial mycelium. Grown on oatmeal agar at 28°C for 21 d.

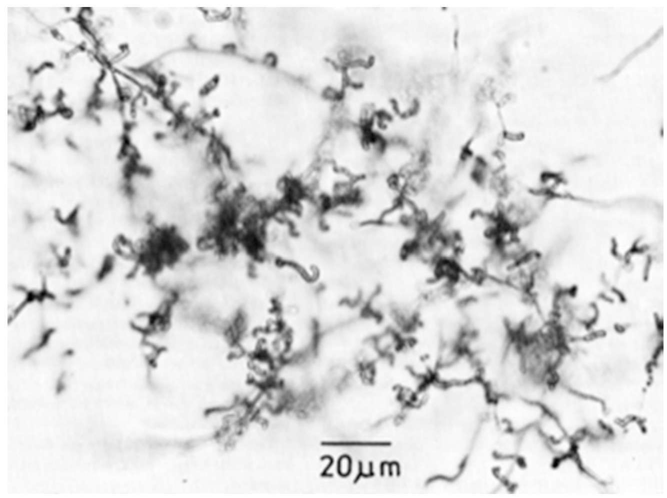

FIGURE 379. *Nonomuraea ferruginea*, type strain IMET 9567. Sporulating aerial mycelium. Grown on oatmeal agar at 28°C for 18 d.

Type strain: ATCC 33516, BCRC 12546, DSM 43674, NBRC 14680, INMI 104, JCM 3321, KCTC 9268, VKM Ac-804.

Sequence accession no. (16S rRNA gene): U48844.

10. **Nonomuraea ferruginea** corrig. (Meyer 1981) Zhang, Wang and Ruan 1998b, 419[VP] (*Actinomadura ferruginea* Meyer 1981, 215; *Microtetraspora ferruginea* Kroppenstedt, Stackebrandt and Goodfellow 1990a, 148)

fer.ru.gi′ne.a. L. fem. adj. *ferruginea* rusty brown, referring to the orange-brown-colored substrate mycelium.

Spore chains short, hooked, or irregular spirals of 1–2 turns, arranged monopodially on long aerial hyphae, 4–9 spores per chain (Figure 379). Spore surface smooth or irregularly folded. Optimum temperature: 28–30°C.

Inorganic salts-starch agar: poor growth, surface leathery, aerial mycelium absent, substrate mycelium color-

less to brownish, no diffusible pigment. Oatmeal agar: growth abundant, surface farinaceous, aerial mycelium white to pale pink, substrate mycelium pink, no diffusible pigment. Oatmeal-nitrate agar: growth abundant, surface dusty, aerial mycelium white to pale pink, substrate mycelium orange-pink, no diffusible pigment. Peptone-glucose medium: growth moderate, surface cartilaginous, aerial mycelium absent, substrate mycelium light brown, no diffusible pigment. Yeast extract-malt extract agar: growth abundant, surface farinaceous, aerial mycelium orange-pink, substrate mycelium bright orange-brown, no diffusible pigment.

Cell hydrolysates contain galactose, madurose, and ribose. Diphosphatidylglycerol, phosphatidylinositolmannosides, ninhydrin and sugar positive phospholipids, hydroxylated phosphatidylethanolamine, and uncharacterized glycolipids are present; phosphatidylethanolamine, phosphatidylglycerol, and phosphatidylinositol are absent.

Additional chemotaxonomic data are given in Table 284, and the fatty acid pattern of the type strain is shown in Table 285.

Positive for arginine dihydroloase, acetoin, citrate lyase, β-galactosidase, gelatinase, lysine decarboxylase, ornithine decarboxylase, and urease activities. L-Arabinose, D-fructose, D-mannose, L-rhamnose, sucrose, and D-xylose are utilized as sole carbon sources, but cellobiose, *meso*-inositol, and D-raffinose are not.

DNA G+C content (mol%): 68.1 (T_m).

Type strain: 14094, ATCC 35575, CCM 3424, BCRC 12537, CIP 106925, DSM 43553, NBRC 14094, IMET 9567, JCM 3283, KCTC 9269, NCIMB 11630, NRRL B-16096, VKM Ac-854.

Sequence accession no. (16S rRNA gene): U48845.

11. **Nonomureae helvata** corrig. (Nonomura and Ohara 1971a) Zhang, Wang and Ruan 1998b, 419[VP] (*Actinomadura helvata* Nonomura and Ohara 1971a, 904; *Microtetraspora ferruginea* Kroppenstedt, Stackebrandt and Goodfellow 1990a, 148)

hel.va′ta. N.L. fem. adj. *helvata* honey-yellow, referring to the color of the substrate mycelium.

Spore chains with spirals of 3–5 turns are seen, or hooks and pseudosporangia on long aerial hyphae. About 10 spores per chain. Spore surface smooth.

Inorganic salts-starch agar: moderate growth, surface farinaceous, aerial mycelium white to cream colored, substrate mycelium yellowish brown, no diffusible pigment. Oatmeal agar: moderate growth, surface leathery, aerial mycelium a filmy cover of sterile hyphae, substrate mycelium yellowish brown, no diffusible pigment. Oatmeal-nitrate agar: good growth, surface farinaceous, aerial mycelium white, substrate mycelium cream colored, no diffusible pigment. According to Nonomura and Ohara (1971a), B vitamins are essential for growth. Yeast extract-malt extract agar: good growth, surface farinaceous, aerial mycelium yellowish white, substrate mycelium yellowish brown, no diffusible pigment.

Cell hydrolysates contain galactose, madurose, ribose, and traces of mannose. Diphosphatidylglycerol, phosphatidylglycerol, phosphatidylinositolmannosides, ninhydrin and sugar positive phospholipids, hydroxylated phosphatidylethanolamine, and uncharacterized glycolipids are present; phosphatidylethanolamine and phosphatidylinositol are absent. Esculin is hydrolyzed. Shows arginine dihydrolase, β-galactosidase, gelatinase, lysine decarboxylase, ornithine decarboxylase, and urease activities, but does degrade casein, gelatin, hypoxynthine, starch, tyrosine, or xanthine. Cellobiose, *meso*-inositol, sucrose, and D-xylose are utilized as sole carbon sources.

Further physiological characteristics are given in Table 286.

Additional chemotaxonomic data are given in Table 284, and the fatty acid pattern of the type strain is shown in Table 285.

DNA G+C content (mol%): not determined.

Type strain: ATCC 27295, CBS 259.72, BCRC 13402, DSM 43142, NBRC 14681, IMET 9584, JCM 3143, KCTC 9274, NCIMB 11115, NRRL B-16123, PCM 2199.

Sequence accession no. (16S rRNA gene): U48975.

12. **Nonomuraea kuesteri** Kämpfer, Kroppenstedt and Grün-Wollny 2005, 848[VP]

ku.es´te.ri. N.L. gen. masc. n. *kuesteri* of Küster, in honor of Eberhard Küster, a German microbiologist, in recognition of his numerous contributions to the taxonomy of actinomycetes.

Spiral spore chains with 3–5 turns. Spore ornamentation not determined. Yellow substrate mycelium, a trace of aerial mycelium, but no soluble pigments formed on oatmeal agar.

Cell hydrolysates contains madurose. Diphosphatidylglycerol, phosphatidylethanolamine, phosphatidylglycerol, phosphatidylinositol, phosphatidylinositolmannosides, ninhydrin-and sugar-positive phospholipids, hydroxylated phosphatidylethanolamine, and uncharacterized glycolipids are present.

Additional chemotaxonomic data are given in Table 284, and the fatty acid pattern of the type strain is shown in Table 285.

Does not produce acid from adonitol, L-arabinose, arabitol, cellobiose, dulcitol, erythritol, glucose, *meso*-inositol, lactose, maltose, D-mannitol, melibiose, methyl D-glucoside, raffinose, L-rhamnose, salicin, sorbitol, sucrose, trehalose, and D-xylose. *N*-Acetyl-D-glucosamine, adonitol, L-aspartate (weak), L-arabinose, D-cellobiose, fumarate (weak), D-fructose, D-galactose, D-glucose, DL-lactate, malate, D-mannitol, D-mannose, D-melibiose, 2-oxoglutarate, L-proline, L-rhamnose, sucrose, D-trehalose, and D-xylose are used as sole carbon sources after 7 d, but acetate, *N*-acetyl-D-galactosamine, *cis*-aconitate, *trans*-aconitate, adipate, 4-aminobutyrate, L-alanine, β-alanine, *p*-arbutin, azelate, citrate, D-gluconate, glutarate, L-histidine, DL-3-hydroxybenzoate, DL-4-hydroxybenzotae, DL-3-hydroxybutyrate, itaconate, *meso*-inositol, L-leucine, maltitol, D-maltose, mesaconate, L-ornithine, L-phenylalanine, L-phenylacetate, putrescine, propionate, pyruvate, D-ribose, salicin, L-serine, sorbitol, suberate, and L-tryptophan are not.

Further physiological characteristics are given in Table 286.

DNA G+C content (mol%): not determined.

Type strain: GW 14-1925, DSM 44753, JCM 13854, NRRL B-24325.

Sequence accession no. (16S rRNA gene): AJ746362.

13. **Nonomuraea longicatena** Chiba, Suzuki and Ando 1999, 1628[VP]

lon.gi.ca.te′na. L. adj. *longus* long; L. n. *catena* chain; N.L. n. *longicatena* long chain, referring to long spore chains.

Straight spore chains with 10–30 spores per chain. Spore ornamentation smooth. No growth at 45°C.

Oatmeal agar: substrate mycelium growth is ochre, white aerial mycelium, no soluble pigment. Yeast extract-malt extract agar: abundant growth, ochre substrate mycelium, white aerial mycelium.

Cell hydrolysates contain madurose. Diphosphatidylglycerol, phosphatidylethanolamine, ninhydrin and sugar positive phospholipids, hydroxylated phosphatidylethanolamine, and uncharacterized glycolipids are present.

Additional chemotaxonomic data are given in Table 284, and the fatty acid pattern of the type strain is shown in Table 285.

Esculin is hydrolyzed; nitrate is not reduced. Degrades casein, elastin, hypoxanthine, and starch.

L-Arabinose, D-mannose, and D-raffinose are utilized as sole carbon sources, but cellobiose, *meso*-inositol, and L-rhamnose are not. Positive for arginine dihydrolase, β-galactosidase, lysine decarboxylase, ornithine decarboxylase, and urease activities. Production of gelatinase is negative; Grows in the presence of up to 3% NaCl.

DNA G+C content (mol%): 71.7 (HPLC).

Type strain: K-252, NBRC 16462, JCM 11136, NRRL 15532.

Sequence accession no. (16S rRNA gene): AB018787.

14. **Nonomuraea maheshkhaliensis** Ara, Kudo, Matsumoto, Takahashi and Ōmura 2007c, 2449[VP] (Effective publication: Ara, Kudo, Matsumoto, Takahashi and Ōmura 2007a, 164.)

ma.hesh.kha.li.en.sis. N.L. fem adj. *maheshkhaliensis* of or pertaining to Maheshkhali, Bangladesh.

Forms a well-developed, branched substrate mycelium. Abundant aerial mycelia are present. Spore chains are spiral with 2–4 turns (17–20 spores). Sporangia are not detected. Spores are spherical to cylindrical and spore surface is folded and rough.

Good growth occurs on Bennett's agar, glucose-yeast extract agar, Hickey–Tresner agar, oatmeal agar (ISP3), oatmeal-nitrate agar, Waksman no. 1 agar, and yeast extract-malt extract agar (ISP2); moderate growth on glycerol-asparagine agar, inorganic salts-starch agar (ISP4), nutrient agar, sucrose-nitrate agar, tyrosine agar (ISP-7) water agar, and yeast extract-malt extract agar; poor growth on glucose-asparagine agar.

Substrate mycelium color is mustard to light wheat color on various agar media.

White aerial mycelia and sporulation occur on ISP3, ISP4 agar, oatmeal-nitrate agar and water agar after 14 d of incubation at 28°C.

A soluble pigment is not produced. Aerobic, Gram-stain-positive, and able to grow at pH 5.0–9.0.

The temperature range for growth is between 15 and 37°C. Production of melanoid pigments is negative. No growth on 4% NaCl.

The isomer of A_2pm was *meso* and galactose, glucose, madurose, mannose, ribose (trace) in the whole-cell hydrolysates. The *N*-acyl type of muramic acid is acetyl. Mycolic acids are absent. The polar lipids include phosphatidylethanolamine and phospholipids of unknown structure containing glucosamine. Phosphatidylcholine is absent.

The menaquinone is MK-9(H_4). Fatty acids with major amounts of $C_{16:0}$ (15.5%), $C_{16:0}$ iso (13.8%), and $C_{17:0}$ 10 methyl (9.6%).

Source: mangrove rhizosphere soil in Maheshkhali, Bangladesh.

DNA G+C content (mol%): 72.0 (HPLC).

Type strain: 16-5-14, JCM 13929, MTCC 8545.

Sequence accession no. (16S rRNA gene): AB290014.

15. **Nonomuraea polychroma** corrig. (Galatenko, Terekhova and Preobrazhenskaya 1981) Zhang, Wang and Ruan 1998b, 419[VP] (*Actinomadura polychroma* Galatenko, Terekhova and Preobrazhenskaya 1981, 803; *Microtetraspora polychroma* Kroppenstedt, Stackebrandt and Goodfellow 1990a, 148)

po.ly.chro′ma. Gr. adj. *polus* many; Gr. n. *chroma* color; N.L. n. *polychroma* intended to mean that the bacterium produces many colors (Note: the epithet *polychroma* is not correct, however, it is not possible to correct it.)

Short spore chains are produced in the form of spirals or pseudosporangia. Spore surface smooth.

Colorless to brown substrate mycelium, traces of white aerial mycelium, but no diffusible pigments formed on oatmeal agar.

The cultures of this species have no antagonistic activity with respect to various test-microbes.

Cell hydrolysates contain madurose and mannose. Diphosphatidylglycerol, phosphatidylethanolamine, ninhydrin and sugar positive phospholipids, hydroxylated phosphatidylethanolamine, and uncharacterized glycolipids are present, as are traces of phosphatidylinositolmannosides.

Additional chemotaxonomic data are given in Table 284, and the fatty acid pattern of the type strain is shown in Table 285.

Does not use L-arabinose, cellobiose, fructose, *meso*-inositol, mannose, raffinose rhamnose, sucrose, or D-xylose as sole carbon sources. Esculin is hydrolyzed, nitrate is not reduced. Elastin, gelatin, and hypoxanthine are degraded. Acetoin, arginine dihydrolase, lysine decarboxylase, and ornithine decarboxylase activities are positive.

DNA G+C content (mol%): not determined.

Type strain: ATCC 49500, DSM 43925, NBRC 14345, IMET 9743, INA 2755, JCM 6834, KCTC 9277, NRRL B-16243, VKM Ac-1084.

Sequence accession no. (16S rRNA gene): U48977.

16. **Nonomuraea recticatena** corrig. (Gauze, Terekhova, Galatenko, Preobrazbenskaya, Borisova and Fedorova 1984) Zhang, Wang and Ruan 1998b (*Actinomadura recticatena* Gauze, Terekhova, Galatenko, Preobrazbenskaya,

Borisova and Fedorova 1984, 3; *Microtetraspora recticatena* Kroppenstedt, Stackebrandt and Goodfellow 1990a, 148)

rec.ti.ca.te′na. L. part. adj. *rectus* straight; L. n. *catena* a chain; N.L. n. *recticatena* (nominative in apposition), a straight chain.

Straight chains of 4–20 spores. Spore ornamentation smooth. The aerial mycelium develops white to cream color on oatmeal agar; substrate mycelium growth is dark yellow to brown; no soluble pigment found.

Cell hydrolysates contain galactose and traces of ribose. Diphosphatidylglycerol, phosphatidylethanolamine, ninhydrin and sugar positive phospholipids, hydroxylated phosphatidylethanolamine, uncharacterized glycolipids, and traces of phosphatidylinositol are present; phosphatidylglycerol and phosphatidylinostol mannosides are absent.

Further chemotaxonomic data are given in Table 284, and the fatty acid pattern of the type strain is shown in Table 285.

Positive for esculin hydrolysis and nitrate reductase. Degrades elastin, gelatin, hypoxanthine, and starch. Positive for acetoin and urease activities. Arginine dihydrolase, citrate lyase, β-galactosidase, lysine decarboxylase, and ornithine decarboxylase are not produced. Further physiological characteristics are given in Table 286. L-Arabinose, *meso*-inositol, and L-rhamnose used as sole carbon sources but cellobiose, D-fructose, D-mannose, D- raffinose, sucrose, and D-xylose are not.

DNA G+C content (mol%): not determined.

Type strain: DSM 43937, NBRC 14525, INA 308, JCM 6835, KCTC 9279, VKM Ac-940.

Sequence accession no. (16S rRNA gene): AJ404230, U48979.

17. **Nonomuraea rosea** Kämpfer, Busse, Tindall, Nimtz and Grün-Wollny 2010, 1123[VP]

ro.se′a. L. fem. adj. *rosea* rose-colored or rosy, referring to the pinkish color of the colonies.

Forms an extensive branched substrate mycelium. A white aerial mycelium is visible on oatmeal agar. Spore chains are spiral; sporangia are not detected. Gram-stain-positive and oxidase positive; shows oxidative metabolism.

Good growth occurs after 3 d incubation on oatmeal agar and nutrient agar (Oxoid) at 25–30°C.

The polar lipids include the major compound diphosphatidylglycerol, moderate amounts of phosphatidylmonomethylethanolamine, phosphatidylethanolamine, hydroxy-phosphatidylethanolamine, hydroxy-phosphatidylmonomethylamine, and an unknown aminophosphoglycolipid. Phosphatidylinositol-mannosides and phosphatidylinositol are present along with small amounts of an unknown phospholipid.

Does not produce acids from the following carbon sources: glucose, lactose, sucrose, D-mannitol, dulcitol, salicin, adonitol, inositol, sorbitol, L-arabinose, raffinose, L-rhamnose, maltose, D-xylose, trehalose, cellobiose, methyl D-glucoside, erythritol, melibiose, and arabitol. The following carbon sources are utilized (after 7 d incubation): *N*-acetyl-D-galactosamine, *N*-acetyl-D-glucosamine, D-cellobiose, D-fructose, D-galactose, D-glucose, D-maltose, D-mannose, L-rhamnose, D-trehalose, D-xylose, adonitol, inositol, 4-aminobutyrate, citrate, fumarate (weak), DL-malate, L-aspartate (weak), and L-proline. The following carbon sources are not utilized: *p*-arbutin, D-gluconate, D-melibiose, D-ribose, sucrose, salicin, maltitol, D-mannitol, sorbitol, putrescine, acetate, propionate, *cis*-aconitate, *trans*-aconitate, adipate, azelate, glutarate, DL-3-hydroxybutyrate, itaconate, mesaconate, pyruvate, DL-lactate, suberate, 2-oxoglutarate, L-alanine, β-alanine, L-histidine, L-leucine, L-ornithine, L-phenylalanine, L-serine, L-tryptophan, DL-3-hydroxybenzoate, DL-4-hydroxybenzoate, and L-phenylacetate.

The fatty acids comprise mainly iso- and 10-heptadecanoic-branched fatty acids. Smaller amounts of unsaturated fatty acids are also detected.

Source: a soil sample.

DNA G+C content(mol%): not determined.

Type strain: GW 12687, CCUG 56107, DSM 45177.

Sequence accession no. (16S rRNA gene): FN356742.

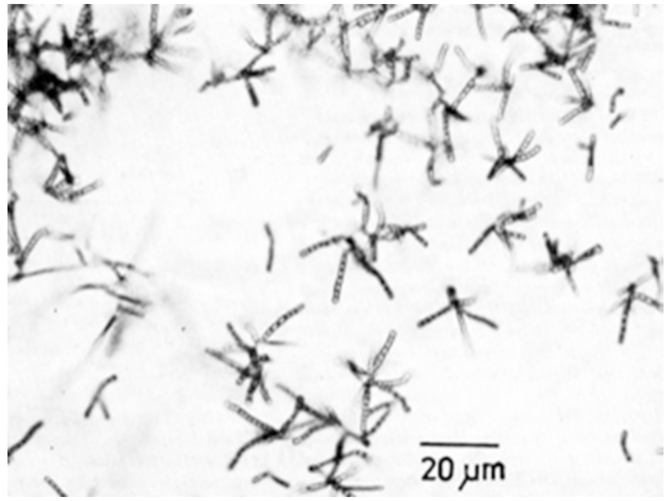

FIGURE 380. *Nonomuraea roseola*, type strain INA 1671. Sporulating aerial mycelium. Grown on oatmeal-nitrate agar at 28°C for 12 d.

18. **Nonomuraea roseola** corrig. (Lavrova and Preobrazhenskaia 1975) Zhang, Wang and Ruan 1998b, 420[VP] (*Actinomadura roseola* Lavrova and Preobrazhenskaya 1975, 483; *Microtetraspora roseola* Kroppenstedt, Stackebrandt and Goodfellow 1990a, 148)

ro.se.o′la. L. adj. *roseus* rose-colored; L. fem. suff. *-ola* diminutive ending; N.L. fem. dim. adj. *roseola* intended to mean with a rosy tinge, referring to the rose-colored aerial mycelium.

Spore chains with spirals of 3–5 turns, or straight chains with 6–30 spores per chain. Spore chains may be stalked or in clusters emerging directly from the agar (Figure 380). Spores elliptical. Spore surface folded.

Czapek sucrose agar: good growth, surface farinaceous, aerial mycelium white, substrate mycelium colorless, no diffusible pigment. Glycerol-asparagine agar: good growth, surface farinaceous, aerial mycelium white to pale pink, substrate mycelium pink to orange colored, no diffusible pigment. Oatmeal agar: abundant growth, surface farinaceous, aerial mycelium pink, substrate mycelium brownish red, no diffusible pigment. Peptone-glucose medium:

good growth, surface leathery, aerial mycelium absent, substrate mycelium yellow to orange colored, no diffusible pigment. Yeast extract-malt extract agar: abundant growth, surface farinaceous, aerial mycelium pink, substrate mycelium rusty brown, no diffusible pigment.

Cell hydrolysates contain galactose, madurose, and ribose; variable for mannose. Diphosphatidylglycerol, phosphatidylethanolamine, ninhydrin and sugar positive phospholipids, hydroxylated phosphatidylethanolamine, and uncharacterized glycolipids are present, as are traces of phosphatidylglycerol; phosphatidylinositol and phosphatidylinositolmannosides are absent.

Additional chemotaxonomic date are given in Table 284, and the fatty acid pattern of the type strain is shown in Table 285.

Esculin is hydrolyzed and nitrate reduced. Degrades gelatin, hypoxanthine, and tyrosine. Positive for arginine dihydrolase, citrate lyase, β-galactosidase, lysine decarboxylase, ornithine decarboxylase, and urease. *meso*-Inositol, L-rhamnose and sucrose are utilized as sole carbon source, but L-arabinose, cellobiose, D-fructose, D-mannose, D-raffinose, and D-xylose are not.

Additional physiological characteristics are given in Table 286.

DNA G+C content (mol%): 66.2 (T_m).

Type strain: ATCC 33579, DSM 43767, NBRC 14685, IMET 9576, INA 1671, JCM 3323, KCTC 9282, VKM Ac-1180.

Sequence accession no. (16S rRNA gene): AJ278221, U48980.

19. **Nonomuraea roseoviolacea** (Nonomura and Ohara 1971a) *Nonomuria roseoviolacea* Zhang, Wang and Ruan 1998b, 420[VP] (*Actinomadura roseoviolacea* Nonomura and Ohara 1971a, 909; *Microtetraspora roseviolacea* Kroppenstedt, Stackebrandt and Goodfellow 1990a, 148)

ro.se.o.vi.o.la′ce.a. L. adj. *roseus* rosy; L. adj. *violaceus* violet colored; N.L. fem. adj. *roseoviolacea* rosy, violet colored, referring to the color of the substrate mycelium (Note: the epithet *roseoviolacea* is not correct, however, it is not possible to correct it.).

Pseudosporangia spore chains with 4–20 spores per chain. Spore ornamentation smooth.

Glucose-asparagine agar: moderate growth, surface slightly farinaceous on the edge of the colonies, substrate mycelium brownish red, pale violet diffusible pigment. Glycerol-asparagine agar: good growth, surface leathery, aerial mycelium absent, substrate mycelium brick red, no diffusible pigment. Inorganic salts-starch agar: good growth, surface farinaceous, aerial mycelium white, substrate mycelium white to pale pink, no diffusible pigment. Oatmeal agar: good growth, surface farinaceous, aerial mycelium pale pink to violet, substrate mycelium pale violet, pale violet diffusible pigment. Yeast extract-malt extract agar: good growth, surface farinaceous to granular, aerial mycelium white to pale pink, substrate mycelium dark purple, no diffusible pigment.

Cell hydrolysates contain madurose, mannose, and ribose. Diphosphatidylglycerol, phosphatidylinositolmannosides, ninhydrin and sugar positive phospholipids, hydroxylated phosphatidylethanolamine, and uncharacterized glycolipids are present; phosphatidylethanolamine, phosphatidylglycerol, and phosphatidylinositol are absent.

Additional chemotaxonomic data are given in Table 284, and the fatty acid pattern of the type strain is shown in Table 285.

Esculin hydrolyzed and nitrate reduced; DNA and hypoxanthine are degraded. L-Arabinose, D-fructose, D-mannose, D-raffinose, L-rhamnose and sucrose, are utilized as sole carbon sources, but cellobiose is not. Positive for acetoin, arginine dihydrolase, lysine decarboxylase, ornithine decarboxylase, and urease activities, but negative for β-galactosidase activity.

DNA G+C content (mol%): 68.5 (T_m).

Type strain: AS 4.1072, ATCC 27297, CBS 260.72, CCM 3491, BCRC 13406, CIP 106924, DSM 43144, NBRC 14098, IMET 9751, JCM 3145, KCTC 9283, NCIMB 11117, NRRL B-16127, VKM Ac-909.

Sequence accession no. (16S rRNA gene): AB039959, AB043101.

Additional comments: in their original description, Nonomura and Ohara (1971a) reported on antibacterial activity of the type strain against *Staphylococcus aureus*. Further investigation of this strain (J. Meyer, unpublished) revealed an active agent similar to daunomycin.

19a. **Nonomuraea roseoviolacea subsp. roseoviolacea** (Nonomura and Ohara 1971a) *Nonomuria roseoviolacea* Zhang, Wang and Ruan 1998b, 420[VP] (*Actinomadura roseoviolacea* Nonomura and Ohara 1971a, 909; *Microtetraspora roseviolacea* Kroppenstedt, Stackebrandt and Goodfellow 1990a, 148)

The original description of *Nonomuraea roseoviolacea* subsp. *roseoviolacea* given by Nonomura and Ohara (1971a) is unchanged. In addition, this subspecies shows reduction of nitrate and liquefies gelatin. Sucrose is not utilized as a sole carbon source. DNA–DNA similarity to *Nonomuraea roseoviolacea* subsp. *carminata* ranges from 49–60%.

DNA G+C content (mol%): 68.5 (T_m).

Type strain: AS 4.1072, ATCC 27297, CBS 260.72, CCM 3491, BCRC 13406, CIP 106924, DSM 43144, NBRC 14098, IMET 9751, JCM 3145, KCTC 9283, NCIMB 11117, NRRL B-16127, VKM Ac-909.

Sequence accession no. (16S rRNA gene): AB039959, AB043101.

19b. **Nonomuraea roseoviolacea subsp. carminata** (Gauze, Sveshnikova, Ukholina, Gavrilina, Filicheva and Gladkikh 1973) Gyobu and Miyadoh 2001, 887[VP] (*Actinomadura carminata* Gauze, Sveshnikova, Ukholina, Gavrilina, Filicheva and Gladkikh 1973, 675)

car.mi.na′ta. N.L. fem. adj. *carminata* (from Fr. *carmin*), carmine.

Substrate hyphae are long, irregularly branched, and do not fragment into short elements. Aerial hyphae branch monopodially and are 0.3–0.4 μm in diameter. Spore chains are formed on the aerial hyphae in tightly closed spirals. Well-developed spore chains are enveloped in a slimy mass, giving the appearance of sporangia (pseudosporangia). Spores are oval to cylindrical in shape and 0.6–1.2 μm in size, with smooth surfaces.

A pink aerial hyphal mass, and a wine-red soluble pigment is formed on yeast-extract–malt extract agar.

Starch is hydrolyzed. Grows at 42°C. Reduction of nitrate, liquefaction of gelatin, peptonization of milk, and production of melanoid pigments are negative. Grows in the presence of 3% NaCl. L-Arabinose, D-fructose, D-galactose D-glucose, L-rhamnose, sucrose, and D-xylose are used as sole carbon sources, but *meso*-inositol, D-mannitol and raffinose are not.

Source: soil.

DNA G+C content (mol%): not determined.

Type strain: DSM 44170, NBRC 15903, INA 4281, JCM 9946, VKM Ac-1780.

Sequence accession no. (16S rRNA gene): AB039961.

20. **Nonomuraea rubra** corrig. (Sveshnikova, Maksimova and Kudrina 1969) Zhang, Wang and Ruan 1998b, 420^{VP} (*Micromonospora rubra* Sveshnikova, Maksimova and Kudrina 1969, 883; *Actinomadura rubra* Meyer and Sveshnikova 1974, 167)

ru′bra. L. fem. adj. *rubra* red-colored, referring to the color of substrate mycelium.

Spore chains with 4–20 spores per chain form curled hooks, spirals with 1–2 turns or spirals with 3–5 turns. Spore ornamentation smooth. Sporulating aerial mycelium mostly seen only in microscopically visible traces after 4–6 weeks of incubation on oatmeal agar, oatmeal-nitrate agar, and Bennett sucrose agar.

Bennett's sucrose agar: good growth, surface cartilaginous or wrinkled, aerial mycelium only traces and pinkish gray, substrate mycelium red to orange, red diffusible pigment. Oatmeal agar: good growth, surface wrinkled, aerial mycelium very poorly developed and mostly sterile or coremia-like, substrate mycelium bright orange-red, pale red diffusible pigment. Oatmeal-nitrate agar: moderate growth, surface cartilaginous glistening, aerial mycelium only traces and pinkish-gray, substrate mycelium red to orange, no diffusible pigment. Yeast extract-malt extract agar: moderate growth, surface cartilaginous glistening, aerial mycelium only traces of sterile hyphae, substrate mycelium dark red to brown, no diffusible pigment.

Cell hydrolysates contain galactose, madurose, ribose, and traces of mannose. The cell-wall composition of *Nonomuraea rubra* shows a slight difference from type III/B of Lechevalier and Lechevalier (1970b). In addition to *meso*-A_2pm, minor amounts of L-A_2pm and glycine have been found.

Diphosphatidylglycerol, phosphatidylinositol, phosphatidylinositol mannosides, ninhydrin and sugar positive phospholipids, hydroxylated phosphatidylethanolamine, and uncharacterized glycolipids are present; Phosphatidylethanolamine and phosphatidylglycerol are absent.

Additional chemotaxonomic data are given in Table 284, and the fatty acid pattern of the type strain is shown in Table 285.

Nitrate is reduced but esculin is not hydrolyzed. Degrades elastin, gelatin, hypoxanthine, starch, and tyrosine. Positive for acetoin, arginine dihydrolase, citrate lyase, β-galactosidase, gelatinase, lysine decarboxylase, ornithine decarboxylase, and urease activities. L-Arabinose, cellobiose, D-fructose, *meso*-inositol, D-mannose, D-raffinose, L-rhamnose, sucrose, and xylose are utilized as sole carbon sources.

Produces the antibiotic maduramycin, a red pigment with indicator properties (pH <7.0 yellow, pH >7.0 red) which possesses a strong antimicrobial activity against Gram-stain-positive bacteria (Fleck et al., 1978).

DNA G+C content (mol%): not determined.

Type strain: ATCC 27031, CBS 132.76, BCRC 12591, CIP 107008, DSM 43768, NBRC 14070, NBRC 14686, IMET 8181, INA 325, JCM 3234, JCM 3389, KCTC 9284, NRRL B-16083, VKM Ac-615.

Sequence accession no. (16S rRNA gene): AF277200.

21. **Nonomuraea salmonea** corrig. (Preobrazhenskaya, Lavrova, Ukholina and Nechaeva 1975b) Zhang, Wang and Ruan 1998b, 420^{VP} (*Actinomadura salmonea* Preobrazhenskaya, Lavrova, Ukholina and Nechaeva 1975b, 408; *Microtetraspora salmonea* Kroppenstedt, Stackebrandt and Goodfellow 1990a, 148)

sal.mo′ne.a. N.L. fem. adj. *salmonea* from L. n. *salmo* salmon, salmon colored referring to the color of substrate mycelium.

Spore chains with 4–30 spores per chain form curled hooks or spirals with 1–2 turns. Spore ornamentation folded or warty.

Inorganic salts-starch agar: moderate growth, surface wrinkled, aerial mycelium only traces of cream colored mycelium, substrate mycelium pale brown, no diffusible pigment. Oatmeal agar: good growth, surface farinaceous, aerial mycelium pale pink, substrate mycelium dark red, no diffusible pigment. Yeast extract-malt extract agar: moderate growth, surface cartilaginous, aerial mycelium cream colored to pinkish, substrate mycelium light brown, no diffusible pigment.

Cell hydrolysates contain galactose, madurose, mannose, and ribose. Phosphatidylinositol, phosphatidylinositolmannosides, ninhydrin and sugar positive phospholipids, hydroxylated phosphatidylethanolamine, and uncharacterized glycolipids are present; diphosphatidylglycerol, phosphatidylethanolamine, and phosphatidylglycerol absent.

Additional chemotaxonomic data are given in Table 284, and the fatty acid pattern of the type strain is shown in Table 285.

Esculin is hydrolyzed and nitrate reduced. Degrades casein, DNA, elastin, gelatin, hypoxanthine, and tyrosine. Positive for acetoin, arginine dihydrolase, citrate lyase, β-galactosidase, lysine decarboxylase, ornithine decarboxylase, and urease production activities. L-Rhamnose and sucrose are utilized as sole carbon source, but L-arabinose, cellobiose, *meso*-inositol, D-fructose, D-mannose, D-raffinose, and D-xylose are not.

DNA G+C content (mol%): 66.0 (T_m).

Type strain: ATCC 33580, CIP 107009, DSM 43678, NBRC 14687, IMET 9582, INA 2488, JCM 3324, KCTC 9285, PCM 2201, VKM Ac-913.

Sequence accession no. (16S rRNA gene): U48982, X97892.

22. **Nonomuraea spiralis** corrig. (Meyer 1979) Zhang, Wang and Ruan 1998b, 420^{VP} (*Actinomadura spiralis* Meyer 1979, 39; *Microtetraspora spiralis* Kroppenstedt, Stackebrandt and Goodfellow 1990a, 148)

spi.ra′lis. N.L. fem. adj. *spiralis* (from L. n. *spira* coil; and L. suff. *alis -is -e*, suffix denoting pertaining to), coiled, referring to the morphology of the spore chains.

Spore chains in spirals of 2–5 turns, closely packed or more or less loose spirals in pseudoverticillate arrange-

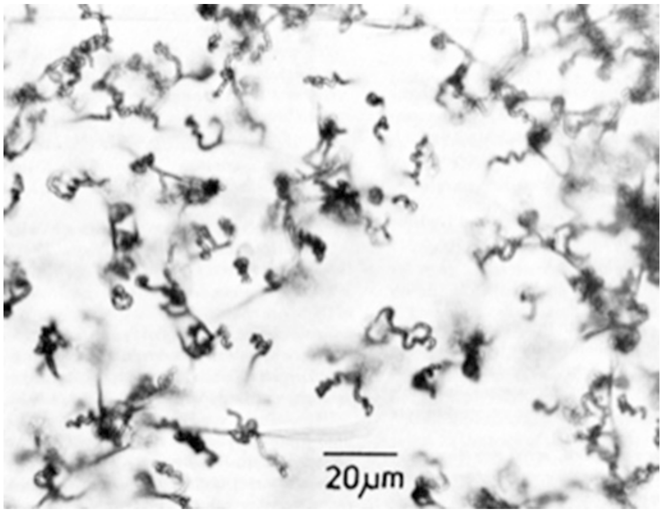

FIGURE 381. *Nonomuraea spiralis*, type strain IMET 9621. Sporulating aerial mycelium. Grown on oatmeal-nitrate agar at 28°C for 18 d.

FIGURE 382. *Nonomuraea spiralis*, type strain IMET 9621. Electron micrograph of spore chain.

ment along short straight aerial hyphae (Figure 381 and Figure 382). 10–15 Spores per chain. Spores spherical to subspherical. Spore surface folded.

Inorganic salts-starch agar: poor growth, surface leathery, aerial mycelium absent, substrate mycelium yellow to yellowish brown, no diffusible pigment. Oatmeal agar: good growth, surface woolly to farinaceous, aerial mycelium white to yellowish white, substrate mycelium yellow to yellowish brown, no diffusible pigment. Oatmeal-nitrate agar: good growth, surface farinaceous, aerial mycelium white, substrate mycelium whitish, no diffusible pigment. Peptone-glucose medium: poor growth, surface leathery, aerial mycelium absent, substrate mycelium yellow to yellowish brown, no diffusible pigment. Yeast extract-malt extract agar: moderate growth, surface farinaceous, aerial mycelium white to yellowish, substrate mycelium yellow to yellowish brown; no diffusible pigment.

Cell hydrolysates contain madurose, mannose, and ribose. Diphosphatidylglycerol, phosphatidylinositolmannosides, ninhydrin and sugar positive phospholipids, and hydroxylated phosphatidylethanolamine are present, but phosphatidylethanolamine, phosphatidylglycerol, and phosphatidylinositol are absent.

Additional chemotaxonomic data are given in Table 284, and the fatty acid pattern of the type strain is shown in Table 285.

Esculin is hydrolyzed and nitrate is reduced. Positive for acetoin and β-galactosidase activities, but negative for arginine dihydrolase, citrate lyase, gelatinase, lysine decarboxylase, ornithine decarboxylase, and urease activities. D-Mannose, L-rhamnose, sucrose, and D-xylose are used as sole carbon sources, but L-arabinose, cellobiose, D-fructose, *meso*-inositol, and D-raffinose are not.

DNA G+C content (mol%): 68.1 (T_m).

Type strain: ATCC 35114, CCM 3426, BCRC 13309, CIP 106923, DSM 43555, NBRC 14097, IMET 9621, JCM 3286, KCTC 9286, NCIMB 11633, NRRL B-16098, VKM Ac-853.

Sequence accession no. (16S rRNA gene): U48983.

23. **Nonomuraea turkmeniaca** corrig. (Terekhova, Galatenko and Preobrazhenskaya 1982) Zhang, Wang and Ruan 1998b, 420[VP] (*Actinomadura turkmeniaca* Terekhova, Galatenko and Preobrazhenskaya 1982, 87; *Microtetraspora turkmeniaca* Kroppenstedt, Stackebrandt and Goodfellow 1990a, 148)

turk.me.ni.a′ca. N.L. fem. adj. *turkmeniaca* pertaining to Turkmen.

Spore chains with spirals of 3–5 turns. Spore ornamentation smooth.

Violet to red substrate mycelium, traces of aerial hyphae, and a pink to violet soluble pigment are formed on oatmeal agar.

Cell hydrolysates contain madurose, mannose, and ribose. Diphosphatidylglycerol, phosphatidylethanolamine, ninhydrin and sugar positive phospholipids, hydroxylated phosphatidylethanolamine, and uncharacterized glycolipids are present, but phosphatidylglycerol, phosphatidylinositol, and phosphatidylinositolmannosides are absent.

Esculin is hydrolyzed and nitrate reduced. Degrades casein, gelatin, hypoxanthine, and starch. Positive for acetoin, arginine dihydrolase, citrate lyase, β-galactosidase, lysine decarboxylase, ornithine decarboxylase, and urease, activities. L-Arabinose, D-fructose, *meso*-inositol, D-mannose, D-raffinose L-rhamnose, sucrose, and D-xylose, are used as sole carbon sources.

DNA G+C content (mol%): not determined.

Type strain: ATCC 49501, CIP 107010, DSM 43926, JCM 6836, NBRC 14348, IMET 9747, INA 3344, KCTC 9287, NRRL B-16246, VKM Ac-852.

Sequence accession no. (16S rRNA gene): AF277201.

Additional organisms

1. **Nonomuraea terrinata** Quintana, Maldonado and Goodfellow 2003, 5[VP]

te.rri.na′ta L. fem. n. *terra* soil; L. part. fem. adj. *nata* born; N.L. fem. part. adj. *terrinata* born from the soil, referring to the source of the isolate.

Spore chains of 8–15 spores, irregular pseudosporangia. Grows well between 20 and 37°C.

A white to ochre substrate mycelium and an abundant aerial mycelium are formed on oatmeal agar, but soluble pigments are not.

Cell hydrolysates contain madurose.

Diphosphatidylglycerol, phosphatidylethanolamine, ninhydrin and sugar positive phospholipids, hydroxylated phosphatidylethanolamine, and uncharacterized glycolipids are present, but phosphatidylglycerol is absent.

Additional chemotaxonomic data are given in Table 284, and the fatty acid pattern of the type strain is shown in Table 285.

Esculin is hydrolyzed, but nitrate is not reduced. Degrades casein, DNA, hypoxanthine, and starch, but not elastin, gelatin, tyrosine, or xanthine.

Source: soil collected in South Korea.

DNA G+C content (mol%): not determined.

Type strain: E626, DSM 44505, NCIMB 13942.

Sequence accession no. (16S rRNA gene): AF302078.

Genus VII. **Planobispora** Thiemann and Beretta 1968, 157[AL]

Gernot Vobis, Nicole Lodders and Peter Kämpfer

Pla.no.bi.spo′ra. Gr. n. *planos* wanderer, tramper; L. adv. num. *bis* twice (double); Gr. fem. n. *spora* a seed, and in biology a spore; N.L. fem. n. *Planobispora* a motile, double-spored organism.

Substrate and aerial mycelia are formed on agar media. Substrate hyphae (0.5–1.0 μm in diameter) are nonfragmenting, branched, and septate. Aerial hyphae (1.0 μm in diameter) are sparsely branched and septate. Gram-stain-positive and non-acid-fast. **Cylindrical to clavate sporangia** (1.0–1.2 μm × 6.0–8.0 μm), each containing a **longitudinal pair of spores**, are developed singly or in bundles on short ramifications of the **aerial hyphae**. The **spores** (zoospores) are oblong and cylindrical with rounded ends, and are **motile** by means of peritrichous flagella. Colonies developed on agar media are flat or occasionally elevated, the **substrate mycelium either without distinctive color or rose colored**. The **aerial mycelium**, which is formed only on certain agar media, is **white or with a light rose tinge**. Strains grow well under aerobic conditions. Chemoorganotrophic and mesophilic, growing well at 28–40°C and pH 6.0–9.0. Cell walls contain *meso*-diaminopimelic acid and whole-cell hydrolysates contain madurose as the diagnostic sugar. Contains diphosphatidylglycerol, phosphatidylethanolamine, hydroxylated phosphatidylethanolamine, and ninhydrin and sugar positive phospholipids as predominant phospholipids. Major menaquinones are MK-9(H_4), MK-9(H_2), and MK-9(H_0). Major types of fatty acids are C_{17} 10-methyl and C_{16} iso-branched fatty acids.

DNA G+C content (mol%): 70–71 (Im).

Type species: **Planobispora longispora** Thiemann and Beretta 1968c, 157[AL].

Further descriptive information

Phylogeny. On the basis of 16S rRNA gene sequence analyses, the genus *Planobispora* is grouped in the family *Streptosporangiaceae*. This is also shown by other 16S rRNA gene sequence studies of this group (Goodfellow et al., 1990b; Goodfellow and Quintana, 2005; Stackebrandt et al., 1997; Zhang et al., 1998b). Within this family, most genera are defined on the basis of distinct chemotaxonomic and morphological properties (Table 275). The genus encompasses two species with validly published names. Representatives of these form a distinct line in the 16S rRNA *Streptosporangiaceae* gene tree (Goodfellow and Quintana, 2005; Figure 383). Their nearest relatives within the family *Streptosporangiaceae* are the genera *Planomonospora*, *Streptosporangium*, and *Sphaerisporangium*. Similarity values between the genera *Planomonospora* (with the exception of *Planomonospora venezuelensis*) and *Planobispora* range from 97.1–96.2%, between the genera *Planobispora* and *Streptosporangium* from 96.5–93.8%, and between the genera *Planobispora* and *Sphaerisporangium* from 96.2–96.1%. As pointed out in the *Planomonospora* chapter, *Planomonospora venezuelensis* shows a closer relationship to *Planobispora* in the phylogenetic tree than to the genus *Planomonospora*. 16S rRNA gene signature nucleotide positions that can be used to differentiate genera of the family *Streptosporangiaceae* are described by Ara and Kudo (2007). Three of the signature nucleotide positions differ between *Planomonospora* (with the exception of *Planomonospora venezuelensis*) and *Planobispora*: 625 (C and G, respectively), 627 (A and G, respectively), 990:1215 (C–G and C–G/U–G, respectively). For two of the three signature nucleotide positions, *Planomonospora venezuelensis* has the same signature as the genus *Planobispora*: 625 (G) and 627 (G).

Cell morphology. The hyphae of the substrate mycelium are 0.5–1.0 μm in diameter, irregularly branched, and occasionally septate. Fragmentation of hyphae is not apparent either on solid agar media or in liquid cultures (Thiemann and Beretta, 1968). The hyphae of the aerial mycelium are 1.0 μm in diameter, long, slender, and wavy, with few lateral branches, and grow more or less parallel to the surface of the substrate. The aerial mycelium is extremely hydrophobic (Thiemann, 1970).

Cylindrical to clavate sporangia are formed on short side branches of aerial hyphae (Figure 384A). They can be arranged singly or in bundles (Suzuki et al., 2001a; Thiemann and Beretta, 1968; Vobis, 1989). Each sporangium contains a longitudinal pair of spores (Figure 384E, F, G).The mean size of a sporangium is 6.0–8.0 μm × 1.0–1.2 μm (Thiemann, 1974a). The sporangia are connected to the main axis of the sporangiophore by short supporting pedicels (Figure 384E, F), which are only 1.0–3.0 μm

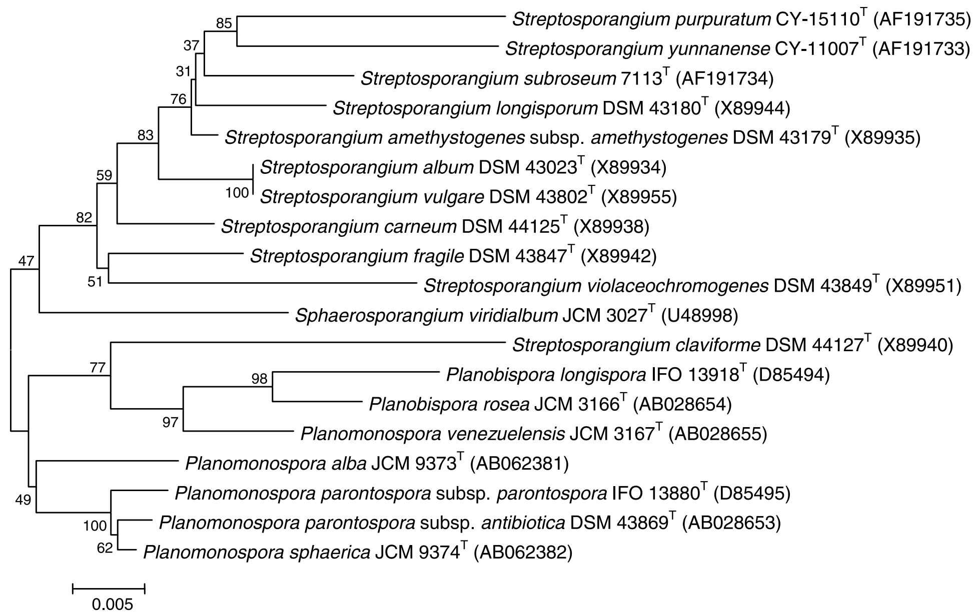

FIGURE 383. Neighbor-joining tree based on 16S rRNA gene sequences of *Planobispora* species and close relatives. The numbers of the nodes indicate the level of bootstrap support (%) based on an analysis of 1000 resampled data sets. The scale bar = 0.5 differences in 16S rRNA gene sequences.

long, very fragile, and collapse easily (Thiemann and Beretta, 1968). As supposed by Bland and Couch (1981), the spores originate from simple transformation of sporogenous hyphae (Figure 384F). The tip of the sporangiophore prolongates until it reaches the length of a ripe sporangium (Figure 384F6). The sporogenous hypha septates from the pedicel and divides into two equal spore-sized segments. The following sporangial primordium continues with the apical outgrowth of the sporangiophore (Figure 384F7). The young spores occupy the sporangial volume (Figure 384G), rounding up later during maturation (Figure 384E, F 1–4). The sporangial envelope is a continuation of the hyphal sheath of the sporangiophore (Figure 384B, C). It is interspersed by longitudinally orientated fibrillar elements, converging like a buttress at the tip of the sporangium (Figure 384B). Between the two spores, a diaphragm is formed transversely, originating from the inner side of the sporangial envelope (Figure 384D, E). In general, the sporangial envelopes are smooth (Thiemann and Beretta, 1968). The studies of Suzuki et al. (2001a) demonstrate various morphological differences in *Planobispora* isolates with clavate to pyriform sporangia, warty sporangial surface, and even three-spored sporangia. The sporangia arranged in bundles form either fan-shaped tufts (Miyadoh et al., 1997), resembling a "half-side" palm leaf pattern like *Planomonospora venezuelensis*, or parallel rows analogous to *Planomonospora parontospora* (Suzuki et al., 2001a).

The spores measure 1.0–1.2 µm × 2.6–4.0 µm, they are oblong with round ends, occasionally slightly curved. The spores (zoospores) are motile by means of peritrichous flagella (Thiemann, 1974a).

The mode of spore liberation was studied by Thiemann (1970). If sporangia of *Planobispora rosea* are dipped into water, the upper spore is released at first through the tip of the sporangium. The lower one remains blocked in the sporangium. Only when the sporangium is broken off at the sporangiophore, the basal spore can be expulsed through the lower end. The central diaphragm (Figure 384D, E), which was called "transverse septum" by Thiemann (1970), occupies an essential function in this process. It separates the two spores by swelling and pushes the spores in opposite directions out of the sporangium. The spores become motile only some time after being dispersed. In spite of good culture conditions, only 2–5% of the spores become motile. The spores germinate with one or two polar germ tubes; occasionally lateral germination is observed (Thiemann and Beretta, 1968).

Cell-wall composition. The peptidoglycan of the cell wall contains *meso*-DAP with madurose (3-*O*-methyl-D-galactose) as the characteristic sugar of whole-cell hydrolysates (Kroppenstedt and Kutzner, 1976, 1978). This chemical cell-wall composition is in accordance with cell-wall type III and sugar pattern B of the classification scheme of Lechevalier and Lechevalier (1970b).

The phospholipids of the cell membranes consist of unknown glucosamine-containing phospholipids, phosphatidylinositol, phosphatidylethanolamine, and diphosphatidylglycerol (Hasegawa et al., 1979), in accordance with phospholipid type IV of Lechevalier et al. (1981).

The fatty acids consist of straight-chain acids, iso- and 10-methyl branched acids, and unsaturated fatty acids. Anteiso-branched fatty acids are not present (Kroppenstedt and Kutzner, 1978), corresponding to fatty acid type 3c of Kroppenstedt (1985).

The two species of *Planobispora* have the menaquinone type 4a of Kroppenstedt (1985) in common, but show variations. *Planobispora rosea* is characterized by di- and tetrahydrogenated menaquinones with nine isoprene units [MK-9(H_2) and MK-9(H_4)] (Whitham et al., 1993), and *Planobispora longispora* by tetra- and dihydrogenated menaquinones with nine units [MK-9(III, VIII-H_4) and MK-9(H_2)], together with MK-9(H_0) (Kudo et al., 1993; Whitham et al., 1993) (Table 287).

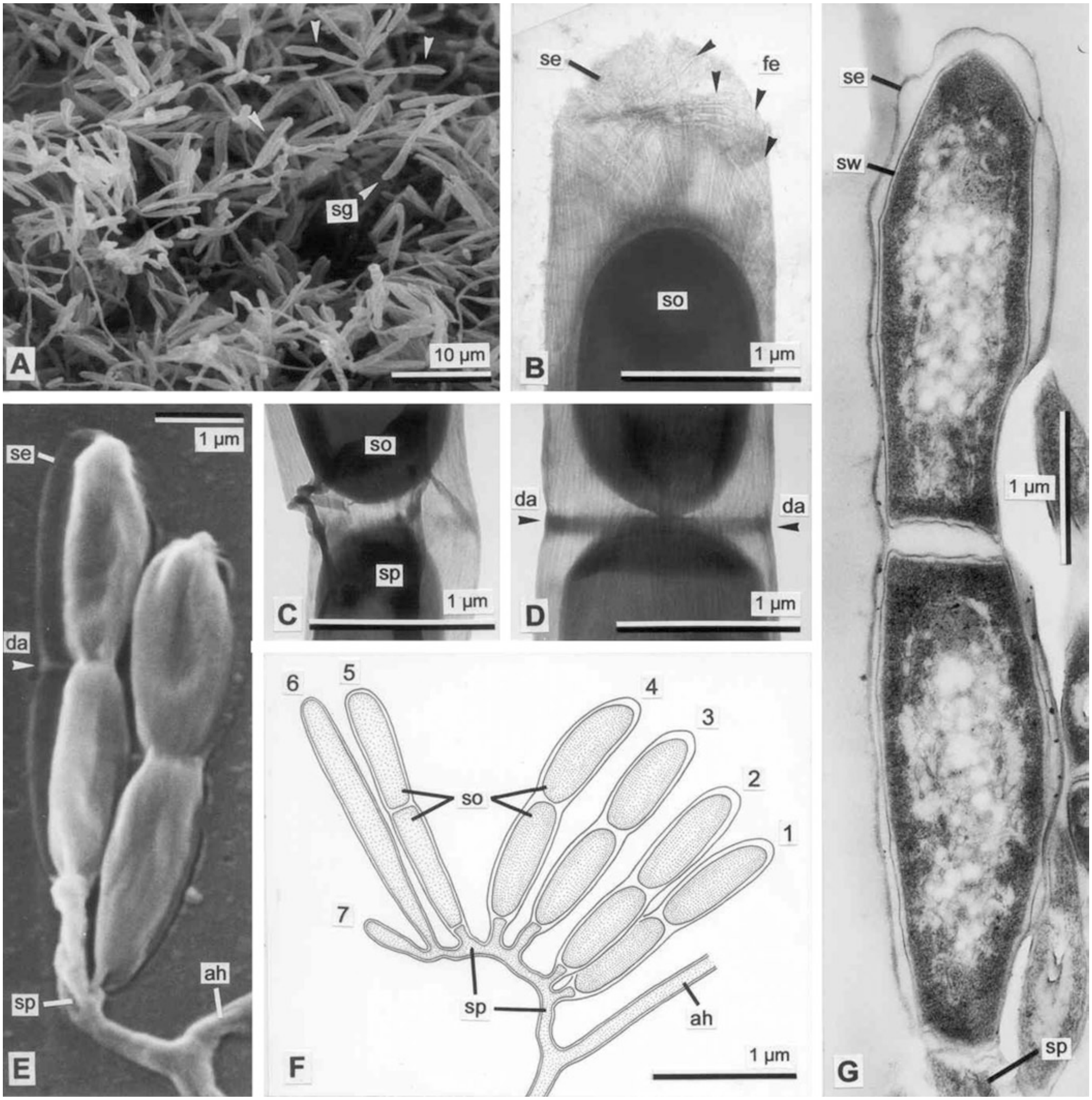

FIGURE 384. Two-spored sporangia of *Planobispora rosea* ATCC 23866 (A, C, D, E, F) and *Planobispora longispora* ATCC 23867 (B, G). A, aerial mycelium with numerous sporangia (SEM); B, tip of the sporangial envelope with fibrous elements (TEM); C, transition from sporangiophore to sporangium (TEM); D, diaphragm in the center (TEM); E, two mature sporangia (SEM); F, sporangiophore with a bundle of 7 sporangia in different stages of development: mature sporangia (1–4), young sporangium (5), immature, still unsegmented sporangium (6) and sporangial primordium (7); G, young sporangium, separated from the sporangiophore and divided in two spore segments (TEM) (see also F 5). Abbreviations: SEM, scanning electron microscope; TEM, transmission electron microscope; ah, aerial hypha; da, diaphragm; fe, fibrous elements; se, sporangial envelope; sg, sporangium; so, spore; sp, sporangiophore; sw, spore wall.

Colony morphology. Substrate and aerial mycelia develop on a variety of agar media. The colonies are mostly flat or occasionally elevated and have a smooth surface. On yeast extract-malt extract agar and on Bennett agar the colonies of *Planobispora longispora* are crusty (Thiemann and Beretta, 1968); *Planobispora rosea* has slightly wrinkled surfaces on Bennett agar and Hickey–Tresner agar (Thiemann, 1970). No distinctive color of the mycelium is formed by *Planobispora longispora* (Thiemann and Beretta, 1968); *Planobispora rosea* produces rose-colored substrate mycelium and white aerial mycelium with

rose tinge (Thiemann, 1970). For *Planobispora longispora*, the development of the aerial mycelium and formation of sporangia are promoted by oatmeal, calcium malate, and soil agars (Thiemann and Beretta, 1968). Hickey–Tresner agar and glycerol-asparagine agar have the same effect on *Planobispora rosea* (Thiemann, 1970).

Planobispora rosea strain ATCC 53773 produces the antibiotic GE 2270, a thiazolyl peptide substance inhibiting bacterial protein synthesis (Selva et al., 1995).

Metabolism. Members of *Planobispora* are aerobic, growing well on the various standard culture media recommended by Waksman (1961) and Shirling and Gottlieb (1966). The type strains of *Planobispora longispora* and *Planobispora rosea* can utilize L-arabinose, cellobiose, fructose, glucose, glycogen, inositol, maltose, mannitol, and starch as sole carbon sources. Adonitol, D-arabinose, erythritol, ethanol, glycerol, inulin, lactose, mannose, α-methyl-D-glucoside, raffinose, sorbitol, and sucrose are not utilized (Goodfellow and Pirouz, 1982; Thiemann, 1974a). Acetamide and serine are not utilized as sole carbon and nitrogen sources (Goodfellow and Pirouz, 1982). Further physiological features and degradation abilities are also shown in Table 288.

TABLE 287. Diagnostic characteristics for *Planobispora* species[a]

Characteristic	*P. longispora*	*P. rosea*
Substrate and aerial mycelium rose-colored[b]	–	+
Sensitive to dimethylchlortetracycline[c]	+	–
Sensitive to gentamicin[c]	–	+
Menaquinones:[d,e]		
MK-9(H_0)	+	–
MK-9(H_2)	+	+
MK-9(H_4)	–	+
MK-9(III, VIII-H_4)	+	–

[a]Symbols: +, >85% positive; –, 0–15% positive.

[b]Data from Thiemann (1970).

[c]Data from Goodfellow and Pirouz (1982).

[d]Data from Kudo et al. (1993).

[e]Data from Whitham et al. (1993).

TABLE 288. Characteristics of the *Planobispora* species[a]

Characteristic	*P. longispora*	*P. rosea*
Utilization of sole carbon sources:		
Adonitol	–	–
Amygdalin	+	–
L-Arabinose	+	+
D-Arabinose	–	–
Cellobiose	+	+
Erythritol	–	–
Ethanol	–	–
Fructose	+	+
Galactose	–	+
Glucose	+	+
Glycerol	–	–
Glycogen	+	+
Inositol	+	+
Inulin	–	–
Lactose	–	–
Maltose	+	+
Mannitol	+	–[b],+[c]
Mannose	–	–
Melezitose	–	+
α-Methyl-D-glucoside	–	–
Raffinose	–	–
Rhamnose	+	–
Salicin	–	+
Sorbitol	–	–
Starch	+	+
Sucrose	–	–
Trehalose	–	–
Xylose	+	+
Degradation tests:		
Adenine	–	–
Arbutin	+	+
Calcium malate	–	–
Casein	+	+
Cellulose	–	–
Chitin	–	+
Elastin	+	+
Esculin	–	+
DNA	+	+
Gelatin	+	+
Guanine	–	–
Hippurate	–	–
Hypoxanthine	+	–
Keratin	+	+
RNA	+	+
Starch	+	+
Testosterone	–	–
Tween 20, 40, 60, and 80	+	+
Tyrosine	–[d], +[c]	+
Xanthine	–	–
Xylan	–	–
Additional physiological properties:		
Diffusible pigments produced	–	–
Melanoid pigments	–	nd
H_2S produced	–	nd
Phosphatase produced	+	+
Reduction of nitrate	+	+
Litmus milk:		
Coagulated	+	–
Peptonized	+	–

[a]Symbols: +, >85% positive; –, 0–15% positive; nd, not determined. Data compiled from Thiemann (1970), Goodfellow and Pirouz (1982), and Thiemann and Beretta (1968). Deviations are indicated by superscript letters.

[b]Data from Thiemann (1970).

[c]Data from Goodfellow and Pirouz (1982).

[d]Data from Thiemann and Beretta (1968).

Strains of *Planobispora* are mesophilic. Good growth occurs at 28–40°C, with no growth at 20°C or 45°C (Goodfellow and Pirouz, 1982; Thiemann, 1974a). Cultures grow well at pH 6.0–9.0. As an exception, *Planobispora longispora* grows at pH 5.0 on oatmeal agar (Thiemann and Beretta, 1968). Strains grow in the presence of brilliant green (up to 0.02%, w/v), crystal violet (up to 0.001%, w/v), and pyronine (0.01%, w/v), but they do not tolerate lysozyme (0.005%, w/v) or NaCl (3.0%, w/v) (Goodfellow and Pirouz, 1982). Nitrate is reduced to nitrite and phosphatase is produced. The type strains of *Planobispora longispora* and

Planobispora rosea are able to degrade arbutin, casein, elastin, DNA, gelatin, keratin, RNA, starch, tyrosine, and Tween 20, 40, 60, and 80. The following compounds are not degraded: adenine, cellulose, guanine, hippurate, testosterone, xanthine, and xylan (Goodfellow and Pirouz, 1982) (see Table 288).

Planobispora longispora and *Planobispora rosea* are sensitive to the antibiotics kanamycin, neomycin, novobiocin, tobramycin, and penicillin. They are not sensitive to cephalosporin, lincomycin, rifampin, and streptomycin. *Planobispora longispora* is sensitive to dimethylchlortetracycline, and *Planobispora rosea* is sensitive to gentamicin (Goodfellow and Pirouz, 1982) (Table 287).

Ecology. The genus *Planobispora* was regarded for a long time as a very rare microorganism, known only from a few localities. Several strains, including the type strains, were isolated from two soil samples collected from a river bank in Venezuela. The pH values of the two samples were 5.3 and 7.6 (Thiemann, 1970). Further strains were isolated by D. Schäfer from a soil sample collected in Namibia (Vobis, 1989). Kizuka et al. (1997) recovered *Planobispora* strains from soil originating from arid regions of South Africa. More recently, Suzuki et al. (2001a) tested 1467 soil samples collected from all parts of the world. Only 3.5% of the samples tested contained *Planobispora* strains. The pH value of the samples ranged from 6.2–8.1, nearly 90% in the small range from 7.0–7.9, indicating that the organisms prefer neutral to slightly alkaline environments. The positive samples were collected in Ecuador, Egypt, French Guiana, India, and Madagascar, whereas no isolates were obtained from samples of temperate areas in Europe, North America, and Oceania. The geographical distribution seems to be restricted to tropical and subtropical zones between latitude 35°N and 35°S (Suzuki et al., 2001a).

Enrichment and isolation procedures

Unfortunately, Thiemann and coworkers never published the isolation procedures they used. The traditional baiting technique as described by Bland and Couch (1981) was successfully applied (Vobis, 1989). Kizuka et al. (1997) could isolate *Planobispora* strains by a centrifugation method. Suzuki et al. (2001a) developed an efficient selective isolation method using humic acid–trace salts gellan gum medium (HSG) containing 0.05% nitrohumic acid, 3 mM $CaCl_2$, 5 mM CHES (*N*-cyclohexyl-2-amino-ethanesulfonic acid), and 0.7% gellan gum, with addition of seven antimicrobial agents (μg/ml): trimethoprim (50), nalidixic acid (50), enoxacin (20), sodium ampicillin (2), streptomycin sulfate (1), cycloheximide (50), and nystatin (50). The presence of trace salts ($FeSO_4 \cdot 7H_2O$, $MnCl_2 \cdot 4H_2O$, $ZnSO_4 \cdot 7H_2O$, and $NiSO_4 \cdot 6H_2O$ with 0.0001% concentration of each), the alkaline environment (pH 9.0), and the incubation temperature of 32–37° C stimulate the formation of sporangia. This morphological characteristic is indispensable to recognize *Planobispora* strains under the microscope. Five hundred milligrams of air-dried soil samples are treated in dry heat at 90°C for 60 min. After cooling, each sample is flooded with 2 ml of a solution of 0.1% skim milk (unsterilized), 0.01% Tween 80 in 5 mM CHES (pH 9.0), and incubated with occasional stirring at 35°C for 1 h. After centrifugation (1000 × *g* for 10 min), 800 μl of the supernatant is diluted with sterile saline, and aliquots are spread onto HSG plates. They are incubated for 14–21 d at 32°C. The colonies grown in these enrichment cultures are controlled microscopically. Pure isolates are obtained by streaking onto HSG and tested for zoospore production by flooding with a solution containing 0.1% skim milk in 5 mM CHES (pH 9.0).

Maintenance procedures

For some weeks or even months, cultures can be stored at room temperature in hermetically closed slant culture tubes on agar medium that support good growth of substrate and arial mycelium and production of sporangia. For long-term preservation, strains must be processed as described for other aerobic actinomycetes. Among others, lyophilization is a recommended method.

Differentiation of the genus *Planobispora* from other genera

The genus *Planobispora* may be confused morphologically with members of genera that can produce pairs of spores on aerial and/or substrate mycelia: *Actinobispora*, *Actinomadura*, *Microbispora*, *Microtetraspora*, *Thermobispora* (Miyadoh et al., 1997; Suzuki, 2001; Wang et al., 1996c; Zhang et al., 1998b), and the former genera *Elytrosporangium*, *Kitasatoa*, and *Microellobosporia*, now belonging to the genus *Streptomyces* (Williams et al., 1989). In opposition to *Planobispora*, the spores of those genera are not produced in a sporangium, and their spores are not motile in aquatic habitats. Confusion with strains of the genus *Dactylosporangium*, which produce motile spores within few-spored, cylindrical to clavate sporangia on substrate hyphae, can finally be avoided by determining the cell-wall chemotype. *Planobispora* with cell-wall type III and sugar pattern B distinguishes from *Dactylosporangium* having chemotype II and sugar pattern D.

Taxonomic comments

The majority of the genera classified in the aggregate group "maduromycetes" (Goodfellow, 1989a), including *Planobispora*, were assigned to the family *Streptosporangiaceae* (Goodfellow, 1992; Goodfellow et al., 1990b). Ward-Rainey et al. (1997) emended the description of the family *Streptosporangiaceae* and included the genera *Herbidospora*, *Microbispora*, *Microtetraspora*, *Planobispora*, *Planomonospora*, and *Streptosporangium*. This taxonomical concept was underpinned by Miyadoh et al. (1997). Subsequently, the family *Streptosporangiaceae* was extended by addition of the genera *Acrocarpospora*, *Nonomuraea*, *Planotetraspora*, and *Sphaerisporangium* corrig. (Ara and Kudo, 2007; Miyadoh et al., 2001).

Differentiation of species of the genus *Planobispora*

The two species presently described cannot be clearly differentiated on the basis of morphology. Tests for utilization of 28 carbon sources and degradation of 21 compounds all show only 10% deviation between the two species (Table 288). Besides other distinguishing physiological and cultural characteristics, the colors of substrate and aerial mycelium (Thiemann, 1970) and the menaquinone composition (Kudo et al., 1993; Whitham et al., 1993) are additional features to differentiate the two species (Table 287).

List of species of the genus *Planobispora*

1. **Planobispora longispora** Thiemann and Beretta 1968, 157[AL]

lon.gi.spo′ra. L. adj. *longus* long; Gr. n. *spora* a seed, and in biology a spore; N.L. n. *longispora* (nominative in apposition) the long spore.

Sporangial development is supported by soil, calcium malate, and oatmeal agars. Spores are straight to slightly curved with rounded ends, measuring 1.0–1.2 × 2.6–4.0 µm. They are motile by peritrichous flagella.

No specific color occurs either in the aerial or in the substrate mycelium. The aerial mycelium is white and the substrate mycelium hyaline to creamy colored. No soluble pigments are produced.

Good growth with abundant aerial mycelium occurs on oatmeal agar. On yeast extract-malt extract, Hickey–Tresner, Bennett, and peptone-beef extract agars, colonies also grow well, but aerial mycelium is not developed.

Amygdalin is used for growth; galactose, melezitose, and salicin are not. Hypoxanthine is degraded; esculin and chitin are not degraded. Melanoid pigments are not produced. Litmus milk is coagulated and peptonized.

Contains tetra- and dihydrogenated menaquinones with nine units [MK-9(III,VIII-H_4) and MK-9(H_2)], together with MK-9(H_0)

DNA G+C content (mol%): 71 (T_m).

Type strain: ATCC 23867, DSM 43041, CBS 115.69, JCM 3092, NBRC 13918, VKM Ac-700.

Sequence accession no. (16S rRNA gene): D85494.

2. **Planobispora rosea** Thiemann 1970, 251[AL]

ro′se.a. L. fem. adj. *rosea* rose-colored.

Sporangial development is promoted by all media on which aerial mycelium is formed, e.g. on soil and Hickey–Tresner agars. The spores are elongated and fusiform, with rounded ends, 1.0–1.2 µm × 3.0–3.5 µm. They are motile by peritrichous flagella.

Substrate mycelium in most media is rose colored; if aerial mycelium is developed, it always has a light rose tinge.

On Bennett, peptone-beef extract and potato plug agars the colonies grow well, are slightly wrinkled or flat and rose colored; no aerial mycelium is developed. Good growth occurs also on Hickey–Tresner agar and the colonies are slightly wrinkled and yellow-amber colored with abundant aerial mycelium. Colonies grow well on oatmeal agar; they are smooth and rose colored and aerial mycelium is moderately developed showing a rose tinge. On glycerol-asparagine agar, growth is moderate and the colonies are smooth, flat, and hyaline; abundant aerial mycelium, white with a rose tinge, is formed.

Galactose, melezitose, and salicin are used for growth; amygdalin is not. Esculin and chitin are degraded, hypoxanthine is not hydrolyzed. Litmus milk is neither coagulated nor peptonized.

Contains di- and tetrahydrogenated menaquinones with nine isoprene units [MK-9(H_2) and MK-9(H_4)].

DNA G+C content (mol%): 70 (T_m).

Type strain: ATCC 23866, DSM 43051, JCM 3166, NBRC 15558, NRRL B-8121, VKM Ac-1318.

Sequence accession no. (16S rRNA gene): AB028654.

Genus VIII. **Planomonospora** Thiemann, Pagani and Beretta 1967, 29[AL]

Gernot Vobis, Nicole Lodders and Peter Kämpfer

Pla.no.mo.no.spo′ra. Gr. n. *planos* wanderer, vagabond; Gr. adj. *monos* alone, single; Gr. fem. n. *spora* a seed, and in biology a spore; N.L. fem. n. *Planomonospora* a motile, single spored organism.

Substrate and aerial mycelium developed on various agar media. Substrate hyphae (0.6–1.0 µm in diameter) are nonfragmenting, irregularly branched, and occasionally septated. Aerial hyphae are sparsely branched and rarely septated. Organisms are Gram-stain-positive and non-acid-fast. **Cylindrical to clavate sporangia** (1.0–1.5 µm × 3.5–5.5 µm) **are formed in bundles on the aerial mycelium**, arranged either in narrow parallel rows resembling rows of bananas or in an open palm leaf pattern. **Each sporangium contains a single spore**. **Spores (zoospores) are oblong to fusiform and motile by means of peritrichous flagella**. Growth under aerobic conditions. Colonies developed on complex agar media are raised or flat with rugose or smooth surfaces. Substrate mycelium may be grayish yellow, light orange, rose, yellowish pink, or violet-brown. Aerial mycelium is white, grayish white, light rose, or pink. Chemoorganotrophic, mesophilic, grows well at 28–37°C and from pH 7.0–8.0.

Cell walls contain *meso*-diaminopimelic acid and whole-cell hydrolysates contain madurose as the diagnostic sugar. Contains diphosphatidylglycerol, phosphatidylethanolamine, hydroxylated phosphatidylethanolamine, and ninhydrin and sugar positive phospholipids as predominant phospholipids. Major menaquinones are MK-9(H_4), MK-9(H_2), and MK-9(H_0). Major types of fatty acids are C_{17} 10 methyl and C_{16} iso branched fatty acids.

DNA G+C content (mol%): 72 (T_m).

Type species: **Planomonospora parontospora** Thiemann, Pagani and Beretta 1967, 29[AL].

Further descriptive information

Phylogeny. 16S rRNA gene sequence analyses place the genus *Planomonospora* in the family *Streptosporangiaceae*. This is confirmed by other 16S rRNA gene sequence studies of this group (Goodfellow et al., 1990b; Goodfellow and Quintana, 2005; Stackebrandt et al., 1997; Zhang et al., 1998b). Within this family, genera are defined on the basis of distinct chemotaxonomic and morphological properties (Table 275). The taxon encompasses four species and two subspecies with validly published names. Representatives of each of these validly described species form a distinct line in the 16S rRNA *Streptosporangiaceae* gene tree (Goodfellow and Quintana, 2005; Figure 383).

TABLE 289. Characteristics of the *Planomonospora* species and subspecies[a,b]

Characteristic	*P. parontospora* subsp. *parontospora*	*P. parontospora* subsp. *antibiotica*	*P. alba*	*P. sphaerica*	*P. venezuelensis*
Utilization of sole carbon sources:					
Adonitol	+	nd	–	–	+
Amygdalin	–	nd	nd	nd	–
D-Arabinose	–	nd	–	–	–
L-Arabinose	+[c], –[d]	+	+	+	+[c], –[d]
Cellobiose	+[c], –[d]	+	+	+	+[c], –[d]
Citrate	+	+	+	+	–
Dextrin	+[e], –[d]	+	+	+	–
Dulcitol	–	nd	–	–	nd
Erythritol	–	nd	–	–	–
Ethanol	–	nd	–	–	–
D-Fructose	+	+	+	+	+[c], d[f], –[d]
D-Galactose	+	+	+	+	+[c], –[d]
D-Glucose	+	+	+	+	+
Glycerol	–	–	+	–	–
Glycogen	+[c], –[d]	+	–	+	+[c], –[d]
Inositol	–	–	–	–	–[c], d[f]
Inulin	+[e], –[c]	nd	–	–	–
Lactose	+	nd	–	–	+
Maltose	–[d,e], +[c]	+	+	+	+[c], –[d]
D-Mannitol	+[c], –[d]	+	+	+	+[c], –[d]
D-Mannose	+[e], –[c,d]	+	+	+	–
Melezitose	+	nd	–	–	–
Melibiose	nd	nd	–	–	nd
α-Methyl-D-glucoside	–	nd	–	–	–
Raffinose	–	–	–	–	–[c], d[g]
L-Rhamnose	+[c,e], –[d]	+[g], –[d]	+	+	+[c,f], –[d]
D-Ribose	–	nd	–	–	nd
Salicin	–	+	–	–	–
D-Sorbitol	–	nd	–	–	–
D-Sorbose	–	nd	–	–	nd
Starch	+[c,e], –[d]	+	+	+	+[c], –[d]
Succinate	–	+	–	+	–
Sucrose	–[d,e], +[c]	+	+	+	+[c], –[d]
Trehalose	+[c], –[d]	+	+	+	+[c], –[d]
D-Xylose	+[c,f], –[d]	+	+	+	+[c,f], –[d]
Sodium salts:					
Acetate	nd	nd	+	+	nd
Benzoate	nd	nd	–	–	nd
Butyrate	nd	nd	+	+	nd
Citrate	nd	nd	–	–	nd
Lactate	nd	nd	+	+	nd
Malate	nd	nd	–	–	nd
Mucate	nd	nd	–	–	nd
Oxalate	nd	nd	–	–	nd
Propionate	nd	nd	–	–	nd
Pyrovate	nd	nd	+	+	nd
Succinate	nd	nd	–	+	nd
Tartrate	nd	nd	–	–	nd
Degradation tests:					
Adenine	–	nd	–	–	–
Arbutin	+	nd	nd	nd	+
Calcium malate	–	–	–	–	–
Casein	+	+	+	+	–[f], +[c]
Cellulose	–	v[g]	–	–	–
Chitin	+	nd	nd	nd	+
DNA	+	nd	+	+	+
Elastin	+	nd	nd	nd	+
Esculin	+	–	–	–	+, –[d]
Gelatin	–[e], +[c]	+	nd	nd	+[c]
Guanine	–	nd	nd	nd	–
Hippurate	–	nd	–	–	–
Hypoxanthine	+, –[f]	–	–	–	+

(continued)

TABLE 289. (continued)

Characteristic	*P. parontospora* subsp. *parontospora*	*P. parontospora* subsp. *antibiotica*	*P. alba*	*P. sphaerica*	*P. venezuelensis*
Keratin	+	nd	nd	nd	+
RNA	+	nd	nd	nd	+
Starch	+	+	nd	nd	+
Testosterone	−	nd	nd	nd	−
Tween (20–80)	+	nd	nd	nd	+
Tyrosine	−[d,e], +[c]	+	+	+	+, −[d]
Xanthine	−	nd	−	−	−
Xylan	−	nd	nd	nd	−
Additional physiological properties:					
H_2S produced	nd	+	nd	nd	+
Gelatinase produced	−	+	+	+	+, −[d]
Litmus milk:					
Coagulated	−	−	nd	nd	−
Peptonized	+	−	nd	nd	−
Melanin produced	−	+	−	−	nd
Nitrate reduced	+[d,e], −[c]	+	−	+	+[d,f], −[c]
Phosphatase produced	+	−	+	+	+
Urease produced	nd	nd	+	+	nd
Growth at:					
15°C	−	+	−	−	−
45°C	−	−	+	−	−
Survives 50°C for 8 h	+	−	+	+	−
Resistance to 5% (w/v) NaCl	−	+	−	−	−

[a]Symbols: +, >85% positive; -, 0–15% positive; nd, not determined; v, strain instability; D, 33–66% of the strains are positive.

[b]Data compiled from [e]Thiemann et al. (1967), [g]Thiemann et al. (1968a), [f]Thiemann, (1970), [c]Goodfellow and Pirouz (1982), and [d]Mertz, (1994). Deviations are indicated by superscript letters.

Planomonospora species and subspecies form a distinct clade in the 16S rRNA gene tree with the exception of *Planomonospora venezuelensis* (Figure 383). Their nearest relatives within the family *Streptosporangiaceae* are the genera *Planobispora*, *Streptosporangium*, and *Sphaerisporangium* corrig. Similarity values between the genera *Planomonospora* (with the exception of *Planomonospora venezuelensis*) and *Planobispora* range from 97.1–96.2%, between the genera *Planomonospora* and *Streptosporangium* from 98.1–95.3%, and between the genera *Planomonospora* and *Sphaerisporangium* from 96.1–95.9%. The 16S rRNA gene sequence of *Planomonospora venezuelensis* differs in 2.2–2.5% from the sequences of the other species of the genus *Planomonospora* (similarity values between the sequences of *Planomonospora venezuelensis* and the other sequences of the genus *Planomonospora* range from 97.5–97.8%). Although similar differences in gene sequences can be found between *Planomonospora venezuelensis* and the genus *Planobispora* (differences of 2.2–2.8%; similarity values range from 97.6–97.8%), *Planomonospora venezuelensis* shows a closer relationship to *Planobispora* in the phylogenetic tree than to the genus *Planomonospora*. 16S rRNA gene signature nucleotide positions that can be used to differentiate genera of the family *Streptosporangiaceae* are described by Ara and Kudo (2007). Three of the signature nucleotide positions differ between *Planomonospora* (with the exception of *Planomonospora venezuelensis*) and *Planobispora*: 625 (C and G, respectively), 627 (A and G, respectively), 990:1215 (C–G and C–G/U–G, respectively). For two of the three signature nucleotide positions, *Planomonospora venezuelensis* has the same signature as the genus *Planobispora*: 625 (G) and 627 (G).

Cell morphology. Strains of *Planomonospora* produce substrate and aerial mycelium on solid media. Substrate hyphae have diameters of 0.6–1.0 μm, and are irregularly branched, septate, and do not fragment on agar media or in liquid-submersed cultures (Mertz, 1994; Thiemann, 1970). Twisting and swelling occurs in *Planomonospora parontospora* (Thiemann et al., 1967, 1968a). Hyphae grow profusely within agar media forming a compact layer on the surface of the substrate mycelium (Thiemann, 1974b). The diameter of aerial hyphae is 0.5–1.0 μm. They are sparsely branched and, in *Planomonospora venezuelensis*, usually long, wavy, and slender (Thiemann, 1970). Hyphae of *Planomonospora sphaerica* develop characteristic sphaerical bodies with diameters up to 5 μm on inorganic salts-starch agar (ISP medium 4) (Mertz, 1994).

The characteristic morphological feature common to all *Planomonospora* species is the monosporous sporangium, which is produced only on the aerial mycelium. Two different morphological arrangements of sporangia can be recognized. In *Planomonospora venezuelensis*, sporangia are bundled like the pinnas of palm leaves ("palm leaf pattern"). The sporangia develop sympodially along the tip of an aerial hypha, which functions as a sporangiophore. The first sporangium originates by differentiation of the final segment of the sporangiophore (Figure 385A) and the primordium of the following sporangium arises as a subterminal protrusion at the base of the last formed sporangium, alternately in each case (Figure 385B). This pattern of development leads to a distichous arrangement of two rows of sporangia on opposite sides (Figure 385C, D). Each sporangium is connected to the common axis by a short stalk. Fibrillar

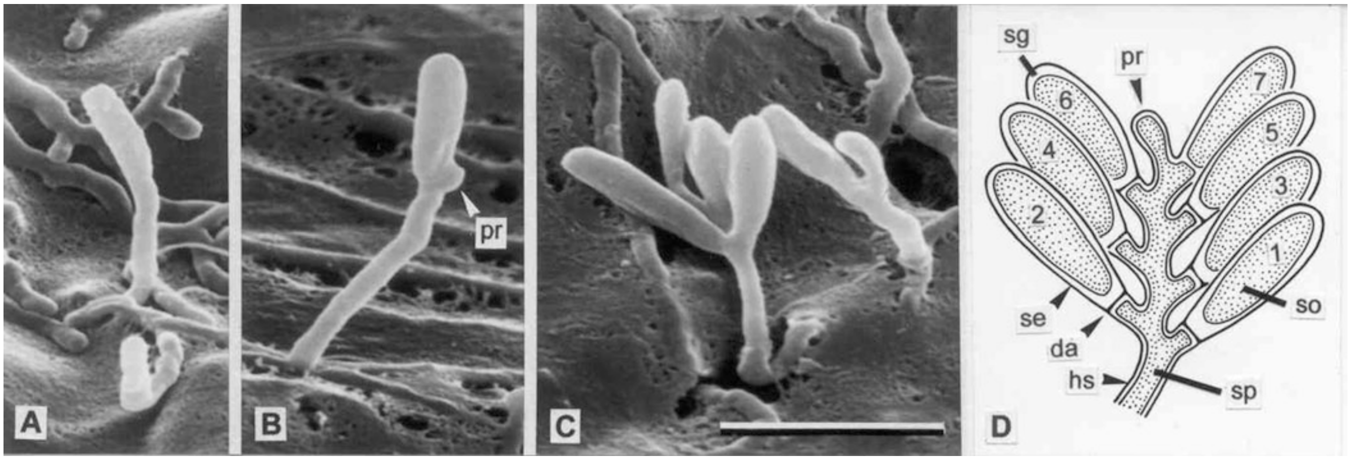

FIGURE 385. Scanning electron micrographs showing ontogenetic development of sporangiophores in *Planomonospora venezuelensis* ATCC 23865 bearing one-spored sporangia in a palm leaf pattern. A, First sporangium produced terminally on an aerial hypha; B, Primordium of the second sporangium protruding at the base of the first sporangium. C, Second sporangium in development (right), sporangiophore with four sporangia (left); D, Sporangiophore with seven sporangia in alternate positions, new sporangial primordium protruding subterminally off the last produced sporangia. Abbreviations: da, diaphragm; hs, hyphal sheath; pr, primordium; se, sporangial envelope; sg, sporangium; so, spore; sp, sporangiophore. Bar = 5 μm.

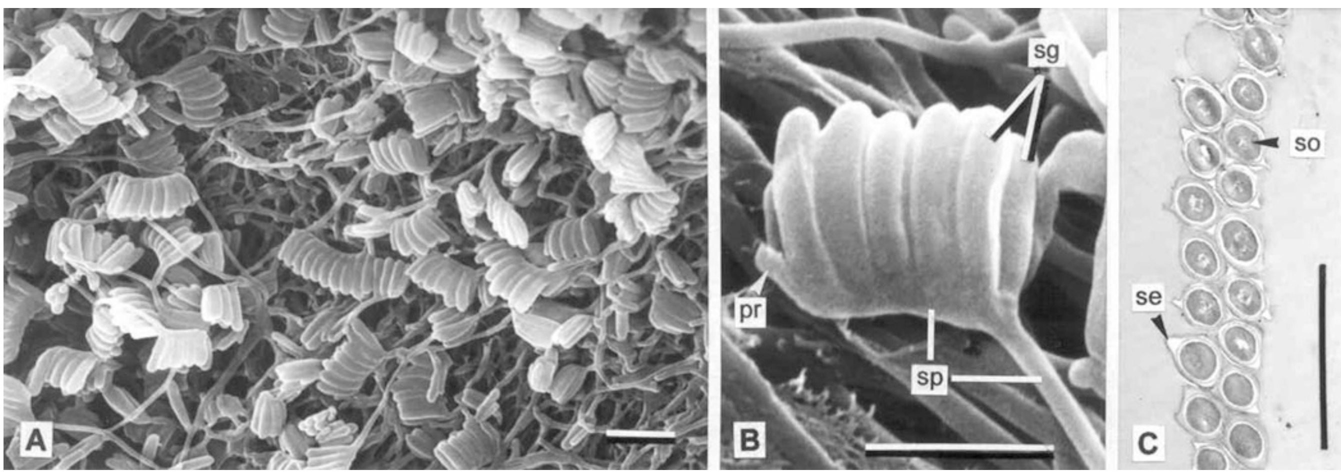

FIGURE 386. Sporangiophores of *Planomonospora parontospora* ATCC 23863. A, Aerial hyphae with numerous sporangial stands; B, sporangiophore bearing a double-row of sessile monosporous sporangia, sporangial primordium protrudes from the sporangiophore apically; C, transverse section of a double row of sporangia, each in alternate position (A, B, scanning electron microscopy; C, transmission electron microscopy). Abbreviations: pr, primordium; se, sporangial envelope; sg, sporangium; sp, sporangiophore. Bars = 5 μm.

elements have been detected on the surface of sporangia (Sharples et al., 1974; Vobis, 1986). The sporangial envelope is a continuation of the hyphal sheath of the sporangiophore (Figure 385D); it retains the hydrophilic character of the aerial hyphae. The morphological appearance of the sporangial strands of *Planomonospora alba*, *Planomonospora parontospora*, and *Planomonospora sphaerica* differ distinctly from those of *Planomonospora venezuelensis*. Sporangia are arranged closely in parallel double rows (Figure 386A). The sporangiophore is slightly bent and can bear up to 60 sporangia, resembling a row of bananas (Mertz, 1994). New sporangia developed from the tip of the sporangiophore (Figure 386B) contribute alternately to each row (Figure 386C). Sporangia are cylindrical with rounded tips, measuring 1.0 μm in diameter and 4.5–5.5 μm in length. Each sporangium is separated from the sporangiophore by a diaphragm (Vobis, 1986). Scanning electron microscope studies show the presence of rostellate structures at the tips of mature and older sporangia, resembling pore-shaped openings or opercula (Locci and Petrolini-Baldan, 1971; Mertz, 1994; Williams, 1970). The fine structure of spore formation was first investigated using transmission electron microscopy by Williams et al. (1973) and Sharples et al. (1974), and reinvestigated by Vobis and Kothe (1985) and Vobis (1986). It can be concluded that sporangial development in *Planomonospora* species follows the same ontogenetical principles as found in other actinomycete genera bearing sporangia on aerial hyphae (Vobis, 1997).

Planomonospora venezuelensis forms fusiform spores, slightly thickened at the terminal end; they are 1.0 μm × 3.0–3.5 μm,

TABLE 290. Diagnostic characteristics for *Planomonospora* species[a]

Characteristic	*P. parontospora*	*P. alba*	*P. sphaerica*	*P. venezuelensis*
Sporangia formed in:				
Parallel rows	+	+	+	–
Palm leaf pattern	–	–	–	+
Production of spherical bodies	–[b]	–	+	–
Color of aerial mycelium:				
White	+[c]	+	–	+
Pink	–	–	+	–
Fatty acids:[d]				
$C_{16:1}$ iso F	–	–	+	–
$C_{16:1}$ iso G	+	+	–	–
$C_{15:0}$ 2-OH	–	+	+	+
$C_{17:1}$	+	+	+	–
$C_{18:2}$ 9c	–	+	+	+

[a]Symbols: +, >85% positive; –, 0–15% positive.

[b]Substrate hyphae with swellings.

[c]With a rose tinge.

[d]Mertz (1994).

filling the sporangium almost completely, and are motile by peritrichous flagella (Thiemann, 1970, 1974b). The spores of *Planomonospora parontospora* are cylindrical to slightly curved or reniform, and are 1.0–1.5 µm × 3.5–4.5 µm. Flagellation is described as lophotrichous (Lechevalier and Lechevalier, 1970a) or peritrichous (Miyadoh et al., 1997; Thiemann, 1974b). Large clavate spores, 10–15 µm long, which move slowly, may be observed (Thiemann et al., 1967). *Planomonospora alba* and *Planomonospora sphaerica* have cylindrical spores, 1.5 × 4.4 µm on mean, which become motile after immersion in water (Mertz, 1994).

The process of spore release begins immediately after sporangia are placed into water. The sporangia change their optical characteristics and become highly opaque (Thiemann, 1970). The spore is pushed upward through the tip, probably due to swelling of material located at the base of the sporangium. The spores become motile by means of peritrichous flagella 30–40 min after they have been expelled (Thiemann et al., 1967). They remain motile for 5–24 h, during which time spore germination may begin (Thiemann, 1970). One or more germ tubes protrude from the spores terminally and subterminally (Miyadoh et al., 1997). In *Planomonospora alba* and *Planomonospora sphaerica*, motility of the sporangiospores has been reported for 30–60 min (Mertz, 1994). The characteristic spherical bodies of *Planomonospora sphaerica* can germinate, producing germ tubes or promycelia (Mertz, 1994); the structures are considered to be atypical-shaped sporangia (Miyadoh et al., 1997).

Cell-wall composition. The peptidoglycan of the cell walls contains *meso*-diaminopimelic acid (Lechevalier and Lechevalier, 1970a; Mertz, 1994) and the characteristic sugar of whole-cell hydrolysates is madurose (3.0-methyl-D-galactose). The sugars galactose and xylose have also been reported (Mertz, 1994) in *Planomonospora alba* and *Planomonospora sphaerica*. *Planomonospora* has a cell-wall chemotype III and a sugar pattern B (Lechevalier and Lechevalier, 1970b).

Planomonospora species show a number of different menaquinone profiles. Di- and tetrahydrogenated menaquinones with nine units [MK-9(H_2) and MK-9(H_4)] are the major components in *Planomonospora parontospora*, whereas tetrahydrogeneted menaquinones with eight isoprene units [MK-8(H_4)] predominate in *Planomonospora venezuelensis* (Collins et al., 1984). In *Planomonospora alba* and *Planomonospora sphaerica*, the major menaquinone detected was MK-9(H_2), though minor amounts of MK-9(H_4) were also present (Mertz, 1994).

The phospholipids of *Planomonospora parontospora* consist of phosphatidylinositol, phosphatidylethanolamine, unknown glucoseamine-containing phospholipids, and diphosphatidylglycerol (DPG) plus lyso-DPG (Hasegawa et al., 1979). The phospholipids present in *Planomonospora alba* and *Planomonospora sphaerica* are diphosphatidylglycerol, phosphatidylglycerol, phosphatidylethanolamine, hydroxyphosphatidylethanolamine, and glucoseamine-containing phospholipids (Mertz, 1994). These polar lipid patterns correspond to phospholipid type IV of Lechevalier et al. (1981).

Planomonospora parontospora and *Planomonospora venezuelensis* have saturated and unsaturated fatty acids; the presence of C_{16} iso and $C_{15/17}$ iso branched fatty acids is variable, but $C_{15/17}$ anteiso is not present (Kroppenstedt, 1985). The presence of 10-methyl branched fatty acids has been demonstrated (Kroppenstedt, 1985; Kroppenstedt and Kutzner, 1978). Differences in fatty acid patterns can be used as diagnostic characteristics for the identification of *Planomonospora* species (Mertz, 1994; Table 290).

Colony morphology. Colonies on agar media are flat or elevated with smooth surfaces, but are occasionally wrinkled or slightly crustose. Abundant aerial mycelium is observed on a few media such as oatmeal agar for *Planomonospora parontospora* (Thiemann et al., 1967) and ATCC medium 172 for *Planomonospora alba* and *Planomonospora sphaerica* (Mertz, 1994). Good or moderately developed aerial mycelium can be observed on Czapek, oatmeal (ISP medium 3), and inorganic salts-starch (ISP medium 4) and potato carrot agar for *Planomonospora alba* and *Planomonospora sphaerica*. In general, *Planomonospora parontospora* and *Planomonospora venezuelensis* develop aerial mycelium moderately or in traces. The color of the aerial mycelium is white with a rose tinge in *Planomonospora parontospora* (Thiemann, 1974b) and white or pink in *Planomonospora sphaerica* (Mertz, 1994). White or grayish white aerial mycelium is a characteristic

feature for *Planomonospora alba* (Mertz, 1994) and *Planomonospora venezuelensis* (Thiemann, 1974b).

The substrate mycelium of *Planomonospora parontospora* is light rose to rose color on oatmeal, skim milk, starch, and tyrosine agars. On Bennett's, potato plug, and nutrient agars, the color of the substrate mycelium is yellowish or creamish to light orange; on Hickey–Tresner and Czapek glucose agars it is hyaline (Thiemann et al., 1967, 1968a). The reverse color of colonies of *Planomonospora sphaerica* is pink on ATCC 172 and on ISP media 3 and 4 and on Czapek, potato-carrot, and tap water agars. The colonies of *Planomonospora alba* show grayish or yellow reverse color on these media (Mertz, 1994). The substrate mycelium of *Planomonospora venezuelensis* produces a violet color on oatmeal and skim milk agars and a brown-violet color on Bennett's, glucose-asparagine, Hickey–Tresner, starch, and yeast extract-malt extract agars. On nutrient agar, the color of the substrate mycelium is light brown, on potato plug agar it is gray, and it is hyaline on calcium malate, glycerol-asparagine and tyrosine agars (Thiemann, 1970).

The production of soluble pigments is restricted in *Planomonospora venezuelensis* to traces of a brown-violet pigment on oatmeal agar and an amber to brown pigment on Hickey–Tresner and glucose-asparagine agars (Thiemann, 1970). A very faint yellow pigment is formed by *Planomonospora parontospora* on oatmeal agar (Thiemann et al., 1967). *Planomonospora parontospora* subsp. *antibiotica* produces a light brown diffusible pigment on tyrosine agar (Thiemann et al., 1968a). *Planomonospora parontospora* subsp. *antibiotica* produces an antibacterial agent, sporangiomycin, under submerged conditions (Thiemann et al., 1968a). A protease inhibitor (antipain) has been isolated from a strain of *Planomonospora parontospora* (Wingender et al., 1975). The type strain of *Planomonospora sphaerica* produces a biological substance which has been identified as the antibiotic thiostrepton. The type strain of *Planomonospora alba* produces a biologically active agent against Gram-stain-positive bacteria and algae; it also exhibits antihelminthic activity and shows inhibition to bovine mastitis (Mertz, 1994).

Metabolism. *Planomonospora* species are aerobic, mesophilic to thermotolerant organisms, growing well on various complex and defined media (Mertz, 1994; Thiemann, 1974b). Their type strains can utilize a number of compounds as sole carbon sources, but in a broad distribution pattern (see Table 289). D-Glucose is used as a sole carbon source by all species.

Members of the genus *Planomonospora* grow at 20–40°C, with a temperature optimum at 28–37°C. *Planomonospora alba* can grow at 52°C (Mertz, 1994). Growth of *Planomonospora parontospora* is consistently good at 22–37°C (Goodfellow and Pirouz, 1982; Thiemann, 1974; Thiemann et al., 1967), but growth is not evident at 10°C. pH values from 6.0–9.0 are tolerated by all species, but growth does not occur at pH 5.0. *Planomonospora parontospora* grows sparsely at pH 6.0 and optimally at pH 7.0–8.0 (Goodfellow and Pirouz, 1982; Thiemann, 1974b; Thiemann et al., 1967). *Planomonospora alba* tolerates 2% (w/v) NaCl, *Planomonospora parontospora* subsp. *parontospora* and *Planomonospora venezuelensis* 3% (w/v) NaCl; only *Planomonospora parontospora* subsp. *antibiotica* is resistant to a concentration of 5% (w/v) NaCl (Mertz, 1994). The presence of lysozyme (0.005% w/v) is partially tolerated (Goodfellow and Pirouz, 1982).The type strains of *Planomonospora parontospora* and *Planomonospora venezuelensis* are sensitive to the antibiotics kanamycin, neomycin, and tobramycin, but are resistant to cephaloridine, lincomycin, novobiocin, rifampin, streptomycin, vancomycin, and penicillin. *Planomonospora venezuelensis* is sensitive to demethylchlortetracycline, but *Planomonospora parontospora* is not (Goodfellow and Pirouz, 1982). *Planomonospora alba* and *Planomonospora sphaerica* are resistant to 2.0 μg of kasugamycin per ml (Mertz, 1994). Further physiological features and degradation abilities are shown in Table 289.

Ecology. Members of the genus *Planomonospora* have a worldwide distribution in temperate, arid, and tropical soils. Thiemann (1970, 1974b) isolated 42 strains from 10 out of 454 soil samples (pH values 5.3–7.8) from Argentina, Chile, India, Italy, Peru, and Venezuela. A further 35 strains were isolated by D. Schäfer (personal communication) from soil samples collected in Ceylon, Egypt, France, Greece, Italy, Mexico, Namibia, Turkey, and the United States (Arizona, Florida, and Texas). The type strains of *Planomonospora sphaerica* and *Planomonospora alba* were isolated from soil collected in India and the Sudan, respectively (Mertz, 1994). Suzuki (2001) reported on the distribution of *Planomonospora* strains in 1200 soil samples collected from 28 countries. Strains of the *Planomonospora parontospora* group were present in 10.9% of the samples from 14 countries. Strains of the *Planomonospora venezuelensis* group were less frequently found and were detected only in 13 samples (1.1%). The latter were collected in tropical or temperate areas of Bolivia, Cyprus, Egypt, Greece, India, Japan, New Caledonia, and Turkey. Nearly all strains of *Planomonospora* (94%) were isolated from neutral to slightly alkaline (pH 7.0–9.0) soil samples (Suzuki et al., 2001b).

Enrichment and isolation procedures

Strains of *Planomonospora* can be enriched from soil by using the baiting technique described by Couch (1954) and Bland and Couch (1981). The soil sample (0.5–1.0 g) is placed into a small sterile Petri dish (4 cm diameter) and flooded with sterile distilled water, up to half the level of the dish, so that the material is well covered. After careful stirring, the soil particles are allowed to sediment. The addition of a very small trace of Tween can be helpful. Natural floating baits like pollen grains of *Pinus* (Schäfer, 1973) or grass blades (Mertz, 1994) are exposed on the surface of the water. Enrichment cultures are incubated in darkness at room temperature and examined after 2 and 4 weeks with a dissecting microscope at high magnification (60×). Typical sporangial bundles, i.e. sporangia on sporangiophores in double rows or in palm leaf pattern, can be picked up with a sterile thin pointed needle (Vobis, 1991) and placed onto the surface of an appropriate agar medium such as artificial soil agar (Henssen and Schäfer, 1971). This medium is transparent and promotes the production of aerial mycelium and sporangial development, thereby allowing the detection and examination of new isolates under the microscope (Cross, 1989). Young colonies can be transferred into slant culture tubes containing nutrient rich agar medium.

A new selective isolation technique developed by Suzuki et al. (2001b) employed a humic acid trace salts gellan gum medium (pH 9.0). A combination of the antimicrobial agents ampicillin (2 μg/ml), enoxacin (20 μg/ml), nalidixic acid, and trimethoprim may be added (Suzuki, 2001). The soil samples can be pretreated by dry heat with 100°C for 60 min (Suzuki, 2001). By using a flooding solution containing 0.1% skim milk in 5 mM *N*-cyclohexyl-2-amino-ethanesulfonic acid buffer

(pH 9.0), high yields of motile spores may be obtained. The flooded soil samples are incubated at 32°C for 90 min, centrifuged at 100 × g for 10 min, and further incubated at 32°C for 60 min after centrifugation (Suzuki et al., 2001b).

Strains of *Planomonospora* can be detected on isolation plates once micromonosporae and streptomycetes have been removed by using a battery of phages (Kurtböke, 2003).

Maintenance procedures

Cultures on agar slants can be stored at room temperature for several weeks. Long-term preservation can be achieved by using procedures recommended for aerobic actinomycetes.

Differentiation of the genus *Planomonospora* from other genera

Planomonospora is related to other genera with cell-wall chemotype III in the family *Streptosporangiaceae*. With the exception of *Herbidospora* Kudo et al. 1993, all of the strains classified in this family produce spores on aerial mycelia, but the sporulation types are highly variable and can be used to differentiate *Planomonospora* from the other genera. All *Planomonospora* strains form zoospores within sporangia. In contrast, the genera *Herbidospora*, *Microbispora*, *Microtetraspora*, and *Nonomuraea* produce nonmotile spores in bisporous, tetrasporous, or oligosporous chains.

The shape of sporangia and the number of spores are of diagnostic value. *Acrosporospora*, *Sphaerisporangium*, and *Streptosporangium* produce spherical and/or club-shaped sporangia which contain numerous nonmotile spores (see Table 275, family *Streptosporangiaceae*), whereas *Planobispora* and *Planotetraspora* strains form cylindrical sporangia which encase 2 and 4 zoospores, respectively (Runmao et al., 1993; Thiemann, 1970; Thiemann and Beretta, 1968). The sporangia of *Planomonospora* are also cylindrical, but each sporangium contains only a single motile spore. The sporangia of this organism are arranged either in parallel double rows (Figure 386) or in a palm leaf pattern (Figure 385), configurations that are unique among sporangia-forming actinomycetes.

Taxonomic comments

Most of the genera classified in the aggregated group "maduromycetes" (Goodfellow, 1989a), including the genus *Planomonospora*, were assigned to the family *Streptosporangiaceae* (Goodfellow et al., 1990b, 1992). Ward-Rainey et al. (1997) emended the description of the family and included six genera in it. The inclusion of the genera *Herbidospora*, *Microbispora*, *Microtetraspora*, *Planobispora*, *Planomonospora*, and *Streptosporangium* in the family was underpinned by Miyadoh et al. (1997). Subsequently, the genera *Acrocarpospora*, *Nonomuraea*, *Planotetraspora*, and *Sphaerisporangium* corrig. were added to the family (Ara and Kudo, 2007; Miyadoh et al., 2001).

Differentiation of species of the genus *Planomonospora*

Planomonospora species can be distinguished by the morphological arrangement of their sporangia (Figure 385 and Figure 386), by their menaquinone (Collins et al., 1984; Mertz, 1994) and fatty acid composition (Mertz, 1994), and by the characteristic color of the mycelium (Mertz, 1994; Thiemann, 1974b). The diagnostic characteristics of the four species are shown in Table 290. Additional differences are given in Table 289 and in the species descriptions.

List of species of the genus *Planomonospora*

1. **Planomonospora parontospora** Thiemann, Pagani and Beretta 1967, 29[AL]

pa.ron.to.spo′ra. Gr. v. *pareimi* to be by or near one, to be side by side; Gr. n. *spora* a seed, and in biology a spore; N.L. n. *parontospora* (nominative in apposition) spores side by side.

The hyphae of the substrate mycelium (0.6–0.8 µm in diameter) are occasionally branched, septated, and twisted, frequently with swellings. The substrate mycelium is rose to light orange in color. The hyphae of the aerial mycelium (1.0 µm in diameter) are sparsely branched. The aerial mycelium is whitish, always with a light rose tinge, and is abundant on oatmeal, Hickey–Tresner, and soil agars. Growth is very good on Bennett's, Hickey–Tresner, oatmeal, and glucose-asparagine agars.

Monosporous sporangia, developed only on aerial hyphae, are arranged in double parallel rows attached directly to a characteristically bent sporangiophore. Mature sporangia are cylindrical, 1.5 µm × 3.5–4.5 µm. Sporangiospores are motile, fusiform, and slightly curved, measuring 1.0–1.5 × 3.5–4.5 µm. The surfaces of the colonies are smooth, with abundantly to sparsely developed aerial mycelium. Moderate growth occurs on Czapek-glucose, nutrient, and soil agars, but growth is poor on glycerol-asparagine, skim milk, starch, and tyrosine agars. Growth is not apparent on calcium malate, cellulose, or peptone-iron agars.

Melanoid pigments are not produced; litmus milk is peptonized; gelatin is not liquified. Growth occurs at 22–37°C; pH optimum of 7.0–8.0.

DNA G+C content (mol%): 72 (T_m).

Type strain: ATCC 23863, DSM 43177, JCM 3093, NBRC 13880, NRRL B-8120, VKM Ac-664.

Sequence accession no. (16S rRNA gene): D85495.

1a. **Planomonospora parontospora subsp. parontospora** Thiemann, Pagani and Beretta 1967, 29[AL]

The subspecies description is the same as for the species.

DNA G+C content (mol%): 72 (T_m).

Type strain: ATCC 23863, DSM 43177, JCM 3093, NBRC 13880, NRRL B-8120, VKM Ac-664.

Sequence accession no. (16S rRNA gene): D85495.

1b. **Planomonospora parontospora subsp. antibiotica** Thiemann, Coronelli, Pagani, Beretta, Tamoni and Arioli 1968a, 528[AL]

an.ti.bi.o′ti.ca. N.L. fem. adj. *antibioticus* (from Gr. prep. *anti* against; Gr. n. *bios* life; L. suff. *-ticus -a -um* suffix of various meanings, but signifying in general made of or belonging to) related to antibiotic, intended to mean producing antibiotic.

Description as for the species. Good growth occurs on inorganic salts-starch and nutrient agars. A light brown diffusible melanoid pigment is formed on tyrosine agar. Gelatin is liquified, litmus milk is not peptonized, and tyrosine is degraded. H_2S is produced.

Produces the antibiotic sporangiomycin.

DNA G+C content (mol%): not determined.

Type strain: ATCC 23864, DSM 43869, JCM 3094, NBRC 15869.

Sequence accession no. (16S rRNA gene): AB028653.

2. **Planomonospora alba** Mertz 1994, 280VP

al′ba. L. fem. adj. *alba* white, referring to the white aerial hyphae.

The hyphae of the substrate mycelium do not fragment and are about 1.0 µm in diameter. Substrate mycelium varies from whitish to yellowish-gray in color. The aerial mycelium is white, and abundant on ATCC medium 172. Aerial hyphae have a diameter of 1.0 µm.

Sporangiophores with long double rows of cylindrical sporangia. Each sporangium contains a single cylindrical to obclavate spore. Spores become motile after 30–60 min immersion in water and are liberated from the sporangial envelope. The mean spore size is 4.4 × 1.5 µm (range 3.5–5.4 × 1.4–1.6 µm).

Abundant growth on ATCC medium 172 and on inorganic salts-starch agar (ISP medium 4); good growth on Aino Henssen agar, Czapek agar, and oatmeal agar (ISP medium 3); and fair growth on potato-carrot agar and tap water agar. Soluble pigments are not produced. Grows at temperatures 20–50°C, tolerates NaCl only at a concentration of 2%, w/v, but is resistant to 2.0 µg of kasugamycin per ml.

Shows biological activity against Gram-stain-positive microorganisms and algae, exhibits antihelminthic activity, and is inhibitory to bovine mastitis.

DNA G+C content (mol%): not determined.

Type strain: A82600, ATCC 51588, DSM 44227, JCM 9373, NRRL 18924.

Sequence accession no. (16S rRNA gene): AB062381.

3. **Planomonospora sphaerica** Mertz 1994, 278VP

spha.e′ri.ca. L. fem. adj. *sphaerica* spherical, referring to the presence of spherical bodies.

Hyphae of the substrate mycelium do not fragment and have a diameter of 1.0 µm. The substrate mycelium is yellowish-pink or pink to a distinctive reddish orange color. The aerial mycelium is pink, but is sometimes white, and is abundant on ATCC medium 172.

Aerial hyphae produce sporangiophores bearing double rows of cylindrical sporangia arranged in parallel. Each sporangium contains a single spore which is released after immersed in water for 30–60 min. Spores are cylindrical to subclavate, mean size 4.4 × 1.5 µm (range 3.5–5.4 × 1.4–1.6 µm), and are motile by flagella. Large spherical bodies are formed on inorganic salts-starch agar. These spherical bodies have the capacity to germinate, forming promycelia.

Growth is abundant on ATCC medium 172 and inorganic salts-starch agar (ISP medium 4); good on Aino Henssen agar, Czapek agar, and potato-carrot agar; and fair on tap water agar. Soluble pigments are not produced. Grows at temperatures of 20–42°C; NaCl is tolerated at a concentration of 2%, w/v; is resistant to 2.0 µg of kasugamycin per ml.

Produces the antibiotic thiostrepton.

DNA G+C content (mol%): not determined.

Type strain: A51460, ATCC 51587, DSM 44632, JCM 9374, NRRL 18923.

Sequence accession no. (16S rRNA gene): AB062382.

4. **Planomonospora venezuelensis** Thiemann 1970, 247AL

ve.ne.zu.e.len′sis. N.L. fem. adj. *venezuelensis* of or pertaining to Venezuela.

Hyphae of the substrate mycelium are 1.0 µm in diameter and are frequently branched and septate. Substrate mycelium is violet-brown on most agar media. The color of the aerial mycelium is white to grayish white. Aerial hyphae (0.5–0.6 µm in diameter) are sparsely branched, long, wavy, and slender.

Monosporous sporangia are developed at the tips of aerial hyphae, alternately on very short lateral ramifications, arranged in two rows on opposite sides, i.e. in a “palm leaf pattern”. Occasionally, sporangia can be produced singly. Sporangia are cylindrical to clavate, 1.0 µm × 4.5–5.5 µm, and are formed abundantly on Hickey–Tresner agar. Spores are motile, fusiform, measuring 1.0 µm in diameter and 3.0–3.5 µm in length.

Good growth on complex media like Bennett’s agar, yeast extract-malt extract, nutrient and potato plug agars. Moderate growth on glycerol-asparagine, oatmeal, skim milk, and tyrosine agars. Optimum temperature for growth is 28–37°C. Traces of a brown-violet soluble pigment are produced on oatmeal agar, and an amber to amber-brown pigment on Bennett’s and Hickey–Tresner agars. Gelatin stabs are scarcely liquified.

DNA G+C content (mol%): not determined.

Type strain: ATCC 23865, DSM 43178, JCM 3167, NBRC 15590, NRRL B-16603, VKM Ac-699.

Sequence accession no. (16S rRNA gene): AB028655.

Genus IX. **Planotetraspora** Runmao, Guizhen and Junying 1993, 468VP emend. Tamura and Sakane 2004, 2055

Zhi-Heng Liu

Pla.no.te.tra. spo′ra. Gr. n. *planos* wanderer; Gr. adj. *tetra* four; Gr. n. *spora* a seed; N.L. fem. n. *Planotetraspora* four-spored organism.

Aerobic, Gram-stain-positive, non-acid-fast actinomycete which forms a branching, nonfragmenting substrate mycelium. **Long, cylindrical sporangia are formed at the ends of short sporangiophores on aerial hyphae. Each sporangium contains a row of four spores which may be cylindrical, oval, or rod-shaped** (0.4–1.4 × 0.8–1.5 µm), and may be motile. Vegetative hyphae tend to be pale yellow to white. Good growth occurs at 25–30°C. Cell walls contain *meso*-diaminopimelic acid, alanine, and glutamic

acid. The peptidoglycan type is A1γ. Muramic acid moieties are *N*-acetylated. Madurose and rhamnose are present in whole-organism hydrolysates. **The major menaquinone is MK-9(H_4), the predominant fatty acid is $C_{18:0}$ 10-methyl, and the diagnostic phospholipid is phosphatidylethanolamine**. Does not contain mycolic acids. Habitat is soil. The phylogenetic position of *Planotetraspora*, as determined by 16S rRNA gene sequence analysis, is in the family *Streptosporangiaceae*.

DNA G+C content (mol%): 71.

Type species: **Planotetraspora mira** Runmao, Guizhen and Junying 1993, 468[VP].

Further descriptive information

The genus *Planotetraspora* contains two species, *Planotetraspora mira* and *Planotetraspora silvatica*, which form a distinct 16S rRNA gene clade within the evolutionary variation encompassed by the family *Streptosporangiaceae*. The closest phylogenetic neighbors of the taxon are the genera *Acrocarpospora* (95.8–97.1%) and *Herbidospora* (95.8–97.1% similarity; Tamura and Sakane, 2004). These workers also found that the type strains of these species share DNA–DNA relatedness levels within the range 38–42%.

Runmao et al. (1993) reported that whole-organism hydrolysates of *Planotetraspora mira* contain arabinose, galactose, mannose, ribose, and xylose, but this was not confirmed in subsequent studies where only madurose and rhamnose were detected as whole-organism sugars (Kudo, 2001; Tamura and Sakane, 2004). In contrast, whole-organism hydrolysates of *Planotetraspora silvatica* NBRC 10041[T] contain galactose, glucose, madurose, 3-*O*-methylmannose, and rhamnose (Tamura and Sakane, 2004).

Planotetraspora mira NBRC 15435[T] grows well and produces aerial mycelia and sporangia on HV agar (Hayakawa and Nonomura, 1989) and on yeast extract-malt extract, oatmeal, inorganic salts-starch, and glycerol-asparagine agars (ISP media 2, 3, 4, and 5, respectively; Shirling and Gottlieb, 1966). The type strain of *Planotetraspora silvatica* also shows good growth on glycerol-asparagine and yeast extract-malt extract agars, and on tyrosine agar (ISP medium 7; Shirling and Gottlieb, 1966).

Enrichment and isolation procedures

The type strain of *Planotetraspora silvatica* was isolated from a sample of forest soil collected from Amami island, Kagashima Prefecture, Japan. The organism was isolated on HV agar (Hayakawa and Nonomura, 1987b) following treatment of the initial dilution with yeast extract and sodium dodecyl sulfate (Hayakawa and Nonomura, 1989). Details were not given on how the *Planotetraspora mira* strains were recovered from a soil sample collected from Wolung village, Sichuan, China (Runmao et al., 1993).

TABLE 291. Phenotypic properties which distinguish between the type strains of *Planotetraspora mira* and *Planotetraspora silvatica*[a,b]

Characteristic	*P. mira* NBRC 15435[T]	*P. silvatica* NBRC 10041[T]
Color of colonies on yeast extract-malt extract agar	White	Yellow
Spores motile	+	−
Acid from:		
Lactose	+	−
Mannitol	+	−
Mannose	+	−
Rhamnose	+	−
Decomposition of:		
Starch	+	+
Xanthine	−	−
Growth on sole carbon sources:		
Glucose	+	−
Mannitol	+	−
Melibiose	−	+
Raffinose	−	+
Xylose	+	−

[a]Symbols: +, >85% positive; −, 0–15% positive.

[b]Data taken from Runmao et al. (1993) and Tamura and Sakane (2004).

Maintenance procedures

Working cultures can be maintained on appropriate standard media such as glucose-asparagine, oatmeal, and yeast extract-malt extract agars. Long-term preservation of strains can be achieved as frozen stocks at −20°C or by using standard lyophilization procedures.

Differentiation of the genus *Planotetraspora* from other genera

Planotetraspora strains can be distinguished from the other genera classified in the family *Streptosporangiaceae* by using a combination of chemotaxonomic and morphological properties (see Table 291 section on the family *Streptosporangiaceae*), notably by their ability to form sporangia which contain a row of four spores. The type strains of the two species form a distinct line of descent within the 16S rRNA *Streptosporangiaceae* gene tree. The signature nucleotides of the 16S rRNA genes of these organisms at positions 502:543 (A–U) and 116:1184 (U–G) are different from those of other members of the family [positions 502:543 (G–C), 1116:1184 (C–G)].

Differentiation of species of the genus *Planotetraspora*

The type strains of *Planotetraspora mira* and *Planotetraspora silvatica* can be distinguished by using a combination of phenotypic properties (Table 291).

List of species of the genus *Planotetraspora*

1. **Planotetraspora mira** Runmao, Guizhen and Jungying 1993, 468[VP]

mi′ra. L. fem. adj. *mira* extraordinary, marvellous.

Forms irregular branching substrate hyphae (0.3–0.6 µm in width) and sparsely branched aerial hyphae (0.2–0.4 µm in width). Cylindrical to clavate sporangia (~2.1–2.7 × 0.6–0.9 µm) are produced on the aerial mycelium either singly or in groups, especially on oatmeal agar; the sporangia are borne on short sporangiophores (0.2–0.8 µm long). Transverse septa divide each sporangium into four spores which at maturity are either cylindrical or spherical (0.8–1.4 × 0.4–0.6 µm). Thirty minutes after being released from the sporangia,

spores become motile by means of polar flagella. The sporangiospores are covered by a thin sporangial wall which is readily seen by transmission electron microscopy, but not by light microscopy. Good growth occurs between 28–37°C, and from pH6.0–8.0.

Milk is slowly peptonized and coagulated.

Little or doubtful growth is observed with L-arabitol, L-arabinose, dulcitol, galactose, inulin, maltose, melezitose, rhamnose, D-ribose, salicin, sorbitol, sorbose, sucrose, and trehalose as sole carbon sources for energy and growth.

Additional phenotypic features are shown in Table 291.

Whole-organism hydrolysates contain madurose and rhamnose. Phosphatidylethanolamine is the diagnostic polar lipid. The predominant menaquinone is MK-9(H_4).

Source: a soil sample collected in Wolung village, Suchuan, China.

DNA G+C content (mol%): not determined.

Type strain: NA9211028, SIIA9201, ATCC 51423, DSM 44359, JCM 9131, NBRC 15435, VKM Ac-2000.

Sequence accession no. (16S rRNA gene): D85496.

Additional comment: the sporangia have been considered to have warty surfaces (Runmao et al., 1993).

2. **Planotetraspora silvatica** Tamura and Sakane 2004, 2055[VP]

sil.va′ti.ca. L. fem. adj. *silvatica* of or belonging to a wood.

Forms an extensively branched substrate mycelium and an aerial mycelium. Long cylindrical sporangia are formed at the ends of short sporangiophores borne on aerial hyphae. Each sporangium contains a single row of oval to rod-shaped spores (0.4–1.4 × 0.8–1.5 µm). A brownish soluble pigment is formed on tyrosine agar (ISP medium 7). The optimal temperature for growth is 25–30°C; growth does not occur at 37°C.

Milk is peptonized and coagulated. Gelatin hydrolysis is negative or weakly positive. Does not degrade calcium malate, or grow in the presence of 4% NaCl.

Galactose, lactose, maltose, melibiose, α-methyl-D-glucoside, and rhamnose are used as sole carbon sources for energy and growth, but adonitol, arabinose, dulcitol, and erythritol are not.

Additional phenotypic features are shown in Table 291.

Muramic acid moieties are *N*-acetylated. Whole-organism hydrolysates contain galactose, glucose, madurose, α-O-methylmannose, and rhamnose. The major fatty acids are $C_{18:0}$ 10 methyl and $C_{16:0}$ iso (>14%). Does not contain mycolic acids. Phosphatidylethanolamine and an unidentified phospholipid containing glucosamine are the diagnostic polar lipids. The predominant menaquinone is MK-9(H_4).

Source: a forest soil in Amami Island, Kagashima Prefecture, Japan.

DNA G+C content (mol%): 71 (HPLC).

Type strain: TT 00-51, DSM 44746, JCM 12867, NBRC 100141.

Sequence accession no. (16S rRNA gene): AB112082.

Genus X. **Sphaerisporangium** Ara and Kudo 2007, 2449[VP] (Effective publication: Ara and Kudo 2007c, 18.)

Martha E. Trujillo, Lorena Carro and Michael Goodfellow

Spha.e.ri.spo.ran′gium. L. n. *sphaera* sphere; N.L. neut. n. *sporangium* sporangia; N.L. neut. n. *Sphaerisporangium* an organism with spherical sporangia.

Aerobic, non-acid-fast, Gram-stain-positive actinomycetes which form branched, non-fragmenting substrate and aerial hyphae. **Single or clustered spherical spore vesicles (~1.5–8.0 µm) are produced on aerial hyphae**. **Vesicles contain coiled chains of non-motile spores which are oval or spherical (0.4–0.9 × 0.6–1.2 µm) with smooth, wrinkled, and prominently ridged surfaces**. Chemo-organotrophic with an oxidative type of metabolism. **The cell wall contains *meso*-diaminopimelic acid. Whole-cell hydrolysates contain galactose, glucose, madurose, mannose, and ribose. The diagnostic phospholipids are phosphatidylethanolamine and ninhydrin-positive phosphoglycolipids. The major fatty acids are $C_{16:0}$ iso and 10-methyl $C_{17:0}$, and the predominant menaquinones, MK-9(H_4) and MK-9(H_6). Phylogenetically related to members of the family *Streptosporangiaceae*.**

DNA G+C content (mol%): 67–72 (HPLC).

Type species: **Sphaerisporangium melleum** corrig. Ara and Kudo 2007c, 2449[VP].

Further descriptive information

Phylogeny. The genus *Sphaerisporangium* forms an independent phyletic branch in the *Streptosporangiaceae* 16S rRNA gene tree (See Figure 360). The species *Thermopolyspora flexuosa* (94.4% sequence similarity) appears to be the closest phylogenetic neighbor forming a deep branch next to *Sphaerisporangium*. The six constituent species form a clade supported by a bootstrap value of 90% (Figure 387). 16S rRNA gene sequence similarity between the six species is 96.3–97.6% and DNA–DNA hybridization values are 32–66% (Cao et al., 2009).

Cell morphology. *Sphaerisporangium* strains form non-fragmenting substrate mycelia and branched aerial hyphae which carry single or clustered spherical spore vesicles (~1.5–8.0 µm) (Figure 388). Spores are nonmotile and are usually formed by the separation of unbranched hyphae within spore vesicles. Spores may be oval, spherical, or pyriform with smooth, wrinkled, and ridged surfaces.

Nutrition and growth conditions. *Sphaerisporangium* strains grow well on standard complex media including Bennett's agar (Jones, 1949), glucose-yeast extract agar (Gordon and Mihm, 1962), oatmeal agar (ISP 3 medium) (Shirling and Gottlieb, 1966), and yeast extract-malt agar (ISP 2 medium). Moderate growth has been observed on glucose-asparagine agar and Hickey–Tresner agar (Hickey and Tresner, 1952), oatmeal-nitrate agar and 1/5 yeast-starch agar. Growth is poor on Czapek's agar (Pridham and Lyons, 1980), glycerol-asparagine agar (ISP 5 medium) (Shirling and Gottlieb, 1966), inorganic salt-starch agar (ISP 4) (Shirling and Gottlieb, 1966), and nutrient agar. All strains are strictly aerobic and chemo-organotrophic with an oxidative metabolism. Most species grow well between 20–37°C and pH 5–9. *Sphaerisporangium cinnabarinum* produces a bright red diffusible pigment.

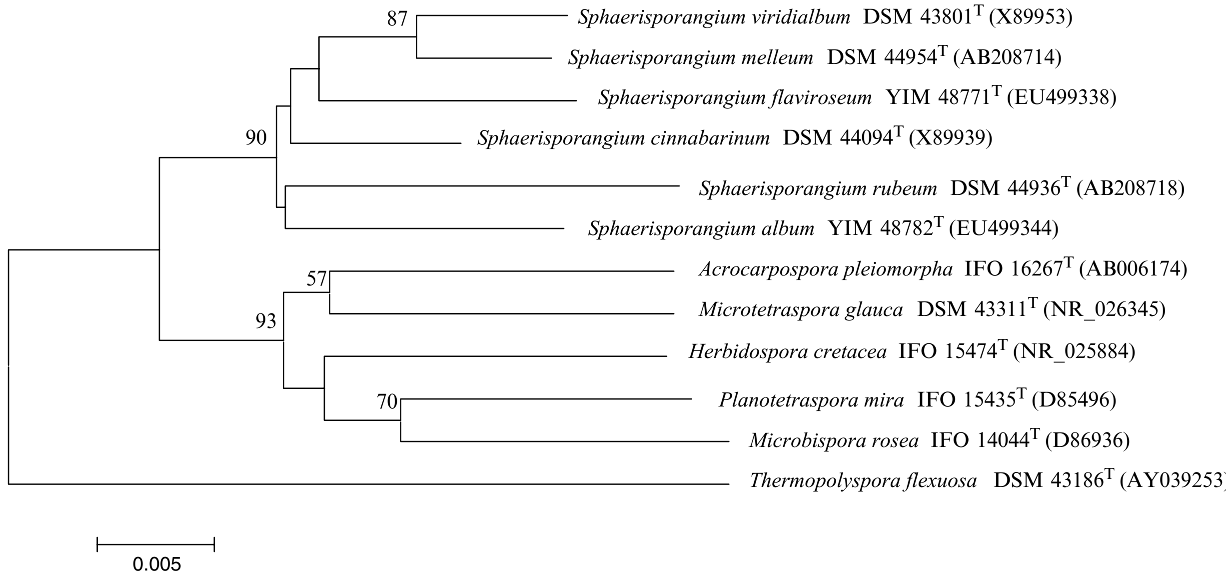

FIGURE 387. Neighbor-joining tree generated from 16S rRNA gene sequences showing relationships between *Sphaerisporangium* species and related taxa. Evolutionary distances were calculated using the Kimura 2-parameter method (Kimura, 1980). Bootstrap values indicated at branching points are expressed as percentages of 1000 replications (only values greater than 50% are shown). Bar = 0.5% sequence divergence.

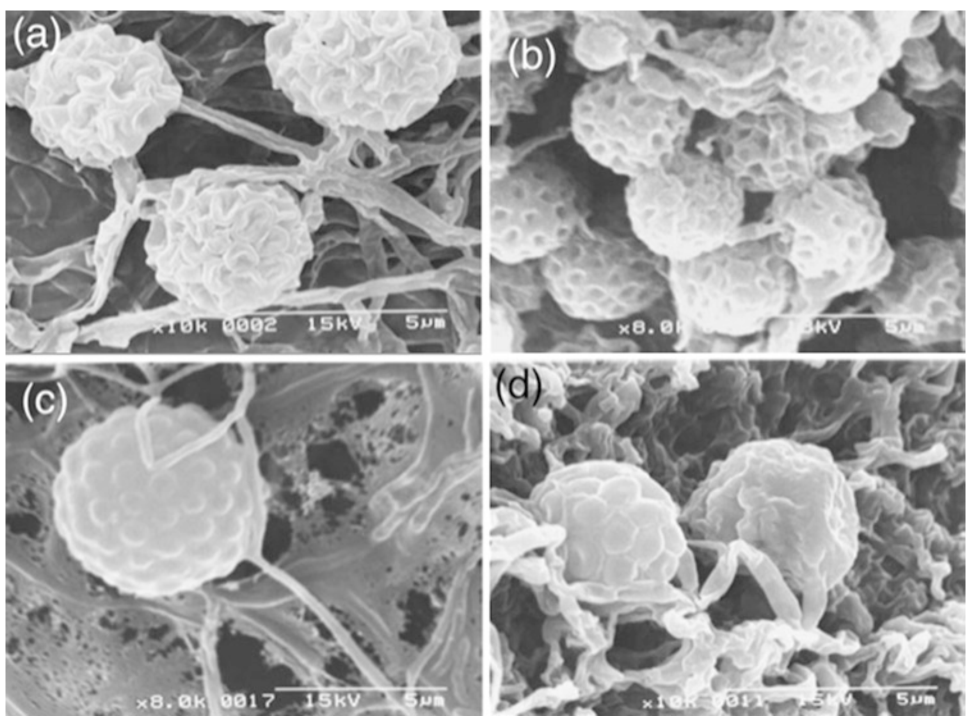

FIGURE 388. Scanning electron micrographs of globose sporangia on aerial mycelia of (a) *Sphaerisporangium melleum* 3-28(8)^{T}; (b) *Sphaerisporangium rubeum* 3D-73(35)^{T}; (c) *Sphaerisporangium cinnabarinum* JCM 3291^{T}, and (d) *Sphaerisporangium viridialbum* JCM 3027^{T} grown on oatmeal-nitrate agar for 21 d at 30°C. (Reproduced with permission from I. Ara and T. Kudo. Society for Actinomycetes, Japan.)

Cell-wall composition. *Sphaerisporangium* species contain *meso*-diaminopimelic acid (*meso*-A_2pm) as the major wall diamino acid and galactose, glucose, madurose, mannose, and ribose which corresponds to a whole-cell sugar pattern B (Lechevalier and Lechevalier, 1970b). The diagnostic sugar madurose is also found in other genera, including *Actinomadura*, *Dermatophilus*, *Microbispora*, *Planobispora*, *Planomonospora*, *Spirillospora*, and *Streptosporangium*. The major phospholipids are

diphosphatidylglycerol, phosphatidylethanolamine, phosphatidylinositol, phosphatidylinositol mannoside, and ninhydrin-positive phosphoglycolipids mannosides. This pattern corresponds to a phospholipid type IV profile (Lechevalier et al., 1981).

The distribution of menaquinones in the genus *Sphaerisporangium* is rather complex. *Sphaerisporangium cinnabarinum*, *Sphaerisporangium melleum*, *Sphaerisporangium rubeum*, and *Sphaerisporangium viridialbum* contain major amounts of MK-9(H_4) and MK-9(H_6) and small amounts of MK-9(H_2), MK-9(H_0), and MK-9(H_8) (Ara and Kudo, 2007). In the case of *Sphaerisporangium flaviroseum* and *Sphaerisporangium album*, the predominant menaquinones are MK-9(H_4), MK-9(H_2), and MK-9, accounting for ~90% of the total composition (Cao et al., 2009). The MK-9 component in these species is 28.1 and 29.0%, respectively; the remaining *Sphaerisporangium* species contain less than 10% of this molecule. Tetrahydrogenation of the menaquinones occurs at sites of isoprene unit III (the third unit from the 2-methyl-1,4–naphthoquinone moiety) and VIII [MK-9(III, VII-H_4)] (Ara and Kudo, 2007).

The major cellular fatty acids are $C_{16:0}$ iso and $C_{17:0}$ 10-methyl with smaller amounts of the saturated, unsaturated, branched and 10-methyl fatty acids, a profile which corresponds to type 3c after Kroppenstedt (1985). Mycolic acids are absent.

Ecology. The primary habitat of *Sphaerisporangium* strains appears to be soil. *Sphaerisporangium album*, *Sphaerisporangium cinnabarinum*, *Sphaerisporangium flaviroseum*, *Sphaerisporangium melleum*, and *Sphaerisporangium rubeum* have been isolated from this source, and *Sphaerisporangium viridialbum*, from acidic volcanic ash. Janso and Carter (2010) found that 19% of 123 endophytic actinomycetes isolated from tropical plants collected from several locations in Papua New Guinea and Mborokua Island (Solomon Islands), belonged to the *Sphaerisporangium* 16S RNA gene tree suggesting that plants, specifically roots, may be a natural habitat for these micro-organisms.

Isolation procedure. Humic acid-vitamin agar (Hayakawa and Nonomura, 1987b) supplemented with cycloheximide (50 mg/ml), nystatin (50 mg/ml), and nalidixic acid was used for the isolation of several *Sphaerisporangium* species using the dilution plate method (Ara and Kudo, 2007). Isolation plates were incubated under aerobic conditions for 21 d at 30°C. *Sphaerisporangium album* and *Sphaerisporangium flaviroseum* were isolated on glycerol-asparagine agar [per liter: glycerol, 10 g; asparagine, 1 g; $K_2HPO_4 \cdot H_2O$, 1 g; $MgSO_4 \cdot 7H_2O$, 0.5 g; $CaCO_3$, 0.3 g; vitamin mixture powder, 3.7 mg (Hayakawa and Nonomura, 1987b); potassium dichromate, 50 mg; agar, 20 g].

Janso and Carter (2010) isolated endophytic *Sphaerisporangium* strains from stems, roots, and leaves by cutting them into pieces using pruning shears or a scalpel following removal of soil and organic debris by rinsing them thoroughly under running tap water. The tissues were successively cut into 2- by 2-mm pieces with a scalpel, placed inside a tea strainer, and immersed in a series of solutions for surface sterilization. Thin or herbaceous tissues (such as leaves, stems, and petioles), were treated with 70% ethanol for 1 min, 50% Clorox bleach (approx. 3% NaOCl) for 3 min, and 70% ethanol for 0.5 min followed by a rinse in sterile water. The procedure was the same for thicker or woody tissues, such as roots and twigs, except that they were immersed in 50% bleach for 5 min. Tissue samples were aseptically transferred to the surface of arginine-vitamin agar (Nonomura and Ohara, 1969a) supplemented with 3% soil extract (Hayakawa et al., 2000) and cycloheximide (100 μg/ml) and nystatin (50 μg/ml). The soil extract was prepared by mixing 100.0 g organic humus (Jolly Gardener Products Inc., Poland, ME) in 100 ml of tap water; the suspension was autoclaved at 121°C for 30 min, transferred to a 1-liter centrifuge bottle, and centrifuged at 4000 r.p.m. for 20 min. The supernatant was filtered through a 0.2-μm cellulose nitrate filter unit (Nalgene). Inoculated Petri dishes were incubated at room temperature (approx. 23–25°C) for up to 8 weeks.

Maintenance procedures

Sphaerisporangium strains can be maintained on yeast-starch agar [per liter: soluble starch, 15.0 g; yeast extract, 4.0 g; $K_2HPO_4 \cdot 7H_2O$, 0.5 g; agar 15.0 g; (pH 7.2)] or ISP 2 agar (Shirling and Gottlieb, 1966) at 4°C for short-term preservation; mycelial fragments in 20% glycerol (v/v) can be maintained at −25°C. Long-term storage can be achieved by lyophilization.

Differentiation of the genus *Sphaerisporangium* from other genera

16S rRNA gene sequencing and the presence of specific signature nucleotides at position 263 (G), 264 (U), 595 (G), 600:638 (U–G), 602:636 (C–G), 603:635 (C–G), 627 (G), 626 (U), 625 (G), 668:738 (C–G), 669:737 (A–U), 671:735 (G–C), 1012:1017 (G–C), and 1263:1272 (G–U) clearly differentiate *Sphaerisporangium* from other genera classified in the family *Streptosporangiaceae* (Ara and Kudo, 2007). The genera *Herbidospora* and *Sphaerisporangium* can be readily distinguished by their menaquinone profiles.

Taxonomic comments

The genus *Sphaerisporangium* was proposed by Ara and Kudo (2007) to accommodate five strains isolated from soil. They also examined "*Streptosporangium cinnabarinum*" JCM 3291 and *Streptosporangium viridialbum* JCM 3027^T which, from the phylogenetic point of view, were considered to be moderately related to the genus *Streptosporangium* (Kemmerling et al., 1993; Tamura et al., 2000; Ward-Rainey et al., 1996). Ara and Kudo (2007) proposed three new species, *Sphaerosporangium melleum*, the type species, *Sphaerosporangium cinnabarinum*, and *Sphaerosporangium rubeum* and, in addition, transferred *Streptosporangium viridialbum* Nonomura and Ohara 1960, to the genus *Sphaerosporangium* as *Sphaerosporangium viridialbum* Nonomura and Ohara 1960 comb. nov. These taxa were included in Validation List no. 118, under the genus *Sphaerisporangium* corrig. as opposed to the earlier incorrect name *Sphaerosporangium* Ara and Kudo 2007. Two additional species, *Sphaerisporangium album* and *Sphaerisporangium flaviroseum*, were recognized by Cao et al. (2009) who also emended the description of the genus.

Differentiation of species of the genus *Sphaerisporangium*

Sphaerisporangium species can be distinguished from one another by using a combination of morphological, nutritional, and physiological characteristics (Table 292).

TABLE 292. Characteristics that differentiate the type strains of *Sphaerisporangium* species[a,b]

Characteristic	*S. melleum*	*S. album*	*S. cinnabarinum*	*S. flaviroseum*	*S. rubeum*	*S. viridialbum*
Substrate mycelium color (ISP 2 agar)	Honey gold	Pale gray	Bamboo	Deep yellow pink	Coral red	Light tan
Substrate mycelium color (ISP 3 agar)	Mustard gold	Yellow white	Light amber	Soft yellow pink	Light coral red	Bamboo
Reduction of nitrate	−	+	−	−	nd	−
Oxidase activity	−	+	−	−	−	−
Degradation of starch	−	+	−	−	−	−
Growth on sole carbon sources:						
L-Arabinose	−	(+)	+	+	−	+
L-Arginine	−	+	+	(+)	−	+
Cellobiose	+	(+)	+	−	−	+
Dextrin	nd	+	+	+	−	−
D-Fructose	nd	(+)	+	+	+	+
Fucose	+	+	+	+	+	−
D-Galactose	+	+	+	+	−	+
L-Histidine	−	+	−	−	+	+
Inositol	−	+	+	(+)	(+)	(+)
Lactose	nd	+	+	(+)	+	+
L-Lysine	−	+	+	−	−	(+)
Maltose	+	+	+	+	−	+
D-Mannitol	nd	+	−	(+)	+	−
DL-Methionine	(+)	−	(+)	−	−	+
L-Proline	−	+	+	(+)	+	+
Raffinose	nd	+	−	(+)	(+)	−
L-Rhamnose	+	+	−	+	−	+
D-Ribose	+	+	−	+	(+)	+
L- Serine	−	+	+	(+)	+	(+)
Sorbose	(+)	−	+	+	−	−
Sucrose	nd	(+)	(+)	+	+	(+)
L-Tryptophan	−	−	(+)	−	−	(+)
Urea	−	+	−	+	−	−
L-Valine	−	+	+	+	−	+
Xanthine	−	−	+	−	(+)	(+)
D-Xylose	+	(+)	+	(+)	−	+
Major menaquinones	MK-9(H_4), MK-9(H_6)	MK-9(H_4), MK-9(H_2), MK-9	MK-9(H_4), MK-9(H_6)	MK-9(H_4), MK-9(H_2), MK-9	MK-9(H_6), MK-9(H_4)	MK-9(H_4), MK-9(H_2)
Major fatty acids	$C_{16:0}$ iso, $C_{17:0}$ 10-methyl	$C_{16:0}$ iso; $C_{17:0}$ 10-methyl	$C_{16:0}$ iso; $C_{17:0}$ 10-methyl	$C_{16:0}$ iso, $C_{17:0}$ 10-methyl	$C_{16:0}$ iso, $C_{17:0}$ 10-methyl, $C_{15:0}$, $C_{17:0}$	$C_{15:0}$ iso, $C_{17:0}$, $C_{16:0}$ iso, $C_{15:0}$
DNA G+C content (mol%)	71	71	70	67.1	70.4	72

[a]Symbols and abbreviations: +, positive; −, negative; nd, not determined; (+), weak growth; MK-9, MK-9(H_2), MK-9(H_4), and MK-9(H_6) designate menaquinones with nine isoprenoic units in the side chain, of which 0, 1, 2, and 3, respectively, are saturated.

[b]Data from: Ara and Kudo (2007); Cao et al. (2009).

List of species of the genus *Sphaerisporangium*

1. **Sphaerisporangium melleum** corrig. Ara and Kudo 2007c, 2449[VP] (Effective publication: Ara and Kudo 2007, 19.)

mel′le.um. L. neut. adj. *melleum* honey-colored.

Branched, non-fragmenting, substrate and aerial hyphae are formed. The vegetative mycelium is light wheat to honey gold in color and the aerial mycelium is white. Spherical spore vesicles which carry coiled spore chains are formed on the aerial hyphae. Abundant sporulation is obtained on inorganic salts-starch, sucrose-nitrate, tap water, and 1/5 yeast extract-starch agars. Good growth is observed on Bennett's, oatmeal, and yeast extract-starch agars; moderate growth on glucose-asparagine, Hickey–Tresner, and 1/5 yeast extract-starch agars, and poor growth on glycerol-asparagine agar, inorganic salts-starch, nutrient, sucrose-nitrate, tap water and tyrosine agars. Diffusible pigments are not produced on these media. Glycerol, erythritol and *myo*-inositol are weakly utilized as sole carbon sources. Other physiological data are given in Table 292 together with information on menaquinone and fatty acid composition.

Source (type strain): soil collected in Bangladesh.

DNA G+C content (mol%): 71.0 (HPLC).

Type strain: 3-28(8), DSM 44954, JCM 13064.

Sequence accession no. (16S rRNA gene): AB208714.

2. **Sphaerisporangium album** Cao, Jiang, Xu, Jiang 2009, 1682[VP]

al′bum. L. neut. adj. *album* white.

Branched, non-fragmenting, substrate and aerial hyphae are formed. Pale-gray substrate and white aerial mycelia are produced. Spherical and pyriform spore vesicles are borne on aerial mycelia. Good growth is observed on oatmeal and yeast extract-malt extract agars, and moderate growth on Czapek's, glycerol-asparagine and inorganic salt-starch agars. Diffusible pigments are not produced on any of these media. Grows in the presence of 2% (w/v) NaCl. Catalase-positive. H_2S is not produced; milk is not coagulated or peptonized. Does not degrade cellulose. Alkaline phosphatase, esterase (C4), esterase lipase (C8), lipase (C14), leucine arylamidase, valine arylamidase, trypsin, α-chymotrypsin, acid phosphatase, naphthol-AS-BI-phosphohydrolase, β-galactosidase, α-glucosidase, β-glucosidase, *N*-acetyl-β-glucosaminidase, and α-mannosidase are produced but not cystine arylamidase, α-fucosidase, α-galactosidase, or β-glucuronidase. L-Arginine, histidine, lysine, ornithine, L-phenylalanine, proline, serine, L-valine, and urea are hydrolyzed, but not glycine, methionine, L-tryptophan, or xanthine. Other physiological data are given in Table 292 together with information on menaquinone and cellular fatty acid composition.

Source (type strain): soil samples collected from virgin forest at Jinbian Rivulet in Hunan Province, China.

DNA G+C content (mol%): 71 (HPLC).

Type strain: CCTCC AA 208026, DSM 45172, YIM 48782.

Sequence accession no. (16S rRNA gene): EU499344.

3. **Sphaerisporangium cinnabarinum** corrig. Ara and Kudo 2007c, 2449[VP] (Effective publication: Ara and Kudo 2007, 19.)

cin.na.ba'ri.num. L. n. *cinnabar -aris*, cinnabar; L. suff. *-inus -a -um*, suffix used with the sense of belonging to; N.L. neut. adj. *cinnabarinum* of cinnabar, referring to the vermilion (bright red) color of the vegetative mycelium and diffusible pigment.

Branched, non-fragmenting, substrate and aerial hyphae are formed. Substrate mycelium is light wheat to melon yellow. White aerial hyphae carry globose spore vesicles containing coiled spore chains. Spores are produced on humic acid-vitamin, oatmeal-nitrate and sucrose-nitrate agars. Good growth is observed on oatmeal and yeast extract-starch; moderate growth on Bennett's, glucose-asparagine, glucose-yeast extract, humic acid-vitamin, inorganic salts-starch, oatmeal-nitrate, and 1/5 yeast extract agars; growth is poor on glycerol-asparagine, Hickey–Tresner, nutrient, tap water, tyrosine, and yeast-extract malt extract agars. Use *myo*-inositol, α-methyl-D-glucoside, and α-D-melibiose as sole carbon sources. Other physiological data are given in Table 292 together with information on menaquinone and cellular fatty acid composition.

Source (type strain): soil collected in the Philippines.

DNA G+C content (mol%): 70 (HPLC).

Type strain: DSM 44094, JCM 3291.

Sequence accession no. (16S rRNA gene): X89939.

4. **Sphaerisporangium flaviroseum** Cao, Jiang, Xu and Jiang 2009, 1682[VP]

fla.vi.ro'se.um. L. adj. *flavus* yellow; L. adj *roseus* rose; N.L. neut. adj. *flaviroseum* yellowish-rose colored.

Branched, non-fragmenting, substrate and aerial hyphae are formed and a yellow-pink substrate and white aerial mycelia produced. Spherical and pyriform spore vesicles are borne on aerial mycelia. Good growth is observed on oatmeal yeast extract-malt extract agars; moderate growth on Czapek's, glycerol-asparagine and inorganic salts-starch agars, ISP 4 agar, and ISP 5 agar. Diffusible pigments are not produced on these media. Grows at pH 8 and in the presence of 1% (w/v) NaCl. Catalase is not produced. Gelatin liquefaction, milk coagulation and peptonization, and production of H_2S are negative. Cellulose is not degraded. Alkaline phosphatase, esterase (C4), esterase lipase (C8), leucine arylmidase, valine arylamidase, trypsin, α-chymotrypsin, acid phosphatase, naphthol-AS-BI-phosphohydrolase, β-galactosidase, α-glucosidase, β-glucosidase, *N*-acetyl-β-glucosaminidase, α-mannosidase, and α-fucosidase are produced, but not cystine arylamidase, α-galactosidase, β-glucuronidase, or lipase (C14) (API ZYM tests). L-Arginine, ornithine, L-phenylalanine, proline, serine, L-valine, and urea are hydrolyzed, but not glycine, histidine, lysine, methionine, L-tryptophan, or xanthine.

Source (type strain): soil collected from Tianzi Mountain in Hunan Province, China.

DNA G+C content (mol%): 67.1 (HPLC).

Type strain: DSM 45170, JCM 16908, KCTC 19393, YIM 48771.

Sequence accession no. (16S rRNA gene): EU499338.

5. **Sphaerisporangium rubeum** corrig. Ara and Kudo 2007c, 2449[VP] (Effective publication: Ara and Kudo 2007, 19.)

ru'be.um. L. neut. adj. *rubeum* red colored.

Branched, non-fragmenting, substrate and aerial hyphae are formed. The vegetative mycelium is light coral pink to coral red and the aerial mycelium is white. Spherical vesicles which carry coiled spore chains are formed on aerial hyphae. Good growth is obtained on yeast extract-starch agar, Bennett's, glucose-yeast extract, Hickey–Tresner, oatmeal and yeast extract-malt extract agars; moderate growth on humic acid-vitamin, inorganic salts-starch, nutrient agar, oatmeal-nitrate, and 1/5 yeast extract-starch agars; growth is poor on glucose-asparagine, glycerol-asparagine, sucrose-nitrate, tap water, and tyrosine agars. Scant sporulation occurs on humic acid-vitamin, oatmeal nitrate, sucrose-nitrate, and water agars.

Adonitol, erythritol, glycerol, and *myo*-inositol are weakly utilized as sole carbon sources. Other physiological data are given in Table 292 together with information on menaquinone and cellular fatty acid composition.

DNA G+C content (mol%): 71 (HPLC).

Type strain: 3D-72(35), DSM 44936, JCM 13067.

Sequence accession no. (16S rRNA gene): AB208718.

6. **Sphaerisporangium viridialbum** (Nonomura and Ohara 1960) corrig. Ara and Kudo 2007c, 2449[VP] (Effective publication: Ara and Kudo 2007, 19.)

vi.ri.di.al'bum. L. adj. *viridis* green; L. neut. adj. *album* white; N.L. neut. adj. *viridialbum* greenish white.

Branched, non-fragmenting, substrate and aerial hyphae are formed. Substrate mycelium is light wheat to bamboo yellow and the aerial mycelium is white. Spherical vesicles which carry coiled spore chains are formed on aerial hyphae. Sporulation occurs on Hickey–Tresner, humic acid-vitamin oatmeal-nitrate, sucrose-nitrate, and 1/5 yeast extract-starch agars. Grows well on Bennett's, glucose-yeast extract, Hickey–Tresner, oatmeal and yeast-extract-starch agars; moderate growth on humic acid-vitamin, nutrient, oatmeal-nitrate, yeast extract-malt extract, and 1/5 yeast extract-starch agars;

growth is poor on glucose-asparagine, glycerol-asparagine, inorganic salts-starch, sucrose-nitrate, tyrosine, and tap water agars. Glycerol and erythritol are weakly utilized as sole carbon sources. Other physiological data are given in Table 292 together with information on menaquinone and cellular fatty acid composition.

Source (type strain): acidic volcanic ash.

DNA G+C content (mol%): 72 (HPLC).

Type strain: ATCC 33328, CBS 432.61, DSM 43801, JCM 3027, KCC A-0027, KCTC 9435, NBRC 13987, NRRL B-2636, VKM Ac-679.

Sequence accession no. (16S rRNA gene): X89953.

Genus XI. **Thermopolyspora** (*ex* Krasil'nikov and Agre 1964) Goodfellow, Maldonado and Quintana 2005, 1980[VP]

Michael Goodfellow

Ther.mo.po.ly.spo′ra. Gr. n. *thermê* heat; Gr. adj. *polu* many; Gr. fem. n. *spora* a seed and, in bacteriology, a spore; N.L. fem. n. *Thermopolyspora* the heat (-loving) many-spored organism.

Aerobic, Gram-stain-positive, non-acid–alcohol-fast, actinomycete which forms non-fragmenting substrate and aerial mycelia. Hooked to irregular spiral chains of 4–10 warty to spiny ornamented spores (1.2–1.5 µm in diameter) are arranged in clusters on long, moderately branched aerial hyphae on potato carrot agar; a light blue aerial mycelium and a brown soluble pigment are also formed on this medium. White to yellowish-white aerial hyphae are borne on a light brown substrate mycelium on oatmeal agar, albeit without the production of diffusible pigments. **Grows from 40–60°C, and optimally between 45 and 55°C. Cell wall contains *meso*-diaminopimelic acid and *N*-acetylated muramic acid. Glucose and ribose are found in whole-organism hydrolysates. The predominant menaquinones are MK-9, MK-9(H_2), and MK-9(H_4), and the major polar lipids are phosphatidylethanolamine, hydroxyphosphatidylethanolamine, phosphatidylinositol mannosides, ninhydrin sugar-positive components, and uncharacterized glycolipids**. The organism is rich in saturated, unsaturated, and branched chain fatty acids, but does not contain mycolic acids. The phylogenetic position of *Thermopolyspora*, as determined by 16S rRNA gene sequence analysis, is in the family *Streptosporangiaceae.*

DNA G+C content (mol%): 77.

Type species: **Thermopolyspora flexuosa** (Meyer 1989) Goodfellow, Maldonado and Quintana 2005, 1982[VP] (*Actinomadura flexuosa* Meyer 1989, 2519).

Further descriptive information

Phylogeny. *Thermopolyspora flexuosa* DSM 43186[T] (formerly *Actinomadura flexuosa*) forms a distinct branch in the *Streptosporangiaceae* 16S rRNA gene tree (see Figure 360 in section on the family *Streptosporangiaceae*). Goodfellow et al. (2005) have shown that this strain produces the PCR product characteristic of members of the family *Streptosporangiaceae* when examined with the family-specific set of oligonucleotide primers developed by Monciardini et al. (2002).

Cell morphology. Strain DSM 43186[T] produces short curved chains of warty to spiny spores on potato carrot agar (Greiner-Mai et al., 1987).

Chemotaxonomy. A comprehensive survey of the chemotaxonomic properties of strain DSM 43186[T] was undertaken by Kroppenstedt et al. (1990a) who found that it produces whole-organism hydrolysates rich in ribose together with a trace of mannose. It also produces complex mixtures of saturated, unsaturated, and branched chain fatty acids and lacks mycolic acids, but contains major amounts of MK-9(H_0), MK-9(H_2), and MK-9(H_4) (points of unsaturation: III and VIII), and minor amounts of MK-9(H_6) (points of unsaturation: II, III and VIII); these components accounted for 41, 31, 25, and 7% of the total menaquinone composition, respectively. The polar lipid pattern was characterized by the presence of major amounts of phosphatidylethanolamine, hydroxylated phosphatidylethanolamine, phosphatidylinositol mannosides, ninhydrin and sugar positive components and uncharacterized glycolipids, and a trace of diphosphatidylglycerol. The diamino acid of the wall peptidoglycan is *meso*-diaminopimelic acid (Becker et al., 1965; Greiner-Mai et al., 1987).

Colony morphology. The organism produces a brown substrate mycelium, a light blue aerial mycelium, and a brown soluble pigment on glucose-yeast extract-malt and potato carrot agars (Greiner-Mai et al., 1987). On oatmeal agar, light brown colonies carry a filmy cover of farinaceous, white to yellowish-white aerial hyphae; sometimes sectors with intensified development of aerial hyphae are seen, but diffusible pigments are not formed (Meyer, 1989). A brown substrate mycelium is formed on peptone-glucose agar, but neither diffusible pigments nor aerial mycelium are produced (Meyer, 1989).

Nutrition and growth conditions. Strain DSM 43186[T] shows good to moderate growth on a range of standard media including glucose-yeast extract-malt extract and oatmeal agars, but grows poorly on Bennett's sucrose and glycerol-asparagine agars (Meyer, 1989). The organism is quite fastidious in its growth temperature and pH requirements; it does not grow below 40°C or above pH 9.0 (McCarthy and Cross, 1984).

Metabolism. Little is known about the metabolic properties of the organism. However, strain DSM 43186[T] was included in a numerical taxonomic study of monosporic actinomycetes and found to be biochemically active with an ability to degrade several complex organic compounds and use a range of sugars as sole carbon sources (McCarthy and Cross, 1984).

Antibiotic sensitivity. The organism is sensitive to a broad range of antibiotics as shown in the species description.

Maintenance procedures

The organism can be maintained on glucose-yeast extract-malt extract agar plates (ISP medium 2; Shirling and Gottlieb, 1966) and as glycerol suspensions of mycelia and spores (20%, v/v) at −20°C. Lyophilization or storage in liquid nitrogen can be used for long-term preservation.

Differentiation of the genus *Thermopolyspora* from other genera

Strain DSM 43186^T can be distinguished from members of other genera classified in the family *Streptosporangiaceae* by using a combination of chemotaxonomic and morphological properties (see Table 275 in section on family *Streptosporangiaceae*), and by 16S rRNA gene sequencing (Goodfellow et al., 2005).

Taxonomic comments

"*Thermopolyspora flexuosa*" was proposed by Krasil'nikov and Agre (1964) for a group of thermophilic actinomycetes which formed short chains of spores on short sporophores. Subsequently, the species had a tortuous taxonomic history as it was included or associated with several taxa, notably the genera *Actinomadura* (Cross and Goodfellow 1973; Lacey et al. 1978), *Micropolyspora* Krasil'nikov et al. 1968, *Nocardia* (Becker et al. 1965; Lechevalier et al. 1966a), and *Nonomuraea* Zhang et al. 1998a. It was classified in the genus *Actinomadura* in the previous edition of *Bergey's Manual of Systematic Bacteriology* (Meyer, 1989). The taxonomic position of the organism was clarified in a polyphasic taxonomic study by Goodfellow et al. (2005) who proposed that the name *Thermopolyspora flexuosa* be revived for the taxon, a proposition advanced by Greiner-Mai et al. (1987) on the basis of chemotaxonomic and morphological data. Goodfellow and his colleagues assigned the genus in the family *Streptosporangiaceae* (Goodfellow et al. 1990b) emend. Ward-Rainey et al. 1997. It is currently classified in the family *Streptosporangiaceae* (Goodfellow et al. 1990b) emend. Zhi et al. 2009.

List of species of the genus *Thermopolyspora*

1. **Thermopolyspora flexuosa** (Meyer 1989) Goodfellow, Maldonado and Quintana 2005, 1982^{VP} (*Actinomadura flexuosa* Meyer 1989, 2519)

fle.xu.o′sa. L. fem. adj. *flexuosa* full of turns or winding, tortuous, flexuous, referring to the morphology of the spore chains.

General chemotaxonomic, colonial, and morphological properties are included in the genus description. Grows well on glucose-yeast extract-malt extract, oatmeal, and peptone-glucose agars. Melanin pigments are formed from tyrosine.

Allantoin and esculin are hydrolyzed, but arbutin and urea are not. Arginine dihydrolase, catalase, citrate lyase, β-galactosidase, β-glucosidase, and oxidase are produced, but acetoin, hydrogen sulfide, lysine decarboxylase, ornithine decarboxylase, and phosphatase are not. Nitrate is reduced.

Agar, casein, DNA, elastin, gelatin, keratin, pectin, starch, testosterone, Tweens 20 and 80, tyrosine, xanthine, and xylan are degraded, but carboxymethylcellulose, chitin, guanine, hypoxanthine, and pectin are not.

L-Arabinose, fructose, galactose, glucose, maltose, mannitol, mannose, sucrose, trehalose, and xylose are used as sole carbon sources for energy and growth, but dulcitol, glycerol, inositol, inulin, lactose, melezitose, melibiose, inositol, raffinose, rhamnose, ribose, sorbitol, and sorbose (all at 1%, w/v) are not. Sodium citrate is used as a sole carbon source, but sodium oxalate (both at 0.1%, w/v) is not.

Alanine, asparagine, arginine, glutamic acid, histidine, tyrosine, and tryptophan are used as sole nitrogen sources, but ammonia salts and nitrates are not.

Grows in the presence of bile salts (0.02%, w/v), crystal violet (0.0,0002%, w/v), potassium tellurite (0.02%, w/v), sodium azide (0.01%, w/v), and thallous acetate (0.001%, w/v), but is sensitive to bile salts (0.5%, w/v), brilliant green (0.0005%, w/v), crystal violet (0.0,0005%, w/v), lysozyme (0.0025%, w/v), sodium azide (0.02%, w/v), sodium chloride (3%, w/v), tetrazolium chloride (0.002%, w/v), and thallous acetate (0.005%, w/v).

Resistant (µg per filter paper disc) to chloramphenicol (25), cephaloridine (5), cephalexin (30), cefoxitin (30), gentamicin sulfate (2), kanamycin sulfate (30), tobramycin sulfate (10), and penicillin (5U), but is sensitive to cephazolin (30), gentamicin sulfate (10), and novobiocin (50 µg/ml).

Major fatty acids (% of total) are $C_{15:0}$ iso (12.5), $C_{15:0}$ (5.7), $C_{16:0}$ iso (13.8), $C_{17:0}$ iso (18.7), $C_{17:0}$ anteiso (12.4), and $C_{17:0}$ 10 methyl-branched (6.9); minor components include $C_{15:0}$ anteiso (4.3), $C_{16:1}$ *cis* (2.8) $C_{15:0}$ iso 2-OH (1.4), $C_{16:0}$ (3.4), $C_{16:0}$ 10 methyl (2.4), $C_{16:0}$ iso 2-OH (2.5), $C_{17:0}$ (2%), $C_{17:0}$ iso 2-OH (3.0), and $C_{18:0}$ (2.5).

Source: soil collected from the Pamir mountains.

DNA G+C content (mol%): 77 (T_m).

Type strain: strain K1132, ATCC 35864, BCRC 12531, CIP 107358, DSM 43186, IMET 9552, JCM 3056, KCC A-0056, KCTC 9270, NBRC 14349, NRRL B-24348.

Sequence accession no. (16S rRNA gene): AY039253.

Additional comments: in the paper by Goodfellow et al. (2005), *Thermopolyspora* is cited as *Thermopolyspora* gen. nov., and *Thermopolyspora flexuosa* as *Thermopolyspora flexuosa* comb. nov., nom. rev. In the abstract of this paper, the strain DSM 43186 is erroneously cited as DSM 41386 [see Erratum. Int. J. Syst. Evol. Microbiol. (2005) *55*:2640]. Basonym: *Actinomadura flexuosa* (*ex* Krasil'nikov and Agre 1964) Meyer 1989, 2519; *Nonomuraea flexuosa* corrig. (Meyer 1989) Zhang et al., 1998a, 420; *Microtetraspora flexuosa* (Meyer 1989) Kroppenstedt et al., 1991, 178; "*Nocardia flexuosa*" (Krasil'nikov and Agre 1964) Becker et al., 1965, 422; "*Thermopolyspora flexuosa*" (Krasil'nikov and Agre 1964) Cross and Goodfellow 1973, 82.

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Family II. **Nocardiopsaceae** (Rainey, Ward-Rainey, Kroppenstedt and Stackebrandt 1996) emend. Zhi, Li and Stackebrandt 2009, 600[VP]

Michael Goodfellow and Martha E. Trujillo

No.car.di.op.sa.ce′a.e. N.L. n. *Nocardiopsis* the type genus of the family;suff. -*aceae* ending to denote a family; N.L. fem. pl. n. *Nocardiopsaceae* the *Nocardiopsis* family.

Aerobic, Gram-stain-positive, non-acid-fast actinomycetes which form an extensively branched substrate mycelium which may bear single spores, sometimes in clusters or spore chains which terminate in pseudosporangia. **Aerial hyphae may carry single spores on dichotomously branched sporangia or differentiate into short or long chains of spores.** All spores are nonmotile. **Some strains are moderate or obligate halophiles.** Whole-cell hydrolysates contain *meso*-diaminopimelic acid as the diagnostic diamino acid. **Contains complex mixtures of saturated, unsaturated and branched-chain fatty acids, but lacks mycolic acids. Polar lipid patterns are complex. Menaquinones have nine, ten, and eleven isoprene units with varying degrees of hydrogenation.** The pattern of 16S rRNA signatures consists of nucleotides at positions 440:497 (U–U), 485 (G), 501:544 (G–C), 502:543 (A–U), 833:853 (U–G) and 1355:1367 (G–C).

Widely distributed, but common in soil, especially saline and hypersaline soils.

DNA G+C content (mol%): 64–76.

Type genus: **Nocardiopsis** (Brocq-Rousseu 1904) Meyer 1976, 487[AL].

Further descriptive information

Phylogeny. The four genera classified in the family *Nocardiopsaceae* form a distinct phyletic line in the 16S rRNA actinobacterial tree (Cai et al., 2008, 2009; Cui et al., 2001; Tang et al., 2008). In addition to *Nocardiopsis*, the type genus, the family contains the genera *Haloactinospora* Tang et al. (2008), *Streptomonospora* corrig. Cui et al. 2001 and *Thermobifida* (Zhang et al. 1998) Yang et al. 2008b. Representatives of these taxa form distinct branches in the 16S rRNA *Nocardiopsaceae* tree (Figure 389) though the position of *Nocardiopsis arabia* in the *Streptomonospora* clade is more apparent than real. This organism has chemical and morphological properties typical of *Nocardiopsis* strains (Hozzein and Goodfellow, 2008) and its position in the *Nocardiopsaceae* tree needs to be re-examined in light of the acquisition of a full 16S rRNA gene sequence. Members of all four genera share a pattern of 16S rRNA signatures (Zhi et al., 2009), as cited in the family description. The family belongs to the order *Streptosporangiales* which also encompasses the families *Streptosporangiaceae* and *Thermomonosporaceae*.

Morphology. *Nocardiopsaceae* strains typically form an extensively branched substrate mycelium, abundant aerial hyphae and nonmotile spores. However, members of the constituent genera show a range of morphological features of diagnostic value (Table 294). The aerial hyphae of *Nocardiopsis* strains, for instance, are either long and moderately branched, straight and flexuous or irregularly zig-zagged, completely fragmenting into oval to elongated, rod-shaped smooth-surfaced spores (Chen et al., 2008; Hozzein and Goodfellow, 2008; Meyer, 1976, 1989). Short straight to flexuous chains of oval, cylindrical or rod-shaped spores are formed on the aerial mycelium of *Streptomonospora* strains (Cai et al., 2008, 2009; Cui et al., 2001; Li et al., 2003b) and long chains of cylindrical spores with smooth surfaces on the aerial mycelium of *Haloactinospora alba* YIM 90648^T, the sole member of this genus (Tang et al., 2008). In contrast, the aerial mycelium of *Thermobifida* strains carry oval to round, single spores on dichotomously branched sporophores which give the appearance of spore clusters; the latter may also be seen occasionally on the substrate mycelium (Yang et al., 2008b; Zhang et al., 1998). Characteristic chains of wrinkled spores terminating in pseudosporangia are borne on the substrate mycelium of the *Haloactinospora alba* strain (Tang et al., 2008) while single, oval or round spores with wrinkled surfaces are formed on either sporophores or dichotomously branching sporophores on the substrate mycelium of members of the genus *Streptomonospora* (Cai et al., 2008, 2009; Cui et al., 2001; Li et al., 2003b).

Chemotaxonomy. Members of the family contain *meso*-diaminopimelic acid as the wall diamino acid and hence have a wall chemotype III *sensu* Lechevalier and Lechevalier (1970b). *Nocardiopsis* and *Thermobifida* strains lack diagnostic sugars (Kroppenstedt and Evtushenko, 2006) whereas *Streptomonospora* strains contain galactose or arabinose and galactose as a characteristic wall sugar (Cai et al., 2008, 2009; Cui et al., 2001) and *Haloactinospora* galactose and ribose (Tang et al., 2008). Members of the family have complex polar lipid patterns with *Streptomonospora* and *Thermobifida* exhibiting a type II and *Haloactinospora* and *Nocardiopsis* a type III profile after Lechevalier et al. (1977, 1981). All four genera are characterized by complex menaquinone (Table 294) and fatty acid profiles; the latter typically contain large proportions of 14-methylpentadecanoic ($C_{16:0}$ iso) and 14-methylhexadecanoic acid ($C_{17:0}$ anteiso) (Kroppenstedt and Evtushenko, 2006; Tang et al., 2008). The menaquinone composition of some *Streptomonospora* strains is influenced by the growth media (Li et al., 2003b); medium-dependent shifts in menaquinone profiles have been reported for *Micrococcus luteus* (Hiraishi and Komagata, 1989) and age-dependent ones in *Streptomyces cyaneus* (Saddler et al., 1986). Teichoic acids are valuable taxonomic markers for the subgeneric classification of *Nocardiopsis* species (Kroppenstedt and Evtushenko, 2006; Naumova et al., 2001). The cell walls of *Thermobifida fusca* also contain teichoic acids (Potekhina et al., 2003) and a polyglycerophosphate lipoteichoic acid (Rahman et al., 2009).

Differentiation of the genera of the family *Nocardiopsaceae*

The genera classified in the family *Nocardiopsaceae* can be distinguished by using a combination of chemotaxonomic, morphological and physiological criteria (Table 294), by 16S rRNA gene signature nucleotides (Tang et al., 2008) and by comparisons of 16S rRNA gene sequenced data (Figure 389). Standard chemotaxonomic procedures can be used for the detection of diagnostic amino acids and sugars in whole-cell hydrolysates (Hasegawa et al., 1983; Staneck and Roberts, 1974), cellular fatty acids (Kroppenstedt, 1985; Sasser, 1990), and polar lipid patterns (Minnikin et al., 1984). Detailed

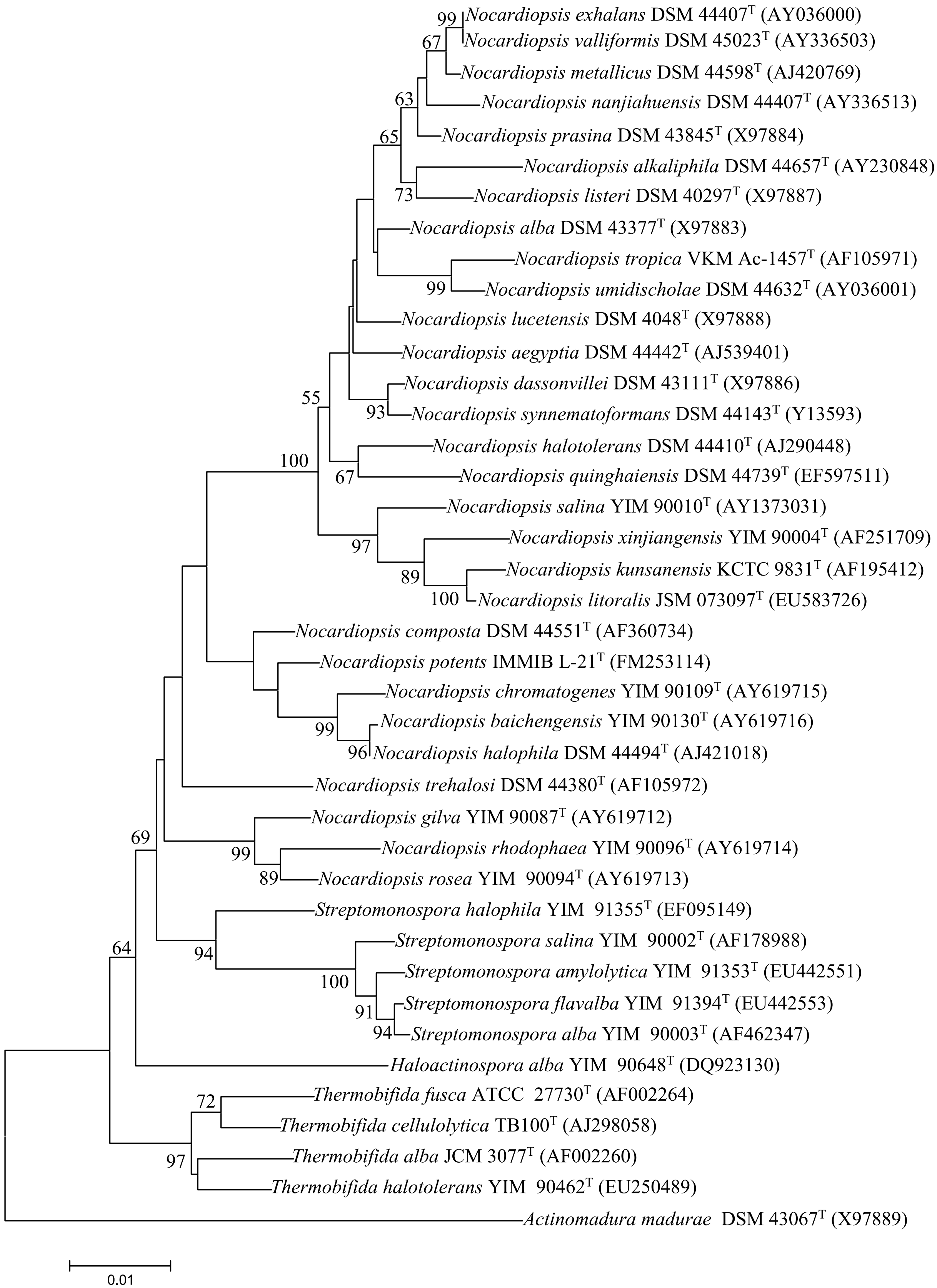

FIGURE 389. Neighbor-joining tree based on 16S rRNA gene sequences showing relationships between taxa classified in the family *Nocardiopsaceae*. Evolutionary distances were calculated using the Kimura two-parameter method of Kimura (1980). Numbers at the nodes are bootstrap values based on 1000 resampled datasets, only values above 50% are given. Bar, 1 nucleotide difference per 100 nucleotides.

TABLE 294. Properties that distinguish between the genera classified in the family *Nocardiopsaceae*[a]

Characteristic	*Nocardiopsis*	*Haloactinospora*	*Streptomonospora*	*Thermobifida*
Spores formed on:				
Aerial mycelium	Long chains	Long chains	Long chains	Single spores on dichotomously branched sporangia
Substrate mycelium	None	Spore chains terminating in pseudosporangia	Single	Occasional single spores borne on dichotomously branched sporangia
Temperature range (°C)	20–45	7–45	20–45	35–60
Growth on NaCl (%)	0–20	9–21	5–25	0–5
Fatty acid type[b]	3d	3d	nd	3e
Phospholipid pattern[c]	DPG, PC, PE, PG, (PI), PME, (PIM)	DPG, PC, PG, PIM	DPG, PC, (PE), PG, PI, (PIMS), (PME), PS)	DPG, PC, (PE) PG, PI, PME
Predominant menaquinones	10(H_2, H_4, H_6), 11(H_2, H_4, H_6, H_8)	11(H_6, H_8)	9(H_2, H_4, H_6, H_8), 10(H_2, H_4, H_6)	10(H_6, H_8)
DNA G+C content (mol%)	64–76	68	69–71	64–69

[a]Data taken from Cui et al. (2001), Cai et al. (2008, 2009), Kroppenstedt and Evtushenko (2006), Li et al. (2003b), Tang et al. (2008) and Zhang et al., (1998).

[b]Fatty acid types according to Kroppenstedt (1985); nd, not determined.

[c]Abbreviations for phospholipids: DPG, diphosphatidylglycerol; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; PI, phosphatidylinositol; PIM, phosphatidylinositol mannoside(s); PME, phosphatidylmethylmonoethanolamine; PS, phosphatidylserine; (), variable distribution.

analytical procedures are available for establishing menaquinone profiles (Kroppenstedt, 1982, 1985) (Collins, 1994), including the integrated method introduced for the analysis of bacterial isoprenoid quinones and polar lipids (Minnikin et al., 1984). Genus-specific primers are available for the PCR identification of novel *Streptomonospora* strains (Cai et al., 2009; Zhi et al., 2006).

Genus I. **Nocardiopsis** Meyer 1976, 487[AL]

Wael N. Hozzein and Martha E. Trujillo

No.car.di.op′sis. N.L. fem. n. *Nocardia* a genus of the order *Actinomycetales*; Gr. fem. n. *opsis* appearance; N.L. fem. n. *Nocardiopsis* that which has the appearance of *Nocardia*.

Gram-stain-positive, aerobic, chemo-organotrophic, **non-acid-fast**, nonmotile filamentous actinomycetes. Substrate mycelium is well developed and hyphae are long and densely branched. **Fragmentation into coccoid and bacillary elements may occur.** Aerial mycelium is well developed and sparse-to-abundant; **aerial hyphae are long**, branched, **straight to flexuous, or irregularly zig-zagged**, **completely fragmenting into spores of various lengths. Spore surface is smooth. Wall peptidoglycan** contains ***meso*-diaminopimelic acid** and the muramic acid of the peptidoglycan is acetylated. No diagnostic sugars are found in whole-organism hydrolysates. **Mycolic acids are absent.** Major phospholipids are **phosphatidylcholine**, phosphatidylmethylethanolamine, phosphatidylglycerol, and phosphatidylinositol, with small amounts of diphosphatidylglycerol. Menaquinones are predominantly variably hydrogenated with ten isoprene units **(MK-10)**, with minor amounts of the **MK-9** and/or **MK-11** series. The main fatty acids are branched and **10-methyl-branched fatty acids, 14-methyl-heptadecanoic acid, and 14-methyl-hexadecanoic acid.** Growth temperature range is 10–45°C. Widely distributed in saline and alkaline soils, and found in compost, vegetable matter, indoor environments, and clinical material of animal and human origin.

DNA G+C content (mol%): 64–69.

Type species: **Nocardiopsis dassonvillei** (Brocq-Rousseau 1904) Meyer 1976, 487[AL].

Further descriptive information

Phylogeny. The genus *Nocardiopsis* is the type genus of the family *Nocardiopsaceae* (Rainey et al., 1996) emend. Zhang et al. (1998), which also includes the genera *Haloactinospora* Tang et al. (2008), *Streptomonospora* (Cui et al., 2001) emend. Li et al. (2003b), and *Thermobifida* (Zhang et al., 1998) emend. Yang et al. (2008b). Phylogenetically, these genera form a distinct and coherent phyletic line in the 16S rRNA gene tree (Cui et al., 2001; Kroppenstedt and Evtushenko, 2006; Tang et al., 2008; Zhang et al., 1998) and can be clearly distinguished from the other two families comprising the suborder *Streptosporangineae* Ward-Rainey et al. 1997, elevated to order *Streptosporangiales* in the present volume, namely the families *Streptosporangiaceae* and *Thermomonosporaceae* (Goodfellow and Quintana, 2006; Gyobu, 2001; Kudo, 2001). The pattern of 16S rRNA signatures consists of nucleotides at positions 440:497 (C–G), 501:544 (C–G), 502:543 (G–C), 831:855 (G–G) 843 (U), 844 (A), and 1355:1367 (A–U) (Zhi et al., 2009).

The genus *Nocardiopsis*, which currently contains 30 species, does not appear to be monophyletic according to 16S rRNA

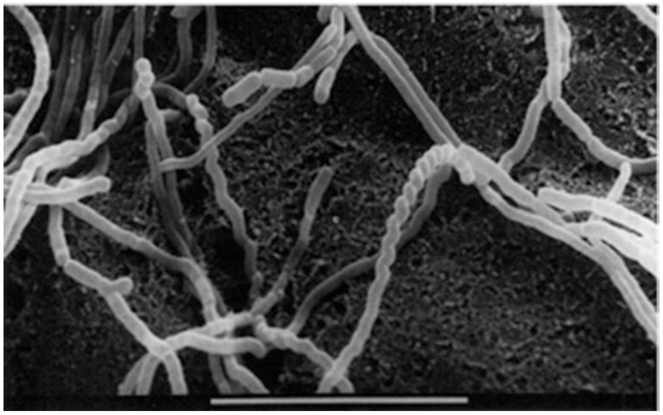

FIGURE 390. Scanning electron micrograph of strain *Nocardiopsis lucentensis* A5-1T^{T}, showing zig-zag hyphae and spore chain with a smooth surface. The culture was grown on ISP medium 4 supplemented with 10% NaCl for 14 d at 35°C. Bar = 1 μm. (Reproduced with permission from Yassin et al., 1993. Int. J. Syst. Bacteriol. *43*: 266–271.)

gene sequence phylogeny (see Figure 389). *Nocardiopsis* species are divided in four phyletic groups, the largest, with 20 species, includes the type species *Nocardiopsis dassonvillei*. On the other hand, a single member cluster is formed by *Nocardiopsis trehalosi*.

Cell morphology. Members of the genus *Nocardiopsis* characteristically form a well-developed and branched substrate mycelium which may fragment into coccoid and bacillary elements. Aerial mycelium production varies from sparse to abundant. The aerial hyphae formed by some strains may not be visible to the unaided eye, requiring careful observation under a microscope. Other cultures are thickly covered with a powdery to velvety aerial mycelium simulating cultures of *Streptomyces griseus* (cf. Gordon and Horan, 1968) because of the yellowish-gray color. Aerial hyphae are long, moderately branched, straight, or flexuous. Initiation of sporulation is often characterized by twisted hyphae, which by examination at higher magnification, reveal a zig-zag arrangement of the developing spores (Figure 390). The elongated spores are smooth and can divide subsequently into smaller spores of irregular size by cross-wall formation. Spores are enclosed within a fibrillar sheath and have thickened polar walls (Williams et al., 1974). *Nocardiopsis synnemataformans* is the only species known to form synnemata from spiral aerial hyphae that wrap together to form long ropes that subsequently fragment into small rod-shaped elements (Figure 391) (Yassin et al., 1997). *Nocardiopsis* strains do not produce sporangia, sclerotia, or motile elements.

The morphology of *Nocardiopsis* resembles that of other arthrospore-forming actinomycetes. However, members of the genus can be presumptively recognized based on various specific macroscopic and microscopic features. Although many *Nocardiopsis* strains resemble some *Streptomyces* strains macroscopically (Gordon and Horan, 1968; Shirling and Gottlieb, 1972), the two genera can be distinguished by direct microscopic comparison of cultures based on their type of life cycle and spore formation. In *Streptomyces*, spores are delimited basipetally almost simultaneously, whereas in *Nocardiopsis*, the cross

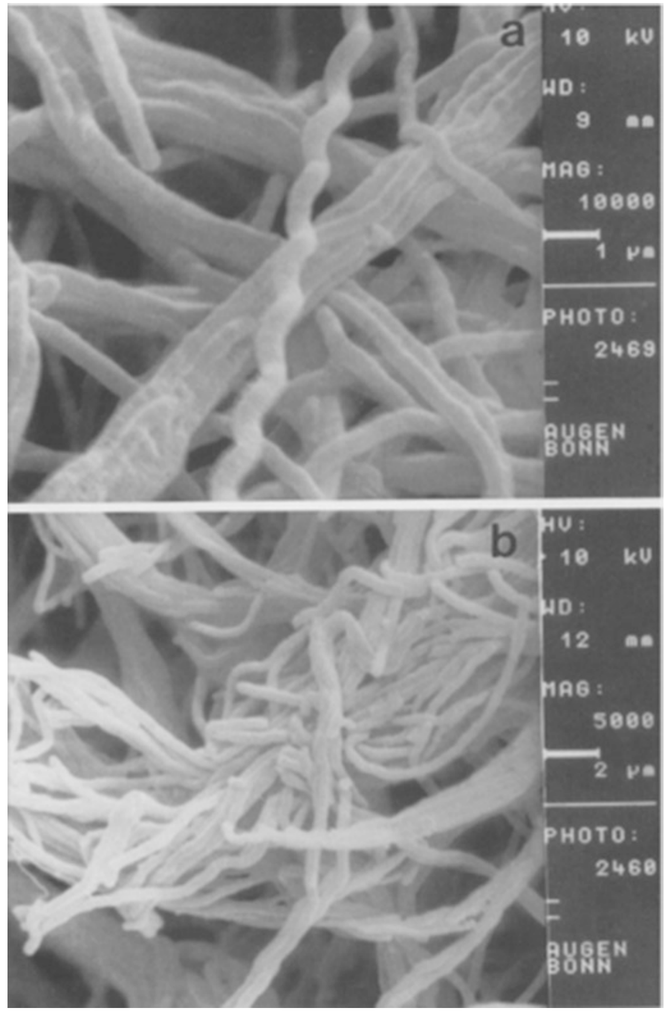

FIGURE 391. Scanning electron micrographs of *Nocardiopsis synnemataformans* strain IMMIB D-1215^{T}, showing spiral hyphae wrapped together to form synnemata (a), which fragment into small rod-shaped elements (b). (Reproduced with permission from Yassin et al., 1997. Int. J. Syst. Bacteriol. *47*: 983–988.)

walls are formed in a relatively uncoordinated manner resulting in spores of various lengths. *Nocardiopsis* species can also differentiated by their nocardioform life cycle.

Cell-wall composition. Members of the genus *Nocardiopsis* have cell-wall chemotype III, which corresponds to peptidoglycan type A1 (Schleifer and Kandler, 1972), i.e. strains contain 2,6-*meso*-diaminopimelic acid (*meso*-DAP), alanine, and glutamic acid in their peptidoglycan. The muramic acid of the peptidoglycan is acetylated (Kroppenstedt, 1987). In whole-cell hydrolyzates, glucose and galactose are detected, but no diagnostic sugars have been found in any of the species analyzed. Cell-wall teichoic acids are present and composed of glycerol and other constituents, like ribitol, glucosamine, galactosamine, succinic, and propionic acids (Naumova et al., 2001). Teichoic acid polymers are considered a valuable chemotaxonomic marker for the intrageneric taxa of *Nocardiopsis* (Kroppenstedt and Evtushenko, 2006; Streshinskaya et al., 1989, 1996, 1998; Tul'skaya et al., 1993, 2000).

Polar lipids. Members of the genus *Nocardiopsis* show a characteristic phospholipid type III profile (Lechevalier et al., 1977) with phosphatidylcholine (PC) as the characteristic polar lipid, plus phosphatidylmethylethanolamine (PME), phosphatidylglycerol (PG), phosphatidylinositol (PI), and small amounts of diphosphatidylglycerol (DPG). Additionally, two spots of glycolipids, identified as monomannosyl diglyceride and monoacetylated glucose, right below the PME spot and two to four unidentified phospholipids above the DPG spot can be detected on two-dimensional thin-layer chromatography plates as additional lipid markers (Grund, 1987; Grund and Kroppenstedt, 1990; Kroppenstedt and Evtushenko, 2006; Minnikin et al., 1977, 1984; Mordarska et al., 1983, 1998).

Menaquinone composition. Members of the genus *Nocardiopsis* show complex menaquinone profiles containing predominant amounts of MK-10(H_0) to MK-10(H_8) and small amounts of the MK-9 and/or MK-11 series (Fischer et al., 1983; Grund and Kroppenstedt, 1990; Minnikin et al., 1978).

Fatty acid profile. *Nocardiopsis* strains are characterized by terminally branched and 10-methyl-branched fatty acids (with chain lengths of 14–18 carbons), but hydroxy fatty acids have never been detected (Grund and Kroppenstedt, 1990; Kroppenstedt and Evtushenko, 2006). Among the terminally branched fatty acids, 14-methyl-heptadecanoic acid ($C_{16:0}$ iso) and 14-methyl-hexadecanoic acid ($C_{17:0}$ anteiso) are the main components. Smaller amounts of the 10-methyl branched tuberculostearic acid, i.e. 10-methyl-octadecanoic acid ($C_{18:0}$10-methyl), and its precursor the unsaturated *cis*9,10 octadecenoic acid ($C_{18:1}$ *cis*) are also found (Grund and Kroppenstedt, 1990). This fatty acid profile belongs to fatty acid type 3d according to Kroppenstedt (1985).

Cultural characteristics. *Nocardiopsis* species tend to grow well on both complex and defined media. Most strains show good to abundant growth on modified Bennett's (Jones, 1949), Czapek–Dox, nutrient (Waksman, 1961b), and glucose-yeast extract-malt extract (Athalye et al., 1981) agars, and on formulations of the International *Streptomyces* Project (ISP media; Shirling and Gottlieb, 1966). Several *Nocardiopsis* species may require NaCl for optimal growth. The halotolerant or moderately halophilic species, *Nocardiopsis halophila*, *Nocardiopsis kunsanensis*, *Nocardiopsis salina*, and *Nocardiopsis xinjiangensis* grow optimally on most ISP media in the presence of 10–15% (w/v) NaCl (Al-Tai and Ruan, 1994; Chun et al., 2000; Li et al., 2003a, 2004).

Nutrition and growth conditions. *Nocardiopsis* strains are strictly aerobic and chemo-organotrophic with an oxidative metabolism. Most species grow well at 10–40°C with an optimum growth temperature of 28–30°C; in addition, several *Nocardiopsis* species are considered thermotolerant organisms and may grow at 45–50°C, with an optimal growth temperature of 37°C, namely, *Nocardiopsis baichengensis*, *Nocardiopsis chromatogenes*, *Nocardiopsis composta*, *Nocardiopsis kunsanensis*, *Nocardiopsis rhodophaea*, and *Nocardiopsis rosea* (Chun et al., 2000; Kämpfer et al., 2002; Li et al., 2006). One thermophilic *Nocardiopsis* isolate, KMD/8, which is able to grow at 65°C with an optimal growth temperature of 50°C, was reported by Kempf (1995).

Nocardiopsis strains can tolerate NaCl concentrations up to 20% (w/v). Halotolerant species include *Nocardiopsis baichengensis*, *Nocardiopsis chromatogenes*, *Nocardiopsis composta*, *Nocardiopsis gilva*, *Nocardiopsis halotolerans*, *Nocardiopsis rhodophaea*, and *Nocardiopsis rosea*, which can grow in media supplemented with 15–18% (w/v) NaCl (Al-Zarban et al., 2002; Kämpfer et al., 2002; Li et al., 2006). Only *Nocardiopsis halophila*, *Nocardiopsis kunsanensis*, *Nocardiopsis xinjiangensis*, and *Nocardiopsis salina* (Al-Tai and Ruan, 1994; Chun et al., 2000; Li et al., 2003a, 2004) are considered as true halophilic species as NaCl (at least 3%, w/v) is necessary for growth, with an optimal concentration of 10–15% (w/v).

Most *Nocardiopsis* strains are characterized by their alkaliphilic behavior as they prefer mild alkaline conditions and some can even grow at pH 13. Alkaliphilic species include *Nocardiopsis alkaliphila*, *Nocardiopsis ganjiahuensis*, *Nocardiopsis kunsanensis*, *Nocardiopsis litoralis*, *Nocardiopsis metallicus*, *Nocardiopsis prasina*, and *Nocardiopsis valliformis* (Chen et al., 2009; Hozzein et al., 2004; Miyashita et al., 1984; Schippers et al., 2002; Yang et al., 2008c; Zhang et al., 2008).

Nocardiopsis strains can utilize a wide range of carbohydrates as carbon sources. They also hydrolyze and degrade diverse compounds. A list of these substrates is given in Table 295.

Pigments. Most *Nocardiopsis* strains do not produce diffusible pigments. However, Gerber (1966) reported that various *Nocardiopsis dassonvillei* strains produced purple colored crystals which were identified as iodinin crystals. Gordon and Horan (1968) also reported the production of yellowish, greenish-yellow, or brown diffusible pigments by strains of the same species. *Nocardiopsis tropica* produces yellow-orange soluble pigments on oatmeal agar or glycerol-yeast extract agar (Evtushenko et al., 2000), whereas *Nocardiopsis chromatogenes* was found to produce a diffusible yellowish-pink pigment on Czapek and ISP 5 agar (Li et al., 2006).

Genetics. Similarity values between the 16S rRNA gene sequences of strains of *Nocardiopsis* species with validly published names range from 93.6 to 99.9%; 41.3% of the similarity values are ≥97%. Although many type strains in the genus share a 16S rRNA gene sequence similarity of 99%, DNA–DNA reassociation values are well below 70%. A value of 55.9% between *Nocardiopsis halophila* and *Nocardiopsis baichengensis* has been reported (Li et al., 2006) and the DNA–DNA values of *Nocardiopsis metallicus* with *Nocardiopsis exhalans* and *Nocardiopsis prasina* are 18.2% and 44.1%, respectively (Schippers et al., 2002).

The *gyrB*, *sod*, and *rpoB* partial gene sequences of strains of 24 *Nocardiopsis* species with validly published names have been determined; the mean similarities of the *gyrB*, *sod* and *rpoB* genes of *Nocardiopsis* species were 87.7%, 87.3%, and 94.1% (that of the 16S rRNA gene of *Nocardiopsis* species studied was 96.6%). The topology of the *gyrB* gene tree showed a high consistency with that of the 16S rRNA gene tree suggesting that the *gyrB* gene could be used as a taxonomic phylogenetic marker with a higher degree of discrimination between the species studied (Yang et al., 2007a, 2008a).

Genes encoding industrially important enzymes such as β-1,3-glucanase and chitinases ChiA and ChiB, from two alkaliphic *Nocardiopsis* strains (F96 and *Nocardiopsis prasina* OPC-131, respectively), have been cloned, sequenced, and expressed in *Escherichia coli* (Masuda et al., 2003; Tsujibo et al., 2003). Sequencing of the whole genome of *Nocardiopsis dassonvillei* DSM 43111[T] is underway (Project ID: 19709 at DOE Joint Genome Institute).

TABLE 295. Differentiating characters for *Nocardiopsis* species[a]

Character	*N. dassonvillei*	*N. aegyptia*	*N. alba*	*N. alkaliphila*	*N. arabia*	*N. baichengensis*	*N. chromatogenes*	*N. composta*	*N. exhalans*	*N. ganjiahuensis*	*N. gilva*	*N. halophila*	*N. halotolerans*	*N. kunsanensis*	*N. listeri*
Color of:[b]															
AM	w	w	w	yw	yw	w	w	w	w	gw	py	w	gy	w	–
SM	b	br	py	yb	gy	oy	rb	yb	y	py	b	yr	b	y	b
Synnemata	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Carbon source utilization:															
L-Arabinose	+	+	–	+	+	+	+	+	+	+	+	+	–	+	+
Cellobiose	+	nd	+	+	+	+	+	+	nd	–	+	w	nd	+	+
D-Galactose	(+)	+	+	w	+	+	+	+	–	nd	+	+	+	v	d
Inositol	–	+	–	w	nd	–	+	+	–	nd	+	+	nd	v	d
D-Lactose	–	+	–	w	nd	–	+	nd	–	–	+	nd	nd	v	d
Maltose	(+)	nd	+	+	+	+	+	–	+	nd	–	+	nd	–	–
Mannitol	+	+	–	–	+	+	+	+	+	nd	+	d	nd	v	d
Melibiose	–	nd	–	nd	nd	nd	nd	nd	–	nd	nd	nd	+	+	+
L-Rhamnose	+	+	–	w	nd	+	+	–	+	+	–	d	nd	+	+
Sucrose	(+)	+	–	+	+	+	+	–	+	–	+	+	+	v	d
Trehalose	+	nd	+	nd	+	nd	nd	+	+	nd	nd	+	nd	–	–
D-Xylose	+	+	–	+	–	–	+	–	+	+	+	+	–	+	+
Degradation of:															
Casein	+	nd	+	+	+	nd	nd	nd	nd	nd	nd	+	nd	+	+
Tyrosine	(+)	nd	+	+	–	nd	nd	nd	nd	nd	nd	–	nd	+	+
Tween 80	(–)	nd	+	nd	nd	nd	nd	nd	nd	+	nd	+	nd	nd	–
Tween 85	(+)	nd	+	nd	nd	nd	nd	nd	nd	–	nd	nd	nd	nd	–
Nitrate reductase	(–)	nd	(–)	nd	nd	–	–	nd	nd	+	+	nd	nd	–	+
Urease	(–)	nd	(+)	nd	nd	–	–	nd	nd	+	–	+	nd	+	–
Growth at/in:															
pH (optimal)[c]	8	nd	9	9.5	nd	7.2	7.2	nd	nd	9.0	7.2	nd	nd	9	8
0% NaCl	+	+	+	+	+	+	+	+	+	+	+	d	+	–	+
10% NaCl	+	–	–	+	+	+	+	+	+	–	+	+	+	+	–
20% NaCl	–	–	–	–	–	–	–	–	–	–	–	+	–	+	–
10°C	(–)	+	–	+	+	–	–	–	+	+	+	–	+	nd	+
42°C	(+)	nd	(+)	+	–	+	+	+	–	–	–	–	–	nd	–
45°C	(–)	–	–	+	–	+	+	+	–	–	–	–	–	nd	–

Character	*N. litoralis*	*N. lucentensis*	*N. metallicus*	*N. potens*	*N. prasina*	*N. quinghaiensis*	*N. rhodophaea*	*N. rosea*	*N. salina*	*N. synnemataformans*	*N. trehalosi*	*N. tropica*	*N. umidischolae*	*N. valliformis*	*N. xinjiangensis*
Color of:[b]															
AM	w	w	w	w	og	w	pi	pi	w	w	w, gy	w	w	yw	w
SM	yw	y	yb	py	lo	py	rb	pi	py	p	lo, yr	oy	y	y	y
Synnemata	–	–	–	–	–	–	–	–	–	+	–	–	–	–	–
Carbon source utilization:															
L-Arabinose	–	–	nd	+	+	+	+	+	–	–	+	+	+	+	nd
Cellobiose	–	+	+	+	d	+	–	–	–	+	–	+	+	nd	–
D-Galactose	–	–	+	+	–	–	–	–	–	+	+	+	+	nd	–
Inositol	nd	+	nd	+	–	+	+	–	–	–	–	–	–	–	nd
D-Lactose	–	–	nd	+	–	–	–	+	nd	–	+	–	nd	+	nd
Maltose	–	+	+	+	–	–	–	+	–	+	+	+	+	nd	–
Mannitol	–	+	+	+	–	+	–	–	–	+	+	+	+	nd	–
Melibiose	–	–	+	nd	–	–	nd	nd	–	nd	+	+	+	nd	nd
L-Rhamnose	–	+	+	–	–	–	–	+	–	+	+	+	+	nd	–
Sucrose	+	+	+	+	–	+	–	+	+	–	–	+	+	–	+
Trehalose	–	+	+	+	–	+	nd	nd	–	–	+	+	+	nd	nd
D-Xylose	+	–	+	–	+	nd	–	–	–	+	+	+	+	+	v
Degradation of:															
Casein	–	+	nd	nd	+	+	nd	nd	nd	+	+	–	nd	nd	nd
Tyrosine	+	+	nd	nd	+	nd	nd	nd	nd	+	–	–	nd	nd	nd
Tween 80	–	–	nd	nd	–	+	nd	nd	nd	nd	+	+	nd	nd	nd
Tween 85	nd	–	nd	nd	–	nd	nd	nd	nd	nd	–	+	nd	nd	nd
Nitrate reductase	–	+	nd	nd	–	–	–	+	+	+	–	–	nd	+	–
Urease	–	–	nd	nd	–	–	–	–	–	+	+	+	nd	nd	+
Growth at/in:															
pH (optimal)[c]	8.5	7.5	8.5	8.5	10	7.0	7.2	7.2	7.2	nd	nd	nd	nd	9.5	7.2
0% NaCl	+	d	+	+	+	+	+	+	–	+	+	+	+	+	nd
10% NaCl	+	–	+	+	–	+	+	+	+	+	–	–	–	–	+
20% NaCl	–	–	–	–	–	–	–	–	+	–	–	–	–	–	nd
10°C	–	+	+	+	–	+	–	–	–	–	–	d	+	–	nd
42°C	–	–	–	–	+	–	+	+	–	–	+	–	–	–	nd
45°C	–	–	–	–	–	–	+	+	–	–	+	–	–	–	nd

[a]Modified from Kroppenstedt and Evtushenko (2006). Data from: Al-Tai and Ruan (1994); Al-Zarban et al. (2002); Chen et al. (2008, 2009); Chun et al. (2000); Evtushenko et al. (2000); Grund and Kroppenstedt (1990); Hozzein et al. (2004); Hozzein and Goodfellow (2008); Kämpfer et al. (2002); Li et al. (2003a, 2006); Peltola et al. (2001); Sabry et al. (2004); Schippers et al. (2002); Yang et al. (2008c); Yassin et al. (1993, 1997, 2009); Zhang et al. (2008). Symbols: +, positive; (+) most strains of the species are positive; –, negative; (–), most strains of the species are negative; nd, not determined; v, variable; d, doubtful result.

[b]AM, aerial mycelium; SM substrate mycelium; –, no aerial mycelium usually formed; b, beige-brown; br, brown; gw, gray-white; gy, gray-yellow; lo, light olive; og, olive-green; oy, orange-yellow; p, pimento; pi, pink; py, pale-yellow; rb, red-brown; w, white; y, yellow; yb, yellow-brown; yr, yellow-red; yw, yellow-white.

Phages. Four *Nocardiopsis* phages were reported by Prauser (1981); however, information about their taxon specificity is still lacking. Clearing effects caused by soil-isolated polyvalent *Streptomyces* phages on *Nocardiopsis dassonvillei* raised speculations about the relationship of these two taxa (Prauser, 1984). However, these two genera can be clearly differentiated and this may only be due to the phage's specificity.

Pathogenicity. The isolation of *Nocardiopsis* strains from human or animal clinical material is well documented (Goodfellow, 1998; Schaal and Beaman, 1984). Like most other actinomycete pathogens, *Nocardiopsis* species are opportunistic rather than invasive pathogens. Liegard and Landrieu (1911) reported the isolation of a strain matching the description of Brocq-Rousseau (1904) from a case of ocular conjunctivitis where they gave it the name *Nocardia dassonvillei*, which later became *Nocardiopsis dassonvillei* (Meyer, 1976). Gordon and Horan (1968) reported that 15 of the 26 *Nocardiopsis* strains in their collection originated from clinical isolates. They speculated that similar cultures from other clinical sources might usually be discarded in laboratories because of their macroscopic resemblance to the genus *Streptomyces*.

The implication of *Nocardiopsis dassonvillei* in skin infections has also been reported (Philip and Roberts, 1984; Singh et al., 1991). The first case of mycetoma caused by *Nocardiopsis dassonvillei* was published by Sindhuphak et al. (1985), who repeatedly isolated *Nocardiopsis dassonvillei* strains from nodules and draining sinuses from the leg of a 39-year-old man. Ajello et al. (1987) confirmed the presence of *Nocardiopsis dassonvillei* in actinomycetoma cases. Furthermore, *Nocardiopsis dassonvillei* is involved in broncho-pulmonary infections (Bernatchez and Lebreux, 1991; Gugnani et al., 1998; Mordarska et al., 1998) and has also been recovered from blood samples (Beau et al., 1999). Although the pathogenicity of *Nocardiopsis synnemataformans* isolated from the sputum of a kidney transplant patient could not be verified, this species is regarded as a potential pathogen.

Antibiotic sensitivity. Very little is known about antibiotic sensitivity of clinical *Nocardiopsis* strains and only a few studies have been carried out. Philip and Roberts (1984) reported that a cutaneous infection caused by *Nocardiopsis dassonvillei* in an elderly man responded to oral treatment with trimethoprim-sulfamethoxazole. Yassin et al. (1997) examined *Nocardiopsis synnemataformans* for its sensitivity by using the agar dilution technique. They reported the following minimum inhibitory concentrations (μg/ml) for: mezlocillin (2.0); amoxycillin plus clavulanic acid (2.0); imipenem (2.0); erythromycin (8.0); clindamycin (>128); tetracycline (≤0.2); vancomycin (≤0.2); gentamicin (≤0.2); tobramycin (≤0.2); amikacin (≤0.2); ciprofloxacin (2.0); and ofloxacin (8.0). In an earlier study, Yassin et al. (1993) also reported that *Nocardiopsis lucentensis* is resistant to lincomycin (128 μg/ml), penicillin G (128 μg/ml), gentamicin (64 μg/ml), streptomycin (64 μg/ml), and neomycin (4 μg/ml), but not to rifampin (128 μg/ml). Similarly, Al-Tai and Ruan (1994) used the paper disk-diffusion method to assess the susceptibility of *Nocardiopsis halophila* and found that it was resistant to amoxycillin, clindamycin, bacitracin, cephalexin, and oxacillin, but susceptible to carbenicillin, chloramphenicol, erythromycin, and novobiocin.

Secondary metabolites. *Nocardiopsis* strains are also known for their potential to produce bioactive metabolites. A disaccharide antibiotic (3-trehalosamine) active against Gram-stain-positive bacteria was reported to be synthesized by *Nocardiopsis trehalosi* (Dolak et al., 1980, 1981). Phenazine antibiotics were obtained from the alkaliphilic *Nocardiopsis* strain OPC-15, which produced different phenazine antibiotics under different culture conditions (Tsujibo et al., 1988). In addition, 1,6-dihydroxyphenazine was obtained from the mycelium after incubation for 6–8 d at 27°C, whereas 1,6-dihydroxyphenazine-5,10-dioxide, known as iodinin, was isolated after incubation for 6 d at 27°C followed by further incubation for 2 d at 4°C. The production of a new indole alkaloid, pendolmycin, by *Nocardiopsis* strain SA 1715, isolated from soil collected in a river near Shanghai, was reported by Yamashita et al. (1988). Members of the genus also produce apoptolidins, new apoptosis inducers in transformed cells (Kim et al., 1997; Wender and Longcore, 2009; Wender et al., 2005).

In the last decade, some bioactive compounds have been discovered from *Nocardiopsis* strains isolated from marine samples. Kahakamides A and B, two new neosidomycin antibiotics (a group of rare indole-*N*-glycosides) were isolated from a *Nocardiopsis dassonvillei* strain (Schumacher et al., 2001). Also, a novel cyclic tetrapeptide was obtained from the culture broth of a marine *Nocardiopsis* isolate (Shin et al., 2003).

A marine-derived actinomycete, *Nocardiopsis* sp. (CMB-M0232), obtained from a sediment sample collected at a depth of 55 m off the coast of Brisbane, Australia, yielded two new FKBP12-binding macrolide polyketides named nocardiopsins A and B (Raju et al., 2010).

As expected, alkalitolerant/alkaliphilic *Nocardiopsis* strains can synthesize many different alkaline enzymes. These compounds have been reported from different *Nocardiopsis* strains (Moreira et al., 2002, 2003). In addition, three chitinases from the alkaliphilic *Nocardiopsis prasina* OPC-131 (Tsujibo et al., 2003), a keratinolytic protease from the alkaliphilic *Nocardiopsis* sp. strain TOA-1 (Mitsuiki et al., 2004), an alkaline protease from *Nocardiopsis* sp. (Monteiro et al., 2005), an endo-1,3-β-glucanase from the alkaliphilic *Nocardiopsis* sp. strain F96 (Fibriansah et al., 2006), and an endo-β-1,4-D-glucanase from the alkalitolerant *Nocardiopsis* sp. SES28 (Walker et al., 2006) have also been isolated. Moreover, a thermostable α-amylase from *Nocardiopsis* sp. was reported by Stamford et al. (2001) and an acid-resistant protease enzyme produced by a strain of *Nocardiopsis alba* was studied by Kelch et al. (2007).

Degradation of organic polymers and transformation of organic compounds have also been reported as applications of some *Nocardiopsis* species. Ghanem et al. (2005) published the first report on the degradation of polyesters, poly(3-hydroxybutyrate) and its copolymers with poly(3-hydroxyvalerate) [poly(3-hydroxybutyrate-co-10–20%-hydroxyvalerate)], by the marine species *Nocardiopsis aegyptia*. Moreover, in a review on transformation of steroids by actinobacteria, Donova (2007) reported that representatives of *Nocardiopsis* are capable of hydroxylating dehydroepiandrosterone.

Ecology. The natural habitat of *Nocardiopsis* strains appears to be the soil where they are frequently isolated together with other actinobacteria (Dolak et al., 1980, 1981; Mikami et al., 1982; Mishra et al., 1987; Wang et al., 1999; Xu et al., 1998). Zitouni et al. (2005) isolated 86 strains from soil samples collected from the Algerian Sahara (desert) and found that 54 isolates belonged to the genus *Nocardiopsis*, whereas Hozzein and colleagues isolated novel *Nocardiopsis* species from desert and sand dune soil samples from Egypt (Hozzein and Goodfellow,

2008; Hozzein et al., 2004). Many *Nocardiopsis* strains have also been found in soils with high salt concentrations (Al-Tai and Ruan, 1994; Al-Zarban et al., 2002; Chen et al., 2008; Chun et al., 2000; Li et al., 2004, 2006; Yassin et al., 1993). Alkaliphilic soils are also a good source for the recovery of *Nocardiopsis* strains. Jiang and Xu (1998) reported that 9 of 49 actinomycetes isolated from alkaline soils were *Nocardiopsis* strains, all grew at pH 12, but six isolates were obligate alkaliphilic strains growing at pH 8–12 but not at pH 7.

In addition, *Nocardiopsis* species have also been reported from other sources including: cotton waste and hay (Lacey, 1977); an Antarctic glacier (Abyzov et al., 1983); deep-sea sediments (Zhang and Zeng, 2008); ovaries of puffer fish (Wu et al., 2005); indoor environments (Peltola et al., 2001); a composting facility (Kämpfer et al., 2002); and a cattle barn (Andersson et al., 1998).

Distribution and abundance of members of the genus *Nocardiopsis* in marine habitats was reported recently (Sabry et al., 2004; Schumacher et al., 2001; Shin et al., 2003). In studies on the diversity of culturable actinobacteria isolated from the marine sponges, *Hymeniacidon perleve*, *Haliclona* sp., and *Iotrochota* sp., *Nocardiopsis* strains were frequently found and it was the second dominant genus after *Streptomyces* in most cases (Jiang et al., 2007, 2008; Zhang et al., 2006).

The presence and isolation of *Nocardiopsis* strains from clinical material including actinomycetomas is well documented (Ajello et al., 1987; Beau et al., 1999; Gugnani et al., 1998; Liegard and Landrieu, 1911; Mordarska et al., 1998; Sindhuphak et al., 1985; Singh et al., 1991; Yassin et al., 1997).

Enrichment and isolation procedures

No specific enrichment or isolation procedures have been recommended for *Nocardiopsis* (Meyer, 1989) and isolation protocols described for streptomycetes are commonly used (Korn-Wendisch and Kutzner, 1992). Most strains grow readily on a variety of media, especially those recommended by the International Streptomycetes Project (ISP); those that have proved to be most effective include yeast extract-malt extract (ISP medium 2), oatmeal (ISP medium 3), glycerol-asparagine (ISP medium 5) (Shirling and Gottlieb, 1966) and glucose-yeast extract-peptone (Naumova et al., 1980) agars and most strains may be recovered from agar plates after incubation at 28–30°C for 7–14 d. Horikoshi (1971) devised a special method for the isolation of alkaliphic *Nocardiopsis* strains: a small amount of soil is suspended in 1 ml sterilized water, 100 µl aliquots of the suspension are spread on dry agar plates, and the plates are incubated for 7–14 d at 27°C. In general, for isolation of alkaliphilic *Nocardiopsis* strains, the pH has to be adjusted to 10 or above with sterile sodium carbonate or sodium hydroxide after autoclaving the isolation medium and the plates should be incubated for 7–14 d at 28°C (Hozzein et al., 2004; Miyashita et al., 1984; Yang et al., 2008c; Zhang et al., 2008).

Various media supplemented with 5–20% (w/v) NaCl have been used to isolate halophilic *Nocardiopsis* strains (Al-Tai and Ruan, 1994; Al-Zarban et al., 2002; Chun et al., 2000; Li et al., 2006; Li et al., 2004; Yassin et al., 1993).

Maintenance procedures

Nocardiopsis strains can be maintained on appropriate media agar slants at 4°C and transferred every 4 months for short-term preservation. To prevent the agar from drying, the tubes should be tightly sealed with silicone stoppers. Medium-term preservation for up to 4 years can be achieved by preparing spore suspensions and/or homogenized mycelia in glycerol (45%, v/v) and keeping them at −25°C (Wellington and Williams, 1978). Storage in liquid nitrogen and lyophilization can be used for long-term storage. For lyophilization, spores and mycelia are suspended in a suitable suspension agent such as skimmed milk (10%, w/v). Storage in liquid nitrogen can be achieved by inoculation of the micro-organisms into small vials containing an appropriate medium which are then incubated until satisfactory growth is visible; the tubes are then tightly sealed and placed in a liquid nitrogen container. Alternatively, a simple, reliable and time-saving method is liquid nitrogen cryopreservation of living cells in small PVC tubes at −196°C (Hoffman, 1989a, 1989b).

Taxonomic comments

The strain that gave its name to the type species of the genus *Nocardiopsis* was originally isolated from mildewed grain and named *Streptothrix dassonvillei* by Brocq-Rosseau (1904), but unfortunately this strain was lost. In 1911, Liegard and Landrieu isolated a micro-organism from a case of ocular conjunctivitis that they found to be similar to the lost strain; however, these authors proposed to include the novel isolate in the genus *Nocardia* as *Nocardia dassonvillei*. Knowledge concerning *Nocardia dassonvillei* was scarce until Gordon and Horan (1968) discovered that the macroscopic appearance and a number of the physiological characters of *Nocardia dassonvillei* were similar to those of *Streptomyces griseus*. Subsequently, Lechevalier and Lechevalier (1970a) transferred *Nocardia dassonvillei* to the genus *Actinomadura* as *Actinomadura dassonvillei*, primarily on the basis of chemical properties.

The genus *Nocardiopsis* was subsequently proposed to accommodate *Actinomadura dassonvillei* as strains of this micro-organism lacked the characteristic whole-organism hydrolysate sugar madurose and formed spores in a distinctive zig-zag formation on aerial hyphae (Meyer, 1976). Additional chemical (Athalye et al., 1984; Collins et al., 1977; Fischer et al., 1983; Lechevalier et al., 1977a; Minnikin et al., 1977; Yamada et al., 1977) and numerical phenetic data (Alderson and Goodfellow, 1979; Athalye et al., 1985; Goodfellow et al., 1979; Goodfellow and Pirouz, 1982) strongly supported the recognition of the genus *Nocardiopsis* with *Nocardiopsis dassonvillei* as the type species.

16S rRNA gene sequence data confirmed the separation between *Nocardiopsis dassonvillei* and *Actinomadura madurae* and showed *Nocardiopsis* to be most closely related to the genera *Microtetraspora* and *Streptomyces* (Goodfellow et al., 1988; Kroppenstedt et al., 1990), whereas the nocardioform soil isolate *Saccharothrix australiensis* (Labeda et al., 1984), which resembles *Nocardiopsis dassonvillei* morphologically and in some chemotaxonomic characters, was found to be closely related to members of the family *Pseudonocardiaceae* (Bowen et al., 1989).

Six of the eight *Nocardiopsis* species with validly published names were subsequently considered to be misclassified (Grund and Kroppenstedt, 1989, 1990). *Nocardiopsis coeruleofusca*, *Nocardiopsis flava*, *Nocardiopsis longispora*, *Nocardiopsis mutabilis*, and *Nocardiopsis syringae* were classified in the genus *Saccharothrix* (Grund and Kroppenstedt, 1989; Labeda and Lechevalier, 1989) as *Saccharothrix coeruleofusca*, *Saccharothrix*

flava, *Saccharothrix longispora*, *Saccharothrix mutabilis*, and *Saccharothrix syringae*, respectively, and *Nocardiopsis africana*, was assigned to the genus *Microtetraspora* as *Microtetraspora africana* (Goodfellow et al., 1988; Kroppenstedt et al., 1990; Poschner et al., 1985). The remaining two species, *Nocardiopsis alba* and *Nocardiopsis dassonvillei*, stayed in the genus *Nocardiopsis* (Fischer et al., 1983; Grund, 1987). *Saccharothrix flava* was later transferred to the genus *Lechevalieria* (Labeda et al., 2001) and *Microtetraspora africana*, together with most other species of the genus *Microtetraspora*, to *Nonomuraea* (Zhang et al., 1998).

Based on phenetic data, Meyer concluded that the remaining single species, *Nocardiopsis antarctica*, was a synonym of *Nocardiopsis dassonvillei* (Meyer, 1989); these results were confirmed by 16S rRNA gene sequences and DNA–DNA hybridization results and *Nocardiopsis antarctica* was transferred to *Nocardiopsis dassonvillei* (Yassin et al., 1997).

Molecular and phenotypic data showed that all tested strains of *Nocardiopsis dassonvillei* should be divided into two species (Fischer et al., 1983; Grund, 1987). On the basis of these results and those of some additional studies, a novel species, *Nocardiopsis alba*, was created (Grund and Kroppenstedt, 1990). This study showed in addition that *Nocardiopsis dassonvillei* subsp. *prasina* was actually more closely related to *Nocardiopsis alba* than to *Nocardiopsis dassonvillei*. This subspecies was therefore transferred to *Nocardiopsis alba* with the revision of its name to *Nocardiopsis alba* subsp. *prasina*. Yassin et al. (1997) subsequently described *Nocardiopsis alba* subsp. *prasina* as *Nocardiopsis prasina* based on 16S rRNA gene sequences and DNA–DNA hybridization studies.

Cell wall studies carried by Pridham and Lyons (1961) revealed that some *Streptomyces* species did not belong to the genus *Streptomyces* because these strains contained *meso*-DAP. The so-called "*meso*-DAP" streptomycetes were further characterized by Grund (1987). Two members of this group, "*Streptomyces alborubidus*" and "*Streptomyces listeri*", were reclassified in the genus *Nocardiopsis* as *Nocardiopsis alborubida* and *Nocardiopsis listeri* (Grund and Kroppenstedt, 1990). Later, Yassin and co-workers found high similarities between the 16S rRNA gene sequences of *Nocardiopsis alborubida* DSM 40465^T and *Nocardiopsis dassonvillei* (Yassin et al., 1997). As DNA–DNA similarity studies revealed 77% similarity, *Nocardiopsis alborubida* was transferred to *Nocardiopsis dassonvillei* as a later subjective synonym of this species. Later, strain VKM Ac-1882^T (= DSM 40465^T) was reclassified as *Nocardiopsis dassonvillei* subsp. *albirubida* (Evtushenko et al., 2000).

Phylogenetic results based on 16S rRNA gene sequences revealed that members of the genus *Nocardiopsis* represent a distinct lineage apart from all other actinomycetes (Goodfellow et al., 1988; Kroppenstedt et al., 1990; Rainey et al., 1996). Therefore, Rainey et al. (1996) proposed the creation of the family *Nocardiopsaceae*, a member of the suborder *Streptosporangineae* Ward-Rainey et al. 1997, now reclassified as the order *Streptosporangiales*, for the genus *Nocardiopsis*.

Since the last edition of *Bergey's Manual*, the systematics of the genus *Nocardiopsis* has undergone many changes and, at present, the genus harbors 30 species with validly published names; it seems unlikely that current members will suffer further reclassification.

Differentiation of the genus *Nocardiopsis* from other genera

The genus *Nocardiopsis* can be readily separated from the genera *Haloactinospora*, *Streptomonospora*, and *Thermobifida* included in the family *Nocardiopsaceae* and from other closely related genera using a combination of morphological, chemotaxonomic, and 16S rRNA gene sequence phylogenetic data. Differential characteristics of the genus *Nocardiopsis* and closely related taxa are presented in Table 294 in the treatment of the family *Nocardiopsaceae*.

Differentiation of the species of the genus *Nocardiopsis*

Differential characteristics of *Nocardiopsis* species are given in Table 295, although most phenotypic data gathered are based on single isolates.

Acknowledgements

The authors are indebted to Dr J. Meyer and to Dr R.M. Kroppenstedt and Dr L.I. Evtushenko as they drew upon material they wrote for the previous edition of this *Manual* and for *The Prokaryotes*, respectively.

List of species of the genus *Nocardiopsis*

1. **Nocardiopsis dassonvillei** (Brocq-Rousseau 1904) Meyer 1976, 487^{AL} (*Streptothrix dassonvillei* Brocq-Rousseau 1904, 228)

das.son.vil′le.i. N.L. gen. masc. n. *dassonvillei* of Dassonville, named after Charles Dassonville (a French microbiologist and veterinarian at the Pasteur Institute).

Good or abundant growth is found on peptone-glucose medium, oatmeal agar (ISP 3), inorganic salts-starch agar (ISP 4), and Bennett's sucrose agar. Colonies have dense and filamentous margins. The aerial mycelium, when present, is farinaceous, white, or yellowish to grayish. Color of substrate mycelium on the media mentioned is either yellowish-brown or olive-colored to dark brown. Soluble pigments, if produced, are yellowish, greenish-yellow, or brown. Melanoid pigments are not produced on ISP 6 or tyrosine agar (ISP 7).

Aerial mycelium hyphae are long, moderately branched, and, at the beginning of sporulation, more or less zig-zag-shaped (Figure 392). They then divide into long segments that subsequently subdivide into smaller spores of irregular size. Spores are elongated and smooth.

L-Arabinose, D-fructose, D-glucose, glycerol, maltose, D-mannitol, D-mannose, L-rhamnose, sucrose, and D-xylose are utilized as carbon sources. Nitrite may or may not be produced from nitrate. Acid is produced from L-arabinose, galactose, mannitol, L-rhamnose, sucrose, and xylose. Lactate, oxalate, and propionate are decarboxylated. Adenine, casein, DNA, esculin, gelatin, guanine, hypoxanthine, starch, tyrosine, and xanthine are degraded, but testosterone is not. Optimum growth temperature is 28–37°C.

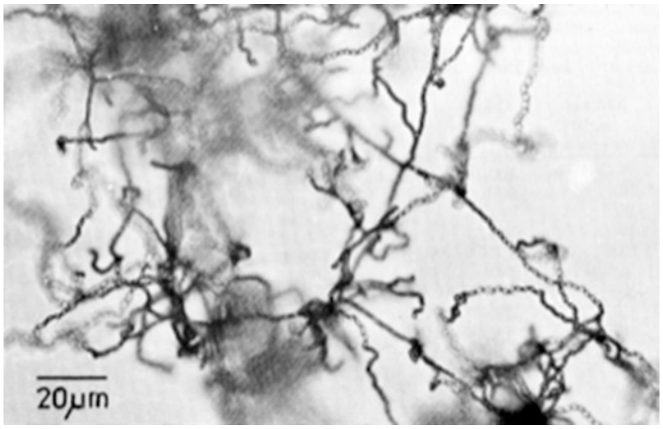

FIGURE 392. *Nocardiopsis dassonvillei* ATCC 23219. Aerial mycelium totally sporulated. Grown on Czapek sucrose agar for 7 d.

Major menaquinones are MK-10(H_6), MK-10(H_4), and MK-9(H_4). Major polar lipids are PME and PC.

Source: the original strain of *Nocardiopsis dassonvillei* was isolated from mildewed grain but this strain was lost. The current type strain was isolated from a case of ocular conjunctivitis.

Type strain: ATCC 23218, DSM 43111, IFO 14626, JCM 7437, NCTC 10488, NRRL B-5397, CIP 107115, IMRU 509, VKM Ac-797.

DNA G+C content (mol%): 69 (T_m).

Sequence accession no. (16S rRNA gene): X97886.

Additional comments: this species currently encompasses two subspecies, *Nocardiopsis dassonvillei* subsp. *dassonvillei* and *Nocardiopsis dassonvillei* subsp. *albirubida*. Yassin et al. (1997) reported that *Nocardiopsis alborubida* corrig. Grund and Kroppenstedt 1990 and *Nocardiopsis antarctica* corrig. Abyzov et al. 1984 were subjective synonyms of *Nocardiopsis dassonvillei* (Brocq-Rousseu 1904) Meyer 1976. Later, Evtushenko et al. (2000) provided evidence for the proposal of the new subspecies, *Nocardiopsis dassonvillei* subsp. *albirubida* comb. nov. (Grund and Kroppenstedt 1990) Evtushenko et al. 2000, and in their paper they corrected the name *alborubida* (*sic*) to *albirubida*.

2. **Nocardiopsis aegyptia** Sabry, Ghanem, Abu-Ella, Schumann, Stackebrandt and Kroppenstedt 2004, 454[VP]

a.e.gyp′ti.a. L. fem. adj. *aegyptia* from Egypt, referring to the country of isolation.

Forms dirty white aerial mycelium, becoming light-yellowish gray in old cultures on glucose-yeast extract-malt extract medium. Hyphae of the aerial mycelium are straight to flexuous. In older cultures, hyphae of aerial mycelium disintegrate into spore-like structures. Short straight chains of spores (0.85 μm diameter) are formed on the aerial mycelium. Spore surface is smooth. No endo- or exopigments are produced.

Grows at 10°C and in 5% (w/v) NaCl, but not at 45°C or in 10% (w/v) NaCl. Optimal growth is obtained on glucose-yeast extract-malt extract agar at 28°C. The following sugars are used as carbon sources: L-arabinose, galactose, glycerol, inositol, D-lactose, mannitol, D-mannose, L-rhamnose, sucrose, and D-xylose.

Polar lipids are PC, PME, PI, PG, and DPG. Major menaquinones are MK-10(H_6), MK-10(H_8), and MK-10(H_4).

Source: the type strain was isolated from marine sediment at a depth of 20 cm on the seashore of Abu Qir Bay, west of Alexandria, Egypt.

Type strain: DSM 44442, JCM 13853, NRRL B-24244, SNG49.

DNA G+C content (mol%): not determined.

Sequence accession no. (16S rRNA gene): AJ539401.

3. **Nocardiopsis alba** corrig. Grund and Kroppenstedt 1990, 9[VP]

al′ba. L. fem. adj. *alba* white, referring to the white aerial mycelium.

Light yellow substrate mycelium with white aerial hyphae is formed. The spore chains are recti-flexibilis and, in the early stages of sporulation, a zig-zag-shaped aerial mycelium is observed. The best formation of aerial mycelium appears when the organism is grown on Czapek–Dox agar supplemented with yeast extract (2 g/l), Casamino acids (6.1 g/l), and tryptophan (20 mg/l).

Cellobiose, dextrin, fructose, glucose, and sucrose are used as sole carbon sources. Adonitol, arabinose, lactose, melezitose, melibiose, *myo*-inositol, raffinose, rhamnose, sorbitol, and xylose are not utilized. An alkaline reaction is observed with citrate, lactate, malate, and malonate; no alkaline reaction occurs with quinate as the carbon source. Acid production is variable for mannitol. Lactate and propionate are decarboxylated, but not oxalate. Adenine, esculin, hypoxanthine, tyrosine, and xanthine are hydrolyzed. Calcium oxalate crystals are not solubilized. Benzoic acid, salicylic acid, *meta*-hydroxybenzoic acid, and *para*-hydroxybenzoic acid are not degraded. Growth occurs in the presence of 5% (w/v) NaCl, but not in the presence of lysozyme (50 μg/ml). The optimal growth temperature is 28°C; no growth occurs at 45°C.

Major menaquinones are MK-10(H_6), MK-10(H_4), and MK-9(H_4). Major polar lipids are PME and PC.

Source: the type strain was isolated from drainage of a hip.

Type strain: DSM 43377, IFO 15097, JCM 9419, VKM Ac-1883.

DNA G+C content (mol%): not determined.

Sequence accession no. (16S rRNA gene): X97883.

Additional comments: the original spelling, *Nocardiopsis albus* subsp. *albus* (*sic*) (orthographically incorrect name), proposed by Grund and Kroppenstedt (1990) has been corrected by Yassin et al. (1997) to *Nocardiopsis alba* subsp. *alba*. After the elevation of the other subspecies of *Nocardiopsis alba*, *Nocardiopsis alba* subsp. *prasina* corrig. (Miyashita et al. 1984) Grund and Kroppenstedt 1990 to full species status as *Nocardiopsis prasina* Yassin et al. 1997 comb. nov., *Nocardiopsis alba* subsp. *alba* corrig. Grund and Kroppenstedt 1990 was simply designated *Nocardiopsis alba* corrig. Grund and Kroppenstedt 1990.

4. **Nocardiopsis alkaliphila** Hozzein, Li, Ali, Hammouda, Mousa, Xu and Jiang 2004, 250[VP]

al.ka.li′phi.la. Arabic article *al* the; Arabic n. *kaïda* soda, saltwort; N.L. n. *alkali* alkali; N.L. adj. *philus -a -um* (from Gr. adj. *philos -ê -on*) friend, loving; N.L. fem. adj. *alkaliphila* loving alkaline environments.

White to yellowish-white aerial mycelium and yellow to yellowish-brown substrate mycelium. Mature aerial mycelium fragments to branched and straight spore-chains with elongated, irregular and smooth spores. Diffusible pigments or melanin are not produced.

Arabinose, cellobiose, maltose, raffinose, sucrose, and xylose are utilized as carbon sources, but weak utilization of dulcitol, glucose, galactose, inositol, lactose, rhamnose, sorbitol, sodium citrate, sodium succinate, and xylitol is observed. Fructose, mannose, mannitol, ribose, and sodium acetate are not used as carbon sources. Growth on potassium nitrate, asparagine, phenylalanine, and serine as nitrogen sources is recorded, but cysteine, glycine, histidine, methionine, threonine, and valine are utilized only weakly, whereas growth on arginine or hydroxyproline is not observed. Casein, gelatin, hypoxanthine, starch, tributyrin, and tyrosine are degraded. Catalase is produced, but not H_2S. Growth occurs at 10–45°C, at pH 7–12, and in 0–10% (w/v) NaCl, with optimum growth at 28–30°C, pH 9.5–10, and 2.5% (w/v) NaCl.

Whole-cell hydrolysates contain the sugars glucose and ribose. Major polar lipids are PC, PG, PME, PE, PIM, an unknown glycolipid, and four unknown phospholipids with high R_f values. Major menaquinones are MK-10(H_6), MK-10(H_8), MK-11(H_2), MK-9(H_6), MK-9(H_{10}), and MK-10(H_4).

Source: the type strain was isolated from desert soil in Egypt.

Type strain: CCTCC AA 001031, DSM 44657, YIM 80379.

DNA G+C content (mol%): 65.8 (T_m).

Sequence accession no. (16S rRNA gene): AY230848.

5. **Nocardiopsis arabia** Hozzein and Goodfellow 2008, 2523[VP]

a.ra′bi.a. L. fem. adj. *arabia* pertaining to Arabia, referring to the isolation of the type strain in Egypt.

Pale-yellow to deep grayish yellow substrate mycelium that carries white aerial hyphae formed on ISP media 1–5. Forms branched substrate mycelium that fragments into coccoid and rod-shaped elements. Aerial hyphae differentiate into straight to flexuous chains of rod-shaped spores with smooth surfaces. Diffusible pigments are not produced.

Arabinose, *meso*-erythritol, D-salicin, and trehalose are used as sole carbon sources, but L-arabitol, melezitose, and xylitol are not. Degrades casein and hypoxanthine, but not adenine, guanine, tyrosine, uric acid, or xanthine. Growth occurs in the presence of NaCl at 0–15% (w/v) (optimally at 5%), and at 10–40°C (optimally at 28°C).

Whole-organism hydrolysates contain the sugars galactose and glucose. The major polar lipids are DPG, PC, PG, PI, PIM, PE, two unknown glycolipids, and two unknown phospholipids with high R_f values. Major menaquinones are MK-10(H_4) and MK-10(H_6).

Source: the type strain was isolated from a sand-dune soil collected at Borg El-Arab, Egypt.

Type strain: CGMCC 4.2057, DSM 45083, S186.

DNA G+C content (mol%): not determined.

Sequence accession no. (16S rRNA gene): EF095149.

Additional comments: given the short sequence obtained for *Nocardiopsis arabia* (1377 bp), phylogenetic analyses place this strain within the radiation of the genus *Streptomonospora*; however, morphological and chemical properties are consistent with its assignment to the genus *Nocardiopsis.*

6. **Nocardiopsis baichengensis** Li, Kroppenstedt, Wang, Tang, Lee, Park, Kim, Xu and Jiang 2006, 1095[VP]

bai.cheng.en′sis. N.L. fem. adj. *baichengensis* pertaining to Baicheng, a county of Xinjiang Province in the west of China where the type strain was collected.

Aerial mycelium is white to yellow-white and the substrate mycelium is light yellow to deep orange-yellow. Vegetative hyphae are well developed and fragmented. Long spore chains are borne on the aerial hyphae; these are smooth-surfaced and nonmotile. No diffusible pigments are produced.

L-Arabinose, cellobiose, D-fructose, D-galactose, D-glucose, glycerol, maltose, D-mannitol, D-mannose, L-rhamnose, D-ribose, sodium acetate, sodium citrate, and sucrose are used as carbon sources, but *myo*-inositol, D-lactose, raffinose, D-sorbitol, starch, D-xylitol, and D-xylose are not. Alanine, arginine, asparagine, glycine, histidine, lysine, phenylalanine, proline, threonine, tryptophan, and valine can be used as sole nitrogen sources, but adenine, cystine, glutamic acid, hydroxyproline, and methionine are not utilized. Milk coagulation, milk peptonization, starch hydrolysis, H_2S production, nitrate reduction, and urease activity are negative, but gelatin liquefaction and melanin production are positive. Optimum growth is at 37–40°C and pH 7.2 with 5–8% (w/v) NaCl. Temperature, pH, and NaCl tolerance ranges are 20–50°C, pH 6–9, and 0–18% NaCl, respectively.

Polar lipids present are PME, PC, PI, PG DPG, and PIM, together with an unknown phosphoglycolipid (PGL) and an unknown phospholipid (PL). Major menaquinones are MK-10(H_2), MK-10(H_4), and MK-10(H_6).

Source: the type strain was isolated from a saline soil sample in the west of China.

Type strain: CCTCC AA 2040016, DSM 44845, KCTC 19009, YIM 90130.

DNA G+C content (mol%): 73.2 (T_m).

Sequence accession no. (16S rRNA gene): AY619716.

7. **Nocardiopsis chromatogenes** Li, Kroppenstedt, Wang, Tang, Lee, Park, Kim, Xu and Jiang 2006, 1094[VP]

chro.ma.to′ge.nes. Gr. n. *chroma -atos* color; Gr. v. *gennaio* to produce; N.L. part. adj. *chromatogenes* producing color.

Aerial mycelium is white and the substrate mycelium is light reddish brown to deep reddish brown on most media. Vegetative hyphae are well developed and fragmented. Long spore chains are borne on the aerial hyphae. Spores are smooth-surfaced and nonmotile. Yellowish pink pigments are produced on Czapek agar and inorganic salts-starch agar (ISP 4) media.

L-Arabinose, cellobiose, D-fructose, D-galactose, D-glucose, glycerol, *myo*-inositol, D-lactose, maltose, D-mannitol, D-mannose, raffinose, L-rhamnose, D-ribose, sodium acetate, sodium citrate, sucrose, and D-xylose are utilized as carbon sources, but D-sorbitol, starch, and D-xylitol are not utilized. Alanine, arginine, asparagine, glycine, histidine, lysine, phenylalanine, proline, threonine, tryptophan, and valine are used as sole nitrogen sources, but adenine,

cystine, glutamic acid, hydroxyproline, and methionine are not utilized. Milk coagulation, milk peptonization, gelatin liquefaction, nitrate reduction, H_2S production, and urease activity are negative, but starch hydrolysis and melanin production are positive. Optimum growth is at 37–40°C and pH 7.2, with 5–8% (w/v) NaCl. Temperature, pH, and NaCl tolerance ranges are 20–60°C, pH 6–9, and 0–18% (w/v), respectively.

The polar lipid profile includes PME, PC, PI, PG, DPG, and PIM, together with an unknown PGL and an unknown PL. Major menaquinones are MK-10, MK-10(H_2), and MK-10(H_4).

Source: the type strain was isolated from a saline soil sample in the west of China.

Type strain: CCTCC AA 2040015, DSM 44844, KCTC 19008, YIM 90109.

DNA G+C content (mol%): 71.8 (T_m).

Sequence accession no. (16S rRNA gene): AY619715.

8. **Nocardiopsis composta** corrig. Kämpfer, Busse and Rainey 2002, 627[VP]

com.pos′ta. N.L. fem. adj. *composta* originating from compost.

White aerial mycelium with colorless to brownish-yellow substrate mycelium. Long, branched substrate hyphae fragment into nonmotile elements. Melanin is not produced on CASO agar (casein soy peptone agar).

N-Acetyl-D-glucosamine, L-arabinose, *p*-arbutin, D-fructose, D-galactose, D-glucose, D-mannose, D-ribose, salicin, trehalose, adonitol, *i*-inositol, maltitol, putrescine, acetate, propionate, 4-aminobutyrate, azelate, citrate, fumarate, glutarate, DL-lactate, oxoglutarate, pyruvate, suberate, L-alanine, β-alanine, L-aspartate, L-histidine, L-phenylalanine, L-proline, L-serine, L-tryptophan, and phenylacetate are used as carbon sources. Maltose, L-rhamnose, sucrose, D-xylose, L-malate, mesaconate, L-leucine, and 4-hydroxybenzoate are not used as carbon sources. Degrades esculin, *para*-nitrophenyl (*p*NP)-α-glucopyranoside, *p*NP-β-D-glucopyranoside, L-alanine-*para*-nitroanilide (*p*NA), and L-proline-*p*NA. No growth occurs in NaCl concentrations of 15% or higher. pH values of 6.5–9.5 are tolerated, but no growth occurs at pH 4.5. No growth is observed at a temperature of 10°C, but growth occurs up to 50°C.

Polar lipids present are PME, PC, DPG, PG, and two unknown PLs. Major menaquinones are MK-10(H_8), MK-11(H_8), MK-10(H_6), and MK-12.

Source: the type strain was isolated from the air surrounding a composting facility in Kassel, Germany.

Type strain: DSM 44551, JCM 11768, KS9, NBRC 100345, NRRL B-24145.

DNA G+C content (mol%): not determined.

Sequence accession no. (16S rRNA gene): AF360734.

Additional comments: the original spelling of the epithet *compostus* (*sic*) has been corrected (List Editor, 2002a). In Latin, *compostus* means brought together, placed together, collected, united, joined, connected, aggregated…, not originating from compost as cited in the paper by Kämpfer et al. (2002).

9. **Nocardiopsis exhalans** Peltola, Andersson, Kämpfer, Auling, Kroppenstedt, Busse, Salkinoja-Salonen and Rainey 2002, 3[VP] (Effective publication: Peltola, Andersson, Kämpfer, Auling, Kroppenstedt, Busse, Salkinoja-Salonen and Rainey 2001, 4302.)

ex.ha′lans. L. part. adj. *exhalans* emitting odors, fumes, toxins, etc.

Yellow vegetative mycelium with white aerial hyphae that are slightly spiral shaped, that fragment to form rod-shaped, nonmotile spores (0.5 µm in diameter). No soluble pigment is produced.

Arabinose, cellobiose, fructose, gluconate, glucose, maltose, mannose, rhamnose, ribose, sucrose, trehalose, xylose, mannitol, putrescine, acetate, *cis*-aconitate, 4-aminobutyrate, azelate, citrate, fumarate, glutarate, 3-hydroxybutyrate, pyruvate, suberate, L-alanine, phenylalanine, proline, serine, 4-hydroxybenzoate, and phenylacetate are used as carbon sources, but not *N*-acetyl-D-glucosamine, arbutin, galactose, melibiose, salicin, adonitol, inositol, maltitol, sorbitol, propionate, adipate, itaconate, lactate, malate, mesaconate, oxoglutarate, β-alanine, aspartate, histidine, leucine, ornithine, tryptophan, or 3-hydroxybenzoate. Esculin is not hydrolyzed. Phosphatase and α-glucosidase are produced. Grows in the presence of 10% (w/v) NaCl; grows at 10, 28, and 37°C, but not at 50°C.

Predominant menaquinones are MK-10(H_6), MK-10(H_8), and MK-10(H_4). The polar lipid profile includes PC, PI, PG, PME, and DPG.

Source: the type strain was isolated from the indoor air of the basement of a water-damaged building of which the occupants suffered from nonspecific health symptoms.

Type strain: DSM 44407, ES10.1, JCM 11759, NBRC 100346, NRRL B-24123.

DNA G+C content (mol%): not determined.

Sequence accession no. (16S rRNA gene): AY028325.

10. **Nocardiopsis ganjiahuensis** Zhang, Zhang, Yang, Shi, Lu, Chen, Jiang and Xu 2008, 198[VP]

gan.ji.a.hu.en′sis. N.L. fem. adj. *ganjiahuensis* pertaining to Ganjiahu Natural Reserve, Xinjiang, north-west China, where the type strain was isolated.

Gray-white powdery aerial mycelium with light yellow substrate hyphae. The aerial mycelium divides into rod-shaped, irregular-sized spores (0.3–0.5 × 1.0–2.0 µm), which are smooth and nonmotile. The substrate mycelium is long, well-developed, and branched. No diffusible pigments are produced.

L-Arabinose, glycerol, *myo*-inositol, L-rhamnose, and D-xylose are utilized as sole carbon sources, but not cellobiose, lactose, or sucrose. Positive for urease production and nitrate reduction. Tween 80 can be degraded, but not Tween 85. Growth occurs at pH 8.5–13, with optimum at pH 8.5–9.5. No growth at pH 7.0. Grows optimally at 28–32°C; growth occurs at 10°C, with no growth at 42°C. Grows well on media supplemented with 0, 1, 3, and 5% (w/v) NaCl; no growth occurs in 10% NaCl.

Major menaquinones are MK-10(H_2) and MK-10(H_4). Polar lipids are DPG, PC, and PME.

Source: the type strain was isolated from soil.

Type strain: CGMCC 4.3500, DSM 45031, HBUM 20038.

DNA G+C content (mol%): 71.1 (HPLC).

Sequence accession no. (16S rRNA gene): AY336513.

11. **Nocardiopsis gilva** Li, Kroppenstedt, Wang, Tang, Lee, Park, Kim, Xu and Jiang 2006, 1093[VP]

gil′va. L. fem. adj. *gilva* pale yellow.

Aerial mycelium is pale yellow to yellow-white and the substrate mycelium is pale yellow to pale greenish-yellow on media tested. Vegetative hyphae are well developed and fragmented. Spiral spore chains are short and are borne on the aerial hyphae. Spores are smooth-surfaced and nonmotile. No diffusible pigments are produced.

L-Arabinose, cellobiose, D-fructose, D-galactose, D-glucose, glycerol, *myo*-inositol, D-lactose, D-mannitol, raffinose, sodium acetate, sodium citrate, D-sorbitol, starch, sucrose, and D-xylose are used as carbon sources, whereas maltose, D-mannose, L-rhamnose, D-ribose, and D-xylitol are not used. Alanine, arginine, asparagine, glycine, histidine, lysine, proline, and threonine are used as sole nitrogen sources, but adenine, cystine, glutamic acid, hydroxyproline, methionine, phenylalanine, tryptophan, and valine are not used. Milk coagulation, milk peptonization, gelatin liquefaction, starch hydrolysis, H_2S production, urease activity, and melanin production are negative, but nitrate reduction is positive. Optimal growth is at 28–30°C and pH 7.2, with 5–8% (w/v) NaCl. Temperature, pH, and NaCl tolerance ranges are 10–40°C, pH 6–9, and 0–18%, respectively.

The polar lipid pattern contains PME, PC, PI, PG, and DPG. Major menaquinones are MK-11(H_4), MK-11(H_6), and MK-11(H_8).

Source: the type strain was isolated from a saline soil sample in the west of China.

Type strain: CCTCC AA 2040012, DSM 44841, KCTC 19006, YIM 90087.

DNA G+C content (mol%): 68.1 (T_m).

Sequence accession no. (16S rRNA gene): AY619712.

12. **Nocardiopsis halophila** Al-Tai and Ruan 1994, 477[VP]

ha.lo′phi.la. Gr. n. *hals halos* salt; Gr. adj. *philos* loving; N.L. fem. adj. *halophila* loving salt.

Cream yellow to coral red substrate mycelium that rarely fragments. Forms a long and extensively branched substrate mycelium. The aerial mycelium is irregularly branched with very long hyphae in zig-zags which fragment into elongated spores with smooth surfaces. No diffusible pigments are produced.

The following carbon sources are used: arabinose, galactose, glucose, fructose, inositol, maltose, mannitol, mannose, raffinose, rhamnose, ribose, sucrose, and xylose. Acid is produced from arabinose, fructose, inositol, maltose, mannitol, ribose, rhamnose, and xylose. Hydrolyzes urea and Tween 80, but does not degrade carboxymethylcellulose, starch, tyrosine, or xanthine. The optimum growth temperature is 30°C. Tolerates NaCl concentrations up to 20% (w/v).

Resistant to lysozyme, amoxycillin, clindamycin, bacitracin, cephalexin, and oxacillin, but susceptible to carbenicillin, chloramphenicol, erythromycin, and novobiocin.

Predominant phospholipids are PC, lyso-DPG, and DPG. Major menaquinones present are MK-10(H_6) and MK-10(H_8).

Source: the type strain was isolated from a saline soil obtained from Iraq.

Type strain: CCIM A.S.4.1195, DSM 44494, IQ-H3, JCM 9892.

DNA G+C content (mol%): not determined.

Sequence accession no. (16S rRNA gene): AF195411.

13. **Nocardiopsis halotolerans** Al-Zarban, Abbas, Al-Musallam, Steiner, Stackebrandt and Kroppenstedt 2002, 528[VP]

ha.lo.to′le.rans. Gr. n. *hals halos* salt; L. part. adj. *tolerans* tolerating; N.L. part. adj. *halotolerans* salt-tolerating, referring to the ability to tolerate high salt concentrations.

The color of substrate mycelium is beige to brown. Dirty-white to yellow-gray aerial mycelium is formed which shows the typical zig-zag formation prior to sporulation. The long-branched substrate hyphae fragment into nonmotile elements. Diffusible pigments are not produced. Melanin is not observed on either peptone-yeast-iron agar or tyrosine agar (ISP media 6 and 7).

Galactose, glucose, glycerol, D-mannose, melibiose, and sucrose are used as sole carbon sources, but not adonitol, L-arabinose, or D-xylose. Able to degrade feathers. Optimal growth is obtained on starch mineral agar supplemented with 10% (w/v) NaCl at 28°C. Grows at 28–35°C and in NaCl concentrations of 0–15%.

The polar lipid pattern is PC, PI, PG, PME, DPG, and three unknown PLs with high R_f values. The major menaquinones are MK-10, MK-10(H_2), and MK-10(H_4).

Source: the type strain was isolated from salt marsh soil at Al-Khiran, Kuwait.

Type strain: DSM 44410, F100, JCM 11760, NBRC 100347, NRRL B-24124.

DNA G+C content (mol%): 68 (HPLC).

Sequence accession no. (16S rRNA gene): AJ290448.

14. **Nocardiopsis kunsanensis** Chun, Bae, Moon, Jung, Lee and Kim 2000, 1911[VP]

kun.sa.nen′sis. N.L. fem. adj. *kunsanensis* pertaining to Kunsan, a location in Korea where the species was isolated.

Yellow substrate mycelium is formed that bears white aerial hyphae which fragment into elongated nonmotile spores with smooth surfaces. Fragmentation of substrate mycelium has not been observed. Diffusible pigments are not formed.

Utilizes D-glucose and sucrose as sole carbon sources, but not acetate, cellobiose, D-galactose, gluconate, *p*-hydroxybenzoate, *myo*-inositol, maltose, mannitol, paraffin, raffinose, L-rhamnose, or trehalose. L-Alanine is used as sole carbon and nitrogen source, but not gelatin, proline, or serine. Degrades adenine, casein, gelatin, hypoxanthine, starch, tyrosine, urea, and xanthine but not esculin. Nitrate is not reduced to nitrite. H_2S is not produced. Resistant to sodium azide (0.01%), crystal violet (0.0001%), and potassium tellurite (0.0001%); sensitive to phenol (0.1%). Grows optimally at 37°C and pH 9, with 10% (w/v) NaCl.

The polar lipids found are PC, PG, and DPG. Major menaquinone is MK-10(H_8).

Source: the type strain was isolated from a saltern sample collected from Kunsan, Republic of Korea.

Type strain: DSM 44524, HA-9, JCM 10721, KCTC 9831, NBRC 100348.

DNA G+C content (mol%): 71 (T_m).

Sequence accession no. (16S rRNA gene): AF195412.

15. **Nocardiopsis listeri** Grund and Kroppenstedt 1990, 10[VP]

lis′te.ri. N.L. gen. masc. n. *listeri* of Lister, named after Joseph Lister (1827–1912), the father of antiseptic surgery.

Substrate mycelium is colorless and clear in all media tested. Usually no aerial mycelium is formed unless the strain is grown on Hickey–Tresner agar (Hickey and Tresner, 1952), on which a white aerial mycelium is present.

Arabinose, cellobiose, dextrin, fructose, glucose, rhamnose, sucrose, and xylose are used as carbon sources, but not adonitol, *myo*-inositol, lactose, melezitose, melibiose, raffinose, or sorbitol. An alkaline reaction is observed with citrate, lactate, and malate, but not with quinate or malonate. Acid is produced from L-arabinose, galactose, L-rhamnose, and xylose; production from D-lactose and melibiose is variable. Lactate and propionate are decarboxylated, but not oxalate. Adenine, esculin, tyrosine, and xanthine are hydrolyzed, but not calcium oxalate or hypoxanthine. Benzoic acid, salicylic acid, *meta*-hydroxybenzoic acid, and *para*-hydroxybenzoic acid are not degraded.

Growth occurs in the presence of 5% (w/v) NaCl, but not in the presence of lysozyme (50 μg/ml). The optimal growth temperature is 28°C; no growth occurs at 45°C.

Polar lipids present include PC and PME; major menaquinones are MK-10 and MK-10(H_2).

Source: the type strain was isolated from a human clinical specimen.

Type strain: ATCC 27442, CBS 661.72, DSM 40297, IF0 13360, ISP 5297, JCM 4782, KCC S-0782, NCTC 434, RIA 1321, VKM Ac-1881.

DNA G+C content (mol%): not determined.

Sequence accession no. (16S rRNA gene): X97887.

16. **Nocardiopsis litoralis** Chen, Wang, Zhang, Tang, Liu, Xiao, Xu, Cui and Li 2009, 2711[VP]

li.to.ra′lis. L. fem. adj. *litoralis* of or belonging to the seashore.

White aerial mycelium and white to yellow-white substrate mycelium with straight to flexuous hyphae are formed. Substrate hyphae are well developed and fragment with age. Long, straight to flexuous spore chains are borne on aerial hyphae, which fragment into elongated nonmotile spores with smooth surfaces. Good growth occurs on most of the media tested. Diffusible pigments or melanin are not produced.

D-Glucose, sucrose, and xylose are used as carbon sources, but L-arabinose, cellobiose, dextrin, D-fructose, D-galactose, D-lactose, maltose, D-mannose, melezitose, melibiose, raffinose, L-rhamnose, D-ribose, D-salicin, trehalose, adonitol, acetate, citrate, gluconate, L-arginine, L-asparagine, L-glutamic acid, glycine, L-histidine, hydroxy-L-proline, L-isoleucine, L-leucine, L-methionine, L-phenylalanine, L-proline, L-serine, L-threonine, and L-valine are not used as carbon sources. Grows optimally at 25°C and pH 8.5, and in the presence of 5–7% (w/v) total salts. No growth is observed in absence of salts. Positive for catalase, but negative for oxidase. Does not reduce nitrate to nitrite. Hydrogen disulfide is not produced. Adenine, gelatin, hypoxanthine, tyrosine, and xanthine are degraded but not casein, cellulose, chitin, DNA, esculin, starch, Tweens 20, 40, 60, and 80, or urea.

The predominant menaquinones are MK-10(H_4), MK-10(H_6), and MK-10(H_8). Polar lipids comprise DPG, PC, and PG.

Source: the type strain was isolated from homogenates of a sea anemone collected from a tidal flat on Naozhou Island in the South China Sea, near Zhanjiang City, Southern China.

Type strain: DSM 45168, JSM 073097, KCTC 19473.

DNA G+C content (mol%): 70.4 (HPLC).

Sequence accession no. (16S rRNA gene): EU583726.

17. **Nocardiopsis lucentensis** Yassin, Galinski, Wohlfarth, Jahnke, Schaal and Trüper 1993, 268[VP]

lu.cen.ten′sis. N.L. fem. adj. *lucentensis* referring to Lucentum, the ancient Latin name of Alicante, a city in Spain, where the type strain was isolated.

The aerial spore mass color is predominantly white. The reverse side of culture growth is yellow to yellowish brown. An extensive substrate mycelium is produced which fragments. Aerial hyphae are branched, long, and well developed on media supplemented with 5–10% (w/v) NaCl and, at the beginning of sporulation, are more or less zig-zag-shaped. The zig-zag-shaped hyphae subdivide into smaller spores which are elongated and have smooth surfaces (Figure 392). Pigments or melanin are not produced. Cultures grow well on both complex and defined media.

The following carbon sources are used on ISP medium 9 supplemented with 5–10% NaCl: D-fructose, glucose, glycerol, *myo*-inositol, maltose, mannitol, mannose, raffinose, rhamnose, sucrose, and trehalose. On the same medium, the following carbon sources are not used: L-arabinose, D-galactose, lactose, and D-xylose. Acid is produced from glucose, inositol, mannitol, raffinose, and rhamnose. Citrate, malate, succinate, acetate, pyruvate, and propionate are decarboxylated, but lactate and oxalate are not. Adenine, hypoxanthine, tyrosine, and xanthine are degraded; casein, starch, gelatin, and esculin are hydrolyzed, but not allantoin, arbutin, or urea. Produces catalase and phosphatase, but not β-galactosidase or β-glucosidase. Nitrate is reduced to nitrite.

Resistant to (μg/ml) gentamicin (64), lincomycin (128), penicillin G (128), neomycin (4), and streptomycin (64), but not to rifampin (128). The organism is susceptible to lysozyme and tolerates NaCl at levels up to and including 10%.

Phospholipids present are PC, PME, PG, and DPG. Major menaquinones include MK-10(H_8), MK-10(H_6), and MK-10(H_{10}).

Source: the type strain was isolated from a soil sample collected in a salt marsh area near Alicante, Spain.

Type strain: A5-1, ATCC 51300, DSM 44048, IFO 15854, JCM 9420, VKM Ac-1962.

DNA G+C content (mol%): 71 (T_m).

Sequence accession no. (16S rRNA gene): X97888.

18. **Nocardiopsis metallicus** Schippers, Bosecker, Willscher, Spröer, Schumann and Kroppenstedt 2002, 2294[VP]

me.tal′li.cus. L. masc. n. *metallicus* the miner, referring to the ability to mobilize metals from slag.

Yellow-brown substrate mycelium bearing white aerial mycelium with straight spore chains.

Acetate, cellobiose, D-galactose, gelatin, gluconate, D-glucose, maltose, mannitol, proline, L-rhamnose, sucrose, trehalose, and D-xylose are used as carbon sources. The pH range for growth is 7.0–10.5 with optimal growth at pH 8.5. Grows well at 30°C, weakly at 10°C, but not at 40°C. Growth occurs on media supplemented with up to 10% (w/v) NaCl.

The polar lipid profile contains DPG, PG, PE, PME, PI, PIM, PC, and unknown PLs with R_f values above DPG.

Source: the type strain was isolated from an alkaline slag dump associated with metallurgical processing in Germany.

Type strain: DSM 44598, JCM 12409, KBS6, NBRC 101841, NRRL B-24159.

DNA G+C content (mol%): 70.8 (HPLC).

Sequence accession no. (16S rRNA gene): AJ420769.

19. **Nocardiopsis potens** Yassin, Spröer, Hupfer, Siering and Klenk 2009, 2732[VP]

po′tens. L. part. adj. *potens* potent, powerful, pertaining to the metabolic activities of the organism.

Pale yellow to brown-yellow substrate mycelium that carries white aerial hyphae is formed on ISP 2–4 agar. Forms a branched substrate mycelium that fragments into coccoid and rod-shaped elements. Aerial hyphae differentiate into straight to flexuous chains of rod-shaped spores with smooth surfaces. Diffusible pigments or melanoid pigments are not produced.

Hydrolyzes casein, elastin, esculin, guanine, hypoxanthine, testosterone, tyrosine, and urea, but not adenine, gelatin, or xanthine. Assimilates acetate, adonitol, adipate, iso-amyl alcohol, L-arabinose, 2,3-butanediol, cellobiose, citrate, *meso*-erythritol, D-galactose, D-gluconate, D-glucose, *myo*-inositol, L-lactate, lactose, maltose, D-mannitol, melezitose, sucrose, and trehalose as carbon sources, but not *m*-hydroxybenzoate, *p*-hydroxybenzoate, 1,2-propanediol, raffinose, L-rhamnose, D-sorbitol, or D-xylose. Utilizes L-alanine, arginine, gelatin, proline, ornithine, and serine, but not acetamide as simultaneous carbon and nitrogen sources. Grows in the presence of 12% NaCl and at 20–37°C, but not at 10 or 42°C.

Major phospholipids are DPG, PC, PG, PI, PIM, and PE. Major menaquinones are MK-11(H_8), MK-11(H_6), MK-11(H_4), MK-11(H_2), MK-10(H_8), MK-10(H_6), MK-10(H_4), MK-10(H_2), MK-9(H_8), MK-9(H_6), MK-9(H_4), and MK-9(H_2).

Source: the type strain was isolated from household waste.

Type strain: CCUG 56587, DSM 45234, IMMIB L-21.

DNA G+C content (mol%): not determined.

Sequence accession no. (16S rRNA gene): FM253114.

20. **Nocardiopsis prasina** (Miyashita, Mikami and Arai 1984) Yassin, Rainey, Burghardt, Gierth, Ungerechts, Lux, Seifert, Bal and Schaal 1997, 987[VP] (*Nocardiopsis dassonvillei* subsp. *prasina* Miyashita, Mikami and Arai 1984, 408)

pra.si′na. N.L. fem. adj. prasina (from Gr. adj. *prasina*) leek green (referring to the color of the mature aerial mycelium).

The substrate mycelium is colorless and the aerial mycelium is white to pale pink; a greenish shade is occasionally observed. The sporophore morphology is recti-flexibilis and a zig-zag-shaped aerial mycelium is observed at the beginning of sporulation. Excellent growth and abundant aerial mycelium formation appear at pH 9.0 and above.

Arabinose, cellobiose, dextrin, fructose, glucose, and glycerol are used as carbon sources, but not adonitol, *myo*-inositol, lactose, melezitose, melibiose, raffinose, rhamnose, sucrose, sorbitol, or xylose. An alkaline reaction is observed with citrate, lactate, and malate. No alkaline reaction occurs with quinate or malonate as carbon sources. Acid is produced from galactose, mannitol, and sucrose. Lactate, oxalate, and propionate are decarboxylated. Adenine, esculin, hypoxanthine, and xanthine are hydrolyzed, but tyrosine is not. Benzoic acid, salicylic acid, *meta*-hydroxybenzoic acid, and *para*-hydroxybenzoic acid are not degraded. Growth occurs in the presence of 5% (w/v) NaCl, but not in the presence of lysozyme (50 μg/ml). The optimal growth temperature is 28°C; no growth occurs at 45°C.

Diagnostic phospholipid is PC; major menaquinones are MK-10(H_8), MK-10(H_6), and MK-10(H_4).

Source: the type strain was isolated from soil from Japan.

Type strain: ATCC 35940, DSM 43845, IFO 14423, JCM 3336, VKM Ac-1880.

DNA G+C content (mol%): not determined.

Sequence accession no. (16S rRNA gene): X97884.

21. **Nocardiopsis quinghaiensis** Chen, Cui, Kroppenstedt, Stackebrandt, Wen, Xu and Jiang 2008, 703[VP]

quing.hai.en′sis. N.L. fem. adj. *quinghaiensis* pertaining to Qinghai, a province of China in which the sample was collected.

The color of the substrate mycelium is white to pale yellow. Forms white aerial mycelium. Substrate hyphae are well developed and fragment with age. Long spore chains are borne on aerial hyphae. Spores are rod-shaped, smooth, and nonmotile. Diffusible pigments are not produced.

L-Arabinose, cellobiose, citrate, D-fructose, D-glucose, glycerol, *myo*-inositol, D-mannitol, D-mannose, D-ribose, raffinose, sucrose, starch, and trehalose are used as sole carbon and energy sources, whereas acetate, adonitol, D-galactose, D-lactose, maltose, melibiose, D-rhamnose, salicin, D-sorbitol, D-xylitol, and D-xylose cannot be used for growth. L-Alanine, L-asparagine, L-glycine, L-histidine, hydroxy-L-proline, L-methionine, L-proline, L-threonine, and L-tyrosine can be utilized as sole nitrogen sources, whereas adenine, L-arginine, L-cystine, glutamic acid, L-lysine, phenylalanine, L-tryptophan, and L-valine cannot be used. Positive in tests for hydrolysis of casein, chitin, starch, Tween 20, and Tween 80, but negative for hydrolysis of cellulose and gelatin. Nitrate is not reduced to nitrite. H_2S, melanin, and urease are not produced. Optimal growth occurs on marine agar 2216 and Czapek agar with 3% (w/v) NaCl at 28°C and pH 7.0. The temperature, pH, and NaCl concentration ranges for growth are 10–37°C, pH 6.0–8.0, and 0–10%, respectively.

The polar lipid pattern is composed of PC, PG, and DPG. The major menaquinones are MK-10, MK-10(H_2), MK-11(H_2), MK-11, and MK-9(H_4).

Source: the type strain was isolated from a sample of saline soil collected from the Qaidam Basin, Qinghai Province, north-west China.

Type strain: CGMCC 4.3494, DSM 44739, YIM 28A4.

DNA G+C content (mol%): 67.1 (T_m).

Sequence accession no. (16S rRNA gene): EF597511.

22. **Nocardiopsis rhodophaea** Li, Kroppenstedt, Wang, Tang, Lee, Park, Kim, Xu and Jiang 2006, 1094[VP]

rho.do.pha′e.a. Gr. n. *rhodos* the rose; Gr. adj. *phaeos* brown; N.L. fem. adj. *rhodophaea* rose-brown (after the color of the substrate mycelium).

Aerial mycelium is pale pink to light reddish brown and the substrate mycelium is light reddish brown to deep reddish brown on media tested. Vegetative hyphae are well developed and fragmented. Short spore chains are borne on the aerial hyphae. Spores are smooth-surfaced and nonmotile. No diffusible pigments are produced.

L-Arabinose, D-glucose, glycerol, *myo*-inositol, sodium acetate, and D-ribose can be utilized as carbon sources, but not cellobiose, D-fructose, D-galactose, D-lactose, maltose, D-mannose, D-mannitol, raffinose, L-rhamnose, sodium citrate, D-sorbitol, starch, sucrose, D-xylitol, or D-xylose. Alanine, arginine, asparagine, glycine, histidine, proline, and valine are used as sole nitrogen sources, whereas adenine, cystine, glutamic acid, hydroxyproline, lysine, methionine, phenylalanine, threonine, and tryptophan are not utilized. Milk coagulation, gelatin liquefaction, starch hydrolysis, H_2S production, urease activity, nitrate reduction, and melanin production are negative, but milk peptonization is positive. Optimum growth is at 37–40°C and pH 7.2, with 5–8% (w/v) NaCl. Temperature, pH, and NaCl tolerance ranges are 20–60°C, pH 6–9, and 0–18%, respectively.

The polar lipid pattern is composed of PME, PC, PI, PG, DPG, and PIM, together with some unknown PGLs and unknown PLs. Major menaquinones are MK-11(H_6) and MK-11(H_8).

Source: the type strain was isolated from a saline soil sample in the west of China.

Type strain: CCTCC AA 2040014, DSM 44843, KCTC 19049, YIM 90096.

DNA G+C content (mol%): 67.1 (T_m).

Sequence accession no. (16S rRNA gene): AY619714.

23. **Nocardiopsis rosea** Li, Kroppenstedt, Wang, Tang, Lee, Park, Kim, Xu and Jiang 2006, 1094[VP]

ro′se.a. L. fem. adj. *rosea* rose colored.

Aerial mycelium is pink-white to pale pink and the substrate mycelium is pale pink to moderate red on media tested. Vegetative hyphae are well developed and fragmented. Spore chains are borne on the aerial hyphae. Spores are smooth-surfaced and nonmotile. No diffusible pigments are produced.

L-Arabinose, D-fructose, D-glucose, D-lactose, maltose, L-rhamnose, D-ribose, sodium acetate, sucrose, and starch can be utilized as carbon sources, but cellobiose, D-galactose, glycerol, *myo*-inositol, D-mannitol, D-mannose, raffinose, sodium citrate, D-sorbitol, D-xylitol, and D-xylose cannot be utilized. Alanine, arginine, asparagine, glycine, histidine, and proline can be used as sole nitrogen sources, but adenine, cystine, glutamic acid, hydroxyproline, lysine, methionine, phenylalanine, threonine, tryptophan, and valine cannot be utilized. Milk coagulation, milk peptonization, gelatin liquefaction, starch hydrolysis, H_2S production, urease activity, and melanin production are negative; nitrate reduction is positive. Optimum growth is at 37–40°C and pH 7.2, with 5–8% (w/v) NaCl. Temperature, pH, and NaCl tolerance ranges are 20–60°C, pH 6–9, and 0–18%, respectively.

The polar lipid pattern is composed of PME, PC, PI, PG, and DPG, together with some unknown PGLs and unknown PLs. Major menaquinones are MK-11, MK-11(H_2), and MK-11(H_4).

Source: the type strain was isolated from a saline soil sample in the west of China.

Type strain: CCTCC AA 2040013, DSM 44842, KCTC 19007, YIM 90094.

DNA G+C content (mol%): 67.9 (T_m).

Sequence accession no. (16S rRNA gene): AY619713.

24. **Nocardiopsis salina** Li, Park, Tang, Wang, Lee, Xu, Kim and Jiang 2004, 1808[VP] emend. Li, Kroppenstedt, Wang, Tang, Lee, Park, Kim, Xu and Jiang 2006, 1093

sa.li′na..L. fem. adj. *salina* salty, saline.

The color of the aerial mycelium is white on most media tested and the substrate mycelium is pale yellow to light orange-yellow or yellow-white. The vegetative hyphae are long, well developed, and fragmented. Long or short spore chains are borne on the aerial hyphae. Spores (0.4–0.66 × 8–1.2 µm) are rod-shaped, smooth, and nonmotile. No diffusible pigments are produced.

Fructose, raffinose, ribose, sodium acetate, sodium citrate, and sucrose are utilized as carbon sources, whereas arabinose, cellobiose, galactose, glucose, inositol, maltose, mannitol, melibiose, rhamnose, trehalose, xylitol, and xylose and are not used. Adenine, arginine, asparagine, glycine, histidine, hydroxyproline, hypoxanthine, methionine, phenylalanine, proline, serine, threonine, and valine are used as nitrogen sources. Negative in tests for milk coagulation, milk peptonization, starch hydrolysis, H_2S production, urease activity, and melanin production. Doubtful result for gelatin liquefaction; positive for nitrate reduction. Grows optimally at 28°C and pH 7.2, with 10% (w/v) NaCl; the temperature, pH, and NaCl tolerance ranges are 20–40°C, 6–9, and 3–20% (w/v), respectively.

Polar lipids present are PC, PG, PI, DPG, PE, PME, and four small PL spots above DPG. Main menaquinones include MK-9(H_8) and MK-10(H_8).

Source: the type strain was isolated from a saline soil sample in the west of China.

Type strain: CCTCC AA 204009, JCM 13364, KCTC 19003, YIM 90010.

DNA G+C content (mol%): 73.1 (T_m).

Sequence accession no. (16S rRNA gene): AY373031.

25. **Nocardiopsis synnemataformans** Yassin, Rainey, Burghardt, Gierth, Ungerechts, Lux, Seifert, Bal and Schaal 1997, 986[VP]

syn.ne.ma.ta.for′mans. Gr. adv. *syn* together; Gr. n. *nema* thread; N.Gr. n. *synnema* threads wrapping together; L. v.

formare to form; L. pres. part. *formans* forming; N.L. part. adj. *synnemataformans* synnema forming, referring to the ability of the organism to form synnemata.

The substrate mycelium is deep pimento colored, penetrates the agar, and bears aerial mycelia; the aerial mycelium is well developed (0.35–0.45 µm in diameter) with zig-zag or spiral forms and is white with a slight pimento touch, which may be reflected color from the substrate mycelium. Different spirals are wrapped together to form synnemata. The mycelia of a synnema fragment in later stages to form rod-shaped elements. No soluble pigment is produced. Melanoid pigments are not produced on either ISP media 6 or 7.

Cellobiose, citrate, galactose, gluconate, glucose, maltose, mannitol, rhamnose, and xylose are used as carbon sources, but not acetate, adipate, adonitol, arabinose, benzoate, erythritol, *m*-hydroxybenzoate, *p*-hydroxybenzoate, inositol, isoamyl alcohol, lactate, lactose, melezitose, paraffin, raffinose, sorbitol, sucrose, trehalose, 2,3-butanediol, or 1,2-propanediol. Alanine, gelatin, and proline are used as sole carbon and nitrogen sources, but not acetamide or serine. Acid is produced from adonitol, galactose, inositol, D-lactose, mannitol, D-mannose, melibiose, L-rhamnose, and sucrose. Lactate is not decarboxylated. Adenine, casein, elastin, esculin, gelatin, hypoxanthine, tyrosine, urea, and xanthine are hydrolyzed; guanine, keratin, and testosterone are not hydrolyzed. Nitrate reductase, β-glucosidase, β-galactosidase, and phosphatase are produced. Grows in the presence of 10% (w/v) NaCl. Growth occurs at 20, 30, and 37°C but not at 42°C.

Main phospholipids are PC, PE, PG, and DPG. Major menaquinones present are MK-10 and MK-10(H_2).

Source: the type strain was isolated from the sputum of a 35-year-old male who had received a renal transplant.

Type strain: DSM 44143, IMMIB D-1215, JCM 10456, NBRC 102581.

DNA G+C content (mol%): 74.1 (HPLC).

Sequence accession no. (16S rRNA gene): Y13593.

26. **Nocardiopsis trehalosi** nom. rev. (*ex* Dolak, Castle and Laborde 1981) Evtushenko, Taran, Akimov, Kroppenstedt, Tiedje and Stackebrandt 2000, 79[VP] (*Nocardiopsis trehalosei* Dolak, Castle and Laborde 1981)

tre.ha.lo′si. N.L. gen. n. *trehalosi* of trehalose, referring to 3-trehalosamine, an aminoglycoside antibiotic that is produced by the type strain of the species.

The color of the substrate mycelium ranges from pale olive-brownish to pale orange-yellow. The aerial mycelium is white to cream or yellowish gray. Aerial hyphae are zig-zag or twisted-ribbon-like at the beginning of sporulation; spores are irregularly sized (mostly elongated) and have a smooth surface. Long-branched substrate hyphae fragment into nonmotile elements. A light yellow-brownish or light orange-yellow soluble pigment is produced on some media. No melanin is observed on peptone-yeast extract-iron agar (ISP 6).

L-Arabinose, D-fructose, D-galactose, D-glucose, glycerol, lactose, maltose, D-mannitol, D-mannose, L-rhamnose, and D-xylose are used for growth as sole carbon sources, but not adonitol, i-inositol, melibiose, D-sorbitol, or sucrose. Acids are produced from L-arabinose, D-fructose, glycerol, lactose, maltose, D-mannitol, D-mannose, and D-xylose, but not from adonitol, D-galactose, i-inositol, melibiose, L-rhamnose, D-sorbose, or sucrose. Acetate, citrate, formate, fumarate, lactate, malate, malonate, pyruvate, propionate, sebacate, succinate, and tartrate are utilized; no alkaline reaction occurs with aconitate, benzoate, salicylate, or oxalate. Acid is produced from L-arabinose, D-lactose, mannitol, D-mannose, and xylose. Lactate and propionate are decarboxylated, but not oxalate. Casein, calcium oxalate crystals, hypoxanthine, xanthine, Tween 80, and urea are degraded, but not tyrosine, Tween 40, Tween 60, or Tween 85. Esculin hydrolysis is variable. Nitrates are not reduced to nitrites.

Resistant to lysozyme (50 mg/ml). Growth occurs on media supplemented with 5% (w/v) NaCl, 2.5% (v/v) methanol, and 3% (v/v) ethanol, but no growth occurs in 10% (w/v) NaCl or in 0.5% (v/v) butanol. Growth temperature ranges between 18 and 45°C; no growth occurs at 10°C. Optimum growth is at 28–37°C.

Major menaquinones are MK-10(H_4) and MK-10(H_6). Diagnostic phospholipid is PC.

Source: the type strain was isolated from experimental biofilters filled with tree bark compost.

Type strain: CIP 106425, DSM 44380, IFO 14201, JCM 3357, NRRL 12026, VKM Ac-942.

DNA G+C content (mol%): not determined.

Sequence accession no. (16S rRNA gene): AF105972.

27. **Nocardiopsis tropica** Evtushenko, Taran, Akimov, Kroppenstedt, Tiedje and Stackebrandt 2000, 79[VP]

tro′pi.ca. L. fem. adj. *tropica* tropical, of or pertaining to the tropic(s), referring to the tropical region where the type strain was isolated.

Substrate mycelium is colorless to olive-yellow or red-orange on the different media tested. The aerial mycelium is white; however, it has a cream color on Czapek agar. Aerial hyphae are long and zig-zag shaped at the beginning of sporulation, fragmenting into elongated spore-like structures which subsequently subdivide into short rod-shaped spores of irregular size. Spore surface is smooth. Long-branched substrate hyphae fragment into nonmotile elements. Soluble pigments are yellow-orange to yellow on oatmeal agar or glycerol-nitrate agar. Melanin is not produced on peptone-yeast extract-iron agar (ISP 6).

L-Arabinose, D-fructose, D-galactose, D-glucose, glycerol, maltose, mannitol, D-mannose, melibiose, L-rhamnose, sucrose, and D-xylose are used as sole carbon sources for growth; adonitol, dulcitol, i-inositol, lactose, D-ribose, and D-sorbose are not used. Acids are produced from L-arabinose, D-fructose, D-galactose, D-glucose, glycerol, maltose, D-mannitol, melibiose, L-rhamnose, and sucrose, but not from adonitol, dulcitol, i-inositol, lactose, D-ribose, sorbose, or D-xylose. An alkaline reaction is observed with citrate, formate, fumarate, lactate, malate, malonate, oxalate, propionate, pyruvate, sebacate, succinate, and tartrate, but no alkaline reaction occurs with acetate, aconitate, benzoate, or salicylate. Lactate, oxalate, and propionate are decarboxylated. Xanthine, hypoxanthine, calcium oxalate crystals, urea, and Tweens

40, 60, 80, and 85 are degraded; casein and tyrosine are not. Nitrates are not reduced to nitrites. Growth occurs on media supplemented with 10% (w/v) NaCl, 1.5% (v/v) butanol, 2.5% (v/v) methanol, and 3% (v/v) ethanol, but no growth occurs with 12% (w/v) NaCl or lysozyme (50 mg/ml). Growth occurs at 37°C but not at 10°C or 42°C; optimum growth is between 28 and 30°C.

Major menaquinones are MK-10(H_6) and MK-10(H_8). Diagnostic phospholipid is PC.

Source: the type strain was isolated from soil in the rhizosphere of *Casuarina* sp. (Seychelles).

Type strain: CIP 106426, DSM 44381, JCM 10877, VKM Ac-1457.

DNA G+C content (mol%): not determined.

Sequence accession no. (16S rRNA gene): AF105971.

28. **Nocardiopsis umidischolae** Peltola, Andersson, Kämpfer, Auling, Kroppenstedt, Busse, Salkinoja-Salonen and Rainey 2002, 3[VP] (Effective publication: Peltola, Andersson, Kämpfer, Auling, Kroppenstedt, Busse, Salkinoja-Salonen and Rainey 2001, 4303).

u.mi.di.scho′la.e. L. adj. *umidus* moist; L. fem. n. *schola* school; N.L. gen. fem. n. *umidischolae* of a moist school.

The vegetative hyphae are yellowish and penetrate the agar. The aerial hyphae are white, 0.2 µm in diameter, and form thick bundles. Soluble pigments are not produced.

Acetate, *cis*-aconitate, aspartate, β-alanine, L-alanine, arabinose, arbutin, 4-aminobutyrate, 3-hydroxybutyrate cellobiose, citrate, fructose, fumarate, galactose, *N*-acetyl-D-glucosamine, gluconate, glucose, glutarate, histidine, lactate, malate, maltitol, maltose, mannitol, mannose, melibiose, oxoglutarate, phenylacetate, phenylalanine, proline, propionate, pyruvate, rhamnose, ribose, serine, sucrose, trehalose, and xylose are used as carbon sources, but not adipate, adonitol, azelate, inositol, 3-hydroxybenzoate, 4-hydroxybenzoate, itaconate, leucine, mesaconate, ornithine, putrescine, salicin, sorbitol, suberate, or tryptophan. Esculin is not hydrolyzed. Produces α- and β-glucosidases, phosphatase, and peptidases. Grows in the presence of 7.5% (w/v) NaCl. Growth occurs at 10, 28, and 37°C, but not at 50°C.

The polar lipid pattern is composed of PC, PI, PG, PME, and DPG. The menaquinone composition includes MK-10(H_6), MK-10(H_4), MK-10(H_8), and MK-10(H_6).

Source: the type strain was isolated from the indoor dust of a water-damaged school.

Type strain: 66/93, DSM 44362, JCM 11758, NBRC 100349, NRRL B-24122.

DNA G+C content (mol%): not determined.

Sequence accession no. (16S rRNA gene): AY036001.

29. **Nocardiopsis valliformis** Yang, Zhang, Guo, Shi, Lu and Zhang 2008c, 1544[VP]

val.li.for′mis. L. n. *vallum* palisade; L. adj. suffix *-formis -is -e* (from L. n. *forma* figure, shape, appearance) like, in the shape of; N.L. fem. adj. *valliformis* shaped like a palisade, referring to the characteristic mycelium, which is often arranged in a shape like a palisade.

Substrate mycelium is yellow to light-brown and is often arranged in a shape like a fence or palisade. Aerial mycelium is abundant and white to yellowish, and fragments into rod-shaped, smooth-surfaced and nonmotile spores (0.3–0.5 × 1.2–2.5 µm). Melanin is not observed on either tyrosine agar or peptone-yeast-iron agar (ISP media 6 and 7).

L-Arabinose, glycerol, lactose, and D-xylose are used as carbon sources, but not *myo*-inositol or sucrose. Nitrate reduction and gelatin liquefaction are positive; H_2S production is negative. Optimal growth temperature is 28°C; no growth occurs at 10, 42 or 45°C. Optimal pH for growth is pH 9.5–13; has a broad range of growth pH, from pH 8.0 to 14.0. No growth at pH 7.0. Growth occurs in the absence of NaCl and in 1, 3, and 5% (w/v) NaCl; no growth in 10% NaCl.

Major phospholipids include DPG and PC. Major menaquinones are MK-10(H_2), MK-10(H_4), and MK-10(H_6).

Source: the type strain was isolated from a soil sample collected from an alkali lake in Xinjiang, China.

Type strain: CGMCC 4.2135, DSM 45023, HBUM 20028.

DNA G+C content (mol%): 70.6 (HPLC).

Sequence accession no. (16S rRNA gene): AY336503.

30. **Nocardiopsis xinjiangensis** Li, Li, Xu, Cui, Xu and Jiang 2003a, 319[VP]

xin.ji.ang.en′sis. N.L. fem. adj. *xinjiangensis* pertaining to Xinjiang, the province of western China in which the sample was collected.

Fragmented substrate and aerial mycelia are well developed on ISP 4, ISP 5, potato agar, and nutrient agar. Short spore chains are borne on the aerial mycelium; spores are rod-shaped with a smooth surface. Diffusible pigments are not produced.

Alanine, cellobiose, galactose, proline, and serine are used as carbon sources, but not glucose, maltose, mannitol, raffinose, rhamnose, sucrose, or xylose. Gelatin liquefaction, urease activity, and melanin production are positive. Milk coagulation, milk peptonization, starch hydrolysis, nitrate reduction, and H_2S production are negative. Optimum growth temperature is 28°C; optimum salt concentration for growth is 10% (w/v); optimum pH for growth is 7.2.

Predominant menaquinones are MK-10(H_2) and MK-10(H_4). Diagnostic phospholipids are PG and PI.

Source: the type strain was isolated from a saline soil sample in the west of China.

Type strain: CCRC 16285, CCTCC AA 99004, DSM 44589, JCM 12328, YIM 90004.

DNA G+C content (mol%): 74.3 (T_m).

Sequence accession no. (16S rRNA gene): AF251709.

Genus II. **Haloactinospora** Tang, Tian, Zhi, Cai, Wu, Yang, Xu and Li 2008, 2078[VP]

Martha E. Trujillo and Michael Goodfellow

Ha.lo.ac.ti.no.spo′ra. Gr. n. *hals halos* salt; Gr. n. *aktis -inos* a ray; Gr. n. *spora* a seed, and in biology, a spore; N.L. fem. n. *Haloactinospora* salt-loving and spored ray, referring to a halophilic and spore-forming actinomycete.

Aerobic, Gram-stain-positive, moderately halophilic filamentous actinomycetes which form well-developed, non-fragmented hyphae. **Long chains of cylindrical spores with smooth surfaces are formed on the aerial mycelium. Short spore chains with wrinkled surfaces and terminal pseudosporangia are formed on the substrate mycelium.** Chemo-organotrophic with an oxidative type of metabolism. **Whole-cell hydrolysates contain *meso*-diaminopimelic acid, galactose, and ribose. Cellular fatty acids are rich in branched, straight-chain, and methyl fatty acids. Major polar lipids are diphosphatidylglycerol, phosphatidylglycerol, phosphatidylcholine, and phosphatidylinositol mannoside.** The predominant menaquinones are MK-10(H_8), MK-11(H_6), and MK-11(H_8).

DNA G+C content (mol%): 68 (HPLC).

Type species: **Haloactinospora alba** Tang, Tian, Zhi, Cai, Wu, Yang, Xu and Li 2008, 2078[VP].

Further descriptive information

Phylogeny. The genus *Haloactinospora* contains one species, *Haloactinospora alba*. The taxon is classified in the family *Nocardiopsaceae*, which also includes the genera *Nocardiopsis*, *Streptomonospora*, and *Thermobifida*. *Haloactinospora alba* forms an independent branch in the 16S rRNA tree between the genera *Streptomonospora* and *Thermobifida*. 16S rRNA gene sequence similarities between *Haloactinospora alba* and members of the family *Nocardiopsaceae* range from 93.3 to 95%. 16S rRNA gene signature nucleotides are consistent with those described for the family *Nocardiopsaceae* (Zhi et al., 2009) (See Figure 389 in the treatment of the family *Nocardiopsaceae*).

Cell morphology. *Haloactinospora alba* forms a well-developed, non-fragmenting substrate mycelium which carries aerial hyphae. Mature aerial hyphae differentiate into long chains of nonmotile cylindrical spores (0.4–0.6 × 1.0–1.2 µm) with smooth surfaces; spore chains with wrinkled surfaces and terminal pseudosporangia are observed on the substrate mycelium (Figure 393).

Nutrition and growth conditions. *Haloactinospora alba* grows well on Czapek's and yeast extract-malt extract agars (ISP 2 medium; Shirling and Gottlieb, 1966); it shows moderate growth on salts-starch, glycerol-asparagine, and nutrient agars, but grows poorly on oatmeal agar (ISP 3 agar; Shirling and Gottlieb, 1966). The color of the aerial mycelium is white to yellow-white and that of the substrate mycelium is pale yellow to light or deep yellow. Diffusible pigments are not produced on any of these media. Temperature, pH, and NaCl tolerance ranges are 20–45°C (optimum 37°C), pH 6–9 (optimum 7–8), and 9–21% (w/v) NaCl (optimum 15%, w/v), respectively.

Cell-wall composition. *Haloactinospora alba* contains *meso*-diaminopimelic acid as the major wall diamino acid. Whole-cell hydrolyzates also contain galactose, ribose, and minor amounts of glucose. The polar lipid pattern of *Haloactinospora alba* YIM 90648[T] includes diphosphatidylglycerol, phosphatidylglycerol, phosphatidylcholine, phosphatidylinositol mannoside, and various unidentified lipids (ninhydrin reagent-positive, Dittmer and Lester reagent-negative); hence, it has phospholipid type III *sensu* Lechevalier et al. (1977, 1981). Menaquinone composition is complex and includes a mixture

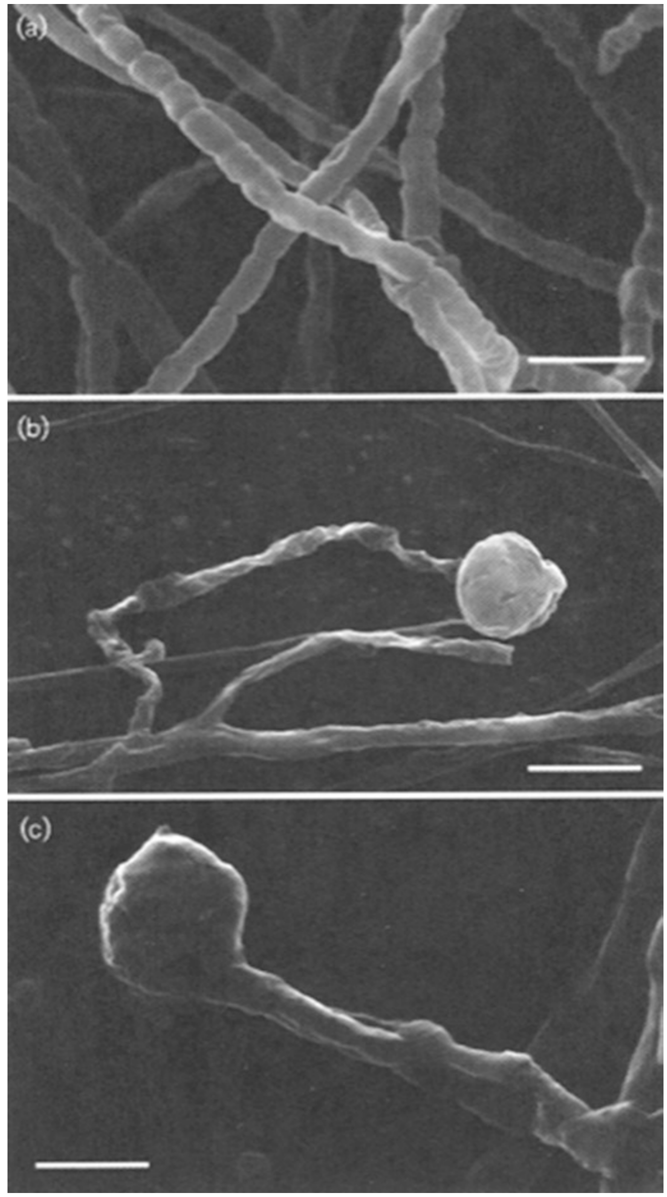

FIGURE 393. Scanning electron micrographs of spore chains and pseudosporangia of *Haloactinospora alba* strain YIM 90648[T] grown on ISP 2 agar supplemented with 15% (w/v) NaCl for 4 weeks at 37°C. (a) Long spore chains on the aerial mycelium; (b, c) substrate mycelium bearing a short spore chain with a terminal pseudosporangium. Bars, 2 µm (a, b) and 1 µm (c). (Reproduced with permission from Tang et al., 2008. Int. J. Syst. Evol. Microbiol. *58*: 2075–2080.)

of MK-10(H_8), MK-11(H_4), MK-11(H_6), and MK-11(H_8), which account for about 52% of the total composition. The fatty acid profile contains major amounts of branched fatty acids and minor amounts of straight-chain and methyl fatty acids; the major fatty acids are 14-methyl-heptadecanoic (iso-16:0) and 14-methyl-hexadecanoic (anteiso-17:0) acids. This fatty acid profile places the organism in fatty acid type 3 d according to Kroppenstedt (1985).

Ecology. The only strain currently representing the genus *Haloactinospora* was isolated from a salt lake in China. Like most members of the family *Nocardiopsaceae*, *Haloactinospora alba* is moderately halophilic; it is likely that additional representatives of this taxon are present in saline habitats.

Isolation procedures

Haloactinospora alba YIM 90648^T was isolated from a salt lake sample by inoculating it onto cellulose-casein-multisalts agar (10 g microcrystalline cellulose, 0.3 g casein, 0.2 g KNO_3, 0.5 g K_2HPO_4, 0.02 g $CaCO_3$, 0.01 g $FeSO_4$, 100 g NaCl, 30 g $MgCl_2 \cdot 6H_2O$, 20 g KCl, and 15 g agar, 1000 ml distilled water). The salts solutions (NaCl, KCl, and $MgCl_2$) should be sterilized separately and added to the medium and the pH should be adjusted to 7.5 with 1 M NaOH. Inoculated plates should be incubated for 3 weeks at 37°C.

Maintenance procedures

Strain YIM 90648^T may be maintained on inorganic salts-starch agar slants (ISP 4 agar; Shirling and Gottlieb, 1966) supplemented with 15% (w/v) NaCl at 4°C for short-term preservation; mycelial fragment suspensions in 20% glycerol (v/v) are recommended for freezing at −25°C.

Differentiation of the genus *Haloactinospora* from other genera

A combination of chemotaxonomic, phenotypic, and phylogenetic procedures can be used to differentiate the genus *Haloactinospora* from related genera. The production of pseudosporangia on substrate mycelium separates *Haloactinospora* from the genera *Nocardiopsis*, *Streptomonospora*, and *Thermobifida*. *Haloactinospora* can be distinguished from the genus *Thermobifida* by differences in growth temperature and tolerance to NaCl. Menaquinone and polar lipid patterns are useful markers for distinguishing between *Haloactinospora* and the three other genera classified in the family *Nocardiopsaceae*. 16S rRNA gene sequencing clearly differentiates *Haloactinospora* from other actinobacterial taxa.

Differentiation of the species of the genus *Haloactinospora*

At present, there is only one species in the genus *Haloactinospora*.

List of species of the genus *Haloactinospora*

1. **Haloactinospora alba** Tang, Tian, Zhi, Cai, Wu, Yang, Xu and Li 2008, 2078^{VP}

al′ba. L. fem. adj. *alba* white.

Aerial and substrate mycelia are well developed and non-fragmented. The color of the aerial mycelium is white to yellow-white and that of the substrate mycelium is pale yellow to light yellow or even deep yellow. Aerial hyphae differentiate into long chains of cylindrical spores which have smooth surfaces. Chains of wrinkled spores with terminal pseudosporangia are formed on the substrate mycelium. Good growth is observed on media supplemented with 15% (w/v) NaCl, notably on Czapek's, yeast extract-malt extract, and potato agars; moderate growth occurs on inorganic salts-starch, glycerol-asparagine agar, and nutrient agars, but growth is poor on oatmeal agar. Diffusible pigments are not produced on these media.

L-Arabinose, D-arabitol, cellobiose, D-fructose, D-galactose, D-glucose, inulin, raffinose, D-ribose, sucrose, D-tagatose, and D-xylose are used as sole carbon sources, but not D-adonitol, D-arabinose, dulcitol, erythritol, glycogen, inositol, D-lactose, maltose, D-mannitol, D-mannose, melezitose, L-rhamnose, L-sorbose, D-sorbitol, trehalose, xylitol, or L-xylose.

Positive for milk peptonization and coagulation. Tests for gelatin liquefaction, production of H_2S and melanin pigments, and for hydrolysis of cellulose, starch and urea are negative.

Temperature, pH, and NaCl tolerance ranges are 20–45°C, pH 6–9, and 9–21% (w/v) NaCl, respectively. Major fatty acids are $C_{16:0}$ iso (23.8%) and $C_{17:0}$ anteiso (36%).

Source: the type strain was isolated from a salt lake in Xinjiang Province, north-west China.

DNA G+C content (mol%): 68 (HPLC).

Type strain: CCTCC AA 206008, DSM 45015, YIM 90648.

Sequence accession no. (16S rRNA gene): DQ923130.

Genus III. **Streptomonospora** corrig. Cui, Mao, Zeng, Li, Zhang, Xu and Jiang 2001, 362^{VP} emend. Li, Xu, Zhang, Tang, Cui, Mao, Xu, Schumann, Stackebrandt and Jiang 2003b, 1424

Xiao-Long Cui

Strep.to.mo.no.spo′ra. Gr. adj. *streptos* pliant, bent; Gr. adj. *monos* single, solitary; Gr. fem. n. *spora* a seed, spore; N.L. fem. n. *Streptomonospora* indicating that this organism forms two types of spores, with wrinkled surfaces, on aerial mycelium and substrate mycelium.

Gram-stain-positive, **aerobic organisms with branching hyphae**, 0.5–0.8 μm in diameter. **Spores are of two types.** The aerial mycelium, at maturity, forms **short chains of nonmotile spores**, which may be oval- to rod-shaped (0.3–1.0 × 0.4–2.0 μm) with wrinkled surfaces. Substrate mycelium is extensively branched with non-fragmenting hyphae. **Single, nonmotile, oval to round spores** (1.4–1.6 μm) are borne on sporophores or dichotomously branched sporophores of substrate hyphae. Optimum

growth occurs in media supplemented with **NaCl at a concentration of 10–15% (w/v), at 28–37°C, and at pH 7.0. Peptidoglycan contains *meso*-diaminopimelic acid as diagnostic diamino acid. Cell walls contain galactose or galactose plus arabinose.** The phospholipid pattern is complex, consisting of phosphatidylglycerol, phosphatidylethanolamine, phosphatidylcholine, phosphatidylinositol, and diphosphatidylglycerol; methylphosphatidylethanolamine, phosphatidylinositol mannosides and phosphatidylserine may occur. The menaquinone composition may depend on the growth medium and consists mainly of menaquinones with nine, ten, or eleven isoprenoid chains and various degrees of hydrogenation. The genus *Streptomonospora* belongs to the family *Nocardiopsaceae* (Rainey et al., 1996). **Inhabit saline environments.**

DNA G+C content (mol%): 71.2–74.4 (HPLC).

Type species: **Streptomonospora salina** corrig. Cui, Mao, Zeng, Li, Zhang, Xu and Jiang 2001, 362[VP].

Further descriptive information

Phylogeny. Based on 16S rRNA gene sequence comparisons of members of the family *Nocardiopsaceae*, members of the genus *Streptomonospora* can be distinguished clearly from those of the genera *Haloactinospora*, *Nocardiopsis*, and *Thermobifida*. The five species currently classified in the genus *Streptomonospora* form two 16S rRNA subclusters: one cluster contains the single species *Streptomonospora halophila*, whereas the second cluster includes the species *Streptomonospora alba*, *Streptomonospora amylolytica*, *Streptomonospora flavalba*, and *Streptomonospora salina* (Figure 394). Both clusters are supported by high bootstrap values indicating significant branch stability. The 16S rRNA gene sequence similarities within the type strains of these species range from 96.7 to 99.6%. In addition, DNA–DNA relatedness values between species tested range from 25.1 to 47.3% (Cai et al., 2009). The signature nucleotides of members of the genus *Streptomonospora*, which differentiate them from those of the other genera in the family *Nocardiopsaceae*, are as follows: 81:88 (G–U), 82:87 (G–C), 185:192 (A–G), 196 (C), 197 (A), 207:212 (G–A), 208 (U), 210 (G), 229 (G), 508 (C), 607 (U), 662:743 (C–G), 998:1043 (G–C), 1025:1036 (U–G), 1026:1035 (G–C), 1042 (G).

Cell morphology and cultural characteristics. Species of the genus *Streptomonospora* produce white to pale yellow colonies with well-developed aerial and substrate hyphae (0.5–0.8 µm in diameter) on nutrient agar, potato agar, Czapek's agar, and most ISP media tested (International *Streptomyces* Project medium; Shirling and Gottlieb, 1966). Species do not produce diffusible pigments on any of these media except for *Streptomonospora flavalba* YIM 91394[T], which produces deep yellow diffusible pigments on potato extract agar (pH 7.0) supplemented with 10% (w/v) NaCl. Morphological observation of 7 to 28-d-old cultures grown on glycerol/asparagine agar (ISP medium 5) or inorganic salt/starch agar (ISP medium 4) containing 10–15% (w/v) NaCl revealed that the vegetative hyphae with irregular branches are well developed, but not fragmented (Figure 395). The aerial mycelium, at maturity, forms short chains of spores that are oval- to rod-shaped (0.3–1.0 × 0.4–2.0 µm) with wrinkled surfaces (Figure 395a). Substrate mycelium is extensively branched with non-fragmenting hyphae. Single spores, which are oval to round and 1.4–1.6 µm in diameter, are borne on sporophores of substrate mycelium or dichotomously branched sporophores, and the surfaces of single spores are wrinkled (Figure 395b). Therefore, there are two types of spores, both of which are nonmotile.

Cell-wall composition. All *Streptomonospora* species contain *meso*-diaminopimelic acid (*meso*-DAP) as the diagnostic amino acid; in addition, *Streptomonospora alba* and *Streptomonospora salina* contain alanine, glutamic acid, muramic acid, acetylglucosamine, and di- and tripeptides typical of peptidoglycan type A1γ (Schleifer and Kandler, 1972). In the initial analysis, Cui et al. (2001) reported that the cell wall of *Streptomonospora salina* YIM 90002[T] contained *meso*-DAP, DD-DAP, glycine, and aspartic acid as cell wall amino acids. A second analysis of the cell wall of this micro-organism (Li et al., 2003b) revealed that the cell-wall composition was similar to that of *Streptomonospora alba*, i.e. peptidoglycan of type A1γ. These authors concluded that the additional amino acids reported previously for *Streptomonospora salina* were probably constituents of a protein tightly attached to the peptidoglycan. All *Streptomonospora* species contain galactose as the diagnostic whole-cell sugar. In addition, *Streptomonospora alba* also contains arabinose. *Streptomonospora alba* YIM 90003[T] and *Streptomonospora salina* YIM 90002[T] have been reported to contain glucose as determined by GC-MS analyses (Chen et al., 2000).

Phospholipid composition. The phospholipid composition of *Streptomonospora* strains is quite complex and varies depending on the species. All strains analyzed contain phosphatidylglycerol, phosphatidylcholine, and phosphatidylinositol. *Streptomonospora alba* and *Streptomonospora salina* also contain phosphatidylethanolamine and methylphosphatidylethanolamine (Cai et al., 2008; Cui et al., 2001; Li et al., 2003b). The former also contains phosphatidylserine. An unidentified phospholipid has also been reported for *Streptomonospora alba*, *Streptomonospora halophila*, and *Streptomonospora salina*. In addition, *Streptomonospora amylolytica*, *Streptomonospora flavalba* (Cai et al., 2009), and *Streptomonospora halophila* contain phosphatidylinositol mannosides.

Quinones. The menaquinone composition may depend on the growth medium and consists mainly of menaquinones with nine, ten, or eleven isoprenoid chains and various degrees of hydrogenation: i.e. a combination of one or more representative(s) of the series [MK-9(H_2), (H_4), (H_6), (H_8)], [MK-10(H_2), (H_4), (H_6), (H_8)], and [MK-11(H_4), (H_6), (H_8), (H_{10})]. Following the procedures of Groth et al. (1999), the predominant menaquinones found in *Streptomonospora alba* YIM 90003[T] grown on vitamin-enriched ISP 2 medium were MK-10(H_2), MK-10(H_4), and MK-9(H_8). The composition changed, however, when cells grown on glucose/yeast extract medium were analyzed: the major menaquinone was MK-9(H_4), whereas MK-9, MK-10(H_4), MK-9(H_2), MK-9(H_6) occurred as minor compounds (Li et al., 2003b). Similar results were obtained when *Streptomonospora salina* YIM 90002[T] was analyzed using cultures grown on different media (Li et al., 2003b). The reason for this change has not been investigated, but medium- and age-dependent shifts in menaquinone composition have been reported previously for *Actinobacteria* (Hiraishi and Komagata, 1989; Saddler et al., 1986).

Fatty acid composition. *Streptomonospora* species contain the following major fatty acids: $C_{17:0}$ anteiso, $C_{16:0}$ iso, $C_{15:0}$ iso, $C_{15:0}$ anteiso, $C_{17:0}$ iso, 10-methyl $C_{17:0}$, $C_{16:0}$, $C_{17:0}$, 10-methyl $C_{18:0}$, $C_{18:0}$, $C_{18:0}$ iso, and $C_{14:0}$ iso.

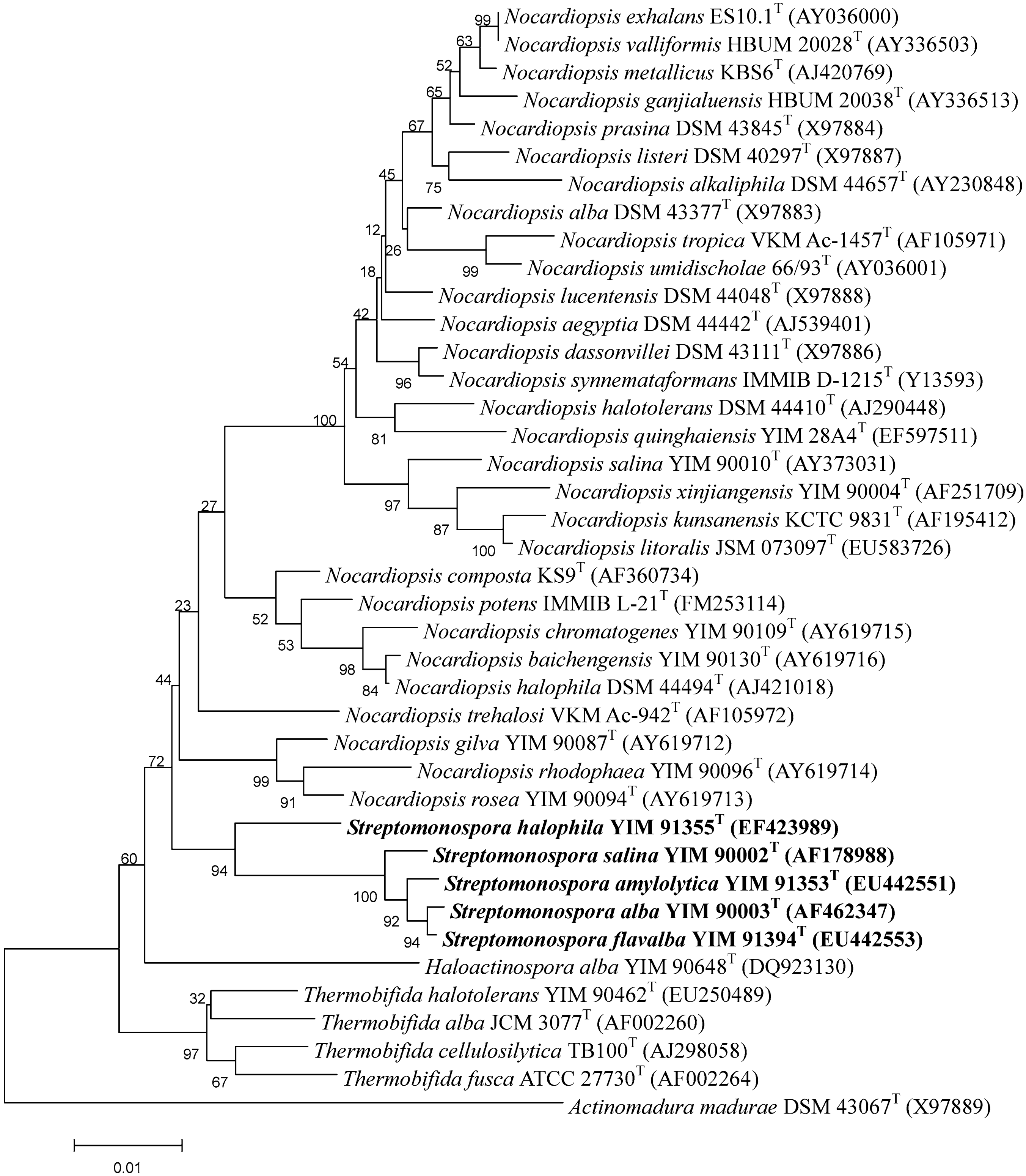

FIGURE 394. Neighbor-joining tree showing the phylogenetic relationships among members of the genera *Streptomonospora*, *Nocardiopsis*, *Haloactinospora*, and *Thermobifida* in the family *Nocardiopsaceae*. *Actinomadura madurae* was used as the outgroup. Bootstrap values from 1000 analyses are shown at the nodes of the tree. Bar = 1 nt substitution per 100 nt of 16S rRNA gene sequence.

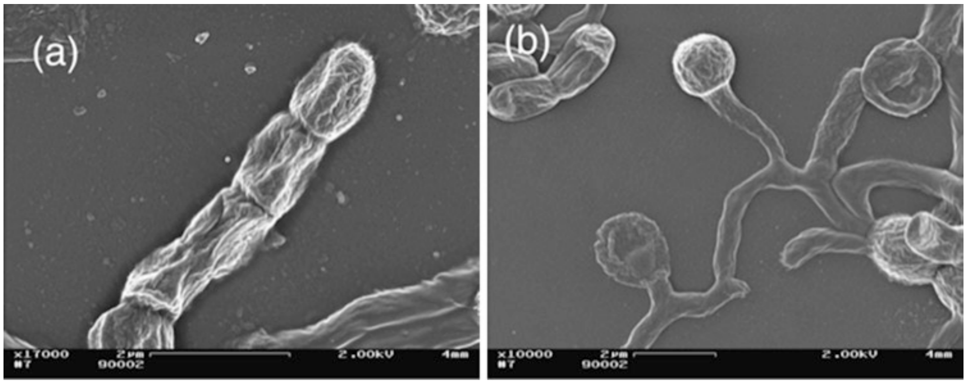

FIGURE 395. Scanning electron micrographs of *Streptomonospora salina* YIM 90002^{T} grown on ISP medium 5 for 28 d at 28°C, showing a short chain of spores (a) and single spores (b). Bars = 2 µm.

Nutrition and growth conditions. Members of the genus *Streptomonospora* are aerobic, heterotrophic, halophilic actinomycetes that require, for optimal growth, complex media adjusted to a pH of 7.0 and supplemented with 10–15% (w/v) NaCl. The optimal growth temperature for *Streptomonospora alba* and *Streptomonospora salina* is 28°C, whereas *Streptomonospora amylolytica*, *Streptomonospora flavalba*, and *Streptomonospora halophila* show optimal growth at 37°C. Good growth is observed on yeast extract/malt extract agar (ISP 2), inorganic salt/starch agar (ISP 4), and glycerol/asparagine agar (ISP 5), and, in most cases, oatmeal agar (ISP 3). *Streptomonospora alba* YIM 90003^{T} grows well on ISP 5 medium, nutrient agar, and Czapek's agar. *Streptomonospora amylolytica* YIM 91353^{T} and *Streptomonospora flavalba* YIM 91394^{T} show good growth on ISP 3 and 2 media, respectively, while both grow well on nutrient agar. *Streptomonospora halophila* YIM 91355^{T} grows very well on modified ISP 5 (Cai et al., 2008). *Streptomonospora salina* YIM 90002^{T} shows good growth on ISP 2 and 5, nutrient agar, Czapek's agar, and potato agar, all supplemented with 15% (w/v) NaCl.

Ecology. All halophilic actinomycetes of the genus *Streptomonospora* have been isolated from hypersaline soil samples from Aiding salt lake in Xinjiang Province in Western China.

Enrichment and isolation procedures

Complex media such as starch/casein agar and modified ISP 5 agar (containing 0.1% K_2HPO_4, 0.1% L-asparagine, 1.0% glycerol, 0.5% yeast extract, 0.5% KNO_3, 10.0% NaCl, 2.0% agar in 1 liter distilled water; pH 7.0) have been used for isolation of *Streptomonospora* strains using dilution plate procedures. Isolation plates are usually incubated at 28 or 37°C for 3 to 4 weeks. Pure cultures may be subsequently obtained on ISP 3 and 4 media, Czapek's agar, potato agar, and nutrient agar, all supplemented with 10–15% (w/v) NaCl.

Maintenance procedures

Streptomonospora cultures may be maintained on ISP 2, ISP 4, and ISP 5 agar slants supplemented with 10–15% (w/v) NaCl at 4°C for short-term preservation. For longer term preservation, all strains can be maintained in 20% (v/v) glycerol suspensions at −80°C. Alternatively, lyophilization is also recommended.

Differentiation of the genus *Streptomonospora* from other genera

Streptomonospora species form two types of spores, which make them easily differentiated from members of the genera *Nocardiopsis*, *Haloactinospora*, and *Thermobifida* of the family *Nocardiopsaceae*. In addition, one set of *Streptomonospora*-specific primers was designed by Zhi et al. (2006, 2007) that can be used to detect/differentiate, by PCR amplification, the large numbers of *Streptomonospora* strains from those of other genera that are obtained from various environments, especially from saline environments.

Taxonomic comments

The genus *Streptomonospora* was first described by Cui et al. (2001) to accommodate a halophilic strain isolated from a salt lake in China; however, the name of the genus was written as "*Streptimonospora*". The original spelling *Streptimonospora* (*sic*) has been corrected by the List Editor, IJSEM (2001), as *Streptomonospora* corrig. Cui et al. 2001, gen. nov.

Differentiation of the species of the genus *Streptomonospora*

Although members of the genus *Streptomonospora* have high 16S rRNA gene sequence similarity values (96.7–99.6%) and share similar morphological properties, low DNA–DNA hybridization values together with differential chemotaxonomic markers, as well as physiological and biochemical characteristics, are useful for differentiating all species (Table 296).

TABLE 296. Phenotypic characteristics that differentiate the type strains of *Streptomonospora* species[a]

Characteristic	*S. salina* YIM 90002[T]	*S. alba* YIM 90003[T]	*S. amylolytica* YIM 91353[T]	*S. flavalba* YIM 91394[T]	*S. halophila* YIM 91355[T]
Media for growth:					
ISP 2	Good	Moderate	Moderate	Good	Good
ISP 3	Poor	Moderate	Good	Moderate	Good
ISP 4	Moderate	Moderate	Moderate	Moderate	Moderate
ISP 5	Good	Good	–	Poor	Good
Czapek's agar	Good	Good	–	Poor	Moderate
Nutrient agar	Good	Good	Good	Good	Poor
Potato agar	Good	Moderate	Moderate	Moderate	Good
Aerial mycelium color	Pale white, white	White	Yellow-white	Yellow-white	Yellow-white
Substrate mycelium color	Mid-yellow	Orange-yellow	Yellow-gray	Pale yellow	Pale yellow
Optimum temperature for growth (°C)	28	28	37	37	37
Diffusible pigment	–	–	–	+ (deep yellow)	–
NaCl concentration for growth (%):					
Range	5–20	5–25	5–20	5–25	5–20
Optimum	15	10–15	10	10	10
Milk peptonization	–	–	+	+	+
Nitrate reduction	–	+	–	–	–
Production of:					
H_2S	–	–	+	–	–
Oxidase	+	–	–	–	–
Melanin	+	–	–	+	–
Amylase	+	–	+	+	–
Cellulase	–	–	–	–	+
Carbon source utilization:					
Arabinose	+	–	+	+	–
Cellobiose	–	–	+	–	–
Glucose	+	+	+	+	–
Inositol	–	–	–	–	+
Maltose	+	–	–	+	+
Rhamnose	–	–	+	+	+
Sorbitol	–	–	+	–	–
Alanine	–	–	–	+	+
Arginine	–	–	+	+	–
Glycine	–	–	–	+	–
Histidine	+	–	–	+	+
Hydroxyproline	–	–	+	–	–
Lysine	–	–	+	–	–
Phenylalanine	–	–	–	+	+
Proline	–	–	+	+	+
Serine	–	–	–	+	–
Threonine	–	–	+	+	+
Tryptophan	–	–	+	+	–
Tyrosine	–	–	–	+	–
Cell-wall sugars	Galactose	Galactose, arabinose	Galactose	Galactose	Galactose
Predominant menaquinones (>10%)	MK-10(H_8) (39.4%), MK-10(H_6) (22.6%), MK-10(H_4) (11.8%)	MK-10(H_8) (50.6%), MK-10(H_6) (15.8%)	MK-10(H_8) (35.8%), MK-10(H_6) (16.4%), MK-9(H_8) (15.3%)	MK-10(H_8) (41.5%), MK-9(H_8) (27.7%), MK-10(H_6) (12.6%)	MK-10(H_8) (43.2%), MK-10(H_6) (17.7%), MK-11(H_8) (10.4%)
Major phospholipids	PG, PI, PC, 2MPE, PL	PG, PE, PI, DPG, MPE, PS, PC, PL	DPG, PC, PG, PI, PIM	DPG, PC, PG, PI, PIM	DPG, PG, PC, PIM, PI, PL
Major fatty acids	$C_{15:0}$ iso (30.1%), $C_{16:0}$ iso (16.5%), 9-methyl $C_{16:0}$ (11.7%), $C_{17:0}$ anteiso (6.7%)	$C_{17:0}$ anteiso (26.0%), $C_{16:0}$ iso (25.1%), $C_{16:0}$ (8.6%), $C_{15:0}$ anteiso (8.0%)	$C_{17:0}$ anteiso (42.4%), $C_{18:0}$ (11.2%)	$C_{17:0}$ anteiso (28.0%), $C_{16:0}$ iso (24.6%)	$C_{16:0}$ iso (39.8%), $C_{17:0}$ anteiso (12.5%), 10-methyl $C_{17:0}$ (10.2%), 10-methyl $C_{18:0}$ (11.1%)
DNA G+C content (mol%)	72.9	74.4	71.2	72.5	72.1

[a]Data are from Cui et al. (2001), Li et al. (2003b) and Cai et al. (2008, 2009). +, Positive; –, negative; DPG, diphosphatidylglycerol; MPE, methylphosphatidylethanolamine; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; PI, phosphatidylinositol; PIM, PI mannosides; PL, unidentified phospholipid; PS, phosphatidylserine.

List of species of the genus *Streptomonospora*

1. **Streptomonospora salina** corrig. Cui, Mao, Zeng, Li, Zhang, Xu and Jiang 2001, 362[VP] emend. Li, Xu, Zhang, Tang, Cui, Mao, Xu, Schumann, Stackebrandt and Jiang 2003b, 1424

sa.li′na. L. fem. adj. *salina* salted, saline, referring to the saline habitat of the micro-organism.

White aerial mycelium, at maturity, forms short chains of spores that are oval- to rod-shaped (1.5–2 × 1 µm) with wrinkled surfaces. Mid-yellow substrate mycelium is extensively branched with non-fragmenting hyphae. Single spores, which are oval to round (1.4 × 1.6 µm), are borne on sporophores or dichotomously branched sporophores of substrate hyphae; the surfaces of the spores are wrinkled. Both spore types are nonmotile. Grows in 5–20% (w/v) NaCl. Optimum growth occurs in media supplemented with 15% (w/v) NaCl, at 28°C, and at pH 7.0. Diffusible pigments are not produced.

Glucose, sucrose, maltose, arabinose, raffinose, starch, glycerol, mannitol, and histidine are used as sole carbon sources. Positive for catalase, starch hydrolysis, and melanin production, but negative for oxidase, milk coagulation, peptonization, hydrolysis of cellulose, H_2S production, nitrate reduction, and gelatin liquefaction.

The cell wall contains *meso*-DAP, alanine, glutamic acid, muramic acid, acetylglucosamine, and di- and tripeptides typical of peptidoglycan type A1γ; the cell-wall sugar is galactose. The major menaquinones are MK-10(H_8) (39.4%), MK-10(H_6) (22.6%), and MK-10(H_4) (11.8%). The phospholipid pattern consists of phosphatidylglycerol, phosphatidylinositol, phosphatidylcholine, two methylated phosphatidylethanolamines, and an unidentified phospholipid. The major fatty acids are $C_{15:0}$ iso (30.1%), $C_{16:0}$ iso (16.5%), 9-methyl $C_{16:0}$ (11.7%), and $C_{17:0}$ anteiso (6.7%).

Source: the type strain was isolated from a hypersaline environment (a salt lake in China).

DNA G+C content (mol%): 72.9 (HPLC).

Type strain: YIM 90002, CCRC 16284, CCTCC 99003, DSM 44593.

Sequence accession no. (16S rRNA gene): AF178988.

2. **Streptomonospora alba** Li, Xu, Zhang, Tang, Cui, Mao, Xu, Schumann, Stackebrandt and Jiang 2003b, 1424[VP]

al′ba. L. fem. adj. *alba* white, referring to the color of colonies on most media.

Aerial and substrate mycelia are well developed, but not fragmented on most media. White aerial mycelium forms short chains of spores at maturity that are straight to flexuous; spores are oval- to cylindrical-shaped (0.4–0.7 × 0.8–1.6 µm) with wrinkled surfaces and are nonmotile. Single, round to oval spores are borne on substrate mycelium. Color of the substrate mycelium is white (ISP 4, ISP 5, Czapek's agar), gray-white (ISP 3), moderate orange-yellow (ISP 2), deep orange-yellow (potato agar), or brilliant orange-yellow (nutrient agar). Growth occurs in 5–25% (w/v) NaCl (optimum 10–15%), at 28°C, and at pH 7.0. Diffusible pigments are not produced.

Except for glucose, carbon source utilization is not easy to determine because of negative reactions caused by extremely poor growth in basal media. Positive for catalase and nitrate reduction, but negative for oxidase, starch hydrolysis, milk peptonization, H_2S production, hydrolysis of cellulose, and melanin production.

The diagnostic diamino acid of peptidoglycan is *meso*-DAP, with galactose and arabinose as cell-wall sugars. The predominant menaquinones are MK-10(H_8) (50.6%) and MK-10(H_6) (15.8%). Major phospholipids are phosphatidylglycerol, phosphatidylethanolamine, phosphatidylinositol, diphosphatidylglycerol, methylphosphatidylethanolamine, phosphatidylserine, phosphatidylcholine, and an unidentified phospholipid. The major fatty acids are $C_{17:0}$ anteiso (26.0%), $C_{16:0}$ iso (25.1%), $C_{16:0}$ (8.6%), and $C_{15:0}$ anteiso (8.0%).

Source: the type strain was isolated from soil in a hypersaline habitat in Xinjiang Province, western China.

DNA G+C content (mol%): 74.4 (HPLC).

Type strain: YIM 90003, CCTCC AA 001013, DSM 44588, JCM 12680.

Sequence accession no. (16S rRNA gene): AF462347.

3. **Streptomonospora amylolytica** Cai, Tang, Chen, Li, Zhang and Li 2009, 2474[VP]

a.my.lo.ly′ti.ca. Gr. n. *amulon* starch; N.L. adj. *lyticus -a -um* (from Gr. adj. *lutikos -ê -on*) able to loosen, able to dissolve; N.L. fem. adj. *amylolytica* producing lysis of starch.

Aerial and substrate mycelia are not fragmented. White to yellow-white aerial mycelia form spore chains at maturity, with nonmotile, oval to cylindrical-shaped spores (0.3–0.4 × 0.4–0.6 µm). Single, oval spores are borne on sporophores of substrate mycelium. Substrate mycelia are yellow-gray on inorganic salt/starch agar, yeast extract/malt extract agar, and oatmeal agar, pale yellow on potato extract agar, and ivory olive brown on nutrient agar medium. Grows well on oatmeal agar and nutrient agar media supplemented with 10% (w/v) NaCl, but no growth occurs on Czapek's agar or glycerol/asparagine agar supplemented with 10% (w/v) NaCl. Diffusible pigments are not produced on any of the media tested. Growth occurs in 5–20% (w/v) NaCl (optimum 10%), at 20–45°C (optimum 37°C), and at pH 5.0–9.0 (optimum pH 7.0).

Arabinose, cellobiose, glucose, rhamnose, and sorbitol are used as sole carbon and energy sources; arginine, hydroxyproline, lysine, proline, threonine, and tryptophan are used as sole nitrogen, carbon, and energy sources. Positive for catalase, hydrolysis of starch, H_2S production, milk coagulation, and peptonization, but negative for oxidase, gelatin liquefaction, hydrolysis of cellulose and urea, melanin production, and nitrate reduction.

The diagnostic amino acid of the cell-wall peptidoglycan is *meso*-DAP and galactose is the major diagnostic sugar in whole-cell hydrolysates. The predominant menaquinones are MK-10(H_8) (35.8%), MK-10(H_6) (16.4%), and MK-9(H_8) (15.3%). The polar lipid profile consists of diphosphatidylglycerol, phosphatidylcholine, phosphatidylglycerol, phosphatidylinositol, and phosphatidylinositol mannosides. The major fatty acids are $C_{17:0}$ anteiso (42.4%) and $C_{18:0}$ (11.2%).

Source: the type strain was isolated from saline soil collected from a salt lake in Xinjiang Province, north-west China.

DNA G+C content (mol%): 71.2 (HPLC).

Type strain: YIM 91353, CCTCC AA 208048, DSM 45171.
Sequence accession no. (16S rRNA gene): EU442551.

4. **Streptomonospora flavalba** Cai, Tang, Chen, Li, Zhang and Li 2009, 2474[VP]

fla.val′ba. L. adj. *flavus -a -um* yellow; L. adj. *albus -a -um* white; N.L. fem. adj. *flavalba*, yellowish-white, referring to the color of the aerial mycelium.

Aerial and substrate mycelia are not fragmented. Yellow-white aerial mycelia form spore chains at maturity, with nonmotile, oval to cylindrical-shaped spores (0.5–0.6 × 0.7–0.9 µm). Single, oval spores are borne on sporophores of substrate mycelium. Substrate mycelia are: pale-yellow on inorganic salt/starch agar, oatmeal agar, yeast extract/malt extract agar, glycerol/asparagine agar, and nutrient agar; yellow-white on Czapek's agar; and ivory olive brown on potato extract agar. Growth occurs in 5–25% (w/v) NaCl (optimum 10%), at 20–45°C (optimum 37°C), and at pH 5.0–9.0 (optimum pH 7.0). Grows well on yeast extract/malt extract agar and nutrient agar media supplemented with 10% (w/v) NaCl, but poor growth occurs on Czapek's agar and glycerol/asparagine agar supplemented with 10% (w/v) NaCl. A yellow diffusible pigment is produced on potato extract agar (pH 7.0) supplemented with 10% (w/v) NaCl.

Arabinose, glucose, maltose, and rhamnose are used as sole carbon sources; alanine, arginine, glycine, histidine, phenylalanine, proline, serine, threonine, tryptophan, and tyrosine are used as sole nitrogen, carbon, and energy sources. Positive for catalase, hydrolysis of starch, melanin production, milk coagulation, and peptonization; negative for gelatin liquefaction, hydrolysis of cellulose and urea, H_2S production, nitrate reduction, and oxidase.

The diagnostic amino acid of the cell-wall peptidoglycan is *meso*-DAP and galactose is the major diagnostic sugar in whole-cell hydrolysates. The predominant menaquinones are MK-10(H_8) (41.5%), MK-9(H_8) (27.7%), and MK-10(H_6) (12.6%). The polar lipids consist of diphosphatidylglycerol, phosphatidylcholine, phosphatidylglycerol, phosphatidylinositol, and phosphatidylinositol mannosides. The major fatty acids are $C_{17:0}$ anteiso (28.0%) and $C_{16:0}$ iso (24.6%).

Source: the type strain was isolated from saline soil collected from a salt lake in Xinjiang Province, north-west China.

DNA G+C content (mol%): 72.5 (HPLC).

Type strain: YIM 91394, CCTCC AA 208047, DSM 45155.
Sequence accession no. (16S rRNA gene): EU442553.

5. **Streptomonospora halophila** Cai, Zhi, Tang, Zhang, Xu and Li 2008, 1559[VP]

ha.lo′phi.la. Gr. n. *hals halos* salt; N.L. adj. *philus -a -um* (from Gr. adj. *philos -ê -on*) friend, loving; N.L. fem. adj. *halophila* salt-loving, referring to the ability to grow at high NaCl concentrations.

Aerial and substrate mycelia are well developed, but not fragmented. Aerial mycelium forms straight to flexuous spore chains at maturity; spores are oval to cylindrical (0.4–0.5 × 0.8–1.0 µm) and nonmotile. Single, oval to round spores (0.8–1.0 µm diameter) are borne on sporophores of substrate mycelium. Colors of the substrate mycelium are: pale yellow on Czapek's agar, nutrient agar, potato agar, modified ISP 5 medium, and oatmeal agar; pale yellow-white on inorganic salts/starch agar; and yellowish brown on yeast extract/malt extract medium. Diffusible pigments are not produced. Ranges of NaCl concentration (w/v), pH, and temperature for growth are 5–20% (optimum 10%), pH 6.0–9.0 (optimum pH 7.0), and 20–45°C (optimum 37°C), respectively.

Inositol, maltose, and rhamnose are used as sole carbon sources; alanine, histidine, phenylalanine, proline, and threonine are used as sole nitrogen, carbon, and energy sources. Positive for milk peptonization, catalase, and hydrolysis of cellulose, but negative for oxidase, melanin production, amylase, nitrate reduction, and production of H_2S.

The diagnostic amino acid is *meso*-DAP; galactose is the main cell-wall sugar. The predominant menaquinones are MK-10(H_8) (43.2%), MK-10(H_6) (17.7%), and MK-11(H_8) (10.4%). Phospholipids are diphosphatidylglycerol, phosphatidylglycerol, phosphatidylcholine, phosphatidylinositol mannosides, phosphatidylinositol, and an unidentified phospholipid. The major fatty acids are $C_{16:0}$ iso (39.8%), $C_{17:0}$ anteiso (12.5%), 10-methyl $C_{17:0}$ (10.2%), and 10-methyl $C_{18:0}$ (11.1%).

Source: the type strain was isolated from a hypersaline soil in Xinjiang Province, north-west China.

DNA G+C content (mol%): 72.1 (HPLC).

Type strain: YIM 91355, DSM 45075, KCTC 19236.
Sequence accession no. (16S rRNA gene): EF423989.

Genus IV. **Thermobifida** Zhang, Wang and Ruan 1998, 417[VP] emend. Yang, Tang, Zhang, Zhi, Wang, Xu and Li 2008b, 1824

Martha E. Trujillo and Michael Goodfellow

Ther.mo.bi′fi.da. Gr. adj. *thermos* hot; L. adj. *bifidus* cleft; N.L. fem. n. *Thermobifida*, the heat (-loving) cleft (sporophores).

Aerobic, Gram-stain-positive, non-acid-fast, nonmotile, chemoorganotrophic actinomycetes that form an extensively branched, **non-fragmenting substrate mycelium**. **White aerial hyphae are produced. Single spores, oval to round (0.5–2.0 µm in diameter) are borne on dichotomously branched sporophores, resulting in spore clusters on the aerial mycelium and sometimes on the substrate mycelium. Spores have a smooth or scaly surface and may be heat sensitive. Cultures grow at 35–60°C and at pH 7–9. Many strains degrade cellulose**. **Cell walls contains *meso*-diaminopimelic acid**. Glucose, galactose, and xylose are found in whole-organism hydrolysates. **The major menaquinones are MK-10(H_6) and MK-10(H_8) and the predominant fatty acids are 14-methylpentadecanoic and 14-methylhexadecanoic acids**, but mycolic acids are not formed. The polar lipid pattern is

complex and includes diphosphatidylglycerol, phosphatidylcholine, phosphatidylglycerol, phosphatidylinositol, phosphatidylmethylethanolamine, and an unknown phospholipid; the presence of phosphatidylethanolamine is variable. The phylogenetic position of the genus, as determined by 16S rRNA gene sequence analysis, is in the family *Nocardiopsaceae*.

DNA G+C content (mol%): 66–72 (HPLC, T_m).

Type species: **Thermobifida alba** (Locci, Baldacci and Petrolini 1967) Zhang, Wang and Ruan 1998, 418[VP].

Further descriptive information

Phylogeny. The genus *Thermobifida* belongs to the family *Nocardiopsaceae* together with the genera *Haloactinospora* (Tang et al., 2008), *Nocardiopsis*, and *Streptomonospora* (Cui et al., 2001; Yang et al., 2008b). Based on 16S rRNA gene sequences, the four *Thermobifida* species form a homogeneous cluster supported by a high bootstrap value (see Figure 389 in the treatment of the family *Nocardiopsaceae*). *Thermobifida alba* (previously *Thermomonospora alba*), *Thermobifida cellulosilytica*, *Thermobifida fusca* (previously *Thermomonospora fusca*), and *Thermobifida halotolerans* share 16S rRNA gene sequence similarities between 97.5 and 98.2%. The type strains of the four species show DNA–DNA reassociation values between 33 and 50%.

Cell morphology. *Thermobifida* strains form highly branched, non-fragmenting substrate mycelia and produce white aerial mycelium on nutrient agar. Sporulation occurs by septation of sheathed, branched aerial hyphae and this leads to the formation of single spores (Crawford and Gonda, 1977). The latter, which are oval to round (0.5–2.0 µm in diameter), are borne at the tips of repeatedly branched sporophores, and form spore clusters on the aerial mycelium and sometimes on the substrate mycelium (Figure 396). Spore surfaces are smooth in all species, except for *Thermobifida cellulosilytica* where they have a scale-like appearance resembling pine-cones (Figure 397). Heat-sensitive spores have been reported for *Thermobifida alba*, *Thermobifida cellulosilytica*, and *Thermobifida fusca*.

Nutrition and growth conditions. Members of the genus *Thermobifida* are aerobic and chemo-organotrophic. The four type strains grow well on yeast extract-malt extract agar (ISP medium 2) but poorly on oatmeal, inorganic salts-starch and glycerol-asparagine agars (ISP media 3, 4 and 5; Shirling and Gottlieb, 1966); they also grow well on nutrient and potato agar plates. *Thermobifida alba* and *Thermobifida halotolerans* grow between 20 and 50°C, whereas *Thermobifida cellulosilytica* and *Thermobifida fusca* grow between 28 and 60°C. All of the strains grow between pH 6 and 9. Most thermobifidae grow in the presence of 3% NaCl, but *Thermobifida halotolerans* can grow in media containing 10% (w/v) NaCl (Yang et al., 2008b).

Cell-wall composition. *Thermobifida* strains contain *meso*-diaminopimelic acid (*meso*-A_2pm) as the wall diamino acid, but lack diagnostic sugars (Kroppenstedt and Evtushenko, 2006; Yang et al., 2008b), that is, they have a wall chemotype III *sensu* Lechevalier and Lechevalier (1970b). They contain MK-10(H_6) and MK-10(H_8) as predominant menaquinones and additional components, such as MK-10(H_{10}), MK-11(H_6), and MK-11(H_8), which distinguish between the four species (Kroppenstedt and Goodfellow, 1992; Kukolya et al., 2002; Yang et al., 2008b). Differences are also found between the fatty acid profiles of the four species although, in all cases, the major components are 14-methylpentadecanoic acid ($C_{16:0}$ iso) and 14-methylhexadecanoic acid ($C_{17:0}$ anteiso) (Yang et al., 2008b). The cellular polar lipid patterns include diphosphatidylglycerol, phosphatidylcholine, phosphatidylglycerol,

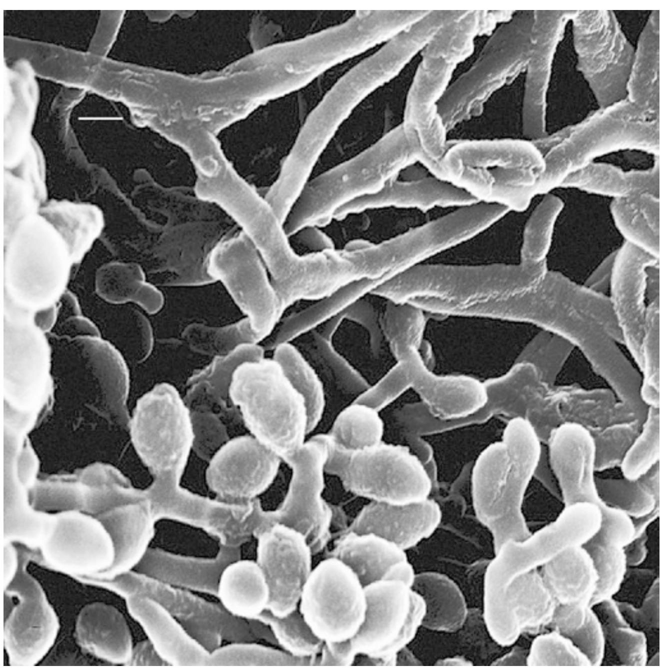

FIGURE 396. Spore formation in *Thermobifida fusca* strain TM51. Bar = 2 µm. (Reproduced with permission from Kukolya et al., 2001. Acta Biol. Hung. *52*: 211–221.)

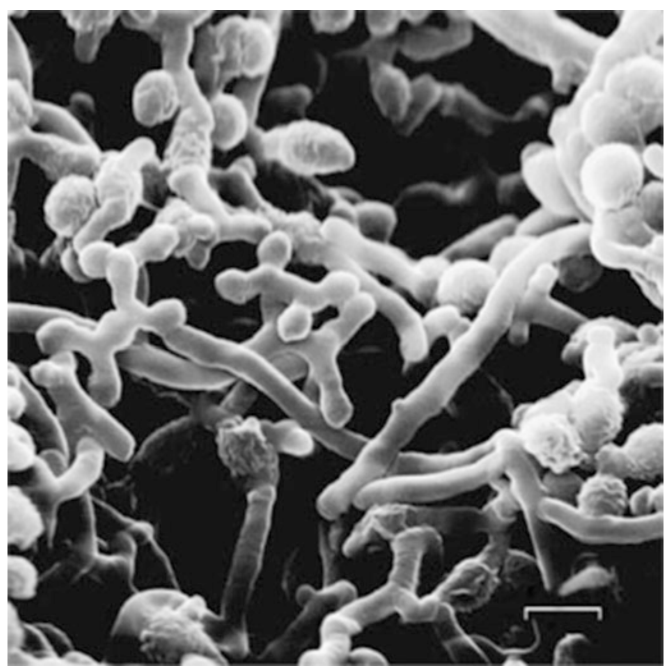

FIGURE 397. Spores of strain *Thermobifida cellulosilytica* TB100[T] formed on aerial hyphae, at the tips of branched sporophores. Bar = 1 µm. (Reproduced with permission from Kukolya et al., 2002. Int. J. Syst. Evol. Microbiol. *52*: 1193–1199.)

phosphatidylinositol, phosphatidylmethylethanolamine, and an unknown phospholipid; *Thermobifida cellulosilytica*, *Thermobifida fusca*, and *Thermobifida halotolerans* also contain phosphatidylethanolamine (Kroppenstedt and Evtushenko, 2006; Yang et al., 2008b). The cell wall of *Thermobifida fusca* contains two teichoic acids, namely, unsubstituted 1,3-poly(glycerol phosphate) and β-glucosylated 1,3-poly(glycerol phosphate) (Potekhina et al., 2003), and a polyglycerophosphate lipoteichoic acid (Rahman et al., 2009).

Metabolism. *Thermobifida* strains are aerobic, catalase-positive, chemo-organotrophic actinomycetes that have an oxidative metabolism. They metabolize a broad range of organic substrates and use diverse sugars as sole carbon sources (Crawford and McCoy, 1972; Kroppenstedt and Evtushenko, 2006; Kukolya et al., 2002; McCarthy and Cross, 1984a; Yang et al., 2008b). Preliminary data suggest that they can cleave a range of 7-amino-4-methylcoumarin and 4-methylumbelliferone-conjugated fluorogenic compounds (Trujillo and Goodfellow, 2003). However, thermobifidae are best known as a source of multiple thermostable enzymes, notably extracellular cellulases (Ball and McCarthy, 1988; Lao et al., 1991; McCarthy, 1987; McGrath and Wilson, 2006; Wilson, 2004) and hemicellulases (McCarthy et al., 1985, 1988).

Thermobifida fusca cellulases have been studied extensively because of their thermostability, broad pH range, and high activity (Beadle et al., 1999; Bellamy, 1973; Irwin et al., 2000; Spiridonov and Wilson, 1998; Wilson, 2004). This organism also has the capacity to degrade plant cell-wall polymers with the exception of lignin. *Thermobifida fusca* YX, a primary degrader of plant cell walls in heated organic matter, grows at 50°C in minimal medium containing cellulose, mannan, starch, or xylan as sole carbon sources and is used as a model system for establishing the complex interactions of glucanases involved in biomass hydrolysis (Warren, 1996).

Extensive studies on *Thermobifida fusca* have led to the identification, purification, and characterization of six extracellular cellulases (Irwin et al., 1993, 1994, 2000, 2003; Wilson et al., 1988; Zhang et al., 1995). In addition, an intracellular β-1,3-glucosidase that degrades cellulose to glucose (Spiridonov and Wilson, 2001), a mannanase (Beki et al., 2003; Hilge et al., 1998, 2001), an extracellular xyloglucanase (Irwin et al., 2003), and the three xylanases (Kim et al., 2004) have been detected, as has the protein CelR, which regulates the induction of cellulases and related enzymes (Wilson, 2004). The structures for three catalytic domains, Cel5Acd, Cel6Acd (Spezio et al., 1993), and Cel9A68 (Sakon et al., 1997), have been elucidated, and the three dimensional structure of *Thermobifida fusca* Cel6A catalytic domain has been determined by X-ray crystallography (Spezio et al., 1993). Components of the *Thermobifida fusca* xylan-degrading system have been purified and characterized (Bachmann and McCarthy, 1989, 1991; Ball and McCarthy, 1989; McCarthy et al., 1985).

The six extracellular enzymes act synergistically to degrade cellulose to cellobiose, traces of cellotriose, and other sugars (Irwin et al., 1993). The released cellobiose is transported into cells where it is converted to glucose by intracellular β-glucosidase (Ball and McCarthy, 1988; Ozaki and Yamada, 1991). Cellulase synthesis in *Thermobifida fusca* is regulated in two ways, induction by cellulose and cellobiose, and carbon source repression (Lin and Wilson, 1987, 1988; Spiridonov and Wilson, 1998). Induction increases cellulase levels about 12-fold and carbon source repression about 9-fold. Other major extracellular proteins induced by cellobiose include a xylanase and two unknown proteins (Chen and Wilson, 2007). *Thermobifida fusca* strains are also very active against arabinoxylan (McCarthy et al., 1985) and can degrade lignocellulosic pulps (Crawford, 1974).

Secreted cellulases are a source of single cell protein (Crawford, 1988; Wood, 1985), while the breakdown of biomass into fermentable sugars has potential for the industrial production of ethanol (Lynd et al., 2002, 2005; Mielenz, 2001; Rubin, 2008). Thermobifidae and other monosporic actinomycetes have been evaluated as a source of single cell protein (Bellamy, 1974, 1977; Crawford, 1988) and ethanol (Ball and McCarthy, 1988; Hägerdahl et al. 1979, 1980; Lee and Humphrey, 1979). Indeed, *Thermobifida fusca* has been used to convert high cellulose, low lignin pulp mill waste into single cell protein (Crawford et al., 1973) that was used as a feed supplement in the diet of chickens (Harkin et al., 1974). The thermostable mannanase produced by this organism has a temperature optimum of 80°C; it hydrolyzes the *O*-glucosidic bonds of mannan and has potential use in pulp and paper production (Hilge et al., 1998; Hilge et al., 2001).

Thermophilic actinomycetes have been used in processes designed to achieve the saccharification of purified cellulose (Bachmann and McCarthy, 1991; Wood, 1985). The cellulases of *Thermobifida fusca* YX, for instance, saccharify finely ground, acid-swollen Avicel, a purified chemically pretreated cellulose (Ferchak, 1980; Su and Paulavicius, 1975). In subsequent studies, Ferchak and Pye (1983) established a process for the saccharification of cellulose using the *Thermobifida fusca* strain and a cellulose/cellobiose mixture; 15–20% glucose syrups were generated from acid-swollen Avicel after 7 d at 50°C. It can be anticipated that the commercial potential of such processes will be improved by the use of cellulase-overproducing *Thermobifida* strains (Meyer and Humphrey, 1982). A composting process for aquatic plants has been developed that involves the use of *Thermobifida fusca* and *Ureibacillus thermosphaericus*, a Gram-stain-negative organism that is unable to ferment sugars (Okuda et al., 2008).

The genome of *Thermobifida fusca* YX has been sequenced (Lykidis et al., 2007), a development that will be of further interest in the metabolism and biotechnological exploitation of this and related organisms. Lykidis and colleagues have shown that the *Thermobifida fusca* genome encodes 45 hydrolytic enzymes, all of the enzymes needed for the glycolytic degradation of monosaccharides and for the biosynthesis of fatty acids, purines and pyrimidines, and enzymes for the *de novo* biosynthesis of all amino acids, apart from arginine. Additionally, glucose utilization is effected through the Entner–Doudoroff pathway. The organism possesses two protein secretion systems, the *sec* general secretion system and the twin-arginine translocation system. Indeed, several of the secreted cellulases have sequence signatures, which indicate that their secretion may be mediated through the twin-arginine translocation system. Extensive transport systems have been detected for import of carbohydrates coupled to transcriptional regulators that control the expression of the transporters and glycosylhydrolases.

Few characterized natural products have been isolated from either archaea or thermophilic bacteria (Donadio et al., 2007).

It is interesting, therefore, that *Thermobifida fusca* YX contains a siderophore biosynthetic gene cluster and has genes that encode a siderophore transport system (Lykidis et al., 2007). This organism also contains a family of structurally related novel nonribosomal peptide siderophores, the fuscachelins, which are products of an orphan gene cluster (Dimise et al., 2008). Fuscachelin A is the first secondary metabolite isolated from *Thermobifida fusca* and the natural product gene cluster is one of only a few detected from a thermophilic micro-organism.

Thermobifida strains produce medically important compounds, as exemplified by the production of topostatin, a novel inhibitor of topoisomerases 1 and 11 (Suzuki et al., 1998a, 1998b). Isoaurastatin, which inhibits topoisomerase 1, has been characterized and shown to be 6,4-dehydroxyisoaurone (Suzuki et al., 2001). A novel non-heme, iron-containing, extracellular peroxidase has been isolated from *Thermobifida fusca* BD25 and its catalytic mechanism has been characterized (Rob et al., 1995, 1996, 1997a, 1997b).

Genetics. In general, genetic studies on *Thermobifida fusca* have been designed to gain an insight into the cellular mechanisms that control the expression and secretion of plant cell wall-degrading enzymes (Wilson, 2004). Genes encoding endoglucanases, exoglucanases, mannosidases, and xylanases have been cloned, sequenced, and expressed in one or more heterologous hosts, namely *Bacillus subtilis* (Ghangas and Wilson, 1987), *Escherichia coli* (Chen and Wilson, 2007; Ghangas et al., 1989; Ghangas and Wilson, 1987, 1988; Irwin et al., 1994, 2000, 2003; Jung et al., 1993; Moser et al., 2008; Spiridonov and Wilson, 2000, 2001), *Streptomyces lividans* (Beki et al., 2003; Ghangas et al., 1989; Ghangas and Wilson, 1988; Irwin et al., 1998, 2000; Zhang et al., 2000; Zhang and Wilson, 1997), and *Pichia pastoris* (Cheng et al., 2005), as a first step towards constructing vehicles for the production of high levels of thermostable enzymes. The xylanase productivity of the *Pichia pastoris* transformant (pPICxXYL) was nearly 70-fold higher than that of the *Thermobifida fusca* strain when cultured in a 5-l fermenter.

Genes encoding two different endo-β-1,4-xylanases have been cloned and expressed in a *Thermobifida alba* strain (Blanco et al., 1997). One of them, *xylA*, was sequenced, subcloned, and overexpressed in *Streptomyces lividans*, and found to encode a protein of 482 amino acids with a deduced molecular mass of 48,456 Da. This protein bound strongly to cellulose and degraded xylans from different origins, producing xylobiose and traces of xylose. There is evidence that chaplin genes are present in *Thermobifida fusca* (Elliot et al., 2003); chaplin amyloids on the surfaces of actinomycetes mediate attachment to hydrophobic surfaces and are responsible for making the surfaces of hyphae and spores hydrophobic (Gebbink et al., 2005).

Thermobifida fusca strains from self-heated organic matter have been found to degrade several diverse biodegradable polyesters, including an aliphatic-aromatic co-polyester (Kleeberg et al., 1998). The gene encoding the *Thermobifida fusca* hydrolase responsible for degrading the co-polyester has been cloned and expressed in *Bacillus megaterium* (Yang et al., 2007b) and *Escherichia coli* (Dresler et al., 2006). In addition, a polyhydroxyalkanoate gene in a *Thermobifida* isolate has been cloned and expressed as a recombinant enzyme (PhaZ-Th) in *Pichia pastoris* (Phithakrotchanakoon et al., 2009). This enzyme is active against *p*-nitrophenyl alkanoates; surface plasmon resonance analysis indicated that PhaZ-Th catalyzed the degradation of poly[(R)-3-hydroxybutyrate] (PHB) films. Surface deterioration of PHB films was observed following exposure to PhaZ-Th by atomic force microscopy.

The genome sequence of *Thermobifida fusca* YX, which consists of a circular chromosome (3.6 Mb), provides the means for elucidating the cellular mechanisms that underpin the metabolism of the organism, including those that control the expression and secretion of cellulases, pectinases, and xylanases (Lykidis et al., 2007). The organism appears to use the TAT secretion system for the export of these enzymes to the extracellular space and produces multiple transcription factors that regulate the expression of glycosylhydrolases and oligo/polysaccharide transport systems. To date, 68% of the 3117 predicted coding sequences have been assigned a function, with 106 genes (3.3%) appearing to be unique to *Thermobifida fusca*. Comparative genome approaches indicate that *Thermobifida fusca* and "*Streptomyces coelicolor*" share a number of developmental genes (Chater and Chandra, 2006; Li et al., 2007; Traag and van Wezel, 2008). *Thermobifida fusca* is one of the few actinobacterial species that does not contain a 5 amino acid insert in glutamyl-tRNA synthase (Gao and Gupta, 2005), an enzyme that plays an essential role in protein synthesis (Woese et al., 2000).

Antibiotic sensitivity. Most *Thermobifida* strains are sensitive to a broad range of antibacterial antibiotics (Kukolya et al., 2002; McCarthy and Cross, 1984a; Trujillo and Goodfellow, 2003) though comparative taxonomic studies on representatives of *Thermobifida* species with validly published names are needed. The antibiotic sensitivity profiles of individual *Thermobifida* species, where known, are provided in the species descriptions.

Pathogenicity. There is no definitive evidence that *Thermobifida* strains, like some other thermophilic actinomycetes, can cause allergic respiratory disorders, though the cause of mushroom workers' disease, a form of allergic alveolitis, is still not settled (Lacey, 1988) It has been claimed that *Thermobifida* aleuriospores may act as allergens (Gusek et al., 1991). In addition, there is evidence that suggests that *Thermobifida alba* and *Thermobifida fusca* may contribute to the occurrence of mushroom workers' lung disease (Van den Bogart et al., 1993).

Ecology. *Thermobifida* strains form an integral part of the authochthonous microflora of overheated plant materials, such as composts, manure heaps, stored hay, and organic household waste (Lacey, 1973; Steger et al., 2007). They are particularly abundant in mushroom composts (Fergus, 1964; Lacey, 1974, 1978; McCarthy and Cross, 1984a, 1984b; McCarthy and Broda, 1984; Song et al., 2001) and in composting anaerobic sludge containing high concentrations of fibers and lipids (Nakasaki et al., 2009). The thermostable, extracellular, cellulolytic (Ball and McCarthy, 1988; McCarthy, 1987), and/or hemicellulolytic (McCarthy et al., 1985, 1988) enzymes produced by *Thermobifida alba* and *Thermobifida fusca* allow them to become dominant in overheated plant materials. *Thermobifida fusca* may be involved in the formation of humic substances in soil (Trigo and Ball, 1994).

Biofilm formation may play a major role in the developmental cycle of *Thermobifida fusca* (Alonso et al., 2008). These workers found that cellulose was specifically colonized by aleuriospores, which germinated, grew, degraded cellulose, and finally differentiated into biofilms encased in a carbohydrate-containing

exopolymeric matrix. Cellulose degradation and expression of *celE*, which encodes endoglucanase E5, was similar for *Thermobifida fusca* biofilms and mycelial pellets. *Thermobifida fusca* cells were also found to grow as biofilms attached to both nutritive and non-nutritive surfaces. Alonso and colleagues speculated that biofilm formation might enable *Thermobifida fusca* to adhere to substrates and obtain nutrients, thereby gaining a selective advantage over competitors in composts. They concluded that *Thermobifida fusca* might prove to be an effective model for filamentous bacterial biofilm research.

Enrichment and isolation procedures

Biocomposted paper (Kempf, 1995), mushroom solid waste compost (Resz et al., 1977; Stutzenberger, 1971), mushroom compost (Fergus, 1964), and self-heated grass cuttings (Kutzner, 2000) are rich sources of *Thermobifida* strains. Isolation of strains from such materials can be achieved by suspending 1 g air-dried sample and 10 g sterile plastic beads in 100 ml Ringer's solution followed by shaking at 180 r.p.m. for 30 min to release spores and mycelial fragments from compost particles. Next, 0.1 ml aliquots of a 10-fold dilution series are plated onto modified starch-casein-nitrate agar plates (Kroppenstedt and Evtushenko, 2006); this medium should be predried to reduce swarming of aerobic, endospore-forming bacilli. Sodium propionate (4 g/l) may be added to the medium to reduce the number of unwanted fast-growing bacteria (Lacey, 1974), although this practice may lead to a reduction in the counts of actinomycetes which form aerial mycelia. *Thermobifida* strains can usually be detected after 3–5 d incubation at 50°C.

Thermobifida strains can be isolated using nonselective agar media, but recovery is poor due to the rapid competing growth of thermophilic aerobic endospore-forming bacilli and thermoactinomycetes. The most effective isolation methods are those based on the use of a sedimentation chamber and Andersen air sampler (Lacey and Dutkiewicz, 1976; McCarthy and Broda, 1984). Dried samples are agitated within the sedimentation chamber to create an aerosol of particles which, after 1–2 h of sedimentation, still contains many actinomycete spores, but comparatively few bacteria. *Thermobifida* strains are isolated from this spore suspension using an Andersen sampler loaded with half-strength tryptone soy agar plates supplemented with cycloheximide (50 µg/ml) to prevent growth of fungi. The inoculated plates are incubated at 50°C for 5 d. The isolation of *Thermobifida* strains may be further improved by adjusting the isolation medium to pH 11.0 (Cross, 1981). Cellulolytic isolates can be identified by incorporating cellulose powder or ball-milled straw into the agar (McCarthy and Broda, 1984; Stutzenberger et al., 1970).

Thermobifida halotolerans was isolated from a salt mine sample after incubation for 3 weeks at 45°C on inorganic salts-starch agar (ISP medium 4; Shirling and Gottlieb, 1966) supplemented with 10% NaCl (Yang et al., 2008b). Putatively novel *Thermobifida* strains have been isolated from mushroom compost on R8 agar plates incubated at 50°C for 3–5 d (Song et al., 2001).

Maintenance procedures

Working cultures of *Thermobifida* can be maintained as sporulating cultures at 4°C for up to 4 months on Czapek–Dox-yeast extract-Casamino acids agar at pH 8.0. Drying out of agar can be prevented by tightly sealing tubes with silicone stoppers. Medium-term preservation for up to 4 years can be achieved by preparing spore suspensions or homogenized mycelia in 20% (v/v) glycerol and storing at −20°C (Wellington and Williams, 1978). For long-term preservation, lyophilization of spores and mycelia suspended in 10% skim milk is a convenient method.

Differentiation of the genus *Thermobifida* from other genera

Thermobifida strains can be readily differentiated from members of the three other genera classified in the family *Nocardiopsaceae* by using a combination of chemotaxonomic, growth, morphological, and physiological properties (Table 297, see also

TABLE 297. Differential characteristics of the type strains of members of the genus *Thermobifida*

Character	*T. alba* DSM 43795^T	*T. cellulosilytica* DSM 44535^T	*T. fusca* DSM 43792^T	*T. halotolerans* YIM 90462^T
Aerial mycelium on glucose-yeast extract-malt extract agar	−	+	−	−
Hydrolysis of:				
Arbutin	+	+	+	−
Gelatin	+	+	+	−
Coagulation of milk	+	+	+	−
Peptonization of milk	+	+	+	−
Nitrate reduction	−	−	−	+
Growth on sole carbon sources:				
D-Arabinose	+	−	−	+
D-Fructose	+	−	+	−
Glycerol	+	−	+	+
Lactose	+	−	+	−
Maltose	+	+	+	−
D-Mannose	+	+	+	−
L-Rhamnose	+	+	−	−
D-Ribose	−	+	−	−
L-Sorbose	+	−	−	−
Xylitol	+	−	−	−
Temperature growth range (°C)	20–50	28–55	28–55	20–50
Presence of phosphatidylethanolamine (diagnostic polar lipid)	−	+	+	+
DNA G+C content (mol%)	71.2	70.4	66.2	69.0

treatment of the family *Nocardiopsaceae*) and by 16S rRNA gene sequence studies (see Figure 389 in the treatment of the family *Nocardiopsaceae*). They can be easily separated from their nearest phylogenetic neighbor, the genus *Nocardiopsis*, as members of this taxon form long chains of spores on aerial hyphae and produce a substrate mycelium that tends to fragment. It has also been shown that the genus-specific primers used for PCR identification of *Nocardiopsis* strains do not produce amplification with DNA from *Thermobifida alba* (Salazar et al., 2002).

Taxonomic comments

The taxonomic history of actinomycetes classified in the genus *Thermobifida* as *Thermobifida alba* and *Thermobifida fusca* is complex, confused, and controversial, as is apparent from early reviews on the classification of monosporic actinomycetes (Cross and Lacey, 1970; Cross and Goodfellow, 1973). Indeed, actinomycetes that formed single spores were assigned somewhat unpredictably to a number of taxa, notably to the genera *Actinobifida* Krasil'nikov and Agre 1964, *Micromonospora* Ørskov 1926, *Saccharomonospora* Nonomura and Ohara 1971, *Thermoactinomyces* Tsiklinsky 1899, and *Thermomonospora* Henssen 1957. All of these taxa were essentially morphological in concept. The dangers of over-relying on morphological features was illustrated by Cross and Lacey (1970), who found an almost continuous range of morphological variation between two extremes represented by *Thermomonospora viridis* and *Thermoactinomyces vulgaris*. Between these two extremes, they observed a range of morphological types that overlapped generic boundaries. They also noted that spore arrangement on the aerial mycelium could be influenced by medium composition and incubation temperature.

The rejection of the genus *Actinobifida* as an invalid name (Cross and Goodfellow, 1973; Skerman et al., 1980) and improvements in the classification of the genus *Thermoactinomyces* (Cross and Goodfellow, 1973) helped to define *Thermomonospora* at the genus level. Subsequently, the application of chemotaxonomic and numerical taxonomic methods showed the genus *Thermomonospora* to be a markedly heterogeneous taxon (Kroppenstedt and Evtushenko, 2006; Kroppenstedt and Goodfellow, 1992; McCarthy and Cross, 1984a, 1984b). The detailed taxonomic changes that ensued are presented elsewhere in this volume (see treatment of the family *Thermomonosporaceae*).

Thermomonospora alba DSM 4395^T, the first viable member of the genus *Thermomonospora*, was isolated and described by Locci et al. (1967), who classified it as *Actinobifida alba*. The organism was transferred to the genus *Thermomonospora* as it formed heat-sensitive spores on substrate and aerial hyphae (Cross and Goodfellow, 1973). Subsequently, *Thermomonospora mesouviformis* Nonomura and Ohara 1974 was proposed as a synonym of *Thermomonospora alba* McCarthy and Cross 1984a.

Thermomonospora fusca was proposed on the basis of distinctive morphological properties observed in contaminated preparations (Henssen, 1957). The organism was omitted from the Approved Lists of Bacterial Names (Skerman et al., 1980), even though a *Thermomonospora fusca* strain had been isolated and described in detail (Crawford, 1975; Crawford and Gonda, 1977). *Thermomonospora fusca* was later proposed as a validly published name (McCarthy and Cross, 1984a) and added to the Approved Lists of Bacterial Names (Moore et al., 1985).

Kroppenstedt and Goodfellow (1992) assigned *Thermomonospora* strains to three distinct groups, based on the discontinuous distribution of chemical markers, and raised the prospect that *Thermomonospora alba* (including *Thermomonospora mesouviformis*) and *Thermomonospora fusca* merited generic status. Zhang et al. (1998) found that the type strains of *Thermomonospora alba* and *Thermomonospora fusca* were more closely related to *Nocardiopsis* strains than to other *Thermomonospora* species on the basis of 16S rRNA gene sequence data. These results, together with associated chemotaxonomic and phenotypic data, led them to propose the transfer of *Thermomonospora alba* (Locci et al. 1967) Cross and Goodfellow 1973 and *Thermomonospora fusca* (*ex* Henssen 1957) McCarthy and Cross 1984a to the new genus *Thermobifida* as *Thermobifida alba* Locci et al., 1967 comb. nov. and *Thermobifida fusca* McCarthy and Cross 1984a comb. nov. *Thermobifida alba* was designated the type species of the genus as it had been validated before *Thermobifida fusca* (by Henssen, 1957); *Thermobifida cellulosilytica* Kukolya et al. 2002 and *Thermobifida halotolerans* Yang et al. 2008b were subsequently added to the genus.

Differentiation of the species of the genus *Thermobifida*

Thermobifida species can be distinguished by using a combination of chemotaxonomic, morphological, nutritional, and physiological properties (Table 297). The two most studied species, *Thermobifida alba* and *Thermobifida fusca*, have different temperature and pH requirements (Kroppenstedt and Evtushenko, 2006; McCarthy and Cross, 1984a), give different patterns of enzymes in cell-free extracts (Greiner-Mai et al., 1987), and show quantitative differences in fatty acid profiles (Kroppenstedt and Evtushenko, 2006; Yang et al., 2008b). Additionally, unlike *Thermobifida alba*, most *Thermobifida fusca* strains form spores on the substrate mycelium (McCarthy and Cross, 1984a).

List of species of the genus *Thermobifida*

1. **Thermobifida alba** (Locci, Baldacci and Petrolini 1967) Zhang, Wang and Ruan 1998, 418^{VP} [*Actinobifida alba* Locci, Baldacci and Petrolini 1967, 88; heterotypic synonym: *Thermomonospora mesouviformis* Nonomura and Ohara 1974; basonym: *Thermomonospora alba* (Locci, Baldacci and Petrolini 1967) Cross and Goodfellow 1973.]

   al'ba. L. fem. adj. *alba* white.

   Forms an extensively branched substrate mycelium and abundant white aerial hyphae on agar media. Large numbers of single spores are borne on dichotomously branched or unbranched sporophores on aerial hyphae in dense clusters. Does not produce melanin pigments on tyrosine agar. The optimal temperature for growth and sporulation is 40–45°C; growth occurs at 30°C and at pH 10 and 11.

Positive for esculin, catalase, β-galactosidase, β-glucosidase, and phosphatase, but negative for oxidase. Degrades agar, carboxymethylcellulose, casein, DNA, keratin, pectin, RNA, tributyrin, Tween 60, and xylan, but not guanine, hypoxanthine, testosterone, tyrosine, or xanthine. Variable for elastin. Acid is produced from glucose. Glycogen and mannitol are used as sole carbon sources for energy and growth, but not adonitol, L-arabinose, cellobiose, glucosamine, glycerol, *myo*-inositol, mannitol, melezitose, melibiose, raffinose, or salicin (all at 1%, w/v). Similarly, lactic acid is used as a sole carbon source, but not L-arginine, benzamide, L-cystine, ethanolamine, L-glutamine, glycine, L-histidine, L-lysine, L-phenylalanine, sodium hippurate, sodium propionate, sodium pyruvate, or L-valine.

Grows in the presence of [% (w/v) except where indicated]: bile (0.02), phenol (0.01), phenyl ethanol (0.1, v/v), and thallous acetate (0.001), but is inhibited by brilliant green (0.0005), crystal violet (0.00002), phenol (0.1), phenyl ethanol (0.2%, v/v), potassium tellurite (0.0005), sodium azide (0.01), sodium chloride (3), tetrazolium chloride (0.002), and thallous acetate (0.005). Sensitive to (μg per filter paper disc): ampicillin (5), carbenicillin (25), cephaloridine (2), cephalotin (20), cephamandole (20), kanamycin (25), lincomycin (25), neomycin (3), novobiocin (40), oleandomycin (20), rifampin (1), streptomycin (16), tetracycline (20), and tobramycin (5).

Additional phenotypic properties are shown in Table 297. The major fatty acids are $C_{16:0}$ (24.4%) and $C_{17:0}$ anteiso (29.1%).

Source: the type strain was isolated from garden soil.

DNA G+C content (mol%): 71.2 (HPLC).

Type strain: CIP 105591, DSM 43795, IMET 9528, IPV, JCM 3077, KCCA-0077, NBRC 16095.

Sequence accession no. (16S rRNA gene): AF002260.

Additional comments: cleaves a broad range of fluorogenic substrates based on 7-amino-4-methylcoumarin and 4-methylumbelliferone (Trujillo and Goodfellow, 2003). McCarthy (1989) pointed out that the distinction between *Thermobifida alba* and *Thermobifida mesouviformis* had been maintained only because the latter was regarded as a mesophilic species unable to grow at 50–55°C (Cross, 1981; Kurup, 1979; Nonomura and Ohara, 1974). He also noted that the type strain of *Thermobifida mesouviformis* can show poor growth at 50°C; hence, these species should be considered to be synonymous. However, Kroppenstedt and Evtushenko (2006) believe that DNA–DNA hybridization studies are needed to determine the relationship between the two species. *Thermobifida alba* strains do not form a well-delineated species and may be low-temperature variants of *Thermobifida fusca* (see McCarthy and Cross, 1984a; McCarthy, 1989).

2. **Thermobifida cellulosilytica** corrig. Kukolya, Nagy, Láday, Tóth, Oravecz, Márialigeti and Hornok 2002, 1198[VP]

cel.lu.lo.si.ly′ti.ca. N.L. n. *cellulosum* cellulose; N.L. fem. adj. *lytica* (from Gr. fem. adj. *lutikê*) able to loosen, able to dissolve; N.L. fem. adj. *cellulosilytica* cellulose-dissolving.

Forms an extensively branched substrate mycelium and abundant white aerial hyphae on agar media. Sporophores branch repeatedly to form dense spore clusters on the aerial mycelium. Spores have a scale-like surface, are oval (1–1.3 × 0.6 μm) and heat-sensitive (90°C, 10 min). Thermotolerant strains grow between 28 and 45°C and thermophilic ones between 40 and 65°C.

Degrades crystalline cellulose and elastin. Does not produce acid from glucose. Cellobiose, galactose, melezitose, and xylose are used as carbon sources, but not *myo*-inositol, dulcitol, or sorbitol. Cultures grown on lignocellulose substrate produce endoglucanase, cellobiohydrolase, and endoxylanase enzymes and are capable of solubilizing lignocarbohydrates.

Resistant to (μg/ml) erythromycin (20), but sensitive to amoxycillin (10), ampicillin (10), apramycin (2.5), chloramphenicol (10), kanamycin (2.5), novobiocin (10), penicillin (10) streptomycin (2.5), tetracycline (5), and thistrepton (1.25).

Additional phenotypic characteristics are shown in Table 297. The major fatty acids are $C_{16:0}$ (28.7%), $C_{17:0}$ (26.1%), $C_{17:0}$ anteiso (18.3%), and $C_{18:0}$ (12.4%). The cellular polar lipid pattern includes phosphatidylethanolamine, a glycolipid, and unidentified phospholipids. The predominant menaquinones are MK-10(H_6) and MK-10(H_8); the minor components vary, apart from the presence of MK-11(H_6).

Source: isolated from overheated manure compost in Gödöllö, Hungary.

DNA G+C content (mol%): 70–71 (HPLC).

Type strain: DSM 44535, JCM 11767, NCAIM B0, TB100.

Sequence accession no. (16S rRNA gene): AJ298058.

Additional comments: the original spelling *Thermobifida cellulolytica* (*sic*) was corrected to *Thermobifida cellulosilytica* in Validation List no. 37 by the List Editor, IJSEM (2002b).

3. **Thermobifida fusca** (McCarthy and Cross 1984a) Zhang, Wang and Ruan 1998, 418[VP] (McCarthy and Cross 1984a, 22) [Basonym: *Thermomonospora fusca* (*ex* Henssen 1957) McCarthy and Cross 1984a].

fus′ca. L. fem. adj. *fusca* dark, tawny.

Forms an extensively branched substrate mycelium and abundant white aerial hyphae on agar media. Large numbers of single spores in dense clusters are usually produced on the aerial mycelium. The colony reserve pigment on CYC agar is pale yellow. Does not form melanin pigments on tyrosine agar. Grows at pH 10–11 and at 35–53°C, but not at 30°C; some strains grow at 60°C.

Positive for esculin, catalase, β-galactosidase, β-glucosidase, and phosphatase, but negative for oxidase. Degrades agar, carboxymethylcellulose, casein, elastin, DNA, keratin, pectin, RNA, tributyrin, Tween 60, and xylan, but not chitin, guanine, hypoxanthine, testosterone, tyrosine, or xanthine. Produces acid from glucose. Cellobiose, galactose, glycogen, and melezitose are used as carbon sources for energy and growth, but not adonitol, L-arabinose, glucosamine, *myo*-inositol, mannitol, melibiose, raffinose, or xylose (all at 1%, w/v). Similarly, sodium propionate and sodium pyruvate are used as sole carbon sources, but not L-arginine, benzamide, ethanolamine, L-cystine, L-glutamine, glycine, lactic acid, L-histidine, L-lysine, L-phenylalanine, sodium hippurate, or L-valine.

Grows in the presence of (%, w/v) bile (0.05), crystal violet (0.00002), phenol (0.01), phenyl ethanol (0.2%, v/v), potassium tellurite (0.02), sodium azide (0.01), tetrazolium chloride (0.02), and thallous acetate (0.01), but is inhibited

by phenol (0.1), phenyl ethanol (0.3%, v/v), sodium azide (0.02), tetrazolium chloride (0.005), and thallous acetate (0.005). Sensitive to (µg per filter paper disc): ampicillin (5), carbenicillin (25), cephaloridine (2), cephalothin (20), cephamandole (20), kanamycin (25), lincomycin (10), neomycin (3), novobiocin (10), oleandomycin (20), rifampin (1), streptomycin (4), tetracycline (20), ticarcillin (70), and tobramycin (0.05).

Additional phenotypic characteristics are shown in Table 297. Major fatty acids are $C_{16:0}$ (38.5%) and $C_{17:0}$ anteiso (15.2%).

Source: isolated from composts and overheated fodders. The type strain was isolated from soil.

DNA G+C content (mol%): 66.2 (HPLC).

Type strain: ATCC 27730, CIP 105594, DSM 43792, IFO (now NBRC) 14071, JCM 3263, NCIB 11185, NRRL B-8184, 190 Th, VKM Ac-.

Sequence accession no. (16S rRNA gene): AF002264.

Additional comments: cleaves a broad range of fluorogenic substrates based on 7-amino-4-methylcoumarin and 4-methylumbelliferone (Trujillo and Goodfellow, 2003). *Thermobifida fusca* was not originally isolated in pure culture (Henssen, 1957) and the unavailability of cultures led to the species being cited as a *nomen dubium* in the 8th edition of the *Manual*. Furthermore, a wholly inaccurate description of *Thermobifida fusca* Henssen, given by Waksman (1961a), led to the use of this name for strains that probably belong to *Thermomonospora chromogena* (Cross and Lacey, 1970; Fergus, 1964; Locci et al., 1967; Nonomura and Ohara, 1969). This conclusion was removed by the subsequent description of what is now the type strain of *Thermobifida fusca* Crawford 1975, although this did not result in validation of the name. Proposals that *Thermobifida fusca* Crawford 1975 be regarded as a synonym of *Thermobifida alba* Cross 1981 or vice versa (Kurup, 1979) are not in agreement with numerical phenetic data (McCarthy and Cross, 1984a), as the respective type strains were recovered in separate clusters.

4. **Thermobifida halotolerans** Yang, Tang, Zhang, Zhi, Wang, Xu and Li 2008b, 1824[VP]

ha.lo.to′le.rans. Gr. n. *hals halos* salt; L. part. adj. *tolerans* tolerating; N.L. part. adj. *halotolerans* referring to the ability to tolerate high salt concentrations.

Forms an extensively branched substrate mycelium and grows well on nutrient, potato, and glucose-yeast extract-malt extract agars, but does not grow on Czapek's agar. Single, smooth-ridged spores are formed at the tips of dichotomously branched sporophores borne on aerial hyphae. Does not form melanin pigments on tyrosine agar. Grows well at 45°C and at pH 7.0 and 8.0. Cellobiose, galactose, glucose, glycerol, raffinose, and xylose are used as sole carbon sources for energy and growth, but not *myo*-inositol or sorbitol.

Additional characteristics are found in Table 297. Diagnostic sugars are glucose, galactose, and xylose. Major fatty acids are $C_{16:0}$ iso (51.1%) and $C_{17:0}$ anteiso (11.7%). The cellular polar lipid pattern contains diphosphatidylglycerol, phosphatidylcholine, phosphatidylethanolamine, phosphatidylglycerol, phosphatidylinositol, phosphatidylmethylethanolamine, and an unknown phospholipid. The predominant menaquinones are MK-9(H_6) and MK-10(H_8).

Source: the type strain was isolated from a saline soil sample collected in Yunnan Province, south-west China.

DNA G+C content (mol%): 69.0 (HPLC).

Type strain: DSM 44931, KCTC 3, YIM 90462.

Sequence accession no. (16S rRNA gene): EU250489.

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Family III. **Thermomonosporaceae** Rainey, Ward-Rainey and Stackebrandt 1997, 486[VP] emend. Zhang, Wang and Ruan 2001, 381 emend. Zhi, Li and Stackebrandt 2009, 600

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Ther.mo.mo.no.spo.ra.ce′a.e. N.L. fem. n. *Thermomonospora* type genus of family; L. suff. *-aceae* ending to denote family; N.L. fem. pl. n. *Thermomonosporaceae*, the *Thermomonospora* family.

Aerobic, Gram-stain-positive, non-acid–alcohol-fast, chemoorganotrophic actinomycete. A stable, branched substrate mycelium carries an aerial mycelium. **Aerial hyphae differentiate into single or short chains of arthrospores or into spore vesicles which release motile spores**. **Wall peptidoglycan contains *meso*-diaminopimelic acid**, ***N*-acetylmuramic acid and is of the A1γ type**. **Major phospholipids are phosphatidylglycerol, phosphatidylinositol, and phosphatidylinositol mannosides**. **Contains hydrogenated menaquinones with nine isoprene units**. Rich in mixtures of straight chain and branched fatty acids. Lacks mycolic acids. **Whole-organism hydrolysates usually contain madurose**. The pattern of 16S rRNA signatures consists of nucleotides at positions 440:497 (C–G), 501:544 (C–G), 502:543 (G–C), 831:855 (G–G), 843 (U), 844 (A), and 1355:1367 (A–U) (Zhi et al., 2009). A few species are pathogenic for animals, including man. Widely distributed in composts, manures, overheated fodder, and soil.

DNA G+C content (mol%): 66–73.

Type genus: **Thermomonospora** Henssen 1957, 398[AL] emend. Zhang, Wang and Ruan 1998b, 418.

Further descriptive information

The family *Thermomonosporaceae* (Rainey et al. 1997) emend. Zhang et al. 2001 encompasses the genera *Actinocorallia* (Iinuma et al. 1994) emend. Zhang et al. 2001, *Actinomadura* (Lechevalier and Lechevalier 1970a) emend. Kroppenstedt et al. 1990, *Spirillospora* Couch 1963, and *Thermomonospora* (Henssen 1957) emend. Zhang et al. 2001. Members of these taxa form a distinct phyletic line in the 16S rRNA gene tree (Zhang et al., 1998b, 2001) and can thereby be distinguished from the families *Nocardiopsaceae* and *Streptosporangiaceae* (Goodfellow and Quintana, 2006; Goodfellow et al., 1990; Gyobu, 2001; Kudo, 2001), the other two families that comprise the suborder *Streptosporangineae* Rainey et al. 1997. *Actinomadura*, *Spirillospora*, and *Thermomonospora* strains are phylogenetically intermixed as can be seen in the *Thermomonosporaceae* 16S rRNA gene tree (Figure 398).

Differentiation of the genera of the family *Thermomonosporaceae*

Members of the genera *Actinocorallia*, *Actinomadura*, *Spirillospora*, and *Thermomonospora* can be distinguished from one another using a combination of chemotaxonomic and morphological markers (Table 298).

Taxonomic comments

Cross and Goodfellow (1973) proposed the family *Thermomonosporaceae* for a morphologically diverse group of sporoactinomycetes classified in the genera *Actinomadura*, *Microbispora*, *Microtetraspora*, *Saccharomonospora*, and *Thermomonospora*. They recognized that the taxon was an artificial construct, but also noted that the constituent genera had more in common than the assortment of taxa assigned to the family *Nocardiaceae*, a well known dumping ground for aerobic actinomycetes (Lechevalier, 1976). The new family encompassed mesophilic and thermophilic organisms that produced heat sensitive, motile or nonmotile spores borne singly, in pairs, or as short chains on aerial hyphae and substrate mycelia, or formed spore vesicles as in the genus *Spirillospora*. All but one of the genera had a wall chemotype III, that is, they contained *meso*-diaminopimelic acid (*meso*-A_2pm) but no other characteristic amino acids or sugars (Lechevalier and Lechevalier, 1970, 1970c). The exception, the genus *Saccharomonospora* had a wall chemotype IV, which meant that it had *meso*-A_2pm as the diamino acid of the wall peptidoglycan and a polysaccharide fraction rich in arabinose and galactose. Other wall chemotype III sporoactinomycetes subsequently thought to be related to the genus *Thermomonospora* included the genera *Actinobifida*, *Actinosynnema*, *Nocardiopsis*, *Saccharothrix*, and *Streptoalloteichus* (Goodfellow and Cross, 1984; McCarthy, 1989).

The family *Thermomonosporaceae sensu* Cross and Goodfellow 1973 was not included in the Approved Lists of Bacterial Names (Skerman et al., 1980) nor was it recognized in *Bergey's Manual of Systematic Bacteriology*, Volume 4 (Williams, 1989). Indeed, the difficulties of formally recognizing actinomycete taxa above the genus level led Goodfellow and Cross (1984) to propose "aggregate groups", the composition of which was based on the application of modern taxonomic methods, notably on chemotaxonomic and numerical taxonomic studies. One of the nine "aggregate groups", the maduromycetes, contained the genera *Actinomadura*, *Excellospora*, *Microbispora*, *Microtetraspora*, *Planobispora*, *Planomonospora*, *Spirillospora*, and *Streptosporangium*. The maduromycetes were not conceived as a natural group but were designed to provide a temporary home for organisms that had chemotaxonomic and morphological features in common (Goodfellow, 1989). In the 1st edition of *Bergey's Manual of Systematic Bacteriology*, McCarthy (1989) recognized another artificial group, "*Thermomonospora* and Related Taxa", which encompassed the genera *Actinosynnema*, *Nocardiopsis*, *Streptoalloteichus*, and *Thermomonospora*. These organisms all had a wall chemotype II but were markedly morphologically diverse.

The introduction and application of chemotaxonomic and molecular systematic procedures provided reliable data for establishing relationships between actinomycete taxa above the genus level (Embley and Stackebrandt, 1994; Stackebrandt and Woese, 1981; Stackebrandt et al., 1997; Zhang et al., 2001). It was apparent from such studies that morphological features previously weighted for the assignment of actinomycete genera to families had little predictive value (Goodfellow, 1989; Stackebrandt and Schleifer, 1984). These developments underlined the artificial nature of previously delineated suprageneric groups (Goodfellow and Cross, 1984; Williams, 1989) and provided a baseline for the circumscription of actinomycete taxa above the genus level (Stackebrandt et al., 1997). In addition, the genus *Actinomadura* was seen to encompass two groups of organisms that had little in common (Fischer et al., 1983; Fowler et al., 1985; Goodfellow et al., 1988; Poschner et al., 1985). This division was formally recognized by Kroppenstedt et al. (1990) who proposed that the genus *Actinomadura* be retained for *Actinomadura madurae* and related species and that the *Actinoma-*

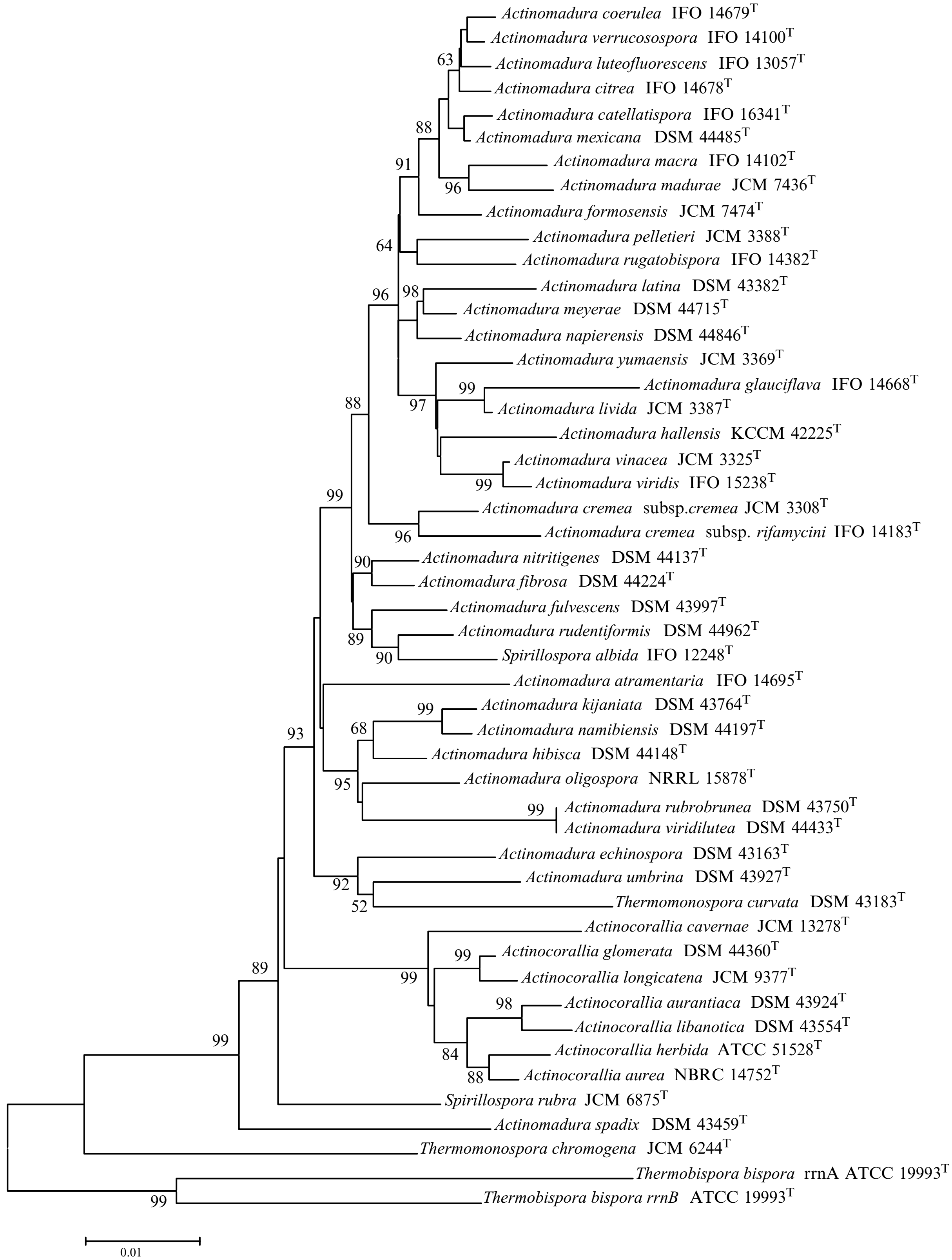

FIGURE 398. Neighbor-joining tree generated from 16S rRNA gene sequences showing relationships between taxa classified in the family *Thermomonosporaceae*. Evolutionary distances were calculated with the Jukes and Cantor method (Jukes and Cantor, 1969). Bootstrap values indicated at branching points are expressed as percentages of 1000 replications (only values greater than 40% are shown). Bar = 1 substitution per 100 nucleotides.

TABLE 298. Phenotypic characteristics separating genera classified in the family *Thermomonosporaceae*

Characteristics	*Thermomonospora*	*Actinocorallia*	*Actinomadura*	*Spirillospora*
Morphology:				
Single spores[a]	+	−	+	−
Chains of spores	+	+	+	−
Spore vesicles	−	−	−	+
Motile spores	−	−	−	+
Chemotaxonomy:				
Phospholipid type[b]	I	II	I(IV)[c]	I/II
Diagnostic sugar	None	Madurose	Madurose	Madurose
Sugar type[d]	C	B	B	B
DNA G+C content (mol%)	nd	66–73	66–73	71–73

[a]Single spores found in *Actinomadura formonsensis* and *Thermomonospora curvata*.

[b]Phospholipid types according to Lechevalier et al. (1977, 1981). Characteristic phospholipids: I, nitrogenous phospholipids absent (phosphatidylglycerol variable); II, only phosphatidylethanolamine; III, phospholipids containing glucosamine (with phosphatidylethanolamine variable) and IV, phospholipids containing glucosamine (with phosphatidylethanolamine variable).

[c]*Actinomadura kijaniata, Actinomadura namibiensis* and *Actinomadura napierensis* have a phospholipid type IV; the phospholipids of *Spirillospora albida* ATCC 14541 were found to be type II (Hasegawa et al., 1979).

[d]Whole-organism sugar patterns of actinomycetes containing *meso*-A_2pm: A, arabinose and galactose; B, madurose (3-*O*-methyl-D-galactose); C, no diagnostic sugars; D, arabinose and xylose (Lechevalier et al., 1970, 1971; Lechevalier and Lechevalier, 1970c).

dura pusilla group be transferred to the genus *Microtetraspora*. The *Actinomadura pusilla* group was subsequently given generic status as *Nonomuraea* Zhang et al. 1998, a proposition raised but never implemented by Goodfellow et al. (1988). Four members of the *Actinomadura madurae* group, *Actinomadura aurantiaca*, *Actinomadura glomerata*, *Actinomadura libanotica*, and *Actinomadura longicatena*, were subsequently transferred to the genus *Actinocorallia* by Zhang et al. (2001).

Other genera associated with the genus *Thermomonospora* were assigned to new families. The genera *Microbispora*, *Microtetraspora*, *Planobispora*, and *Streptosporangium* were classified in the family *Streptosporangiaceae* Goodfellow et al. 1990, the genera *Actinosynnema* and *Saccharothrix* in the family *Actinosynnemataceae* Labeda and Kroppenstedt 2000, and the genus *Saccharomonospora* in the family *Pseudonocardiaceae* Embley et al. 1988; the genus *Streptoalloteichus* is closely associated with the latter two families (Labeda and Kroppenstedt, 2000). The genus *Nocardiopsis* can be sharply separated from the genus *Thermomonospora* (Fowler et al., 1985; Kroppenstedt et al., 1990) and is now the type genus of the family *Nocardiopsaceae* which also includes the genera *Streptomonospora* Cui et al. 2001 and *Thermobifida* Zhang et al. 1998. The genus *Thermobifida* provided a home for three species previously classified in the genus *Thermomonospora*, namely, *Thermobifida alba*, *Thermobifida fusca*, and *Thermobifida mesouviformis*. The genus *Excellospora* Agre and Guzeva 1975 was included in the Approved Lists of Bacterial Names with *Excellospora viridilutea* as the type species (Skerman et al., 1980). Members of this taxon have been transferred to the genus *Actinomadura* (Kroppenstedt et al., 1990, 1991; Zhang et al., 2001) thereby underpinning the view that excellosporae should be seen as thermophilic actinomadurae (Meyer, 1989a). These developments leave the genera *Actinocorallia*, *Actinomadura*, *Spirillospora*, and *Thermomonospora* as members of the family *Thermomonosporaceae* Rainey et al. 1997 emend. Zhang et al. 2001 emend. Zhi et al. 2009.

Genus I. **Thermomonospora** Henssen 1957, 398[AL] emend. Zhang, Wang and Ruan 1998, 418[VP]

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Ther.mo.mo.no.spo′ra. Gr. n. *thermê* heat; Gr. adj. *monos* single, solitary; Gr. fem. n. spora seed; N.L. fem. n. *spora* a spore; N.L. fem. n. *Thermomonospora* the heat (-loving) single-spored (organism).

Aerobic, Gram-stain-positive, non-acid–alcohol-fast, chemoorganotrophic actinomycetes which form branched substrate/aerial mycelia. **Single spores with spiny surfaces are borne at the tips of short sporophores arising from the aerial mycelium**. Substrate mycelium may be yellow-orange to brown. White or light brown aerial mycelium is produced. **Temperature growth range 40–55°C**. **Cell wall contains *meso*-diaminopimelic acid but no diagnostic sugars**. Predominant menaquinones are MK-9(H_4), MK-9(H_6), and MK-9(H_8). Major phospholipids are diphosphatidylglycerol, phosphatidylglycerol, phosphatidylinositol, and phosphatidylinositol mannoside. **Rich in straight chain**, **iso- and anteiso- fatty acids, but lack mycolic acids. Isolated from soil, but is common in composts, manures, and overheated fodders**.

DNA G+C content (mol%): not determined for any members of the genus.

Type species: **Thermomonospora curvata** Henssen 1957, 401[VP].

Further descriptive information

Phylogeny. *Thermomonospora* species show a close phylogenetic relationship with members of the genus *Actinomadura*. Comparative 16S and 23S rRNA gene sequence analyses (Zhang et al., 1998, 2001) indicate that *Thermomonospora curvata*, the type species, *Actinomadura echinospora*, and *Actinomadura umbrina* form a moderately stable phyletic line which is separated from other *Actinomadura* species (see Figure 399). The phylogenetic position of *Thermomonospora chromogena* is not clear though it is evident that this organism is not closely related with other taxa in the family *Thermomonosporaceae* (Yap et al., 1999; Zhang et al., 1998). The uncertain phylogenetic relationship between *Thermomonospora chromogena* and related actinomycetes may be a reflection of its two distinct types of rRNA operons (Yap et al., 1999).

Cell morphology. The substrate and aerial mycelia of *Thermomonospora* strains consist of branching nonfragmenting hyphae. Single spores are borne on branched and unbranched sporophores on aerial mycelia. Spore clusters in *Thermomonospora chromogena* are formed by sequential sporulation on incurving hyphae. Spore arrangement on the aerial mycelium can be influenced by medium composition and incubation temperatures (Cross and Lacey, 1970). The spores are heat-sensitive and spore surfaces are spiny.

Nutrition and growth conditions. Members of the genus *Thermomonospora* are strictly aerobic, chemo-organotrophic micro-organisms and are moderately thermophilic as they grow well at 50°C. Some strains grow up to 60°C, but aerial mycelium production and sporulation are often poor at high temperatures. *Thermomonospora chromogena* shows little or no growth below 40°C with optimal growth and sporulation occurring on slightly alkaline nutrient media incubated under aerobic conditions. *Thermomonospora curvata* grows well and sporulates at pH 11.0. Most *Thermomonospora* strains can utilize fructose, glucose, mannose, and trehalose as sole carbon sources, but not dulcitol, inulin, raffinose, sorbitol, or sorbose. Enzymes produced by *Thermomonospora* strains include carboxymethylcellulase, catalase, β-galactosidase, and β-glucosidase; casein, gelatin, and keratin are degraded (McCarthy and Cross, 1984b; Trujillo and Goodfellow, 2003).

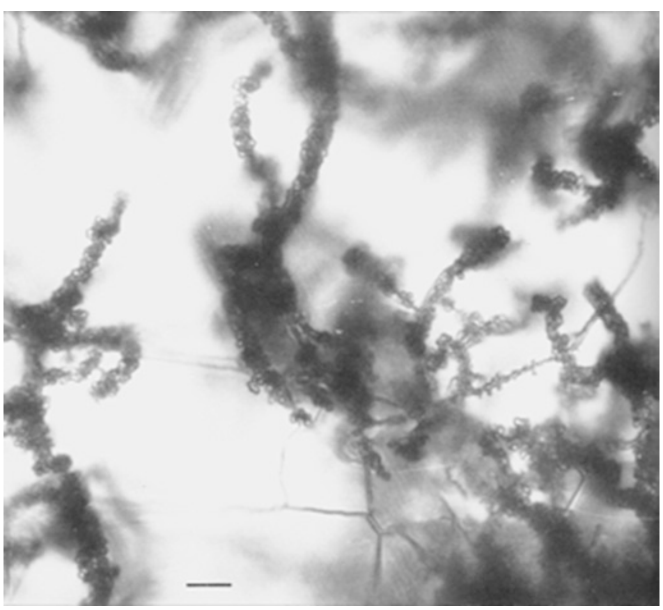

FIGURE 399. *Thermomonospora curvata.* Single spores borne laterally along aerial hyphae on branched and unbranched sporophores. Bar = 10 μm.

Genetics. Genetic studies on *Thermomonospora chromogena* ATCC 43196[T] (Yap et al., 1999) show that the genome of this strain contains six rRNA operons (*rrn*), four of which are complete and two incomplete. Comparative sequence analyses demonstrated that five of the operons had almost identical sequences whereas that of the *rrnB* operon displayed a sequence difference of 6–10%. These studies also revealed a close evolutionary relationship between the *rrnB* operon of *Thermomonospora chromogena* and the *rrnA* operon of *Thermobispora bispora*. Based on these data, Yap et al. (1999) raised the possibility that *Thermomonospora chromogena* acquired the *rrnB* operon from either *Thermobispora bispora* or from a related micro-organism via horizontal gene transfer. Plasmids have been isolated from *Thermomonospora chromogena* (McCarthy, 1989) while cellulolytic *Thermomonospora* strains can be infected by various bacteriophages (Lawrence et al., 1986).

Cell-wall composition. The cell-wall peptidoglycan of *Thermomonospora* strains contain *meso*-A_2pm, but no characteristic sugars (wall chemotype III *sensu* Lechevalier and Lechevalier, 1970b). Long-chain fatty-acid composition has been reported to be a mixture of straight-chain and branched-chain *iso*- and *anteiso*- components (Goodfellow and Cross, 1984), that is, a fatty acid pattern type 3a (Kroppenstedt, 1985). The cell wall envelope does not contain mycolic acids. The predominant menaquinones of *Thermomonospora curvata* have side chains with nine isoprene units that are hexa- or octahydrogenated whereas *Thermomonospora chromogena* contains major amounts of MK-9(H_4) (Collins et al., 1982). Thermomonosporae have a phospholipid type I pattern (Lechevalier et al., 1977, 1981), that is, one characterized by the presence of diphosphatidylglycerol, phosphatidylglycerol, phosphatidylinositol, and phosphatidylinositol mannoside.

Ecology. Thermophilic thermomonosporae are found in overheated substrates such as baggasse, composts, fodders, and manures. They are especially abundant in mushroom compost (Fergus, 1964; Lacey, 1974, 1977; McCarthy and Cross, 1981, 1984b). Thermomonosporae secrete thermostable extracellular enzymes which enable them to become established as dominant populations during high temperature composting of plant residues and other wastes (Bernier and Stutzenberger, 1988; Fergus, 1964; George et al., 2001; Stutzenberger, 1971). Furthermore, *Thermomonospora* strains contribute significantly to the degradation of cellulose and lignocellulose residues from agricultural and urban wastes (McCarthy, 1987). *Thermomonospora curvata* is active in the decomposition of municipal waste compost (Stutzenberger and Sterpu, 1971, 1978). The growth of thermophilic actinomycetes in high temperature environments leads to the release of spores than can cause allergic alveolitis. At present, there are no grounds for implicating the spores of *Thermomonospora* species in such respiratory disorders (Lacey, 1988) though the cause

of mushroom worker's disease, a form of allergic alveolitis, is still a matter for conjecture.

Isolation procedures

Thermomonospora strains can be isolated from composts and overheated vegetable materials by dilution plating on nonselective media, but recovery is poor owing to the rapid growth of *Bacillus* and *Thermoactinomyces* strains. The most effective isolation methods are those based on the use of a sedimentation chamber and an Andersen air sampler (Lacey and Dutkiewicz, 1976; McCarthy and Broda, 1984; McCarthy and Cross, 1981). Dried environmental samples are shaken within the sedimentation chamber to create an aerosol of propagules which after 1–2 h of sedimentation still contain many actinomycete spores but relatively few bacteria. Actinomycetes are isolated from such spore suspensions using an Andersen sampler loaded with 1/2 strength tryptone soy agar plates supplemented with cycloheximide (50 µg/ml) to prevent the growth of fungi. *Thermomonospora* colonies can usually be recognized after incubation for 3–5 d at 50°C (Kroppenstedt and Goodfellow, 2006; McCarthy, 1989). *Thermomonospora chromogena* is readily isolated on selective media supplemented with either kanamycin (25 µg/ml; McCarthy and Cross, 1981) or rifampin (5 µg/ml; Athalye et al., 1981). Mixtures of organic matter and soil inoculated in partially sealed polyethylene bags yield samples enriched in thermophilic actinomycetes, including thermomonosporae (McCarthy, 1989).

Maintenance procedures

Thermomonospora strains can be maintained as sporulating cultures on Czapek–Dox-yeast extract-Casamino acids agar at pH 8.0, stored at 4°C, and subcultured every 4 weeks. Medium-term preservation for up to four years can be achieved by preparing spore suspensions or homogenized mycelia in 10–20% (v/v) glycerol and storing at –25°C (Zippel and Neigenfind, 1988). Lyophilization of spore and mycelia suspended in 10% skim milk provides a convenient method for long-term storage.

Differentiation of the genus *Thermomonospora* from other genera

The major characteristics that distinguish members of the genus *Thermomonospora* from other genera classified in the family *Thermomonosporaceae* are presented in Table 299, Family *Thermomonosporaceae*. Members of the redefined genus *Thermomonospora* share chemical markers in common with *Actinomadura* strains, i.e. a wall chemotype III, phospholipid type I, menaquinone type 4B2, and fatty acid type 3a (Collins et al., 1982; Kroppenstedt, 1985; Lechevalier et al., 1981, 1977, Lechevalier and H.A. Lechevalier, 1970a). The integrity of the group containing *Thermomonospora chromogena* and *Thermomonospora curvata* is also supported by fatty acid and menaquinone data. Variations in quantitative fatty acid composition and in menaquinone profiles of *Thermomonospora chromogena* and *Thermomonospora curvata* compared with other members of family *Thermomonosporaceae* may be attributed to differences in their temperature requirements for growth (Kroppenstedt et al., 1990). The lack of madurose (sugar type C; Lechevalier and Lechevalier, 1970b) might be an effect of the elevated temperature for growth. As in members of thermophilic *Actinomadura* species (formerly *Excellospora* strains) this sugar is found only in trace amounts.

TABLE 299. Characteristics differentiating between the species of the genus *Thermomonospora*[a, b]

Characteristic	*T. curvata*	*T. chromogena*
Substrate mycelium color	Yellow/orange	Brown
Aerial mycelium color	White	Light brown
Spores on aerial mycelium:		
In clusters	–	+
Single on branched and unbranched sporophores	+	–
Biochemical test:		
Oxidase	–	+
Degradation of:		
Agar	+	–
Cellulose powder (MN300)	+	–
Elastin	–	+
Hypoxanthine	–	+
Starch	+	–
Tyrosine	–	+
Xanthine	–	+
Growth at:		
35°C	+	–
pH 11	+	–
Growth on sole carbon sources (1%, w/v):		
Galactose	–	+
Sucrose	+	–
Growth in the presence of (µg/ml):		
Kanamycin (25)	–	+

[a]Symbols: +, 90% or more strains positive; –, 10% or less strains positive.

[b]Data from: McCarthy (1989), McCarthy and Cross (1984a, 1984b), and Trujillo and Goodfellow (2003).

Taxonomic comments

The genus *Thermomonospora* was proposed by Henssen (1957) for thermophilic actinomycetes isolated from composted stable manure. Initially the taxon encompassed three species with members that produce single spores on aerial hyphae. All of these organisms formed colorless to pale yellow colonies and a white aerial mycelium, but were distinguished from one another according to aerial mycelium morphology and by the type of branching shown by substrate hyphae. *Thermomonospora curvata*, the only species isolated and maintained in pure culture, subsequently became the type species of the genus (Henssen and Schnepf, 1967). The description of the remaining two species, *Thermomonospora fusca* and *Thermomonospora lineata*, was based on morphological properties in contaminated preparations. Neither of these species were included in the Approved Lists of Bacterial Names (Skerman et al., 1980) despite the fact that *Thermomonospora fusca* had been isolated in pure culture and described in detail (Crawford, 1975; Crawford and Gonda, 1977). *Thermomonospora fusca* was subsequently validated (Moore et al., 1985) but was later transferred to the genus *Thermobifida* based on 16S rRNA gene sequencing data (Zhang et al., 1998).

A mesophilic monosporic actinomycete classified in the genus as *Thermomonospora mesophila* Nonomura and Ohara 1971a was later transferred to the genus *Microbispora* as *Microbispora mesophila* Miyadoh et al. 1990. A second mesophilic species, *Thermomonospora mesouviformis* Nonomura and Ohara 1974, was assigned to the genus *Thermobifida* as a synonym of *Thermobifida alba* Zhang et al. 1998. A third mesophilic species, *Thermomonospora formosensis*,

proposed by Hasegawa et al. (1986) was cited as a species *incertae sedis* in *Bergey's Manual of Systematic Bacteriology* (McCarthy, 1989). This species was subsequently transferred to the genus *Actinomadura* as *Actinomadura formosensis* Zhang et al. 1998.

Krasil'nikov and Agre (1964) proposed the genus *Actinobifida* for actinomycetes that formed single spores on dichotomously branched sporophores, but they failed to acknowledge that dichotomous branching had been observed previously in members of the genera *Thermomonospora* (Henssen, 1957) and *Micromonospora* (Jensen, 1930, 1932; Krasil'nikov, 1941). The following year these workers proposed a second species, *Actinobifida chromogena*, thereby implying that all actinomycetes showing dichotomous branching should be transferred to the genus *Actinobifida*. A third species, *Actinobifida alba*, was introduced by Locci et al. (1967). *Actinobifida dichotomica*, the type species of the genus was later transferred to the genus *Thermoactinomyces* as it produced endospores and *Actinobifida alba* to the genus *Thermomonospora* as it formed heat sensitive spores on substrate and aerial hyphae (Cross and Goodfellow, 1973).

A comprehensive numerical taxonomic study of the genus *Thermomonospora* and related organisms (McCarthy and Cross, 1984b, 1984a) confirmed the status of *Thermomonospora curvata* and provided strong evidence for the formal recognition of *Thermomonospora fusca*. In contrast, *Thermomonospora mesouviformis* was considered to be a synonym of *Thermomonospora alba*. These taxa, termed the "white *Thermomonospora* group" because of their white aerial mycelium, were sharply distinguished from organisms which included the type strain of *Actinobifida chromogena* (Krasil'nikov and Agre 1965; "*Thermomonospora falcata*" Henssen 1970, and similar actinomycetes isolated from mushroom compost (McCarthy and Cross, 1981). The "*chromogena*" strains with reddish-brown colonies and a light brown aerial mycelium had been provisionally assigned to the genus *Thermomonospora* Cross 1981 based on wall composition and morphological features. In the meantime, *Thermomonospora viridis* Küster and Locci 1963 had been transferred to the genus *Saccharomonospora* as *Saccharomonospora viridis* Nonomura and Ohara 1971a.

It is clear that the genus *Thermomonospora* has undergone many changes since it was proposed by Henssen (1957). The application of molecular systematic techniques has led to significant improvements in the taxonomy of this taxon, but it seems likely that in future further changes will be necessary. It also seems likely that as more data becomes available, *Thermomonospora chromogena* will be seen to merit generic status.

Differentiation of the species of the genus *Thermomonospora*

Thermomonospora curvata and *Thermomonospora chromogena* can be distinguished using a combination of phenotypic properties as shown in Table 299.

List of species of the genus *Thermomonospora*

1. **Thermomonospora curvata** Henssen 1957, 401$^{\mathrm{VP}}$

cur.va'ta. L. v. *curvo* to curve; L. part. fem. adj. *curvata* curved.

Colonies on agar media have a yellow to orange reverse color and bear an abundant white aerial mycelium. Single spores are borne laterally along aerial hyphae on branched and unbranched sporophores (Figure 399). Spores are not formed on the substrate mycelium. Grows in the range 30–53°C, but not at 60°C. Growth observed at pH 11.

Catalase, β-galactosidase and β-glucosidase are produced. Nitrate is reduced. Degrades carboxymethylcellulose, esculin, keratin, Tweens 20 and 80, and xylan, but not chitin, DNA, guanine, or pectin.

Fructose, maltose, mannose, and trehalose are used as sole carbon sources, but not L-arabinose, glycerol, *meso*-inositol, lactose, mannitol, melibiose, or L-rhamnose. Additional phenotypic characteristics are shown in Table 299.

DNA G+C content (mol%): not determined.

Type strain: ATCC 19995, CIP 105592, DSM 43183, HAMBI 1549, IFO (now NBRC) 15933, JCM 3096.

Sequence accession no. (16S rRNA gene): AF002262, D86945.

Sequence accession no. (23S rRNA gene): AF116236.

Sequence accession no. (16S–23S rRNA ITS): AF134112.

2. **Thermomonospora chromogena** (*ex* Krasil'nikov and Agre 1965) McCarthy and Cross, 1984a, 356$^{\mathrm{VP}}$ (Effective publication: McCarthy and Cross 1984b, 22.)

chro.mo.ge'na. Gr. n. *chroma* color; Gr. v. *gennaio* to produce; N.L. adj. *chromogenes* color producing.

Colonies on agar media are small, entire, raised, and dark reddish brown to light brown. Aerial mycelium white to light brownish, turning blue-gray on prolonged incubation. A dark brown soluble pigment is often produced. Spore clusters on the aerial mycelium are formed by sequential sporulation on incurving hyphae (Figure 400). Spores are not formed on the substrate mycelium. Grows in the range 40–53°C, but not at 60°C. Does not grow at pH 11.

β-Galactosidase and β-glucosidase are produced, but not catalase. Nitrate is reduced. Degrades arbutin, carboxym-

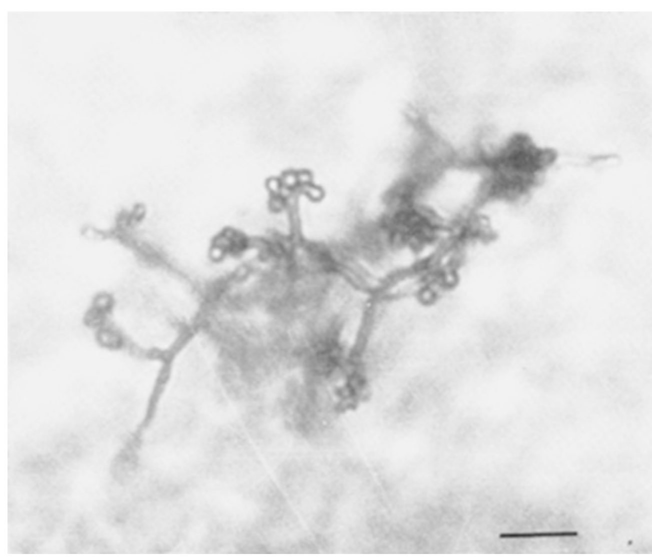

FIGURE 400. *Thermomonospora chromogena*. Single spores produced sequentially on incurving hyphae. Bar = 10 μm.

ethylcellulose, esculin, keratin, pectin, Tweens 20 and 80, tyrosine, and xylan but not chitin or DNA.

Glucose, mannose, and trehalose are used as sole carbon sources, but not L-arabinose, dulcitol, glycerol, inulin, melezitose, melibiose, sorbose, or xylose. Additional phenotypic characteristics are presented in Table 299.

DNA G+C content (mol%): not determined.

Type strain: strain Agre no. 577, ATCC 43196, DSM 43794, NBRC 16096, JCM 6244, NCIB (now NCIMB) 10212, NRRL B-16983.

Sequence accession no. (16S rRNA gene): AF116558.

Genus II. **Actinocorallia** Iinuma, Yokota, Hasegawa and Kanamuru 1994, 233^VP emend. Zhang, Kudo, Nakajima and Wang 2001, 381^VP

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Ac.ti.no.co.ral′li.a Gr. n. *actis actinos* a ray; L. n. *corallium* coral; N.L. fem. n. *Actinocorallia* (*sic*) intended to mean a microorganism (actinomycete) that forms sporophores resembling coral.

Aerobic, Gram-stain-positive, nonmotile actinomycete which forms branched non-fragmenting vegetative hyphae. **Spore chains are formed on the aerial mycelium**. Spores are cylindrical or rod-shaped. **Spore surface smooth or warty**. Substrate mycelium pale yellowish to brown. Mature aerial mycelium is pale yellow or pink. Chemo-organotrophic. Temperature growth range is 7–42°C. Melanin pigments are not produced. Does not grow in the presence of 5% (w/v) NaCl. Cell wall contains *meso*-2,6-diaminopimelic acid and *N*-acetylated muramic acid. **Major phospholipids are diphosphatidylglycerol, phosphatidylethanolamine, and phosphatidylinositol**. Predominant menaquinones are MK-9(H_4), MK-9(H_6), and MK-9(H_8). Hexadecanoic, 14-methylpentadecanoic, octadecanoic, and 19-methyloctadecanoic acids are the predominant fatty acids. Mycolic acids are absent.

DNA G+C content (mol%): 66.2–73.0 (T_m, HPLC).

Type species: **Actinocorallia herbida** Iinuma, Yokota, Hasegawa and Kanamuru 1994, 233^VP.

Further descriptive information

Phylogeny. The genus *Actinocorallia* encompasses seven species, *Actinocorallia aurantiaca*, *Actinocorallia aurea*, *Actinocorallia cavernae*, *Actinocorallia glomerata*, *Actinocorallia herbida*, *Actinocorallia libanotica*, and *Actinocorallia longicatena*, which form a distinct phyletic line in the *Thermomonosporaceae* 16S rRNA gene tree (Figure 398). Members of this taxon share 16S rRNA gene similarities within the range 97.3–99.4%. Representatives of the genus contain 16S rRNA signatures characteristic of the family *Thermomonosporaceae* (Stackebrandt et al., 1997). The type strains of *Actinocorallia glomerata* and *Actinocorallia longicatena*, the two most closely related strains in the 16S rRNA phyletic line, share DNA:DNA relatedness values of 36% and 39% in reciprocal crosses (Itoh et al., 1995). The 16S–23S internal transcribed spacer regions of representative strains share high nucleotide sequence similarities and form a well delineated taxon based on partial sequence analyses of 23S rRNA genes (Zhang et al., 2001).

Cell morphology. *Actinocorallia* strains form well developed, branched, nonfragmenting substrate hyphae, features they have in common with members of the genus *Actinomadura*. Aerial hyphae differentiate into long chains of spores after 10–14 d growth on a range of media, including glycerol-asparagine, oatmeal, tyrosine and yeast extract-malt extract agars. *Actinocorallia herbida* produces characteristic coralloid sporophores on glycerol-asparagine, humic acid-vitamins, oatmeal, and yeast extract-malt extract agars. These arise from the substrate mycelium and differentiate into long chains of non-motile spores (more than 30 spores per chain) at the tips; coremia have been observed, albeit rarely, on yeast extract-malt extract agar. The spores of *Actinocorallia aurantiaca* and *Actinocorallia libanotica* are connected by intersporal pads (Figure 401). *Actinocorallia glomerata* forms distinctive globular or amorphous spore masses (pseudosporangia-like structures), 1.5–5.0 µm in diameter, in which hyphae are coiled or intertwined (Figure 402). *Actinocorallia longicatena* is characterized by straight to flexuous chains bearing arthrospores (more than 20 spores) (Figure 403).

Nutrition and growth conditions. *Actinocorallia* strains are aerobic and chemo-organotrophic with an oxidative metabolism. They grow well on oatmeal agar and have no specific requirements for minerals or vitamins. Members of the genus

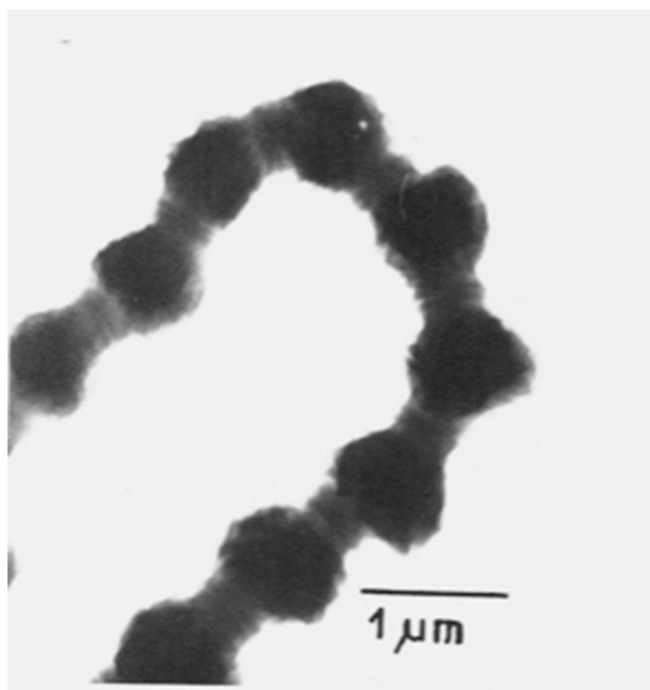

FIGURE 401. *Actinocorallia libanotica* strain IMET 9618. Spore chain with "intersporal pads". Electron micrograph (16,500×).

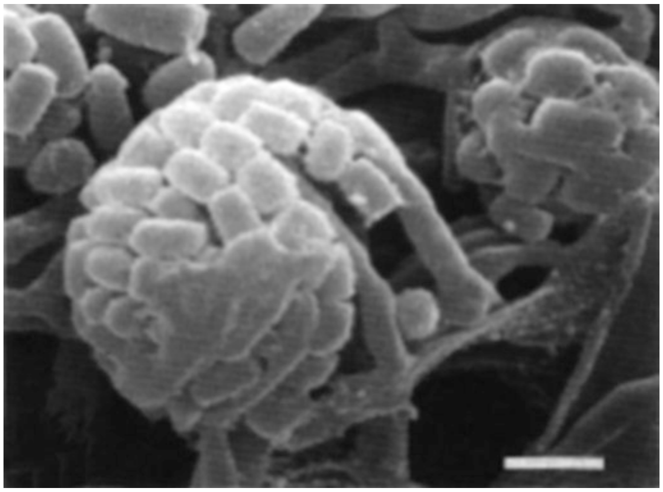

FIGURE 402. *Actinocorallia glomerata* strain I-226. Pseudosporangium-like structures. The micro-organism was incubated on oatmeal agar at 28°C for 2–3 weeks. Electron micrograph (bar = 1 µm). (Reproduced with permission from Itoh et al., 1995. Actinomycetologica *9*: 167.)

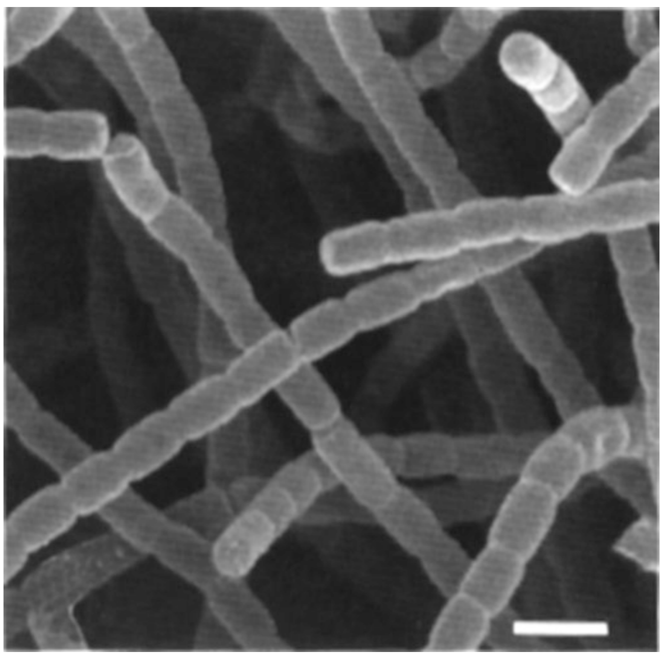

FIGURE 403. *Actinocorallia longicatena* strain I-497. Chains of arthrospores. The micro-organism was incubated on a tenth strength yeast extract-malt extract agar at 28°C for 2–3 weeks. (bar = 1 µm). (Reproduced with permission from Itoh et al., 1995. Actinomycetologica *9*: 167.)

are metabolically active (Table 300), but the descriptions of most species are based on relatively limited studies on single isolates. In contrast, *Actinocorallia aurantiaca* and *Actinocorallia libanotica* strains were included in extensive numerical taxonomic studies on *Actinomadura* and related taxa (Athalye et al., 1985; Trujillo and Goodfellow, 2003).

Cell-wall composition. *Actinocorallia* strains contain *meso*-diaminopimelic acid (*meso*-A_2pm) as the major wall diamino acid and, with the exception of *Actinocorallia cavernae*, contain madurose, that is, they have a wall chemotype III B *sensu* Lechevalier and Lechevalier (1970b). The diagnostic sugar madurose is found in other genera, including *Actinomadura*, *Dermatophilus*, *Microbispora*, *Planobispora*, *Planomonospora*, *Spirillospora*, and *Streptosporangium*. Whole-organism hydrolysates of *Actinocorallia glomerata* and *Actinocorallia longicatena* also contain galactose, glucose, mannose, and ribose (Itoh et al., 1995), and those of *Actinocorallia cavernae* contain glucose, mannose, and minor amounts of galactose (Lee, 2006).

The major phospholipids of *Actinocorallia* strains are diphosphatidylglycerol, phosphatidylethanolamine, and phosphatidylinositol, a profile which corresponds to phospholipid pattern type II *sensu* Lechevalier et al. (1981). The predominant menaquinones are MK-9(H_4) and MK-9(H_6) with smaller amounts of MK-9(H_8). *Actinocorallia aurantiaca* also contains a small proportion of MK-9(H_2) and *Actinocorallia libanotica* small amounts of MK-9(H_0) and MK-9(H_2) (Kroppenstedt et al., 1990). *Actinocorallia* strains contain complex mixtures of straight chain saturated, unsaturated, iso-branched, anteiso-branched and 10-methylated branched fatty acids, that is, a type 3a pattern *sensu* Kroppenstedt (1985); the predominant components are hexadecanoic, 14-methylpentadecanoic, octadecenoic, and 14-methyloctadecanoic acids.

Ecology. *Actinocorallia* strains are common in soil (Iinuma et al., 1994; Lavrova and Preobrazhenskaia, 1975; Lee, 2006; Meyer, 1979) where they probably play a role in the turnover of organic matter. They have also been isolated from a decayed leaf of a deciduous tree and from the root of a herbaceous plant (Itoh et al., 1995).

Isolation procedures

Actinocorallia strains can be isolated using methods described for *Actinomadura*. *Actinocorallia aurantiaca* was isolated from soil by Lavrova and Preobrazhenskaya (1975) using Gauze's medium 2 supplemented with rubromycin (0.5, 1.0, or 2.0 µg/ml), an antibiotic that inhibits the growth of common streptomycetes on isolation plates thereby allowing the development of "rare" actinomycetes. Iinuma et al. (1994) isolated *Actinocorallia herbida* by inoculating serial dilutions of soil onto colloidal chitin-vitamins agar and incubating the inoculated plates at 28°C for up to 3 weeks. *Actinocorallia glomerata* and *Actinocorallia longicatena* were isolated from plant material after washing with sterile water and drying it at 28°C for 7 d. The dried samples were blended in sterile water prior to incorporation into yeast extract agar (0.02%, w/v) supplemented with cycloheximide and nystatin (each at 50 µg/ml) and incubated at 25°C for up to 3 weeks (Itoh et al., 1995).

Maintenance procedures

Short-term storage can be achieved by serial transfer on modified Bennett's or yeast extract-malt extract agars, and long-term storage by lyophilization or storage in liquid nitrogen. For lyophilization, spores and mycelia are suspended in a suitable fluid such as 7.5% (w/v) glucose serum or 10% skimmed milk. Suspensions of spores and mycelium in glycerol (20%, v/v) can be stored in liquid nitrogen at –80°C.

Differentiation of the genus *Actinocorallia* from other genera

Actinocorallia strains can be distinguished from the other three genera classified in the family *Thermomonosporaceae* using a combination of chemotaxonomic, morphological, and

TABLE 300. Characteristics differentiating the species of the genus *Actinocorallia*[a,b]

Characteristic	1. *A. herbida*	2. *A. aurantiaca*	3. *A. aurea*	4. *A. cavernae*	5. *A. glomerata*	6. *A. libanotica*	7. *A. longicatena*
Morphology	Straight coralloid sporophores	Hooks, spirals	Straight to flexuous	Straight or flexuous	Straight, pseudosporangia	Hooks, curled	Straight or flexuous
Spore surface	Smooth	Warty	nd	Smooth	Smooth	Folded, warty	Smooth
Growth on yeast-extract-malt extract agar:							
Degree of growth	Moderate	Poor	Moderate	Good	Good	Abundant	Good
Aerial mycelium	White	White	Green-yellow	Sparse	White	Pale pink	White
Substrate mycelium	Honey gold	Yellowish brown	Light yellow	Olive black	Dull gold	Yellowish-brown	Oak-brown
Esculin hydrolysis	–	–	nd	+	nd	+	nd
Nitrate reductase	–	+	+	–	nd	+	nd
Degradation of:							
Casein	–	–	nd	+	+	–	+
Gelatin	–	+	+	+	–	–	–
Hypoxanthine	nd	–	nd	+	+	–	–
Starch	–	–	+	+	+	+	+
Tyrosine	nd	–	nd	+	–	–	–
Xanthine	nd	–	nd	–	–	–	–
Sole carbon sources:							
Adonitol	nd	+	nd	–	–	+	+
L-Arabinose	–	–	nd	+	+	+	+
Fructose	+	–	nd	+	+	–	+
Glycerol	nd	–	nd	–	+	+	+
Maltose	nd	–	nd	+	+	+	+
Mannose	nd	+	nd	+	+	–	–
Melibiose	nd	–	nd	–	–	–	–
Salicin	nd	–	nd	+	+	+	+
Sucrose	+	–	–	+	+	–	+
Ribose	nd	–	nd	+	–	–	–
Xylose	–	+	nd	+	+	+	+
Tolerance to 1% (w/v) NaCl	–	+	nd	+	+	+	+
Madurose in whole-cell sugars	+	+	+	–	+	+	+

[a]Symbols: +, positive; –, negative; nd, not determined.

[b]Data from: Athalye et al. (1985), Iinuma et al. (1994), Itoh et al. (1995), Lavrova and Preobrazhenskaya (1975), Lee (2006), Meyer (1979), Tamura et al. (2007), Trujillo and Goodfellow (2003) and Zhang et al. (2001).

phylogenetic data (Table 298). They can be separated readily from members of the genus *Actinomadura* as they have a phospholipid type I as opposed to a phospholipid type II pattern (Kroppenstedt and Goodfellow, 2006).

Taxonomic comments

The genus *Actinocorallia* Iinuma et al. 1994 was proposed to accommodate a single species, *Actinocorallia herbida*. The single representative of this taxon produced characteristic coralloid sporophores and was considered to have a wall chemotype III/C, that is, it contained *meso*-A_2pm but no diagnostic sugars. Zhang et al. (1998) found that *Actinocorallia herbida*, *Actinomadura aurantiaca*, and *Actinomadura libanotica* formed a 16S rRNA gene clade that was well separated from one that included the rest of the *Actinomadura* species; these findings raised the question whether the latter two species should be transferred to the genus *Actinocorallia*.

Following an extensive chemotaxonomic and phylogenetic study of members of the family *Thermomonosporaceae*, Zhang et al. (2001) emended the description of the genus *Actinocorallia* in order to accommodate four species previously classified in the genus *Actinomadura*. Thus, *Actinomadura aurantiaca*, *Actinomadura glomerata*, *Actinomadura libanotica*, and *Actinomadura longicatena* were transferred to the genus *Actinocorallia* as *Actinocorallia aurantiaca*, *Actinocorallia glomerata*, *Actinocorallia libanotica*, and *Actinocorallia longicatena*, respectively. Zhang and his colleagues also detected major amounts of diphosphatidylglycerol, phosphatidylethanolamine, and phosphatidylinositol in *Actinocorallia libanotica* and the presence of madurose in *Actinocorallia herbida*. These findings mean that *Actinocorallia libanotica* has a phospholipid type II, not I, as reported by Kroppenstedt et al. (1990) and that *Actinocorallia herbida* has a sugar type B, not C, as reported by Iinuma et al. (Iinuma et al., 1994). It was clear from these findings that six of the seven *Actinocorallia* species contain madurose and have a phospholipid pattern type II *sensu* Lechevalier et al. (1977, 1981). The type strain of *Actinocorallia cavernae* lacks madurose but has a phospholipid pattern type II (Lee, 2006).

Differentiation of the species of the genus *Actinocorallia*

The differential characteristics of the species of *Actinocorallia* are given in Table 300. However, identification of members of the genus is not easy as most of the phenotypic data are based on sole representatives of species and even then there are inconsistencies between data from different studies.

Acknowledgements

The authors are grateful to Dr Olga Genilloud for reading the manuscript and providing helpful comments.

List of species of the genus *Actinocorallia*

1. **Actinocorallia herbida** Iinuma, Yokota, Hasegawa and Kanamuru 1994, 233[VP]

her'bi.da. L. fem. adj. *herbida* grassy, referring to the formation of aerial mycelia like grass.

Spores borne in long straight chains or on coralloid sporophores.

Glycerol-asparagine agar: poor growth, sparse white aerial mycelium, substrate mycelium light ivory, and no diffusible pigment. Inorganic salts-starch agar: poor growth, no aerial mycelium, substrate mycelium light wheat color, and no diffusible pigment. Oatmeal agar: moderate growth, aerial mycelium light yellow, substrate mycelium yellow, and no diffusible pigment. Tyrosine agar: poor growth, no aerial mycelium, substrate mycelium light ivory color, and dusty coral-colored diffusible pigment. Yeast extract-malt extract agar: moderate growth, sparse white aerial mycelium, substrate mycelium honey gold color, and no diffusible pigment. The temperature range for growth is 12–38°C, the optimal range 24–32°C. Glucose is used as a sole carbon source, but not inositol, mannitol, or raffinose. Grows in the presence of 3% but not 5% (w/v) NaCl. Antibiotic resistance (μg/ml) is shown towards cefsulodin (100), but not against gentamicin (100), novobiocin (20), rifampin (50), streptomycin (100), or vancomycin (50). Additional phenotypic properties are shown in Table 300. The predominant fatty acids are $C_{16:0}$ (25.8%), $C_{16:1}$ (15.4%), $C_{17:1}$ (16.0%), and $C_{18:1}$ (26.5%).

Source: soil sample collected in Bangkok, Thailand.

DNA G+C content (mol%): 73.0 (HPLC) (type strain).

Type strain: AL-50780, ATCC 51528, DSM 44254, NBRC 15485, JCM 9647, NCIMB 13337, VKM Ac-1994.

Sequence accession no. (16S rRNA gene): D85473.

Sequence accession no. (23S rRNA gene): AF134086.

Sequence accession no. (16S–23S rRNA ITS): AF134109.

2. **Actinocorallia aurantiaca** (Lavrova and Preobrazhenskaia 1975) Zhang, Kudo, Nakajima and Wang 2001, 381[VP] (*Actinomadura aurantiaca* Lavrova and Preobrazhenskaya 1975, 485[AL])

au.ran.ti'a.ca. N.L. fem. adj. *aurantiaca* orange-colored, referring to the gold-colored substrate mycelium.

Spore chains (of 4–8 spores), hooked or in spirals (one turn), in small clusters emerging directly from the agar. Spore surface warty. Spores connected by intersporal pads. Inorganic salts-starch agar: moderate growth; sparsely scattered dots of sporulating hyphae; aerial mycelium whitish, substrate mycelium cream-colored to orange, and no diffusible pigment. Oatmeal agar: moderate growth; surface farinaceous, aerial mycelium cream-colored to pink; substrate mycelium yellowish white, and no diffusible pigment. Peptone-glucose medium: moderate growth; surface farinaceous, aerial mycelium cream-colored; substrate mycelium yellow to orange, and no diffusible pigment. Yeast extract-malt extract agar: poor growth; sparsely scattered dots of sporulating hyphae; aerial mycelium whitish, substrate mycelium yellow brown, and no diffusible pigment. The temperature range for growth is 10–30°C, the optimal range is 25–28°C. The pH growth range is 6.0–10.0.

Degrades arbutin, DNA, testosterone, and Tweens 20, 40, 60, and 80, but not chitin, elastin, pectin, RNA, or xylan. Galactose, glucose, rhamnose, and trehalose are used as sole carbon sources, but not lactose, mannitol, mannose, melezitose, sorbitol, or xylitol. Antibiotic resistance (µg/ml) is shown towards ampicillin (10), benzylpenicillin (25), carbenicillin (120), cephaloridine (2), cephalothin (20), cephamandole (30), lincomycin (10), oleandomycin (20), and ticarcillin (70), but not against streptomycin (2), tobramycin (0.05), or tetracycline (20). Additional phenotypic properties are shown in Table 300.

Source: soil.

DNA G+C content (mol%): not determined.

Type strain: DSM 43924, IFO (now NRBC) 15554, IMET 9577, INA 1933, JCM 8201, KCTC 9554.

Sequence accession no. (16S rRNA gene): AF134066, AJ293701.

Sequence accession no. (23S rRNA gene): AF134072.

Sequence accession no. (16S–23S rRNA ITS): AF134090.

3. **Actinocorallia aurea** Tamura, Hatano, Suzuki 2007, 2054[VP]

au're.a. L. fem. adj. *aurea* golden.

Straight to flexuous chains of aerial spores are formed on the tip of coralloid sporophores. Spores are short, non-motile rods (0.6–0.9 × 1.0–1.5 mm). Substrate mycelium is yellow on maltose-Bennett's agar, yeast extract-malt-extract agar, inorganic salts-starch agar, glycerol-asparagine agar, and peptone-yeast extract-iron agar, and grayish white on oatmeal and tyrosine agars. The aerial spore mass is yellow on yeast extract-starch agar, maltose-Bennett's agar, and on 1/4 strength oatmeal and yeast extract-malt extract agars. Optimum temperature range for growth is 20–30°C. Grows at 37°C, but not 45°C. Does not grow on 4% (w/v) NaCl. Gelatin is liquefied, starch is hydrolyzed, and nitrate is reduced. Glucose, maltose, rhamnose, and xylose are used as sole carbon sources. Additional phenotypic properties are shown in Table 300. The major fatty acids are $C_{16:0}$ (21%), $C_{18:1}$ (19%), 10-methyl $C_{18:0}$ (10%), and $C_{17:1}$ (13%).

Source: soil.

DNA G+C content (mol%): 71–73 (HPLC).

Type strain: DSM 14752, NBRC 44434.

Sequence accession no. (16S rRNA gene): AB006177.

4. **Actinocorallia cavernae** Lee 2006, 1087[AL]

ca.ver'na.e. L. gen. n. *cavernae* of a cavern, the site from which the type strain was isolated.

Aerial hyphae differentiate into straight to flexuous spores chains. Spore surface smooth. Substrate mycelium is olive black in color on yeast extract-malt extract agar, dark yellow brown on oatmeal agar, and cream on inorganic salts-starch agar. Aerial mycelium is abundant and grayish white on inorganic salts-starch and oatmeal agars, but sparse on yeast extract-malt extract agar. Diffusible pigments are not formed. Grows optimally in the temperature range 25–30°C, but not at 10° or 37°C. Grows in the pH range 5.1–10.1, but not at pH 4.1. Does not degrade DNA or elastin. Urea is hydrolyzed. H_2S is not produced. D-Arabinose, 2,3-butandiol, cellobiose, galactose, glucose, lactose, melezitose, rhamnose, and trehalose are used as sole carbon sources, but not dulcitol, *meso*-erythritol, inositol, inulin, mannitol, methyl-D-glucoside, methyl-D-mannoside, 1,2 propandiol, raffinose, sorbitol, sorbose, or xylitol. Additional phenotypic properties are shown in Table 300. The predominant fatty acids are $C_{16:0}$ (25.9%), $C_{18:1}$ (19.2), $C_{17:0}$ (11.7%), 10-methyl $C_{18:0}$ (tuberculostearic acid, 9.4%), *ai*-$C_{17:0}$ (7.4%), and $C_{18:0}$ (6.7%).

Source: soil sample taken from inside a natural cave on Jeju Island, Republic of Korea.

DNA G+C content (mol%): 70.1 (HPLC).

Type strain: N3-7, DSM 45040, JCM 13278, NRRL B-24429.

Sequence accession no. (16S rRNA gene): AY966427.

5. **Actinocorallia glomerata** (Itoh, Kudo, Oyaizu and Seino 1995) Zhang, Kudo, Nakajima and Wang 2001, 381[VP] (*Actinomadura glomerata* Itoh, Kudo, Oyaizu and Seino 1995, 173)

glo.me.ra'ta. L. fem. part. adj. *glomerata* (from L. v. *glomerare* to form into ball, glomerate) gathered into a mass, referring to the pseudosporangium-like structures.

Rod-shaped spores (0.6–0.8 × 0.4–0.5 µm) are formed in slimy globular, pseudosporangia-like clusters (Figure 402) or occasionally in straight chains. Spore surface smooth. Glycerol-asparagine and tyrosine agars: moderate growth; aerial mycelium white; substrate mycelium pearl-colored, and no diffusible pigment. Inorganic salts-starch agar: moderate growth; no aerial mycelium; substrate mycelium pearl-colored, and no diffusible pigment. Oatmeal agar: good growth; aerial mycelium white; substrate mycelium sand-colored, and no diffusible pigment. Yeast-extract-malt extract agar: good growth; aerial mycelium white; substrate mycelium dull gold color, and no diffusible pigment. Temperature range for growth is 7–41°C, optimal range 28–30°C. Adenine is not degraded. Galactose, glucose, rhamnose, and trehalose are used as sole carbon sources, but not dulcitol, erythritol, inositol, mannitol, raffinose, or sorbitol. Antibiotic resistance (µg/ml) is shown towards novobiocin (20), rifampin (50), streptomycin (50), and vancomycin (50). Additional phenotypic properties are shown in Table 300. Predominant fatty acids are $C_{16:0}$ (28%), $C_{18:1}$ (20%), and 10-methyl $C_{19:0}$ (15%).

Source: a decayed leaf of a deciduous tree collected from a pond in Saitama, Japan.

DNA G+C content (mol%): 71.7 (HPLC) (type strain).

Type strain: DSM 44360, IFO 15960, JCM 9376, NBRC 15960.

Sequence accession no. (16S rRNA gene): AF134068, AJ293704.

Sequence accession no. (23S rRNA gene): AF134077.

Sequence accession no. (16S–23S rRNA ITS): AF134098.

6. **Actinocorallia libanotica** (Meyer 1981) Zhang, Kudo, Nakajima and Wang 2001, 381[VP] (*Actinomadura libanotica* Meyer 1981, 215[VP])

li.ba.no'ti.ca. L. n. *Libanus* Lebanon; L. fem. suff. -*tica*, suffix denoting made of or belonging to; N.L. fem. adj. *libanotica* referring to Lebanon (the country in which the soil sample was taken).

Spores chains in hooks or curled, arranged in clusters on short hyphae on the aerial mycelium, 5–12 spores per chain: spores subspherical, and spore surface folded or warty. Spores are connected by intersporal pads (Figure 401). Inorganic

salts-starch agar: moderate growth; only a flimsy cover of aerial mycelium; substrate mycelium grayish, and no diffusible pigment. Oatmeal agar: abundant growth; surface farinaceous; aerial mycelium white to pale pink; substrate mycelium yellowish brown, and no diffusible pigment. Oatmeal-nitrate agar: abundant growth; surface farinaceous; aerial mycelium white to pale pink; substrate mycelium yellowish brown, and no diffusible pigment. Peptone-glucose medium: moderate growth; surface granular; aerial mycelium white; substrate mycelium yellowish brown, and no diffusible pigment. Yeast extract-malt extract agar: abundant growth; surface farinaceous; aerial mycelium pale pink; substrate mycelium yellowish brown, and no diffusible pigment. The temperature range for growth is 10–37°C, and the optimal range is 28–30°C. Does not produce allantoinase or urease. Degrades arbutin, RNA, testosterone, Tweens 20, 40, 60, and 80 and xylan, but not chitin, elastin, guanine, pectin, or xylan. Cellobiose, dextrin, galactose, glycogen, rhamnose, and trehalose are used as sole carbon sources, but not glucosamine, lactose, mannitol, raffinose, sorbitol, or xylitol. Antibiotic resistance (μg/ml) is shown towards ampicillin (10), benzylpenicillin (10), carbenicillin (90), cephaloridine (2), demeclocycline (2), lincomycin (10), neomycin (1), oleandomycin (3), and ticarcillin (30), but not against cephalotin (20), cephamandole (20), gentamicin (4), neomycin (3), rifampin (2), streptomycin (4), tetracycline (20), tobramycin (0.05), or vancomycin (0.25). Additional phenotypic properties are shown in Table 300.

Source: soil.

DNA G+C content (mol%): 66.2 (T_m).

Type strain: ATCC 35576, DSM 43554, IMET 9616, NBRC 14095, JCM 10696, NCIB 11686, NRRL B-16097, VKM Ac-939.

Sequence accession no. (16S rRNA gene): U49007.

Sequence accession no. (23S rRNA gene): AF134078.

Sequence accession no. (16S–23S rRNA ITS): AF134100.

7. **Actinocorallia longicatena** (Itoh, Kudo, Oyaizu and Seino 1995) Zhang, Kudo, Nakajima and Wang 2001, 381[VP] (*Actinomadura longicatena* Itoh, Kudo, Oyaizu and Seino 1995, 175)

lon.gi.ca.te′na. L. adj. *longus* long: L. fem. n. *catena* chain; N.L. fem. n *longicatena* a long chain.

Straight to flexuous chains of 20 or more spores are borne on aerial hyphae. Spores are rod-shaped (0.6–0.9 × 0.4 × 0.5 μm). Spore surface smooth (Figure 403). Glycerol-asparagine agar: growth moderate; sparse white aerial mycelium; substrate mycelium colorless, and no diffusible pigment. Inorganic salts-starch agar: growth poor to moderate; sparse white aerial mycelium; substrate mycelium pearl, and no diffusible pigment. Oatmeal agar: growth good; sparse aerial mycelium; substrate mycelium sandy-colored, and no diffusible pigment. Tyrosine agar: growth moderate; aerial mycelium white; substrate mycelium pearl-colored, and pale brown diffusible pigment. Yeast extract-malt extract agar: good growth; aerial mycelium white; substrate mycelium oak brown, and no diffusible pigment. Temperature range for growth is 7–37°C. Adenine is not degraded. Galactose, glucose, rhamnose, and trehalose are used as sole carbon sources, but not dulcitol, erythritol, inositol, mannitol, raffinose, or sorbitol. Antibiotic resistance (μg/ml) is shown against novobiocin (20), rifampin (50), streptomycin (50), and vancomycin (50). Additional phenotypic properties are shown in Table 300. Predominant fatty acids are $C_{16:0}$ (24%), $C_{18:1}$ (16%), and 10-methyl $C_{19:0}$ (14%).

Source: root of a herbaceous plant (*Bidens* sp.) grown in Saitama, Japan.

DNA G+C content (mol%): 70.9 (HPLC) (type strain).

Type strain: I-497, CIP 105488, DSM 44361, NBRC 15961, JCM 9377.

Sequence accession no. (16S rRNA gene): AF163117, AJ293707.

Sequence accession no. (23S rRNA gene): AF163128.

Sequence accession no. (16S–23S rRNA ITS): AF163139.

Genus III. **Actinomadura** Lechevalier and Lechevalier 1970a, 400[AL] emend. Kroppenstedt, Stackebrandt and Goodfellow 1990, 156

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Ac.ti.no.ma.du′ra. Gr. n. *actis actinos* a ray; N.L. n. *Madura* Madura, name of a province in India; N.L. fem. n. *Actinomadura* referring to a micro-organism first described as the causative agent of "Madura foot" disease.

Gram-stain-positive, non-acid–alcohol-fast, nonmotile actinomycetes that form an **extensively branched non-fragmenting, substrate mycelium**. **Aerial mycelium moderately developed or absent**. When present, aerial hyphae carry up to 50 arthrospores. **Aerial mycelium at maturity forms short or occasionally long chains of arthrospores**. **Spore chains straight, hooked** (**open loops**), **or irregular spirals (1–4 turns)**. **Spore surface folded, irregular, rugose, smooth, spiny, or warty**. Color of mature sporulated aerial mycelium: blue, brown, cream, gray, green, pink, red, white, or yellow. **Colonies have a leathery or cartilaginous appearance** when aerial mycelium is lacking. Aerobic, chemo-organotrophic with an oxidative type metabolism. Temperature growth range 10–60°C. **Cell wall contains *meso*-2,6-diaminopimelic acid as the major diamino acid and *N*-acetylated muramic acid**. Whole-cell hydrolysates contain galactose, glucose, madurose, mannose, and ribose. **The major phospholipids are diphosphatidylglycerol and phosphatidylinositol**. **Menaquinones are predominantly hexahydrogenated with nine isoprene units saturated at sites II, III, and VIII**. Complex fatty acid profile is rich in branched saturated and unsaturated fatty acids, including tuberculostearic acid. Mycolic acids are absent. Widely distributed in soil. Some strains are pathogenic for animals, including man.

DNA G+C content (mol%): 66–73 (T_m, HPLC).

Type species: **Actinomadura madurae** (Vincent 1894) Lechevalier and Lechevalier 1970a, 400[AL].

Further descriptive information

Phylogeny. The genus *Actinomadura* is classified in the family *Thermomonosporaceae* Rainey et al. 1997 emend. Zhang et al. 2001 emend. Zhi et al. 2009 together with the genera *Actinocorallia* Iinuma et al. 1994 emend. Zhang et al. 2001, *Spirillospora* Couch 1963 and *Thermomonospora* Henssen 1957 emend. Zhang et al. 2001. These taxa form a distinct phyletic line in the 16S rRNA gene tree (Zhang et al., 1998, 2001) and can thereby be distinguished from the other two families that comprise the order *Streptosporangiales*, namely the families *Nocardiopsaceae* and *Streptosporangiaceae* (Goodfellow and Quintana, 2006; Gyobu, 2001; Kudo, 2001). Members of the genera *Actinomadura*, *Spirillospora*, and *Thermomonospora* are phylogenetically intermixed, but can be distinguished using morphological properties (Kroppenstedt and Goodfellow, 2006; Zhang et al. 1998, 2001). The complex topology of the *Thermomonosporaceae* 16S rRNA gene tree is shown in Figure 398.

Cell morphology. *Actinomadura* strains characteristically form nonfragmenting, extensively branched, substrate mycelia and aerial hyphae which carry 1–50 arthrospores. *Actinomadura formosensis* forms single spores on both aerial and substrate mycelia (Hasegawa et al., 1986) whereas *Actinomadura echinospora* and *Actinomadura rugatobispora* usually produce two spores per chain, sometimes three, on aerial hyphae (Miyadoh et al., 1990). Longer spore chains are characteristic of most other *Actinomadura* species though spores have not been detected in *Actinomadura fibrosa* or *Actinomadura latina* strains. Spores are borne in curled, hooked, spiral, or straight chains. Spore sufaces may be folded, irregular, rugose, smooth, shiny, or warty (Table 301).

Some strains, notably those from clinical sources, lack aerial mycelium with colonies exhibiting a cartilaginous or leathery appearance. However, members of most species form a spore-bearing, powdery aerial mycelium on media such as inorganic salts-starch, oatmeal and yeast extract-malt extract agars after cultivation for 10–14 d. At maturity, aerial mycelia may be blue, cream, gray, green, pink, yellow, or white thereby differing little from streptomycetes. Superficial similarity to streptomycetes is also reinforced by the morphology of the sporophores. *Actinomadura spadix* forms pseudosporangia-like structures, i.e. tightly spiraled spore chains embedded in a dry to slimy mass thereby simulating the colonial appearance of *Streptosporangium* strains when examined microscopically at low magnification. Actinomadurae and streptomycetes can be distinguished by direct microscopic comparison of cultures. Most *Actinomadura* strains have spores noticeably larger than the diameter of their hyphae, whereas streptomycetes have spores and hyphae of similar diameter.

Cell-wall composition. The genus *Actinomadura* was defined chemically (Lechevalier and Lechevalier, 1970a) to encompass former *Nocardia* species with cell-wall type III, i.e. with *meso*-A_2pm in the peptidoglycan, but no arabinogalactan polymer, which is in contrast to *Nocardia* species. Members of some species also contain small amounts of the LL-isomer. Whole-organism hydrolysates contain galactose, glucose, mannose, ribose, and madurose (3-*O*-methyl-D-galactose; Lechevalier and Gerber, 1970), though the latter may be found in trace amounts. Madurose also occurs in other wall type III taxa, including *Actinocorallia*, *Dermatophilus*, *Microbispora*, *Nonomuraea*, *Planobispora*, *Planomonospora*, *Spirillospora*, and *Streptosporangium*. Differentiation from these genera is based on molecular and morphological characteristics.

Polar lipid and menaquinone composition. Most *Actinomadura* species, including *Actinomadura madurae*, the type species, have a phospholipid type I pattern (Lechevalier et al., 1977, 1981), which is characterized by the presence of diphosphatidylglycerol, phosphatidylinositol, and phosphatidylinositol mannosides. In contrast, *Actinomadura kijaniata*, *Actinomadura namibiensis*, and *Actinomadura napierensis* have a phospholipid pattern type PIV, which is characterized by the presence of diphosphatidylglycerol, phosphatidylethanolamine, phosphatidylinositol, phosphatidylinositolmannoside, phosphatidylmethylamine, and unknown glucosamine-containing phospholipids (Cook et al., 2005; Wink et al., 2003). *Actinomadura* strains can be differentiated from the genus *Actinocorallia* on the basis of polar lipid profiles. Members of the genus *Actinomadura* contain mainly MK-9(H_6) saturated at sites II, III, and VIII, small amounts of MK-9(H_4) saturated at sites II, III, and VIII, and small amounts of MK-9(H_8) (Kroppenstedt et al., 1990). Differentiation from other wall chemotype III genera, such as *Microbispora* or *Nonomuraea*, is difficult as members of these taxa have similar menaquinone profiles (Athalye et al., 1984; Kroppenstedt et al., 1990; Poschner et al., 1985).

Fatty acid profile. Actinomadurae contain complex mixtures of fatty acids with hexadecanoic (16:0), 14-methylpentadecanoic (iso-16:0), and 10-methyloctadecanoic (tuberculostearic) acids predominating, that is, they have a fatty acid type 3a (Kroppenstedt, 1985). *Actinomadura rubrobrunea*, a thermophilic organism, can be separated from mesophilic actinomadurae by differences in fatty acid composition (Agre and Guzeva, 1975). It contains relatively high proportions of iso-branched fatty acids (high melting point) and low amounts of 10-methyl branched acids (low-melting-point) in line with its thermophilic nature. Hydroxylated fatty acids have been detected in *Actinomadura atramentaria*.

Cultural characteristics. Actinomadurae are slower-growing than streptomycetes and tend to grow well on modified Bennett's (Jones, 1949) and glucose-yeast extract agars (Athalye, 1981), and on formulations that were found to be useful in the International *Streptomyces* Project (ISP; Shirling and Gottlieb, 1966). Most strains show abundant growth on oatmeal agar (ISP 3 medium) at 28–30°C, but *Actinomadura kijaniata* and *Actinomadura macra* grow better on yeast extract- malt extract agar (ISP 2 medium). *Actinomadura vinacea* grows well on glucose-peptone agar but poorly on these media. *Actinomadura spadix* requires vitamin B12 for good growth (Nonomura and Ohara, 1971d). *Actinomadura rubrobrunea* grows well on oatmeal and peptone maize agars at 50°C, and *Actinomadura yumaensis* grows poorly on media containing inorganic nitrogen sources. *Actinomadura latina*, *Actinomadura madurae*, and *Actinomadura pelletieri*, the three recognized pathogenic species, show moderate to good growth on Czapek–Dox Casamino acids (Cross and Attwell, 1974; Trujillo, 1994).

Nutrition and growth conditions. All *Actinomadura* strains are strictly aerobic and chemo-organotrophic with an oxidative metabolism. Most species grow well at 25–40°C, but *Actinomadura formosensis*, *Actinomadura rubrobrunea*, and *Actinomadura viridilutea* are thermophilic, with optimal growth temperatures

TABLE 301. Morphological characters separating *Actinomadura* species[a]

Species	Color on oatmeal agar: Aerial mycelium	Color on oatmeal agar: Substrate mycelium	Diffusible pigments	Spore chain type	Spore surface type
A. madurae	Trace, white specks	Colorless	None	Hooks to spirals	Warty
A. atramentaria	White	Colorless	Inky brown[b]	Straight	Smooth
A. catellatispora	Yellow	Light yellow	None	Straight	Smooth
A. citrea	White to blue	Lemon yellow	Yellow	Hooks or curled	Irregular or warty
A. coerulea	Pink to blue	Colorless to pale pink	None	Hooks to spirals	Warty
A. cremea subsp. *cremea*	White to yellow	Colorless	None	Hooks to spirals	Warty
A. cremea subsp. *rifamicini*	White to yellow	Colorless	None	Hooks to spirals	Warty
A. echinospora	Yellow to pink	Orange	Yellow	Paired spores	Spiny
A. fibrosa	White	Orange	Brown	No spores formed	–
A. formosensis	White	Light apricot	None	Single	Warty
A. fulvescens	White	Red-brown	Red-brown	Spirals	Smooth
A. glauciflava	Bluish green	Yellow to brown	Yellow	Hooks, spirals	Warty
A. hallensis	White	Brown	None	Hooks, spirals	Warty
A. hibisca	Light gray	Red violet	Carmine red	Straight	Smooth
A. kijaniata	Trace, white specks	Pine green	None	Spirals	Smooth
A. latina	Absent	Cream to pink	None	No spores formed	–
A. livida	Trace	Gray to pink	Pale violet	Hooks, spirals	Uneven
A. luteofluorescens	Yellow to blue	Yellow to green	Yellow-green	Hooks, curled	Warty
A. macra	Cream to pink	Cream to pink	None	Hooks, curled	Smooth
A. mexicana	Absent	Cream to yellow	None	Hooks	Warty
A. meyerae	Sparse	Cream to yellow	None	Hooks	Warty
A. namibiensis	None	Salmon pink	None	Spirals	Smooth
A. napierensis	White	Grey	None	Spirals	Smooth
A. nitritigenes	Brown	Colorless to brown	None	Hooks	Smooth
A. oligospora	Trace,white	Gray	None	Straight, hooked	Smooth
A. pelletieri	Absent	Pink to brownish red	None	Hooks to spirals	Warty
A. rubrobrunea	Grayish-blue	Orange	None	Hooks to spirals	Spiny
A. rudentiformis	White	Cream	None	No spores formed	No spores formed
A. rugatobispora	Light to dusty green	Pastel yellow	None	Paired spores	Rugose with vertical ridges
A. spadix	Yellow/brown	Reddish gray/grayish brown	Reddish gray	Pseudosporangia	Smooth
A. umbrina	Scanty, white	Colorless	None	Straight, hooks, spirals	Smooth
A. verrucosospora	Pink to blue	Orange to pink	None	Hooks to spirals	Warty
A. vinacea	Absent	Pink to red	None	Straight	Irregular
A. viridilutea	Blue green	Orange to yellow	None	Hooks to spirals	Spiny
A. viridis	Green	Yellow-brown to green	None	Straight	Smooth
A. yumaensis	Gray to yellow	Gray to yellow	None	Hooks	Smooth

[a]Data from Athalye et al. (1985), Lu et al. (2003), Mertz and Yao (1990), Miyadoh et al. (1985), Meyer (1989a, 1989b), Quintana et al. (2003), Wink et al. (2003), Cook et al. (2005), Lee and Jeong (2006), and Le Roes and Meyers (2007).

[b]Pigment found on tyrosine agar and yeast extract–malt extract agar.

between 45°C and 65°C. *Actinomadura* strains can metabolize a wide range of amino acids and sugars as carbon sources for growth (Table 302); proteolytic activity is shown by the capacity of most strains to hydrolyze arbutin, casein, elastin, gelatin, testosterone, and Tweens 60 and 80 (Kroppenstedt and Goodfellow, 1992; Trujillo and Goodfellow, 2003). On the other hand, xylan is not degraded (Table 303). Pectinase activity has been reported for *Actinomadura mexicana*, *Actinomadura napierensis*, and *Actinomadura verrucosospora* (Cook et al., 2005; Trujillo and Goodfellow, 2003). Pathogenic strains of *Actinomadura madurae* produce collagenolytic enzymes (Rippon, 1968). Most actinomadurae grow in the presence of NaCl 3% (w/v). *Actinomadura atramentaria* tolerates NaCl concentrations of 7% (w/v).

Pigments. *Actinomadura madurae* and *Actinomadura pelletieri* produce prodigiosin-like pigments (Gerber, 1971, 1973; Lechevalier et al., 1971) that are similar to those of *Serratia marcescens*. Members of these species isolated from patients produce prodiginines characterized by a tripyrrole skeleton and identified as cyclononylprodiginine, nonylprodiginine, and undecylprodiginine. *Actinomadura atramentaria* produces an inky brown pigment on several media, especially in submerged cultures (Miyadoh et al., 1987); under alkaline conditions the pigment is dark green-brown and is more soluble in water. In contrast, under acidic conditions, it is dark violet and more soluble in organic solvents.

Genetics. Very little is known about the genetics of *Actinomadura* strains though extensive phylogenetic studies based on the 16S, 23S rRNA, and intergenic spacer regions have been carried out (Wang et al., 1996; Zhang et al., 1998, 2001). 16S rRNA gene sequence similarity of the type strains of all 35 validly named *Actinomadura* species is in the range 93.5–99.6%, suggesting a moderate degree of heterogeneity. Members of the taxon contain the 16S rRNA nucleotide sequence signatures characteristic of members of the family *Thermomonosporaceae* (Stackebrandt et al., 1997).

TABLE 302. Carbon source utilization for *Actinomadura* species[a, b]

Carbon source	*A. madurae*	*A. atramentaria*	*A. catellatispora*	*A. citrea*	*A. coerulea*	*A. cremea*	*A. echinospora*	*A. fibrosa*	*A. formosensis*	*A. fulvescens*	*A. glauciflava*	*A. hallensis*	*A. hibisca*	*A. kiijanata*	*A. latina*	*A. livida*	*A. luteofluorescens*	*A. macra*	*A. mexicana*	*A. meyerae*	*A. namibiensis*	*A. napierensis*	*A. nitritigenes*	*A. oligospora*	*A. pelletieri*	*A. rubrobrunea*	*A. rudentiformis*	*A. rugatobispora*	*A. spadix*	*A. umbrina*	*A. verrucosospora*	*A. vinacea*	*A. viridilutea*	*A. viridis*	*A. yumaenesis*
Adonitol	+	–	nd	+	+	–	–	+	nd	+	nd	–	–	+	–	–	+	–	+	+	nd	+	nd	+	–	nd	+	nd	+	nd	+	nd	nd	+	–
Arabinose	d	–	nd	+	–	+	+	+	–	+	nd	+	–	–	+	–	–	–	+	+	+	+	+	+	–	nd	nd	v	+	nd	+	nd	nd	–	–
Cellobiose	+	+	nd	+	+	+	+	+	nd	+	nd	+	+	+	+	+	+	+	+	+	nd	nd	+	+	+	nd	+	nd	+	nd	+	nd	nd	+	+
Fructose	+	–	nd	+	–	+	+	+	–	–	nd	+	nd	+	+	+	+	–	–	+	–	+	+	+	–	nd	+	+	+	nd	+	nd	nd	+	+
Galactose	+	–	nd	–	+	+	–	+	+	+	nd	–	nd	+	+	+	+	–	–	–	nd	+	+	–	–	+	+	nd	+	nd	–	nd	+	+	+
Glucose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	nd	nd	+	+	nd	+	+	+	nd	+	nd	nd	+	+
Glycerol	+	+	nd	+	–	+	+	+	–	+	nd	+	–	–	+	–	+	+	+	+	nd	+	+	+	–	+	nd	+	+	nd	+	nd	+	+	+
Glycogen	+	–	nd	+	–	+	+	+	nd	+	nd	nd	nd	+	d	+	+	–	+	+	nd	nd	nd	+	+	nd	nd	nd	+	nd	+	nd	nd	+	+
Lactose	–	+	nd	–	–	–	+	+	–	+	nd	–	–	+	d	–	–	+	–	–	nd	nd	–	+	d	+	+	nd	+	nd	–	nd	+	–	–
Maltose	+	–	nd	+	–	+	+	+	–	–	nd	+	nd	+	+	–	+	–	–	+	nd	+	–	–	d	+	nd	nd	+	nd	+	nd	+	+	+
Mannitol	+	–	nd	+	–	+	+	–	+	–	nd	+	–	–	+	+	+	–	–	+	v	+	nd	–	–	+	+	+	+	nd	+	nd	+	+	–
Mannose	d	+	nd	–	+	+	+	+	–	+	nd	–	nd	+	d	+	–	+	–	–	nd	nd	+	+	–	nd	nd	nd	+	nd	–	nd	nd	–	+
Melezitose	–	–	nd	–	–	–	–	–	nd	–	nd	–	nd	–	+	–	–	–	–	–	nd	+	nd	–	–	nd	+	nd	+	nd	d	nd	nd	–	+
Melibiose	–	+	nd	–	–	–	–	–	nd	–	nd	–	nd	–	–	–	–	–	–	–	nd	–	nd	–	–	nd	+	nd	+	nd	–	nd	nd	–	–
Raffinose	–	–	nd	–	–	–	–	+	–	–	nd	–	nd	–	–	–	–	–	–	–	–	+	nd	–	–	+	+	–	+	nd	–	nd	+	–	–
Rhamnose	d	–	nd	+	–	+	+	+	–	–	nd	–	nd	+	+	–	+	–	+	+	+	+	+	–	d	+	+	+	+	nd	+	nd	+	+	–
Ribose	–	–	nd	+	–	+	–	+	nd	d	nd	nd	nd	+	d	+	–	–	–	+	nd	nd	nd	+	–	nd	+	nd	+	nd	–	nd	nd	+	+
Salicin	–	–	nd	–	–	–	–	–	nd	–	nd	+	nd	+	–	–	–	–	–	–	nd	nd	nd	+	–	nd	+	nd	–	nd	d	nd	nd	–	–
Sorbitol	–	+	nd	–	–	–	–	nd	–	–	nd	–	nd	–	–	–	–	–	–	–	nd	nd	nd	–	–	nd	nd	nd	+	nd	–	nd	nd	–	–
Trehalose	+	+	nd	+	+	+	+	+	–	+	nd	+	nd	+	+	–	+	+	+	+	nd	+	+	+	d	nd	+	nd	+	nd	+	nd	nd	+	+
Xylitol	+	+	nd	–	–	–	–	–	nd	–	nd	–	nd	–	–	–	–	–	–	+	nd	nd	nd	–	–	nd	+	nd	+	nd	–	nd	nd	–	–
Xylose	d	–	nd	+	+	+	+	–	–	+	nd	+	nd	+	+	+	+	–	+	+	v	+	+	+	d	nd	+	–	+	nd	+	nd	nd	+	–
L-Arginine	–	–	nd	+	+	–	–	nd	nd	–	nd	nd	nd	–	d	–	+	–	–	+	nd	nd	nd	–	–	nd	+	nd	–	nd	d	nd	nd	+	+
L-Glutamine	–	–	nd	–	+	–	–	nd	nd	+	nd	nd	nd	+	–	–	+	–	+	–	nd	nd	nd	–	–	nd	nd	nd	–	nd	–	nd	nd	+	+
Glycine	d	–	nd	+	–	–	–	nd	nd	–	nd	nd	nd	+	d	–	–	–	–	–	nd	nd	nd	–	d	nd	nd	nd	–	nd	d	nd	nd	+	–
L-Histidine	d	–	nd	+	+	–	–	nd	nd	+	nd	nd	nd	+	–	+	+	–	–	–	nd	nd	nd	–	d	nd	+	nd	–	nd	d	nd	nd	+	–
L-Leucine	–	+	nd	–	+	–	–	nd	nd	–	nd	nd	nd	+	–	–	+	+	–	–	nd	nd	nd	+	–	nd	nd	nd	–	nd	–	nd	nd	+	–
L-Methionine	–	–	nd	–	–	–	–	nd	nd	–	nd	nd	nd	+	–	–	–	–	–	–	nd	nd	nd	–	–	nd	+	nd	–	nd	–	nd	nd	+	+
L-Phenylalanine	d	–	nd	+	+	–	–	nd	nd	–	nd	nd	nd	+	+	+	+	+	–	–	nd	nd	nd	–	d	nd	+	nd	+	nd	–	nd	nd	–	–
L-Valine	d	+	nd	–	+	–	–	nd	nd	d	nd	nd	nd	+	–	–	–	+	–	–	nd	nd	nd	–	–	nd	+	nd	+	nd	–	nd	nd	+	+

[a]Symbols: +, 90% or more strains positive; –, 10% or less strains positive; d, 11–89% of strains positive; nd, not determined.

[b]Data from Athalye (1981), Athalye et al. (1985), Goodfellow et al. (1979), Horan and Brodsky (1982), Labeda et al. (1985), Lipski and Altendorf (1995), Lu et al. (2003), Mertz and Yao (1986, 1990), Quintana et al. (2003), Trujillo and Goodfellow (1997a, 2003), Kim (1999), Cook et al. (2005), Lee and Jeong (2006), Le Roes and Meyers (2007), and Wink et al. (2003).

TABLE 303. Physiological characteristics differentiating the species of *Actinomadura*[a, b]

Character	*A. madurae*	*A. atramentaria*	*A. catellatispora*	*A. citrea*	*A. coerulea*	*A. cremea*	*A. echinospora*	*A. fibrosa*	*A. formosensis*	*A. fulvescens*	*A. glauciflava*	*A. hallensis*	*A. hibisca*	*A. kijanata*	*A. latina*	*A. livida*	*A. luteofluorescens*	*A. macra*	*A. mexicana*	*A. meyerae*	*A. namibiensis*	*A. napierensis*	*A. nitritigenes*	*A. oligospora*	*A. pelletieri*	*A. rubrobrunea*	*A. rudentiformis*	*A. rugatobispora*	*A. spadix*	*A. umbrina*	*A. verrucosospora*	*A. vinacea*	*A. viridilutea*	*A. viridis*	*A. yumaenesis*
Arbutin hydrolysis	+	+	nd	+	−	+	+	nd	nd	+	nd	nd	nd	+	+	−	+	+	+	+	nd	+	+	+	−	−	+	nd	−	nd	+	nd	nd	+	+
Casein	+	+	−	+	+	+	+	+	nd	+	+	+	+	+	+	+	+	+	+	+	nd	+	+	+	+	nd	+	nd	+	nd	+	+	nd	+	+
Chitin	−	−	nd	−	−	−	−	nd	nd	+	nd	nd	nd	−	−	−	−	−	−	−	nd	nd	nd	−	−	+	nd	nd	−	nd	nd	nd	nd	−	−
DNA	+	+	nd	+	−	−	+	nd	nd	+	nd	+	nd	+	−	+	+	−	nd	nd	nd	nd	nd	+	−	nd	nd	nd	+	nd	+	−	nd	+	+
Esculin hydrolysis	+	+	+	+	+	+	+	+	nd	+	+	+	+	+	+	+	+	+	+	+	nd	nd	+	+	−	+	nd	nd	+	nd	+	+	nd	+	+
Elastin	+	+	nd	+	−	+	+	+	+	+	nd	nd	nd	+	d	−	−	+	+	−	nd	nd	+	−	+	nd	nd	nd	+	nd	+	nd	nd	+	+
Gelatin	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	+	+	+	+	+	+	+	+	nd	+	+	+	+	+
Guanine	+	−	nd	−	+	−	nd	−	nd	nd	nd	nd	nd	−	d	+	+	−	nd	nd	nd	nd	−	−	−	nd	nd	nd	−	nd	+	nd	nd	nd	−
Hypoxanthine	+	+	−	−	−	+	−	+	nd	+	+	−	+	+	−	−	+	+	−	+	nd	+	+	−	+	−	−	nd	−	nd	+	−	nd	−	+
Nitrate reduction	+	+	+	+	+	+	−	+	−	nd	+	−	−	−	nd	+	+	+	nd	nd	nd	nd	+	−	+	+	−	−	+	nd	−	−	+	nd	+
Starch	−	−	−	−	−	+	−	+	−	d	+	+	−	+	+	+	+	−	−	+	nd	+	−	−	−	+	+	+	−	nd	−	−	+	+	+
Testosterone	−	+	nd	+	−	+	−	+	nd	+	nd	nd	nd	+	d	−	+	−	−	nd	nd	nd	+	−	−	nd	nd	nd	−	nd	+	−	nd	−	−
Tributyrin	+	+	nd	+	+	+	−	nd	nd	+	nd	nd	nd	+	+	+	−	−	+	+	nd	nd	nd	+	+	nd	nd	nd	+	nd	+	nd	nd	+	−
Tyrosine	+	nd	−	+	−	+	−	+	nd	−	−	−	+	+	+	−	+	+	−	−	nd	+	d	−	+	−	−	nd	−	nd	+	−	nd	−	+
Urease production	−	−	−	−	−	+	nd	+	nd	nd	nd	+	−	+	nd	−	−	−	−	nd	−	nd	nd	+	−	nd	−	nd	−	nd	−	nd	nd	nd	nd
Xanthine	−	−	−	−	−	−	−	−	nd	−	−	−	+	+	+	−	−	−	−	−	nd	−	−	−	−	−	−	−	+	nd	−	−	nd	+	+

[a]Symbols: +, 90% or more strains positive; −, 10% or less strains positive; d, 11–89% of strains positive; nd, not determined.

[b]Data from Athalye (1981), Athalye et al. (1985), Goodfellow et al. (1979), Horan and Brodsky (1982), Kim (1999), Labeda et al. (1985), Lipski and Altendorf (1995), Lu et al. (2003), Mertz and Yao (1986, 1990), Quintana et al. (2003), Trujillo and Goodfellow (1997a, 2003), Cook et al. (2005), Lee and Jeong (2006), Le Roes and Meyers (2007), and Wink et al. (2003).

Fischer et al. (1983) carried out extensive DNA–DNA hybridization studies on *Actinomadura* species and recorded homology values in the range 18–100%. The highest values were between *Actinomadura madurae* strains (96%) and *Actinomadura pelletieri* strains (85–100%) and between two strains of *Actinomadura verrucosospora* (96%); homology values found between strains of *Actinomadura citrea*, *Actinomadura madurae*, *Actinomadura pelletieri*, and *Actinomadura verrucosospora* ranged from 25% to 44%. In a continuation of these studies Poschner et al. (1985) found that homology values between *Actinomadura citrea*, *Actinomadura coerulea*, *Actinomadura cremea*, *Actinomadura madurae*, and *Actinomadura pelletieri* were in the range 27–48%. *Actinomadura glauciflava* shares DNA–DNA relatedness values of 53% with *Actinomadura citrea*, 62% with *Actinomadura luteofluorescens* and *Actinomadura verrucosospora*, and 38% with *Actinomadura coerulea* (Lu et al., 2003).

Recent descriptions of *Actinomadura* species where DNA–DNA hybridization studies were perfomed underline the complex genetic relationships encompassed by members of this taxon. The type strains of *Actinomadura kijaniata* and *Actinomadura namibiensis* share a DNA relatedness value of 72% but can be distinguished using phenotypic data, especially on the basis of diagnostic carbon-utilization and enzyme patterns (Wink et al., 2003). Lee and Jeong (2006) reported that *Actinomadura hallensis* contains an additional extended helix (22 nt in length) between *Escherichia coli* positions 453 and 479 with a unique nucleotide composition suggesting that sequences of this region may prove useful for species identification.

Phages. The occurrence of *Actinomadura* phages has been reported in organic mulches used to support the growth of avocado trees in Australia (Kurtböke et al., 1993).

Pathogenicity. *Actinomadura latina*, *Actinomadura madurae*, and *Actinomadura pelletieri* cause human actinomycetoma in tropical and subtropical areas, particularly on the African and American continents. The natural habitat of pathogenic actinomadurae is thought to be the environment, namely the surface layers of soil. Actinomadurae from soil invade the human body. The disease, which is often induced by implantation of organisms into tissues by sharp objects, such as thorns or soil contaminated splinters, is characterized by a progressive swelling of the infected area, distortion of the normal anatomy, and multiple draining sinuses and fistulae. Purulent discharge containing the causative agent in the form of granules is characteristic of advanced stages of the disease. Almost 80% of infections enter through the lower extremities of the body. This probably explains the etiology of "Madura foot" described by Gill of Madura in South India in 1842 (Gill, 1842). However, other sites of the human body may be affected such as the back and the head. Non-mycetomic infections caused by *Actinomadura madurae* have been reported (Wust et al., 1990), including one involving an immunocompromised patient (McNeil et al., 1992).

Pathogenic actinomadurae are thought to have a worldwide distribution, but the disease is only a major health problem in tropical and subtropical countries (Fahal, 2006). It is endemic in localities lying between latitudes 15°S and 30°N, an area which includes India, Mali, Mexico, Senegal, Somalia, Sudan, and much of Central and South America (Fahal, 2006; Fahal and Hassan, 1992). There is evidence that the distribution of the causal organisms varies. In India, *Actinomadura madurae* is the predominant causal agent (Klokke et al., 1968; Venugopal and Venugopal, 1991) whereas on the African continent the prevalent organism is *Actinomadura pelletieri* (Develoux et al., 1988; Fahal, 2006; Yu et al., 1993). Infections by *Actinomadura madurae* have been reported from temperate climatic regions, such as Greece, the Netherlands, and the USA (Davis et al., 1999; de Hoog et al., 1993; Ispoglou et al., 2003). Climatic conditions may play a major part in the distribution of the causal agents of actinomycetoma (Develoux et al., 1988; Serrano et al., 1988). The endemic regions have a rainy season of 4–6 months with a relative humidity of 60–80% and temperatures of 30–37°C followed by a dry season of 6–8 months with a relative humidity of 12–30% and daytime temperatures of 45–60°C, which fall to 15–18°C at night (Magaña, 1984).

In general, the disease is four times more frequent in males than females and mainly affects 16–40-year-old adults, but cases involving children and elderly men do occur (El Moghraby, 1971; Lopez, 1993; Serrano et al., 1986). The disease typically affects farmers and herdsmen, though people in occupational activities such as carpentry, housework, and industry can be infected. The lower and upper extremities of the body are the main sites of infection, as the skin in these areas is more likely to be exposed to soil and puncture injuries with contaminated thorns (Goodfellow, 1996; Gumaa et al., 1986; Serrano et al., 1986).

Little information is available on the pathogenicity mechanisms of *Actinomadura*. According to Pulverer and Schaal (1978) it has not been possible to demonstrate the pathogenicity of these actinomadurae for laboratory animals. However, Rippon (1968) reported that virulent strains of *Actinomadura madurae* produce a collagenase that has a significant role in the pathogenicity of the organism.

Antibiotic sensitivity. Cases of actinomycetoma caused by pathogenic actinomadurae have been treated with a range of antibiotics, including amikacin, dapsone, streptomycin, and trimethoprim-sulfamethoxazole (Welsh et al., 1987). Boiron et al. (1992) used the disk diffusion method to assess the susceptibility of *Actinomadura madurae* and *Actinomadura pelletieri* strains against 29 microbial agents and found that they were particularly sensitive to aminocycline, amikacin, imipenem, tobramycin, and vancomycin. Vera-Cabrera et al. (2004) examined 24 *Actinomadura madurae* strains isolated from patients with actinomycetoma for their sensitivity to new quinolones, including garenoxacin, gatifloxacin, moxifloxacin, and two oxazolidinones, linezolid and the compound, DA-7867, and found that all but one of the strains showed high susceptibility to all of these agents; they were most active to DA-7867 showing a minimum inhibitory concentration of 0.06 µg/ml. However, further work needs to be done to determine whether these results can be extrapolated to antibiotic activity in subcutaneous abscesses and granulomas that are formed in cases of mycetoma. Trujillo and Goodfellow (2003) found that most *Actinomadura* strains were resistant to ampicillin and rifampin, and apart from *Actinomadura oligospora* and *Actinomadura yumaensis* were susceptible to tetracycline.

Ecology. Most *Actinomadura* species have been isolated from soil where they probably have a role in organic matter turnover (Galatenko and Preobrazhenskaya, 1981; Horan and Brodsky, 1982; Labeda et al., 1985; Lavrova et al., 1972; Lee

and Jeong, 2006; Lu et al., 2003; Meyer, 1979, 1989a, 1989b; Miyadoh et al., 1987, 1989; Nonomura and Ohara, 1971c; Preobrazhenskaya et al., 1975b; Quintana et al., 2003; Wink et al., 2003). Preobrazhenskaya and her colleagues (Chormonova and Preobrazhenskaya, 1981; Galatenko et al., 1987; Galatenko and Preobrazhenskaia, 1981) studied the occurrence and frequency of *Actinomadura* species in different soils. A comparison of chernozem, dark chestnut, and sierozem soils in Kazakhstan and Turkmenistan showed that the number of *Actinomadura* strains was higher in cultivated than in uncultivated soils. The highest numbers of actinomadurae were isolated from chernozem soil (Kazakhstan) though *Actinomadura citrea* was the most prevalent species followed by *Actinomadura cremea* and *Actinomadura verrucosospora*. The dark chestnut soil (cultivated) contained approximately half the number of actinomadurae found in the chernozem soil, but the species diversity of the two soils was similar. The lowest numbers of actinomadurae were isolated from sierozem soil (Turkmenistan). The frequency of actinomadurae appears to depend upon the humus content of the soil. *Actinomadura rubrobrunea* was isolated from Egyptian soils in which maize and rice had been cultivated (Agre and Guzeva, 1975), and *Actinomadura nitritigenes* was isolated from a laboratory scale biofilter that contained tree bark compost as the packing material and which was supplied with ammonia (Lipski and Altendorf, 1995). There is evidence that clinically and ecologically significant actinomadurae are underspeciated (Trujillo and Goodfellow, 2003).

Actinomadura latina, *Actinomadura madurae*, and *Actinomadura pelletieri* strains have been isolated mainly from clinical material, though there is evidence that members of *Actinomadura madurae* are widespread in organically rich soils. *Actinomadura madurae* strains isolated from environmental samples tend to lack the red endopigment of clinical isolates and sporulate more readily (Gerber, 1971, 1973; Kroppenstedt and Goodfellow, 1992; Lechevalier et al., 1971). *Actinomadura latina* and *Actinomadura pelletieri* have only been found in clinical specimens.

Enrichment and isolation procedures

Many different media have been employed for the isolation of *Actinomadura* strains, especially from soil samples. Those that have proved to be the most effective include egg albumin (Lawson and Davey, 1972), glycerol-asparagine (ISP medium 5; Shirling and Gottlieb, 1966), oatmeal (ISP medium 3; Shirling and Gottlieb, 1966), inorganic salts-starch (ISP medium 4; Shirling and Gottlieb, 1966) and yeast extract-malt extract (ISP medium 2; Shirling and Gottlieb, 1966) agars. Strains may be isolated from agar plates after incubation for up to 6 weeks. Enrichment of actinomadurae from soil can be achieved using pretreatment regimes and selective media. Nonomura and Ohara (1971d) reduced the number of unwanted micro-organisms by air-drying soil, applying dry heat at 100°C for 1 h before plating diluted soil suspensions onto several media, notably arginine-vitamins (AV) and mineral-glucose-asparagine (MGA) agars and incubating for several weeks at 28–30°C. Isolation media should be supplemented with antifungal antibiotics such as cycloheximide to inhibit fungal growth.

Lavrova et al. (1972) and Preobrazhenskaya et al. (1975b) increased the number of actinomadurae isolated from soils by the addition of antibiotics to medium 2 of Gauze et al. (1957); these antibiotics inhibited the growth of bacteria and more frequently occurring streptomycetes thereby providing more favorable conditions for the slow-growing actinomadurae. Bruneomycin (0.5, 1.0, or 2.0 μg/ml), rubomycin (5.0, 10.0, or 20.0 μg/ml), and streptomycin (0.5, 1.0, or 2.0 μg/ml) proved to be the most effective antibiotics. Athalye et al. (1981) combined drying and heat pretreatment regimes with the use of rifampin (5 μg/ml) as the selective agent for the isolation of *Actinomadura* strains from diverse environmental samples. The mean numbers of actinomadurae recovered were higher using this treatment than obtained with untreated samples. Trujillo and Goodfellow (2003) isolated novel *Actinomadura* species from environmental samples collected from diverse geographical locations (Hong Kong, Kenya, Mexico, South Africa, and Venezuela) providing further evidence that the selective isolation procedure recommended by Athalye et al. (1981) is effective.

Pathogen *Actinomadura* species can be isolated from clinical samples, such as pus and biopsy material, using Brain Heart Infusion (Schaal, 1972), Sabouraud glucose (Gordon, 1974) and yeast extract agars (Pridham et al., 1956, 1957). All cultures should be incubated aerobically at 25–27°C and at 36°C for up to 3 weeks and examined both macroscopically and microscopically for growth every 2 d (Schaal, 1984). Actinomadurae can be recognized by their filamentous appearance, leathery colonies, and by the production of red prodiginine pigments. Actinomycetoma granules should be washed in sterile tap water before they are crushed to obtain material for inoculation of culture media.

Maintenance procedures

Heavily sporulating cultures are needed to maintain high viability irrespective of the preservation method. The highest survival rates for nonsporulating cultures are achieved using cells from the exponential growth phase. The culture age of the actinomadurae to be preserved is very important, especially for the preservation of thermophilic strains, as these lyse at the stationary phase of growth. Sporulated actinomadurae can be maintained on oatmeal agar, or other appropriate media, at 4°C and transferred every four months for short-term preservation; tubes should be sealed with silicone stoppers to prevent the agar drying out. Medium-term preservation for up to four years can be achieved by mixing spore suspensions for homogenized mycelia with glycerol (45%, v/v) and storing at –25°C (Zippel and Neigenfind, 1988). Long-term storage can be achieved by lyophilization or storage in liquid nitrogen. For lyophilization, spores and mycelia are suspended in a suitable fluid, such as 7.5% (w/v) glucose serum or 10% skimmed milk. For storage in liquid nitrogen, the micro-organisms are inoculated into small vials containing an appropriate medium, incubated until satisfactory growth is visible when the tubes are tightly sealed and placed in a liquid nitrogen container. An alternative simple and practical method involves nitrogen cryopreservation of material in small polyvinyl chloride tubes ("straws") at –196°C (Hoffmann, 1989a, 1989b).

Differentiation from closely related taxa

Differentiating characteristics of the genus *Actinomadura* and other closely related taxa are presented in Table 298, Family *Thermomonosporaceae*. The genus *Actinomadura* can be readily separated from other members of the family *Thermomonosporaceae*

and from other closely related taxa using chemotaxonomic, morphological, and 16S rRNA gene sequence data. The phospholipid pattern is an important criterion for differentiating between members of the genera *Actinomadura* and *Actinocorallia*.

Taxonomic comments

The genus *Actinomadura* was described by Lechevalier and Lechevalier (1970) to accommodate aerobic actinomycetes that had a wall chemotype III and formed a branched substrate mycelium with or without a secondary mycelium that may bear chains of arthrospores. The type species was defined as *Actinomadura madurae* (Vincent 1894) Lechevalier and Lechevalier 1970. *Actinomadura dassonvillei* (Brocq-Rousseu 1904) Lechevalier and Lechevalier 1970, and *Actinomadura pelletieri* (Laveran 1906) Lechevalier and Lechevalier 1970 were also included in the genus.

Actinomadura madurae was first described in 1894 by Vincent as *Streptothrix madurae*, based on isolates from an Algerian case of Madura foot. This combination proved to be illegitimate and Blanchard (1896) transferred the organism to the genus *Nocardia* Trevisan, hence *Nocardia madurae* (Vincent 1894) Blanchard 1896 is the oldest legitimate name of this micro-organism. The organism was subsequently assigned to many genera, including *Streptomyces* (Lacey et al., 1978). The inclusion of *Actinomadura madurae* in either *Nocardia* or *Streptomyces* became untenable when it was shown that whole-organism hydrolysates contained *meso*-A_2pm and a previously unknown sugar, madurose, later identified as 3-*O*-methyl-D-galactose (Becker et al., 1965; Lechevalier, 1968; Lechevalier and Gerber, 1970). In contrast, members of the genus *Nocardia* contained A_2pm, arabinose, and galactose, and *Streptomyces* strains the LL isomer of A_2pm. *Actinomadura dassonvillei* and *Actinomadura pelletieri* have similar taxonomic histories, although *Actinomadura pelletieri* was first placed in the genus *Micrococcus* by Laveran (1906) because the colonies fragmented into cocci suggesting a *Rhodococcus* species. *Actinomadura pelletieri* was subsequently classified in the genus *Nocardia* Pinoy 1912 then in the genus *Streptomyces* Waksman and Henrici 1948. *Actinomadura dassonvillei* was seen to differ from the other two members of the genus *Actinomadura* in its greater vigor and sporulation and lack of madurose. The genus *Nocardiopsis* was subsequently proposed to accommodate *Actinomadura dassonvillei* strains (Meyer, 1976).

Numerical phenetic (Alderson and Goodfellow, 1979; Athalye et al., 1985; Goodfellow, 1971; Goodfellow et al., 1979; Goodfellow and Pirouz, 1982), chemical (Athalye et al., 1984; Collins et al., 1977; Fischer et al., 1983; Lechevalier et al., 1977; Minnikin et al., 1977; Yamada et al., 1977), and 16S rRNA gene sequence data (Goodfellow et al., 1988; Kroppenstedt et al., 1990) confirmed the separation between the genus *Actinomadura* and the genera *Nocardia* and *Nocardiopsis*, and suggested that *Actinomadura madurae* and *Actinomadura pelletieri* were heterogeneous taxa. There was also evidence that the taxon could be separated into two aggregate taxa, the *Actinomadura madurae* and the *Actinomadura pusilla* groups.

Fischer et al. (1983) provided compelling evidence that the genus *Actinomadura* was heterogeneous when they assigned representative strains to two aggregate groups defined using chemotaxonomic and nucleic acid pairing data. rRNA partial oligonucleotide sequencing (Fowler et al., 1985; Goodfellow et al., 1988), menaquinone (Athalye et al., 1984), polar lipid (Agre et al., 1975; Lechevalier et al., 1977), numerical taxonomic (Athalye et al., 1985; Goodfellow and Pirouz, 1982), and DNA homology studies (Poschner et al., 1985) all underlined this division. *Actinomadura madurae* and related species were seen to have a closer affinity to *Thermomonospora curvata* than to *Actinomadura pusilla* and allied taxa that were found to be related to *Streptosporangium roseum* (Fowler et al., 1985).

The division of the genus *Actinomadura* into two aggregate groups was formally recognized by Kroppenstedt et al. (1990) who proposed that the genus *Actinomadura* Lechevalier and Lechevalier 1970 be retained for *Actinomadura madurae* and related species, and that the *Actinomadura pusilla* group be reclassified in the genus *Microtetraspora* Thiemann et al. 1968. This division was supported by a polyacrylamide gel electrophoresis analysis of the ribosomal protein AT-L30 which exhibits electrophoretic mobility that is specific at the genus level (Ochi et al., 1991).

By 1991, the genus *Actinomadura* contained 34 validly described species recognized primarily by chemical and morphological features (Mertz and Yao, 1990; Meyer, 1989a, 1989b; Miyadoh et al., 1987; Terekhova et al., 1987). A phylogenetic analysis based on 16S rRNA gene sequences of *Actinomadura* type strains was carried out by Wang et al. (1996) who supported Kroppenstedt et al. (1990) in classifying members of the *Actinomadura pusilla* group in the genus *Microtetraspora*. These workers also proposed the transfer of *Microbispora echinospora* and *Microbispora viridis* to the genus *Actinomadura* as *Actinomadura echinospora* and *Actinomadura rugatobispora*, respectively (Miyadoh et al., 1990). Subsequently, the genus *Nonomuraea* was proposed for membership in the *Actinomadura pusilla* group following extensive comparative 16S rRNA gene sequencing studies (Zhang et al., 1998), a proposition first raised by Goodfellow et al. (1988).

Zhang and his colleagues also demonstrated that *Thermomonospora formosensis* was related to several *Actinomadura* species on the basis of chemotaxonomic and 16S rRNA gene sequence data (Goodfellow, 1989; Kroppenstedt et al., 1990; Kroppenstedt and Goodfellow, 1992; Kudo, 1997). In a continuation of these studies, Zhang et al. (2001) proposed that *Actinomadura aurantiaca*, *Actinomadura glomerata*, *Actinomadura libanotica*, and *Actinomadura longicatena* be transferred to the new genus *Actinocorallia* as *Actinocorallia aurantiaca*, *Actinocorallia glomerata*, *Actinocorallia libanotica*, and *Actinocorallia longicatena*.

Zhang et al. (2001) considered that *Excellospora viridilutea* should be reclassified in the genus *Actinomadura* as *Actinomadura viridilutea*. They also noted that *Spirillospora albida*, the type species of the genus, was closely related to some *Actinomadura* species on the basis of 16S rRNA gene sequence data. Further studies are needed to unravel relationships between *Actinomadura* and *Spirillospora* species. In contrast, *Actinomadura echinospora* and *Actinomadura umbrina* form a clade with *Thermomonospora curvata* based on 16S and 23S rRNA gene sequence data; further comparative taxonomic investigations are needed to resolve the status of these taxa. Finally, numerical taxonomic (Athalye et al., 1985; Trujillo and Goodfellow, 2003) and 16S and 23S rRNA gene sequence data (Zhang et al., 2001) of *Actinomadura*-related actinomycetes together with phylogenetic data (Zhang et al., 2001) indicate that *Actinomadura spadix* is sharply separated from other *Actinomadura* species, a result that is supported by polyacrylamide gel electrophoresis analysis of

the ribosomal protein AT-L30 (Ochi et al., 1991). It seems likely that *Actinomadura spadix* will be found to merit recognition as a new genus.

In summary, the systematics of the genus *Actinomadura* has been markedly improved since the 2nd edition of *Bergey's Manual of Systematic Bacteriology*. The taxon currently encompasses 35 validly published species, though it seems likely that some of the present members will be reclassified in the future.

Differentiation of the species of the genus *Actinomadura*

Published descriptions of *Actinomadura* species are often incomplete since different investigators emphasize some phenotypic features and omit others. This makes identification difficult. Nevertheless, most species can be separated using a combination of morphological and physiological properties (Table 301, Table 302, and Table 303), though in most cases only the type strain has been studied. However, even when several strains have been studied (e.g. *Actinomadura madurae*, *Actinomadura pelletieri*), the results tend to be variable or inconsistent when those from the literature are compared.

Enzymic substrates based on the fluorophores 4-methylumbelliferone (4-MU) and 7-amino-methylcoumarin (7-AMC) were carried out by Trujillo and Goodfellow (2003) to differentiate *Actinomadura* species. Encouraging results were obtained for differentiating between pathogenic *Actinomadura* species (Table 304) (Goodfellow et al., 1995; Trujillo and Goodfellow, 2003). Enzymic tests were demonstrated by Wink et al. (2003) to differentiate *Actinomadura kijaniata* from *Actinomadura namibiensis*, which share a DNA homology higher than 70%.

TABLE 304. Phenotypic characters separating pathogenic *Actinomadura* species[a,b]

Characteristic	*A. latina*	*A. madurae*	*A. pelletieri*
Degradation tests:			
Arbutin	+	+	−
Esculin	+	+	−
Starch	+	−	+
Cleavage of 7-amino-4-methylcoumarin (7AMC-) substrates:			
Glutaryl-L-phenylalanine-7AMC	−	+	+
L-Pyroglutamide-7AMC	+	+	−
Cleavage of 4-methylumbelliferone (4MU-) substrates:			
4MU-*N*-acetyl-D-galactosoamide	−	d	+
4MU-α-L-arabinopyranoside	+	+	−
4MU-β-D-cellopyranoside	+	+	−
4MU-β-D-galactoside	−	+	−
4MU-β-D-glucoside	+	+	−
4MU-β-D-glucuronide	+	+	−
4MU-β-D-lactoside	−	+	−
4MU-sulfate	−	+	−
4MU-xyloside	+	+	−
Growth on sole carbon sources (1%, w/v):			
Adonitol	−	+	−
Fructose	+	+	−
Galactose	+	+	−
Glycerol	+	+	−
Mannitol	+	+	−
Melezitose	+	−	−

[a]Symbols: +, 90% or more strains positive; −, 90% or more strains negative; d, 11–89% of strains positive.

[b]Data from Trujillo and Goodfellow (1997a, 2003).

Acknowledgements

The authors are particularly indebted to Dr J. Meyer as they drew upon material from the section on *Actinomadura* she wrote for the previous edition of this Manual. They also acknowledge help from Dr L.I. Evtushenko and Dr J. Euzéby for translating some material written in Russian and for revising the etymology of some names, respectively. We also thank Dr P.R. Meyers and Dr E.T. Quintana for reading the manuscript.

List of species of the genus *Actinomadura*

1. **Actinomadura madurae** (Vincent 1894) Kroppenstedt, Goodfellow and Stackebrandt, 1990, 156[AL] (*Streptothrix madurae* Vincent 1894, 132)

ma.du'ra.e. N.L. gen. n. *madurae* of Madura, name of a district in India.

Spore chains short (3–12 spores per chain), hooked or curled, in clusters directly emerging from the agar surface or borne on long aerial hyphae. Spores are elliptical to round. Spore surface warty (Figure 404). Inorganic salts-starch agar*: poor growth; surface granular; aerial mycelium absent; substrate mycelium grayish white, and no diffusible pigment. Oatmeal agar: good growth; surface leathery; aerial mycelium absent or sparse white specks; substrate mycelium colorless center, edge often red, and no diffusible pigment. Peptone-glucose medium: poor growth; surface cartilaginous; aerial mycelium absent; substrate mycelium dark pink to red, and no diffusible pigment. Yeast extract-malt extract agar: moderate growth; surface cartilaginous; aerial mycelium absent; substrate mycelium dark pink to brownish violet, and no diffusible pigment. Growth at 10–45°C, optimally at 28–37°C. Tweens 20, 40, and 60 are degraded. Resistant to ampicillin (5 μg/ml) and carbenicillin (120 μg/ml), but susceptible to lincomycin (10 μg/ml) and tobramycin (1 μg/ml). Additional phenotypic data are shown in Table 302 and Table 303.

Source: clinical specimens (mycetoma) and from soil.

DNA G+C content (mol%): 66.0–68.2 (T_m) (type strain, 68.2).

Type strain: ATCC 19425, CCM 136, CCUG 32944, CECT 3043, CIP 105487, DSM 43067, HAMBI 1926, IAM 14277, IFM 0585, NBRC 14623, JCM 7436, IMET 9585, IMRU 1190, KCTC 9192, NCIMB 13469, NCTC 5654, NRRL B-3843, VKM Ac-809.

*Media used are the following: glycerol-asparagine agar (ISP 5), inorganic salts-starch agar (ISP 4), oatmeal agar (ISP 3), tyrosine agar (ISP 7), and yeast extract-malt extract agar (ISP 2) (Shirling and Gottlieb, (1966)); glucose-asparagine agar (Lindenbein, 1952), inorganic salts-starch agar (Gauze 1) (Gauze et al., 1957);

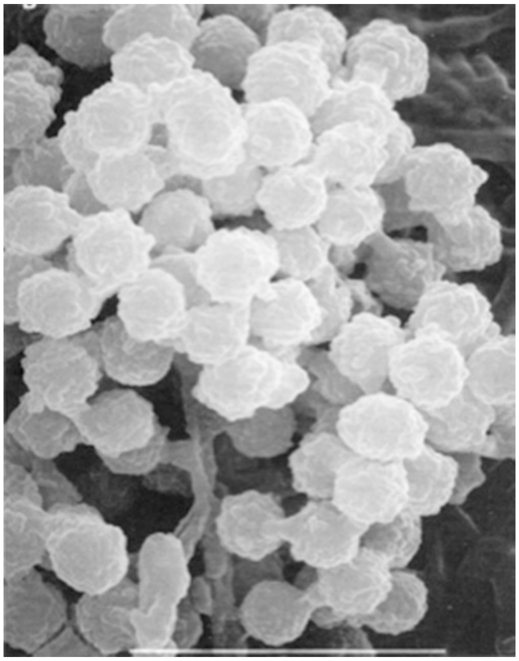

FIGURE 404. *Actinomadura madurae*, strain RG 1091. Spore chain, electron micrograph (16,500×).

Sequence accession no. (16S rRNA gene): U58527, X97889.
Sequence accession no. (23S rRNA gene): AF1162290.
Sequence accession no. (16S–23S rRNA ITS): AF134103.

2. **Actinomadura atramentaria** Miyadoh, Amano, Tohyama and Shomura 1987, 345[VP]

a.tra.men.ta′ri.a. N.L. fem. adj. *atramentaria* (from L. n. *atramentum* any black liquid, ink) inky, referring to the inky brown diffusible pigments.

Spores are borne in longitudinal pairs or in straight chains of three or rarely four spores. Chains develop into thick tufts on the aerial hyphae. Straight chains of three to five spores are occasionally observed at the tips of sporulating aerial hyphae. Spores are oval to ellipsoidal (0.6–0.8 × 0.8–1.5 µm). Spore surface smooth (Figure 405). Glycerol-asparagine agar: good growth; moderate white aerial mycelium; substrate mycelium dark brown, and pale brown diffusible pigment. Inorganic salts-starch agar: poor growth; moderate white aerial mycelium; substrate mycelium colorless, and no diffusible pigment. Oatmeal agar: poor growth; thin white aerial mycelium; substrate mycelium colorless, and no diffusible pigment. Tyrosine agar: moderate growth; moderate white aerial mycelium; substrate mycelium dark brown, and inky brown soluble pigment. Yeast extract-malt extract agar: good growth; moderate white aerial mycelium; substrate mycelium dark brown, and inky brown diffusible pigment. Growth at 15–42°C and optimally at 28–37°C. Nitrate is reduced, milk is peptonized. Grows in the presence of NaCl 7%, w/v and at pH 12. Resistant to ampicillin (5 µg/ml), carbenicillin (100 µg/ml) and ticarcillin (80 µg/ml), but susceptible to tetracycline (20 µg/ml). Additional phenotypic data are presented in Table 302 and Table 303.

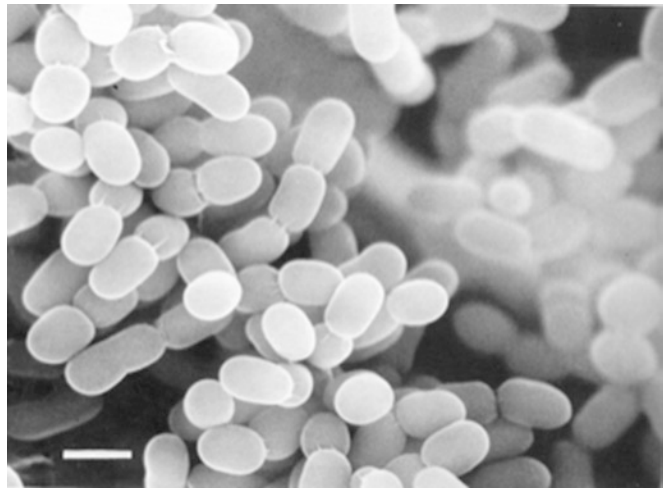

FIGURE 405. Electron micrograph of *Actinomadura atramentaria* strain SF 2197. Bar = 1 µm. (Reproduced with permission from S. Miyadoh, S. Amano, and T. Shomura. Atlas of Actinomycetes, Society for Actinomycetes Japan.)

Source: soil.
DNA G+C content (mol%): 72 (T_m).
Type strain: SF2197, DSM 43919, HUT 6547, NBRC 14695, JCM 6250, KCTC 9620, NCIMB 12618.
Sequence accession no. (16S rRNA gene): U49000.
Sequence accession no. (23S rRNA gene): AF134071.
Sequence accession no. (16S–23S rRNA ITS): AF134089.

3. **Actinomadura catellatispora** Lu, Wang, Zhang, Shi, Liu, Quintana and Goodfellow 2003, 140[VP]

ca.tel.la.ti.spo′ra. L. n. *catella* small chain; Gr. n. *spora* a seed, and in biology a spore; N. L. fem. n. *catellatispora* organism forming small chains of spores.

Short straight chains of spores (0.85 µm diameter) formed on the aerial mycelium. Spore surface smooth. Light-yellow substrate mycelium and a yellow aerial mycelium formed on yeast extract-malt extract agar. Diffusible pigments are not formed. Grows at 18–35°C. Additional phenotypic properties are shown in Table 302 and Table 303.

Source: a mud sample taken from a sewage ditch in Southern China.
DNA G+C content (mol%): 70.8 (T_m).
Type strain: 3.24, AS 4.1522, DSM 44772, NBRC 16341, JCM 10667.
Sequence accession no. (16S rRNA gene): AF154127.

4. **Actinomadura citrea** Lavrova, Preobrazhenskaya and Svesnikova 1972, 967[AL]

ci′tre.a L. fem. adj. *citrea* of or pertaining to the citrus-tree, intended to mean lemon-yellow; referring to the lemon-yellow color of the substrate mycelium.

Spore chains (3–9 spores per chain) short, hooked or curled, in sparse clusters on moderately branched aerial mycelium. Spore surface irregular or warty. Inorganic salts-starch agar: moderate growth; surface leathery; traces of yellow aerial mycelium turning blue with age; substrate mycelium yellowish brown, and diffusible pigment yellow.

Oatmeal agar: abundant growth; surface leathery; traces of yellowish aerial mycelium turning blue with age; substrate mycelium lemon yellow, and diffusible pigment yellow. Peptone-glucose medium: good growth; surface leathery; surface filmy cover of aerial mycelium consisting of sterile hyphae and coremia; substrate mycelium yellow, and no diffusible pigment. Yeast extract-malt extract agar: moderate growth; surface leathery; filmy cover of aerial mycelium consisting of sterile hyphae; substrate mycelium yellowish brown, and no diffusible pigment. Grows at 10–37°C and optimally at 28–30°C. RNA is degraded, but not xylan. Glycogen, maltose, and trehalose are used as carbon sources. Resistant to ampicillin (5 μg/ml), rifampin (10 μg/ml), and ticarcillin (80 μg/ml). Other phenotypic characteristics are shown in Table 302 and Table 303.

Source: soil.

DNA G+C content (mol%): 67.6 (T_m).

Type strain: ATCC 27887, BCRC (formerly CCRC) 13352, DSM 43461, NBRC 14678, IMET 9573, INA 1849, JCM 3295, KCTC 9617, NRRL B-16121, VKM Ac-1119.

Sequence accession no. (16S rRNA gene): U49001, AJ420139.

Sequence accession no. (23S rRNA gene): AF116216.

Sequence accession no. (16S–23S rRNA ITS): AF134091.

5. **Actinomadura coerulea** Preobrazhenskaya, Lavrova, Ukholina and Nechaeva 1975b, 404[AL]

co.e.ru′le.a. L. fem. adj. *coerulea* azure, blue, dark blue, referring to the blue aerial mycelium.

Spore chains curled, hooked or in spirals of one turn, arranged in tufts on long aerial hyphae. Spore surface warty. Inorganic salts-starch agar (Gauze 1): poor growth; surface slightly farinaceous; aerial mycelium pale blue; substrate mycelium colorless, and no diffusible pigment. Oatmeal agar: good growth; surface slightly farinaceous; aerial mycelium pale pink turning blue at maturity; substrate mycelium colorless or pale pink, and no diffusible pigment. Oatmeal-nitrate agar: good growth; surface slightly farinaceous; aerial mycelium white; substrate mycelium white, and no diffusible pigment. Yeast extract-malt extract agar: moderate growth; surface granular with a filmy cover of aerial mycelium consisting of sterile hyphae and coremia; substrate mycelium pale brown, and no diffusible pigment. Grows at 10–37°C and optimally at 28–30°C. RNA is degraded. Arginine and glutamine are used as sole carbon sources. Resistant to ampicillin (10 μg/ml), cephaloridine (10 μg/ml), cephalotin (35 μg/ml), cephamandole (40 μg/ml), and ticarcillin (80 μg/ml), but is susceptible to tetracycline (20 μg/ml). Other phenotypic properties are shown in Table 302 and Table 303.

Source: soil.

DNA G+C content (mol%): 67.0 (T_m).

Type strain: ATCC 33576, DSM 43675, NBRC 14679, IMET 9580, INA 765, JCM 3320, KCTC 9337, VKM Ac-1511.

Sequence accession no. (16S rRNA gene): U49002

Sequence accession no. (23S rRNA gene): AF116217.

Sequence accession no. (16S–23S rRNA ITS): AF134092.

6. **Actinomadura cremea** Preobrazhenskaya, Lavrova, Ukholina and Nechaeva 1975b, 404[AL]

cre′me.a. N.L. fem. adj. *cremea* cream-colored (referring to the color of the aerial mycelium).

Spore chains short, in hooks or spirals of one turn, 3–8 spores per chain arranged in clusters. Spore surface warty. Inorganic salts-starch agar: poor growth; surface granular; aerial mycelium cream-colored to pale pink; substrate mycelium colorless, and no diffusible pigment. Oatmeal agar: moderate growth; surface farinaceous; aerial mycelium white to yellowish white; substrate mycelium colorless, and no diffusible pigment. Peptone-glucose medium: good growth; surface granular; aerial mycelium white to cream-colored; substrate mycelium brown, and no diffusible pigment. Yeast extract-malt extract agar: moderate growth; surface farinaceous; aerial mycelium white; substrate mycelium light brown, and no diffusible pigment. Grows at 20–37°C. Resistant to ampicillin (15 μg/ml), carbenicillin (120 μg/ml), cephaloridine (10 μg/ml), cephalotin (35 μg/ml), cephamandole (40 μg/ml), and ticarcillin (80 μg/ml), but is susceptible to tetracycline (20 μg/ml). Additional phenotypic properties are shown in Table 302 and Table 303.

Source: soil.

DNA G+C content (mol%): 68.0 (T_m).

Type strain: ATCC 33577, BCRC (formerly CCRC) 13394, DSM 43676, NBRC 14182, IMET 9578, INA 292, JCM 3308, NRRL B-16605, VKM Ac-912.

Sequence accession no. (16S rRNA gene): AF134067.

Sequence accession no. (23S rRNA gene): AF134073.

Sequence accession no. (16S–23S rRNA ITS): AF134094.

Additional comments: Gauze et al. (1975) isolated a rifamycin-producing strain (INA 1349) which they described as *Actinomadura cremea* subsp. *rifamycini*. This name was subsequently attributed to Gauze et al. (1987). Euzéby and Kudo (2001) pointed out that this taxon should be attributed to Gauze et al. (1975). However, apart for the production of rifamycin, there are no characteristics that distinguish this strain from *Actinomadura cremea*. Rifamycin is also produced by some *Nocardia* and *Streptomyces* strains.

7. **Actinomadura echinospora** (Nonomura and Ohara 1971c) Miyadoh, Amano, Tohyama and Shomura 1989, 1909[VP] (Basonym: *Microbispora echinospora* Nonomura and Ohara 1971c, 891[AL].)

e.chi.no.spo′ra. L n. *echinus* sea urchin; Gr. n. *spora* a seed, and in biology a spore; N.L. fem. n. *echinospora* spiny spore.

Aerial hyphae differentiate into a non-sporogenous main axis which bears spore-bearing side branches. Spores are formed in clusters. Initially two but sometimes three spores per chain. Spore surface spiny (Figure 406). A yellowish orange to dark yellow substrate mycelium and soluble yellow or yellowish-brown pigments are formed on oatmeal agar. A yellowish pink aerial spore mass and a yellowish brown substrate mycelium are produced on inorganic salts-starch agar-V. The temperature range for optimal growth is 35–40°C; growth is very poor at 25°C. Tween 60 is degraded. Resistant to ampicillin (15 μg/ml), carbenicillin (90 μg/ml), and streptomycin (16 μg/ml), but is susceptible to ticarcillin (70 μg/ml) and tetracycline (20 μg/ml). Requires vitamin B for growth. Additional phenotypic properties are shown in Table 302 and Table 303.

Source: soil.

DNA G+C content (mol%): 74 (T_m).

Type strain: ATCC 27300, BCRC (formerly CCRC) 12547, DSM 43163, HUT 6548, NBRC 14042, JCM 3148, KCTC 9313.

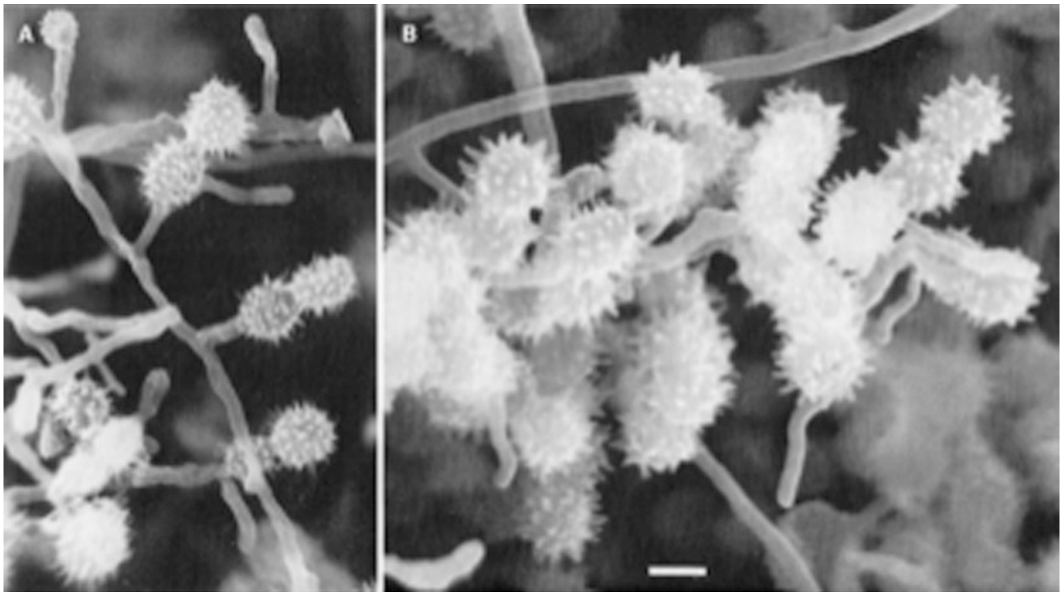

FIGURE 406. Electron micrograph of *Actinomadura echinospora* JCM 3148T. Aerial hyphae bear lateral branches where spores are produced. Spore surface is spiny. Bar = 1 μm. (Reproduced with permission from S. Amano and S. Miyadoh (A) and M. Hayakawa, H. Iino, and H. Nonomura (B). Atlas of Actinomycetes, Society for Actinomycetes Japan.)

Sequence accession no. (16S rRNA gene): U49004, AJ420135.

Sequence accession no. (23S rRNA gene): AF134075.

Sequence accession no. (16S–23S rRNA ITS): AF134095.

8. **Actinomadura fibrosa** Mertz and Yao 1990, 31VP

fi.bro′sa. L. n. *fibra* a fiber, filament; L. suff. *-osus -a -um* suffix used with the sense of full of, prone to; N.L. fem. adj. *fibrosa* fibrous, referring to the fibrous appearance of the aerial hyphae.

An extensive, reddish brown to reddish orange substrate mycelium is formed. Aerial hyphae are rarely produced except on modified Bennett's, Bennett tomato paste, oatmeal, and yeast extract-malt extract agars. When produced, the aerial mycelium is pink to white. Does not usually form soluble pigments. Grows well on modified Bennett's, glycerol-asparagine, inorganic salts-starch, tyrosine, and yeast extract-malt extract agars. Forms thick fibers. Has not been observed to produce spores. Grows at 20–45°C and optimally at 37°C. Does not degrade guanine. Produces catalase and phosphatase, and liquefies gelatin and skim milk. Acetate, formate, lactate, propionate, pyruvate, and sucrose are used as sole carbon sources. Resistant to cephalotine (30 μg/ml), lincomycin (2 μg/ml), rifampin (5 μg/ml), and lysozyme (50 μg/ml), but is susceptible to bacitracin (10 U), gentamicin (10 μg/ml), and tetracycline (30 μg/ml). Grows in the presence of NaCl (5%, w/v). Produces esterase (C_4), esterase (C_8), leucine arylamidase, naphthol-AS-BI-phosphohydrolase, β-glucosidase, and trypsin, but is negative for cystine arylmidase (API ZYM tests). Additional phenotypic properties are shown in Table 302 and Table 303.

Source: soil collected in West Africa.

DNA G+C content (mol%): not determined.

Type strain: A82810.1, ATCC 49459, DSM 44224, DSM 44224, JCM 9371, NRRL 18348.

Sequence accession no. (16S rRNA gene): AF163114, AJ293702.

Sequence accession no.(23S rRNA gene): AF163136.

Sequence accession no. (16S–23S rRNA ITS): AF163125.

9. **Actinomadura formosensis** (Hasegawa, Tanida and Ono 1986) Zhang, Wang and Ruan 1998, 418VP (*Thermonospora formosensis* Hasegawa, Tanida and Ono 1986, 22VP)

for.mo.sen′sis. N.L. fem. adj. *formosensis* of or belonging to Formosa (Republic of China).

Single spores are formed on unbranched sporophores on both aerial and substrate hyphae. The spores (diameter, about 1 μm) are heat sensitive, nonmotile, and globose. Spore surface warty. Glycerol-asparagine agar: moderate growth; aerial mycelium absent; substrate mycelium light apricot, and no diffusible pigment. Inorganic salts-starch agar: poor growth; aerial mycelium absent; substrate mycelium shell-colored, and no diffusible pigment. Oatmeal agar: poor or moderate growth; aerial mycelium poor and white when present; substrate mycelium light apricot, and no diffusible pigment. Peptone-yeast extract-iron agar: moderate growth; aerial mycelium absent; substrate mycelium cinnamon-colored, and no diffusible pigment. Tyrosine agar: moderate growth; aerial mycelium poor and white to flesh pink, and no diffusible pigment. Yeast extract-malt extract agar: aerial mycelium poor and white to flesh pink; substrate mycelium light to dusty orange, and no diffusible pigment. Growth temperature range is 23–41°C. Soluble starch and sucrose are used as sole carbon sources. Growth occurs on media supplemented with up to 2%, w/v NaCl. Susceptible to chloramphenicol, erythromycin, penicillin, tetracycline, and streptomycin. The type strain produces rifamycins O and S and is resistant to rifampin. Additional phenotypic properties are shown in Table 302 and Table 303.

Source: soil.

DNA G+C content (mol%): 72.0 (T_m).

Type strain: C-36820, ATCC 49059, CIP 105595, DSM 43997, NBRC 14204, NBRC 15870, JCM 7474, KCTC 9647, NCIMB 12773, NRRL B-16984, VKM Ac-1954.

Sequence accession no. (16S rRNA gene): AF002263, AJ293703.

Sequence accession no. (23S rRNA gene): AF116218.

Sequence accession no. (16S–23S rRNA ITS): AF134096.

10. **Actinomadura fulvescens** Terekhova, Galatenko and Preobrazhenskaya 1987, 179[VP] (Effective publication: Terekhova, Galatenko and Preobrazhenskaya 1982, 87.)

ful.ves′cens. N.L. part. adj. *fulvescens* becoming reddish-yellow.

Spore chains spiral. Spore surface smooth. Scanty white aerial mycelium, and a colorless or yellowish substrate mycelium is formed on synthetic media and a brownish-yellow substrate mycelium and soluble pigment of the same color on organic media. Grows well on modified Bennett's, Czapek's, oatmeal, and yeast extract-malt extract agars. Grows at 10–45°C. Degrades Tweens 20, 40, 60, and 80. Glutamine and histidine are used as sole carbon sources. Resistant to ampicillin (15 µg/ml), carbenicillin (100 µg/ml), cephalotin (35 µg/ml), and streptomycin (16 µg/ml), but is susceptible to tetracycline (20 µg/ml) and tobramycin (0.5 µg/ml). Additional phenotypic properties are shown in Table 302 and Table 303.

DNA G+C content (mol%): not determined.

Type strain: DSM 43923, NBRC 14347, IMET 9745, INA 3321, JCM 6833, KCTC 9339, NCTC B-16245, NRRL B-16245, VKM Ac-938.

Sequence accession no. (16S rRNA gene): U49005, AJ420137.

Sequence accession no. (23S rRNA gene): AF134076.

Sequence accession no. (16S–23S rRNA ITS): AF134097.

11. **Actinomadura glauciflava** Lu, Wang, Zhang, Shi, Liu, Quintana and Goodfellow 2003, 140[VP]

glau.ci.fla′va. L adj. *glaucus* bluish-green; L. adj. *flavus* yellow; N.L. fem. adj. *glauciflava* bluish-green yellow.

Curled, hooked or spiral spore chains. Spore surface, warty. A yellow-brown, non-fragmenting substrate mycelium is produced. An abundant bluish-green aerial mycelium and a yellow diffusible pigment are formed on yeast extract-malt extract agar. Grows at 18–35°C. Casein, esculin, gelatin, hypoxanthine, and starch are degraded, but not tyrosine or xanthine. Nitrate is reduced.

Source: soil collected in Yunnan Province, China.

DNA G+C content (mol%): 72.0 (T_m).

Type strain: strain 80-60, AS 4.1202, CIP 107902, DSM 44770, DSM 44770, NBRC 14668, JCM 6161, VKM Ac-1273.

Sequence accession no. (16S rRNA gene): AF153881, AB184612.

12. **Actinomadura hallensis** Lee and Jeong 2006, 262[AL]

hal.len′sis. N.L. fem. adj. *hallensis* pertaining to Mt Halla, Jeju Island, Republic of Korea, the origin of the soil sample from which the type strain was isolated.

Short hooked or spiral spore chains. Spore surface warty (Figure 407). Substrate mycelium is grayish reddish purple on inorganic salts-starch agar, and brown on oatmeal agar and blackish purple on yeast-extract-malt extract agar. A moderate amount of white aerial mycelium is formed on inorganic salts-starch and oatmeal agars. Good growth is formed on all of these media. The temperature range for growth is 20–45°C. Produces catalase. H_2S is produced. Does not degrade Tween 80. Sucrose is used as a sole carbon soruce. Other phenotypic properties are shown in Table 302 and Table 303.

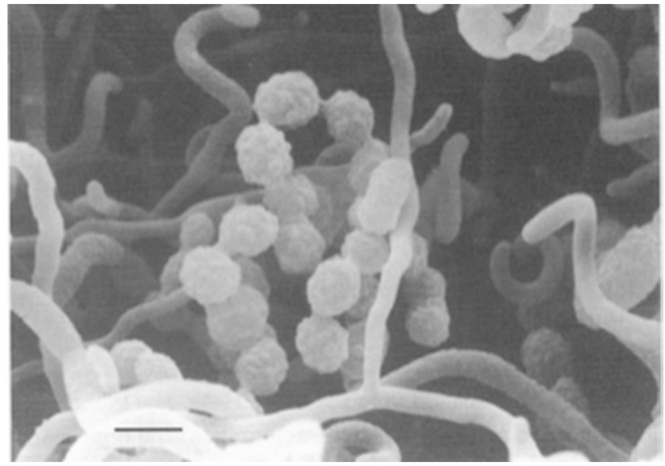

FIGURE 407. Electron micrograph of *Actinomadura hallensis* 647-1[T] grown on ISP 4 medium for 3 weeks. Bar = 0.8 µm. (Reproduced with permission from Lee and Jeong, 2006. Int. J. Syst. Evol. Microbiol. *56*: 259–264.)

Source: soil sample collected from Mt Halla, Jeju Island, Republic of Korea.

DNA G+C content (mol%): 67.5 (HPLC).

Type strain: H647-1, DSM 44987, IMSNU 50760, JCM 13882, KCCM 42245, KCTC 9992, NBRC 102110, NRRL B-24436.

Sequence accession no. (16S rRNA gene): DQ076484.

13. **Actinomadura hibisca** Tomita, Nishio, Saitoh, Yamamoto, Hoshino, Ohkuma, Konishi, Miyaki and Oki 1991, 758[AL] (Effective publication: Tomita, Nishio, Saitoh, Yamamoto, Hoshino, Ohkuma, Konishi, Miyaki and Oki 1990, 758.)

hi′bis.ca. N.L. n. *hibisca* (from Gr. n. *ibiskos* marsh mallow), rose mallow, a plant with reddish flower referring to the production of red diffusible pigments.

Long straight spore-chains (10–50 oblong spores per chain). Spore surface smooth. Coiled spore chains are occasionally formed at the tip of long chains. Pinkish white, deep red, or light brown substrate mycelium is produced on glycerol-asparagine, tyrosine and yeast-extract malt extract agars, respectively. A scant white to light pink aerial mycelium is formed on all of these media. Abundant growth is obtained on glycerol-asparagine agar, and moderate growth on glucose-asparagine and yeast extract-malt extract agars. A reddish pigment due to the production of pradimicins is produced abundantly on peptone-yeast extract-iron and yeast extract-malt extract agars. Produces melanoid pigment on tyrosine agar though growth is poor. Grows at 18–40°C. Adenine is degraded. Acid is produced from cellobiose and glucose. Produces pradimicins A, B, and C.

Enzymic activities (API ZYM system) are positive for cystine arylmidase, esterase (C_4), esterase (C_8), leucine arylamidase, naphthol-AS-BI-phosphohydrolase, β-glucosidase, and trypsin. Additional phenotypic properties are presented in Table 302 and Table 303.

DNA G+C content (mol%): not determined.

Type strain: P157-2, ATCC 53557, DSM 44148, NBRC 15177, JCM 9627, NCIMB 13253.

Sequence accession no. (16S rRNA gene): AF163115, AJ293705.

Sequence accession no. (23S rRNA gene): AF163137.

Sequence accession no. (16S–23S rRNA ITS): AF163126.

14. **Actinomadura kijaniata** Horan and Brodsky 1982, 195[VP]

ki.ja.ni′a.ta. N.L. fem. adj. *kijaniata* derived from "kijani" (the Swahili word for green).

Spore chains in long, open spirals with 10 or more spores per chain. Spores are elliptical (1.0–1.5 µm diameter by 1.5–2.0 µm long). Spore surface smooth. Inorganic salts-starch agar: moderate growth; surface flat and granular; aerial mycelium absent; substrate mycelium center slate-green and periphery light tawny, and faint green diffusible pigment. Oatmeal agar: moderate growth; surface flat to granular; aerial mycelium only white specks; substrate mycelium dark pine-green, and no diffusible pigment. Tyrosine agar: good growth; surface raised and folded; aerial mycelium abundant white; substrate mycelium lead gray, and no diffusible pigment. Yeast extract-malt extract agar: good growth; surface raised and folded; aerial mycelium only white specks; substrate mycelium center dark jade-green and periphery biscuit-colored, and no diffusible pigment. Grows at 10–60°C. RNA and Tweens 20, 40, 60, and 80 are degraded. Glutamine, glycine, histidine, leucine, methionine, phenylalanine, and valine are used as sole carbon sources. Resistant to ampicillin (15 µg/ml), cephaloridine (10 µg/ml), cephamandole (40 µg/ml), neomycin (7 µg/ml), streptomycin (16 µg/ml), rifampin (10 µg/ml), and tobramycin (5 µg/ml), but is susceptible to tetracycline (20 µg/ml). Additional phenotypic data are shown in Table 302 and Table 303.

DNA G+C content (mol%): 69.7 (T_m).

Type strain: 13-363, ATCC 31588, CCRC 13146, DSM 43764, NBRC 14229, IMET 9741, JCM 3306, KCTC 9129, NCIMB 13755, NRRL 12069, NRRL B-16121, SCC 1256, VKM Ac-874.

Sequence accession no. (16S rRNA gene): U49006, X97890.

Sequence accession no. (23S rRNA gene): AF116219.

Sequence accession no. (16S–23S rRNA ITS): AF134099.

Additional comments: Actinomadura kijaniata differs from other *Actinomadura* species as it forms a deep green substrate mycelium and long spore chains on the aerial mycelium. Whole-cell hydrolysates contain *meso*-A_2pm together with a trace of the L-isomer (Horan and Brodsky, 1982). Furthermore, this species produces a complex of acid enol antibiotics, the major component of which was designated kijanimicin. This antibiotic has an unusual *in vitro* spectrum of activity against Gram-stain-positive and anaerobic microorganisms. *In vivo* activity has been shown against *Plasmodium berghei* and *Plasmodium chabaudi* in mice (Waitz et al., 1981).

15. **Actinomadura latina** Trujillo and Goodfellow 1997b, 917[VP] (Effective publication: Trujillo and Goodfellow 1997a, 230.)

la.ti′na. L. fem. adj. *latina* latin; named after *America Latina*, since many clinically significant strains of actinomadurae have been isolated in Latin America.

Moderate growth on Czapek, oatmeal and yeast extract-malt extract agars. Colonies cream to pink, convex, and wrinkled. Aerial mycelium absent or rare. No diffusible pigments. Grows at 10–37°C. Degrades Tweens 20, 40, and 60. Phenylalanine is used as a sole carbon sources. Resistant to rifampin (6 µg/ml), but is susceptible to ticarcillin (70 µg/ml). Additional phenotypic data are shown in Table 302 and Table 303.

Source: a patient with actinomycetoma in the arm.

DNA G+C content (mol%): 67 (T_m).

Type strain: A10, ATCC BAA-277, DSM 43382, IFM 0961, JCM 10674.

Sequence accession no. (16S rRNA gene): AY035998.

16. **Actinomadura livida** Lavrova and Preobrazheskaya 1975, 483[AL]

li′vi.da. L. fem. adj. *livida* livid (referring to the grayish-violet color of the substrate mycelium).

Spore chains in hooks or spirals of one turn. Spore surface irregular. Inorganic salts-starch agar: good growth; surface cartilaginous glistening; aerial mycelium only microscopically visible hyphae; substrate mycelium yellowish brownish, and pale violet diffusible pigment. Oatmeal agar: good growth; surface cartilaginous dull; aerial mycelium microscopically visible hyphae; substrate mycelium pale grayish pink, and pale violet diffusible pigment. Peptone-glucose medium: good growth; surface cartilaginous; aerial mycelium absent; substrate mycelium yellowish brown, and no diffusible pigment. Yeast extract-malt extract agar: moderate growth; surface cartilaginous; aerial mycelium absent; substrate mycelium pale brownish, and no diffusible pigment. Grows at 20–45°C. Histidine and phenylalanine are used as sole carbon sources. Resistant to cephaloridine (10 µg/ml), lincomycin (25 µg/ml), oleandomycin (20 µg/ml), rifampin (10 µg/ml), and ticarcillin (80 µg/ml), but is sensitive to ampicillin (5 µg/ml), benzylpenicillin (25 µg/ml), and tetracycline (20 µg/ml). Additional phenotypic data are shown in Table 302 and Table 303.

Source: soil.

DNA G+C content (mol%): not determined.

Type strain: ATCC 33578, DSM 43677, NBRC 14682, IMET 9575, INA 1678, JCM 3387, VKM Ac-908.

Sequence accession no. (16S rRNA gene): AF163116, AJ293706.

Sequence accession no. (23S rRNA gene): AF163138.

Sequence accession no. (16S–23S rRNA ITS): AF163127.

17. **Actinomadura luteofluorescens** (Shinobu 1962) Preobrazhenskaya and Lavrova in Preobrazhenskaya, Lavrova and Blinov 1975a, 526[AL] (*Streptomyces luteofluorescens* Shinobu 1962, 115)

lu.te.o. flu.o.res′cens. L. adj. *luteus* yellow; N.L. part. adj. *fluorescens* fluorescing; N.L. part. adj. *luteofluorescens* yellow and

fluorescing, referring to the greenish tinge of the diffusible pigment produced by the organism.

Short spore chains in hooks or curled, arranged in clusters. Spore surface warty. Inorganic salts-starch agar: poor growth; surface leathery; aerial mycelium microscopically visible hyphae; substrate mycelium orange to brown, and yellow diffusible pigment. Oatmeal agar: good growth; surface farinaceous; aerial mycelium yellowish pink turning blue with age; substrate mycelium pale greenish yellow, and greenish yellow diffusible pigment. Peptone-glucose medium: good growth; surface wrinkled leathery; aerial mycelium absent; substrate mycelium orange to brown, and yellow diffusible pigment. Yeast extract-malt extract agar: moderate growth; surface farinaceous; aerial mycelium yellowish white; substrate mycelium dark yellow, and pale yellow diffusible pigment. Grows at 10–45°C. RNA is degraded. Resistant to ampicillin (15 μg/ml), carbenicillin (120 μg/ml), cephaloridine (10 μg/ml), cephalotin (35 μg/ml), cephamandole (40 μg/ml), rifampin (10 μg/ml), and streptomycin (16 μg/ml), but is susceptible to tetracycline (20 μg/ml). Additional phenotypic data are shown in Table 302 and Table 303.

Source: soil.

DNA G+C content (mol%): not determined.

Type strain: ATCC 25469, CBS 702.69, CGMCC AS 4.1382, CIP 105484, DSM 40398, NBRC 13057, ISP 5398, JCM 4203, JCM 4491, KCC S-0203, KCC S-0491, NRRL B-12327, RIA 1249, VKM Ac-1509.

Sequence accession no. (16S rRNA gene): U49008.

Sequence accession no. (23S rRNA gene): AF134079.

Sequence accession no. (16S–23S rRNA ITS): AF134101.

18. **Actinomadura macra** Huang 1980, 565[VP]

ma′cra. L. fem. adj. *macra* lean (referring to the poor, thin growth of this organism).

Sporulation is extremely rare and delayed. A few short spore chains (flexuous, hooked, or straight; 4–15 spores) have been observed after 5 weeks of incubation on isolation agar (Jensen, 1930) and Czapek sucrose agar. Spores oval to elliptical (0.8–1.0 × 1.2–2.0 μm). Spore surface smooth. Czapek sucrose-nitrate agar: poor growth; surface smooth; aerial mycelium scant and pale cream-colored; substrate mycelium colorless, and no diffusible pigment. Inorganic salts-starch agar: very scanty growth; surface smooth; aerial mycelium absent; substrate mycelium colorless or pale grayish, and no diffusible pigment. Oatmeal agar: moderate growth; surface smooth; aerial mycelium scant and cream to faint pink; substrate mycelium cream to faint pink, and no diffusible pigment. Yeast extract-malt extract agar: good growth; surface raised and wrinkled; aerial mycelium white to grayish; substrate mycelium black, and brown diffusible pigment. Grows at 10–45°C. Tweens 20, 40, 60, and 80 are degraded. Lactose, phenylalanine, and valine are used as sole carbon sources. Resistant to ampicillin (15 μg/ml), cephalotin (30 μg/ml), and carbenicillin (100 μg/ml), but is susceptible to benzylpenicillin (25 μg/ml), ticarcillin (70 μg/ml), rifampin (2 μg/ml), and tetracycline (20 μg/ml).

Source: soil.

DNA G+C content (mol%): not determined.

Type strain: Pfizer FD 25934, ATCC 31286, CIP 105532, BCRC (formerly CCRC) 13378, DSM 43862, NBRC 14102, IMET 9754, JCM 3287, KCC A-0287, KCTC 9342, NRRL B-16124.

Sequence accession no. (16S rRNA gene): U49009.

Sequence accession no. (23S rRNA gene): AF134080.

Sequence accession no. (16S–23S rRNA ITS): AF134102.

19. **Actinomadura mexicana** Quintana, Trujillo and Goodfellow 2004, 307[VP] (Effective publication: Quintana, Trujillo and Goodfellow 2003, 514.)

me.xi.ca′na. N.L. fem. adj. *mexicana*, referring to Mexico, the country of origin of the soil sample from which the organism was isolated.

Aerial hyphae are rare but when present may differentiate into hooked chains of spores (1 μm). Spore surface warty. Extensively branched non-fragmenting, light yellow substrate mycelium and yellow diffusible pigment formed on yeast extract-malt extract agar. Pink to palid red, convex colonies with a wrinkled morphology formed on modified Bennett's agar. Grows at 10–37°C. L-Glutamine is used as sole carbon sources. Resistant to ampicillin (15 μg/ml), benzylpenicillin (25 μg/ml), and lincomycin (25 μg/ml), but is susceptible to neomycin (1 μg/ml), streptomycin (2 μg/ml), cephalotine (20 μg/ml), and tetracycline (20 μg/ml). Additional phenotypic data are shown in Table 302 and Table 303.

Source: soil collected in Mexico.

DNA G+C content (mol%): not determined.

Type strain: A290, DSM 44485, JCM 13236, NRRL B-24203.

Sequence accession no. (16S rRNA gene): AF277195.

20. **Actinomadura meyerae** Quintana, Trujillo and Goodfellow 2004, 307[VP] (Effective publication: Quintana, Trujillo and Goodfellow 2003, 515.)

me.ye′ra.e. N.L. gen. fem. n. *meyerae* of Meyer, named after the German microbiologist Jutta Meyer in recognition of her contributions to actinomycete systematics.

Aerial hyphae are rare but when present may differentiate into hooks and spiral chains of spores. Spore surface warty. Cream to light yellow, convex colonies with a wrinkled morphology formed on modified Bennett's agar, and a light yellow substrate mycelium on yeast extract-malt extract agar. Diffusible pigments not produced. Grows at 25–45°C and pH 7–9. RNA is degraded, but not pectin. Arginine is used as a carbon source. Resistant to ampicillin (15 μg/ml), benzylpenicillin (25 μg/ml), lincomycin (25 μg/ml), and oleandomycin (20 μg/ml), but is susceptible to rifampin (2 μg/ml) and tetracycline (20 μg/ml). Additional phenotypic data are shown in Table 302 and Table 303.

Source: garden soil collected in Mexico.

DNA G+C content (mol%): not determined.

Type strain: A288, DSM 44715, JCM 13237, NRRL B-24247.

Sequence accession no. (16S rRNA gene): AY273787.

21. **Actinomadura namibiensis** Wink, Kroppenstedt, Seibert and Stackebrandt 2003, 723[VP]

na.mi.bi.en′sis. N.L. fem. adj. *namibiensis* of or belonging to Namibia from where the type strain was isolated.

Spiral spore chains of about 20 spores are formed after about 20 d. Spore surface smooth. Substrate mycelium is salmon pink on glycerol-asparagine, inorganic salts-starch, oatmeal, tyrosine, and yeast extract-malt extract agars and colorless on peptone-yeast extract-iron agar. Soluble pigments are not produced on any of these media. White aerial hyphae are formed on glycerol-asparagine inorganic salts-starch and tyrosine agars. Enzymic activities (API ZYM system) are shown for cystine arylmidase, leucine arylamidase, naphthol-AS-BI-phosphohydrolase, β-glucosidase, and trypsin, but not for esterases (C_4) and (C_8). Other phenotypic properties are shown in Table 302 and Table 303.

Source: soil sample collected in Namibia.

DNA G+C content (mol%): not determined.

Type strain: HAG 010767, CIP 108365, DSM 44197, FH-A 1198, JCM 13238, NRRL B-24153.

Sequence accession no. (16S rRNA gene): AJ420134.

22. **Actinomadura napierensis** Cook, le Roes and Meyers 2005, 705[VP]

na.pi.e.ren′sis. N.L. fem. adj. *napierensis* of or belonging to Napier in South Africa, the source of the type strain.

Spiral spore chains are formed. Spore surfaces smooth. Grey aerial and brown-gray substrate mycelia formed on Czapek, Middlebrook 7H9, and yeast extract-malt extract agars. White aerial and substrate mycelia produced on inorganic salts-starch agar. A faint blue diffusible pigment is produced on tyrosine agar. Tween 80 is degraded, but not allantoin, guanine, hippurate, or xylan. Shows a weak H_2S reaction. Dextrin is used as a sole carbon source. Grows in the presence of 2% but not 5% (w/v) NaCl.

Source: soil in Napier, Western State Province, South Africa.

DNA G+C content (mol%): 70 (T_m).

Type strain: B60, DSM 44846, JCM 13850, NRRL B-24319.

Sequence accession no. (16S rRNA gene): AY568292.

23. **Actinomadura nitritigenes** Lipski and Altendorf 1995, 722[VP]

ni.tri.ti′ge.nes. N.L. n. *nitris -itis* nitrite; N.L. suff. *-genes* (from Gr. v. *gennaô* to produce) producing; N.L. adj. *nitritigenes* nitrite producing.

Oval to ellipsoidal spores produced in straight or hook-like chains that are up to seven spores long. Spore surface smooth. Substrate mycelium is colorless to brown on glycerol-asparagine, inorganic salts-starch, oatmeal, peptone-yeast extract-iron, tyrosine, and yeast extract-malt extract agars. Good growth is obtained on all these media except for peptone-yeast extract-iron agar. Diffusible pigments are not produced. Aerial mycelium is either white or brown. Grows at 45°C. Tweens 20 and 80 are degraded, but not adenine, guanine, or xylan. H_2S is not produced. Starch and sucrose are used as sole carbon sources, but not alanine, propionate, pyruvate, or serine. Resistant to neomycin (3 μg/ml), but is susceptible to gentamicin (4 μg/ml) and streptomycin (4 μg/ml). Enzymic activity (API ZYM system) shown for cystine arylmidase, esterase (C_8), β-glucosidase, leucine arylamidase, naphthol-AS-BI-phosphohydrolase, and trypsin, but not for esterase (C_4). Other phenotypic properties are shown in Table 302 and Table 303.

Source: experimental biofilters filled with tree bark compost.

DNA G+C content (mol%): 74 (HPLC).

Type strain: L46, DSM 44137, NBRC 15918, JCM 10104, NCIMB 13456.

Sequence accession no. (16S rRNA gene): AY035999.

24. **Actinomadura oligospora** Mertz and Yao 1986, 180[VP]

o.li.go.spo′ra. Gr. adj. *oligos* few; Gr. n. *spora* seed; N.L. n. *oligospora* organism forming few spores, referring to the relative absence of sporophores in this organism.

Aerial mycelia are absent except for trace amounts produced on inorganic salts-starch and sodium butyrate agars. Substrate mycelia give rise to occasional sparse twisted oyster white aerial hyphae that differentiate into flexuous or hooked chains of 10–50 spores. Spores are oblong (0.5–0.7 × 0.9–1.3 μm). Spore surface smooth. Colonies grow slowly (10–20 d) and are convex with filamentous margins. Growth is best on complex media. Colony surfaces and reverse surfaces are yellowish gray to brown. Soluble pigments are produced on inorganic salts-starch and yeast-glucose agars. Grows at 20–37°C. Does not degrade adenine, calcium malate, guanine, hippurate, or keratin. Leucine is used as a sole carbon source, but not dextran or inulin. Resistant to bacitracin (10 U), cephaloridine (10 μg/ml), cephalotin (35 μg/ml), cephamandole (40 μg/ml), rifampin (10 μg/ml), and tetracycline (20 μg/ml), but is susceptible to gentamicin (10 μg/ml), streptomycin (10 μg/ml), tobramycin (10 μg/ml), and vancomycin (30 μg/ml). Produces a polyether antibiotic. Does not grow at pH 8. Additional phenotypic data are shown in Table 302 and Table 303.

Source: soil collected in Karnataka, India.

DNA G+C content (mol%): not determined.

Type strain: A80190.1, ATCC 43269, JCM 10648, NRRL 15878.

Sequence accession no. (16S rRNA gene): AF163118

Sequence accession no. (23S rRNA gene): AF163140.

Sequence accession no. (16S–23S rRNA ITS): AF163129.

25. **Actinomadura pelletieri** (Laveran 1906) Lechevalier and Lechevalier 1970a, 400[AL] (*Micrococcus pelletieri* Laveran 1906, 341)

pel.le.ti′e.ri. N.L. gen. masc. n. *pelletieri* of Pelletier, named after T. Pelletier, who first isolated this species.

Spore chains short (2–6 spores per chain), hooked or in spirals of two to three turns. Spores subspherical. Spore surface warty. Oatmeal agar: moderate growth; surface cartilaginous; aerial mycelium absent; substrate mycelium pink to brownish red, and no diffusible pigment. Oatmeal-nitrate agar: moderate growth; surface cartilaginous; traces of sporulating hyphae on aerial mycelium; substrate mycelium colorless, and no diffusible pigment. Peptone-glucose medium: moderate growth; surface cartilaginous; aerial mycelium absent; substrate mycelium brownish red, and no diffusible pigment. Yeast extract-malt extract agar: moderate growth; surface cartilaginous; traces of sterile hyphae on aerial mycelium; substrate mycelium pink to brownish red, and no diffusible pigment. Grows at 25–37°C. Tweens 20, 40, and 60 are degraded, but not adenine. Starch hydrolysis is variable. Collagenase is produced and nitrate reduced.

Cellobiose, glucose, and glycogen are used as sole carbon sources, but not adonitol, arabinose, erythritol, inositol, raffinose, or sorbitol. Does not grow in the presence of 4%, w/v, NaCl. Additional phenotypic properties are shown in Table 302 and Table 303.

Source: actinomycetoma of the arm. Most strains classified as *Actinomadura pelletieri* have been isolated from clinical samples.

DNA G+C content (mol%): 65.5–67.3 (T_m).

Type strain: ATCC 33385, CCUG 38891, CIP 105483, DSM 43383, HUT 6549, IAM 12634, IMET 9693, JCM 3388, KCTC 9110, NBRC 103052, NCTC 4162, NRRL B-3997.

Sequence accession no. (16S rRNA gene): AF163119, AJ293710.

Sequence accession no. (23S rRNA gene): AF163141.

Sequence accession no. (16S–23S rRNA ITS): AF163130.

Additional comments: Cummins (1962), who was the first to isolate *meso*-A_2pm from whole-organism hydrolysates of members of this species, also found a small amount of L-isomer and glycine in some strains.

26. **Actinomadura rubrobrunea** (*ex* Krasil'nikov, Agre and El-Registan 1968) Kroppenstedt, Goodfellow and Stackebrandt 1991, 178[VP] (*Excellospora rubrobrunea* Agre and Guzeva 1975, 522)

ru.bro.bru′ne.a. L. adj. *ruber, bra brum* red; N.L. adj. *bruneus a um* brown; N.L. fem. adj. *rubrobrunea* reddish brown.

Coiled to spiral chains of 2–20 spores, some simple. Spore surface spiny. Reddish-brown substrate mycelium. Grows at 37–65°C and optimally at 45–55°C. Does not degrade adenine or cellulose. Additional phenotypic properties are shown in Table 302 and Table 303.

Source: soil.

DNA G+C content (mol%): not determined.

Type strain: ATCC 49883, CIP 105486, DSM 43750, NBRC 15178, IMET 9705, INMI 2991, JCM 7345, KCTC 9493, VKM Ac-1470.

Sequence accession no. (16S rRNA gene): AF134069, EU637008.

Sequence accession no. (23S rRNA gene): AF134081.

Sequence accession no. (16S–23S rRNA ITS): AF134104.

27. **Actinomadura rudentiformis** le Roes and Meyers 2007, 48[AL]

ru. den. ti. for′mis. L. masc. n. *rudens* rope; L. adj. suff. *-formis* like, in the shape of; N.L. fem. adj. *rudentiformis* shaped like a rope.

Spores have not been detected. Cream-colored substrate mycelium with a sparse white aerial mycelium formed on oatmeal agar. Wrinkled, cream-colored substrate mycelium, but no sporulation on yeast extract-malt extract agar. White substrate mycelium but does not sporulate on inorganic salts-starch agar. Pigments are not produced on glycerol-asparagine, peptone-yeast extract-iron, or tyrosine agars. Rope-like or fiber-like growth visible under scanning electron microscopy. Growth occurs at 30, 37, and 45°C, but not at 4°C or pH 4.3. Degrades adenine, Tween 80, and xylan. Guanine and starch are weakly degraded. Hydrolyzes hippurate, but not allantoin, pectin, or urea. Lecithinase, lipase, and proteolytic activities are not observed on egg-yolk agar. Does not produce H_2S or reduce nitrate. Inositol, inulin, melezitose (weak), ribose (weak), salicin (weak), sodium acetate, and sucrose are used as sole carbon sources, but not sodium citrate. DL-α-Amino-*n*-butyric acid, L-arginine, L-cysteine, L-histidine, L-hydroxyproline, L-methionine, L-phenylalanine, potassium nitrate, L-servine, L-threonine, and L-valine are used as sole carbon and nitrogen sources. Resistant to kanamycin (10 μg/ml), neomycin (30 μg/ ml), oleandomycin (100 μg/ml), penicillin G (10 IU), rifampin (50 μg/ml), and tobramycin (50 μg/ml), but is sensitive to cephaloridine (100 μg/ml), gentamicin (100 μg/ml), lincomycin (100 μg/ml), streptomycin (100 μg/ml), and vancomycin (50 μg/ml). Additional phenotypic properties are shown in Table 302 and Table 303.

Source: soil taken from the banks of the Gamka River in the Swartberg Nature Reserve, Western Cape Province, South Africa.

DNA G+C content (mol%): not determined.

Type strain: HMC1, DSM 44962, JCM 14907, NRRL B-24458.

Sequence accession no. (16S rRNA gene): DQ285420.

28. **Actinomadura rugatobispora** (Miyadoh, Tohyama, Amano, Shomura and Niida 1985) Miyadoh, Amano, Tohyama and Shomura 1990, 1909[VP] (*Microbispora viridis* Miyadoh, Tohyama, Amano, Shomura and Niida 1985, 281[VP])

ru.ga.to.bi.spo′ra. L. part. adj. *rugatus* wrinkled; Gr. pref. *bi* twice; Gr. n. *spora* a seed and, in biology, a spore; N.L. n. *rugatobispora* organism forming wrinkled paired-spores, referring to spore morphology.

Aerial hyphae differentiate into a non-sporogenous main axis which bears spore-bearing side branches. Spores are oval (1.9–1.2 × 1.4–2.0 μm). Usually two but sometimes three spores per chain. Spore surface rugose with vertical ridges (Figure 408). Aerial mycelium is grayish green and the substrate mycelium pastel yellow on oatmeal agar. The substrate mycelium is pale yellowish brown on inorganic salts-starch agar. Grows at 25–35°C. Does not require vita-

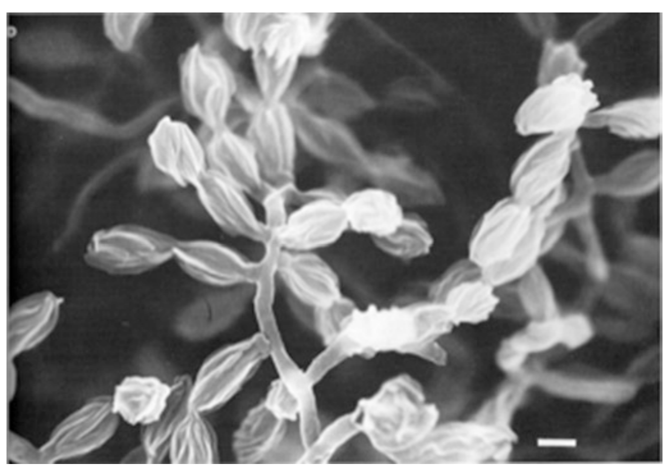

FIGURE 408. SEM of *Actinomadura rugatobispora* SF 2240[T] (IFO 14382[T]). Usually two but sometimes three spores per chain. Spore surface rugose with vertical ridges. Bar = 1 μm. (Reproduced with permission from S. Miyadoh, S. Amano, and T. Shomura. Atlas of Actinomycetes, Society for Actinomycetes Japan.)

min B for growth. Additional phenotypic properties are shown in Table 302 and Table 303.

Source: soil.

DNA G+C content (mol%): 73 (T_m).

Type strain: SF2240, ATCC 51643, CIP 105482, DSM 44130, NBRC 14382, JCM 3366, NRRL B-16566.

Sequence accession no. (16S rRNA gene): U49010.

Sequence accession no. (23S rRNA gene): AF134082.

Sequence accession no. (16S–23S rRNA ITS): AF134105.

29. **Actinomadura spadix** Nonomura and Ohara 1971d, 911[AL]

spa′dix. L. fem. adj. *spadix* chestnut-colored (referring to the color of the substrate mycelium).

Short spore chains with 5–10 spores in small, round spore masses (pseudosporangia) formed on long aerial hyphae. Sporulation observed on soil extract agar and partly on oatmeal-yeast-glucose agar. Spore surface smooth (Figure 409). Glycerol-asparagine agar: aerial mycelium absent; substrate mycelium grayish brown, and diffusible pigment reddish-gray or grayish brown. Oatmeal agar: aerial mycelium light grayish-yellowish brown; substrate mycelium grayish brown, and diffusible pigment reddish-gray or grayish brown. Yeast extract-malt extract agar: aerial mycelium absent; substrate mycelium dark brown, and diffusible pigment brown. Grows at 30°C, but not at 25 or 37°C. Does not degrade Tweens 20, 40, 60, and 80. Phenylalanine and valine are used as sole carbon sources, but not arginine or glutamine. Resistant to rifampin (10 µg/ml), but is susceptible to ampicillin (5 µg/ml), benzylpenicillin (25 µg/ml), carbenicillin (90 µg/ml), tobramycin (0.05 µg/ml), and tetracycline (20 µg/ml). Additional phenotypic properties are shown in Table 302 and Table 303.

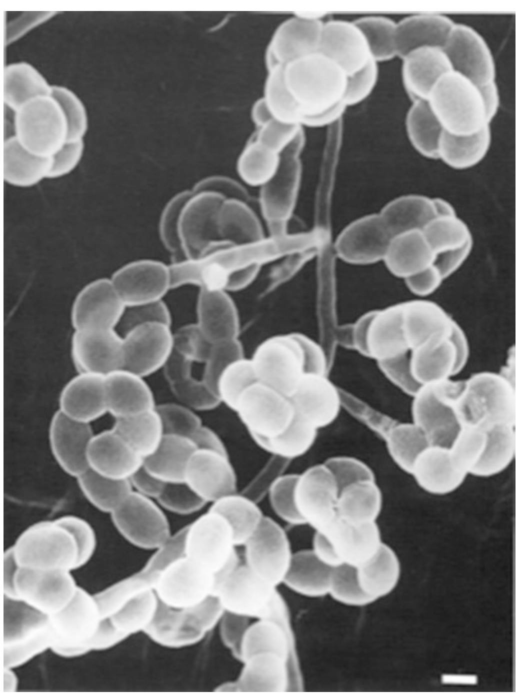

FIGURE 409. SEM of *Actinomadura spadix* MH 193-16 F4. Short spore chains with 5–10 spores in small, round spore masses (pseudosporangia) formed on long aerial hyphae. Spore surface smooth. Bar = 1 µm. (Reproduced with permission from M. Hamada and T. Shomura. Atlas of Actinomycetes, Society for Actinomycetes Japan.)

Source: soil.

DNA G+C content (mol%): 66.4 (T_m).

Type strain: ATCC 27298, CBS 261.72, BCRC (formerly CCRC) 13386, CIP 105479, DSM 43459, NBRC 14099, IMET 9752, JCM 3146, KCC A-0146, KCTC 9252, NCIB 11118, NRRL B-16128.

Sequence accession no. (16S rRNA gene): AF163120, AB364581.

Sequence accession no. (23S rRNA gene): AF163142.

Sequence accession no. (16S–23S rRNA ITS): AF163131.

Additional comments: according to Nonomura and Ohara (1971c), Actinomadura spadix requires B vitamins for growth. Phylogenetic data (16S and 23S rRNA) suggests that this strain is misclassified in the genus *Actinomadura* and may represent a new genus though additional data are needed to clarify its taxonomic status.

30. **Actinomadura umbrina** Galatenko, Terekhova and Preobrazhenskaya 1987, 179[VP] (Effective publication: Galatenko, Terekhova and Preobrazhenskaya 1981, 803.)

um.bri′na. N.L. fem. adj. *umbrina* wood brown.

Short spore chains may be straight, hooked or spiral, often branching. Smooth spore surface. Substrate mycelium is brown or black as are soluble pigments. White scanty aerial mycelium produced. Good growth occurs on mineral (Gauze 1), Czapek's, glycerol-nitrate, glycerol asparagine, and inorganic salts-starch agars. Does not grow on carbon utilization agar supplemented with different sugars, including glucose. Additional phenotypic properties are shown in Table 302 and Table 303.

Source: soil.

DNA G+C content (mol%): not determined.

Type strain: ATCC 49502, CIP 105485, DSM 43927, NBRC 14346, IMET 9746, INA 2309, JCM 6837, NRRL B-16244, VKM Ac-1086.

Sequence accession no. (16S rRNA gene): AF163121, AJ293713.

Sequence accession no. (23S rRNA gene): AF123143.

Sequence accession no. (16S–23S rRNA ITS): AF163132.

31. **Actinomadura verrucosospora** Nonomura and Ohara 1971d, 908[AL]

ver.ru.co.so.spo′ra. L. fem. adj. *verrucosa* warty; Gr. n. *spora* seed; N.L. n. *verrucosospora* organism forming warty spores, referring to the warty surface of the spores.

Spore chains (5–12 spores per chain) hooked or curled or spirals of one turn, arranged in tufts on long aerial hyphae. Spores elliptical. Spore surface warty. Glycerol-asparagine agar: moderate growth; aerial mycelium absent; substrate mycelium bright red, and no diffusible pigment. Inorganic salts-starch agar: poor growth; surface farinaceous to granular; aerial mycelium white; substrate mycelium yellowish white, and no diffusible pigment. Oatmeal agar: good growth; surface farinaceous; aerial mycelium pink turning blue with maturity; substrate mycelium orange to pink, and no diffusible pigment. Oatmeal-nitrate agar: good growth; surface slightly farinaceous; aerial mycelium white to grayish; substrate mycelium white to pink, and no

diffusible pigment. Yeast extract-malt extract agar: moderate to good growth; surface farinaceous; aerial mycelium white; substrate mycelium yellowish, and no diffusible pigment. Grows at 10–37°C. Does not degrade RNA or xylan. Resistant to ampicillin (10 µg/ml), benzylpencillin (25 µg/ml), carbenicillin (120 µg/ml), and rifampin (10 µg/ml), but is susceptible to lincomycin (10 µg/ml) and tetracycline (20 µg/ml). Additional phenotypic properties are shown in Table 302 and Table 303.

Source: soil in Japan.

DNA G+C content (mol%): 69.0 (T_m).

Type strain: ATCC 27299, CBS 258.72, CCM 3492, BCRC (formerly CCRC) 13408, CIP 105480, DSM 43358, DSM 43550, IMET 9588, JCM 3147, KCC A-0147, NCIMB 11119, NRRL B-16129, RIA 1503, VKM Ac-668.

Sequence accession no. (16S rRNA gene): U49011.

Sequence accession no. (23S rRNA gene): AF134083.

Sequence accession no. (16S–23S rRNA ITS): AF134106.

32. **Actinomadura vinacea** Lavrova and Preobrazhenskaya 1975, 486[AL]

vi.na′ce.a. L. fem. adj. *vinacea* of or belonging to wine, referring to the brownish-red color of the substrate mycelium.

Short, straight spore chains, arranged in small clusters on moderately branched hyphae of the aerial mycelium. Spore surface irregular. Czapek-sucrose agar: poor growth; aerial mycelium pink or absent; substrate mycelium brownish red, and diffusible pigment pale lilac to red. Inorganic salts-starch agar (Gauze 1): poor growth; surface slightly velvety; aerial mycelium white to gray; substrate mycelium colorless, and diffusible pigment pale pink. Oatmeal agar: poor growth; surface dull leathery; aerial mycelium absent; substrate mycelium pink to red, and no diffusible pigment. Peptone glucose medium: poor to moderate growth; surface cartilaginous; aerial mycelium absent; substrate mycelium brownish red, and diffusible pigment dark red. Yeast extract-malt extract agar: poor growth; surface leathery; aerial mycelium absent; substrate mycelium dark brown-red, and diffusible pigment red. Additional phenotypic properties are shown in Table 302 and Table 303.

Source: soil.

DNA G+C content (mol%): not determined.

Type strain: ATCC 33581, CIP 105481, DSM 43765, NBRC 14688, IMET 9574, IMET 9673, INA 1682, JCM 3325, KCC A-0325, KCTC 9344.

Sequence accession no. (16S rRNA gene): AF134070.

Sequence accession no. (23S rRNA gene): AF134084.

Sequence accession no. (16S–23S rRNA ITS): AF134107.

33. **Actinomadura viridilutea** (Agre and Guzeva 1975) Zhang, Kudo, Nakajima and Wang 2001, 381[VP] (*Excellospora viridilutea* Agre and Guzeva 1975)

vi.ri.di.lu′te.a. L. adj. *viridis* green; L. adj. *luteus* yellow; N.L. fem. adj. *viridilutea* green-yellow.

Spore chains hooked to spiral. Spore surface spiny. Substrate mycelium is yellow; aerial mycelium is bluish green. Grows at 37–65°C and optimally at 45–55°C. Does not degrade cellulose. The following carbon sources were used when assayed in Czapek's agar without sucrose as basal medium: acetate, arabinose (weak), citrate (weak), dulcitol (weak), galactose, glucose, glycerol, inositol, lactose, maltose, mannitol, raffinose, rhamnose, starch, sucrose, and xylose (weak). Asparagine, NH_4, urea, and tyrosine are used as nitrogen sources. Does not show antibacterial or antifungal activities against *Bacillus mycoides*, *Bacillus subtilis*, *Escherichia coli*, *Sarcina lutea*, *Serratia marcescens*, *Staphylococcus aureus*, *Streptomyces globisporus*, *Streptomyces fluorescens*, *Streptomyces griseus*, or *Streptomyces levoris* or against *Aspergillus niger*, *Fusarium oxysporum*, *Penicillium notatum*, and *Saccharomyces cerevisiae*. Additional phenotypic properties are shown in Table 302 and Table 303.

DNA G+C content (mol%): not determined.

Type strain: ATCC 33925, BCRC (formerly CCRC) 13638, DSM 44433, NBRC 14480, IMET 9742, INMI 187, JCM 3398, JCM 7346.

Sequence accession no. (16S rRNA gene): D86943.

Sequence accession no. (23S rRNA gene): AF134087.

Sequence accession no. (16S–23S rRNA ITS): AF134110.

Additional comments: Actinomadura viridilutea can be differentiated from *Actinomadura rubrobrunea* by the solubility, light absorbance, and pH color change of its pigments, by its antimicrobial response, and the color of its substrate mycelium.

34. **Actinomadura viridis** (Nonomura and Ohara 1971b) Miyadoh, Anzai, Amano and Shomura 1989, 156[VP] (*Microtetraspora viridis* Nonomura and Ohara 1971b, 5[AL])

vi′ri.dis. L. fem. adj. *viridis* green.

Spores in straight chains. Spore surface smooth. Substrate mycelium pale yellowish-brown on Hickey–Tresner agar. Good sporulation on inorganic salts-starch and MGA agars, but none on Czapek oatmeal or soil extract agars. Grows at 20–45°C. Resistant to neomycin (1 µg/ml), streptomycin (4 µg/ml), cephalotin (35 µg/ml), ampicillin (15 µg/ml), and rifampin (10 µg/ml), but is susceptible to benzylpenicillin (25 µg/ml) and tetracycline (20 µg/ml). Additional phenotypic properties are shown in Table 302 and Table 303.

Source: soil.

DNA G+C content (mol%): 72 (T_m).

Type strain: ATCC 27103, CBS 833.70, BCRC (formerly CCRC) 13398, CIP 105478, DSM 43175, NBRC 15238, IMET 9546, JCM 3112, KCC A-0112, KCTC 9290, VKM Ac-1315.

Sequence accession no. (16S rRNA gene): D85467, AJ420141.

Sequence accession no. (23S rRNA gene): AF134085.

Sequence accession no. (16S–23S rRNA ITS): AF134108.

35. **Actinomadura yumaensis** Labeda, Testa, Lechevalier and Lechevalier 1985, 333[AL]

yu.ma.en′sis. N.L. fem. adj. *yumaensis* of or belonging to Yuma County, Arizona, USA, the source of the soil sample from which the type strain was isolated.

Spore chains are short (mean, 20 spores per chain), loosely coiled and usually borne on branched, and almost verticillate aerial sporophores. Spores are ovoid and measure 0.6–0.8 × 1.0–1.4 µm. Spore surface smooth (Figure 410). Bennett-sucrose agar: good growth; aerial mycelium absent; convoluted substrate mycelium yellowish gray to grayish brown; substrate mycelium dark grayish-yellowish brown, and diffusible pigment orange. Inorganic salts-starch agar: poor growth; white aerial mycelium; substrate

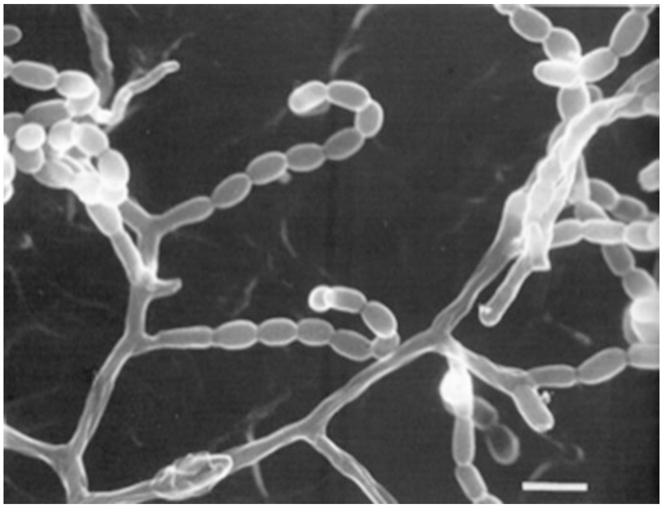

FIGURE 410. Electron micrograph of *Actinomadura yumaensis* IFO 14689[T]. Spore chains are coiled and usually borne on branched, almost verticillate aerial sporophores. Bar = 2 µm. (Reproduced with permission from S. Miyadoh and S. Amano. Atlas of Actinomycetes, Society for Actinomycetes Japan.)

mycelium colorless, and no diffusible pigment. Oatmeal agar: moderate growth; surface flat waxy; grayish yellow; moderate white aerial mycelium; substrate mycelium grayish yellow, and no diffusible pigment. Yeast-extract-malt extract agar: good growth; surface raised waxy; convoluted colonies yellowish gray to grayish-yellowish brown; aerial mycelium absent; substrate mycelium dark yellowish brown, and diffusible pigment orange. Grows at 10–45°C. Arginine and sucrose are used as sole carbon sources. Resistant to ampicillin (15 µg/ml), benzylpenicillin (25 µg/ml), cephaloridine (10 µg/ml), cephalotin (35 µg/ml), cephamandole (30 µg/ml), neomycin (7 µg/ml), rifampin (10 µg/ml), streptomycin (16 µg/ml), and tetracycline (40 µg/ml). Additional phenotypic features are shown in Table 302 and Table 303.

Source: soil sample collected in Yuma County, Arizona.

DNA G+C content (mol%): not determined.

Type strain: LL-C23024, ATCC 43060, CIP 105436, NBRC 14689, JCM 3369, KCTC 9495, NRRL 12515.

Sequence accession no. (16S rRNA gene): AF163122.

Sequence accession no. (23S rRNA gene): AF163144.

Sequence accession no. (16S–23S rRNA ITS): AF163133.

Species *incertae sedis*

a. **"Actinomadura azurea"** Nakamura and Isono 1983, 1468

Produces cationomycin.

Type strain: ICM 2033.

b. **"Actinomadura luzonensis"** Tomita, Hoshino, Sasahira and Kawaguchi 1980, 1098

Produces the antitumor antibiotic complex BBM-928.

Type strain: ATCC 31491 (G455–101).

c. **"Actinomadura pulveracea"** Iwami, Kiyoto, Nishikawa, Terano, Kohsaka, Aoki and Imanaka 1985, 835

Produces antitumor antibiotics FR-900405 and FR-900406.

Type strain: No. 6049.

d. **"Actinomadura albolutea"** Tohyama, Miyadoh, Ito, Shomura, Ito and Ishikawa 1984, 1144

The placement of this species in the genus *Actinomadura* is doubtful. Morphologically, Actinomadura albolutea resembles *Nocardiopsis* however, the presence of maduroe in whole-cell hydrolysates, phospholipids of type PIV, and MK-9(H_4) as major menaquinones indicate that this micro-organism is clearly differentiated from *Nocardiopsis*. Produces an indole-*N*-glycoside antibiotic SF-2140.

Type strain: FERM-BP 386 (SF2140).

Genus IV. **Spirillospora** Couch 1963, 61[AL]

Michael Goodfellow and Martha E. Trujillo

Spi.ril. lo.spo′ra. L. n. *spira* a spiral; N.L. dim. neut. n. *spirillum* a short spiral; Gr. n. *spora* a seed and, in biology, a spore; N.L. fem. n. *Spirillospora* an organism with spores in spirals.

Aerobic, Gram-stain-positive, chemo-organotrophic, mesophilic actinomycetes which **form spherical to vermiform spore vesicles** (5.0–24.0 µm in diameter) **on aerial mycelia**. **Spore vesicles contain numerous spores arranged in coiled and branched chains. Spores are rod-shaped or curved** (0.5–0.7 × 2.0–6.0 µm) **and are motile by means of one to seven subpolar flagella**. The hyphae of the substrate and aerial mycelia are 0.2–1.0 µm thick, branched, and septate. **The substrate mycelium is white to pale yellow or pale buffy pink to red; the aerial mycelium is usually white. Cell-wall peptidoglycan contains *meso*-diaminopimelic acid; madurose is the characteristic sugar of whole-organism hydrolysates.** Grows at 18–35°C. Predominant menaquinones are MK-9(H_4) and MK-8 (H_4).

DNA G+C content (mol%): 71.0–73.0 (T_m, Bd).

Type species: **Spirillospora albida** Couch 1963, 61[AL].

Further descriptive information

Phylogeny. Members of the genus *Spirillospora* were originally classified in the family *Streptosporangiaceae*, but it is evident from chemotaxonomic, nucleic acid reassociation, and comparative 16S rRNA sequencing studies that the genus is a *bona fide* member of the family *Thermomonosporaceae* (Stackebrandt et al., 1997, 1981; Zhang et al., 2001). However, the type strains of the two constituent species, *Spirillospora albida* and *Spirillospora rubra*, are well separated in the *Thermomonosporaceae* 16S rRNA gene tree

(Figure 398 in *Thermomonosporaceae* chapter). These organisms share a 16S rRNA gene similarity of 96.8%, a value which corresponds to 58 differences out of a total of 1444 nucleotides. *Spirillospora albida* IFO 12248$^{\mathrm{T}}$ and *Spirillospora rubra* JCM 6875$^{\mathrm{T}}$ share the highest 16S rRNA gene similarity with *Actinomadura rudentiformis* DSM 44962$^{\mathrm{T}}$ (97.7% and 97.2%, respectively). The type strains of *Spirillospora albida* and *Spirillospora rubra* have also been shown to be distantly related in an analysis of 23S rRNA gene sequences (Zhang et al., 2001). In addition, the type strain of *Spirillospora albida* shares a 70% 16S–23S rRNA internal transcribed spacer (ITS) sequence identity with several *Actinomadura* species, a value much higher than those (>40%) reported between many *Actinomadura* species (Zhang et al., 2001). In contrast, Zhang and his colleagues found that the type strain of *Actinomadura rubra* exhibits only 30–40% 16S–23S ITS sequence similarity to those of all other species in the family *Thermomonosporaceae*.

Cell morphology. *Spirillospora* strains form substrate and aerial mycelia. The hyphae are branched and septate and are 0.2–1.0 µm in diameter (Couch and Bland, 1974). Spore vesicles (sporangia) produced on aerial mycelia are usually spherical with a diameter of 5.0–24.0 µm; the mean diameter is 10 µm (Figure 411). Subspherical to elongated or club-shaped and vermiform spore vesicles are also formed (Couch, 1963). At the initiation of spore vesicle development, the end of an aerial hypha winds into a coil that is enclosed in a common sheath (Bland and Couch, 1981; Lechevalier et al., 1966; Locci and Petrolini-Baldan, 1971; Vobis, 1985). In some cases it appears that the first coils are temporarily free (Vobis and Kothe, 1985). The coiled sporogenous hyphae are branched and fragment into oblong segments that differentiate into spore-size segments (Lechevalier et al., 1966; Vobis, 1985).

The spores are short to long rods, and are frequently curved (0.5–0.7 × 2–7 µm) (Couch and Bland, 1974). A subpolarly inserted tuft of one to seven flagella gives the spores a slight motility which can become more vigorous in the presence of an energy source (Higgins et al., 1967). In addition to the spores enclosed in spore vesicles, free, exposed spores in regular or irregular coils may be found among the aerial hyphae. When flooded with water, the coils break up into rod-shaped to curved spores that subsequently become motile. Conidia-like structures in moniliform arrangement may be produced by the substrate mycelium (Couch, 1963). The septa involved in spore formation are double-layered [cross wall type 2 of Williams et al. (1973)]. Aerial hyphae are additionally covered with a thick sheath from which the spore vesicular envelope originates (Lechevalier et al., 1966; Vobis and Kothe, 1985). *Spirillospora albida* forms spore vesicles on Czapek, peptone-Czapek, and oatmeal agars and *Spirillospora rubra* on cornmeal-soil agar (Vobis and Kothe, 1989) and artificial soil agar (Henssen and Schäfer, 1971).

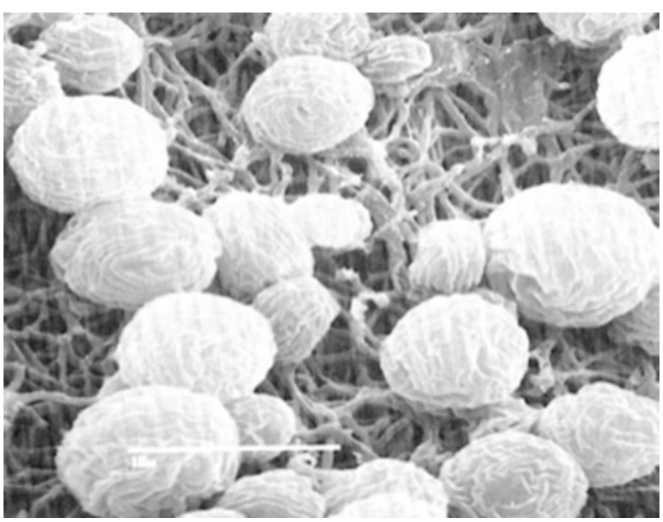

FIGURE 411. *Spirillospora albida* ATCC 15331. Scanning electron micrograph of spherical spore vesicles formed on the aerial mycelium after incubation for 15 d (3200×). Bar = 10 µm.

Nutrition and growth conditions. Spirillosporae can be cultured on various complex media. They grow well on Czapek, peptone-Czapek, and oatmeal agars and moderately on casein and tyrosine agars. Colonies are compact and elevated, sometimes with protruberances on casein, peptone-Czapek, and tyrosine agars. Flattish or confluent colonies are formed on Czapek and oatmeal agars. Some strains produce a blue confluent pigment called spirillomycin, which shows antibiotic activity against some Gram-stain-positive bacteria (Domnas, 1968; McInnis and Domnas, 1970). The pigment producing strains use amylopectin, galactose, glucose, maltose, and sucrose as carbon sources for growth (Domnas, 1970). *Spirillospora* grows well at 18–35°C (Couch and Bland, 1974).

Cell-wall composition. The cell-wall peptidoglycan of *Spirillospora* strains contain *meso*-diaminopimelic acid, and whole-organism hydrolysates contain the diagnostic sugar madurose (Lechevalier and Lechevalier, 1970c; Yamaguichi, 1965); this means that they have a wall chemotype III and a sugar pattern B according to the scheme of Lechevalier and Lechevalier (1970b). The type strain of *Spirillospora albida* contains diphosphatidylglycerol, phosphatidylinositol, and phosphatidylinositol mannosides as major polar lipids (Zhang et al., 2001) and hence has a type 1 phospholipid pattern (Lechevalier et al., 1981) though previous workers reported a type II pattern for this organism (Hasegawa et al., 1978). The predominant menaquinones are MK-9(H_4) and MK-9(H_6) (Collins et al., 1984). Spirillosporae contain major amounts of hexadecanoic ($C_{16:0}$; 20–26% of total fatty acids), octadecenoic ($C_{18:1}$; 21–27%), 14-methylpentadecanoic ($C_{16:0}$ iso; 5–11%), and 10-methyloctadecanoic (tuberculostearic) acids (Zhang et al., 2001), that is, they have a fatty acid type 3a pattern *sensu* Kroppenstedt (1985).

Ecology. *Spirillospora* strains have been isolated from soil, albeit infrequently, by using baits (pollen for the isolation of *Spirillospora albida* and hair for the recovery of *Spirillospora rubra*). However, these organisms accounted for less than 1% of the "sporangiate" actinomycetes isolated from soil using baits (Schäfer, 1973). One part of the life cycle of spirillosporae is adapted to the aquatic milieu. Motile zoospores are able to colonize pollen grains that float on the surface of water and constitute their natural substrate. Mycelia are developed, forming spore vesicles when in contact with air. The spore vesicles are considered to be resistant stages against desiccation. When flooded with water, the zoospores are released from the spore vesicles through either a rupture in the envelope or through a large, irregular pore (Couch, 1963).

Isolation procedures

Spirillosporae have been isolated from soil by baiting with natural substrates (Bland and Couch, 1981; Couch, 1954; Schäfer, 1973). A small amount of soil, approximately one level teaspoonful, is placed in a sterile Petri dish and flooded with sterile water

(distilled water or filtered soil or charcoal water extracts may be used). Various types of pollen [from members of the genera *Liquidamber*, *Pinus and Sparanium*; (Schäfer, 1973)] or other natural substrates such as hair, snakeskin, or boiled grass are added as baits and after incubation for 1–4 weeks the baits are examined with a binocular dissecting microscope (×100). Aerial mycelium and spore vesicles can be recognized at the surface of the water by their glistening appearance. Pollen baits bearing spore vesicles are picked up with a thin needle and transferred to the surface of agar media in small Petri dishes. Individual spore vesicles are separated from the baits and rolled on the agar surface to free them from contaminating bacteria. The cleaned spore vesicles are used as an inoculum for pure cultures. Various complex media support the growth of *Spirillospora* strains. *Spirillospora albida* grows well on Czapek, peptone-Czapek, and oatmeal agars whereas *Spirillospora rubra* prefers half-concentrated corn meal agar (Difco) supplemented with sterilized garden soil (50 g/liter) or half concentrated skim milk agar (Difco; Schäfer, 1973).

Maintenance procedures

Subcultures should be made after a period of 12 weeks. For long-term preservation, the organisms must be lyophilized by using standard procedures employed for aerobic actinomycetes, preferably using well-sporulating cultures. A simple, reliable, and quick method involves nitrogen cryopreservation of living cells in small polyvinyl tubes ("straws") at –196°C (Hoffman, 1989a, 1989b).

Differentiation of the genus *Spirillospora* from other genera

Spirillospora strains can be distinguished from the other three genera classified in the family *Thermomonosporaceae* using a range of chemotaxonomic, morphological and phylogenetic data (Table 298, see chapter on Family *Thermomonosporaceae*). Members of the genus can be easily confused on morphological grounds with those of some species of *Streptosporangium*. All strains belonging to these taxa have multispored, usually spherical, spore vesicles borne on aerial hyphae. However, members of the two genera can be distinguished on the basis of menaquinone and polar lipid composition (Collins et al., 1984; Zhang et al., 2001). In addition, spirollosporae, unlike streptosporangiae, produce zoospores.

Taxonomic comments

Spirillospora albida, the type species of the genus, is closely related to many *Actinomadura* species (Figure 398). The 16S rRNA and 23S rRNA gene sequences of *Spirillospora albida* and some *Actinomadura* strains exhibit much higher similarity than the sequence similarity between most representatives of *Actinomadura* species, as is the case with 16S–23S ITS sequences (Zhang et al., 2001). These data strongly suggest that *Spirillospora albida* has a very close evolutionary relationship with some *Actinomadura* species. In contrast, corresponding data on *Spirillospora rubra* shows that this organism is only distantly related to *Spirillospora albida* and to most other taxa classified in the family *Thermomonosporaceae*, which suggests that this species may merit generic status. However, further comparative taxonomic studies are needed on additional *Spirillospora* strains to clarify their relationships with one another and with members of other genera assigned to the family *Thermomonosporaceae*, notably the genus *Actinomadura*.

Differentiation of species of the genus *Spirillospora*

The two *Spirillospora* species can be distinguished by the characteristic colors of their substrate mycelia. The colonies of *Spirillospora albida* are white to pale yellow whereas those of *Spirillospora rubra* are red to reddish brown.

List of species of the genus *Spirillospora*

1. **Spirillospora albida** Couch 1963, 65[AL]

al′bi. da. L. fem. adj. *albida* whitish.

Spherical spore vesicles (mean, 10 µm in diameter; range, 5.0–24.0 µm) are formed at the tips of aerial hyphae on Czapek, peptone-Czapek, and oatmeal agars. Subspherical to elongated or club-shaped and vermiform spore vesicles are also formed. Spores are rod-shaped, frequently curved (0.5–0.7 × 2.0–6 µm), and weakly motile.

The color of the substrate mycelium is white to pale yellow or buffy pink; the aerial mycelium is white. After 8 weeks, colonies reach diameters up to 13 mm on Czapek, peptone-Czapek, and oatmeal agars, and diameters 5–10 mm on casein and tyrosine agars. A pale yellowish soluble pigment is produced on casein agar and a clay-colored pigment on tyrosine agar. Some strains produce a blue soluble pigment on peptone-Czapek agar. Good growth occurs at 18–35°C and optimum growth at 25°C. Degrades casein and L-tyrosine.

Source: soil.

DNA G+C content (mol%): 70.0–72.0 (T_m) for the type strain and 72.9 (B_d) for strain UNCC 761.

Type strain: ATCC 15331, CBS 291.04, DSM 43034, NBRC 12248, IMET 9031, JCM 3041, NRRL B-3350, VKM Ac-926.

Sequence accession no. (16S rRNA gene): D85498.

Sequence accession no. (23S rRNA gene): AF134088.

Sequence accession no. (16S–23S rRNA ITS): AF134111.

2. **Spirillospora rubra** (*ex* Schäfer 1973) nom. rev. *Spirillospora rubra* Schäfer 1973, 199[AL]

ru′bra. L. fem. adj. *rubra* red.

Hyphae of substrate mycelium are branched (0.4–0.9 µm in diameter), as are aerial hyphae (0.6–1.2 µm in diameter). Spherical spore vesicles, with a diameter of 10.0–25.0 µm, are formed at the tips of aerial hyphae on artificial soil agar and on cornmeal-soil agar.* Rod-shaped spores are frequently slightly curved (0.8 × 1.8–2.8 µm), and weakly motile.

Substrate mycelium is red to reddish brown; the aerial mycelium is white. Good growth occurs on casein and yeast extract-starch agars, and moderate growth on oatmeal-yeast extract, peptone, starch, and tyrosine agars. Grows well at 20–37°C. Degrades casein, but not L-tyrosine. Nitrate is not reduced.

Source: soil.

DNA G+C content (mol%): not determined.

Type strain: CBS 571.75, JCM 6875.

Sequence accession no. (16S rRNA gene): AF163123

Sequence accession no. (23S rRNA gene): AF163145.

Sequence accession no. (16S–23S rRNA ITS): AF163134.

oatmeal-nitrate agar (Prauser and Bergholz, 1974) and peptone-glucose medium ~~(Prauser and Falta,~~ 1968).

*Difco cornmeal agar: cornmeal, 8.5 g, agar, 7.5 g, sterile garden soil, 50 g, distilled water, 1000 ml.

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Order XVI. **Incertae sedis**

Previously classified within the *Pseudonocardiaceae* (Garrity et al., 2005), this placement of *Thermobispora* is not consistent with chemotaxonomic markers or subsequent rRNA gene analyses. The cell-wall diamino acid is *meso*-diaminopimelic acid; whole-cell hydrolysates contain the sugars madurose and galactose; the major menaquinone is MK-9(H_0); and the phospholipid pattern is type PIV. These properties distinguish it from the *Pseudonocardiaceae*. Moreover, in analyses of the 16S rRNA genes, *Thermobispora* appears as a deep lineage within the *Streptosporangiales* but without a clear association to any of the described families (Ludwig et al., 2012). Given the ambiguities, this genus is reclassified in an order *incertae sedis* in this volume.

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Genus I. **Thermobispora** Wang, Zhang and Ruan 1996, 937[VP]

Seung Bum Kim

Ther.mo.bi.spo′ra. Gr. adj. *thermos* hot; Gr. adj. *bis* two; Gr. fem. n. *spora* seed; N.L. fem. n. *Thermobispora* high temperature, two-spored organism.

Gram-stain-positive, aerobic, non-acid-fast, **thermophilic** organisms that produce substrate hyphae (0.5–0.8 µm in diameter). **The aerial mycelia branch monopodially and bear longitudinal pairs of spores**, usually arranged alternately on side branches arising from the main hyphae. **The smooth-surfaced spores are oval to spherical and nonmotile**. Fragmentation of mycelia is not observed on agar or in liquid media. Neither sclerotia, sporangia, nor any other special structures are formed. The cell wall contains a major amount of ***meso*-diaminopimelic acid**, and **the whole-cell hydrolysates contain madurose and galactose**. **MK-9(H_0) is the major menaquinone**. **The phospholipid pattern is type PIV**, including phosphatidylethanolamine and a small amount of glucosamine-containing phospholipids. The major fatty acid is 14-methylpentadecanoic acid ($C_{16:0}$ iso).

DNA G+C content (mol%): 71.

Type species: **Thermobispora bispora** (Henssen 1957) Wang, Zhang and Ruan 1996, 937[VP].

Further descriptive information

Thermobispora is classified with an order *incertae sedis* within this volume. The spores of the type and only species, *Thermobispora bispora*, are usually 1.2–2.0 µm in diameter, but in liquid media spores with a diameter of 3 µm have been observed. The aerial mycelium is white, and the substrate mycelium yellow or yellowish brown on ISP 4 agar medium. Soluble pigments are not produced. The predominant menaquinone of *Thermobispora bispora* is MK-9(H_0), with MK-9(H_2) and MK-9(H_4) present in smaller amounts. The strains of *Thermobispora bispora* can grow at 65°C, but not at 35°C or lower temperatures. The 16S rRNA gene sequences of two *Thermobispora bispora* strains (strains ATCC 19993[T] and JCM 3082) were determined by Wang et al. (1996).

Enrichment and isolation procedures

Thermobispora strains have been isolated from (decaying) manure samples. Colonies can be obtained using selective media for actinobacteria and incubation at 50°C or higher temperatures.

Maintenance procedures

Thermobispora bispora can be maintained at temperatures between 50 and 65°C on ISP medium 4, Bennett's agar, oatmeal agar or rolled oats mineral medium (DSM medium no. 84). For long-term preservation, storage of the mycelial or spore suspensions in 20% (v/v) glycerol at −20°C, or lyophilization is recommended.

Differentiation from closely related taxa

The production of aerial spores in longitudinal pairs from monopodially branching aerial mycelia distinguishes *Thermobispora* from the related genera of the family *Pseudonocardiaceae*. The presence of menaquinone MK-9(H_0) in *Thermobispora* is also a useful marker to distinguish the genus from other genera of the family. Unlike most genera of the family *Pseudonocardiaceae* that have type II or III phospholipids, *Thermobispora* has a type IV phospholipid profile. In addition, strains of *Thermobispora* are obligate thermophiles, growing at temperatures between 50 and 65°C, which is comparable to the growth range (20–62.5°C) of another thermophilic genus within the family, *Thermocrispum*. *Thermobispora* can also be differentiated from related genera using the 16S rRNA gene restriction fragment patterns, as suggested by Cook and Meyers (2003).

Taxonomic comments

Thermobispora bispora was originally described as "*Thermopolyspora bispora*" Henssen 1957, and later transferred to *Microbispora* as "*Microbispora bispora*" Lechevalier 1965. However, the strains classified as "*Microbispora bispora*" differed in many ways from the other validly named species of *Microbispora*, in the levels of DNA homology, growth temperature, predominant menaquinones, phospholipid type, major fatty acid profiles and the electrophoretic pattern of ribosomal proteins (Miyadoh et al., 1990; Ochi et al., 1993). Finally, Wang et al. (1996) sequenced the 16S rRNA gene of strains "*Microbispora bispora*" JCM 3082 and "*Microbispora bispora*" ATCC 19993, and combined these results with chemotaxonomic and DNA–DNA hybridization data to classify the two strains in the genus *Thermobispora*. In addition, Wang and colleagues (1996) highlighted the thermophilic lifestyle of *Thermobispora bispora* to support the reclassification of these strains.

Previously, *Thermobispora* was assigned to the family *Pseudonocardiaceae*, with *Pseudonocardia* and *Actinopolyspora* as neighboring genera (Garrity et al., 2005). However, this assignment was

not supported by chemotaxonomic markers and analyses of the 16S rRNA genes performed for this volume suggested that *Thermobispora* appeared as a deep lineage within the *Streptosporangiales*, but without a clear association to any of the described families (Ludwig et al., 2012). Because this assignment was not strongly supported by chemotaxonomic markers, the genus is placed in an order *incertae sedis*.

Miscellaneous comments

Thermobispora bispora ATCC 19993^T was reported to have four copies of rRNA operons, containing two distinct types of 16S rRNA genes, both transcriptionally active (Wang et al., 1997). Considerable numbers of nucleotide differences between the two gene sequences exist, but the invariable or rarely invariable regions of both genes remain unchanged.

List of species of the genus *Thermobispora*

1. **Thermobispora bispora** (Henssen 1957) Wang, Zhang and Ruan 1996, 937^{VP} [*Thermopolyspora bispora* Henssen 1957, 395; *Microbispora bispora* (Henssen 1957) Lechevalier 1965, 141]

   bi.spo′ra. Gr. adj. *bis* two; Gr. fem. n. *spora* seed; N.L. fem. adj. *bispora* two spores.

   Substrate and aerial mycelia are formed, and longitudinal pairs of spores are borne on the branches of the aerial mycelium. The diameter of the spores is usually 1.2–2.0 μm, but occasionally spores with a diameter of 3 μm are observed in liquid culture. The aerial mycelium is white and the substrate mycelium is yellow or yellowish brown on ISP medium 4 and IFO328 medium. Soluble pigments have not been observed. Starch is not hydrolyzed. Negative for iodinin production and nitrate reduction. Inositol and rhamnose are utilized for growth, but not arabinose or glycerol. The type strain grows between 50 and 65°C but not at 35°C.

   Habitat: decaying manure.

   DNA G+C content (mol%): 71 (T_m).

   Type strain: ATCC 19993, CBS 139.67, NBRC 14880.

   Sequence accession no. (16S rRNA gene): U58523 (ATCC 19993).

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Class II. **Acidimicrobiia** class. nov.

Paul R. Norris

A.ci.di.mi.cro.bi′i.a. N.L. neut. n. *Acidimicrobium* type genus of the type order; suff. *-ia* ending to denote a class; N.L. pl. neut. n. *Acidimicrobiia* the *Acidimicrobiales* class.

The class *Acidimicrobiia* was delineated on the basis of 16S rRNA gene sequence analyses and taxon-specific 16S rRNA signature nucleotides as for the subclass *Acidimicrobidae* Stackebrandt et al. 1997. The pattern of 16S rRNA signature nucleotides was revised as 242:284 (U–G), 291:309 (U–A), 316:337 (C–G), 819 (U), 952:1229 (C–G), and 1115:1185 (U–G) (Zhi et al., 2009). The class contains a single order, the type order *Acidimicrobiales*. The nomenclatural type is the genus *Acidimicrobium* Clark and Norris 1996, 1189^{VP} (Effective publication: Clark and Norris 1996, 790.)

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Zhi, X.-Y., W.-J. Li and E. Stackebrandt. 2009. An update of the structure and 16S rRNA gene sequence-based definition of higher ranks of the class *Actinobacteria*, with the proposal of two new suborders and four new families and emended descriptions of the existing higher taxa. Int. J. Syst. Evol. Microbiol. *59*: 589–608.

Order I. **Acidimicrobiales** Stackebrandt, Rainey and Ward-Rainey 1997, 483[VP]

Paul R. Norris

A.ci.di.mi.cro.bi′a.les. N.L. neut. n. *Acidimicrobium* type genus of the order; suff. *-ales* ending to denote an order; N.L. fem. pl. n. *Acidimicrobiales* the *Acidimicrobium* order.

In 2009, the emended description of the order *Acidimicrobiales* (Zhi et al., 2009) noted a single family, the *Acidimicrobiaceae*, with the pattern of 16S rRNA signature nucleotides as given for the subclass *Acidimicrobidae*, now the class *Acidimicrobiia*. Also in 2009, a second family of the order was proposed, the *Iamiaceae* (Kurahashi et al., 2009). Nucleotides U–A at positions 952:1229 in the type species of *Iamia*, the single genus in the family *Iamiaceae*, expand the pattern of 16S rRNA signature nucleotides given for the class *Acidimicrobiia* and the order *Acidimicrobiales*, which also includes C–G at positions 952:1229 (Zhi et al., 2009).

A large number of uncultured bacteria of the class *Actinobacteria* have been designated potential members of the *Acidimicrobiia* and *Acidimicrobiales* through analysis of their cloned 16S rRNA genes. Three genera have been described (*Acidimicrobium*, *Ferrimicrobium*, and *Ferrithrix*), which contain species from acidic mine waters or geothermal sites. The rRNA gene sequences of these species are relatively closely related among more divergent sequences found in some of the uncultured *Acidimicrobiia*. Another acidophilic, iron-oxidizing species, related to *Acidimicrobium ferrooxidans* has been isolated and will require placement in a new genus ("*Acidithiomicrobium*" sp.). These genera share their acidophilic phenotype (optimum growth at about pH 2) and a capacity for ferrous iron oxidation. In contrast, the type species of the genus *Iamia* (*Iamia majanohamensis*) is a neutrophile (optimum growth at pH 7; pH range 6–9). It was isolated from the epidermis of sea cucumber and is unlikely to oxidize ferrous iron. The 16S rRNA gene sequence of *Iamia majanohamensis* indicates a phylogenetic placement between the acidophiles of the *Acidimicrobiales* and representatives of the relatively closely related orders of the *Actinobacteria* (sequences from the type species of the type genera of the type families of the *Rubrobacteriales*, *Bifidobacteriales*, and *Coriobacteriales* were used in phylogenetic tree construction; Figure 412). Further consideration of the content, limits, and possible subdivision of the *Acidimicrobiales*, and any concurrence of these aspects with phenotype, will be facilitated by isolation and characterization of some of the large number of uncultured "*Acidimicrobiia*" that are phylogenetically peripherally related to the classified acidophiles of the order.

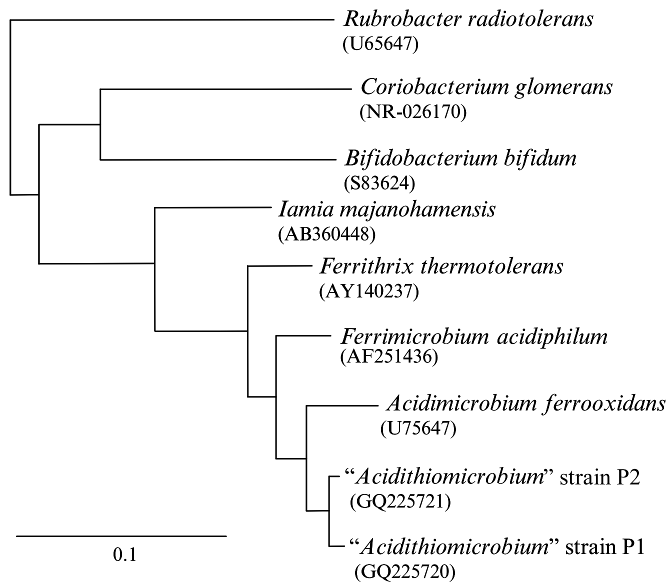

FIGURE 412. Phylogenetic relationships of members of the *Acidimicrobiales* and the most closely related orders of the *Actinobacteria*. Maximum-likelihood evolutionary tree of 16S rRNA gene sequences (GenBank accession nos in parentheses) from type species of the type genera of the orders *Rubrobacteriales* (the outgroup), *Bifidobacteriales* and *Coriobacteriales*, from the type species of the type genus of the family *Iamia*, and from the cultured, ferrous iron-oxidizing actinobacteria of the *Acidimicrobiales*. Bar = 0.1 substitutions per site. Bootstrap values from 100 replicates are shown.

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Family I. **Acidimicrobiaceae** Stackebrandt, Rainey and Ward-Rainey 1997, 483[VP]

Paul R. Norris

A.ci.di.mi.cro.bi.a.ce′a.e. N.L. neut. n. *Acidimicrobium* type genus of the family; suff. *-aceae* ending to denote a family; N.L. fem. pl. n. *Acidimicrobiaceae* the *Acidimicrobium* family.

Small rods, sometimes occur as filaments. Acidophilic, mesophilic, or moderately thermophilic, with the capacity for ferrous iron oxidation. All species are capable of heterotrophic growth. Some species are also autotrophic. The genera of this family (*Acidimicrobium*, *Ferrimicrobium*, and *Ferrithrix*) have single species representatives that, along with the unclassified "*Acidithiomicrobium* sp.", have the pattern of 16S rRNA signatures proposed for the class *Acidimicrobiia*.

Type genus: **Acidimicrobium** Clark and Norris 1996, 1189[VP] (Effective publication: Clark and Norris 1996, 790.).

Genus I. **Acidimicrobium** Clark and Norris 1996, 1189[VP] (Effective publication: Clark and Norris 1996, 790.)

A.ci.di.mi.cro′bi.um. N.L. neut. n. *acidum* (from L. adj. *acidus* sour) an acid; Gr. adj. *mikros* small; Gr. masc. n. *bios* life; N.L. neut. n. *microbium*, a microbe; N.L. neut. n. *Acidimicrobium* referring to a small bacterium from acidic environments.

Thermotolerant or **moderately thermophilic, acidophilic bacterium**. **Small, rod-shaped cells**, which may be in filaments of variable length. **Optimum growth occurs at 45–50°C** and approximately pH 2. Heterotrophic growth occurs on yeast extract, during which cells are motile. **Autotrophic growth occurs on ferrous iron. Most rapid growth is observed aerobically with ferrous iron and yeast extract.** Found in warm, acidic, mineral sulfide-rich environments.

DNA G+C content (mol%): 67–68 (T_m and HPLC).

Type species: **Acidimicrobium ferrooxidans** Clark and Norris 1996, 1189[VP] (Effective publication: Clark and Norris 1996, 790.).

Further descriptive information

The phylogenetic relationships of *Acidimicrobium* and related species are illustrated in Figure 412. The rod-shaped cells (0.35–0.4 × 1–1.5 µm) are sometimes found in long filaments, depending on the strain and growth conditions (Figure 413). The major fatty acids of total cell hydrolysates are $C_{16:0}$ iso (83%) and $C_{17:0}$ anteiso (8%) and the major menaquinone is MK-9(H_8) (Kurahashi et al., 2009).

Autotrophic growth occurs with ferrous iron as substrate in the presence of low concentrations of reduced sulfur compounds such as tetrathionate, which might provide a source of reduced sulfur for biosynthesis. Heterotrophic growth occurs with yeast extract (0.02%, w/v). Most rapid growth occurs in the presence of ferrous iron and yeast extract. Pyrite is rapidly oxidized during growth in the presence of yeast extract, but growth on elemental sulfur has not been described. Anaerobically, reduction of ferric iron occurs in the presence of organic substrates (yeast extract and glycerol).

Found in warm, acidic, iron-, sulfur- or mineral-sulfide-rich environments, including industrial commercial copper leach dumps, coal mine drainage, and natural geothermal springs.

Enrichment and isolation procedures

The type strain of the type species (*Acidimicrobium ferrooxidans* strain ICP[T]) was isolated from enrichment cultures grown in a mineral salts medium which contained (g/l): $MgSO_4 \cdot 7H_2O$ (0.4), $(NH_4)_2SO_4$ (0.2), K_2HPO_4 (0.1), $FeSO_4 \cdot 7H_2O$ (13.9), and yeast extract (0.2). The phosphate concentration was reduced (K_2HPO_4, 0.01 g/l) in medium solidified with phytagel (0.4%, w/v) for single colony isolation.

Maintenance procedures

Cultures can be maintained for several months at room temperature without shaking in medium (initially pH 2) containing pyrite (50–100 µm diameter particle size) and yeast extract (0.02%, w/v). The type strain can also be maintained vacuum dried and at −70°C in the presence of 7% (v/v) DMSO (Hallberg and Johnson, 2007).

Differentiation of the genus *Acidimicrobium* from other genera

The DNA G+C content and 16S rRNA gene sequence differentiate bacteria of the *Acidimicrobium* and related genera of iron-oxidizing actinobacteria (Table 305, Figure 412), whereas most other features are common to more than one genus. The single species representatives of the genera *Acidimicrobium*, *Ferrimicrobium*, and *Ferrithrix* (and the unclassified "*Acidithiomicrobium*" species) have the pattern of 16S rRNA signatures proposed for the *Acidimicrobiia*, *Acidimicrobiales*, and *Acidimicrobiaceae*, with nucleotides at positions 242:284 (U–G), 291:309 (U–A), 316:337 (C–G), 819 (U), 952:1229 (C–G), and 1115:1185 (U–G).

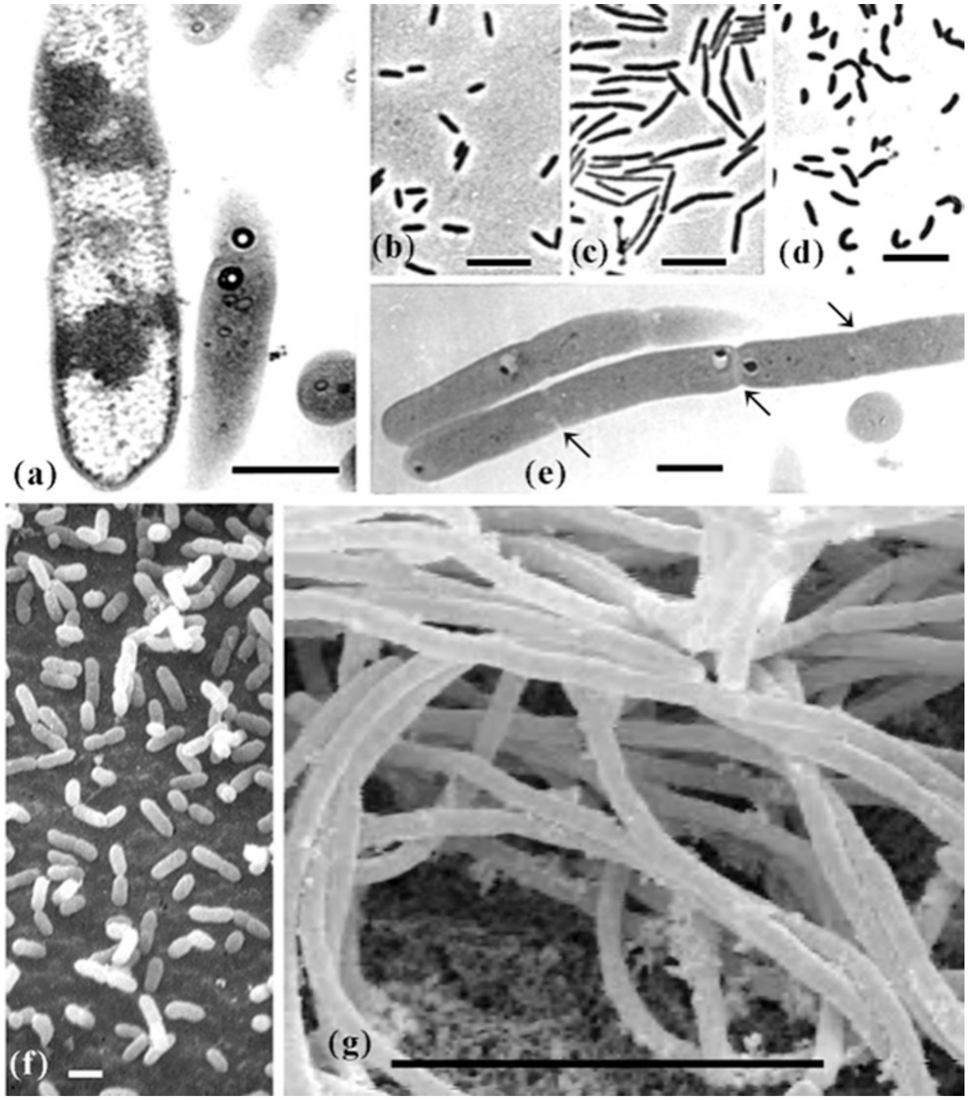

FIGURE 413. Morphology of ferrous-iron-oxidizing actinobacteria. (a) Electron micrograph of thin sections of a mixed culture of iron-oxidizing moderate thermophiles from which the type strain of *Acidimicrobium ferrooxidans* (strain ICP[T]) was isolated, showing small *Acidimicrobium*-like bacteria with a larger *Sulfobacillus* sp. (bar = 0.5 µm**).** Light microscopy of the type strain of *Acidimicrobium ferrooxidans* grown (b) autotrophically on ferrous iron, (c) on ferrous iron and yeast extract, and (d) heterotrophically on yeast extract (bars = 5 µm). (e) Thin sections of *Acidimicrobium ferrooxidans* strain TH3 grown on ferrous iron and yeast extract, with arrows indicating the cross walls in long filaments of cells (bar = 0.5 µm). Scanning electron micrographs of the type strains of (f) *Ferrimicrobium acidiphilum* (bar = 1 µm) and (g) *Ferrithrix thermotolerans* (bar = 5 µm), each grown on ferrous iron and yeast extract. [(a–e) reproduced with permission from Clark and Norris, 1996. Microbiology *142:* 785–790; (f–g) kindly provided by D.B. Johnson, University of Wales, Bangor, UK.]

TABLE 305. Differential characteristics of *Acidimicrobium ferrooxidans* and related, acidophilic, ferrous-iron-oxidizing bacteria[a]

Characteristic	*Acidimicrobium ferrooxidans*	*Ferrimicrobium acidiphilum*	*Ferrithrix thermotolerans*	"*Acidithiomicrobium*" strains P1 and P2
DNA G+C content (mol%)	68	55	50	52–55
16S rRNA identity to *Acidimicrobium ferrooxidans* (%)	100	93	91	95
Motility	+	+	−	+
Growth temperature (°C)[b]	48/57	35/37	43/50	50/59
Sulfur oxidation	−	−	−	+
Autotrophy	+	−	−	+

[a]+, Positive; −, negative.

[b]Optimum growth temperature/maximum growth temperature.

List of species of the genus *Acidimicrobium*

1. **Acidimicrobium ferrooxidans** Clark and Norris 1996, 1189[VP] (Effective publication: Clark and Norris 1996, 790.)

fer.ro.o′xi.dans. L. n. *ferrum* iron; N.L. v. *oxido* to oxidize; N.L. part. adj. *ferrooxidans* iron-oxidizing.

Cells are small and rod-shaped and may be in filaments of variable length. In contrast to the type strain, ICP[T], strain TH3 generally grows in filaments (Figure 413).

The optimum temperature for growth is about 48°C, with good activity maintained at 30°C and the maximum temperature for growth is about 57°C. Autotrophic growth on ferrous iron is weak compared to that of most autotrophic iron-oxidizing acidophiles. Heterotrophic growth occurs with yeast extract. Most rapid growth occurs in the presence of ferrous iron and yeast extract, where iron oxidation rapidly goes to completion. In the presence of yeast extract, pyrite is extensively oxidized during growth. In contrast, only weak acidification of the medium has been observed during growth with yeast extract and sulfur, and a capacity for sulfur oxidation has not been established.

Source: the type strain, ICP[T], was isolated from a pyrite enrichment culture established with a sample from an Icelandic geothermal site. Previously, strain TH3 of the same species was isolated from a copper mine leach dump in the USA (Norris and Barr, 1985). The TH3 designation was used previously for the original isolate of the species which came from the same source, but was not retained in culture (Brierley, 1978).

DNA G+C content (mol%): 67–68 (T_m and HPLC).

Type strain: ICP, DSM 10331.

Sequence accession no. (16S rRNA gene): U75647.

Genus II. **Ferrimicrobium** Johnson, Bacelar-Nicolau, Okibe, Thomas and Hallberg 2009, 1087[VP]

Paul R. Norris

Fer.ri.mi.cro′bi.um. L. n. *ferrum* iron; N.L. neut. n. *microbium* microbe; N.L. neut. n. *Ferrimicrobium* iron microbe, referring to its capacity for ferrous iron oxidation.

Motile rods, Gram-stain-negative. **Acidophilic**, mesophilic, and obligately heterotrophic. Capable of **oxidation of ferrous iron** and pyrite, and reduction of ferric iron. Grows with yeast extract or a limited range of organic compounds.

DNA G+C content (mol%): 55 (T_m).

Type species: **Ferrimicrobium acidiphilum** Johnson, Bacelar-Nicolau, Okibe, Thomas and Hallberg 2009, 1087[VP].

Further descriptive information

All information, including 16S rRNA phylogeny (Figure 412), pertains to the type strain of *Ferrimicrobium acidiphilum*, T23[T] (Johnson et al., 2009).

Colonies of the type species (strain T23[T]) display a "fried egg"-like morphology on solid medium (Johnson et al., 2009). Cells are rod-shaped and motile, with chains of three to five cells common during exponential growth. *meso*-Diaminopimelic acid is present in the peptidoglycan, which is of type A1γ. The major fatty acids are $C_{16:0}$ iso and $C_{14:0}$ iso (acetone extraction of whole-cell lysate). The dominant menaquinone is MK-8(H_{10}).

Optimum growth is observed at 35°C (no growth at 45°C) and pH 2.0 (minimum pH 1.4). Heterotrophic growth occurs on yeast extract and a limited range of defined organic substrates, including glycerol, citric acid, and glutamic acid.

16S rRNA gene sequences almost identical to that of *Ferrimicrobium acidiphilum* have been cloned from DNA extracted from various acidic mine waters or soils worldwide, including from the UK, the USA, Spain, and China.

Enrichment and isolation procedures

Ferrimicrobium acidiphilum T23[T] was isolated from pyrite enrichment cultures inoculated with mine water from the abandoned Cae Coch sulfur mine, North Wales, UK, by plating on a ferrous iron-containing overlay solid medium (Johnson and Hallberg, 2007).

Maintenance procedures

Ferrimicrobium acidiphilum T23[T] is routinely subcultured in liquid media containing 10 mM ferrous sulfate and 0.02% (w/v) yeast extract at pH 2.0 and 30°C. Cultures can be maintained for several months at room temperature without shaking in medium (initially pH 2) containing pyrite (50–100 µm particle size diameter) and yeast extract (0.02%, w/v). The type strain can also be maintained at −70°C in the presence of 7% (v/v) DMSO (Hallberg and Johnson, 2007).

Differentiation of the genus *Ferrimicrobium* from other genera

Among the single species-containing genera of iron-oxidizing actinobacteria, *Ferrimicrobium acidiphilum* is the only mesophile and can also be distinguished by its DNA composition and 16S rRNA gene sequence (Table 305).

List of species of the genus *Ferrimicrobium*

1. **Ferrimicrobium acidiphilum** Johnson, Bacelar-Nicolau, Okibe, Thomas and Hallberg 2009, 1087[VP]

a.ci.di′phi.lum. N.L. neut. n. *acidum* (from L. adj. *acidus* sour) an acid; N.L. adj. *philus -a -um* (from Gr. adj. *philos -ê -on*) friend, loving; N.L. neut. adj. *acidiphilum* acid-loving.

Cells are rod-shaped, 1–3 µm in length and 0.5 µm in diameter, and motile. Gram-stain-negative. Forms gelatinous colonies of approximately 1–3 mm diameter and with ferric iron deposits in the center on ferrous iron overlay plates. Mesophilic, optimum growth is at 35°C (maximum 37°C), and acidophilic, optimum growth is at pH 2 (minimum pH 1.4). Obligately heterotrophic with growth on yeast extract, glycerol, citric acid, and glutamic acid. Capable of oxidation of ferrous iron and pyrite, and, in the presence of glycerol, reduction of ferric iron. *meso*-Diaminopimelic acid is present in the peptidoglycan, which is of type A1γ. The major fatty acids are $C_{16:0}$ iso (64%), $C_{14:0}$ iso (11%), $C_{17:1}$ ω6*c* (5.97%), $C_{15:0}$ anteiso (5.14%), $C_{15:0}$ iso (5.03%), $C_{17:0}$ anteiso (3.18%), $C_{16:1}$ iso H (2.49%), and $C_{14:0}$ iso 3-OH (1.20%). The dominant menaquinone is MK-8(H_{10}), with minor amounts of MK-8 and MK-8(H_{10}) containing one or two methyl groups (Johnson et al., 2009).

Source: found in acidic iron-rich environments associated with the oxidation of sulfide minerals, such as acid mine drainage waters.

DNA G+C content (mol%): 55 (T_m).

Type strain: T23, DSM 19497, ATCC BAA-1647.

Sequence accession no. (16S rRNA gene): AF251436.

Genus III. **Ferrithrix** Johnson, Bacelar-Nicolau, Okibe, Thomas and Hallberg 2009, 1088[VP]

Paul R. Norris

Fer.ri.thr′ix. L. n. *ferrum* iron; Gr. fem. n. *thrix* hair, thread; N.L. fem. n. *Ferrithrix* iron thread, referring to filamentous nature and capacity for ferrous iron oxidation.

Grows as filaments (occasionally as single rods). Gram-stain-negative. **Acidophilic, thermotolerant**, or moderately thermophilic, with optimum growth at 43°C. **Obligately heterotrophic**. Grows on yeast extract, glycerol, or ethanol. Capable of **ferrous iron oxidation** and ferric iron reduction. Unknown growth factor(s) from yeast extract is (are) required for growth.

DNA G+C content (mol%): 50 (T_m).

Type species: **Ferrithrix thermotolerans** Johnson, Bacelar-Nicolau, Okibe, Thomas and Hallberg 2009, 1088[VP].

Further descriptive information

All information (see list of species) relates to the type strain (Y005[T]) of the single species (Johnson et al., 2009). Its phylogenetic relationship to other acidophilic, iron-oxidizing actinobacteria is illustrated in Figure 412.

Ferrithrix thermotolerans has not yet been isolated from outside of geothermal regions of Yellowstone National Park, USA, but closely related 16S rRNA gene sequences have been cloned from acid mine drainage in the UK and Spain.

Enrichment and isolation procedures

Ferrithrix thermotolerans was isolated from overlay solid medium containing ferrous iron that was streaked with a sample of a ferrous iron/yeast extract enrichment culture established with an acidic sample from the Beryl Spring/Gibbon River area of Yellowstone National Park (Johnson et al., 2003).

Maintenance procedures

Cultures can be maintained for at least 1 year at room temperature in medium (initially pH 2) containing pyrite (50–100 µm diameter particle size) and yeast extract (0.02%, w/v). The type strain can also be maintained at −70°C in the presence of 7% (v/v) DMSO (Hallberg and Johnson, 2007).

Differentiation of the genus *Ferrithrix* from other genera

Among the single species-containing genera of iron-oxidizing actinobacteria, *Ferrithrix thermotolerans* can be distinguished by its optimum growth temperature, which is between that of the mesophilic *Ferrimicrobium acidiphilum* and the moderately thermophilic *Acidimicrobium ferrooxidans*, and by its DNA composition and 16S rRNA gene sequence (Table 305).

List of species of the genus *Ferrithrix*

1. **Ferrithrix thermotolerans** Johnson, Bacelar-Nicolau, Okibe, Thomas and Hallberg 2009, 1088[VP]

ther.mo.to′le.rans. Gr. n. *thermê* heat; L. part. adj. *tolerans* tolerating; N.L. part. adj. *thermotolerans* heat-tolerating, able to tolerate high temperatures.

Grows as filaments (Figure 413), which form visible flocs of approximately 1–3 mm diameter, and occasionally as nonmotile, rod-shaped cells of 3–4 × 0.5 µm. Gram-stain-negative. Colonies are small (1–2 mm in diameter), rhizoidal, and iron-encrusted on ferrous iron, ferrous iron/tetrathionate

solid overlay media. The peptidoglycan type is A1γ. The major fatty acid is $C_{16:0}$ iso, with other fatty acids present in minor amounts. Optimal growth is at 43°C (maximum 50°C). Acidophilic, with optimal growth at pH 1.8 (minimum pH 1.6). Obligately heterotrophic, capable of growth with yeast extract, glycerol, and ethanol. An unknown growth factor(s) supplied by yeast extract is (are) required for growth. Also able to oxidize ferrous iron and reduce ferric iron.

Source: found in geothermal springs.

DNA G+C content (mol%): 50 (T_m).

Type strain: Y005, DSM 19514, ATCC BAA-1645.

Sequence accession no. (16S rRNA gene): AY140237.

Other bacteria

An *Acidimicrobium*-like bacterium, which was found in a pyrite-enrichment mixed culture with *Acidimicrobium ferrooxidans*, was initially referred to as "*Acidimicrobium*" species 2 (Cleaver et al., 2007). This bacterium was subsequently obtained in pure culture, but it has not yet been officially named. A closely related bacterium was isolated from an acidic, geothermal soil in Greece. These moderately thermophilic acidophiles have been described as "*Acidithiomicrobium*" strains P1 and P2, respectively (Norris et al., 2011; 16S rRNA gene sequence accession nos GQ225720 and GQ225721, respectively). They are noted here as close relatives of *Acidimicrobium ferrooxidans* and as species which increase the metabolic diversity of this family of actinobacteria. They grow strongly autotrophically on ferrous iron or pyrite in contrast to *Acidimicrobium ferrooxidans* and grow strongly autotrophically with elemental sulfur as the sole substrate (Davis-Belmar and Norris, 2009). They grow at slightly higher temperatures than *Acidimicrobium ferrooxidans* (Table 305). These features and their phylogenetic relationship to the other iron-oxidizing actinobacteria are noted (Table 305, Figure 412).

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Family II. **Iamiaceae** Kurahashi, Fukunaga, Sakiyama, Harayama and Yokota 2009, 872[VP]

Paul R. Norris

I.a.mi.a.ce′a.e. N.L. fem. n. *Iamia* type genus of the family; suff. *-aceae* ending to denote a family; N.L. fem. pl. n. *Iamiaceae* the *Iamia* family.

The family *Iamiaceae* was created for the type species *Iamia majanohamensis* in recognition of the evolutionary distance of its 16S rRNA gene sequence from that of *Acidimicrobium ferrooxidans*, the type species of the type genus of the *Acidimicrobiaceae*.

The pattern of 16S rRNA gene sequence signature nucleotides for the family consists of 408:434 (G–C), 722:733 (G–G), 1118:1155 (U–A), 443:491 (U–A), 1165:1171 (G–C), and 1263:1272 (A–U).

Type genus: **Iamia** Kurahashi, Fukunaga, Sakiyama, Harayama and Yokota 2009, 871[VP].

Genus I. **Iamia** Kurahashi, Fukunaga, Sakiyama, Harayama and Yokota 2009, 871^{VP}

Paul R. Norris

I.a′mi.a. N.L. fem. n. *Iamia* arbitrary name formed from the acronym of the Institute of Applied Microbiology at the University of Tokyo, which has made significant contributions to microbiology.

Gram-stain-positive rods. Aerobic. Oxidase- and catalase-positive. Mesophilic. The predominant menaquinone is MK-9(H_6). The major whole-cell fatty acids are $C_{17:0}$, $C_{17:1}$ ω8*c*, $C_{15:0}$, and $C_{16:0}$. Isolated from the epidermis of a sea cucumber and grown on marine agar.

DNA G+C content (mol%): 74.

Type species: **Iamia majanohamensis** Kurahashi, Fukunaga, Sakiyama, Harayama and Yokota 2009, 871^{VP}.

Further descriptive information

The phylogenetic relationship of the single species of *Iamia* to those of the *Acidimicrobiaceae*, the other family of the *Acidimicrobiales*, is illustrated in Figure 412. Further descriptive information is as for the single species, *Iamia majanohamensis* (Kurahashi et al., 2009).

Enrichment and isolation procedures

Iamia majanohamensis was obtained from the abdominal epidermis of a sea cucumber, *Holothuria edulis*, which was collected off the coast of Aka Island, Okinawa prefecture, Japan (Kurahashi and Yokota, 2004). Cultures were maintained routinely on marine agar at 25°C.

List of species of the genus *Iamia*

1. **Iamia majanohamensis** Kurahashi, Fukunaga, Sakiyama, Harayama and Yokota 2009, 871^{VP}

ma.ja.no.ha.men′sis. N.L. fem. adj. *majanohamensis* pertaining to Majanohama, the site on the coast of Aka Island, Japan, where the type strain was isolated.

Gram-stain-positive rods, 1.2–1.7 by 0.3–0.5 µm. Aerobic. Oxidase- and catalase-positive. Colonies on marine agar (which contains peptone and yeast extract) are small, white, circular, convex, smooth, shiny, and 0.2–0.3 mm in diameter after 3 weeks incubation at 30°C. Optimal growth temperature is 28–30°C. No growth occurs below 10°C or above 45°C. Hydrolysis of gelatin is observed. Positive for arginine dihydrolase, lysine decarboxylase, ornithine decarboxylase, production of acetoin and gelatinase, and reduction of nitrate to N_2, but negative for β-galactosidase, urease, tryptophan deaminase, utilization of citrate, production of H_2S and indole, and assimilation of amygdalin, arabinose, D-glucose, inositol, D-mannose, melibiose, rhamnose, sucrose, and sorbitol.

Source: the type strain was isolated from the ventral epidermis of the sea cucumber *Holothuria edulis* at Aka Island, Okinawa, Japan.

DNA G+C content (mol%): 74.4 (HPLC).

Type strain: F12, NBRC 102561, DSM 19957.

Sequence accession no. (16S rRNA gene): AB360448.

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Class III. **Coriobacteriia** class. nov.

Helmut König

Co.ri.o.bac.te.ri′i.a. N.L. neut. n. *Coriobacterium* type genus of the type order; suff. *-ia* ending to denote a class; N.L. pl. neut. n. *Coriobacteriia* the *Coriobacteriales* class.

The class is formed by elevation of the subclass *Coriobacteridae* Stackebrandt, Rainey and Ward-Rainey 1997. The subclass *Coriobacteridae* was circumscribed on the basis of phylogenetic analysis of the 16S rRNA gene sequences (Figure 414). The 16S rDNA/rRNA signature pattern is as that of the family *Coriobacteriaceae*.

Type order: **Coriobacteriales** Stackebrandt, Rainey and Ward-Rainey 1997, 483^{VP}.

Reference

Stackebrandt, E., F.A. Rainey and N.L. Ward-Rainey. 1997. Proposal for a new hierarchic classification system, *Actinobacteria* classis nov. Int. J. Syst. Bacteriol. *47*: 479–491.

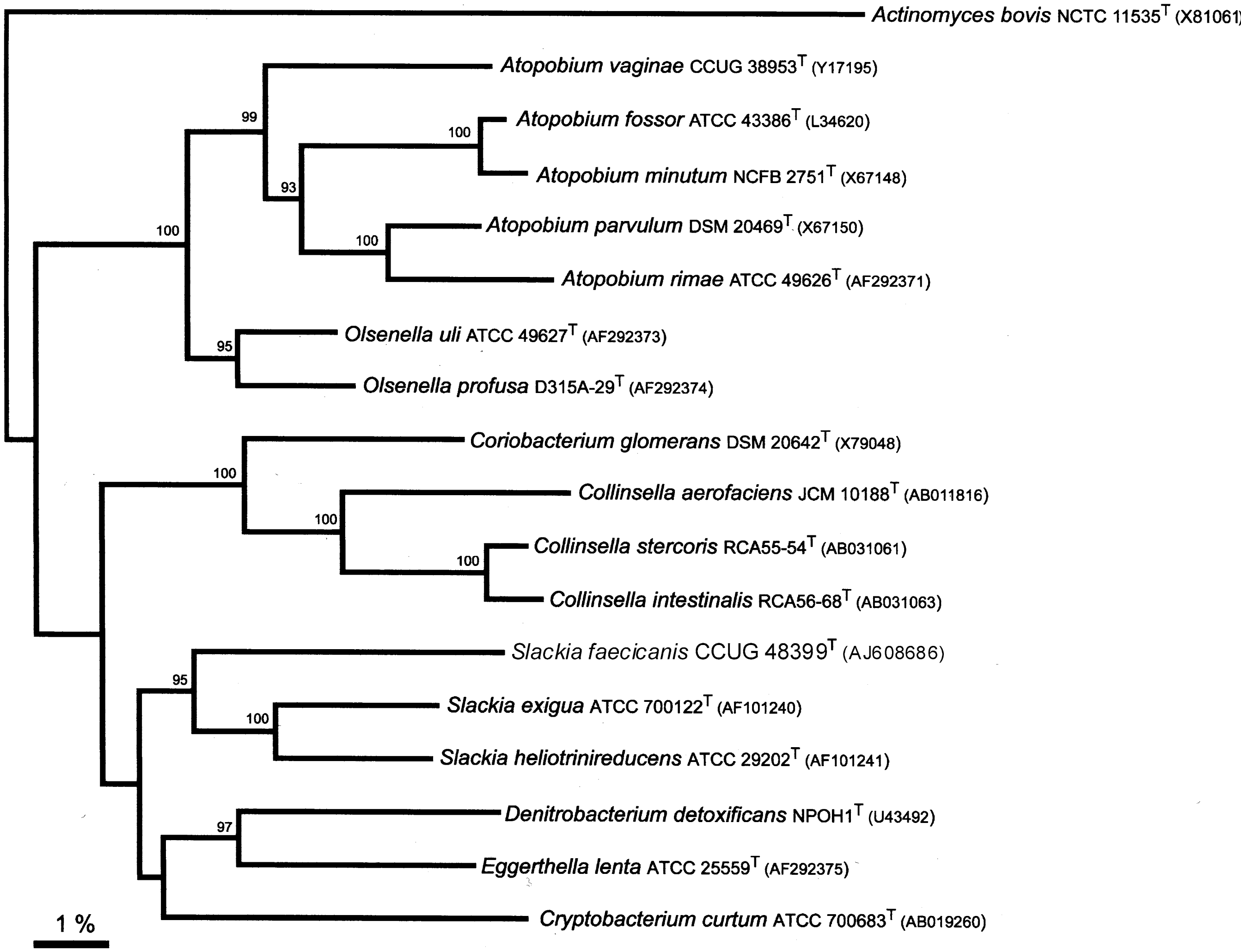

FIGURE 414. Unrooted tree of the species of the family *Coriobacteriaceae*. The tree was constructed using the neighbor-joining method based on a comparison of approximately 1330 nucleotides. Bootstrap values, expressed as a percentage of 1000 replications, are given at branching points. Bar = 1% sequence divergence. (Reprinted with permission from Lawson et al., 2005. Int. J. Syst. Evol. Microbiol. *55*: 1243–1246.)

Order I. **Coriobacteriales** Stackebrandt, Rainey and Ward-Rainey 1997, 483[VP]

Helmut König

Co.ri.o.bac.te.ri.a′les. N.L. neut. n. *Coriobacterium*, type genus of the order, suff. *-ales* ending to denote an order; N.L. fem. pl. n. *Coriobacteriales* the *Coriobacterium* order.

The order contains the type family *Coriobacteriaceae*. The 16S rDNA/rRNA gene signature pattern is as that of the family *Coriobacteriaceae*. The order *Coriobacteriales* was circumscribed on the basis of phylogenetic analysis of the 16S rRNA gene sequences.

Type genus: **Coriobacterium** Haas and König 1988, 383[VP].

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Family I. **Coriobacteriaceae** Stackebrandt, Rainey and Ward-Rainey 1997, 483[VP]

Helmut König

Co.ri.o.bac.te.ri.a.ce′a.e. N.L. neut. n. *Coriobacterium* type genus of the family: suff. *-aceae* ending to denote a family; N.L. fem. pl. n. *Coriobacteriaceae* the *Coriobacterium* family.

The pattern of 16S rDNA/rRNA signature nucleotides (base positions relative to *Escherichia coli*) of members of the family consists of 113:314 (C–G), 294:303 (G–C), 295:302 (U–A), 407:435 (C–G), 613:627 (G–C), 670:736 (G–C), 771:808 (U–A), 772:807 (A–U), 823:877 (A–U), 941:1342 (A–U), 950:1231 (U–G), 1120:1153 (G–C), 1148 (C), 1165:1171 (C–G), 1242:1295 (G–C), 1313:1324 (G–C), 1321 (C), 1410:1490 (A–U), 1415:1485 (C–G) and 1416:1484 (C–G). The family contains the genera *Coriobacterium*, *Atopobium*, *Collinsella*, *Cryptobacterium*, *Denitrobacterium*, *Eggerthella*, *Olsenella*, and *Slackia*. The genera were circumscribed on the basis of phylogenetic analysis of the 16S rDNA/rRNA gene sequences.

Rod-shaped or coccoid cells occur singly, in pairs or as rods. Spores and flagella are absent. Gram-stain-positive. Strictly or facultatively anaerobic growth. Sugars may or may not be fermented. The family *Coriobacteriaceae* was circumscribed on the basis of phylogenetic analysis of the 16S rDNA/rRNA gene sequences (Figure 414).

Type genus: **Coriobacterium** Haas and König 1988, 383[VP].

Genus I. **Coriobacterium** Haas and König 1988, 383[VP]

Helmut König

Co.ri.o.bac.ter′i.um. Gr. fem. n. *koris* bug; Gr. neut. n. *bakterion* a small rod; N.L. neut. n. *Coriobacterium*, rodlet associated with bugs.

The cells form chains of **pear-shaped** to irregularly shaped rods. The bacteria are **obligately anaerobic**. The cells stain **Gram-positive**. Spores and **flagella are absent**. Under strictly anaerobic conditions, the **fermentation products** of glucose are **acetic acid**, **L-lactic acid**, and **ethanol**. The **peptidoglycan** belongs to the **Lys–Asp type** (A4α). The bacteria occur in the **intestinal tracts** of insects.

DNA G+C content (mol%): 60–61.

Type species: **Coriobacterium glomerans** Haas and König 1988, 383[VP].

Further descriptive information

The fermentation products from glucose are L-lactic acid, acetic acid, and ethanol under strict anaerobic conditions with N_2/CO_2 as gas phase (using the culture technique of Balch et al. (1979). Under certain conditions in TPY medium (Scardovi, 1981), CO_2 and H_2 are also produced.

Enrichment and isolation procedures

A Gram-stain-positive anaerobic bacterium was regularly found in great numbers in histological sections and anaerobic cultures of the third bulbous mid-gut portion of the red soldier ant *Pyrrhocoris apterus* (L.). It formed long chains of irregular pear-shaped cells with large spherical involutions. All histological sections showed bundles of Gram-stain-positive chain-forming bacteria attached to the epithelium of the intestine at the beginning of the third bulbous portion of the mid-gut. The chains could reach more than 150 μm into the lumen of the gut.

Flattened, dry-looking colonies of chain-forming rod-like bacteria, with filamentous and irregular edges, could be detected when the gut contents were incubated anaerobically on blood-agar or supplemented Schaedler agar plates after 5–10 d at an incubation temperature of 25–37°C. The bacteria were further purified on TPY agar plates. The colonies consisted of long filaments, bent into hairpins, curls, and loops. In fluid TPY medium, bacteria formed a flocculent, woolly sediment with a clear supernatant.

Maintenance procedures

The bacteria can be maintained on TPY agar plates at 37°C. For longer periods, cultures can be stored in liquid nitrogen in the presence of 10% glycerol.

Differentiation of the genus *Coriobacterium* from other genera

Coriobacterium glomarans, the only species of this genus, is distinguished from members of the genus *Lactobacillus* by its higher G+C content (strain PW2[T], 60–61 mol%; *Lactobacillus*, 32–53 mol%) and by the molar ratio of the fermentation products.

Coriobacterium glomarans shares some characteristics with the genus *Bifidobacterium* (e.g. G+C content of the DNA 55–67 mol%) and it possesses the type 6 murein of this genus (Scardovi, 1981). Based on its ability to ferment carbohydrates, it does not correspond to any species in this group. It differs from the genus *Bifidobacterium* by its lack of a positive test for fructose-6-phosphate phosphoketolase and by production of hydrogen. The high amount of ethanol produced cannot be explained by the hexose fermentation pathway in bifidobacteria.

Strain PW2[T] is distinguished from *Eubacterium* by the G+C mol% content of its DNA (*Eubacterium*, 30–55 mol%), usually by its fermentation products, and by its murein composition. Several members of the genus *Eubacterium* produce hydrogen, and acetic and lactic acids as the sole major acid products, but among

these species, where the G+C content of the DNA is known, it is about 45 mol%; also, those species of *Eubacterium* that have been investigated possess the quite different type B murein.

The genus *Lachnospira* has been defined as containing curved-to-helical subterminally flagellated rods.

Coriobacterium glomarans is immotile, and flagella have not been observed by electron microscopy. *Lachnospira* but not *Coriobacterium glomarans* forms formic acid from glucose.

Taxonomic comments

Coriobacterium glomerans Haas and König 1988, 383^{VP} is the only species so far described in the genus *Coriobacterium*, but other strains/clones have been described (strain CCUG 33917, AJ131149; strain CCUG 33918, AJ131150; strain EKSO3, AJ245921; strain 3WC8.1, AJ586811; strain SG1214, AY669285; clone CC1015, AY669270).

List of species of the genus *Coriobacterium*

1. **Coriobacterium glomerans** Haas and König 1988, 383^{VP}

glo′me.rans. L. part. adj. *glomerans* agglomerating.

The cells form flocculent, wooly sediments with a clear supernatant in fluid media. They are regular to pear-shaped, forming cell chains under strictly anaerobic conditions (using the culture technique of Balch et al. (1979). They form irregularly shaped rods in older cultures or after culture in the GasPak Anaerobic System. The bacteria are strictly anaerobic. The cells are Gram-stain-positive (Kopeloff modification of the Gram stain).

The cells have an electron-dense Gram-stain-positive cell wall, 40 nm wide. The peptidoglycan belongs to the Lys–Asp type (A4α). The peptide subunit is composed of Asp, Glu, Gly, Ala, and Lys in a molar ratio of 0.91:1.00:0.45:1.96:1.01.

The length of the cells varies (0.44–1.80 μm). Spherical involution forms with a diameter of up to 198 μm are common. The bacteria form chains more than 150 μm long (Figure 415).

The colonies consist of long filamentous chain-forming bacteria, bent into hairpins, curls, and loops. The colony size is about 0.6 mm. The organisms grow on blood agar (Columbia agar base [BBL]), supplemented Schaedler agar (BBL), and TPY agar at 25°C and 30°C. When grown in TPY medium, the bacteria produce acetic acid, L-lactic acid, and ethanol from glucose in a molar ratio of 1.16:1.00:0.95. CO_2 and H_2 are also produced. D-Lactic acid, formic acid, volatile short-chain alcohols, or other volatile fatty acids are not formed. Under more stringent anaerobic conditions with N_2–CO_2 (80:20) as the gas phase, but lacking H_2, the molar ratio of acetic acid to L-lactic acid to ethanol is 1.20:1.00:0.23. This indicates that in the presence of the higher concentrations of molecular hydrogen (produced by the commonly used GasPack anaerobic system) strain PW2T forms increased amounts of ethanol.

The cells ferment L-arabinose, cellobiose, glucose, maltose, mannitol, melibiose, raffinose, mannose, ribose, salicin, sucrose, and D-xylose. Inositol, inulin, lactose, melibiose, raffinose, and starch are not fermented.

FIGURE 415. Electron micrograph of *Coriobacterium glomerans* strain PW2. Platinum shadowing of a cell chain showing irregular cells.

Source: the third bulbous midgut portion of all stages of the red soldier ant (*Pyrrhocoris apterus*; Heteroptera: Pyrrhocoridae), except the egg stage. The filamentous cell chains are attached to the epithelia of the intestines.

DNA G+C content (mol%): ca. 61 (T_m and nucleotide analysis)

Type strain: PW2, ATCC 49209, DSM 20642, JCM 10262.

DNA G+C content (mol%): X79048 (EMBL).

Genus II. **Atopobium** Collins and Wallbanks 1993, 188^{VP} (Effective publication: Wallbanks and Collins 1992, 239.)

Paul A. Lawson

A.to.po′bi.um. Gr. adj. *atopos* having no place, strange; Gr. neut. part. used as a noun *bion* living thing; N.L. neut. n. *Atopobium* strange living thing.

Cells consist of short rods, often with central swellings, or small cocci that may appear to be elliptical. They occur singly, in pairs, and in short chains. Gram-stain-positive, does not form spores, and is nonmotile. **Obligately or facultatively anaerobic and catalase-negative.** Nitrate is not reduced. **The major fermentation products from glucose are lactic acid together with acetic and formic acids; trace amounts of succinic acid may be formed.** Hydrogen is not produced. Growth is stimulated by

TABLE 306. Differential characteristics among members of the genus *Atopobium*[a,b]

Characteristic	*A. minutum*	*A. fossor*	*A. parulum*	*A. rimae*	*A. vaginae*
Acid produced from:					
Cellobiose	−	−	+	+	nd
Glucose	+	+	+	+	−
Lactose	−	−	+	−	−
Maltose	−	−	+	+	−
Mannose	+	+	+	+	−
Ribose	−	−	−	+	−
Sucrose	−	−	+	+	−
Enzyme production:					
Acid phosphatase	−	nd	+	+	+
Alanine arylamidase	−	nd	+	−	−
Arginine dihydrolase	+	nd	−	−	+
Arginine arylamidase	+	nd	+	−	+
Histidine arylamidase	−	nd	−	−	+
β-Galactosidase	−	nd	+	−	−
Leucine arylamidase	−	nd	+	−	+
Proline arylamidase	+	nd	−	−	+
Pyroglutamic acid arylamidase	d	nd	+	+	−
Glycine arylamidase	−	nd	+	−	+
Serine arylamidase	−	nd	−	−	+
Tyrosine arylamidase	−	nd	+	−	−
Fermentation products from glucose	L, A, F, s	L, A	L, A, F, s	L, A, F, s	nd
DNA G+C content (mol%)	44	44	39	45	44
Source	Human blood, abdominal wounds, pelvis abscesses	Horse pharynxes and root abscesses	Human gingival crevices	Human gingival crevices, periodontal pockets	Human vagina

[a]Symbols and abbreviations: −, negative; + positive; d, differences between strains; nd, not determined; a, acetic acid; f, formic acid; l, lactic acid; s, succinic acid; minor products are indicated by lower-case letters.

[b]Data from Rodriguez et al. (1999) and Kageyama et al. (1999b).

Tween 80. Growth may occur in the presence of 6.5% w/v NaCl. Gelatin is not liquefied, meat is not digested and indole is not produced. Isolated from human and animal sources.

DNA G+C content (mol%): 35–46 (T_m).

Type species: **Atopobium minutus** (Hauduroy, Ehringer, Urbain, Guillot and Magrou 1937) Collins and Wallbanks 1993, 188[VP] (Effective publication: Wallbanks and Collins 1992, 239.) (*Lactobacillus minutus* Hauduroy, Ehringer, Urbain, Guillot and Magrou 1937; Moore and Holdeman 1972, 63[AL] emend. Olsen, Johnson, Moore and Moore 1991).

Further descriptive information

In addition to being Gram-stain-positive and non-sporeforming rods to cocci, cells may contain central swellings. A variety of peptidoglycan types are evident, *Atopobium minutus* contains L-Orn–L-Ser–D-Glu (A4β), *Atopobium parvulum* L-Lys–D-Asp (A4α), and *Atopobium fossor* and *Atopobium rimae* L-Ala–D-Glu–LL-Dpm-Gly (A3γ). The cell-wall structure of *Atopobium vaginae* has not been determined. *Atopobium* species possess some unifying traits, but can be distinguished by a number of tests that include carbohydrate fermentation patterns, the presence or absence of metabolic enzymes, and the end products of glucose metabolism (see Table 306).

Atopobium spp. have been isolated from a number of human and animal sources. *Atopobium parulum* and *Atopobium rimae* were originally isolated from the human gingival crevice and *Atopobium vaginae* from the human vagina. Numerous studies employing both culture and culture-independent methods based on the sequencing of 16S rRNA genes have shown that *Atopobium* and other members of the family *Coriobacteriaceae* contribute towards the normal flora of the gastrointestinal tract and are readily recovered from human feces (Harmsen et al., 2000; Holdeman et al., 1976). Harmsen and colleagues developed a specific probe to two clusters that include *Coriobacterium* and *Collinsella* (S-*-Cor-0653-a-A18 (COR653) 5′ CCTCCC(/C) TACCGGACCC) and *Atopobium* S-*-Ato-0291-a-A-17 (ATO291) 5′ GGTCGGTCTCTCAACCC) which also includes the *Coriobacterium* group. These studies suggested that the diversity of *Atopobium* strains present in feces appeared to increase with the age of the individual. Although considered to be part of the normal flora of the GI tract, *Atopobium* may under particular conditions act as an opportunistic pathogen and contribute towards disease processes. *Atopobium rimae* and *Atopobium parvulum*, for example, have been identified as agents of chronic periodontitis (Kumar et al., 2005; Olsen et al., 1991).

Kumar et al. (2005) used a quantitative 16S clonal analysis to investigate biofilms associated with chronic periodontitis and periodontal health. Their data demonstrated that in addition to a number of other genera, *Atopobium* phylotypes were elevated in disease tissue. Furthermore, Angelakis et al. (2009)

reported an unusual case of human bacteremia caused by *Atopobium rimae* that was directly responsible for the septicemia.

Perhaps the most compelling evidence for the association of *Atopobium* with disease comes from investigations involving *Atopobium vaginae* with bacterial vaginosis (BV). Studies employing specific PCR primers towards *Atopobium* suggest that as many as 70% of women with BV harbor *Atopobium vaginae* and that postmenopausal women are particularly susceptible to BV (Burton et al., 2004). Although the exact etiology is still not clear, a possible explanation is that the available fermentable carbohydrate glycogen from vaginal epithelial cells decreases during the onset of menopause (Boskey et al., 1999; Burton et al., 2004; Cauci et al., 2002) promoting the use of other metabolic sources (i.e. proteins) for energy.

Atopobium fossor, the only non-human species of this genus, was originally isolated from horses; it is often recovered as a common component of the bacterial mixed-culture of the pharynx in addition to tooth root abscesses. Moreover, in a recent study of the lower respiratory tract of horses diagnosed with pneumonia, Racklyeft and Love (2000) found that 32% of the animals harbored *Atopobium fossor* using culture-based methods. Although they found a number of obligate anaerobes in co-culture, these authors were unable to suggest an association between any particular species and disease.

Isolation procedures

Complex media recommended for anaerobes by the Anaerobe Laboratory at the Virginia Polytechnic Institute (Holdeman et al., 1976) and the Wadsworth-KTL Anaerobe Laboratory Manual (Jousimies-Somer et al., 2002b) are frequently used as basal media. Supplements that include hemin and vitamin K are routinely added along with Tween 80, blood, or serum to enhance growth. There is no enrichment or selective medium for these organisms. *Atopobium minutum*, *Atopobium parvulum*, *Atopobium rimae*, and *Atopobium vaginae* have been recovered from human sources while *Atopobium fossor* has only been isolated from horses.

Maintenance procedures

For short-term storage, *Atopobium* strains can be maintained in chopped meat medium and DSMZ medium 104 PYG-medium (http://www.dsmz.de). For long-term preservation, strains can be maintained in a medium containing 15–20% glycerol at −70°C or lyophilized.

Taxonomic comments

Based on phylogenetic considerations and associated phenotypic features, the novel genus *Atopobium* was proposed by Collins and Wallbanks (1992) to accommodate misclassified *Lactobacillus* and *Streptococcus* species isolated from either human gingival crevices or from abscesses, blood, and wounds (Olsen et al., 1991). The genus originally contained three species, namely *Atopobium minutum*, *Atopobium parvulum*, and *Atopobium rimae*. Kageyama et al. (1999b) transferred *Eubacterium fossor* to the genus *Atopobium* based on phylogenetic and phenotypic evidence. This organism was recovered from the normal flora of the pharynx and from necrotic diseases of tissue surrounding the teeth of horses.

Some initial confusion existed on the precise phylogenetic position of *Atopobium*; the genus was originally described in a study that included organisms with phenotypic traits of lactic acid producing bacteria and other low G+C Gram-stain-positive organisms. However, Stackebrandt and Ludwig (1994) in a more comprehensive study showed that *Atopobium* formed a phyletic line in the *Actinomycetes* line of descent. In addition, Rainey et al. (1994) demonstrated the genera *Atopobium* and *Coriobacterium* were close phylogenetic neighbors which shared a number of phenotypic characteristics; members of these taxa were Gram-stain-positive, nonmotile, and non-spore-forming and gave end products of metabolism that included acetic and lactic acids. The close relationship between the two genera was underpinned in the hierarchic classification system of Stackebrandt et al. (1997) who classified these taxa in the family *Coriobacteriaceae* as they shared 16S rDNA/rRNA signature nucleotides, namely 113:314 (C–G), 294:303 (G–C), 295:302 (U–A), 670:736 (G–C), 771:808 (U–A), 772:807 (A–U), 823:877 (A–U), 941:1342 (A–U),950:1231 (U–G), 1120:1153 (G–C), 1148 (C), 1165:1171 (C–G), 1242:1295 (G–C), 1313:1324 (G–C), and 1410:1490 (A–U). Furthermore, the 16S rDNA loop 1357–1364 of these genera consists of only eight nucleotides while all other taxa in the *Actinomycetes* line of descent contain an additional nucleotide (Rainey et al., 1994).

Differentiation of the genus *Atopobium* from other genera

In addition to differentiation by 16S rRNA gene sequence analysis (Figure 416), *Atopobium* can be readily distinguished from its closest phylogenetic relatives using a combination of biochemical and chemotaxonomic criteria (Table 307).

List of species of the genus *Atopobium*

1. **Atopobium minutum** (Hauduroy, Ehringer, Urbain, Guillot and Magrou 1937) Collins and Wallbanks 1993, 188[VP] (Effective publication: Wallbanks and Collins, 1992, 239.) (*Lactobacillus minutus* Hauduroy, Ehringer, Urbain, Guillot and Magrou 1937; Moore and Holdeman 1972, 63[AL] emend. Olsen, Johnson, Moore and Moore 1991)

mi.nu′tum. L. neut. adj. *minutum* minute, small.

Strictly anaerobic, small, elliptical, nonmotile, Gram-stain-positive rods (approx. 0.6–1.0 × 0.8–3.0 µm) occur singly, in pairs, and in short chains. Colonies on brain heart blood agar are pinpoint to 1 mm in diameter, raised or low convex, entire, translucent to transparent, and buff, gray, light yellow, or white after 5 d of anaerobic incubation at 37°C. Growth is stimulated by 0.02 (v/v) Tween 80 and may be enhanced when 10% (v/v) rabbit serum is added to culture media. Six out of 10 tested strains grew at 45°C. Four out of 11 strains grew in the presence of 6.5% (w/v) NaCl.

The fermentation end products from glucose are lactic acid (0.2–4.5 meq/100 ml of culture; mean for 21 strains, 2.3 meq/100 ml), acetic acid (0–0.9 meq/100 ml; mean for

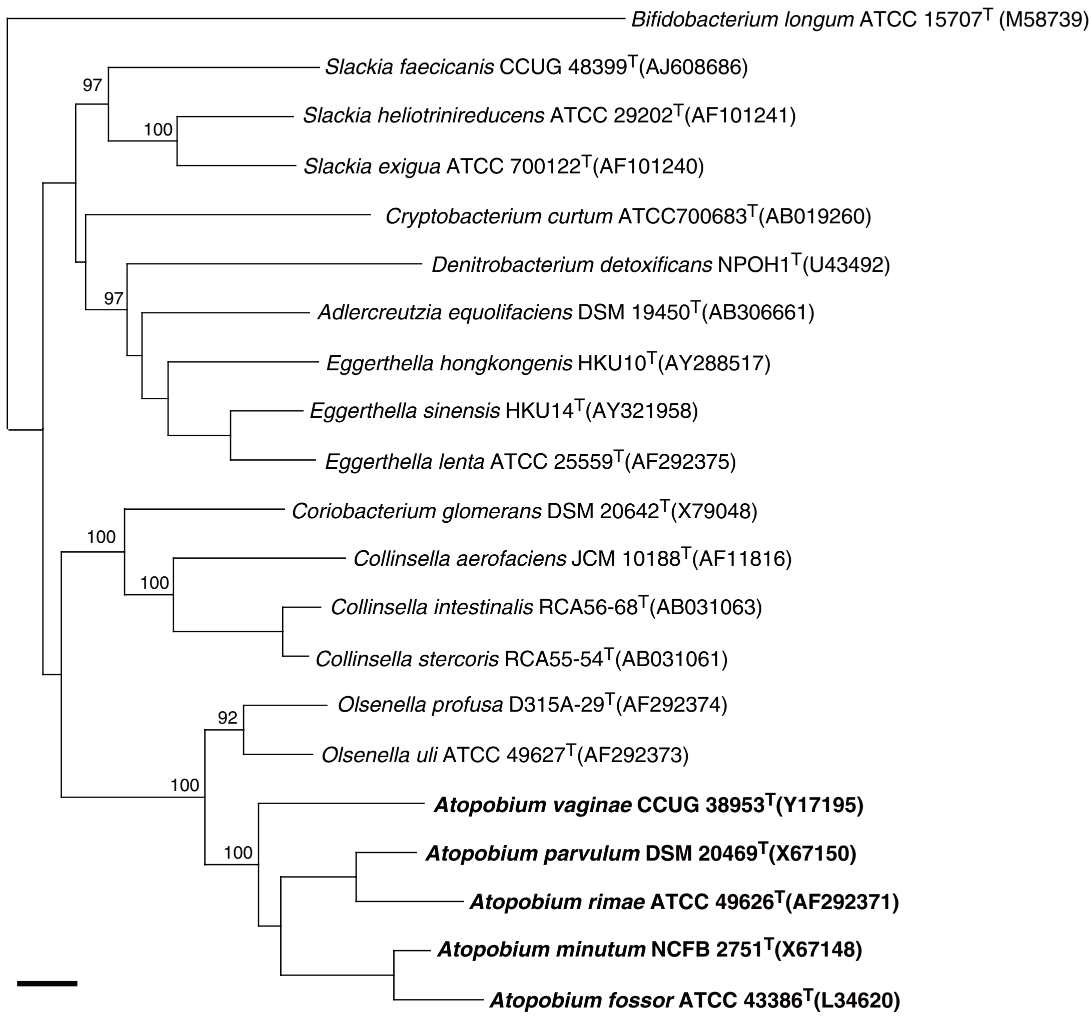

FIGURE 416. Unrooted neighbor-joining tree depicting the estimated phylogenetic relationships of members of the genus *Atopobium* and other members of the family *Coriobacteriaceae. Bifidobacterium longum* was used as the outgroup. The numbers on the branches refer to bootstrap values determined from 1000 replications. Only values about 90% are shown. Bar = 1% sequence divergence.

21 strains, 0.3 meq/100 ml), formic acid (0–0.7 meq/100 ml), and occasionally trace amounts of succinic acid. Little, if any, gas is detected in deep agar cultures. Hydrogen is not produced. The terminal pH range of PYG-Tween 80 broth cultures is 4.5–5.8; the mean terminal pH for 20 tested strains was 5.1.

A few strains (3 out of 27) weakly ferment starch (pH 5.5–5.7), but not amygdalin, erythritol, esculin, glycerol, glycogen, inositol, mannitol, melezitose, melibiose, raffinose, rhamnose, sorbitol, and xylose.

Gelatin is not liquefied, meat is not digested, indole is not produced, and nitrate is not reduced. Reactions are negative for bile-esculin, catalase, DNase, and hippurate hydrolysis.

Susceptibility to antibiotics varies: 1 out of 14 strains were resistant (μg per ml) to chloramphenicol (12), clindamycin (1.6), and erythromycin (3); 2 out of 14 strains were resistant to penicillin G (2U), and 3 out of 14 strains to tetracycline (6).

The major cellular fatty acid derivatives are $C_{18:1}$ *cis*9 fatty acid methyl ester and $C_{18:1}$ dimethylacetyl.

Source: abdominal wounds, blood, pelvic abscesses, and similar human infections.

DNA G+C content (mol%): 44 (T_m).

Type strain: ATCC 33267, CCUG 31167, DSM 20586, JCM 1118, LMG 9439, NCIMB 702751 (formerly NCFB 2751), VPI 9428.

Sequence accession no. (16S rRNA gene): M59059, X67148.

2. **Atopobium fossor** Kageyama, Benno and Nakase 1999d, 1325[VP] (Effective publication: Kageyama, Benno and Nakase 1999b, 394.) (*Eubacterium fossor* Bailey and Love 1986, 386[VP])

fos′sor. L. n. *fossor* a digger, delver.

TABLE 307. Characteristics that are useful in distinguishing the genera of the *Coriobacteriaceae* and *Adlercreutzia*[a,b]

Characteristic	1. *Atopobium*	2. *Adlercreutzia*	3. *Collinsella*	4. *Coriobacterium*	5. *Cryptobacterium*	6. *Eggerthella*	7. *Denitrobacterium*	8. *Olsenella*	9. *Slackia*
End products of metabolism	a, L, (s)	None	a, f, H, l	a, l	None	(a, l, s)[c]	nd	a, L, (s)	(a, iv)
Glucose fermentation	+	–	+	+	–	–	–	+	–
Growth stimulated by:									
Tween 80	+	–	+	nd	–	–	nd	+	–
Arginine	–	+	nd	nd	+	+	nd	–	+
Growth in 20% bile	–	–	nd	nd	–	+[b]	nd	d	–
Hydrogen production	–	–	+	+	nd	–	+	d	nd
Peptidoglycan type	A3γ, A4α, A4β	A1γ	A4	A4α	nd	A4γ	nd	A4β, A4α	nd
Major cellular fatty acid(s)	$C_{18:1}$ *cis9*	$C_{18:1}$ *cis9*	nd	nd	nd	$C_{14:0}$ br, $C_{15:0}$ br[c,d]	$C_{14:0}$ br, $C_{16:0}$ DMA	$C_{18:1}$ *cis9*[e]	$C_{18:0}$ br, $C_{18:1}$
DNA G+C content (mol%)	35–46	64.1–66.5	60–65	60–61	50–51	61.1–64.9	56–60	63–64	60–64
Source	Human oral cavity, blood, abdominal wounds. Horse pharynxes and root abscesses	Human and rat intestine	Human intestine	Red soldier beetle intestine	Human oral cavity	Human intestine and blood culture	Bovine rumen	Human oral cavity	Human oral cavity and dog intestine

[a]Abbreviations: a, acetic acid; f, formic acid; h, hydrogen; iv, isovaleric acid; l, lactic acid; s, succinic acid; minor products are indicated by lower-case letters, products in parentheses may or may not be formed; nd, not determined; br, branched. Symbols: –, negative; + positive; d, differences between strains.

[b]Data from Dewhirst et al. (2001) and Maruo et al. (2008).

[c]Determined for *Eggerthella lenta* but not for *Eggerthella hongkongensis* or *Eggerthella sinensis.*

[d]Maruo et al. (2008) found to be $C_{16:0}$ br.

[e]Determined for *Olsenella uli* but not for *Olsenella profuse.*

Obligately anaerobic, Gram-stain-positive, nonmotile cells (0.5–0.9 × 0.3 μm) that vary from coccobacillary to rod-shaped with parallel sides and rounded ends. Polar pili are present. On SBA, colonies are minute after 24 h, enlarging to 1 mm after 48 h when they appear to be set in small pits (0.3 mm deep and 3 mm wide) in the agar. After 4 d, colonies have umbonate to raised centers (1 mm) with either a roughened depressed region or a series of concentric rings radiating outward 1 to 2 mm from the center of colonies. Hemolysis does not occur. Growth in broth is improved by the addition of Tween 80 (0.75%, v/v) or serum (5%, v/v). Dense growth with uniform turbidity and a fine granular deposit is evident when organisms are grown in either prereduced peptone-yeast extract-glucose broth or cooked meat-glucose broth.

Acetic and lactic acids (lactic acid > acetic acid) are the major fermentation products. Gas is not detected in PYT-glucose deep agar cultures, although ammonia, hydrogen, and sulfide are produced.

Acid is formed from mannose but not from adonitol, amygdalin, arabinose, cellobiose erythritol, esculin, fructose, glycogen, inositol, lactose, maltose, mannitol, melezitose, melibiose, raffinose, rhamnose, ribose, salicin, sorbitol, starch, sucrose, trehalose, or xylose.

Most strains, including the type strain, ferment glucose weakly. Acetylmethylcarbinol, catalase, indole, lecithinase, lipase, and urease are not produced nor are esculin, starch, Tween 20, Tween 40, or Tween 80 hydrolyzed. Nitrate is not reduced. There is no reaction in milk and no digestion of gelatin. Bile neither stimulates nor inhibits growth. Cells do not survive heating for 10 min at 80°C.

The organism is susceptible (μg per ml) to ampicillin (4), carbenicillin (100), doxycycline (6), chloramphenicol (12), erythromycin (3), and to penicillin (2U per ml).

Source: the pharynx of a healthy horse.

DNA G+C content (mol%): 44 (T_m).

Type strain: ATCC 43386, CIP 106638, DSM 15642, JCM 9981, NCTC 11919, VPB 2127.

Sequence accession no. (16S rRNA gene): AB015945, L34620.

3. **Atopobium parvulum** (*ex* Weinberg, Nativelle and Prévot 1937) Collins and Wallbanks 1993, 188[VP] (Effective publication: Collins and Wallbanks 1992, 239.) (*Streptococcus parvulus ex* Weinberg, Nativelle and Prévot 1937, Cato 1983, 83)

par′vu.lus. L. neut. dim. adj. *parvulus* somewhat small.

Obligately anaerobic, nonmotile, nonspore-forming, Gram-stain-positive cocci (approx. 0.3–0.6 μm in diameter) that occur singly, in pairs, in clumps, and in short chains. Occasional elongate cells, some with a central swelling, may occur in stained preparations prepared from growth in media without Tween 80. Colonies on brain heart blood agar are pinpoint to 1 mm in diameter, raised or low convex,

circular, entire, translucent to transparent, and colorless to gray after 2–5 d of anaerobic incubation at 37°C. Growth is stimulated (30 out of 30 tested strains) in culture media supplemented with 0.02% (v/v) Tween 80 and with 10% (v/v) rabbit serum. Of 72 strains tested, 58 grew at 45°C, and 6 out of 67 strains grew in the presence of 6.5% (w/v) NaCl.

The fermentation products from glucose are lactic acid (0.7–6.3 meq/100 ml of culture; mean for 40 strains, 4.2 meq/100 ml), acetic acid (0.1–0.6 meq/100 ml; mean for 40 strains, 0.3 meq/100 ml), formic acid (0–0.3 meq/100 ml) and occasionally trace amounts of succinic acid. Little, if any, gas is detected in deep agar cultures. Hydrogen is not produced. The terminal pH range in PYG-Tween 80 broth cultures is 4.0–5.1; the mean terminal pH for 32 strains was 4.4.

A total of 11 out of 82 tested strains fermented esculin; 1 out of 68, glycerol; 1 out of 82, melibiose; and 16 out of 82, xylose. Amygdalin, erythritol, glycogen, inositol, mannitol, melezitose, raffinose, rhamnose, sorbitol, and starch are not fermented.

Starch is not hydrolyzed in the absence of added serum (which contains amylase). Gelatin is not liquefied, meat is not digested, indole is not produced, and nitrate is not reduced. Reactions are usually negative for bile-esculin (none out of 68 tested strains were positive), DNase (1 out of 68 strains positive), and hippurate hydrolysis (none of 68 strains positive). Catalase activity is not detected.

Susceptibility to antibiotics varies; 1 out of 82 tested strains were resistant (μg per ml) to chloramphenicol (12), 10 out of 82 to clindamycin (1.6), 5 out of 82 to erythromycin (3), 6 out of 82 to penicillin G (2U), and 1 out of 82 to tetracycline (6).

The major cellular fatty acid derivatives are $C_{18:1}$ *cis*9 fatty acid methyl ester and $C_{18:1}$ dimethylacetyl.

Source: human gingival crevices and, according to Prévot et al. (1967), from patients with pulmonary gangrene, tonsillitis, sinusitis, otitis, and dental and thoracic abscesses.

DNA G+C content (mol%): 39 (T_m).

Type strain: ATCC 33793, CCUG 32760, CIP 102970, DSM 20469, IPP 1246, JCM 10300, VPI 0546.

Sequence accession no. (16S rRNA gene): S44206, X67150.

4. **Atopobium rimae** (Olsen, Johnson, Moore and Moore 1991) Collins and Wallbanks 1993, 188[VP] (Effective publication: Wallbanks and Collins 1992, 239; *Lactobacillus rimae* Olsen, Johnson, Moore and Moore 1991, 264.)

ri′mae. L. gen. n. *rimae* of a fissure, pertaining to the gingival crevice.

Strictly anaerobic, small, elliptical, nonmotile, Gram-stain-positive rods occur singly, in pairs, and in short chains. Central swellings may occur, particularly in cells grown on solid medium. Colonies on brain heart blood agar are 1–2 mm in diameter, raised or low convex, entire, and translucent to transparent after 5 d of anaerobic incubation at 37°C. Growth is stimulated by 0.02% (v/v) Tween 80 and usually when 10% (v/v) rabbit serum (45 out of 53 strains) is added to culture media. Of 33 tested strains, 21 grew at 45°C. There is no growth in the presence of 6.5% (w/v) NaCl.

The fermentation products from glucose are lactic acid (1–10 meq/100 ml of culture; mean for 33 strains, 4.7 meq/100 ml), acetic acid (0.2–0.6 meq/100 ml; mean for 33 strains, 0.3 meq/100 ml), formic acid (0–0.4 meq/100 ml; mean, 0.05 meq/100 ml), and occasionally trace amounts of succinic acid. Little, if any, gas is detected in deep agar cultures. Hydrogen is not produced. The range of terminal pH of PYG-Tween 80 broth cultures is 3.8–4.9; the mean terminal pH for 30 strains was 4.3.

Glycogen is fermented only when serum (which contains amylase) is added to the medium. In the absence of serum, starch is seldom fermented (1 out of 66 strains positive) or hydrolyzed (11 out of 66 strains positive). Amygdalin, erythritol, and esculin are rarely fermented (1 out of 76 strains positive), glycerol, inositol, mannitol, melezitose, melibiose, raffinose, rhamnose, sorbitol, and xylose are not fermented.

Gelatin is not liquefied, meat is not digested, indole is not produced, and nitrate is not reduced. Reactions are negative for bile-esculin, DNase, and hippurate hydrolysis. Catalase activity is not detected.

Susceptibility to antibiotics: none of the 70 tested strains were resistant (μg per ml) to chloramphenicol (12), clindamycin (1.6), erythromycin (3), or tetracycline (6); 5 out of 70 strains were resistant to penicillin G (2U).

The major cellular fatty acid derivatives are $C_{18:1}$ *cis*9 fatty acid methyl ester and $C_{18:1}$ dimethylacetyl.

Source: human gingival crevices, especially in patients with periodontitis.

DNA G+C content (mol%): 45 (T_m).

Type strain: ATCC 49626, CCUG 31168, DSM 7090, NBRC 15546, NCFB 2896, JCM 10299, LMG 11476, VPI D140H-11A.

Sequence accession no. (16S rRNA gene): S44205, AF292371.

Additional comments: formerly known as *Lactobacillus* DO2.

5. **Atopobium vaginae** Rodriguez-Jovita, Collins, Sjödén and Falsen 1999, 1575[VP]

va.gi′nae. L. n. *vagina* vagina; L. gen. n. *vaginae* of the vagina.

Facultatively anaerobic, small-elongated Gram-stain-positive cocci occur singly, in pairs, or short chains. Tiny pin-head colonies are formed on blood agar at 37°C.

Acid is not produced from mannose or raffinose.

Positive for arginine dihydrolase, arginine arylamidase, acid phosphatase, glycine arylamidase, histidine, arylamidase, leucine arylamidase, proline arylamidase and serine arylamidase, but negative for alanine arylamidase, α-arabinosidase, cystine arylamidase, chymotrypsin, ester lipase C8, esterase C4, α-fucosidase, α- and β-galactosidase, β-galactosidase 6-phosphate, α- and β-glucosidase, glutamic acid decarboxylase, glutamylglutamic acid arylamidase, β-glucuronidase, α-mannosidase, β-*N*-acetylglucosaminidase, lipase C14, pyroglutamic acid arylamidase, tyrosine arylamidase, trypsin, and urease. Weak activity for phenylalanine arylamidase may be detected. Indole-negative. Nitrate is not reduced. Gelatin and esculin are not hydrolyzed.

Source: vaginal flora of a healthy person. Habitat is unknown.

DNA G+C content (mol%): 44 (T_m).

Type strain: ATCC BAA-55, CCUG 38953, CIP 106431, DSM 15829.

Sequence accession no. (16S rRNA gene): Y17195.

Genus III. **Collinsella** Kageyama, Benno and Nakase 1999c, 564$^{\mathrm{VP}}$

Akiko Kageyama and Yoshimi Benno

Col.lin.sel′la. N.L. fem. dim. ending *-ella*; N.L. fem. n. *Collinsella* named to honor Matthew D. Collins, a contemporary English microbiologist, for his outstanding contributions to microbial systematics.

Gram-stain-positive, obligatory anaerobic organism which forms chains of rods (0.3–0.7 × 1.2–4.3 μm). **Does not form spores or flagella**. Colonies are produced in 2 d at 37°C on EG agar in an anaerobic jar containing 100% CO_2. **Fermentation products of glucose are ethanol, formate, hydrogen, and lactate**. Cell wall contains an A4 type peptidoglycan. The 16S rDNA sequence analysis shows that the organism is a member of the family *Coriobacteriaceae*, which also includes the genera *Atopobium*, *Coriobacterium*, *Cryptobacterium*, *Denitrobacterium*, *Eggerthella*, *Olsenella*, and *Slackia*.

DNA G+C content (mol%): 60–65 (HPLC).

Type species: **Collinsella aerofaciens** (Eggerth 1935) Kageyama, Benno and Nakase 1999c, 564$^{\mathrm{VP}}$.

Further descriptive information

16S rRNA gene sequence data show that the genus *Collinsella* is closely related to the genus *Coriobacterium* of the family *Coriobacteriaceae*, class *Coriobacteriales* Stackebrandt et al. 1997 (Figure 417). Almost complete 16S rRNA gene sequences (positions 28–1491; *Escherichia coli* numbering system) have been determined for *Collinsella* strains. The range of sequence similarity values is 96.6–97.8% for *Collinsella aerofaciens* strains, and 98.2–99.5% for *Collinsella stercoris* strains; *Collinsella intestinalis* strains share a 99.6% sequence similarity.

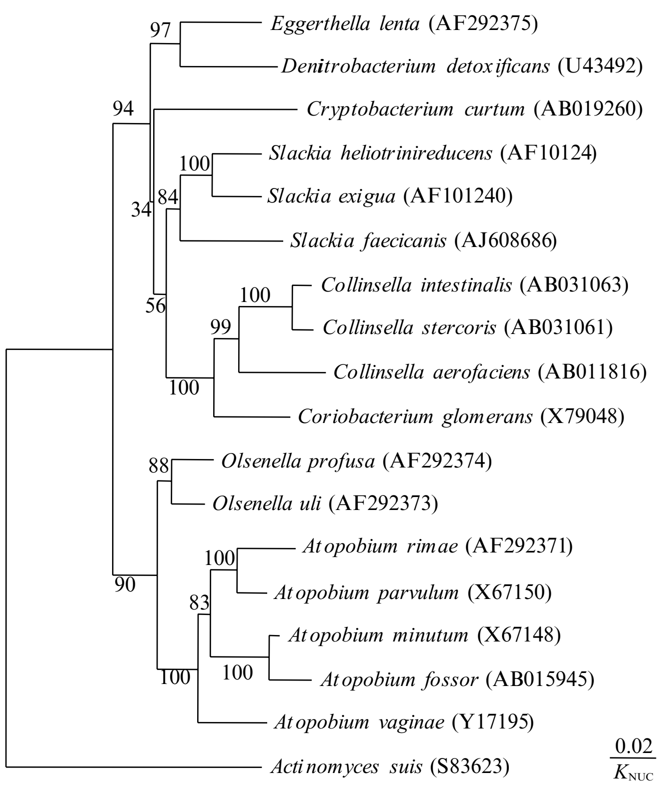

FIGURE 417. Phylogenetic tree derived from 16S rRNA gene sequences of the members of the family *Coriobacteriaceae* and constructed using the neighbor-joining method and K_{nuc} values. Numbers at branching points are bootstrap values (1000 resamplings). The tree was unrooted and *Actinomyces suis* was used as an outgroup.

Species-specific primers have been designed for the three *Collinsella* species. *Collinsella aerofaciens*-specific primers AERO-F (5′-CTTTCAGCAGGGAAGAGTCAA-3′; positions 436–466; *Escherichia coli* numbering system) and AERO-R (5′-AGCCATGCACCACCTGTATGG-3′; positions 1060–1039; *Escherichia coli* numbering system) generate an amplicon of about 590 bp; optimum PCR results are obtained at 63°C after 25 cycles. Similarly, *Collinsella intestinalis*-specific primers INTE-F (5′-CTTACCAGGGCTTGACATGA-3′; positions 980–999; *Escherichia coli* numbering system) and INRE-R (5′-TTAGGCGCCTCCCCCCAGAT-3′; positions 1469–1450; *Escherichia coli* numbering system) yield an amplicon of about 486 bp; optimum PCR results are obtained at 58°C after 25 cycles. Finally, *Collinsella stercoris*-specific primers STER-F (5′-TGCAAGTCGAACGGCACCCG-3′; positions 56–75; *Escherichia coli* numbering system) and STER-R (5′-CCGTCTGGGCTTTGCCGGCC-3′; positions 223–204; *Escherichia coli* numbering system) produce an amplicon of about 158 bp; optimum PCR results are obtained at 58°C and after 25 cycles.

Collinsella strains have an A4-type peptidoglycan (Schleifer and Kandler, 1972). *Collinsella aerofaciens* has an A4β-type peptidoglycan with a (L-Ala)–D-Glu–L-Orn–L-Asp peptide subunit and an interpeptide bridge which only contains L-asparagine moieties; *Collinsella intestinalis* an A4α-type peptidoglycan with a (L-Ala)–D-Glu–L-Lys–D-Glu peptide subunit and interpeptide bridge consisting of D-glutamic acid moieties, and *Collinsella stercoris* an A4β-type peptidoglycan with a (L-Ala)–D-Glu–L-Orn–D-Asp peptide subunit and an interpeptide bridge containing D-asparagine moieties.

The antibiotic sensitivity pattern of *Collinsella* strains, including 32 strains of *Collinsella aerofaciens*, eight strains of *Collinsella stercoris*, and two strains of *Collinsella intestinalis*, has been determined (Table 308). *Collinsella* strains are sensitive to various antibiotics, but not to ceftazidime or cefotetan (MIC: 12.5–25 μg/ml), or to aztreonam or carumonam (MIC: <100 μg/ml).

Collinsella aerofaciens is the most abundant bacterium in the human intestine [about 10^{10} cells (g feces)/min], and is found in more than 90% of human intestines (Benno et al., 1986; Finegold and Sutter, 1978; Moore and Holdeman, 1974). *Collinsella intestinalis* and *Collinsella stercoris* are also present in the human intestine. None of these three species exhibit pathogenicity.

Enrichment and isolation procedures

Strains of *Collinsella* species can be isolated from human feces of patients with colon cancer, Crohn's disease, and ulcerative colitis, and from healthy individuals on EG agar medium* and

*Premixed EG agar (Eiken Chemical Co., Ltd.) supplemented with 5% horse blood contains 3 g of beef extract, 5 g of yeast extract, 10 g of peptone, 1.5 g of glucose, 0.5 g of L-cysteine HCl, 0.2 g of L-cystine, 4 g of Na_2HPO_4, 0.5 g of soluble starch,, 0.5 g of polysorbate 80, 0.5 g of silicone, and 15 g of agar in 1000 ml of distilled water, pH 7.7.

TABLE 308. Susceptibilities of *Collinsella aerofaciens*, *Collinsella intestinalis*, and *Collinsella stercoris* to antimicrobial agents

Organism and antimicrobial agent	MIC (mg/ml) Range	50%	90%	MIC (mg/ml) for type strain
C. aerofaciens (32 strains)				
Ampicillin	0.025–0.78	0.2	0.2	0.2
Erythromycin	0.05–0.39	0.05	0.1	0.1
Tetracycline	0.39–50	0.78	1.56	0.78
Cephalothin	0.39–6.25	0.78	1.56	3.13
Cefuroxime	0.78–3.13	1.56	3.13	3.13
Ceftazidime	3.13–25	12.5	25	25
Cefotetan	6.25–50	12.5	25	50
Aztreonam	12.5–100	50	50	100
Carumonam	50–400	100	200	200
C. stercoris (8 strains)				
Ampicillin	0.05	0.05	0.05	0.05
Erythromycin	0.05	0.05	0.05	0.05
Tetracycline	0.39–0.78	0.39	0.78	0.78
Cephalothin	1.56	1.56	1.56	1.56
Cefuroxime	0.78–3.13	1.56	3.13	1.56
Ceftazidime	12.5	12.5	12.5	12.5
Cefotetan	25	25	25	25
Aztreonam	100–200	200	200	200
Carumonam	100–200	100	200	200
C. intestinalis (2 strains)				
Ampicillin	0.2	0.2	0.2	0.2
Erythromycin	0.78	0.78	0.78	0.78
Tetracycline	0.78	0.78	0.78	0.78
Cephalothin	12.5	12.5	12.5	12.5
Cefuroxime	1.56–3.13	1.56	3.13	1.56
Ceftazidime	12.5	12.5	12.5	12.5
Cefotetan	50	50	50	50
Aztreonam	12.5	12.5	12.5	12.5
Carumonam	100	100	100	100

in EGF broth.* Organisms are cultivated for 2 d at 37°C in an anaerobic chamber using these media.

Maintenance procedures

Collinsella strains can be maintained for several weeks on EG agar plates in an anaerobic jar containing 100% CO_2. Long-term storage can be achieved at −80°C in 10% glycerol or by lyophilization in the presence of stabilizers such as 10% skim milk.

Differentiation of the genus *Collinsella* from other genera

A number of characteristics that can be used to distinguish the genus *Collinsella* from other genera classified in family *Coriobacteriaceae* are shown in Table 307. These properties include fermentation end products. Specific primers can also be used to distinguish *Collinsella* strains from the other genera.

*Contains 2.4 g of Lab-Lemco powder (Oxoid), 10 g of proteose peptone No.3 (Difco), 5 g of yeast extract (Difco), 4 g of Na_2HPO_4, 40 ml of sterilized horse blood, 5 g of glucose, 0.5 g of soluble starch (Sigma) and 0.5 g of L-cysteine HCl in 960 ml of distilled water, pH 7.6.

Taxonomic comments

Eubacterium aerofaciens was reclassified as *Collinsella aerofaciens* by Kageyama et al. (1999c). Moore et al. (1971) had reported that these organisms belonged to the genus *Eubacterium* because they were Gram-stain-positive, obligatorily anaerobic, non-spore-forming rods that did not produce propionic acid as a major product (*Propionibacterium*); lactic acid as a sole major acid (*Lactobacillus*); large amounts of succinic (in the presence of CO_2) and lactic acids, sometimes with acetic and formic acids (*Actinomyces*); or acetic and lactic acids (acetic > lactic), with or without formic acid, as sole major acid products (*Bifidobacterium*). The genus *Eubacterium* had a broad definition and acted as a repository for large numbers of phenotypically diverse species (Andreesen, 1992).

The type species of the genus *Eubacterium* is *Eubacterium limosum*. It was evident from several studies that *Eubacterium barkeri*, *Eubacterium callanderi*, and *Eubacterium limosum* (Mountfort et al., 1988) formed the nucleus of the redefined genus *Eubacterium*. Based on the characteristics of this group, a preliminary working definition of *Eubacterium sensu stricto* was proposed: Gram-stain-positive, rod-shaped nonmotile obligatory anaerobic organisms which may form endospores, are saccharoclastic, produce acetate, butyrate, lactate, and H_2, and formate or CO_2 as products of glucose fermentation. The cell wall has a type B peptidoglycan and the G+C content of the DNA is within the range 45–47 mol% (Willems and Collins, 1996).

Some species classified in the genus *Eubacterium*, but not assigned to *Eubacterium sensu stricto*, have been transferred to established or new genera (Kageyama et al., 1999b, 1999c; Ludwig et al., 1992; Wade et al., 1999; Willems et al., 1997). *Eubacterium aerofaciens* was reclassified to the novel genus *Collinsella* based on phylogenetic characteristics and cell-wall peptidoglycan type (Kageyama et al., 1999c). Subsequently, two new *Collinsella* species were proposed, *Collinsella intestinalis* and *Collinsella stercoris*, and the description of genus *Collinsella* was emended (Kageyama and Benno, 2000).

Differentiation of species of the genus *Collinsella*

Properties which can be used to distinguish between *Collinsella* species are shown in Table 309 and Table 310. Species specific primers also can be used for species differentiation.

TABLE 309. Differences in antibiotic susceptibilities between *Collinsella* species

	MIC_{90} (mg/ml)[a]		
	C. aerofaciens	*C. stercoris*	*C. intestinalis*
Erythromycin	0.1	0.05	0.78
Cephalothin	1.56	1.56	12.5
Cefotetan	25	25	50
Aztreonam	50	200	12.5

[a]MICs at which 90% of strains are inhibited.

TABLE 310. Differential characteristics of *Collinsella* species[a,b]

	C. aerofaciens	*C. stercoris*	*C. intestinalis*
Acid from:			
Lactose	+	+	−
Maltose	+	+	−
Enzyme activity:			
Alkaline phosphatase	−	w	w
Acid phosphatase	w	+	+
Leucine arylamidase	w	+	+
N-Acetyl-β-glucosaminidase	−	+	+

[a]Data based on reactions of six strains of *Collinsella aerofaciens*, two strains of *Collinsella intestinalis*, and eight strains of *Collinsella stercoris*.

[b]Symbols: +, positive; w, weakly positive; −, negative.

List of species of the genus *Collinsella*

1. **Collinsella aerofaciens** (Eggerth 1935) Kageyama, Benno and Nakase 1999c, 564[VP] [*Eubacterium aerofaciens* (Eggerth 1935) Prévot 1938, 295[AL]]

ae.ro.fa′ci.ens. Gr. n. *aer*, *aeros* air, gas; L. v. *facere* to make, to produce; N.L. part. adj. *aerofaciens* gas-producing.

Gram-stain-positive, obligatory anaerobic organism which occur in chains of 6–120 cells (0.3–0.7×1.2–4.3 µm). Spores and flagella are absent. Colonies are white in the center with clear surroundings and exhibit protuberances on EG agar. Can be cultivated in 2 d at 37°C on EG agar in an anaerobic jar containing 100% CO_2. Acid is produced from fructose, galactose, glucose, lactose, maltose, and mannose, but not from arabinose, erythritol, glycogen, inositol, mannitol, melezitose, soluble starch, sorbitol, or xylose. Has an A4β-type peptidoglycan with a (l-Ala)–d-Glu–l-Orn–l-Asp peptide subunit and interpeptide bridge composed of l-asparagine moieties.

Source: human feces.

DNA G+C content (mol%): 61 (HPLC).

Type strain: JCM 10188, ATCC 25986, CCUG 28087, DSM 3979, NCTC 11838, VPI 1003.

Sequence accession no. (16S rRNA gene): AB011816.

2. **Collinsella intestinalis** Kageyama and Benno 2000, 1773[VP]

in′test.in.alis. n. *intestinum* gut, intestine; L. suff. *-alis* suffix denoting pertaining to; N.L. masc. adj. *intestinalis* pertaining to the intestine.

Gram-stain-positive, obligate anaerobic organism that occur in chains of 2–20 cells (0.3–0.5 × 1.2–2.2 µm). Colonies are white or gray in the center with clear margins on EG agar. Spores and flagella are absent. Grows on EG agar after 2 d at 37°C in an anaerobic jar containing 100% CO_2. Acid is produced from cellobiose, fructose, galactose, glucose, mannose, ribose, and salicin, but not from amygdalin, l-arabinose, erythritol, esculin, glycogen, inositol, lactose, maltose, mannitol, melezitose, raffinose, rhamnose, sorbitol, starch, sucrose, trehalose, or d-xylose. Positive for leucine arylamidase, acid phosphatase, naphthol-AS-BI-phosphohydrolase, and *N*-acetyl-β-glucosaminidase, but negative for chymotrypsin, cystine arylamidase, α-fucosidase, α-galactosidase, β-glucuronidase, lipase (C4), α-mannosidase, trypsin, and valine arylamidase. Has an A4α type peptidoglycan with a (l-Ala)–d-Glu–l-Lys–d-Glu peptide subunit, and interpeptide bridge composed of d-glucose moieties.

Source: human feces.

DNA G+C content (mol%): 64.4 (HPLC).

Type strain: JCM 10643, DSM 13280, RCA56-68, CCUG 45296, CIP 106914.

Sequence accession no. (16S rRNA gene): AB031063.

3. **Collinsella stercoris** Kageyama and Benno 2000, 1773[VP]

ster′co.ris. L. n. *stercus -oris* dung, excrement, feces; L. gen. n. *stercoris* of feces, referring to source of the isolate.

Gram-stain-positive, obligate anaerobic organism which occurs in chains of 2–20 cells (0.3–0.4 × 1.3–2.4 µm). Colonies are white or gray in the center with clear margins on EG agar. Spores and flagella are absent. Grows on EG agar in 2 d at 37°C in an anaerobic jar containing 100% CO_2.

Acid is produced from cellobiose, fructose, galactose, glucose, lactose, maltose, mannose and salicin, but not from amygdalin, L-arabinose, erythritol, esculin, glycogen, inositol, mannitol, melezitose, raffinose, rhamnose, ribose, sorbitol, starch, sucrose, trehalose or D-xylose. Positive for acid phosphatase, β-galactosidase, leucine arylamidase, naphthol-AS-BI-phosphohydrolase, and *N*-acetyl-β-glucosaminidase-positive, but negative for chymotrypsin, cystine arylamidase, α-fucosidase, α-galactosidase, β-glucuronidase, lipase (C4), α-mannosidase, trypsin, or valine arylamidase. Has an A4β type peptidoglycan with a (L-Ala)–D-Glu–L-Orn–D-Asp peptide subunit, and interpeptide bridge consisting only of D-asparagine moieties.

Source: human feces.

DNA G+C content (mol%): 61.2 (HPLC).

Type strain: JCM 10641, DSM 13279, RCA55-54, CCUG 45295, CIP 106913.

Sequence accession no. (16S rRNA gene): AB031061.

Genus IV. **Cryptobacterium** Nakazawa, Poco, Ikeda, Sato, Kalfas, Sundqvist and Hoshino 1999, 1199VP

Futoshi Nakazawa

Crypt.o.bac.te′ri.um. Gr. n. *kruptos* hidden; Gr. n. *bakterion* a small rod; N.L. neut. n. *Cryptobacterium* a hidden rod-shaped bacterium.

Cells are very short rods, 0.4 × 0.8–1.0 µm. Gram-stain-positive, occasionally Gram-stain-negative in stationary phase, having a cell wall typical of Gram-stain-positive bacteria (Figure 418). **Nonmotile, non-sporing, and obligately anaerobic. Catalase-negative. Asaccharolytic and no volatile end product in peptone-yeast extract-glucose broth**. Positive for arginine hydrolysis, negative for nitrate reduction and inert in most conventional biochemical tests. Isolated from the periodontal pocket of an adult human with periodontal disease, and from necrotic dental pulp.

DNA G+C content (mol%): 50–51 (T_m).

Type species: **Cryptobacterium curtum** Nakazawa, Poco, Ikeda, Sato, Kalfas, Sundqvist and Hoshino 1999, 1199VP.

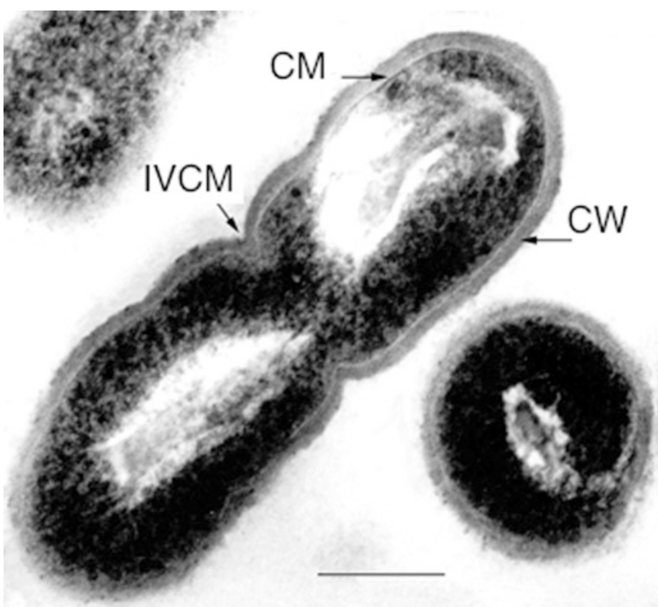

FIGURE 418. Transmission electron microphotograph of *Cryptobacterium curtum*. CM, cytoplasmic membrane; CW, cell wall, IVCM, invagination of cytoplasmic membrane.

Enrichment and isolation procedures

Growth in broth media is very poor with or without carbohydrates. *Cryptobacterium* forms circular, convex, and translucent colonies on BHI-blood agar plates, which are about 0.3–0.5 mm in diameter. Colonies are less than 1 mm in diameter even after prolonged incubation. Hemolysis does not occur around colonies on BHI-blood agar plates.

Differentiation of the genus *Cryptobacterium* from other genera

According to the results of 16S rRNA gene sequence analyses, the genus *Cryptobacterium* belongs to the family *Coriobacteriaceae* which contains anaerobic, asaccharolytic, Gram-stain-positive rods. DNA–DNA hybridization studies indicate that the genus *Cryptobacterium* shows low levels (1–2%) of DNA relatedness against closely related taxa such as the genera *Collinsella*, *Eggerthella* (Nakazawa and Hoshino, 2004), *Mogibacterium* (Nakazawa et al., 2000, 2002), and *Slackia*. The genus *Cryptobacterium* can also be distinguished by restriction fragment length polymorphism (RFLP) analysis of 16S rDNA using the endonucleases *Alu*I and *Smf*. SDS-PAGE analysis of whole-cell proteins and Western blotting reactions with rabbit antisera also allow the genus *Cryptobacterium* to be distinguished from related genera. In addition, the genus can be differentiated from the genera *Collinsella*, *Eggerthella*, and *Slackia* by metabolic end products, arginine hydrolysis, and nitrate reduction.

Taxonomic comments

Initially organisms now classified as *Cryptobacterium* species were isolated from the human oral cavity as anaerobic, asaccharolytic, *Eubacterium*-like isolates. However, these isolates were inert in most conventional biochemical tests and could not be assigned to previously established bacterial species. The genus *Cryptobacterium*, with *Cryptobacterium curtum* as the type strain, was described on the basis of a polyphasic taxonomic study, notably by using 16S rRNA gene sequence and DNA–DNA hybridization data.

List of species of the genus *Cryptobacterium*

1. **Cryptobacterium curtum** Nakazawa, Poco, Ikeda, Sato, Kalfas, Sundqvist and Hoshino 1999, 1199[VP]

cur′tum. L. neut. adj. *curtum* shortened (a shortened cell of this organism).

Cells are very short, Gram-stain-positive, obligately anaerobic, nonmotile, non-sporing rods which occur singly or in masses, and which sometimes stain Gram-negative in older cultures. On BHI-blood agar plates they form minute circular, convex, translucent colonies less than 1 mm in diameter, even after prolonged incubation in an anaerobic glove box. Growth in broth media is poor with or without carbohydrates. Hemolysis does not occur on BHI-blood agar plates. They are inert in most biochemical tests. Neither esculin nor starch are hydrolyzed. Does not reduce nitrate or liquefy gelatin. Negative for catalase, indole, and urease. Ammonia is produced from arginine. Nonfermentative and do not utilize adonitol, amygdalin, arabinose, cellobiose, erythritol, esculin, fructose, galactose, glucose, glycogen, inositol, lactose, maltose, mannitol, mannose, melezitose, melibiose, rhamnose, ribose, salicin, sorbitol, starch, sucrose, trehalose, or xylose. No metabolic end product is detected in peptone-yeast extract medium supplemented with glucose or peptone-yeast extract-glucose broth.

Source: the periodontal pocket of an adult human with periodontal disease, and from necrotic dental pulp.

DNA G+C content (mol%): 50–51 (HPLC).

Type strain: 12-3, ATCC 700863.

Sequence accession no. (16S rRNA gene): AB019260.

Genus V. **Denitrobacterium** Anderson, Rasmussen, Jensen and Allison 2000, 633[VP]

Robin C. Anderson and Thaddeus B. Stanton

De.nit.ro.bac.te′ri.um. L. pref. *de* from; L. n. *nitro* nitrocompound; Gr. neut. dim. n. *bakterion* a small rod; N.L. neut. n. *Denitrobacterium* named for a nitrocompound-reducing rod-shaped bacterium.

Rod-shaped cells (0.5–1.0 × 1.0–1.5 µm). Cells may possess irregular, bulbous ends. **Gram-stain-positive, non-sporulating**, and **nonmotile**. Strictly **anaerobic** chemo-organotroph; growth is supported by suitable electron acceptors and is proportional to amount of acceptor reduced. **Nonfermentative.** Growth occurs in anaerobic media containing clarified ruminal fluid, peptone, and a suitable electron acceptor. **Dimethyl sulfoxide, nitroethane, 3-nitropropan-1-ol, 2-nitropropan-1-ol, 3-nitropropionate, trimethyl amine-oxide and, by some strains, nitrate, may be used as electron acceptors and support good growth**. **Nitroethanol, 2-nitrobutane, and 1-nitropropane also may be used as electron acceptors, but growth with these compounds is marginal, presumably due to the accumulation of toxic end products. Azide, chlorate, fumarate, nitrite, 2-nitrobenzene, perchlorate, sulfate, and sulfite do not support growth.** Little if any acid is produced during growth in medium with hydrogen or formate as electron donor. **Hydrogen, formate, and (DL)-lactate can be used as electron donors**; acetate is produced when grown with lactate. **Gelatin is not hydrolyzed, indole and H_2S are not produced**. Palmitic acid ($C_{14:0}$) is the predominant cellular fatty acid of cells grown in modified brain heart infusion medium, although an abundance of other straight and branched chain fatty acids, aldehydes, and dimethyl acetals are present. Cells contain a *c*-type cytochrome. Isolated from bovine ruminal contents. A single species of *Denitrobacterium*, *Denitrobacterium detoxificans*, has been characterized. The results of 16S rRNA gene sequence analysis indicate *Denitrobacterium* belongs to the *Actinobacteria* (Figure 419).

DNA G+C content (mol%): 56–61 (T_m).

Type species: **Denitrobacterium detoxificans** Anderson, Rasmussen, Jensen and Allison 2000, 633[VP].

Further descriptive information

Based on 16S rRNA gene sequence analysis, the genus *Denitrobacterium* falls within class *Actinobacteria*, subclass *Coriobacteridae*, order *Coriobacteriales*, suborder *Coriobacterineae*, family *Coriobacteriaceae*. A single species, *Denitrobacterium detoxificans*, has been named. *Denitrobacterium* is most closely related to the genus *Eggerthella* (Figure 419).

Cells of *Denitrobacterium detoxificans* are rod-shaped (0.5–1.0 × 1.0–1.5 µm), stain Gram-positive, and do not react with 3% KOH to yield a slimy interface typical of Gram-stain-negative cell types (Gregersen, 1978). Electron micrographs of thin sections reveal Gram-stain-positive cell-wall structures and the presence of unidentified inclusion bodies (Anderson et al., 1997). Stains for spores or known types of inclusion bodies such as polyphosphate granules, glycogen, starch, or poly β-hydroxybutyrate are all negative. Cells do not survive treatment for 10 min at 80°C (Anderson et al., 1997). Flagella are not observed (Anderson et al., 1997).

Palmitic acid ($C_{14:0}$) is the predominant fatty acid present in cells grown in modified brain heart infusion medium; however, considerable amounts of other straight and branched chain fatty acids (C_{12} to C_{18}), as well as aldehydes and dimethyl acetals, are also present (Anderson et al., 1997, 2000). Cells contain a *c*-type cytochrome (Anderson et al., 1997).

Denitrobacterium detoxificans obtains energy for growth exclusively via anaerobic respiration, coupling the oxidation of hydrogen, formate, or lactate to the reduction of suitable anaerobic electron acceptors such as dimethyl sulfoxide, nitroethane, 3-nitropropan-1-ol, 2-nitropropan-1-ol, 3-nitropropionate, nitroethanol, 1-nitropropane, 2-nitrobutane, and trimethyl amine-oxide. Nitrate is also used as an electron acceptor and is reduced to ammonia by the type strain *Denitrobacterium*

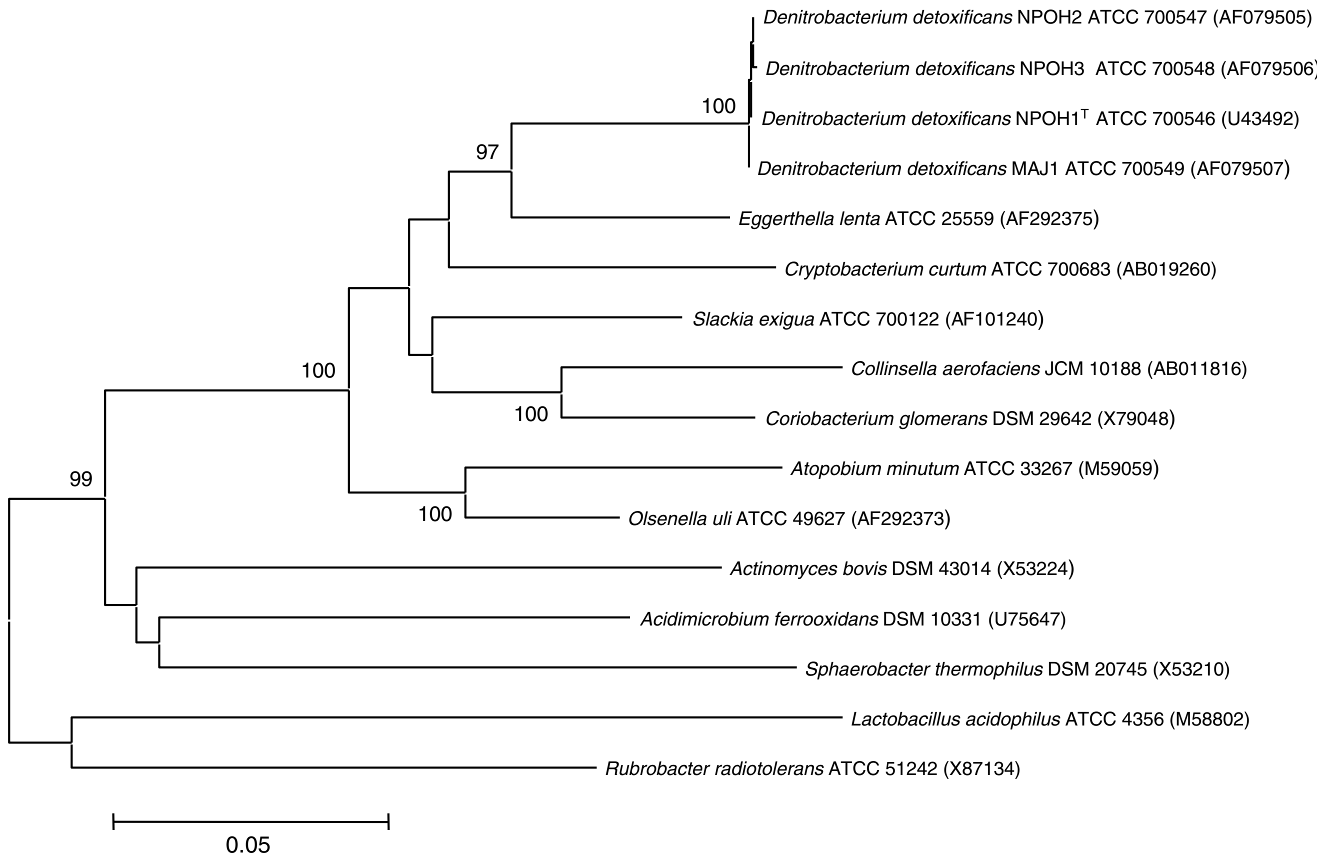

FIGURE 419. Phylogenetic tree based on 16S rRNA gene sequences of *Denitrobacterium detoxificans* strains and type strains of their closest relatives. Phylogenetic and molecular evolutionary analyses were conducted using MEGA version 2.1 (Kumar et al., 2001). Sequence alignments were based on 1592 nucleotide positions and compared by Jukes–Cantor analysis. The tree was constructed using the neighbor-joining method. Scale bar represents a 5% difference in nucleotide sequence. Bootstrap values greater than 95 and based on 1000 replicates are indicated near branch points.

detoxificans NPOH1^T, but not by any of the other three strains tested, NPOH2, NPOH3, and MAJ1 (Anderson et al., 2000).

Denitrobacterium strains are routinely cultured in anaerobic medium B* (Anderson et al., 1996) supplemented with an appropriate source of reductant and electron acceptor. Reductant (provided as 16 mM sodium formate) and acceptor (provided as 3-nitropropionate; generally 5–10 mM) support good growth and are convenient to prepare, filter-sterilize, and to add to previously prepared medium. Dimethyl sulfoxide, nitroethane, 3-nitropropan-1-ol, trimethyl amine-oxide, and, in the case of strain NPOH1^T, nitrate also support good growth, whereas nitroethanol, 2-nitrobutane, and 1-nitropropane do not. Reductant can also be provided as H_2 in the gas phase or as lactate (Anderson et al., 1997). Undefined nutritional factors in phytone are important for maximum growth of all four strains tested.

Cells of *Denitrobacterium detoxificans* NPOH1^T exhibit a positive reaction for arginine aminopeptidase and a weakly positive alkaline phosphatase reaction in the AN-IDENT test system; they exhibit positive reactions for phosphatase acid and naphthol-AS-BI-phosphorohydrolase and a weak positive reaction for lipase-C14 in the API ZYM test system (Anderson et al., 1997). Reactions to all other AN-IDENT and API ZYM tests are negative.

Of the four strains of *Denitrobacterium detoxificans* analyzed, all share >99% 16S rRNA gene sequence similarity.

Denitrobacterium detoxificans strain NPOH1^T exhibits sensitivity to 6 μg/ml cephalothin, 12 μg/ml, chloramphenicol, 1.6 μg/ml clindamycin, and 3 μg/ml erythromycin, and is resistant to 4 μg/ml ampicillin, 100 μg/ml carbenicillin, 2 U/ml penicillin, and 6 μg/ml tetracycline (Anderson et al., 1997).

Denitrobacterium detoxificans strains were isolated from bovine ruminal contents enriched for enhanced rates of 3-nitropropionic acid or 3-nitropropan-1-ol metabolism (Anderson et al.,

*Medium B contains clarified ruminal fluid at 8% (v/v) and (mg/liter): K_2HPO_4, 225.0; KH_2PO_4, 225.0; $(NH_4)_2SO_4$, 450.0; NaCl, 450.0; $MgSO_4 \cdot 7H_2O$, 45.0; $CaCl_2$, 22.5; thiamine, 2.0, pantothenate, 2.0; nicotinamide, 2.0; pyridoxine-HCl, 2.0; riboflavin, 2.0; *p*-aminobenzoic acid, 1.0; biotin, 0.05; folic acid, 0.05; lipoic acid, 0.05; vitamin B-12, 0.02; resazurin, 1.0; cysteine-HCl, 500.0; Na_2CO_3, 4000.0; phytone peptone, 8000.0. Clarified ruminal fluid is prepared by autoclaving freshly collected ruminal fluid that had been strained through cheese cloth or nylon paint strainers and then centrifuging at least twice at 10,000 *g* to remove particulate matter. Cysteine-HCl and Na_2CO_3 are added after the medium containing the other ingredients is adjusted to pH 6.8. The complete medium is boiled and then cooled on ice while being flushed with oxygen-free CO_2 or H_2CO_2 (50:50). When cooled, the medium is anaerobically distributed to culture tubes (Bryant, 1972) which are immediately closed with stoppers and autoclaved.

1996, 1997). 3-Nitropropionic acid or 3-nitropropan-1-ol are toxic constituents of some forages, notably many *Astragalus* species (milkvetches), known to cause poisoning of grazing ruminants (Anderson et al., 2005). Strain NPOH1^{T} was isolated from ruminal contents from a cow reared at the National Animal Disease Center (NADC) in Ames, Iowa; strains NPOH2 and NPOH3 were isolated likewise from ruminal contents from a different cow reared at the same facility and strain MAJ1 was isolated from ruminal contents from a cow reared in British Columbia. Rates of ruminal nitrotoxin metabolism are markedly increased when cattle are fed sublethal amounts of nitro-containing forage or nitroethane, a nontoxic analog, thus implicating a selection mechanism to enrich for bacteria like *Denitrobacterium detoxificans*, which can confer protection to the host (Anderson, 1998; Majak, 1992b, 1992a; Majak et al., 1986). *Denitrobacterium detoxificans*-like 16S rDNA has been detected in a carious dental sample (Chhour et al., 2005).

Enrichment and isolation procedures

Denitrobacterium can be isolated by enriching anaerobic nitro-respiring bacteria. *Denitrobacterium detoxificans* was isolated from ruminal contents enriched from 3×10^4 cells/ml ruminal fluid to $>10^7$ cells/ml during consecutive (24 h intervals at 39°C) batch culture in a minimal ruminal fluid medium supplemented with gradually increasing concentrations (5–10 mM) of 3-nitropropionic acid or 3-nitropropan-1-ol (Anderson et al., 1996). Concentrations of other bacteria decreased concurrently during the enrichment to approximately 10^7 cells/ml. Anaerobic techniques for both broth and agar (2%) medium (Bryant, 1972) were used during enrichment and colony isolation. Hydrogen (50% in CO_2) in the gas phase served as reductant during the isolations although 16 mM formate, which supports nearly equal amounts of growth (Anderson et al., 1997), may work equally well. Isolates picked at random from roll tubes containing solidified medium B were cultured in nitro-supplemented medium B broth. The isolates that grew and reduced nearly all of the added 3-nitropropionic acid or 3-nitropropan-1-ol, as determined via colorimetric analysis of spent culture fluid (Anderson et al., 1993), were indicative of *Denitrobacterium detoxificans*.

Maintenance procedures

All strains of *Denitrobacterium detoxificans* can be maintained by serial transfer, at 3–4 d intervals, in anaerobically prepared medium B (Anderson et al., 1996) supplemented with 16 mM formate and 9 mM 3-nitropropionic acid. Anaerobic *Denitrobacterium detoxificans* cultures preserved as slants or broths in 20% glycerol can be stored at −80°C for at least 10 years.

Differentiation of the genus *Denitrobacterium* from other genera

Denitrobacterium species can be differentiated from other anaerobic respiring organisms by their obligate respiratory metabolism and their ability to respire on nitroalkane compounds such as 3-nitropropan-1-ol, 2-nitropropan-1-ol, 3-nitropropionic acid, nitroethanol, nitroethane, 1-nitropropane, and 2-nitrobutane but not on other acceptors such as O_2, chlorate, fumarate, perchlorate, sulfate, or sulfite. *Denitrobacterium* species can also be differentiated from other genera by 16S rRNA gene sequence analysis.

Differentiation of species of the genus *Denitrobacterium*

The genus contains a single species, *Denitrobacterium detoxificans*.

List of species of the genus *Denitrobacterium*

1. **Denitrobacterium detoxificans** Anderson, Rasmussen, Jensen and Allison 2000, 633[VP]

   de.tox.if'i.cans. L. pref. *de* from; L. n. *toxicum* poison; L. neut. n. *detoxificans* poison reducer.

   The species description is as stated above for the genus. Isolated from ruminal contents enriched for enhanced rates of 3-nitropropan-1-ol metabolism, the toxic aglycone of miserotoxin (3-nitro-1-propyl-β-D-glucopyranoside). All four strains examined share close (>99%) 16S rRNA gene sequence similarity, and have been deposited in the American Type Culture Collection. The type strain differs from the others (strain NPOH2, ATCC 700547; strain NPOH3, ATCC 700548 and MAJ1, and ATCC 700549) in that it has the ability to reduce nitrate.

   DNA G+C content (mol%): 56–61 (T_m).

   Type strain: NPOH1, ATCC 700546, CCUG 5674.

   Sequence accession no. (16S rRNA gene): U43492.

Genus VI. **Eggerthella** Wade, Downes, Dymock, Hiom, Weightman, Dewhirst, Paster, Tzellas and Coleman 1999, 599[VP]

William G. Wade and Floyd E. Dewhirst

Egg.er.thel'la. N.L. fem. n. named to honor Arnold H. Eggerth (1889–1962), who first described the organism later named *Eubacterium lentum*.

Gram-stain-positive obligately **anaerobic bacilli**, **which** are **nonmotile** and do not produce endospores. **Growth** is **stimulated by arginine**. **Acid is not formed** from sugars. The cell wall contains an A4γ-type peptidoglycan with an (L-Ala)–D-Glu–*m*-Dpm–D-Glu peptide subunit with an interpeptide bridge that consists only of D-Glu.

DNA G+C content (mol%): 61–65 (HPLC).

Type species: **Eggerthella lenta** Wade, Downes, Dymock, Hiom, Weightman, Dewhirst, Paster, Tzellas and Coleman 1999, 599[VP].

Further descriptive information

Members of this genus and related genera tend to be unreactive in the biochemical tests commonly used for identification and therefore have historically been a difficult group to characterize. The phenotypic description of *Eggerthella lenta*, in particular, has long been known to define a heterogeneous group of organisms isolated from the large intestine of humans and animals. In one study investigating this heterogeneity, 32 strains were divided into 4 groups on the basis of growth stimulation by arginine, production of H_2S, catalase, and bile acid-degrading enzymes (MacDonald et al., 1979). Groups A–C degraded steroids and related molecules, while members of Group D did not display this activity and had a cellular fatty acid composition distinct from the other groups (Verhulst et al., 1987). The type strain was subsequently shown to belong to the Group A–C cluster (Mosca et al., 1998), and the colonies of most strains were shown to fluoresce red under UV light (Mosca et al., 1993). Whether the three groups in this cluster correspond to the three currently validly published species remains to be established. Given the marked phenotypic differences between Group D and Groups A–C, it is likely that Group D strains do not belong to this genus. In addition, the use of a commercially available identification kit has frequently resulted in the mis-identification of oral *Slackia exigua* isolates as *Eggerthella lenta* (Wade et al., 1990). A systematic polyphasic study of a large collection of strains identified as *Eggerthella* by a combination of sequence analysis of 16S rRNA genes and the presence of the characteristic peptidoglycan described above is required. Once this has been done, it will be possible to construct more precise descriptions of the three *Eggerthella* species described to date. It is also likely that additional novel species, and perhaps novel genera, would be detected by such an approach.

Eggerthella species are found in the human large intestine and in clinical specimens from a wide variety of infections including those of the blood and postoperative wounds and various kinds of abscesses found in different parts of the body such as the brain and associated with the rectum, scrotum, and pelvis (Chan and Mercer, 2008; Lattuada et al., 2005; Lau et al., 2004c; Moore et al., 1971; Schwiertz et al., 2000). *Eggerthella lenta* is one of a number of Gram-stain-positive anaerobic bacilli, including *Collinsella aerofaciens*, another member of the *Coriobacteriaceae*, capable of inducing arthritis in a rat model (Severijnen et al., 1990).

Enrichment and isolation procedures

Strains of all species grow readily on horse blood agar forming colonies at least 0.5 mm in diameter after a 48-h anaerobic incubation at 37°C. The inclusion of 0.5% arginine in agar and broth media stimulates the growth of all species.

Maintenance procedures

Strains can be maintained on blood agar incubated anaerobically at 37°C and subcultured weekly. Lyophilization of cultures in the early stationary phase of growth is recommended for long-term storage of most strains. Strains can also be stored at −70°C in Brain Heart Infusion broth supplemented with 10% glycerol.

Procedures for testing special characters

The general methods described for the characterization of anaerobes in the VPI *Anaerobe Laboratory Manual* (Holdeman et al., 1977b) and the *Wadsworth-KTL Anaerobic Bacteriology Manual* (Jousimies-Somer et al., 2002a) are suitable for the study of members of this genus. The RapidID 32A anaerobe identification kit (bioMérieux) includes tests useful for the differentiation of the species of this genus. However, as mentioned above, identification by 16S rRNA gene sequencing is the most reliable method for this group and will also detect as yet un-named taxa.

Differentiation of the genus *Eggerthella* from other genera

Key characteristics of *Eggerthella* and related genera are shown in Table 307.

Taxonomic comments

Eggerthella lenta was originally described as a member of the genus *Eubacterium*, but was shown by 16S rRNA gene sequence analysis to belong to the family *Coriobacteriaceae* within the phylum *Actinobacteria*, while *Eubacterium limosum*, the type species of *Eubacterium*, is in the *Firmicutes* (Kageyama et al., 1999a; Wade et al., 1999). A new genus, *Eggerthella*, was proposed to accommodate this species (Wade et al., 1999) and two additional species were subsequently described (Lau et al., 2004b). The phylogenetic relationship between the genus *Eggerthella* and related genera within the family *Coriobacteriaceae* can be seen in the tree constructed from 16S rRNA gene comparisons in Figure 420.

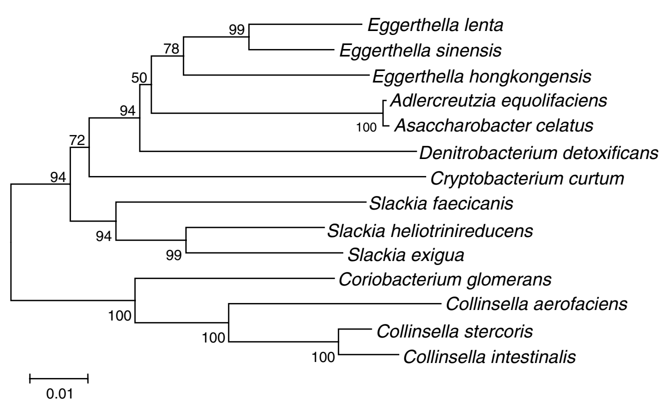

FIGURE 420. Neighbor-joining tree based on 16S rRNA gene sequence comparisons over 1367 aligned bases showing relationships between *Eggerthella* species and between them and related genera. Numbers at the nodes represent bootstrap values for each branch based on data for 100 trees; only values greater than 50% are shown. Bar = 0.01 substitutions per nucleotide position.

Differentiation of species of the genus *Eggerthella*

A scheme for the differentiation of *Eggerthella* species is shown in Table 311.

TABLE 311. Phenotypic characteristics of *Eggerthella lenta*, *Eggerthella hongkonensis*, and *Eggerthella sinensis*[a,b,c]

Chaacteristic	*E. lenta*	*E. hongkongensis*	*E. sinensis*
Arginine arylamidase	–	v	+
β-Glucosidase	–	+	–
Leucine arylamidase	–	v	–
Nitrate reduction	+	–	–

[a]Symbols: +, present; –, absent; v, variable.

[b]Data from Maruo et al. (2008).

[c]Tests performed using the Rapid ID 32A kit (bioMérieux).

Further reading

Moore W.E.C., E.P. Cato and L.V. Holdeman. 1971. *Eubacterium lentum* (Eggerth) Prévot 1938: emendation of description and designation of the neotype strain. Int. J. Syst. Bacteriol. *21*: 299–303.

List of species of the genus *Eggerthella*

1. **Eggerthella lenta** Wade, Downes, Dymock, Hiom, Weightman, Dewhirst, Paster, Tzellas and Coleman 1999, 599[VP]

len′ta. L. fem. adj. *lenta* slow.

Cells from PYG broth cultures are 0.2–0.4 × 0.2–2.0 µm and occur singly and in pairs and short chains. Colonies on horse blood agar are 0.5–2.0 mm, circular, entire to erose, raised to low convex, translucent to semiopaque, dull to shiny, smooth, and sometimes with a mottled appearance. Most strains grow at 30 and 45°C; some grow at 25°C. Arginine enhances growth. Gas is not detected in PYG agar deep cultures. Ammonia is produced from arginine. Acid is not produced from sugars. Hydrogen sulfide is produced in the butt of TSI (triple-sugar iron) slants incubated anaerobically, but not in SIM medium. Catalase is produced by strains grown on agar medium containing 1%, w/v arginine. Hippurate is not hydrolyzed.

The metabolic end products from glucose are acetate with trace amounts of lactate or succinate. H_2 is not produced.

Source: human large intestine, feces, blood and clinical infections, primarily wounds and abscesses.

DNA G+C content (mol%): 61 (HPLC) for the type strain.

Type strain: ATCC 25559, CCUG 17323 A, CIP 106637, DSM 2243, JCM 9979, NCAIM B.01418, NCTC 11813.

Sequence accession no. (16S rRNA gene): AF292375.

Additional comments: arginine is degraded via the arginine dihydrolase pathway (Sperry and Wilkins, 1976a). The type strain contains cytochromes *a*, *b*, and *c* and a carbon monoxide-binding pigment (Sperry and Wilkins, 1976b).

Eggerthella lenta has been shown to transform a wide range of compounds, including steroids and lignans, primarily via dehydrogenases and dehydroxylase. Bile acids such as cholic and chenedeoxycholic acids are degraded by the action of 3α-, 7α-, and 12α- dehydrogenases; the 7α- activity is only demonstrable under anaerobic conditions (Hirano and Masuda, 1981; MacDonald et al., 1977). *Eggerthella lenta* strains also degrade corticosteroids (Bokkenheuser et al., 1977, 1979), linoleic acid, and related unsaturated long-chain fatty acids (Verhulst et al., 1986). The biological activity of the cardiac glycosides digitoxin and digoxigenin are destroyed by *Eggerthella lenta*-mediated reduction of a critical double bond (Dobkin et al., 1982; Robertson et al., 1986). Significant destruction of digoxigenin is thought to occur in 10% of treated patients owing to carriage of high concentrations of *Eggerthella lenta* in their gut microbiota (Chandrasekaran et al., 1987). *Eggerthella lenta* can participate in the metabolism of phyto-estrogens such as secoisolariciresinol diglucoside to produce the estrogen-like compounds enterodiol and enterolactone, which may play a role in the prevention of cancer, atherosclerosis, and diabetes (Clavel et al., 2005, 2006; Jin et al., 2007).

The gene encoding the tetracycline efflux pump, TetK, conferring resistance to this antimicrobial, has been detected in *Eggerthella lenta* (Roberts et al., 1991)

2. **Eggerthella hongkongensis** Lau, Woo, Woo, Fung, Wong, Chan, Tam and Yuen 2006, 2025[VP] (Effective publication: Lau 2004b, 255.)

hong.kong′en′sis. N.L. fem. adj. *hongkongensis* pertaining to Hong Kong, the city where the bacterium was discovered.

Cells are obligately anaerobic, nonsporing, nonmotile Gram-stain-positive coccobacilli which occur in chains. Colonies on blood agar are gray-white and 0.5 mm in diameter after a 48-h anaerobic incubation at 37°C. Catalase, arginine dihydrolase and β-glucosidase are produced, but production of alanine arylamidase, arginine arylamidase, leucine arylamidase, and lysine arylamidase is variable. Alkaline phosphatase, indole, and urease are not produced and nitrate is not reduced. Acid is not produced from glucose or other sugars.

Source: blood.

DNA G+C content (mol%): 61 (HPLC) for the type strain.

Type strain: HKU10, DSM 16106, JCM 14552, LMG 22124.

Sequence accession no. (16S rRNA gene): AY288517.

3. **Eggerthella sinensis** Lau, Woo, Woo, Fung, Wong, Chan, Tam and Yuen 2006, 2025[VP] (Effective publication: Lau 2004b, 255.)

sin′en′sis. N.L. gen. n. *sinae* of China; N.L. fem. adj. *sinensis* pertaining to China, the country where the bacterium was discovered.

Cells are obligately anaerobic, non-sporing, nonmotile, Gram-stain-positive, coccobacilli which occur in chains. Colonies on blood agar are gray-white, and 0.5 mm in diameter after a 48-h anaerobic incubation at 37°C. Catalase, arginine dihydrolase, arginine arylamidase and lysine arylamidase are produced but not alkaline phosphatase, indole, urease, or β-glucosidase. Nitrate is not reduced and acid is not produced from sugars.

Source: blood.

DNA G+C content (mol%): 65 (HPLC) for the type strain.

Type strain: HKU14, DSM 16107, JCM 14551, LMG 22123.

Sequence accession no. (16S rRNA gene): AY321958.

Genus VII. **Olsenella** Dewhirst, Paster, Tzellas, Coleman, Downes, Spratt and Wade 2001, 1802[VP]

Floyd E. Dewhirst and William G. Wade

Ol.sen.el′la. L. fem. dim. ending -*ella*; N.L. fem n. *Olsenella* of Olsen, named to honor Ingar Olsen, a contemporary Norwegian microbiologist who first described *Lactobacillus uli.*

Cells are small, **elliptical rods** which occur singly, in pairs or short chains. Nonsporeforming. Nonmotile. Gram-stain-positive. **Obligate anaerobes**. Glucose fermented to **acetate and lactate**. Does not produce catalase, urease or indole, or reduce nitrate.

DNA G+C content (mol%): 63–64 (HPLC).

Type species: **Olsenella uli** (Olsen, Johnson, Moore and Moore 1991) Dewhirst, Paster, Tzellas, Coleman, Downes, Spratt and Wade 2001, 1802[VP] (*Lactobacillus uli* Olsen, Johnson, Moore and Moore 1991, 263.).

Further descriptive information

The major fatty acid of the cell membrane is $C_{18:1}$ (*cis*9). Strains have been isolated from the human oral cavity (Dewhirst et al., 2001; Munson et al., 2004; Olsen et al., 1991; Siqueira et al., 2005), human bacteremia (Lau et al., 2004a), and cow rumen (GenBank AJ251324).

Enrichment and isolation procedures

Strains of *Olsenella* species have been recovered using agar media commonly used for the cultivation of anaerobes, such as Brain Heart Infusion, Peptone Yeast Glucose, and Fastidious Anaerobe Agar, supplemented with 5% horse or sheep blood. Specific enrichment procedures have not been reported for the genus. The growth of *Olsenella uli*, but not that of *Olsenella profusa*, is markedly stimulated by addition of Tween 80 (0.02%, v/v).

Maintenance procedures

Stock cultures of *Olsenella* species can be maintained under anaerobic conditions by weekly transfer on Blood Agar plates. Cultures can be stored by lyophilization or freezing at −80°C in Brain Heart Infusion Broth supplemented with 10% glycerol.

Differentiation of the genus *Olsenella* from other genera

Characters that differentiate *Olsenella* from closely related genera are shown in Table 307. *Olsenella* can be differentiated from *Atopobium*, *Cryptobacterium*, and *Denitrobacterium* by G+C content of the DNA, from *Adlercreutzia*, *Denitrobacterium*, *Eggerthella*, and *Slackia* by sugar fermentation, and from *Collinsella*, *Coriobacterium*, and *Eggerthella* by site of origin. Molecular methods, such as sequencing of the 16S rRNA gene, provide the most definitive identification.

Taxonomic comments

Prior to the use of 16S rRNA-based phylogenetic analysis, strains of species in the genus *Olsenella* were misclassified in the genera *Eubacteria* and *Lactobacillus* of the phylum *Firmicutes*. The genus *Olsenella* is now recognized as one of nine genera classified in the family *Coriobacteriaceae* in the phylum *Actinobacteria* (Dewhirst et al., 2001; Wade et al., 1999) as shown in Figure 421.

Using 16S rRNA gene sequence analysis, examination of oral strains in the Virginia Polytechnic Institute Anaerobe

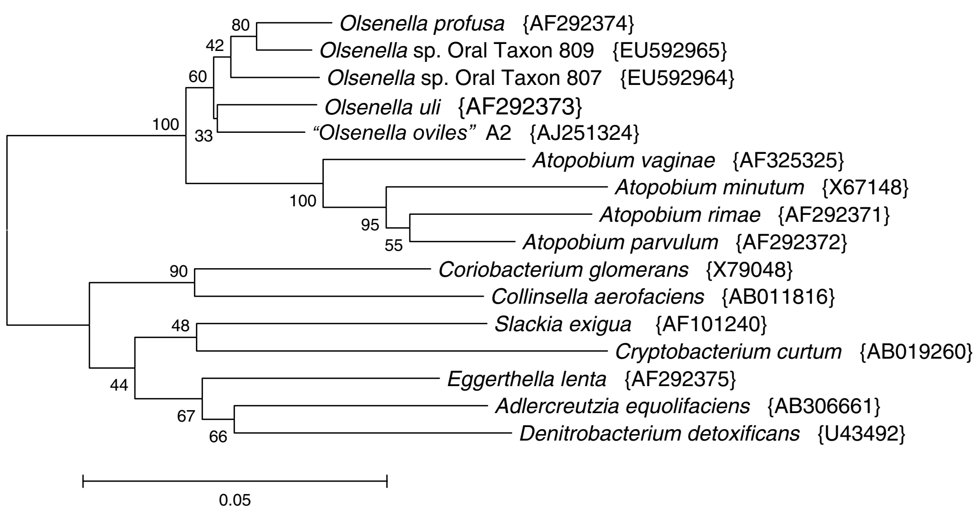

FIGURE 421. Neighbor-joining tree (Saitou and Nei, 1987) showing relationships between *Olsenella* species and representatives of other genera classified in the family *Coriobacteriaceae*. The optimal tree with the sum of branch length = 0.581 is shown. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) is shown next to the branches (Felsenstein, 1985). The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed using the Jukes–Cantor method (Jukes and Cantor, 1969) and are expressed as the number of base substitutions per site. All positions containing alignment gaps and missing data were eliminated only in pairwise sequence comparisons (pairwise deletion option). There were a total of 1491 positions in the final dataset. Phylogenetic analyses were conducted in MEGA4 (Tamura et al., 2007).

TABLE 312. Differential characteristics of *Olsenella uli* and *Olsenella profusa*[a]

Test	*O. uli* (48 strains)	*O. profusa* (2 strains)
Rapid ID 32A profile	2012 0337 05	4516 0537 05
Growth in PYG broth	Poor (1+)	Good (3–4+)
Growth stimulation by Tween 80	Marked	Slight
Arginine hydrolysis	+	−
Fermentation of:		
Arabinose	−	+
Mannitol	−	+
Melibiose	−	+
Raffinose	−	+
Colony morphology[b]	Raised, grey, semi-translucent	Pyramidal, cream, opaque

[a]Data from Olsen et al. (1991) and Dewhirst et al. (2001).

[b]After incubation for 7 d on Fastidious Anaerobe Agar.

Laboratory collection of W.E.C. Moore and L.V. Holderman Moore has identified two additional *Olsenella* species (F.E. Dewhirst, unpublished studies). These strains were designated Coccus D49 and *Lactobacillus* D19 by the Moores, and are now designated *Olsenella* sp. Oral Taxon 807 and *Olsenella* sp. Oral Taxon 809 as shown in Figure 421 and described in the Human Oral Microbiome Database (www.homd.org; Dewhirst et al., 2008). A rumen strain was isolated and informally called "*Atopobium oviles*" in a GenBank submission (AJ251324), but the name has not been formally published. Because the rumen strain has 97% mean similarity to *Olsenella uli* and *Olsenella profusa*, and only 93.5% mean similarity to *Atopobium* species, we would suggest the informal designation should be "*Olsenella oviles*". Several dozen clone sequences in GenBank, from a variety of sources, are greater than 90% similar to species in the genera *Olsenella* and *Atopobium*. Some of these clones come from environmental sources, but may represent bacteria shed from mammalian hosts.

Acknowledgements

Floyd E. Dewhirst was supported by NIH grant DE106937 from the National Institute of Dental and Craniofacial Research.

Differentiation of species of the genus *Olsenella*

Biochemical characteristics useful in distinguishing the species of genus are listed in Table 312. The two species are easily differentiated from one another by using Rapid ID32A anaerobe identification kits (bioMérieux) and on the basis of 16S rRNA gene sequences.

List of species of the genus *Olsenella*

1. **Olsenella uli** (Olsen, Johnson, Moore and Moore 1991) Dewhirst, Paster, Tzellas, Coleman, Downes, Spratt and Wade 2001, 1802[VP] (*Lactobacillus uli* Olsen, Johnson, Moore and Moore 1991, 263)

u'li. Gr. n. *oulon* the gums; N.L. gen. n. *uli* of the gum.

This description comes primarily from Olsen et al. (1991) with additional information from Dewhirst et al. (2001). Cells are small elliptical rods, which occur singly, in pairs, or short chains and may show central swellings. Nonmotile. Colonies on brain heart blood agar are 1–2 mm in diameter, raised or low convex, and entire after 5 d of anaerobic incubation.

Obligate anaerobe. Grows in Peptone Yeast extract Glucose Hemin vitamin K (PYG-HK) broth (Holdeman et al., 1977a). Growth is stimulated by Tween 80 (0.02%, v/v).

The major fermentation products from glucose are acetate and lactate with occasional minor amounts of succinate. Acid is produced from fructose, glucose, maltose, mannose, salicin, and sucrose, but not from amygdalin, erythritol, esculin, glycerol, inositol, mannitol, melezitose, melibiose, raffinose, rhamnose, sorbitol, or xylose. Indole is not produced and nitrate is not reduced. Negative reaction for bile-esculin, DNase, and hippurate hydrolysis. The Rapid ID32A profile is 2012 0337 05. The major cell wall fatty acid is $C_{18:1}$ (*cis*9).

Source: human gingival crevices of patients with periodontitis (Olsen et al., 1991) and from patients with endodontic infections (Siqueira et al., 2005). Has been recovered from human bacteremia (Lau et al., 2004a).

DNA G+C content (mol%): 64 (HPLC) for type strain.

Type strain: VPI D76D-27C, ATCC 49627, CCUG 31166, DSM 7084, JCM 12494, LMG 11480.

Sequence accession no. (16S rRNA gene): AF292373.

Additional comments: the species was previously designated *Lactobacillus* D01 in the Virginia Polytechnic Institute Anaerobe Laboratory collection of W.E.C. Moore and L.V. Holdeman Moore and initially named *Lactobacillus uli* by Olsen et al. (1991). The phenotypic description of the species

needs validation as some strains used in the description of *Lactobacillus uli* have been identified recently as belonging to other *Olsenella* species and to *Atopobium* species by 16S rRNA gene sequence analysis (Dewhirst, unpublished observation).

2. **Olsenella profusa** Dewhirst, Paster, Tzellas, Coleman, Downes, Spratt and Wade 2001, 1802^{VP}

pro.fus′a. L. adj. *profusus* profuse, referring to the good growth of the organism.

The description is based primarily on Dewhirst et al. (2001). Cells are short rods 0.6 × 0.8–2.0 µm, which occur singly or in chains. Nonmotile. After incubation for 7 d on FAA plates, colonies are approximately 1–1.3 mm in diameter, circular, entire, pulvinate to pyramidal, cream colored, and opaque when viewed under a plate microscope. Growth in broth media is good, and addition of Tween 80 does not significantly enhance growth.

Acetate and lactate are major end products of metabolism. Arabinose, cellobiose, fructose, glucose, lactose, maltose, mannitol, mannose, melibiose, raffinose, salicin, sucrose, and trehalose are fermented, but not melezitose, or rhamnose. Esculin is hydrolyzed, but not arginine or starch. Gelatin is not liquified meat is not digested. Does not produce catalase, indole, or H_2S or reduce nitrate.

Source: human subgingival plaque of patients with periodontitis (Dewhirst et al., 2001) and dental caries (Munson et al., 2004). Detected in endodontic infections (Rôças and Siqueira, 2005).

DNA G+C content (mol%): 64 (HPLC) for the type strain.

Type strain: VPI D315A-29, DSM 13989, CCUG 45371, CIP 106885, DSM 13989, JCM 14553.

Sequence accession no. (16S rRNA gene): AF292374.

Additional comments: the species was previously designated *Eubacterium* D52 in the Virginia Polytechnic Institute Anaerobe Laboratory Collection of W.E.C. Moore and L.V. Holderman Moore.

Genus VIII. **Slackia** Wade, Downes, Dymock, Hiom, Weightman, Dewhirst, Paster, Tzellas and Coleman 1999, 598^{VP}

William G. Wade and Floyd E. Dewhirst

Slack′ia. N.L. fem. n. named to honor Geoffrey Slack, distinguished British microbiologist and dental researcher.

Cells are Gram-stain-positive **cocci, coccobacilli, or short bacilli**, which are obligately **anaerobic**, nonmotile, and do not produce endospores. **Arginine is hydrolyzed**; catalase, urease, and indole are not produced and esculin is not hydrolyzed. **Acid is not produced from sugars**. Metabolic end products from glucose are either **acetate or not detectable**.

DNA G+C content (mol%): 60–64 (HPLC).

Type species: **Slackia exigua** Wade, Downes, Dymock, Hiom, Weightman, Dewhirst, Paster, Tzellas and Coleman 1999, 598^{VP}.

Further descriptive information

The predominant long chain cellular fatty acids in *Slackia faecicanis* have been shown to be $C_{16:0}$, $C_{16:1}$ (*cis*9), $C_{18:0}$, $C_{18:1}$ (*cis*9), and $C_{19:1}$ iso (*cis*9) (Lawson et al., 2005) but comparable data are not available for *Slackia exigua* or *Slackia heliotrinireducens*. *Slackia exigua* degrades arginine by the arginine deaminase pathway, as does *Cryptobacterium curtum*, another member of the family *Coriobacteriaceae* (Uematsu et al., 2006).

Strains corresponding to *Slackia exigua* were referred to as *Eubacterium* D6 in the Virginia Polytechnic Institute Anaerobe Laboratory collection of W.E.C. Moore and L.V. Holdeman Moore, "*Eubacterium* Cluster 2" (Wade et al., 1990) and "*Eubacterium* S-group" (Sato et al., 1993) prior to the formal description of the species, initially as "*Eubacterium exiguum*" (Poco et al., 1996). *Slackia exigua* is part of the human oral microbiota and has been associated with periodontitis (Moore et al., 1983), endodontic infections (Hashimura et al., 2001; Sato et al., 1993), and dentoalveolar abscesses (Downes et al., 2001; Wade et al., 1994). In patients with periodontitis, the species is particularly associated with deep pockets suggesting that this is its preferred habitat (Booth et al., 2004). In addition, patients with rapidly progressive periodontitis and refractory adult periodontitis have been shown to have raised antibody titers to *Slackia exigua*, suggesting a role in the pathogenesis of the disease (Smith and Wade, 1999). *Slackia exigua* has also been isolated from a case of bacteremia as part of a polymicrobial infection (Woo et al., 2004).

Ingestion of ragwort by grazing animals can lead to death due to hepatic cirrhosis up to 2 years after ingestion (Muth, 1968). However, sheep are resistant to the toxic substances in ragwort while horses and cattle are susceptible. It is the presence of *Slackia heliotrinireducens* and other as yet uncharacterized bacteria in the sheep rumen that is thought to confer resistance by degrading the pyrrolizidine alkaloids responsible for toxicity (Craig et al., 1992). *Slackia heliotrinireducens* can degrade heliotrine and lasiocarpine but not jacobine or seneciphylline (Hovermale and Craig, 2002).

Enrichment and isolation procedures

Slackia exigua has been successfully grown on Brain Heart Infusion agar and Fastidious Anaerobe Agar, both supplemented with 5% horse or sheep blood, while *Slackia faecicanis* can be cultured on Bacteroides agar (Holdeman et al., 1977a). Strains of all species are slow-growing, but *Slackia exigua* is particularly slow, forming colonies no greater than 1 mm in diameter, even after prolonged incubation.

Enrichment for *Slackia heliotrinireducens* can be achieved by culture in sheep rumen contents supplemented with heliotrine (2 mg/ml) and chloral hydrate (0.2 mg/ml) in an atmosphere of H_2-CO_2 (4:1) for 24–30 h, followed by successive subcultures

in a medium containing 30% rumen fluid, 0.2% heliotrine, and salts. Dilutions from the final enrichment can be cultured in roll tubes in medium containing 30% rumen fluid, 0.1% heliotrine, salts, and 1% (w/v) Ionagar (Oxoid no. 2). After incubation for 7–8 d at 38°C, colonies resembling those of *Slackia heliotrinreducens* can be selected and subcultured (Lanigan, 1976). Methods for assay of the alkaloids and their metabolites are given by Lanigan and Smith (1970).

Maintenance procedures

Strains can be maintained on Blood Agar plates, or tryptone-yeast-mineral salts (TYM; Lanigan, 1976) for *Slackia heliotrinireducens*, incubated anaerobically at 37°C, and subcultured weekly. Lyophilization of cultures is recommended for long-term storage, although strains have been successfully stored at −70°C in Brain Heart Infusion Broth + 10% glycerol.

Differentiation from closely related taxa

A simple scheme for the differentiation of the three *Slackia* species, primarily based on a commercial kit for the identification of anaerobic bacteria, is shown in Table 313. Given the slow growth and general unreactivity of members of this group and related *Actinobacteria*, molecular methods such as sequencing of the 16S rRNA gene are recommended for unambiguous identification.

Taxonomic comments

The phylogenetic relationship between the genus *Slackia* and related genera within the family *Coriobacteriaceae* can be seen in the tree constructed from 16S rRNA gene comparisons in Figure 420.

TABLE 313. Differential characteristics of *Slackia* species[a,b]

Characteristic	*S. faecicanis*	*S. exigua*	*S. heliotrinireducens*
Cell shape	Rods	Rods	Cocci
Nitrate reduction	+	−	+
Fermentation products from glucose	nd	nd	Acetate (trace)
API Rapid ID32A system tests:			
Alanine arylamidase	−	+	+
Arginine arylamidase	−	+	−
Glycine arylamidase	−	+	+
Histidine arylamidase	−	+	+
Proline arylamidase	−	+	+
Serine arylamidase	−	+	+
Tyrosine arylamidase	−	+	+

[a]Symbols and abbreviations: +, present; −, absent; nd, none detected.

[b]Data from Lawson et al. (2005).

Acknowledgements

Martin Slayne, Sarah Cheeseman, Sarah Hiom, Dave Dymock, and Julie Downes are thanked for their scientific and technical contributions to the work described in this chapter. Paul Lawson is thanked for discussions clarifying characteristics of *Slackia faecicanis.*

List of species of the genus *Slackia*

1. **Slackia exigua** (*Eubacterium exiguum* Poco, Nakazawa, Ikeda, Sato, Sato and Hoshino 1996) Wade, Downes, Dymock, Hiom, Weightman, Dewhirst, Paster, Tzellas and Coleman 1999, 598[VP]

ex.igu′a. L. fem. adj. *exigua* scanty, small, referring to the scanty or poor growth of the organism.

Cells are short Gram-stain-positive rods that are obligately anaerobic, nonmotile, and nonsporing. Individual cells are 0.5 × 1.0 mm, and the cells occur singly or in clumps. Sometimes cells from older cultures stain Gram-negative. On BHI-blood agar plates, the cells form minute, circular, convex, translucent colonies that are less than 1 mm in diameter even after prolonged incubation in an anaerobic glove box. Growth in broth media is poor with or without carbohydrates but is moderately enhanced in the presence of 5% bovine serum, 0.2% lysine, or 0.2% arginine. Hemolysis does not occur around colonies on BHI blood agar plates. The cells are inert in most biochemical tests. Starch and esculin are not hydrolyzed, and nitrate is not reduced. Does not liquefy gelatin. Catalase-, indole- and urease-negative. Ammonia is produced from arginine. All strains are nonfermentative and do not utilize adonitol, amygdalin, arabinose, cellobiose, erythritol, esculin, fructose, galactose, glucose, glycogen, inositol, lactose, maltose, mannitol, mannose, melezitose, melibiose, rhamnose, ribose, salicin, sorbitol, starch, sucrose, trehalose, or xylose. No detectable metabolic end products are produced in peptone-yeast extract medium supplemented with glucose or peptone-yeast extract-glucose broth.

Source: human necrotic pulp samples, periapical infections, and acute dento-alveolar abscesses.

DNA G+C content (mol%) of the type strain: 60 (HPLC).

Type strain: S-7, ATCC 700122, CIP 105133, JCM 11022.

Sequence accession no. (16S rRNA gene): AF101240.

2. **Slackia heliotrinireducens** Wade, Downes, Dymock, Hiom, Weightman, Dewhirst, Paster, Tzellas and Coleman 1999, 599[VP]

he.li.o.trin.i.re.duc.ens. N.L. n. *heliotrinum* derived from heliotrine, a pyrrolizidine alkaloid; L. part. adj. *reducans* leading back, bringing back and in chemistry converting to a different oxidation state; N.L. part. adj. *heliotrinireducens* referring to the organism's ability to bring about oxidative cleavage of the heliotrine molecule.

Cells are cocci (0.5–0.7 µm in diameter), that occur in pairs, in small clusters and short chains. Subsurface colonies in TYM agar are lenticular with an entire margin and smooth surface. After incubation for 7–10 d, colonies are 0.6–0.8 mm

in diameter, grayish-white, and translucent. Surface colonies are 1–2 mm in diameter, effuse, colorless, and transparent with an entire edge. Optimum growth temperature is between 38°C and 42°C with some growth between 30°C and 46°C. Maximal growth occurs between pH 6.5 and 7.0 with some growth between pH 6.2 and 7.2. The presence of 2% NaCl is inhibitory to growth in TYM medium. Growth is enhanced by arginine (10–25 mM), but not by 1% (v/v) serum or 1% (v/v) Tween 80, while glycine (40 mM) is inhibitory. Addition of alanine, glutamate, histidine, ornithine, proline, serine, threonine, and combinations of alanine plus ornithine or alanine plus proline do not affect growth. Ammonia is formed from adenine, arginine, tryptone, yeast extract, and uracil. Nitrates are completely reduced to ammonia if sufficient electron donor (H_2 or formate) is present. Sulfates are not reduced. Creatinine is not hydrolyzed. Does not form gas in TYM-agar medium.

Only a trace of acetate is detected in PY-glucose culture by gas chromatographic analysis. Small amounts of H_2 and CO_2 are produced. Fumarate is reduced to succinate if formate or H_2 is present. Lactate, malate, and pyruvate are not metabolized. Pyrrolizidine alkaloids (europine, heleurine, heliotrine, lasiocarpine, and supinine) are reduced to 1-methylene derivatives in the presence of H_2 or formate (electron donors). Some macrocyclic diesters are metabolized, but more slowly than the monoesters. Anacrotine, cynaustraline, heliotridine, retrorsine, and sarracine are not metabolized. A *c*-type cytochrome is present in ultrasonic extracts of cells. Growth is inhibited in media containing 10 units/ml penicillin.

Source: sheep rumen.

DNA G+C content (mol%) of the type strain: 61 (HPLC).

Type strain: RHS1, ATCC 29202, NCTC 11029, CCUG 47954, JCM 14554, DSM 20476.

Sequence accession no. (16S rRNA gene): AF101241.

3. **Slackia faecicanis** Lawson, Greetham, Gibson, Giffard, Falsen and Collins 2005, 1245[VP]

fae.ci.ca′nis. L. n. *faex*, *faecis* feces; L. gen. n. *canis* dog; N.L. gen. n. *faecicanis* from dog feces.

Cells are short Gram-stain-positive rods (0.5 × 1–2 µm). Colonies after a 48-h anaerobic incubation at 37°C are 1–2 mm in diameter, translucent to gray, with an uneven surface with irregular edges. Strains are obligate anaerobes. Nitrate is reduced to nitrite. Catalase and indole are not produced. Acid is not produced from glucose, lactose, maltose, mannose, mannitol, melibiose, ribose, starch, or sucrose. Using the API Rapid ID32A system, only arginine dihydrolase is positive. The predominant long-chain cellular fatty acids consist of $C_{16:0}$, $C_{16:1}$ (*cis*9), $C_{18:0}$, $C_{18:1}$ (*cis*9), and $C_{19:1}$ iso (*cis*9).

Source: the primary habitat is unknown but is probably the intestinal tract of the dog.

DNA G+C content (mol%) of the type strain: 61 (HPLC).

Type strain: 5WC12, CCUG 48399, CIP 108281, JCM 14555.

Sequence accession no. (16S rRNA gene): AJ608686.

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Class IV. **Nitriliruptoria** class. nov.

WOLFGANG LUDWIG, JEAN EUZÉBY AND WILLIAM B. WHITMAN

Ni.tri.li.rup.tor′i.a. N.L. masc. n. *Nitriliruptor* type genus of the type order; suffix *-ia* ending to denote a class; N.L. pl. neut. n. *Nitriliruptoria* the *Nitriliruptorales* class.

This class is formed by elevation of the subclass *Nitriliruptoridae* Kurahashi et al. 2010, which was delineated on the basis of 16S rRNA gene sequence analyses and the distinctive phyletic lineage of the type order.

Type order: **Nitriliruptorales** Sorokin, van Pelt, Tourova and Evtushenko 2009, 252[VP].

Reference

Kurahashi, M., Y. Fukunaga, Y. Sakiyama, S. Harayama and A. Yokota. 2010. *Euzebya tangerina* gen. nov., sp. nov., a deeply branching marine actinobacterium isolated from the sea cucumber *Holothuria edulis*, and proposal of *Euzebyaceae* fam. nov., *Euzebyales* ord. nov. and *Nitriliruptoridae* subclassis nov. Int. J. Syst. Evol. Microbiol. *60*: 2314–2319.

Order I. **Nitriliruptorales** Sorokin, van Pelt, Tourova and Evtushenko 2009, 252[VP]

The Editorial Board

Ni.tri.li.rup.tor.a′les. N.L. masc. n. *Nitriliruptor* type genus of the order; suffix -*ales* ending to denote an order; N.L. fem. pl. n. *Nitriliruptorales* the order of the genus *Nitriliruptor*.

Description is the same as the family *Nitriliruptoraceae*.

Type genus: **Nitriliruptor** Sorokin, van Pelt, Tourova and Evtushenko 2009, 251[VP].

Reference

Sorokin, D.Y., S. van Pelt, T.P. Tourova and L.I. Evtushenko. 2009. *Nitriliruptor alkaliphilus* gen. nov., sp. nov., a deep-lineage haloalkaliphilic actinobacterium from soda lakes capable of growth on aliphatic nitriles, and proposal of *Nitriliruptoraceae* fam. nov. and *Nitriliruptorales* ord. nov. Int. J. Syst. Evol. Microbiol. *59*: 248–253.

Family I. **Nitriliruptoraceae** Sorokin, van Pelt, Tourova and Evtushenko 2009, 251[VP]

The Editorial Board

Ni.tri.li.rup.to.ra.ce′a.e. N.L. masc. n. *Nitriliruptor* type genus of the family; suffix -*aceae*, ending to denote a family; N.L. fem. pl. n. *Nitriliruptoraceae* the family of the genus *Nitriliruptor*.

Rods that stain Gram-positive. **Aerobic heterotrophs with the ability to degrade organic nitriles** as well as organic acids and sugars. **Alkaliniphilic and moderately salt-tolerant**. The description is otherwise the same as the type genus.

Type genus: **Nitriliruptor** Sorokin, van Pelt, Tourova and Evtushenko 2009, 251[VP].

Genus I. **Nitriliruptor** Sorokin, van Pelt, Tourova and Evtushenko 2009, 251[VP]

The Editorial Board

Ni.tri.li.rup′tor. N.L. n. *nitrilum* nitrile, nitrile group; L. masc. n. *ruptor* breaker; N.L. masc. n. *nitriliruptor* nitrile-breaker.

Short rods that **stain Gram-positive**. Aerobes that **utilize short-chain organic acids, amides, and aliphatic nitriles** as energy and carbon sources. The cell envelopes contain *meso*-diaminopimelate and saturated C_{14}–C_{16} fatty acids.

DNA G+C content (mol%): 70.8 (T_m).

Type species: **Nitriliruptor alkaliphilus** Sorokin, van Pelt, Tourova and Evtushenko 2009, 251[VP].

Further descriptive information

This description of the genus is based upon a single isolate of a single species and must necessarily be preliminary. Cells are short rods (0.4 × 1.5–3.0 µm) and nonmotile. Colonies develop very slowly over a period of 1 month and are flat, colorless, and spreading. The cells stain Gram-positive and contain alanine,

glutamate, and *meso*-diaminopimelate in a 2:1:1 molar ratio. Small amounts of other amino acids as well as the sugars glucose, galactose and glycerol are also present. Fatty acids include $C_{16:0}$ (19%), C_{14} (17%), $C_{16:1}$ ω7 (16%), $C_{14:0}$ (13%), C_{16} (8%), $C_{17:1}$ ω8 (5%), and $C_{18:1}$ ω9 (4%).

Growth is aerobic, and anaerobic growth on sugars with or without nitrate as an electron acceptor is not observed. Isobutyronitrile is completed degraded as a sole carbon and nitrogen source. Other nitriles utilized include propionitrile, butyronitrile, valeronitrile, and capronitrile, but not acetonitrile. Simple monocarboxylic and dicarboxylic organic acids, pyruvate, malate, fumarate, and citrate are also utilized. Sugars and sugar alcohols are utilized, including D-glucose, D-fructose, maltose, D-mannose, melezitose, α,α-trehalose, sucrose, D-arabinose, inositol, cellobiose, and glycerol. Yeast extract and peptone also support slow growth.

Isolated from a soda lake, the pH optimum and range are 9.0–9.4 and 8.4–10.4, respectively. The NaCl optimum and range are 0.2–0.3 M and 0.1–2.0 M, respectively. A mesophile, the temperature optimum is 32°C.

The type species was isolated from an isobutyronitrile-degrading consortium initiated from soda lake sediments of the Kulunda Steppe, Altai, Russia in pH 10 medium containing a total Na concentration of 0.6 M (Sorokin et al., 2007). Environmental clones with ~95 % sequence similarity have been isolated from sediments of other soda lakes and from saline and other soils.

List of species of the genus *Nitriliruptor*

1. **Nitriliruptor alkaliphilus** Sorokin, van Pelt, Tourova and Evtushenko 2009, 251[VP]

al.ka.li′phi.lus. N.L. n. *alkali* soda ash; Gr. adj. *philos* loving; N.L. adj. *alkaliphilus* alkali-loving.

The description is the same as for the genus.

DNA G+C content (mol%): 70.8 (T_m).

Type strain: ANL-iso2, DSM 45188, NCCB 100119, UNIQEM U239.

Sequence accession no. (16S rRNA gene): EF422408.

References

Sorokin, D.Y., S. van Pelt, T.P. Tourova and G. Muyzer. 2007. Microbial isobutyronitrile utilization under haloalkaline conditions. Appl. Environ. Microbiol. *73*: 5574–5579.

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Order II. **Euzebyales** Kurahashi, Fukunaga, Sakiyama, Harayama and Yokota 2010, 2318[VP]

THE EDITORIAL BOARD

Eu.ze.by′a.les. N.L. fem. n. *Euzebya* type genus of the family; *-ales* ending to denote an order; N.L. fem. pl. n. *Euzebyales* the order of the genus *Euzebya*.

The description of the order is the same as for the genus and based upon the distinct 16S rRNA gene phyletic lineage.

Type genus: **Euzebya** Kurahashi, Fukunaga, Sakiyama, Harayama and Yokota 2010, 2318[VP].

Reference

Kurahashi, M., Y. Fukunaga, Y. Sakiyama, S. Harayama and A. Yokota. 2010. *Euzebya tangerina* gen. nov., sp. nov., a deeply branching marine actinobacterium isolated from the sea cucumber *Holothuria edulis*, and proposal of *Euzebyaceae* fam. nov., *Euzebyales* ord. nov. and *Nitriliruptoridae* subclassis nov. Int J Syst Evol Microbiol *60*: 2314–2319.

Family I. **Euzebyaceae** Kurahashi, Fukunaga, Sakiyama, Harayama and Yokota 2010, 2318[VP]

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Eu.ze.by.a.ce′a.e. N.L. fem. n. *Euzebya* type genus of the family; *-aceae* ending to denote a family; N.L. fem. pl. n. *Euzebyaceae* the family of the genus *Euzebya*.

The description of the family is the same as for the genus and based upon the distinct 16S rRNA gene phyletic lineage.

Type genus: **Euzebya** Kurahashi, Fukunaga, Sakiyama, Harayama and Yokota 2010, 2318[VP].

Genus I. **Euzebya** Kurahashi, Fukunaga, Sakiyama, Harayama and Yokota 2010, 2318[VP]

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Eu.ze′by.a. N.L. fem. n. *Euzebya* named for Jean Paul Marie Euzéby, a French microbiologist who has contributed significantly to microbial systematics, including the Latinization of microbial names.

Rods that stain Gram-positive and do not form endospores. Aerobic chemo-organotrophs, oxidase- and catalase-positive. **Sodium chloride is required** for growth. **MK-9(H_4)** is the predominant menaquinone. The cell envelopes contain ***meso*-diaminopimelate, rhamnose, and galactose.** The major fatty acids are $C_{16:1}$ ω7*c*, $C_{16:0}$, and $C_{17:1}$ ω8*c*. The major polar lipid is phosphatidylglycerol.

DNA G+C content (mol%): 68.3 (HPLC).

Type species: **Euzebya tangerina** Kurahashi, Fukunaga, Sakiyama, Harayama and Yokota 2010, 2318[VP].

Further descriptive information

This description of the genus is based upon a single isolate of a single species and must necessarily be preliminary. Cells are rods (0.6–0.8 × 1.5–6.0 µm) and nonmotile. They stain Gram-positive and do not form endospores. Colonies develop very slowly over a period of 5 weeks and are pulvinate with an entire edge, tangerine in color, nearly opaque, and have a hard texture. The cells wall contains alanine, glutamate, and *meso*-diaminopimelate in a 2:1:1 molar ratio as well as the sugars rhamnose and galactose. Fatty acids include $C_{16:1}$ ω7*c* (35%), $C_{16:0}$ (19%), $C_{17:1}$ ω8*c* (18%), $C_{18:1}$ ω9*c* (8%), $C_{17:0}$ (5%), and 10-methyl $C_{17:0}$ (4%). The predominant menaquinone is MK-9(H_4).

Growth is aerobic, and nitrate is not reduced. Grows on complex media with peptone and yeast extract as carbon sources, such as marine agar or broth 2216 and TYSW medium (2 g tryptone and 1 g yeast extract in 1 l artificial seawater). Also assimilates L-arabinose and melibiose, but not amygdalin, D-glucose, inositol, D-mannose, rhamnose, sucrose, and sorbitol. Positive for hydrolysis of gelatin and urea and production of catalase, oxidase and acetoin. Negative for the production of β-galactosidase, arginine dihydrolase, lysine decarboxylase, ornithine decarboxylase, tryptophan deaminase, H_2S, and indole. Unable to utilize citrate.

Isolated from a marine source, the NaCl range for growth is 0.5–12%. The temperature optimum and range are 20–28°C and >10 and <40°C, respectively. The pH range is 7–9. Stock cultures are maintained in marine broth 2216 with 5% DMSO at −80°C.

The type species was isolated from the abdominal epidermis of a sea cucumber, *Holothuria edulis*, which had been collected from the coast of Aka Island, Okinawa prefecture, Japan, at a depth of 6 m (Kurahashi and Yokota, 2004; Valenzuela-Encinas et al., 2009). Environmental clones with greater than ~98% sequence similarity have been isolated from dolomite rock (Horath and Bachofen, 2009), haloalkaline soil (Valenzuela-Encinas et al., 2009), and anthracene-contaminated soils.

List of species of the genus *Euzebya*

1. **Euzebya tangerina** Kurahashi, Fukunaga, Sakiyama, Harayama and Yokota 2010, 2318[VP]

   tan.ge.ri′na. N.L. fem. adj. *tangerina* tangerine-colored, referring to the colony color.

   The description is the same as for the genus.

   DNA G+C content (mol%): 68.3 (HPLC).

   Type strain: F10, NBRC 105439, KCTC 19736.

   Sequence accession no. (16S rRNA gene): AB478418.

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Class V. **Rubrobacteria** class. nov.

KEN-ICHIRO SUZUKI

Ru.bro.bac.te′ri.a. N.L. masc. n. *Rubrobacter* type genus of the type order; suff. *-ia* ending to denote a class; N.L. pl. neut. n. *Rubrobacteria* the *Rubrobacterales* class.

The subclass *Rubrobacteridae* was established by Rainey et al. (1997) for the phylogenetic construction of the hierarchical structure of the class *Actinobacteria*. At the time, only the genus *Rubrobacter* was known, and the subclass contained the single order *Rubrobacterales*, which was represented by the family *Rubrobacteraceae* for the genus *Rubrobacter*. Although the genus *Thermoleophilum* had already been described (Zarilla and Perry, 1984), it was not included in the study. Later, the genus *Thermoleophilum* was reported to be the member of the subclass *Rubrobacteridae* (Yakimov et al., 2003). Almost at the same time, the new genera *Conexibacter* (Monciardini et al. 2003) and *Solirubrobacter* (Singleton et al. 2003) were described. Although their classification at the higher ranks was discussed, a formal recommendation was not made. In 2005, the *Manual* tentatively assigned the genera *Conexibacter*, *Solirubrobacter*, and *Thermoleophilum* to the family *Rubrobacteraceae*, which was the only family validly published at the time (Appendix 2 of Garrity et al., 2005). Stackebrandt (2004, 2005) reanalyzed the relationship of these genera to the subclass *Rubrobacteridae* and proposed the families *Conexibacteraceae*, *Solirubrobacteraceae* and *Thermoleophilaceae* with the emendation of the subclass *Rubrobacteridae* and the family *Rubrobacteraceae*. The results of Stackebrandt (2004, 2005) were considered in the later study for reconstruction of the class *Actinobacteria* based on the 16S rRNA gene sequences (Zhi et al., 2009). Almost at the same time, Reddy and Garcia-Pichel (2009) proposed the orders *Thermoleophilales* and *Solirubrobacterales*, which reflected the low sequence similarity of the 16S rRNA genes between these families and *Rubrobacter* spp.

Lastly, Ludwig et al. (2012) found little phylogenetic or phenotypic evidence for an association between *Rubrobacter* and the orders *Thermoleophilales* and *Solirubrobacterales*. For this reason, they proposed the classification of these groups into the novel classes *Rubrobacteria* and *Thermoleophilia*.

The rRNA gene signatures for the families of the classes *Rubrobacteria* and *Thermoleophilia* are given in Table 314 and differential signatures are given in Table 315.

Type order: **Rubrobacterales** Rainey, Ward-Rainey and Stackebrandt 1997, 483[VP] emend. Zhi, Li and Stackebrandt 2009, 593.

TABLE 314. Signature sequences of the members of the classes *Rubrobacteria* and *Thermoleophilia*[a]

	Rubrobacterales	*Solirubrobacterales*			*Thermoleophilales*
Position(s)	*Rubrobacteraceae*	*Solirubrobacteraceae*	*Conexibacteraceae*	*Patulibacteraceae*	*Thermoleophilus*
52:359	G–C	C–G	U–A	C–G	C–G
63:104	**C–G**	G–C	G–C	G–C	G–C
70:98	**A–U**	G–C	G–C	G–C	G–C
127:234	G–C	G–C	G–C	G–C	G–C
139:224	**U–A**	A–U	G–C	G–C	G–C
144:178	**G–C**	C–G	U–A	C–G	C–G
291:309	U–A	U–A	U–A	U–A	U–A
370:391	C–G	C–G	C–G	C–G	**G–C**
408:434	G–C	G–C	A–U	G–C	G–C
580:776	U–A	U–A	U–A	U–A	**C–G**
590:649	C–G	C–G	U–A	U–A	C–G
600:638	U–G	C–G	U–G	U–G	C–G
657:749	**G–C**	U–A	U–A	U–A	U–A
670:736	A–U	A–U	A–U	A–U	**G–C**
681:709	C–G	**U–A**	**U–A**	**U–A**	C–G
823:877	G–C	G–C	G–C	A–U	G–C
906	A	A	A	A	A
941:1342	A–U	A–U	A–U	A–U	**G–C**
953:1228	**U–A**	G–C	G–C	G–C	G–C
954:1226	**C–G**	G–C	G–C	G–C	G–C
955:1225	U–A	U–A	U–A	U–A	U–A
999:1041	U–A	U–A	G–U	U–A	**A–U**
1051:1207	**C–G**	G–C	G–C	G–C	G–C
1115:1185	C–G	C–G	C–G	C–G	C–G
1118:1155	C–G	**U–A**	**U–A**	**U–A**	C–G
1311:1326	A–U	A–U	A–U	A–U	**G–C**
1410:1490	U–A	U–A	U–A	A–U	U–A

[a]The order-characteristic signatures are indicated in bold letters (Reddy and Garcia-Pichel, 2009).

TABLE 315. Signature sequences that differentiate the families of the classes *Rubrobacteria* and *Thermoleophilia* (modified from Zhi et al., 2009)

	52:359	63:104	144:178	145:177	242:284	291:309	293:304	316:337	370:391	377:386
Rubrobacteraceae		C–G	G–C	G–C	C–G	U–A	G–U	C–G		G–C
Conexibacteraceae			U–A	U–A			G–C			C–G
Patulibacteraceae	C–G		C–G				G–C			C–G
Solirubrobacteraceae	C–G		C–G	C–G			G–C			
Thermoleophilaceae	C–G		C–G	C–G			G–U		G–C	C–G

	408:434	409:433	418:425	502:543	590:649	600:638	657:749	670:736	681:709	722:733
Rubrobacteraceae		C–G					G–C		C–G	G–G
Conexibacteraceae	A–U		U–A		U–A	U–G				
Patulibacteraceae				G–C	U–A	U–G				
Solirubrobacteraceae					C–G	C–G				
Thermoleophilaceae					C–G	C–G		G–C	C–G	

	819	941:1342	952:1229	953:1228	954:1226	1051:1207	1115:1185	1311:1326	1313:1324	1354:1368
Rubrobacteraceae	A		U–A	U–A	C–G	C–G	C–G		U–A	
Conexibacteraceae										
Patulibacteraceae										U–G
Solirubrobacteraceae										
Thermoleophilaceae		G–C						G–C		

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Order I. **Rubrobacterales** Rainey, Ward-Rainey and Stackebrandt 1997, 483[VP] emend. Zhi, Li and Stackebrandt 2009, 593

Ken-ichiro Suzuki

Ru.bro.bac.te′ra.les. N.L. masc. n. *Rubrobacter* type genus of the order; suff. *-ales* ending to denote an order; N.L. fem. pl. n. *Rubrobacterales* the *Rubrobacter* order.

The description of the order is the same as the family *Rubrobacteraceae*. It is defined on the basis of the 16S rRNA gene sequences (see Table 314 and Table 315) and supported by similarities in phenotypic properties.

Type genus: **Rubrobacter** Suzuki, Collins, Iijima and Komagata 1989, 44[VP].

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Family I. **Rubrobacteraceae** Rainey, Ward-Rainey and Stackebrandt 1997, 483^{VP} emend. Zhi, Li and Stackebrandt 2009, 593

Ken-ichiro Suzuki

Ru.bro.bac.te.ra.ce′a.e. N.L. masc. n. *Rubrobacter* type genus of the family; suff. *-aceae* ending to denote a family; N.L. fem. pl. n. *Rubrobacteraceae* the *Rubrobacter* family.

The description of the family is the same as the genus *Rubrobacter*. It is defined on the basis of the 16S rRNA gene sequences (see Table 314 and Table 315) and supported by similarities in phenotypic properties.

Type genus: **Rubrobacter** Suzuki, Collins, Iijima and Komagata 1989, 44^{VP}.

Genus I. **Rubrobacter** Suzuki, Collins, Iijima and Komagata 1989, 93^{VP} (Effective publication: Suzuki, Collins, Iijima and Komagata 1988, 38.)

Ken-ichiro Suzuki

Ru.bro.bac′ter. L. adj. *ruber* red; N.L. masc. n. *bacter* equivalent of the Gr. neut. n. *baktron* a rod; N.L. masc. n. *Rubrobacter* red rod.

Gram-stain-positive irregular rods. Nonmotile and non-sporforming. Cells are generally 0.8–1.0 by 1.0–4.0 µm. Cells in old culture present shorter coccoid form. Aerial mycelia are not formed. **Obligately aerobic**. Catalase and cytochrome oxidase reactions are positive. Nitrite is produced from nitrate.

Where known, the **diamino acid of the cell-wall peptidoglycan is L-lysine** (cell-wall type A3α). However, there is no data for the species *Rubrobacter taiwanensis*. Predominant respiratory quinone is menaquinone with eight isoprene units (**MK-8**). The polar lipids consist of phospholipids including diphosphatidylglycerol, phosphatidylglycerol and one phosphoglycolipid. Some additional phospholipids and glycolipids are also found. Predominant cellular fatty acids are internal branched acids such as **12-methyl hexadecanoic acid and/or 14-methyl octadecanoic acid**. Mycolic acid is not present.

DNA G+C content (mol%): 65–69 (HPLC, T_m).

Type species: **Rubrobacter radiotolerans** (Yoshinaka, Yano and Yamaguchi 1973) Suzuki, Collins, Iijima and Komagata 1989, 93^{VP} (Effective publication: Suzuki, Collins, Iijima and Komagata 1988, 38.).

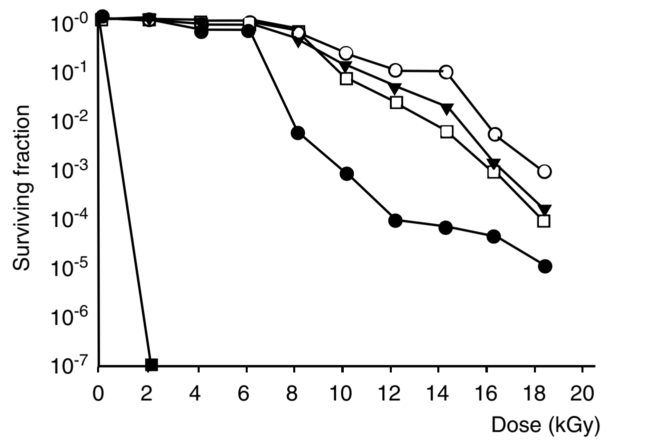

FIGURE 422. Gamma-radiation resistance of species of the genus *Rubrobacter* (from Chen et al., 2004). *Rubrobacter radiotolerans* DSM 5868 (○); *Rubrobacter taiwanensis* BCRC 17173 (▼); *Rubrobacter xylanophilus* DSM 9441 (●): *Deinococcus radiodurans* DSM 20539 (□); *Escherichia coli* K-12 (■).

Further descriptive information

Tolerance to γ-irradiation is the most distinctive feature of the genus *Rubrobacter*, although this characteristic is not specified in the original description of the genus (Yoshinaka et al., 1973). All the described species of the genus *Rubrobacter* show resistance against gamma-radiation (Chen et al., 2004). In addition, the species described so far are moderate thermophiles growing optimally at 46–60°C.

Resistance to γ-irradiation. The type strain of *Rubrobacter radiotolerans*, the type species of the genus, was isolated from a hot spring with high background radiation, and the sample was exposed to γ-radiation prior to isolation. Later, the strain was found to be extremely radiotolerant, comparable to that of species of the genus *Deinococcus* which is also strongly radiatino resistant. For *Rubrobacter radiotolerans*, the shoulder dose, or the dose at which killing is first observed, of exponentially growing cells is 6 kGy, and the D_{10}, or the dose necessary for 10% survival, is 10.0 kGy in phosphate buffer on aerated condition (Ito et al., 1983). The strains of the other species are also extremely radiotolerant, similar to that of *Deinococcus* species whose radiotolerance is well known (Figure 422, from Chen et al., 2004; Ferreira et al., 1999; Saito et al., 1994).

Cellular fatty acids. Strains of the genus *Rubrobacter* contain a large proportion of fatty acids which have methyl branch at a characteristic position in the middle of acyl chain. Such branched fatty acids widely distributed in actinobacteria are 10-methyl acids. 10-Methyl acids are known as the derivatives of corresponding monounsaturated fatty acids such as oleic acid

($C_{18:0}$ ω9). The predominant fatty acids of the genus *Rubrobacter* are 12-methyl hexadecanoic acid (12-Me $C_{16:0}$) and 14-methyl octadecanoic acid (14-Me $C_{18:0}$). Their methyl branch is at a unique position, the 5th carbon from the omega or apolar end of the fatty acid. They are possibly biosynthesized by methylation of corresponding unsaturated acids, namely $C_{16:0}$ ω5 and $C_{18:0}$ ω5, respectively (Suzuki et al., 1993). These unsaturated acids are of the same series of fatty acids with double bonds between the 4th and 5th carbons from the omega end of the acyl chain. Furthermore, 12-Me $C_{16:0}$ is the predominant fatty acid for *Rubrobacter radiotolerans* (Suzuki et al., 1988), and 14-Me $C_{18:0}$ is that for *Rubrobacter taiwanensis* and *Rubrobacter xylanophilus*. The optimal growth temperature of *Rubrobacter radiotolerans* is 45°C, in contrast to 60°C for the latter two species. It is expected that strains growing at higher temperatures have longer cellular fatty acids. Suzuki et al. (1988) reported that $C_{19:0}$ anteiso and $C_{17:0}$ 2-OH were also present in the cells of *Rubrobacter radiotolerans*,. However, these fatty acids were not found by subsequent investigators (Carreto et al., 1996; Chen et al., 2004). It will be interesting if there is found to be some relationship between these distinctive fatty acids and radiation resistance.

Enrichment and isolation procedures

The three species of the genus *Rubrobacter* are characterized by their strong resistance to γ-irradiation. The type strain of *Rubrobacter radiotolerans* was isolated from a sample pre-treated with 1.7 kGy irradiation by Cs^{137} for 5 h (Yoshinaka et al., 1973). Furthermore, the samples for isolation were collected at a radioactive hot spring. Although *Rubrobacter radiotolerans* is a moderate thermophile, it grows slowly. The characteristic pink colonies were picked up after 1-week cultivation at 37°C on an agar plate on which the irradiated sample was spread. Some faster-growing bacteria appeared after only a few days prior to the small pink colonies of the genus *Rubrobacter*.

The strains of the other two species were isolated from samples without radiation pretreatment and background radioactivity. These *Rubrobacter* strains were isolated from samples incubated at 50°C for growth of moderate thermophiles. In general, the isolation procedure involved spreading samples on agar plates and incubating at 50°C for 5–7 d. Pink colonies that appear slowly are picked (Carreto et al., 1996; Chen et al., 2004).

The strains of the genus *Rubrobacter* grow on various media, such as nutrient agar, tryptic soy broth, R medium, and *Thermus* medium. *Thermus* medium gives the best growth. The strains of the genus *Rubrobacter* are enriched by strong γ-irradiation and aerobic cultivation at 45–60°C.

Maintenance procedures

The strains of the genus *Rubrobacter* are preserved by freezing at lower than −70°C suspensions in *Thermus* medium containing 15% glycerol. Lyophilization in 10% skimmed milk containing 1% monosodium glutamate is also useful for long-term preservation and shipment.

Differentiation of the genus *Rubrobacter* from other genera

The genus *Deinococcus*, which also shows very strong radiation resistance, is classified in the phylum *Deinococci* and clearly differentiated by 16S rRNA gene sequences.

Taxonomic comments

Rubrobacter radiotolerans was firstly proposed by Yoshinaka et al. (1973) as a new species of the genus *Arthrobacter* because of the pleomorphic cell morphology, absence of acid production from sugars, and cell-wall peptidoglycan containing L-lysine. Later, Suzuki et al. (1988) proposed a new genus *Rubrobacter* for the species on the basis of the distinctive chemotaxonomic features in addition to the unusual physiological characteristics. The distinctiveness of this organism was further supported by phylogenetic analyses of the 16S rRNA gene sequences of the class *Actinobacteria* by Stackebrandt et al. (1997). The genus *Rubrobacter* was found to form a deep lineage, and it was classified outside of the subclass *Actinobacteridae*, which accommodated the order *Actinomycetales* in the class *Actinobacteria*. Stackebrandt et al. (1997) classified the single-genus taxon in the subclass *Rubrobacteridae*, order *Rubrobacteriales*, and family *Rubrobacteriaceae*. Later, the genera *Thermoleophilum* Yakimov et al. 2003 and *Solirubrobacter* Singleton et al. 2003 were classified in this subclass. Although the genus *Conexibacter* was closely related to *Solirubrobacter*, it was not included (Monciardini et al., 2003). Subsequently, these genera were reclassified into independent families, namely *Conexibacteraceae*, *Solirubrobacteraceae*, and *Thermoleophilaceae* (Stackebrandt 2004; Zhi et al. 2009), and novel orders, *Solirubrobacterales* and *Thermoleophilales*. The order *Solirubrobacterales* contains the families *Conexibacteraceae*, *Patulibacteraceae*, and *Solirubrobacter* (Reddy and Garcia-Pichel 2009). The order *Thermoleophilales* accommodates the family *Thermoleophilaceae*. In the absence of clear evidence of relatedness, these genera were finally reclassified in a novel class, *Thermoleophilia*, as well (Ludwig et al., 2012).

Currently, three species have been described in the genus *Rubrobacter*, and they share common chemotaxonomic, biochemical and physiological features. Because only one or two strains have been described for each species, the variability within species is not known. The species classification has also been confirmed by DNA relatedness studies (Chen et al., 2004).

Differentiation of the species of the genus *Rubrobacter*

Three species share many phenotypic and chemotaxonomic features, such as isoprenoid quinones and cell-wall peptidoglycan type (Table 316). Likewise, the cellular fatty acid profiles are very similar. Features that distinguish the species include the optimum growth temperature and a number of other physiological and biochemical characteristics (Table 316). The DNA relatedness and 16S rRNA gene sequence differences also strongly support the differentiation.

The common biochemical and physiological characteristics for the three species of the genus *Rubrobacter* are as follows. Positive reaction: catalase, cytochrome oxidase, β-glucosidase, β-galactosidase, growth in 5% NaCl and utilization of D-cellobiose, D-raffinose, D-trehalose, D-arabinose, D-fructose, D-mannose, lactose, L-glutamate, and pyruvate. Negative reaction: β-glucosidase, β-galactosidase, hydrolysis of starch, casein, cellulose, and tributyrin, and utilization of D-sorbitol. Characteristics variable among species are shown in Table 316.

TABLE 316. Phenotypic characteristics of *Rubrobacter* species[a,b]

Characteristic	1. *R. radiotolerans*[a]	2. *R. taiwanensis*[b]	3. *R. xylanophilus*[c]
Colony color	Reddish pink	Light pink	Light pink
Cell size (μm)	0.8–1.0 × 1.0–4.0	0.9–1.0 × 1.0–4.0	0.9–1.0 × 1.0–3.0
Optimum growth temperature (°C)	46–48	60	60
Cell-wall peptidoglycan	L-Lys–L-Ala	nd	L-Lys–L-Ala
Menaquinones	MK-8	MK-8	MK-8
Cellular fatty acids (at cultivation temp.)	12Me-$C_{16:0}$ (71%), $C_{19:0}$ anteiso (12%)*, and 2OH-$C_{17:0}$ (17%)* (at 37°C)	14Me-$C_{18:0}$ anteiso (31–33%), 12Me-$C_{17:0}$ (13%) and 12Me-$C_{16:0}$ (12%) (at 60°C)	14Me-$C_{18:0}$ (53%), $C_{18:0}$ (20%),12Me-$C_{16:0}$ (12%), and others (at 60°C)
DNA G+C content (mol%)	67.9 (HPLC)	67.9–68.5 (HPLC)	67.6 (T_m)
Nitrate reduction to nitrite	+	nd	+
Ammonium as a sole N source	+	nd	+
Resistance to γ-irradiation	+++	++	+
Hydrolysis of:			
Gelatin	−	+	+
Xylan	−	−	+
DNA	−	+	−
Esculin	+	−	+
Utilization of:			
D-Glucose	+	d	+
D-Galactose	−	+	+
D-Xylose	−	+	+
D-Melibiose	−	+	+
Rhamnose	+	d	+
Glycerol	+	−	−
Galactitol	−	nd	w
D-Mannitol	+	−	−
Ribitol	+	−	−
myo-Inositol	−	+	+
Acetate	−	nd	w
Malate	+	−	+
Succinate	−	−	+
L-Asparagine	+	d	+
L-Glutamine	−	+	w
L-Serine	−	+	−
Acetamide	−	nd	+

[a]Symbols : +, positive; −, negative; d, different in strains; nd, no data.

[b]The data are those of type strains except *Rubrobacter taiwanensis* (two strains) cited from Yoshinaka et al. (1973), Suzuki et al. (1988), Carreto et al. (1996), and Chen et al. (2004).

List of species of the genus *Rubrobacter*

1. **Rubrobacter radiotolerans** (Yoshinaka, Yano and Yamaguchi 1973, 2273[AL]) Suzuki, Collins, Iijima and Komagata 1989, 93[VP] (Effective publication: Suzuki, Collins, Iijima and Komagata 1988, 38.) (Basonym: *Arthrobacter radiotolerans* Yoshinaka, Yano and Yamaguchi 1973, 2273[AL]).

ra.di.o.to′le.rans. L. n. *radius* a beam or ray; N.L. pref. *radio*- pertaining to radiation; L. pres. part. *tolerans* tolerating; N.L. part. adj. *radiotolerans* (γ-ray) radiation-tolerating.

Gram-stain-positive irregular rod. Cells are 0.8–1.0 × 1.0–4.0 μm in size. Coccoid cells are often found in older cultures. Nonmotile. Colonies on nutrient agar are circular, smooth, entire convex or umbonate, glistening, opaque and reddish-pink in color. Colonies are 0.5–1.0 mm in diameter after 2 weeks, increasing in size to 4–5 mm after 4 weeks.

Xylose, arabinose, glucose, fructose, maltose and glycerol are utilized for growth. Galactose and mannose are weakly utilized. Sucrose, lactose, sorbitol and citrate are not utilized. Acid is not produced from any of carbon sources tested. Gelatin is not liquefied. Neither indole nor acetylmethylcarbinol is produced. Methyl red test negative. Ammonium sulfate is utilized as a sole nitrogen source.

Optimal temperature for growth is 46–48°C. Optimal pH for growth is 7.0–7.4. Growth is observed at 6% NaCl and lower but not at 10% NaCl in the culture medium.

The peptidoglycan structure is L-Lys–L-Ala. The cellular fatty acids are composed of more than 70% of 12-methyl branched octadecanoic acid, 12% of 16-methyl branched octadecanoic acid and 17% of 2-hydroxy heptadecanoic acid. Instead of these two acids, Carreto et al. (1996) report the presence of 14-Me $C_{18:0}$ and some long-chain alcohol.

The type strain was isolated from the radioactive hot spring at Misasa, Tottori, Japan after treatment of samples with γ-irradiation. Highly resistance to γ-irradiation. Shoulder dose of logarithmic growing cells is 6×10^5 rad and D_0 is 1×10^6 rad in phosphate buffer on aerated conditions.

Source of type strain: radioactive hot spring at Misasa, Tottori, Japan

DNA G+C content (mol%): 68 (HPLC).

Type strain: Yoshinaka strain P-1 = ATCC 51242 = CIP 106991 = DSM 5868 = IAM 12072 = IFO (now NBRC) 14777 = JCM 2153 = VKM Ac-1989.

Sequence accession no. (16S rRNA gene): D45058.

2. **Rubrobacter taiwanensis** Chen, Wu, Lin, Lu, Lin, Chang and Tsay 2004, 1853^VP

tai.wan.en′sis. N.L. masc. adj. *taiwanensis* of Taiwan, where the micro-organism was first isolated.

Cells are irregular short rods, 0.9–1.0 µm wide to 1.0–3.0 µm long. Colonies on *Thermus* medium grown at 60°C for 7 d are 1.6–2.2 mm in diameter, circular, convex, smooth, opaque, and light pink. The temperature range for growth is 30–70°C. Optimal growth is at 60°C. The pH range for growth is 6–11. Optimal growth is at pH 8.0. Growth occurs at NaCl concentrations of 5% and below in *Thermus* medium. Cytochrome oxidase and β-galactosidase are positive. Gelatin and DNA are hydrolyzed. The carbon sources utilized are shown in Table 316. 14-Methyl octadecanoic acid is the predominant cellular fatty acid (31–33%). Other fatty acids present are 12-methyl heptadecanoic acid and 12-methyl hexadecanoic acid. The strains of this species show strong resistance to γ-irradiation.

Source of type strain: thermally heated water/soil/mud samples of Lu-shan hot springs, Nantou, Taiwan.

DNA G+C content (mol%): 67.9–68.5 (HPLC).

Type strain: strain LS-293 = ATCC BAA-406 = BCRC 17173 = JCM 12932.

Sequence accession no. (16S rRNA gene): AF465803 (type strain).

3. **Rubrobacter xylanophilus** Carreto, Moore, Nobre, Wait, Riley, Sharp and Da Costa 1996, 463^VP

xy.la.no′phi.lus. N.L. n. *xylanum* xylan; Gr. adj. *philos* liking, friendly to; N.L. masc. adj. *xylanophilus* liking xylan.

Forms pleomorphic short rod-shaped cells that are 0.9–1.0 µm wide and 1.0–3.0 µm long; coccoid cells are also present. Colonies on *Thermus* medium incubated at 60°C for 7 d are 1.5–2.0 mm in diameter, circular, convex, smooth, opaque and light pink. Grows optimally at 60°C, and no growth is observed below 40°C and above 70°C. Optimal pH for growth is between 7.5 and 8.0, and no growth occurs below 6.0 and above 10.0. Growth occurs at NaCl concentrations of 6% and below in *Thermus* medium. β-Galactosidase-positive. Xylan is hydrolyzed. Growth occurs on various sugars, organic acids and amino acids (Table 316). The cell-wall peptidoglycan is L-Lys–L-Ala (type A3α). The major fatty acid is 14-methyl octadecanoic acid. Octadecanoic acid and by 12-methyl hexadecanoic acid are also present in cells grown at either 60°C or 45°C.

Source of type strain: thermally polluted run-off (temperature 50°C) from a carpet factory, Wilton, Wiltshire, UK.

DNA G+C content (mol%): 67.6 (T_m) for type strain.

Type strain: strain PRD-1 = CIP 105412 = DSM 9941 = NBRC 16129 = JCM 11954 = NBRC 100952.

Sequence accession no. (16S rRNA gene): CP000386.

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Class VI. **Thermoleophilia** class. nov.

KEN-ICHIRO SUZUKI AND WILLIAM B. WHITMAN

Ther.mo.le.o.phi′li.a. N.L. neut. n. *Thermoleophilum* type genus of the type order; suff. *-ia* ending to denote a class; N.L. pl. neut. n. *Thermoleophilia* the *Thermoleophilales* class.

The class is defined on the basis of the 16S rRNA gene sequences (Table 314 and Table 315) and is supported by similarities in phenotypic properties. Although initial phylogenetic analyses described an affiliation of the families *Thermoleophilaceae*, *Conexibacteraceae*, *Patulibacteraceae*, and *Solirubrobacteraceae* within the order *Rubrobacterales* (Zhi et al., 2009), more recent analyses do not provide strong support for this association (Ludwig et al., 2012) (Figure 423). For this reason, these families were reclassified in their own class.

Type order: **Thermoleophilales** Reddy and Garcia-Pichel 2009, 91^VP^.

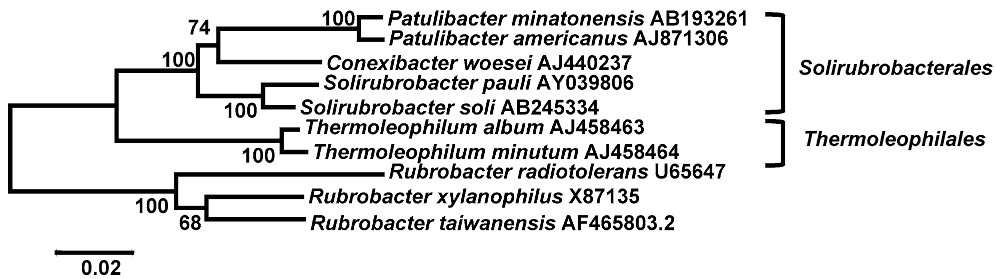

FIGURE 423. Phylogeny of the 16S rRNA genes of the representatives of the class *Thermoleophilia*. Members of the class *Rubrobacteria* serve as an outgroup. The phylogenetic tree was calculated with the Minimum Evolution algorithm of MEGA4. Evolution distance, calculated by the Kimura two-parameter model, is shown on the scale bar. Numbers represent the percentage bootstrap support for the indicated nodes based upon 1000 replicates.

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Order I. **Thermoleophilales** Reddy and Garcia-Pichel 2009, 91^VP^

KEN-ICHIRO SUZUKI

Ther.mo.le.o.phi′la.les. N. L. neut. n. *Thermoleophilum* type genus of the order; suff. *-ales* ending to denote an order; N. L. fem. pl. n. *Thermoleophilales* the *Thermoleophilum* order.

Type genus: **Thermoleophilum** Zarilla and Perry 1986, 355^VP^ (Effective publication: Zarilla and Perry 1984, 290.).

The order is defined on the basis of 16S rRNA gene sequences (Table 314) and supported by similarities in phenotypic properties. The properties are the same as for the family *Thermoleophilaceae*.

References

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Family I. **Thermoleophilaceae** Stackebrandt 2005, 548[VP] (Effective publication: Stackebrandt 2004.) emend. Zhi, Li and Stackebrandt 2009, 594

Ken-ichiro Suzuki

Ther.mo.le.o.phi.la.ce′a.e. N.L. neut. n. *Thermoleophilum* type genus of the family; suff. *-aceae* ending to denote a family; N.L. fem. pl. n. *Thermoleophilaceae* the *Thermoleophilum* family.

Type genus: **Thermoleophilum** Zarilla and Perry 1986a, 355[VP] (Effective publication: Zarilla and Perry 1984, 290).

The family is defined on the basis of the 16S rRNA gene sequences (see Table 314) and supported by similarities in phenotypic properties. Members of the family are small, nonmotile rods which stain Gram-positive. Described species are moderately thermophilic aerobes that utilize *n*-alkanes, such as *n*-heptadecane, and a few other compounds as sole carbon sources.

Genus I. **Thermoleophilum** Zarilla and Perry 1986a, 355[VP] (Effective publication: Zarilla and Perry 1984, 290.)

Jerome J. Perry*

Ther.mo.le.o′phi.lum. Gr. n. *therme* heat; L. n. *oleum* oil; Gr. adj. *philos* loving; N.L. neut. n. *Thermoleophilum* heat- and oil-loving microbe.

Very small rods, 0.3–0.4 µm in diameter and 0.7–1.5 µm in length. Regular in shape. **No resting stage or endospores are observed**, Gram-stain-negative. **The major diamino acid in the cell wall is diaminopimelic acid.** Obligately **aerobic.** Nonmotile. **Optimum temperature for growth is 55–62°C** (minimum 45°C, maximum 70°C). pH optimum is 6–7.5. **Chemo-organotrophic, utilizing *n*-alkanes from 13 to 20 carbons in length; no other growth substrates are utilized.** Oxygen is terminal electron acceptor. Catalase-positive. Unpigmented. Growth is not stimulated by increased oxygen tension (shaking). **Generation time is 6–9 h.** Isolated from both thermal and non-thermal environments.

DNA G+C content (mol%): 68.8–70.4 (T_m).

Type species: **Thermoleophilum album** Zarilla and Perry 1986a, 355 (Effective publication: Zarilla and Perry 1984, 290.).

Further descriptive information

The sequence of bases in the 16S rRNA of *Thermoleophilum album* strain NM indicated that this micro-organism should be placed in a separate "phylum" within the *Eubacteria* (C. Woese, personal communication). Further study (Brown and Haas, 1997) provided evidence that the genus *Thermoleophilum* is related to and should be grouped with the green non-sulfur bacteria. A more recent study analyzing the morphology and genotype of the three strains of *Thermoleophilum album* and *Thermoleophilum minutum* indicated that the genus should be placed in the *Rubrobacter* subdivision of the *Actinobacteria* (Yakimov et al., 2003). These authors suggest that *Thermoleophilum* is a member of the group 2 subclass as the sole known culturable genus. On the basis of 16S rRNA gene sequence analysis, the type strains of *Thermoleophilum album* and *Thermoleophilum minutum* form a distinct lineage within the class *Actinobacteria*. Figure 424 shows a maximum-likelihood tree of selected members of the class *Actinobacteria*. The closest relatives of *Thermoleophilum album* and *Thermoleophilum minutum* are *Conexibacter woesei* (sequence similarities of 90.1% and 90.2%, respectively), *Solirubrobacter soli* (90% sequence similarity), *Solirubrobacter pauli* (sequence similarities of 89.8% and 89.9%, respectively), and *Patulibacter minatonensis* (90% sequence similarity). The genus *Thermoleophilum* is currently the only member of the family *Thermoleophilaceae*.

Six strains of *Thermoleophilum* have been isolated and all are small Gram-stain-negative rods that are regular in shape and occur singly. There is no evidence of flagella or motility (Zarilla and Perry, 1984). The major diamino acid in the cell walls is diaminopimelic acid, with lesser amounts of lysine or ornithine in some strains (Merkel et al., 1978a). Electron micrographs of thin sections reveal internal structures (Kennedy and Finnerty, 1975) that may be involved in alkane uptake and storage (Figure 425). Growth on agar surfaces is inconsistent and single colonies are rare. Growth is generally confluent, whitish, and flat. The optimum growth temperature is 60°C at pH 7.0. All strains are strict aerobes and grow solely on *n*-alkanes from *n*-tridecane to *n*-eicosane; optimal growth is on *n*-heptadecane (Zarilla and Perry, 1984, 1986b). Growth occurs on a mineral salts medium with *n*-alkane added at 0.1% (v/v) with NH_4 as preferred nitrogen source (Perry, 1992). Addition of growth factors (B vitamins, amino acids, etc.) does not increase growth rate or total cell yield. The total cell yield under putatively optimal conditions is 0.3–0.6 g/l. The generation time varies among strains and is 6–9 h. Addition of ^{14}C-labeled acetate to the growth medium (mineral salts + *n*-heptadecane) results in little incorporation of radiolabel into the resultant cell mass (<5% of total carbon). *Thermoleophilum* strains lack 2-oxoglutarate dehydrogenase and apparently do not utilize the tricarboxylic acid cycle in energy generation. They do possess the enzymes of the glyoxylate cycle (Weaver et al., 1987).

The DNA G+C content is 68.8–70.4 mol%. Thermal elution profiles of *Thermoleophilum* duplexes affirm that there are two species within the genus. Reassociation experiments with DNA from *Thermoleophilum* strains confirm that this genus has no relationship to *Thermus* sp., *Thermomicrobium roseum*, or thermophilic hydrocarbon-utilizing bacilli (Zarilla and Perry, 1987). *Thermoleophilum* has a novel respiratory quinone, a tetrahydrogenated menaquinone, 2-methyl-3-VI,VII-tetrahydroheptaprenyl-1,4-naphthoquinone (Collins et al., 1986). Although these organisms are strict aerobes, they do not respond favorably to increased aeration (Allgood and Perry, 1985). Examination of the enzymes involved in defense against toxic products of oxygen reduction, i.e. superoxide dismutase, peroxidase, and catalase, indicate that only catalase responds favorably to increased

*Deceased 17 May 2011.

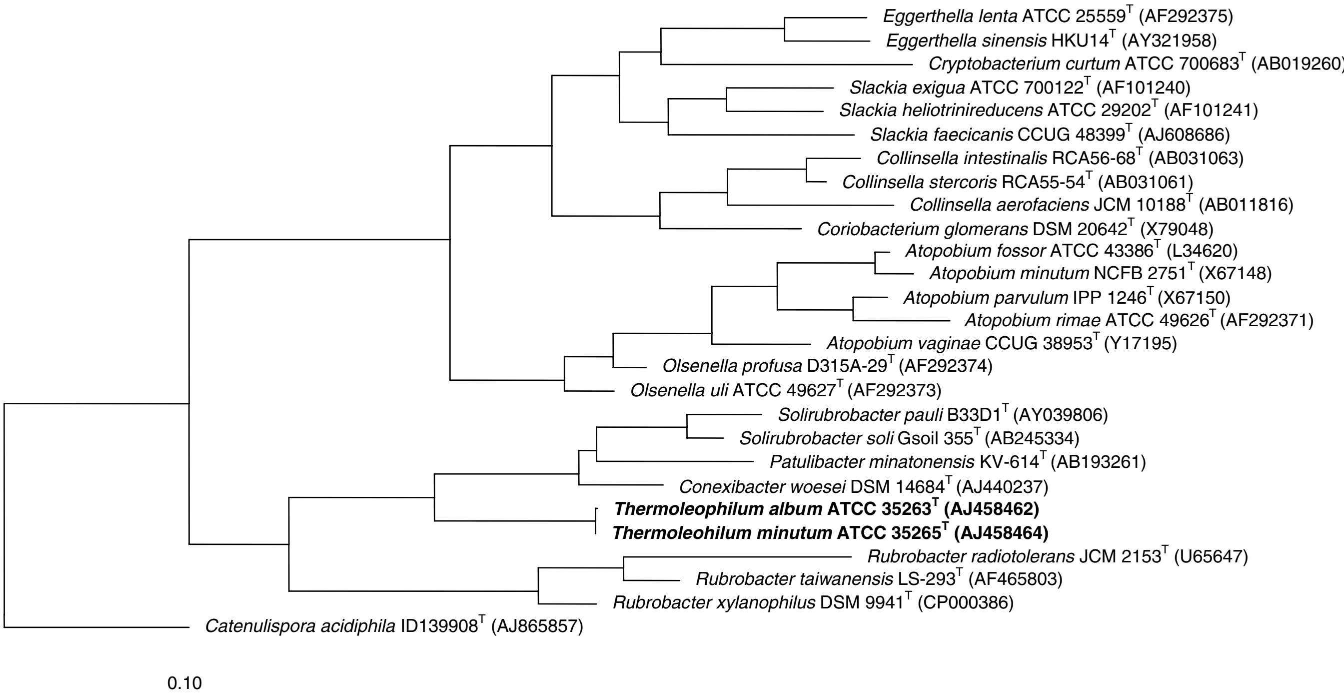

FIGURE 424. Phylogenetic analysis based on 16S rRNA gene sequences available from the EMBL data library (accession numbers are given in parentheses). The phylogenetic tree was constructed using the ARB software package (December 2007 version; Ludwig et al., 2004) and the corresponding SILVA SSURef 95 database (July 2008 version; Pruesse et al., 2007). Tree building was performed using the maximum-likelihood method with fastDNAml (Olsen et al., 1994) and no conservatory filter. Bar = 0.10 nucleotide substitutions per nucleotide position.

FIGURE 425. Electron micrograph of a thin section of *Thermoleophilum album* with clear areas containing stored *n*-alkane. Magnification = 31,000×.

oxygenation. Catalase was recovered and purified from *Thermoleophilum album* NM and is a heat-stable, manganese-containing enzyme with an M_r of 141,000 (Allgood and Perry, 1986). The manganese catalase gene from *Thermoleophilum album album* NM has been cloned and the nucleotide sequence has been determined (Phucharoen et al., 2001). The gene consists of 88 bp and encodes 294 amino acids with a molecular mass of 32,500 Da.

Electrophoretic mobility of selected enzymes on polyacrylamide gel indicated that enzymes from *Thermoleophilum* strains differ in mobility from equivalent enzymes in other species. An enzyme from each *Thermoleophilum* species was purified and characterized: malate dehydrogenase from *Thermoleophilum album* and isocitrate dehydrogenase from *Thermoleophilum minutum* (Novotny and Perry, 1990, 1991). The results indicate that the difference in mobility of these enzymes was related to some differences in amino acid composition and to the conformation of the protein. P RNA from *Thermoleophilum album* NM has been characterized and the RNase P is a type A ribonuclease (Brown and Haas, 1997).

The minimum antibiotic concentrations (μg/ml) that were inhibitory to all strains are as follows: chlortetracycline (25–50), neomycin (5–10), chloramphenicol (5–10), penicillin (5–25), and novobiocin (5–10).

Enrichment and isolation procedures

Six strains of *Thermoleophilum* have been obtained in axenic culture. These strains can be separated into two species: *Thermoleophilum album* and *Thermoleophilum minutum*. The six strains were isolated by enrichment from soil or mud obtained from across the USA (Table 317). Mud was added to a basal salts medium

TABLE 317. Source of the soil sample from which the obligately thermophilic strains of *Thermoleophilum* were isolated

Species	Source (in the USA)	Strain
T. album	Hot Springs, AR	HS-5^T
	Faywood Hot Springs, NM	NM
	Yellowstone National Park, WY	YS-3
	Roanoke Rapids, NC	RR-D
T. minutum	Yellowstone National Park, WY	YS-4^T
	Beaufort, NC	PTA-1

(Leadbetter and Foster, 1958) with *n*-heptadecane as sole carbon source (Merkel et al., 1978b). The enrichment was incubated at 60°C for 1–2 weeks and a transfer was made from media having visible turbidity to a sterile medium of the same composition. After several transfers in liquid medium, axenic cultures were obtained by streaking on a solid medium (2.0% agar) with *n*-heptadecane (0.2 ml) added in the cover of the inverted plate. *Thermoleophilum*, as the sole culturable genus in the subclass, serves as a prime example of the exacting culture conditions required for the isolation of a fastidious species from nature.

An exceedingly limited substrate range, high growth temperature, and sparse growth on a solid substrate offer ample evidence that culturing many "unculturable" microbes from nature is a difficult task.

Differentiation of the genus *Thermoleophilum* from other genera

The distinct and readily determinable characteristic of members of the genus *Thermoleophilum* is their restriction to growth with *n*-alkanes as substrate. Several characteristics that separate *Thermoleophilum* species from other Gram-stain-negative thermophiles are listed in Table 318. Under phase-contrast microscopy, *Thermomicrobium* cells appear pleomorphic, whereas *Thermus* cells are long, thin, regular rods and the cells of *Thermoleophilum* species are very small and regular in shape. The only other thermophilic hydrocarbon-utilizing aerophiles known are members of the genus *Bacillus* and can be distinguished by Gram-stain and production of endospores.

TABLE 318. Differential characteristics of Gram-stain-negative thermophilic rods

Characteristic	*Thermus* strains	*Thermomicrobium roseum*	*Thermoleophilum* strains
Peptidoglycan major diamino acid	Ornithine	None	Diaminopimelic acid
Cell morphology	Rods, 5–10 µm	Pleomorphic rods, 3–6 µm	Short rods, 0.7–1.5 µm
n-Alkane utilization	–	–	+
Growth on complex media	+	+	–
Growth on glucose	+	–	–
Generation time	20–60 min	5.5 h	6–9 h
Pigment	Cream, yellow, orange, pink	Pink	None
Respiratory quinone	MK-8	MK-8	MK-7(H_4)
Temperature range (°C)	60–70	70–75	55–65

List of species of the genus *Thermoleophilum*

1. **Thermoleophilum album** Zarilla and Perry 1986a, 355[VP] (Effective publication: Zarilla and Perry 1984, 290).

al′bum. L. neut. adj. *album* white.

Rod-shaped cells, 0.9 µm in length, nonmotile, nonsporeforming, Gram-stain-negative, aerobic, catalase-positive, and non-pigmented. Forms very small translucent to white colonies on agar surfaces. Grows solely with *n*-alkanes from 13 to 20 carbons in length at temperatures from 45 to 70°C. Addition of growth factors or increased aeration do not result in an increase in the growth rate or total cell yield.

Source: mud samples taken from both thermal and nonthermal environments.

DNA G+C content (mol%): 70.4 (T_m).

Type strain: HS-5, ATCC 35263.

Sequence accession no. (16S rRNA gene): AJ458462.

Further comments: additional strains of this species are NM (= ATCC 35266), YS-3 (= ATCC 35264), and RR-D (= ATCC 35267).

2. **Thermoleophilum minutum** Zarilla and Perry 1986b, 16[VP]

mi.nu′tum. L. neut. adj. *minutum* small, referring to cell size.

Rod-shaped cells, 1–1.5 µm in length. Nonmotile, nonsporeforming. Gram-stain-negative, aerobic, and catalase-positive. Nonpigmented. Forms small translucent to white colonies on agar surfaces. Grows solely with *n*-alkanes from 13 to 20 carbons in length at temperatures from 45 to 70°C. Addition of growth factors or increased aeration do not result in an increase in the growth rate or total cell yield. This species can be differentiated from *Thermoleophilum album* solely by thermal elution profile of DNA/DNA duplexes (Zarilla and Perry, 1986b).

Source: the type strain was isolated from a hot spring in Yellowstone National Park, USA.

DNA G+C content (mol%): 70.0 (T_m).

Type strain: YS-4, ATCC 35265.

Sequence accession no. (16S rRNA gene): AJ458464.

Further comments: an additional strain of this species is PTA-1 (= ATCC 35268), isolated from a mud sample from Beaufort, NC, USA.

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Order II. **Solirubrobacterales** Reddy and Garcia-Pichel 2009, 91[VP]

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So.li.ru.bro.bac′te.ra.les. N.L. masc. n. *Solirubrobacter* type genus of the order; suff. *-ales* ending to denote an order; N.L. fem. pl. n. *Solirubrobacterales* the *Solirubrobacter* order.

The order *Solirubrobacterales* contains three families, *Solirubrobacteraceae*, *Conexibacteraceae*, and *Patulibacteraceae*. The order is defined on the basis of 16S rRNA gene sequence (see Table 314) and phenotypic similarities (Table 319). Although only a few genera have been described, cloning and sequencing of environmental DNA suggest that many more related taxa exist in nature. Currently described species are all Gram-stain positive, mesophilic and sometimes psychrotolerant, and contain *meso*-diaminopimelate as the diamino acid in their peptidoglycan.

Type genus: **Solirubrobacter** Singleton, Furlong, Peacock, White, Coleman and Whitman 2003, 489[VP].

TABLE 319. Characteristics of the genera in the order *Solirubrobacterales*[a]

Properties	*Solirubrobacter*[b]	*Conexibacter*[c]	*Patulibacter*[d]
Morphology (length in μm)	Rod (1.4)	Rod (0.9–1.2)	Rod (1.2–1.5)
Motility	Nonmotile	Motile	Motile
Temperature optimum (°C)	28–30	28–37	24–27
pH range	6–7.7	7–7.5	6–8
Oxidase	Negative	Positive	Negative
Major fatty acid	$C_{16:0}$ iso	$C_{18:1}$ ω9*c*	$C_{18:1}$ ω9*c*
Diamino acid in peptidoglycan	nd	*meso*-DAP	*meso*-DAP
Isoprenoid quinone	MK-7(H_4)	MK-7(H_4)	DMK-7, MK-7(H_4)

[a] Abbreviations: *meso*-DAP, *meso*-diaminopimelate; iso, iso methyl-branched; DMK, demethylmenaquinone; MK, menaquinone; nd, not determined.

[b] Data from Singleton et al. (2003) and Kim et al. (2007).

[c] Data from Monciardini et al. (2003).

[d] Data from Takahashi et al. (2006) and Reddy and Garcia-Pichel (2009).

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Takahashi, Y., A. Matsumoto, K. Morisaki and S. Ōmura. 2006. *Patulibacter minatonensis* gen. nov., sp. nov., a novel actinobacterium isolated using an agar medium supplemented with superoxide dismutase, and proposal of *Patulibacteraceae* fam. nov. Int. J. Syst. Evol. Microbiol. *56*: 401–406.

Family I. **Solirubrobacteraceae** Stackebrandt 2005, 548^{VP} emend. Zhi, Li and Stackebrandt 2009, 594

William B. Whitman and Ken-ichiro Suzuki

So.li.ru.bro.bac′te.ra.ce.ae. N. L. masc. n. *Solirubrobacter* type genus of the family; suff. *-aceae* ending to denote a family; N. L. fem. pl. n. *Solirubrobacteraceae* the *Solirubrobacter* family.

Type genus: **Solirubrobacter** Singleton, Furlong, Peacock, White, Coleman and Whitman 2003, 489^{VP}.

The family is defined on the basis of 16S rRNA gene sequences (see Table 314) and supported by similarities in phenotypic properties. Members of the family are nonmotile rods which stain Gram-positive. Described species are aerobes that utilize many sugars and a few other compounds as sole carbon sources. For a comparison to related families, see the order description.

Genus I. **Solirubrobacter** Singleton, Furlong, Peacock, White, Coleman and Whitman 2003, 489^{VP}

William B. Whitman

So.li.ru.bro.bac′ter. L. n. *solum* soil; L. adj. *ruber* red; N.L. n. *bacter* the masculine equivalent of the Gr. neut. n. *bakterion* a rod; N.L. masc. n. *Solirubrobacter* a soil red rod.

Gram-stain positive, **rod** of medium length. **Nonmotile.** Spores are not formed. **Aerobe** and mesophile. Grows well on common sugars, a few amino acids and other compounds as sole carbon source. The **major phospholipid fatty acids (PFLAs) are $C_{16:0}$ iso and $C_{18:1}$ ω9*c***, and **MK-7(H_4) is the predominant menaquinone** in the only species that has been tested. Some species are sensitive to desiccation.

DNA G+C content (mol%): 71–72 (Lc).

Type species: **Solirubrobacter pauli** Singleton, Furlong, Peacock, White, Coleman and Whitman 2003, 489^{VP}.

Further descriptive information

This genus is represented by two species, each one represented by a single strain, and the description must necessarily be preliminary. Clones of 16S rRNA genes with high sequence similarity to members of this genus are frequently encountered in clone libraries of agricultural, pasture and forest soils; insect guts; plant-associated bacteria; and aerosols (Brodie et al., 2007; Fall et al., 2007; Gremion et al., 2003; Joseph et al., 2003; Singleton et al., 2003; Wang et al., 2008; Youssef et al., 2009; Yu et al., 2008). Presumably, this genus is representative of a group of abundant but largely uncharacterized microorganisms.

For the type strain of *Solirubrobacter pauli* B33D1^T, cells are short rods, especially in older cultures (Singleton et al., 2003). Much longer cells are occasionally observed in fresh cultures. In liquid culture, cells often grow in long chains that wrap around each other producing large aggregates (Figure 426). Even in rich medium, only a low cellular density (maximum turbidity, near 0.1 at 600 nm) is obtained. Colonies are usually pink in color. Plates incubated at higher temperatures (≥28°C) often produce less pigment initially, although a deep pink color appears over time. The color resembles that of *Rubrobacter* species, and the pigments like those of *Rubrobacter* species are not easily extracted using traditional methods. However, methanol can extract a small amount of pigment, which has absorption maxima at 466, 493, and 526 nm. These maxima are similar to those reported for pigments extracted from *Rubrobacter radiotolerans* (Saito et al., 1994).

The menaquinone composition has only been examined in *Solirubrobacter soli*, where it was found to be MK-7(H_4) (Kim et al., 2007). The major phospholipid fatty acids (PFLAs) are $C_{16:0}$ iso and $C_{18:1}$ ω9*c*. Smaller amounts of $C_{18:3}$ ω6*c*, $C_{17:1}$ ω6*c*, $C_{19:0}$, $C_{18:0}$ iso, and $C_{16:0}$ may also be present. Related organisms *Conexibacter woesei*, *Patulibacter minatonensis*, and *Patulibacter americanus* also

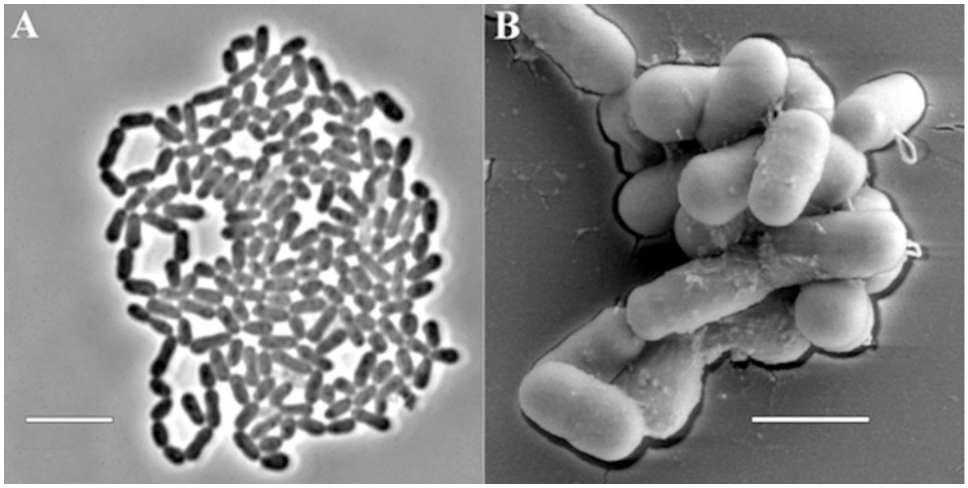

FIGURE 426. Morphology of *Solirubrobacter pauli*. Photomicrographs of strain B33D1 grown in nutrient broth. (A) Phase-contrast image of an aggregate of cells. Scale bar = 5 µm. (B) Scanning electron micrograph of an aggregate. Scale bar = 1 µm. (Reprinted with permission from Singleton et al., 2003. Int. J. Syst. Evol. Microbiol. *53*: 485–490.)

possess high levels of $C_{18:1}$ ω9*c* (Monciardini et al., 2003; Reddy and Garcia-Pichel, 2009; Takahashi et al., 2006).

Enrichment and isolation procedures

The type strain B33D1^T of *Solirubrobacter pauli* was isolated from a burrow of the epigeic earthworm *Lumbricus rubellus* in an agricultural soil in Athens, GA, USA (Furlong et al., 2002). Fresh soil was diluted to extinction and plated on medium composed of 50% Nutrient Broth (Difco, pH 7.0, ~23°C). Isolates were allowed to grow for 2 weeks before colonies were picked. A single pink colony was selected and maintained on the same medium. It was the only representative of this taxon of the more than 200 isolates screened from these soils. At present, no evidence suggests that its isolation from an earthworm burrow has significance. Subsequent studies indicated that spread plates greatly underestimate the viability of strain B33D1, and that pour plates containing 1.5% agar enhance plating efficiency.

The type strain Gsoil 355^T of *Solirubrobacter soli* was isolated from soil of a ginseng field in Daejon, Korea (Kim et al., 2007). Soil suspensions were serially diluted and plated on R2A medium (Difco). After 5 d of incubation at 30°C, colorless colonies were transferred to fresh medium.

Maintenance procedures

The organism has been stored at −70°C in medium containing 15% glycerol.

Taxonomic comments

Upon its isolation, *Solirubrobacter* was affiliated with the 16S rRNA group 2 of the *Rubrobacteridae* subclass of the *Actinobacteria* phylum, as defined by Holmes et al. (2000). Aside from the thermophilic and radiation-resistant species of *Rubrobacter*, group 2 at that time was based entirely on gene sequences obtained directly from soil DNA. Concurrent with the description of *Solirubrobacter*, another mesophile *Conexibacter woesei* isolated from soil and the moderate thermophiles *Thermoleophilum album* and *Thermoleophilum minutum* were also recognized as members of group 2 (Monciardini et al., 2003; Yakimov et al., 2003). Although originally described in the 1980s, the relatedness of *Thermoleophilum* to the *Actinobacteria* was not known until sequencing of its 16S rRNA genes. Subsequently, additional related genera and species were isolated from soil, including *Patulibacter minatonensis*, *Patulibacter americanus*, and *Solirubrobacter soli* (Kim et al., 2007; Reddy and Garcia-Pichel, 2009; Takahashi et al., 2006).

Based upon the low degree of sequence similarity of their 16S rRNA genes and phenotypic differences, the genera *Thermoleophilum*, *Solirubrobacter*, *Conexibacter*, and *Patulibacter* were classified in separate families (Stackebrandt, 2004; Takahashi et al., 2006; Zhi et al., 2009). In recognition of the differences between *Thermoleophilum* and other members of the group, the families were subsequently classified into two orders, *Thermoleophilales* and *Solirubrobacterales* (Reddy and Garcia-Pichel, 2009). More recently, phylogenetic analyses of the 16S rRNA genes failed to provide strong support for the relatedness of these groups to *Rubrobacter* (Ludwig et al., 2012). For that reason, they are currently classified within the class *Thermoleophilia*. Although represented by only a few cultivated bacteria, the large number of genes cloned from mesophilic soils suggests that these are abundant and diverse groups in nature. However, it is very speculative to infer the properties of the uncultivated members at this time.

Differentiation of species of the genus

The descriptions of *Solirubrobacter pauli* and *Solirubrobacter soli* were based upon descriptions of single strains with growth properties measured under somewhat different growth conditions. Therefore, the reliability of the reported differences in phenotype is not certain. With that caveat, *Solirubrobacter pauli* but not *Solirubrobacter soli* utilizes acetate, D-sorbitol, and L-alanine as sole carbon sources. *Solirubrobacter soli* but not *Solirubrobacter pauli* grows in 1% (w/v) NaCl.

List of species in the genus *Solirubrobacter*

1. **Solirubrobacter pauli** Singleton, Furlong, Peacock, White, Coleman and Whitman 2003, 489[VP]

pau'li. N.L. gen. n. *pauli* of *Paulus*; named for the prominent soil microbiologist Eldor Alvin Paul.

Cells are rods (ca. 1.4 µm × 0.7 µm). Much longer cells are occasionally observed in fresh cultures, and older cultures generally have uniformly shorter rods. In liquid culture, cells often grow in long chains that wrap around each other to produce large aggregates. A capsule is not present. Colonies grown on Difco Nutrient Broth agar plates are round, convex, with entire edges, and usually pink in color. Catalase-positive and oxidase-negative. Casein and Tween 80 are not hydrolyzed. No hemolysis is observed on blood agar. In minimal medium, a variety of sugars serve as sole carbon sources including fructose, galactose, glucose, lactose, mannose, sorbitol, sucrose, and xylose. Cellobiose and mannitol do not support growth. Among the organic acids, pyruvate supports good growth. Acetate supports weak growth. Citrate, malate, and succinate do not support growth. Casamino acids provide good growth. When tested individually, only the amino acids alanine, arginine, and lysine support growth. Glycerol is the only alcohol tested that supports growth. Alcohols that do not support growth are methanol, ethanol, 1-propanol, 2-propanol, butanol, isobutyl alcohol, and iso-amyl alcohol. Of the aromatic compounds tested, only chlorogenic acid supports growth. Anthranilic acid, benzoic acid, catechol, protocatechuic acid, *p*-coumaric acid, gentisic acid, ferulic acid, *p*-hydroxybenzoic acid, syringic acid, and vanillic acid do not produce observable growth after 2 weeks. Ammonium will serve as a sole nitrogen source, and Casamino acids will serve as a sole carbon and nitrogen source. Nitrate and urea are not nitrogen sources. Optimal growth at 28–30°C and pH 6.0–6.5. The temperature and pH ranges for growth are 19–38°C and 6–7.5. It fails to grow anaerobically and requires at least 1% (v/v) air for growth. No growth is observed in the presence of 1% NaCl. Sensitive to polymyxin, ampicillin, tetracycline, and streptomycin.

DNA G+C content (mol%): 71.8 (Lc).

Type strain: B33D1, ATCC BAA-492, DSM 14954, JCM 13025.

Sequence accession no. (16S rRNA gene): AY039806.

2. **Solirubrobacter soli** Kim, Na, Lee, Im, Soung and Yang 2007, 1454[VP]

so'li. L. neut. gen. n. *soli* of the soil, the source of the type strain.

Cells are short rods, 1–3 µm in length. Colonies are not pigmented following growth at 30°C on R2A agar. Weakly positive for oxidase. Gelatin is hydrolyzed. Sugars serve as sole carbon sources, including L-arabinose, L-fucose, D-glucose, D-maltose, D-melibiose, L-rhamnose, D-ribose, sucrose, and glycogen. Other utilized compounds include adipate, gluconate, *myo*-inositol, L-proline, *N*-acetyl-D-glucosamine, and salicin. Compounds that do not support growth as sole carbon source include 2-ketogluconate, 3-hydroxybenzoate, 4-hydroxybenzoate, 3-hydroxybutyrate, 5-ketogluconate, acetate, caprate, citrate, itaconate, lactate, L-malate, malonate, phenylacetate, propionate, suberate, *N*-valerate, D-mannitol, D-sorbitol, L-alanine, and L-histidine. In the API test systems, the following reactions were positive: *N*-acetyl-β-glucosaminidase, acid phosphatase, alkaline phosphatase, cystine arylamidase, esterase (C4), esterase (C8), α-glucosidase, β-glucosidase, β-galactosidase, leucine arylamidase, and valine arylamidase. Nitrate is not reduced to nitrite or N_2. Optimal growth at 30°C, slow growth at 15°C, and no growth at 37°C. Growth is observed in 0–1.5% (w/v) NaCl.

DNA G+C content (mol%): 71.5 (Lc).

Type strain: Gsoil 355, JCM 14923, KCTC 12628, LMG 23485.

Sequence accession no. (16S rRNA gene): AB245334.

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Family II. **Conexibacteraceae** Stackebrandt 2005, 547^{VP} (Effective publication: Stackebrandt 2004, 457.) emend. Zhi, Li and Stackebrandt 2009, 594

Peter Schumann

Co.ne.xi.bac.te.ra.ce′a.e. N.L. masc. n. *Conexibacter* type genus of the family; suff. -*aceae* ending to denote a family; N.L. fem. pl. n. *Conexibacteraceae* the *Conexibacter* family.

The pattern of 16S rRNA gene sequence signatures consists of nucleotides at positions: 144:178 (U–A), 145:177 (U–A), 293:304 (G–C), 377:386 (C–G), 408:434 (A–U), 418:425 (U–A), 590:649 (U–A), and 600:638 (U–G) (Zhi et al., 2009). The family *Conexibacteraceae* is a member of the order *Solirubrobacterales* Reddy and Garcia-Pichel (2009).

Type genus: **Conexibacter** Monciardini, Cavaletti, Schumann, Rohde and Donadio 2003, 574^{VP}.

Taxonomic comments

The family *Conexibacteraceae* Stackebrandt 2005 contains the genus *Conexibacter* Monciardini et al., 2003 and belongs, together with the families *Solirubrobacteraceae* Stackebrandt 2005 emend. Zhi, Li and Stackebrandt 2009 and *Patulibacteraceae* Takahashi, Matsumoto, Morisaki and Ōmura 2006 emend. Zhi, Li and Stackebrandt 2009, to the order *Solirubrobacterales* Reddy and Garcia-Pichel 2009 (Reddy and Garcia-Pichel, 2009; Stackebrandt, 2004; Takahashi et al., 2006; Zhi et al., 2009). The members of the order *Solirubrobacterales* represent a separate cluster in the phylogenetic tree (http://www.arb-silva.de/projects/living-tree/) and are coherent with respect to the set of 16S rRNA gene signature nucleotides, the positive Gram-staining, the occurrence of *meso*-diaminopimelic acid in the peptidoglycan and seven isoprenoid units in the side chain of the respiratory quinones as well as to the psychrotolerant to mesophilic growth (Reddy and Garcia-Pichel, 2009). The affiliation of the family *Conexibacteraceae* to the order *Rubrobacterales* Rainey et al. 1997 emend. Zhi, Li and Stackebrandt 2009 as proposed by Stackebrandt et al. (1997) and Zhi et al. (2009) is neither supported by the topology of the phylogenetic tree (http://www.arb-silva.de/projects/living-tree/) nor by phenotypic characteristics. The families *Conexibacteraceae*, *Rubrobacteraceae*, *Solirubrobacteraceae*, *Thermoleophilaceae*, and *Patulibacteraceae* contain only few culturable strains and the family *Conexibacteraceae* is based on a single strain only. However, many 16S rRNA gene sequences of clones from soils of different regions of the world (e.g. Rheims et al., 1996; Rheims and Stackebrandt, 1999; Yakimov et al., 2003) and of unidentified soil isolates (Joseph et al., 2003; Sangwan et al., 2005) show high similarities to cultured organisms of this group (see Figure 427). It is expected that additional strains will be isolated after elaboration of appropriate cultivation techniques, which will give a better insight into the phenotypic diversity of members of the orders *Rubrobacterales*, *Thermoleophilales*, and *Solirubrobacterales*.

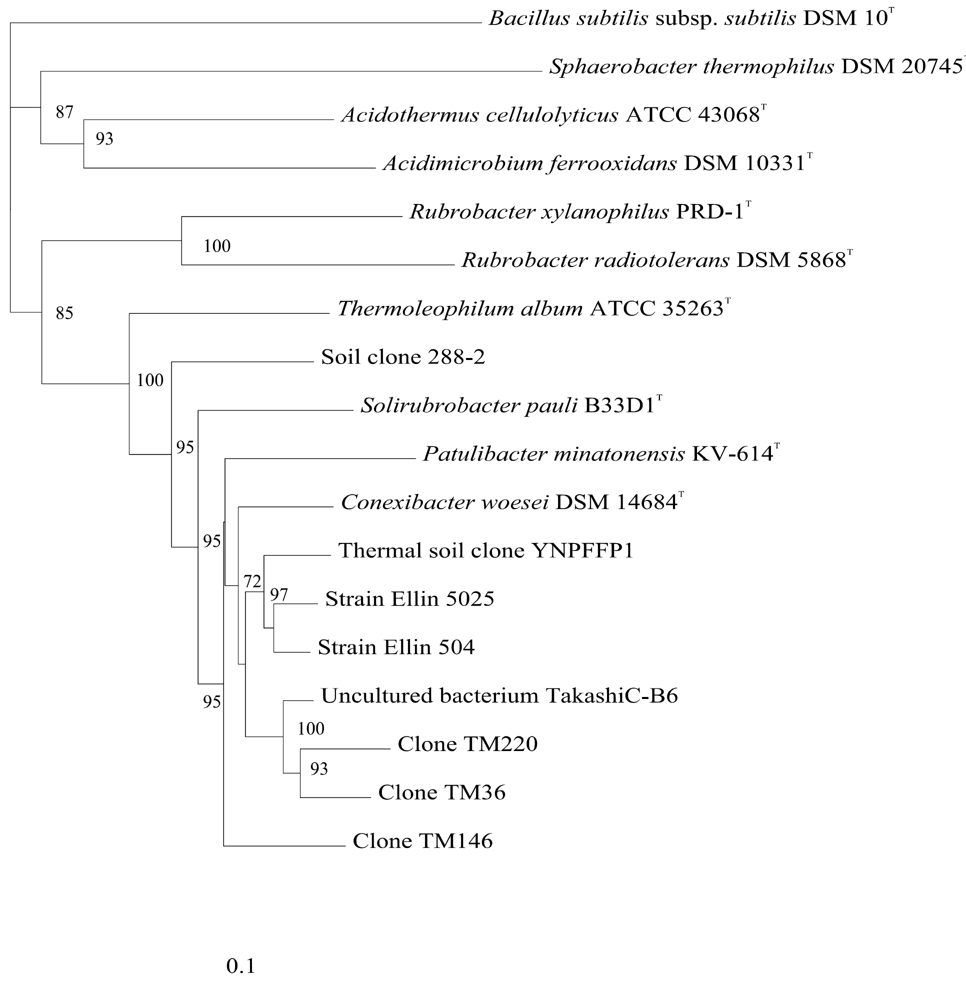

FIGURE 427. 16S rRNA gene sequence neighbor-joining tree (Felsenstein, 1993) of *Conexibacter woesei* DSM 14684^T among representatives of the class *Rubrobacteria*, including species with validly published names, unidentified strains, and clones obtained from environmental DNA. Bootstrap values (>70%) of 1000 resamplings (Felsenstein, 1985) are indicated at nodes. *Bacillus subtilis* subsp. *subtilis* DSM 10^T served as root. Bar = 10 nt substitution per 100 nt.

Genus I. **Conexibacter** Monciardini, Cavaletti, Schumann, Rohde and Donadio 2003, 574^{VP}

Peter Schumann

Co.nex.i.bac′ter. L. part. adj. *conexus* bound, tied; N.L. masc. n. *bacter* rod; N.L. masc. n. *Conexibacter* a rod that is bound.

Cells are small rods (0.6–0.7 × 0.9–1.2 µm), occurring singly or in pairs. **Gram-stain-positive** and **non-sporulating**. **Motile by long, peritrichous flagella.** Aerobic. **Catalase- and oxidase-positive**. The **peptidoglycan** is of the **A1γ type** (based on ***meso*-diaminopimelic acid**, direct cross-linkage). Mycolic acids are absent. The major isoprenoid quinone is menaquinone **MK-7(H_4)**. The **polar lipid** pattern consists of **phosphatidylinositol** and an unidentified phospholipid; amino-functional lipids and glycolipids are absent. The **fatty acid profile** is dominated by **oleic, 14-methyl-pentadecanoic, hexadecanoic, and ω6*c*-heptadecenoic acids**. Phylogenetically, the genus is a member of the family *Conexibacteraceae* Stackebrandt 2005 emend. Zhi, Li and Stackebrandt 2009, order *Solirubrobacterales* Reddy and Garcia-Pichel 2009.

DNA G+C content (mol%): 71 (HPLC).

Type species: **Conexibacter woesei** Monciardini, Cavaletti, Schumann, Rohde and Donadio 2003, 574^{VP}.

Further descriptive information

The genus *Conexibacter* was established to accommodate an isolate from forest soil that showed only remote 16S rRNA gene sequence similarity to validly named taxa (Monciardini et al.,

2003). *Conexibacter woesei* shows the 16S rRNA signature nucleotide A at position 906, but displays U instead of A or C at position 955, a substitution that is typical of members of the sublasses *Rubrobacteridae* and *Sphaerobacteridae* (Reddy and Garcia-Pichel, 2009; Stackebrandt et al., 1997).

An insertion of approximately 100 nt within domain III of the 23S rRNA genes found in members of the subclass *Actinobacteridae* but absent in *Atopobium minutum* (Embley and Stackebrandt, 1994) was also not detected in *Conexibacter woesei* strain ID131577^T, suggesting that the deep-branching lineages of the class *Actinobacteria* might differ in this feature from members of the subclass *Actinobacteridae* (Monciardini et al., 2003).

The closest phylogenetic neighbors of *Conexibacter woesei* ID131577^T with validly published names are *Solirubrobacter soli* Gsoil 355^T (Kim et al., 2007), *Patulibacter americanus* (Reddy and Garcia-Pichel, 2009), *Patulibacter minatonensis* JCM 12834^T (Takahashi et al., 2006) and *Solirubrobacter pauli* ATCC BAA-492^T (Singleton et al., 2003) with 94.3, 93.5, 93.5, and 93.3% 16S rRNA gene sequence similarities, respectively (according to a search using the EzTaxon server; Chun et al., 2007). Only moderate 16S rRNA gene sequence similarity values are shown between *Conexibacter woesei* ID131577^T and strains of other members of the subclass *Rubrobacteridae* Rainey et al. 1997 emend. Stackebrandt 2004 (Stackebrandt, 2004; Stackebrandt et al., 1997) (see Figure 427), e.g. 90.2 and 90.1% similarity, respectively, with *Thermoleophilum minutum* and *Thermoleophilum album* (Zarilla and Perry, 1984, 1986), and 84.5% with *Rubrobacter xylanophilus* (Carreto et al., 1996). The 16S rRNA gene sequences of the isolate and its phylogenetic neighbors *Solirubrobacter soli*, *Solirubrobacter pauli, Patulibacter americanus*, and *Patulibacter minatonensis* fell into a cluster consisting of clones of hitherto-uncultured bacteria obviously occurring worldwide in different soils (Rheims et al., 1996; Rheims and Stackebrandt, 1999; Yakimov et al., 2003) and strains of the subclass *Rubrobacteridae* isolated from pasture soil (Ellinbank, Victoria, Australia) by cultivation on modified VL55 medium (Sait et al., 2002) and incubation at 25°C for up to 3 months (Joseph et al., 2003; Sangwan et al., 2005). The highest binary similarity value (95.8%) was found between *Conexibacter woesei* ID131577^T and strain Ellin 504 (Sangwan et al., 2005).

The isolation and cultivation of representatives of this phylogenetic cluster offered the opportunity to gain an insight into the phenotypic characteristics of these organisms. Colonies of strain ID131577^T are smooth, mucoid, and of whitish-creamy color on Todd–Hewitt agar. They are sticky and difficult to disintegrate. Cells of *Conexibacter woesei* ID131577^T are short, Gram-stain-positive rods (0.6–0.7 × 0.9–1.2 µm), occurring singly or in pairs. Cells are motile by long peritrichous flagella (Figure 428). After approximately 1 week of cultivation in liquid media, rigid nonmotile spiral bodies are observed by phase-contrast microscopy (shown by Monciardini et al., 2003). Electron microscopic investigation revealed that these bodies were formed by self-aggregation of flagella (Figure 428) in a way that cells may become entangled in flagellar networks (Figure 428). Discarded flagella give rise to the formation of undulating or spiral bodies with a regular periodicity of approximately 2–3 µm (Figure 428). This aggregation of cells via a flagellar network appears to be a unique feature of *Conexibacter woesei*.

Strain ID131577^T shows peptidoglycan of type A1γ based on directly cross-linked *meso*-diaminopimelic acid, whereas mycolic acids are absent. A unique feature of strain ID131577^T is the occurrence of the tetrahydrogenated menaquinone MK-7(H_4), which has not been found as a single respiratory quinone in other bacteria before. Phosphatidylinositol and an additional unknown phospholipid are components of the polar lipid pattern, but not amino-functional lipids or glycolipids. The fatty acid profile of strain ID131577^T is composed of $C_{18:1}$ ω9*c* (41.4%), $C_{16:0}$ iso (16.3%), $C_{17:1}$ ω6*c* (13.9%), $C_{16:0}$ (12.7%), $C_{16:1}$ ω7*c* (1.9%), $C_{18:0}$ (1.6%), $C_{17:1}$ ω8*c* (1.5%), $C_{19:1}$ ω6*c* (1.5%) and $C_{17:0}$ iso (1.2%) (fatty acids representing less than 1% of the total fatty acids are not reported). The DNA G+C content of the type strain of *Conexibacter woesei* is 71 mol%.

Strain ID131577^T grows on brain-heart infusion (BHI; Difco), trypticase soy-yeast extract medium (medium no. 92, www.dsmz.de), and Todd–Hewitt agar at 28–37°C and pH 7–7.5 under aerobic conditions. No growth occurs under anaerobic conditions or at NaCl concentrations higher than 2% (w/v). Physiological features and susceptibility to antibiotics are given in the species description.

Isolation and maintenance procedures

Strain ID131577^T was detected as a contaminant during the isolation of filamentous actinomycetes from a soil sample of a wooded area in Gerenzano, Italy. Soil was plated on half-strength HV medium (Hayakawa and Nonomura, 1987) following dilution in water. Colonies were serially transferred on new half-strength HV medium, ISP3 medium (Shirling and Gottlieb, 1966), and Todd–Hewitt medium (Difco) until pure colonies were obtained.

Serial transfers at 4-week intervals followed by maintenance at 4°C and storage of cells as 20% (w/v) glycerol suspensions at −20°C and at −80°C were suitable provisions for preservation. Long-term preservation methods are freeze-drying in skim milk and maintenance in liquid nitrogen at −196°C.

Differentiation of the genus *Conexibacter* from related genera

Signature nucleotides that differentiate the genus *Conexibacter* from members of the families *Patulibacteraceae* and *Solirubrobacteraceae* are the following: 52:359 (U–A), 144:178 (U–A), 408:434 (A–U) and 999:1041 (G–U) (Reddy and Garcia-Pichel, 2009). The genus *Conexibacter* can be differentiated from other culturable members of the classes *Thermoleophilia* and *Rubrobacteridae* by 16S rRNA gene sequence similarity values of <95%. The phenotypic characteristics of *Conexibacter woesei* ID131577^T and its closest phylogenetic relatives *Solirubrobacter pauli* B33D1^T, *Solirubrobacter soli* Gsoil 355^T, *Patulibacter americanus* CP177-2^T and *Patulibacter minatonensis* KV-614^T are compiled for comparison in Table 320.

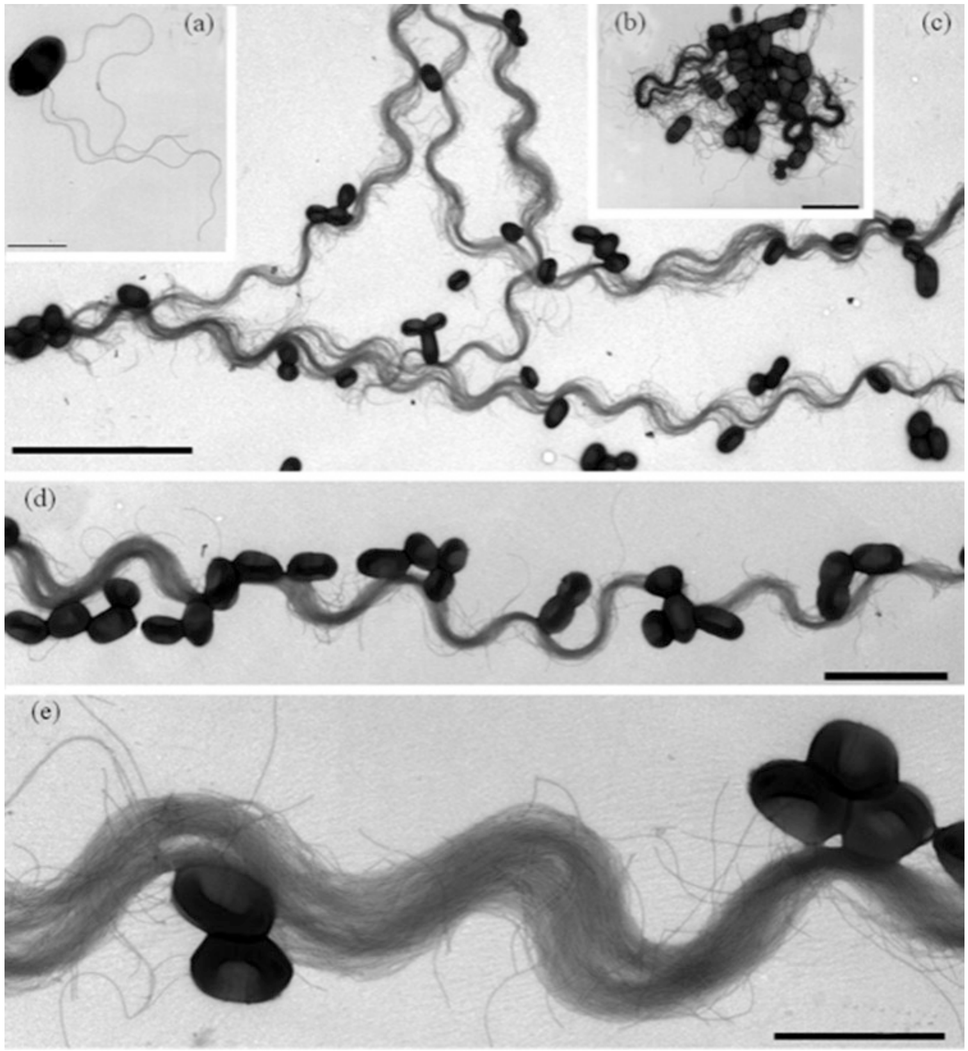

FIGURE 428. Electron micrographs of negatively stained cells of strain ID131577^{T}. Individual bacteria exhibit peritrichous long flagella (a). After a longer period of growth, bacteria form aggregates (b) and, due to self-aggregation of flagella, they exhibit an undulating network-like structure with entangled bacteria (c–e). Bars: (a, e), 1 μm; (b, d), 3 μm; (c) 5 μm. (Reprinted with permission from Monciardini et al., 2003. Int. J. Syst. Evol. Microbiol. *53*: 569–576.)

List of species of the genus *Conexibacter*

1. **Conexibacter woesei** Monciardini, Cavaletti, Schumann, Rohde and Donadio 2003, 574^{VP}

woe′se.i. N.L. gen. masc. n. *woesei* of Woese, named to honor Carl R. Woese for his pioneering work on the use of 16S rRNA in phylogenetic analysis.

In addition to the properties described for the genus, colonies on Todd–Hewitt agar are smooth, mucoid to sticky, and of white to cream color. Nitrate is reduced to nitrite. Gelatin and esculin are hydrolyzed. Urea is not decomposed. NaCl is not tolerated at concentrations of 2% (w/v) or above. The type strain is able to utilize the following substrates: glycerol, L-arabinose, D-ribose, D-xylose, acetic acid, α-ketovaleric acid, propionic acid, pyruvic acid (Biolog GP microplate), methylpyruvate, β-hydroxybutyric acid, α-ketoglutaric acid, and α-ketovaleric acid (Biolog GN microplate). The type strain shows the following enzyme activities: esterase for 2-naphthyl caprylate and 2-naphthyl butyrate, leucine arylamidase, acid phosphatase, and naphthol-AS-BI-phosphohydrolase (API ZYM test). The following tests of the Biolog substrate panels, API 20NE gallery, and API ZYM enzyme assay are negative: mannan, α-cyclodextrin, dextrin, glycogen, Tween 40, Tween 80, *N*-acetyl-D-galactosamine, *N*-acetyl-D-glucosamine, adonitol, D-arabitol, cellobiose, iso-erythritol, D-fructose, L-fucose, D-galactose, gentiobiose, α-D-glucose, *myo*-inositol, α-lactose, α-D-lactose, lactulose, maltose, D-mannitol, D-mannose, melibiose, methyl β-D-glucoside, raffinose, L-rhamnose, D-sorbitol, sucrose, trehalose, turanose, xylitol, monomethyl succinate, *cis*-aconitic acid, citric acid, formic acid, D-galactonic acid lactone, D-galacturonic acid, D-gluconic acid, D-glucosaminic acid, D-glucuronic acid, α-hydroxybutyric acid, γ-hydroxybutyric acid, *p*-hydroxyphenylacetic acid, itaconic acid, α-ketobutyric acid, DL-lactic acid, malonic acid, quinic

TABLE 320. Differential phenotypic properties of *Conexibacter woesei* DSM 14684[T], *Patulibacter minatonensis* KV-614[T], *Patulibacter americanus* CP177-2[T], *Solirubrobacter pauli* DSM 14954[T] and *Solirubrobacter soli* Gsoil 355[Ta]

Characteristic	*C. woesei* DSM 14684[T]	*P. minatonensis* KV-614[T]	*P. americanus* CP177-2[T]	*S. pauli* DSM 14954[T]	*S. soli* Gsoil 355[T]
Cell size (μm)	0.6–0.7 × 0.9–1.2	0.6–0.7 × 1.2–1.5	nd	0.7 × 1.4	1.0-3.0 (length)
Cell arrangement	Singly or in pairs	Singly	Singly	Long chains	Singly
Motility	Motile by long peritrichous flagella	Motile by long flagella	Variable (jerking)	None	None
Optimal growth temperature (°C)	28–37	24–27	25	28–30	30
pH range	7.0–7.5	6–8	5–9, optimal 7	6.0–7.7, optimal 6.5	nd
Oxidase	Positive	Negative	Negative	Negative	Weakly positive
Substrates utilized	Glycerol, C_2–C_5 acids, pentoses	Broad range of sugars, alcohols, acids and few amino acids	Few sugars, arginine	Common sugars, few amino acids	Common sugars
Major fatty acids (>10%)	$C_{18:1}$ ω9*c*, $C_{16:0}$ iso, $C_{17:1}$ ω6*c*, $C_{16:0}$	$C_{18:1}$ ω9*c*, $C_{15:0}$ anteiso	$C_{18:1}$ ω9*c*, $C_{15:0}$ anteiso	$C_{16:0}$ iso, $C_{18:1}$ ω9*c*	$C_{16:0}$ iso, $C_{18:1}$ ω9*c*
Diamino acid of the peptidoglycan	*meso*-A_2pm	*meso*-A_2pm	*meso*-A_2pm	nd	nd
Isoprenoid quinone	MK-7(H_4)	DMK-7	MK-7(H_2)	nd	MK-7(H_4)
DNA G+C content (mol%)	71	72	72	71.8	71.5
Isolation site	Temperate forest soil	Soil	Biological soil crusts	Burrow of earthworm	Soil

[a]All strains are Gram-stain-positive, aerobic, catalase-positive and do not form endospores. Data from Monciardini et al. (2003), Takahashi et al. (2006), Reddy and Garcia-Pichel (2009), Singleton et al. (2003) and Kim et al. (2007). A_2pm, 2,6-Diaminopimelic acid; nd, not determined.

acid, D-saccharic acid, sebacic acid, succinic acid, bromosuccinic acid, succinamic acid, glucuronamide, alaninamide, D-alanine, L-alanine, L-alanyl glycine, L-asparagine, L-aspartic acid, L-glutamic acid, glycyl L-aspartic acid, glycyl L-glutamic acid, L-histidine, hydroxy-L-proline, L-leucine, L-ornithine, L-phenylalanine, L-proline, L-pyroglutamic acid, D-serine, L-serine, L-threonine, DL-carnitine, γ-aminobutyric acid, urocanic acid, inosine, uridine, thymidine, phenylethylamine, putrescine, 2-aminoethanol, 2,3-butanediol, DL-α-glycerol phosphate, glucose 1-phosphate, glucose 6-phosphate, β-cyclodextrin, inulin, *N*-acetylmannosamine, amygdalin, arbutin, lactulose, maltotriose, melezitose, methyl α-D-galactoside, 3-methyl glucose, methyl α-D-glucoside, methyl α-D-mannoside, palatinose, salicin, sedoheptulosan, stachyose, D-tagatose, lactamide, D-lactic acid methyl ester, L-lactic acid, D-malic acid, L-malic acid, methyl succinate, *N*-acetyl-L-glutamic acid, adenosine, 2′-deoxyadenosine, AMP, TMP, UMP, fructose 6-phosphate, caprate, adipate, phenylacetate, degradation of tryptophan, fermentation of glucose, arginine dihydrolase, urease, alkaline phosphatase, lipase, valine arylamidase, cystine arylamidase, trypsin, chymotrypsin, α-galactosidase, β-galactosidase, β-glucuronidase, α-glucosidase, β-glucosidase, *N*-acetyl-β-glucosaminidase, α-mannosidase, and α-fucosidase. Good growth occurs at pH 7.0–7.5 and at 28–37°C. The type strain is susceptible to amikacin (30 μg), gentamicin (10 μg), nitrofurantoin (300 μg), novobiocin (30 μg), polymyxin B (300 IU), and teicoplanin (30 μg) and only weakly susceptible to chloramphenicol (30 μg), erythromycin (15 μg), tetracycline (30 μg), and vancomycin (30 μg). Cells are resistant to ampicillin (10 μg), aztreonam (100 μg), ceftazidime (30 μg), ciprofloxacin (5 μg), clindamycin (2 μg), kanamycin (30 μg), methicillin (5 μg), norfloxacin (10 μg), oxacillin (1 μg), rifampin (30 μg), streptomycin (10 μg), trimethoprim (5 μg), and tobramycin (10 μg).

Source: temperate forest soil.

DNA G+C content (mol%): 71 (HPLC).

Type strain: ID131577, DSM 14684, JCM 11494.

Sequence accession no. (16S rRNA gene): AJ440237.

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Family III. **Patulibacteraceae** Takahashi, Matsumoto, Morisaki and Ōmura 2006, 405^VP

Yōko Takahashi

Pa.tu.li.bac.te.ra.ce′a.e. N.L. masc. n. *Patulibacter* type genus of the family; suff. -*aceae* ending to denote a family; N.L. fem. pl. n. *Patulibacteraceae* the *Patulibacter* family.

Cells are rods, Gram-stain-positive, non-endospore-forming. Cell-wall peptidoglycan is based upon *meso*-diaminopimelic acid. The main isoprenoid quinone is demethylmenaquinone. Mycolic acids are absent. The signature nucleotides in the 16S rRNA gene sequence (*Escherichia coli* sequence; Brosius et al., 1978) of members of the family are: 52:359 (C–G), 98 (A), 139 (G), 144:178 (C–G), 370:391 (C–G), 580:761 (U–A), 590:649 (U–A), 600:638 (U–G), 670:736 (A–U), 953:1228 (G–C), 954:1226 (G–C), 999:1041 (U–A), 657:749 (U–A), 681:709 (U–A), 941:1342 (A–U), 1051:1207 (G–C), and 1311:1326 (A–U). Phylogenetically, the family is a member of the phylum *Actinobacteria*, class *Thermoleophilia*, order *Solirubrobacterales*.

DNA G+C content (mol%): 72.0 (HPLC).

Type genus: **Patulibacter** Takahashi, Matsumoto, Morisaki and Ōmura 2006, 405^VP emend. Reddy and Garcia-Pichel 2009, 91.

Further descriptive information

In the present volume, the order *Solirubrobacterales* Reddy and Garcia-Pichel 2009 contains three families, *Conexibacteraceae*, *Solirubrobacteraceae*, and *Patulibacteraceae* (Takahashi et al., 2006). The family *Patulibacteraceae* can be distinguished within the order *Solirubrobacterales* on the basis of 16S rRNA signature nucleotides (Stackebrandt et al., 1997; Reddy and Garcia-Pichel, 2009).

The family *Patulibacteraceae* comprises the sole genus *Patulibacter*.

Genus I. **Patulibacter** Takahashi, Matsumoto, Morisaki and Ōmura 2006, 405^VP emend. Reddy and Garcia-Pichel 2009, 91

Yōko Takahashi

Pa.tu.li.bac′ter. L. adj. *patulus* spreading; N.L. masc. n. *bacter* from Gr. n. *baktron* a rod; N.L. masc. n. *Patulibacter*, spreading growth-bacterium.

Cells are rods, Gram-stain-positive, and non-endospore-forming. Bacterial respiration is aerobic. Cell-wall peptidoglycan contains ***meso*-diaminopimelic acid (DAP)**, alanine, and glutamic acid. Cell-wall type A1γ (based on *meso*-DAP, direct cross-linkage). The acyl type of the peptidoglycan is acetyl. The fatty acid profile is dominated by **$C_{18:1}$ ω9*c***, $C_{15:0}$ anteiso, and $C_{17:0}$ anteiso. Mycolic acids are absent. The predominant isoprenoid quinone is **demethylmenaquinone with seven isoprene units (DMK-7).**

DNA G+C content (mol%): 72.0 (HPLC).

Type species: **Patulibacter minatonensis** Takahashi, Matsumoto, Morisaki and Ōmura 2006, 405^VP.

Further descriptive information

Patulibacter minatonensis forms flat and nearly transparent colonies with a white color on one-fifth strength nutrient agar (1/5 NA, Difco), ISP 3 medium (Shirling and Gottlieb, 1966), and R2A agar (Difco) consisting of 0.05% yeast extract, 0.05% proteose peptone, 0.05% Casamino acids, 0.05% glucose, 0.05% soluble starch, 0.03% sodium pyruvate, 0.03% dipotassium phosphate, 0.005% magnesium sulfate, and 1.5% agar, and with a pale yellow color on heart infusion agar and Todd–Hewitt agar (Difco) consisting of 0.9% beef heart digest, 1.1% pancreatic digest of casein, 0.3% soy bean peptone, 0.2% glucose, 0.25% sodium carbonate, 0.05% monosodium phosphate, 0.2% sodium chloride, and 1.5% agar. Bacterial cells are rod-shaped (1.2–1.5 × 0.6–0.7 μm), Gram-stain-positive, show negative oxidase reaction and reduce nitrate to nitrite. Cells possess motility due to the presence of long flagella (Figure 429). The strain does not grow on media containing ≥2% (w/v) NaCl. The pH range for growth is 6–8. The temperature range and optimum temperature for growth is 16–28°C and 24–27°C, respectively.

Enrichment and isolation procedures

Patulibacter minatonensis KV-614 was isolated from a soil sample using an agar medium supplemented with superoxide dismutase (SOD) following the method of Takahashi et al. (2003). Glucose-peptone-meat extract agar (GPM agar) medium consisting of 1% D-glucose (Wako Pure Chemical Industry), 0.5% peptone (Kyokuto Seiyaku Co.), 0.5% meat extract (Kyokuto), 0.3% NaCl and 1.2% agar (Wako) was used for isolation. Solid GPM agar medium supplemented with SOD (Sigma) was prepared by spreading SOD (300 U/plate) on the surface. Then 100-μl samples of soil suspensions diluted with sterilized water were spread on dishes of these solid media, which were incubated at 27°C for 14 d. The strain was cultured on 1/5 NA, ISP 3 medium, heart infusion agar, R2A agar, Todd–Hewitt agar (Difco), and yeast extract-glucose agar medium containing 1.0% yeast extract (Difco), 1.0% glucose, and 1.2% agar.

The appropriate cultivation conditions have not been found for many unknown micro-organisms. While searching for factors that promote bacterial colony growth, it was found that using isolation agar medium supplemented with SOD increases the number of colonies from a soil sample and that addition of SOD and catalase increases it still further (Takahashi et al., 2003).

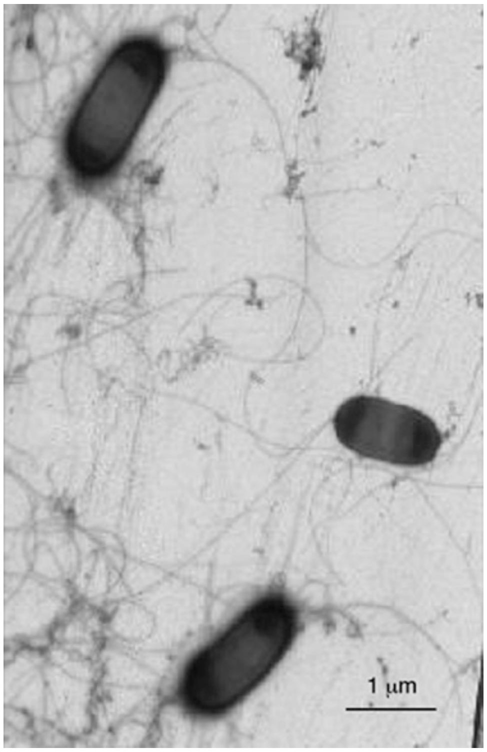

FIGURE 429. Transmission electron micrograph of negatively stained cells with long flagella of *Patulibacter minatonensis* KV-614. (Reprinted with permission from Takahashi et al., 2006. Int. J. Syst. Evol. Microbiol. *56*: 401–406.)

Maintenance procedures

Cultures can be maintained in the laboratory by transfer to the same media used for isolation and stored for a short period. One-fifth strength nutrient agar medium is recommended for maintenance. Recommended conditions for long-term preservation are storage at −80°C with 20% glycerol or lyophilization in the presence of 10% skim milk as stabilizer. The lyophilized ampoules should be stored at 5°C.

Differentiation of the genus *Patulibacter* from other genera

Figure 430 shows a phylogenetic tree constructed by the neighbor-joining method on the basis of 16S rRNA gene sequences of *Patulibacter minatonensis* KV-614 and closely related taxa. *Patulibacter* forms a distinct branch within the order *Rubrobacterales* (Stackebrandt et al., 1997), and is associated with the genera *Conexibacter* (Monciardini et al., 2003) and *Solirubrobacter* (Singleton et al., 2003). The 16S rRNA gene sequence similarity of the type strain KV-614 to *Conexibacter woesei* DSM 14684 is 92.4% and to *Solirubrobacter pauli* B33D1 is 89.7%. The genus *Conexibacter* has one species. The new species of *Solirubrobacter*, *Solirubrobacter soli* Gsoil 355, reported in 2007, also has a low 16S rRNA gene sequence similarity (91.8%) to the type strain (Kim et al., 2007).

Table 320 shows the phenotypic characteristics of *Patulibacter minatonensis* and two related genera, *Conexibacter* and *Solirubrobacter*. *Conexibacter woesei* also contains *meso*-DAP in the cell wall peptidoglycan and produces long flagella, but clearly differs from *Patulibacter minatonensis* in the major menaquinone MK-7(H_4) and predominant fatty acids. *Solirubrobacter pauli* and *Solirubrobacter soli* are also different from *Patulibacter minatonensis* by being non-motile cells and having different fatty acid composition.

Miscellaneous comments

The signature nucleotides in the 16S rRNA gene sequence of members of the subclass *Rubrobacteridae* (Stackebrandt, 2004), which has been elevated to class *Rubrobacteria*, 127:234 (G–C), 291:309 (U–A), 955:1225 (U–A), 1115:1185 (C–G), and 1410:1490 (A–U) (*Escherichia coli* sequence; Brosius et al., 1978) is also present in *Patulibacter*. An A residue at position 906 and a U residue at position 955 are characteristics of members of the class *Rubrobacteridae* (Stackebrandt et al., 1997).

Figure 431 shows a phylogenetic tree constructed with 16S rRNA gene sequences of strain *Patulibacter minatonensis* KV-614 and those of its closest clonal and cultured relatives retrieved from the GenBank database. Clones prefixed "TM" were isolated from a peat bog (Rheims et al., 1999, 1996), and strains prefixed "Ellin" were isolated from Australian soils by culture on simple solid media at 25°C for up to 3 months (Joseph et al., 2003; Sait et al., 2002). The sequences of clones YNPFFP1 and 1959-7 are available from public databases. The closest sequence is that of the bacterium Ellin 5025 (93.2%). This reveals that the strain is closely related to a phylogenetic cluster consisting of uncultured bacteria from terrestrial environments and unidentified bacterial strains.

DMK-7 detected in *Patulibacter minatonensis* KV-614 is a unique isoprenoid quinone and has been known to occur in *Enterococcus faecalis* (Hiraishi, 1988), *Pasteurella*, and *Haemophilus* (Kroppenstedt and Mannheim, 1989). Demethylmenaquinones hitherto have been detected in two genera *Patulibacter* and *Demequina* (Yi et al., 2007) in the class *Actinobacteria*.

Since this chapter was prepared, a second species, *Patulibacter americanus*, was published (Reddy and Garcia-Pichel, 2009); see Table 320 in *Conexibacteraceae*, above, for distinguishing characteristics.

Acknowledgements

The author thanks Atsuko Matsumoto for help with phylogenetic tree construction, and also Ken-ichiro Suzuki for reading the manuscript.

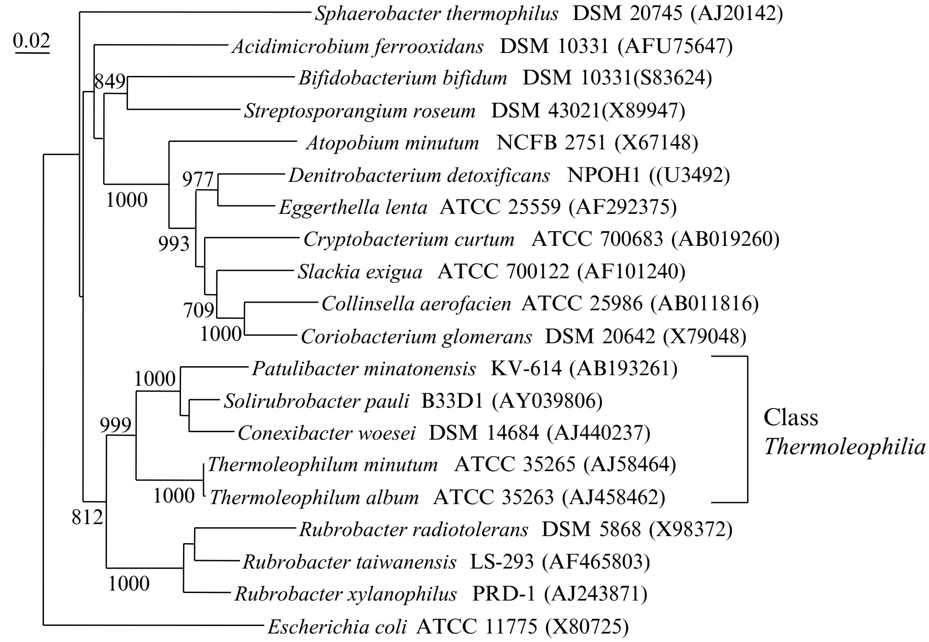

FIGURE 430. Phylogenetic tree showing the position of *Patulibacter* and the other members of the order *Rubrobacterales* based on 16S rRNA gene sequences. Numbers at nodes indicate the level of bootstrap support based on neighbor-joining analysis of 1000 resampled datasets. Only values higher than 700 are shown. Bar = 2 inferred nucleotide substitutions per 100 nt. The tree was rooted with *Escherichia coli.*

List of species of the genus *Patulibacter*

1. **Patulibacter minatonensis** Takahashi, Matsumoto, Morisaki and Ōmura 2006, 405[VP]

mi.na.to.nen′sis. N.L. masc. adj. *minatonensis* pertaining to Minato-ku, the ward of Tokyo, Japan, where the species originated.

Patulibacter minatonensis forms flat and nearly transparent colonies with whitish or pale yellow color. Cells are 1.2–1.5 × 0.6–0.7 μm in size, motile by long flagella, aerobic, Gram positive, and non-endospore-forming, catalase-positive, and oxidase-negative. Cell-wall peptidoglycan contains *meso*-DAP, alanine, and glutamic acid. The acyl type is acetyl. Mycolic acids are absent. The predominant isoprenoid quinone is DMK-7. Cellular fatty acid components are ω9c octadecenoic acid (oleic acid, $C_{18:1}$ ω9*c*, 63%), 12-methyl tetradecanoic acid ($C_{15:0}$ anteiso, 10%), 14-methyl hexadecanoic acid ($C_{17:0}$ anteiso, 8%), hexadecanoic acid ($C_{16:0}$, 5%), and octadecanoic acid ($C_{18:0}$, 4%). The strain does not grow on media containing 2% (w/v) NaCl. The pH range for growth is 6–8. The temperature range for growth is 16–28°C.

The type strain is able to utilize the following substrates: dextrin, inulin, mannan, *N*-acetyl-D-mannosamine, L-arabinose, D-arabitol, cellobiose, D-fructose, L-fucose, D-galactose, D-galacturonic acid, α-D-glucose, *m*-inositol, maltose, maltotriose, D-mannitol, D-mannose, D-melezitose, α-methyl-D-galactoside, β-methyl-D-glucoside, α-methyl-D-glucoside, α-methyl-D-mannoside, D-psicose, L-rhamnose, salicin, sedoheptulosan, turanose, α-hydroxybutyric acid, lactamide, D-lactic acid methyl ester, L-lactic acid, L-serine, 2,3-butanediol, adenosine, inosine, uridine, adenosine-5′-monophosphate, fructose-6-phosphate, glucose-1-phosphate, glucose-6-phosphate, DL-α-glycerol phosphate, D-ribose, D-tagatose, D-xylose, acetic acid, propionic acid, D-alanine, putrescine, maltose, potassium gluconate, adipic acid, and L-malic acid. The type strain shows the following enzyme activities: alkaline phosphatase, elastase (C4), elastase lipase (C8), lipase (C4), leucine arylamidase, acid phosphatase, and naphthol-AS-BI-phosphohydrolase.

In the disc susceptibility test, cells are susceptible to tetracycline (30 μg/disc), polymyxin B (300 IU/disc), amikacin (30 μg/disc), chloramphenicol (30 μg/disc), erythromycin (15 μg/disc), ciprofloxacin (5 μg/disc), tobramycin (10 μg/disc), vancomycin (30 μg/disc), kanamycin (30 μg/disc), gentamicin (10 μg/disc), ampicillin (10 μg/disc), and imipenem (10 μg/disc). Conversely, cells are resistant to aztreonam (30 μg/disc), ceftazidime (30 μg/disc), nalidixic acid (1 μg/disc), norfloxacin (10 μg/disc), and oxacillin (1 μg/disc).

DNA G+C content (mol%): 72.0 (HPLC).

Type strain: KV-614, NRRL B-24346, JCM 12834, NBRC 100761.

Sequence accession no. (16S rRNA gene): AB193261.

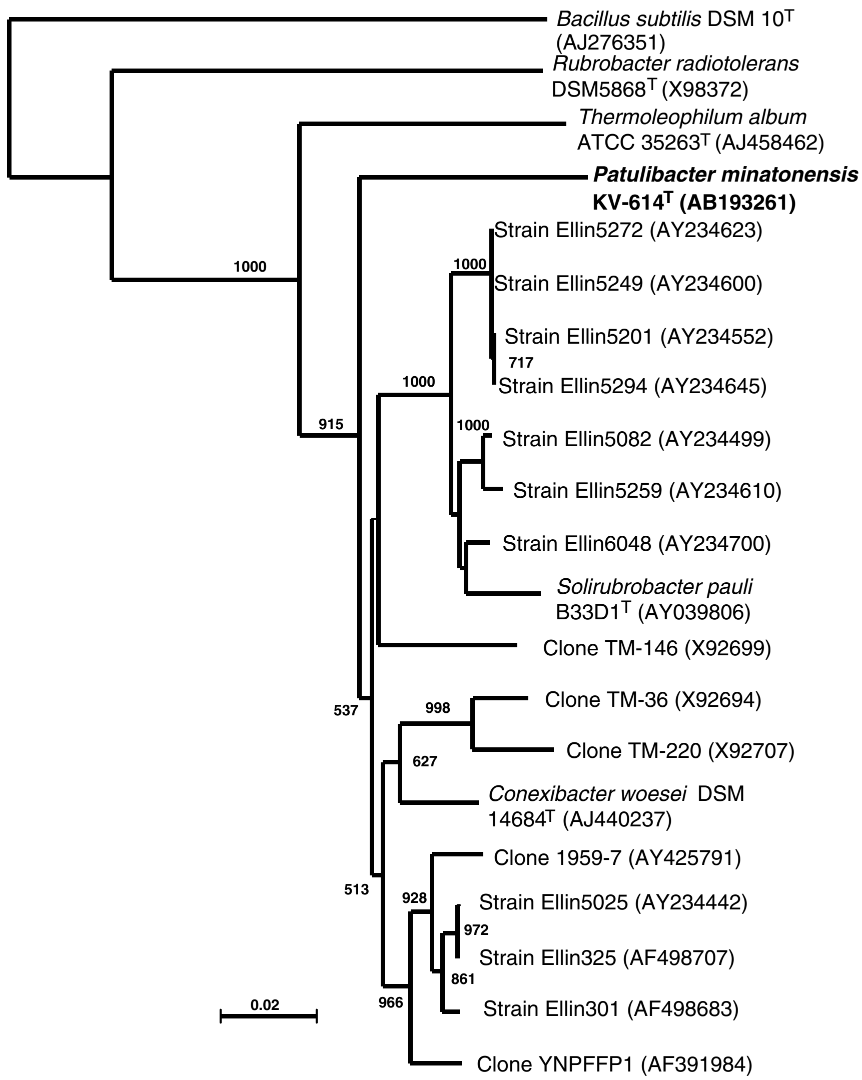

FIGURE 431. Phylogenetic tree constructed based on 16S RNA gene sequences of *Patulibacter minatonensis* KV-614 and its closest clonal and cultured relatives. Numbers at nodes are bootstrap values based on 1000 resamplings; only values higher than 500 are shown. Bar = 2 inferred nucleotide substitutions per 100 nt. The tree was rooted with *Bacillus subtilis*. (Reprinted with permission from Takahashi et al., 2006. Int. J. Syst. Evol. Microbiol. *56*: 401–406.)

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